

Article

Advertisement Call Variation of Two Frog Species along an Urban–Rural Gradient in Shanghai, China

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Abstract: Urbanization has a significant influence on amphibian communities, populations, and individuals because of habitat loss, degradation of habitat quality, and habitat fragmentation. Advertisement calls of anurans are used for communication and play an important role in reproduction; however, how anthropogenic noise and habitat change caused by urbanization affect anuran advertisement calls is less well known. In this study, we examined changes in the advertisement calls of two frog species, the ornamented pygmy frog (*Microhyla fissipes*) and rice frog (*Fejervarya multistriata*), during the breeding season along an urban–rural gradient in Shanghai, China. We used the percentage of impervious area (including buildings and roads) in each 2 km-radius landscape to represent the urbanization index of 34 study sites. We then measured five advertisement call parameters (call duration, call interval, call rate, pulse rate, and dominant frequency) of these two species (100 males of *F. multistriata* and 89 males of *M. fissipes*) in each study site from May to July in 2020 and 2021. We explored how the urbanization index and other variables (air temperature, snout–vent length and calling situation (one frog species calling, or two frog species calling together)) in each study site affected these call parameters. The results showed that the dominant frequency of *M. fissipes* was significantly negatively related to urbanization index, and call duration and call interval were significantly negatively related to air temperature; furthermore, call rate and pulse rate were significantly positively related to air temperature, and call duration was also significantly positively related to snout–vent length. For *F. multistriata*, pulse rate was significantly negatively related to urbanization index, snout–vent length, and situation (two frog species calling together), but significantly positively related to air temperature; by contrast, call interval was significantly positively related to snout–vent length. We suggested that these two frog species had different responses to urbanization, which could help us to better understand the change of anuran breeding behaviors and reproductive life-history strategies in the face of rapid urbanization.

Keywords: anuran; behavior change; reproductive strategies; sound communication; urbanization



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1. Introduction

A major hallmark of the Anthropocene is the rapid transformation of more natural biomes into human-dominated landscapes because of rapid population growth and accelerated urbanization [1,2], with the latter having a significant impact on biodiversity [3–5]. Rapid urbanization has resulted in phenotypic, genetic trait, and breeding behavior changes in species in response to new environments [6,7], with variations in acoustic signals being

the most obvious. Many insects, anurans, birds, and mammals use acoustic signals to attract mates and protect their territory [8,9]. However, anthropogenic noise, anthropogenic substrate-borne vibrations, light pollution, and habitat change resulting from urbanization affect the production, transmission, and reception of acoustic signals [10–13]. For example, studies reported that birds vocalized at higher dominant frequencies and sound levels in urban areas than in forests because of background noise [14–16]. Kempenaers et al. [17] also found that, in areas with artificial light, the dawn chorus of blue tits (*Cyanistes caeruleus*) started earlier compared with birds in naturally dark areas.

Amphibians are facing a rapid decline worldwide. The latest International Union for Conservation of Nature (IUCN) Red List reported that 41% of amphibian species are threatened with extinction, rendering them the most threatened vertebrates compared with mammals (26%) and birds (14%) [18]. Habitat loss, degradation of habitat quality, and habitat fragmentation have a significant negative impact on amphibian communities, populations, and individuals [19]. For example, Gagné and Fahrig [20] reported that frog species richness in urban landscapes was lower than in forested and agricultural landscapes in Canada. Similarly, Lourenço-de-Moraes et al. [21] found that anuran communities in the urban matrix are nested subsets of non-urban matrix sites.

Acoustic signals are the main form of communication of most adult male frogs and have an important role in sexual selection and reproductive behaviors [22,23]. The signals of frogs are usually divided into three types (reproductive, aggressive, and defensive), with advertisement calls being a form of reproductive signal that are usually considered species specific [24,25]. Most research focuses on spectral parameters (e.g., dominant frequency (DF)) and temporal parameters (including call duration (CD), call interval (CI), call rate (CR), and pulse rate (PR)) of advertisement calls in anurans [24].

The environment context and habitat structure can constrain acoustic transmission when long-distance sound communication is used [26] and, according to the acoustic adaptation hypothesis (AAH) [27], animals change their acoustic signals to improve their transmission through the environment. Increasing anthropogenic noise caused by urbanization is the main factor affecting advertisement calls of anurans [28]. Many studies found that anthropogenic noise had a significant effect on frog-calling parameters and behaviors in the urban environment. For example, Higham et al. [10] found that traffic noise increased the call pitch of brown tree frogs (*Litoria ewingii*) in urban areas. Similarly, anthropogenic noise in urban areas also altered the call rate or call duration of frog species [29–32]. Vargas-Salinas et al. [33] found that American toads (*Anaxyrus americanus*) and gray tree frogs (*Hyla versicolor*) changed their calling behavior (gap calling) to ameliorate the effects of traffic noise. In addition, habitat change caused by urbanization also influences frog call parameters. For example, Tan et al. [12] found that human-made drainage ditches in urban areas increased the call intensity and call duration of Mientien tree frogs (*Kurixalus idiootocus*). Ambient temperature also impacts the parameters of advertisement calls in many frog species, especially their temporal parameters, including CD, CI, CR, and PR [34,35]. Additionally, the spectral parameter (DF) of some frog species is constrained by their body size [36,37].

Our previous studies indicated that habitat loss and fragmentation caused by urbanization had significant effects on the population dynamics [38–40], body size [41], and genetic diversity [42] of frogs in Shanghai, which is the most-urbanized city in China; however, it is less clear whether frogs have altered advertisement calls in response to anthropogenic noise and habitat change caused by urbanization. In this study, we recorded the advertisement calls of two frog species (*Fejervarya multistriata* and *Microhyla fissipes*) in 34 study sites along an urban–rural gradient from May to July of 2020 and 2021 in Shanghai, analyzing five characters of advertisement calls: temporal parameters, including CD, CI, CR, and PR; and a spectral parameter, DF. We also measured air temperature, individual snout–vent length, and calling situation (one frog species calling or two frog species calling together), which have previously been considered as potential variables influencing frog advertisement calls. We aimed to explore how the urbanization index (UI) and other variables (air temperature,

snout–vent length, and calling situation) affected these call parameters. We predicted that the spectral parameter (DF) and some temporal parameters (CD, CR, and PR) of the two frog species would increase, whereas CI would decrease along the urban–rural gradient in Shanghai. By increasing the spectral parameter, frogs are able to reduce the frequency overlap between calls and anthropogenic noise [30]; additionally, by increasing some temporal parameters (CD, CR, and PR) while decreasing CI, they are able to increase call detectability by conspecifics [43]. The results of this study will help us to better understand changes in anuran breeding behaviors and reproductive life-history strategies in the face of rapid urbanization.

2. Methods and Materials

2.1. Study Area and Study Sites

Shanghai ($30^{\circ}40''$ N to $31^{\circ}53''$ N and $120^{\circ}51''$ E to $122^{\circ}12''$ E) is located in eastern China in the southeastern Yangtze River Delta, with a total area of 6340.5 km² and a population of 24.3 million [44]. It is one of the largest cities in the world and continues to undergo rapid urbanization [44,45]. Shanghai has many rivers, lakes, and paddy rice fields that are suitable for breeding amphibians [39]. We investigated 22 and 20 study sites for *M. fissipes* and *F. multistriata*, respectively, from May to July in 2020 and 2021 (*M. fissipes*: 14 study sites; *F. multistriata*: 12 study sites, with another eight sympatric study sites) (Figure 1). The distance between each site was >1 km to ensure spatial independence [46,47].

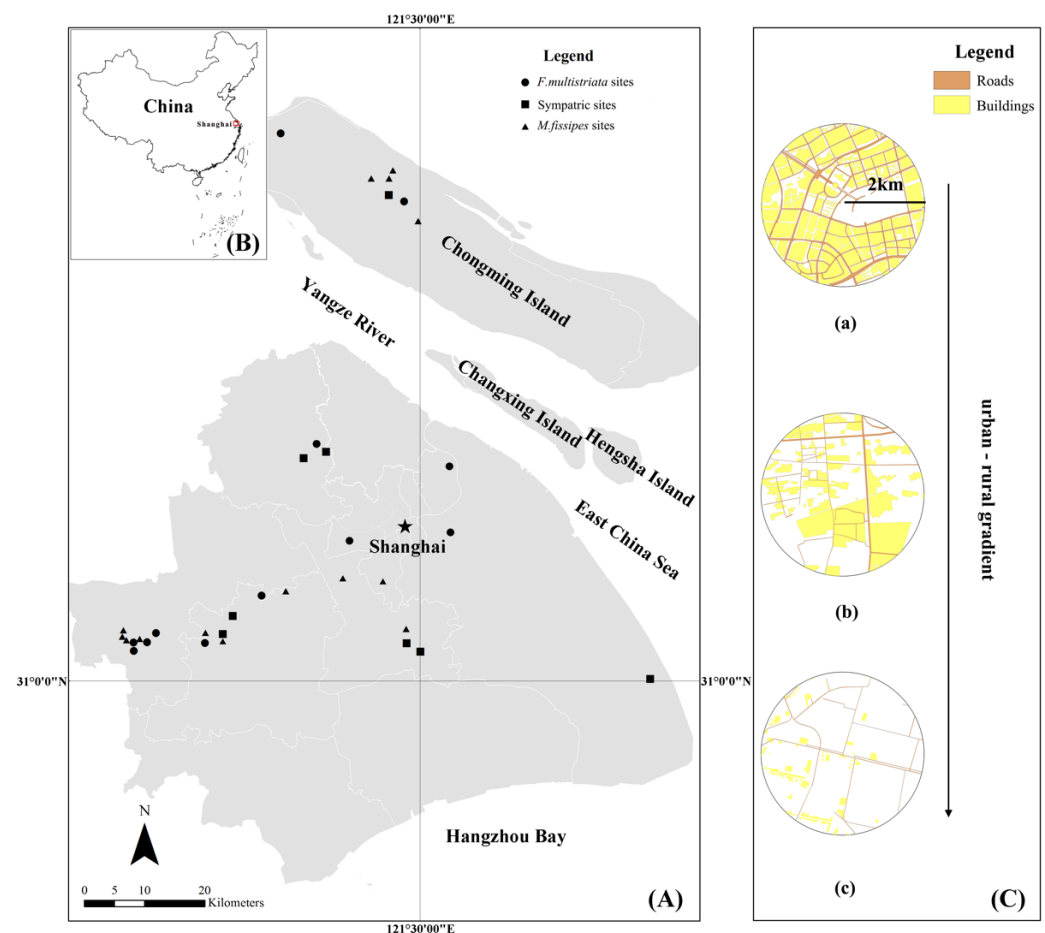


Figure 1. Locations of the 34 study sites along an urban–rural gradient in Shanghai (A), China (B). In total, 22 and 20 study sites were used for *M. fissipes* and *F. multistriata*, respectively, with eight sympatric sites. Three examples of study sites within a 2 km radius of different UI sites along an urban–rural gradient in Shanghai (a: 0.812; b: 0.469; c: 0.042) (C).

2.2. Study Species

In this study, we focused on the ornamented pygmy frog (*M. fissipes*) and rice frog (*F. multistriata*), which are abundant species inhabiting both urban and rural areas in Shanghai and, thus, provide a suitable number of frogs for use in this study. However, over the past several decades, the richness and abundance of both species in Shanghai have decreased rapidly because of urbanization [39,40]. The ornamented pygmy frog inhabits paddy fields, pools, and grasses and is distributed across southeast China, Sri Lanka, Myanmar, and South Asia. The rice frog lives in paddy fields, stagnant water (e.g., swamps, ponds, and ditches), and non-irrigated farmland south of the Yellow River in China and some areas in Southeast Asia. Both frog species breed from April to September each year, especially after rainfall [48], and their advertisement call parameters have been reported previously [36,37].

2.3. Urbanization Index

To define the urbanization level of each study site, we obtained landscape data within a 2 km radius of each study site from satellite images of Formosat-2 (June 2012; 2 m resolution) and Google Earth Pro 7.3.2 (Google), and combined aerial photographs with ground surveys to distinguish the land-use types if they were hard to define [39,42]. The land-use types determined included buildings (including commercial buildings, residential buildings, parking lots, and other anthropogenic building sites) and roads (including highways, arterial roads, elevated ring-roads, and other hardened roads) (Figure 1); the percentage of each land-use type in each study site was calculated using ArcMap 10.3 (Environment Systems Research Institute) and Fragstats 4.2 [49] (Table S1). The percentage of impervious area (including buildings and roads) in each study site was then scaled to a score between 0 and 1 (i.e., subtracting the minimum value and dividing by the range) as the UI: the higher the UI, the more urbanized the study site [20,38,50].

2.4. Field Recordings

The advertisement calls of individual males from the two frog species were recorded between 19:00 and 24:00 from May to July in 2020 and 2021 during their breeding season. The call of each individual male frog was recorded for at least 5 min, using a directional microphone (Sennheiser ME66, Vidmark, Germany) attached to a field recorder (Marantz PMD661, Shirakawa, Japan) with a sampling rate of 44.1 kHz and 16-bit resolution [33,37]. The distance to each male frog was 0.5–1 m [36,51]. All calling frogs were recorded as being on land in this study.

At each study site, we also measured air temperature, the SVL of each frog individually, and the calling situation (only one frog species calling (*M. fissipes*: 14 study sites; *F. multistriata*: 12 study sites), or two species calling together (eight sympatric study sites)), which have previously been considered as potential variables influencing frog advertisement calls [36,52]. After field recordings, each individual calling male was captured and their SVL measured with electronic digital calipers (DL91150, Ningbo, China) (± 0.01 mm). Air temperature was measured at the position of the calling males by using a digital temperature meter (HYELEC MS6252B, Dongguan, China) (± 0.1 °C).

No frogs were harmed during this study and all were released after these measurements were taken.

2.5. Bioacoustics Analysis

We transferred field recordings from the field recorder to a computer with Adobe Audition 2020 (version 13.0) [24]. To reduce the background noise, a black background sound recording of each recording was used as a template for overall noise reduction [36]. The edited files were saved in a “wav” format. We then used Praat 6.1.08 [53] to analyze the parameters of the advertisement calls (Figure 2) by using a spectral parameter, including DF, and temporal parameters, including CD, CI, CR, and PR (Table S2). These parameters were defined and illustrated previously by Kohler et al. [24] and Ziegler et al. [54] (Table 1).

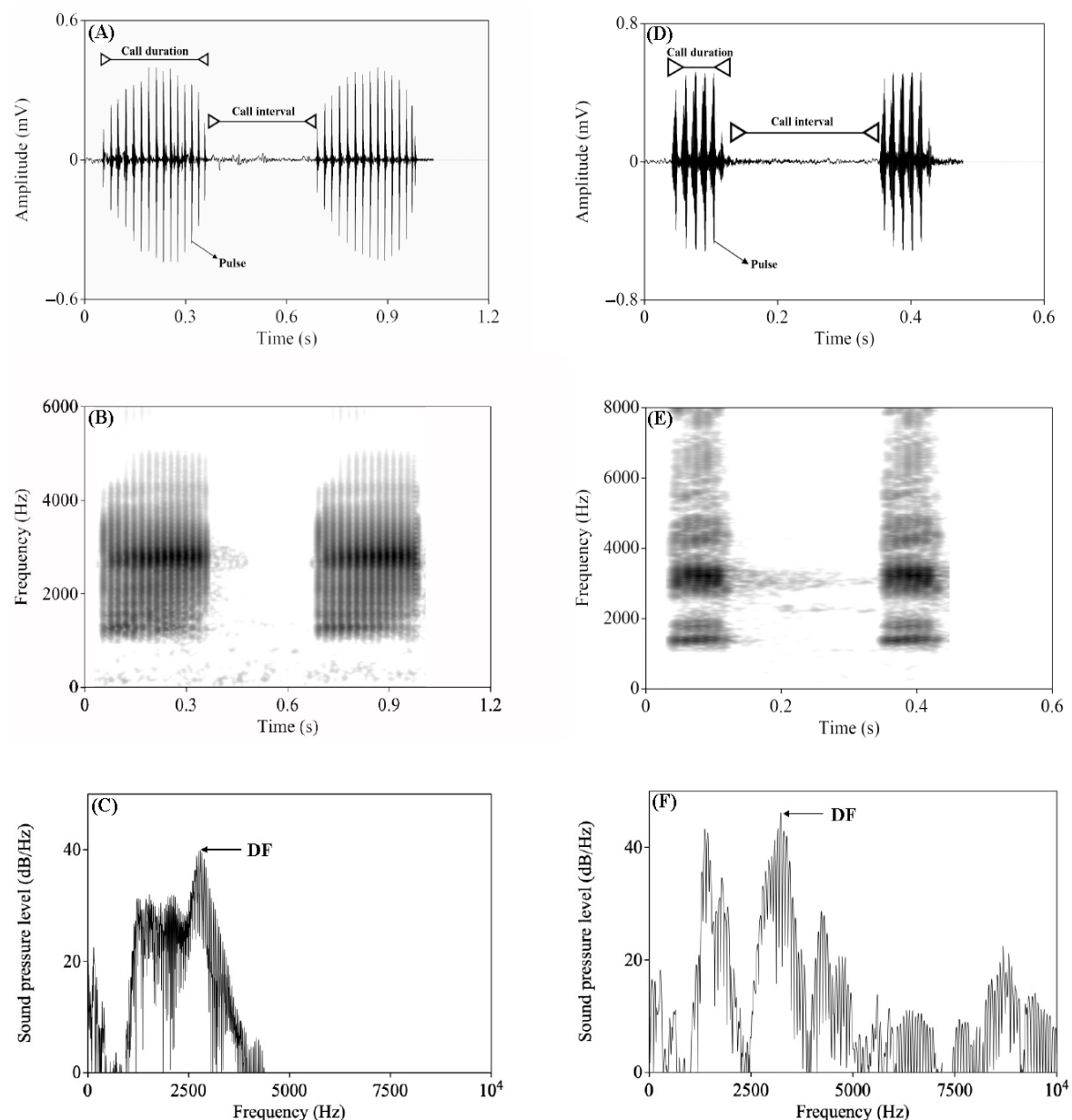


Figure 2. Oscillogram (A), spectrogram (B), and power spectrum (C) of a 1.2 s call of *M. fissipes*, and oscillogram (D), spectrogram (E), and power spectrum (F) of a 0.6 s call of *F. multistriata*.

Table 1. Description of frog advertisement call parameters used in the current study.

Frog Call Parameter	Definition
Call duration (CD)	Duration of a single call, regardless of whether comprising single or multiple notes; measured from beginning to the end of the call (in s).
Call interval (CI)	Interval between two consecutive calls, measured from the end of the call to the beginning of the next call (in s).
Call rate (CR)	Number of calls emitted in 1 min (calls/min).
Pulse rate (PR)	Number of pulses repeated in 1 s within a note (pulses/s).
Dominant frequency (DF)	Peak frequency of the call (i.e., the frequency containing the highest sound energy, in Hz).

2.6. Statistical Analyses

We selected ten advertisement calls per call sequence, which were used to calculate the mean of advertisement call parameters for each male frog; the parameters of the

advertisement calls were calculated based on the mean of these parameters from all frog individuals at each study site [24]. A general linear model (GLM) was used to test the effects of UI and other variables (air temperature, SVL, and calling situation) on advertisement call parameters (i.e., CD, CI, CR, PR, and DF) in each study site. We included five of the advertisement call parameters as response variables and four variables as predictor variables (UI, air temperature, and SVL were continuous variables, whereas calling situation was a categorical variable). The normality of advertisement call parameters was tested using the Shapiro–Wilk test. For the advertisement call parameters of *M. fissipes* and *F. multistriata*, we used a Gaussian distribution with an identity link function. Call rate of *F. multistriata* was $\log_{10}$ transformed to fit the model assumptions.

To ensure predictor variables were independent, we estimated their variance inflation factors (VIFs). A VIF > 4 indicated a possible collinearity [55,56].

We ranked all possible candidate models based on their Akaike information criterion value with a correction (AICc) for small sample size [57]. All models with the $\Delta\text{AICc} < 2$ were considered to be equally suitable for making inferences. We also calculated Akaike weights (W_i), which can be used to estimate the probability that any model is the best model among the entire candidate models. The summed W_i was calculated to measure the probability and the model-average coefficients [57]. If the null model was analyzed as the model with the lowest AICc in the model sets with $\Delta\text{AICc} < 2$, all other models were disregarded.

All statistical analyses were performed using R 4.0.2 [58]. The “glmulti” [59] and “MuMIn” [60] packages were used for model selection and averaging. Differences were significant at $p < 0.05$.

3. Results

In total, 100 male *M. fissipes* and 89 male *F. multistriata* were recorded. The percentage of impervious area of the study sites ranged from 3.05% to 79.77% (mean 27.66 ± 23.29 SD) (Table S1). We also determined the call parameters of the two frog species at each study site (Table 2). The air temperature during the recordings ranged from 21.4 °C to 29.7 °C for *M. fissipes* and from 22.3 °C to 28.9 °C for *F. multistriata* (Table S2). The SVL of *M. fissipes* ranged from 19.11 mm to 26.83 mm (mean 22.95 ± 1.59 SD) in *M. fissipes*, and from 32.79 mm to 44.07 mm (mean 38.48 ± 2.15 SD) in *F. multistriata* (Table S3).

Table 2. Advertisement call parameters of *M. fissipes* and *F. multistriata* in 34 study sites along an urban–rural gradient in Shanghai.

Frog Call Parameter	Species	Min–Max	Mean	SD	Sample Size
Call duration (s)	<i>M. fissipes</i> (N = 22)	0.204–0.346	0.284	0.041	100
	<i>F. multistriata</i> (N = 20)	0.131–0.338	0.225	0.062	89
Call interval (s)	<i>M. fissipes</i> (N = 22)	0.305–0.646	0.443	0.099	100
	<i>F. multistriata</i> (N = 20)	0.067–0.117	0.086	0.011	89
Call rate (calls/min)	<i>M. fissipes</i> (N = 22)	68.703–119.648	93.070	16.308	100
	<i>F. multistriata</i> (N = 20)	182.683–340.424	240.573	44.049	89
Pulse rate (pulses/s)	<i>M. fissipes</i> (N = 22)	42.739–72.172	53.577	7.809	100
	<i>F. multistriata</i> (N = 20)	69.483–95.361	81.704	8.145	89
Dominant frequency (Hz)	<i>M. fissipes</i> (N = 22)	1854.546–3208.715	2560.587	327.888	100
	<i>F. multistriata</i> (N = 20)	1402.905–2693.004	2034.459	408.559	89

The VIFs for the predictor variables were all < 4, which suggested no severe collinearity between the four predictor variables in the analysis (Table S4).

Air temperature and SVL were two main predictors in the top two models ($\Delta\text{AICc} < 2$) for CD of *M. fissipes* (Table S5). Model average coefficients showed that the CD of *M. fissipes* was significantly negatively related to air temperature (estimated mean $\pm$ standard error (SE) = -0.012 ± 0.003 , 95% confidence interval (CI) [-0.018 , -0.005], $p < 0.001$), but was significantly positively correlated to SVL (estimated mean $\pm$ SE = 0.016 ± 0.007 , 95% CI

[0.002, 0.030], $p = 0.026$) (Table 3). Air temperature was the most important predictor in the top three models ($\Delta\text{AICc} < 2$) for CI of *M. fissipes* (Table S5). Model average coefficients showed that CI of *M. fissipes* was significantly negatively correlated to air temperature (estimated mean $\pm$ SE = -0.033 ± 0.009 , 95% CI $[-0.050, -0.016]$, $p < 0.001$) (Table 3). Air temperature was the most important predictor in the top two models ($\Delta\text{AICc} < 2$) for CR of *M. fissipes* (Table S5). Model average coefficients showed that CR of *M. fissipes* was significantly positively correlated to air temperature (estimated mean $\pm$ SE = 5.608 ± 1.382 , 95% CI $[2.899, 8.318]$, $p < 0.001$) (Table 3). Air temperature was the most important predictor in the top two models ($\Delta\text{AICc} < 2$) for PR of *M. fissipes* (Table S5). Model average coefficients showed that PR of *M. fissipes* was significantly positively correlated to air temperature (estimated mean $\pm$ SE = 2.970 ± 0.536 , 95% CI $[1.920, 4.021]$, $p < 0.001$) (Table 3). UI was the most important predictor in the top four models ($\Delta\text{AICc} < 2$) for DF of *M. fissipes* (Table S5). Model average coefficients showed that DF of *M. fissipes* was significantly negatively correlated to UI (estimated mean $\pm$ SE = -631.460 ± 212.250 , 95% CI $[-1047.458, -215.453]$, $p = 0.003$) (Table 3).

Table 3. Model-averaged estimates of the coefficient of predictors in the best models ($\Delta\text{AICc} < 2$) between advertisement call parameters and variables (urbanization index, air temperature, snout–vent length, calling situation). Estimated coefficients, standard errors (SE), upper and lower 95% confidence intervals (CIs) and p value (P) in the most parsimonious models are shown for each species. Bold type indicates significant variables.

Species	Call Parameter	Variable	Estimate	SE	Lower 95% CI	Upper 95% CI	p
<i>M. fissipes</i> (N = 22)	Call duration	Intercept	0.224	0.202	−0.171	0.620	0.266
		Air temperature	−0.012	0.003	−0.018	−0.005	<0.001
		Snout–vent length	0.016	0.007	0.002	0.030	0.026
	Call interval	Calling situation					
		Two species	−0.015	0.014	−0.043	0.013	0.296
		Intercept	1.382	0.373	0.650	2.113	<0.001
		Air temperature	−0.033	0.009	−0.050	−0.016	<0.001
		Urbanization index	−0.070	0.063	−0.192	0.053	0.264
	Call rate	Snout–vent length	−0.019	0.019	−0.057	0.019	0.332
		Intercept	−51.132	35.893	−121.481	19.217	0.154
		Air temperature	5.608	1.382	2.899	8.318	<0.001
		Calling situation					
	Pulse rate	Two species	5.565	6.186	−6.559	17.689	0.368
		Intercept	−6.514	31.503	−68.260	55.231	0.836
		Air temperature	2.970	0.536	1.920	4.021	<0.001
		Snout–vent length	−1.633	1.169	−3.925	0.659	0.162
	Dominant frequency	Intercept	4070.500	1632.030	871.772	7269.226	0.012
		Urbanization index	−631.460	212.250	−1047.458	−215.453	0.003
		Snout–vent length	−108.500	65.120	−236.121	19.127	0.096
		Calling situation					
		Two species	152.470	122.600	−87.821	392.770	0.214
<i>F. multistriata</i> (N = 20)	Call interval	Intercept	−0.056	0.065	−0.183	0.071	0.388
		Snout–vent length	0.004	0.002	0.000	0.007	0.031
		Urbanization index	0.009	0.008	−0.008	0.025	0.299
	Pulse rate	Intercept	23.529	19.198	−14.098	61.156	0.220
		Urbanization index	−7.579	3.018	−13.493	−1.665	0.012
		Air temperature	3.025	0.630	1.790	4.260	<0.001
		Snout–vent length	−1.072	0.524	−2.100	−0.044	0.041
		Calling situation					
		Two species	−3.727	1.637	−6.936	−0.517	0.023

Table 3. Cont.

Species	Call Parameter	Variable	Estimate	SE	Lower 95% CI	Upper 95% CI	<i>p</i>
	Dominant frequency	Intercept	3646.220	2319.860	−900.619	8193.063	0.116
		Urbanization index	−530.060	350.130	−1216.290	156.179	0.130
		Snout–vent length	−92.710	67.360	−224.729	39.319	0.169
		Air temperature	−64.040	58.480	−178.667	50.582	0.273

There were null models with the lowest AICc in the best models sets of CD and CR of *F. multistriata* (Table S5). SVL was the most important predictor in the top two models ($\Delta\text{AICc} < 2$) for CI of *F. multistriata* (Table S5). Model average coefficients showed that CI of *F. multistriata* was significantly positively correlated to SVL (estimated mean $\pm$ SE = 0.004 ± 0.002 , 95% CI (0.000, 0.007), $p = 0.031$) (Table 3). UI, air temperature, SVL, and calling situation were the four main predictors in the top two models ($\Delta\text{AICc} < 2$) for PR of *F. multistriata* (Table S5). Model average coefficients showed that PR of *F. multistriata* was significantly positively correlated to air temperature (estimated mean $\pm$ SE = 3.025 ± 0.630 , 95% CI (1.790, 4.260), $p < 0.001$), but significantly negatively correlated to UI (estimated mean $\pm$ SE = -7.579 ± 3.018 , 95% CI (−13.493, −1.665), $p = 0.012$), SVL (estimated mean $\pm$ SE = -1.072 ± 0.524 , 95% CI (−2.100, −0.044), $p = 0.041$), and calling situation (estimated mean $\pm$ SE = -3.727 ± 1.637 , 95% CI (−6.936, −0.517), $p = 0.023$) (Table 3).

4. Discussion

In this study, we found that two frog species have different responses to levels of urbanization in Shanghai. The DF of *M. fissipes* and PR of *F. multistriata* were significantly negatively correlated to UI. Temporal parameters of *M. fissipes* (i.e., CD, CI, CR, and PR) were all significantly impacted by air temperature. Call duration of *M. fissipes* and CI of *F. multistriata* were significantly positively related to SVL, whereas the PR of *F. multistriata* was also significantly impacted by air temperature, SVL, and calling situation.

Dominant frequency is not only a static trait that reflects body size, but also a plasticity trait under the influence of social context, including competition with other male frogs and avoidance of environmental noise in many frog species [26]. Previous studies found that DF was negatively correlated with SVL of calling males in many frog species [36,37,61]. In this study, DFs of *M. fissipes* and *F. multistriata* were also negatively correlated with SVL, although this was not significant for *F. multistriata* (Table 3). Previous studies suggested that some frog species increase the DF to avoid overlapping and signal masking caused by anthropogenic noise [33,62,63]. Some studies also found that frog species decreased DF when exposed to anthropogenic noise [64,65]. We found that the DF of *M. fissipes* decreased significantly with increasing UI, which was not consistent with our hypothesis. Physical barriers (buildings) in the environment may influence the propagation of acoustic signals, contributing to a greater attenuation and degradation of the signal [22]. Therefore, the environmental effects on sound propagation depend on the structural complexity of the environment. Lower frequency sounds propagate more efficiently in the environment [8,66]. Animals that live in more heterogeneous habitats may present songs with a lower dominant frequency to improve signal transmission based on the acoustic adaptation hypothesis [12,27,67]. It is possible that habitat changes caused by urbanization have had a significant effect on the DF of *M. fissipes*. However, other measures such as microhabitat characteristics and anthropogenic noise of frog calling sites would be important variables to be included in future analyses.

Pulse rate is an important trait for taxonomy and represents the most important call property in mate recognition in some anuran species [68,69]. Previous studies found that PR was more dependent on the air temperature in some frog species [54,70], but has only rarely been suggested to be influenced by body size [71]. In this study, we found that the PR of *F. multistriata* was not only affected by air temperature, but also related to UI, SVL,

and calling situation (two species calling together). In fact, *F. multistriata* individuals in urban areas mainly live in urban parks with microhabitats of high vegetation coverage and relatively low air temperature according to our measurements, which may further impact their PR as a result of urban landscaping [72]. Thus, we suggest that the decreasing PR of *F. multistriata* with increasing UI may also be affected by the local environment and individual body size. Besides, the changes of land use and anthropogenic noise caused by urbanization need to be further considered to analyze whether they would impact frog PR.

Calling is an energy-intensive activity because of muscular contractions; therefore, ambient temperature has a dramatic effect on such calls [24,73]. In this study, the CD and CI of *M. fissipes* were negatively correlated to air temperature (Table 3), which is in agreement with results of studies in eastern frogs (*Crinia signifera*) [35] and Puerto Rican coqui frogs (*Eleutherodactylus coqui*) [34]. We also found that the CR of *M. fissipes*, and PR of both *M. fissipes* and *F. multistriata*, were positively correlated to air temperature (Table 3), consistent with other studies [52,61]. In addition, the CD of *M. fissipes* and CI of *F. multistriata* were positively related to SVL (Table 3), which is consistent with previous research [74]. The calling situation was only significant negatively correlated to PR of *F. multistriata*, but there was also no conclusive relation between PR and heterospecific anurans in a previous study [75,76]. Therefore, we suggest that air temperature and SVL also affect changes in the temporal call parameters of these two frog species along the urban–rural gradient in Shanghai.

In conclusion, our results showed that both frog species changed their advertisement calls along an urban–rural gradient in Shanghai, with populations of *M. fissipes* reducing their DF, and populations of *F. multistriata* reducing their PR. Air temperature and SVL were also the main factors related to their temporal call parameters (CD, CI, CR, and PR). However, more research is required to determine the effects of other factors (i.e., vegetation cover at the call site, anuran community structure, and genetic diversity of each population in different sites) in relation to urbanization on the advertisement calls of these frog species.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d14070550/s1>, Table S1: Proportion of land-use types in 34 study sites of *M. fissipes* and *F. multistriata* (2-km radius); Table S2: The advertisement call parameters of *M. fissipes* and *F. multistriata* of each study site along an urban–rural gradient in Shanghai; Table S3: The snout-vent length and air temperature of *M. fissipes* and *F. multistriata* of each study site along an urban–rural gradient in Shanghai; Table S4: Variance inflation factor (VIF) values for the variables used for the multiple linear regression model (MLR) of *M. fissipes* and *F. multistriata*; Table S5: The best models ($\Delta AIC_C \leq 2$) examining relationships between variables (urbanization index, air temperature, snout-vent length, calling situation) and advertisement call parameters (call duration, call interval, call rate, pulse rate, dominant frequency) of *M. fissipes* and *F. multistriata*.

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