Phenotypic divergence and eco-evolutionary dynamics in moor frog tadpoles

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Abstract

In the face of rapid environmental change, persistence of natural populations often relies on evolutionary rescue. Such rapid evolution can, in turn, affect ecosystem properties (i.e. cause evo-to-eco effects), as recently documented across taxa and ecosystems. Amphibians often act as keystone species, making them ideal candidates for studying eco-evolutionary dynamics, yet empirical studies remain rare. We aimed to bridge this gap in the moor frog (*Rana arvalis*), known for adaptive phenotypic plasticity and local adaptation to environmental acidification. To investigate context-dependent phenotypic divergence and potential for ecosystem feedbacks we performed an outdoor mesocosm experiment on *R. arvalis* tadpoles. We used a full-factorial design, rearing tadpoles from two different population origins (pH 4 vs 7) in two contrasting environments (pH 4.3 vs 8.4). Additionally, we included no-tadpole mesocosms in each pH environment to assess population origin effects relative to a no-tadpole baseline. We found that tadpoles exhibited substantial phenotypic plasticity in physiological and life-history traits (higher corticosterone levels, faster development, larger metamorphic size, and different diets in the pH 4.3 as compared to pH 8.4 environments) and environment dependent divergence between populations: acid-origin tadpoles had higher survival in the pH 4.3 environment, larger metamorphic size in both pH environments, and relatively longer guts in the pH 8.4 environment than neutral-origin tadpoles. Additionally, tadpole presence reduced light penetration in pH 4.3, and decreased zooplankton density while increasing phytoplankton density in pH 8.4. Finally, in the pH 4.3 environment, acid-origin tadpoles reduced phytoplankton (relative to neutral origin tadpoles), whereas in the pH 8.4 environment neutral origin tadpoles reduced periphyton while acid origin tadpoles reduced vegetation biomass (relative to the no-tadpole baseline). These results demonstrate marked phenotypic plasticity and divergence in *R. arvalis* tadpoles under ecologically relevant conditions, the central ecological role of tadpoles in ecosystems, and the potential for their evolutionary divergence of tadpoles to alter ecosystem function. These results indicate that plastic and/or evolved trait divergence of tadpoles can reshape eco-evolutionary dynamics in freshwater ecosystems. In light of global amphibian declines, understanding such context-dependent eco-evolutionary dynamics is vital for predicting ecosystem responses and informing conservation strategies.

Introduction

Amidst the ongoing environmental changes observed worldwide, wild populations face substantial challenges, as evidenced by the global biodiversity crisis (Sala et al., 2000; Brondízio et al., 2019). 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 (Ghalambor et al., 2007; Chevin et al., 2010). Importantly, while phenotypic evolution may permit evolutionary rescue in face of environmental change (Bell and Gonzalez, 2011; Carlson et al., 2014), phenotypic changes can also influence ecosystem structure and function and, when heritable, give rise to evo-to-eco effects (Hendry, 2017). Therefore, to understand how natural populations respond to environmental changes, we 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 across space is commonly observed in the form of adaptive divergence and local adaptation (Kawecki & Ebert, 2004; Räsänen & Hendry, 2008). Local adaptation, defined as the evolution of locally adapted phenotypes through natural selection, resulting in local genotypes outperforming immigrant genotypes (Williams, 1966; Kawecki & Ebert; 2004), is common in the wild (Hereford, 2009) and influences how populations and species respond to environmental changes (Hoffmann & Hercus, 2000; Bijlsma & Loeschcke, 2005; Meek et al., 2023). Notably, local adaptation can evolve at ecological timescales (Bell & Gonzalez, 2011), implying that rapid adaptive divergence of ecologically relevant traits has the potential to affect ecological processes (Hairston et al., 2005; Harmon et al., 2009; Hanski, 2012; Walsh et al., 2012; Matthews et al., 2016; Des Roches et al., 2017). When divergent selection acts on ecologically relevant traits, particularly in keystone species, phenotypic evolution may influence ecosystem structure and function and, in turn, shape the selection pressures acting on the focal organisms and their surrounding community (Hairston et al., 2005; De Meester et al., 2019), resulting in eco-evolutionary feedback loops (Hendry, 2017). Understanding the drivers and context dependency of such 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 the potential for rapid evolution to alter ecosystem properties 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 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. Thus far such studies are mostly conducted on a few model species (e.g., *Daphnia*, *Drosophila,* guppies and stickleback, reviewed in De Meester et al., 2019; De Meester & Pantel, 2014), while in many other ecologically important species showing 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 effects is still rarely assessed in eco-evolutionary dynamics studies in the context of environmental change (e.g. Hendry, 2017)

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

(Relyea, 2002; Laugen et al., 2003; Pfennig et al., 2010; Hangartner et al. 2011), provide an excellent model system. First, given the important role of tadpoles in freshwater ecosystems (Gibbons et al. 2006, Hocking and Babbitt 2014), and their high potential for genetic and plastic phenotypic divergence (Rose 2005, Conover et al. 2009, Richter-Boix et al. 2011, Urban et al. 2014), understanding the environmental basis of phenotypic divergence and subsequent ecological effects of tadpoles provides valuable insight into 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 (e.g., Petranka & Kennedy, 1999; Schiesari et al., 2009; Parlato & Mott, 2023) and indirect (e.g. competitive release and nutrient cycling; Davic, 1983; DuRant & Hopkins, 2008) effects. Hence, phenotypic divergence of tadpoles in any traits influencing such 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 properties, studies examining evo-to-eco feedbacks of tadpoles remain sparse.

Here we use the moor frog (*Rana arvalis*) as an empirical model. A series of laboratory studies on *R. arvalis* tadpoles have demonstrated substantial phenotypic plasticity and adaptive divergence among populations inhabiting an environmental acidification gradient in Sweden (Räsänen et al., 2003; Hangartner et al., 2011, 2012a, b; Egea-Serrano et al., 2014, Mausbach et al., 2022; 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 and changes in natural selection (Haines, 1981; Hangartner et al., 2011). Particularly relevant here, *R. arvalis* tadpoles from acidic *versus* neutral origin populations have diverged in physiology (corticosterone levels), larval life-history as well as predator defence traits (Hangartner et al., 2011, 2012a; Egea-Serrano et al., 2014; Mausbach et al., 2022). However, studies on adaptive divergence of *R. arvalis* tadpoles in response to different pHs 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 for ecosystem feedbacks).

To bridge these gaps, we conducted a semi-realistic outdoor mesocosm experiment using *R. arvalis* tadpoles from two populations (originating from an acidic vs. a neutral pH pond) to i) investigate the extent of adaptive divergence under contrasting ecologically realistic environments, ii) gauge the ecological role of *R. arvalis* tadpoles (indicative of potential to act as a keystone species), iii) test whether tadpoles from the two contrasting population origins have different effects on ecosystem parameters (i.e. evo-to-eco effects), and iii) assess the context dependency of these effects. We conducted a self-sustaining outdoor 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 an acidic (pH 4) and a neutral (pH 7) origin population reared from early larval stages to metamorphosis in two contrasting environments (pH 4.3 and 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 acidic *versus* neutral pH origin tadpoles have differential effects on ecology, and to what extent these may depend on the environmental context (pH 4.3 *versus* 8.4), we assessed key components of freshwater ecosystems structure (amount of periphyton and phytoplankton, vegetation biomass, and zooplankton density) and function (net primary productivity and light penetration). We made several key predictions. First, given prior evidence for genetically based phenotypic divergence and substantial trait plasticity in these populations in laboratory conditions (Hangartner et al., 2012b; Egea-Serrano et al., 2014; Mausbach et al., 2022), we predicted that the populations should be phenotypically divergent 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 environment (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 environment acid origin tadpoles outperform neutral origin tadpoles if they have generally higher stress tolerance, or that neutral origin tadpoles outperform acid origin tadpoles if they have a broader pH tolerance in the alkaline range (due to potential local adaptation to pH 7 environment). Finally, under the assumption that phenotypic divergence influences ecological function of tadpoles, we predicted that the two populations should have different effects on ecosystem variables, and that these effects could 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; Räsänen et al., 2003, 2005; Hangartner et al., 2011; Egea-Serrano et al., 2014; Mausbach et al., 2022).

In this study, we used two *R. arvalis* populations from southwestern 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 its 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 (Scaramella et al., 2022; Mausbach et al., 2022) to behavioural and morphological predator-induced defences (Egea-Serrano et al., 2014; Scaramella et al., 2022) and larval life-history (e.g., Hangartner et al., 2012a). 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 *versus* absence) owing to phenotypic plasticity. Notably, dietary traits of these populations have not been previously investigated.

Field sampling

Between April 18 and 23 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 (~ 6°C) until transfer to the laboratory at the Evolutionary Biology Centre of Uppsala University (Uppsala, Sweden), on April 23. Once in the laboratory, the embryos were maintained in groups of ~ 50 embryos by clutch (family) in 0.8 L polypropylene (PP) containers with 0.7L RSW. RSW 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, 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 environments (pH 4.3 and pH 8.4) × two populations (acid and neutral origin) and five replicate tanks (N= 20 mesocosms). In addition, three tanks of each pH environment were set up without tadpoles as “no-tadpoles baseline” (total N= 26 mesocosms). This addition bears notable benefits as it allows to assess the effects of tadpole presence (independent of population origin) on ecosystem parameters. Moreover, and quite critically in our view, it allows the comparison of the two populations to a no-tadpoles baseline, providing a more nuanced interpretation of the magnitude and direction of potential evo-to-eco effects, as the magnitude induced by population origin can now be compared to a baseline level characterised by an environment without tadpoles. Specifically, the presence of no-tadpoles baseline could allow us to detect more subtle effects 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. On the other hand, computing the magnitude at which each population affects a given environmental variable (relative to the no-tadpoles baseline) and opposing these two magnitudes of effects obtained independently from each other could allow the detection of more subtle effects that should be characteristics of early stages of divergence. This may thus allow an earlier detection of the onset of emergence of evo-to-eco effects, as well as insights onto the temporal dynamic of eco-evolutionary dynamics at large.

Experimental setup

Between April 16 and 18 2018, we filled the tanks (680L PP tanks, external measures 120 × 100 × 83cm (length × width × height, INT200, Accon) with ~525L of sand-filtered water from the nearby lake Mälaren (pH = ~ 7.5), resulting in ~ 50 cm water depth. The tanks were organised in three rows of 9 mesocosms (with an empty spot in the middle), each tank being about 60cm distant from the nearest neighbour. We then randomly assigned each tank to a given pH environment, with an overall check of balanced distribution of pH environments across space. In the nominal pH 4.3 environment tanks, we added 300mL of 1M H_2SO_4 to each of the mesocosms as we filled them with water, and later another 100mL 1M H2SO4 to reach pH of 4.3. Our initial aim for the two contrasting pH environments was to match the average pH of the source ponds of each population. The pH of water from lake Mälaren was initially ~ 7.5 (soon after spring snow melt) but over the following days, the pH in all mesocosms aimed to have a nominal pH 7.5 had increased to ~ 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 ~7.5, with the idea that later addition of various type of inoculum (see below) would at least partially contribute to bring the pH down, as well as for logistic reasons. 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 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 (1.4 ×1.6 mm mesh size) to prevent immigration by large predatory invertebrates.

On April 21, we collected surface water filtrates, pond sediment and aquatic shoreline vegetation in both source ponds. The rationale was to sample planktonic fauna, flora and microorganisms as to create a sustainable environment for the tadpoles. 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. We used a shovel to sample bottom sediment over its first 10cm in accessible shallow areas near the shoreline. 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 of *Calliergon cordifolium* and *Calliergonella cuspidate* mosses. Upon collection, we screened all vegetation for potential tadpole predators (mainly larvae of diving beetles, dragonflies and damselflies, and backswimmer nymphs*)* and removed them upon sight. Despite careful screening, a few early-instar predators evaded this step in the pH 4.3 tanks (see datafile) and these were removed when sighted during the experiment. 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.

All tanks were inoculated on the same day (April 21) with 1L of pond sediment, 1L (equivalent to 600g) of tightly pressed shoreline vegetation, and a filtrate equivalent of ~40L of surface water originating from the study 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). 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 exchanged water between tanks within each pH environment to homogenise conditions across mesocosms. To allow later assessments of amount of periphyton (see Environmental parameters below), we attached a 6 cm wide strip of yellow polyethylene to one side of each mesocosm on May 4. The strips were vertically oriented and ran from surface to bottom (length ~ 60cm) 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 Gosner stage 25 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., 2012b), 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. The allocation of either origin (or absence of tadpoles) across the mesocosms was done randomly within each pH treatment. 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, we estimate that the starting tadpole biomass was 1.7 × higher (approximately 1.99 g) for the acid origin than for the neutral origin (1.19 g) 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, pH averaged 4.35 ± 0.02 (SE) in the pH 4.3 environment and 8.41 ± 0.01 in pH 8.4 environment (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 environment tanks, we added 230 g of dry *Sphagnum* and 270g of peat pellets and to the pH 8.4 environment 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. We also measured water temperature every 2 to 3 days, and water temperature averaged 21.05 °C ± 0.09 (SE) over the course of the experiment, with 15.6°C as a minimum value and 28.1°C as a maximum value across all mesocosms (data not shown). Measures of dissolved oxygen were taken on five occasions and averaged 9.56 ± 0.04 (SE) mg/L, with 8.21 mg/L as a minimum and 12.11mg/L as a maximum value across all mesocosms (data not shown), providing a very oxygen rich environment for the tadpoles (Sparling 2009).

Tadpole data 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).

On May 31 (day 26), we found first individuals that had reached metamorphosis (G42: emergence of forelimbs) and commenced to take-down the experiment. On day 27, we collected data on ecosystem variables (as detailed below). On day 28, we initiated the takedown of all mesocosms and assessed survival, developmental stage and body mass for 20 to 29 individuals per mesocosm (when survival allowed, starting day 28), as detailed below. 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).

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 (Altwegg & Reyer, 2003), key mediators of multitrait variation (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 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). We individually weighed the first 20 tadpoles (or fewer if survival was lower) sampled from each mesocosm by gently drying the tadpoles 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 pH × population combinations, as well as replicates. We gently caught each tadpole with a hand-held fish net, transferred it into a container filled with ~ 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 for each individual tadpole sampled. A more complete description of the process can be found in the Appendix S1: Materials and Methods.

Gut length - To assess gut length, 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. Each individual was photographed with a digital camera (Olympus C-5060) by placing the tadpole on its side on a Petri dish covered with millimetre paper for scale. To measure gut length, the whole gut was surgically removed and the intestinal coil was subdivided into smaller fragments (see Diaz-Paniagua, 1985). 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 size (length from 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 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 environment 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 screened 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, “other 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 (“clump”). Fifth, rare items (“rare”), including dinoflagellates, zooflagellates, fragments of macrophytes, pollen, rotifers and crustacean zooplankton.

Given that we did not standardize the amount of gut content screened (i.e. 30 microscopy fields 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.

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 determinants - as well as indicators - of environment state and are logistically feasible to monitor given the experimental design.

Light penetration - On day 27, we estimated the penetration of photosynthetically active radiation (PAR in µmol . m^{-2} . s^{-1}) 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 due to evaporation along the experiment. To account for variation in ambient light variation, we simultaneously recorded incident PAR using a separate sensor held ~80cm above the water surface (LI190, LI-COR Biosciences). All measures were obtained through the automated averaging of PAR over 5 seconds and duplicated for each tank. For each duplicate, we computed the ratio of PAR measured at 20 cm depth to incident PAR (expressed as percentage). We then computed the average out of these two ratios and used this as the response variable in statistical analyses.

Periphyton and phytoplankton densities - To estimate the amount of periphyton produced over the course of the experiment, on day 27 we collected the lowest 10 cm of the polyethylene strip set up on day -1. 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 estimate 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 h; 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 densities (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 strip (for periphyton samples, 7.5mL 95% EtOH) or filter (for phytoplankton samples, 15mL 95% EtOH), and keeping these falcon tubes at 4°C for 12 hours (Jespersen & Christoffersen, 1987). In the case of chl-*a* extracts from periphyton samples, the two sets of extracts (from the two pieces of strips) from a given mesocosm were pooled together into the same 15mL falcon tube. We then filtered the solutions containing extracted chl-*a* through 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 each sample's 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* density mg chl-*a* per m^3 using the formula according to Lorenzen (1967). However, 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 & Moss, 1998), by influencing water quality (Dhote & Dixit, 2007), the availability of nutrients for planktonic primary producers (Moore et al., 1984; Søndergaard & Moss, 1998, Dhote & Dixit, 2007) 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 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 & Woodward, 1998), and hence a core measure of ecosystem function (Walsh et al., 2012). NPP is affected by nutrient levels and phytoplankton density (Smith & 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 (~ 03:15 am) and immediately after sunset (~ 22:00) in the centre of each mesocosm at ~ 10 cm water depth, using a luminescent/optical DO sensor probe (Intellical™ LDO 10105 with HQ40D, Hach). We computed daily DO production for each mesocosm on day 27 as $DO_{\text{after sunset}} – DO_{\text{before sunrise}}$ as a proxy for NPP.

Zooplankton density - To estimate the abundance of zooplankton, we sampled two litres of surface water from each mesocosm in the late afternoon (~ 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.

Zooplankton was identified using Sandhall & Berggren (2001) and 40 x optical microscope. All planktonic individuals encountered were crustaceans. We identified Ostracods down to the class, Copepods to the order (Cyclopoida, Calanoida, Harpacticoida), and all other to the genus (*Bosmina*, *Daphnia*, *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 & Weisberg, 2019). We checked, both visually and statistically, that the statistical models fitted their respective 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 & 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, in most cases also dealt with the non-normal distribution as well as autocorrelation of residuals. In the remaining cases, we log-transformed our data when residuals appeared non-normally distributed.

We initially analysed survival with a generalised linear mixed model (using a binary observation per individual tadpole) but due to multiple issues with model fit, we switched to an analysis of survival using 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 environment (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, with the exception of the model on survival data, 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 environment x population treatment x body length interaction together with the pH environment 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 environment) on gut length had 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 environments x population origins. However, the variability in developmental stage combined to the non-linear, 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 Appendix S1: Table S1 for detail on the distribution of these tadpoles across pH environment 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 environment, population origin, sampling time (categorical, two levels: first and second sampling) and the pH environment 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 Appendix S1: Table S2.

Due to inherent differences between the pH environments induced by the contrasting starting conditions, we chose to compare the effects of tadpole population origin on several environmental variables within each the two pH environments (pH 4.3 and pH 8.4). In these analyses, we compared three levels within each pH environment: tanks containing acid origin tadpoles (5 replicates), tanks containing neutral origin tadpoles (5 replicates) and tanks containing no-tadpoles (3 replicates). For each environmental variable, and within each pH environment, we fitted a general linear model with "population" (categorical, three levels: acid origin, neutral origin and no-tadpoles baseline) as sole fixed effect. 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 & 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 Appendix S1: Table S3.

We used effect size analyses as a complementary approach to get further insight onto potential effects of tadpole presence on the ecosystem, as well as contrasting ecosystem feedback effects originating from evolutionary divergence. 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 & Feinn, 2012). We used Hedges' 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* no tadpoles and c) mesocosms with neutral origin tadpoles *versus* no tadpoles within each of our two pH environments. Hedges' g effect size estimate is particularly suited for small sample sizes due to its correction for bias inherent in Cohen's d (Hedges, 1981; Cohen, 2009). We used the bootES package in R (Kirby & Gerlanc, 2013) to calculate Bias-Corrected and accelerated (BCa) bootstrapped 95% confidence interval, non-overlap with zero being indicative of practical significance. We increased the number of iterations up to 5000 bootstrap resamples so that convergence was reached. We plotted the estimated Hedges' g for each pairwise comparison, along with their 95% confidence intervals, for each measured ecosystem parameter within pH 4.3 and pH 8.4 environments. This allowed us to determine the direction the magnitude of population specific effects on ecosystem parameters.

Results

Tadpole responses

Survival - Survival of tadpoles ranged from 30 to 100% across tanks over the 28 days duration of the experiment (Figure1a), and there was a significant pH environment x population origin interaction effect ($F_{1,16}$ = 11.34, P= 0.004, Figure1a). Post-hoc pairwise comparisons showed that acid origin tadpoles had substantially higher survival than neutral origin tadpoles in the pH 4.3 environment (~ 85% vs ~ 60% on average; z = 5.26, P< 0.001, Figure1a), whereas there was no difference between origins in the pH 8.4 environment where both populations had high survival (z = 0.49, *P*= 0.621, Figure 1a).

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

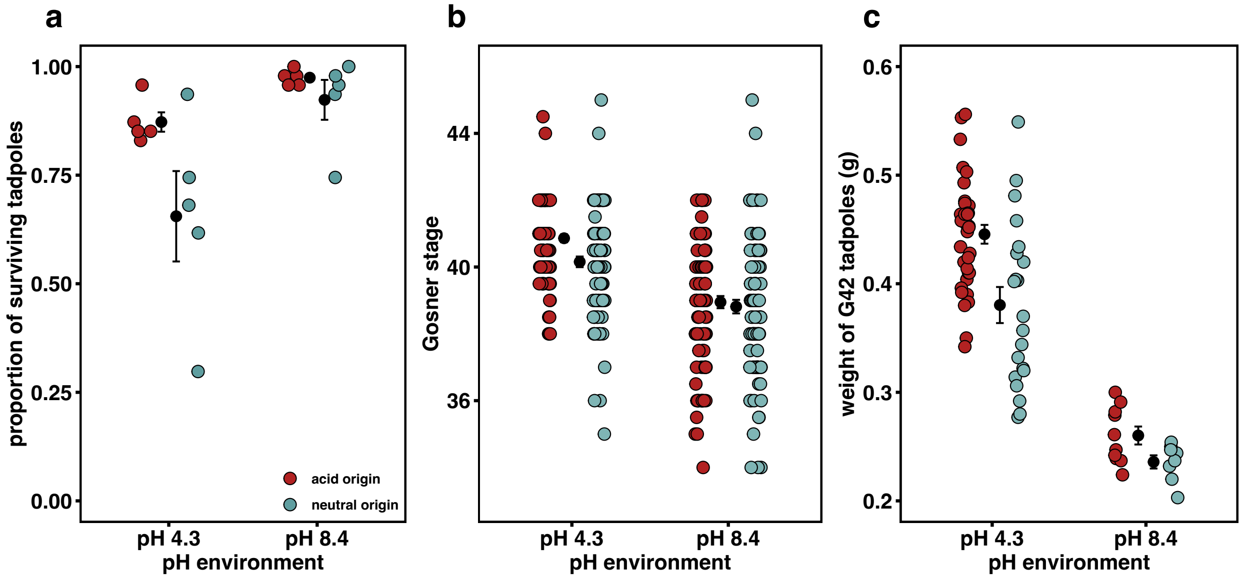

Figure 1: a) Survival, b) Gosner stage at mesocosm takedown and c) weight of G42 tadpoles for the acid origin (red-filled circles) and neutral origin (blue-filled circles) population of *R. arvalis* in two pH environments (pH 4.3 vs pH 8.4). Group specific means (± 1 SE) are represented inwards relative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

G42 stage metamorphs were ca. 70% (acid origin: 71%, neutral origin 69.5%) larger in the pH 4.3 than the pH 8.4 environment (pH environment: χ^2_1= 94.86, *P*< 0.001, Figure 1c). Acid origin metamorphs were substantially larger than neutral origin metamorphs in both pH environments indicated by a significant population origin (χ^2_1= 5.75, *P*= 0.017, Figure 1c), but there was no significant pH environment x population origin effect (χ^2_1= 0.71, *P*= 0.398, Figure 1c).

Corticosterone – Corticosterone levels at the mid-larval stage were substantially higher in the pH 4.3 than the pH 8.4 environment (pH environment: χ^2_1= 13.17, *P*< 0.001, Figure 2a), but there was no significant population origin (χ^2_1= 0.28, *P*= 0.595, Figure 2a) or pH x population origin interaction effect (χ^2_1= 1.12, *P*= 0.291, Figure 2a).

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 environment x population origin x body length interaction (χ^2_1= 8.60, *P*= 0.003, Figure 2b) in the full model (see Appendix S1: Results for model details), and we therefore conducted analyses within each of the two pH environments separately. Analyses within each pH environment found a highly significant population origin x body length interaction in the pH 8.4 environment (χ^2_1= 1.204, *P*< 0.001, Figure 2c), but no significant population origin x body length interaction in the pH 4.3 environment (χ^2_1= 0.02, *P*= 0.887, Figure 2b). These effects arose because in the pH 8.4 environment, neutral origin tadpoles had relatively shorter guts at larger size (Figure 2c), whereas in the pH 4.3 environment both populations showed similar slopes (Figure 2b).

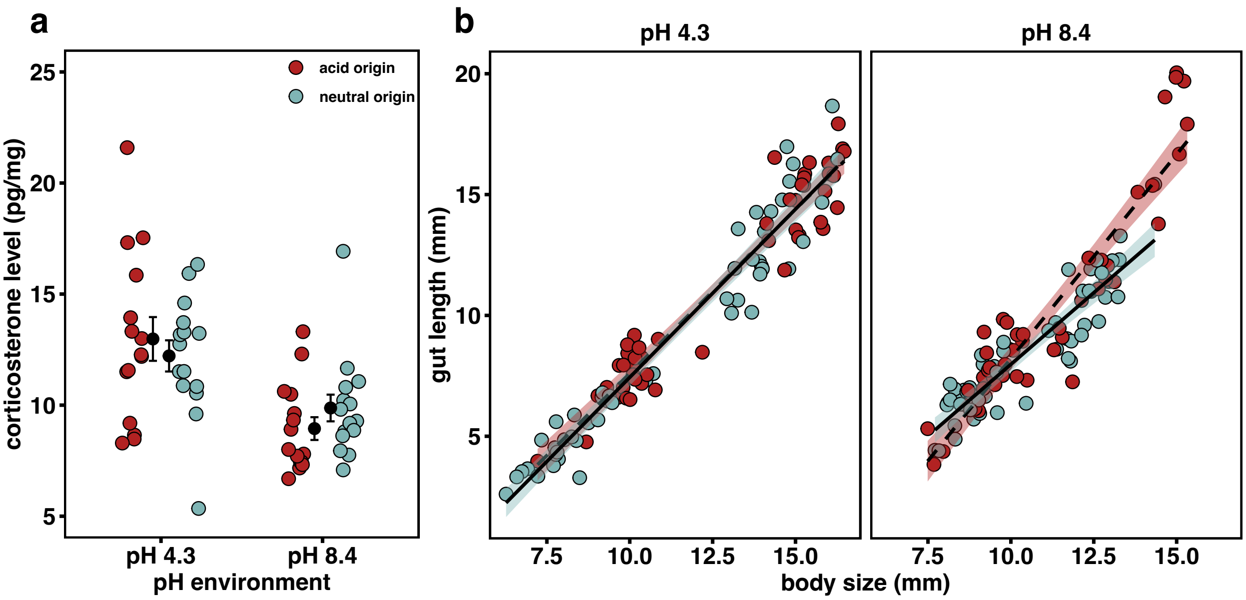

Figure 2: a) Corticosterone level in two pH environments (pH 4.3 vs pH 8.4), and b) tadpole gut length as a function of body size in pH 4.3 (left-side panel) and pH 8.4 (right-side panel) for the acid origin (red-filled circles) and neutral origin (blue-filled circles) *R. arvalis* tadpoles. Red-filled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

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

Environmental parameters

The general linear models revealed no significant effects of population treatment ("acid origin", "neutral origin", "no-tadpoles") 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, Appendix S1: Figure S2a), periphyton (pH 4.3: $F_{2,10}$= 2.02, P= 0.183; pH 8.4: $F_{2,10}$= 1.79, P= 0.216, Figure 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, Figure 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, Figure 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, Appendix S1: Figure S2b). While there was no significant effect of the population treatment on zooplankton density within the pH 4.3 environment ($F_{2,10}$= 1.37, P= 0.297), population treatment affected zooplankton density in the pH 8.4 environment ($F_{2,10}$= 5.08, P= 0.030, Figure 3d). This effect arose because there was a significant difference between the no-tadpole baseline and the acid origin treatment (t = -3.21, P= 0.028, Figure 3d), as well as between the no-tadpole baseline and the neutral origin treatment (t = -2.65, P= 0.037, Figure 3d), but acid and neutral origin treatments did not differ from each other (t = -0.65, P= 0.532, Figure 3d). These results indicate that tadpole presence *per se* decreases zooplankton density (Figure 3d).

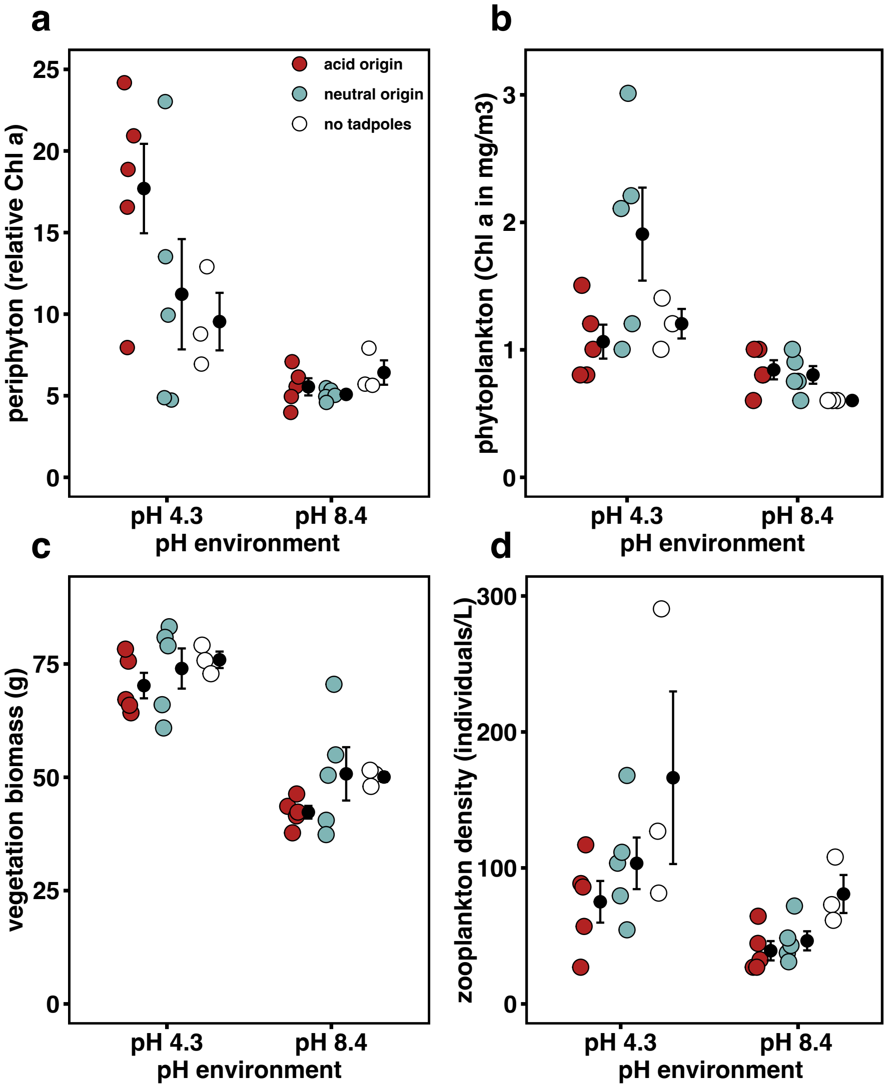

Figure 3: a) periphyton level, b) phytoplankton level, c) vegetation biomass and d) zooplankton density in the presence of either acid origin (red-filled circles) vs neutral origin (blue-filled circles) tadpoles or absence of tadpoles (open circles) in pH 4.3 vs pH 8.4 environments. Each single observation 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 Hedges' g estimates ranged from 0.02 to 2.43 in absolute values (Figure 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 (bootstrapped 95% confidence interval non-overlapping zero; Figure 4a,b).

Acid versus neutral origin – In the pH 4.3 environment, the presence of acid origin tadpoles decreased phytoplankton abundance more than the presence of neutral origin tadpoles (Hedges' g = -1.24, 95%CI [-3.29, -0.09]; (Figure 4a), but tadpole population origin did not affect differentially any other environmental parameters (Figure 4a,b).

Acid origin versus no tadpoles, neutral origin versus no tadpoles - Here we estimated to what extent does acid origin increase/decrease a given variable relative to the no-tadpoles baseline, and compared it to the extent to which the neutral origin does relative to the baseline. In two cases acid origin and neutral origin tadpoles had different effects on ecosystem variables as compared to the no-tadpole baseline. In the pH 8.4 environment, neutral origin tadpoles decreased periphyton levels relative to the baseline (Hedges' g = -1.45, 95% CI [-2.27, -0.99], Figure 4b), whereas the acid origin tadpoles did not (g = -0.63 [-2.11, 0.84], Figure 4b). Conversely, acid origin tadpoles decreased vegetation biomass relative to the baseline (g = -2.43 [-4.14, -1.15], Figure 4b), whereas the neutral origin tadpoles did not (g = 0.06 [-1.65, 1.31], Figure 4b). These results indicate that acid and neutral origin tadpoles differ in their ecological functions and consequently ecosystem feedbacks.

We also found cases where the different tadpole population origins analogously affected ecosystem variables, but did not differ from each other in the magnitude of the effects. In the pH 4.3 environment, both populations decreased PAR light penetration relative to the baseline (acid vs no tadpoles: g = -1.92 [-2.89, -1.13]; neutral vs no tadpoles: g = -1.13 [-3.28, -0.19], Figure 4a). In the pH 8.4 environment, both populations increased phytoplankton (acid vs no tadpoles: G = 1.53 [0.48, 3.11]; neutral vs no tadpoles: g = 1.38 [0.48, 2.55], Figure 4b), but decreased zooplankton density relative to the baseline (acid vs no tadpoles: g = -1.9 [-4.02, -0.61]; neutral vs no tadpoles: g = -1.58 [-3.63, -0.12], Figure 4b). These results indicate that tadpole presence (irrespective of origin) affected different ecosystem variables in the two pH environments.

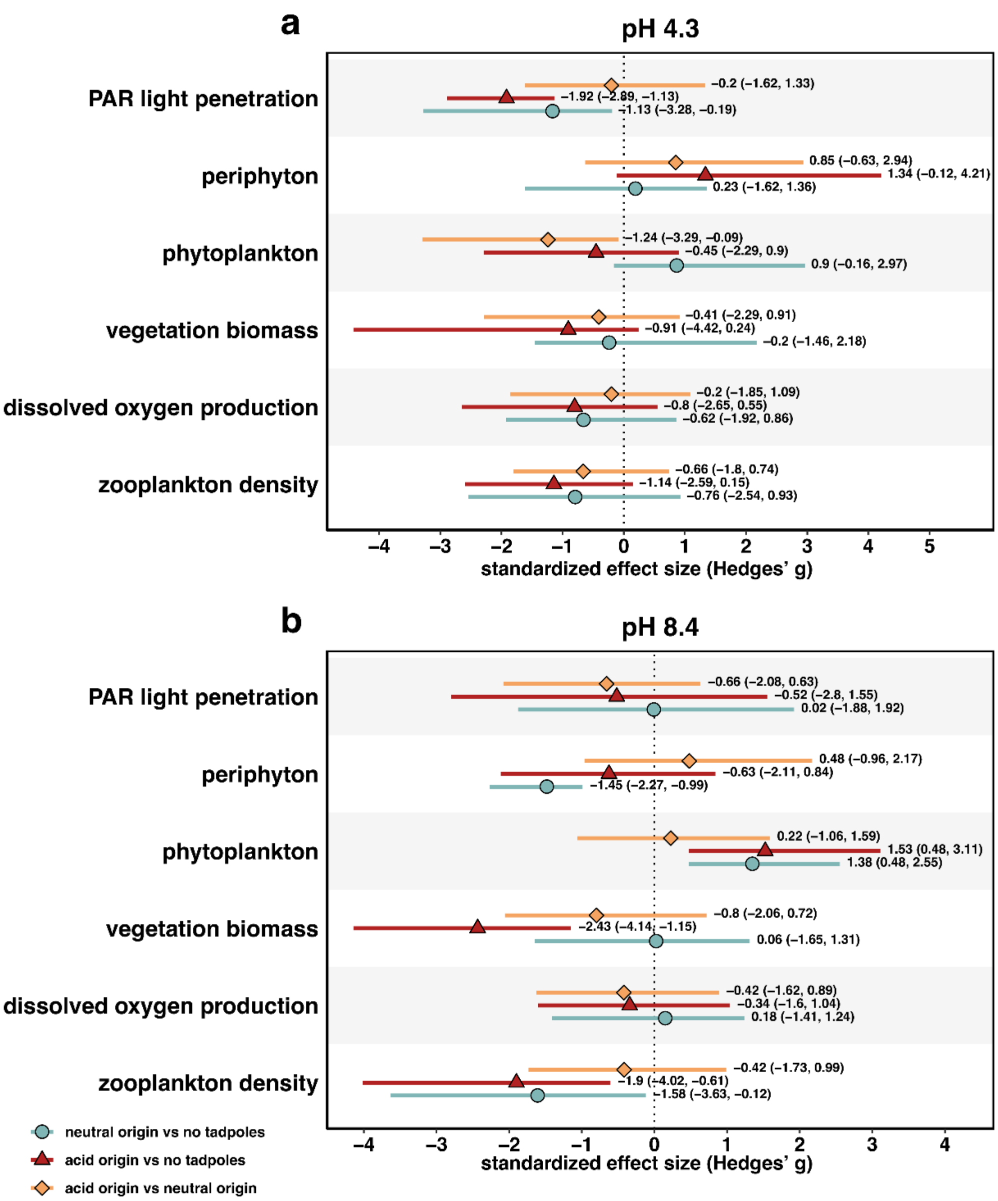

Figure 4: Standardized effect size (Hedges' g) with 95% Bias-Corrected and accelerated bootstrap confidence intervals for pairwise comparisons (diamond: acid vs neutral; triangle: acid vs no tadpoles; circle: neutral vs no tadpoles) within pH 4.3 environment (a) and pH 8.4 environment (b). Positive values 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, acid origin tadpoles decrease the level of phytoplankton compared to neutral origin tadpoles within pH 4.3 (i.e. Hedges' g = -1.24 [-3.29, -0.09], see panel a).

Discussion

Phenotypic plasticity and genetic divergence are both important components of rapid phenotypic evolution and have the potential to feedback to ecology (Hendry, 2016). Results of our semi-realistic outdoor mesocosm experiment, where *R. arvalis* tadpoles from an acidic and neutral pH origin population were reared in two contrasting pH environments from early larval stages to metamorphosis, support the view of substantial phenotypic plasticity in both populations: tadpoles displayed higher corticosterone levels and a more diverse diet at mid-larval stages, and developed faster and to a larger metamorphic size in the pH 4.3 than the pH 8.4 environment. In line with previous laboratory studies on these populations (Hangartner et al., 2011; Egea-Serrano et al., 2014), we found evidence for population divergence in a key fitness component as acid origin tadpoles reached a higher body mass at metamorphosis in both pH environments. We also found evidence suggestive of environment specific adaptive plasticity in dietary morphology and local adaptation: acid origin tadpoles had relatively longer guts at large body sizes than neutral origin tadpoles in the pH 8.4 environment and survived better than neutral origin tadpoles in the pH 4.3 environment. In terms of ecological function, we found clear evidence for the ecological role of *R. arvalis* tadpoles as their presence (independent of origin) decreased light penetration (in the pH 4.3 environment), and reduced zooplankton density while increasing phytoplankton density (in the pH 8.4 environment). Finally, our results suggest potential for context-dependent ecosystem feedbacks deriving from recent population divergence (Räsänen et al., 2003). In the pH 4.3 environment, acid origin tadpoles reduced phytoplankton levels relative to the neutral origin population. In the pH 8.4 environment, acid origin tadpoles reduced vegetation biomass, while neutral origin tadpoles reduced periphyton levels instead (relative to the no-tadpoles baseline). Overall, these results demonstrate high phenotypic plasticity yet notable population divergence in *R. arvalis* tadpoles, as well as substantial potential for these tadpoles to act as keystone species, and for their evolutionary divergence to yield contrasting ecosystem feedback

Environment dependent phenotypic variation

We found strong environmental effects (i.e. differences in mean trait value between the pH 4.3 and 8.4 environments) on all phenotypic traits studied, including developmental stage and size at metamorphosis) as well as physiology (corticosterone level) and dietary morphology (gut length), with gut length also indicating genotype-by-environment (G x E) interactions. The timing of and size at metamorphosis are key fitness components in anuran amphibians, with early metamorphosis at a large body size resulting typically in higher terrestrial growth and survival (Altwegg & Reyer, 2003). 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 a larger metamorphic size in the pH 4.3 than the pH 8.4 environment. While acidic pH is known to be physiologically stressful in broad range of taxa in standardized laboratory conditions (e.g. Merilä et al., 2004; Weber & Pirow, 2009; Guan & Liu, 2020), in our ecologically more complex environment, the pH 4.3 environment nevertheless provided better growth conditions than pH 8.4 suggesting that the effects of pH strongly depend on the environmental context. In our specific case, this could be because the pH 4.3 environment was more productive and resource rich, as indicated by net primary productivity, phytoplankton level and vegetation biomass being higher than in the pH 8.4 environment (see further discussion below and Appendix S1: Results).

Interestingly, however, we found that corticosterone levels were higher in the pH 4.3 than in the pH 8.4 environment. While higher level of corticosterone (as observed here in the pH 4.3

environment) is often assumed to indicate elevated stress levels (reviewed in Denver, 2009), corticosterone level more generally reflects metabolic activity independently of stress (Jimeno et al., 2018). As acidic environments may select for higher energy uptake by tadpoles (Liess et al., 2015), elevated corticosterone levels may relate to the higher growth rates in the pH 4.3 environment in our study. Despite apparently better growth conditions, however, tadpole survival (especially for the neutral origin population) was lower in the pH 4.3, suggesting suboptimal conditions for some aspects of performance (further discussed below).

From an environmental perspective, the two pH environments were clearly distinct, and hence likely modified any direct effects of pH per se. First, the two pH environments received inoculum for zooplankton, micro-organisms, macrophytes and bottom sediment from the acid *versus* neutral origin ponds, respectively, hence starting with different resource base and habitat structure. While starting biomass of wet vegetation was equal, dry biomass at the end of the experiment was much lower in the pH 8.4 environment. Therefore, the higher biomass in the pH 4.3 environment over the course of the experiment, combined with the bushier morphology of *Sphagnum* moss (compared to *Calliergonella* and *Calliegon* mosses in the pH 8.4 environment) likely provided larger surface for epiphytic growth within pH 4.3 environment and, hence, higher food availability for tadpoles which primarily feed on periphyton (Montaña et al., 2019). Moreover, as a strong ecosystem engineer (van Breemen, 1995; Svensson, 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 & Beckvar, 1980).

This supposed higher dietary resource availability and diversity was somewhat reflected in the gut content of the tadpoles: tadpole diet consisted of a more even combination of green/blue algae and diatoms (~ 40% for each) in the pH 4.3 environment than in the pH 8.4 environment (which was mostly dominated by small centric diatom *Cyclotella*, M. 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 environment. A possibly more favourable protein-carbonate ratio provided by a mixture of green algae and diatoms in the pH 4.3 environment (Kupferberg, 1997; Richter-Boix et al., 2007) could have allowed tadpoles to allocate energy towards growth and development rather than foraging (e.g. Pfennig, 1990) – hence explaining the quicker development and larger metamorphic size in the pH 4.3 environment. While the differences in gut content likely reflect largely dietary availability, it should be noted however that tadpoles may display selective foraging (Richter-Boix et al., 2007). Further mesocosm and field studies, concomitant to behavioural studies, are hence needed to more accurately characterise diet and potential for selective foraging in *R. arvalis* tadpoles in acidic and neutral ponds.

The lower survival of tadpoles in the pH 4.3 environment, especially for the neutral origin population (range for acid origin: 80-95%; range for neutral origin: 30-90%, see below), compared to high survival in the pH 8.4 for both populations (range 75-100%) despite the apparently more favourable growth conditions can have several reasons. In addition to physiological stress of acidic pH, the unintended presence of a small number of predators in some of the pH 4.3 tanks (mostly small larvae of dragonflies, damselflies and diving beetles, see datafile for quantitative detail) likely contributed to the lower survival. Post-hoc analyses within pH 4.3, however, found no significant correlation between 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 the pH 8.4 environment, despite appearing resource-limited, only led to sub-lethal effects on *R. arvalis* tadpoles. As discussed above, the higher corticosterone levels in the pH 4.3 environment compared to pH 8.4 (for both *R. arvalis* populations) may reflect higher physiological stress of acidic than alkaline pH, and/or higher metabolic activity linked with faster growth and development (Guillette et al., 1995; Sapolsky et al., 2000).

Phenotypic divergence between the populations

The higher survival of the acid versus the neutral origin tadpoles in the pH 4.3 environment in our study strengthen evidence for local adaptation of the acidic population (e.g., Egea-Serrano et al. 2014). However, we found no difference between the populations in the pH 8.4 environment, and hence no support for our two hypotheses that, first, adaptation of acid origin tadpoles to physiologically more stressful conditions may provide them with a generally better ability to cope with stressful conditions or, second, that neutral origin tadpoles may outperform acid origin tadpoles as pH 8.4 is closer to their native pH. This absence of survival differences between the populations and survival high overall in the pH 8.4 environment indicates that slightly alkaline pH was not a major challenge for survival of *R. arvalis* tadpoles, even under the low-resource conditions we observed at this pH. Despite a net improvement in ecological realism in our study compared to several previous standardized laboratory studies on these *R. arvalis* populations (e.g., Hangartner et al., 2011), our study here may still not reflect the full extent of local adaptation. In nature many other factors, such as ability of tadpoles to cope with - and escape - predation (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 (Räsänen et al., 2005; Hangartner et al., 2011; Egea-Serrano et al., 2014), we found that acid origin tadpoles were larger at metamorphosis than neutral origin tadpoles, both in the pH 4.3 (13% increase, on average) and the pH 8.4 (12% increase, on average) environment. This difference is likely due to both genetic and maternal effects (Räsänen et al., 2005: Hangartner et al. 2011, 2012a, b). Notably, the acid origin tadpoles in our study were 65% heavier than the neutral origin tadpoles at the beginning of the experiment (average tadpole weight 0.033g vs 0.020g, respectively) indicative of differences in maternal investment in egg size (Räsänen et al., 2008; Hangartner et al., 2012b). For this reason, the observed difference in metamorphic mass between the populations likely reflects a combination of variation in maternal investment and genetically higher growth during the experiment (Räsänen et al., 2005; Hangartner et al. 2012a, b). In terms of physiological responses our results for corticosterone levels at mid-larval stages contrast with a previous laboratory study, where acid origin tadpoles had lower average corticosterone levels than neutral origin tadpoles (Mausbach et al., 2022), as we found no population differences in corticosterone levels. 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.

Resource availability and quality are major selective agents in nature, having repeatedly driven the evolution of resource polymorphism in a range of taxa (Skulason & 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 environment suggests population divergence in diet induced plasticity. The optimal digestion theory predicts longer guts in environments with low quantity and quality food resources (Sibly, 1981). Given that resource quality differs along acidification gradients (Eriksson et al., 1980; Geelen & Leuven, 1986; DeNicola 2000), resource variation may thus have favoured differential diet-induced plasticity between our *R. arvalis* populations. 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 *Lithobates sylvaticus* (Stoler & Relyea, 2013) and *Scaphiopus multiplicatus* tadpoles (Pfennig, 1990). Such dietary plasticity is common in a wide range of taxa (e.g. 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 (Lindgren & Laurila, 2005; Liess et al., 2015). Hence, it is possible that the apparently lower food quantity and/or quality in our pH 8.4 environment led to the expression of adaptive gut length plasticity to improve energy uptake (Sibly, 1981).

A hypothetical link could also be made between the increased gut length of acid origin tadpoles and our finding of the reduced vegetation biomass within pH 8.4 environment in presence of acid origin tadpoles. It is possible that tadpoles fed directly on macrophytes, as evidenced by fragments of macrophytes found in the guts of tadpoles (M. Kaiser, pers. obs.) and as acid origin tadpoles are larger this effect could have been more pronounced than in the presence of neutral origin tadpoles. Generally, while our analyses revealed that gut content differed strongly between the two pH environments, we found no differences in diet between the populations. 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 quantify the extent of population-specific adaptive developmental plasticity in dietary traits of *R. arvalis* tadpoles (Liess et al., 2015).

The ecological footprint of Rana arvalis *tadpoles*

Tadpoles are often key players in freshwater ecosystems, influencing various aspects of ecosystem function (Wood & Richardson, 2010; Whiles et al., 2013; Corline et al., 2025). Our experimental design included no-tadpole mesocosms for both pH environments, which allowed the comparison of all variables of interest in the presence of tadpoles (either acidic or neutral origin) and in their absence (no-tadpoles baseline), and thus to make inferences on the functional role of *R. arvalis* tadpoles. In the pH 4.3 environment, we found that tadpole presence *per se* (independent of tadpole origin) decreased light penetration. One of the main parameters known to affect light penetration is phytoplankton density (Fleming-Lehtinen & Laamanen, 2012). However, we found no effect of tadpole presence on phytoplankton density in the pH 4.3 environment, suggesting that it is unlikely that tadpoles indirectly affected light penetration through effects on phytoplankton. Alternatively, the effects may have come from tadpoles re-suspend bottom sediment particles as they forage (bioturbation; Ranvestel et al., 2004). While we have no direct observations on the impact of tadpole activity on the sediment, it is possible that reduced light-penetration was a result of tadpoles engaging in more explorative behaviour and/or higher availability of sediment (which was not assessed) in the pH 4.3 environment. Further studies are needed to gain mechanistic understanding of the impact of tadpole presence on light penetration.

In the pH 8.4 environment, we found that tadpole presence increased phytoplankton but reduced zooplankton density. This complements previous studies that documented that anuran tadpole presence can affect phytoplankton growth (Osborne & McLachlan, 1985; Mallory & Richardson, 2005), and more generally act on lower trophic levels simultaneously via direct and/or indirect effects (Rowland et al., 2017). One possibility for the increased phytoplankton and reduce zooplankton levels may be that, in the presumably more resource-limited environment of the pH 8.4 treatment, tadpoles have more actively consumed zooplankton through direct predatory behaviour and thus affected their density, which in turn led to indirect effects release of zooplankton pressure on phytoplankton. Previous research has shown that tadpoles can strongly affect zooplankton density via both consumptive and non-consumptive effects (Parlato & Mott, 2023). While the diet of *R. arvalis* has been generally little studied (but see Montaña et al., 2019 for a review of anuran tadpoles), ranid tadpoles have been shown to impact zooplankton density through direct and indirect pathways, including predatory behaviour (Petranka & Kennedy, 1999), nutrient cycling (Osborne & McLachlan, 1985) and competition for resources (Seale, 1980; Leibold & Wilbur, 1992). Our gut content analyses here showed that tadpoles fed mostly diatoms and green algae, but also other rarer items (including zooplankton), such as bacteria and pollen (Appendix S1: Results, M. Kaiser, pers. obs.). Support for tadpole consumption of zooplankton in our study comes from zooplankton body parts being found in the gut of the tadpoles (M. Kaiser, pers. obs.). Given the direct trophic link between zooplankton and phytoplankton (Levine et al. 1999), trophic cascades induced by the active foraging of tadpoles on zooplankton may have led to facilitation of phytoplankton growth. Such cascading effects have been documented in *Rana kukunoris*, as its predation on detritivorous insects led to reduced plant biomass through altered decomposition of dung (Wu et al., 2014).

Ecosystem feedbacks

When phenotypic divergence influences ecologically relevant traits, there is a potential for feedbacks from evolution to ecology (evo-to-eco), a process coined ecosystem feedback (Hendry, 2017). In this study, we found evidence for phenotype-to-ecology feedbacks in both pH environments. First, acid origin tadpoles reduced phytoplankton level relative to the neutral origin tadpoles in the pH 4.3 environment. Given that anuran tadpoles can feed, among others, on suspended particles in the water (Seale, 1980; Montaña et al., 2019), one straightforward explanation for this result could be that tadpoles have actively fed on phytoplankton material in pH 4.3. Or, as discussed above, consumption of zooplankton may have facilitated increases in phytoplankton. As to potential mechanisms leading to this population difference within pH 4.3 environment, one possibility is that the lower survival of neutral-origin tadpoles, and hence reduced density and biomass, is one driver of these effects. Differences in survival between populations are themselves population-specific responses to environmental conditions (whether linked to pH stress, predation, or competition) and thus are an integral part of population-specific effects on environmental parameters. Survival differences may, however, overshadow other mechanistic pathways leading to population differences, highlighting the need for further studies to disentangle them. Another complementary driver of the population specific effects on phytoplankton levels, may be the inherent size differences of tadpoles from the contrasting origins: acid origin tadpoles are substantially larger than the neutral origin tadpoles in common garden conditions (Hangartner et al., 2011; Egea-Serrano et al., 2014; Mausbach et al., 2022; this study), which may lead to higher energy uptake requirements and higher consumption of phytoplankton.

Second, in the pH 8.4 environment neutral origin tadpoles decreased the level of periphyton more than acid origin tadpoles did (relative to the no-tadpole baseline). In parallel, acid origin tadpoles decreased vegetation biomass within the pH 8.4 environment relative to the no-tadpoles baseline, which neutral origin tadpoles did not. One hypothetical scenario explaining the combined population specific effects on periphyton (reduced more by neutral origin tadpoles) and macrophytes (reduced more by acid origin tadpoles, see above) could be that in low resource environments (here pH 8.4), the larger size of acid origin tadpoles may have allowed them to feed and digest rough plant material (which hence may also have induce the longer guts, see above), whereas small sized neutral origin tadpoles may need to feed primarily on periphyton. This hypothesis could be tested experimentally in follow-up studies aimed at identifying the exact mechanisms through which divergent populations affect the environment they inhabit.

In the pH 8.4 environment, survival was generally high for both populations (over 90% on average) and did not differ significantly between populations, therefore we can exclude differences in density to contribute to population specific effects observed. These differences in tadpole effects were thus more likely related to the slightly lower overall biomass of the neutral-origin population, in addition to other functional traits (as discussed above). All this being said, it is possible that genetically based differences in energy demands and assimilation abilities may have led, for instance, to contrasting growth rates and nutrient excretion capacity, such as in the case of latitudinally divergent *R. temporaria* tadpoles (Liess et al., 2015). The observed differences in gut length (indicative of assimilation efficacy) and/or metamorphic size (indicative of energy demands) may complementarily come into play and yield higher nutrient excretion from a larger biomass of tadpoles (i.e. here acid origin tadpoles) which, in turn, may provide resources for primary producers and lead to effects on phytoplankton (in pH 4.3) and periphyton (in pH 8.4). In our study, the population specific effects on ecosystem parameters are most likely the result of multiple interacting factors, including tadpole size, developmental stage, and survival, which are factors that represent common ecological processes in natural populations. 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 environment, 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, because the acid origin population decreased vegetation (macrophyte) biomass within the pH 8.4 environment, we may have expected NPP results to mirror those of vegetation biomass, but this was not the case.

A potential factor influencing the results in our study is the occurrence of an extreme heatwave during the latter half of the experiment, which led to elevated water temperatures (mean ~ 21 C°). While this did not seem to affect survival (which was generally high), it did speed up tadpole growth and developmental rates compared to our typical laboratory assessments at 17 C°, where reaching metamorphoses in these populations takes roughly 60-70 days (Hangartner et al., 2012), versus ~ 42 days here. Hence, any phenotypic variation (including metabolic activity, developmental and growth rates) as well as effects on ecosystem functioning may have been over- or underestimated relative to more average environmental conditions typical at this latitude. Importantly, however, as the occurrence of such heatwaves is increasing rapidly due to climate change, future studies assessing role of temperature conditions in evo-to-eco effects would be valuable.

Conclusions

The 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 ecosystem feedbacks of anuran tadpoles. In general, our study supports previous findings for adaptive divergence in larval life-history traits in these populations, as well as partially evidence for local adaptation (Kawecki & Ebert, 2004, Hereford, 2009). 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.

Keystone species, which tadpoles are often suggested to be 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., 2017). 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 metamorphic size and gut length and, significantly, our study provides first evidence for ecosystem feedbacks emerging from rapid divergence of these acid and neutral origin populations, and that these effects were context dependent (different ecosystem parameters were affected by the two populations in the two environments). More studies investigating the consequences of contemporary adaptive divergence and rapid evolution are needed for better understanding of ecosystem functioning 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 collected all remaining data, with substantial help from MK and KR. QC analysed the data and produced the figures. QC wrote the first draft of the manuscript and developed it with substantial help from KR and AL. All authors read, provided comments on earlier versions, and validated the last version of the manuscript.

Conflict of interest statement

The authors declare no conflicting interests.

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Appendix S1: Supplementary Information

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.

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

pH environment	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

We extracted model estimated means from our model on tadpole developmental stage as to quantify the difference between environments 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.

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. "rlm" standards for robust linear model. "lmm" stands for linear mixed model.

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 (environment)	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 environment (pH 4.3 vs pH 8.4). *PAR light penetration* stands for Photosynthetically Active Radiation light penetration. "lm" stands for linear model.

	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						

Results

Gut length - We found a highly significant pH environment 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. This same initial model indicated a significant effect of the environment x body length interaction (χ^2_1= 19.092, *P*<0.001, Fig. 2b), of the environment 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 environment (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).

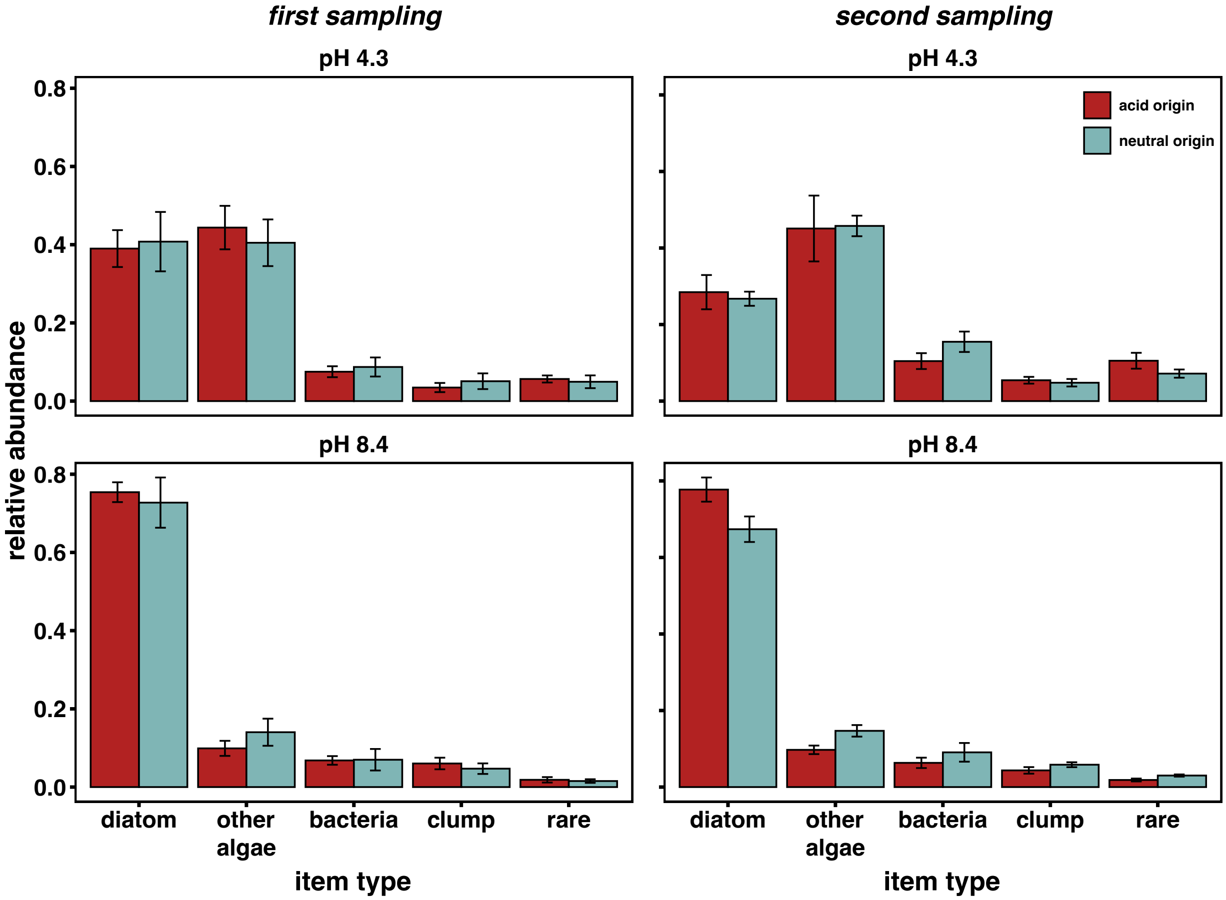

Figure S1: gut content of either population (acid origin vs neutral origin) across pH environments (pH 4.3 vs pH 8.4) and sampling times (first vs second sampling).

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 environment as a categorical predictor.

We found a significant effect the pH environment 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 environment 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.

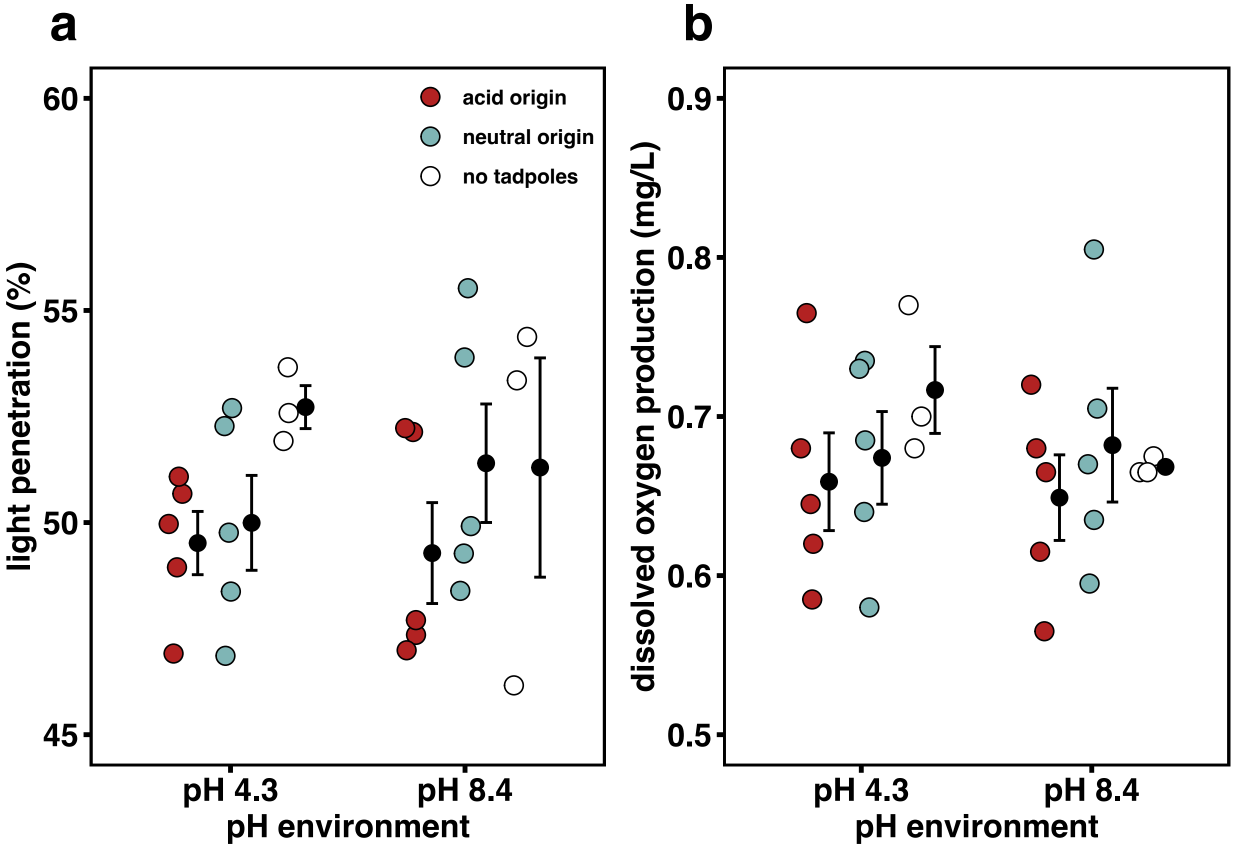

Figure S2: light penetration and daily dissolved oxygen production in the presence of either population (acid or neutral origin) or in the absence of tadpoles, across both pH environments (pH 4.3 vs pH 8.4).