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7 **Integrating transport pressure data and species distribution models to estimate invasion**
8 **risk for alien stowaways**

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Abstract

The number of alien species transported as stowaways is steadily increasing, and new approaches are urgently needed to tackle this emerging invasion pathway. We introduce a general framework for identifying high-risk transport pathways and receiving sites for alien species that are unintentionally transported via goods and services. This approach combines the probability of species arrival at transport hubs with the likelihood that the environment in the new region can sustain populations of that species. We illustrate our approach using a case study of the Asian black-spined toad (*Duttaphrynus melanostictus*) in Australia, a species that is of significant biosecurity concern in Australasia, Indonesia, and Madagascar. A correlative model fitted to occurrence data from the native geographic range of *D. melanostictus* predicted high environmental suitability at locations where the species has established alien populations globally. Applying the model to Australia revealed that transport hubs with the highest numbers of border interceptions and on-shore detections of *D. melanostictus* were environmentally similar to locations within the species' native range. Numbers of *D. melanostictus* interceptions and detections in Australia increased over time, but were unrelated to indices of air and maritime trade volume. Instead, numbers of interceptions and detections were determined by the country of origin of airplanes (Thailand) and ships (Indonesia). Thus, the common assumption that transport pressure is correlated with invasion risk does not hold in all cases. Our work builds on previous efforts to integrate transport pressure data and species distribution models, by jointly modelling the number of intercepted and detected stowaways, while incorporating imperfect detection and the environmental suitability of receiving hubs. The approach presented here can be applied to any system for which historical biosecurity data are available, and provides an efficient means to allocate quarantine and surveillance efforts to reduce the probability of alien species establishment.

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48 Keywords: biosecurity; *Duttaphrynus melanostictus*; establishment; prioritization; risk

49 assessment; species distribution model

Introduction

Invasive alien species are a growing threat to biodiversity worldwide (Bellard et al. 2016, Early et al. 2016). Increasing global connectivity through human transportation networks is unintentionally moving species well beyond their native geographic ranges (Banks et al. 2015, Cope et al. 2016). Indeed, stowaway transport is an emerging pathway for the movement of alien species worldwide, and one that is expected to increase in magnitude over the coming decades, due to the continuing trend of globalisation (Banks et al. 2015, Hulme 2015).

The sheer quantity of goods and services moved around the world makes it challenging to address the risk posed by the stowaway pathway (Hulme et al. 2008, Hulme 2015, Jarrad et al. 2015). Common activities aimed at mediating the stowaway pathway involve the inspection of arriving people, baggage, goods, and services, to ensure that no alien individuals are passively transported into the recipient jurisdiction (Beale et al. 2008, Jarrad et al. 2011, 2015, Hulme 2015, Cope et al. 2016). However, such inspections are labour- and resource-intensive (Jarrad et al. 2011). A protocol for identifying high-risk transport pathways and receiving sites, based on the risk of transport and establishment of alien species at those sites, would provide a sound basis to focus and optimize the deployment of biosecurity efforts targeting the arrival of stowaways (Yemshanov et al. 2015, McGeoch et al. 2016). Such a protocol could be used, for example, to predict the risk of marine invasions due to ballast water discharge (Seebens et al. 2013); to estimate the probability of insects hitchhiking and establishing due to the transport of firewood (Koch et al. 2012); or to forecast the impacts of recreational boats on the movement and establishment of aquatic species (Bossenbroek et al. 2007).

Numerous studies have sought to identify general biological traits (e.g., generation time, fecundity) of establishment success in alien species to prioritise biosecurity efforts, but progress to date has been limited. One of the reasons for this lack of progress is that effects of biological traits can be mediated or confounded by extrinsic introduction characteristics (Hayes and Barry 2008, Duncan et al. 2014). Two such characteristics are the extent to which environmental conditions at an introduction location match those within a species' native geographic range (environmental matching) and surrogates for propagule pressure (the number of distinct introduction events and the number of individuals released in each event). Unlike many biological traits, environmental matching and propagule pressure correlate with establishment success in a wide array of plant, vertebrate, and invertebrate taxa (Hayes and Barry 2008, Lockwood et al. 2013, Blackburn et al. 2015).

A common approach for predicting the environmental suitability of a given introduction location is to fit a statistical model to distribution and environmental data from a species' native geographic range (Elith 2011, Jiménez-Valverde and Peterson 2011, Tingley et al. 2014). This correlative approach (broadly referred to as species distribution modelling – SDM) is used in risk assessment schemes in a range of countries (e.g., Australia, New Zealand, USA, South Africa); when combined with species introduction effort, SDM can provide an efficient means to prioritize receiving sites for quarantine and surveillance (Jiménez-Valverde and Peterson 2011, Guisan et al. 2013). Predicting spatial heterogeneity in propagule pressure typically requires the use of proxies (Caley et al. 2015), such as human population density, macroeconomic parameters such as gross domestic product, or information on transport networks (Pyšek et al. 2010, et al. 2016). The critical assumption with this approach is that higher values of these metrics increase propagule pressure – an assumption that may be scale-dependent (Drake et al. 2015), and is rarely tested.

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101 In this paper, we employ data on an Asian toad species (*Duttaphrynus melanostictus*;
102 Schneider, 1799), unintentionally transported to Australia, to reveal how SDM can be
103 combined with maritime shipping and airline transport data to estimate the joint probability
104 of arrival and survival of alien stowaways. *Duttaphrynus melanostictus* has a broad native
105 geographic range that extends from northern Pakistan east to southern China, and south to
106 Indonesia. The species has also established alien populations on numerous tropical islands
107 globally, where it has caused substantial environmental and economic impacts (Kraus 2009,
108 IUCN 2012, O'Shea et al. 2015, Measey et al. 2016, Wogan et al. 2016). Recent border
109 interceptions and on-shore detections of this species in Australia have raised significant
110 biosecurity concern, as the species has the potential to cause similarly devastating ecological
111 impacts to those produced by the invasive cane toad (*Rhinella marina*) in Australia (Shine
112 2010).

113

114 We adopted an integrated approach to investigate the following questions: (i) what is the
115 probability that individuals of *D. melanostictus* are intercepted at the Australian border and
116 detected on-shore; (ii) does the total number of *D. melanostictus* arriving into each State
117 correlate with specific trade routes or transport pressure; and (iii) which Australia airports
118 and maritime shipping ports are at greatest risk of *D. melanostictus* establishment? Our case
119 study of *D. melanostictus* in Australia provides a proof of concept of the utility of our
120 approach to inform quarantine and surveillance efforts aimed at managing the risk of arrival
121 and establishment of alien species via the stowaway pathway (Jarrad et al. 2015, Yemshanov
122 et al. 2015, McGeoch et al. 2016). Given the increasing availability of data on historical
123 introductions globally (Kraus 2009, Essl et al. 2015, Chapple et al. 2016, Dyer et al. 2016),
124 this approach is readily generalizable to a wide array of countries and taxonomic groups.

Methods

Biosecurity data

We collated data on *D. melanostictus* detected at the border (interceptions) and on-shore (detections) in Australia, during 1988–2013 (Appendix S1). Data were available since 1986, but no toad was recorded until 1988. The final dataset contained 113 records of *D. melanostictus* in Australia, with detailed information on: (i) life stage (adult or juvenile); (ii) date; (iii) Australian city where the toad was found; (iv) pathway of transport into Australia (mail, shipping, or aviation); (v) country of origin of the package, ship, or airplane; and (vi) whether it was a border interception or on-shore detection. For border interceptions, each data source explicitly reported whether the animal was intercepted at a port or an airport (i.e., at a Quarantine Intervention Point). For on-shore detections (i.e., post-quarantine detections), each data source explicitly reported whether the animal originated from a ship or an airplane (e.g., as reported by importers of commodities). All intercepted toads were found hitchhiking in shipments of commodities that were not associated with the import of live animals into Australia, confirming that toads were unintentionally transported. *Duttaphrynus melanostictus* is also not a common pet species in Australia, ruling out intentional or unintentional releases of pets. There are no additional known pathways of introduction for *D. melanostictus* into Australia.

The recent rise in the profile of *D. melanostictus* as an invasive species elsewhere in the world (e.g., Madagascar; Kolby, 2014) might have increased awareness in Australia, contributing to an increased number of *D. melanostictus* identified and reported by authorities. This could mask real trends in the number of *D. melanostictus* interceptions and detections over time. We therefore compiled information on all other species of the family Bufonidae detected and

intercepted at Australian ports and airports (five species: *Sclerophrys capensis*, *Bufo stomaticus*, *B. viridis*, *Rhinella marina*, *R. spinulosa*). Data for these species were obtained from the same data sources as for *D. melanosticus*, and for the same period.

Transport pressure data

Connectedness with source regions where an alien species occurs, when combined with information on trade volumes from those regions, is an important predictor of the likelihood of interception and detection. Highly connected areas that receive more goods and services from source regions are also more likely to be at risk of an introduction (Hulme 2009, Banks et al. 2015, Cope et al. 2015, 2016). We considered all countries where *D. melanosticus* is known to occur (either native or introduced) as the potential source pool of toads to be transported unintentionally into Australia (Kraus 2009, IUCN 2012).

We gathered information on the total number of direct flights (including both cargo and passenger flights), and the total number of seats on those flights, from every country inhabited by *D. melanosticus* (Kraus 2009, IUCN 2012) to destination airports in Australia for the period 1996–2012 (from OAG Aviation Worldwide Ltd: <http://analytics.oag.com/home/>). We excluded air traffic data for Tasmania and the Northern Territory, because there were only two and three records of airplanes originating from countries inhabited by *D. melanosticus*, respectively (3.6% and 5.4% of all original records, respectively). For the international shipping network, we similarly collated data on the number of ships per Australian port from each country inhabited by *D. melanosticus*. Data on shipping traffic were obtained from the Australian Department of Agriculture and Water Resources, period 1999–2012 (Cope et al. 2015, 2016), excluding Australian port facilities used exclusively for coal trade. For each State in Australia, we calculated the combination of

the total number of airplanes and seats (airports), and ships (ports) arriving from each country inhabited by *D. melanosticus* to each Australian State and Territory for the whole time period for which we had data (1996–2012 for airports; 1999–2012 for ports). We refer to these estimates of total transport pressure as State (State airports- or State ports-) specific routes hereafter.

Pathway modelling

We quantified the role of pathway-level State-specific routes on interceptions and detections of *D. melanosticus* in Australia by matching State-specific numbers of toads to our measures of State-specific routes. This approach resulted in 99 and 39 matched combinations of port- and airport-specific routes, respectively, and toad detections and interceptions (one per destination port or airport in Australia per country of origin of the airplane or ship). We standardized, by subtracting the mean and scaling by the standard deviation, all State-specific traffic measurements prior to running the analyses.

We used the hierarchical approach developed in García-Díaz et al. (2016) for modelling whole-State air traffic and shipping traffic transport pathways in Australia, including interceptions and detections. This hierarchical modelling approach allowed us to jointly model relationships between the numbers of *D. melanosticus* detected and intercepted, and estimate probabilities of interception at the border and of detection on-shore. For this work, we have tailored the model to the case of interceptions and detections of *D. melanosticus* in each Australian State (denoted by i) originating from each country inhabited by toads, j :

$$Nb_{i,j} \sim \text{Binomial}(N_{\text{tot}_{i,j}}, pb_j) \quad (1)$$

$$Nos_{i,j} \sim \text{Binomial}(N_{\text{tot}_{i,j}} - Nb_{i,j}, pos_i) \quad (2)$$

200

201 where $Nb_{i,j}$ is the number of toads intercepted at State i from country of origin j ; $N.tot_{i,j}$ is the
202 total number of *D. melanosticus* arriving into State i from country of origin j ; pb_j is the
203 probability of interception at the border in shipments from country of origin j ; $Nos_{i,j}$ is the
204 number of *D. melanosticus* detected on-shore which bypassed quarantine at State i from
205 country of origin j ; and pos_i is the probability of detection on-shore in State i . We assumed
206 that only $Nb_{i,j}$ and $Nos_{i,j}$ have been directly recorded in our database, while $N.tot_{i,j}$ was
207 considered as an unobserved variable because imperfect probabilities of interception and
208 detection prevent it from being observed directly (Kéry and Royle 2016). $N.tot_{i,j}$ is therefore
209 estimated by assuming that the total number of toads arriving into each State from each
210 country of origin is a function of transport pressures and differences between countries of
211 origin due to factors other than transport pressure. After 2010, the agency managing border
212 biosecurity implemented a risk-assessment based strategy for determining inspection targets
213 (A. Broadley, com. pers, Department of Agriculture and Water Resources, Australian
214 Government). We accounted for this change by estimating origin-specific probabilities of
215 interception at the border. We do not name the individual States to avoid providing sensitive
216 information on their biosecurity capabilities that may be used by people, such as wildlife
217 smugglers, for fostering illicit activities.

218

219 We modelled the relationship between $N.tot_{i,j}$ and the State-specific routes using negative
220 binomial models to account for overdispersion in our data (Lindén and Mäntyniemi 2011).
221 We included a country of origin intercept in the negative binomial models, to account for
222 differences in the number of toads transported from different countries due to reasons other
223 than varying transport pressures. We estimated the total number of toads to have escaped in
224 the wild in each State (total 1996–2012 and 1999–2012 for air- and ship-routes, respectively)

by subtracting the numbers intercepted and detected from the estimated total number arriving into each State, $N_{totij} - Nb_{ij} - Nos_{ij}$, and summing across countries of origin.

We fitted the models independently for the air traffic and shipping State-specific routes using Markov Chain Monte Carlo methods as implemented through the R statistical software (R Development Core Team 2015) interface to the JAGS software (Plummer 2003). Additional model fitting details and code for running the models can be found in Appendix S2.

We measured the fit of the regularized models via Bayesian p -values (Gelman et al. 2013, Kéry and Royle 2016). Bayesian p -values measure the discrepancy between the value of a model fit statistic (e.g., the Freeman-Tukey test) for the observed data, y^{obs} , and the fit statistic obtained from a replicated dataset if the model is true, y^{rep} (Kéry & Royle, 2016). Bayesian p -values range from 0 to 1. A poor fitting model is indicated by extreme values (e.g., 0.05 or 0.95), whereas an excellent fitting model has a Bayesian p -value relatively close to 0.5 (Gelman et al. 2013, Kéry and Royle 2016). Following Kéry & Royle (2016), we used the Freeman-Tukey statistic as our fit statistic, and we estimated it and Bayesian p -values for both the number of toads detected at the border, Nb_{ij} , and intercepted on-shore, Nos_{ij} .

Species distribution modelling

Data on the global distribution of *D. melanostictus* were collated from a variety of sources (Appendix S1). Records with missing geographic coordinates, which contained enough textual information to identify a specific location with reasonable spatial certainty (e.g., suburbs, parks), were georeferenced using the ggmap package in R 3.2.2 (R Development Core Team 2015). Records with only coarse locality descriptions (e.g., towns, cities, counties, rivers) were excluded. All georeferenced locations were manually vetted for

accuracy. Removing duplicate records within 10' grid cells (the resolution of our environmental data; see below) resulted in 420 unique native-range records and 42 alien records for analysis.

Energy and water availability are key determinants of amphibian distributions at broad spatial scales (Araújo et al. 2006). We used four variables (10' resolution; 1961 – 1990) from the CliMond database (Kriticos et al. 2012) that reflect energy and water availability to model the native geographic range of *D. melanostictus*: (i) mean temperature of the warmest quarter; (ii) mean temperature of the coldest quarter; (iii) mean moisture index of the warmest quarter; and (iv) isothermality. We also used the human influence index (Gallardo et al. 2015), as *D. melanostictus* is associated with human settlements throughout much of its native range (Wogan et al. 2016). The human influence index integrates human population density, land use and infrastructure, and human access into a single metric of anthropogenic impact (Wildlife Conservation Society - WCS 2005). This variable was available at a higher spatial resolution than the variables in the CliMond database, and so we resampled it to a 10' resolution using bilinear interpolation.

We used Maxent (v. 3.3.3k) to model the native distribution of *D. melanostictus*, and to project its potential distribution globally. We used the default settings of Maxent, except that we excluded threshold and product features, and increased the regularization multiplier (beta=2.5) to create smoother response curves (Elith et al. 2010, Tingley et al. 2014). Maxent contrasts records where a species has been detected with background records across a region of interest. Ideally, the extent of this region should be based on a species' biology, and only include locations that are likely to have been sampled and that were accessible to the species (Barve et al. 2011). *Duttaphrynus melanostictus* primarily occurs throughout temperate and

tropical forests in its native range, and its western range limit is tightly aligned with the transition between deciduous forests and arid desert and thorn scrub landscapes. In addition, the Himalayas presumably represent a dispersal barrier at the northern range limit. We therefore drew random background records (n=10,000) from temperate, tropical, and subtropical ecoregions within the Indo-Malaysian and eastern Palaearctic biogeographic realms, which only included areas south of the Himalayas (Olson et al. 2001, Keith et al. 2014). To examine whether projecting our Maxent model globally required extrapolating response curves into un-sampled environmental space, we used multivariate environmental similarity surfaces (Elith et al. 2010).

Predictive performance of the Maxent model was evaluated in two ways. First, we used five-fold cross validation and the area under a receiver operating characteristic curve (AUC) to assess the model's ability to discriminate between native-range occurrence records and random background points (as defined above). The second approach involved assessing how well the model predicted alien populations of *D. melanostictus*. Assessing predictive performance of SDMs for alien species is challenging, because measures such as AUC use absence (or background) data, and thus assume that species have colonized all suitable environments (Elith 2011, Tingley et al. 2016). We therefore visually assessed whether our SDM assigned high relative likelihoods of occurrence to locations where *D. melanostictus* has established alien populations. We also assessed whether the model accurately predicted alien populations by converting relative likelihoods of occurrence into presence-absence predictions using the highest threshold that captured 90% of native range records (0.322). These latter results should be treated cautiously, however, given problems associated with calculating thresholds on presence-background data (Guillera-Arroita et al. 2015).

Results

Border interceptions and on-shore detections

Numbers of border interceptions and on-shore detections in Australia have increased over time (Fig. 1). Prior to 1999, *D. melanostictus* was only detected once at the border (in Darwin, Northern Territory in 1988), and no on-shore detections were reported. In contrast, 88 border interceptions and 24 on-shore detections have been reported since 1999.

Comparing trends in *D. melanostictus* interceptions and detections with other species of Bufonidae in Australia revealed similar patterns for the two groups (Fig. 1; Fig. S1).

Duttaphrynus melanostictus were found alive in 91% (81/89) of border interceptions and 88% (21/24) of on-shore detections. Seven border interceptions involved two or more toads (maximum=3), whereas only one on-shore detection involved two individuals. All other records were of a single toad (n=69), or did not contain information on the number of animals detected. Thirty-two percent (n=36) of records contained information on toad age; 78% (n=28) of these were of adult toads (border interceptions: 24/31; on-shore detections: 4/5). The remaining eight individuals were classified as juveniles (border interceptions: 7/31; on-shore detections: 1/5).

Transportation pathways and effects of transport pressure on invasion risk

Most stowaway toads arrived from Indonesia (63% of 104 records with information on the origin of stowaways), although toads were also transported from Thailand (15%), China (7%), Malaysia (6%), India (3%), Brunei (1%), Hong Kong (1%), Papua New Guinea (1%), Singapore (1%), and Taiwan (1%). One animal that was intercepted at the border originated from Christmas Island, Australia, where *D. melanostictus* has not established. Most animals

were detected at maritime shipping ports (79% of the 110 records that reported vessel origin), rather than at airports or in the mail.

Models developed for the transport pathways fitted the interception and detection data adequately, as indicated by Bayesian p -values (Table 1). Country of origin was the main driver of the number of *D. melanosticus* transported to Australia for both air and shipping pathways, with important differences in the posterior estimates for the different countries of origin (Fig. 2; see Table S1). Ships originating from Indonesia and airplanes originating from Thailand were more likely to be transporting toads into Australia than ships and airplanes departing from other countries within the geographic range of *D. melanosticus*. The three transport pressure covariates included in the pathway models had small posterior effects on toad interceptions and detections in Australia, with all posterior estimates centred around zero (Table 1).

Estimated posterior probabilities of interception at the border were variable across shipments originating from different countries (Table S2), but overall were marginally higher for toads arriving in airplanes than in ships (median $\pm$ SE: 0.51 ± 0.09 and 0.47 ± 0.06 , respectively; Table 1). Median probabilities of on-shore detection were similar across States and transport pathways (Table 1); however, posterior estimates spanned a wide range of values, from 0.01 to 0.98, across the seven Australian States (Table 1). Toads may have been introduced into some States and not been intercepted or detected by biosecurity activities, with median posterior estimates <1.00 in all cases (Table 1). Summing all posterior estimates across States and Territories suggests that it is likely that *D. melanosticus* have been introduced in Australia (shipping routes: 22.54 ± 23.43 ; air traffic routes: 9.92 ± 7.89 ; Table 1).

Environmental suitability

Mean temperature of the coldest quarter had the strongest effect on the species' native distribution in the Maxent model (permutation importance = 60.2%), followed by mean temperature of the warmest quarter (19.7%), isothermality (11.5%), the human influence index (4.8%), and the mean moisture index of the warmest quarter (3.8%). The predicted native distribution of *D. melanostictus* closely aligned with the species' observed distribution (Fig. 3). Indeed, the model assigned higher relative likelihoods of occurrence to native-range populations than it did to random background sites (mean AUC $\pm$ SD = 0.817 $\pm$ 0.018; Fig. 4a). Alien populations were also assigned high relative likelihoods of occurrence (Figs. 3, 4b). Reclassifying model outputs into binary predictions revealed that 98% (41/42) of alien populations were predicted to be environmentally suitable (Fig. 4b). The only alien location that was assigned a low relative likelihood of occurrence was a site in Papua New Guinea, where the species has established a population in a semi-urban garden (<http://www.projectnoah.org/spottings/319316056>). Projecting the model onto the species' alien range did not require the model to extrapolate into novel environmental space (Fig. S2).

Projecting the model globally revealed further scope for invasion in all of the regions where *D. melanostictus* has established alien populations, including Madagascar and Indonesia. In Australia, the model predicted that landscapes surrounding many airports and maritime shipping ports are environmentally suitable (Fig. 4c, d), and that the species could potentially colonise substantial portions of southern, eastern, and northern parts of the continent (Fig. 3). Many of the locations where *D. melanostictus* has been intercepted at the border and detected on-shore are predicted to be highly suitable (cf. Figs. 3 & 5).

DISCUSSION

Combining transport pathway data and SDM predictions can provide an informative means to identify risky transport routes and environmentally suitable receiving hubs. The widespread availability of species occurrence data globally, coupled with ongoing efforts to collate data on historical introductions and pathways (Kraus 2009, Essl et al. 2015, Chapple et al. 2016, Dyer et al. 2016), means that this approach can be readily applied to other regions and taxa to prioritize quarantine and surveillance efforts for species unintentionally transported via goods and services.

It is clear that opportunities have existed for *D. melanosticus* individuals to be introduced in Australia in recent times (i.e., 1996-2012 and 1999-2012 for shipping and air traffic routes, respectively). We are not aware of any extant population of this species in the country, but our results suggest a potential risk for introductions. Plausibly, our finding that *D. melanosticus* has been repeatedly transported to Australia reflects the fact that the species is particularly abundant in anthropogenic habitats that facilitate unintentional transport, such as shipping ports and tourist resorts. *Duttaphrynus melanosticus* is also nocturnal, and potentially seeks shelter in anthropogenic retreats (e.g., shipping crates, luggage) during the day, when most shipping activity occurs (White and Shine 2009). Additional research on the behaviour of *D. melanosticus* at shipping hubs is needed to further clarify mechanisms of introduction.

Our models for the ship- and air-specific transport routes revealed that vehicles coming from only two countries within the range of *D. melanosticus* – Indonesia and Thailand, respectively – are major drivers of the number of *D. melanosticus* detected in Australia. Collaborating with authorities from these two countries would likely enhance detection capacities at the sources, reducing the likelihood of unintentionally shipping toads.

399

400 We also found low to moderate probabilities of interception at the border, and of on-shore
401 detection in some States; actions aimed at increasing these probabilities would further reduce
402 the likelihood of introduction and establishment. Our results suggest that maintaining high
403 inspection targets may be one way to increase border interceptions, as an increase in
404 inspection targets after 2001 (100% of incoming containers being visually, externally
405 inspected), corresponded with marked increases in the number of toad interceptions in
406 subsequent years. Future studies should evaluate whether the focused risk-assessment based
407 inspection system implemented by the Australian Government in 2010 reduces the
408 probability of *D. melanostictus* interception.

409

410 Interestingly, we found no evidence that geographic biases in the origins of stowaway toads
411 were driven by transport pressure. After accounting for country of origin and probabilities of
412 interception and detection, relationships between *D. melanostictus* numbers and transport
413 pressure at Australian ports and airports were weak and/or uncertain. From a biosecurity
414 perspective, the reasons underlying State-level variation in on-shore detection probabilities
415 warrant further study; one possibility is that they simply relate to different on-shore
416 biosecurity surveillance efforts (García-Díaz et al. 2016).

417

418 We found that a SDM fitted to the native geographic range of *D. melanostictus* accurately
419 predicted locations of both native and alien populations, providing support for our
420 assumption that locations in Australia that are environmentally similar to the species' native
421 range represent biosecurity priorities. Projecting the SDM across Australia revealed that a
422 large number of airports and maritime shipping ports where *D. melanostictus* has not yet been
423 intercepted or detected are predicted to be environmentally suitable. Furthermore, airports

and maritime shipping ports that have received greater numbers of *D. melanostictus* to date are some of the most environmentally suitable locations in the country. Thus, propagule pressure data and SDM predictions identified similar biosecurity priorities in our case study. In other systems, however, these two components of the invasion process may differ more markedly, and should therefore always be jointly considered.

For systems in which transport pressure correlates more strongly with numbers of stowaways, our approach could be extended to predict spatial variation in both environmental suitability and propagule pressure. For example, a positive correlation between shipping volumes and honeybee interceptions in Australia enabled Heersink et al. (2015) to combine shipping volumes with an SDM to estimate spatially-explicit likelihoods of establishment. However, other studies that have combined transport pressure data and SDMs have simply assumed positive relationships between transport pressure and the likelihood of establishment (Herborg et al. 2007, Crafton 2015, Fletcher et al. 2016). Applying our framework to the case of *D. melanostictus* in Australia illustrates that this common assumption, while supported by several empirical studies (e.g., García-Díaz et al., 2016; Heersink et al., 2016), does not hold in all cases (also see Caley et al., 2015). The lack of proportionality between the number of stowaway toads and trade volumes may have been due to geographic biases in the origins of stowaway toads, and low levels of proactive and reactive capacity to tackle biosecurity threats in these centres of origin (Early et al. 2016). Regardless of the precise mechanism, our results suggest that drivers of transport may vary depending on the species and/or taxonomic group being passively transported.

In the absence of correlations between transport pressure and numbers of *D. melanostictus* stowaways in Australia, our results suggest that border inspection efforts should be focused

on airplanes coming from Thailand and ships coming from Indonesia, at recipient transport hubs with high environmental suitability. Deploying greater on-shore detection resources at receiving sites with higher predicted environmental suitability will also enable early detection in the event of an on-shore introduction, and hence reduce the probability of *D. melanostictus* establishing in a suitable environment.

It is worth noting that our predictions of environmental suitability for *D. melanostictus* in some regions of Australia differ considerably from those used in a previous risk assessment (Page et al. 2008). In particular, our SDM predicts lower environmental suitability in north-western Australia, but much higher suitability in southern regions. This disparity between model predictions in southern regions is crucially important in light of our finding that cities such as Perth, Sydney, and Melbourne are subject to high levels of transport pressure. High predictions in southern areas also represent a biodiversity concern, as these regions are predicted to lie outside the broad fundamental niche of the only established toad species in Australia: the cane toad *Rhinella marina* (Kearney et al. 2008). This species, like *D. melanostictus*, possesses a potent cardiotoxic steroid, and has had devastating effects on native frog-eating predators (Shine 2010). High predictions of environmental suitability in southern Australia were not due to model extrapolation (Fig. S2), to the contribution of any one particular environmental variable (Fig. S3), or to differences in the environmental variables used to parameterize the two models (Fig. S4). Regardless of the reason for the disparity between predictions, our results suggest that the ‘serious’ establishment risk previously assigned to this species (the 2nd highest possible rating) may, in fact, be an underestimate. Our predictions are, however, in line with a more recent assessment based on alternative threat categories, which assigned the species as having an ‘extreme’ risk of establishment (Massam et al. 2010).

Our SDM also predicts significant overlap between suitable environments for *D. melanosticus* and numerous global biodiversity hotspots that contain high species richness and endemism, and are subject to significant threat (Mittermeier et al. 2011). For example, our model predicted high environmental suitability in Toasmina in eastern Madagascar, a region where *D. melanosticus* has recently established alien populations (Kolby 2014, Moore et al. 2015). Madagascar, like Australia, lacks native toad species, placing numerous species at risk (Kolby 2014). Our model also predicts that there is scope for further spread in eastern parts of Madagascar, concurring with a previous SDM based on the species' native range (Pearson 2015).

Conclusion

The number of alien species transported as stowaways is expected to increase as connectivity between regions of the world increases with globalized trade (Banks et al. 2015, Hulme 2015, Heersink et al. 2016). Furthermore, increases in trade volume along established routes could increase the likelihood of low-probability hitchhiking events. The modelling approach presented here provides a flexible framework to tackle this emerging pathway. Our work builds on previous efforts to integrate transport pressure data and SDMs, by jointly modelling the number of intercepted and detected stowaways, while incorporating imperfect detection and the environmental suitability of recipient hubs. We have illustrated the applicability of our framework using the case study of *D. melanosticus* in Australia, but we expect our work to have broad applicability given the large number of alien species globally transported (Kraus 2009); the increasing importance of stowaway pathways for the movement of alien species (García-Díaz and Cassey 2014); and current efforts to synthesize information on historical introduction pathways globally (Essl et al. 2015, McGeoch et al. 2016).

499

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644 **Supplementary Material**

645 **Appendix S1.** Sources of detection, interception, and occurrence data.

646 **Appendix S2.** R code for pathway models and model fitting details.

647 **Table S1.** Posterior estimates of the country of origin intercepts, relating the total number of
648 *Duttaphrynus melanosticus* transported into seven Australian States via shipping and air
649 routes.

650 **Figure S1.** Relationships between the number of interceptions and detections per year of
651 *Duttaphrynus melanosticus* and other Bufonidae species in Australia (1986-2013).

652 **Figure S2.** SDM prediction uncertainty due to model extrapolation and due to the data used
653 to train the SDM.

654 **Figure S3.** Exploration of a SDM prediction in Melbourne, Australia.

655 **Figure S4.** Difference between SDM predictions from a model based on the 16 climate
656 variables used in a previous risk assessment for *Duttaphrynus melanosticus*, and a model
657 based on the five environmental variables used in the current study.

658 **Table captions**

659 **Table 1.** Model fit (Bayesian p -values) and posterior parameter estimates (median $\pm$ standard
660 error, and 95% Credible Intervals) of the models for the transport of *D. melanosticus* into
661 Australia. The average probability of interception was estimated across all the countries of
662 origin of ships and flights arriving in Australia (origin-specific estimates presented in Table
663 S2). The total number introduced in Australia was estimated by summing estimates for all
664 Australian States. Covariates: (1) flights: total number of direct flights from all countries
665 inhabited by the species; (2) seats: total number of seats on the direct flights; and, (3) ships:
666 total number of ships from all countries inhabited by the species. Covariates were
667 standardized. β_i , slopes of the models. Posterior estimates of the origin-specific intercepts are
668 provided in Fig. 2, and in Table S1.

	Shipping traffic routes	Air traffic routes
Bayesian p -value interceptions	0.31	0.72
Bayesian p -value detections	0.45	0.57
$\beta_{flights}$	NA	-0.11 ± 0.48 (-1.06, 0.90)
β_{seats}	NA	-0.19 ± 0.42 (-1.09, 0.60)
β_{ships}	-0.60 ± 0.63 (-2.19, 0.24)	NA
Average probability of interception at the border (pb)	0.47 ± 0.06 (0.35, 0.60)	0.51 ± 0.09 (0.34, 0.68)
Probability of detection on-shore (pos_i)		
State 1	0.62 ± 0.24 (0.16, 0.98)	0.44 ± 0.26 (0.06, 0.95)
State 2	0.40 ± 0.29 (0.01, 0.96)	NA
State 3	0.18 ± 0.22 (0.02, 0.81)	0.56 ± 0.24 (0.13, 0.97)
State 4	0.63 ± 0.24 (0.14, 0.98)	0.34 ± 0.27 (0.01, 0.93)
State 5	0.44 ± 0.29 (0.02, 0.97)	NA
State 6	0.60 ± 0.25 (0.13, 0.98)	0.57 ± 0.24 (0.14, 0.98)
State 7	0.57 ± 0.25 (0.11, 0.98)	0.61 ± 0.24 (0.16, 0.98)
Number of toads introduced		
State 1	0.24 ± 1.33 (0.00, 3.00)	0.25 ± 0.83 (0.00, 3.00)
State 2	0.07 ± 0.56 (0.00, 1.00)	NA
State 3	0.83 ± 5.90 (0.00, 8.00)	0.21 ± 0.82 (0.00, 2.00)
State 4	0.09 ± 0.63 (0.00, 1.00)	0.31 ± 0.89 (0.00, 3.00)
State 5	0.05 ± 0.48 (0.00, 1.00)	NA
State 6	0.11 ± 0.68 (0.00, 1.00)	0.31 ± 1.38 (0.00, 4.00)
State 7	0.14 ± 0.86 (0.00, 2.00)	0.18 ± 0.66 (0.00, 2.00)
Australia	22.54 ± 23.43 (3.00, 80.00)	9.92 ± 7.89 (1.00, 30.00)

Figure captions

Fig. 1. Number of on-shore detections and border interceptions of *Duttaphrynus melanostictus* and other species of the family Bufonidae in Australia over time.

Fig. 2. Posterior estimates (median and 95% Credible Intervals) of the country of origin intercepts, from a model relating the total number of *Duttaphrynus melanostictus* transported into seven Australian States via shipping and air routes. Note, not all countries with shipping routes also had air routes into Australia. Estimates were obtained from a negative binomial model relating the total number of toads to State-specific transport pressure covariates. Posterior estimates are also provided in Table S1.

Fig. 3. Environmental suitability for *Duttaphrynus melanostictus* globally (a), and across Australia (b), Madagascar (c), the species' native geographic range (d), and the species' alien range in Indo-Malaysia (e). Hollow circles in panels (b), (c) and (e) represent *D. melanostictus* occurrence records.

Fig. 4. Environmental suitability of native-range occurrence records of *Duttaphrynus melanostictus* (a); alien records of *D. melanostictus* (b); Australian airports (c); and Australian maritime shipping ports (d).

Fig. 5. Number of border interceptions (a, c) and post-border detections (b, d) of *Duttaphrynus melanostictus* at maritime shipping ports and airports from 1986–2013 (log10 scale). Also shown in (b) are two individuals detected in the wild (Lang Lang and Middle Swan, VIC).

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.