

EFFECTS OF TEMPERATURE AND BODY WEIGHT ON GASTRIC EVACUATION RATES OF ALPINE NEWT (*TRITURUS ALPESTRIS*) LARVAE

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We present different polynomial functions to describe gastric evacuation rates of alpine newt larvae in relation to temperature and body dry weight. Gastric evacuation rates increased with increasing temperature and decreased with increasing body weight during early development. Gastric evacuation rates measured in the laboratory were low compared to those estimated under field conditions.

INTRODUCTION

The driving force behind gastric evacuation studies in fish has been the need to estimate daily food consumption (Bromley, 1994). Elliott & Persson (1978) developed a now widely-used method for calculation of daily food consumption. The Elliott & Persson model, although initially developed for fish, is equally applicable to other poikilothermic vertebrate predators, and it has been used to estimate the feeding rates of alpine newts on zooplankton prey (Schabetsberger & Jersabek, 1995). When urodeles are top predators in lentic communities, they may depress the population size of certain crustacean species (Dodson, 1970). However, their impacts on zooplankton communities seem low relative to those of planktivorous fish (Strohmeier & Crowley, 1989; Holomuzki *et al.*, 1994; Schabetsberger *et al.*, 1995). Knowledge of amounts of food consumed by urodeles during development will be important to understand the role of urodele larvae as a structuring force in fishless lakes and ponds. Yet little information on gastric evacuation rates is available for urodeles (Schabetsberger, 1994). The aim of this paper is to present mathematical functions which allow the calculation of gastric evacuation rates in relation to temperature and body weight. The fits of a linear and two exponential models were compared.

METHODS

EGG COLLECTION AND REARING OF LARVAE

One hundred and twenty adult (60 females, 60 males) newts (*Triturus alpestris*) were collected in mid-June from breeding ponds in the north-eastern calcareous Alps of Austria (Totes Gebirge, 1650 m altitude). In the laboratory groups of six females and six males were kept individually in aquaria (56 x 36 x 10cm), and strips of floating plastic (polyethylene, 30 cm long, 0.8 cm wide) were provided as substrate for oviposition. Newts were held at 20°C under a light:dark cycle of 17:7 hr (light from 0500 hr to 2200 hr) and fed with tubificid worms. Eggs were collected each day and moved to three aerated 200

litre tanks kept at 8°C. On completion of egg collection, adults were returned to breeding ponds. Temperature in the incubation tanks was raised to 22°C within two days and a light:dark cycle of 12:12 hr was imposed (light from 0800 hr to 2000 hr). This regime ensured that most of the larvae hatched within 9-10 days. Developing larvae were fed daily on finely-cut tubificid worms. The worms were active during feeding and triggered prey capture in the newt larvae. Animals in the rearing tanks and those used in the experiments were held at the same temperature regime.

FEEDING EXPERIMENTS

During the development of the larval cohort three series of experiments were conducted in turn with different size classes of larvae (small: 1.7-2.2 mg body dry weight; medium: 8.3-13.3 mg; large: 29.3-39.9 mg). For each series the size class was tested consecutively at four experimental temperatures (8, 12, 16 and 20°C, Fig. 1).

Before the start of each series the temperature in the climate chamber was lowered to 8°C within three days (Fig. 1). For each experimental temperature a subset of 150-180 larvae was removed from the tanks and held individually in 50 ml jars to avoid gill damage due to aggressive behaviour of starving conspecifics. The jars were covered with a 1 mm mesh and placed in aerated aquaria (56 x 36 x 10 cm) in the climate chamber. Then the animals were starved for a certain interval to ensure gastric emptying, which was faster than in the subsequent experiments due to less stomach contents of animals in the rearing tanks (small size class at 8, 12, 16 and 20°C for 48 hr; medium size class at 8 and 12°C for 72 hr and at 16 and 20°C for 48 hr; large size class at 8 and 12°C for 96 hr

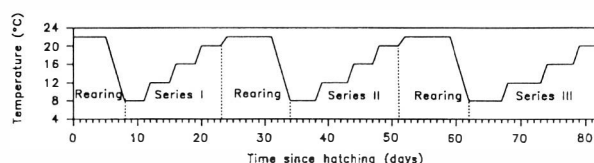


FIG. 1. Diagram of the experimental design.

and at 16 and 20°C for 72 hr). Following food deprivation finely-cut tubificids were provided to the newt larvae for three hours around noon. Food was then removed and thereafter 30 larvae were anaesthetized in MS 222 and fixed in 4% formaldehyde at 3-5.5 hr intervals. Following the feeding experiment at 8°C the next subset of larvae was removed from the rearing tanks and the temperature in the climate chamber was raised to 12°C within 24 hr. The same experimental procedure as described above was then repeated at 12, 16 and 20°C. After finishing a series of experiments the temperature in the climate chamber was raised to 22°C for seven days to get the larvae at larger size (Fig. 1).

Gastric evacuation rates of larvae in the natural habitat were available from a previous study (Schabetsberger, 1994). Briefly, 150 to 200 larvae were collected in an alpine lake during September and October. Food consisted of crustacea and chironomid larvae. Ten to fifteen larvae were placed in food-free cages and exposed in 3 m depth at the lake bottom. Changes in water temperature were within $\pm 1^\circ\text{C}$. Every 4 hr 15-30 larvae were then removed, anaesthetized with MS 222 and fixed in 4% formaldehyde. Seven different experiments were conducted at temperatures between 3.9 and 10.6°C. All the larvae used in the experiments would have died in the natural habitat due to anoxic conditions under the ice cover (Jersabek & Schabetsberger, 1996).

After eight weeks in the fixative, stomach contents of five (small and medium sized larvae) or three (large larvae) individuals were pooled, placed in preweighed aluminum boats, dried to constant weight at 60°C (48 to 96 hr) and weighed to the nearest μg . Larvae were dried and weighed in the same way and stomach contents were expressed as percentage body dry weight.

DATA ANALYSIS

Gastric evacuation rates. An exponential model of the form:

$$S_t = S_0 e^{-Rt} \quad (1)$$

was fitted to geometric means of stomach contents using a non-linear least squares technique (Marquardt-Levenberg algorithm; Conway, Glass & Wilcox 1970). S_t is stomach content at time t (% body dry weight), S_0 is the amount of food in the stomach at the beginning of the experiment (% body dry weight) and the exponent R is the instantaneous gastric evacuation rate (h^{-1}). In a previous study (Schabetsberger, 1994) this simple exponential function was found to describe gastric evacuation curves in adult and larval alpine newts and it was shown that R increased exponentially with increasing temperature T (°C) (coefficients \pm SE):

$$R = 0.0163(\pm 0.0037)e^{0.07967(\pm 0.0131)T} \quad (2)$$

The time for total emptying of the stomach (A_p, h) can be estimated as the time when 99% of the initial meal is evacuated (Elliott, 1972):

$$A_p = \frac{\ln 100}{R} \quad (3)$$

but due to the asymptotic approach of the evacuation curves to the abscissa small variations in R will result in large changes in the estimates of gastric evacuation times. A_p has been found to increase exponentially with increasing body dry weight W (mg) in preliminary field experiments (Schabetsberger, 1994):

$$A_p = 27.63(\pm 2.35)e^{0.0265(\pm 0.0021)W} \quad (4)$$

Jobling *et al.* (1977), Flowerdew & Grove (1979) and Dos Santos & Jobling (1991) characterized the relationship between A_p and body weight of fish as:

$$A_p = aW^b \quad (5)$$

with the exponent b ranging from 0.364-0.389 for fish fed a fixed proportion of their body weight. Consequently R is expected to decrease with increasing body weight according to either equation (6) or (7):

$$R = ae^{-bW} \quad (6)$$

$$R = aW^{-b} \quad (7)$$

To describe the dependence of gastric evacuation rate on the independent variables temperature and body dry weight ($R = f(T, W)$) the fit of a linear regression plane and two different polynomial surface functions to the data triplets was tested (Dependence of R on T and W either in a double-linear, double-exponential and exponential-root relation):

$$R = a + bT - cW \quad (8)$$

$$R = e^{-a + bT - cW - d} \quad (9)$$

$$R = ae^{bT}W^{-c} - d \quad (10)$$

The numerical values of the constants $a-d$ are different in equations 5-10. Fitting was performed on a DECstation 5000/33 using the non-linear least squares method implemented in *Mathematica* 2.2. The Marquardt-Levenberg method minimizes the χ^2 merit function (Press *et al.*, 1992):

$$\chi^2 = \sum_{T=T_{\min}}^{T=T_{\max}} \sum_{W=W_{\min}}^{W=W_{\max}} (R(T, W) - f_R(T, W))^2 \quad (11)$$

where $T_{\min} = 8^\circ\text{C}$, $T_{\max} = 20^\circ\text{C}$, $W_{\min} = 1.75 \text{ mg}$, $W_{\max} = 39.89 \text{ mg}$, $R(T, W)$ is the gastric evacuation rate measured under the given experimental conditions and $f_R(T, W)$ is the R -value calculated with the polynomial in question. The χ^2 val-

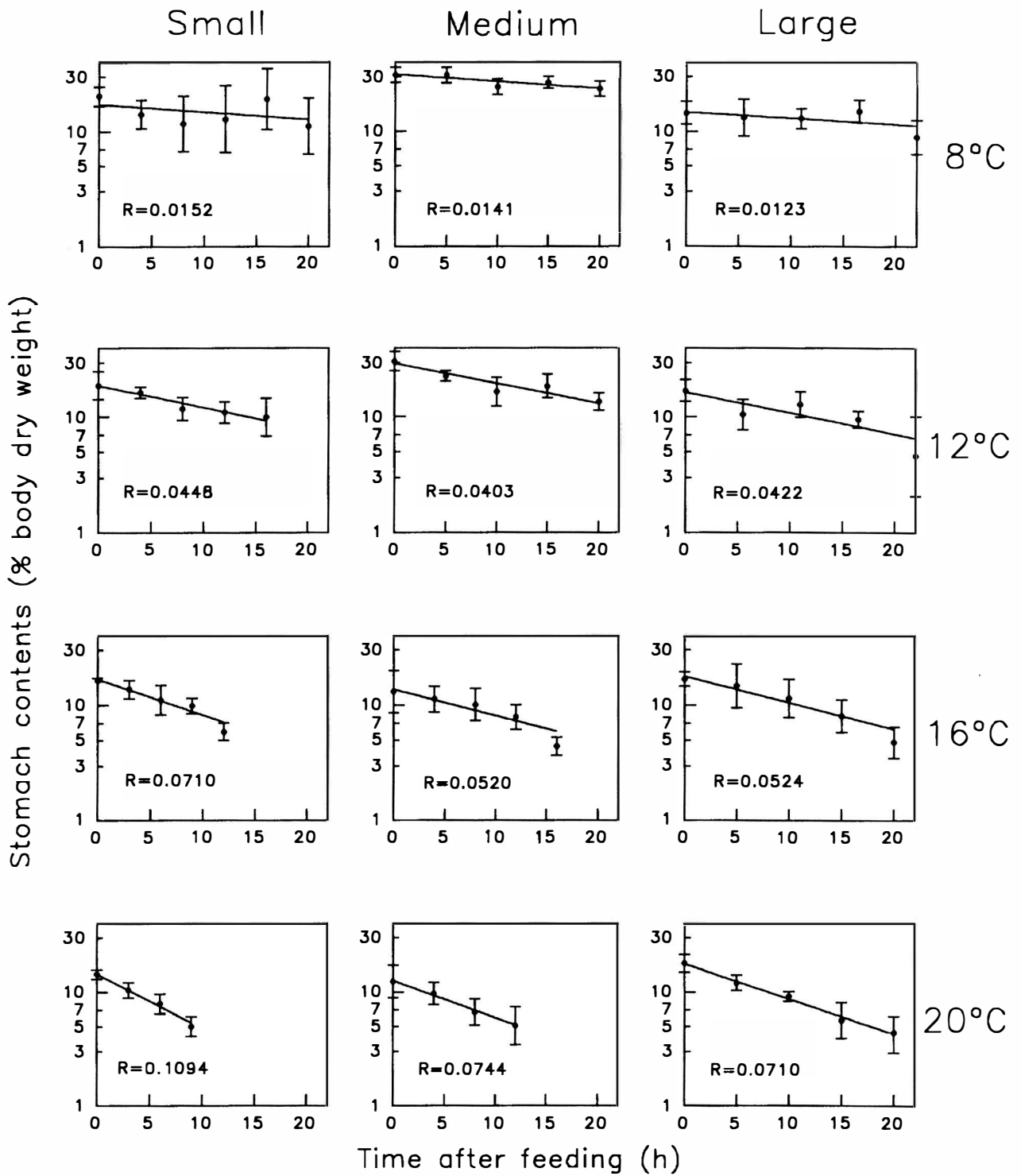


FIG. 2. Semi-log plots of mean stomach contents (% body dry weight) of small, medium and large alpine newt larvae at 4 experimental temperatures in relation to time of starvation (h). Points are geometric means ($n=30$) \pm 95% CL. Gastric evacuation rates (R, h^{-1}) are shown in each panel.

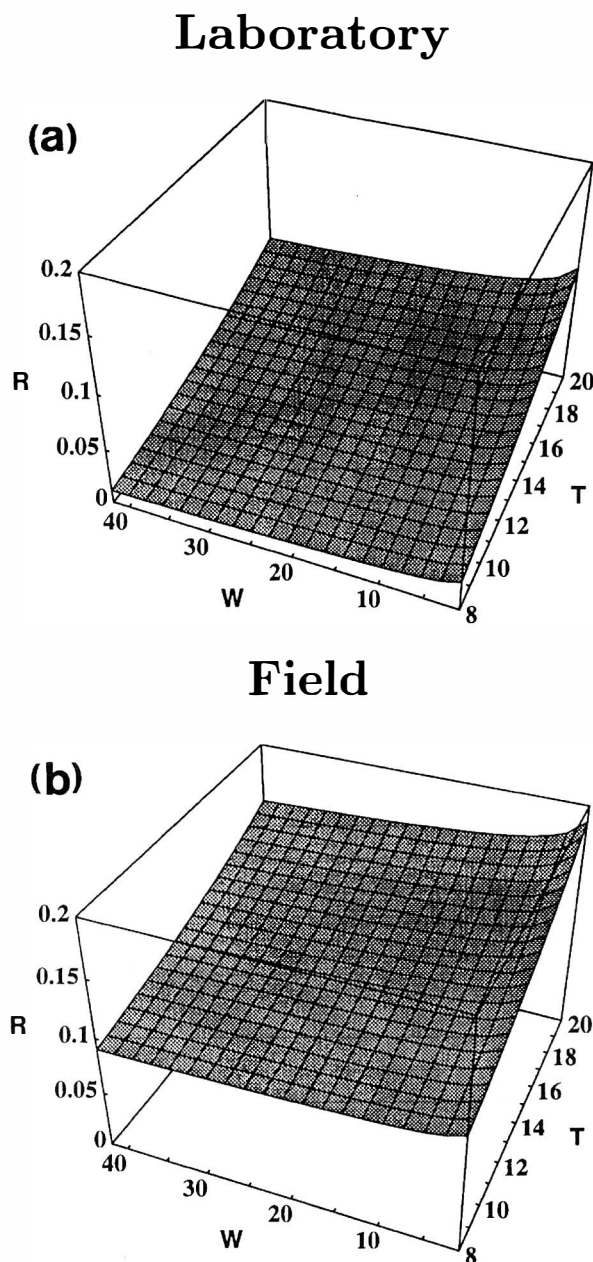


FIG. 3. Three-dimensional grid plots of gastric evacuation rate R (h^{-1}) in relation to temperature T ($^{\circ}\text{C}$) and body dry weight W (mg) of alpine newt larvae. a: laboratory conditions, b: field conditions.

ues were used to compare goodness of fit of the different models.

Growth of larvae. Stage of development was determined according to Gallien & Bidaud (1959). They distinguish 56 stages of development between fertilization and metamorphosis. Total length was measured with a vernier calliper. Due to aggressive behaviour of conspecifics, the thin tail tips were frequently damaged under both laboratory and field conditions. Only in the field were we able to collect enough larvae with intact tail tips to perform a geometric mean regression (Ricker, 1975) of dry weight against total length:

$$\log(W) = \log(a) + (b/r)\log(L) \quad (12)$$

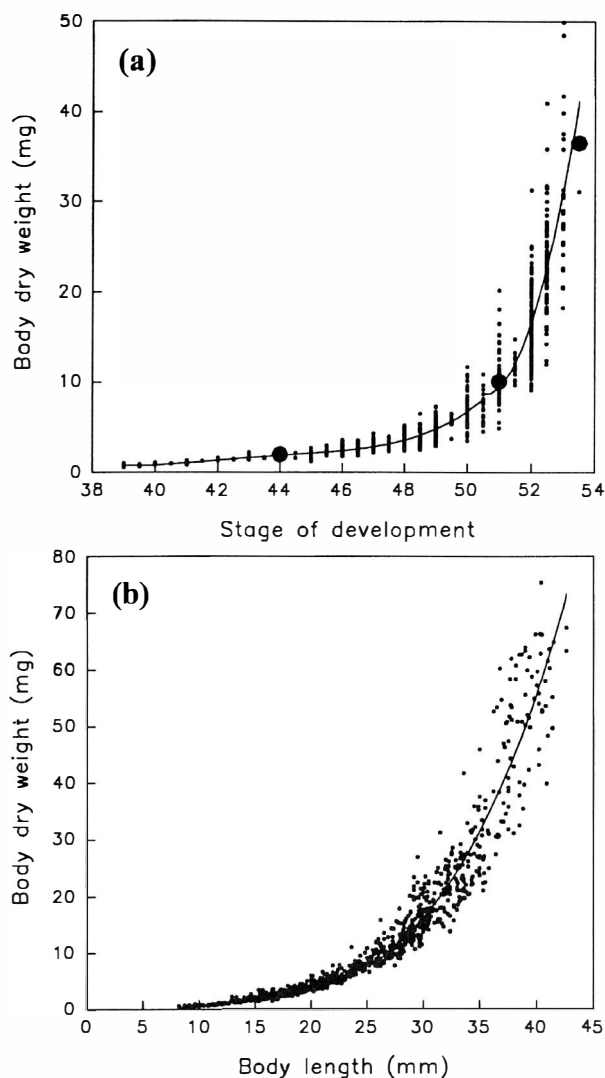


FIG. 4. Growth of larvae. a: Stage of development after Gallien and Bidaud (1959) in relation to body dry weight (mg) of alpine newt larvae collected in the natural habitat ($n = 948$). Mean dry weight of the three size classes of larvae used in the laboratory experiments are shown (\bullet). b: Total length (mm) in relation to body dry weight (mg) of alpine newt larvae collected in the natural habitat ($n = 1066$).

where W = body dry weight without stomach contents (mg), a and b are the constants for regression of log weight on log length, r is the correlation coefficient between log weight and log length, and L is the total body length of larvae (mm). Only larvae with undamaged gills and limbs were used in the feeding experiments.

RESULTS

GASTRIC EVACUATION RATES

For all size classes there was a significant increase in gastric evacuation rate with increasing temperature (small larvae: ANCOVA, $F=6.82$, $P<0.01$; medium sized larvae: $F=8.26$, $P<0.01$; large larvae: $F=5.28$, $P<0.05$; Fig. 2). A significant decrease in gastric evacuation rate with increasing body weight was recorded for the larvae held at 20°C ($F=12.61$, $P<0.01$).

Gastric evacuation rates as a function of temperature and body weight are shown in a grid plot in Fig. 3a. The

relationship was best described by a function of the form given by equation (10) ($\chi^2=0.00056$, coefficients \pm SE):

$$R=0.0301(\pm 0.0049)e^{0.0756(\pm 0.0054)T} \times W^{-0.0846(\pm 0.0061)} - 0.0286(\pm 0.0061) \quad (13)$$

Gastric evacuation rates measured under field conditions were approximately three times faster than those determined in the laboratory (Schabetsberger, 1994), so the function was raised by a constant term to fit the field data ($\chi^2=0.00673$, Fig. 3b):

$$R=0.0301(\pm 0.0049)e^{0.0756(\pm 0.0054)T} \times W^{-0.0846(\pm 0.0061)} + 0.0503(\pm 0.0038) \quad (14)$$

A function of the form given by equation (9):

$$R=e^{-2.5698(\pm 0.2225)+0.0433(\pm 0.0058)T-0.0030(\pm 0.0004)W} - 0.0072(\pm 0.0019) \quad (15)$$

gave a similar fit to the field data ($\chi^2=0.00631$) as that of equation (10).

GROWTH OF LARVAE

Larvae raised in the laboratory gained weight according to Fig. 4a, describing the relationship between developmental stage and body dry weight of larvae caught in the natural habitat (Schabetsberger & Jersabek, 1995). We fitted fourth and second order polynomials to larvae less and more advanced than stage 51 respectively.

Body dry weight of larvae (mg) caught in the natural habitat could be predicted from total length (mm) using equations (16) and (17) (coefficients \pm SE). The data were best described by separate equations for larvae of less and greater than 26 mm total length (Fig. 4b):

$$<26 \text{ mm: } W=1.4011 \cdot 10^{-3}(\pm 0.0653) L^{2.6776(\pm 0.0276)} \quad (16)$$

$$>26 \text{ mm: } W=7.6231 \cdot 10^{-6}(\pm 0.0781) L^{4.2844(\pm 0.0657)} \quad (17)$$

DISCUSSION

Temperature is a major factor controlling digestion rate in newts. Like fish (Bromley, 1994) gastric evacuation rate increases exponentially with increasing temperature in adult and larval alpine newts (Schabetsberger, 1994; this study). Body weight and the accompanying development of the digestive system had comparatively little effect on rates of gastric clearance in alpine newt larvae. Only during early development did gastric evacuation rates decrease with increasing body weight (Fig. 3), and in larvae >10 mg body dry weight, R -values were unaffected by body size. Similar results have been obtained in studies on fish larvae and juveniles, with the results being attributed to a growing functionality of the digestive system (Mills *et al.* 1984, Madon & Culver, 1993; Hayward &

Bushmann, 1994). In general R appears to decrease during development (Doble & Eggers, 1978; Perera & De Silva, 1978; De Silva & Owoyemi, 1983), but in some cases no effect of body weight on digestion rates has been found (Pedersen, 1984; Talbot *et al.*, 1984). Care should be taken not to confuse the exponential rate R , having the dimension of h^{-1} , with the absolute rate of gastric evacuation ($mg \cdot h^{-1}$). Although the exponential rate decreases, the absolute amount of food evacuated per unit of time increases during development.

Gastric evacuation rate did not decrease exponentially with increasing body weight as was suspected in a previous study (Schabetsberger, 1994), but a model having the general form of equation (10) also gave a good fit to the field data. A constant had, however, to be introduced in order to account for the faster gastric evacuation of food in the field than in the laboratory (Fig. 3b). The discrepancy between gastric evacuation rates measured under field and laboratory conditions probably resulted from several reasons. In the field experiments the larvae had fed continuously on a variety of prey items such as different species of cladocerans, copepods and chironomids (Schabetsberger & Jersabek, 1995). The meals were comparatively small (3-7 % body weight) and predigested before the start of the experiments (Schabetsberger, 1994). On the other hand the laboratory animals ingested large meals after a period of food deprivation. In two experiments larvae ate more than 30 % of their own body weight (Fig. 2). The long tubificid worms are normally not included in the natural diet. However, we cannot conclude that gastric evacuation rate is generally faster in the wild, because we did not test the laboratory animals at low levels of stomach contents in the range exhibited by wild fish.

These contrary feeding regimes probably resulted in evacuation rates which lie at the upper and lower limits of the physiologically possible. Calculating daily food consumption with the Elliott & Persson model (Elliott & Persson, 1978) using R -values obtained from equation (13) probably produces minimum estimates, whereas equations (14) and (15) might be used to estimate maximum food consumption in the field. The experiments in the field yielded less precise results than those conducted under controlled laboratory conditions. However, we have still included the weaker field data for basically two reasons. Published estimates of gastric evacuation rates are likely to be used by other researchers to assess the maximum food intake of larvae and their impact on prey populations, as it has been done in many fish studies (Bromley, 1994). Estimating R from equation (14) at least yields rough estimates of maximum food intake, whereas using equation (13) would probably underestimate food consumption for the reasons mentioned above. Further, equation (14) provides a good fit to all field data ($\chi^2 = 0.0067$), although the underlying R values were obtained from seven independent experiments with different sized larvae feeding on a variety of food items. We therefore find

that this function describes natural digestion rates sufficiently well.

Although these simplistic equations are strictly speaking only valid under the given experimental conditions, they could be applied to calculate rough estimates of daily rations of other urodele species with similar body size until more information for the species in question is available. A study of digestion in *Notophthalmus viridescens* (Jiang & Claussen, 1993) suggests that evacuation rates are similar within urodeles. However, digestion and evacuation rates of alpine newt larvae appear to be low compared to those of young fish (Govoni *et al.*, 1986) which may consume up to 140% of their own body weight per day (Marmulla & Rösch, 1990; Troschel & Rösch, 1991).

Using equations (16) and (17) it is possible to avoid the tedious determination of dry weight. At hatching larvae grew primarily in length, then later gained considerably more weight per unit length prior to metamorphosis (Fig. 4). This growth pattern may be interpreted as an adaptation to grow out of the food size spectrum of predatory insect larvae (Tejedo, 1993), but more information is necessary before this growth pattern can be explained. Care should be taken when estimating body dry weight from total length using the relationship shown in Fig. 4b, and checks should be made to ensure that the population in question follows the published growth curves. For example, low environmental temperatures retard development and individuals may attain large body size prior to metamorphosis, especially when they overwinter in a pond (Bizer, 1978). If the population in question exhibits different growth rates, *R*-values could be estimated by omitting body weight in equations (13) and (14).

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