Urban plums and toads: do fleshy fruits affect the post-metamorphic growth of amphibians?

Mikołaj Kaczmarski, Piotr Tryjanowski and Anna Maria Kubicka

Department of Zoology, Poznań University of Life Sciences, Poznań, Wielkopolska, Poland

ABSTRACT

Background: The main aim of the study was to analyse the influence of fleshy fruits (plums) on the post-metamorphic growth and feeding behaviour of the green toad *Bufotes viridis*. We tested the following two hypotheses: (1) juveniles of the green toad are characterised by faster growth in conditions involving fallen plums *Prunus cerasifera* due to the associated presence of more varied food such as invertebrates; (2) green toads exhibit more active feeding behaviour in the presence of fleshy fruits.

Methods: A total of 120 fresh metamorphs of the green toad were randomly assigned to one of four groups: two experimental groups with fleshy plums and two other groups as controls (without fruits). Each group was kept in an enclosure to which wild invertebrates had free access. Each individual toad was measured for snout-vent length (mm) and body mass (g) every other day for 30 days. In order to determine whether fallen plums influence the feeding behaviour of toads, the number of active and hidden (under an artificial shelter) individuals was also noted.

Results: The results showed that green toads from both enclosures with plums were characterised by more rapid growth than individuals from the control treatments. Simultaneously, in the enclosure with fleshy fruits, greater species richness of wild invertebrates was observed. No differences in active feeding behaviour were noted between control groups and groups with plums.

Discussion: Fleshy fruits, upon falling, attract many types of invertebrates; thus they may represent good dietary supplements for fresh amphibian metamorphs. Therefore, the presence of fruit trees close to a breeding site might influences the post-metamorphic growth of amphibians, but not their feeding behaviour. The presence of insects associated with fallen fruit seems to favour the occurrence of amphibian populations, which is particularly important, since, due to political and social pressure, numbers of fruit trees are currently being reduced. To our knowledge, no previous study has investigated the potential influence of the presence of fruit trees on the growth and behaviour of anurans.

Corresponding author
Mikołaj Kaczmarski,
traszka.com@gmail.com

INTRODUCTION

Usually, fruit trees lie outside the area of interest of ecologists, however, on the landscape level, the number of fruit trees is decreasing steadily (*Orłowski & Nowak, 2007*;

Plieninger et al., 2012). Very few studies in this area show that the distribution of the greatest numbers of fleshy-fruited species is closely related to man-made components of the environment such as roads (*Suhonen et al., 2017*), orchards, rural areas (*Suhonen & Jokimäki, 2015*), and even electricity pylons (*Dylewski et al., 2017*).

Nonetheless fleshy fruits, as an attractive source of nutrients, are consumed by humans (*Clark & Nicholas, 2013*), invertebrates (*Sallabanks & Courtney, 1992*; *Bieber, Silva & Oliveira, 2013*; *Rodríguez, Alquézar & Peña, 2013*) and by many vertebrates such as lizards (*Pietrzak, Steindorff de Arruda & Cechin, 2014*), mammals (*Virgós et al., 2010*; *Kurek, 2015*; *Lim et al., 2017*), and, especially, birds (*Jordano, 1994*; *Lázaro, Mark & Olesen, 2005*; *Jordaan, Johnson & Downs, 2011*; *Suhonen & Jokimäki, 2015*; *Suhonen et al., 2017*). However, while birds and bats may eat ripe fruit directly from trees (*Suhonen et al., 2017*), fruit becomes an available resource for the majority of mammals or lizards only after it falls. Fruits lying on the ground decompose and attract numerous invertebrates by odour (*Sallabanks & Courtney, 1992*; *Rodríguez, Alquézar & Peña, 2013*) and colour (*Fadzly & Burns, 2010*). Moreover, some indirect interactions have also been described whereby predators (birds or mammals) deliberately choose fruits infested by insects. These tritrophic interactions are often unpredictable and difficult to measure (*Rodríguez, Alquézar & Peña, 2013*); however, fallen or rotten fruits lying on the ground may have implications for the functioning of the ecosystem.

Fallen fruits near amphibian breeding sites (e.g. different kinds of water bodies) may have important ecological effects and may constitute an undervalued component of the environment. This is because fruits attract insects (*Sallabanks & Courtney, 1992*) that can be eaten by amphibians. Following metamorphosis, which is a critical stage of life, amphibians begin intensive feeding prior to their first hibernation (*Hartel et al., 2010*; *Schmidt, Hodl & Schaub, 2012*). Physical growth during the first month of terrestrial life, as well as, in effect, post-metamorphosis size, affect life performance in the subsequent stage (*Werner, 1986*). Furthermore, body length and body mass are often used as indices of condition, which is directly related to the survival pattern of amphibians (*Sinsch & Schäfer, 2016*). Therefore, the quality and amount of nutrition can influence the subsequent longevity and fecundity of adults, and additional resources in the form of insects attracted by fruits may play a major role. To the best of our knowledge, little information is available on the potential role of fruit trees in facilitating food acquisition by juvenile anurans.

The main driving force of global amphibian extinction is human activity ranging from direct catching for consumption, road mortality, and destruction of habitats to indirect effects such as the spread of diseases or the growth of the ozone hole (*Stuart et al., 2005*; *Elżanowski et al., 2009*; *Brühl et al., 2013*; *Hocking & Babbitt, 2014*; *Saenz, Hall & Kwiatkowski, 2014*; *Ficetola et al., 2015*). Also, in Europe, the plight of many common species is becoming alarming due to growing human impact, despite extensive knowledge on the subject and the introduction of restrictive laws protecting amphibians (*Denoël, 2012*; *Denoël et al., 2013*; *Beebee, 2014*; *Petrovan & Schmidt, 2016*). One of the most common amphibian species in Europe, occurring along a gradient from heavily urbanised to rural, is the green toad *Bufotes viridis* (*Kuzmin, 2001*). This species is

undergoing a decline mainly on the fringes of its range in Europe (it is listed as least concern, according to *IUCN, 2018*) despite its widely-known high level of adaptation to living in proximity to humans (*Kuzmin, 2001*; *Aghasyan et al., 2015*).

Considering the above information, we investigated, by conducting an enclosure experiment, whether the presence of windfalls of fleshy fruits (plums, *Prunus cerasifera*) which offer a higher level of availability to insects might have a positive effect on the post-metamorphic growth and feeding behaviour of the green toad. We tested the two following hypotheses: (1) green toad juveniles display greater growth in treatments with rotten plums *P. cerasifera* due to the presence of more varied food such as wild invertebrates; (2) green toad juveniles will be more active in the presence of fleshy fruits by increasing foraging movements as a response to prey attracted by plums.

MATERIALS AND METHODS

Study species

The green toad *Bufotes viridis* (Laurenti, 1768) (synonym *Bufo viridis*; Bufonidae) is a widely distributed species inhabiting most of Europe, as well as central Asia as far as western Kazakhstan (*Kuzmin, 2001*; *Aghasyan et al., 2015*). In some areas, the green toad is considered a synanthropic species (see *Ogielska & Kierzkowski, 2010*) which prefers habitats created by humans (*Mollov, 2011*), rural sites, and urban habitats (*Ensabella et al., 2003*; *Kovács & Sas, 2010*; *Zawadzki, Flesch & Kaczmarski, 2017*). *Bufotes viridis* is known to be attracted by artificial lights and consumes insects near street lamps (*Covaciu-Marcov et al., 2010*). Generally, this species feeds on the most abundant potential prey in the feeding territory (and thus is considered an opportunistic predator); however, its main prey are terrestrial invertebrates, in particular ants (*Baruš & Oliva, 1992*; *Covaciu-Marcov et al., 2010*). This is why we chose it as a model species for our research.

Prunus cerasifera is commonly known as the cherry plum or the myrobalan plum. Native to Southeast Europe and Western Asia, it was introduced into the rest of Europe and nowadays is classified as a neophyte. Wild types include large shrubs or small trees between 8 and 12 m in height. It is one of the first European trees to flower in spring, often starting to bloom before the leaves have opened. The cherry plum (yellow or red edible drupe two to three cm in diameter) is a popular ornamental tree for garden and landscaping use; numerous cultivars have been developed (*Zohary & Hopf, 2000*). Spontaneous spreading trees often occur in ruderal post-industrial areas, near railroads, on the borders of fields, and as hedges or on forest edges (*Colic et al., 2003*; *Nikolic & Rakonjac, 2007*).

All of the green toad individuals used in this study derived from one population in the centre of Poznań, Wielkopolska region, Poland, 75 m a.s.l. (52°24′4″N, 16°54′54″E) (*Zawadzki, Flesch & Kaczmarski, 2017*). The climate is within the transition zone between humid continental and oceanic, with average summer temperatures of 18.1 °C in July, winter temperatures of −1.6 °C in January, and average annual precipitation of 517 mm (WOS 2010, after *Majkowska et al., 2017*). In these conditions, the annual breeding season of *B. viridis* runs from April to early summer (*Kaczmarski, Szala & Kloskowski, in press*). Freshly metamorphosed individuals begin to leave the breeding pond in high
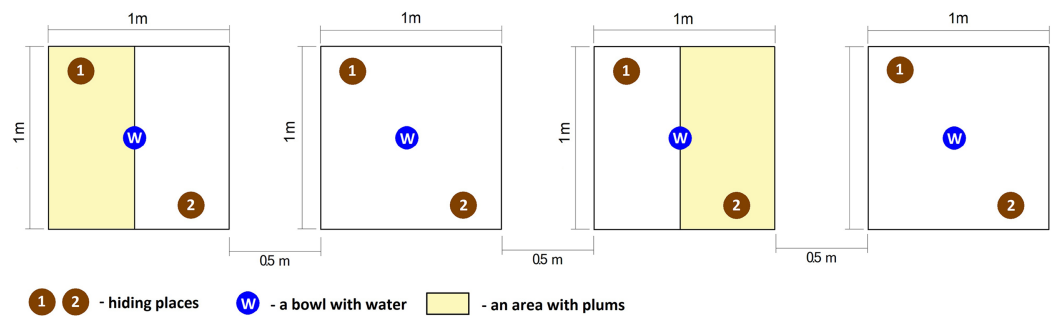
Figure 1 Graphical representation of experimental enclosures.

densities, depending on the time the eggs are deposited, from mid-July until the end of August. At the same time, plums ripen and start to fall, from early July to mid-September.

Experimental design and procedures

Two treatments, replicated twice were used in the study. The first treatment was with fruit absent (control) in turn, the second type was with fruit present (fruit treatment). Freshly metamorphosed of green toads (120 individuals) were collected from the pond located in Poznań the same day, on 30 July 2017, at the peak of dispersion. This ensured that all individuals had emerged from the pond at the same time, signifying that they were approximately the same age. Next, toads were randomly assigned to one of four experimental groups, with 30 fresh metamorphs per group. Each group was placed in a separate enclosure with the following dimensions: 1 × 1 × 1 m. According to *Kuzmin (2001)*, the maximum number of fresh metamorphs per one m^2 should not exceed several dozen individuals; thus, the density during the experiment was acceptable. Each enclosure contained two numbered hiding places (built from plastic stands) and one bowl with water. The enclosures were composed of wooden frame and covered by agrotextiles in turn, the ground was filled with moist soil which created conditions appropriate for amphibians. All enclosures were covered by lids composed of the wooden frame and net with four cm mesh size. The lids protected amphibians from predators such as birds and feral cats but allowed invertebrates (mostly flying insects) to get in and out of the enclosures.

Additionally, two enclosures included plums, which were strewn about in half of the experimental area. The number of fleshy fruits in the enclosures was estimated based on the mean number of plums per one m^2 calculated from 20 areas located in Poznań. The mean number of fruits per one m^2 was then divided in half, since plums were strewn only on one side of the enclosure and covered 0.5 m^2. This approach ensured us that the number of fruits reflected reality. All enclosures were placed alternately along a line in a partly shaded place so that each pair of enclosures with plums was separated by an experimental area without fleshy fruits. The distance between each pair of enclosures was 0.5 m (Fig. 1).

Throughout the experiment, body mass was recorded to the nearest 0.01 g with a portable electronic balance; snout-vent length (SVL) was measured to the nearest 0.01 mm

with a digital calliper. Measurements were carried out at noon, every other day, for 30 days, and were conducted on all individuals from all groups. Both parameters were measured and recorded by the same observer during the whole experiment. Additionally, other parameters, such as temperature, humidity, numbers of active and hidden toads, numbers of missing toads, and presence of invertebrates, were noted every other day for all enclosures separately. Since the green toads were kept in enclosures, a feeding procedure was established to exclude the death from starvation of the individuals from the control group during the duration of the experiment. All groups had access to an equal amount of food. The diet comprised invertebrates such as crickets, *Callosobruchus* spp. and *Shelfordella lateralis* (with vitamin supplements). Toads were fed ad libitum. This kind of food is often used in terraria to feed frogs and toads or other insectivores (*Finke & Winn, 2004*; *Mc Williams, 2008*). The water was changed at feeding times, after all individuals were measured.

Green toad is under strict species protection in Poland; thus, the permission of the Regional Director of Environmental Protection has been obtained (No. WPB—II.6401.346.2017.AC). Therefore, we followed all applicable institutional and national guidelines for the care and use of animals. Moreover, the observer (MK) responsible for the feeding and measurement of green toads was trained by the Polish Laboratory Animals Science Association. All animals were released at the site of capture in accordance with the permission granted us.

Statistical analysis

In order to estimate measurement error, the body mass and SVL of 30 randomly selected green toads were measured twice by one observer during the 21st day of the experiment (MK). Then, the relative technical error of measurement (relative TEM) for MK was calculated. Relative TEM expresses the quality of measurement in percentages; the lower the index of relative TEM, the greater the accuracy of the measured parameters (*Perini et al., 2005*). Repeated measurements were performed the same day with a 5-h break between the first and second rounds. Both measurements were performed the same day in order to avoid the influence of the toads' development.

To check whether living conditions were the same in all experimental cages, temperature and humidity were tested for normality and homogeneity of variances using the Shapiro–Wilk and Fligner–Killeen tests, respectively. Next, differences in temperature and humidity among four cages were analysed using a paired Student's t-test for which the significant level was established at $p < 0.004$ after the Bonferroni correction in order to counteract the problem of multiple comparisons.

The body mass index (BMI) was calculated according to the following equation: body mass (g)/SVL (mm)^2 (*Labocha, Schutz & Hayes, 2014*). Body mass, SVL, and BMI were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) for each group separately. In the case of *B. viridis*, the parameters could not be assigned to particular individuals due to changes in pattern and colouration during post-metamorphic growth (similar to the natterjack toad *Epidalea calamita*, according to (*Meyer & Grosse, 1997*)). Therefore, correlation between three parameters (SVL, body mass, and

Table 1 Pearson correlation coefficients of SVL, body mass, and BMI.

	N	SVL	Body mass
Body mass	60	0.883*	
BMI	60	0.959*	0.975*

Notes:
SVL, snout-vent length, BMI, body mass index.
* Indicates significant Pearson correlation coefficient at $p < 0.05$.

Table 2 Student's *t*-test between the control and experimental groups for the first day of the experiment.

	N	Mean (exp)	Mean (cont)	df	t	p
SVL	120	18.71	18.83	118	−0.5787	0.5639
Body mass	120	0.64	0.63	114.7	0.3219	0.7481
BMI	120	225.55	226.84	117.4	−0.13122	0.8958

Notes:
SVL, snout-vent length; BMI, body mass index; Mean (exp), a mean value for the experimental group; Mean (cont), a mean value for the control group.

BMI) was carried out using the Pearson correlation coefficient on the mean values for all groups in aggregate.

An unpaired Student's *t*-test for BMI was conducted to determine whether individual green toads had been randomly divided into control and experimental groups. In order to test the first hypothesis (i.e. juvenile toads display greater growth in the presence of rotten plums), general linear mixed models were used. The presence of fleshy fruits was the criterion for consideration as a treatment group. Individual measurements and day of the experiment were the random variables. Moreover, time (days of the experiment) was crossed with the presence of fleshy fruits and designated a fixed effect (*Bates, 2010*). In order to test the second hypothesis (i.e. juvenile toads will be more active in the presence of fleshy fruits), analysis of variance (ANOVA) was carried out. The number of active toads (individuals who were visible to the observer and did not hide in the hiding places) for every day of the experiment and the experimental areas (two enclosures with fruits and two enclosures without fruits) were considered dependent and independent variables, respectively. All statistical tests were significant at the level $p < 0.05$ and were carried out using R software (version 3.4.2, *R Development Core Team, 2008*).

RESULTS

The relative TEM index was acceptable for both parameters (body mass = 1.43%; SVL = 2.18%). Differences in living conditions (temperature and humidity) between enclosures were not significant (all $p > 0.004$; paired Student's *t*-test after the Bonferroni correction). Collecting all green toad juveniles was not possible twice during the experiment. Descriptive statistics of SVL, body mass, and BMI for each group during the whole experiment are included as a supplement (Tables S1–S3). All Pearson correlation coefficients were significant (Table 1). The differences in SVL, body mass, and BMI between control and experimental groups were not significant for the first day of the experiment (Table 2).

Table 3 Results of GLMM for SVL, body mass, and BMI of green toads.

	Estimate	Standard error	df	Z-value	p
SVL					
Intercept	19.537	0.086			
Time	0.082	0.005	16.465	15.879	<0.001*
Exp	0.072	0.121	4.866	0.598	0.577
Time*Exp (fe)	0.046	0.007	16.555	6.223	<0.001*
Individual (r)	<0.001	0.010			
Time (r)	<0.001	<0.001			
Body mass					
Intercept	246.245	7.315			
Time	5.184	0.528	4.768	9.829	<0.001*
Exp	4.938	10.338	12.005	0.478	0.641
Time*Exp (fe)	3.617	0.747	4.782	4.845	0.005*
Individual (r)	15.690	3.961			
Time (r)	0.220	0.469			
BMI					
Intercept	64.209	1.015			
Time	0.563	0.073	4.419	7.746	0.001*
Exp	1.637	1.435	8.347	1.141	0.286
Time*Exp (fe)	0.370	0.103	4.430	3.597	0.019*
Individual (r)	0.483	0.695			
Time (r)	0.005	0.069			

Notes:
SVL, snout-vent length; time, next days of the experiment; Exp, type of treatment (control or experimental group of green toad juveniles); BMI, body mass index; fe, a variable as a fixed effect; r, variables as a random effect.
* Indicates significant variable at $p < 0.05$.

The quadratic effect of time was insignificant for all dependent variables (for all $p > 0.05$); therefore, we dropped these effects from the final models. For all tested parameters (SVL, body mass, and BMI), fixed effects (time and the presence of fleshy fruits) were significant (Table 3). A graphical presentation of changing SVL, body mass, and BMI over time is shown in Fig. 2. The number of active toads did not differ significantly different between enclosures (ANOVA: $F = 0.076$, $p = 0.972$). Invertebrates were first observed in the enclosures containing fruits on Day 4 and occurrence continued through Day 28. In contrast, invertebrates were not observed in control enclosures until Day 10, and on many later occasions afterwards no invertebrates were seen. Table S4 contains the list of taxa noted during the experiment.

DISCUSSION

All of the analysed parameters (SVL, body mass, and BMI index) were greater in individuals from the two experimental groups (with fruits) than from the control groups (without fruits). Thus, the results confirmed our first hypothesis: that post-metamorphs of the green toad kept with fallen fruits (*P. cerasifera*) were characterised
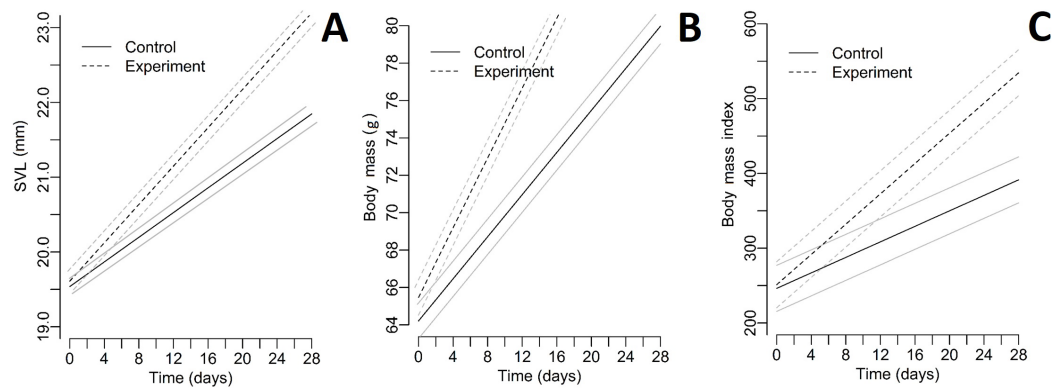
Figure 2 Plots representing the measurements and BMI index of toads during the experiment. (A) SVL, (B) body mass, (C) body mass index. Experiment—groups with fleshy fruits (plums); Control—groups without fleshy fruits (plums); grey line—95% confidence interval; black line—fitted line.

by faster growth than those kept without fruits. We suggest that these differences may be due to the presence of more varied food. Invertebrates are attracted generally by fallen fruits not only plums. All toads had unrestricted access to wild invertebrates, but we observed much more frequent presence of wild invertebrates in the experimental enclosures with fruits (Table S4 in the Supplemental Files). The persistence of ripe fleshy fruits on the ground is strictly dependent on the presence of frugivores and the kind/species of fruit (*Tang, Corlett & Hyde, 2005*). Fallen fruits start to decompose and to become attractive to many taxa of insects, such as Lepidoptera, Hemiptera, Coleoptera, Hymenoptera, or Diptera (*Sallabanks & Courtney, 1992*; *Rodríguez, Alquézar & Peña, 2013*). Fruits, when they fall from trees, are usually superabundant and may limit the intensity of competition between juvenile toads. Similarly, in experimental conditions inside enclosures insects associated with these fruits occur regularly, especially fruit flies *Drosophila* (see Table S4 in the Supplemental Files). Adult *Drosophila* are very numerous and known to be attracted by fruits (*Sallabanks & Courtney, 1992*). In particular, it is worth noting that fruit flies are often the primary ingredient in the diets of young amphibians bred in captivity (*Dugas, Yeager & Richards-Zawacki, 2013*). These invertebrates may also supplement amphibian diets which stimulate growth, especially where a significant decline in the number of insects is observed (which is disturbing, considering the important role of insectivores in the global circulation of matter) (*Hallmann et al., 2017*). Moreover, the great numbers of *P. cerasifera* in the environment and the lack of human interest in their fruits as a resource causes considerable periodic accumulations of fruits on the ground (between July and mid-September). Exactly at the same time, freshly metamorphosed individuals begin to leave breeding ponds in great numbers.

In our study conditions, differences in the numbers of active juvenile green toads between the experimental and control groups were not significant. Therefore, the second hypothesis, that *B. viridis* exhibits more active feeding behaviour in the presence of fleshy fruits than in their absence, could not be confirmed. It is difficult to refer to data on

diet of juvenile toads in natural conditions because this type of information is very scarce and studies have usually been carried out on adults (*Baruš & Oliva, 1992*; *Covaciu-Marcov et al., 2005*, *2010*). Little is known about the diet of juvenile green toads, but it is certainly similar to that preferred by adults, differing mostly in size, which is connected with a change in the foraging strategy during growth (*Kuzmin, 2001*). Adult green toads forage and feed intensively in the summer, unaffected by drought or high temperatures. *Bufotes viridis* consumes almost exclusively terrestrial prey (including flying insects), but also accidentally eats aquatic prey animals, meaning that the green toad can be considered a strong generalist (*Covaciu-Marcov et al., 2005*). Therefore, the insects attracted by fallen fruits can be treated as normal source of food for toads without affecting feeding behaviour. Windfall flashy fruit (in a broader sense, *Malus, Prunus, Pyrus, Cerasus,* and others) may be important in the lives of amphibians within a limited but crucial time period in temperate climate zones (similarly as in the case of mammals; see: *Kurek, 2015*).

This study is characterised by certain limitations. The first is that parameters were not assigned to particular individuals. This approach was carried out due to changes in pattern and colouration during the post-metamorphic growth of *B. viridis*. Similar changes have been observed in other toads, for example, *Epidalea calamita* (*Meyer & Grosse, 1997*). Assigning each parameter to individual toads might yield more information about variations in development dynamics. Therefore, in future, similar studies should also focus on analysis on an individual level in species where the assignment of parameters to particular specimens is possible. The second limitation is that no controls were carried out during the night. Fallen fruits can attract, for example, nocturnal Lepidoptera such as Catocalinae. The green toad is a nocturnal species which can be attracted by artificial lights and which consumes insects near street lamps (*Covaciu-Marcov et al., 2010*). Thus, night control might have yielded information about feeding behaviour and possible prey. On the other hand, additional checks at night might have been stressful and might have negatively influenced the toads' behaviour and body condition. Moreover, the lack of night controls did not influence the obtained results, which showed faster growth in individuals from the experimental group (with plums).

Numerous studies are being undertaken to explain how urbanisation affects species (*Johnson & Munshi-South, 2017*). However, while the need to protect breeding ponds for the preservation of amphibian populations is well documented, it is necessary to conduct research verifying the significance of individual components of the environment, for example, constructions of embankments under tracks, in the life history of particular amphibians (*Kaczmarski & Kaczmarek, 2016*). Similar studies have been carried out on birds (*Morelli et al., 2014*). Intensively urbanised space and intensive-use agricultural and forest areas have become the dominant forms of land use. This leads to the fragmentation of natural environmental patches (*Curado, Hartel & Arntzen, 2011*). Introducing practical knowledge, such as a system of ecological buffers around breeding sites or key habitats, may be an effective solution (*Semlitsch & Bodie, 2003*; *Scheele et al., 2014*).

Fruit trees are an important component of suburban or rural flora, (1) from both an aesthetic (as an ornamental tree) and practical points of view; (2) because they constitute

food resources; and in a broader sense, (3) because they provide ecosystem services (*Giergiczny & Kronenberg, 2014*; *Lafontaine-Messier, Gélinas & Olivier, 2016*; *Larondelle & Strohbach, 2016*). Some fruit tree species can attract pests, for example, rats (*Rattus*), flies (*Musca*), and wasps (*Vespa* and *Vespula*); on the other hand, the appearance of masses of insects attracted by fermenting fruit also affects other predators on invertebrates, such as mammals, birds, and lizards (*Sallabanks & Courtney, 1992*; *Rodríguez, Alquézar & Peña, 2013*). Our study showed that green toad juveniles can also benefit from the presence of invertebrates attracted by fermenting fruits. However, fallen fruits and the species associated with them are treated by city administrators, as well as residents themselves, as a problem (e.g. shade, odour, the need to remove leaves) (*Kronenberg, 2012*) and even as a biological threat (e.g. pathogen vectors, painful stings). In our opinion, fruit trees, along with the insects they periodically attract, should begin to serve as a kind of keystone for urbanised and ruderal areas. This aspect is important, especially since trees are currently being removed from the urban environment as a result of political and social pressure to maintain cleanliness (*Kronenberg, 2012*; *Andrew & Slater, 2014*; *Conway, 2016*).

As far as we know, this study is the first to focus on the indirect role of fruit trees as a source of food for amphibians at a critical moment of their development, that is, after metamorphosis and during the period of intensive growth preceding the first hibernation. This issue suggests several additional possibilities concerning the influence of fallen fruits on growth dynamics on an individual level and the distribution of amphibians in the environment. However, fruit lying on the ground can constitute an ecological trap: amphibians attracted by insects can be eaten by predators, such as wild boars (*Sus scrofa*) feeding on fallen fruits. Similarly, predation by ground beetles (Carabid) may be a threat to group of amphibians feeding on fruits (*Wizen & Gasith, 2011*; *Schaefer et al., 2018*). Particularly important is the question of whether the distribution of fruit trees causes periodic aggregations of amphibians and affects their post-breeding dispersion. If so, properly spaced fruit trees (greenery design) can contribute to reducing the road mortality of amphibians by redirecting them. This kind of hypothetical effect may have enormous potential for the conservation of amphibians. Moreover, in a broader sense, indicating a 'new' function for trees (*sensu stricto*: ecosystem services) may have a positive impact on local biodiversity in urban and rural areas.

CONCLUSION

Toads from experimental enclosures with *P. cerasifera* were characterised by faster growth than individuals from the experimental area without fleshy fruits. The falling fleshy fruits attract many types of invertebrates that can be eaten by toads; therefore, the presence of fruit trees close to a breeding site might influence positively the post-metamorphic growth or dispersion of amphibians. However, it is necessary to test these assumptions in natural conditions and in other amphibian species. No differences in active feeding behaviour were noted between experimental groups with and without *P. cerasifera* fruits, which means that fallen fruits can be treated as an attractor of food (e.g. insects) without affecting feeding behaviour. To our knowledge, no previous study has

investigated the potential influence of the presence of fruit trees on the growth and behaviour of anurans.

ACKNOWLEDGEMENTS

The authors would like to thank the head of Dendrological Garden from the Poznań University of Life Sciences for sharing a place to conduct the experiment, Łukasz Myczko, PhD from the Poznań University of Life Sciences for technical support and Łukasz Jankowiak, PhD from the University of Szczecin for fruitful discussion and help in the statistical analyses. The authors are also grateful Mr Marek Zawadzki from Polish Ecological Club and Foundation on the Guard of Nature for monitoring of the green toad breeding site. In the end, we also thank two reviewers for their useful comments which greatly improved the article.

Funding

Mikołaj Kaczmarski received funding from the National Science Centre research grant no UMO-2017/27/N/NZ8/01996. Anna Maria Kubicka received funding from BIOVEINS 'Connectivity of green and blue infrastructures: living veins for biodiverse and healthy cities' – grant no 2016/22/Z/NZ8/00004. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
National Science Centre research: UMO-2017/27/N/NZ8/01996.
BIOVEINS 'Connectivity of green and blue infrastructures: living veins for biodiverse and healthy cities': 2016/22/Z/NZ8/00004.

Competing Interests

Piotr Tryjanowski is an Academic Editor for PeerJ.

Author Contributions

- Mikołaj Kaczmarski conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Piotr Tryjanowski conceived and designed the experiments, analysed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Anna Maria Kubicka conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e. approving body and any reference numbers):

Permission of Regional Director of Environmental Protection has been obtained (No. WPB—II.6401.346.2017.AC).

Field Study Permissions

The following information was supplied relating to field study approvals (i.e. approving body and any reference numbers):

Experiments were approved by the Regional Director of Environmental Protection (permission no. WPB—II.6401.346.2017.AC).

Data Availability

The raw measurements are provided in the raw data file provided in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.6337#supplemental-information.

REFERENCES

Aghasyan A, Avci A, Tuniyev B, Crnobrnja-Isailovic J, Lymberakis P, Andrén C, Cogalniceanu D, Wilkinson J, Ananjeva N, Üzüm N, Orlov N, Podloucky R, Tuniyev S, Kaya U, Stöck M, Khan M, Kuzmin S, Tarkhnishvili D, Ishchenko V, Papenfuss T, Degani G, Ugurtas I, Rastegar-Pouyani N, Disi A, Anderson S, Beebee T, Andreone F. 2015. Bufotes viridis (errata version published in 2016). *The IUCN Red List of Threatened Species* **2015**: e.T155333A86444583 DOI 10.2305/IUCN.UK.2015-1.RLTS.T155333A74514442.en.

Andrew C, Slater D. 2014. Why some UK homeowners reduce the size of their front garden trees and the consequences for urban forest benefits as assessed by i-Tree ECO. *Arboricultural Journal: The International Journal of Urban Forestry* **36(4)**:197–215 DOI 10.1080/03071375.2014.994388.

Baruš V, Oliva O. 1992. *Obojžživelníci (Amphibia)*. Praha: Academia.

Bates D. 2010. *lme4: mixed-effects modeling with R*. New York: Springer.

Beebee TJC. 2014. Amphibian conservation in Britain: a 40-Year History. *Journal of Herpetology* **48(1)**:2–12 DOI 10.1670/12-263.

Bieber AGD, Silva PSD, Oliveira PS. 2013. Attractiveness of fallen fleshy fruits to ants depends on previous handling by frugivores. *Écoscience* **20(1)**:85–89 DOI 10.2980/20-1-3573.

Brühl CA, Schmidt T, Pieper S, Alscher A. 2013. Terrestrial pesticide exposure of amphibians: an underestimated cause of global decline? *Scientific Reports* **3(1)**:1135 DOI 10.1038/srep01135.

Clark KH, Nicholas KA. 2013. Introducing urban food forestry: a multifunctional approach to increase food security and provide ecosystem services. *Landscape Ecology* **28(9)**:1649–1669 DOI 10.1007/s10980-013-9903-z.

Colic S, Zec G, Marinkovic D, Jankovic Z. 2003. Genetic and phenotypic variability of cherry plum (*Prunus cerasifera* Ehrh) pomological characteristics. *Genetika* **35(3)**:155–160 DOI 10.2298/GENSR0303155C.

Conway TM. 2016. Tending their urban forest: residents' motivations for tree planting and removal. *Urban Forestry & Urban Greening* **17**:23–32 DOI 10.1016/j.ufug.2016.03.008.

Covaciu-Marcov SD, Cupşa D, Ferenti S, David A, Dimancea N. 2010. Human influence or natural differentiation in food composition of four amphibian species from Histria fortress, Romania? *Acta Zoologica Bulgarica* **62(3)**:307–313.

Covaciu-Marcov S-D, Sas I, Cupşa D, Rois R, Bogdan H. 2005. The trophic spectrum of a population of *Bufo viridis* (Amphibia) from the campus of the University of Oradea, Romania. *Biota* **6**:5–12.

Curado N, Hartel T, Arntzen JW. 2011. Amphibian pond loss as a function of landscape change—a case study over three decades in an agricultural area of northern France. *Biological Conservation* **144(5)**:1610–1618 DOI 10.1016/j.biocon.2011.02.011.

Denoël M. 2012. Newt decline in Western Europe: highlights from relative distribution changes within guilds. *Biodiversity and Conservation* **21(11)**:2887–2898 DOI 10.1007/s10531-012-0343-x.

Denoël M, Perez A, Cornet Y, Ficetola GF. 2013. Similar local and landscape processes affect both a common and a rare newt species. *PLOS ONE* **8(5)**:e62727 DOI 10.1371/journal.pone.0062727.

Dugas MB, Yeager J, Richards-Zawacki CL. 2013. Carotenoid supplementation enhances reproductive success in captive strawberry poison frogs (*Oophaga pumilio*). *Zoo Biology* **32(6)**:655–658 DOI 10.1002/zoo.21102.

Dylewski Ł, Kurek P, Wiatrowska B, Jerzak L, Tryjanowski P. 2017. Man-made perching sites—electricity pylons accelerate fleshy-fruited plants succession in farmlands. *Flora* **231**:51–56 DOI 10.1016/j.flora.2017.04.004.

Elżanowski A, Ciesiołkiewicz J, Kaczor M, Radwańska J, Urban R. 2009. Amphibian road mortality in Europe: a meta-analysis with new data from Poland. *European Journal of Wildlife Research* **55(1)**:33–43 DOI 10.1007/s10344-008-0211-x.

Ensabella F, Loriga S, Formichetti P, Isotti R, Sorace A. 2003. Breeding site selection of *Bufo viridis* in the city of Rome (Italy). *Amphibia-Reptilia* **24(3)**:396–400 DOI 10.1163/156853803322440853.

Fadzly N, Burns KC. 2010. What weta want: colour preferences of a frugivorous insect. *Arthropod-Plant Interactions* **4(4)**:267–276 DOI 10.1007/s11829-010-9109-0.

Ficetola GF, Rondinini C, Bonardi A, Baisero D, Padoa-Schioppa E. 2015. Habitat availability for amphibians and extinction threat: a global analysis. *Diversity and Distributions* **21(3)**:302–311 DOI 10.1111/ddi.12296.

Finke M, Winn D. 2004. Insects and related arthropods: a nutritional primer for rehabilitators. *Journal of Wildlife Rehabilitation* **27(3)**:14–27.

Giergiczny M, Kronenberg J. 2014. From valuation to governance: using choice experiment to value street trees. *Ambio* **43(4)**:492–501 DOI 10.1007/s13280-014-0516-9.

Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, Stenmans W, Müller A, Sumser H, Hörren T, Goulson D, De Kroon H. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLOS ONE* **12(10)**:e0185809 DOI 10.1371/journal.pone.0185809.

Hartel T, Sas I, Pernetta A, Geltsch IC. 2010. The reproductive dynamics of temperate amphibians: a review. *North-Western Journal of Zoology* **3**:127–145.
Hocking D, Babbitt K. 2014. Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology* **9(1)**:1–17.

IUCN. 2018. *The IUCN red list of threatened species.* Version 2018-1. *Available at* http://www.iucnredlist.org (accessed 6 November 2018).

Johnson MTJ, Munshi-South J. 2017. Evolution of life in urban environments. *Science* **358(6363)**:eaam8327 DOI 10.1126/science.aam8327.

Jordaan LA, Johnson SD, Downs CT. 2011. The role of avian frugivores in germination of seeds of fleshy-fruited invasive alien plants. *Biological Invasions* **13(8)**:1917–1930 DOI 10.1007/s10530-011-0013-z.

Jordano P. 1994. Spatial and temporal variation in the Avian-Frugivore assemblage of Prunus Mahaleb: patterns and consequences. *Oikos* **71(3)**:479–491 DOI 10.2307/3545836.

Kaczmarski M, Kaczmarek JM. 2016. Heavy traffic, low mortality—tram tracks as terrestrial habitat of newts. *Acta Herpetologica* **11(2)**:227–231.

Kaczmarski M, Szala K, Kloskowski J. Early onset of breeding season in the green toad *Bufotes viridis* in Western Poland. *Herpetozoa* (in press) **32**.

Kovács É, Sas I. 2010. Aspects of breeding activity of *Bufo viridis* in an urban habitat: a case study in Oradea, Romania. *Biharean Biologist* **4(1)**:73–77.

Kronenberg J. 2012. Barriers to preserving urban trees and ways of overcoming them. *Sustainable Development Applications* **3**:31–49.

Kurek P. 2015. Consumption of fleshy fruit: are central European carnivores really less frugivorous than southern European carnivores? *Mammalian Biology* **80(6)**:527–534 DOI 10.1016/j.mambio.2015.10.001.

Kuzmin SL. 2001. *Bufotes viridis: green Toad. Available at* http://amphibiaweb.org/species/312 (accessed 1 July 2018).

Labocha MK, Schutz H, Hayes JP. 2014. Which body condition index is best? *Oikos* **123(1)**:111–119 DOI 10.1111/j.1600-0706.2013.00755.x.

Lafontaine-Messier M, Gélinas N, Olivier A. 2016. Profitability of food trees planted in urban public green areas. *Urban Forestry & Urban Greening* **16**:197–207 DOI 10.1016/j.ufug.2016.02.013.

Larondelle N, Strohbach MW. 2016. A murmur in the trees to note: urban legacy effects on fruit trees in Berlin, Germany. *Urban Forestry & Urban Greening* **17**:11–15 DOI 10.1016/j.ufug.2016.03.005.

Lázaro A, Mark S, Olesen JM. 2005. Bird-made fruit orchards in northern Europe: nestedness and network properties. *Oikos* **110(2)**:321–329 DOI 10.1111/j.0030-1299.2005.13500.x.

Lim VC, Clare EL, Littlefair JE, Ramli R, Bhassu S, Wilson JJ. 2017. Impact of urbanisation and agriculture on the diet of fruit bats. *Urban Ecosystems* **21(1)**:61–70 DOI 10.1007/s11252-017-0700-3.

Majkowska A, Kolendowicz L, Półrolniczak M, Hauke J, Czernecki B. 2017. The urban heat island in the city of Poznań as derived from Landsat 5 TM. *Theoretical and Applied Climatology* **128(3–4)**:769–783 DOI 10.1007/s00704-016-1737-6.

Mc Williams DA. 2008. *Nutrition recommendations for some captive amphibian species (Anura and Caudata).* Guelph: GARN-NARG.

Meyer F, Grosse W. 1997. Populationsökologische Studien an Amphibien mit Hilfe der fotografischen Individualerkennung: Übersicht zur Methodik und Anwendung bei der Kreuzkröte (Bufo calamita). *Mertensiella* **7**:79–92.

Mollov I. 2011. Habitat distribution of the amphibians and reptiles in the city of Plovdiv, Bulgaria. *Biharean Biologist* **5(1)**:25–31.

Morelli F, Beim M, Jerzak L, Jones D, Tryjanowski P. 2014. Can roads, railways and related structures have positive effects on birds?—A review. *Transportation Research Part D: Transport and Environment* **30**:21–31 DOI 10.1016/j.trd.2014.05.006.

Nikolic D, Rakonjac V. 2007. Divergence of myrobalan (*Prunus cerasifera* Ehrh.) types on the territory of Serbia. *Genetika* **39(3)**:333–342 DOI 10.2298/gensr0703333n.

Ogielska M, Kierzkowski P. 2010. Long term data on the amphibians of Wrocław. *Fragmenta Faunistica* **53(2)**:195–212 DOI 10.3161/00159301ff2010.53.2.195.

Orłowski G, Nowak L. 2007. The importance of marginal habitats for the conservation of old trees in agricultural landscapes. *Landscape and Urban Planning* **79(1)**:77–83 DOI 10.1016/j.landurbplan.2006.03.005.

Perini TA, Oliveira GL, Ornellas JDS, Oliveira FP. 2005. Technical error of measurement in anthropometry. *Revista Brasileira de Medicina do Esporte* **11**:86–90.

Petrovan SO, Schmidt BR. 2016. Volunteer conservation action data reveals large-scale and long-term negative population trends of a widespread amphibian, the common toad (*Bufo bufo*). *PLOS ONE* **11(10)**:e0161943 DOI 10.1371/journal.pone.0161943.

Pietrzak C, Steindorff de Arruda JL, Cechin SZ. 2014. Frugivory and seed dispersal by Tropidures torquatus. *Herpetological Journal* **23**:75–79.

Plieninger T, Schleyer C, Mantel M, Hostert P. 2012. Is there a forest transition outside forests? Trajectories of farm trees and effects on ecosystem services in an agricultural landscape in Eastern Germany. *Land Use Policy* **29(1)**:233–243 DOI 10.1016/j.landusepol.2011.06.011.

R Development Core Team. 2008. *R: a language and environment for statistical computing.* Vienna: The R Foundation for Statistical Computing. *Available at* http://www.R-project.org.

Rodríguez A, Alquézar B, Peña L. 2013. Fruit aromas in mature fleshy fruits as signals of readiness for predation and seed dispersal. *New Phytologist* **197(1)**:36–48 DOI 10.1111/j.1469-8137.2012.04382.x.

Saenz D, Hall TL, Kwiatkowski MA. 2014. Effects of urbanization on the occurrence of *Batrachochytrium dendrobatidis*: do urban environments provide refuge from the amphibian chytrid fungus? *Urban Ecosystems* **18(1)**:333–340 DOI 10.1007/s11252-014-0398-4.

Sallabanks R, Courtney SP. 1992. Frugivory, seed predation, and insect-vertebrate interactions. *Annual Review of Entomology* **37(1)**:377–400 DOI 10.1146/annurev.en.37.010192.002113.

Schaefer AM, Schaefer F, Wagner T, Sinsch U. 2018. Carabid predation on *Bombina variegata* metamorphs: size at and timing of metamorphosis matter. *Salamandra* **54(3)**:222–228.

Scheele BC, Boyd CE, Fischer J, Fletcher AW, Hanspach J, Hartel T. 2014. Identifying core habitat before it's too late: the case of *Bombina variegata*, an internationally endangered amphibian. *Biodiversity and Conservation* **23(3)**:775–780 DOI 10.1007/s10531-014-0624-7.

Schmidt BR, Hodl W, Schaub M. 2012. From metamorphosis to maturity in complex life cycles: equal performance of different juvenile life history pathways. *Ecology* **93(3)**:657–667 DOI 10.1890/11-0892.1.

Semlitsch RD, Bodie JR. 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology* **17(5)**:1219–1228 DOI 10.1046/j.1523-1739.2003.02177.x.

Sinsch U, Schäfer AM. 2016. Density regulation in toad populations (*Epidalea calamita*, *Bufotes viridis*) by differential winter survival of juveniles. *Journal of Thermal Biology* **55**:20–29 DOI 10.1016/j.jtherbio.2015.11.007.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues SL, Fischman DL, Waller WW. 2005. Status and trends of amphibian declines and extinctions worldwide. *Science* **309(5743)**:1999c DOI 10.1126/science.1113265.

Suhonen J, Jokimäki J. 2015. Fruit removal from rowanberry (*Sorbus aucuparia*) trees at urban and rural areas in Finland: a multi-scale study. *Landscape and Urban Planning* **137**:13–19 DOI 10.1016/j.landurbplan.2014.12.012.

Suhonen J, Jokimäki J, Lassila R, Kaisanlahti-jokimäki M. 2017. Effects of roads on fruit crop and removal rate from rowanberry trees (*Sorbus aucuparia*) by birds in urban areas of Finland. *Urban Forestry & Urban Greening* **27**:148–154 DOI 10.1016/j.ufug.2017.08.001.

Tang AMC, Corlett RT, Hyde KD. 2005. The persistence of ripe fleshy fruits in the presence and absence of frugivores. *Oecologia* **142(2)**:232–237 DOI 10.1007/s00442-004-1730-0.

Virgós E, Cabezas-Díaz S, Mangas JG, Lozano J. 2010. Spatial distribution models in a frugivorous carnivore, the stone marten (*Martes foina*): is the fleshy-fruit availability a useful predictor? *Animal Biology* **60(4)**:423–436 DOI 10.1163/157075610X523297.

Werner EE. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *American Naturalist* **128(3)**:319–341 DOI 10.1086/284565.

Wizen G, Gasith A. 2011. An unprecedented role reversal: ground beetle larvae (Coleoptera: Carabidae) lure amphibians and prey upon them. *PLOS ONE* **6(9)**:e25161 DOI 10.1371/journal.pone.0025161.

Zawadzki M, Flesch A, Kaczmarski M. 2017. New breeding site of the green Toad *Bufotes viridis* Laurenti, 1768 in the downtown of Poznań. *Przegląd Przyrodniczy* **28**:109–114.

Zohary D, Hopf M. 2000. *Domestication of plants in the old world.* Vol. 531. Third Edition. Oxford: University Press, 206.