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The influence of artificial light at night on the development of tadpoles of the European green toad (Bufotes viridis)

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Abstract

Light pollution has increased 6% per year over the last six decades, impacting over 20% of the Earth's surface and leading most of the human population to live in light polluted areas. Past research indicates a variety of effects of artificial light at night (ALAN) on the physiology, morphology and behavior of different vertebrates and invertebrates. Amphibians are already the most threatened vertebrate group, and as mostly nocturnal animals the disruption of the natural light-dark cycle may endanger them even more. In this study, we investigated the influence of ALAN on the survival, development and growth as well as shape of European green toad tadpoles. Individuals were raised under laboratory conditions. The experimental group was subjected to constant light exposure, with stronger illumination during the day and dimmed light at night, whereas the control group received identical daytime lighting but no light during the night. Larvae reared under continuous light had a significantly higher mortality over time than larvae that were kept in full darkness at night. Additionally, tadpoles from the light group exhibited longer bodies and lower body-to-tail ratios compared to individuals from the control group. Our findings support previous studies and highlight the impact light pollution has on early anuran development, potentially affecting adult individuals and whole populations.

Keywords: Artificial Light at Night, Light Pollution, Amphibians, *Bufotes viridis*, Tadpoles, Geometric Morphometrics

Zusammenfassung

Lichtverschmutzung hat in den letzten sechs Jahrzehnten um 6% pro Jahr zugenommen, betrifft über 20% der Erdoberfläche und führt dazu, dass die Mehrheit der menschlichen Bevölkerung in lichtverschmutzten Gebieten lebt. Bisherige Studien weisen auf eine Vielzahl von Auswirkungen von künstlichem Licht in der Nacht (ALAN) auf die Physiologie, Morphologie und das Verhalten diverser Wirbeltiere und Wirbelloser hin. Amphibien gelten bereits als die am stärksten bedrohte Wirbeltiergruppe und als überwiegend nachtaktive Tiere könnte die Störung des natürlichen Hell-Dunkel-Zyklus sie weiter gefährden. In dieser Studie untersuchten wir den Einfluss von ALAN auf das Überleben, die Entwicklung, das Wachstum sowie die Körperform von Kaulquappen der Wechselkröte. Die Tiere wurden unter Laborbedingungen aufgezogen. Die experimentelle Gruppe wurde kontinuierlichem Licht ausgesetzt, mit stärkerer Beleuchtung am Tag und gedimmten Licht in der Nacht, während die Kontrollgruppe eine identische Tagesbeleuchtung, aber nachts kein Licht erhielt. Larven, die unter Dauerlicht aufgezogen wurden, wiesen eine signifikant höhere Mortalität im Laufe des Experiments auf als die Larven, die nachts in völliger Dunkelheit gehalten wurden. Darüber hinaus zeigten die Kaulquappen aus der Lichtgruppe längere Körper und niedrigere Verhältnisse von Körper zu Schwanz im Vergleich zur Kontrollgruppe. Unsere Ergebnisse unterstützen frühere Studien und verdeutlichen den Einfluss von Lichtverschmutzung auf die frühe Entwicklung von Anuren, der sich potenziell auf adulte Individuen sowie ganze Populationen auswirken kann.

Schlagwörter: Künstliches Licht in der Nacht, Lichtverschmutzung, Amphibien, *Bufotes viridis,* Kaulquappen, Geometrische Morphometrie

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Introduction

Amphibians are experiencing the fastest decline of any vertebrate group, with 41% of species threatened with extinction according to the IUCN Red List (IUCN, 2025; Re:wild et al., 2023). The major threats are habitat loss, including degradation, the destruction of breeding waters and fragmentation of the landscape, as well as climate change (Gollmann, 2007) and diseases such as Chytridiomycosis (Tobler et al., 2010). Another endangering factor, which was ignored for a long time but has been increasingly investigated in recent years is artificial light at night (Rich & Longcore, 2006). With the growth of the human population and the rapid urbanization (Cinzano et al., 2001), light pollution becomes a more widespread problem with a variety of impacts on the environment and wildlife (Rich & Longcore, 2006).

Light pollution describes the changes in luminance at night due to the use of artificial light (Cinzano et al., 2001) with the main sources being lit buildings, lighting of streets and public places such as pedestrian zones and parks as well as lights on vehicles (Longcore & Rich, 2004). The new World Atlas of artificial night sky brightness points out that light pollution is not limited to developed countries. Almost everybody living in the European Union and the United States (more than 99%) as well as over 80% of the world population inhabit regions where the night sky exceeds the polluted status (i.e. over 8 % above natural nighttime brightness ≙ >14 µcd/m^2) (Falchi et al., 2016).

Effects of artificial light at night (ALAN) have been observed in many organisms, including vertebrates, invertebrates and plants. These effects could result in ecological and evolutionary consequences for entire ecosystems (Hölker et al., 2010). The increase of light pollution is especially challenging for nocturnal species (Rich & Longcore, 2006), as those animals adapted most of their sensory systems – vision, hearing, olfaction and touch – to darkness (Le Duc & Schöneberg, 2016). Light at night causes disorientation and entrapment of migratory birds, resulting in exhaustion, collisions and capture by predators (Evans Ogden, 1996; Longcore & Rich, 2004). Female sea turtles have shown changes in the selection of nest locations, avoiding illuminated beach areas (Salmon, 2003). Moreover, lights near beaches interfere with natural sea finding behavior of young turtles, resulting in disorientation of hatchlings leaving their nests (Salmon et al., 1995). The hatchlings end up desiccated, being easier targets to predators, or crushed under wheels (Salmon, 2006). Regarding the effects of light pollution on animal development, van Geffen et al. (2014) have shown that male caterpillars of *Mamestra*

brassicae reared under white light at night pupate earlier with a lower pupal mass than individuals with a natural light-dark cycle. In a study involving mice, exposure to ALAN affected weight gain and resulted in slower growth during early development (Borniger et al., 2014).

Amphibians, particularly anurans, are exposed to a variety of environmental conditions due to the diverse microhabitats they inhabit during different life stages. As predominantly nocturnal amphibians with complex life cycles and limited mobility, frogs and toads may be particularly vulnerable to light pollution. Light at night can lead to changes in aggregation and antipredator behavior as well as alterations in mating habits and foraging (Buchanan, 2006). Anurans from the Brazilian wetlands which were exposed to ecological light pollution displayed changes in their seasonal and diel calling activity (Dias et al., 2019). Moreover, effects on egg and larvae development as well as tadpoles and their behavior have been detected, caused by the changes in the length of the photophase (light phase – day) and scotophase (dark phase – night) (Buchanan, 2006). Constant light reduced growth in larvae of the American clawed frog (*Xenopus laevis*) and Painted frog (*Discoglossus pictus*) (Delgado et al., 1987; Gutierrez et al., 1984). A recent study on tadpoles of the European tree frog (*Hyla arborea)* showed opposite results, with ALAN promoting growth in larvae (Petrović et al., 2025). Wood frog (*Rana sylvatica*) tadpoles reared under constant light conditions showed contradictory results: Shidemantle et al. (2022) reported smaller individuals, whereas May et al. (2019) observed larger individuals compared to control groups. Regarding metamorphosis, the exposure of tadpoles of the American toad (*Anaxyrus americanus)*, the Northern leopard frog (*Rana pipiens*) and the European tree frog (*Hyla arborea*) to ALAN resulted in stimulation of metamorphosis and shortening of larval duration (Dananay & Benard, 2018; Eichler & Gray, 1976; Petrović et al., 2025), in larvae of *Xenopus laevis* and *Discoglossus pictus* however it led to a delayed metamorphosis (Delgado et al., 1987; Gutierrez et al., 1984). Exposure to artificial light at night can interrupt the production of N-Acetyltransferase (NAT), an enzyme involved in the production of melatonin in young frogs (Lee et al., 1997). Melatonin is a hormone regulated by the light-dark cycle, with peak secretion occurring at night. It plays a crucial role in the regulation of the circadian rhythm, the body's internal clock, which governs a wide range of physiological processes, including the sleep-wake cycle, body temperature, heart rate and hormonal secretion over an about 24-hour cycle (Begemann et al., 2025; Korkmaz et al., 2009).

All the studies mentioned point out that artificial light pollution influences the development of anurans significantly, but the findings are inconclusive, as some studies report that ALAN promotes growth and accelerates development in larvae while others describe reduced growth and slower development. This study aimed to investigate the influence of constant light on the development of European green toad larvae. The European green toad *Bufotes viridis* (Laurenti, 1768) is a predominantly nocturnal species with its primary habitats being open dry steppes and wild river floodplains (Stöck et al., 2008; Landler et al., 2023). Due to its distinct pioneering character, the European green toad can be found in agricultural and ruderal landscapes as well as within villages and big cities (Stöck et al., 2008; Konowalik et al., 2020; Sistani et al., 2021). Understanding the influence of artificial light at night on the development of *Bufotes viridis* tadpoles is crucial, as this synanthropic species frequently inhabits illuminated urban habitats, where light pollution may impact its physiology, behavior, and ultimately population viability.

In order to analyze effects of light pollution, tadpoles of *Bufotes viridis* were raised under constant light, variables such as survival, development and growth were then measured and compared to a control group. By setting landmarks and using geometric morphometrics, alterations in the tadpoles' shapes were investigated as well. According to findings from earlier studies on tadpoles of other anuran species, larvae from the light group were expected to be smaller than larvae from the control group (Delgado et al., 1987; Gutierrez et al., 1984; Shidemantle et al., 2022). We also hypothesized that the duration of the larval stage would be shorter with tadpoles reaching the stage of metamorphosis faster, compared to the control animals (Dananay & Benard, 2018; Eichler & Gray, 1976; Petrović et al., 2025).

Material & Methods

Light chamber and settings

Larvae were raised in plastic containers (measurements: 26 x 17 x 14 cm), filled with aged tap water and covered with perforated lids inside a climate chamber, which was subdivided into eight compartments with each one holding two containers (Figure 1). The temperature was set to a constant 20°C during the whole process (Appendix A). The experimental group was exposed to continuous light, with stronger illumination by day and dimmed light by night (12L strong/12L dimmed), while the control group received identical lighting during the day but no light at night (12L/12D). Across levels, groups were assigned to either the left or the right side in a counterbalanced manner to control for potential side-specific differences. Light intensity was measured with a light meter (LI-250A, LI-COR Environmental GmbH, Bad Homburg, Germany) as photosynthetic photon flux density (PPFD, µmol/m^2/s), which quantifies the number of photons in the photosynthetically active range (PAR, 400-700 nm) reaching a square meter of surface per second (Zhen et al., 2022). The visual sensitivity of the Common toad (*Bufo bufo*), representative of Bufonidae, has been reported to cover a wavelength range of approximately 360-650 nm (Yovanovich et al., 2019). The light conditions during the day varied between 7.22 µmol/m^2/s and 19.7 µmol/m^2/s, which equals about 275-749 lux (unit of illuminance), in the chambers (Table 1). The light spectrum of the fluorescent tube used during the day covered the visible range. However, the spectral composition was uneven with pronounced peaks in the blue (420-490 nm), green (490-575 nm) and red (650-700 nm) regions (Figure 2). The dimmed light at night measured 0.01-0.03 µmol/m^2/s, which is equivalent to approximately 1 lux, in the compartments containing the experimental groups and 0 µmol/m^2/s in the control group chambers (Table 2). The LED light spots used in the experimental groups at night reflected the full spectrum of visible light with a flat, uniform distribution (Figure 3).

Spawn collection and storage

On 11 April 2024, during the breeding season of *Bufotes viridis*, sections from five different strands of spawn were collected from ponds at Rudolf Bednar Park in the second district in Vienna. The eggs were transferred to BOKU University, where the experiment was performed. The eggs were divided according to their origin strand and stored in plastic containers filled with water from the spawning site. The containers were kept in a climate chamber at BOKU University with a 12L/12D photoperiod until the larvae hatched.

Figure 1: Experimental setup. *The modified fridge contained 16 containers (A) in eight chambers. The temperature was set to 20°C, oxygen supply was ensured using air pumps (B) and associated air stones. During the day the light tubes attached to the door were on. At night small LED plates (C) were activated in the chambers of the experimental groups, the present LED plates in the chambers of the control groups were covered. When closed, the fridge was additionally secured with lashing straps.*

Table 1: Light conditions inside the compartments during the day. *Measured light conditions (in µmol/m2/s) in the compartments inside the climate chamber during the day. The measurements were carried out on 12 January 2024. Colors indicate the treatment group (D = 12h light/12h dark = white; L = 12h strong light/12h dimmed light = orange).*

First row	Back	9.52 µmol/m²/s	7.22 µmol/m²/s	Back
	Front	9.52 µmol/m²/s	7.22 µmol/m²/s	Front
Second row	Back	18.62 µmol/m²/s	17.13 µmol/m²/s	Back
	Front	18.62 µmol/m²/s	17.13 µmol/m²/s	Front
Third row	Back	19.1 µmol/m²/s	19.7 µmol/m²/s	Back
	Front	19.1 µmol/m²/s	19.7 µmol/m²/s	Front
Fourth row	Back	17.26 µmol/m²/s	15.22 µmol/m²/s	Back
	Front	17.26 µmol/m²/s	15.22 µmol/m²/s	Front

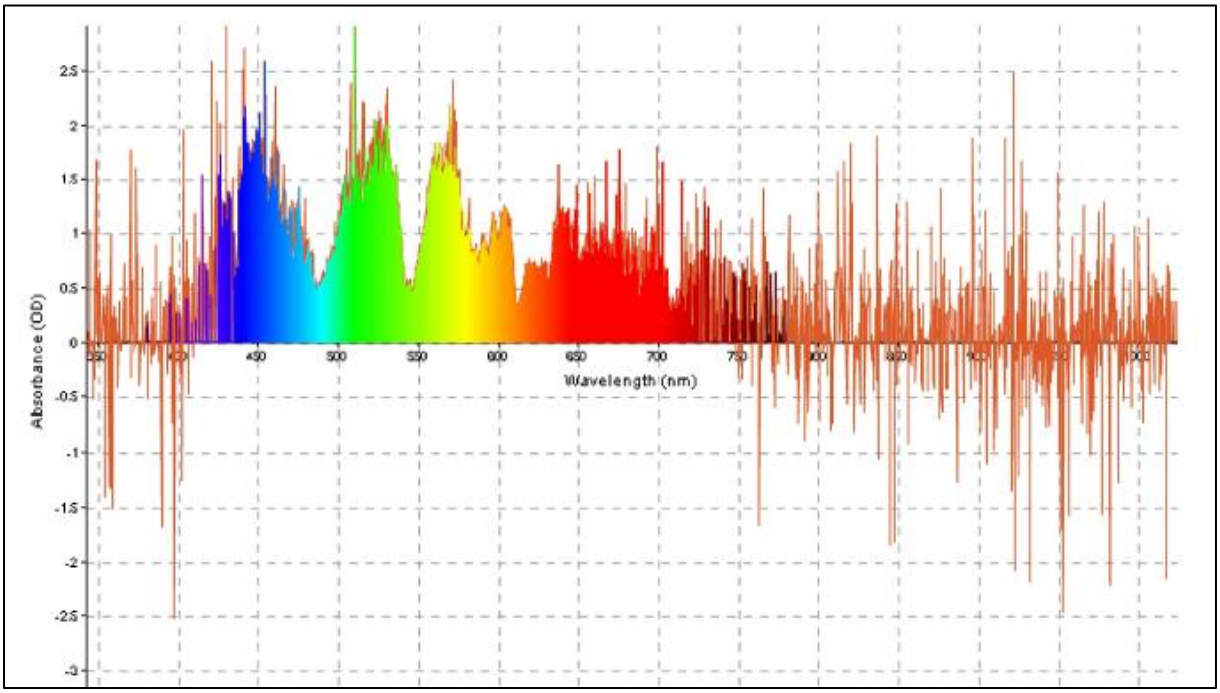

Figure 2: Light spectrum of a fluorescent tube used during the day. *The x-axis indicates wavelength (nm) in the range of 350-1000 nm and the y-axis shows absorbance (OD). The background is color-coded according to the visible spectrum from violet to red. The spectral measurement of the fluorescent tube used during daytime covered the range of visible light, but not evenly. Distinct emission lines were visible in the blue (420-470 nm), green (490-575 nm) and red (650-780 nm) regions. Increased fluctuations in the short-wavelength region below 420 nm and in the long-wavelength region above 730 nm can be attributed to the low sensitivity of the measuring device outside the PAR.*

Table 2: Light conditions inside the compartments during the night. *Measured light conditions (in µmol/m2/s) in the compartments inside the climate chamber at night. The measurements were carried out on 12 January 2024. Colors indicate the treatment group (D = 12h light/12h dark = white; L = 12h strong light/12h dimmed light = orange).*

First row	Back	0 µmol/m²/s	0.01 µmol/m²/s	Back
	Front	0 µmol/m²/s	0.01 µmol/m²/s	Front
Second row	Back	0.03 µmol/m²/s	0 µmol/m²/s	Back
	Front	0.03 µmol/m²/s	0 µmol/m²/s	Front
Third row	Back	0 µmol/m²/s	0.03 µmol/m²/s	Back
	Front	0 µmol/m²/s	0.03 µmol/m²/s	Front
Fourth row	Back	0.03 µmol/m²/s	0 µmol/m²/s	Back
	Front	0.03 µmol/m²/s	0 µmol/m²/s	Front

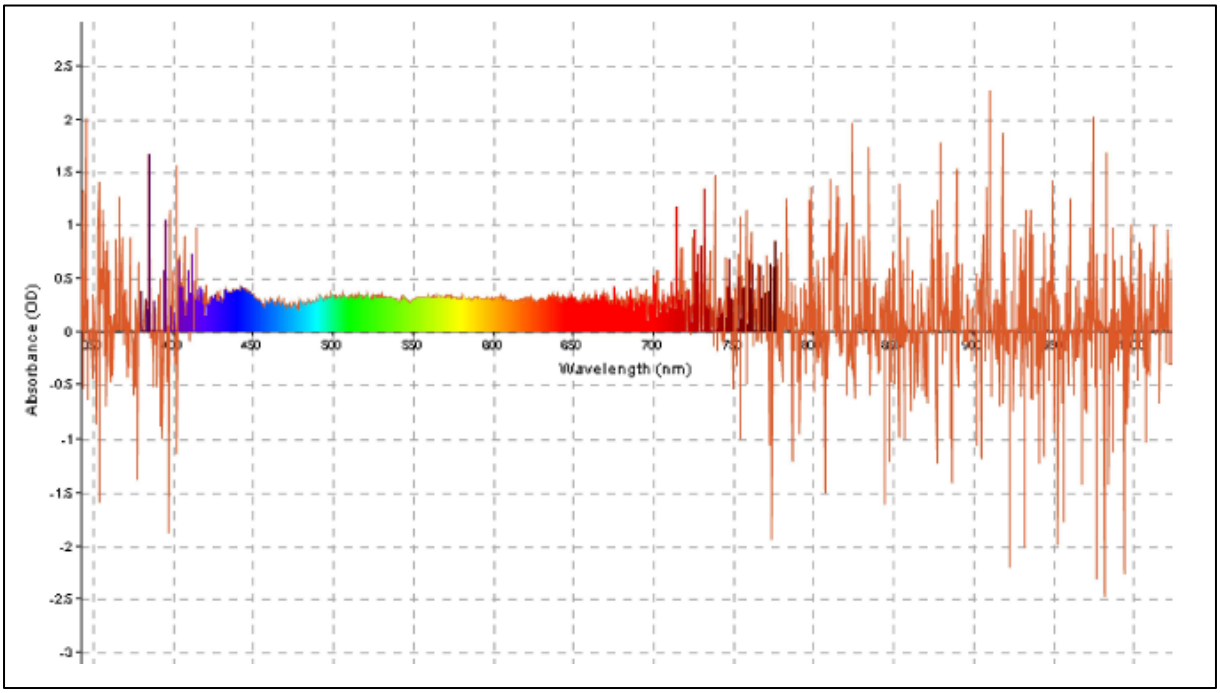

Figure 3: Light spectrum of a light spot used at night. *The x-axis indicates wavelength (nm) in the range of 350-1000 nm and the y-axis shows absorbance (OD). The background is color-coded according to the visible spectrum from violet to red. The spectral measurement of the white LED spots used during nighttime in the experimental groups reveals a predominantly continuous distribution without any peaks Increased fluctuations in the short-wavelength region below 420 nm and in the long-wavelength region above 730 nm can be attributed to the low sensitivity of the measuring device outside the PAR.*

Water change and oxygenation

Water changes were performed every one to two weeks, depending on the accumulation of body waste from the tadpoles and the water turbidity. Towards the end of the experiment, the water in containers holding single individuals was changed less frequently. To help stabilize the aquatic environment in the containers, approximately 0.5 ml of aquarium bacterial starter (JBL Denitrol, JBL GmbH & Co. KG, Neuhofen, Germany) was added per liter of water. The solution contains bacteria strains which support the degradation of ammonium and nitrate, toxic chemical compounds of larval feces. To ensure a steady oxygen supply, air pumps

connected to air stones were used, with each container having the same ventilation intensity set.

Larvae allocation

The experiment started on 16 April 2024 with the allocation of the free-swimming larvae into their containers. Five tadpoles, each one from another spawn string, were put into each container. Overall, we had 16 containers with a total of 80 individuals which were then placed randomly into the modified fridge. In each chamber there were two containers, one in the front and one in the back (Table 3). Within the first ten days of the experiment a total of nine tadpoles died, three in the experimental and six in the control group. To keep the sample size high, the nine dead individuals were replaced with randomly selected larvae from the remaining spawn, as we assumed that the fatalities were not induced by the light conditions but were rather caused by the acclimatization process.

Table 3: Distribution of test containers within the light chamber. *Every row has two chambers, each containing two containers (back and front). Numbers indicate the placement in the light chamber; letters and colors indicate the treatment group (D = 12h light/12h dark = white; L = 12h strong light/12h dimmed light = orange).*

First row	Back	61 D	63 L	Back
	Front	62 D	64 L	Front
Second row	Back	65 L	67 D	Back
	Front	66 L	68 D	Front
Third row	Back	69 D	71 L	Back
	Front	70 D	72 L	Front
Fourth row	Back	73 L	75 D	Back
	Front	74 L	76 D	Front

Feeding

The tadpoles were fed three times a week with fish food (JBL NovoBel, JBL GmbH & Co. KG, Neuhofen, Germany). For the first three weeks 0.01 g of fish flakes were added per individual. After that, the amount of food was increased to 0.05 g per individual due to the elevated demand during growth. In addition to the fish flakes, one catfish pellet (0.2 g; JBL PRONOVO PLECO WAFER M, JBL GmbH & Co. KG, Neuhofen, Germany) per container per week was added.

Growth, metamorphosis and survival: data collection and analysis

To assess the total body lengths of the tadpoles, photographs from above the containers, with a ruler placed underneath, were taken at four times during the experiment (day 0 – day 20 – day 30 – day 52). The measurements were performed on the photos using the image processing software ImageJ (Schneider et al., 2012). The larvae's rumps and tails were measured separately in order to calculate the body-to-tail ratio as well as the total body length (Figure 4). In addition, the developmental stages according to the Gosner table were determined by using dorsal and lateral photographs from 16 May (day 30), with the breakthrough of forelimbs at stage 42 indicating the beginning of metamorphosis (Gosner, 1960).

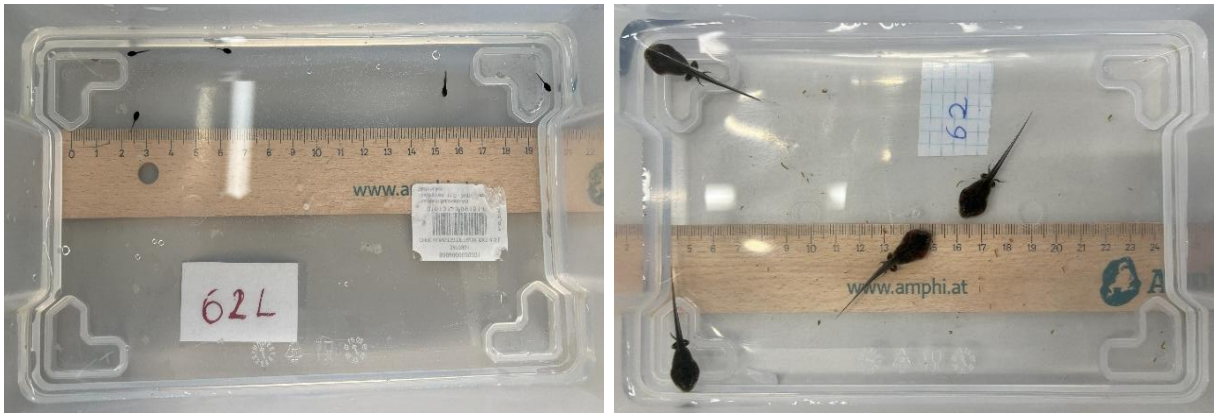

Figure 4: Examples of pictures used for measurements of total body length and body-to-tail ratio. *Photographs were taken from above the containers, a ruler placed underneath served as a scale to measure the tadpoles' rumps and tails separately using the software ImageJ. Total body length and body-to-tail ratio were calculated based on the measurements. Picture on the left was taken on 16 April (day 0), picture on the right was taken on 16 May (day 30).*

All statistical analyses were performed in R version 4.5.1 (R Core Team, 2025). Total length, body-to-tail ratio, metamorphosis timepoints and Gosner stages were analyzed using separate Linear Mixed-Effects Models fitted with the glmmTMB function of the glmmTMB package (Brooks et al., 2017). The models included the respective morphological or developmental parameter as the response variable, treatment as a fixed effect and containers as a random effect. Survival rates were assessed using a binomial Logistic Mixed-Effects Model fitted with the glmmTMB function from the glmmTMB package, with treatment as the predictor and containers as a random factor. Model predictions were calculated for total length, body-to-tail ratio and survival rates using the function ggpredict from the ggeffects package (Lüdecke, 2018). Data visualization was performed using the ggplot function of the ggplot2 package (Wickham, 2016) and the ggline function from the ggpubr package (Kassambara, 2025), with additional plots generated using base R.

Shape: data collection and analysis

For the geometric morphometric analyses, photographs of the tadpoles were taken on day 30 of the experiment. For this purpose, the larvae were put individually into small glass cuvettes filled with aged tap water and positioned inside a photo light box. Pictures were taken with a smartphone camera (iPhone 13 Pro) from a dorsal and lateral point of view (Figure 5). One individual was excluded from both dorsal and lateral analysis because it was already undergoing metamorphosis (Gosner stage 44) and its markedly reduced tail would have skewed the results.

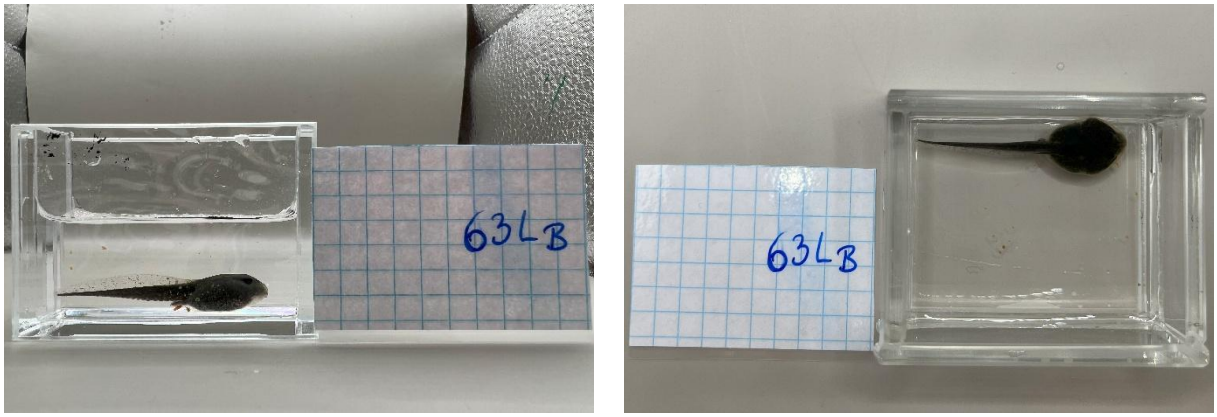

Figure 5: Examples of pictures used for geometric morphometric analysis. *The glass cuvettes were placed inside the photo light box. Photographs were taken on day 30 from a lateral (left) and dorsal (right) point of view.*

In order to set the landmarks, the images were first loaded into the program TpsUtil (Rohlf, 2023), where they were flipped, so the tadpoles would face in the same direction, and then transformed into a suitable format for further use. The landmarking was performed using the software TpsDig (Rohlf, 2021) with the lines on the graph paper serving as a scale. On the lateral images 11 anatomic landmarks and 49 semilandmarks were placed (Figure 6). On the dorsal images 6 anatomic landmarks and 16 semilandmarks were placed (Figure 7). The 2D landmark data files were subsequently imported into the statistical software R. Before further processing, the dorsal coordinates were mirrored across the x-axis and then averaged to remove any tail curvatures of the tadpoles which may have influenced the shape of the individuals. By using a Generalized Procrustes Analysis (GPA) all information about size, position and orientation from the landmark configurations was eliminated, creating Procrustes shape coordinates which only contain information about the shape (Bookstein, 1996; Mitteroecker & Gunz, 2009). A Principal Components Analysis (PCA) was applied to the Procrustes shape coordinates of both perspectives to summarize major patterns of shape variations, with PC1 representing the axis which explains the most variance. To identify effects of the treatments on the tadpoles' shapes, linear models were fitted for the first three principal components indi-

vidually. The Generalized Procrustes Analysis and the Principal Component Analysis, including the plots, were performed using the geomorph package (Baken et al., 2021).

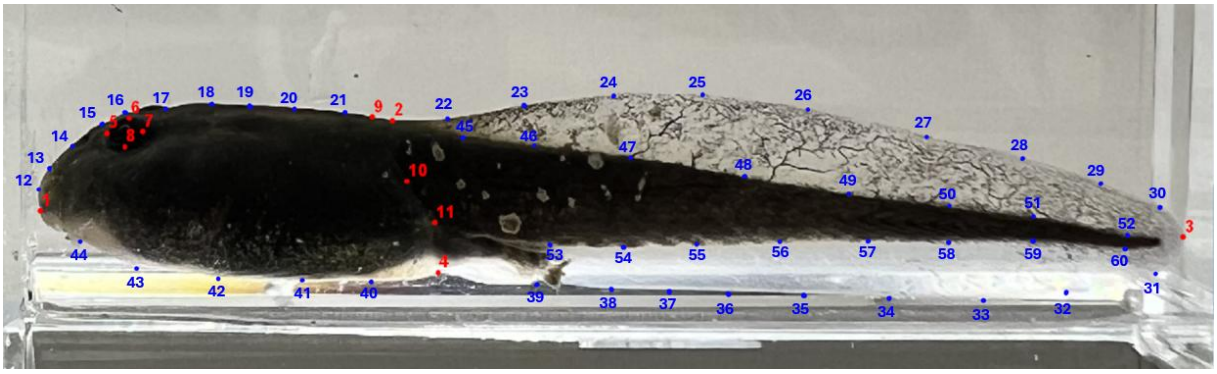

Figure 6: Anatomic landmarks (1 -11, red) and semilandmarks (12–60, blue) for the lateral view. *1: most rostral point of upper lip; 2: juxtaposition of dorsal tail fin and body; 3: tail tip; 4: juxtaposition of ventral tail fin and body; 5: most rostral point of the eye; 6: most dorsal point of the eye; 7: most caudal point of the eye; 8: most ventral point of the eye; 9: most dorsal point of juxtaposition of body and tail musculature; 10: most cranial point of border between dorsal and ventral myomeres of tail musculature; 11: most ventral point of juxtaposition of body and tail musculature. Arrows indicate the direction of numbering for the semi-landmarks. 12-21: between 1 and 2 (dorsal side of head and body); 22-30: between 2 and 3 (dorsal margin of tail fin); 31-39: between 3 and 4 (ventral margin of tail fin); 40-44: between 4 and 1 (ventral side of head and body); 45-52: between 9 and 3 (dorsal margin of tail musculature); 53-60: between 11 and 3 (ventral margin of tail musculature) (Kofler, 2014).*

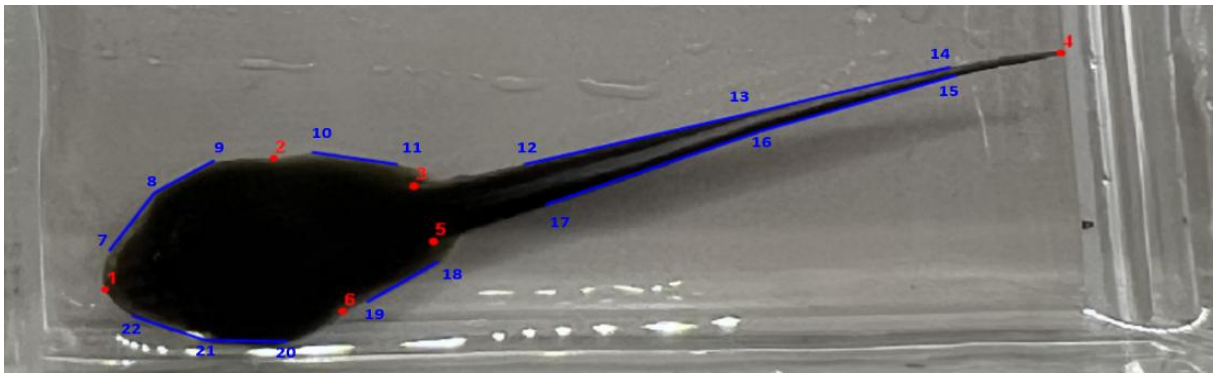

Figure 7: Anatomic landmarks (1-6, red) and semilandmarks (7-22, blue) for the dorsal view. *1: most rostral point of the head; 2: on body outline opposite of 6; 3: intersection of torso and tail on the right side of the body; 4: tail tip; 5: intersection of torso and tail on the left side of the body; 6: juxtaposition of body and spiracle (Theil, 2024).*

Relocation of larvae and end of experiment

Toads undergoing metamorphosis, starting at Gosner stage 42, were relocated to transition containers outside of the light chambers. To ensure the needs of the animals during metamorphosis, both aquatic and terrestrial habitat components were provided. The experiment lasted 157 days and ended on 20 September 2024.

Ethical statement

The collection of specimens was conducted under permit number MA22 – 230917-2020 issued by the Municipal Department 22 (Environmental Protection), City of Vienna, Austria. The experiment was approved by the Institutional Review Board of the Federal Ministry of Women, Science and Research (protocol code: 2022-0.343.864).

Results

Survival Rate

Out of 40 individuals in each treatment group, 27 tadpoles (67.5 %) survived in the control group and 18 tadpoles (45 %) survived in the experimental group. The mean tadpole survival per container in the control group was 3.375 whereas in the experimental group the mean survival was 2.25 (Figure 8). Tadpoles reared under continuous light exhibited a higher mortality rate over time than the tadpoles raised under a 12L/12D cycle.

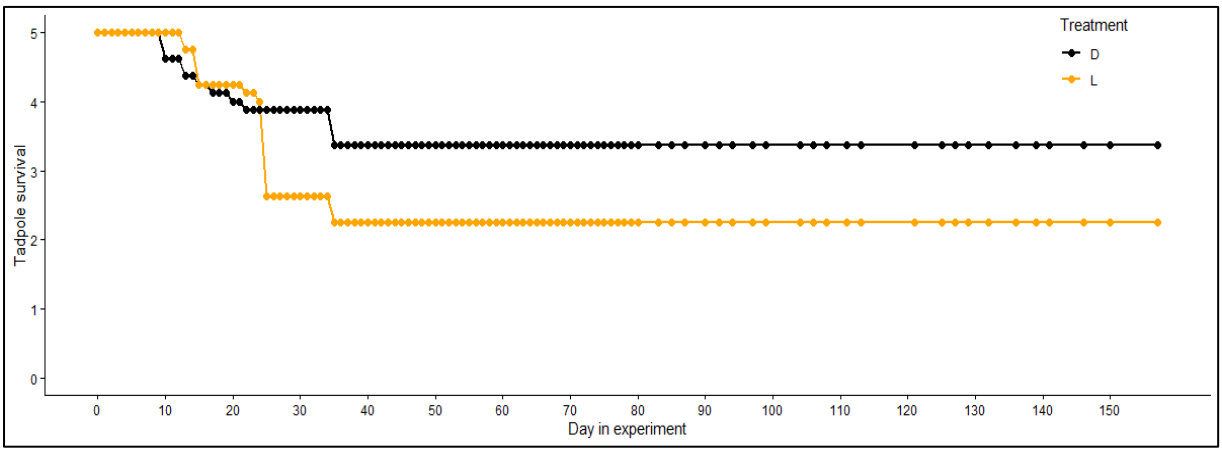

Figure 8: Mean tadpole survival per container. *Mean survival of tadpoles per container over the duration of the experiment for the treatments (D = 12h light/12h dark; L = 12h strong light/12h dimmed light).*

Model-based predictions showed a gradual decrease in survival probability over the course of the experiment for both treatments, but the decline differed considerably between the groups (Figure 9). Individuals in the control group had a survival probability of 89% on day 52 of the experiment, whereas the predicted survival rate in the experimental group was 61%.

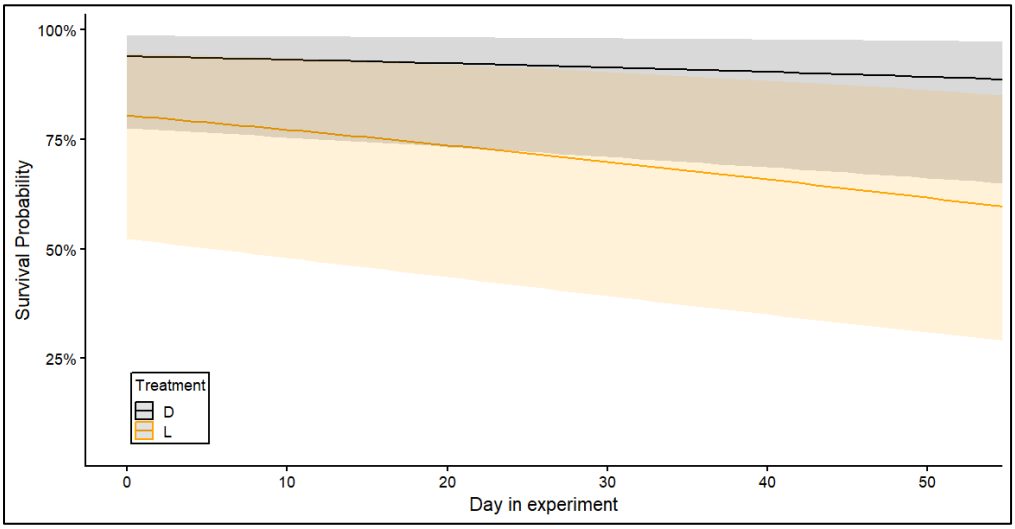

Figure 9: Survival probability. *Predicted survival rate for the tadpoles of both treatment groups (D = 12h light/12h dark; L = 12h strong light/12h dimmed light) till day 52.*

The analysis showed that the day of the experiment and the interaction between the treatment and the day of the experiment had a highly significant influence on the survival rate of the tadpoles ($p < 0.001$). Treatment as a main prediction factor alone had no significant effect on the survival of the tadpoles ($p = 0.214$; Table 4).

Table 4: Effects of the treatment (L = 12h strong light/12h dimmed light) and the day of the experiment on the survival rate of the tadpoles.

Parameter	Coefficient	95% CI	z	P	Std. Coef.	Std. Coef. 95% CI	Fit
Intercept	3.010484	[1.43, 4.59]	3.734	< 0.001***	2.27	[0.70, 3.85]	
Treatment [L]	-1.387274	[-3.58, 0.80]	-1.243	0.213946	-1.86	[-4.04, 0.32]	
Day of experiment	-0.012864	[0.01, -0.01]	-12.138	< 0.001***	-0.50	[-0.59, -0.42]	
Treatment [L] x Day of experiment	-0.008307	[0.01, -0.01]	-5.537	< 0.001***	-0.33	[-0.44, -0.21]	
R2 (conditional)							0.90
R1 (marginal)							0.20

Total body length and body-to-tail ratio

As expected, individuals of both treatment groups showed an increase in total length over the course of the experiment. On day 52 of the experiment the average body length of the tadpoles from the control group was 4.69 cm (SD ± 0.88), the individuals from the light group were significantly longer with a mean body length of 5.11 cm (SD ± 0.46; Figure 10A). Model predictions were consistent with the results, indicating a greater increase in total body length of larvae from the experimental group compared to larvae from the control group (Figure 10B). The average body-to-tail ratio decreased over time in both treatment groups, however, while the mean ratio in the light group remained unchanged towards the end of the experiment, there was a slight increase in the mean ratio of the control group between day 30 and 52. At the last measurement, the mean body-to-tail ratio was lower in the light group than in the control group (Figure 10C,D).

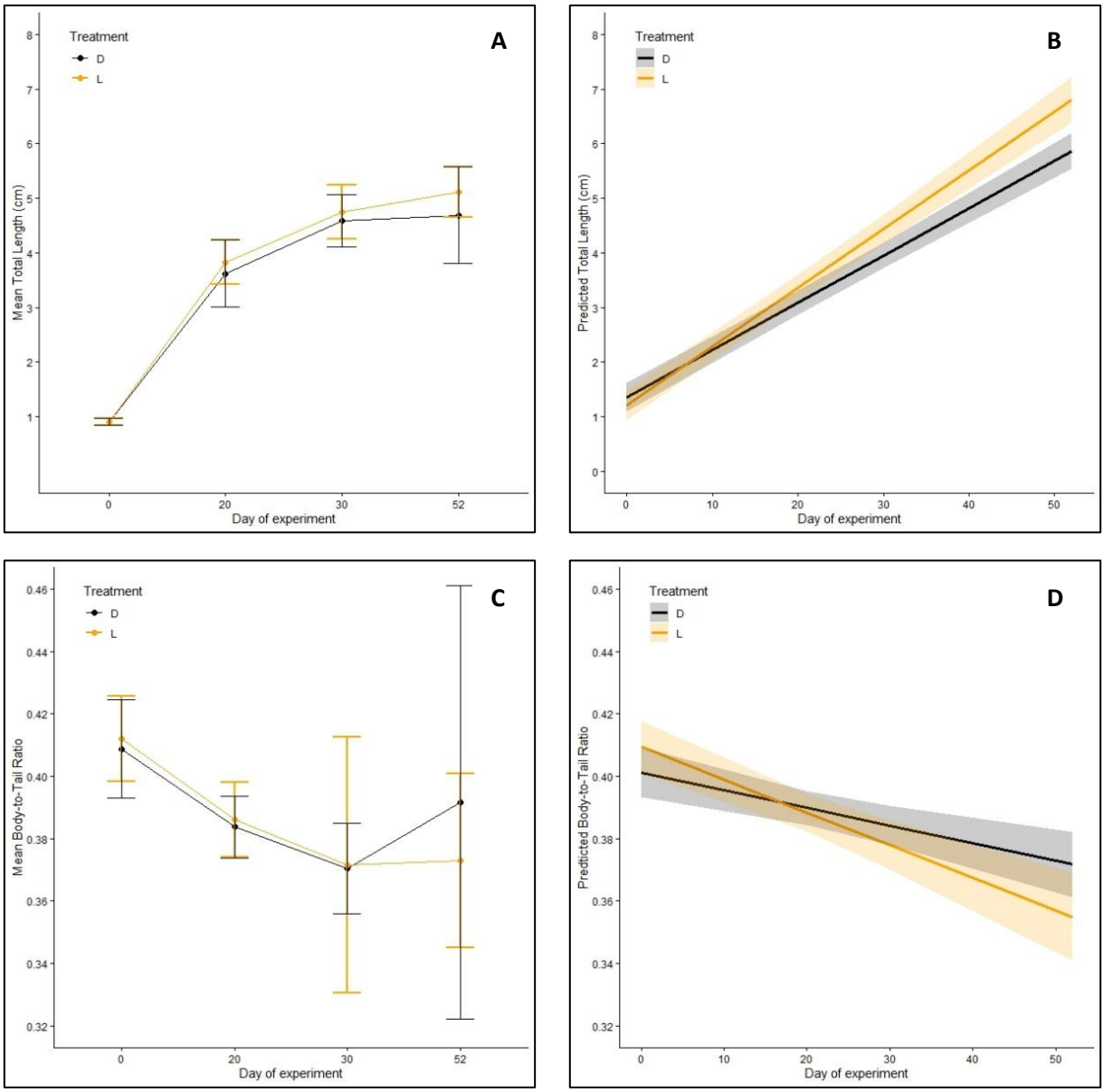

Figure 10: Total length and body-to-tail ratio during the experiment for both treatment groups. (A) *Mean total body lengths (in cm).* ***(B)*** *Predicted mean total body lengths (in cm).* ***(C)*** *Mean body-to-tail ratios.* ***(D)*** *Predicted mean body-to-tail-ratios. Letters and colors indicate the treatment group (D = 12h light/12h dark; L = 12h strong light/12h dimmed light).*

The statistical analysis revealed a highly significant effect of the day of the experiment on the total body lengths of the larvae ($p < 0.001$), but no significant effect of the treatment alone on the lengths of the tadpoles was found ($p = 0.465$). Nevertheless, a highly significant interaction between the day of the experiment and the treatment on the length of the larvae was identified ($p < 0.001$; Table 5).

Table 5: Effects of the treatment (L = 12h strong light/12h dimmed light) and the day of the experiment on the total length of the tadpoles.

Parameter	Coefficient	95% CI	z	P	Std. Coef.	Std. Coef. 95% CI	Fit
Intercept	1.356362	[1.09, 1.62]	9.982	< 0.001***	-0.05	[-0.18, 0.08]	
Treatment [L]	-0.141639	[-0.52, 0.24]	-0.731	0.464857	0.14	[-0.05, 0.33]	
Day of experiment	0.086708	[0.08, 0.09]	22.758	< 0.001***	0.84	[0.76, 0.91]	
Treatment [L] x Day of experiment	0.020768	[0.01, 0.03]	3.401	< 0.001***	0.20	[0.08, 0.32]	
R2 (conditional)							0.83
R1 (marginal)							0.81

There was a highly significant effect from the day of the experiment ($p < 0.001$) as well as a significant interaction between the day of the experiment and the treatment ($p < 0.05$) as to the body-to-tail ratio of the tadpoles. When considered as the sole main predictor, treatment had no effect on the body-to-tail ratio ($p = 0.159$; Table 6).

Table 6: Effects of the treatment (L = 12h strong light/12h dimmed light) and the day of the experiment on the body-to-tail ratio of the tadpoles.

Parameter	Coefficient	95% CI	z	P	Std. Coef.	Std. Coef. 95% CI	Fit
Intercept	0.4012116	[0.39, 0.41]	99.50	< 0.001***	-0.00935	[-0.19, 0.17]	
Treatment [L]	0.0081521	[0.00, 0.00]	1.41	0.1593	-0.02	[-0.29, 0.25]	
Day of experiment	-0.0005667	[0.00, 0.00]	-3.95	< 0.001***	-0.31	[-0.46, -0.16]	
Treatment [L] x Day of experiment	-0.0004800	[0.00, 0.00]	-2.08	<0.05*	-0.26	[-0.51, -0.02]	
R2 (conditional)							0.20
R1 (marginal)							0.18

Gosner stages

Tadpole development did not differ between the treatments. On day 30 of the experiment the mean Gosner stage of the tadpoles in the control group was 36 (SD ± 3.06) while in the light group the average Gosner stage was 37 (SD ± 3.66; Figure 11).

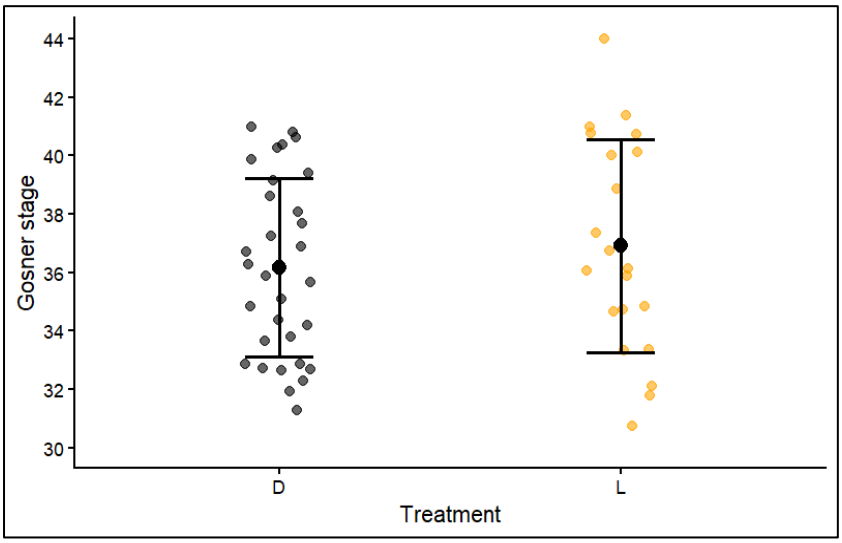

Figure 11: Gosner stages of tadpoles by treatment on day 30 of the experiment. *Individuals are represented by small dots (D = 12h light/12h dark = black; L = 12h strong light/12h dimmed light = orange). Large points and error bars indicate treatment means ± standard deviation.*

The day of the experiment had a highly significant influence on the developmental stages of the tadpoles ($p < 0.001$), whereas no effect of the treatment was identified (Table 7).

Table 7: Effect of the light treatment (L = 12h strong light/12h dimmed light) on the developmental stages of the tadpoles on day 30.

Parameter	Coefficient	95% CI	z	P	Std. Coef.	Std. Coef. 95% CI	Fit
Intercept	35.8924	[33.78, 38.00]	33.34	< 0.001***	-0.17	[-0.81, 0.47]	
Treatment [L]	0.6844	[-2.42, 3.79]	0.43	0.665	0.21	[-0.73, 1.15]	
R2 (conditional)							0.83
R1 (marginal)							0.01

Although no differences were found between the experimental and control group, there was a great variation across all containers regarding the developmental stages of the larvae. In addition, differences in Gosner stages were observed within the individual treatment groups (Figure 12).

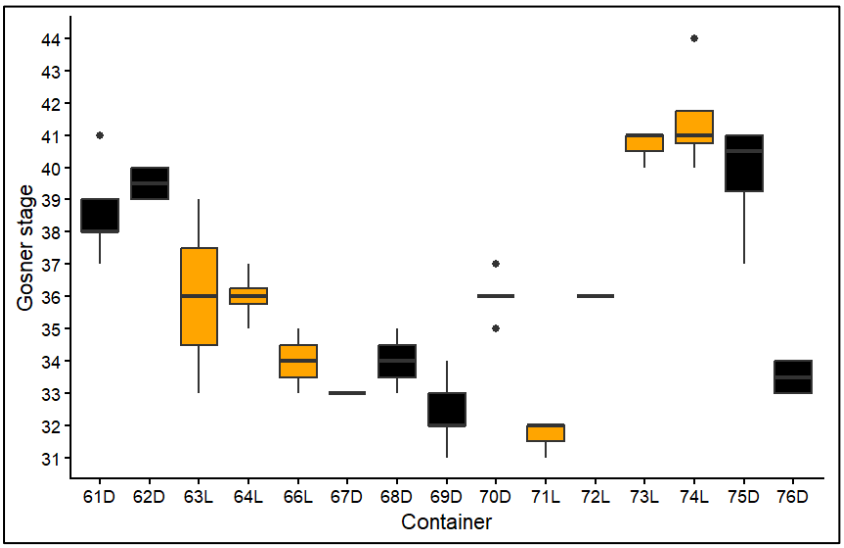

Figure 12: Gosner stages of tadpoles for each container. *Boxplots show the distribution of developmental stages on day 30 of the experiment per container. Colors and container names indicate the treatment (D = 12h light/12h dark = black; L = 12h strong light/12h dimmed light = orange).*

Metamorphosis

Among the tadpoles that survived until the end of the experiment, 17 out of 18 in the experimental group (94.4%) and 25 out of 27 in the control group (92.6%) reached metamorphosis (Gosner stage 42). The larvae from the experimental group had a mean time to metamorphosis of 51.8 days (SD ± 28.6) while the individuals from control group reached metamorphosis in 65.5 days (SD ± 32.8) on average (Figure 13).

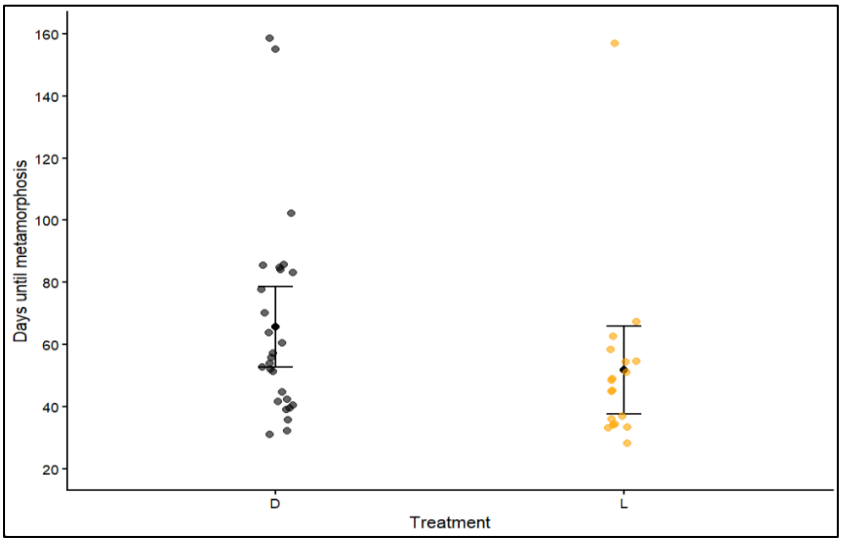

Figure 13: Days until metamorphosis for tadpoles of both treatments. *The points represent individual metamorphosis times of the tadpoles from the two treatments (D = 12h light/12h dark = black; L = 12h strong light/12h dimmed light = orange). Large points and error bars indicate treatment means ± standard deviation.*

A highly significant effect of the day of the experiment on the timing of metamorphosis ($p < 0.001$) was identified, whereas no significant effect of the treatment on metamorphosis time was observed ($p = 0.928$; Table 8).

Table 8: Effect of the light treatment (L = 12h strong light/12h dimmed light) on the tadpoles' metamorphosis timing.

Parameter	Coefficient	95% CI	z	P	Std. Coef.	Std. Coef. 95% CI	Fit
Intercept	64.159	[41.69, 86.63]	5.596	< 0.001***	0.13	[-0.58, 0.84]	
Treatment [L]	-1.584	[-36.04, 32.87]	-0.090	0.928	-0.05	[-1.14, 1.04]	
R2 (conditional)							0.82
R1 (marginal)							0.00052

Shape

The first three principal components for the dorsal view accounted for 76.34% of the observed shape variation, however, the second principal component (PC2) was discarded, as it was most likely based on an artefact (i.e. shortening of bent tails by mirroring). For the lateral view the first three principal components explained 73.78% of the registered shape variation (Table 9). Treatment groups overlapped in both dorsal and lateral PCA spaces, showing no significant clusters in either PCA plot (Figure 14).

Table 9: Summary of variance explained by the first three principal components (PC) following PCA.

PCA – Dorsal	PCA – Lateral
PC 1: 36.74%	PC 1: 37.68%
PC 2: 25.34% (discarded)	PC 2: 21.45%
PC 3: 14.26%	PC 3: 14.65%

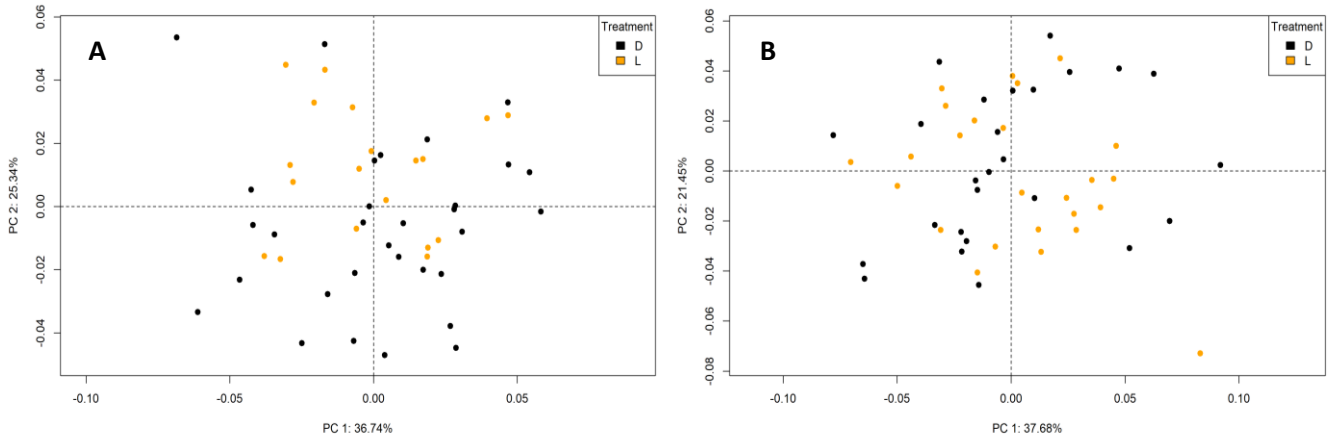

Figure 14: Principal Component Analysis of the Procrustes shape coordinates. (A) *PC1 and PC2 (discarded) from the dorsal view.* ***(B)*** *PC1 and PC2 from the lateral view. Colors and letters indicate the treatment (D = 12h light/12h dark = black; L = 12h strong light/12h dimmed light = orange).*

For the dorsal view, the first principal component (PC1) described the variation in the body-tail junction, reflecting differences in the relative width of the tadpoles' tail bases. PC3 depicted the variation in the relative width of the trunk along the anterior-posterior axis (Figure 15). The statistical analyses revealed no significant effect of the treatment on neither PC1 ($p = 0.55$) nor PC3 ($p = 0.74$) of the dorsal data (Table 10). In the lateral view, PC1 represented the variety in head position, PC2 reflected the different heights of the trunk compared to the tail and PC3 described variation in the body-to-tail transition (Figure 16). The separate linear models performed on the first three lateral principal components showed that treatment had no significant effect on any of the PCs ($p > 0.2$; Table 10).

Table 10: Effect of the light treatment (L = 12h strong light/12h dimmed light) on the principal components (PC) for the dorsal and lateral view.

View	PC	Coefficient	SE	t-value	P	Multiple R^2	Adjusted R^2
Dorsal	PC1	-0.005186	0.00866	-5.99	0.55	0.00726	-0.013
Dorsal	PC3	-0.001817	0.00551	-0.33	0.74	0.00221	-0.018
Lateral	PC1	-0.005059	0.01110	-0.456	0.65	0.00422	-0.016
Lateral	PC2	0.000707	0.00839	0.084	0.93	0.00014	-0.020
Lateral	PC3	0.007785	0.00684	1.137	0.26	0.02572	0.006

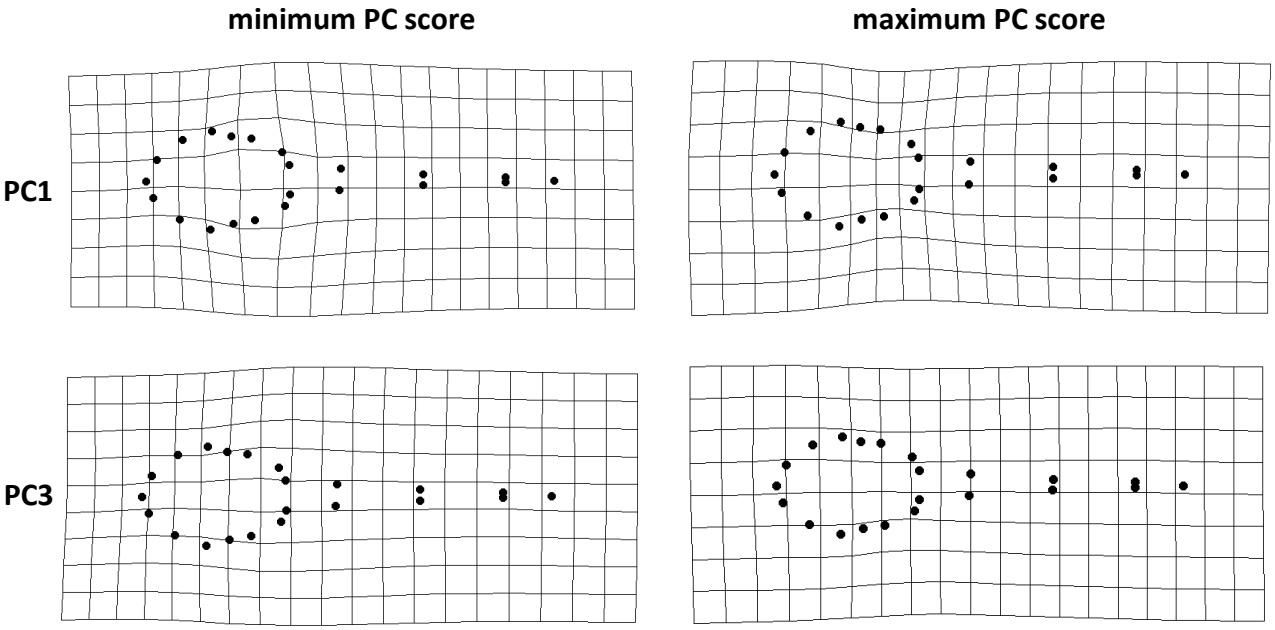

Figure 15: Dorsal deformation grids. *Deformation grids illustrate shape variation along the first (PC1) and third (PC3) principal components of the dorsal dataset. For each component, the grids represent the minimum and maximum shape configurations relative to the mean shape.*

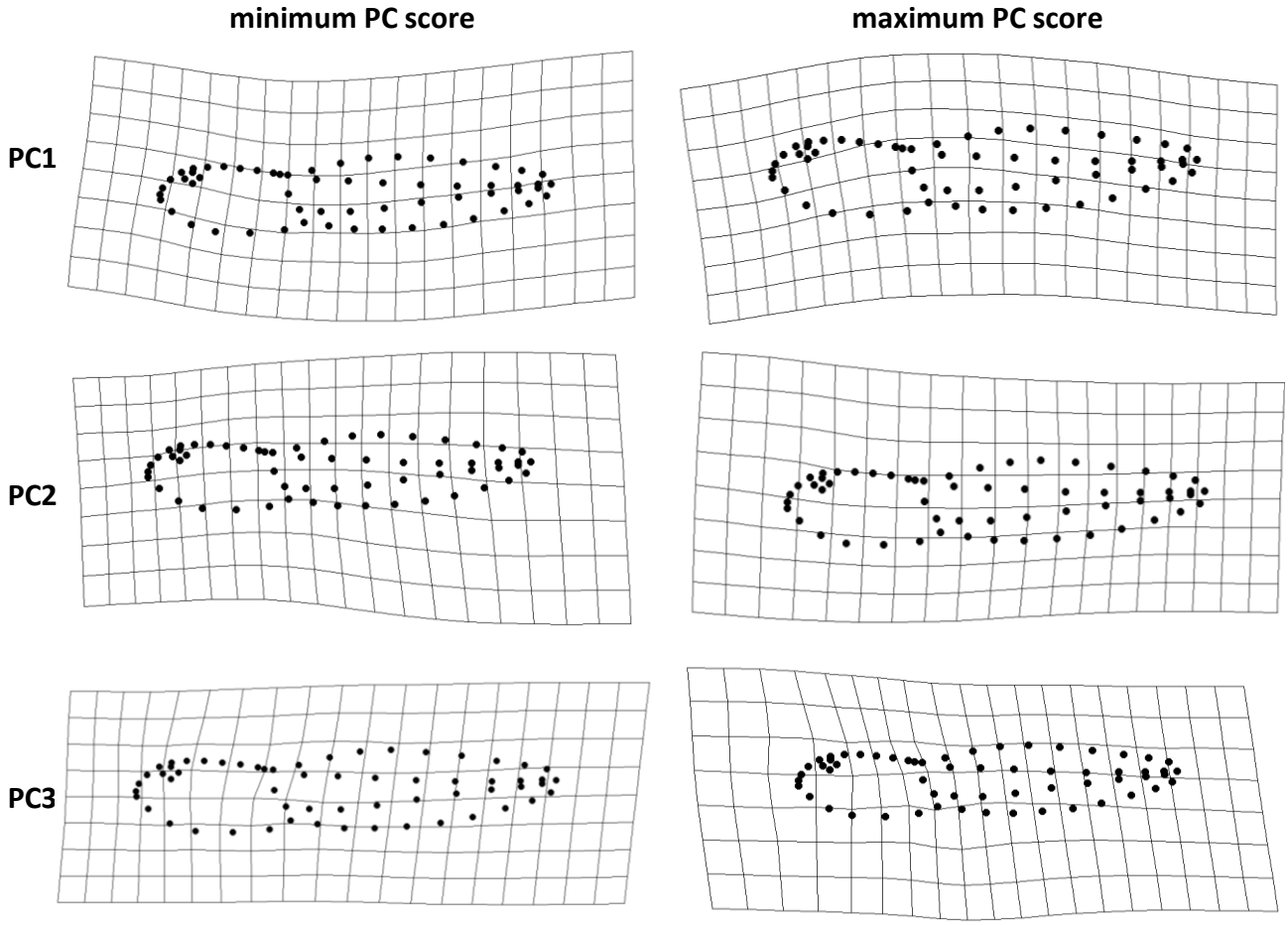

Figure 16: Lateral deformation grids. *Deformation grids illustrate shape variation along the first three principal components (PC1-PC3) of the lateral dataset. For each component, the grids represent the minimum and maximum shape configurations relative to the mean shape.*

Discussion

Our study showed that ALAN had a significant impact on tadpoles of the European green toad undergoing metamorphosis. The results demonstrated a negative influence of ALAN on the survival probability of tadpoles. As the experiment progressed, the survival rate in the experimental group declined significantly, resulting in a higher mortality rate in tadpoles raised under constant illumination compared to tadpoles that were kept in darkness at night. Overall, the survival probability in the light group was 50% lower than in the control group. Previous studies on ALAN and its impact on amphibian larvae did not indicate reduced survival rates (Petrović et al., 2025; May et al., 2019; Dananay & Benard, 2018). In contrast, increases in mortality rates caused by ALAN have been described in fish larvae as well as free-swimming larvae of mussels and barnacles (Blanco-Vives et al., 2010; Tidau et al., 2023).

Against our hypothesis, tadpoles reared under constant light conditions had longer bodies than tadpoles raised under a 12L/12D cycle. The animals in the experimental group were significantly larger on day 52 of the experiment than the ones in the control group, but differences in size were already detectible on day 20. Similar outcomes regarding the total body length of tadpoles were found in the European tree frog and Wood frog (Petrović et al., 2025; May et al., 2019). In addition to body measurements, May et al. (2019) performed behavioral assays, in which tadpoles reared under ALAN exhibited a reduced swimming activity by more than 50 % compared to a control group. A decrease in locomotor activity would lead to lower energy expenditure, which could result in more energy being available for growth. Moreover, research indicates that exposure to ALAN can impair the synthesis of melatonin, a hormone critically involved in the regulation of circadian rhythms. The body's internal clock regulates the timing of insulin secretion and glucocorticoid production, both of which play central roles in metabolic regulation (Begemann et al., 2025; Korkmaz et al., 2009). Consequently, the disruption of the circadian rhythm by ALAN can indirectly alter metabolic processes. Furthermore, melatonin is recognized as an antagonist of thyroid hormone secretion. Thyroid hormones, in turn, regulate a wide range of physiological and morphological processes across nearly all tissues of premetamorphic tadpoles and are essential for the initiation of metamorphosis (Tata, 1998; Brown & Cai, 2007). Thus, the elongated bodies of the tadpoles from the light group may result from interrupted circadian signaling and enhanced thyroid hormone activity due to suppressed melatonin production, promoting growth during the larval stage.

By analyzing the body-to-tail ratio we confirmed that larvae from the ALAN group had significantly longer tails which resulted in smaller body-to-tail ratios compared to the larvae from the control group. Eichler & Gray (1976) described a similar outcome in their experiment, although no statistical analyses were conducted. Proportionally longer tails have been brought into context with poor growth performance in tadpoles of the Common frog (*Rana temporaria)*, as longer tails indicate a reduced growth of the body (Merilä et al., 2004). This assumption does not align with our results since there was a positive correlation between the length of the head-trunk region and the interaction of the day of the experiment and the treatment as well, it just was not as clear as the tail length (Appendix B). Since this trade-off can be ruled out, an elongated tail may appear as an advantageous shift in the tadpoles' morphology. However, Arendt (2010), who performed his own experiments on five species of Spadefoot toad tadpoles (Scaphiopodidae) and reviewed multiple studies on tadpoles of other anuran species, including the genera *Rana* and *Bombina*, showed that longer tails do not consistently improve swimming performance and that the effect is species-specific.

In terms of the Gosner stages and the timing of metamorphosis, we did not identify a significant effect of artificial light at night. Nevertheless, the constant illumination group showed a lower variability in the mean time to metamorphosis compared to the tadpoles raised with a 12/12 light-dark cycle. The findings of previous studies concerning developmental changes due to ALAN are ambiguous. While tadpoles of *Hyla arborea* and *Anaxyrus americanus* exhibited an accelerated development (Petrović et al., 2025; Dananay & Benard, 2018), larvae of *Xenopus laevis* and *Discoglossus pictus* had a decelerated development (Delgado et al., 1987; Gutierrez et al., 1984). These contradictory effects of ALAN on the timing of metamorphosis and the developmental stages of tadpoles may be attributed to differences in illuminance regimes among studies, including variation in light intensity, spectral composition and light source type. Nonetheless, May et al. (2019) described comparable results to ours as individuals raised under constant light were longer at metamorphosis while neither the timing of metamorphosis nor the Gosner stages differed between the light and control group.

After applying the approach of Geometric Morphometrics to identify potential changes in the shape of tadpoles, we did not find any evidence of ALAN modifying the body form of premetamorphic green toads. Environmental variables such as temperature or the presence of predators are known for their ability to influence morphological traits of tadpoles (Vences et al.,

2002). Moreover, a study investigating the effects of the heavy metal copper on the development of European green toad tadpoles described significantly narrower rumps and smaller tails fins in individuals exposed to copper (Theil, 2024). These findings demonstrate that environmental pollutants may alter larval morphology. Given that ALAN represents a widespread form of anthropogenic pollution, it may function as a stressor with the potential to influence morphological traits in tadpoles. However, in this study no significant effects of ALAN on the shape of the animals were identified, which could be explained by the timing of image acquisition, potentially missing a critical developmental window.

Although we did not find an effect of constant light on the development in contrast to studies with similar approaches (Dananay & Benard, 2018; Petrović et al., 2025), we did detect an influence on the survival rate and the growth of anuran larvae. Research on the topic of light pollutions effects on larval development has increased in recent years but the available evidence is inconclusive. These discrepancies may be attributable to the different light sources and settings used across studies, whose comparison is further complicated by the lack of standardized descriptions of light setups. Most of the studies referred to above described significant but diverse effects of ALAN on the development of tadpoles, whereas research investigating the impact of different light spectra seems to agree on the assumption that blue light (420-490 nm) affects the development and growth of larvae in a positive and red light (620-780 nm) in a negative way (Ruchin, 2021; Ji et al., 2025; Borah et al., 2022). Taking this information into account, it is possible that the spectral composition of light is the more relevant factor in the development of anuran larvae rather than the photoperiod itself and should be considered in future studies.

Exposure to ALAN during early development may have cascading effects on life-history trajectories, potentially influencing fitness-related traits and long-term population dynamics. A larger body size at metamorphosis, as we experienced it in the tadpoles of the experimental group, correlated positively with a higher survival rate of juveniles in multiple anuran species (Székely et al., 2020; Altwegg & Reyer, 2003; Berven, 1990). Individuals of the mole salamander (*Ambystoma talpoideum*) which exhibited bigger bodies at metamorphosis were larger at first reproduction and females reproduced at a younger age compared to salamanders with smaller sizes at metamorphosis (Semlitsch et al., 1988). The larger sizes of tadpoles induced by constant light may have a positive effect on the future fitness of the individuals, but ALAN

may still influence whole populations negatively. Hatching success was significantly lower in eggs of the Wood frog when raised under light pollution (May et al., 2019), in the common clownfish (*Amphiprion ocellaris*) constant light was responsible for a hatch rate of zero percent (Fobert et al., 2019). Predation risk in adults of the Convict tang (*Acanthurus triostegus*) was elevated when raised under light conditions (O'Connor et al., 2019) and a constantly illuminated environment during the premetamorphic stage of Wood frog tadpoles led to a higher susceptibility to trematodes as well as salt in adulthood (May et al., 2019). Overall, the influence of ALAN on anuran larvae appears diverse and the consequences for populations remain difficult to predict.

Our study demonstrated that artificial light at night increases mortality as well as body length and decreases the body-to-tail ratio of premetamorphic anurans under laboratory conditions. Aside from our results, literature reports contradictory effects of ALAN on amphibian development and comparison remains challenging. Future research should quantify and report key experimental parameters such as light intensity and spectral composition to improve comparability across studies. Nonetheless, translating results from such studies to free-living individuals is complex. Recent research conducted on wild populations suggests that animals in natural environments may respond differently to light pollution than under laboratory conditions (Cheynel et al., 2025), highlighting the importance of investigating the impact of light pollution on amphibians in their natural habitats, including its interactions with other environmental factors and pollutants to better understand potential consequences for populations and ecosystems.

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Appendix

Appendix A: Average temperatures during experiment

Table A1: Average temperatures (in Celsius) in top and bottom chambers during the experiment.

Week	Temperature - Top	Temperature - Bottom
25.04.-28.04.2024	19.57987952	21.07268781
29.04.-05.05.2024	18.96011707	20.84897304
06.05.-12.05.2024	19.15529964	21.13928329
13.05.-19.05.2024	19.11853679	21.31290668
20.05.-26.05.2024	19.10037103	21.30615481
27.05.-02.06.2024	18.94171656	21.23145826
03.06.-09.06.2024	18.97201234	21.18410673
10.06.-16.06.2024	19.03024626	21.38937577
17.06.-23.06.2024	19.08991518	21.36267466
24.06.-30.06.2024	19.36531216	21.40612536
01.07.-07.07.2024	19.38681833	21.28704129
08.07.-14.07.2024	19.47182259	21.13243609
15.07.-21.07.2024	19.38578839	21.15971628
22.07.-28.07.2024	19.39710859	21.22074063
29.07.-04.08.2024	19.40370775	21.19230048
05.08.-11.08.2024	19.41053935	21.1936111
12.08.-18.08.2024	19.44099176	21.07842897
19.08.-25.08.2024	19.41234487	20.98835625
26.08.-01.09.2024	19.40849702	21.1016821
02.09.-08.09.2024	19.41097741	21.12628784
09.09.-15.09.2024	19.42299906	21.15652083
16.09.-22.09.2024	20.64539714	21.51758132

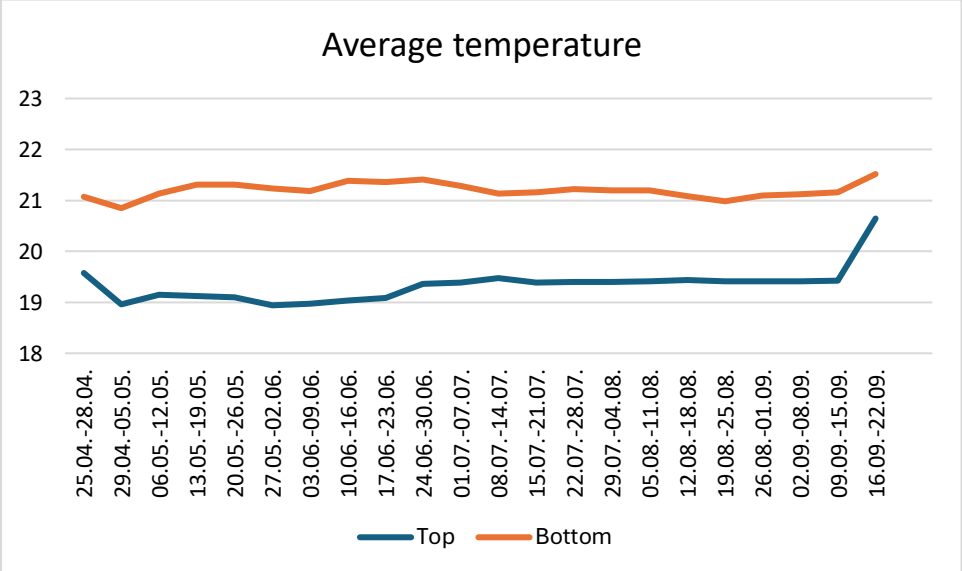

Figure A1: Average temperatures (in Celsius) in top and bottom chambers during the experiment.

Appendix B: Separate analysis of body and tail lengths

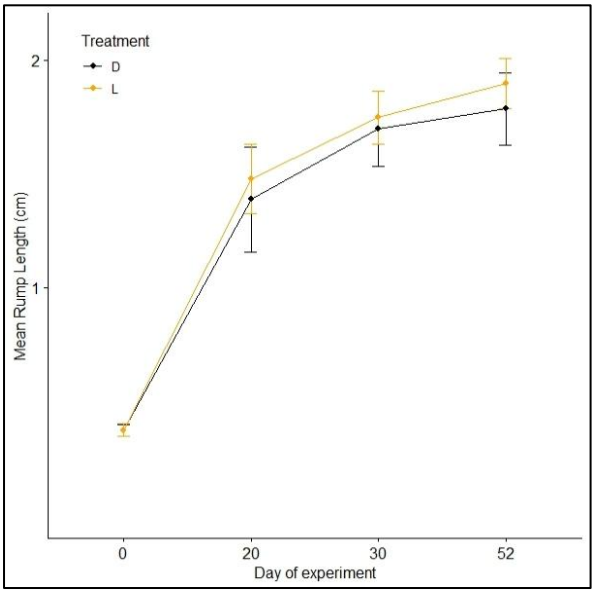

Figure B1: Mean rump lengths (in cm) during the experiment for both treatments (D = 12h light/12h dark = black; L = 12h strong light/12h dimmed light = orange).

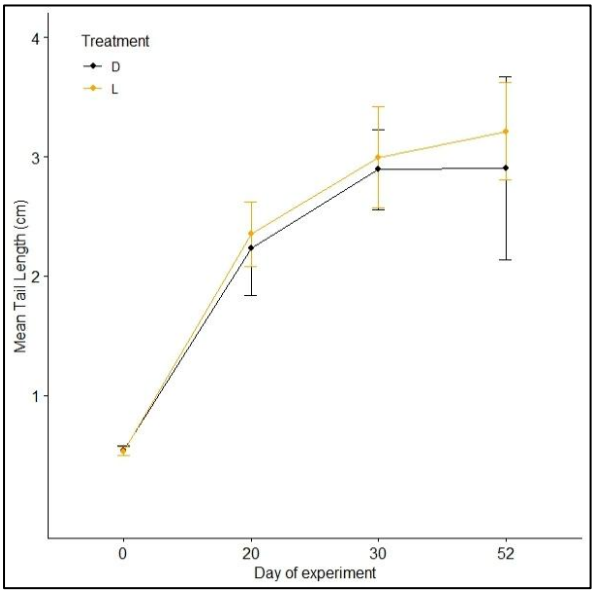

Figure B2: Mean tail lengths (in cm) during the experiment for both treatments (D = 12h light/12h dark = black; L = 12h strong light/12h dimmed light = orange).