Journal of Animal Ecology

British Ecological Society

Journal of Animal Ecology 2015, **84,** 1744–1756 doi: 10.1111/1365-2656.12426

Cool tadpoles from Arctic environments waste fewer nutrients – high gross growth efficiencies lead to low consumer-mediated nutrient recycling in the North

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Summary

1. Endothermic organisms can adapt to short growing seasons, low temperatures and nutrient limitation by developing high growth rates and high gross growth efficiencies (GGEs). Animals with high GGEs are better at assimilating limiting nutrients and thus should recycle (or lose) fewer nutrients. Longer guts in relation to body mass may facilitate higher GGE under resource limitation.

2. Within the context of ecological stoichiometry theory, this study combines ecology with evolution by relating latitudinal life-history adaptations in GGE, mediated by gut length, to its ecosystem consequences, such as consumer-mediated nutrient recycling.

3. In common garden experiments, we raised *Rana temporaria* tadpoles from two regions (Arctic/Boreal) under two temperature regimes (18/23 °C) crossed with two food quality treatments (high/low-nitrogen content). We measured tadpole GGEs, total nutrient loss (excretion + egestion) rates and gut length during ontogeny.

4. In order to maintain their elemental balance, tadpoles fed low-nitrogen (N) food had lower N excretion rates and higher total phosphorous (P) loss rates than tadpoles fed high-quality food. In accordance with expectations, Arctic tadpoles had higher GGEs and lower N loss rates than their low-latitude conspecifics, especially when fed low-N food, but only in ambient temperature treatments. Arctic tadpoles also had relatively longer guts than Boreal tadpoles during early development.

5. That temperature and food quality interacted with tadpole region of origin in affecting tadpole GGEs, nutrient loss rates and relative gut length, suggests evolved adaptation to temperature and resource differences. With future climate change, mean annual temperatures will increase. Additionally, species and genotypes will migrate north. This will change the functioning of Boreal and Arctic ecosystems by affecting consumer-mediated nutrient recycling and thus affect nutrient dynamics in general. Our study shows that evolved latitudinal adaption can change key ecosystem functions.

Key-words: assimilation efficiency, consumer-mediated nutrient recycling, digestive efficiency, ecological stoichiometry, latitudinal adaptation, *Rana temporaria*

Introduction

Consumers regulate nitrogen (N) and phosphorus (P) availability through differential nutrient excretion and through nutrient egestion, a process called consumer-mediated nutrient recycling (CNR). CNR is a key mechanism driving nutrient turnover and primary productivity in many ecosystems and is thus an integral part of ecosystem function (Vanni *et al.* 2002; Liess & Hillebrand 2004; Leroux & Loreau 2010). Even though the concept of CNR has been considerably improved via the application of ecological stoichiometry theory (Sterner & Elser 2002), our understanding of the underlying *adaptive* mechanisms affecting CNR is poor. Current ecological CNR models predict that the relative proportions at which animals

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recycle nutrients depend on the elemental mismatch between the animal and its food (Sterner 1990; Evans-White & Lamberti 2006). This means that animals ingesting N-deficient food should decrease their N recycling and increase their P recycling compared to animals feeding on nutritionally balanced food.

However, in addition to consumer-food nutrient imbalances, nutrient excretion and egestion rates also depend on nutrient assimilation efficiency (Rothlisberger, Baker & Frost 2008; Liess 2014). This consumer trait can be genetically fixed or phenotypically plastic and can have strong implications for CNR. For example, high consumer nutrient assimilation efficiency should lead to a lower consumer nutrient loss rate and thus lower CNR. CNR may thus differ between species and genotypes, temporally and geographically due to adaptations or due to environmentally induced trait plasticity. Species and populations often adapt their life-history traits (such as growth and development rate) to their local environmental conditions. For example, in time-constrained environments, it is advantageous to grow quickly and efficiently and complete development before conditions deteriorate (Lind, Persbo & Johansson 2008). Short growing seasons impose strong natural selection pressure on organisms (Lovelock *et al*. 2007), and growing quickly during the short period when conditions are favourable is one strategy for surviving in such environments (Danks 2006). High-latitude habitats have short growing seasons and are cold; thus, organisms are time limited, and in ectothermic animals, growth rates are generally restricted. Consequently, high-latitude species can compensate by shifting their temperature optima towards lower temperatures (Yamahira & Conover 2002), and by evolving a higher capacity for growth or development, especially at lower temperatures (Elser *et al*. 2000; Laugen *et al*. 2003). Thus, high-latitude organisms generally have higher growth rates and are more efficient in using ingested food for growth than their low-latitude conspecifics (Elser *et al*. 2000; Lindgren & Laurila 2005), even under low temperatures.

For an animal to be as efficient as possible in converting food into growth, that is in attaining its highest possible gross growth efficiency (GGE), even under nutrient-limiting conditions, it requires a maximization of its nutrient assimilation efficiency. Therefore, high-latitude populations have adapted to attain high nutrient assimilation efficiencies (Elser *et al*. 2000). High assimilation efficiency is indicative of maximizing nutrient uptake in the gut and minimizing nutrient loss through excretion and egestion. Thus, under nutrient limitation, GGE should be inversely related to the loss rate of the limiting nutrient. Therefore, high-latitude organisms should maximize GGE by having lower nutrient loss rates (i.e. lower CNR) than their low-latitude conspecifics, especially when food quality is low. Animals may be able to attain these higher nutrient assimilation efficiencies and GGEs by increasing their relative gut lengths (Savory & Gentle 1976; Sibly 1981; Yang & Joern 1994; Relyea & Auld 2004). Therefore, high-latitude animals should have relatively longer guts than their low-latitude conspecifics in order to maximize GGE, especially under low-quality food.

With future climate change, mean annual temperatures will increase disproportionally in Arctic and Boreal regions (between 2 and 5 °C by the end of this century, ICCP 2007) and likely lead to heat stress in resident cold-adapted populations as well as lead to a northward migration of species/genotypes (Chen *et al*. 2011). This will change Boreal and Arctic ecosystems and have consequences for key ecosystem functions, such as CNR. In order to understand how CNR is affected by adaptation to high latitudes, we used common frog (*Rana temporaria*) tadpoles from two regions (Arctic/Boreal) and raised these under two temperature regimes (18 °C/23 °C) crossed with two food quality treatments (high/low-N content) in two common garden experiments. In *experiment 1*, we raised all tadpoles until metamorphosis and tested the effects of our experimental variables on nutrient loss rates in relation to life-history parameters (growth rate, GGE). In *experiment 2*, we terminated tadpoles across ontogeny to be able to test how experimental variables and region of origin affected gut length in relation to body size during tadpole development. *Experiment 1* tested hypotheses I–III, and *experiment 2* tested hypothesis IV, as follows:

- **I.** Low-N food leads to stronger N limitation of tadpole growth, and tadpoles thus have lower N loss rates and higher P loss rates (excretion + egestion) in order to maintain a stoichiometrically balanced growth.
- **II.** Arctic tadpoles have higher growth rates and higher GGEs than Boreal tadpoles under ambient temperature conditions.
- **III.** a. GGE should be inversely related to the loss rate of the limiting nutrient.
  b. Thus, in order to maximize GGE, Arctic tadpoles have lower recycling (loss) rates of the limiting nutrient than Boreal tadpoles, especially when food quality is low.
- **IV.** In order to maximize GGE, Arctic tadpoles have evolved relatively longer guts than Boreal tadpoles, especially when fed low-quality food.

Materials and methods

MODEL SPECIES AND REGION OF ORIGIN

We used tadpoles of the common frog *R. temporaria* from two regions. Abisko (the Arctic site) is situated above the Arctic Circle in northern Sweden (68°20′N), whereas Umeå (the Boreal site, 63°50′N) is located 500 km south of Abisko (for more site information, see Liess *et al*. 2013). These two regions were selected since genetic differentiation due to habitat specific adaptation (Palo *et al*. 2003) has been shown, and we collected eggs from four ponds (populations) per region. High-latitude (Arctic) tadpoles are adapted to shorter growing seasons and colder temperatures (for ambient pond water temperature in summer 2012, see

Fig. S1, Supporting information) and have developed higher growth rates and nutrient assimilation efficiencies (Lindgren & Laurila 2005) allowing them to be better competitors (Lindgren & Laurila 2010), whereas lower latitude tadpoles (in the Uppsala region) were adapted to warmer and more variable conditions as well as to higher predation pressure (Laurila, Lindgren & Laugen 2008). Environmental constraints, life-history adaptation to latitude, trade-offs and likely consequences for CNR are presented in Table 1.

EGG COLLECTION AND EGG SIZE

Eggs were collected in May (10 May 2010 and 14 May 2012) in the Boreal and in June (1 June 2010 and 6 June 2012) in the Arctic region. About 20–50 eggs were taken from each sampled egg clutch (family), from each population (pond, four per region), and brought to the laboratory. Eggs were photographed, and the diameter of 10 eggs from each family was 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.

STUDY DESIGN

We conducted two experiments in consecutive years. In both experiments, we used tadpoles from the same ponds, the same food quality treatments and the same temperature treatments. In both experiments, we crossed the treatments food quality (high/low-N content) with temperature of incubation (ambient/warm) in a full factorial design. In *experiment 1,* we sampled five families (egg clutches) from each of four populations (ponds) in each region (Arctic/Boreal). 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 (see Liess *et al.* 2013). For *experiment 2,* we collected eggs from as many egg clutches (families) as possible from our four study populations (ponds) described above, hatched all eggs from each region together, and then haphazardly selected 20 tadpoles from each region for each treatment combination, leading to 80 tadpoles per region and 160 experimental units in total.

TREATMENT VARIABLES

The temperature treatments were 18 °C and 23 °C. Pond water temperature was measured during 2012 (stowaway Tidbit underwater data loggers, Onset HOBO data loggers, Borne, USA) and showed that even during the particularly cold June of that year (mean temperature −1 °C, colder than average in the studied regions, see SMHI 2015), all Boreal ponds and half the Arctic ponds reached 18 °C pond water temperature at some point during the tadpole larval period, whereas only the shallowest Boreal pond reached a water temperature of over 23 °C (Fig. S1). Thus, the temperature treatment of 23 °C is considered as a stressful condition. The different food quality treatments were chosen to contain the same proportions of carbon (C) (42%) and P (0·9%) per dry mass (DM) but differ in N content. High-N food contained 6% N per DM, whereas low-N food contained only 3% N per DM. N was chosen as the limiting nutrient, since previous studies indicated N to be the limiting nutrient for benthic consumer growth in central (Liess & Hillebrand 2005, 2006), and especially in the northern Sweden (Liess, Drakare & Kahlert 2009). Food was a mixture of finely ground fish food and rabbit chow (as used by Lind, Persbo & Johansson 2008), mixed in different proportions (fish food: rabbit chow dry weight at 3 : 1 and 1 : 3 for high-N and low-N food, respectively). Since ranid tadpoles are omnivores, this type of food is comparable to their natural food (Schiesari, Werner & Kling 2009; Caut *et al.* 2013).

EXPERIMENTAL PROCEDURE

After collection, eggs from each family were placed in plastic containers, fully submerged in water (~10 cm deep) and left to hatch. For *experiment 1,* each egg clump was kept separate, whereas for *experiment 2* all eggs from one region were pooled. When the tadpoles reached Gosner stage 23 (free swimming, Gosner 1960), a control group of 20 tadpoles (10 haphazardly chosen tadpoles from each region) was frozen to estimate start DM. For *experiment 1*, 16 tadpoles from each family were haphazardly chosen, and for *experiment 2*, 20 tadpoles from each region were haphazardly chosen (see *Study design*). These tadpoles were put into experimental units (for details, see Liess *et al.* 2013) for the corresponding treatments, and transferred to their respective

Table 1. Depiction of the connections between the different environmental constraints, the physiological and life-history adaptations, the trade-offs and the envisioned ecosystem consequences of regional adaptation. Superscripts on adaptations and trade-offs indicate by which experiment each adaptation was investigated

Region	Constraint	Adaptation	Trade-off	Ecosystem consequence
Arctic	Short growing season Low temperature *But:* Low predation pressure	High growth (and dev.) rate[a,c] Small size at metamorphosis[c] High growth efficiency[a] Long guts[b] Low nutrient loss rates[a] Low temperature optima	Low defences against predation	Nutrients are cycled slowly
Boreal	High predation pressure Variable conditions High temperatures	Slow growth (and dev.) rate[a,c] Large size at metamorphosis[c] Small bodies, but wide tails (predator defence) High temperature optima	Short guts (low assimilation efficiency)[b] High nutrient loss (high consumer-mediated nutrient recycling)[a]	Nutrients are cycled quickly

[a]Examined in *experiment 1* to answer hypotheses I–III. [b]Examined in *experiment 2* to answer hypothesis IV. [c]Examined in *experiment 1* and presented in Liess *et al.* (2013).

climate rooms with a photoperiod of 6-h: 18-h dark: light. Each of the 640 experimental units (*experiment 1*) or 160 units (*experiment 2*) 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 (15 mg on day 0 and 4, 30 mg on day 8, 45 mg on day 12, 60 mg on day 16 and 75 mg every 4th day thereafter). These amounts of food were sufficiently little, so that all food was consumed, prior to the subsequent feeding. Containers were cleaned and water was replaced every fourth day, directly prior to feeding. For *experiment 1*, 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 to determine dry weight and body nutrient composition (data presented in Liess *et al*. 2013). For *experiment 2*, two tadpoles from each treatment were terminated on days 2, 5, 9, 15, 17 and 21. In addition, 2 more tadpoles from each treatment were terminated when tadpoles reached Gosner stage 42 and when tadpoles reached Gosner stage 46 (complete absorbance of tail, Gosner 1960). All tadpoles (*experiments 1* and *2*) were killed by placing them individually in the anaesthetic MS-222 (ethyl 3-aminobenzoate methanesulphonate). The tadpoles were then transferred to a Petri dish containing water, rinsed, frozen and then freeze-dried.

LIFE-HISTORY RESPONSE VARIABLES

We examined tadpole age, size, growth, GGE and gut length. For *experiment 1,* we photographed tadpoles at age 2, 8, 14 and 16 days and estimated their length (L in cm) using digital image analyses (IMAGEJ, version 1.45s, Softonics, Barcelona, Spain). We calculated tadpole DM (in mg) using temperature- and region-specific length–mass regressions, from *experiment 2*, since region ($P = 0{\cdot}031$) and the temperature × region interaction ($P = 0{\cdot}024$) affected variables a and b in equation 1.

$$\mathrm{DM\,(mg)} = a^{b \times L(\mathrm{cm})} \qquad \text{eqn 1}$$

where estimated values for variables a and b were 0·5 and 1·03 for warm Arctic tadpoles; 0·4 and 1·04 for warm Boreal tadpoles; 0·6 and 1·06 for ambient Arctic tadpoles; and 0·3 and 1·07 for ambient Boreal tadpoles, respectively. We then used tadpole DM (mg) from day 2 ($\mathrm{DM_{d2}}$), day 8 ($\mathrm{DM_{d8}}$) and day 16 ($\mathrm{DM_{d16}}$) to calculate tadpole growth rate ($\mathrm{day^{-1}}$) and GGE (%) using equations 2 and 3.

$$\mathrm{Growth\ rate_{d2-d16}}\left(\mathrm{day^{-1}}\right) = \frac{\ln \mathrm{DM_{d2}} - \ln \mathrm{DM_{d16}}}{t(\mathrm{d})} \qquad \text{eqn 2}$$

where t time in days from day 2 to day 16 and ln the natural logarithm.

$$\mathrm{GGE_{d8-d16}}(\%) = \frac{\mathrm{DM_{d8}} - \mathrm{DM_{d16}}}{I} \times 100, \qquad \text{eqn 3}$$

where I is the ingested food (mg) from day 8 to day 16 (75 mg, see Experimental Procedure).

For *experiment 2*, we determined Gosner stage (Gosner 1960) in relation to age for all terminated tadpoles and photographed them with a digital camera. We then dissected these tadpoles to determine their gut length (only possible from day 9). The gut was removed, placed in a small Petri dish, arranged, without stretching it, and then photographed. Tadpole and gut lengths were measured using digital image analyses (IMAGEJ, version 1.45s). After dissection, tadpoles and guts were freeze-dried and weighed. Using tadpole length and DM, we established growth curves and the above-described length–mass regressions.

EXCRETION AND EGESTION RATES

Since tadpoles recycled or 'lose' N and P through dissolved excretion and faecal pellet egestion, we conducted excretion trials according to Liess (2014) on day 14 of *experiment 1*. Tadpoles were transferred to a container with 20 mL of pre-filtered aerated water with known concentrations of soluble reactive P (SRP) and dissolved inorganic N (DIN). Tadpoles were left to excrete without food for 1 h (according to Vanni *et al*. 2002). One hour was long enough to attain reliable estimates for nutrient excretion and egestion rates, and short enough for excretion and egestion rates not to start decreasing due to tadpole starvation. After excretion trials, tadpoles were photographed and DM was estimated (see above). Water with excretion products and faecal pellets was divided into two parts and filtered onto two pre-combusted (540 °C, 4 h) GF/C (Whatman) filters to separate fluid excretion from faecal pellet egestion. Filtered water was immediately analysed for $NO_3 + NO_2$ and NH_4^+ (DIN) and SRP. DIN was analysed with the sulphanilamide method and SRP with the ammonium–molybdate method (Grasshoff, Ehrhardt & Kremling 1983) in a Flow Injection Analyzer (FIA). Filters with attached faecal pellets were dried and frozen for the measurement of egested particulate CN and egested particulate P. P was measured as phosphate after hydrolysis with heating and potassium persulphate (Grasshoff, Ehrhardt & Kremling 1983), and CN was measured with a CHN analyzer (LECO CHN-932). Using estimated day 14 tadpole DM, we calculated tadpole N and P excretion and egestion rates ($\mathrm{h^{-1}}$).

STATISTICAL ANALYSES

For *experiment 1*, 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 nutrient excretion and egestion rates, using population (nested within region) and family (nested within population) as random factors (Tables 2 and 3). All models were implemented in a Bayesian MCMC framework using *MCMCglmm* (Hadfield 2009). All models (iterations: 50 000; burn in: 5000; thinning interval: 100) used parameter-expanded priors (centred at 0; variance of 1000), which are non-informative in relation to the data but have proper distributions (Gelman 2006). We performed model simplification according to deviance information criterion (DIC; Bayesian equivalent to Akaike's Information Criterion) to determine the best model (lowest DIC) for each response variable (Tables 2 and 3).

For *experiment 2*, tadpoles were sampled randomly from all families and populations within each region (see Experimental Procedure); thus, we could not estimate family and population effects. We used ANCOVAS, with region, temperature and food quality as fixed factors and Gosner stage as a continuous predictor variable to control for the effect of developmental stage (Table 4). For the covariate Gosner stage, we subtracted 23 units for a meaningful 0-intercept, since experiments started at Gosner stage 23. We also used three-way ANOVAS to determine the effects

Table 2. Best minimal models to explain response variables N and P excretion rates (μg mg^{-1} h^{-1}) as well as N and P loss rates (μg mg^{-1} h^{-1}) of *experiment 1* using deviance information criteria (DIC). 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. pMCM < 0.05 are printed in bold

Predictor variables			*Predictor variables*		
Response variable: N excretion rate			*Response variable*: N excretion and egestion rate (=N loss rate)		
(Best model with lowest DIC)	post.mean	pMCMC	(Best model with lowest DIC)	post.mean	pMCMC
Intercept (Arctic, ambient, high)	−0·93	**< 0·001**	Intercept (Arctic, ambient, high)	0·47	**0·002**
Region (Arctic → Boreal)	0·14	0·188	Region (Arctic → Boreal)	0·21	**0·046**
Temperature (ambient → warm)	0·0021	0·968	Temperature (ambient → warm)	0·23	**< 0·001**
Food quality (high → low)	−0·40	**< 0·001**	Food quality (high → low)	0·11	**0·008**
Region × Temp	−0·091	**0·081**	Region × Temp	−0·35	**< 0·001**
Region × Food quality	0·082	0·126	Temp × Food quality	−0·19	**0·004**
Temp × Food quality	−0·23	**< 0·001**			**0·002**
Response variable: P excretion rate			*Response variable*: P excretion and egestion rate (=P loss rate)		
(Best model with lowest DIC)			(Best model with lowest DIC)		
Intercept (Arctic, ambient, high)	−2·1	**< 0·001**	Intercept (Arctic, ambient, high)	−0·62	**< 0·001**
Region (Arctic → Boreal)	0·033	0·744	Region (Arctic → Boreal)	0·16	0·134
Temperature (ambient → warm)	0·28	**< 0·001**	Temperature (ambient → warm)	0·22	**0·002**
Food quality (high → low)	−0·28	**< 0·001**	Food quality (high → low)	0·34	**< 0·001**
Region × Food quality	−0·15	**0·018**	Region × Temp	−0·12	0·214
Temp × Food quality	−0·16	**0·014**	Region × Food quality	0·29	**0·004**
			Temp × Food quality	−0·17	0·062
			Region × Temp × Food quality	−0·30	**0·028**

of region of origin, temperature and food quality on tadpole life-history response variables on experimental day 14. Model simplification was performed when necessary, using AIC. All described effects were significant (pMCMC <0·05 or $P < 0·05$) unless otherwise specified. We conducted all statistics in the statistical package *R* 2.14.0 (R Development Core Team 2011).

Results

TADPOLE NUTRIENT LOSS RATES (EXPERIMENT 1)

On average, 81% (±11) (±standard deviation, SD) of total N loss (excretion + egestion) happened through faecal pellet egestion (hatched areas, Fig. 1a,b), whereas only 19% of recycled N was lost in dissolved form. Region of origin affected total N loss rate, which was higher in Boreal than in Arctic tadpoles, but only under ambient temperature (Fig. 1a,b, Table 2). Additionally, total N loss rate was higher under low-N food, but only in ambient treatments (Fig. 1a,b, temperature × food quality interaction, Table 2). In contrast, when considering only dissolved nutrients loss, N excretion rates were lower, when tadpoles were fed lower N food, especially under warm conditions (Fig. 1c,d, temperature × food quality interaction, Table 2).

On average, 80% (±SD) (±8·9) of total P loss (excreted + egested) was lost faecal pellet form (hatched area, Fig. 2a,b) and only 20% as dissolved excretions. Under low-N food, total P loss rate was higher in Boreal than in Arctic tadpoles, but only under ambient temperature (Fig. 2a,b, three-way interaction, Table 2). Lower N food in general led to increased total P loss rates, especially under ambient conditions (Fig. 2a,b, food quality × temperature interaction, Table 2). In contrast, dissolved P excretion rates were reduced when tadpoles fed on low-N food, especially under warm conditions (Fig. 2c,d, temperature × food quality interaction, Table 2). Last, food quality affected P excretion rates more strongly in Boreal than in Arctic tadpoles (Fig. 2c, d, region × food quality interaction, Table 2).

TADPOLE GROWTH RATE AND GGE (EXPERIMENT 1)

Growth rate was higher in Arctic than in Boreal tadpoles in ambient treatments, but similar in warm treatments (Fig. 3a,b, best minimal model in Table 3). In general, higher temperature and higher food quality led to higher tadpole growth rates (Fig. 3a,b, Table 3). Tadpole GGE was higher in Arctic than in Boreal tadpoles under ambient conditions, whereas this pattern was reversed under warm conditions, indicating geographic adaptation. In addition, low food quality and warm temperatures reduced GGE (Fig. 3c,d, best minimal model without covariate in Table 3). When we included the covariate total N loss rate in addition to the fixed factors in the model for predicting GGE (see Table 3), our model improved significantly (DIC decreased by 25%). There was a negative relationship between N loss rate and GGE, especially in ambient treated Boreal tadpoles

(Fig. 4; Table 3: N loss × region × temperature interaction), which was steeper in high-N food (Fig 4a,c) compared to low-N food treatments (B and D; Table 3: N loss × region × food quality interaction).

TADPOLE AGE, SIZE AND GUT LENGTH DURING DEVELOPMENT (EXPERIMENT 2)

We used the covariate developmental (Gosner) stage in all models and tested treatment effects on tadpole life-history parameters corrected for developmental stage (Table 4). At each Gosner stage, Boreal tadpoles were older than Arctic tadpoles and tadpoles from ambient treatments were older than tadpoles from warm treatments (Fig. 5a). This effect became stronger at later Gosner stages, that is for more developed tadpoles (Gosner stage × region,

Table 3. Best minimal models for *experiment 1* with covariates to explain the response variables tadpole growth rate and tadpole gross growth efficiency (GGE) using deviance information criteria (DIC). 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. pMCMC < 0.05 are printed in bold

Predictor variables	post.mean	pMCMC
Response variable: Growth rate		
(Best model with lowest DIC, no covariate)		
Intercept (Arctic, ambient, high) −1·8	**< 0·001**	
Region (Arctic → Boreal)	−0·042	0·096
Temperature (ambient → warm)	0·027	**0·004**
Food quality (high → low)	−0·10	**< 0·001**
Region × Temperature	0·055	**< 0·001**
Response variable: GGE		
(Best model with lowest DIC, no covariate)		
Intercept (Arctic, ambient, high)	3·3	**< 0·001**
Region Arctic → Boreal)	−0·17	**0·036**
Temperature (ambient → warm)	−0·11	**0·006**
Food quality (high → low)	−0·35	**< 0·001**
Region × Temperature	0·28	**< 0·001**
Region × Food quality	0·091	0·110
Response variable: GGE		
(Best model with lowest DIC, covariate: N loss)		
Intercept (Arctic, ambient, high)	3·2	**< 0·001**
Covariate: N loss	−0·0099	0·848
Region (Arctic → Boreal)	0·30	**0·042**
Temperature (ambient → warm)	0·31	**0·012**
Food quality (high → low)	−0·0028	0·982
N loss × Region	−0·22	**0·002**
N loss × Temperature	−0·18	**0·006**
Region × Temperature	−0·17	**< 0·001**
N loss × Food quality	−0·23	0·126
Region × Food quality	−0·39	**0·012**
Temperature × Food quality	−0·058	0·320
N loss × Region × Temperature	0·21	**0·012**
N loss × Region × Food quality	0·26	**< 0·001**

Table 4. Best minimal models for *experiment 2* with covariate Gosner stage to explain tadpole age (day), tadpole dry mass until G 37 (mg) and tadpole relative gut length (m g^{-1}) during tadpole ontogeny using Akaike's Information Criteria (AIC). *t*-Values indicate the strength and direction of the main effects. *t*-Values of the interaction terms signify the direction and strength of the combined effects of the parameters involved in the interaction. The adjusted R^2 values ($R^2_{adj.}$), degrees of freedom (d.f.), *P*-values and parameter estimates are presented. *P*-values < 0.05 are printed in bold

Predictor variables	Parameter estimate	*t*-value	*P*-value
Response variable: Tadpole age			
(Best model with lowest AIC, $R^2_{adj.}$ = 0·96, d.f. = 128)			
Intercept (Boreal, ambient, high)	−1·037	−1·8	0·073
Covariate: Gosner stage	1·698	40	**< 0·001**
Region (Arctic → Boreal)	2·129	3·5	**< 0·001**
Temperature (ambient → warm)	0·024	0·04	0·969
Food quality (high → low)	0·106	0·30	0·762
Gosner stage × Region	0·216	4·5	**< 0·001**
Gosner stage × Temperature	−0·398	−8·4	**< 0·001**
Response variable: Tadpole DM until Gosner stage 37			
(Best model with lowest AIC, $R^2_{adj.}$ = 0·92, d.f. = 128)			
Intercept (Boreal, ambient, high)	−6·916	−58	**< 0·001**
Covariate: Gosner stage	0·276	20	**< 0·001**
Region (Arctic → Boreal)	0·245	2·1	**< 0·001**
Temperature (ambient → warm)	0·220	1·9	0·065
Food quality (high → low)	0·008	0·66	0·513
Gosner stage × Region	0·022	1·4	**< 0·001**
Gosner stage × Temperature	−0·064	−4·4	**< 0·001**
Gosner stage × Food quality	−0·026	−1·9	0·067
Response variable: Tadpole relative gut length			
(Best model with lowest AIC, $R^2_{adj.}$ = 0·86, d.f. = 71)			
Intercept (Boreal, ambient, high)	1737·616	8·8	**< 0·001**
Covariate: Gosner stage	−91·272	−7·4	**< 0·001**
Region (Arctic → Boreal)	−194·58	−0·75	0·454
Temperature (ambient → warm)	764·39	2·3	**0·025**
Food quality (high → low)	388·07	1·6	0·108
Gosner stage × Region	8·829	0·58	0·563
Gosner stage × Temperature	−42·103	−2·0	**0·049**
Region × Temperature	−994·486	−2·8	**0·007**
Gosner stage × Food quality	−26·378	−1·5	0·134
Region × Food quality	−330·179	−1·3	0·191
Temperature × Food quality	−722·455	−1·9	0·061
Gosner stage × Region × Temperature	62·614	2·6	**0·012**
Gosner stage × Region × Food quality	28·242	1·3	0·192
Gosner stage × Temperature × Food quality	55·584	1·9	0·063
Region × Temperature × Food quality	937·523	2·1	**0·039**
Gosner stage × Region × Temp. × Food quality	−71·640	−2·1	**0·042**

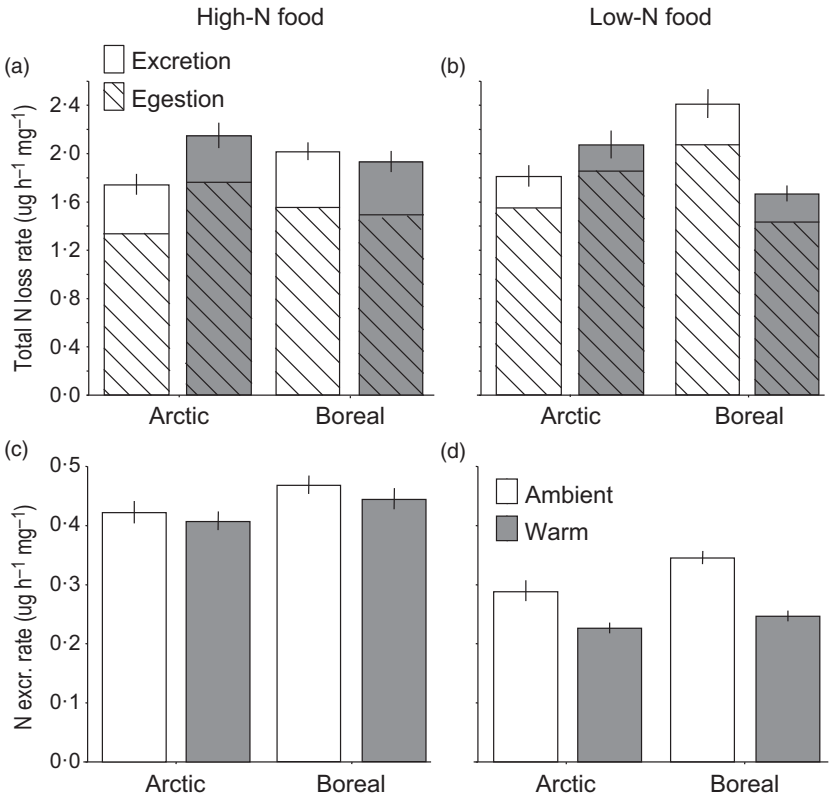

Fig. 1. (a, b) Mean (±SE) total N excretion and egestion rates (μg h^{-1} mg^{-1}), and (c, d) mean (±SE) N excretion rate (μg h^{-1} mg^{-1}) of Boreal and Arctic tadpoles during early development (day 14) in the different temperature treatments of the (a, c) high-N food treatment and (b, d) the low-N food treatment (*experiment 1*).

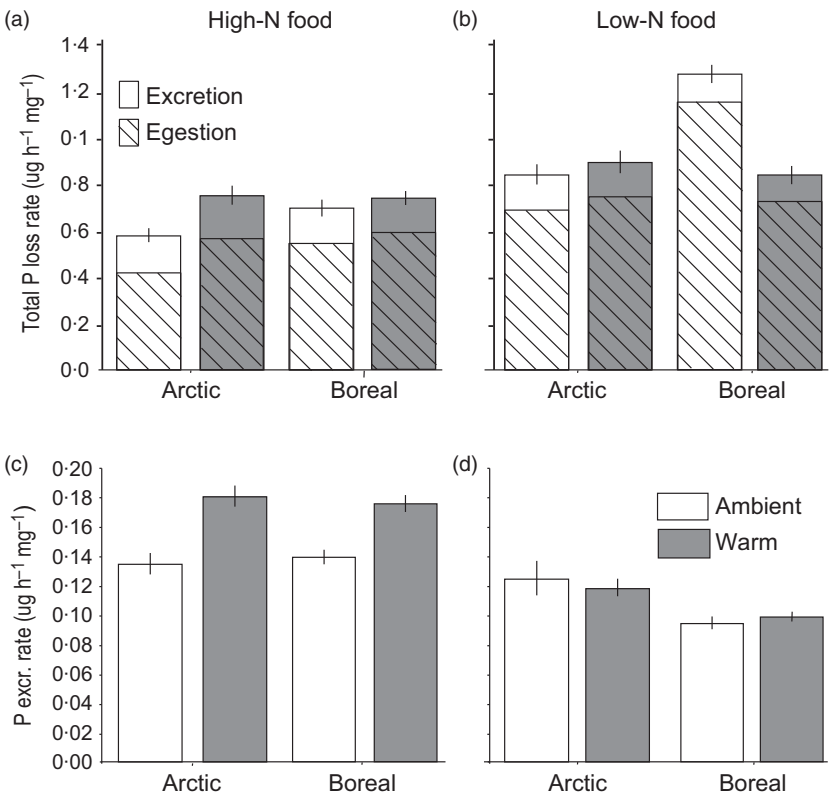

Fig. 2. (a, b) Mean (±SE) total P excretion and egestion rates (μg h^{-1} mg^{-1}), and (c, d) P excretion rate (μg h^{-1} mg^{-1}) of Boreal and Arctic tadpoles during early development (day 14) in the different temperature treatments of the (a, c) high-N food treatment and (b, d) the low-N food treatment (*experiment 1*).

Gosner stage × temperature interactions, Table 4). On day 14, Arctic tadpoles had reached higher Gosner stages than Boreal tadpoles and Arctic tadpoles were more developed in the warm compared to ambient temperature treatments (inlay in Fig. 5a; region × temperature interaction, Table 5).

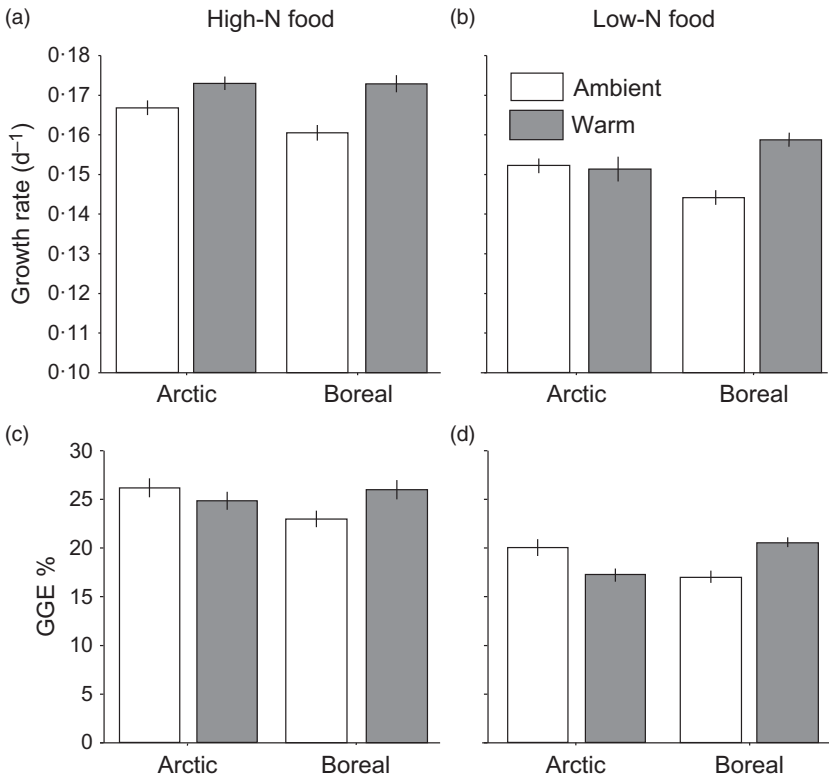

Fig. 3. (a, b) Mean (±SE) growth rate (day^{-1}), and (c, d) mean (±SE) gross growth efficiency (GGE) (%) of Boreal and Arctic tadpoles during early development in the different temperature treatments of the (a, c) high-N food treatment and (b, d) the low-N food treatment (*experiment 1*). Growth rates are calculated for the interval of day 2 to day 16 and GGE for day 8 to day 16 (*experiment 1*).

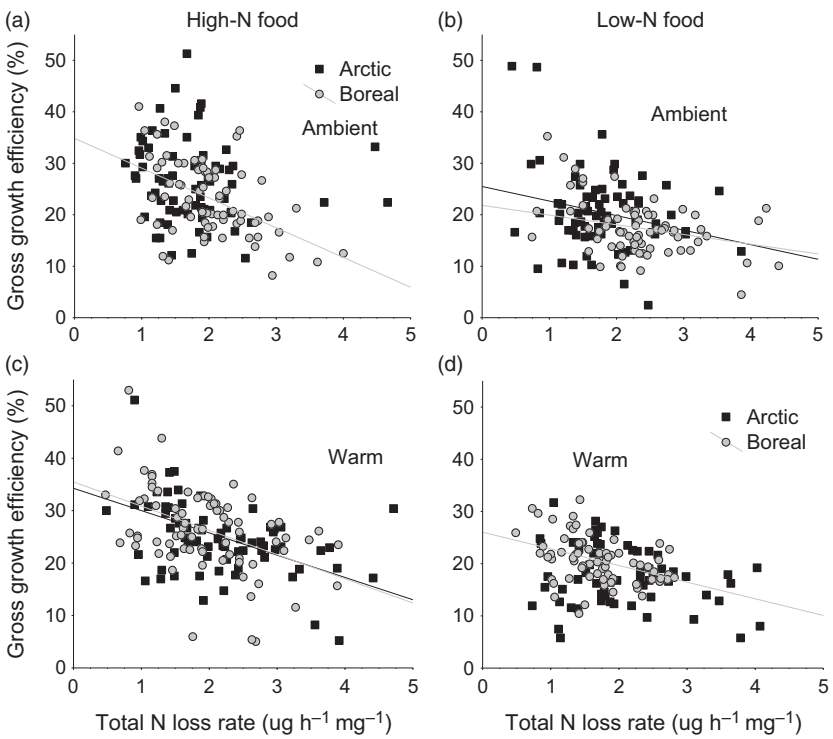

Fig. 4. Gross growth efficiency (GGE; %) of tadpoles in the ambient (a, b) and in the warm (c, d) climate rooms plotted against total N loss (N excretion + egestion) rate (μg h^{-1} mg^{-1}) for both Arctic (black squares) and Boreal (white circles) tadpoles for (a, c) high-N and (b, d) low-N food treatments. GGEs are calculated for the interval of day 8 to day 16 and N loss rates were measured on day 14 (*experiment 1*).

Boreal tadpoles were larger than Arctic tadpoles, and tadpoles from ambient treatments were larger than tadpoles from warm treatments, especially at higher Gosner stages (Gosner stage × temperature interaction, Table 4, Fig. 5b). On day 14, Arctic tadpoles were heavier than Boreal ones, warm-treated tadpoles were heavier than ambient ones, and tadpoles fed high-N food were heavier than those fed low-N food (inlay in Fig. 5b, Table 5). These effects were, however, not additive, since temperature effects were strongest in Arctic tadpoles fed low-N

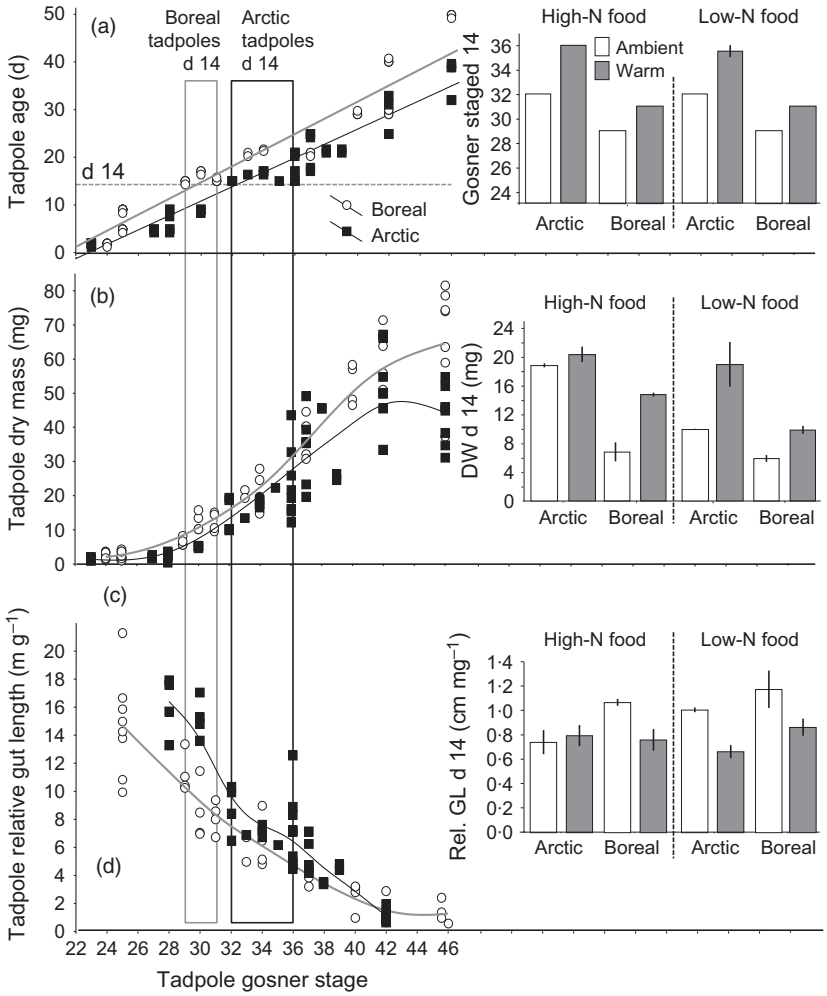

Fig. 5. (a) Tadpole age, (b) dry mass (DM) and (c) relative gut length plotted against tadpole Gosner stage (23–46) for Boreal (white circles) and Arctic (black squares) (*experiment 2*), pooled for all temperature and food quality treatments. Lines are added to for illustrative purposes as a (a) linear regression lines, and (b) and (c) as distance weighted least squares regression lines, and are not connected to our statistical models. The encircled areas mark the developmental stages when Boreal tadpoles (grey line) and Arctic tadpoles (black line) were 14 days old. Inlays represent mean (±SE) (a) tadpole developmental stage, (b) DM and (c) relative gut length on day 14 of *experiment 2* of Boreal and Arctic tadpoles in different temperature and food quality treatments.

food and in Boreal tadpoles fed high-N food (three-way interaction, Table 5).

Tadpole gut length corrected for body size (relative gut length in m g^{-1}) decreased during ontogeny. The effect of region, food quality and the strong positive effect of temperature on relative gut length depended on the interaction between all factors (best model, Table 4, Fig. 5c). Arctic tadpoles had relatively longer guts than Boreal tadpoles, especially in warm treatments and when fed high-N food (see interactions, Table 4). Reduced-N food increased relative tadpole gut length especially in ambient Arctic tadpoles (region × temperature × food quality interaction, Table 4), whereas warmer temperatures led to increased relative gut length, but only in Arctic tadpoles and especially during early developmental stages (Gosner stage × temperature × region interaction, Table 4). Lastly, Arctic tadpoles had relatively longer guts than Boreal tadpoles during early development, especially in the warm, high-N food treatment (four-way interactions, Table 3). On day 14, the best model included a non-significant three-way interaction between region, temperature and food quality (Table 5, inlay Fig 5c). Guts were relatively longer in Boreal than in Arctic tadpoles, while the effect of temperature depended on food quality; tadpoles under ambient temperatures had relatively longer guts than warm tadpoles, and this effect was stronger in tadpoles fed low-N food.

Discussion

OVERVIEW

We found that *R. temporaria* tadpoles were able to plastically respond to temperature and food quality changes by adjusting their gut lengths. They were also able to differentially excrete and egest nutrients in order to maintain their elemental balance. However, this ability depended on their region of origin. Higher latitude populations showed adaptations that led to reduced nutrient loss under nutrient limitation, especially when growing in ambient temperature conditions. Our results suggest that evolved adaptations in nutrient assimilation efficiency may be beneficial for maintaining high growth rates among time-stressed high-latitude populations, and such adaptation may thus reduce nutrient turnover at the ecosystem level. This study emphasizes the importance of within species genetic diversity and adaptation for ecosystem processes.

Table 5. Best minimal models to explain tadpole age (day), tadpole dry mass (mg) and tadpole relative gut length (m g^{-1}) on day 14 of *experiment 2* using Akaike's Information Criteria (AIC). t-Values indicate the strength and direction of the main effects. *t*-Values of the interaction terms signify the direction and strength of the combined effects of the parameters involved in the interaction. The adjusted R^2 values ($R^2_{adj.}$), degrees of freedom (d.f.), *P*-values and parameter estimates are presented. *P*-values < 0.005 are presented in bold

Predictor variables	Parameter estimate	*t*-value	*P*-value
Response variable: Tadpole Gosner stage on day 14			
(Best model with lowest AIC, $R^2_{adj.}$ = 0·99, d.f. = 11)			
Intercept (Boreal, ambient, high)	32·063	65	**< 0·001**
Region (Arctic → Boreal)	−3·000	−17	**< 0·001**
Temperature (ambient → warm)	3·750	21	**< 0·001**
Food quality (high → low)	−0·125	−1	0·339
Region × Temperature	−1·750	−7	**< 0·001**
Response variable: Tadpole weight on day 14			
(Best model with lowest AIC, $R^2_{adj.}$ = 0·91, d.f. = 8)			
Intercept (Boreal, ambient, high)	−3·967	−40	**< 0·001**
Region (Arctic → Boreal)	−1·029	−7·4	**< 0·001**
Temperature (ambient → warm)	0·076	0·55	0·599
Food quality (high → low)	−0·634	−4·6	**0·002**
Region × Temperature	0·710	3·6	**0·007**
Region × Food quality	0·507	2·6	**0·033**
Temperature × Food quality	0·552	2·8	**0·023**
Region × Temperature × Food quality	−0·827	−3·0	**0·018**
Response variable: Tadpole relative gut length on day 14			
(Best model with lowest AIC, $R^2_{adj.}$ = 0·62, d.f. = 8)			
Intercept (Boreal, ambient, high)	745·730	8·762	**< 0·001**
Region (Arctic → Boreal)	327·840	2·724	**0·026**
Temperature (ambient → warm)	53·170	0·442	0·670
Food quality (high → low)	264·060	2·194	0·060
Region × Temperature	−361·490	−2·124	0·066
Region × Food quality	−156·850	−0·921	0·384
Temperature × Food quality	−395·440	−2·323	**0·049**
Region × Temperature × Food quality	392·520	1·631	0·142

More specifically, we show that in order to maintain nutritionally balanced growth, tadpoles feeding on low-N food had lower N excretion rates (but not lower overall N loss rates) and higher total P loss (excretion + egestion) rates than tadpoles feeding on high-N food (partly supporting hypothesis I). Arctic tadpoles had higher growth rates and higher GGEs than Boreal tadpoles, but only under lower temperature (partly supporting hypothesis II). GGE was inversely related to the loss rate of the limiting nutrient (supporting hypothesis IIIa); however, this relationship depended on region of origin and temperature. In order to maximize GGE, Arctic tadpoles had lower nutrient loss rates than Boreal tadpoles, especially under low-N food, but only in ambient treatments (partially supporting hypothesis IIIb), and Arctic tadpoles had relatively longer guts during early development than Boreal tadpoles (supporting hypothesis IV).

NUTRIENT EXCRETION AND EGESTION

In order to maintain nutritionally balanced growth, animals regulate nutrient loss rates (Sterner 1990; Elser & Urabe 1999). Due to physiological constraints, nutrient loss is usually regulated through differential nutrient excretion in dissolved form (Sterner & Elser 2002), whereas faecal pellet egestion is more likely a function of food digestibility. Thus, when feeding on low-N food, tadpoles excreted less N. However, despite reduced N excretion rates, total N loss rates increased slightly in tadpoles feeding on N-deficient food, probably due to the lower digestibility of low-N food (more plant material). Therefore, less N was assimilated in the gut, which increased N losses via faecal pellets. In order to maintain their stoichiometric balance, P loss rates were much higher in tadpoles fed low-N food compared to tadpoles fed high-N food. However, animals can also cope with nutritionally imbalanced food by storing nutrients, thus changing their overall body nutrient stoichiometry (Sterner & Elser 2002). Arctic tadpoles had a slightly more flexible nutrient stoichiometry than Boreal tadpoles (Liess *et al.* 2013), although there was no clear evidence, indicating that tadpoles were able to store excess P when feeding on low-N food (Liess *et al.* 2013). We conclude that stoichiometric principles (Elser & Urabe 1999) can explain CNR, when both dissolved excretion products and egestion in faecal pellet form are considered (Liess 2014).

REGION OF ORIGIN-SPECIFIC GROWTH RATE AND GGE DEPENDS ON TEMPERATURE

Arctic tadpoles had higher growth rates and GGEs than Boreal tadpoles only in the ambient treatments, indicating geographic adaptation. High-latitude genotypes can compensate for lower temperatures by shifting their temperature optima, ensuring maximum growth rates and GGEs are attained at lower temperatures (Yamahira & Conover 2002). The ambient treatment was likely closer to the optimal temperature for Arctic than for Boreal tadpoles, whereas the warm treatment probably restricted Arctic growth due to heat stress, but did not restrict Boreal tadpole growth to the same extent. Thus, as documented previously for *R. temporaria* tadpoles, although using lower temperatures than in the present study (Lindgren & Laurila 2005), latitude of origin and temperature interacted in determining tadpole growth rates and GGEs.

GGE AND NUTRIENT LOSS

Since GGE depends on efficient nutrient uptake and incorporation into body mass, GGE should be inversely related to the loss rate of the limiting nutrient, N. High

GGE means maximizing nutrient uptake in the gut, but also minimizing nutrient loss. In accordance with this, we found that GGE was inversely related to N loss rate; however, this relationship depended on tadpole region of origin and temperature treatment. Due to the strong selection pressure for rapid development rates in Arctic tadpoles as a consequence of an abbreviated growing seasons (Laugen *et al.* 2003; Palo *et al.* 2003), Arctic tadpoles have consistently higher development rates than their Boreal counterparts, often coupled with higher growth rates and GGEs (Lindgren & Laurila 2005). In order for Arctic tadpoles to grow more efficiently (than Boreal tadpoles), they should therefore lose fewer limiting nutrients. In accordance with this, we found that in the ambient temperature treatment, Arctic tadpoles had higher GGEs and at the same time lower N loss rates than Boreal tadpoles. However, in the warm treatments, the situation was reversed, likely due to increased heat stress experienced by Arctic tadpoles. Lindgren & Laurila (2005) also reported that temperature affected region of origin-specific GGE, with reduced effects of latitude on GGE under higher temperatures. Applied to our study, this suggests that Boreal tadpoles were less stressed under warmer temperatures than Arctic tadpoles. Probably, warm incubated Arctic tadpoles were stressed (as indicated by Liess *et al.* 2013) and suffered from less efficient nutrient uptake in the gut, possibly connected to digestive enzyme adaptation to lower temperatures (Angilletta 2009), resulting in higher N losses under heat stress. Our results show clearly that GGE and the limiting nutrient loss rate were inversely connected and that temperature effects on tadpole GGE and nutrient loss rates depend on region of origin.

AGE, SIZE AND GUT LENGTH THROUGH ONTOGENY

Arctic tadpoles developed faster than Boreal tadpoles, especially under warmer temperatures. Tadpoles under ambient temperatures were larger and older at each developmental stage than warm-treated tadpoles, especially for those originating from lower latitudes (consistent with Laugen *et al.* 2003; Lindgren & Laurila 2005). However, in contrast to Liess *et al.* (2013), where tadpoles at metamorphosis were larger when fed higher quality food, we found no food quality effect on tadpole size at earlier developmental stages (<Gosner stage 38), indicating that food quality effects possibly take some time to manifest in body size.

We estimated GGE during early tadpole development (pre-Gosner stage 38), when growth rates were highest, to test whether longer guts were the mechanism behind higher GGEs in Arctic compared to Boreal tadpoles. Previous studies have shown that gut length was longer in amphibians originating from higher compared to lower altitudes (Naya, Veloso & Bosinovic 2009; Lou *et al.* 2013) and latitudes (Lindgren & Laurila 2005), possibly due to adaptation to lower food quality/availability at higher altitudes and latitudes necessitating more efficient resource use. We also found that relative tadpole gut length decreased during tadpole development. Gut length decrease during later larval stages is common in *Rana* species (Pretty, Naitoh & Wassersug 1995; and references therein) and may be connected with the switch from a mainly vegetarian to a more carnivorous diet (Wickramasinghe, Oseen & Wassersug 2007). However, despite documented *Rana* gut length plasticity due to food quality (Relyea & Auld 2004; Stoler & Relyea 2013), reducing food quality only had weak effects on gut length in our study. In the Arctic ambient treatment, relative gut length was higher at lower food quality. Thus, Arctic tadpole gut length plasticity in response to food quality might be adaptive, since high-latitude tadpoles are dependent on efficient growth during the short summer. In addition, predation pressure at higher latitudes is generally low (Laurila, Lindgren & Laugen 2008), enabling high-latitude tadpoles to forage more activity (Laurila, Lindgren & Laugen 2008) and invest more resources into gut tissue (this study, Lindgren & Laurila 2005). Conversely, Boreal tadpoles producing longer guts would divert resources away from predator defences, since a longer gut means a larger body at the cost of a shorter tail, thus decreasing the chance of escape from predation (Van Buskirk & Relyea 1998). Our results support the hypothesis that low-latitude tadpoles have relatively shorter guts than high-latitude tadpoles. This was also found by Lindgren & Laurila (2005), when comparing Arctic *R. temporaria* tadpoles with southern conspecifics from the Uppsala region. However, relative tadpole gut length did not always covary with GGE. Thus, our results confirm findings from earlier experiments with *R. temporaria* tadpoles, where increased gut length was found to contribute to higher GGEs in high-latitude tadpoles, but was likely not the sole adaptive mechanism (Lindgren & Laurila 2005).

Our results show that Arctic tadpoles aged 14 days (especially under warmer temperatures) were more developed than Boreal tadpoles. It is not clear how these differences in developmental stage across experimental treatments influenced tadpole nutrient assimilation efficiency and GGE. However, differences in GGE during these early developmental stages (during maximum tadpole growth rates) are likely relatively small. Since relative gut length is higher in less developed tadpoles, we assume that (if there are any differences in GGE) less developed tadpoles may have slightly higher GGEs and lower nutrient loss rates than more developed tadpoles. If this is true, then latitudinal differences between tadpoles of similar developmental stages would be even stronger than the differences found in this study.

ECOSYSTEM CONSEQUENCE OF ADAPTATION TO LATITUDE: SLOWER NUTRIENT CYCLING IN THE NORTH?

Evolutionary and ecological mechanisms are interdependent, and the slower consumer-mediated nutrient turnover

rates at high latitudes, in addition to low anthropogenic nutrient inflow, may be one of the major factors that help to maintain the unproductive, oligotrophic state of a majority of Arctic ponds and lakes. If we consider our findings in the light of global warming, it may be expected that high-latitude populations/genotypes of dispersive species (such as *R. temporaria*) will become heat stressed and will eventually be replaced by lower latitude genotypes migrating northwards as temperatures rise. Both the potential northward migration of southern genotypes (Valladares *et al.* 2014) and plastic life-history changes due to higher temperatures (this study, Lindgren & Laurila 2005) may result in higher CNR rates. In addition to the predicted future amplification of the global N cycle (Galloway *et al.* 2004), this may increase the productivity of these high-latitude clear-water oligotrophic Arctic ecosystems, consequently changing their appearance, as well as altering both biogeochemical cycling and ecological functioning.

Acknowledgements

Egg collection was carried out with permission from the county administrative boards of Västerbotten (permit nr.: 522-7145-2010) and Norra Norrland (permit nr.: 522-8175-10). AL and OR were financed by the LEREC project, granted by The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS) and MIL by the Swedish Research Council (VR). Funding was provided by a grant of the Oscar and Lili Lamms Minnes foundation and the Umeå University Young Researchers Award to AL. We thank Tyler Logan and the Climate Impact Research Centre for field support, Jan Johansson, Gustaf Thomsson and Elena Heusner for help in the laboratory and Frank Johansson, Helena Johansson and Anssi Laurila for discussing *Rana temporaria* adaptations with us. Last, we sincerely thank Ane T. Laugen for her many helpful comments.

Data accessibility

The data sets supporting this article are available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.dt63p (Liess *et al.* 2015).

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Received 24 February 2015; accepted 23 July 2015
Handling Editor: Peter Hambäck

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Ambient water temperature for the tadpole ponds of origin during summer 2012.