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## **Evaluating the effectiveness of footprint platforms to detect invasive mammals: coypu (*Myocastor coypus*) as a case study**

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### **ABSTRACT**

Effective and easy-to-apply monitoring techniques are necessary to detect alien species at their first stage of invasion, allowing rapid removal or delimitation of the invaded range for eradication or routine control actions. Monitoring tools should be effective in detecting the target species, reduce false absences and allow an early detection. The coypu (*Myocastor coypus*), is a large semi-aquatic rodent native to subtropical and temperate South America, introduced all over the world for its valuable fur. We tested tracking plates in the framework of a coypu occupancy study in order to take into account false absences and define a standardized monitoring protocol for the species with a limited engagement of staff. We set 60 tracking plates in linear transects along artificial waterbodies within the rice district in northwestern Italy and checked them for six consecutive days. For the analyses we fitted single-season occupancy models to our detection history data. We detected coypu presence at least once in 29 out of the 60 investigated transects (48%). When modelling occupancy and detection probability constant in time and space, the estimate  $\Psi$  was 0.48 and detection probability  $p$  was 0.60. A minimum of four consecutive visits to the transects provided reliable detection. Coypu's probability of presence was significantly driven by the amount of surface covered by rice plantations around the investigated water courses. The proposed method may function as a tool for the rapid detection of coypu on large scale monitoring projects and in case of new colonization, and as a basis for subsequent prompt control actions.

### *Keywords:*

Tracking plates; *Myocastor coypus*; Italy; Occupancy models; Detection

### **Introduction**

Biological invasion is one of the greatest threats to biodiversity conservation and a leading driver of extinction for many taxa (Bellard et al., 2016), as well as a major cause of economic losses (Pimentel et al., 2005). It is therefore urgent to develop effective management strategies aimed at containing these impacts. To implement early warning and rapid response systems or management actions, monitoring is pivotal. Effective monitoring techniques are necessary to detect species at their first stage of invasion, allowing a rapid removal, or to

delimit the invaded range for eradication or control (Byers et al., 2002; Bogich et al., 2008; Simberloff et al., 2013).

Monitoring tools should be effective in detecting the target species, even at low densities, and preferably be cheap and easy to apply. A common sampling problem, especially for elusive species, is the failure to detect a species' presence despite it being present, generating false absences. It usually happens when the population size is small, the individuals are difficult to sample, or sampling effort is limited (Gu and Swihart, 2004). Imperfect detection might neglect sites where the species is inaccurately deemed to be absent, limiting the effectiveness of management actions (Ancillotto et al., 2018). Given the importance of an accurate detection, especially for introduced species, there is a need to design and test new monitoring techniques that are inexpensive, require low effort, reduce false absences and provide an early detection.

The coypu (*Myocastor coypus*), is a large semi-aquatic rodent native to subtropical and temperate South America, introduced all over the world for its valuable fur (Carter and Leonard, 2002; Bertolino et al., 2012). While perceived in some regions as a valuable resource, in most of the countries this species is considered a pest because of its impact on ecosystems, crops and irrigation systems (Carter and Leonard, 2002; Panzacchi et al., 2007; Bertolino et al., 2012). In the invaded range, coypus can alter natural habitats because their feeding activities destroy marsh vegetation (Boorman and Fuller, 1981; Carter et al., 1999) or directly impact animals such as waterbirds (Bertolino et al., 2011). Moreover, this rodent can damage cereal crops, especially in agricultural areas connected by canals to aquatic environments (Abbas, 1988; Panzacchi et al., 2007), and undermine water control structures through their burrows (Carter and Leonard, 2002; Panzacchi et al., 2007). Consequently, the species is managed in many countries to reduce its negative impacts, including Italy where it is widespread (Bertolino et al. 2015; Loy et al. 2019).

The methods commonly used to detect the presence of coypu include the direct observations of animals (Marini et al., 2011) or the detection of active burrows (D'Adamo et al., 2000), feeding signs (Corriale et al., 2006) or paths used by the animals (Balestrieri et al., 2016). All these methods require a search in the field that could be time expensive. Furthermore, they have not been tested against presence and absence of the species, but see Balestrieri et al. (2016) for a quantitative approach. A technique not yet tested with coypu, but widely used to detect many other mammal species (Zielinski and Truex, 1995; Gillies et al., 2003; Connors et al., 2005; DeSa et al., 2012), is the use of tracking plates: plates covered by a plastic medium used to detect the footprints of animals.

In this study we tested the effectiveness of tracking plates for coypu detection. The method was tested in the framework of an occupancy model in order to take into account false absence and evaluate covariates that influence coypu presence. The purpose of this research was the definition of a standardized monitoring protocol for the species with a limited engagement of staff.

## **Materials and Methods**

### *Study area*

Our study was conducted within the River Po Valley in northwestern Italy (Piedmont region, from 45°11'N 8°06'E to 45°08'N 8°20'E; Fig. 1). The climate in this area is subcontinental with hot summers and cold winters. Abundant rainfall occurs mainly in spring and fall, with occasional snowfalls in winter. Part of the territory falls within the Park of the Rivers Po and Orba, a system of natural reserves established to protect residual wetlands and freshwater habitats.

The landscape is dominated by the Po riverbed and its riparian ecosystem, intensive rice growing and dense artificial drainage network on the left of the river and hilly broadleaved forests, grassland and crop fields on its right (Fig. 1). The paddy fields water network consists of narrow, long canals subjected to seasonal regulated drying out (autumn/winter) and flooding (spring/summer), functional to rice growth. Permanent water bodies not undergoing flow rate regulation also occur. These are artificial ponds, quarry lakes and major channels. Residual vegetation on the banks is mainly represented by spontaneous grass and herbs, with sparse woody vegetation, common reed *Phragmites australis* and broadleaf cattail *Typha latifolia*. Riparian vegetation on both sides of the Po riverbed hosts assemblages with different species of willows (*Salix* spp.), poplars (*Populus* spp.), European alder (*Alnus glutinosa*) and hygrophilous herbs, shrubs and bushes. On the right of the Po river the majority of water bodies include artificial ponds and lakes, and minor rivers.

### Field Survey

We selected 60 areas to survey along artificial canals within the paddy fields, the banks of the Po and other small rivers, ponds and other water bodies. In each area we set a linear transect with 3 tracking plates placed at 100 m intervals, in close proximity and in parallel to water bodies (Fig. 2). Tracking plates were made of squared (60 x 60 cm) plywood covered with a thin layer of vaseline in order to better hold the overlying thicker layer of sand, necessary to tracks impression, and baited with a few pieces of carrot. Between June to September 2018, 15 transects were set up at a time and inspected once a day for 6 consecutive days, and then moved to other areas. During each inspection, detected tracks (Fig. 2) were identified, measured, photographed and then deleted, the plates were reactivated by adding sand and renewing bait when necessary.

One of the assumptions of occupancy models requires that the target species is not misidentified. Coypu is much larger than other rodent species present in the area, like rats (*Rattus rattus* and *R. norvegicus*) and Italian water voles (*Arvicola italicus*, Castiglia et al., 2016); it has also webbed hind feet that make the foot-print easily recognizable.

Transects were set up at a distance of at least 1.5 Km from each other, with the aim of avoiding transects being visited by the same individual (Doncaster and Micol, 1989; Reggiani et al., 1993). Transects were placed regardless of the boundaries of Natural Reserves, in order to homogeneously survey the study area.

During the survey we also recorded the presence of footprints (outside the tracking plates), paths, and burrows along each transect, as well as any observed animals.

### Statistical analyses

Analyses on the effectiveness of tracking plates and of factors influencing coypu's distribution were performed using occupancy models developed by MacKenzie et al. (2002). These models allow the estimation of the species probability of presence ( $\psi$ ) and detection ( $p$ ) at a given sampling site, taking into account imperfect detection, i.e., failure in detecting a species when present, resulting in false absences (MacKenzie et al., 2006).

For each transect, we compiled the detection history: non-detection, 0; detection, 1. We then fitted single-season occupancy models (MacKenzie et al., 2002) using the program PRESENCE 12.25 (Hines, 2006). We selected 6 covariates that we hypothesized could influence the probability of coypu presence (Table 1). We expected the probability of presence in a given transect to be associated with the water body type (lentic/lotic) and width at water level, water speed (zero, low, high), the cover of vegetation (tall grasses, shrubs and trees) taller than 80 cm on water bodies banks. Tall vegetation was included in the analysis because it represents potential shelter from local predators, e.g. canids, mustelids and herons

(Bertolino et al., 2012). We also calculated the surface covered by paddy fields and other crops in a buffer area. The mean canal lengths (linear extension) in adult coypu individual home ranges is estimated to be about 1190 m (Doncaster and Micol, 1989), while, according to Reggiani et al. (1993), in the Mediterranean area coypus avoid crops beyond a 100 m distance from water bodies. We then built in QGIS software rectangular buffers of 20 ha (1000 m long, 200 m wide) centered on each transect in order to measure the cover of the two landscape covariates. Quantitative covariates were standardized to z-scores. Covariates were tested for correlations before their inclusion in the models.

Considering the limited sample size ( $n=60$ ) we used the corrected AICc (Burnham and Anderson, 2002). This value is not provided in the PRESENCE output, so this and related statistics were computed using the formulas reported in Symonds and Moussalli (2011) and Richards et al. (2011). The goodness of fit was assessed using the measure for overdispersion ( $\hat{c}$ ) with 2000 bootstraps, correcting with quasi-AICc (QAICc) if necessary (Hines, 2006).

We evaluated the minimum number of visits necessary to be confident of the coypu absence from an area, using the formula  $N = \ln(\alpha\text{level})/\ln(1-p)$  (Reed, 1996), where  $\alpha$  represents the probability of type I error (fixed at 0.05) and  $p$  the detection probability.

We then evaluated the influence of the six variables hypothesized to be related to site occupancy by building all possible models. We held detection probability ( $p$ ) constant, because landscape covariates could not change within the 6-days survey. Models were ranked according to AICc (or QAICc) values and selected with a 2-steps process. First, we considered all models with  $\Delta AICc < 6$  from the model with the lowest AICc (Richards et al., 2011). Then, we considered if in nested models adding 1 or more parameters increased the fit, as measured by the log-likelihood value (Burnham and Anderson, 2002).

We also used Akaike weights ( $w_{gt}$ ) to assess the strength of evidence in support of each model (Srivathsa et al., 2014). Finally, model-averaging was used to obtain parameter estimates and associated standard errors for covariates present in two or more selected models (Burnham and Anderson, 2002). Model-averaging was computed using the spreadsheet developed by Brian R. Mitchell ([www.uvm.edu/~bmitchel/software.html](http://www.uvm.edu/~bmitchel/software.html)) and using the second formula proposed by Burnham and Anderson (2002; formula 6.12, p. 345). Averages were computed over all models, with regression coefficient considered to be zero in models that do not contain the corresponding covariate and normalizing Akaike weights by dividing by their sum. Effect sizes of covariates were computed as regression coefficients after model averaging divided by their standard errors (Burnham and Anderson 2002)

## Results

We detected coypu presence at least once in 29 out of the 60 investigated transects (naive estimate 0.48). When modelling occupancy and detection probability constant in time and space, the estimate  $\Psi$  was 0.48 and detection probability  $p$  was 0.60. With  $p(t)$  AICc was higher and therefore  $p$  was kept constant in the subsequent modelling stages. The minimum number of visits per site necessary to establish coypu's absence from an area was 3.2.

We also recorded animals' paths along 16 transects, footprints along 8 transects and burrows in 3, while animals were observed only in 1 transect. These data could not be analysed with occupancy models because they were too limited (footprints and observed animals) or the framework was not applicable for the short duration of the survey (no change in paths and burrows expected in 6 days).

Models including covariates outperformed the reference constant model. Since overdispersion was found in the most general model the value was corrected ( $\hat{c} = 2.35$ ). The results for the first ten and the constant model are reported in Table 2; all other models have higher  $\Delta QAIC$ .

Eight models were included within  $\Delta QAICc < 6$  and nearly all included Rice as a covariate (Table 2). However, models from 4 to 8 add more covariates without improving the fit of the models. Therefore, we retained only the first three models, which included also Other crops and Vegetation as covariates. Models 1 and 3 are nested with the simplest model 2 which has only one parameter; however, the increase in the parameters (1 and 2 respectively) are compensated by a decrease in the likelihood and therefore there is support also for these models.

Model averaged values (Table 3) indicated that the effect size of rice is 2.5 times greater than Othercrops and 10 times greater than Vegetation, supporting the overwhelming importance of this covariate. The relation between rice cover and  $\Psi$  is explained by a cubic curve (Fig. 3).

## Discussion

The results of our research demonstrate that tracking plates constitute an efficient and rapid indirect method for the effective detection of coypu's presence. This allows the definition of a standardized protocol for large scale and long-term monitoring of the species, feasible in the face of limited resources. In the landscape investigated, with the estimated probability of detection, a minimum of four consecutive visits was necessary to ensure that in the presence of coypu non detection will only occur with a probability of 5% or less.

On the other hand, during the surveys we recorded only few signs of the species presence and animals were observed only along a transect. Though it was not possible to apply occupancy models to these data for a proper statistical comparison, the most common signs of presence, the paths used by animals, were recorded only in 55% of the transects where coypu were recorded with tracking plates.

The positive association between coypu's occurrence and rice fields cover confirms what was previously known on the species habitat preferences. It inhabits a wide range of freshwater habitats, e.g. rivers, streams, channels, including those more artificial like rice fields (Reggiani et al., 1993; D'Adamo et al., 2000; Bertolino and Ingegno, 2009). Rice fields could be considered as a partial surrogate of natural aquatic habitats, which however are flooded during plants growth and dried out after harvest. The paddy drainage network seems to promote the population's spatial segregation in favor of rice plantations, despite seasonal flooding and drying out of fields and canals. As hypothesized by Bertolino et al. (2005), the drying out of canals from September to April is probably compensated by permanent wetlands, e. g. scattered quarry lakes, large channels, and artificial ponds, which may act as shelter areas and guarantee the long-term persistence of vital populations and subsequent recolonization in spring (Bertolino and Ingegno, 2009). Our results seem to confirm the observed descending gradient in species distribution at increasing distance from the rice plantations. Along the Po river banks and in the landscape on the right of the river where the water network is less extended and land use heterogeneity at the landscape scale increases, coypu is sporadic. Further research on this area may focus on the study of potential seasonal shifting of the species ranges as a consequence of drainage network management (canals flooding and drying out), also investigating potential source-sink dynamics.

The coypu has huge impacts on ecosystems through clearing of aquatic vegetation, with indirect negative effects on animal species requiring plants for shelter and their reproduction (Willner et al., 1979; Foote and Johnson, 1993; Bertolino et al., 2005). Furthermore, it has important economic costs associated to crops and drainage systems damage (Abbas, 1988; Panzacchi et al., 2007). For this reason, the species is included in the Union list of invasive species under the EU Regulation on IAS (1143/2014). The regulation requires member states to monitor listed invasive alien species and report their distribution every six years.

Furthermore, species on this list should be managed to avoid spreading and to mitigate impacts.

The tracking plates method developed here could be used to investigate coypu distribution where the species is spreading or where it is managed for spatial containment or population control. The proposed protocol allows to model probability of detection and together with Reed's formula the minimum effort necessary to reduce false absences. The method is easy to apply and cheap and could easily inform and direct control activities, allowing a better evaluation of their effects, and be used as an effective tool for implementing an early warning system. In this respect, further calibration in other habitats may be useful to demonstrate the general validity of tracking plates for coypu monitoring.

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**Table 1**

List of predictor covariates used to model the probability of presence ( $\Psi$ ) parameter.

<b>Covariate</b>	<b>Description</b>
Vegetation	The percent amount of tall grasses (> 80 cm) and shrubs/trees cover on banks (%)
Water width	Width of canals at water level (m)
Water type	Lentic/lotic
Water speed	Zero, low, high
Rice fields	Surface covered by rice fields within a 20 ha buffer
Other crops	Surface covered by other cultivated land within a 20 ha buffer

**Table 2**

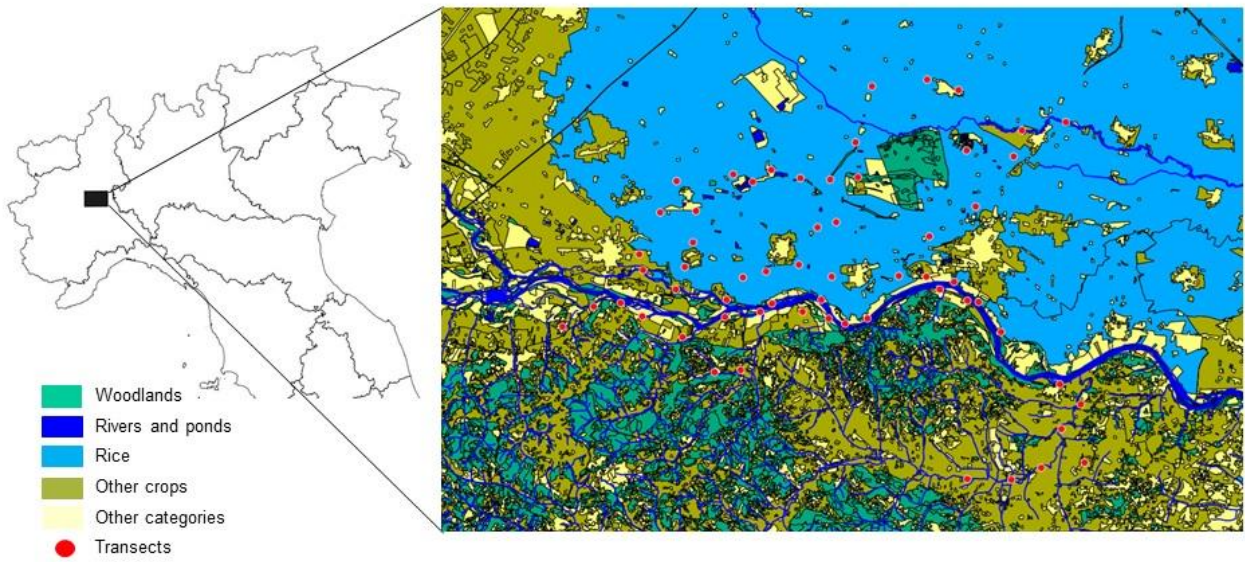
First top ranked single-season occupancy models of coyru detection.

<b>ID</b>	<b>Model</b>	<b>QAICc</b>	<b><math>\Delta</math>QAICc</b>	<b>QAICc wgt</b>	<b>No.Par.</b>	<b>-2LogLike</b>
1	psi(Rice+Othercrops) p(.)	123.25	0.00	0.30	4	269.39
2	psi(Rice) p(.)	123.66	0.41	0.25	3	275.77
3	psi(Rice+Vegetation+Othercrops) p(.)	125.20	1.95	0.11	5	268.38
4	psi(Rice+Waterspeed) p(.)	125.47	2.22	0.10	4	274.62
5	psi(Rice+Waterwidth) p(.)	125.55	2.30	0.10	4	274.81
6	psi(Rice+Watertype) p(.)	125.89	2.64	0.08	4	275.60
7	psi(Rice+Waterwidth+Vegetation) p(.)	127.58	4.33	0.03	5	273.98
8	psi(Othercrops) p(.)	127.80	4.55	0.03	3	285.52
9	psi(Waterwidth) p(.)	135.84	12.59	0.00	3	304.42
10	psi(Watertype+Waterwidth) p(.)	138.45	15.20	0.00	4	305.16
11	psi(.) p(.)	138.79	15.54	0.00	2	316.59

**Table 3**

Model-averaged parameter estimates for variables associated with occupancy by coypus and their effect sizes.

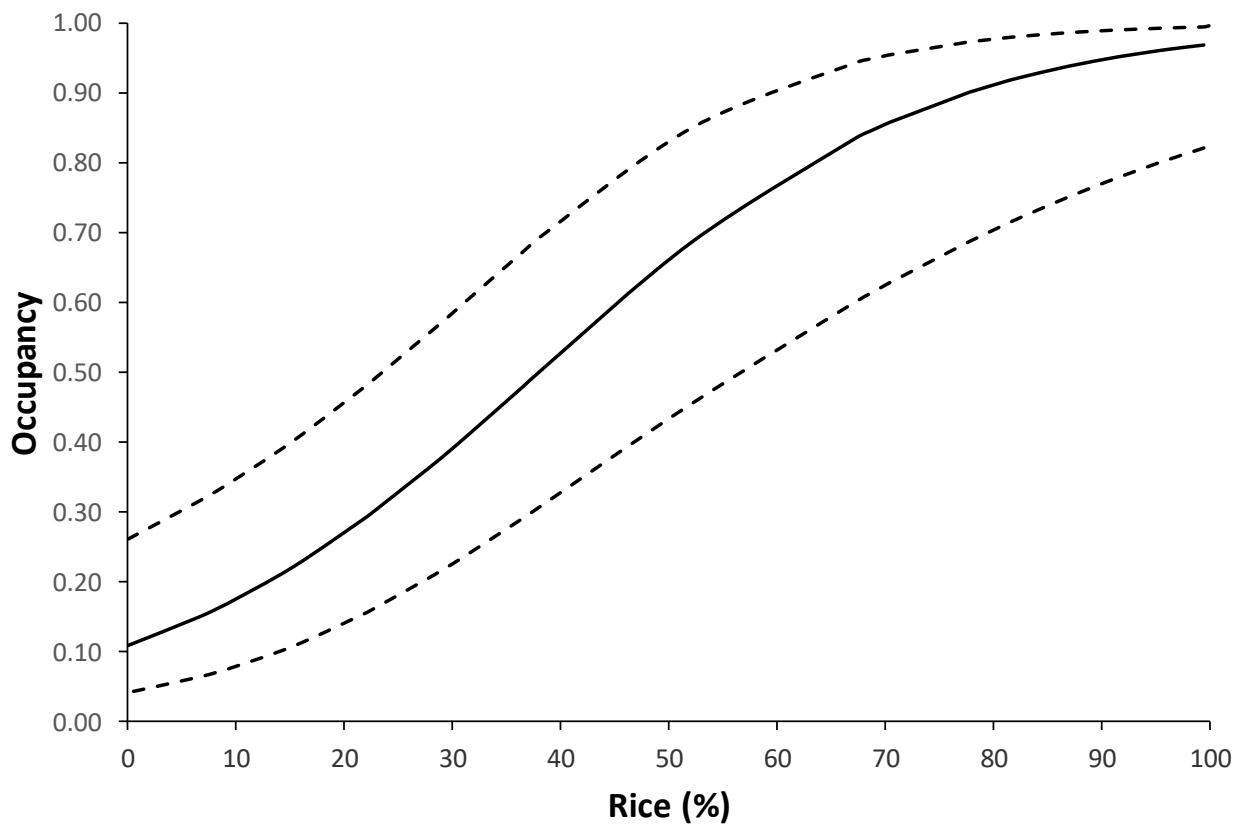
<b>Variable</b>	<b><math>\beta</math></b>	<b>SE</b>	<b>Lower 95% CI</b>	<b>Upper 95% CI</b>	<b>Effect size</b>
Rice	1.943	0.589	0.789	3.098	3.299
Othercrops	1.147	1.101	-0.575	2.871	1.042
Vegetation	-0.218	0.661	-3.260	1.080	0.330



**Fig. 1.** Study area (shaded area, left) within Italy and map with sampling sites (red circles, right) and land cover categories.



**Fig. 2.** On the left, tracking plate set on the bank of a canal; on the right, feet and tail tracks imprinted in the sand.



**Fig. 3.** Coypus occupancy as a function of the percent surface covered by rice plantation within the buffer built around each focal transect. Continuous line: occupancy (psi); dotted lines: 95% Confidence interval.