Title:

Abundance, distribution and spread of the invasive Asian toad *Duttaphrynus melanostictus* in eastern Madagascar

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Running title:

Distribution and abundance of Asian toad in Madagascar

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Abstract

The Asian toad, *Duttaphrynus melanostictus*, was accidentally introduced to Toamasina (Eastern Madagascar) around 2010, and since then has spread at a substantial rate across a larger area. This study documents the expansion of the invasive range of this species, calculates the invasion spread rate, and it further estimates the toad abundance and habitat preferences. Updates of the distribution range revealed a fivefold increase of the invaded area during three years, and a doubling of the rate of spread, showing a shift of the invasion towards the North-West, most probably because of the absence of ecological barriers. We used N-mixture models to estimate toad's abundance on the basis of repeated count data of six areas in Toamasina and its surrounding countryside. Toad distribution shows heterogeneous density across the distribution range, with an average abundance of 184 toads ha^{-1} (95% CI, 132–263). The toad's abundance was highest in sites with the presence of organic waste, and was negatively related to the density of road networks in the proximity of study sites. The rapid expansion of the Asian toad in the Toamasina region suggests that this toad is an increasing threat for Madagascar. We identify immediate management actions that could limit the spread of alien toads in this megadiverse country.

Key words

distribution range; spreading rate; abundance estimation; *Duttaphrynus melanostictus*; N-mixture models; Toamasina

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Introduction

Madagascar is one of the most celebrated biodiversity hotspots (Myers et al. 2000), where long geographical isolation and geological stability have favoured the evolution of a unique biodiversity (Goodman and Benstead 2003; Vences et al. 2003, Crottini et al. 2012; Holt et al. 2013; Ganzhorn et al. 2014). Besides the well-known conservation issues resulting from significant environmental degradation (Green and Sussman 1990; Vallan 2002; Harper et al. 2007), in 2014, a series of scientific communications drew attention to a new challenge for nature conservation in Madagascar (Andreone et al. 2014; Crottini et al. 2014; Kolby 2014; Kull et al. 2014; Moore et al. 2015): an invasive population of the Asian common toad (*Duttaphrynus melanostictus*, hereafter referred to as "Asian toad") was reported in Toamasina, the second city of the country for size and population and the major seaport of the island, located in eastern Madagascar. The ecological similarities with the dramatic invasion history of the cane toad *Rhinella marina*, which determined the decline of multiple endemic vertebrates across Australia (Shine 2010), have raised fears of a similar predicted ecological catastrophe for Madagascar.

The *D. melanostictus* complex is native to South and South East Asia, and has already succeeded in invading multiple regions including Bali, New Guinea, Sulawesi, Timor-Leste and other islands, mostly between Sundaland and Wallacea (Reilly et al. 2017). Recent phylogeographical analyses revealed the Asian toad to be a complex of species, in need of taxonomic revision (Wogan et al. 2016), and the toad lineage identified in Madagascar belongs to the clade distributed in South East Asia. The individuals that first established in Madagascar probably came from an area between Cambodia and Vietnam (Vences et al. 2017).

The Madagascar invasion probably started as early as 2010 (Moore et al. 2015), most likely with the accidental introduction of a few toads via commercial containers from South East Asia (McClelland et al. 2015). After a typical lag phase of a few years, the Asian toad population has grown exponentially, taking advantage of the suitable climate, especially the annual rainy season that likely matches the conditions occurring in the native area of this species (Vences et al. 2017), and directly triggers reproduction in this species (Ngo and Ngo 2013).

Although in its native range this species often lives in disturbed anthropogenic habitats, species distribution models suggested that the Asian toad can potentially spread across the lowlands of the eastern and northern coasts of Madagascar (Pearson 2015; Vences et al. 2017), thus raising the fear that it could threaten native rainforest communities.

The ongoing and unpredictable spread of the Asian toad is likely to pose major threats to the Malagasy native ecosystems. The most feared effect related to this biological invasion is the poisoning of naïve predators (Brown et al. 2016, Marshall et al. 2018), as Asian toads can release a cardiotoxic toxin that can be fatal to predators if ingested (Chen and Kovarikova 1967; Marshall et al. 2018), since the vast majority of native potential predators seems to be non-resistant to bufotoxins (Marshall et al. 2018). Other potential negative effects include the transmission of pathogens to native amphibian species, and the competition for breeding sites and feeding resources (Mahapatra et al. 2017), given its explosive breeding behaviour (Fan et al. 2013) and the sizeable clutches of up to ten thousand eggs (Licata et al. in preparation).

Currently, published information on the Asian toad distribution in Madagascar has been based on data collected in 2014, when the Asian toad distribution range covered an area of about 108 km^2 across Toamasina and its immediate vicinities (Moore et al. 2015), with maximum densities (of up to 51 toads per 100 m^2) reported for the urban area (McClelland et al. 2015). A more recent report provided wide abundance estimates across different habitats, with average densities ranging from 325 to 987 toads per hectare, and the highest value recorded in urban areas (1800 toads/ha; Reardon et al. 2018).

Although extremely useful to have a first impression of this invasion, these estimates were based on extrapolated simplistic counting approaches which did not take into account the detection probability of the species. It is therefore necessary to apply a more robust statistical approach to assess overall abundance of the species and update its distribution.

With this study we provide an update on the distribution, abundance and characterize the invasion spread of the Asian toad in the Toamasina Province. Updating the invaded range and the collection of accurate presence-absence data will enable the implementation of robust predictive models of potential distribution of the species. Integrating these data with estimates of abundance across different invaded environments (which also depends on the carrying capacity of the colonised habitats) can further enable the identification of the factors determining species densities, and provide information on the type of habitat preferentially selected (or contraselected) by the species. Finally, the spread capacity of the invasive species needs to be characterized as it may change across time and space due to the influence of topographic landscape features or due to the evolution of specific dispersal and life-history traits (Perkins et al. 2013). All these different types of information could be used to better assess the risk that this invasive species is posing to the native communities. It is also critical information for any planned implementation of delimitation and containment measures (Stohlgren and Schnase 2006), which will be fundamental to any effort to prevent the spread of Asian toads into ecologically sensitive areas or for attempting eradication of smaller satellite populations.

Materials and Methods

Study area

To update the distribution map and assess the invasion spread, broad scale monitoring was performed, trying to cover the whole Province of Toamasina (Toamasina I, Toamasina II, and Brickaville Districts). The northernmost locality visited was Mahavelona (60 km from Toamasina), while the southernmost was Brickaville (80 km from Toamasina) (Fig. 1a).

We opportunistically selected 6 localities where the toad was detected to estimate species abundance and relationships between abundance and environmental features (Fig. 1b). The chosen sites represented the two main habitat types where the species is currently reported: *a*) urban and *b*) agricultural/seminatural localities (*sensu* Moore et al. 2015). The three urban localities are within the border of the city of Toamasina: district of Mangarano (18°8'46"S 49°22'47"E), Amparihilava (18°10'7"S 49°22'7"E), and Barikadimy Campus of the University of Toamasina (18°7'49"S 49°22'42"E). Two localities predominantly associated with an agricultural landscape are located in Farafaty Village (18°8'33"S 49°21'27"E) and in Ambodibonara Village (18°8'21"S 49°20'30"E). Here the area is characterized by the presence of rice paddies, mixed fruit trees, sugar cane plantations, and surrounded by degraded forest and mixed shrubland (locally called "savoka"). The last locality is a palm oil plantation located at about 10 km South of Toamasina, next to the village of Mahasoa (18°13'51"S 49°18'18"E).

At the time of the first delimitation survey that took place in 2014 (Moore et al. 2015), both Mahasoa and Ambodibonara were located at the fore front of the toad invasion area.

Farafaty was selected among the sites that were used to test the efficiency of different control methods (Reardon et al. 2018). These trials took place between mid-January and April 2016, and were conducted for the purpose of assessing the efficacy of potential eradication tools to inform on the technical feasibility of Asian toad eradication.

Invaded area and dispersal rate

A total of 104 localities were inspected between November 2016 and September 2017 (Online Resource 1), reporting the presence or non-detection of toads. Coordinates were recorded by means of a Garmin 60CSx GPS device. Localities were selected opportunistically, based on received notifications of possible toad occurrences to the toll-free hotline number instituted by the local NGO "Madagascar Fauna and Flora Group" (MFG), and through selection of sites that were outside the recorded known distribution of the toad in 2014 (Moore et al. 2015), that were chosen to identify the invasion front and carry out MFG's awareness-raising activities concerning the toad. Upon arrival, a field crew of ca. 10 people conducted interviews with local residents, followed by visual encounter surveys (VES) that took place after dusk (from 18:00─21:00) (as per Moore et al. 2015).

The invaded area was evaluated by calculating the minimum convex polygon (Worton 1987), using the convex hull tool of ArcGIS 10.2 (ESRI 2011). To estimate the maximum spreading rate of the invasion, we considered the maximum linear distance between the presumed introduction point (i.e., the centroid calculated in 2014; Moore et al. 2015) and the most distant location recorded, representing the invasion front, divided by the time difference between the date of first detection in that locality and the estimated start date of the invasion. We chose arbitrarily the midpoint of May 2010 as the start date of the invasion, which represents the first reported estimate of when the toad was first seen in Toamasina (Moore et al. 2015), assuming no multiple introductions occurred as suggested by Vences et al. (2017), who presented compelling genetic evidence for a single introduction event.

We calculated the mean spread rate by dividing the distances between all invasion front localities and the presumed introduction point by the time difference between the date of first detection of the toad in the locality and the presumed start date of the invasion, and averaging the results. This datum was then compared with the data available from the study of Moore et al. 2015.

Estimation of abundance and environmental features

The study sites were spaced at least 1 km apart from each other. Within each locality we selected five quadrat plots (20 x 20 m), spaced at least 100 m from each other. Authorization was sought from residents and landowners prior to establishing plots. Each plot was individually marked with plastic flags and georeferenced. 30 quadrat plots were established in total, covering an area of 12000 m^2.

From 12^{th} of December 2016 to 21^{st} of January 2017, two experienced observers visited each site four times at regular time intervals (7-8 days), starting the counting soon after dusk (18:00─21:00), when toads are most active. Each count took approximately 10 minutes to inspect the whole plot area, and any sighted toad was removed during the survey period. For each quadrat plot, we measured four environmental variables: *Road*, the distance from the nearest road (to investigate the effect on toad abundances, which could be both positive (Brown et al. 2006) or negative (Andrews et al. 2008; Fahrig et al. 1995)); *Waste*, the presence of organic waste (i.e. household rubbish or agricultural waste) within the plot or along its borders was noted in light of the fact that these sites, and the arthropods community inhabiting therein (Frankie and Ehler 1978; McIntyre 2000), might represent forage hotspots for this species, typically associated with anthropogenically degraded habitats (vanDijk et al. 2004); *Buildings*, the number of buildings within a radius of 100 m; *Tree*, the percentage of tree cover within a radius of 100 m.

To determine building density and the percentage of tree canopy cover, we analysed Google Earth satellite imagery of 2017 using the software imageJ (as per Ricotta et al. 2014). Two weather conditions were obtained from the local meteorological station located at Toamasina airport: precipitation during the 24 hours before the survey (mm) and temperature at the time of the survey.

Statistical analysis

We used N-mixture models to estimate toad abundance on the basis of repeated count data, and to identify the environmental variables determining variation of abundance. This class of models allows for estimating animal abundance from repeated surveys at fixed sites, without marking individuals to identify them (Royle 2004). Some studies highlighted that the joint estimation of abundance and detection probability can be problematic, and that these models are sensitive to violations of their assumptions (Barker et al. 2018; Duarte et al. 2018). Nevertheless, recent analyses of real-world data suggested that this approach can provide reliable estimates and can be extremely useful to measure the abundance of wild animals (Kéry 2017; Ficetola et al. 2018).

Our sampling was performed during 40 days, thus we assumed that populations were closed. N-mixture models were fitted using a Zero-Inflated Poisson error distribution (ZIP), as ZIP models consistently showed lower Akaike's Information Criterion (i.e., AIC, Burnham and Anderson 2002) than Poisson models. Negative binomial models were not considered because of their convergence issues (Kéry 2018). In N-mixture models, the upper bound to approximate an infinite summation in the likelihood was “100 + the maximum observed species abundance”, as preliminary analyses and simulations suggest that this value provides robust and stable estimates (Ficetola et al. 2018).

We built models including weather conditions as variables influencing toad detection, and environmental variables of sites as potential predictors of toad abundance. We built models including all potential combinations of independent variables, ranking them on the basis of their AICc values, using a model selection approach that excludes from the confidence set the overly complex models (Richards et al. 2011). Models with a ΔAICc>10 were excluded from candidate model set (Burnham and Anderson 2002). Spearman’s rank correlation coefficient for site covariates indicated lack of significant correlation among the ecological parameters considered, allowing us to use all covariates in the model building procedure.

Data were analysed using the software R (version 3.5.0) with the package Unmarked (Fiske and Chandler 2011) and AICcmodavg, to assess the strength of the different models tested (Mazerolle 2011). We used the package spdep (Bivand et al. 2005) to assess the spatial autocorrelation of the residuals of the global model. The results of this analysis suggested the absence of spatial autocorrelation (Moran’s I statistics= -0.03, P = 0.48).

Results

Distribution

The Asian toad was detected in 50 out of 104 visited localities (Online Resource 1), resulting in a minimum convex polygon of 549 km^2 (Fig. 2). The current centroid is located 6.75 km and 295° north-west with respect to the last calculated centroid (Moore et al. 2015).

The maximum distance between the putative introduction point and the furthest location so far recorded is 21.9 km. Assuming that no other introductions have occurred, the maximum spread rate of toads is 3.3 km year^{-1}, while the average spread rate based on all invasion front localities was of 2.5 ± 0.6 km year $^{-1}$ (Fig. 3).

Abundances

During our surveys, we detected 363 Asian toads (range: 0-23 individuals per survey per plot). Toads were not detected in 20% of surveyed plots over 4 visits.

The model with zero-inflated Poisson distribution (ZIP) showed the lowest AICc values and was employed as the global model. The best AICc model (Table 1) suggested that weather conditions did not influence the detection of toads, and among the environmental covariates, waste presence (*Waste*) and road proximity (*Road*) were the most important variables in describing toad abundance. The covariate coefficient showed that the presence of waste had a strong positive effect leading to increased toad densities (B = 2.93, P < 0.001), which were higher in urban and agricultural degraded areas with open-air dumps, while road proximity had a negative relationship (B = 0.44, P < 0.001). The estimated per-individual detection probability was 0.455 ± 0.04 (mean ± SE), which corresponds to a site-level detection probability ranging between 0.62 and >0.99, depending on species abundance. The estimated mean abundance per plot was 7.4 individuals (median = 4.2; 95% CI, 5.3–10.5), with estimated numbers of individuals per plot ranging from 0 to 59. The total abundance across the 30 study plots (total surface: 12,000 m^2) was 221 individuals (95% CI, 158–316), which means a density of 184 toads ha^{-1} (95% CI, 132–263). The abundance showed strong variation across the surveyed sites, and no strong differences were detected between urban and seminatural localities (Fig. 4). The highest abundances were recorded at the palm oil plantation (Mahasoa, see Fig. 4), where the estimated abundance was 559 toads ha^{-1} (95% CI, 445–695).

Discussion

Although Asian toads have successfully invaded several regions of the world, there is a general lack of studies on the ecology of this species (Reilly et al. 2017). Our work provides a detailed analysis of the invasive potential of the Asian toad in eastern Madagascar, updating the ongoing invasive process and identifying the habitat features related to the variation of species abundance.

Rate and patterns of invasion

Our study highlights a rapid advancement of the invasion front, with an expansion of the toad particularly towards North-West (Fig. 2), probably due to the absence of ecological barriers (Brown et al. 2015). Conversely, it seems that the northward toad invasion has been slowed down probably by the presence of the River Ivoloina, suggesting that this river functioned as a partial barrier, as already reported in literature (Leblois et al. 2000; Li et al. 2009; Zao et al. 2009). Contrary to this, the river Ivondro in the South does not seem to play the same role in arresting the invasion; this might be due to the presence of multiple man-made canals and waterways, primarily used for transportation and fishing, that could have further facilitated the invasion, increasing the connectivity between previously isolated biota (Crooks and Suarez 2006; Rahel 2007). Nonetheless, due to the lack of a rigorously defined sampling designs for assessing the invasive range, our results may be subject to spatial and detection biases. It should, therefore, be noted that the current distribution update is not exhaustive and is likely to be very conservative.

Our estimate of spread rate (2.5 – 3.3 km / year) is two-times higher than the previously reported values (Moore et al. 2015) (Fig. 3). This may be real or it may be the result of an underestimate by Moore et al. (2015). An acceleration of invasion rate has been extensively reported during the invasion of cane toads in Australia, where it has been associated with the rapid adaptive changes in morphological and behavioural traits, and promoted by the absence of ecological pressures (Phillips et al. 2006). Available data suggest that the Asian toad population in Madagascar lives in environments strongly similar to the ones occupied by the species in its native range (Vences et al. 2017) and, apart from sporadic killings by local people and some limited population control efforts in very small targeted areas, they are probably not exposed to strong predation pressure because of their toxicity (Marshall et al. 2018), even though they could still suffer attacks from naive predators during the initial stages of colonization. There is an urgent need to verify if the observed increase of the spread rate reported for Madagascar is facilitated by favourable local environmental conditions (as observed in Australia with cane toads; Urban et al. 2008), or if it is related to man-mediated introductions to new regions. The monitoring of domestic trade of building materials (e.g. concrete blocks or wood piles) and the translocation of agricultural products (e.g. palm oil empty fruit bunches) should be prioritized, since these substrates are often used as shelters by the Asian toads (FL, pers. obs.), and human-mediated movement of these goods could inadvertently contribute to the species range expansions into new areas.

Abundance and habitat preferences

Abundance is a major factor determining the impact of invasive species (Leung et al. 2012), and identifying the influencing factors is relevant to understanding the causes of its spread and persistence in the invaded area. The mean abundance herein reported finds both discrepancies and similarities with data available from other invasive amphibian species. Studies on *Rhinella marina* showed that populations can reach very high densities in newly colonized habitats (>2000 toads / ha, Freeland 1986), probably because of high prey availability and reduced competition with conspecifics during the first stages of invasion (Brown et al. 2013). Interestingly, in Papua New Guinea, Zug et al. (1975) reported consistent shifts in abundances across different habitats, where 30 cane toads / ha could be found in savannahs, whereas in forested areas the number was ten times lower.

Overall, our results showed that the Asian toad in Madagascar shows a strong variation of abundance (Fig. 2), and abundances are positively correlated to the presence of rubbish dumps. This relationship is probably due to the high edaphic humidity, the presence of favourable shelters and the high suitability of these sites for Asian toad potential prey species (Frankie and Ehler 1978; McIntyre 2000). Conversely, toad abundance is lowest near to roads. Although roads can be used as dispersal corridors by invasive species (e.g. in cane toad; Brown et al. 2006), in our study sites it seems that roads act as a source of stress for amphibians, as already reported by Fahrig et al. (1995) and Andrews et al. (2008). Here too, due to the higher detectability, they might be killed by local people more easily or they might be more exposed to the risk of roadkill.

The relationships between invasive toads and human disturbance has already been observed in the cane toad, where the invasive species actively selects breeding sites where anthropogenic disturbances have occurred. For instance, in the Solomon Islands, cane toads showed densities 3.11 times higher in disturbed (versus non-disturbed) habitats (Pikacha et al. 2015). In our study, we found no difference in abundance between urban and seminatural sites but interestingly, we found the highest densities at the palm oil plantation locality of Mahasoa (Fig. 4), where, in a quadrat plot located in close proximity to an open-air rubbish dump filled with agricultural waste, up to 23 individuals were counted. Here, the high-carrying capacity could be enhanced by local management practices. Indeed, tons of plant remains (e.g. empty fruit bunches) are used as mulch for local plantations (Hamdan et al. 1998). Asian toads often exploit this substrate and it will be important to verify if, during the relocation of the mulch across plantations, toads are also moved into new palm oil parcels.

The relationship between toad abundance and canopy cover should be further investigated, even though no significant relationships were found during this study. The canopy cover percentage obtained by satellite imagery does not provide a quantitative measure of habitat features within the surveyed area, as it overlooks differences among habitats (e.g., differences between plantation vs. secondary forest). Indeed, in several plots located in highly forested areas toads were absent, suggesting that those habitats might have lower suitability, as already reported for several bufonids species that typically prefer poorly vegetated or open areas (Guerry and Hunter 2002; Tucker and Simmons 2009), but a finer study using radiotelemetry should be applied to better assess the suitability of forested habitats.

It should be noted that the ecological range and replication of sites where abundance was investigated in this study remains small and so further replications and sampling of ecological variables are needed to enhance the robustness of our estimates.

Conclusions

The rapid range expansion of the Asian toad in the Toamasina region suggests that this toad is an increasing threat for Madagascar (Fig. 2), with an observed occupied area at least fivefold larger and an observed doubled spread rate than recorded in the last surveys (Moore et al. 2015). Further, we provide estimates of the abundance of this species across different selected sites. Estimates obtained from the semi-natural site of Farafaty may be slightly lower due to the small-scale eradication trials conducted 8 months prior to this study that might have affected the abundance estimation, even if reinvasion into the control trial areas is likely to have been occurring in the intermediary period. Finally, the analysis of relationships between toad abundance and environmental variables provides important management indications. The strong correlation between waste presence and toad abundance suggests that dump sites should be prioritized for the implementation of control measures, , as proper waste control, management and disposal programs could help in reducing feeding resources and shelters for this invasive species. For instance, the simple relocation of urban waste disposal sites outside the invaded area can help, over time, to limit the proliferation of the Asian toad in the city.

Madagascar is one of the most biodiverse countries in the world, and this invasion represents a major threat for native biodiversity and for the country's economy, which largely depends on wildlife ecotourism (Duffy 2006). Such rich and unique ecosystems are already threatened by multiple anthropogenic pressures, and biological invasions are rapidly becoming a major new threat to biodiversity and ecological robustness (Crowl et al. 2008; Bellard et al. 2014, 2016), highlighting the need for Madagascar to develop and establish biosecurity protocols.

An eradication plan now seems to be very difficult to implement given the large scale of the invasion (McClelland et al. 2015; Reardon et al. 2018), worsened by the difficulty to make such activities a priority in a country as economically impoverished as Madagascar. Notwithstanding, it is important to intesify the research on the parameters influencing the biology and habitat occupancy of this species, as this will help inform delimitation and containment protocols for Asian toads in any future mitigation efforts. For example, Parc Ivoloina represents an important amphibian biodiversity stronghold (Crottini et al. 2013), and lies in close proximity to the city of Toamasina.

This site represents a perfect pilot study site to develop containment measures to exclude the toad and represents an opportunity to develop protective methods that can later be applied at the Betampona Strict Nature Reserve (ca. 30 km north west from Toamasina; Fig. 1), an important microendemism hotspot which hosts an incredibly high number of microendemic frog species (Rosa et al. 2012) that are directly threatened by the continued spread of the Asian toad.

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CAPTIONS FOR FIGURES

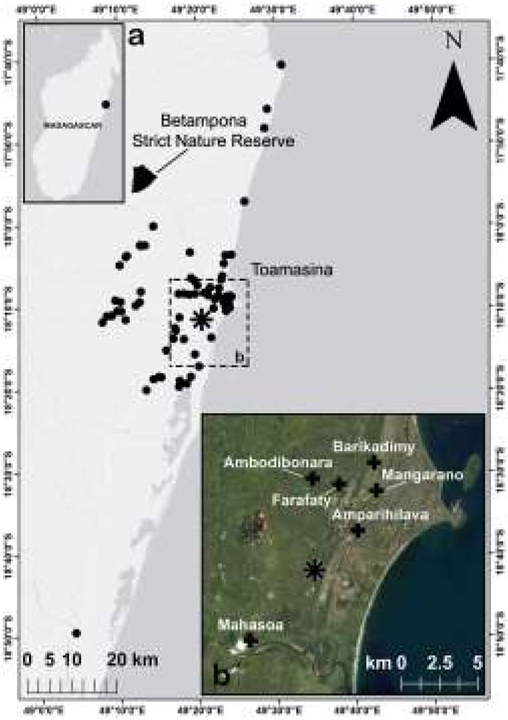

Fig. 1 a) Location of study area with sites visited (*filled circle*) and the presumed introduction point calculated for data collected in 2014 (*star*; Moore et al. 2015) to update the distribution range of the Asian toad in Toamasina (Madagascar). The map also shows the position of Betampona Strict Nature Reserve, the protected area nearest to the Asian toad invasive range; b) Sites selected for the study on toad abundance (*greek crosses*) (December 2016–January 2017)

Fig. 2 Map of the current distribution range (*solid line*) of Asian toad showing sites of detection (*filled circles*) and non-detection (*crosses*). The *dotted line* indicates the 2014 distribution range (Moore et al. 2015). The *filled triangle* represents the current distribution centroid, located 6.75 km W of the last calculated centroid (*star*; Moore et al. 2015)

Fig. 3 Box-plot showing the invasion spread rates, comparing the estimates of this study with data available from Moore et al. (2015)

Fig. 4 Histogram of toad abundances across surveyed sites. Lines represent 95% confidence intervals