Context dependency of phenotypic divergence and eco-evolutionary feedback: insight from a mesocosm experiment on moor frog tadpoles.

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Abstract

Rapid environmental change is driving global biodiversity declines, challenging species to persist through genetic adaptation and phenotypic plasticity. These responses can also feed back onto ecosystems ecology, a process called eco-evolutionary feedbacks, potentially reshaping both selective environments and ecosystem properties. However, how phenotypic divergence and potential eco-evolutionary feedbacks depend on the environmental context is rarely assessed in ecologically realistic settings. Here, we used an outdoor mesocosm experiment to investigate context-dependent phenotypic divergence and ecological feedbacks in amphibian tadpoles, which are key players in their native ecosystems, and show strong potential for local adaptation and phenotypic plasticity. Specifically, we assessed the extent of i) phenotypic divergence and ii) differential effects on ecosystem properties between two divergent populations of the moor frog (*Rana arvalis*) in ecologically contrasting conditions. To this end, we conducted a full factorial experiment rearing tadpoles from two contrasting pH populations (acidic *versus* neutral origin) in two contrasting pH environments (pH 4.3 *versus* 8.4). To assess the effects of tadpole presence *per se*, and the relative effects of within species phenotypic divergence on key ecosystem properties, we complemented the design with no-tadpole control mesocosms. In terms of parallel responses to the contrasting environments, both population origins showed substantial phenotypic plasticity. Tadpoles had higher corticosterone levels, developed faster and to a larger metamorphic size in the pH 4.3 than the pH 8.4 treatment. Diet also differed between pH treatments. Regarding phenotypic divergence, acid-origin tadpoles had higher survival in the pH 4.3 treatment and reached a larger metamorphic size than neutral-origin tadpoles (in both treatments). We also found genotype-by-environment interactions in dietary morphology: acid-origin tadpoles had relatively longer guts than neutral-origin tadpoles in the pH 8.4 treatment, suggesting potential for divergence in

diet-mediated ecological effects. Finally, several key findings emerged from the ecological effects of tadpoles. Tadpole presence per se (relative to no-tadpole controls) influenced several ecosystem parameters (i.e. light penetration, phyto- and zooplankton abundance). While no population-origin effects were observed in the pH 4.3 treatment, the two populations had different effects on periphyton and phytoplankton abundance and vegetation biomass in the pH 8.4 treatment. These findings highlight the potential for within-species divergence in amphibians to alter ecosystem properties and call for further investigation into the context dependency of eco-evolutionary dynamics in face of the ongoing environmental changes.

Introduction

Amidst the ongoing environmental changes, wild populations face substantial challenges, evidenced by the global biodiversity crisis (Brondízio et al. 2019; Sala et al. 2000). Simultaneously, environmental stress arising from such rapid environmental change can induce natural selection and swift evolutionary responses (e.g. Bijlsma & Loeschcke, 2005; Hoffmann & Hercus, 2000). How well species can cope with these environmental challenges is influenced by their capacity to genetically adapt and display adaptive phenotypic plasticity (Chevin et al. 2010; Ghalambor et al. 2007). Importantly, while phenotypic changes may permit evolutionary rescue in face of environmental change (Bell and Gonzalez 2011; Carlson et al. 2014), phenotypic changes can also influence ecosystem processes via eco-evolutionary feedbacks (Hendry 2017), hence altering the ecological and selective environment. Therefore, to understand how natural populations respond to environmental changes, we first need to understand both how phenotypic variation is expressed in ecologically relevant environments, but also what the ecological consequences of within species phenotypic change are.

Adaptation of natural populations to different environments is commonly observed across space in the form of adaptive divergence and local adaptation (Kawecki and Ebert 2004; Räsänen and Hendry 2008). Local adaptation is defined as the evolution of locally adapted phenotypes through natural selection, resulting in local genotypes outperforming immigrant genotypes (Kawecki and Ebert 2004; Williams 1966). Local adaptation is common in the wild (Hereford 2009) and influences the way populations and species respond to environmental changes (Bijlsma and Loeschcke 2005; Hoffmann and Hercus 2000; Meek et al. 2023). Notably, local adaptation can evolve at ecological timescales (Bell and Gonzalez 2011), implying that adaptive divergence of ecologically relevant traits has the potential to affect ecological processes (Des Roches et al. 2017; Hanski 2012; Harmon et al. 2009; Matthews et al. 2016;

Walsh et al. 2012). When divergent selection acts on ecologically relevant traits, particularly in keystone species, evolution may in turn influence ecosystem structure and function, which in turn shapes the selection pressures acting on the focal organisms and their surrounding community (De Meester et al. 2019; Hairston et al. 2005), resulting in eco-evolutionary dynamics (Hendry 2017). Understanding the drivers and context dependency of eco-evolutionary dynamics is crucial for comprehending the far-reaching consequences of rapid environmental change for community equilibrium and ecosystem function (Hanski 2012; Hendry 2017)

One way to assess potential for eco-evolutionary feedbacks is by studying ecological effects of phenotypically divergent morphotypes or locally adapted populations (e.g. Harmon et al., 2009; Palkovacs & Post, 2009). Here, local adaptation can be used as a proxy for potential to evolve over time (space-for-time) and hence used to inform about the potential direction of change in response to a given environmental change, as well as potential for phenotypic change to influence ecology. Such studies are mostly conducted on a few model species (e.g. *Daphnia*, *Drosophila,* guppies and sticklebacks, reviewed in De Meester et al., 2019; De Meester & Pantel, 2014), while for many ecologically important species that show adaptive divergence, such as amphibians, such assessments are largely missing. Likewise, how the environmental context influences potential for expression of trait divergence and evo-to-eco feedbacks is still rarely assessed in eco-evolutionary dynamics studies in the context of environmental change (e.g. Hendry 2015).

Anuran tadpoles, which are valuable model organisms for studying local adaptation and phenotypic plasticity in response to a range of natural and human induced selective agents (Hangartner et al. 2011; Laugen et al. 2003; Pfennig et al. 2010; Relyea 2002), provide an excellent model system. Given the important role of tadpoles in freshwater ecosystems, and potential for genetic and plastic phenotypic divergence, understanding the potential of environmental context and ecological effects provides valuable insight onto how environmental changes influence intraspecific diversity and potential feedbacks to ecology. Specifically, tadpoles can influence plant growth, community composition, and nutrient cycling (Kupferberg 1997; Loman 2001; Montaña et al. 2019), as well as regulate prey density and biomass through both direct (Parlato and Mott 2023; Petranka and Kennedy 1999; Schiesari et al. 2009) and indirect effects (e.g. competitive release and nutrient cycling; Davic, 1983; DuRant & Hopkins, 2008). Hence, divergence of tadpoles in any traits influencing these ecosystem parameters has the potential to feedback to ecology. While these multifaceted roles make anuran tadpoles well-suited models for investigating how adaptive divergence may influence ecosystem processes, studies examining eco-evolutionary feedbacks via tadpoles remain sparse.

Here we use the moor frog (*Rana arvalis*) as an empirical model. Particularly, a series of laboratory studies on *R. arvalis* tadpoles has demonstrated substantial phenotypic plasticity and adaptive divergence among populations inhabiting an environmental acidification gradient (Egea-Serrano et al. 2014; Hangartner et al. 2011; 2012b; 2012a; Mausbach et al. 2022; Räsänen et al. 2003; Scaramella et al. 2022). Environmental acidification, whether through anthropogenic or natural processes (e.g. Lacoul et al., 2011), is a potent agent of natural selection, and has been shown to influence phenotypic expression from physiology and morphology to behaviour and life-history traits in a wide range of taxa (e.g. Driscoll et al., 2001; Lacoul et al., 2011; Räsänen & Green, 2009). Importantly, while acidic pH (as a physiological stressor) acts as a central driver of phenotypic expression, several other correlated environmental changes occur simultaneously during acidification and, hence, alter biological communities (e.g. lead to shifts in predator communities, population densities or resource availability and quality (Haines 1981; Hangartner et al. 2011). Particularly relevant here is that *R. arvalis* tadpoles from acidic *versus* neutral origin populations have diverged in physiology (corticosterone levels), larval life-history as well as predator defence traits (Egea-Serrano et al.

2014; Hangartner et al. 2011; 2012b; Mausbach et al. 2022). However, studies on adaptive divergence of *R. arvalis* tadpoles in response to pH have been mostly conducted under highly standardized laboratory conditions (but see Egea-Serrano et al., 2014), limiting understanding of adaptive divergence as expressed in more complex environments. Moreover, despite the expected ecological role of *R. arvalis* tadpoles, it is not known whether this observed phenotypic divergence has ecological consequences (i.e., potential evo-to-eco effects).

To bridge these gaps, we conducted a semi-realistic outdoor mesocosm experiment using *R. arvalis* tadpoles from two populations (originating from an acidic *versus* a neutral pH pond) to i) investigate the extent of adaptive divergence, ii) test whether tadpoles from of the two contrasting population origins have different effects on ecosystem parameters and iii) assess what is the context dependency of these effects. We used a mesocosm experiment because it offers a promising approach to bridge the gap between controlled laboratory studies, which may lack ecological realism, and observational studies in natural settings, where identifying causal pathways can be challenging (Stewart et al. 2013). We conducted a 2 x 2 factorial experiment, with tadpoles from the two populations reared from early larval stages to metamorphosis in two contrasting environments (pH 4.3 and pH 8.4).

To estimate adaptive divergence, we assessed stress physiology (corticosterone), dietary morphology (gut length), life-history traits (developmental stage and metamorphic size) and survival. To estimate if the tadpoles from the two origins have differential effects on ecology (indicative of evo-to-eco effects), and to what extent these may depend on the environmental context (pH 4.3 *versus* 8.4), we assessed key ecosystem parameters of freshwater ecosystems (light penetration, amount of periphyton and phytoplankton, vegetation biomass, net primary productivity, and zooplankton density). We made several key predictions. First, given prior evidence for genetically based phenotypic divergence and substantial trait plasticity in these populations in laboratory conditions (Egea-Serrano et al. 2014; Hangartner et al. 2012a;

Mausbach et al. 2022), we predicted that the populations should indeed show divergence but that the magnitude and direction may be deviate from lab-based observations due to the more complex and semi-natural setting. Second, in terms of local adaptation, the acid origin tadpoles should outperform neutral origin tadpoles in the pH 4.3 treatment (e.g. display higher survival, faster developmental time and/or higher mass at metamorphosis). Within the pH 8.4 environment predictions are less straightforward, as this environment did not fully correspond to the native environment of either population (see Materials and methods). It is possible, for example, that in the pH 8.4 treatment acid origin tadpoles outperform neutral origin tadpoles if they have generally higher stress tolerance. Alternatively, neutral origin tadpoles could outperform acid origin tadpoles if they have a broader pH tolerance in the alkaline range (due to potential local adaptation to pH 7.5 environment). Finally, under the assumption that (genetic or plastic) phenotypic divergence influences ecological function of the tadpoles, we predicted that the two populations have different effects on ecosystem variables, and that these effects would differ between the two environmental settings.

Materials and Methods

Study species and populations

Rana arvalis is a semiaquatic ranid frog distributed over most of Northern, Central and Eastern Europe and parts of Siberia (Glandt 2006; IUCN SSC Amphibian Specialist Group 2023). It breeds in freshwater ponds and lakes in a variety of habitats and acidification levels, from pH 4 to pH 8 (Glandt 2006). *R. arvalis* shows remarkable adaptive divergence to acidic *versus* neutral conditions during both embryonic and larval life stages (Andrén et al. 1989; Egea-Serrano et al. 2014; Hangartner et al. 2011; Mausbach et al. 2022; Räsänen et al. 2003; 2005).

In this study, we used two *R. arvalis* populations from south-western Sweden that inhabit contrasting pH environments, and have been extensively studied for adaptive divergence along an acidification gradient (e.g. Egea-Serrano et al., 2014; Hangartner et al., 2012a; Mausbach et al., 2022). The two locations are permanent ponds influenced to a varying degree by anthropogenic and natural acidification (Hangartner et al. 2011). Tottajärn (57°60N, 12°60E; pH ~4.0, henceforth acid origin) is influenced by both natural acidification and human induced acid rain, whereas Stubberud (58°46N, 13°76E; pH ~7.3, henceforth neutral origin) is more resilient to natural and anthropogenic acidification due to limestone bedrock (Hangartner et al. 2011). For further details on the characteristics of these two sites, see Hangartner et al. (2011).

R. arvalis tadpoles from these two populations differ in their phenotype and performance in an environment-specific way indicating both genetic and plastic sources of phenotypic divergence among populations. The multi-trait divergence of *R. arvalis* tadpoles extends from their physiology (Mausbach et al. 2022; Scaramella et al. 2022) to behavioural and morphological predator-induced defences (Egea-Serrano et al. 2014; Scaramella et al. 2022) and larval life-history (eg. Hangartner et al., 2012). Specifically, in laboratory experiments, the acid origin population had on average lower corticosterone levels, deeper tails and better ability to evade predation, and slower larval growth rates, but reached metamorphosis at larger size than the neutral origin population (Hangartner et al. 2011, Egea-Serrano et al. 2014, Mausbach et al. 2022). Importantly, the magnitude of phenotypic divergence depends on rearing conditions (i.e. acidic *versus* neutral pH and predator presence or absence) due to phenotypic plasticity. (Note: Dietary traits of these populations have not been previously investigated).

Field sampling

From April 18 to 23 in 2018, we collected 10 freshly laid clutches from each of the two study sites. Upon collection, the eggs were maintained in reconstituted soft water (henceforth RSW - deionized water with 61.4 $mg.L^{-1}$ $MgSO_4$ X $7H_20$, 48 $mg.L^{-1}$ $NaHCO_3$, 30 $mg.L^{-1}$ $CaSO_4$ X $2H_20$ and 2 $mg.L^{-1}$ KCl; APHA, 1985) at pH 7.5 and cool temperature (ca. 6°C) until transfer to the laboratory at the Evolutionary Biology Centre of Uppsala University, Uppsala, Sweden, on April 23rd. Once in the laboratory, the embryos were maintained in groups of ca. 50 embryos by clutch (family) in 0.8 L polypropylene (PP) containers with 0.7L RSW. Water was renewed every three days. The embryos were reared in a walk-in climate room at ~17°C under a 17:7 day/light photoperiod until reaching Gosner stage 25 (start of independent feeding, Gosner, 1960). At this point, tadpoles were provided a finely ground spinach and spirulina mix *ad libitum* as food for 2-3 days (i.e. until a sufficient number of individuals was available from each family for the mesocosm experiment).

Experimental design

To assess phenotypic differences and potential ecosystem feedbacks in contrasting environments, we set up an outdoor mesocosm experiment at the Institute of Freshwater

Research, Swedish Agricultural University, Sweden (Drottningholm; 59°33N, 17°87E). The experimental design was fully factorial with two pH treatments (pH 4.3 and pH 8.4) x two populations (acid and neutral origin) and five replicate tanks (N= 20 mesocosms). In addition, three tanks of each pH treatment were set up without tadpoles as "no-tadpole controls" (henceforth control) (Total N= 26 mesocosms). This addition bears notable benefits as it allows to assess a) effects of tadpole presence (independent of population origin) on ecosystem parameters and b) the context dependency of effects of the two populations on ecosystem parameters. Moreover, and quite critically in our view, it allows the comparison of the two populations to a no-tadpole control, providing a more nuanced interpretation of the magnitude and direction of potential evo-to-eco trends, as a difference in a given environmental parameter induced by population origin can now be compared to a baseline level characterised by an environment without tadpoles. Specifically, the presence of no-tadpole controls could allow us to detect more subtle effects that may otherwise be blurred out by noise and whose detection would be impeded by low statistical power (which is one of the central downside of more realistic mesocosm experiments; Sasaki et al., 2025). For instance, phenotypic divergence at early stages of evolution may only lead to effects of low magnitude that might not be detected via the traditional way of opposing means of two populations. Hence, the no-tadpole control allows to independently compare the magnitude at which each population affects a given environmental variable.

Experimental setup

Between 16 and 18 April, 2018, we filled all 26 tanks (680L polypropylene tanks, external measures 120cm long x 100cm wide x 83cm high (length x width x height, INT200, Accon) with ~525L of sand-filtered water from the nearby lake Mälaren (pH = ~ 7.5), resulting in ca. 50 cm water depth. In the nominal pH 4.3 treatment tanks, we added 300mL of 1M H_2SO_4 to each of the mesocosms during filling of the tanks with water and later another 100mL 1M H2SO4 to reach pH of 4.3. Note that the initial aim for the two contrasting pH environments was to match the average pH of the source ponds of each population (i.e. pH= 4.3 and pH= 7.5). Note that the source water from lake Mälaren was initially pH= ~ 7 (soon after spring snow melt) but over the following days, the pH in all mesocosms aimed to be a nominal pH 7.5 had increased to pH= ~ 9.4 (likely due to microbial activity in the source water from lake Mälaren). At this point we decided to not alter the natural pH fluctuations in the mesocosms initially aimed at pH = ~7.5, with the idea that later addition of various type of inocula (see below) would at least partially contribute to bring the pH down. This proved to be true and several days after inoculation with vegetation, pond sediment and water filtrate (see below), the pH of the mesocosms initially aimed at 7.5 stabilised around pH = 8.4. While pH= 8.4 is higher than the pH of the neutral origin source population, it is within the range of pH naturally inhabited by *R. arvalis* (Glandt 2006). After the tanks were filled with water, we covered them with white fibreglass mesh (1.4x1.6 mm mesh size) to reduce immigration by predatory invertebrates.

On 21 April, we collected surface water filtrates, pond sediment and aquatic shoreline vegetation as inoculum from each source ponds. The pH = 4.3 tanks were inoculated with material from the acidic pond (Tottatjärn), and the pH 8.4 tanks with material from the neutral pond (Stubberud). To account for within-pond spatial heterogeneity, we collected all material from three equidistant locations spread across the respective ponds and pooled them together before distributing the inocula evenly across the tanks. The surface water filtrates were obtained using a 60 µm bongo net dragged over several meters of water surface and rinsing the filtrate off to obtain the filtered content of the water. The rationale was to sample planktonic fauna, flora and microorganisms as to create a sustainable environment for the tadpoles. We used a shovel to sample bottom sediment over its first 10cm in accessible shallow areas with little water coverage (near the shore). Aquatic plants were sampled by hand, with the aim to sample viable macrophytes (i.e. plants sampled whole, with roots). The acid pond vegetation inoculum was dominated by *Sphagnum (S. cuspidatum*, *S. magellanicum)* and *Warnstorfia spp.* The neutral pond vegetation inoculum consisted mainly *Calliergon cordifolium and Calliergonella cuspidate* mosses. Upon collection, we screened all vegetation for potential tadpole predators (mainly larvae of predaceous diving beetles, dragonflies, damselflies, as well as backswimmers*)* and removed them upon sight. Despite careful screening, a few early-instar predators evaded this step in the pH= 4.3 treatment (see datafile) and these were removed when sighted during the experiment.

All tanks were inoculated on the same day (April 21) with 1L of pond sediment, 1L (equivalent to 600g) of tightly pressed shoreline macrophytes, and a filtrate equivalent of ~40L of surface water originating from the study ponds. Following inoculation, we allowed mesocosms to stabilise for 14 days to allow establishment of communities and ensuring a self-sustaining environment before the experiment commenced and tadpoles were added. During this time, we mixed the waters between tanks within each pH treatment to homogenise conditions across mesocosms. To allow later assessments of amount of periphyton (see Environmental parameters section), we attached a 6 cm wide strip of yellow polyethylene to one side of each mesocosm on May 4 (day -1; i.e. the day before adding the tadpoles to the tanks). The strips were vertically oriented and ran from surface to bottom (ca. 60cm long) along the side of the mesocosm. To maintain the strip vertical, we ballasted the base of this plastic strip by gluing a 6x5cm piece of ceramic tile to the bottom part of the plastic strip.

On May 5, the experiment was initiated by introducing 60 approximately G25 tadpoles to each mesocosm (day 0). To make sure we captured genetic as well as maternal effect variation inherently present within each population (Hangartner et al. 2012a), we randomly selected six individual tadpoles from each of the 10 clutches (i.e. families) and pooled them together to be assigned to a specific tank. (We repeated this procedure 10 times for each population to have the initial tadpoles for each of the 20 mesocosm with tadpoles). The experimental tadpoles were transported to the experimental site in 20L plastic containers containing RSW and then gently transferred to the outdoor mesocosms. To assess starting biomass of the two populations known to differ in larval body size (e.g. Hangartner et al., 2011), which likely has ecological importance (see Discussion), we weighed three separate subsets of 10 individuals (one randomly selected individual per clutch) as a batch. The acid origin tadpole subset of 10 tadpoles weighed (mean ± SE) 0.331 ± 0.011 g, and the neutral origin subset 0.198 ± 0.005 g. Given that there were 60 individuals in each tank, estimate that the starting tadpole biomass was 1.7 x higher (approximately 1.99 g) for the acid origin than for the neutral origin (1.19 g) for neutral origin mesocosms.

Experimental procedures

After the tadpoles were added, we monitored the mesocosms daily for well-being of the tadpoles, and no issues were observed. We took pH measurements every 2 to 3 days to ensure that pH would remain stable. Over the course of the experiment (see below for detail on span of the experiment), pH averaged (mean ± SE) 4.35 ± 0.02 in the pH 4.3 treatment and 8.41 ± 0.01 in pH 8.4 treatment (geometric mean across mesocosms; data not shown). As pH tended to increase at the start of the experiment, on May 14 (day 9) we added *Sphagnum* moss (Solmull Naturtorv, Hasselfors Garden) and peat pellets (Torfpellets - art. ZB-01270, Zoobest) in lingerie washing bags (0.3mm mesh size, Persson et al., 2007) to each mesocosm to stabilize the pH of the mesocosms. To each of the pH 4.3 treatment tanks, we added 230 g of dry *Sphagnum* and 270g of peat pellets and to the pH 8.4 treatment tanks 23 g of dry *Sphagnum* and 27 g of peat pellets. This procedure also provided the mesocosms with humic compounds present in natural conditions and presents variation in natural ponds. We also took measures of water temperature every 2 to 3 days, and water temperature averaged (mean ± SE) 21.05 °C ± 0.09, with maximum at 28.1 and minimum at 15.6°C (data not shown). Measures of dissolved oxygen were taken on five occasions and averaged (mean ± SE) 9.56 ± 0.04 mg/L, with maximum at 12.11 and minimum at 8.21 mg/L (data not shown).

Tadpole parameters were sampled at different time points during the experiment. On day 16, we sampled mid-larval stage tadpoles for corticosterone (three individuals/mesocosm, total N = 60), and on days 14 and 20, we sampled tadpoles for gut length and diet (five individuals/mesocosm x 2 time points, total N = 200). The experiment ended after the first tadpoles reached metamorphosis (Day 26). On the following days, we assessed survival, developmental stage and body mass for 20 to 29 individuals per mesocosm (when survival allowed, starting Day 28).

On May 31 (day 26), we found first individuals that had reached metamorphosis (G42: emergence of forelimbs) and hence commenced to end the experiment. On day 27, we collected data on ecosystem variables (as detailed below). On day 28, we initiated the takedown of all mesocosms. For logistic reasons, we spread the tadpole collection from each of the mesocosms over two days (days 28 and 29). We collected no more than ~30 individuals per mesocosm on day 28, to roughly spread the sampling evenly across the two days for all mesocosms. Tadpoles and metamorphs were gently captured using a small hand-held fish net, transported in groups to the laboratory, deeply anaesthetised and sacrificed using 2 g/L MS222 (Sigma Aldrich, E10521).

Data collection

A - Tadpole responses

As tadpole response variables, we assessed survival, corticosterone level, gut length, gut content, tadpole developmental stage at the end of the experiment, and body mass of G42 individuals at end of the experiment. These variables were chosen because they are important performance measures and fitness components (survival, development and metamorphic size, Altwegg & Reyer, 2003), key mediators of multitrait variation (corticosterone, Mausbach et al., 2022, see below), and indicative of dietary ecology (Sibly 1981; Stoler and Relyea 2013).

Survival and life history traits - Survival within a given tank was defined as the proportion of tadpoles that survived until the end of the experiment (from day 0 until day 29) out of the 47 individuals per mesocosm (i.e. we subtracted the 13 tadpoles that were sampled earlier for corticosterone and dietary morphology from the original 60 individuals in each tank). When survival allowed, we individually weighed the first 20 tadpoles sampled from each mesocosm by gently drying the tadpoles/froglets on paper towel and weighing them to the closest 0.001g using a digital scale (Mettler, Type PM200). The tadpoles were then photographed with a digital camera (Olympus C-5060) and their developmental stage assessed from the digital images (following Gosner, 1960).

Corticosterone - Corticosterone is a key mediator of stress and metabolic responses in tadpoles (Denver 2009), and the main biologically relevant glucocorticoid in *R. arvalis* tadpoles with potential to influence the multivariate phenotype *(Mausbach et al. 2022)*. On day 16, when tadpoles had reached mid-larval stage (~ G34), we sampled three individuals per mesocosm for whole body corticosterone. We chose the mid-larval stage as the population differences are clearest at this stage in laboratory conditions *(Mausbach et al. 2022)*. As corticosterone varies according to the circadian rhythm *(Pancak and Taylor 1983)*, we sampled one individual at the time per mesocosm in order to equally distribute sampling time across treatments and replicates. We gently caught each tadpole with a hand-held fish net, transferred it into a container filled with ca. 500mL of water from its own mesocosm, and then transported it to the laboratory for processing (3 to 5 minutes procedure).

In the laboratory, the tadpoles were deeply anaesthetized with 2 g/L MS222 dissolved in RSW (Sigma Aldrich, E10521). Each tadpole was gently dry-blotted using a paper towel, and individually weighed to nearest of 0.001 mg with a digital scale (Mettler, Type PM200). Each individual was subsequently snap-frozen in a sterile 3.5 mL PP tube (60.549.001, Sarstedt), which was placed for 10 minutes on a dry ice-96% ethanol slurry. The samples were transported on dry ice to Uppsala University, Uppsala, and stored at -80°C until hormonal extraction. Corticosterone level assessment was conducted according to Mausbach et al. (2022). Briefly, we conducted organic phase extraction with Ethyl acetate, and standard Enzyme Immuno Assays (EIA, Arbor assays) hormonal assessments (adapted from Burraco et al., 2015), resulting in a measure of corticosterone expressed in pg per mg of tadpole tissue (correcting for differences in tadpole body mass), for each individual tadpole sampled. A more complete description of the process can be found in the supplementary materials.

Gut length - To assess gut length (and diet, ~ gut content), we sampled five individuals per mesocosm at two time points during the experiment: when tadpoles in the experiment were on average at stage G30 (day 14) and G35 (day 20). We chose these time points to represent potential developmental plasticity in dietary morphology. Tadpoles were collected from each tank using a handheld dipnet, gently dry-blotted using a paper towel and immediately assessed for the developmental stage visually *(Gosner 1960)*. We sacrificed the tadpoles using MS222

(2g/L) dissolved in RSW and stored them in 96 % ethanol for later measurements of gut length and diet assessment. To measure gut length, the whole gut was surgically removed and the intestinal coil was subdivided into smaller fragments (see Diaz-Paniagua, 1985). Each individual was photographed with a digital camera (Olympus C-5060) by placing the tadpole on its side on a Petri dish equipped with millimetre paper for scale. The gut fragments of each individual were placed on millimetre paper and photographed with the digital camera. From these digital images, we extracted tadpole body length (snout to hindlimb bud) and gut length to the nearest 0.01 mm using ImageJ (version 1.54k). Total gut length for each individual tadpole was calculated by summing the length of all fragments of an individual´s gut.

Gut content - Among the five tadpoles per mesocosm that were sampled for gut length, we randomly selected one individual for analyses of gut content (i.e. five independent biological replicates per pH treatment x population origin x sampling time combination), for each of the two time points (day 14 and day 20). We used microscopy (Nikon eclipse 800i, x40 magnification) to assess the main components found in the guts (following Diaz-Paniagua, 1985). All identification was done by a single person (MK). We a used a total of 30 field of views per individual and initially identified 30 distinct item types in the guts based on Streble & Krauter (2006). Due to the rarity of several of the initially established categories, we collapsed them into five main categories. First, diatoms, which included items initially categorised as: *Eunotia, Navicula, Frustulia, Tabellaria, Asterionella, Pinnularia, Cyclotella*, *Melosira,* unidentified large and small diatoms. Second, algae which included items originally categorised as: *Desmidiales, Bambusina, Scenedesmus, Tetraedron,* round green algae, unknown green, green filament, green fragment, Third, bacteria, which included items initially categorised as: *Chroococcales*, unidentified bacteria, non-green filament, non-green particle. Fourth, unidentified clumps of various sizes. Fifth, rare items, including dinoflagellates, zooflagellata, fragments of macrophytes, pollen, rotifers and crustacean zooplankton.

Given that we did not standardize the amount of gut content screened (i.e. 30 microscopy field per individual), we divided the number of items from each category by the total number of items found for a given individual to calculate the relative abundance of each item for each tadpole. We used the relative abundance of the five item categories as the response variables in the statistical analyses.

B - Environmental parameters

As environmental parameters, we assessed light penetration, amount of periphyton and phytoplankton, vegetation biomass, net primary productivity and zooplankton density. These were chosen because they are parameters likely to be affected either directly or indirectly by tadpoles, are critical determinant - as well as indicators - of environment state and are logistically feasible to monitor given the experimental design.

Light penetration - On day 27, we estimated light penetration of photosynthetically active radiation (PAR) in each mesocosm by measuring photosynthetic photon flux density using a LI-1000 datalogger (LI-COR Biosciences). We recorded PAR around midday (clear sky conditions) at 20 cm depth using an underwater quantum sensor (LI192, LI-COR Biosciences). Water depth in the tanks was then ~40 cm, even though it initially was ~50 cm, due to some evaporation along the experiment. To account for variation in ambient light variation, we simultaneously recorded incident light intensity using a separate sensor held ~80cm above the water surface (LI190, LI-COR Biosciences). All measures were obtained through the automated averaging of light intensity over 5 seconds and done in duplicates for each tank. We used the average of the duplicate ratio of light intensity measured at 20 cm depth to incident light intensity (expressed as percentage) as the response variable in statistical analyses.

Periphyton and phytoplankton densities - To estimate the amount of periphyton produced over the course of the experiment, we collected the lowest 10 cm of the polyethylene strip on day 27. The 10 cm strip was divided into two equal-size pieces, which were placed in separate 15mL falcon tubes wrapped in tin foil (to prevent light damage on chlorophyll) and immediately stored at -20°C until chlorophyll extraction 15 days later (see below).

To assess the relative amount of phytoplankton in each mesocosm at the end of the experiment, we collected a 500mL water sample from each mesocosm at 20 cm depth in the afternoon of day 27. The water was collected by filling and sealing amber high-density polyethylene bottles (414004-120, VWR) underwater. The samples were immediately stored in the dark at 4°C, until filtration (within 18 hours, Dye, 2023) when the samples were passed through glass microfiber filter (0.7 μm mesh size, 25 mm diameter, Whatman) using a 60mL handheld syringe. Of each initial 500mL water sample, we filtered 120 to 240 mL (depending on the efficiency of the water sample to cover the filter, assessed visually by gradual coloration of the filter). We recorded the total volume (V) of the water filtered for each sample to calculate the relative density of phytoplankton (see below). Following filtration, we immediately placed the filters in 15 mL falcon tubes wrapped in tin foil (to prevent light damage on chlorophyll) and stored the filters at -20°C until extraction (15 days later, see below). We used chlorophyll-*a* (chl-*a*) concentration in periphyton and phytoplankton samples to estimate their respective density (Kalchev et al. 1996). Extraction took place 15 days after collection of the samples, ensuring a nearly null potential for chlorophyll degradation (Dye 2023). We extracted the chl-*a* from the samples by adding 95% ethanol directly into each falcon tube containing the plastic strips (for periphyton samples, 7.5mL 95% EtOH) or filters (for phytoplankton samples, 15mL 95% EtOH), and keeping these falcon tubes at 4°C for 12 hours (Jespersen and Christoffersen 1987). In the case of chl-*a* extracts originating from periphyton samples, the two sets of extracts (from the two pieces of strips) originating from a given mesocosm were pooled together into the same 15mL falcon tube. We then filtered the solutions containing extracted chl-*a* (0.7 µm glass fiber filter, 25mm diameter; 1825-025, Whatman) to eliminate extraction debris. We used spectrophotometry (UV-1800, Shimadzu) to simultaneously determine sample absorbance at 665 nm and 750 nm, using the same 50 mm length quartz glass high-performance cuvette (100-QS, Hellma Analytics) for all samples. We blanked the spectrophotometer with 95% ethanol before processing each sample. We calculated chl-*a* densities using the formula adapted from Lorenzen (1967):

$$Ca = 10^3 . (D_{665} - D_{750}) . v . 83^{-1} . l\text{-}1 . V^{-1}$$

where Ca= chl-*a* concentration (mg.m-3), D_{665}= absorbance at 665 nm after correction by the cell-to-cell blank, D_{750}= absorbance at 750 nm after correction by the cell-to-cell blank, v = volume of ethanol used for extraction (mL), 83 = absorption coefficient in 96% ethanol, l = cell (cuvette) length (cm) and V =volume of filtered water (L). The amount of phytoplankton is expressed directly as mg chl-*a* per m^3. However, because our estimate of periphyton density reflects absolute amount of chl-a present on the plastic strip from which chl-a was extracted, the measuring units of the formula above do not apply to periphyton estimates. Instead, we use “relative chl-a” for periphyton, representative of the amount of chlorophyll on the surface of the plastic strip (i.e. thus in mg of chl-a per $120cm^2$). We used these measures of periphyton and phytoplankton density in the statistical analyses.

Vegetation biomass - Macrophytes can play a substantial role in freshwater ecosystems (Søndergaard and Moss 1998), by influencing water quality (Dhote and Dixit 2007), the availability of nutrients for planktonic primary producers (Dhote and Dixit 2007; Moore et al. 1984; Søndergaard and Moss 1998) and providing habitat structure favouring density of aquatic organisms such as tadpoles (Landi et al. 2014). Previous studies have demonstrated a positive correlation between epiphytic material removal by anuran tadpoles and macrophyte growth (Kupferberg 1997). To assess whether tadpoles may directly (e.g. through grazing) or indirectly (e.g. facilitation, competitive release, nutrient input) affect macrophyte growth, we measured dry plant biomass at the end of the experiment. On day 30, we collected all plant material (macrophytes, including roots) from each mesocosm using a 1mm mesh size sieve. We then manually strained as much water as possible off the plant material, and stored it in opaque plastic bags in a dark room at ~4°C. Within 15 days, we placed the content of each bag in aluminium trays and dried it at 60°C for 72 hours. We ensured complete drying of the plant material by following the weight loss of several trays during the process, and these had reached a stable weight ahead of the 72h drying. We subsequently weighed the content of each tray to the nearest 0.01g to obtain mesocosm-specific vegetation biomass (dry mass).

Net Primary Productivity - Net Primary Productivity (NPP) is a measure of the rate of carbon assimilation and productivity of an aquatic ecosystem (Cao and Woodward 1998), and hence a core measure of ecosystem function (Walsh et al. 2012). NPP is affected by nutrient levels and phytoplankton density (Smith and Piedrahita 1988). On day 27, we measured dissolved oxygen (DO) concentration (mg/L) as a proxy for NPP (Harmon et al. 2009). DO was measured immediately before sunrise (ca. 03:15 am) and immediately after sunset (ca. 22:00) in the centre of each mesocosm at ca. 10 cm water depth, using a luminescent/optical DO sensor probe (Intellical™ LDO 10105 with HQ40D, Hach). We computed DO production for each mesocosm on day 27 as $DO_{after\ sunset} - DO_{before\ sunrise}$ as a proxy for daily NPP.

Zooplankton density - To estimate the abundance of zooplankton, we sampled two litres of surface water from each mesocosm in the late afternoon (ca. 17:00) of day 27. We ladled out one litre from two opposite corners using 1L PP containers, and filtered the water through a 100µm mesh size sieve. The filtrate was transferred into a 50mL falcon tube by rinsing it off the sieve using tap water, and stored at -20°C until later processing. Freezing the zooplankton directly in tap water used for rinsing of the mesh proved very appropriate, as we were able to identify all items in these samples.

All planktonic individuals encountered were crustaceans. We identified them based on external characteristics (Sandhall and Berggren 2001) using a x40 magnification optical microscope. We identified Ostracods down to the class, Copepods to the order (Cyclopoida, Calanoida, Harpacticoida), and *Chydoridae* to the family, and all other to the genus (*Bosmina*, *Holopedium*, *Daphnia*, *Ceriodaphnia*, *Chydorus*, *Polyphemus*, *Scapholeberis*, *Simocephalus*, and *Diaphanosoma*). We counted the total number of individuals belonging to each taxon. Zooplankton diversity being rather low (N = 13 taxa), and some taxa being sometimes only represented by a few individuals, we summed the number of all individuals in a sample to compute the absolute number of crustaceans encountered. We used this measure of zooplankton density (individuals/L) as a response variable in the statistical analyses.

Statistical analyses

We conducted all statistical analyses and produced all plots in R version 4.2.0 (R Core Team 2022). We used the “ggplot2” package for all plots (Wickham 2016). Data were analysed using general and generalized linear mixed models, or non-parametric tests (detailed below). We fitted all general linear models using the “stats” package (R Core Team 2022) and all general linear mixed models using the “lme4” package (Bates et al. 2015). We analysed all the models fitted through a type 3 analysis of variance using the “car” R package (Fox and Weisberg 2019). We checked, both visually and statistically, that the statistical models fitted model assumptions using the “performance” R package (Lüdecke et al. 2019). In the presence of one (or more) clear outlier(s) based on cooks distance > 0.5, we alpha-winsorized at 0.05 in order to conservatively deal with the outlier(s), using the “psych” R package (Revelle 2007). When alpha-winsorizing did not prove effective at dealing with outliers, we fitted a robust linear model using the ‘MASS’ package (Ripley and Venables 2009). We used weighted least square linear regression models in cases of residuals heteroskedasticity. Specifically, we extracted the absolute values of residuals-vs-fitted from the initial heteroskedastic models and used them as weights in a new model using the same data and keeping the same structure (Rosopa et al. 2013). This method proved effective in all cases and, complementarily, in most cases also dealt with the non-normal distribution of as well as autocorrelation of residuals. In the remaining cases, we log-transformed our data when residuals appeared non-normally distributed.

A- Tadpole responses

We initially analysed survival with a generalised linear mixed model (with a binary observation per individual tadpole) but due to issues with model fit, we switch to analyses of survival as the percentage of surviving tadpoles (one value per mesocosm) as a response variable fitting a robust linear model to our data. We fitted general linear mixed models to our data on developmental stage, body size of G42 individuals, corticosterone level and gut length. All these models included pH treatment (categorical, two levels: pH 4.3 and pH 8.4), population origin (categorical, two levels: acid origin, neutral origin) as well as the interaction between the two formers as fixed effects predictor. These models also included tank ID as random effect predictor. The model on gut length was slightly more complex and also included body length (continuous) as a fixed effect predictor, to control for variability in body length, as well as the pH treatment x population treatment x body length interaction together with the pH treatment x body length and the population treatment x body length interactions. Additionally, this model also included sampling time (categorical, two levels: first and second sampling) as a random effect predictor. Post-hoc models (within pH treatment) on gut length has a much simpler structure and included population treatment, body length, as well as the population treatment x body length interaction as fixed effect predictors. These two post-hoc models also included tank ID and sampling time as random effects. We initially aimed to compare the average body mass of tadpoles across pH treatments x population origins. However, the variability in developmental stage combined to the non-linear and environment-specific relationship between body mass and developmental stage complexified this analysis, and we chose to analyse the body mass of G42 tadpoles instead (see table S1 for detail on the distribution of these tadpoles across pH treatment x origin and tanks). For the analyses of corticosterone level and gut content, we proceeded to stepwise model reduction based on non-significance, starting with the 3-way interaction before continuing with the 2 ways interactions. In the case of corticosterone level, we ended up deleting the “stage” covariate entirely as it had no nearly significant effect. We used permutational multivariate analysis of variance with 9999 permutations via Bray-Curtis method using the vegan package (Oksanen et al. 2001) to analyse gut content, with the relative abundance of each food item as the response variable. This model included pH treatment, population origin, sampling time (categorical, two levels: first and second sampling) and the pH treatment x population origin as fixed effect predictors, as well as tank ID as a random effect predictor. We used residuals vs fitted as weight in the models fitted to developmental rate, body mass and corticosterone levels, to deal with model heteroskedasticity. Specific details on model structure can be found in table S2.

B - Environmental parameters

Due to inherent differences between the pH treatments induced by the contrasting starting conditions, we compared the effects of tadpole population origin on several environmental variables within each the two pH treatments (pH 4.3 and pH 8.4), separately. In these analyses, we compared three levels within each pH treatment: tanks containing acid origin tadpoles (5 replicates), tanks containing neutral origin tadpoles (5 replicates) and tanks containing no-tadpoles (3 replicates).

We conducted general linear models, with “population” with three levels (acid origin, neutral origin and no-tadpole control) as fixed effects. If there was an overall statistically significant effect between these three groups, we proceeded to pairwise post-hoc t-tests comparing model-estimated group-specific means using the “emmeans” package (Lenth 2025). We used Benjamini & Hochberg multiple comparison adjustment in these post-hoc tests (Benjamini and Hochberg 1995). Initial models on phytoplankton and zooplankton density within pH 4.3 were highly heteroskedastic; we fixed this by using the absolute values of residuals-vs-fitted from these respective initial models as weights in a new model, which proved effective. Specific details on model structure can be found in table S3.

We used effect size analyses as a complementary approach to get further insight to potential evo-to-eco effects. Effect size analysis provides a nuanced understanding of the magnitude and direction of observed differences, particularly in studies with low replication and thus limited statistical power (Sullivan and Feinn 2012). We used Hedge’s G as our standardized effect size metric to quantify differences between a) mesocosms with acid *versus* neutral origin tadpoles, b) mesocosms with acid origin tadpoles *versus* control mesocosms (i.e. no tadpoles) and c) mesocosms with neutral origin tadpoles *versus* control mesocosms (i.e. no tadpoles) within each of our two pH treatments. Hedge's G effect size estimate is particularly suited for small sample sizes due to its correction for bias inherent in Cohen’s (Cohen 2009; Hedges 1981). We used the bootES package in R (Kirby and Gerlanc 2013) to calculate Bias-Corrected and accelerated (BCa) bootstrapped 95% confidence interval, whose non-overlap with zero is indicative of practical significance. We increased the number of iterations up to 5000 bootstrap resamples so that convergence was reached. We plotted the estimated Hedge’s G for each pairwise comparison, along with their 95% confidence intervals, for each measured ecosystem parameter within either pH 4.3 and pH 8.4 treatments. This allowed us to determine the direction the magnitude of population specific effects on ecosystem parameters.

Results

A - Tadpole responses

Survival - Survival of tadpoles ranged from 30% to 100% across tanks during the experiment (i.e. 28 days, see Fig.1a). There was a significant pH treatment x population origin interaction on tadpole survival ($F_{1,16}$ = 11.34, P= 0.004, Fig.1a). Post-hoc pairwise comparisons showed that acid origin tadpoles had substantially higher survival (ca. 85%) than neutral origin tadpoles (ca. 60%) at pH 4.3 (z = 5.26, P< 0.001, Fig.1a), whereas both populations had high survival at pH 8.4 and there was no difference between origins (z = 0.49, P= 0.621, Fig.1a). This result indicates that pH 4.3 was more stressful and that the acid origin tadpoles had higher tolerance.

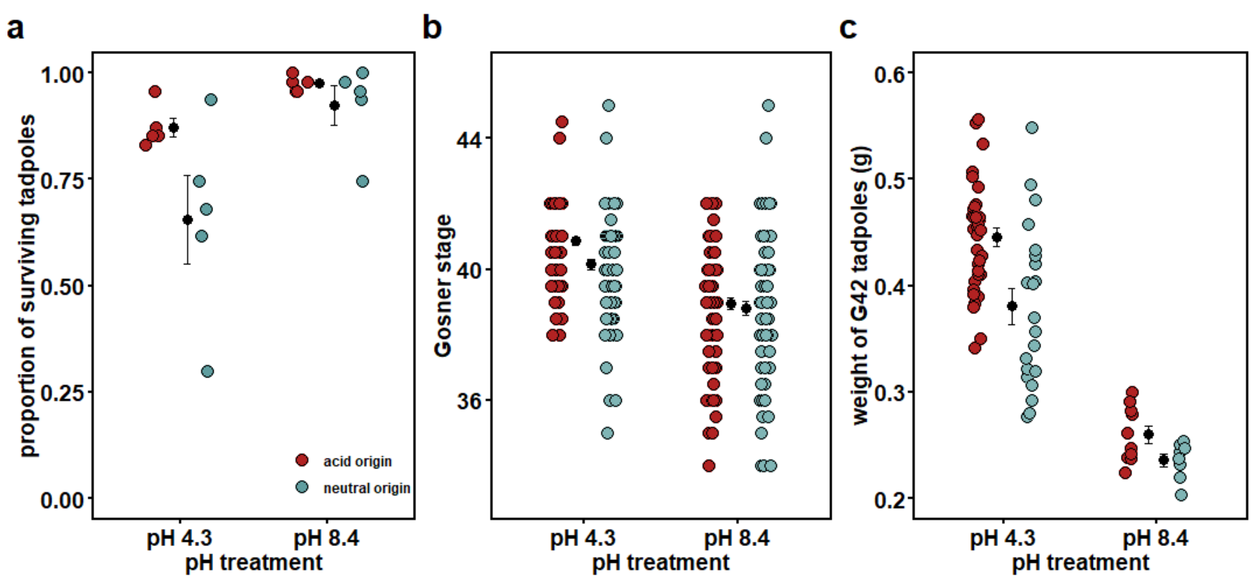

Figure 1: a) Survival, b) Gosner stage at mesocosm takedown and c) weight of G42 tadpoles for the acid origin (red circles) and neutral origin (blue circles) population of *R. arvalis* in two pH treatments (pH 4.3 vs pH 8.4). Group specific means (± 1 SE) are represented inwards relative to single observations.

Life-history traits - Tadpole developmental stage at the end of the experiment ranged from G34 to G45 (Fig. 1b). Tadpoles generally developed faster at pH 4.3 than at pH 8.4 (pH treatment: χ^2_1= 13.27, *P*< 0.001, Fig. 1b). However, there was no significant effect of population origin (χ^2_1= 2.84, *P*= 0.092, Fig. 1b) or treatment x population interaction (χ^2_1= 0.95, *P*= 0.330, Fig. 1b) in developmental stage, indicating that both populations developed at a comparable speed.

G42 stage metamorphs were larger at pH 4.3 than at pH 8.4 (pH treatment: χ^2_1= 94.86, *P*< 0.001, Fig. 1c). Acid origin metamorphs were 71 % and neutral origin metamorphs 69.5% larger at metamorphosis in pH 4.3 treatment than in the pH 8.4 treatment (Fig. 1c). Acid origin metamorphs were substantially larger than neutral origin metamorphs in both pH treatments indicated by a significant population origin (χ^2_1= 5.75, *P*= 0.017), but no significant pH treatment x population origin (χ^2_1= 0.71, *P*= 0.398) effect (Fig. 1c). These results indicate that the pH 4.3 treatment provided more favourable conditions for development and growth of the tadpoles, and that acid origin tadpoles have genetically higher growth rate (independent of pH treatment).

Corticosterone – Corticosterone levels, taken at mid-larval staged, were substantially higher at pH 4.3 than at pH 8.4 treatment (pH treatment: χ^2_1= 13.17, *P*< 0.001, Fig. 2a), but there was no significant population origin (χ^2_1= 0.28, *P*= 0.595, Fig. 2a) or pH x population origin interaction effect (χ^2_1= 1.12, *P*= 0.291, Fig. 2a). This result indicates that the pH 4.3 treatment was more physiologically stressful or metabolically demanding, but that there was no differences between the populations in their physiological responses.

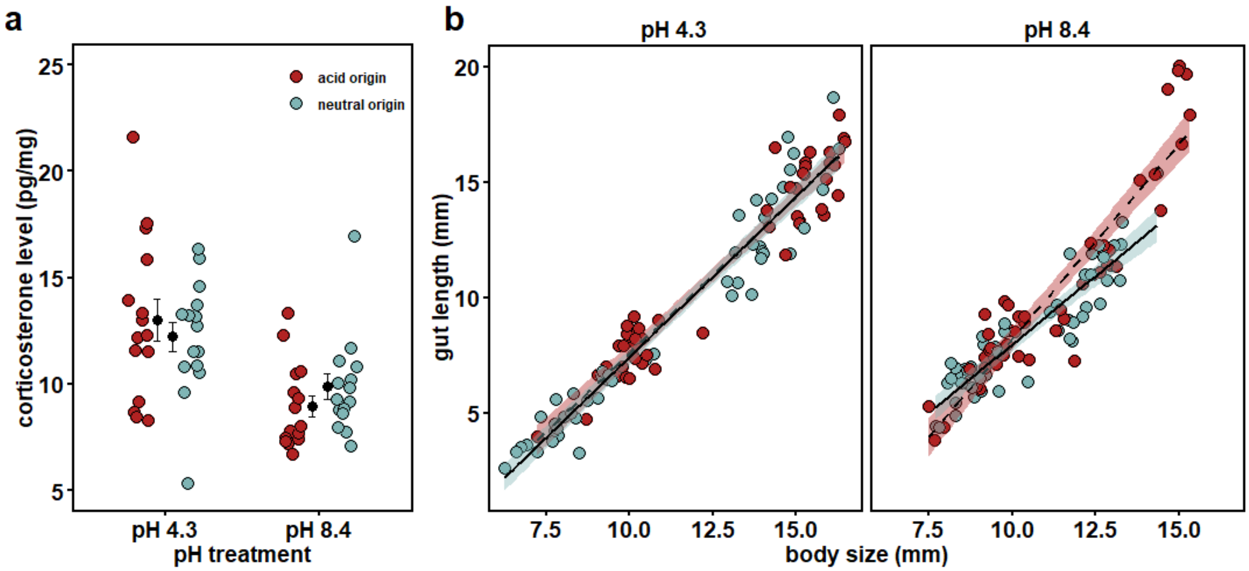

Figure 2: a) corticosterone level in two pH treatments (pH 4.3 vs pH 8.4), and b) tadpole gut length as a function of body size in pH 4.3 (left side panel) and pH 8.4 (right side panel) for the acid origin (red circles) and neutral origin (blue circles) *R. arvalis* tadpoles. Red-filled circles represent acid origin tadpoles, blue-filed circles represent neutral origin tadpoles. In a), group specific means (± 1 SE) are represented in addition to single observations. In b), the lines represent the least squares linear regression lines of the acid origin (dashed line) and neutral origin (solid line) data points and shaded areas represent 95% confidence intervals.

Gut length - Developmental stage of tadpoles sampled for dietary traits on day 14 ranged from G28 to G33, and on day 20 from G32 to G38 (data not shown). There was a highly significant pH treatment x population origin x body length interaction (χ^2_1= 8.60, P= 0.003, Fig. 2b) in the full model (see supplementary results for output of each term), and we therefore next conducted analyses within each of the two pH treatments separately. Analyses within each pH treatment found a highly significant population origin x body length interaction in the pH 8.4 treatment (χ^2_1= 1.204, P< 0.001), but no significant population origin x body length interaction in the pH

4.3 (χ^2_1= 0.02, P= 0.887, Fig. 2b,c) treatment. These effects arose because in the pH 8.4 treatment, neutral origin tadpoles had relatively shorter guts at larger size (Fig. 2c), whereas in the pH 4.3 treatment both populations showed similar slopes (Fig. 2b). These results indicate environment dependent developmental plasticity in gut length.

Gut content - Multivariate analysis of tadpole gut content detected a significant effect of pH treatment ($F_{1,35}$= 91.39, P< 0.001, Fig. S1), but no significant effects of sampling time ($F_{1,35}$= 2.82, P= 0.084, Fig. S1), population origin ($F_{1,35}$= 0.90, P= 0.356, Fig. S1) or pH treatment x population origin interaction ($F_{1,35}$= 0.39, P= 0.608, Fig. S1). These effects arose because tadpoles fed mostly on diatoms in the pH 8.4 treatment (over 75% of their diet, on average), whereas they fed a comparable proportion of diatoms and larger algae (~40% on average) in the pH 4.3 treatment (Fig S1). This was the case for both tadpole origins. These results indicate that dietary resources differed between the treatments, but there is no evidence for population differentiation in diet.

B - Environmental parameters

The formal statistical analyses revealed no significant effects of “population” (“acid origin”, “neutral origin” , “no-tadpole control”) on PAR light penetration (pH 4.3: $F_{2,10}$= 2.77, P= 0.110; pH 8.4: $F_{2,10}$= 0.62, P= 0.557, Fig. S2a), periphyton (pH 4.3: $F_{2,10}$= 2.02, P= 0.183; pH 8.4: $F_{2,10}$= 1.79, P= 0.216, Fig. 3a), phytoplankton density (pH 4.3: $F_{2,10}$= 2.17, P= 0.164; pH 8.4: $F_{2,10}$= 2.79, P= 0.109, Fig. 3b), vegetation biomass (pH 4.3: $F_{2,10}$= 0.60, P= 0.566; pH 8.4: $F_{2,10}$= 1.41, P= 0.289, Fig. 3c) or NPP (pH 4.3: $F_{2,10}$= 0.79, P= 0.480; pH 8.4: $F_{2,10}$= 0.34, P= 0.719, Fig. S2b),

We found no significant effects of the population treatment zooplankton density within the pH 4.3 treatment ($F_{2,10}$= 1.37, P= 0.297), however population treatment affected zooplankton density in pH 8.4 ($F_{2,10}$= 5.08, P= 0.030, Fig. 3d). This effect arose because there was a significant difference between the no-tadpole control and the acid origin treatment (t = -3.21, P= 0.028, Fig. 3d), as well as between no-tadpole control and the neutral origin treatment (t = -2.65, P= 0.037, Fig. 3d), but acid and neutral origin treatments did not differ from each other (t = -0.65, P= 0.532, Fig. 3d). These results indicate that tadpole presence can decrease zooplankton density (Fig. 3d).

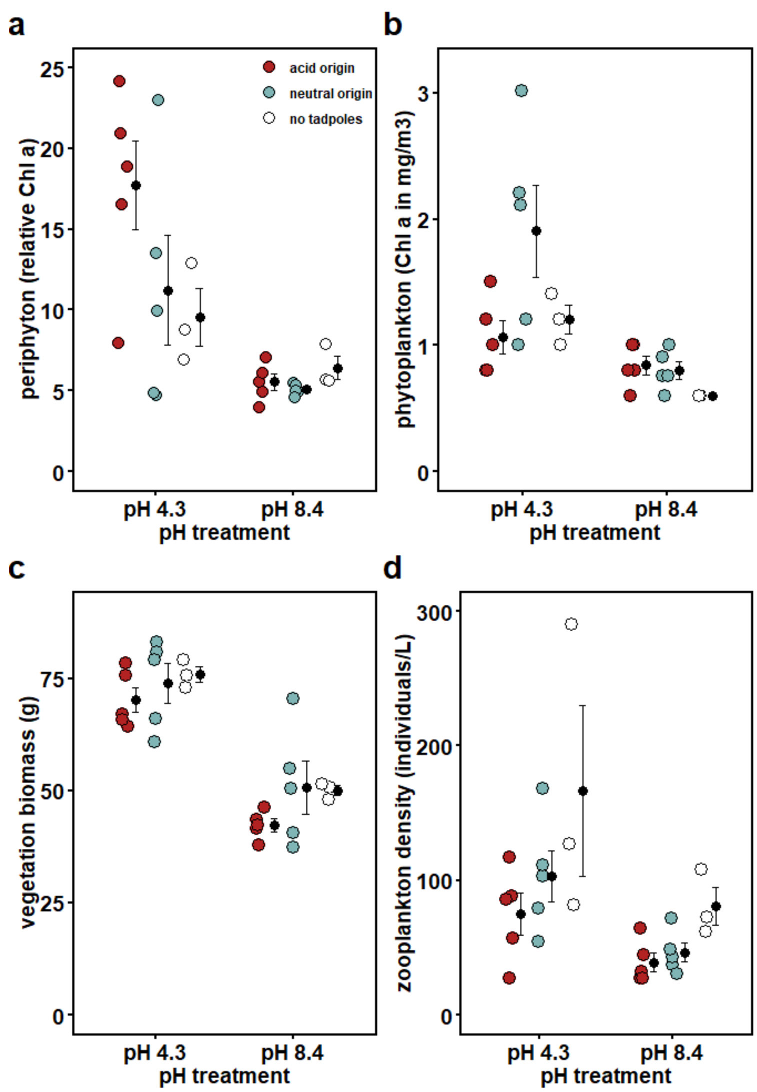

Figure 3: a) periphyton level, b) phytoplankton level, c) vegetation biomass and d) zooplankton density in the presence of either acid origin (red circles) vs neutral origin (blue circles) tadpoles or absence of tadpoles (no tadpole control, open circles) in pH 4.3 vs pH 8.4 treatments. Each single observation represents one measure per mesocosm. Group specific means (± 1 SE) are displayed to the right side of the single observations they summarise.

Effect size estimates

Standardized Hedge's G estimates ranged from 0.02 to 2.43 in absolute values (Fig. 4a,b). 16 out of the 36 calculated effect sizes were of large magnitude ($G \geq 0.8$; Cohen, 1988) and in 10 instances the effect size were of practical significance (BCa bootstrap 95% non-overlapping zero; Fig. 4a,b).

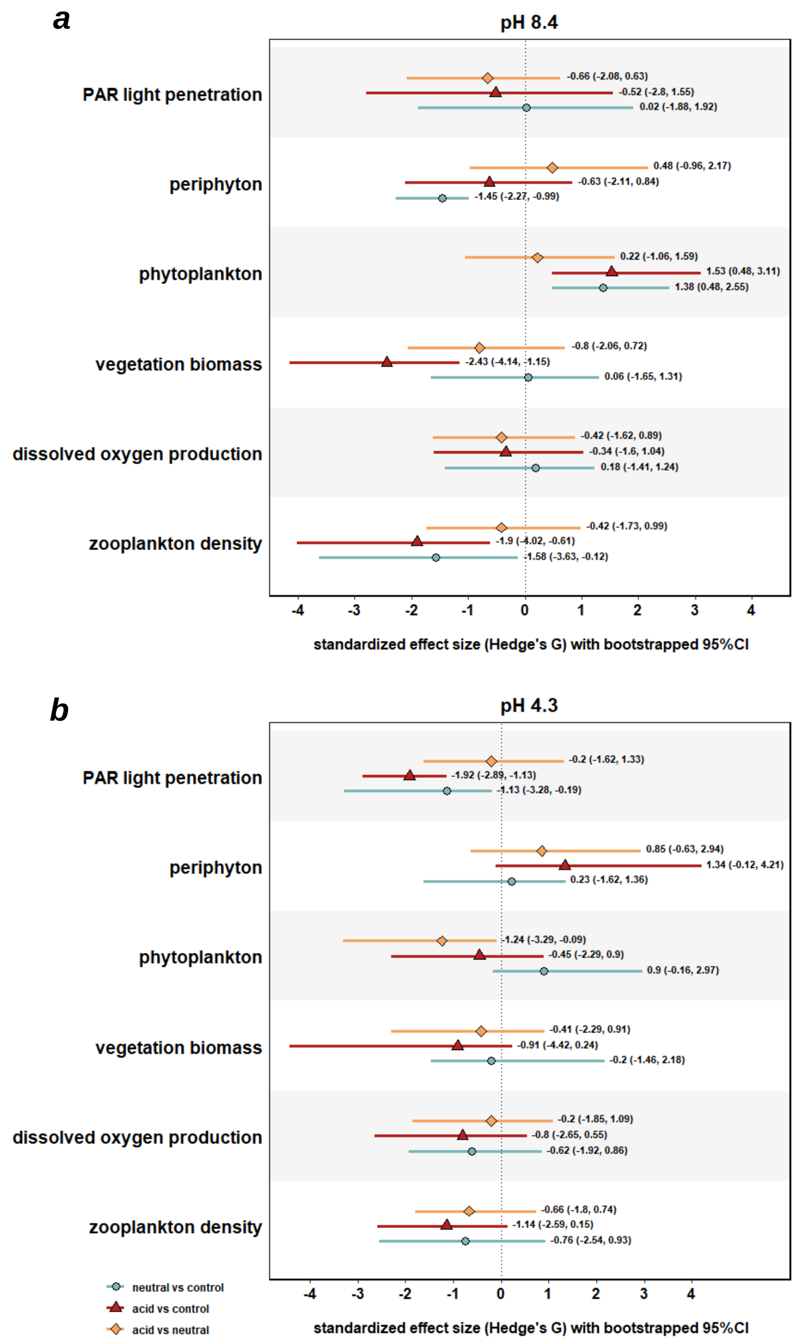

Figure 4: Standardized effect size (Hedge's G) with 95% BCa bootstrap confidence intervals for pairwise comparisons (diamond: acid vs neutral; triangle: acid vs control; circle: neutral vs control) within pH 4.3 treatment (a) and pH 8.4 treatment (b). Positive values mean that, within a pairwise comparison, the former group increases the level of the given variable relative to the latter, and vice versa. For instance, pairwise effect size estimates comparisons suggests that

acid origin tadpoles decrease the level of phytoplankton compared to neutral origin tadpoles within pH 4.3 (i.e. Hedge's G = -1.24 [-3.29, -0.09], see panel b).

Acid vs neutral origin – In the pH 4.3 treatment, the presence of acid origin tadpoles decreased phytoplankton level relative to the neutral origin tadpoles (Hedge's G = -1.24 [-3.29, -0.09]; (Fig. 4a), but did not affect differentially any other environmental parameters (Fig. 4a).

Acid vs control, neutral vs control - In two cases acid origin and neutral origin differently affected ecosystem variables, and population specific effect size differed from each other (i.e. to what extent does acid origin increase/decrease a given variable relative to the no-tadpole control, compared to the extent to which the neutral origin does relative to the control). In the pH 8.4 treatment, neutral origin tadpoles decreased periphyton levels relative to the control (Hedge's G = -1.45; 95% CI [-2.27, -0.99], Fig. 4b), whereas the acid origin tadpoles did not (G = -0.63 [-2.11, 0.84], Fig. 4b). Conversely, acid origin tadpoles decreased vegetation biomass relative to the control (G = -2.43 [-4.14, -1.15], Fig. 4b), whereas the neutral origin tadpoles did not (G = 0.06 [-1.65, 1.31], Fig. 4b). These results indicate that acid and neutral origin tadpoles have different ecological functions.

We also found cases where populations analogously affected ecosystem variables, but did not differ from each other in the magnitude of the effects. At pH 4.3, both populations decreased PAR light penetration relative to the control (acid vs control: G = -1.92 [-2.89, -1.13]; neutral vs control: G = -1.13 [-3.28, -0.19], Fig. 4a). At pH 8.4, both populations increased phytoplankton (acid vs control: G = 1.53 [0.48, 3.11]; neutral vs control: G = 1.38 [0.48, 2.55], Fig. 4b), but decreased zooplankton density relative to the control (acid vs control: G = -1.9 [-4.02, -0.61]; neutral vs control: G = -1.58 [-3.63, -0.12], Fig. 4b). These results indicate that tadpole presence (irrespective of origin) affected different ecosystem variables in the two pH treatments.

Discussion

Environmental stress influences trait expression, and is a strong driver of adaptive divergence at contemporary timescales (Bijlsma and Loeschcke 2005; Hoffmann and Hercus 2000). When trait divergence is ecologically relevant, in particularly in keystone species, it has the potential to mediate feedbacks between ecology and evolution (i.e. *eco-evolutionary dynamics* reviewed in Hendry, 2017). While such eco-evolutionary feedbacks have been demonstrated in several model species, there have been limited tests on how such feedbacks depend on environmental context and to what extent amphibians, being keystone species in many freshwater ecosystems, may cause eco-evolutionary feedbacks. Using tadpoles from two *R. arvalis* populations originating from an acidic *versus* a neutral pH ponds, and showing substantial phenotypic divergence under standardized laboratory conditions in multiple traits (e.g. tadpole morphology, life-history traits and corticosterone levels; Egea-Serrano et al., 2014; Hangartner et al., 2012a; Mausbach et al., 2022), we tested the potential for this trait divergence to impact ecological function, and its context dependency.

Our semi-realistic outdoor mesocosm experiment, where tadpoles were reared in two contrasting pH treatments from early larval stages to metamorphosis, supported the view of substantial phenotypic plasticity in both populations: tadpoles developed quicker, were heavier at metamorphosis, and displayed higher corticosterone levels in the pH 4.3 compared to the pH 8.4 treatment. In addition, tadpoles from both populations fed more on a mix of diatoms and larger algae in pH 4.3. treatment, whereas tadpoles in the pH 8.4. fed primarily on small diatoms, suggestive of differential resource availability and high plasticity in diet composition.

Second, in parallel with previous laboratory studies, acid origin tadpoles reached a higher body mass at metamorphosis (G42) in both pH treatments, indicative of genetic divergence in this key fitness component. In contrast to laboratory studies, however, we found little evidence for divergence in developmental rates under these conditions. We further found environment specific performance differences. In terms of phenotypic divergence, acid origin tadpoles had relatively longer guts at large body sizes than neutral origin in pH 8.4 (there was no difference in pH 4.3), indicative of adaptive plasticity in dietary morphology. Moreover, in line with a previous laboratory experiment (Egea-Serrano et al. 2014), acid origin tadpoles survived better than neutral origin tadpoles in the pH 4.3 treatment (while there was no such difference in pH 8.4), indicative of local adaptation of the acid origin population to acidic conditions.

Third, our effect size analyses provided clear evidence for the ecological role of tadpoles: tadpole presence *per se* (independent of tadpole origin) decreased light penetration (in the pH 4.3 treatment), and reduced zooplankton density while increasing phytoplankton density (in the pH 8.4 treatment). Interestingly, while formal statistical analyses provided little evidence for population-to-ecology effects (indicative of evo-to-eco feedback), our effect size estimates suggest context-dependent ecological effects of population divergence: First, in the pH 4.3 treatment, the acid origin tadpoles reduced phytoplankton level (relative to the neutral origin population). Second, in the pH 8.4 treatment, the acid origin tadpoles reduced vegetation biomass, while neutral origin tadpoles reduced the periphyton levels instead (relative to the no-tadpole control). Overall, these results demonstrate high phenotypic plasticity, yet notable divergence and substantial potential for evo-to-eco effects in *R. arvalis* tadpoles adapted to acidic versus neutral pH environments.

Environment dependent phenotypic variation

Timing of metamorphosis and metamorphic size are key fitness components in anuran amphibians, with early metamorphosis along with a large body size resulting in higher terrestrial growth and survival (Altwegg and Reyer 2003). In our study, we found strong environmental effects (i.e. differences in mean trait value between the pH 4.3 and 8.4 treatments) on all phenotypic traits studied here (developmental stage and size at metamorphosis, corticosterone level and gut length), with gut length also indicating genotype-by-environment (G x E) interactions. Somewhat counterintuitively, we found that tadpoles from both populations developed substantially faster (i.e. reached a more advanced stage by end of the experiment) and reached larger metamorphic size in the pH 4.3 than the pH 8.4 treatment. While low pH is known to be physiologically stressful in broad range of taxa in standardized laboratory conditions (Merilä et al. 2004; Weber and Pirow 2009; Guan and Liu 2020), this suggests that in our ecologically more complex environment, the pH 4.3 treatment nevertheless provided better growth conditions than pH 8.4. This could be because the pH 4.3 treatment was more productive and resource rich, whereby net primary productivity, levels of phytoplankton and vegetation biomass were higher than in the pH 8.4 treatment (see discussion below and supplementary results). Alternatively, acidic environments may select for higher energy uptake by tadpoles (Liess et al. 2015). Notably, while higher level of corticosterone (as observed here in the pH 4.3 treatment) is generally assumed to indicate elevated stress levels (reviewed in Denver, 2009), corticosterone level can also reflect higher metabolic activity independently of stress (Jimeno et al. 2018), and thus potentially be linked to growth here. Despite apparently better growth conditions, tadpole survival (especially for the neutral origin population) was lower in the pH 4.3, suggesting suboptimal conditions for some aspects of performance (see below).

From an environmental perspective, the two pH treatments were clearly distinct, and hence likely modified any direct effects of pH per se. First, the two treatments received different inoculum for zooplankton and microbes, as well as starting macrophytes, providing different resource base and habitat structure. While starting biomass of vegetation was equal (in terms of wet mass), biomass (in terms of dry mass) was much lower at the end of the experiment in the pH 8.4 treatment. As a consequence, the higher biomass in the pH 4.3 treatment, combined with bushier morphology of *Sphagnum* (compared to *Calliergonella* and *Calliegon* mosses in the pH 8.4 treatment) likely provided larger platform for epiphytic growth within pH 4.3 treatment and, hence, higher food availability for tadpoles. As a strong ecosystem engineer (Svensson 1995; van Breemen 1995), *Sphagnum* might have induced fundamental changes in various ecosystem processes and acted as a host to an extensive microbial community (Bragina et al. 2012) which *R. arvalis* includes in its diet (Seale and Beckvar 1980). The supposed higher dietary resource availability and diversity was somewhat reflected in the gut content of the tadpoles as tadpole diet appeared to consist of a more even combination of green/blue algae and diatoms (ca. 40% for each) than in the pH 8.4 (which was mostly dominated by small centric diatom *Cyclotella,* Kaiser, pers. obs.). Notably, *Cyclotella* diatoms occur predominantly in oligotrophic lakes (reviewed in Saros & Anderson, 2015), further indicating that resources were more limited in the pH 8.4 treatment. A possibly more favourable protein-carbonate ratio provided by a mixture of green algae and diatoms in the pH 4. 3 treatment (Kupferberg 1997; Richter-Boix et al. 2007) could have allowed tadpoles to allocate energy towards growth and development rather than foraging (eg. Pfennig, 1990) – hence explaining the quicker development and larger metamorphic size in the pH 4.3 treatment. While the differences in gut content likely reflect largely dietary availability, it should be noted that tadpoles may display selective foraging (Richter-Boix et al. 2007). Further mesocosm and field studies are hence needed to assess tadpole diet, and behavioural studies would help to reveal whether *R. arvalis* tadpoles show selective foraging.

While growth conditions seemed more favourable in the pH 4.3 treatment, tadpole survival was lower in the in the pH 4.3 treatment, especially for the neutral origin population (acid origin: 80-95%; neutral origin: 30-90%, see below). Average survival was very high in the pH 8.4 for both populations (75 – 100%, Fig. 1a). In addition to physiological stress of acidic pH, an obvious reason for lower survival in the pH 4.3 treatment in our experiment could the unintended presence of a small number of predators in some of the tanks (mostly small larvae of Aeshnid and Libellulid dragonflies, damselflies and Dytiscid diving beetles, see datafile). Post-hoc analyses within pH 4.3 however found no significant correlation between of predator abundance and tadpole survival (data not shown), suggesting that the quantitative presence of predators was not the main driver of survival differences. Hence, it is possible that pH 4.3 *per se* exerted stronger stress on tadpoles than pH 8.4, ultimately leading to decreased survival (affecting neutral origin tadpoles to a higher extent), while our pH 8.4 treatment, despite appearing resource-limited, only led to sublethal effects on *R. arvalis* tadpoles. Interestingly, corticosterone levels were substantially higher in the pH 4.3 treatment compared to pH 8.4 (in both populations). As corticosterone is a key indicator of stress responses and overall metabolic activity (reviewed in amphibians Denver, 2009), this elevation may reflect higher physiological stress of acidic pH, compared to alkaline stress, and/or higher metabolic activity linked with faster growth and development (Sapolsky et al. 2000; Guillette et al. 1995).

Phenotypic divergence between the populations

The above-mentioned survival differences between the acid and neutral origin tadpoles of *R. arvalis* in our study here strengthen evidence for local adaptation of the acidic population pH 4.3 (Egea-Serrano *et al.* 2014). Despite improvement in ecological realism compared to previous laboratory studies, our study here may however not reflect the full extent of local adaptation, as in nature many other factors, such as ability of tadpoles to cope with and escape predatory pressure (Egea-Serrano et al. 2014), variation in resource availability, as well as intra- and interspecific competition may come into play simultaneously.

In accordance with several previous laboratory studies on this system (Egea-Serrano et al. 2014; Hangartner et al. 2011; Räsänen et al. 2005; Teplitsky et al. 2007), we found that acid origin tadpoles were larger at metamorphosis at both pH 4.3 (13% increase, on average) and pH 8.4 (12% increase, on average) than neutral origin tadpoles. This difference is likely due to both genetic and maternal effects (Hangartner et al. 2011; 2012b; 2012a; Räsänen et al. 2005). It should however be noted that acid origin tadpoles were 65% heavier than neutral origin tadpoles at the beginning of the experiment (average tadpole weight 0.033g vs 0.020g); for this reason it remains uncertain to what extent this observed difference in metamorphic mass is linked to higher growth during the experiment or simply derives from the inherent condition of tadpoles at the beginning of the experiment. In contrast with our previous standardized laboratory study, where acid origin tadpoles had lower average corticosterone levels than neutral origin tadpoles (Mausbach et al. 2022), we found no population differences in corticosterone levels in our study. This may be because of context dependence differences in corticosterone expression of natural populations (Mausbach et al. 2022), and here possibly due to differences in energetic demands and relationship of corticosterone with metabolic activity.

Diet mediated divergence?

Resource availability and quality are major selective agents in nature, having repeatedly driven the evolution of resource polymorphism in a range of taxa (Skulason and Smith 1995), including amphibians (Pfennig et al. 2010). Our finding that acid and neutral origin tadpoles differed in relative gut length at larger body size in the pH 8.4 treatment (Fig. 2c), indicates population divergence in diet induced plasticity. The optimal digestion theory predicts longer guts in environments with low quantity and quality food resources (Sibly 1981), and it is likely that resource quality differs along acidification gradients (DeNicola 2000; Eriksson et al. 1980; Geelen and Leuven 1986). Such resource variation may favour differential diet-induced plasticity in *R. arvalis* along pH gradients.

Earlier evidence for plastic variation in dietary traits in amphibians comes from studies using diet manipulation, which found that food quality and quantity affect gut length and the size of the oral disc in *R. sylvatica* (Stoler and Relyea 2013) and *Scaphiopus multiplicatus* tadpoles (Pfennig, 1990). Such dietary plasticity is common in a wide range of taxa (eg. Olsson et al., 2007; Pfennig, 1990) and enables individuals to maintain growth and functionality in spatiotemporally heterogenous environments. In addition, adaptive divergence in gut length plasticity has been found in *R. temporaria* populations at different latitudes in response to low temperature, and was proposed to influence growth efficiency (Liess et al. 2015; Lindgren and Laurila 2005). It is possible that the apparently lower food quantity and/or quality in our pH 8.4 treatment (See above) led to the expression of adaptive gut length plasticity to improve energy uptake (Sibly 1981).

A very hypothetical link could be made between the increased gut length of acid origin tadpoles within pH 8.4 treatment and the reduction of vegetation biomass. Acid origin tadpoles being larger, it is not unlikely that they fed directly on macrovegetation in the pH 8.4 treatment, in absence of other food sources. In fact, fragments of macrophytes were found in the gut of both population origins and in both treatments. Even though their proportions appeared rather low (below 5%, data not shown), the current data does not allow us to validate or refute this possibility. Generally, while our analyses revealed that gut content differed strongly between the pH treatments, we found no differences in diet between the populations (see above). Whether - and to what extent - gut content reflects resource availability within the mesocosms or the result of selective foraging (Kupferberg 1997) remains to be determined, but our results suggest high dietary plasticity/low selectivity in diet composition in both these populations given the experimental conditions. Further laboratory assays of developmental plasticity would aid in testing how tadpoles respond to combined stressors of pH and resource availability, and to what extent there may be population specific adaptive developmental plasticity in dietary traits of *R. arvalis* tadpoles (Liess et al. 2015).

On the ecological footprint of tadpoles on the environment

Tadpoles are often key players in freshwater ecosystems, influencing various aspects of ecosystem function (Wood and Richardson 2010; Whiles et al. 2013; Corline et al. 2025). Here our comparison of treatments with tadpoles (either acidic or neutral origin) and treatments without tadpoles (no-tadpole control) allows to make inferences about the functional role of tadpoles – which also forms an important baseline for testing predictions about differences among phenotypically divergent genotypes on ecosystem properties (i.e. eco-evo-feedbacks as discussed below). We found that tadpole presence *per se* (independent of tadpole origin) decreased light penetration (in the pH 4.3 treatment), and reduced zooplankton density but increased phytoplankton density (in the pH 8.4 treatment).

The finding that PAR light penetration was reduced in presence of tadpoles in the pH 4.3 treatment result could arise from increased phytoplankton density (Fleming-Lehtinen and Laamanen 2012), and tadpoles could for instance decrease phytoplankton via direct consumption (Seale 1980). However, we found no evidence for an effect of tadpole presence *per se* on phytoplankton density, suggesting that phytoplankton was not the main driver of PAR light penetration in our study. Alternatively, through bioturbation, tadpoles may directly decrease PAR light penetration as the re-suspend bottom sediment particles (Ranvestel et al. 2004). While we have no direct observations on the impact of tadpole activity on the sediment, this effect seems likely and was possibly higher in the pH 4.3, which had higher plant biomass.

Our gut content analyses showed that in this mesocosm experiment *R. arvalis* tadpoles fed on diverse sources from mostly diatoms and green algae to rarer items, such as bacteria and pollen (see Fig. S1). This supports the view that tadpoles have the potential to impact ecosystems through dietary pathways – in particularly directly consumption of various algae (Montaña et al. 2019). We found effects of tadpole presence on phyto- or zooplankton abundance, but these effects where pH treatment dependent and, in some cases, arose only from one of the populations (see below). While the diet of *R. arvalis* has been generally little studied (but see Montaña et al., 2019 for a review of anuran tadpoles), *Rana* species have been shown to impact zooplankton density through direct and indirect pathways, including predatory behaviour (Petranka and Kennedy 1999), nutrient cycling (Osborne and McLachlan 1985) and competition for resources (Leibold and Wilbur 1992; Seale 1980).

Phytoplankton abundance was reduced in presence of acid origin tadpoles only in the pH 4.3 treatment, while zooplankton abundance was reduced but phytoplankton abundance increased by tadpole presence (regardless of origin) in pH 8.4 treatment. While we cannot ascertain the pathways leading to such changes, we may expect that in the more resource limited environment of the pH 8.4 treatment, it is possible that tadpoles may have more actively consumed zooplankton, which subsequently may have released phytoplankton from predation. Partial support for zooplankton consumption comes from zooplankton body parts in the gut of the tadpoles (data not shown).

In general, tadpoles may act on lower trophic levels simultaneously via direct and/or indirect top-down and/or bottom-up effects (Rowland et al. 2017). Given the direct trophic link between zooplankton and phytoplankton (Levine et al. 1999), it is not unlikely there are trophic cascades induced by the active foraging of tadpoles on zooplankton, leading to facilitation of phytoplankton growth. These results support the general idea that anuran tadpoles play a key role in their environment. Further experimental studies are however needed to disentangle the direct from indirect effects of *R. arvalis* tadpoles on ecosystem parameters.

Evo-to-eco effects

When phenotypic divergence influences ecologically relevant traits, especially in keystone species, there is a potential for feedbacks between evolution to ecology (Hendry 2017). We found some evidence for ecological feedbacks from phenotypic divergence in both pH environments: First, in the pH 4.3 treatment, acid origin tadpoles reduced phytoplankton level (relative to the neutral origin population). Second, in the pH 8.4 treatment, acid origin tadpoles reduced vegetation biomass, while neutral origin tadpoles reduced periphyton levels instead (relative to the no-tadpole control). Because acid origin tadpoles from the Tottatjärn population used here are larger compared to neutral origin tadpoles (Hangartner et al. 2011, Egea-Serrano et al. 2014, Mausbach et al. 2022, our study), one of the most straightforward interpretations for these population level differences arise from direct or indirect effects of differences in food consumption. Alternatively, differences in energy demands and assimilation may leading to contrasting growth rates and nutrient excretion capacity, such as in the case of latitudinally divergent *R. temporaria* tadpoles (Liess et al. 2015). Because higher nutrient excretion from a larger biomass of tadpoles (i.e. here acid origin tadpoles) may provide resources for primary producers, the observed differences in gut length (indicative of assimilation efficacy) and metamorphic size (indicative of energy demands) may lead to effects on periphyton and phytoplankton. Complementing previous studies that documented that anuran tadpole presence can affect phytoplankton growth (Mallory and Richardson 2005; Osborne and McLachlan 1985), our study suggest that phenotypic divergence alone can lead to differential effects on phytoplankton, and calls for an assessment of the proximate mechanisms at play.

Based on known differences in behavioural activity between the acid and neutral origin populations (Egea-Serrano et al. 2014), larval growth rates (Hangartner et al., 2011, this study) and dietary morphology (this study) there may be differences between acid and neutral origin tadpoles in foraging. In particular, because periphyton is expected to be the main resource used by Ranid tadpoles (Montaña et al. 2019), differences between populations in grazing effectiveness could be translated to more efficient periphyton removal. Alternatively, populations adapted to acidic environments might cope better with acid stress and hence need less resources, leading to reduced food consumption. This could be assessed experimentally by future research.

Although our statistical analyses detected no significant difference between acid and neutral population origin on periphyton levels in either pH treatment, our effect size analyses indicated that within the pH 8.4 treatment, neutral origin tadpoles decreased the level of periphyton more than acid origin tadpoles did (relative to no-tadpole control tanks). In parallel, acid origin tadpoles decreased vegetation biomass within the pH 8.4 treatment (relative to the no-tadpole control), which neutral origin did not. One scenario explaining population specific effects on periphyton and macrophytes could be that in low resource environments (here: pH 8.4), acid origin tadpoles may be able to digest rough plant material (and which hence may induce longer guts, see above), whereas neutral origin tadpoles may need to feed primarily on periphyton. This hypothesis could be tested experimentally in follow-up experimental studies aimed at identifying the exact mechanisms through which divergent populations affect the environment they inhabit. Irrespective of the mechanism, however, our findings indicate that these two *R. arvalis* populations have different ecological functions – either mediated by their large differences in body mass or – not mutually exclusively, other functionally relevant traits.

Previous studies have demonstrated the potential for indirect effects of higher trophic levels on Net Primary Productivity (NPP). For instance, changes in NPP were shown to originate from divergence in alewife (*Alosa pseudoharengus*) life history, which in turn induced divergence in zooplankton life-histories and consequently phytoplankton density (Walsh et al.

2012). In our study, NPP was not affected by tadpole origin (nor tadpole presence). This is somewhat surprising given that we observed differences between the two *R. arvalis* populations on their impacts on phytoplankton level in the pH 4.3 treatment, and we may have thus expected differences in ecosystem productivity. It may be, however, that pelagic phytomaterial (phytoplankton) represented a small proportion of the overall chlorophyllic biomass compared to the benthic macrophytes. Therefore, any change in NPP due to different levels of pelagic phytomaterial may have been diluted out by the larger contribution of macrophytes. This being said, within the pH 8.4 treatment, the acid origin population tended to decrease vegetation (macrophyte) biomass, and we may have expected NPP results to somewhat mirror those of vegetation biomass, but this was not the case.

One important aspect to address is that the population effect on ecosystem parameters in our study is most likely the result of multiple interacting factors, including tadpole size, developmental stage, and survival. It should be noted that the lower survival of neutral-origin tadpoles in the pH 4.3 treatment (which directly reduced density and biomass), together with their smaller body mass compared to acid-origin tadpoles, may have contributed to the effects observed—factors that represent common ecological processes in natural populations. In contrast, because survival was high for both populations in the pH 8.4 treatment, differences in tadpole effects were more likely related to the slightly lower biomass (data not shown) of the neutral-origin population, in addition to other functional traits, as discussed above. Importantly, however, differences in survival between populations are themselves population-specific responses to environmental conditions (whether linked to pH stress, predation, or competition) and thus do not invalidate the existence of population-specific effects on environmental parameters. They may, however, obscure the mechanistic pathways leading to such differences, highlighting the need for further studies to disentangle them. In addition to differences in survival (density) mediated effects, some other caveats of the study need to be addressed. We did not recreate the pH 7.5 environment that would have been closer to the pH for the neutral origin population. While the pH 8.4 is not allowing to rigorously assess adaptive phenotypic differences, the environmental contrast nevertheless allowed us to assess to what extent phenotypic divergence depends on the ecological context (and it does) or their ecological feedbacks. Furthermore, an unusual heatwave during the 27 days of the experiment led to high water temperatures (mean ± 21C). While these elevated temperatures did not seem to affect survival (which was generally high), they sped up tadpole growth and developmental rates compared to our typical laboratory assessments at 17C, where reaching metamorphoses in these populations takes roughly 60-70 days (e.g. Hangartner et al. 2012). Hence, any phenotypic differences (including metabolic activity, developmental and growth rates) as well as effects on ecosystem functioning may have been underestimated due to the shorter study period. As the occurrence of such heatwaves is increasing rapidly due to climate change, future studies may conduct similar experiments under different – controlled - temperature conditions to assess the dependence of observed effects.

Conclusions

Results from our outdoor mesocosm experiment provide new knowledge about adaptive divergence of *R. arvalis* along an acidification gradient and, in particular, the context dependence of trait divergence and potential for eco-evolutionary feedbacks on ecology in anuran tadpoles. In general, our study supports previous findings for adaptive divergence in larval life-history traits in these *R. arvalis* populations, but provided no evidence for local adaptation *sensu stricto* (Kawecki & Ebert, 2004, Hereford 2009): we did not observe opposite population trends between the pH treatments - the acid origin population was performing better in both environments. A novel finding is evidence for phenotypic plasticity in gut length of *R.*

arvalis tadpoles, indicative of adaptation to different resource conditions (in addition to the well-established adaptation to pH and predators). However, further multifactorial laboratory studies are needed to disentangle the interplay between pH, resource availability/quality and predators in natural populations.

Importantly, our study investigated adaptive divergence of tadpoles in a more realistic context than laboratory-based studies and showed strong acidity mediated effects on tadpole phenotype. The acid and neutral origin tadpoles differed in developmental rate, body size and gut length and, importantly, we found some evidence for eco-evolutionary feedback from adaptive divergence of this acid and neutral origin population and that these effects were context dependent (different ecosystem parameters were affected by the two populations in the two treatments). Keystone species, which tadpoles often present in freshwater ecosystems, can be strong drivers of community dynamics through direct top-down processes resulting in cascading effects at lower trophic levels (Morin 1995) or indirect bottom-up effects (Rowland et al. 2017). Given that the two tadpole origins had differential effects on some of the ecosystem parameters, this also calls for more attention to divergence of natural populations in their ecological functions in the context of conservation biology and ecosystem management (e.g. Des Roches et al. 20xx). More studies investigating the consequences of contemporary adaptive divergence and rapid evolution are needed for better understanding ecosystem function in nature, and the role of amphibians in them.

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Authors contributions

AL and KR conceived the study. QC, AL and KR designed the experiment. QC set up and coordinated the experiment, with substantial help from MK. JM collected the data on corticosterone. MK collected the data on gut length and gut content. QC analysed the data and produced the figures. QC wrote the first draft of the manuscript and developed it with substantial help from KR. All authors read, provided comments on earlier versions, and validated the last version of the manuscript.

Data and code availability

Data and code used to analyse the data and produce the figures will be uploaded in a repository and made available to reviewers at the submission of this manuscript to a peer reviewed journal. The access to data and code will be made public immediately upon publication.

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Supplementary Materials

Materials and Methods

Hormonal extraction - Corticosterone was analysed using standard procedures (Mausbach et al. 2022). We conducted organic phase extraction with Ethyl acetate, and standard Enzyme Immuno Assays (EIA, Arbor assays) hormonal assessments (adapted from Burraco et al. 2015) using an absorbance plate reader (Molecular devices, SpectraMax 190).

Briefly, the samples were defrosted and homogenized for 30 s using a hand-held Qiagen tissue ruptor. Between 0.080-0.099 g of each homogenized sample was pipetted into a sterile 2mL PP screw tube (Sarstedt, 72.693.005) and 1500 µL of Ethyl acetate added (99.8%, Sigma aldrich, 270989). The samples were homogenized for 30 s using a mechanical orbital agitator (VWR Vortex), and transferred to a plate shaker for 30 min at 4°C. The samples were centrifuged at 5000 Rpm (VWR, Micro Star 17) for 15 min and the resulting supernatant (approx. 1450 µL) was transferred into safe lock tubes (2mL, Eppendorf, PP), and immediately stored at -20°C. For extraction, the samples were evaporated at 45°C using a speed vac (SpeedVac plus, SC110A attached to Savant, Gel Pump GP110). The samples were filled with a stream of nitrogen to prevent oxidation, and then sealed with Parafilm for transportation dry at room temperature to the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) in Duebendorf. The samples were reconstituted in 115 µL assay buffer (Arbor Assays Detect X Corticosterone Enzyme Immunoassay Kit, K014-H1/H5) and 5 µl 99% EtOH, thoroughly agitated (VWR Vortex) and stored at -20°C for later EIA analyses. EIA analyses were conducted following the Arbor Assays Detect X Corticosterone Enzyme Immunoassay Kit (K014-H1/H5) instructions. We adapted the standard curve due to the relatively low corticosterone concentration of some samples by using a concentration range from 39.063 to

5000 pg/mL. We measured optical density at 450 nm with a plate reader (Molecular devices, SpectraMax 190) and transformed the values to concentrations (pg/mL) using the provided Arbor Assay software (https://www.myassays.com/). The corticosterone concentrations were corrected for mass of extracted tissue, as well as volume of the sample used for each well, resulting in a measure of corticosterone concentration as pg per mg of tadpole tissue for each individual tadpole.

Statistical analyses

Table S1: Number of tadpoles at G42 per combination of pH treatment x population origin and across tanks:

pH treatment	pH 4.3									pH 8.4									
Population origin	acid origin					neutral origin				acid origin					neutral origin				
Tank id	8	10	16	24	26	4	9	15	25	2	13	14	17	22	7	11	20	21	
# of tadpoles	2	9	6	9	9	1	2	15	3	1	3	2	2	2	1	2	2	3	

Table S2: details on model structure and data transformations for analyses of survival, developmental stage, body mass of G42 individuals, corticosterone level, gut length (including post-hoc tests), and gut content.

We extracted model estimated means from our model on tadpole developmental stage as to quantify the difference between treatments for each population. We used the “emmeans” package (Lenth, 2025) to run pairwise comparison between all groups, and adjusted for multiple comparison using the Benjamini-Hochberg (1995) method.

data	survival	developmental stage	body mass of G42 tadpoles at takedown	corticosterone level	gut length	gut length Post hoc within pH 4.3	gut length Post hoc within pH 8.4	gut content
Type of model	rlm	lmm	lmm	lmm	lmm	lmm	lmm	permanova
Fixed effects								
pH (treatment)	x	x	x	x	x			x
population (origin)	x	x	x	x	x	x	x	x
pH x population	x	x	x	x	x			x
body length					x	x	x	
pH x body length					x			
population x body length					x	x	x	
pH x population x body length					x			
sampling time								x
Random effects								
Tank ID		x	x	x	x	x	x	x
sampling time					x	x	x	
Transformation & statistical practices								
Residuals vs fitted as weights		x	x	x				

Table S3: details on model structure and data transformations for analyses of periphyton, phytoplankton, dissolved oxygen production (NPP), PAR light penetration, vegetation biomass and zooplankton density at the end of the experiment, in either pH treatment (pH 4.3 vs pH 8.4).

	pH 4.3						pH 8.4					
data	periphyton	phytoplankton	dissolved oxygen production	PAR light penetration	vegetation biomass	zooplankton density	periphyton	phytoplankton	dissolved oxygen production	PAR light penetration	vegetation biomass	zooplankton density
Type of model fitted	lm	lm	lm	lm	lm	lm	lm	lm	lm	lm	lm	lm
Fixed effects												
population treatment	x	x	x	x	x	x	x	x	x	x	x	x
Transformation & statistical practices												
Residuals vs fitted as weights		x				x						

Supplementary results

Gut length - Developmental stage of tadpoles sampled for dietary traits on day 14 ranged from G28 to G33, and on day 20 from G32 to G38 (data not shown) at day 20. We found a highly significant a significant pH treatment x population origin x body length interaction effect on tadpole gut length (χ^2_1= 8.605, *P*= 0.003, Fig. 2b) in the full model, and we therefore next conducted analyses within each of the two pH treatments separately, of the treatment x body length interaction (χ^2_1= 19.092, *P*<0.001, Fig. 2b), of the treatment x population origin interaction (χ^2_1= 4.693, *P*= 0.030, Fig. 2b), of the body length (χ^2_1= 498.705, *P*<0.001, Fig. 2b) and of the treatment (χ^2_1= 5.94, *P*= 0.015, Fig. 2b). However, we found no significant effect of the population origin x body length (χ^2_1= 0.180, *P*= 0.671, Fig. 2b) and of the population origin (χ^2_1= 0.869, *P*= 0.351, Fig. 2b).

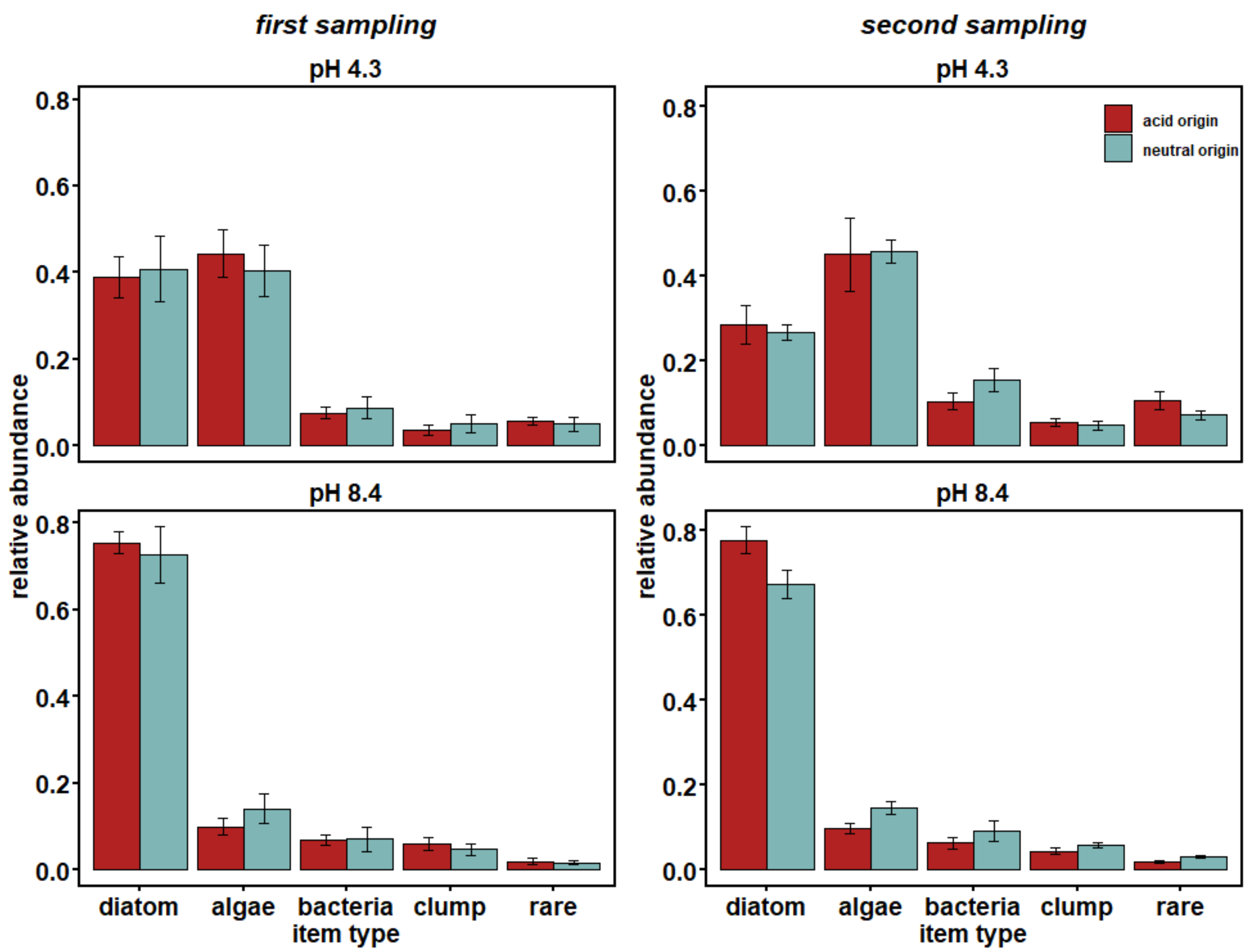
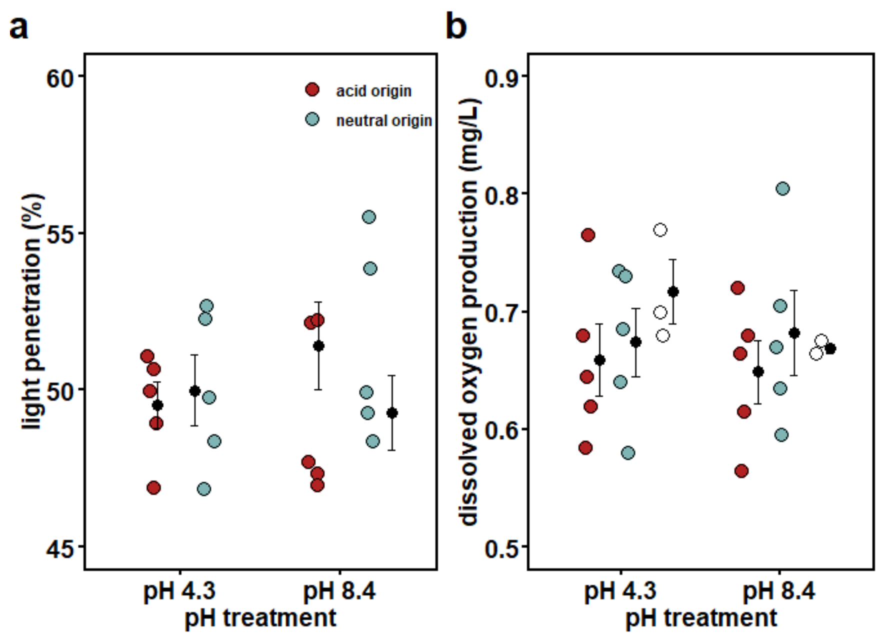


Figure S1: gut content of either population (acid origin vs neutral origin) across pH treatments (pH 4.3 vs pH 8.4) and sampling times (first vs second sampling).

In the discussion, we mention that the pH 4.3 environment appeared more productive and resource-rich than the pH 8.4. As to better understand our results and their implication, we tested whether the environment provided to the tadpoles differed in productivity. To do this, we reduce the data and selected only the mesocosms containing no tadpoles (3 mesocosms at pH 4.3 and 3 mesocosms at pH 8.4), as this will paint the most representative picture of what the tadpoles has access to, and eliminate presence and/or population specific effects. We compared several variables that may reflect productivity, namely: phytoplankton density, periphyton density, vegetation biomass, Net Primary Productivity, zooplankton density. We used non-parametric Kruskal Wallis tests, with each of these variables as a numerical response variable, and pH treatment as a categorical predictor.

We found a significant effect the pH treatment on phytoplankton density (K-W χ^2_1= 4.355, *P*= 0.037, Fig. 3b), on vegetation biomass (K-W χ^2_1= 3.857, *P*= 0.049, Fig. 3c), on NPP (K-W χ^2_1= 3.971, P=0.046, Fig. S2b) as the level of these variables was higher in pH 4.3 than pH 8.4. We found no significant effect of the pH treatment on periphyton density (K-W χ^2_1= 2.333, *P*= 0.127, Fig. 3a) or on zooplankton density (K-W χ^2_1= 2.333, *P*= 0.127, Fig. 3d), despite an apparent trend for these to be higher in pH 4.3.

1502



1503 Figure S2: light penetration and daily dissolved oxygen production in the presence of either
1504 population (acid or neutral origin) or in control (no tadpoles) conditions, across both pH
1505 treatments (pH 4.3 vs pH 8.4).

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