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Hot tadpoles from cold environments need more nutrients – life history and stoichiometry reflects latitudinal adaptation

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Summary

1. High-latitude species (and populations within species) are adapted to short and cold summers. They often have high growth and development rates to fully use the short growing season and mature before the onset of winter.

2. Within the context of ecological stoichiometry theory, this study combines ecology with evolution by relating latitudinal life-history adaptations to their molecular consequences in body nutrient composition in *Rana temporaria* tadpoles.

3. Temperature and food quality were manipulated during the development of tadpoles from Arctic and Boreal origins. We determined tadpole growth rate, development rate, body size and nutrient content, to test whether (i) Arctic tadpoles could realize higher growth rates and development rates with the help of higher-quality food even when food quantity was unchanged, (ii) Arctic and Boreal tadpoles differed in their stoichiometric (and life history) response to temperature changes, (iii) higher growth rates lead to higher tadpole P content (growth rate hypothesis) and (iv) allometric scaling affects tadpole nutrient allocation.

4. We found that especially Arctic tadpoles grew and developed faster with the help of higher-quality food and that tadpoles differed in their stoichiometric (and life history) response to temperature changes depending on region of origin (probably due to different temperature optima). There was no evidence that higher growth rates mediated the positive effect of temperature on tadpole P content. On the contrary, the covariate growth rate was negatively connected with tadpole P content (refuting the growth rate hypothesis). Lastly, tadpole P content was not related to body size, but tadpole C content was higher in larger tadpoles, probably due to increased fat storage.

5. We conclude that temperature had a strong effect on tadpole life history, nutrient demand and stoichiometry and that this effect depended on the evolved life history.

Key-words: allometry, amphibian, C: nutrient ratio, common frog, development rate, growing season, northern Sweden, temperature adaptation, vertebrate stoichiometry

Introduction

Ecosystem ecology and evolutionary ecology are two of the least integrated fields in biology (Elser 2006). Ecological stoichiometry theory may be a tool to bridge this divide (Sterner & Elser 2002), since it has been shown that there is a tight connection between evolutionary processes, biochemistry, nutrient cycling and population dynamics (Elser *et al.* 2000b). Ecological stoichiometry theory postulates that the connection between evolutionary and ecological processes, which is reflected in an organism's body nutrient content and stoichiometry, is mediated by the life history of the organism. This connection is exemplified in the growth rate hypothesis (Elser *et al.* 2000b, 2003), which states that changes in consumer nutrient content are caused by differential cellular allocation to RNA-rich ribosomes. To attain fast growth, organisms

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have to synthesize many proteins. Protein synthesis is conducted by ribosomes, which contain ribosomal nucleic acid (RNA). Fast-growing organisms with a high rate of protein synthesis therefore contain copious RNA. This phosphorous-rich RNA constitutes a large part of the consumer's body phosphorus (P) pool. Thus, according to the growth rate hypothesis, consumers with high growth rates should have high P content and consequently also high P demands to realize these high growth rates (Hessen, Faerovig & Andersen 2002; Sterner & Elser 2002). In vertebrates, a large amount of P is bound up in P rich bone, and an increase in body size should lead to an increase in vertebrate bone proportion (and thus P content), due to allometric scaling (Sterner & Elser 2002), diluting the effect of growth rate on organismal P content. Additionally, organisms with high growth rates should have higher nitrogen (N) contents and demands, since the proteins synthesized during fast growth are N-rich biomolecules (Sterner & Elser 2002). Therefore, in vertebrates, the connection between growth rate and nutrient content might be as strong for N as for P.

According to the theory of life-history evolution, taxa, or even populations within the same species, differ in life-history traits due to evolved adaptations to different habitats. For example, in time-constrained habitats, it is advantageous to grow fast and finish one's life cycle rapidly at the cost of a smaller body size (Abrams *et al.* 1996). Thus, larvae can emerge or eggs be produced before the temporary habitat is disrupted (Wellborn, Skelly & Werner 1996). At high latitudes, short growing seasons impose strong selection pressures on organisms (Lovelock *et al.* 2007), and growing and developing fast during this short period of abundance is one strategy for coping with such environments (Danks 2006). Even within species, individuals from high-latitude populations often have a higher growth capacity than their low-latitude conspecifics (Elser *et al.* 2000a; Lindgren & Laurila 2005). However, growth rate optimization also faces nutritional constraints (DeMott & Pape 2005) as explained in the growth rate hypothesis (see above), especially when growth rates are not limited by other factors such as low temperatures (Persson *et al.* 2011). Since high-latitude populations need to maximize their growth rates, it should be especially important for them to attain high P intake rates or low P excretion rates (as shown for high-latitude *Daphnia*, Elser *et al.* 2000a).

High latitudes are generally characterized by short growing seasons, exerting evolutionary pressure towards higher growth and development rates, but also by low temperature, which generally restricts growth rates in poikilotherms. These two forces oppose each other and may lead to phenotypic similarity of organisms along latitudinal gradients in nature (i.e. countergradient variation, Conover & Schulzt 1995). Across latitudinal gradients of temperature and growing season length, two forms of 'latitudinal compensation' have evolved. First, high-latitude genotypes compensate for shorter growing seasons by evolving a higher capacity for growth (Laugen *et al.* 2003), and secondly, higher latitude genotypes compensate for lower temperatures by shifting their temperature optima (the temperature at which maximum growth rate is attained, given no other growth limitations) towards lower temperatures (Yamahira & Conover 2002). Metabolic rates, biochemical processes (Gillooly *et al.* 2001, 2002) and attack rates (Englund *et al.* 2011) are all temperature dependent. Ambient temperatures during an organism's life, in absolute terms, or in relation to the organism's optimal temperature, might therefore affect its body nutrient stoichiometry. A literature survey showed that increased acclimation temperatures led to reduced levels of proteins and RNA, and thus lowered N and P content in poikilothermic organisms (Woods *et al.* 2003). However, species distributed spatially across temperature gradients were excluded from this survey. Thus, we do not know how conspecifics with different temperature optima differ in their body stoichiometry and stoichiometric response to temperature change. Likely, temperature effects on body stoichiometry depend on the temperature change in relation to the organism's optimal temperature. Our study shows that understanding the factors determining consumer life-history traits and nutrient content across latitudes is important for predicting the consequences of anthropogenic environmental change on nutrient dynamics and nutrient limitation scenarios.

The aim of this study was to examine the connection between life-history traits and organismal stoichiometry in the context of environmental heterogeneity (as recommended by El-Sabaawi *et al.* 2012), by examining life-history adaptations and stoichiometry of high and low-latitude organisms under different temperature and food quality regimes. The common frog *Rana temporaria* is found in shallow fish-free ponds all across Sweden, and tadpoles from its Boreal and Arctic populations were used in laboratory experiments to examine temperature and food quality effects on life history and body nutrient stoichiometry. The following specific hypotheses were tested: (i) Arctic tadpoles can realize their higher growth and development rates with the help of higher-quality food, (ii) Arctic and Boreal tadpoles are adapted to different temperatures and thus differ in their stoichiometric response to temperature, (iii) tadpoles with higher growth rates have higher P content (growth rate hypothesis) and (iv) larger tadpoles have a higher P content (allometric scaling).

Material and methods

STUDY DESIGN

We crossed the treatments of food quality (high and low) and temperature of incubation (cold and warm) in a fully factorial design. From each region (Arctic and Boreal), we sampled five families (egg clutches) from each of four populations (ponds).

Every family-treatment combination was replicated four times, giving a total of 80 tadpoles per region and treatment combination, and 640 experimental units in total.

REGION OF ORIGIN

We selected the region of origin so that there would be genetic differentiation between the populations from the different regions due to adaptation to their specific habitats (Palo *et al.* 2003). Abisko (the Arctic site) lies in the north of Sweden above the Arctic Circle (68°20′N), whereas Umeå (the Boreal site, 63°50′N) is located ca. 800 km south of Abisko. The Abisko region has a growing season of 110–120 days, whereas the Umeå region has a growing season of circa 150 days per year (SMHI 2012). Growing season is here defined as the number of days where daily mean air temperature exceeds +5 °C. We collected eggs from four Arctic ponds (populations) and four Boreal ponds.

EGG COLLECTION AND EGG SIZE

Eggs were collected in May in the Boreal and in June in the Arctic region. About 20–50 eggs were taken from each of five independent egg clutches (families) from each population and brought to the laboratory. They were stored at 4 °C, fully submerged in water for up to 1 week. Eggs were photographed, and egg diameter of 10 eggs from each family measured. There were no differences in egg sizes between the regions (Arctic: 0·222 mm, 95% CI: 0·210–0·233 mm, Boreal: 0·210 mm, 95% CI: 0·180–0·240 mm; pMCMC = 0·12), so maternal effects were not included in the analyses.

TREATMENT VARIABLES

The temperature treatments were 18 °C and 23 °C. The different food quality treatments were chosen to contain the same proportions of carbon (C) (42%) and P (0·9%) per dry mass but differ in N. High-quality food contained 6% N per dry mass, whereas low-quality food contained only 3% N per dry mass. N was chosen as the limiting nutrient in the low-quality treatments since previous Swedish studies indicated N to be the limiting nutrient for benthic consumer growth (Liess & Hillebrand 2005, 2006), especially in the northern parts of Sweden (Liess, Drakare & Kahlert 2009). Food was a mixture of finely ground fish food and rabbit chow (as recommended Lind, Persbo & Johansson 2008), mixed in different proportions [high-quality food, fish food : rabbit chow (3 : 1) and low-quality food, fish food : rabbit chow (1 : 3)].

Experimental procedure

To start the experiment, eggs from each family were left to hatch at room temperature (20 °C). When the tadpoles reached Gosner stage 23 (free swimming, Gosner 1960), a control group of 20 tadpoles (10 random tadpoles from each region) was frozen to estimate start dry mass. A further 16 tadpoles from each family were randomly chosen and put into the corresponding treatments and transferred to their respective climate rooms with a photoperiod of 6 : 18 h dark : light. Each of the 640 experimental units consisted of square plastic containers that held 0·75 L of aged and aerated tap water. The tadpoles were fed a fixed amount of their respective food quality mix every fourth day (for further details, see Lind, Persbo & Johansson 2008). Containers were cleaned and water was replaced every fourth day, prior to feeding. All units were checked twice a day to determine tadpole developmental stage. Each tadpole that reached Gosner stage 42 (front legs visible, Gosner 1960) was killed by placing it in the anaesthetic MS-222 (ethyl-3-aminobenzoate methanesulphonate). The tadpole was then transferred to a Petri dish containing water, rinsed and then dried by dabbing with a tissue. The gut was removed to avoid disruption of nutrient measurements by gut content prior to freezing and freeze drying of the tadpole. The experiment was ended when all tadpoles were processed.

Tadpole growth increment and development rate

Tadpole dry $\text{mass}_{\text{end (E)}}$ was measured at the end of the experiment, and tadpole dry $\text{mass}_{\text{start (S)}}$ was estimated with the help of length–weight regressions for the start of the experiment (Gossner stage 23). Development time (t), the time it took to develop from Gosner stage 23 (start) to Gosner stage 42 (end) was recorded for each tadpole. With the help of these parameters tadpole growth increment (Eqn 1) and development rate (Eqn 2) were calculated as follows:

$$\text{Tadpole growth increment}(\text{mg day}^{-1}) = (\text{tadpole dry mass}_{\text{E}} - \text{tadpole dry mass}_{\text{S}})/t(\text{day}) \quad \text{eqn 1}$$

and

$$\text{Tadpole development rate } (\text{day}^{-1}) = 1/t(\text{day}), \quad \text{eqn 2}$$

where t is development time in days and ln the natural logarithm.

Tadpole body nutrient content

Whole tadpoles were freeze-dried by placing frozen tadpoles in a bench top freeze-drier (Hetosicc, CD *2.5;* Heto Co., Allerod, Denmark) for 4 days. Afterwards, dry tadpoles were weighed and then ground to a fine power using a pestle and mortar. Subsamples of this homogenized powder were taken to estimate P and CN content. P content of each tadpole was measured 2–4 times to achieve a reliable value. P content was measured as phosphate after hot acid hydrolysis with potassium persulfate (Grasshoff, Ehrhardt & Kremling 1983). Samples of 200–1600 μg were used for each P analysis. One sample (500–1000 μg) per tadpole was taken for C and N analysis, measured with a CHN analyser (LECO CHN-932, Carlo–Erba Strumentazione, Milano, Italy). C, N and P contents were calculated as% per dry mass. Food P as well as CN

content was measured on 8 samples per food quality using identical methods.

Statistical analyses

Separate univariate mixed-effect models tested the effect of the fixed factors (temperature, food quality and region of origin) on tadpole life-history traits and stoichiometry, using population (nested within region) and family (nested within population) as random factors. In all these models, we used tadpole start size as a covariate, to test for the importance of differences in initial conditions of tadpoles (Table 1). In the same way, models including life-history traits as covariates (mixed-effect ANCOVAS) tested the effects of life history, region of origin and treatment factors on tadpole nutrient stoichiometry (Table 2). In all these models (Table 2), tadpole start size was also included as a covariate but in no case improved the models. Differences in family-level egg size between the regions were estimated in a mixed-effect model incorporating region as a fixed effect and population as a nested random effect. We performed model simplification according to deviance information criterion (DIC; Bayesian equivalent to Akaike Information Criterion) to determine the best model (lowest DIC) for each response variable (Tables 1 and 2). All described effects were significant (pMCMC < 0·05) unless otherwise specified. All models were implemented in a Bayesian MCMC framework using *MCMCglmm* (Hadfield 2009) in the statistical package *R* 2.14.0 (R Development Core Team 2011; iterations: 50 000; burn in: 5000; thinning interval: 100) and used parameter-expanded priors (centred at 0; variance of 1000), which are noninformative but have proper distributions (Gelman 2006).

Results

LIFE-HISTORY TRAITS

Boreal tadpoles had higher growth increments than Arctic tadpoles, but increased temperature decreased growth increments more in Boreal than in Arctic tadpoles (interaction region × temperature: Table 1, Fig. 1a). Higher food quality increased growth increments, but the food quality effect was slightly stronger in warm than in cold treatments (interaction food quality × temperature: Table 1, Fig. 1a). The covariate tadpole start size was included in this model and was shown to have positive effect on growth increment (Table 1).

Arctic tadpoles developed quicker than Boreal tadpoles, especially when fed high-quality food (interaction region × food quality: Table 1; Fig. 1b). Higher temperature treatment led to quicker development, and these effects were most pronounced in Boreal (as opposed to Arctic) tadpoles (interaction region × temperature: Table 1, Fig. 1b). Higher food quality led to faster development rates, especially in the warm treatment (interaction food quality × temperature: Table 1, Fig. 1b). No covariate was included in this model.

The cold treatment led to larger body sizes at metamorphosis. This effect was especially strong in Boreal tadpoles, which became almost twice as large as Arctic tadpoles under cold conditions (interaction region × temperature: Table 1, Fig. 1c). Tadpoles feeding on high-quality food were larger at metamorphosis than tadpoles feeding on low-quality food, especially in the cold treatment (interaction food quality × temperature) and for boreal tadpoles (three-way interaction: Table 1, Fig. 1c). No covariate was included in this model.

Table 1. Best minimal models (with covariate start weight) to explain response variables (bold) using deviance information criteria (DIC). The intercept signifies the value of the response variable for Arctic tadpoles in the cold temperature and high food quality treatment. The values indicate the strength and direction of the main effects. The values of the interaction terms signify the direction and strength of the combined effects of the parameters involved in the interaction. Predictor variables with pMCMC > 0·05 remained if they reduced model DIC values

Response variables: Predictor variable	Tadpole P (%) DIC: 320† Value	Tadpole N (%) DIC: 305† Value	Tadpole C (%) DIC: 1585† Value	Growth increment (mg day^{-1}) DIC: −359 Value	Development rate (day^{-1}) DIC: −5596 Value	Metamorphic weight (g) DIC: −4367 Value
Intercept (Arctic, cold, high food)	1·681*	10·016*	44·040*	1·5040*	0·0325*	0·05196*
Region (Arctic→Boreal)	0·003	0·193*	0·442*	0·5710*	−0·0084	0·0344*
Temp (cold→warm)	0·160*	0·305*	−0·949*	−0·3288*	0·0098*	−0·0202*
Food quality (high→low)	−0·027	0·065	−0·886*	−0·4489*	−0·0012*	−0·0130*
Region × Temp	−0·077	−0·397*	0·589*	0·0072*	0·0040*	−0·0231*
Region × Food quality	–	−0·107	–	–	0·0010*	−0·0086*
Temp × Food quality	–	−0·134	–	−0·0029*	−0·0015*	0·0075*
Region × Temp × Food quality	–	0·216*	–	–	–	0·0047*
Covariate: Start weight	–	–	–	119·44*	–	–

*Indicates predictor variables with pMCMC < 0·05.
†For better models with other covariates and lower DIC, see Table 2.

Table 2. Best minimal models with covariates to explain response variables (bold) using deviance information criteria (DIC). The covariate start weight only improved one model. The values indicate the strength and direction of the main effects. The values of the interaction terms signify the direction and strength of the combined effects of the parameters involved in the interaction

Predictor variable Response variable: Tadpole P%	Value	Predictor variable	Value
DIC: 317 (best model)		DIC: 322 (2nd best model)	
Intercept (Arctic, cold, high)		Intercept (Arctic, cold, high)	1·870*
Covariate: Development rate	−12·88*	*Covariate:* Growth increment	−0·115
Region (Arctic→Boreal)	−0·090*	Region (Arctic→Boreal)	0·061
Temp (cold→warm)	0.263*	Temp (cold→warm)	0·132*
Food quality (high→low)	−0.049	Food quality (high→low)	−0·079*
		Region × Temp	−0·101
Response variable: Tadpole N%			
DIC: 259 (best model)		DIC: 279 (2nd best model)	
Intercept (Arctic, cold, high)	11·144*	Intercept (Arctic, cold, high)	10·82*
Covariate: Growth increment	−0·680*	*Covariate:* Metamorphic weight	−15·6*
Region (Arctic→Boreal)	−0·190	Region (Arctic→Boreal)	−0·21
Temp (cold→warm)	−0·228	Temp (cold→warm)	−0·022
Food quality (high→low)	−0·208*	Food quality (high→low)	−0·11*
Growth increment × Region	0·336*	Metamorphic weight × Region	10·8*
Growth increment × Temp	0·239	Region × Temp	−0·21*
Region × Temp	0·211		
Growth incr. × Region × Temp	−0·365*		
Response variable: Tadpole C%			
DIC: 1554 (best model)		DIC: 1576 (2nd best model)	
Intercept (Arctic, cold, high)	42·55*	Intercept (Arctic, cold, high)	43·115*
Covariate: Growth increment	0·88*	*Covariate:* Metamorphic weight	18·520*
Region (Arctic→Boreal)	0·035*	Region (Arctic→Boreal)	−0·110
Temp (cold→warm)	−0·720	Temp (cold→warm)	−0·640*
Food quality (high→low)	−0·494*	Food quality (high→low)	−0·659*
Region × Temp	0·748*	Region × Temp	0·956*

*indicates predictor variables with pMCMC < 0·05.

BODY NUTRIENT CONTENT

Tadpole P content was higher in warm than in cold treatments. This was true in all models with and without covariates. When no covariates were used, only temperature significantly affected tadpole P content (Fig. 2a, Table 1). The best model explaining tadpole P content included development rate as covariate (Table 2, Fig. 3a,b). In this model, increasing development rate had a negative effect on tadpole P content. Boreal tadpoles had lower P contents than Arctic tadpoles. And higher temperature had a positive effect on tadpole P content. Including growth increment as a covariate showed no significant correlation and did not improve the model over the original model without covariates (see DIC, Tables 1 and 2). Also adding body size at metamorphosis as a covariate to the model showed no significant correlation and did not improve the model.

Increased temperature led to increased tadpole N content in Arctic populations but to reduced tadpole N content in Boreal populations, in the model without covariates (interaction temperature × region: Table 1, Fig. 2b). In this model, there was also a three-way interaction; higher food quality had positive effects on Arctic tadpole N content under warm conditions and negative effect under cold conditions, but the opposite was true for Boreal tadpoles (three-way interaction: Table 1, Fig. 2b). The model best explaining tadpole N content used tadpole growth increment as covariate (Table 2). In this model, tadpole N content decreased with increasing growth increment; however, this relationship was stronger in Arctic than in Boreal tadpoles, especially under cold temperature conditions (three-way interaction: Table 2, Fig. 4a,b). In the two models using growth increment and metamorphic weight as covariates, high food quality had a positive effect on tadpole N content (Table 2). The second best model used metamorphic weight as a covariate, which had a negative effect on tadpole N content, especially in Arctic tadpoles (interaction metamorphic weight × region:

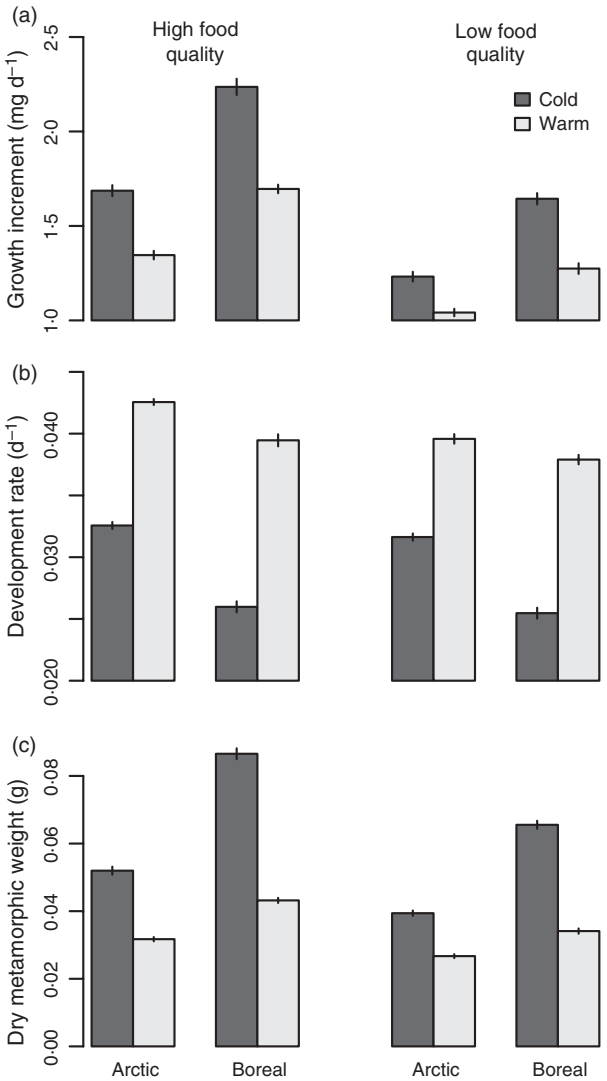

Fig. 1. Tadpole life-history traits: (a) growth increment, (b) developmental rate and (c) metamorphic weight of Arctic and Boreal tadpoles in the different temperature and food quality treatments. Light bars represent warm temperature, dark bars cold temperature treatments, left subpanels present high and right subpanels low food quality treatments. Error bars represent the standard error.

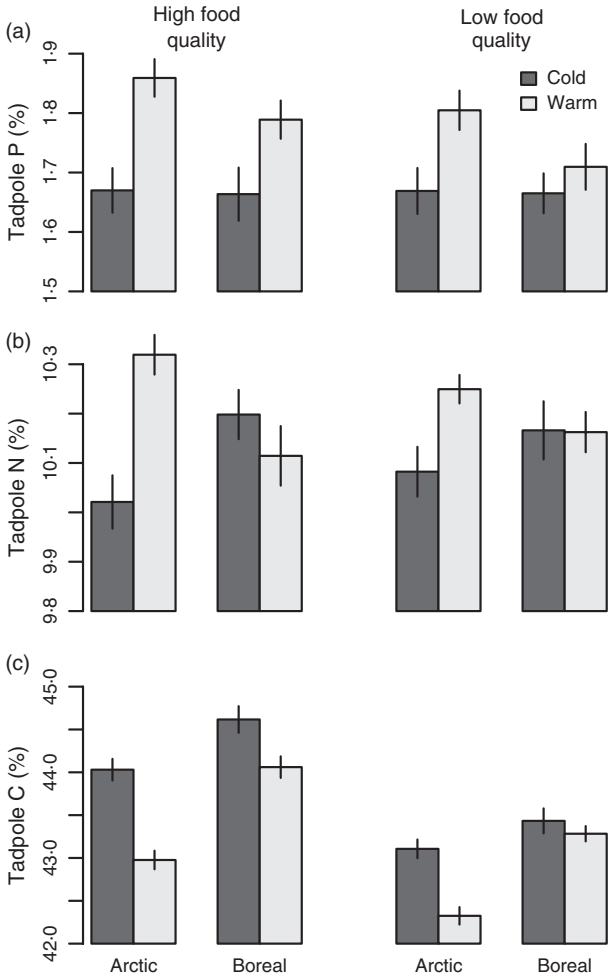

Fig. 2. Tadpole nutrient content in mass per cent per dry weight: (a) P content, (b) N content and (c) C content of Arctic and Boreal tadpoles in the different temperature and food quality treatments. Legends, subpanels and error bars as in Fig. 1.

Table 2, Fig. 4c,d). The interaction between temperature and region was the same as in the models without covariates (interaction temperature × region: Tables 1 and 2, Figs 2b and 4c,d).

When no covariates were used, C content was elevated in cold compared to warm treatments and this effect was stronger in Arctic than in Boreal tadpoles (interaction region × temperature, Table 1, Fig. 2c). In this model, Boreal tadpole C content was higher than Arctic tadpole C content. In all models (with and without covariates), tadpole C content was higher under high-quality food (Tables 1 and 2, Fig. 2c). The best model explaining tadpole C content included tadpole growth increment as a covariate, which had a positive effect on tadpole C content (Table 2, Fig. 5a,b). In this model, Boreal tadpoles had higher C content than Arctic tadpoles, especially under warm conditions (interaction temperature × region: Table 2, Fig. 5a,b). Interestingly, in this model, temperature effects on body C content became insignificant. The second best model explaining tadpole C content used metamorphic weight as a covariate. Larger tadpoles contained more C (Table 2, Fig. 5c,d). All effects in this model were the same as in the model without covariates, except that the main effect of region disappeared (Table 2, Fig. 5c,d).

Discussion

LIFE-HISTORY TRAITS

Arctic tadpoles could realize their higher development rates with the help of higher-quality food (*partially supporting hypothesis I*) as well as with the help of higher temperatures. This indicates that Arctic tadpole development rates might be more sensitive to stoichiometrically

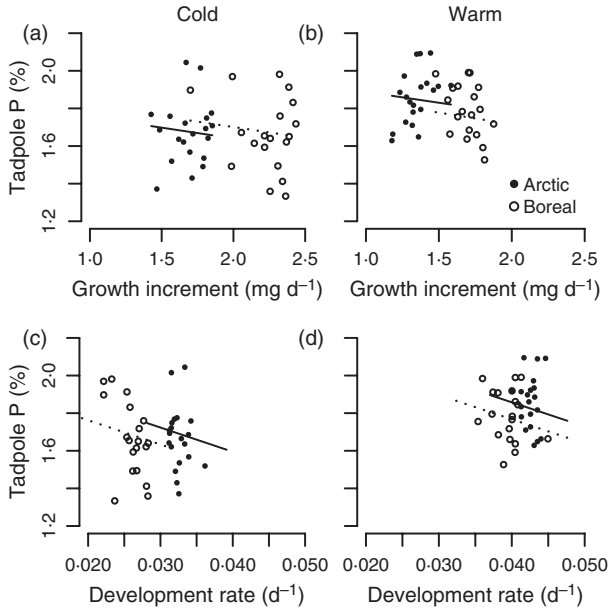

Fig. 3. Tadpole P content in mass per cent per dry weight in relation to (a, b) growth increment and (c, d) development rate. Each individual data point represents the family mean for one treatment combination. Since food quality did not interact with other treatment factors or covariates, only data from the high food quality treatment are presented. Filled circles represent Arctic and open circles Boreal tadpoles.

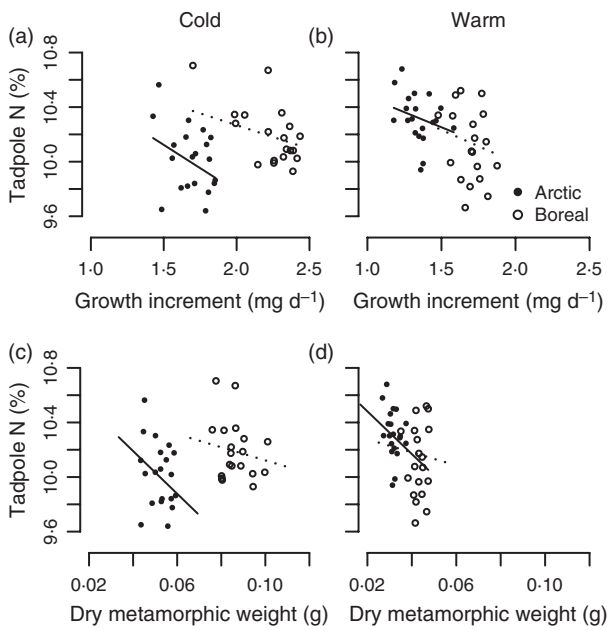

Fig. 4. Tadpole N content in mass per cent per dry weight in relation to (a, b) growth increment and (c, d) metamorphic weight. Each individual data point represents the family mean for one treatment combination. Since food quality did not interact with other treatment factors or covariates, only data from the high food quality treatment are presented. Filled circles represent Arctic and open circles Boreal tadpoles.

imbalanced food. Food quality effects were also dependent on temperature. The effect of high-quality food for maximum growth and development rates

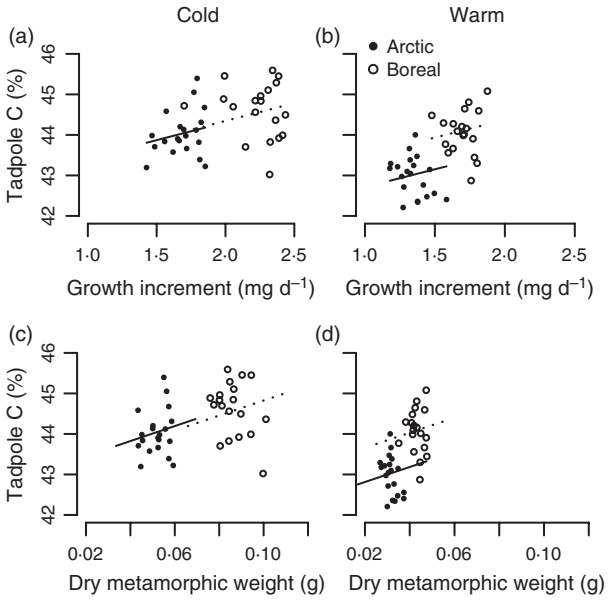

Fig. 5. Tadpole C content in mass per cent per dry weight in relation to (a, b) growth increment and (c, d) metamorphic weight. Each individual data point represents the family mean for one treatment combination. Since food quality did not interact with other treatment factors or covariates, only data from the high food quality treatment are presented. Filled circles represent Arctic and open circles Boreal tadpoles.

were larger in the warm treatments, in accordance with previous studies (DeMott & Pape 2005; Persson *et al.* 2011). A previous study on *R. temporaria* tadpoles showed that food availability had similar positive effects under warm but not under cold conditions (Laugen *et al.* 2003).

We found that Arctic tadpoles consistently had higher development rates than their Boreal counterparts. This is in line with previous studies that showed higher amphibian development rates in time-constrained (Morey & Reznick 2004; Richter-Boix, Llorente & Montori 2006; Lind, Persbo & Johansson 2008) and Arctic (Laugen *et al.* 2003; Palo *et al.* 2003) environments. In accordance with other latitudinal studies on *R. temporaria* (Laugen *et al.* 2003), we found that the high development rates of Arctic tadpoles were not caused by increased maternal provisioning to the egg, since egg sizes were similar in the two regions. In agreement with the temperature–size rule (Angilletta & Dunham 2003; Arendt 2011), we found that tadpoles grew larger in lower temperatures. However in contrast to previous studies with high-latitude populations of zooplankton and fish (Elser *et al.* 2000a; Yamahira & Conover 2002), we found higher growth increments in low than in high-latitude tadpoles, and therefore, tadpoles of the warm adapted low-latitude populations were larger at both temperatures. This may seem surprising, given the common finding that many ectotherms, including amphibians, are larger at higher latitudes (Bergmann's rule, see Atkinson & Sibly 1997). However, our result is

in agreement with a latitudinal survey of body size in wild-collected and laboratory-reared *R. temporaria* in Scandinavia (Laugen *et al.* 2005). Larger body size in low than in high-latitude tadpoles is probably explained by the strong selection pressure for fast development in high-latitude populations (Laugen *et al.* 2003). High development rates in general (Abrams *et al.* 1996), and in time-constrained *R. temporaria* populations in particular (Merilä, Laurila & Lindgren 2004; Lind & Johansson 2007) lead to smaller sizes at metamorphosis. In accordance with Palo *et al.* (2003), we also found a strong temperature effect on tadpole life-history traits. This temperature effect was stronger on Boreal than on Arctic tadpoles, suggesting that Boreal tadpoles were more plastic in their life-history traits in response to ambient temperature than Arctic tadpoles and possibly had a higher temperature optima. The second point is in accordance with a previous study that finds higher temperature optima for lower-latitude populations (Yamahira & Conover 2002).

TADPOLE NUTRIENT STOICHIOMETRY

Arctic and Boreal tadpoles differed in their stoichiometric response to temperature changes (*supporting hypothesis II*). But there was no evidence that higher growth rates mediated the strong effects of increased temperature on tadpole P content (*refuting hypothesis III*). Higher P content in warm than in cold treatments coincided with higher development rates but not with higher growth increments. There are two possible explanations for this increase in P content with temperature. First, a temperature-induced increase in growth augmented tadpole P content, as suggested by the growth rate hypothesis (Elser *et al.* 2003). This did not happen in our experiment. Indeed, a significant negative correlation was found in the model between the covariate growth rate and tadpole P content. Similarly, a highly replicated study with gastropods failed to detect any connection between snail growth rate and snail P content (Liess & Lange 2011). The second explanation is that temperature *per se* influenced tadpole P content. At different temperatures, organisms have different RNA requirements for maintenance processes and thus differ in their P content, as emphasized in a literature review by Woods *et al.* (2003), who found that organismal P content was higher after short-term cold exposure. This is not unlikely, since many body processes are temperature dependent (Gillooly *et al.* 2001, 2002) and whole organism RNA content has previously been assumed to be temperature dependant (Gillooly *et al.* 2005). Our results, however, contradict previous assumptions (Woods *et al.* 2003; Gillooly *et al.* 2005), since we found that higher temperature had consistently positive effects on whole organism P content. One explanation for the difference between our results and previous studies (Elser *et al.* 2000a; Woods *et al.* 2003) may be that the physiological reaction to temperature differs between short-term exposure and long-term growing conditions, especially in complex organisms such as vertebrates. In our study, tadpoles were grown under different temperature conditions from the first free-swimming larval stage until the final aquatic larval stage. During this time, tadpoles increased up to 100-fold in weight. Therefore, temperature may not only have affected processes at the cellular level but whole body morphology of the froglets, such as thickness of bones, storage of body fat and the relative size of internal organs. For example, we found that relative intestine length was shorter under colder temperature conditions (J. Guo, unpublished data), and a study on spadefoot toads showed that storage of body fat could be affected by environmental factors (Kulkarni *et al.* 2011). Morphological changes, in addition to cellular processes, will undoubtedly also reflect in body nutrient stoichiometry.

Tadpole N content showed a region of origin specific response to temperature treatment in both magnitude and direction of the response: Increased temperature increased Arctic tadpole N content but decreased Boreal tadpole N content. Since N is mainly found in proteins (Sterner & Elser 2002), this pattern could potentially be explained by differences in protein synthesis between the two regions. A previous study testing the effect of temperature stress on *R. temporaria* heat stress protein expression also found a strong interaction with region of origin. Less heat stress protein Hsp 70 was expressed in high than in low-latitude populations, despite lower heat tolerance of high-latitude population (Sørensen *et al.* 2009). The authors concluded that an alternate mechanism was at work protecting high-latitude tadpoles from heat stress. However, in our study, maximum temperatures were much lower than in Sørensen *et al.* (2009) (23 °C compared to 32 °C and 36 °C). Thus, Boreal tadpoles in particular did not experience heat stress. Furthermore, it is possible that an alternative heat tolerance mechanism in Arctic tadpoles led to protein production at much lower temperatures, resulting in the observed patterns of tadpole N response to temperature.

Arctic tadpole C content was lower and more variable in response to temperature than Boreal tadpole C content. We assume that the variability in tadpole C content was to a large degree associated with differential storage of fat. Lipids contain mainly C and no N or P (Sterner & Elser 2002). An earlier study on spadefoot toads showed that storage of body fat, regulated by stress hormones, was affected both by environmental factors and by species-specific environmental adaptation (Kulkarni *et al.* 2011). Tadpoles that were adapted to maximize development rate had higher basic levels and a less plastic regulation of this stress hormone, and thus less fat storage. In our study, Arctic tadpoles were plastic in their body fat uptake, whereas Boreal tadpoles seemed to consistently have high levels of body fat. This might suggest a selection pressure towards low stress hormone levels and high fat production in Boreal tadpoles at all times, independent

of temperature, whereas Arctic tadpole development and growth rates in nature were strongly constrained by low temperatures (Laugen *et al*. 2003). In our study, when temperature restrictions were lifted experimentally, Arctic tadpole development sped up and very little fat was stored. Since Arctic tadpoles incorporate fat at low but not at high temperatures, the absence of fat storage at high temperatures could be a maladaptive response to a temperature they are not adapted to, or alternatively a temperature stress response. Boreal tadpoles, however, might have higher temperature optima and therefore have been under selection to slow down their development rates under warmer temperatures to store body fat and improve their chances of winter survival, especially if Boreal survival is affected more by winter starvation than by death through pond drying or freezing. These differences in response will have consequences in a changing climate, where increasing temperature and growth season length in the Arctic could have a detrimental impact on Arctic *R. temporaria* genotypes. When life-history strategies of rapid development, irrespective of circumstances, cease to be optimal, it is plausible that migration of better-suited genotypes from the south may out-compete current Arctic genotypes.

Tadpole P content was not explained by body size; however, tadpole C content increased in larger tadpoles (*refuting hypothesis IV)*. Allometric scaling laws predict that larger vertebrates should have stronger bones to support heavier body weight. Since bone tissue is P rich (Sterner & Elser 2002), larger tadpoles should have higher body P content. However, we found that body size was not related to tadpole P content. Other effects of body size probably counteracted this pattern: Larger and faster growing tadpoles generally stored more body fat (see Discussion above), thus increasing tadpole C content (but not tadpole N or P content). The positive correlation of body size with tadpole C content at the same time 'diluted' body N content with increasing body size and counteracted the allometric effects on tadpole P content. Allometric scaling laws determine an animal's stoichiometric needs, but to clarify this, we need to know to which different body functions nutrient are being allocated as body mass changes.

Temperature had strong effects on tadpole nutrient stoichiometry. On the one hand, temperature effects on P were independent of origin and might thus reflect a physiological or chemical inevitability, rather than an adaptive response. On the other hand, temperature effects on tadpole N and C content may depend on region specific adaptation to temperature optima and length of growing season. However, the physiological mechanisms that mediated these temperature effects still need further investigation. Our data suggest that increased temperature is likely to alter uptake and incorporation of nutrients by consumers and thus their nutrient stoichiometry, further influencing nutrient requirement and nutrient cycling.

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