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A laboratory experimental assessment of the sensibility of *Rana temporaria* tadpoles to the effects of car traffic-associated seismic disturbances

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Abstract. Vibrations generated by road traffic are considered to be a form of physical environmental pollution. Nonetheless, the effect of this disturbance in natural habitats, and in particular on the aquatic stage of amphibians, received very little attention from the scientific community.

This study aims to assess the direct effect of mechanical waves and the consequent water turbidity on the aquatic larval stage of *Rana temporaria* by exposing an experimental group to laboratory-induced vibrations and comparison with a non-exposed control group.

Our results show that this kind of pollution had no significant effect on the development rate, the length of the larval period, and the mortality of the larvae. However, we identified a significant effect on the size of animals, both during the larval period, and at metamorphosis. This result raises concern about the long-term risks to the amphibian population exposed to this type of low-profile pollution.

Key words: development, growth, metamorphosis, amphibian performance, traffic-related pollution

Introduction

Commercial and recreational vehicle traffic in the peri-urban and rural areas is on a constant rise, increasing the pressure on the already deteriorated amphibian populations by supplementing the existing chemical contamination and rising road crossing injuries and mortality (Adlassnig *et al*., 2013; Hamer *et al*., 2015). However, studies on vehicle traffic-related consequences are scarce and focus mostly on nuptial migration-related road-crossing mortality (Elzanowski *et al*., 2009). Referring strictly to Europe (van Gelder, 1973; Hels and Buchwald, 2001; Cooke and Sparks, 2004), this type of disturbance seems to affect the Common toad (*Bufo bufo*) in suburban areas and the Common frog (*Rana temporaria*) in the countryside (Elzanowski *et al*., 2009; Puki, 2006). Furthermore, all mentioned research refers to adult frogs of reproductive age. Studies regarding the impact of car traffic on amphibians in the larval stage are few and refer to indirect effects (noise - Castaneda *et al*., 2022, de-icing chemical contamination - Sanzo and Hecnar, 2006).

When a vehicle strikes an irregularity in a road surface, an impact load is generated, which in turn, gives rise to long-wavelength mechanical waves in the form of seismic vibrations that travel through the soil (Toplak *et al*., 2016). In addition, these waves are exacerbated by obstacles such as potholes and bumps and are considered a form of environmental pollution (Chilton *et al*., 1975; Hunaidi, 2000; Ducarne *et al.,* 2018; Niazmand-Aghdam *et al.,* 2021).

As far as we know, the effect of car tire impact on the soil and water surface on the larval-stage of amphibians hasn't been researched yet, even though the presence of the lateral line indicates their high sensitivity to this type of stimuli (Quinzio and Fabresi, 2014).

Amphibian life history theory predicts that in suboptimal habitats, larvae will accelerate their development and growth-rate coupled with a shorter larval period, to minimize mortality (Newman, 1992; Stearns and Coella, 1986; Wilbur and Collins, 1973). The faster development rate may have repercussions, such as smaller size at metamorphosis and reduced survival rate during adulthood (Arendt, 1997). Therefore, the value of this adaptive strategy depends on the cost-benefit ratio after the metamorphic transition.

This study aimed to evaluate the effects of seismic disturbances and water turbidity produced by car traffic on a *Rana temporaria* population from the Făgetul Clujului - Valea Morii protected area. The area is a Site of Community Importance (SCI,46°42′53″N 23°34′18″E), covering 1.7 ha that includes forest habitats of community-interest and 33 protected plant and animal species (M.M.D.D., 2008).

Although access by motor vehicles in natural protected areas is strictly prohibited and constitutes contravention according to Romanian Law (O.U.G., 2007), the above-mentioned protected area is constantly, and intensively subjected to this type of traffic. During the week, the vehicle traffic is utilitarian in nature (heavy vehicles and tractors), and on weekends, the traffic is recreational (4x4 cars, ATVs, and dirt bikes - personal observations). For the studied species, the Common frog, this traffic can represent a permanent disturbance of the breeding ponds through the indiscriminate and sometimes intentional use of the more rugged off-road tracks, which also happen to create puddles that function as amphibian breeding areas in the spring. In our experimental design, we formulated two hypotheses:

1. Seismic disturbances and increased water turbidity will affect larval development exposed to this type of treatment.
2. The larvae exposed to disturbances will metamorphose faster and have a smaller average size than the control group.

Materials and methods

1 Experimental design

The study was carried out in the laboratory of the University Babeș-Bolyai Vivarium, Cluj-Napoca, Romania, between April and June 2022.

To test our hypotheses, we designed an experiment based on two groups (control and experimental), each consisting of 24 individuals of Rana temporaria larvae.

Simulating the traffic-related vibrations in the laboratory is a rather complicated endeavour, considering the wide range of frequencies in the resulting ground-borne mechanical waves. From the low frequencies produced by the so-called wheel hop (8-16 Hz; Watts, 1992) to the higher frequencies produced by the tire impact forces (800-1500 Hz; Hajek et al., 2006), all these vibrations travel through the soil and water and may influence the natural habitats. A perfect reproduction of the whole range of the mentioned frequencies is, therefore, impossible at the moment. However, we simulated vibrations similar to those produced by the impact of wheels on the soil and water surface of the breeding ponds by placing the experimental rearing containers on a high-frequency vibrating reptile feeder (Exoterra Vivicator) for 15 minutes twice a day (30 min total time/day), three days a week (90 minutes/week). We also simulated the turbidity resulting from these disturbances by agitating the water twice a day, each day, for 1 min, by vigorously moving a paddle along the length of the growth containers.

The animals were collected as eggs (three clutches) on April 3, 2022, from a temporary pond located in Făget forest, Cluj-Napoca, Cluj county, Romania (46°41’48.57’’ N 23 °32’46.80’’E, altitude 682m, Someș river basin). The three clutches were kept separately, in 20L plastic containers filled with dechlorinated water, until the end of the hatching period (April 14, 2022). With the beginning of the larval feeding stage (Smith-Gill and Berven, 1979) of circa Gosner 25 (Gosner, 1960), we selected larvae from each litter (88 in total, 24/treatment) and distributed them randomly to form the two study groups - control and experimental. Larvae excluded from the experiment were released at the site of capture. The study animals were kept in 4L containers (9x17x26cm), with eight larvae/container, in three liters of dechlorinated water (Craioveanu *et al*., 2019). This density corresponds to low densities in natural populations (Glennemeier and Denver, 2002; Rot-Nikcevic *et al*., 2005). A substrate of approximately 1 cm thickness was added to each container, consisting of sterilized forest soil from the collection area of the initial clutches. The water temperature was kept constant at 20 °C (+/- 1) at a natural circadian rhythm. Each container had an oxygen supply provided by electric pumps. Every three days, we changed about 90% of the water with fresh, dechlorinated tap water. We cleaned the organic residues daily by siphoning.

With the emergence of the forelimbs (approx. Gosner 42), we reduced the amount of water to 2 L, and the containers were tilted so that a dry area formed at the raised end (Craioveanu *et al*., 2021). From this point, exposure to the experimental stimuli and feeding ceased. When reaching about Gosner 45 developmental stage, the animals got out of the water towards the dry end of the container and were relocated one by one in containers specific to the terrestrial environment (starting with May 18, 2022), and later released in the collection area.

We considered Gosner stage 45-46 as the moment of metamorphosis, as it corresponds to the total absorption of the larval tail.

The larval diet consisted of Spirulina (Organic Spirulina 500 mg, protein 63.5%, carbohydrates 16.1%, and lipids 8.2%. Origin: China) and pelleted rabbit food (Versele Laga Cuni fit pure, protein 15%, carbohydrates 15% lipids 3%. Origin: Hungary), so that the animals could selectively consume their preferred food type. We used Spirulina to simulate the presence of many protein-rich species of micro-algae (e. g. *Chlorella*, *Anabaena*, *Aphanizomenon*, *Scenedesmus*, *Pediastrum*) (Cărăuș, 2012) in the original ecosystem.

2 Measurements and analysis

Length measurements were performed on digital images using the free image analysis software ImageJ (http://imagej.nih.gov/ij). Images were recorded with a Nikon D3200 camera mounted on a stand 30 cm above the specimens.

For the larval phase of the experiment, we measured the following morphological variables: 1) total body length (the distance from snout to tail tip, in mm), and 2) larval development stages according to the Gosner (1960) model. To establish the correct Gosner-stage we used images taken from several angles. In addition to these variables, we recorded the length of the larval period for each group (days) and mortality at metamorphosis (Craioveanu *et al.,* 2019).

As handling is concerned, the larvae were carefully captured using a shallow net and transferred to a petri dish containing water levels approximately equal to the thickness of the animal (Davis et al., 2008). When photographing, we used a Petri dish with a grid scale. The image was taken only when the subject was not moving, and the entire dorsal part was visible, followed by the prompt return to the original container. Due to the small size and fragility of the larvae at the moment of hatching, batch separation and the first measurements were done only on day 4 (April 14, 2022). We performed five measurements at intervals of approximately one week (April 14, 20, 25, May 4, 11).

In the metamorphosis phase of the experiment, we measured the body length (snout to vent in mm) (Altig, 2007) daily during the entire metamorphosis period (May 20 - June 10). We also recorded the mortality (% of dead animals) during metamorphosis.

The normal distribution of the data was tested with Shapiro-Wilk test. To verify if the larvae grow and develop differently between the two groups (control and experimental), we performed two-sample tests for body length and Gosner-stage, for each larval-stage measurement occasion (between April 14 and May 11). Additionally, we compared the length at metamorphosis and duration of development (defined as days to metamorphosis) between groups. For normally distributed data, we used the two-sample t-test, and for non-normally distributed data, we used the Wilcoxon test. The program RStudio (RStudio Team 2022) was used to perform all analyses.

Results

The first measurement performed at the beginning of the experiment showed no differences between groups regarding their body length ($t = 1.2198$, $df = 43.956$, p-value = 0.229). Gosner stages were identical in both groups as they were selected on this criterion from the beginning.

Three succeeding length measurements (2nd -4th) showed significant differences between groups (Table 1, Figs. 1-3).

In the fifth measurement, we saw no significant differences between the body lengths of the larvae in the control and experiment groups (Table 1).

At metamorphosis, we also found a significant difference between the body lengths of the larvae in the control and experiment groups (Table 1, Fig. 4).

Table 1. Length measurements and statistical tests performed on the two groups of *R. temporaria* larvae.

Measurements	Normality test result (Shapiro-Wilk)	Comparison test result (t-test/ Wilcoxon test)
1st measurement (14th of April)	W = 0.99, p-value = 0.972	t = 1.22, df = 43.96, p-value = 0.229
2nd measurement (20th of April)	W = 0.85, p-value < 0.001	W = 395, p-value = 0.027
3rd measurement (25th of April)	W = 0.83, p-value < 0.001	W = 412, p-value = 0.010
4th measurement (4th of May)	W = 0.80, p-value < 0.001	W = 417, p-value = 0.008
5th measurement (11th of May)	W = 0.89, p-value < 0.001	W = 344, p-value = 0.255
Metamorphosis measurement (20th of May - 10th of June)	W = 0.96, p-value = 0.358	t = 2.10, df = 28.02, p-value = 0.045

In all cases where we recorded significant differences, the control group had higher values than the experiment group.

Gosner's development stages and duration of metamorphosis did not differ significantly between groups on all measurement occasions (in all comparisons p-value > 0.05).

At metamorphosis, the control group had a mortality of 33%, and the experiment group had 37.5%. Before metamorphosis, we recorded no deaths.

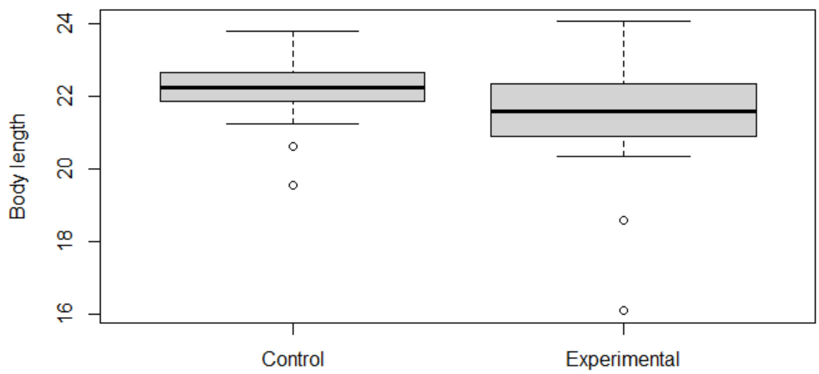

Figure 1. Comparison of body lengths of *R. temporaria* larvae in the second measurement occasion. Boxplots represent median (thick line inside the box), interquartile interval (whiskers) and outlier values (empty dots).

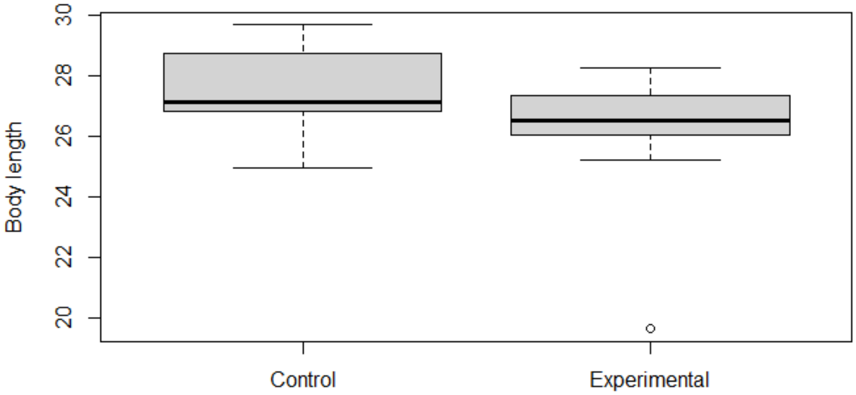

Figure 2. Comparison of body lengths of *R. temporaria* larvae in the third measurement occasion. Boxplots represent median (thick line inside the box), interquartile interval (whiskers) and outlier values (empty dots).

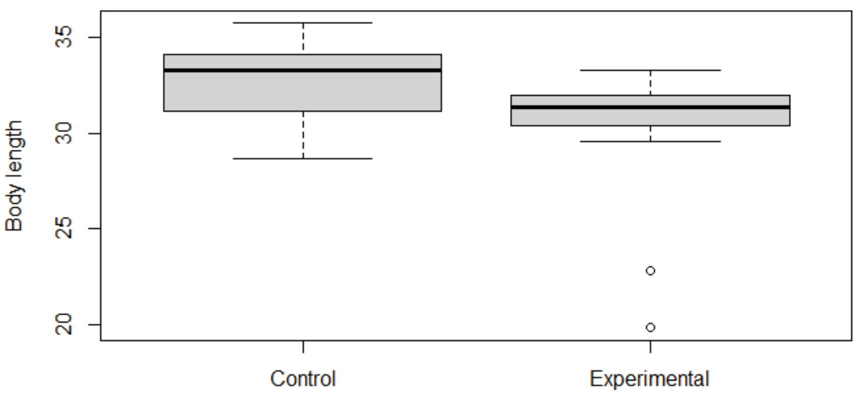

Figure 3. Comparison of body lengths of *R. temporaria* larvae in the fourth measurement occasion. Boxplots represent median (thick line inside the box), interquartile interval (whiskers) and outlier values (empty dots).

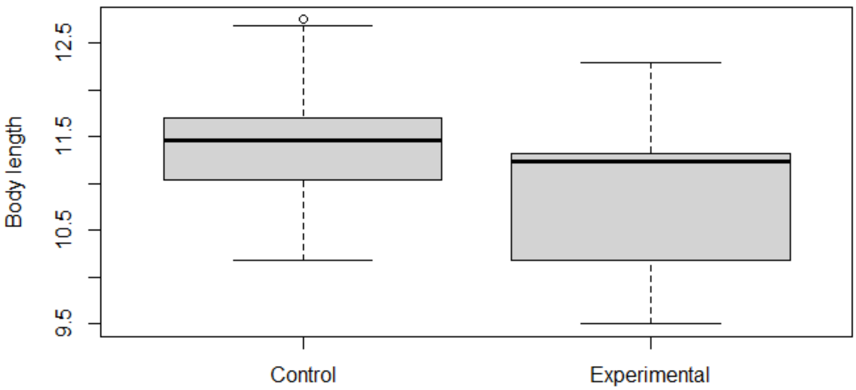

Figure 4. Comparison of body lengths of *R. temporaria* larvae at metamorphosis. Boxplots represent median (thick line inside the box), interquartile interval (whiskers) and outlier values (empty dots).

Discussion

Environmental conditions to which amphibian larvae are exposed can produce delayed effects on the individual performance of postmetamorphic adults. Thus, adult performance is affected by environmental factors long before metamorphosis. The quality of postmetamorphic animals, along with the survival rate at metamorphosis, and postmetamorphic environmental conditions are essential elements that will determine the future dynamics of the entire population (Craioveanu *et al.,* 2019; Beckerman et al., 2002). The metamorphosis is a critical process in the development of amphibians, as they invest the energy acquired and stored as tadpoles in terrestrial maturity, with a complete reconfiguration of their appearance and physiology (Wilbur and Collins, 1973; Semlitsch *et al.,* 1988; Denver *et al.,* 1998).

In this study, we investigated the effect of vibration, mechanical waves, and high turbidity on the larval development of *Rana temporaria*. According to our results, the stimuli we used on the experimental group had no significant effects on the **development rate** (Gosner stages), the **length of the larval period**, and the **mortality at metamorphosis**. The significant difference between the control and experimental groups was the **size of the animals**. Thus, the experimental group had consistently and significantly a smaller size (total length) both during the larval period (measurement sessions 2, 3, and 4) and at metamorphosis (body length).

After reaching the minimum body size required to initiate metamorphosis, anuran larvae are capable of timing the moment of metamorphosis, depending on the growth opportunities, or the risk of mortality in the larval habitat (Wilbur and Collins, 1973). This timing is modulated by the production of thyroid hormone - the main inducer of metamorphosis - along with the production of corticosteroid stress hormones - causing the actual transformation of tissues during metamorphosis. In response to environmental stressors, these two hormones are released earlier and in increased amounts, accelerating metamorphosis (Denver, 2021). Although this mechanism promotes immediate survival in degraded larval habitats, the short and medium-term cost is a reduced tadpole size during the larval period and reduced size at metamorphosis.

The comparisons between our control and experimental groups indicate that this stress-response mechanism was activated as an effect of the experimental treatment.

Although individual size at metamorphosis varies greatly even within the same population (Storer, 1925; Jones *et al.,* 2005; Denver *et al.,* 1998), the smaller size of metamorphs negatively affects performance and survival in the terrestrial stage (Szekely *et al.,* 2020). Thus, smaller specimens are much more affected by predation (Lawlor et al., 1999), cannibalism by conspecific animals of larger size (Alvarez, 2013), and have lower lipid reserves (Scott, 1994) which lead to a decreased ability to cope with variations in the environment.

Our findings largely confirm the initial hypotheses of this study. Seismic-type disturbances and increased water turbidity affected the experimental animals. Also, the experimental group had consistently smaller body sizes than the control group, but the larval period was not significantly different between the two groups.

Conclusion

The stimuli represented by mechanical waves and high water turbidity affected Rana temporaria individuals in the experimental group, resulting in the decreased postmetamorphic performance of the animals.

Even though our laboratory experiment could not precisely reproduce the effects of general traffic, the results indicate that motor-vehicle traffic through the Făgetul Clujului - Valea Morii protected area most likely harms the resident *Rana temporaria* population and can be of major concern regarding its survival in the future.

Our results also intend to raise awareness regarding the effect of traffic-related ground vibrations on amphibian populations.

Limitations of our study

A major limitation of our study is that we only visually quantified the disturbances caused by vehicles in the natural ponds and due to the lack of the proper technology, we were unable to measure them and experimentally reproduce their consequences. Our results, even if experimental, suggest that understanding the 'wavescape' generated and maintained by illegal traffic in the natural systems can be a genuine scientific goal since tadpoles of a common amphibian species responded to such stimuli in laboratory conditions.

References

Adlassnig, W., Sassmann, S., Grawunder, A., Puschenreiter, M., Horvath, A., & Koller-Peroutka, M. (2013). Amphibians in metal-contaminated habitats. *Salamandra.* 49. 149-158.

Altig, R. (2007). A primer for the morphology of anuran tadpoles. *Herpetol. Conserv. Biol.* 2, 71-74.

Alvarez, J.A. (2013). Rana draytonii (California red-legged frog) Cannibalism. *Herpetol. Rev.* 44: 126–127.

Arendt, J.D. (1997). Adaptive intrinsic growth rates: an integration across taxa. *Quat. Rev. Biol.* 72:149–177.

Beckerman, A., Benton, T.G., Ranta, E., Kaitala, V., Lundberg, P. (2002). Population dynamic consequences of delayed life-history effects. *Trend Ecol Evol* 17:263–269

Castaneda, E., Leavings, V.R., Noss, R., & Grace, M. K. (2020). The effects of traffic noise on tadpole behavior and development. *Urban Ecosyst* 23, 245–253. https://doi.org/10.1007/s11252-020-

Cărăuș, I. (2012). Algae of Romania. A distributional checklist of actual algae, *Studii și Cercetări Biologice,* Univ. Bacau 7, 1-809 third revision

Chilton F., Friesz, T., & Chen, E. (1975). Traffic induced vibrations, https://onlinepubs.trb.org/

Craioveanu, O., Craioveanu, C., Ghira, I., Mireşan, V., & Hartel, T. (2019). Does carnivory pay off? Experiments on the effects of different types of diet on growth and development of Bufo bufo (Linnaeus, 1758) tadpoles and carry-over effects after metamorphosis. *Herpetozoa.* 32. 21-31. 10.3897/herpetozoa.32.e35627.

Craioveanu, O., Craioveanu, C., Bențea, M.I., Coroian, C., & Mireşan, V. (2021). Assessing the performance of Alphitobius piceus (Oliver, 1792) as novel feeder insect species for small sized postmetamorphic frogs. *Stud. Univ. Babeş-Bolyai Biol..* 66. 5-20. 10.24193/subbbiol.2021.2.01.

Cooke, A.S., & Sparks, T.H. (2004). Population declines of Common toads (Bufo bufo): the contribution of road traffic and monitoring value of casualty counts. *Herpetol Bull* 88:13–26.

Culley, D.D., Meyers, S.P., & Doucette, A. (1977). A high density rearing system for larval anurans. *Lab.Anim.,* 6: 34-41.

Davis, A.K., ConneL, L.L., A. Grosse, A., & Maerz, J.C. (2008). A Fast, Non-invasive Method of Measuring Growth in Tadpoles Using Image Analysis. *Herpetol. Rev.* 39 (1), 56-58.

Denver, R.J., Mirhadi, N., & Phillips, M. (1998). Adaptive plasticity in amphibian metamorphosis: response of Scaphiopus hammondii tadpoles to habitat desiccation. *Ecology* 79: 1859–1872.

Denver, R.J. (2021). Stress hormones mediate developmental plasticity in vertebrates with complex life cycles. *Neurobiol Stress.* 2021 Feb 2; 14:100301. doi: 10.1016/j.ynstr.2021.100301. PMID: 33614863; PMCID: PMC7879041.

Ducarne, L., Ainalis, D., & Kouroussis, G. (2018). Assessing the ground vibrations produced by a heavy vehicle traversing a traffic obstacle, *Science of The Total Environment,* Volume 612, pp. 1568-1576, doi.org/10.1016/j.scitotenv.2017.08.226.

Elzanowski, A., Ciesiołkiewicz, J., Kaczor, M., Radwańska, J., & Urban, R. (2009). Amphibian road mortality in Europe: A meta-analysis with new data from Poland. *Eur. J. Wildl. Res.* 55: 33-43. doi: 10.1007/s10344-008-0211-x.

Glennemeier, K.A., & Denver, R.J. (2002). Role for corticoids in mediating the response of Rana pipiens tadpoles to intraspecific competition. *J. Exp. Zool.,* 292, 32–40.

Gosner, K., (1960). A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. *Herpetologica* 16 (3), 183-190.

Hajek, J.J., Blaney, C.T., & Hein, D.K. (2006). Mitigation of Highway Traffic-Induced Vibration. Quiet Pavements: Reducing Noise and Vibration. *2006 Annual Conference of the Transportation Association of Canada Charlottetown,* Prince Edward Island

Hamer, A.J., Langton, T.E.S., & Lesbarrères, D. (2015). Making a Safe Leap Forward. *Handbook of Road Ecology,* pp. 261–270. Available at: http://dx.doi.org/10.1002/9781118568170.ch31.

Hels, T., & Buchwald, E. (2001). The effect of road kills on amphibian populations. *Biol Conserv* 99:331–340. doi: 10.1016/S0006-3207 (00)00215-9

Hunaidi, O., (2000). Traffic vibrations in buildings, *National Science Library - National Research Council Canada, Construction Technology Update*; no. 39

Jones, L.L.C., Leonard, W.P., & Olson D.H. (2005). *Amphibians of the Pacific Northwest*. Seattle Audubon Society, Seattle.

Lawler, S.P., Dritz, D., Strange T., & Holyoak M. (1999). Effects of introduced mosquitofish and bullfrogs on the threatened California red-legged frog. *Cons. Biol.* 13: 613–622.

Licht, L.E. (1967). Growth inhibition in crowded tadpoles: intraspecific and interspecific effects. *Ecology,* 48: 736-745.

Mandelli, J., Arruda-Soares, H., Fontanello, D., Penteado, L.A., & Campos, B. (1987). Different sizes of net cages and the weight gains of bullfrog tadpoles. *Rev. Fat. Med. Vet. Zootec.* Univ. S. Paulo, 24: 209-212.

Martínez, I.P., Álvarez, R., & Herráez, M.P. (1996). Growth and metamorphosis of Rana perezi larvae in culture: Effects of larval density. *Aquaculture,* Volume 142, Issues 3–4, 1996, pp. 163-170.

Newman, R.A. (1992). Adaptive plasticity in amphibian metamorphosis. *Bioscience.* 42:671–678.

Niazmand-Aghdam, N., Ranjbarian, M., Khodakarim S., Mohammadian F., & Farhang Dehghan S. (2021). The effects of combined exposure to road traffic noise and whole body vibration on types of attention among men. *Med Lav*. 2021 Oct 28; 112(5):360-369. doi: 10.23749/mdl.v112i5.11772. PMID: 34726664; PMCID: PMC8600575.

Puky, M. (2006). Amphibian road kills: a global perspective. In: Irwin CL, Garrett P, McDermott KP (eds.)*, Proceedings of the 2005 international conference on ecology and transportation. Center for transportation and the environment.* North Carolina State University, Raleigh, NC, pp. 325–338.

Quinzio, F. (2014). The Lateral Line System in Anuran Tadpoles: Neuromast Morphology, Arrangement, and Innervation. *The Anatomical Record,* Vol.297, issue 8, 1508-1522.

Rot-Nikcevic, I., Denver, R.J., & Wassersug, R.J. (2005). The influence of visual and tactile stimulation on growth and metamorphosis in anuran larvae. *Functional Ecology* 19, 1008–1016.

Sanzo, D., & Hecnar, S.J. (2005). Effects of road de-icing salt (NaCl) on larval wood frogs (Rana sylvatica). *Environmental Pollution,* Volume 140, Issue 2, 2006, pp. 247-256, ISSN 0269-7491, https://doi.org/10.1016/j.envpol.2005.07.013.

Scott, D.E. (1994). The effect of larval density on adult demographic traits in Ambystoma opacum. *Ecology* 75:1383–1396

Semlitsch, R.D., & Caldwell, J.P. (1982). Effects of density on growth, metamorphosis, and survivorship in tadpoles of Sccrphiopus holbrooki. *Ecology,* 63: 905-911.

Semlitsch, R.D., Scott, D.E., & Pechmann, J.H. (1988). Time and size at metamorphosis related to adult fitness in Ambystoma talpoideum. *Ecology* 69: 184–192.

Smith-Gill, S.J., & Berven, K.A. (1979). Predicting amphibian metamorphosis. *Am. Nat.,* 113: 563-585.

Stearns, S.C., & Koella, J.C. (1986). The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution.* 40:893–913.

Storer, T.I. (1925). A synopsis of the amphibia of California. *University of California Publications in Zoology* 27: 1–342.

Szekely, D., Cogalniceanu, D., Székely, P., Armijos, D., Espinosa, V., & Denoel, M. (2020). How to recover from a bad start: size at metamorphosis affects growth and survival in a tropical amphibian. *BMC Ecology.* 20. 24. doi: 10.1186/s12898-020-00291-w.

Travis, L. (1981). Control of larval growth variation in a population of Pseuducriu triseriuta (Anura: Hylidae). *Evolution,* 35: 423-432.

Toplak, S., Lujej, S., Ivanic, A., & Lep, M. (2016). The pavement quality and vibrations. Integrated Solutions for Infrastructure Development. ISBN: 978-0-9960437-3-1

Van-Gelder, J.J. (1973). A quantitative approach to the mortality resulting from traffic in a population of Bufo bufo L. *Oecologia* 13:93–95. doi: 10.1007/BF00379622

Watts, G.R. (1992). The generation and propagation of vibration in various soils produced by the dynamic loading of road pavements. *J. Sound Vib.,* Volume 156, Issue 2,1992, Pages 191-206, ISSN 0022-460X, https://doi.org/10.1016/0022-460X(92)90692-Q.

Wilbur, H.M., & Collins, J.P. (1973). Ecological aspects of amphibian metamorphosis. *Science* 182: 1305–1314.

Wilbur, H.M. (1977). Density-dependant aspects of growth and metamorphosis in Bufo umericcmus. *Ecology,* 58: 196-200.