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Developmental plasticity of growth and digestive efficiency in dependence of early-life food availability

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Summary

1. Nutrition is a potent mediator of developmental plasticity. If food is scarce, developing organisms may invest into growth to outgrow size-dependent mortality (short-term benefit) and/or into an efficient digestion system (long-term benefit).

2. We investigated this potential trade-off, by determining the influence of food availability on juvenile body and organ growth, and on adult digestive efficiency in the cichlid fish *Simochromis pleurospilus*.

3. We reared two groups of fish at constant high or low food rations, and we switched four other groups between these two rations at an early and late juvenile period. We measured juvenile growth and organ sizes at different developmental stages and determined adult digestive efficiency.

4. Fish kept at constant, high rations grew considerably faster than low-food fish. Nevertheless, *S. pleurospilus* partly buffered the negative effects of low food availability by developing heavier digestive organs, and they were therefore more efficient in digesting their food as adults.

5. Results of fish exposed to a ration switch during either the early or late juvenile period suggest (i) that the ability to show compensatory growth after early exposure to low food availability persists during the juvenile period, (ii) that digestive efficiency is influenced by varying juvenile food availability during the late juvenile phase and (iii) that the efficiency of the adult digestive system is correlated with the growth rate during a narrow time window of juvenile period.

Key-words: cichlid fish, digestive efficiency, early environment, growth rate, Lake Tanganyika, phenotypic plasticity, plasticity window

Introduction

The ability of organisms to respond to the environmental conditions encountered during ontogeny by phenotypic change ('developmental plasticity') is a pervasive feature of life (Schmalhausen 1949; Bradshaw 1965; Schlichting & Pigliucci 1998; West-Eberhard 2003). It has been demonstrated in virtually all organisms investigated so far, from fungi (Brasier 1987) to humans (Bateson *et al*. 2004), and at all levels of phenotype organization including morphology, life history, behaviour, physiology and the genome (West-Eberhard 2003; Aubin-Horth & Renn 2009). Long-term experiments showed that the early environment can affect a broad range of phenotypic traits persistently. For example, the food availability experienced during early development can shape life-history trajectories (Bashey, 2006, Taborsky 2006a,b; Barrett *et al*. 2009), adult behaviour (Scheuber, Jacot & Brinkhof 2004; Holveck & Riebel 2010) and learning ability (Kotrschal & Taborsky 2010a).

These adaptations to early nutrition are likely to be accompanied by, or even caused by, a reorganization of the underlying physiological function. For example, organisms may cope with harsh food conditions during development by increasing the efficiency of their digestive system to allow for higher energy uptake (e.g. Cox & Secor 2007; Karasov 1996). However, the development of a more efficient digestion is likely to involve physiological costs, such as building and maintaining a larger or more efficient digestive tract (Piersma & Gils 2010). Investment in a higher digestive efficiency early in life should thus compromise other costly body functions such as growth during

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the time when investment into the digestive tract takes place. An early reduction in growth may be particularly critical in organisms where the early survival is strongly determined by body size, such as in many aquatic species (Sogard 1997).

Balancing the costs and benefits of physiological adaptations towards an increased energy uptake are particularly important during the early life stages of organisms that are exposed to negative size-dependent mortality. Individuals growing up in a food-limited environment may thus have to choose between strategies yielding either immediate or delayed survival benefits. Maximizing early growth may allow them to outgrow more quickly the period of highest size-dependent mortality. Investing into a more efficient digestive system may instead force them to grow more slowly at the onset of life, but later on, they can benefit from utilizing sparse food more economically. Here, we test these alternative strategies by investigating how differential resource availability during the juvenile period mediates growth in juveniles and digestive efficiency in adult animals. We use the mouthbrooding cichlid fish *Simochromis pleurospilus* as model species, as it has been shown that the juvenile feeding conditions influence the entire life history of these fish (Taborsky 2006a,b; Kotrschal & Taborsky 2010a).

Because most studies of developmental plasticity expose the study organisms to different environmental conditions during a single period in life only and compare their performance later in life (Taborsky 2006a,b; Barrett *et al*. 2009; Segers & Taborsky 2012), they cannot identify potential critical periods in which individuals are sensitive to environmental change (Dufty, Clobert & Moller 2002). It is likely that the scope of plasticity changes over time, because plasticity should increase due to the increasing amount of information organisms can gather with age, but it should decrease as organisms increasingly specialize on a given environment (Dufty, Clobert & Moller 2002). Such opposing tendencies may give rise to 'plasticity windows', that is, developmental time windows during which an organism is prone to respond phenotypically plastic towards environmental change (Fischer *et al*. 2014; Hoverman & Relyea 2007). The second aim of this study is thus to identify possible plasticity windows for growth and digestive efficiency during the juvenile period.

Material and methods

STUDY SPECIES

Simochromis pleurospilus is a maternally mouthbrooding cichlid of the subfamily Tropheini endemic to Lake Tanganyika, East Africa. It lives along the rocky shores of the lake where it feeds on epilithic turf algae. *S. pleurospilus* reproduce all year-round and adult males defend small, adjoining territories of 2–4 m^2 where females visit them to spawn (Kotrschal & Taborsky 2010b). Juveniles and females are non-territorial and use large home ranges. After spawning, females leave the male territory immediately and care for the clutch on their own (Taborsky 2006b). Four weeks after spawning, the young are independent. Juveniles and adults live partly in sympatry, but juveniles are confined to the shallow areas between 0·5 and 2 m depth, and adults inhabit a greater depth range (Kotrschal *et al*. 2012). As the productivity of turf algae declines rapidly with water depth (Taborsky 1999), both juveniles and adults may encounter substantial variation in food availability, which is paralleled by variation in body reserves (Kotrschal, Fischer & Taborsky 2011).

GENERAL EXPERIMENTAL METHODS

We reared 130 individuals of the F1 generation of wild-caught *S. pleurospilus* in separate 20-litre Plexiglas tanks, each equipped with a layer of sand, a flower pot half for shelter and an internal biological filter. The experimental fish were derived from seven clutches of different females, and siblings were distributed over all treatments in proportion to the treatment sample sizes. Throughout this study, we refer to time points during the experiment in weeks starting from week 0, which denotes the moment when juveniles were placed in their individual holding tanks (Fig. 1). At this time, the fish were about 21 days old.

During their juvenile period, we exposed the fish to six different feeding conditions. Fish either received (i) a high food ration always (abbreviated as N_{HH}, where 'N' stands for 'not switched' and H for 'high'; $n = 40$); (ii) a low (L) food ration always (N_{LL}, $n = 40$); (iii) a high food ration, switched to a low food ration at week 8 (S_{HL8}, where 'S' stands for 'Switched'; $n = 10$); (iv) a high food ration, switched to a low food ration at week 16 (S_{HL16}; $n = 10$); (v) a low food ration, switched to a high food ration at week 8 (S_{HL8}, $n = 10$); or (vi) a low food ration switched to a high food ration at week 16 (S_{LH16}, $n = 10$). Fish were fed 6 days a week with standardized agarose cubes containing Tetramin flake

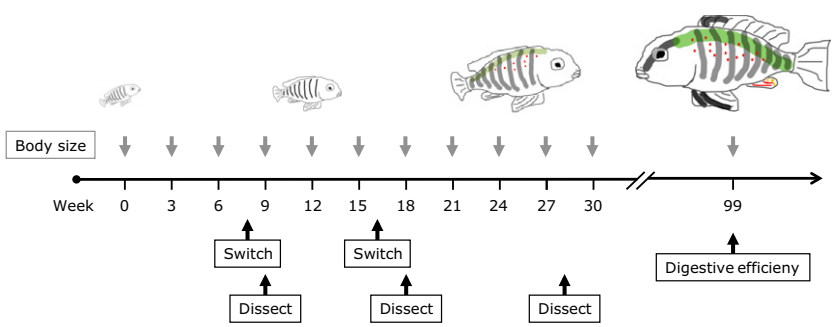

Fig. 1. Timeline of food experiment. At independence (week 0), we separated individual *Simochromis pleurospilus* and reared them in individual tanks. At week 8 and week 16, we switched subsets between treatments. We measured body size (weight and length) of all fish every 3 weeks until week 30, and at week 99; we also dissected a subsample of individuals for organ measurements at week 9, week 18 and week 28, and determined the digestive efficiency (DE) at week 99.

food enriched by 5% *Spirulina* algae. The cubes contained an amount of Tetramin corresponding to 12% (high food or 'H' treatment; corresponds to near *ad libitum* ration) or 4% (low food or 'L' treatment) of mean body weight. All fish of a treatment group received the same food ration, which was based on the mean body mass of fish within this group. We adjusted the food rations to increasing body weight every 2 weeks following a feeding scheme developed for our study species for the entire ontogeny until adulthood by Taborsky (2006b). We stopped adjusting the rations to body weight in N_{HH} fish at week 24, because they no longer depleted the food cubes. We continued to adjust the rations of N_{LL}, S_{LH} and S_{HL} fish to increasing mass with the feeding scheme of Taborsky (2006b) until week 34 when they reached the same body size as N_{HH} fish. Thereafter, all fish were kept on the same N_{HH} food ration, independent of fish size. Fish that were switched between diets received the respective age-specific food rations of N_{LL} and N_{HH} fish. As in other mouthbrooding fish, *S. pleurospilus* females spontaneously spawned without a male being present and kept their eggs in their buccal cavity for some days (cf. Taborsky 2006a). Therefore, we checked females daily for the presence of eggs to obtain an indicator for the onset of female maturation.

We measured lengths and weights of fish every 3 week during the juvenile period (until week 30) and again around week 99 (see Fig. 1). The juvenile period was characterized by near-linear growth and ended at the onset of maturity (Taborsky 2006b, this study). Standard lengths (SL, from the tip of the snout to the end of the caudal peduncle) were read from a measuring board with a 1-mm grid and were estimated to the nearest 0·5 mm by eye. Weights were read to the nearest 1·0 mg from an electronic balance. All measurements were taken before the daily feeding by the same person (AK).

Because a few fish died in the course of the 2 years of our experiment and some samples were lost in various stages of laboratory analyses, we give sample sizes in the Results section where necessary.

MORPHOLOGY

To determine how different food availability may affect the development of the digestive system, we dissected 12 N_{HH} and 12 N_{LL} fish, equally spread over three time points during the juvenile period (week 9, week 18 and week 28). Before dissecting the fish, we measured SL. Then, we took fresh weights of two organs that can be assumed to be unrelated to digestion (heart and eye) and of the two main digestive organs (liver and gut) to the nearest 0·1 mg, and measured gut length after carefully uncoiling the gut. Fish were starved for 48 h prior to dissections to ensure complete gut evacuation. We used five separate general linear models (GLM) with the organ of interest as dependent variable, feeding regime (H or L) as fixed factor, and SL and dissection age as covariates. Because of the limited sample size for each time point, we control for age and body size in the overall model, but in Fig. 2, we show the estimated marginal means of organ sizes for low and high food rations combining the three juvenile age classes. Because we had clear predictions for each one of the organs (gut and liver should be larger in low-food fish; heart and eye should not differ between rations), we kept $\alpha = 0{\cdot}05$ and did not employ multiple testing corrections (Nakagawa 2004). We did not determine organ parameters in adult fish because the fish were needed for other studies.

GROWTH PARAMETERS

Specific growth rate

We determined the specific growth rate of standard length (SGR; % growth per day) per 3-week period of each individual fish during the juvenile period (Fig. 1) as

$$\mathrm{SGR} = \frac{\mathrm{InSl}_2 - \mathrm{InSl}_1}{\mathrm{age}_2 - \mathrm{age}_2} * 100$$

where SL_1, SL_2, age_1 and age_2 are initial and final sizes and ages of two successive measurements (Ricker 1979).

Growth trajectories

To test whether the fish responded to our N_{HH} and N_{LL} food treatments, we compared repeated measures of body sizes during the juvenile period (from week 0 to week 30) in a general linear

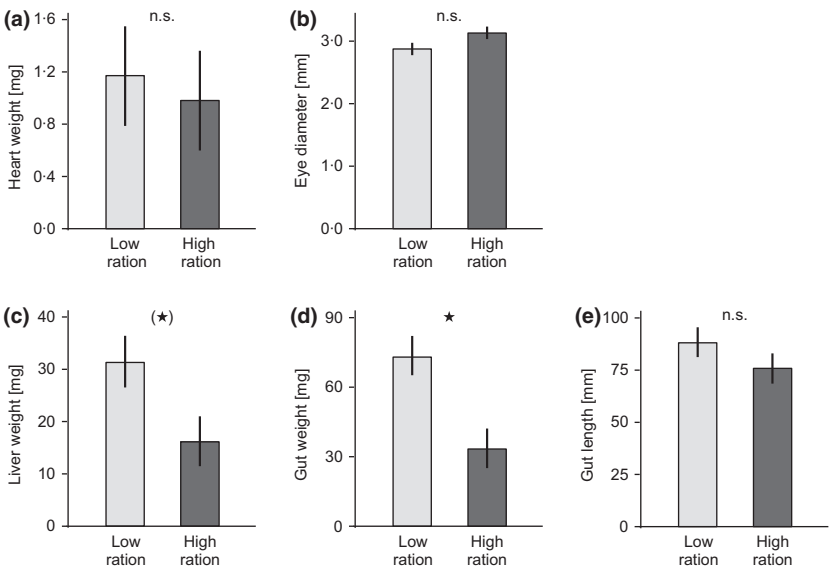

Fig. 2. Size of organs of juvenile *Simochromis pleurospilus* fed low and high food rations. The estimated marginal means (± SE) derived from GLMs controlling for body size and age at dissection are shown. While the heart (a), eyes (b) and gut lengths (e) were unaffected by food availability, gut mass (d) was greater in fish kept on low food rations and liver weights (c) tended to differ in the same direction.*$P < 0.05$; (*) $P > 0.05$, < 0.10; n.s., $P > 0.10$.

mixed model with SL as dependent variable, age as covariate and individual fish as random effect. To determine the growth of switched fish, we used general linear mixed models (GLMMs) to compare the SGRs before and after the ration switches (the triweekly periods that were closest to before and after the switches, but excluding those periods in which the switch took place). We used the SGRs before and after the switch as dependent variable, the time point (before or after the switch) as fixed factor and fish ID as random effect. Because SGR decreases with increasing body size, we also included body size before the switch as covariate. In addition, we determined how many weeks switched fish needed to reach the mean body size of non-switched fish (i.e. time until S_{LH} fish reach the N_{HH} trajectory and time until S_{HL} fish reached N_{LL} trajectory). We considered the trajectories of non-switched fish as being reached, when the body sizes of the respective switch and non-switched fish did not differ significantly from each other anymore (i.e. $P > 0{\cdot}05$, *t*-tests).

DIGESTIVE EFFICIENCY

Digestive efficiency is the ratio of energy assimilated to energy ingested (Flowerdew & Grove 1980), which indicates how much energy of the consumed food can be utilized. To determine digestive efficiency (DE) of adults, we added 2% chromic oxide as inert marker to the food pellets and fed them to the fish on two consecutive days before collection of their faeces in week 99 (Fig. 1). By determining the amount of chromic oxide in the faeces, we could determine how much food the fish ingested (McGinnis & Kasting 1964). Note that we did not determine the fraction of energy in the faeces from sloughed intestinal cells and mucus. Our measures therefore reflect 'apparent digestive efficiency', which represents a minimum value for the energy taken from food (Throckmorton 1973). One evening in week 99, we placed all animals in clean 10 × 10 × 15 cm net cages equipped with a piece of opaque plastic tube as shelter within the holding tanks. The net cages had a mesh size of < 0·2 mm and therefore collected all faeces. The next morning (after 16 h in the cages), we removed the fish from the cages, syphoned all faeces from the cage, dried them at 65 °C for 24 h and weighed them to the nearest 0·1 mg. To assure that 16 h are sufficient for full gut evacuation, we had performed a pilot study. We had fed chromic-oxide-marked food to 10 *S. pleurospilus* that were not part of this experiment and had placed them in net cages for 24 h after feeding. Subsequently, their faeces had been collected every 2 h. Most faeces were excreted 4–8 h post-feeding, and we did not detect any faeces after 16 h post-feeding.

About 1 mg of the dried faeces was used to determine Cr levels: microwave digestion of samples was performed in 7 ml 60:40 nitric acid and hydrogen peroxide mixture (HNO_3 65%, H_2O_2 30%, both suprapure grade). 93 ml of ultrapure water was added, and the solution was separated in two fractions. One fraction was filtered (0·45 μm), and Cr was determined by ICP-OES (inductive coupled plasma optical emission spectrometry) using a Varian Liberty 150 AX Turbo. The other fraction was diluted 1:100 with 3% nitric acid, Co was added as internal standard, and Cr was analysed by ICP-CRI-MS (inductively coupled plasma collision/reaction interface mass spectrometry) with a Varian 820-MS applying 80 mL min^{-1} hydrogen as collision gas to the sampler cone. Cr analyses of ICP-OES and ICP-MS were compared for quality insurance. The rest of the sample (2–10 mg) was used to determine energy content; we determined energy content (J g^{-1}) from food and faeces by bomb calorimetry using an adiabatic calorimeter (IKA 4000, Janke & Kunkel, Staufen, Germany) with a microbomb insert. Following Flowerdew and Grove (1980), digestive efficiency was calculated as:

$$\text{Efficiency}(\%) = 100 * \left(\frac{Cr_2O_3/\text{Energy}_{\text{food}}}{Cr_2O_3/\text{Energy}_{\text{faeces}}}\right)$$

To investigate the influence of juvenile food availability on adult DE, we performed three analyses with increasing specificity. (i) We determined the amount of food received by each individual by calculating the percentage of food mass contained in the food pellets relative to the body mass of individuals using data from our triweekly body mass measurements. We then took the arithmetic mean of these values during the entire juvenile period (i.e. until week 30) as a measure of food available to individual fish and correlated these values with DE. (ii) As DE did not differ between early- and late-switched groups, we pooled the S_8 and S_{16} groups and performed one GLM on the resulting four treatment groups (N_{HH}, N_{LL}, S_{LH} and S_{HL}). For this model, we used food availability before ('early') and after ('late') the switch as factors, full factorial design, log-link, deviance scaling parameter; (Norusis 2007) and DE as dependent variable. Since all interactions were non-significant ($P > 0{\cdot}4$), we excluded them from the final model. (iii) Then, we related the 10 triweekly juvenile growth periods (SGRs, from weeks 0–3 until weeks 27–30) to adult digestive efficiency in 10 separate analyses: To ensure that the natural decrease in SGR during the juvenile period of fish does not bias our results, we performed GLMs with DE as dependent variable, the respective SGR, and the respective body size at the start of every growth period as covariates. In all analyses, DE values were square root-transformed to meet normality criteria. To minimize the chance of a type I error through multiple testing, we applied sequential Bonferroni correction (Holm 1979). All analyses were done with SPSS 19·0, SPSS Inc., Chicago.

Results

MORPHOLOGY

While the organs unrelated to digestion did not differ between N_{HH} and N_{LL} fish (heart weight and eye diameter; Table 1; Fig. 2a,b), gut weight (but not length) was greater in N_{LL} after controlling for body size (Table 1; Fig. 2d,e),

Table 1. Morphometric parameters of organs in relation to ration, age and body size (standard length) of *Simochromis pleurospilus* fed low ($N = 12$) and high food ($N = 12$) rations and dissected at three time points during juvenile development

	Heart mass		Eye mass		Liver mass		Gut mass		Gut length	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Food ration	0·067	0·798	2·471	0·133	3·068	*0·097*	5·680	**0·028**	0·955	0·341
Age	0·126	0·727	3·214	*0·090*	2·392	0·139	4·008	0·061	0·384	0·543
Body size	1·816	0·194	2·635	0·122	13·788	**0·002**	18·317	**< 0·001**	11·819	**0·003**

Model results of five GLMs; *P*-values <0·05 are highlighted in bold; *P*-values <0·1 are highlighted in italics.

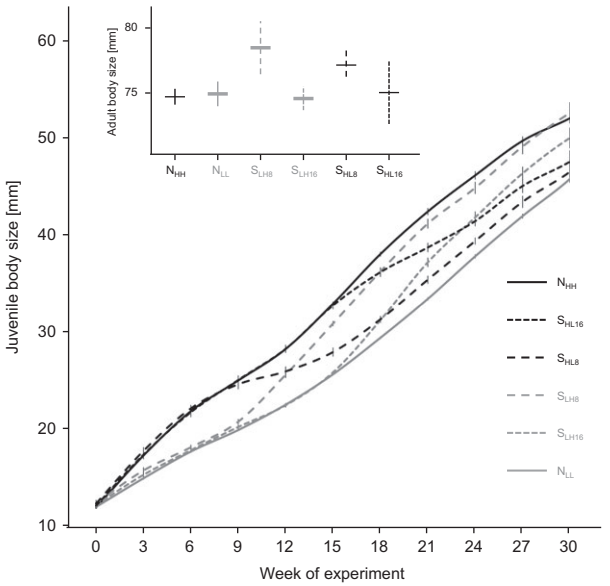

Fig. 3. Growth curves of juvenile *Simochromis pleurospilus* reared on different rations (± SE) until an age of 30 weeks. N_{LL} – constant low food rations; N_{HH} – constant high food rations; S_{LH8} – switched from low to high food at week 8; S_{LH16} – switched from low to high food at week 16; S_{HL8} – switched from high to low food at week 8; S_{HL16} switched from high to low food at week 16. The top left insert depicts the body size of adult fish (± SE) at week 99.

and liver weight tended to differ in the same direction (Table 1; Fig. 2c).

LIFE HISTORY

Juvenile growth of N_{LL} and N_{HH} was almost linear and diverged markedly between treatments. As expected, juvenile size (up to week 30) was significantly larger for any given time in N_{HH} fish, than in N_{LL} fish (GLMM: food ration: $F_{1,71} = 513{\cdot}64$, $P < 0{\cdot}0001$; age: $F_{9,71} = 22134{\cdot}29$, $P < 0{\cdot}0001$; N_{HH}: $N = 31$; N_{LL}: $N = 24$; Fig. 3). Individuals reared on high food rations reached maturity about 2·5 weeks earlier (*t*-test: $t = 5{\cdot}10$, $P < 0{\cdot}001$; age of first spawning: N_{HH}: week 22·1 ± 0·6 SE; $N = 9$; N_{LL}: week 24·5 ± 0·6 SE; $N = 12$) and at smaller body sizes (*t*-test: $t = 6{\cdot}12$, $P < 0{\cdot}001$) than fish reared on low food rations.

As adults (week 99), when all fish were fed the same rations, they were of a similar body size (*t*-test: $t = 0{\cdot}19$, $P = 0{\cdot}85$; N_{HH}: $N = 31$; N_{LL}: $N = 24$) and weight (*t*-test: $t = 1{\cdot}06$, $P = 0{\cdot}29$).

GROWTH TRAJECTORIES

The S_{LH8} fish accelerated their growth rates after the switch (GLMM: time point: $F_{1,12{\cdot}9} = 11{\cdot}70$, $P = 0{\cdot}005$, body size: 24, $F_{1,11{\cdot}2} = 20{\cdot}59$, $P = 0{\cdot}001$; $N = 10$, Fig. 3) and reached the body sizes of fish always fed high food rations at week 28·4 ± 1·9 SE. These fish even outgrew the N_{HH} fish from around week 30 (Fig. 3) and were larger and heavier than N_{HH} fish as adults (SL at week 99, *t*-test: $t = 2{\cdot}39$, $P = 0{\cdot}022$; weight, $t = 2{\cdot}99$, $P = 0{\cdot}005$; N_{HH}: $N = 31$, S_{LH8}: $N = 7$, Fig. 3). The S_{LH16} fish also accelerated their growth rates after the switch (GLMM: time point: $F_{1,14{\cdot}3} = 8{\cdot}09$, $P = 0{\cdot}013$, body size: d.f. = $F_{1,14{\cdot}5} = 6{\cdot}30$, $P = 0{\cdot}024$, $N = 10$), but they did not exceed the N_{HH} trajectory (SL at week 99, *t*-test: $t = 0{\cdot}23$, $P = 0{\cdot}82$, N_{HH}: $N = 31$, S_{LH16}: $N = 10$, Fig. 3). S_{LH8} and S_{LH16} fish took equally long to catch up with the N_{HH} fish (S_{LH8} 17·8 week (± 2·0 SE), S_{LH16} 18·0 week (± 1·7 SE); *t*-test: $t = 0{\cdot}07$, $P = 0{\cdot}946$; Fig. 3).

The growth rate of S_{HL8} fish dropped slightly after the switch (GLMM: time point: $F_{1,11{\cdot}8} = 4{\cdot}65$, $P = 0{\cdot}052$, body size: $F_{1,10{\cdot}2} = 4{\cdot}153$, $P = 0{\cdot}068$. $N = 9$), but then growth accelerated again. Surprisingly, these animals grew faster than N_{LL} fish as in the end (week 99), they had reached body sizes that tended to be even larger than fish kept on a constant *high* food ration (*t*-test: $t = 2{\cdot}25$, $P = 0{\cdot}054$; N_{HH}: $N = 31$, S_{HL8}: $N = 5$, Fig. 3).

The S_{HL16} fish showed no significant reduction in growth rates after the switch (GLMM: time point: $F_{1,15{\cdot}0} = 0{\cdot}02$, $P = 0{\cdot}900$, body size: $F_{1,15{\cdot}0} = 5{\cdot}01$, $P = 0{\cdot}041$, $N = 9$), and they steadily approached the N_{LL} growth trajectory (Fig. 3). S_{HL16} fish tended to reach the N_{LL} growth curve faster than S_{HL8} fish (S_{HL8} 18·9 week (± 1·0 SE), S_{HL16} 17·0 week (± 1·1 SE); *t*-test: $t = 1{\cdot}84$, $P = 0{\cdot}086$).

DIGESTIVE EFFICIENCY

Fish that received the N_{LL} treatment were more efficient in using energy than fish that received N_{HH} (91.5 % vs. 84.3 %; *t*-test, $F = 8{\cdot}28$, $P = 0{\cdot}019$, $N_{HH} = 28$, $N_{LL} = 22$; only fish kept on constant rations). When including all experimental fish (switched and non-switched individuals), the mean juvenile food availability received by each individual correlated negatively with its adult digestive efficiency (Pearson: $r = -0{\cdot}314$, $P = 0{\cdot}011$, $N = 75$). We furthermore found that late, but not early, juvenile food availability influenced adult digestive efficiency (GLM: early food availability: $F_{1,71} = 0{\cdot}03$, $P = 0{\cdot}87$; late food availability: $F_{1,71} = 5{\cdot}0$, $P = 0{\cdot}028$; $N = 28$ (high rations), 22 (low rations), 15 (low-high rations), 10 (high-low rations); Fig. 4). Fish receiving high food rations late in their juvenile period showed lower digestive efficiencies. Furthermore, we identified the particular time window within the juvenile period, which influenced adult digestive efficiency most strongly (see 'Methods' section); DE was strongly negatively correlated with SGR between week 12 and week 15 only, but not with growth rates in all other 3-week periods (Table 2). The faster the fish grew between week 12 and week 15, the less efficient was their adult digestive system.

Discussion

The juvenile growth trajectories of *S. pleurospilus* were strongly determined by food availability. The results of fish with switched rations showed that the potential for growth

Table 2. Using ten separate GLMs, we tested the relationship between specific growth rates (SGRs) during all juvenile triweekly periods and adult digestive efficiency (DE), controlled for the size of the fish at the start of the respective period (standard length – SL) in *Simochromis pleurospilus* (all $N = 75$, significant P value highlighted in bold)

Parameter	d.f.	F	P
SGR week 0–3	1, 72	0·19	0·666
SL week 0	1, 72	1·68	0·199
SGR week 3–6	1, 72	0·27	0·602
SL week 3	1, 72	0·06	0·814
SGR week 6–9	1, 72	0·04	0·849
SL week 6	1, 72	0·02	0·901
SGR week 9–12	1, 72	0·08	0·778
SL week 9	1, 72	0·01	0·911
SGR week 12–15	**1, 72**	**9·09**	**0·004**
SL week 12	1, 72	0·01	0·921
SGR week 15–18	1, 72	0·01	0·911
SL week 15	1, 72	1·21	0·275
SGR week 18–21	1, 72	0·10	0·746
SL week 18	1, 72	1·25	0·268
SGR week 21–24	1, 72	1·11	0·296
SL week 21	1, 72	2·33	0·132
SGR week 24–27	1, 72	0·11	0·737
SL week 24	1, 72	2·05	0·156
SGR week 27–30	1, 72	0·14	0·712
SL week 27	1, 72	2·49	0·120

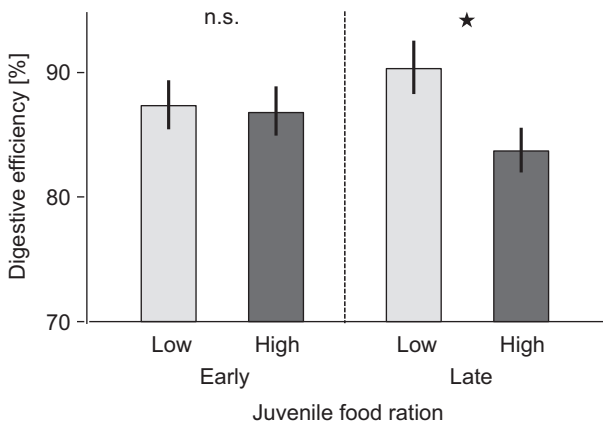

Fig. 4. Effects of juvenile ration on adult digestive efficiency in *Simochromis pleurospilus*. Fish kept on low food rations late during their juvenile period developed a more efficient digestive system as adults (estimated marginal means (± SE) derived from a GLM with digestive efficiency as dependent variable and early and late food ration as fixed effects). $*P < 0.05$; n.s., $P > 0.10$.

compensation is retained during the most of the juvenile period. Fish reared on low food rations had a more efficient digestive system as adults, probably because they developed heavier digestive organs during the juvenile phase, suggesting a long-term adaptation to low food availability. The response of the level of digestive efficiency to juvenile ration appears to occur relatively late during the juvenile phase.

GROWTH TRAJECTORIES

In fish and many other aquatic organisms, mortality usually decreases strongly with increasing body size, because the most important aquatic predators are gape-size limited (Sogard 1997). Therefore, young fish should be expected to prioritize early growth, which is indeed reflected in the early growth patterns of our experimental fish. Fish kept on a higher food availability grew faster (see also Taborsky 2006b), and fish switched from a low to a high food ration showed compensatory growth (Ali *et al.* 2003, Dmitriew, 2011). The groups switched from low to high rations early and late in the juvenile period took similarly long to catch up in size with the N_{HH} fish and showed similarly strong growth acceleration to food increase (cf. Fig. 3). Most likely, fish can benefit from retaining the ability to compensate throughout their juvenile period (this study) or even beyond (Taborsky 2006b), because of the key importance of large size for survival, particularly in aquatic environments (Sogard 1997). In contrast, in S_{HL} fish, changes in growth occurred faster when fish were switched later in life (S_{HL16}), compared to early switched fish. This is surprising, as the S_{HL16} fish probably had stored more energy (fat storage in *S. pleurospilus* increases allometrically with body size with larger animals storing relatively more fat (Kotrschal, Fischer & Taborsky 2011)), which should have enabled them to buffer the sudden food restriction.

DIGESTIVE EFFICIENCY

Simochromis pleurospilus reared on a restricted diet had a higher digestive efficiency than fish fed near *ad libitum* rations, and across all experimental fish, relative juvenile food availability was negatively correlated with adult DE. Taken together, these results suggest that fish reared on low food availability developed a higher DE to buffer the negative effects of an adverse environment. Models derived from digestive theory (Sibly 1981) delineate important gastrointestinal features that influence digestive efficiency: the volume of the gastrointestinal tract, the digestion rates due to levels of pancreatic and intestinal enzymes and microbial activity, the nutrient absorption rate and the digestive retention time. Animals living on poor diets should therefore have larger digestive chambers than those on rich diets. This is the case in animals specializing on food that is difficult to digest, such as snakes specializing on different prey types (Britt, Hicks & Bennett 2006), starlings adjusting to a low-fibre diet (Geluso & Hayes 1999), cichlid species occupying diverse dietary niches during adaptive radiation (Sage & Selander 1975). Similarly, larger digestive chambers are favoured when animals specialize on food that is bulky relative to its nutritional content, such as coprophagous voles (Lee & Houston 1993) or secondarily

herbivorous lizards (Vervust *et al*. 2010). A plastic increase in digestive organ size in response to lower food availability has so far never been reported. Here, we found that *S. pleurospilus* reared on a N_{LL} ration developed heavier guts and tended to have heavier livers, which both are likely to have increased their DE. Because the guts of N_{LL} fish were heavier, but not longer, a higher digestive efficiency might have been achieved by a structural change in gut morphology, such as more microvilli or a better vascularization (Govoni, Boehlert & Watanabe 1986).

Studies investigating the relationship between ration and DE so far mostly focused on short- and medium-term effects: for example, in dab (*Limanda limanda*, Jobling, Gwyther & Grove 1977), in perch (*Perca fluviatilis*, Solomon & Brafield 1972) and in side-blotched lizards (*Uta stansburiana*, Waldschmidt, Jones & Porter 1986), DE decreased with increasing meal size. The only study we are aware of and where animals were reared on different rations to determine their later digestive efficiency (Cox & Secor 2007) found no effect of rearing conditions on DE. These results are not directly comparable to our experiment, however, as Cox & and Secor (2007) measured DE, while the pythons (*Python molurus*) were still kept at the different experimental rations at which they had been reared. Our study thus is the first to show that juvenile food availability can determine the digestive efficiency of similar-sized adults living under identical ecological conditions.

When jointly analysing the data of fish which had and had not experienced a ration switch, we found that food availability determined DE in *S. pleurospilus* particularly during the later juvenile period: In individuals exposed to a food switch, only the ration received after the switch influenced digestive efficiency. This suggests that the late juvenile stage is a critical period when digestive efficiency is determined in *S. pleurospilus*. However, between week 12 and week 15, growth rate was negatively correlated with adult DE, which might indicate the existence of a critical period in the development of DE already slightly earlier in life. Thus, at the current stage of our knowledge, it is difficult to conclude whether or not DE is shaped during a particular critical sensitive period. Theory predicts that in most environments, organisms should not respond to environmental cues right at the beginning of life, but should first collect information about environmental states before making costly phenotypic adjustments (Fischer *et al*. 2014). Even if our results render it difficult to identify a particular critical period in the development of DE, they suggest that the response of DE to the juvenile ration occurs relatively late in the juvenile period, which is in line with these theoretical predictions.

In conclusion, our results show that juvenile food availability exhibits immediate and lifelong effects on growth and digestive efficiency. It is therefore crucial to incorporate early individual dietary history when attempting to explain individual variation in physiology found in natural populations.

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References

Ali, M., Nicieza, A. & Wootton, R.J. (2003) Compensatory growth in fishes: a response to growth depression. *Fish and Fisheries*, **4**, 147–190.

Aubin-Horth, N. & Renn, S.C.P. (2009) Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Molecular Ecology*, **18**, 3763–3780.

Barrett, E.L.B., Hunt, J., Moore, A.J. & Moore, P.J. (2009) Separate and combined effects of nutrition during juvenile and sexual development on female life-history trajectories: the thrifty phenotype in a cockroach. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 3257–3264.

Bashey, F. (2006) Cross-generational environmental effects and the evolution of offspring size in the Trinidadian guppy *Poecilia reticulata*. *Evolution*, **60**, 348–361.

Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., D'udine, B., Foley, R.A. *et al*. (2004) Developmental plasticity and human health. *Nature*, **430**, 419–421.

Bradshaw, A.D. (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, **13**, 115–155.

Brasier, C.M. (1987) *The Dynamics of Fungal Speciation*. Cambridge University Press, Cambridge.

Britt, E.J., Hicks, J.W. & Bennett, A.F. (2006) The energetic consequences of dietary specialization in populations of the garter snake, *Thamnophis elegans*. *Journal of Experimental Biology*, **209**, 3164–3169.

Cox, C.L. & Secor, S.A. (2007) Effects of meal size, clutch, and metabolism on the energy efficiencies of juvenile Burmese pythons, *Python molurus*. *Comparative Biochemistry and Physiology A – Molecular & Integrative Physiology*, **148**, 861–868.

Dmitriew, C.M. (2011) The evolution of growth trajectories: what limits growth rate? *Biological Reviews*, **86**, 97–116.

Dufty, A.M., Clobert, J. & Moller, A.P. (2002) Hormones, developmental plasticity and adaptation. *Trends in Ecology & Evolution*, **17**, 190–196.

Fischer, B., Van Doorn, G.S., Dieckmann, U. & Taborsky, B. (2014) The evolution of age-dependent plasticity. *American Naturalist*, **183**, 108–125.

Flowerdew, M.W. & Grove, D.J. (1980) An energy budget for juvenile thick-lipped mullet, *Crenimugil labrosus* (Risso). *Journal of Fish Biology*, **17**, 395–410.

Geluso, K. & Hayes, J.P. (1999) Effects of dietary quality on basal metabolic rate and internal morphology of European starlings (*Sturnus vulgaris*). *Physiological and Biochemical Zoology*, **72**, 189–197.

Govoni, J.J., Boehlert, G.W. & Watanabe, Y. (1986) The physiology of digestion in fish larvae. *Environmental Biology of Fishes*, **16**, 59–77.

Holm, S. (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, **6**, 65–70.

Holveck, M.J. & Riebel, K. (2010) Low-quality females prefer low-quality males when choosing a mate. *Proceedings of the Royal Society B-Biological Sciences*, **277**, 153–160.

Hoverman, J.T. & Relyea, R.A. (2007) How flexible is phenotypic plasticity? Developmental windows for trait induction and reversal. *Ecology*, **88**, 693–705.

Jobling, M., Gwyther, D. & Grove, D.J. (1977) Some effects of temperature, meal size and body-weight on gastric evacuation time in dab *Limanda limanda* (L). *Journal of Fish Biology*, **10**, 291–298.

Karasov, W.H. (1996) Digestive plasticity and avian energetics in feeding ecology. *Avian Energetics and Nutritional Ecology* (ed C. Carey), pp. 61–84. Chapman Hall, New York, NY.

Kotrschal, A., Fischer, B. & Taborsky, B. (2011) An noninvasive method to determine fat content in small fish based on swim bladder size estimation. *Journal of Experimental Zoology Part a-Ecological Genetics and Physiology*, **315A**, 408–415.

Kotrschal, A. & Taborsky, B. (2010a) Environmental change enhances cognitive abilities in fish. *Public Library of Science - Biology*, **8**, e1000351.

Kotrschal, A. & Taborsky, B. (2010b) Resource defence or exploded lek – A question of perspective. *Ethology*, **116**, 1–10.
Kotrschal, A., Heckel, G., Bonfils, D. & Taborsky, B. (2012) Life-stage specific environments in a cichlid fish: implications for inducible maternal effects. *Evolutionary Ecology*, **26**, 123–137.
Lee, W.B. & Houston, D.C. (1993) The role of coprophagy in digestion in voles (*Microtus agrestis* and *Clethrionomys glareolus*). *Functional Ecology*, **7**, 427–432.
McGinnis, A.J. & Kasting, R. (1964) Colorimetric analysis of chromic oxide used to study food utilization by phytophagous insects. *Journal of Agricultural and Food Chemistry*, **12**, 259–262.
Nakagawa, S. (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behavioral Ecology*, **15**, 1044–1045.
Norusis, M.J. (2007) *SPSS 15.0 Advanced Statistical Procedures Companion*. Prentica Hall Inc., Chicago.
Piersma, T. & Gils, J.A. (2010) *The Flexible Phenotype. Towards a Body-Centred Integration of Physiology, Ecology and Behaviour*. Oxford University Press, Oxford.
Ricker, W.E. (1979) Growth rates and models. *Fish Physiology* (eds W.S. Hoar, D.J. Randall & J.R. Brett), pp. 677–743. Academic Press, New York, NY.
Sage, R.D. & Selander, R.K. (1975) Trophic radiation through polymorphism in cichlid fishes. *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 4669–4673.
Scheuber, H., Jacot, A. & Brinkhof, M.W.G. (2004) Female preference for multiple condition-dependent components of a sexually selected signal. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **271**, 2453–2457.
Schlichting, C.D. & Pigliucci, M. (1998) *Phenotypic Evolution. A Reaction Norm Perspective*. Sinauer associates, Sunderland.
Schmalhausen, I.I. (1949) *Factors of Evolution*. Blakiston, Philadelphia.
Segers, F.H.I.D. & Taborsky, B. (2012) Juvenile exposure to predator cues induces a larger egg size in fish. *Proceedings of the Royal Society B-Biological Sciences*, **279**, 1241–1248.
Sibly, R.M. (1981) Strategies in digestion and defecation. *Physiological Ecology: An Evolutionary Approach to Resource Use* (eds T. Townsend & P. Calow), pp. 109–138. Blackwell, Oxford.
Sogard, S.M. (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science*, **60**, 1129–1157.
Solomon, D.J. & Brafield, A.E. (1972) The energetics of feeding, metabolism and growth of perch (*Perca fluviatils*). *Journal of Animal Ecology*, **41**, 699–718.
Taborsky, B. (1999) Size-dependent distribution in littoral fish: optimization or competitive exclusion? *Behaviour and Conservation of Littoral Fishes* (eds V.C. Almada, R.F. Oliveira & E.J. Goncalves), pp. 351–376. ISPA, Lisboa.
Taborsky, B. (2006a) Mothers determine offspring size in response to own juvenile growth conditions. *Biology Letters*, **2**, 225–228.
Taborsky, B. (2006b) The influence of juvenile and adult environments on life-history trajectories. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 741–750.
Throckmorton, G. (1973) Digestive efficiency in herbivorous lizard *Ctenosaura pectinata*. *Copeia*, **1973**, 431–435.
Vervust, B., Pafilis, P., Valakos, E.D. & Van Damme, R. (2010) Anatomical and physiological changes associated with a recent dietary shift in the lizard *Podarcis sicula*. *Physiological and Biochemical Zoology*, **83**, 632–642.
Waldschmidt, S.R., Jones, S.M. & Porter, W.P. (1986) The effect of body-temperature and feeding regime on activity, passage time, and digestive coefficient in the lizard *Uta Stansburiana*. *Physiological Zoology*, **59**, 376–383.
West-Eberhard, M. (2003) *Developmental Plasticity and Evolution*. Oxford University Press, Oxford.

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