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The direct cost of amphibian metamorphosis: insights from body weight loss in facultative paedomorphs

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Abstract

Background Metamorphosis is a key event in the life history of many organisms, including amphibians, because it can involve abrupt changes in morphology and habitat. However, metamorphosis can also be bypassed by paedomorphic processes that allow reproduction at the larval stage. The evolution of these alternative developmental processes depends on the payoffs of life in larval and adult environments, as well as the cost of transition. Previous studies on the cost of metamorphosis have focused on the larval stage, which is still subject to the achievement of a minimal size threshold as well as growth to adulthood. Therefore, facultatively paedomorphic species could be valuable models for testing the direct costs of metamorphosis. This is because facultative paedomorphs are sexually dimorphic adults that are capable of metamorphosis.

Results By modelling weight loss using an experimental, longitudinal design that manipulated the ecological drivers of metamorphosis in a facultatively paedomorphic amphibian, the palmate newt (*Lissotriton helveticus*), we found that metamorphosis imposes costs in terms of body weight, whereas temperature has a smaller effect. In contrast, newts that do not metamorphose did not lose weight. The costs were sex-related with females at a disadvantage during metamorphosis. Furthermore, the decrease in food consumption associated with metamorphosis resulted in a loss of body weight.

Conclusions Overall, these results emphasise the importance of considering direct costs when studying the evolutionary ecology of metamorphosis. They also show that polymorphic species are suitable models for investigating the drivers of metamorphosis and its loss in micro- and macroevolution.

Keywords Amphibians, Body mass, Feeding, Facultative paedomorphosis, Growth models, Metamorphosis cost, Palmate newt, Polyphenism

Background

Metamorphosis is an abrupt morphological and physiological change that is widespread in the animal kingdom and is thought to play an important role in both micro- and macroevolution [1–4]. Astonishing examples are numerous and diverse, including the complex transformation of planktonic organisms in echinoderms or eels [5, 6], lateral reorganisation in flatfish [7], wing development in holometabolous insects [8], and the transition from aquatic to terrestrial life in amphibians [9]. Changes can be so striking that larval and post-metamorphic

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Fig. 1 Paedomorphic palmate newts retain gills at the adult stage, but also the ability to metamorphose (facultative paedomorphosis). The picture shows a female from a pond in Larzac, France (photographed by Mathieu Denoël)

stages have been misinterpreted as belonging to different taxonomic groups [10]. While common processes can be shared between distant organisms such as vertebrates, tunicates and cephalochordates [11], convergence between larval stages of different taxa can complicate the understanding of their phylogenies [12] whereas different mechanisms can induce metamorphosis in insects compared to vertebrates and some marine invertebrates [4, 13, 14]

By allowing the colonisation of new environments, metamorphosis provides new opportunities for foraging and living: from the oceans to continental rivers in leptocephalous larvae that become glass eels, from pelagic to benthic habitats in echinoderms and flatfish, from land to air in winged insects [8], and from water to land in biphasic amphibians [1]. Amphibians have been particularly well studied due to their rich diversity, which can be studied under both natural and experimental conditions [15, 16]. The timing of metamorphosis is under selection depending on the payoffs of living in aquatic versus terrestrial environments and the resulting growth and survival in these contrasting habitats [17–19]. For example, when density or temperature increases or water levels decrease—all of which indicate a risk of desiccation detrimental to aquatic life—development is accelerated with an earlier onset of metamorphosis—providing the potential to survive on land [20, 21].

At the extremes, where the constraints of the pre- or post-metamorphic habitat outweigh the benefits of transition, there is potential for the evolution of alternative developmental processes [17, 18, 22]. A striking example is paedomorphosis, the acquisition of sexual maturity in a larval somatic morphology [23] (Fig. 1). Its major role in evolution was first highlighted by Garstang [24] and decades later by the conceptual developments of Gould [3] and McKinney and McNamara [25]. In amphibians, paedomorphosis has evolved repeatedly in Caudata and is now found in nine of the ten families [15]. While it is fixed (obligate paedomorphosis) in several families, it remains a polymorphism (facultative paedomorphosis) in many salamandrids, ambystomatids and plethodontids, and exceptionally in hynobiids [26–29]. Two adult phenotypes are then found in populations, the metamorphs, which have lost their gills at metamorphosis and can live on land, and the paedomorphs, which retain somatic larval traits such as gills and pursue an aquatic life [22] (Fig. 1). Although several specific advantages of paedomorphic life have been demonstrated in facultatively paedomorphic species (e.g. early maturation: [30] or open access to new resources: [31]), evidence for its fixation, i.e. obligate paedomorphosis, is suggested by the high constraints of terrestrial environments (e.g. arid lands) [32–34]. In contrast, life on land can also offer advantages for terrestrial phenotypes in terms of survival and dispersal and ultimately lead to direct development [17, 22, 35].

Despite the benefits of habitat change, metamorphosis remains a costly event, relying on stored lipids and causing mass loss [36, 37]. The consequences of shifts in the timing of metamorphosis can also be costly, with carry-over effects in the following life stage, leading to a reduction in survival, performance and reproductive fitness [38–40]. As larvae require a minimum size for metamorphosis and typically a post-metamorphic period to reach maturity [41], growth models of metamorphosis are inherently constrained by a size threshold as well

Table 1 Model-averaged estimates and *wAIC* relative support for each effect implemented in the trend analysis of body weight for (A) the paedomorphic palmate newts that do not metamorphose and (B) those that metamorphose. *S* for sex (contrast male vs female), *T* for temperature treatment (contrast high vs low), *l* for mean linear effect of time and *q* for mean quadratic effect of time. Relative support was only calculated for explanatory terms that included either the linear or quadratic effect of time. In the case of the mean linear effect of time (*l*) for paedomorphic newts that metamorphosed during the experiments, it could not be estimated as this effect was always required to fit the model for this data set

	(A) Not metamorphosing newts		(B) Metamorphosing newts	
Effect	**Estimate ± SE**	**Relative support**	**Estimate ± SE**	**Relative support**
Intercept	1.347 ± 0.028	-	0.984 ± 0.043	-
S	−0.350 ± 0.057	-	−0.282 ± 0.085	-
T	−0.126 ± 0.059	-	−0.135 ± 0.092	-
*S*T*	0.086 ± 0.110	-	0.028 ± 0.113	-
l	−0.308 ± 0.283	1.5	−9.849 ± 1.309	NA
*l*S*	0.317 ± 0.288	1.1	2.114 ± 2.211	3.3
*l*T*	0.118 ± 0.168	0.5	3.953 ± 2.284	3.5
*l*S*T*	0.036 ± 0.060	0.2	−1.227 ± 1.285	0.6
q	1.084 ± 0.132	> 100	−1.411 ± 0.524	> 100
*q*S*	−0.725 ± 0.257	32.6	1.282 ± 0.841	2.63
*q*T*	0.397 ± 0.225	1.8	3.579 ± 1.210	> 100
*q*S*T*	−0.439 ± 0.338	1.3	0.208 ± 0.398	0.5

as the subsequent acquisition of sexual maturity, which often occurs months or years after metamorphosis [17, 18, 22]. Not only size, but also the differentiation rate is a key indicator of metamorphic potential [42]. In this perspective, facultative paedomorphosis could be a valuable model to test the direct costs of metamorphosis. Indeed, paedomorphs that do not metamorphose can be used as controls to be compared with paedomorphs that metamorphose as they already reached the minimal size for metamorphosis and are also already at the reproductive stage. However, such a direct metamorphic cost has not been investigated using facultative paedomorphs as a model system.

Our aim was to determine the direct costs of metamorphosis using facultatively paedomorphic newts as a model system under contrasting aquatic conditions. Our main hypothesis is that paedomorphic newts incur a cost during metamorphosis in terms of body weight, an essential trait as it provides individuals with essential reserves for sustaining activities, survival and reproductive fitness [43, 44]. Specifically, our hypotheses are (1) that newts approaching metamorphosis lose weight in contrast to those that do not (i.e. that stay paedomorphic), as suggested by metamorphosis costs in amphibian larvae [37], (2) that metamorphosis is more costly for females than for males, as males are more likely to metamorphose than females "the male escape hypothesis": [45, 46], (3) that costs are expected to be exacerbated in warm waters through an increased metabolism rate linked to higher temperatures [37, 42, 45], and (4) that degrowth rates (i.e. body weight loss) observed during metamorphosis are also associated with a reduction in food intake—which in turn contributes to the cost of metamorphosis as suggested by research on anuran tadpoles [47]. To test these hypotheses, we analysed the individual growth trajectories through metamorphosis in a facultatively paedomorphic newt according to thermal constraint. To this aim, we induced metamorphosis by reducing the water level, a very strong driver of metamorphosis in this species [45], and we compared the weight trajectory of paedomorphic newts that metamorphosed (i.e. becoming metamorphs) to that of paedomorphic newts that did not metamorphose (i.e. remaining paedomorphs) according to their sex and the thermal condition experienced.

Results

Comparison of the weight trend curves of newts during the experiment

This analysis aimed to show the growth patterns of newts in the two groups: those that metamorphosed (in the low water level) and those that did not (in the high water level), considering the effects of sex and temperature.

For newts that did not metamorphose during the 85 days of the experiment, our trend analysis shows strong support for a convex shape, regardless of sex and thermal treatment, as revealed by a large negative quadratic effect of time, compared to a smaller, positive linear effect ($q = 1.084 \pm 0.132$; Table 1 (A), Fig. 2A and Additional file 1: Table S1 for the detailed AIC model support). As revealed by the interactive effect of sex and

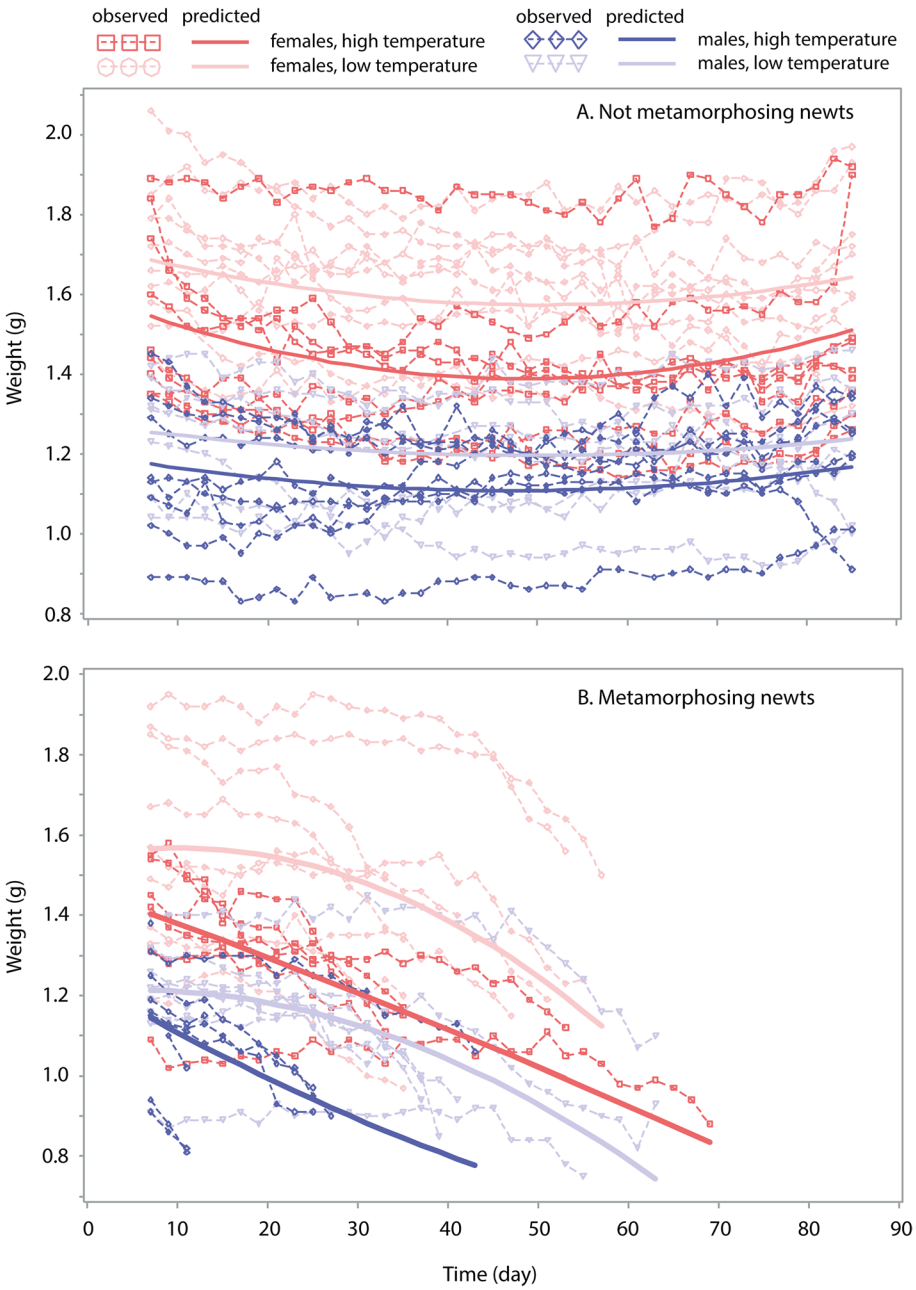

Fig. 2 Weight variation over time in paedomorphic palmate newts: **A** that do not metamorphose; **B** that metamorphose. Thick lines: mean predicted curves obtained by model averaging; dotted lines: individual patterns. The metamorphosing newts were reared in the low water level treatment which causes metamorphosis, while the newts that did not metamorphose were reared in the high water level treatment (see Additional file 4: Fig. S1 and Additional file 5: S2 for the other conditions)

the quadratic term ($q^*S = -0.725 \pm 0.257$, Table 1 (A), Fig. 2A), this convex shape was also more pronounced in females than in males (i.e. quadratic effect of 1.446 vs 0.721, respectively). To a lesser extent, the convex shape was also more pronounced in the high- than in the low temperature treatment (quadratic term of 1.282 vs. 0.885, respectively) as indicated by the positive interactive effect between the temperature treatment and the quadratic term ($q^*T = 0.397 \pm 0.225$, Table 1 (A), Fig. 2A). The results of the analysis restricted to the first 70 days of the experiment (in order to use the same time scale as that used for the metamorphosing newts) are relatively similar, except that the overall weight loss is more pronounced than in the analysis over the 85 days and that

the difference in convex shape is more influenced by temperature than by the sex of the newts (Additional file 2: Table S2). The differences between the two analyses were predictable, given that the newts regain weight after the midpoint of the experiment (Fig. 2A).

All newts that metamorphosed during the experiment experienced significant weight loss until metamorphosis, regardless of sex and temperature, as indicated by the strong negative value of this linear trend (i.e. $l = -9.849 \pm 1.309$; Table 1 (B), Fig. 2B). Moreover, while this weight loss exhibited a well-pronounced downward curvature for newts in the low temperature treatment (as indicated by the relatively large negative quadratic effect of time, i.e. $q = -1.411 \pm 0.524$; Table 1 (B) and Fig. 2B), this curvature was significantly less pronounced for newts in the warm temperature treatment, as revealed by the positive interaction between treatment and the quadratic term (i.e. $q^*T = 3.579 \pm 1.210$; Table 1 (B), Fig. 2B). This downward curvature was also slightly less pronounced for males than for females, as revealed by the positive interaction between the quadratic effect of time and that of sex (i.e. $q^*S = 1.282 \pm 0.841$; Table 1 (B) and Fig. 2B). The body weight loss from the start of the experiment to the end of the experiment is largely higher for metamorphosing than not metamorphosing newts (Additional file 3: Table S3), and this difference in weight loss remains significant even when computed until the mean time of metamorphosis (i.e. day 41) as revealed by Wilcoxon exact tests (Additional file 3: Table S3).

These results therefore showed that the newts that remained paedomorphic underwent an initial period of weight loss, followed by a growth period that partially compensated for the initial weight loss. This trend is also consistent with the percentage of weight loss recorded at the end of the experiment. Conversely, the results for metamorphosing newts show degrowth rates in all groups, and suggest that weight loss was slightly lower in males than in females, and more reduced at low temperatures than at high temperatures.

Weight trajectory curve approaching metamorphosis in newts placed at low water level

This analysis focuses on the metamorphic process itself, allowing key parameters of degrowth rates in both sexes to be retrieved across the two temperature treatments. This analysis has the advantage of not being constrained by water levels, as it focuses on the trigger for metamorphosis (i.e. low water levels).

The results of our Gompertz model indicate that at the end of metamorphosis, the degrowth rate of weight differs significantly between females and males, but not according to the temperature treatment ($t_{31} = -3.54$, $P = 0.0013$ and $t_{31} = -1.17$, $P = 0.2516$, respectively; Table 2, Fig. 3): females experienced a significantly higher degrowth rate than males did just before metamorphosis as indicated by the negative difference between sexes on the maximal degrowth rate parameter (see b_{sex} in Table 2). The mean onset of the maximal degrowth period preceding the metamorphosis (i.e. T_{adp}) was 6 days before the metamorphosis. Given the negative significant interaction between sex and inflexion time (C_{sex} in Table 2), males entered significantly more quickly than females in the maximal degrowth period ($T_{adp} = 9$ days and 4 days respectively for males and females). Finally, since females experienced a longer slow degrowth period (i.e. the period preceding the onset of the maximal degrowth one) than males did and also suffered a higher maximal degrowth rate ($b_{females} = 0.15$ vs $b_{male} = 0.06$ according to the mean degrowth rate and its interactive effect with the sex, Table 2) they metamorphosed later and suffered a larger weight loss than males did. This is well congruent with the weight difference observed between the

Table 2 Parameter estimates of the final Gompertz model for weight loss of metamorphosing newts in the low water treatment

Parameter	Estimate ± SE	IC 95%	t_{31}	*P*
$\boldsymbol{a_0}$	1.4004 ± 0.0404	[1.3179; 1.4829]	34.63	< 0.0001
$\boldsymbol{a_{sex}}$	−0.1746 ± 0.0842	[−0.3463; −0.0028]	−2.07	0.0466
a_{temp}	−0.0520 ± 0.0771	[−0.2092; 0.1051]	−0.68	0.5047
$\boldsymbol{b_0}$	0.1059 ± 0.00614	[0.0933; 0.1184]	17.23	< 0.0001
$\boldsymbol{b_{sex}}$	−0.04716 ± 0.0133	[−0.0744; −0.0200]	−3.54	0.0013
b_{temp}	−0.0148 ± 0.0126	[−0.0406; 0.0110]	−1.17	0.2516
$\boldsymbol{c_0}$	−10.8034 ± 0.500	[−11.8236; −9.7831]	−21.60	< 0.0001
$\boldsymbol{c_{sex}}$	−2.1980 ± 0.996	[−4.2299; −0.1661]	−2.21	0.0349
c_{temp}	−1.4069 ± 0.8880	[−3.2179; 0.4041]	−1.58	0.1232
$\boldsymbol{\sigma^2_{a_{0i}}}$	0.0470 ± 0.0135	[0.0193; 0.0745]	3.47	0.0015
$\boldsymbol{cov_{ab0i}}$	−0.0016 ± 0.0011	[−0.0039; 0.0007]	−1.39	0.1742
$\boldsymbol{\sigma^2_{b_{0i}}}$	0.0005 ± 0.0001	[0.0001; 0.0008]	2.82	0.0083
$\boldsymbol{\sigma^2_{res}}$	0.0007 ± 0.0001	[0.0006; 0.0008]	15.01	< 0.0001

a_0: mean initial weight (i.e. at day 6 before metamorphosis), a_{sex} : effect size for the sex on the initial weight (estimated as the difference between males and females), a_{temp} : effect size for the temperature effect on the initial weight, b_0 : overall maximal degrowth rate, b_{sex} : effect size for sex on degrowth rate (estimated as the difference between males and females), b_{temp} : effect size for temperature effect on the degrowth rate (estimated as the difference between high temperature minus low temperature), c_0 : mean time of inflection, c_{sex} : effect size for sex on time of inflection (estimated as the difference between males and females), c_{temp} : effect size for temperature effect on time of inflection (estimated as the difference between high temperature minus low temperature), $\sigma^2_{a_{0i}}$: subject random effect on initial weight, $\sigma^2_{b_{0i}}$: subject random effect on degrowth rate, cov_{ab0i} : covariance between subject random effects, σ^2_{res} : residual variance. All parameters retained in the final model are shown in bold (i.e. significant fixed effects and random effects). t_{31} indicates the value of the *t* test for each parameter. Note that the time frame is reversed, i.e. from the metamorphosis to the beginning of the experiment, the c_0 value indicates therefore that the average inflexion time is estimated after the metamorphosis (11 days) so that the duration of the maximal degrowth period is no longer in males than in females

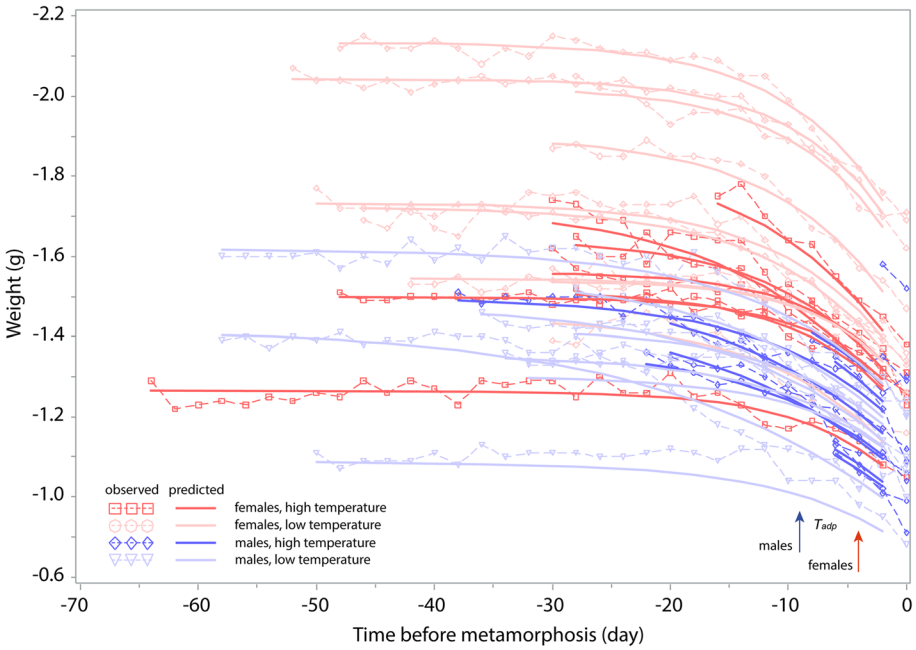

Fig. 3 Body weight degrowth curve of metamorphosing paedomorphic palmate newts according to sex and temperature treatment. Time is given backwards from the end of metamorphosis (i.e. day 0 is the time at which metamorphosis is completed). Thick lines: predicted curve including both subject random and fixed effects; dotted lines: individual patterns. Arrows (T_{adp}) indicate the time at which the degrowth rates increase. See Table 2 for Gompertz curves parameter estimates

start of the experiment and the date of metamorphosis (Additional file 3: Table S3).

Overall, these results are consistent with those of the trend analysis. However, the detailed profiles revealed by the Gompertz model highlight the non-linearity of the weight loss curves, with significantly greater weight loss in the few days before the end of metamorphosis. These results also emphasise sex differences during metamorphosis: males undergo metamorphosis more rapidly than females, who exhibit a longer initial phase of slow weight loss. The accelerated phase of weight loss in the days preceding complete metamorphosis is also more abrupt in females than in males.

Variation in the food intake of the metamorphosing newts

This analysis aims to explore the relationship between food intake and metamorphosis in both sexes when subjected to different temperatures.

The food intake propensity was not significantly affected by the interactive effect of the sex and the temperature treatment (Additional file 6: Table S4). However, both the interactive effect of the period and sex ($F_{1,28.65}=10.34$, $P=0.0032$) as well as the one of period and temperature treatment ($F_{1,43.56}=4.65$, $P=0.0365$) significantly altered the food intake propensity (Additional file 6: Table S4): food intake decreased 8.29 (IC95% [2.16; 31.86]) times more in females than in males (food intake odds ratios between the first and second period respectively for females and males: 115.31 and 13.91, Additional file 6: Table S4) and 5.10 (IC95% [1.11; 23.38]) times more at high than at low temperature (odds ratios of 90.44 and 17.73, respectively) when approaching the completion of metamorphosis (Fig. 4; Additional file 6: Table S4). As shown by contrast tests done separately within each sex and temperature modality (Additional file 7: Table S5), the odds ratios of food intake were higher before the onset of the period of maximum decline than after regardless of the sex and temperature but ranged from 6 times higher for males exposed to low temperature to 260 times higher for females exposed to high temperature.

Overall, these results show that food intake decreases as newts approach metamorphosis. They also indicate that there are effects of sex and temperature with females ingesting less food than males, and with a higher food intake at high temperatures than at low temperatures.

Discussion

Our results show evidence for an immediate cost of metamorphosis and highlight differences across the sexes and thermal environments. By taking advantage of studying metamorphosis in an adult phenotype, it was possible to model weight loss in newts all freed from the constraints of reaching the minimum size for metamorphosis

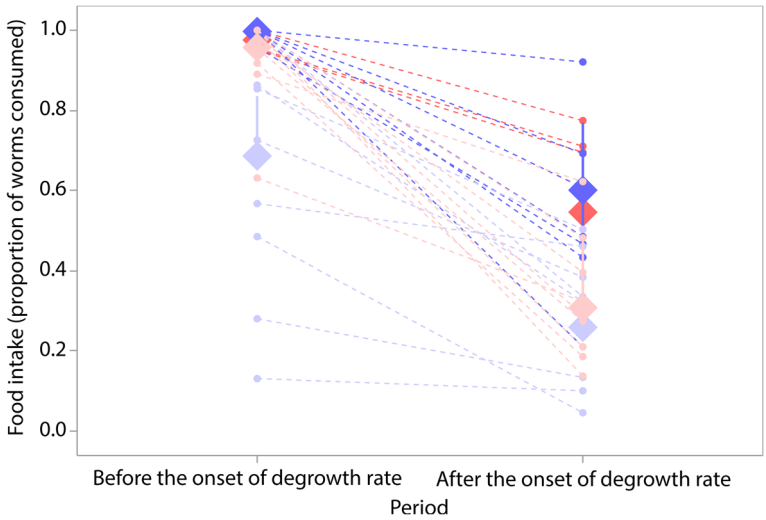

Fig. 4 Food intake (proportion of worms eaten by 48 h) in metamorphosing paedomorphic palmate newts before and after the onset of degrowth rate (weight loss) observed and predicted according to sex and temperature. Dark red: females at high temperature, light red: females at low temperature, dark blue: males at high temperature, light blue: males at low temperature. Diamonds: mean estimates; vertical lines: IC95%; dots: individuals; interrupted lines joint values of the same individuals

and attaining sexual maturity. This also revealed a higher cost in females than in males, providing complementary insights into the sex-specific drivers of life history changes. Overall, these results help us to understand the advantage of remaining paedomorphic by avoiding the cost of metamorphosis, but also that the cost associated with alternative ontogenies differs between the two sexes. More broadly, this shows the importance of considering all developmental steps, from the larval to the post-larval stage, and therefore taking into account all facets of metamorphosis in order to understand the costs and benefits of transition modes, and ultimately their evolution.

Weight loss and metamorphosis

Paedomorphic newts followed two developmental pathways, remaining paedomorphic or undergoing metamorphosis. By remaining paedomorphic, paedomorphs did not lose weight on average despite temporal variation due to acclimation. However, newts that underwent metamorphosis experienced a rapid decrease in body weight (Fig. 3). Although water loss can cause body weight loss through dehydration [48], this is not or less the case for paedomorphic newts that remain aquatic during metamorphosis. Indeed, in paedomorphic newts, body mass is correlated with stored lipids and triglycerides and can therefore be used as a proxy of fat reserves [43]. Given that metamorphosis in this species is largely determined by water level [45], we compared paedomorphic newts that metamorphosed under low water level conditions with paedomorphic newts that did not metamorphose under high water level conditions. It could therefore be argued that the difference in weight curves between metamorphosed and non-metamorphosed individuals results not only from metamorphosis itself, but also from the environmental factor that induced it (i.e. the low water level). Nevertheless, the weight curves of the few newts that do not metamorphose under the low water levels (Additional file 4: Fig S1) are similar to those that do not metamorphose under the high water levels (Fig. 2A), and, those of the few newts that metamorphose under high water levels (Additional file 5: Fig. S2 and Additional file 8: Fig. S3, and Additional file 9: Table S6) are similar to those observed in in newts that metamorphose under low water levels (Figs. 2B and 3).

The observed weight loss suggests that the payoffs of paedomorphosis and metamorphosis should not be considered solely in terms of the advantages of living in water versus on land. Evolutionary models of paedomorphosis and metamorphosis therefore need to incorporate the cost of the transition itself. In this sense, these empirical results are consistent with theoretical models [17, 22]. Previous experimental work has informed us about the major changes that occur during metamorphosis, in terms of morphology related to tissue resorption, remodelling and maintenance, but also in terms of gene expression cascades and physiological booms e.g. hormonal, such as thyroid pathways: [49, 50]. Future analyses, such as using transcriptomic tools, will help us to decipher more closely the different molecular pathways given the complexity of metamorphosis in both sexes and across environmental conditions [14, 51, 52]. Previous research also showed the risk of metamorphosing at a small size

as this leads to carry-over effects later in life. For example, adverse larval conditions affect metamorphosis and later delay gonadal maturation in spadefoot toads [39]. The immature state of anuran tadpoles or caudate larvae makes the outcome of metamorphosis complex, as it has consequences for future reproduction and is constrained by minimum size for metamorphosis. In this respect, paedomorphs proved to be good models for testing body weight variation in being released from such a constraint. Their study therefore completes our understanding of the quantitative direct costs of metamorphosis. Nevertheless, the way in which adult metamorphosis may alter subsequent reproduction remains an essential question to be answered in order to model the relative fitness of paedomorphs and metamorphs.

Sex differences in weight loss

Females lost more weight during metamorphosis than males. They also started losing weight before males. As the study took place during the breeding season, this suggests that females may divert energy directly from their unfertilised eggs. While they are metamorphosing, they would have no advantage in maintaining oviposition, an obligate aquatic behaviour [53], as metamorphosed paedomorphs in the studied species typically left water and stop breeding [45]. By having lost weight, this implies a high expected carry-over effect to their next reproductive season as they would have less energy available to allocate for new egg production. Semlitsch, Scott and Pechmann [54] found that metamorphosis at a large size favoured the onset of the next reproduction in ambystomatid females but not in males, suggesting that weight loss may be particularly detrimental to female fitness. Our results support the "male escape hypothesis" as it costs males less weight than females to complete metamorphosis and avoid detrimental aquatic conditions. Therefore, the transition from water to land may be less constraining for males, allowing them to avoid their low success as paedomorphs, while potentially gaining advantages by returning to water as metamorphs [46].

The growth models of paedomorphosis vs. metamorphosis propose that paedomorphosis can be favoured by contrasting growing conditions. First, under good growth conditions, larvae should postpone metamorphosis and take advantage of local aquatic resources ("paedomorphosis advantage hypothesis") [18, 22]. Although this first model would predict rapid metamorphosis when water conditions deteriorate, if the cost of metamorphosis is too high, larvae may opt for paedomorphosis according to the "best of a bad lot hypothesis" [22]. The fact that adult females in our experimental design lost more weight first but metamorphosed later than males suggests that growth patterns themselves may not be the only important intrinsic factor governing the metamorphic pathway in newts. Alternatively, it cannot be ruled out that growth patterns have a contrasting effect on the ontogenetic pathway followed by each sex.

Overall, the cause of the "male escape hypothesis" may be more related to the lower payoff of a paedomorphic life for a male than for a female rather than to the cost of metamorphosis itself. However, future research should examine variation in sex ratios within and between morphs in different contexts. The integration of sex differences into evolutionary ecology studies of paedomorphosis is rarely done, yet it is an essential component of understanding metamorphosis [55].

Our results on the sex-specific cost of metamorphosis contribute to the examples from other groups. However, they differ in that they are not linked to pathways to adulthood, since both paedomorphic males and females become fully reproductive before possibly undergoing metamorphosis. Although we found a higher cost in females than in males here, sex-related costs may vary considerably across species. For example, in moths, there is a difference in immunity challenge between the sexes, with males emerging smaller than females during metamorphosis [56]. In sepside flies, males spend more time and lose more weight in the pupal stage than females [57]. In anurans, spadefoot toad males have lower survival rates than females [58]. It would be interesting to have direct controls to test metamorphosis in non-caudate species, but this is limited by the need for facultative developmental processes. Facultative paedomorphosis in newts and salamanders therefore offers a unique opportunity to explore and determine direct metamorphic costs across the sexes.

Temperature and the cost of metamorphosis

Analysis of metamorphosing newts showed that weight loss started earlier in the high temperature treatment. However, when looking specifically at drying conditions, i.e. when newts are coping with low water levels, temperature had no effect on weight loss. An effect of temperature was expected as this environmental factor can increase metabolic expenditure in ectotherms [59, 60]. In addition, research on tadpole metamorphosis has shown that higher temperatures are associated with greater weight loss [61, 62]. The effect of temperature is likely to be complex due to acclimation processes, adverse effects of high temperatures and local adaptations [61, 63, 64]. Therefore, our results call for more research on the costs of metamorphosis in varied thermal conditions.

Both temperatures tested here were found in the natural environment of palmate newts [65], with the highest temperature contributing to newt metamorphosis in drying waters [45] and affecting negatively breeding success [65]. Newts also acclimatised rapidly to the experimental conditions, as evidenced by the weight gain of non-metamorphosing newts which partially recover from their initial weight loss.

Food intake and metamorphosis

As they approach metamorphosis, paedomorphic newts reduce their food intake, despite being offered food ad libitum. These results suggest that weight loss during metamorphosis is not only due to physiological processes and tissue remodelling, but also to a lack of energy intake. The lack of food intake was particularly emphasised in anuran tadpoles, as the epithelial shift prevents food intake to allow the transition from a "herbivorous" lifestyle to active carnivorous foraging [47, 66]. This is very different from caudates, which are active predators before and after metamorphosis and therefore do not require major organismal reorganisation. On the other hand, corticotropin-releasing factor, which promotes metamorphosis as a response to environmental stress, also causes an inhibition of food intake [67, 68] and could then explain the pattern found here in paedomorphic newts.

Conclusions

Understanding the causes of persistence and loss of metamorphosis remains one of the most exciting questions in evolutionary biology. Although studying the adaptation to life in the pre- and post-metamorphic environment and the benefits of reproductive potential before and after metamorphosis are keys to unravel the success of alternative ontogenetic pathways, we have shown here that the costs of the transition period should also be considered as they may play a role in the trade-off. Future studies should focus on carry-over effects as well as on individual patterns of metamorphosis in natural situations. Long-term field studies could be of great support in this regard [69]. Metamorphosis is a key process not only in amphibian development and evolution, but also in a large variety of invertebrates and vertebrates [1, 8]. It evolved in response to various environmental pressures and opportunities, resulting in an extraordinary diversity of life forms [70]. Despite common patterns, there is also much variation in the underlying processes and organismic responses, and thus in the concept of metamorphosis [71]. Therefore, more comparative research across taxa could help us to understand how metamorphic costs could affect the evolution of metamorphosis differently or similarly. Finally, it is essential to integrate research in the context of global change as anthropogenic stressors affect metamorphosis in a wide range of organisms [4].

Methods

Study model

Eighty paedomorphic palmate newts (*Lissotriton helveticus*—40 adults of each sex) were captured during the breeding season from a fishless pond where they coexist with metamorphic palmate newts (Bergerie de Tédenat, Larzac, Hérault, France; 43.77° N – 3.45° E, 792 m elevation a.s.l.) as part of a study examining also the environmental drivers of metamorphosis [45]. The sample size was chosen to allow us to test the different hypotheses while not exceeding the number needed given conservation concerns (i.e. declining populations), the small size of natural populations and permit requirements [72]. Larzac plateau is a hotspot for paedomorphosis in this species [73, 74] with populations of paedomorphs inhabiting ponds that can dry up occasionally [75, 76]. A newt was identified as a paedomorph if it exhibited both sexual traits (i.e. a mature cloaca) and the retention of external gills and gill slits [77]. All paedomorphs had a snout-vent length greater than the minimum size for metamorphosis [78]. The sexes are dimorphic, with males having a swollen and smooth cloaca, whereas females have a striated and elongated cloaca [77]. Newts were transported to the laboratory in six 3-l tanks (30×20 cm, 18 cm high), placed inside a cool box.

Experimental design

Maintenance followed our description in Mathiron et al. (2017), where the proximate mechanisms of metamorphosis were investigated. Upon arrival in the laboratory at the University of Liège, newts were individually distributed (20 individuals per treatment) in 80 identical tanks (15×24 cm; 22 cm height): 2 water levels (high: 10 cm vs. low: 1.5 cm, i.e. 3.6 vs. 0.5 l) and 2 temperatures (warm: 21.1 ± 0.03 °C vs. low: 16.7 ± 0.02 °C) [45]. Ten individuals of each sex were evenly distributed within each treatment. The temperatures were in the range of those experienced by palmate newts in Larzac. While the lower temperature is considered favourable for newt aquatic life, the higher temperature is less optimal [45, 65]. The lower water level was used to induce metamorphosis while the higher level was used to maintain newts in a condition more favourable to paedomorphosis, based on our previous research [45, 79]. Specifically, 33 out of 40 newts metamorphosed in shallow waters, whereas only six out of 40 metamorphosed at the higher water level (see [45] and Additional file 10: Table S7 for details). Water was changed every two days. The photoperiod reflected the natural cycle of the newt's origin location (13 h light/11 h dark). The luminosity of each tank was

156 ± 2.8 lx (Lumilux de luxe 2350-lm daylight tube, L36 W/12–950, OSRAM). To avoid a sharp transition between day and night, dawn was simulated for 30 min in the morning and dusk for 30 min in the evening (Daylight L36 W/965 Biolux, OSRAM). Each newt was fed ad libitum (chironomid larvae: 100 mg per 2 days; a typical prey of the study species in Larzac: [31]).

The experiment lasted 85 days and observations were made every 2 days during water changes until the end of the experiment for paedomorphic newts that did not metamorphose (i.e. remained paedomorphs) and were stopped for each newt that had completed its metamorphosis (i.e. become a metamorph) before the end of the experiment. The completion of metamorphosis was assessed by checking the closure of the gill slits. Weight was determined by placing the newts on an Ohaus Scout balance (0.001 g resolution). To assess food intake, the number of worms (i.e. chironomid larvae) distributed for each newt was counted before and after water renewal (i.e. old, unconsumed worms vs. newly provided worms). Unconsumed worms were permanently removed during the water change. All newts survived to the end of the experiment, but one newt died at the completion of metamorphosis.

Statistical procedures

We performed two types of analysis to compare the growth trajectory of the newts, as revealed by their weight changes over the course of the experiment, and according to their sex and the treatments they experienced. First, we used trend analysis to examine the overall trajectory of body weight over the course of the experiment. Since almost all newts metamorphosed in the low water treatment (33 out of 40), whereas few did so in the high water treatment (6 out of 40, see [45] and Additional file 10: Table S7 for details), these analyses were performed separately for newts that metamorphosed in the low water treatment and for newts that did not metamorphose in the high water treatment. Second, we used Gompertz curves to investigate in more detail the weight loss of metamorphosing newts according to sex and temperature treatment in the low water treatment. All these analyses were carried out discarding the first 5 days after the onset of the experiment (i.e. the first three measurements), as large variations in body weight were observed during this period, probably due to an immediate acclimation effect. All analyses were done using SAS 9.4 software [80].

Trend analysis of newt weight variation

We performed these analyses on the paedomorphs that did not metamorphose in the high water treatment ($n = 34$) and on the paedomorphs that metamorphosed in the low water treatment ($n = 33$). Trend analyses were implemented in the MIXED procedure [80] using orthogonal polynomial terms to specify the linear (l) and quadratic (q) effects of time to avoid collinearity between the two effects. Both sexes (S), temperature level (T), their interactive effect as well as the three-way interaction with each time effect were introduced as explanatory terms in the fixed part of the full model, resulting in the following structure: $Intercept + S + T + S*T + l + l*S + l*T + l*S*T + q + q*S + q*T + q*S*T$. In addition, both nominal variables (i.e. sex and temperature treatment) were also introduced as contrast forms to facilitate the calculation of their relative support as well as their model-averaged estimate (i.e. $0.5\ X_i\beta_x - 0.5\ X_j\beta_x$, where X_i and X_j are dummy variables corresponding to each modality i, j of the nominal variable X, and β_x is the slope associated with its effect). The choice of covariance structure on the full model was made using restricted maximum likelihood (REML) optimisation, and two alternative covariance structures were considered (1) with a random intercept on the individual growth curve, and a first-order autoregressive error term for the residual (AIC = −4930.3), and (2) with a first-order autoregressive correlation structure on the individual growth curve (AIC = −5124.8). The second covariance structure was then used for subsequent analyses. All reduced models ($n = 75$) were then constructed by successively removing all explanatory terms from the initial full model and computed using ML optimisation. The AIC weight (wAIC) was calculated for each model and the effect of each explanatory term was assessed based on its relative support, calculated as the ratio between the Akaike weight of the best-supported model including this effect and the wAIC of the best-supported model ignoring this effect. We then computed the model-averaged estimates for each sex and temperature treatment (across all models), which were then used to construct the corresponding predicted curves. Since all newts that metamorphosed did so before day 70, we also performed a complementary trend analysis on newts that did not metamorphose, limiting the duration to 70 days to assess the sensitivity of our analysis to the time scale. We also compared the overall percentage of weight loss between metamorphosing and non-metamorphosing newts, midway through the experiment, which corresponds to the average date of metamorphosis (day 41), in each sex and temperature, using the Wilcoxon exact test.

Curve analyses of weight variation in metamorphosing newts in the low water treatment

The main objective of these analyses was to examine in more detail the acceleration of weight loss as metamorphosis approaches, depending on sex and temperature treatment. Nonlinear growth models are particularly

valuable for this task and, as these models are developed to analyse the growth curves rather than the decline curves, their application can be easily adapted to the present study by reversing the time frame. We therefore reversed the time frame of our dataset and used a Gompertz growth curve to model weight change backwards from the day of metamorphosis completion to the initial weight (i.e. at the start of the experiment, excluding the 5-day acclimation period). The Gompertz model was implemented in the NLMIXED procedure [80] and parameterised as follows (see [81] for the different forms of parametrisation):

$$w(t) = Ae^{\left(-e^{(-Gm(t-Ti))}\right)}$$

where $w(t)$ is the weight as a function of time t (time to completion of metamorphosis), A is the weight at the start of the experiment (i.e. the upper asymptotic weight value), $(-Gm)$ is the maximum degrowth rate and Ti is the time of inflection. To examine the effect of sex and temperature treatment, A, Gm and Ti were themselves expressed as a linear combination of these two explanatory factors. Individual random effects were also introduced for A and Gm. The initial model was of the form:

$$w_{itkl} = \left(a_0 + a_{0i} + \sum_{h=1}^{p} \alpha_h X_{kl}\right) e^{\left(-e^{\left(-\left(b_0+b_{0i}+\sum_{h=1}^{p}\beta_h X_{kl}\right)*\left(t-\left(c_0+\sum_{h=1}^{p}\gamma_h X_{kl}\right)\right)\right)}\right)}$$

where w_{itkl} is the weight of individual i at time t from sex k and temperature treatment l. For each parameter, A, Gm and Ti, these two factors (i.e. sex and temperature treatment) were introduced as additive effects represented by their linear combination X_{kl} using a contrast form to directly estimate their associated effect size (see above for details). The subscript 0 stands for the general mean and $0i$ for the individual random effect following a $N\left(0, \sigma^2\right)$ where σ^2 is specific to the shape parameters A and Gm. All non-significant fixed effects were successively removed from the initial model to obtain the final model. The onset of the period of maximum degrowth rate preceding metamorphosis, i.e. the time when the decay rate increases (calculated as the number of days preceding the metamorphosis), was estimated as a function of the asymptotic deceleration point using the following equation:

$$T_{\text{adp}} = T_i - \frac{\ln(36.8 - 9.77\sqrt{14.06})}{G_m}$$

Finally, we also controlled that the few metamorphosing newts within the high water level experienced a similar weight loss to those exposed to the low water level. To this end, we did not consider the effect of temperature or the sex of the newts because of the small sample size and because the aim of this analysis was to verify that the weight loss was specific to metamorphosing newts and not to the water level experienced (Additional file 8: Fig. S3, Additional file 9: Table S6 and Additional file 11: text S1).

Variation in the food intake of the metamorphosing newts

These analyses were performed on paedomorphic newts that metamorphosed in the low water treatment ($n = 33$). To test whether the weight loss experienced by metamorphosing newts was associated with reduced food intake, we compared food consumption before and after the mean onset of maximal degrowth rate period estimated from the Gompertz model (i.e. 6 days). Thus, we divided the dataset into two periods of equal duration: between day 14 and day 9 preceding the metamorphosis (i.e. before the maximal degrowth rate period), and during the 6 days preceding the metamorphosis (i.e. after the onset of the maximal degrowth rate period). The proportion of worms consumed (measured every two days) was then compared between the two periods using a generalised linear mixed model implemented in the GLIMMIX Procedure [80] controlling for both sex effect and temperature treatment. For this, the proportion of worms consumed was treated as the dependent variable using a logit link and binomial distribution for the error term, and both period (i.e. before and after the onset of degrowth), sex, temperature treatment (low and high temperature) and their first-order interactive effect were included as fixed effects in the model. A subject random effect was also introduced on both the intercept and the period effect, to account for the non-independence of the repeated measurements made on each subject (i.e. three repeated measurements per animal for each period). The significance of each explanatory term introduced into the model was tested with a non-sequential F-test, using the Kenward–Roger approximation to calculate the denominator df. Non-significant terms were then successively removed to obtain the final model, and the odds ratio was calculated for each remaining factor to assess its effect size. Additional contrast tests were also performed to examine the effect of each factor, in case they were involved in an interaction.

Abbreviations

AIC	Akaike information criterion
GLIMMIX	Generalised linear mixed
NLMIXED	Nonlinear mixed
REML	Restricted maximum likelihood
wAIC	Weighted Akaike information criterion

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12915-026-02608-5.

Additional file 1. Table S1 Table of model selection for trend analysis of weight variation of paedomorphic palmate newts during the experiment.

Additional file 2. Table S2 Model-averaged estimates and wAIC relative support for each effect implemented in the trend analysis of body weight for the paedomorphic palmate newts that do not metamorphose during the first 70 days of the experiment.

Additional file 3. Table S3 Overall weight loss observed for metamorphosing and not metamorphosing paedomorphs according to each sex and the temperature treatment.

Additional file 4. Fig. S1 Weight variation over time in paedomorphs that do not metamorphose in the low water level treatment.

Additional file 5. Fig. S2 Weight variation over time in metamorphosing paedomorphs in the high water level treatment.

Additional file 6. Table S4 Food intake of metamorphosing newts (in the low water treatment) as a function of time period (before and after the onset of the maximum decay period), sex, and temperature treatment, as well as their first-order interactive effects.

Additional file 7. Table S5 Results of the contrast tests comparing odds ratio of food intake between the two periods (i.e. before and after the onset of the maximum degrowth period) for each combination of sex and temperature treatment.

Additional file 8. Fig. S3 Body weight degrowth curves of metamorphosing paedomorphic palmate newts in the high water level treatment.

Additional file 9. Table S6 Parameter estimates of the final Gompertz model for the weight loss of metamorphosing newts in the high water treatment.

Additional file 10. Table S7 Number of newts that metamorphosed in each treatment (water level and temperature) for both sexes.

Additional file 11. Text S1 Curve analyses of the weight variation in metamorphosing paedomorphic palmate newts in the high water treatment.

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Authors' contributions
M.D.: conceptualization, investigation, data curation, methodology, visualization, funding acquisition, project administration, supervision, writing—original draft, writing—review and editing; A.G.E.M.: investigation, data curation, writing—review and editing; S.B.: investigation, data curation; J.P.L.: formal analysis, data curation, visualization, writing—review and editing. All authors read and approved the final manuscript.

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Data availability
All data generated or analysed during this study are included in this published article, its supplementary information files and in a publicly available repository. Data and scripts are available in the Zenodo archive at https:/doi.org/10.5281/zenodo.19371630 [82].

Declarations

Ethics approval and consent to participate
The capture permit was issued by the Direction Régionale de l'Environnement, de l'Aménagement et du Logement (DREAL) du Languedoc-Roussillon. All experiments reproduced natural environmental conditions and were conducted in accordance with all relevant guidelines and regulations. They were approved by the animal ethical committee of the University of Liège, Belgium (reference: 1613).

Competing interests
The authors declare no competing interests.

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