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The effect of food on growth and metamorphosis of paedomorphs in *Triturus alpestris apuanus*

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Abstract

The effect of food level on growth and metamorphosis of paedomorphs was examined in an experimental replicated design in *Triturus alpestris apuanus*. Paedomorphosis concerns the retention of larval characteristics in adult individuals. Newts that forgo metamorphosis and then retain gill and gill slits in the adult stage maintain an aquatic life but keep potential for undergoing metamorphosis. Paedomorphs metamorphosed later in treatments with food as a non-limiting factor than in stressful treatments where food was limited. Timing of metamorphosis did not differ between males and females. Food level greatly affected growth rates. These results confirm and extend optimality models that predict paedomorphosis under advantageous aquatic conditions (paedomorph advantage hypothesis) and metamorphosis in stressful aquatic habitats.

Keywords: *Triturus alpestris*, paedomorphosis, metamorphosis, polyphenism, food level

With 3 figures and 1 table

Introduction

Paedomorphosis is the retention of juvenile characters in the reproductive adult (GOULD 1977; MCKINNEY & MCNAMARA 1991; WHITEMAN 1994). This heterochronic process is widespread in newts and salamanders whose larvae can mature without undergoing metamorphosis. While paedomorphosis is the only ontogenetic pathway in some species, it is facultative in others: some larvae become paedomorphs, while others metamorphose and become adult later (SEMLITSCH & WILBUR 1989). In species in which paedomorphosis is facultative, both developmental patterns can occur together, with the relative number of paedomorphs and metamorphs varying across populations (SEMLITSCH et al. 1990).

Facultative paedomorphosis was proven to be adaptive with respect to prey and habitat selection (REILLY & LAUDER 1988; FASOLA 1993; WHITEMAN et al. 1996; DENOËL & JOLY, in press) and age at first mating (KRENZ & SEVER 1995; RYAN & SEMLITSCH 1998; DENOËL & JOLY 2000). Experimental studies dealing with environmental effects on metamorphosis showed that paedomorphosis could be a response to low density (HARRIS 1987; SEMLITSCH 1987), permanent water (SEMLITSCH 1987) and low food level (SPRULES 1974; VOSS 1995). However, other studies revealed that food supply did not affect the frequency of larvae becoming paedomorphic or metamorphic (SEMLITSCH 1987; LICHT 1992).

WILBUR & COLLINS (1973) predicted that under favorable aquatic conditions allowing fast growth, larvae would remain in their habitat and become paedomorphic. WHITEMAN (1994) agreed with this model and called it "the paedomorph advantage hypothesis". Poor aquatic conditions (water drying out, high density) can be considered as stressful environments. In such circunstances, DENVER (1997) demonstrated that tadpole metamorphosis is mediated by a classical neuro-hormonal stress pathway involving corticotropin-releasing hormone.

Because low growth rates can also lead to paedomorphosis, WHITEMAN (1994) gave an alternative hypothesis: the best of a bad lot. This model assumes that facultative paedomorphosis may also evolve when the aquatic environment makes metamorphosis costly or impossible for the slow-growing larvae. The best issue is then to become paedomorphic.

The aim of this study was to test WHITEMAN's hypotheses (1994) on the ontogenetic pathways of adult individuals. We selected two extreme food treatment levels in order to see whether a similar effect (i.e. metamorphosis) existed in both treatments. Particularly, with respect to DENVER's experiments (1997), we expected paedomorphic newts reared in good feeding conditions to remain paedomorphic, while newts reared in a stressful environment (absence of prey) to metamorphose.

Materials and methods

Subjects

On 31 August 1999, 40 (adult) paedomorphic Alpine newts (*Triturus alpestris apuanus*) were caught by dip-netting from a mid-elevation pond at La Pianca

(Tuscany, Italy, UTM: 33TTJ64, 1000 m elevation). The pond is permanent and is surrounded by pastures and deciduous forests. The sample consisted of 20 males and 20 females. Adulthood was determined on the basis of a well-developped cloaca, and sex by the shape of the cloaca.

Experimental procedure

On 1 September 1999, we individually marked newts in the laboratory, by toe-clipping (TWITTY 1966) and distributed them randomly into four aquaria (80 x 40 x 30 cm), 5 males and 5 females in each. We used an equal sex-ratio to test the effect of treatment on both sexes. Toe-clipping is known not to affect survival and body condition of newts (ARNTZEN et al. 1999). The bottoms of the aquaria were covered with gravel, light was 5000 lux (photoperiod: 14 D / 8 N) and temperature was 14° C. Newts were fed with *Chironomus* larvae (1.2 g per aquarium) every 2 days.

The experiment began on 11 September 1999. Two treatments, « food » and « fasting », were chosen. In the « food » treatment, living *Chironomus* larvae (1.2 g per aquarium, i.e. 120 mg per newt) were given daily. Such a food level makes food a non-limiting factor for the newts (DENOËL 2001). This simulates an extremely favourable situation. In the « fasting » treatment, no food was given to the newts in order to simulate an extremely bad situation. Each treatment was replicated twice and called « group 1 » and « group 2 ». The experiment lasted 120 days. We stopped the experiments at day 120 in order to avoid mortality due to the low mass of the starved newts at this date (1 individual death). At days 0, 30, 60, 90, and 120, newts were checked to determine the numbers of paedomorphs and metamorphs, their snout-vent length (to the nearest 1 mm) and body mass (to the nearest 0.1g). A newt was considered to be a paedomorph if gill slits were open, and identified as a metamorph if gill slits were closed. Newts from the « fasting » treatment were fed extensively after the completion of the experiment in order to compensate for their weight loss. They recovered well.

Statistical analysis

The null hypothesis of equal survival in « food » and « fasting » treatments was tested with a generalized Wilcoxon-Gehan test (CHAP 1997; STATSOFT 2000).

A continuous autoregressive moving average model for unequally spaced repeated measurements (JONES 1993) was used to evaluate changes in growth variables in male and female newts during the 120 days of the experiment (data from the two groups were pooled). Linear equations were developed for each measured variable to describe how these variables varied over time. Since we wanted to evaluate the impact of food supply, similar equations were fitted for both treatments (« food » and « fasting »). The Akaike Information Criterion (AIC), i.e. an arithmetic function of the deviance and the number of parameters estimated (SAKAMOTO et al. 1986), was computed for the null model (fitting one single trend for both treatments) and for the covariance model containing the food supply as covariate factor and interaction with time. The difference between the deviances of the two models allows standard chi-square tests to be applied. Non-zero slope was tested in each treatment by a simple linear trend versus time. Standard estimates and standard errors were calculated for the time coefficient, allowing to test whether this coefficient was zero or not with a standard *t*-test.

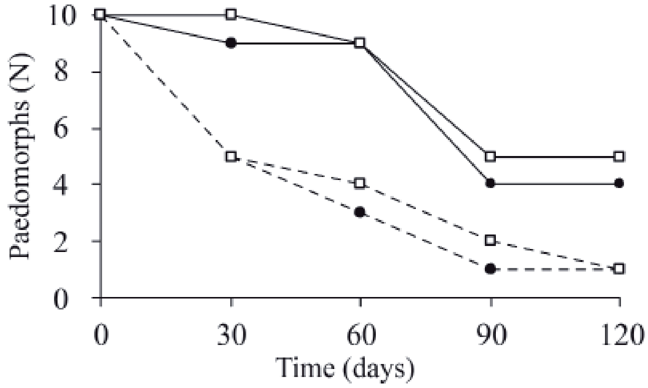

Fig. 1. Effect of food availability on metamorphosis date in paedomorphs of the two replicates. Solid lines: « food » treatments; dashed lines: « fasting » treatments; open boxes: group 1; full circles: group 2.

Results

Metamorphosis

In all treatments, a proportion of the paedomorphs metamorphosed during the experiment (120 days) (Fig. 1). There was no significant difference between the two groups of replicates (« food » treatments: Wilcoxon = -0.40, $p = 0.69$; « fasting » treatments: Wilcoxon = -0.20, $p = 0.84$). The dates of metamorphosis significantly differed between « food » and « fasting » treatments. They were earlier in the « fasting » treatment than in the « food » treatment (Wilcoxon = 3.44, $p < 0.001$) (Fig. 1). When considering the sexes separately, males as well as females appeared to metamorphose faster in the « fasting » treatment than in the « food » treatment (in males: Wilcoxon = 1.97, $p < 0.05$; in females: Wilcoxon = 2.92, $p < 0.01$) (Fig. 2). Although females seemed to metamorphose later than males, the timing of metamorphosis did not significantly differ between the two sexes in the « food » treatment (Wilcoxon = -1.57, $p = 0.12$) and in the « fasting » treatment (Wilcoxon = -0.53, $p = 0.60$) (Fig. 2). Whereas the frequency of metamorphosis was fairly constant in fasting newts, the largest number of newts metamorphosed between days 60 and 90 in the food treatment (Figs 1 and 2).

Growth

Table I summarizes the significance of the difference of growth patterns of newts in the « food » and « fasting » treatments. The covariance model containing the food supply as covariate factor and interaction with time fits the data better than the null model, whatever the variable considered, i.e. snout-vent length and body mass in males and females.

In the « food » treatment, the regression slopes between the growth patterns and time were significantly positive (snout-vent length in females: slope = 0.073, $p < 0.001$; snout-vent length in males: slope = 0.045, $p < 0.001$; body mass in females: slope = 0.016, $p < 0.001$; body mass in males: slope = 0.005, $p < 0.001$) (Fig. 3). During the time of the « food » experiment, individuals gained up to 11 mm in snout-vent length and 3.3 g in body mass.

In the « fasting » treatment, the regression slopes

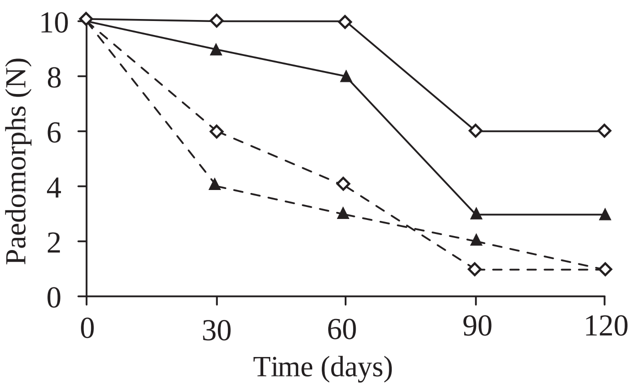

Fig. 2. Effect of food availability on metamorphosis date in paedomorphic males and females. Solid lines: « food » treatments; dashed lines: « fasting » treatments; open rhombuses: females; full triangles: males.

between the growth patterns and time were significantly negative (snout-vent length in females: slope = -0.016, $p < 0.05$; snout-vent length in males: slope = -0.012, $p < 0.05$; body mass in females: slope = -0.014, $p < 0.001$; body mass in males: slope = -0.011, $p < 0.001$) (Fig. 3). Most newts in the "fasting" treatments became smaller (up to 3 mm in snout-vent length) and all lost weight (up to 2.7 g, i.e. up to 71 % of the initial body mass).

Discussion

The manipulation of an environmental factor, food level, directly affected the growth pattern and timing of metamorphosis of paedomorphic Alpine newts. Newts with access to food metamorphosed later and grew faster than those reared without food. Such an environmental effect on the expression of discrete alternatives reveals that paedomorphosis in *Triturus alpestris* is an example of polyphenism (WEST-EBERHARD 1989).

According to the paedomorph advantage hypothesis proposed by WHITEMAN (1994), larvae living in a favourable aquatic environment undergo rapid growth and do not metamorphose, thus benefiting of the good aquatic conditions. Once a larva has become an adult, and thus a paedomorph, metamorphosis is still possible. We can hypothesize, as WHITEMAN (1994) did with larvae, that paedomorphs would metamorphose when aquatic conditions are poor. Our results on adult paedomorphic Alpine newts are in agreement with this hypothesis. Newts reared in good aquatic conditions, i.e. without any limitation of food supply either did not metamorphose during the 120 days of the experiment (45% of the newts studied) or metamorphosed later than the newts of the « fasting » treatment. On the contrary, 90 percent of the newts living in unfavourable aquatic conditions metamorphosed by the end of the experiment. Difference in growth patterns was also considerable. While increase in snout-vent length and body mass was great in the « food » treatment, newts of the « fasting » treatment became shorter and thinner.

Metamorphosis of starved newts is also in favour of ontogenetic pathway switching in stressful environments (DENVER 1997). Absence of food can indeed induce a stress that might be mediated through a classical neurohormonal stress pathway involving corticotropin-releasing hormone as was shown in the case of pond drying in desert toads (DENVER 1997).

In terms of ontogenetic pathway, a paedomorphic newt is confronted with two possibilities: metamorphosing or remaining a paedomorph. It is not possible for a newt to know the quality of both terrestrial and aquatic environments in order to choose the best habitat in which to live. By using only cues from the aquatic habitat, such as the food supply and its own growth rate, a newt may make a beneficial decision for its success. Of course, such decisions would have to be made by selection on generations of individuals experiencing both kind of habitats. Other favourable conditions such as the permanence of water and low density were associated with a tendency of larvae to remain aquatic and not to metamorphose (HARRIS 1987; SEMLITSCH 1987). If aquatic life is highly beneficial, with food as a non-limiting factor as it was in our « food » treatment, a newt would forgo metamorphosis as long as metamorphosis would be costly or paedomorphosis would offer specific advantages.

Nevertheless, some studies on *Ambystoma* revealed that the frequency of individuals that became paedomorphic or metamorphic did not differ between food treatments (SEMLITSCH 1987; LICHT 1992), whereas others showed that high food supply was associated with a tendency to become metamorphs instead of paedomorphs (SPRULES 1974; VOSS 1995). However, in the studies of SEMLITSCH (1987) and LICHT (1992), independently of maturity, larvae metamorphosed later in low food experiments than in high food experiments. The results of Sprules' and Voss' studies are consistent with the best of a bad lot hypothesis. Temperature seems to act also with food level to favour paedomorphosis (VOSS 1995). Our experimental situation was different from those of other studies because we dealt with adult animals instead of larvae. Thus, the challenge for the animal was not same. Indeed, a larva

Table 1. Differences in growth patterns for males and females between the treatments « food » and « fasting »: Akaike Information Criterion (AIC) for the null model (fitting one single trend for both treatments) and for the covariance model containing the food supply as covariate factor and interaction with time. Chi-square tests were computed on the difference of deviance of the two models. SVL = snout-vent length.

Variable	AIC null model	AIC covariance model	χ^2	df	p
SVL females	270.90	217.22	42.44	2	< 0.001
SVL males	248.47	208.03	55.68	2	<0.001
Mass females	174.40	88.14	88.26	2	<0.001
Mass males	114.61	41.73	74.88	**2**	<0.001

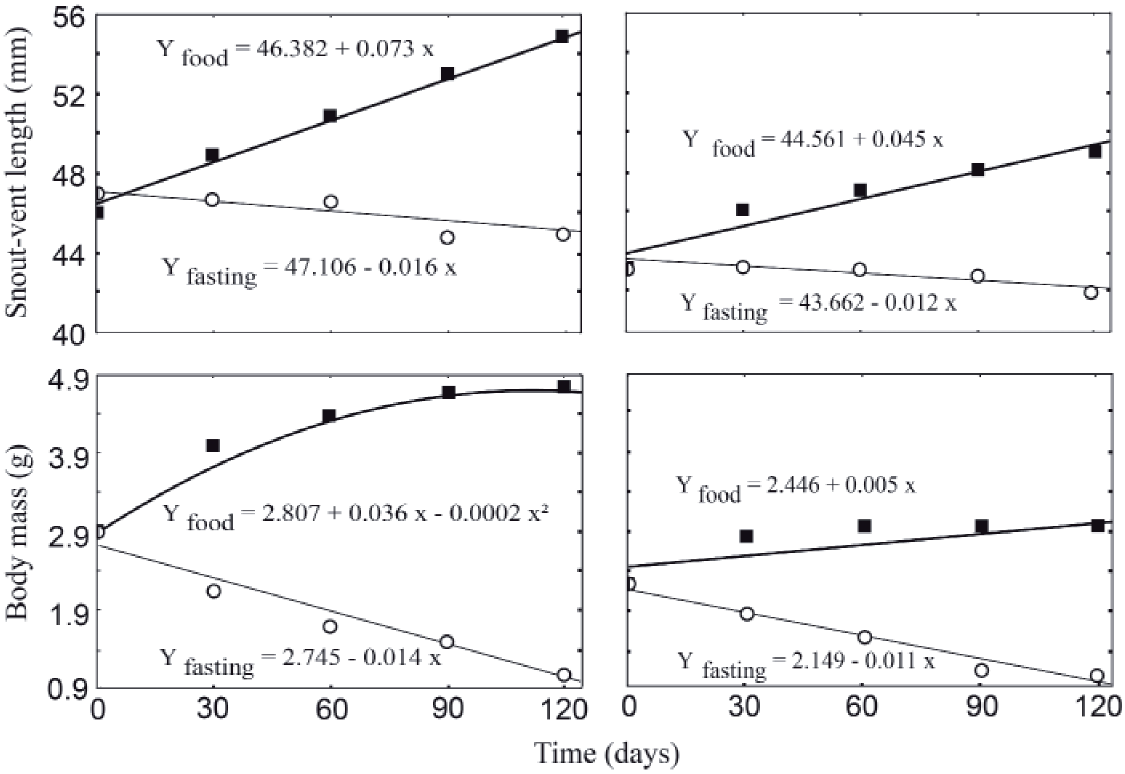

Fig. 3. Linear and quadratic regressions between time and morphology (snout-vent length and body mass) in males and females, in « food » (thick lines, full squares) and « fasting » (thin lines and open circles) treatments. Means of the morphological values are represented (N = 10 for each).

that transforms would experience an unknown terrestrial growth, and then an unknown age at maturity and first reproduction. Moreover, a larva has to grow up to a size threshold before being able to metamorphose. These problems do not occur for a paedomorph. Indeed, paedomorphic Alpine newts are mature and, given their large size, are able to metamorphose. Consequently, these individuals will not experience an undeterminate growth and maturity after metamorphosis. In the case of aquatic habitat deterioration and on the basis of the paedomorph advantage hypothesis, we would thus expect that fast growing larvae would gain more benefit by forgoing the uncertainty of a metamorphic ontogenetic pathway than paedomorphs.

Paedomorphosis appears as a discrete alternative life-history trait that is genetically based (VOSS & SHAFFER 1997), whose genetic underpinning may differ between species (SHAFFER & VOSS 1996) and for which a gene x environment interaction has been demonstrated (VOSS 1995), as several environmental factors can modify its expression (HARRIS 1987; SEMLITSCH 1987; this study). A better understanding of the environmental effects on the maintenance and evolution of paedomorphosis would therefore require studies dealing with different species confronted with a large range of habitats and conditions.

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