Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

An indirect method for assessing the abundance of introduced pest *Myocastor coypus* (Rodentia) in agricultural landscapes

A. Balestrieri[1], M. Zenato[2], E. Fontana[2], P. Vezza[3], L. Remonti[2], F. E. Caronni[4]*, N. Saino[1] & C. Prigioni[2]

1 Department of Biosciences, University of Milan, Milan, Italy
2 Department of Earth and Environmental Sciences, University of Pavia, Pavia, Italy
3 Department of Environment, Land and Infrastructures Engineering, Politecnico of Turin, Turin, Italy
4 Lombardy Region, Agriculture Directorate, Piazza Città di Lombardia 1, Milan, Italy

Keywords
Coypu; nutria; invasive species; introduction range; monitoring; volunteer; pest management; population estimates.

Correspondence
Alessandro Balestrieri, Department of Biosciences, University of Milan, via Celoria 26, 20133 Milan, Italy. Tel: +39 348 3132130
Email: alebls@libero.it

Editor: Jane Waterman
*Current address: Regional Park of the River Ticino Valley, Lombardy Region, via Isonzo 1, 20013 Pontevecchio di Magenta (MI).

Received 13 November 2014; revised 12 June 2015; accepted 1 July 2015

doi:10.1111/jzo.12284

Abstract

Pest management requires the development of robust monitoring tools. In Italy, coypu *Myocastor coypus* (nutria) have been controlled since the early 1990s, but the effectiveness of these measures has never been tested. With the aim of developing a reliable and volunteer-based method for the long-term monitoring of coypu abundance in agricultural landscapes, we calibrated an index based on surveys for coypu paths against density estimates obtained through a standardized mark–recapture technique. Two trapping sessions were performed in winter for each of 12 1-km long stretches of irrigation canals and watercourses using 15 baited cage traps. Trapping sessions lasted 7 days each, with a 10-day break between sessions. Population size was assessed using three methods: Peterson–Lincoln's formula, capwire estimators and accumulation curves. Active coypu paths and five habitat variables were recorded by walking on the edge of both banks. The variables were then related to population size (y) by means of multi-regressive models, testing for the predictive power of the selected models by leave-one-out cross-validation. Multi-regressive models included only the number of coypu paths with the best performances achieved by the model based on Peterson–Lincoln formula, supporting path count as an effective method to assess the abundance of the coypu in agricultural landscapes. Concurrently, to assess the field suitability of the indirect method, surveys for coypu paths were carried out on 122 randomly chosen 3-km long stretches of irrigation canals and watercourses in the central part of the River Po valley (*c*. 15 000 km^2; N Italy). The highest (>8/100 m) mean number of paths was recorded in the central part of the study area. According to the regression models, the overall number of coypu is predicted to range between 350 000 and 1 100 000, raising doubts about the effectiveness of current control measures.

Introduction

Strategies to overcome the impacts of biological invasions adopted by the Convention on Biological Diversity (United Nations, 1992) include prevention, early detection and, wherever eradication fails or is not attempted, direct management and restoration, that is, long-term population control and mitigation of impacts. In all of these strategies, monitoring plays a major role for understanding the invasion process and assessing the status of alien species (Rooney *et al*., 2004) and whether control measures are working (Campbell *et al*., 2002). In Europe, monitoring schemes for invasive species are still inadequate and the development of robust and cost-effective monitoring tools must be a key objective of ongoing strategies (Vilà & García-Berthou, 2010).

Assessing the numbers and trend of mammal populations is often difficult and prohibitively time consuming and expensive, requiring the collection of a large amount of data (Gese, 2001; Pollock, 2006). As a consequence, indirect indices of abundance, based on surveys for field signs, have been often used to assess the presence, relative and absolute abundance of several species (e.g. Gese, 2001), including semi-aquatic rodents (Gray *et al*., 2013). As the attribution of field signs to the target species is often uncertain and animals cannot be individually identified, indirect indices have been the object of some criticism (Kruuk & Conroy, 1987; Jennelle, Runge &

MacKenzie, 2002). Nonetheless, field sign surveys have been frequently implemented because they allow monitoring even of rare or elusive species at relatively low cost, and can be easily repeated to allow for direct comparisons over time (Heinemeyer, Ulizio & Harrison, 2008).

As several factors can influence the relationship between field signs and population abundance, indices need to be calibrated against direct density estimates (Sadlier *et al*., 2004). Few indices have been properly validated with a known population density (Gibbs, 2000). In general, results have supported the use of field sign indices as an effective tool for wildlife monitoring (see Heinemeyer *et al*., 2008 for a review), although some exceptions have been reported (e.g. Sargeant, Johnson & Berg, 1998; Sadlier *et al*., 2004).

Like most semi-aquatic mammals introduced in Europe, the coypu *Myocastor coypus* (or nutria) is a successful colonizer of freshwater ecosystems, substantially disturbing aquatic vegetation through grazing and undermining riverbanks by burrowing (Bertolino & Genovesi, 2007). It is included in the list of the 100 World's Worst Invasive Alien Species (Bertolino, 2009) and in the list of the 10 invasive species with the highest number of impact types on ecosystem services (Vilà *et al*., 2010). In Italy, coypu eat more than 100 plant species (Scaravelli, 2002), including some classified as endangered or vulnerable. Damage to the drainage systems is *c*. 10 times higher than crop losses and, in the period 1995–2000, accounted to about €2 million per year (Panzacchi *et al*., 2007).

Several countries are carrying out permanent coypu population control. In Italy, the progressive increase in damage and economic losses refunded to farmers by the government (Panzacchi *et al*., 2007) suggests that control campaigns may be totally ineffective, but the long-term effects of removal on coypu density have never been tested properly.

Although in South America some attempts to assess coypu abundance by sign (burrow) surveys have been carried out (Zalba, Politi & De La Fuente, 2001; Corriale *et al*., 2008), in its wide introduction range, coypu numbers have been almost exclusively assessed by mark–recapture methods in small study areas (<100 ha; e.g. Reggiani, Boitani & Destefano, 1995; Petralia, 2003). Mark–recapture methods are commonly used for assessing population size in a wide variety of animal species (Amstrup, McDonald & Manly, 2005), but are not practical when large areas need to be sampled.

Conspicuous field signs that may be used for the indirect assessment of coypu abundance include feces, burrows and paths. Although coypu feces can be correctly identified based on morphology and size, seasonal and habitat-related variation in the pattern of deposition and persistence of feces may affect detectability (Sadlier *et al*., 2004). Burrow-based indices require several assumptions about the number of individuals using each underground system, and because burrows are long-lasting features, they cannot represent short-term fluctuations in coypu abundance (Corriale *et al*., 2008). Well-worn paths are traced by coypu on river and canal banks while going into and out of the water. Networks of transects surveyed for tracks are often used to assess the abundance of several mammals (e.g. Wilson & Delahay, 2001; Heinemeyer *et al*., 2008) and these line–intercept indices seem to correlate well with density (Stander, 1998).

At large geographical scale, the routine monitoring of wildlife needs the involvement of teams of volunteers. When performed by volunteers, survey methods must be as simple as possible and easy to learn in order to provide accurate estimates of population parameters (Newman, Buesching & Macdonald, 2003). With the aim of developing a reliable, cost-effective, volunteer-based index of coypu abundance in agricultural landscapes, we tested an indirect method, based on surveys for coypu paths, against population size estimates obtained using a standardized mark–recapture technique.

Then, to assess the field suitability of this indirect method, we carried out a preliminary study across the coypu's range in the central part of the River Po valley (Lombardy region, N Italy). Cross-validated models allowed us, for the first time, to provide population size estimates in different areas of this region.

Materials and methods

Study area

The River Po valley, or Po-Venetian Plain, is the largest Italian lowland (*c*. 46 000 km^2) and one of the most densely populated areas of the country. The pedogenetic and micromorphological characteristics of the soils support high levels of agricultural (rice, maize, wheat, sugar beet, fruit) productivity and cattle farming. Residual woods cover less than 5% of the whole area (Falcucci, Maiorano & Boitani, 2007). A complex network of canals for irrigation and drainage (1.6 km/km^2 of canals, for a total of 24.5×10^3 km) allows coypu to spread across farmland from main rivers. Most canals (*c*. 60%) range from 2 to 6 m in width and, as they are mainly used for irrigation and flood control, aquatic vegetation is periodically removed to ensure optimal hydraulic performance.

Climate is subcontinental temperate, with mean yearly temperature of 12.0°C and mean yearly rainfall of 1000 mm.

Coypu *Myocastor coypus bonariensis* were first imported in 1928, probably from Northern Argentina, for fur farming (Santini, 1978), an activity which, since the 1950s, spread through several small, family-run farms. Several coypu escaped from farms or were deliberately released into the wild, forming self-sustained populations. Currently, the coypu is widespread throughout the Po-Venetian Plain and in central Italy (Cocchi & Riga, 2001).

This study focused on the central part of the valley (*c*. 15 000 km^2; Lombardy region; Fig. 1), where the coypu has been controlled since 1993 by both cage trapping and shooting.

Population size assessment

To derive an estimate of coypu population size, a mark–recapture method was used. Trapping sessions were performed between 21 November 2013 and 4 April 2014 using

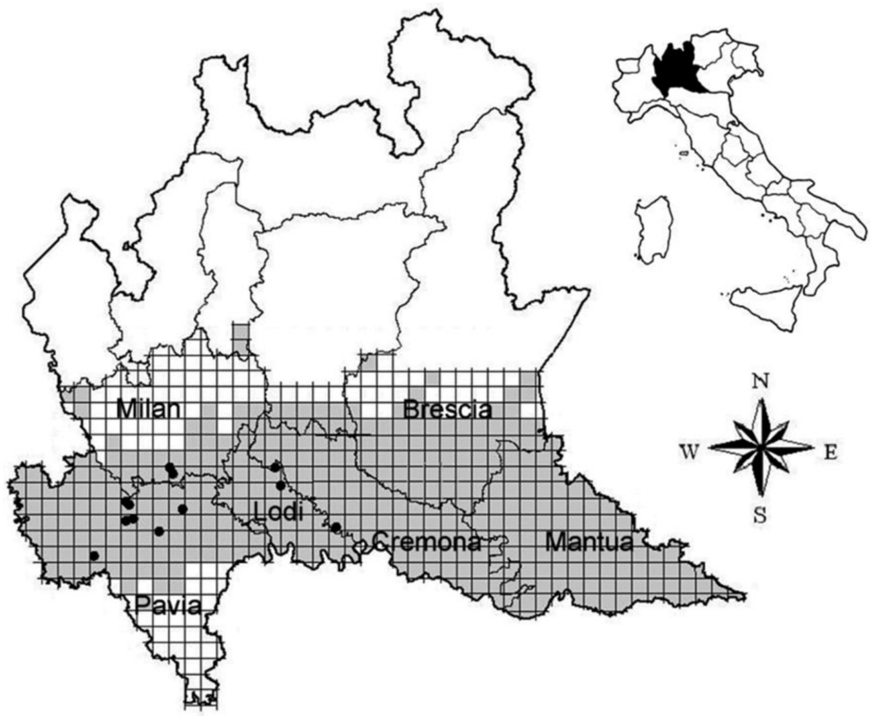

Figure 1 Current coypu range (gray squares) in Lombardy (N Italy) using a 5 × 5 km grid (Prigioni *et al.*, 2013) and location of the stretches of canals and watercourses for which coypu density was assessed by a mark–recapture method.

fifteen 35 × 35 × 80 cm cage traps, baited with a mixture of vegetables (apples, carrots and maize). Traps were placed on 'well-marked' coypu paths, along 12 (Fig. 1) 1-km long stretches of irrigation canals ($n = 10$) and watercourses ($n = 2$), ranging in width between 1.8 and 25 m. Trapping sites were chosen as to sample both natural and artificial watercourses in relation to their respective percent length in the study area.

To meet the 'closed' population assumption, for each sampling stretch, the two trapping sessions lasted 7 days each with a 10-day break between sessions. Traps were neither prebaited nor removed during the 10-day break. It is reasonable to assume that the effects of births/deaths and immigration/emigration did not alter population size significantly in such a short period. While active, traps were checked every morning.

The animals caught were weighed (±50 g) and classified as juveniles (<2 kg), subadults (sexually mature coypu weighing 2.1–3 kg) or adults (≥3 kg; Guichón *et al.*, 2003).

Because of the short length of trapping period, permanent marking was considered unnecessary. Temporary marks reduce animal suffering and potential bodily damage or survival impacts (Silvy, Lopez & Peterson, 2012). In previous studies, coypu had been visually marked by painting on the tails (Kik, 1980), fur cutting (Reggiani *et al.*, 1995), ear punch codes (Lohmeier, 1981) and applying hair dye or ear tags (Guichón *et al.*, 2003). We marked trapped coypu on their back using a commercial bleaching kit, consisting of a dust-free bleach powder and a developer. Bleach was applied on a small area (*c.* 3 cm^2) of the back by inserting a paintbrush through the mesh of the cage walls. Marks needed *c.* 10 min to dry and become durable, and therefore, animals were manipulated for a total of *c.* 15 min before being released. Recaptured individuals were marked twice to avoid double counts.

Trapping effort was expressed as the number of trap nights (i.e. number of traps × number of working nights). For each sampling area, the total number of active coypu paths and the following variables were recorded every 100 m: watercourse width, water depth and speed, bank height and inclination. Paths – that is, the routes traced by coypu climbing up each watercourse bank – were counted by walking on the edge of both banks and considered to be active when showing clear signs of recent coypu passage, that is, fresh feces, tracks or well-worn, tunnel-shaped vegetation. As vegetation on canal banks was rarely thick, paths could be seen throughout their length and both branching out (*Y*-shaped) paths and paths less than 1 m apart were considered as a single path, that is, as a single way in/out the water. When vegetation cover reduced the observer's ability to observe paths, the number of paths reaching the edge of the bank was assumed to correspond to the number of ways in/out the water. The first three watercourse variables could affect the number of coypu using the sampling stretch, whereas the variables bank height and inclination were used to try to assess the ease of access to surrounding grazing areas, potentially influencing the number of coypu paths.

Population size was firstly assessed using the Peterson–Lincoln's formula, modified by Chapman (1951):

$$\hat{N} = \frac{(n_1+1)(n_2+1)}{(m_2+1)} - 1$$

Variance was calculated as:

$$\text{var}\,N = \frac{(n_1+1)(n_2+1)(n_1-m_2)(n_2-m_2)}{(m_2+1)^2(m_2+2)}$$

where n_1 is the animals caught, marked and released in the first trapping session; n_2 is the animals caught in the second session; and m_2 is the marked individuals captured in the second session.

A second estimate of population size was calculated using capwire estimators (Miller, Joyce & Waits, 2005), fitting data to two different models to obtain the maximum likelihood estimate of each population size. Under the equal capture model, all individuals were assumed to have an equal probability of being sampled, whereas under the two-innate rates model the population was assumed to include a mixture of easy-to-capture and difficult-to-capture individuals. The fit of the two models was compared using a likelihood ratio test (LRT); the *P*-value was calculated using a parametric bootstrap approach to estimate the distribution of the LRT for data simulated under the less parameterized equal capture model (Pennell *et al.*, 2013). With this method, data from the two capture sessions were pooled together, so the overall number of recaptures was augmented, including also intra-session recaptures.

Both Peterson–Lincoln's and capwire estimates can be biased by trap response, that is, the capture probability of marked individuals in the second session (Pollock *et al.*, 1990; Ebert *et al.*, 2010). Population size was therefore also assessed by plotting the number of trap nights against the corresponding cumulative number of individuals (i.e. excluding both intra and inter-session recaptures) and then fitting the accumulation curve by non-linear regression to the asymptotic logistic ($y = a/(1 + be^{-cx})$) or Michaelis–Menten ($y = ax/(b + x)$) equations (Colwell & Coddington, 1994). In both equations, y represents the observed number of individuals, x the number of trap nights and a the asymptote or predicted population size. The 95% confidence intervals (CIs) were based on 2000 bootstrap replicates.

Each population abundance estimate (y) was then related to the number of coypu paths and the five watercourse variables (x_i) by means of a multi-regressive model (equation 1).

$$y = \beta_0 + \beta_1 \cdot x_1 + \beta_2 \cdot x_2 + \ldots + \beta_{p-1} \cdot x_{p-1} + \varepsilon \tag{1}$$

where β_i are the regression coefficients estimated by the ordinary least-squares technique and ε is the random error component.

To build multi-regressive models, the procedure reported in Vezza *et al.* (2010) was followed. Non-supervised regional frequency analysis (nsRFA library), available in R, was used for the computation of the models. A combination of all habitat variables was attempted, checking for variable multicollinearity by the variance inflation factor (<5; Montgomery *et al.*, 2001), and homoscedasticity and normality of residuals by diagnostic graphs and Anderson–Darling's test (the test rejects the hypothesis of normality when either $P \le 0.05$ or $A > 0.75$; Laio, 2004). Finally, regression models were discarded whenever one of the independent variables was non-significant according to Student's *t*-test ($\alpha = 0.01$). The adjusted coefficient of determination (R^2_{adj}) was used to assess the descriptive power of each regression.

The generalization performance of selected models was then estimated by a leave-one-out cross-validation (LOOCV) procedure, which is more robust than other techniques for assessing predictive errors and can be applied to regional models (Vezza *et al.*, 2010). LOOCV is a special case of *k*-fold cross-validation where all the data except for a single observation (p–1 in equation 1) are used for training, and the model is tested on that single observation. Based on LOOCV, the value of the classification models for estimating coypu abundance was assessed by both the root mean square error $RMSE_{CV}$ and coefficient of determination R^2_{CV}.

$RMSE_{CV}$ is the square root of the average residual square error V_{CV}:

$$RMSE_{CV} = \sqrt{V_{CV}} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}$$

where $\hat{y}_i$ is the estimated value of the ith dependent variable obtained by a model run with all the observations except the ith one, whereas the coefficient of determination is calculated as:

$$R^2_{CV} = \frac{var(y) - V_{CV}}{var(y)}$$

Regional survey

In the same period, surveys for coypu paths were carried out in 122 (28.7%) out of the 425 5 × 5 km² squares for which coypu presence has been recently confirmed (Fig. 1; Prigioni *et al.*, 2013). Sampling squares were randomly distributed in six Lombardy provinces according to the relative size of their lowland areas; from west to east (Fig. 1): Pavia ($n = 23$), Milan ($n = 12$), Lodi ($n = 10$), Cremona ($n = 32$), Brescia ($n = 20$) and Mantua ($n = 25$).

For each square, a 3-km long stretch of irrigation canals and watercourses was surveyed for active coypu paths on both riversides. Stretches were randomly chosen on 1:10 000 maps, although sometimes obstructions or dried up canals forced redrawing of the transect during the survey. To avoid bias due to variation in sampling experience, all surveys were carried out by the same trained staff (four surveyors). Data were expressed as the mean number of paths per 100 m.

As several 100-m long stretches included no coypu paths, resulting in a distribution too skewed to be normalized, mean numbers of paths per province were compared by the Kruskal–Wallis test with post-hoc Mann–Whitney pairwise comparisons. As a conservative correction for multiple testing, *P*-values were multiplied by the total number of pairs of groups (Bonferroni correction).

A preliminary estimate of coypu abundance in the central River Po valley was then calculated based on the three regression models relating to the number of coypu paths/100 m to population size and on the available information about the length of the hydrographic network (including both watercourses and canals) in the sampled provinces in winter.

Results

Excluding intra-session recaptures ($n = 48$), we achieved a total of 316 captures: 163 (min-max = 5–45) in the first session and 153 (2–25) in the second one. Nineteen per cent of the animals caught in the first session were recaptured in the second one. Age classes were as follows: 13.9% juveniles ($n = 44$), 15.2% subadults ($n = 48$), 70.9% adults ($n = 224$); thus, 86.1% of coypu were sexually mature.

Estimated numbers of coypu ranged between 8 and 196 individuals per stretch (Table 1) and, on average, between 3.9 (accumulation curves) and 6.1 (capwire) individuals per 100 m of watercourse. By capwire, equal capture models performed better than two-innate rates models in 10/12 sites (Table 1). Accumulation curves provided for the narrowest 95% CI (Table 1).

Multi-regressive models included only the number of coypu paths, with values of β_1 ranging between 0.55 and 0.59. The best performances in terms of R^2_{CV} and $RMSE_{CV}$ were achieved by the model based on Peterson–Lincoln estimates of coypu abundance (Fig. 2; Table 2).

In the six sampled provinces, the mean number of coypu paths per 100 m of canal stretch (Table 3) varied significantly ($\chi^2 = 230.4$, $P < 0.0001$, 5 d.f.). Post-hoc pairwise comparisons allowed grouping of the provinces of Milan, Brescia and Mantua, with Pavia, Lodi and Cremona diverging from all the other areas (Table 3).

The highest (>8) mean number of paths per 100 m was recorded in the province of Lodi, in the northern part of the province of Cremona and on the Cremona–Mantua border, broadly coinciding with the downstream course of the River Oglio (Fig. 3).

According to the regression model based on Lincoln–Peterson estimates and the length of the hydrographic network of each province (Table 4), the number of coypu in the study area appears to range between 346×10^3 and 1011×10^3, corresponding to 298–870×10^3 sexually mature individuals. Based on the estimates obtained by either capwire or accumulation curves, the number of coypu should range between 357×10^3 and 1042×10^3 or 372×10^3 and 1085×10^3, respectively.

Discussion

Mark–recapture estimates of abundance and concurrent coypu path counts corresponded well, inferring that this indirect method was robust.

Although the mark–recapture method provided three different, albeit similar, estimates of coypu abundance for each canal stretch, trapping out the entire population would be required to establish actual density (see Corriale *et al*., 2008). Removal trapping was not applied because, to be effective, eradication needs the target population to be closed to immigrants. In the Po-Venetian Plain, this assumption is weakened substantially by the complex network of irrigation canals, which favor the swift recolonization of cleared stretches. As an example, in 2002–2003 coypu culling by cage trapping resulted in a significant decrease of local population size only for 5/18 trapping sites (Prigioni, Remonti & Balestrieri, 2005); coypu removal, making vacant territories available to immigrants, enhanced the rapid recolonization of most trapping sites, resulting in the overestimate of population size (Prigioni *et al*., 2013).

Bleaching provided effective short-term marks without requiring the chemical immobilization of the animals. No adverse effect of bleach, e.g. hair loss, was observed in the study period. Moreover, as both weighing and marking were carried out on restrained animals, this methodology was safe, fast and could be performed by a single researcher.

A wide range of statistical models are available for analyzing mark–recapture data (Amstrup *et al*., 2005). The performance of each model depends on the robustness of the estimators to violations of its underlying assumptions. One critical assumption is whether capture probability is constant

Table 1 Number of coypu paths/100 m, estimated population size and 95% confidence intervals (CIs) as assessed by three different methods for the 12 trap sites (1-km long stretches of watercourses)

	Peterson–Lincoln		Capwire		Accumulation curves		
Stretch code	$\hat{N}$	95% CI	$\hat{N}$	95% CI	$\hat{N}$	95% CI	Paths/100 m
SG	98	29–167	90[b]	51–135	92	84–96	20.1
PS A	55	13–122	53[a]	18–75	16	13–16	10.3
PS B	84	32–143	71[a]	48–120	38	32–38	16.4
FE	21	15–34	15[a]	12–20	12	11–12	5.4
CAR	24	12–43	48[a]	21–100	14	11–14	6.15
SM1	28	26–33	62[a]	37–137	37	27–37	5.9
NB	69	34–106	46[a]	23–45	25	23–25	12.3
SB	29	9–63	15[a]	7–33	8	7–8	5.3
SM2	53	27–83	32[b]	25–37	23	22–23	8.8
CAV	54	14–119	55[a]	18–115	24	23–24	3.8
TOR	152	64–241	196[a]	127–278	171	165–171	23.9
PIZ	15	11–25	42[a]	12–42	12	11–12	3.1

[a]Equal capture model.
[b]Two-innate rates model.

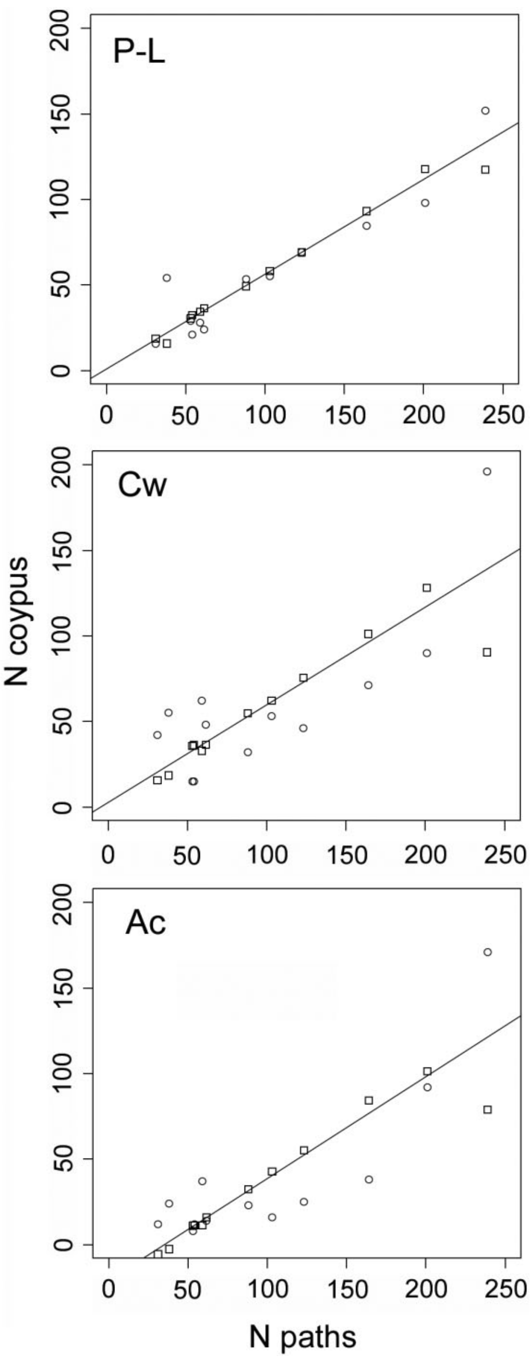

Figure 2 Observed values (dots) and leave-one-out cross-validated estimates (squares) of population size (N coypu) as assessed by three different linear regression models (P-L, Peterson–Lincoln; Cw, capwire; Ac, accumulation curves), plotted vs. the corresponding number of paths (Npaths).

or changes with time or among individuals. Whenever capture probability is expected to vary, models accounting for individual heterogeneity perform best, while Peterson–Lincoln's formula tends to underestimate population size (Grimm, Gruber & Henle, 2014).

While in central Italy, coypu catchability has been reported to vary mainly among individuals (Reggiani *et al*., 1995), in our study area equal capture models performed better than two-innate rates models. Nonetheless, accumulation curves, which did not depend on trap response, yielded the lowest estimates, suggesting that the trap–shy response of marked individuals may have introduced some positive bias in the other two population size estimators (Pollock *et al*., 1990).

The three estimates of population size resulted in similar regression slopes, supporting path counts as an effective method to assess the abundance of coypu in agricultural landscape.

Counting paths allowed us to assess coypu abundance in the six surveyed provinces in a short time (each researcher could easily survey two grid squares per day). The lowest path density found in the province of Pavia, where rice fields are widespread, was unexpected. We postulate that the current distribution of the coypu partially depends on the location of the major sites of release/escape, but further studies are needed to assess the influence of either habitat variables or diseases on the distribution of coypu in the central River Po valley. In this area, in late autumn–winter smaller irrigation/drainage trenches are drained, reducing the length of the hydrographic network and making this the most effective time to survey coypu. Also, low vegetation cover and fallow lands make the network more accessible to surveyors.

Cross-validated models allowed us to calculate a first estimate of coypu population size at regional scale. Considering that, in the study area, on average *c*. 100 000 coypu are removed each year (C. Prigioni, unpubl. data), the most conservative estimate of about 300 000 sexually mature individuals in winter raises doubts about the effectiveness of current management actions for reducing both population size and damage. Although path counts allowed to derive only an approximate estimate of coypu absolute abundance, this method may represent a cost-effective, volunteer-based tool to monitor and compare the relative abundance and trend of coypu populations in areas with different control intensities.

Conclusions

This indirect method, based on path counts

– allows surveying of large areas in a relatively short time and, requiring little training, can be easily carried out by volunteers (see Newman *et al*., 2003); on average, the whole coypu range in Lombardy could be surveyed by two volunteers per province in 20–25 working days;

– is much less expensive than mark–recapture methods and disturbs neither the target species nor other wetland wildlife;

– appears to be sensitive enough to record short-term fluctuations in coypu density, unlike burrow counts, allowing periodic monitoring of the effects of control campaigns.

The intercept and slope of any index–abundance relationship can vary substantially between habitats (Reid, Hansen & Ward, 1966). Because, in Europe, coypu are widespread mainly in agricultural areas, counting paths may be an effective method for monitoring coypu populations over a wide part of its introduction range, provided that paths can be

Table 2 Parameters (R^2, coefficient of determination; RMSE, root mean square error; adj., adjusted; CV, leave-one-out cross-validation) of the selected multi-regressive models ($y = \beta_0 + \beta_1 \cdot x$) relating to the number of coypu paths/100 m (Npath) to population size (y) as assessed by three different methods

Method	Regression model	*P*-value	R^2_{adj}	$RMSE_{CV}$	R^2_{CV}
Peterson–Lincoln	$y = 0.895 + 0.554 \cdot$ Npath	<0.0001	0.88	17.06	0.815
Capwire	$y = 2.672 + 0.571 \cdot$ Npath	<0.0001	0.62	39.65	0.311
Acc. curves	$y = -20.88 + 0.595 \cdot$ Npath	<0.0001	0.70	34.26	0.472

Table 3 Mean numbers of paths/100 m of watercourse (±SE; min–max values in brackets) for the six sampled provinces and Mann–Whitney post-hoc pairwise comparisons

Paths/100 m	Province	Pavia	Milan	Lodi	Brescia	Cremona
4.2 ± 1.25 (0–40)	Pavia	–				
5.9 ± 1.79 (0–30)	Milan	<0.0001	–			
8.9 ± 2.0 (0–36)	Lodi	<0.0001	<0.0001	–		
5.4 ± 1.50 (0–50)	Brescia	<0.001	0.28	<0.0001	–	
7.7 ± 1.54 (0–64)	Cremona	<0.0001	0.051	<0.0001	<0.0001	–
6.2 ± 1.4 (0–41)	Mantua	<0.0001	1	<0.0001	0.025	0.002

SE, standard error.

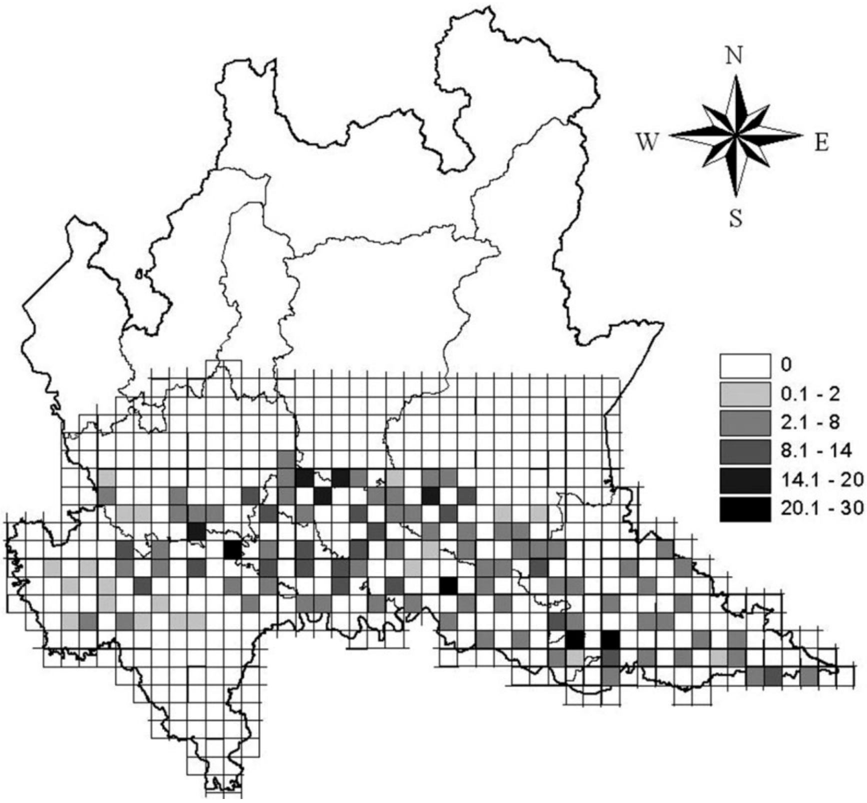

Figure 3 Mean number of coypu paths/square ($10 \times 10\ km^2$; 3-km long stretch of watercourses sampled per square) according to six classes of abundance.

assigned to the target species with certainty. In its current introduction range, the coypu is sympatric with other aquatic mammals that also trace paths on watercourse banks – such as beavers *Castor fiber* and otters *Lutra lutra* – but generally prefer wooded riverbanks. If the ongoing population expansion increases their occurrence in cultivated areas, this method will need further testing to test its effectiveness for assessing coypu numbers in areas of syntopy.

Table 4 Coypu numbers in the central Po plain according to Lincoln–Peterson model (number of coypu/100 m = 0.554 × mean number of paths/100 m + 0.895) with 95% confidence intervals (CIs)

Province	Hydrographic network (km)	Number of coypu	95% CI
Pavia	4724.04	109 658	44 948–174 368
Milan	1298.28	42 796	17 606–67 986
Lodi	2089.86	103 159	56 730–149 588
Brescia	4641.66	137 832	62 661–213 003
Cremona	2772.51	118 885	72 372–165 398
Mantua	4858.18	171 137	92 057–240 606
Total	20 384.53	683 468	346 374–1 010 950

Acknowledgements

This study was funded by Lombardy region and ERSAF (Regional Agency for Agricultural and Forest Services). We are grateful to all rangers, landowners and students who helped with field work. Lesley C. Wright kindly revised the paper for English language use.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Amstrup, S.C., McDonald, T.L. & Manly, B.F.J. (2005). *Handbook of capture-recapture analyses*. Princeton and Oxford: Princeton University Press.

Bertolino, S. (2009). Species account of the 100 of the most invasive alien species in Europe: *Myocastor coypus* (Molina), coypu, nutria (Myocastoridae, Mammalia). In *DAISIE handbook of alien species in Europe. Invading nature – Springer series in invasion ecology*, Vol. 3: 325–329. Springer (Ed.). Dordrecht: Springer.

Bertolino, S. & Genovesi, P. (2007). Semiaquatic mammals introduced into Italy: case studies in biological invasion. In *Biological invaders in inland waters: profiles, distribution and threats*: 175–191. Gherardi, F. (Ed.). Dordrecht: Springer.

Campbell, S.P., Clark, J.A., Crampton, L.H., Guerry, A.D., Hatch, L.T., Hosseini, P.R., Lawler, J.J. & O'Connor, R.J. (2002). An assessment of monitoring efforts in endangered species recovery plans. *Ecol. Appl.* **12**, 674–681.

Chapman, D.G. (1951). Some properties of the hypergeometric distribution with applications to zoological censuses. *Univ. Calif. Publ. Stat.* **1**, 131–160.

Cocchi, R. & Riga, F. (2001). *Guidelines for the control of Coypu* (Myocastor coypus). Quaderni di Conservazione della Natura, 5, Ministero Ambiente and Istituto Nazionale per la Fauna Selvatica, Italy.

Colwell, R.K. & Coddington, J.A. (1994). Estimating terrestrial biodiversity through extrapolation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **345**, 101–118.

Corriale, M.J., Arias, S.M., Bò, R.B. & Porini, G. (2008). A methodology for indirect estimation of the *Myocastor coypus* abundance at an urban wetland in Argentina. *Acta Zool. Sinica* **54**, 164–168.

Ebert, C., Knauer, F., Ilse, S. & Hohmann, U. (2010). Individual heterogeneity as a pitfall in population estimates based on non-invasive genetic sampling: a review and recommendations. *Wildlife Biol.* **16**, 225–240.

Falcucci, A., Maiorano, L. & Boitani, L. (2007). Changes in land-use/land-cover patterns in Italy and their implications for biodiversity conservation. *Landsc. Ecol.* **22**, 617–631.

Gese, E.M. (2001). Monitoring of terrestrial carnivore populations. In *Carnivore conservation*: 327–396. Gittleman, J.L., Funk, S.M., Macdonald, D. & Wayne, R.K. (Eds). Cambridge: Cambridge University Press.

Gibbs, J.P. (2000). Monitoring populations. In *Research techniques in animal ecology*: 213–252. Boitani, L. & Fuller, T.K. (Eds). New York: Columbia University Press.

Gray, M.J., Chamberlain, M.J., Buehler, D.A. & Sutton, W.B. (2013). Wetland wildlife monitoring and assessment. In *Wetland techniques*, Vol. 2, *Secaucus*: 265–318. Anderson, J.T. & Davis, C.A. (Eds). New Jersey: Springer.

Grimm, A., Gruber, B. & Henle, K. (2014). Reliability of different mark-recapture methods for population size estimation tested against reference population sizes constructed from field data. *PLoS ONE* **9**, e98840.

Guichón, M.L., Borgnia, M., Fernández Righi, C., Cassini, G.H. & Cassini, M.H. (2003). Social behavior and group formation in the coypu (*Myocastor coypus*) in the Argentinean pampas. *J. Mammal.* **84**, 254–262.

Heinemeyer, K.S., Ulizio, T.J. & Harrison, R.L. (2008). Natural signs: tracks and scats. In *Noninvasive survey methods for carnivores*: 45–74. Long, R.A., MacKay, P., Zielinski, W.J. & Ray, J.C. (Eds). Washington: Island Press.

Jennelle, C.S., Runge, M.C. & MacKenzie, D.I. (2002). The use of photographic rates to estimate densities of tigers and other cryptic mammals: a comment on misleading conclusions. *Anim. Conserv.* **5**, 19–120.

Kik, P. (1980). De beverrat, *Myocastor coypus* (MOLINA), in Nederland. *Lutra* **23**, 55–64.

Kruuk, H. & Conroy, J.W.H. (1987). Surveying otter *Lutra lutra* populations: a discussion of problems with spraints. *Biol. Conserv.* **41**, 179–183.

Laio, F. (2004). Cramer-von Mises and Anderson-Darling goodness of fit tests for extreme value distributions with unknown parameters. *Water Resour. Res.* **40**, W09308.

Lohmeier, L. (1981). Home range, movements and population density of nutria on a Mississippi pond. *J. Miss. Acad. Sci.* **26**, 50–54.

Miller, C.R., Joyce, P. & Waits, L.P. (2005). A new method for estimating the size of small populations from genetic mark-recapture data. *Mol. Ecol.* **14**, 1991–2005.

Montgomery, D.C., Peck, E.A. & Vining, G.G. (2001). *Introduction to linear regression analysis*. 3rd edn. New York: Wiley.

Newman, C., Buesching, C.D. & Macdonald, D.W. (2003). Validating mammal monitoring methods and assessing the performance of volunteers in wildlife conservation – 'Sed quis custodiet ipsos custodies?'. *Biol. Cons.* **113**, 189–197.

Panzacchi, M., Bertolino, S., Cocchi, R. & Genovesi, P. (2007). Population control of coypu *Myocastor coypus* in Italy compared to eradication in UK: a cost-benefit analysis. *Wildlife Biol.* **13**, 159–171.

Pennell, M.W., Stansbury, C.R., Waits, L.P. & Miller, C.R. (2013). Capwire: a R package for estimating population census size from non-invasive genetic sampling. *Mol. Ecol. Res.* **13**, 154–157.

Petralia, E. (2003). Status della Nutria (*Myocastor corpus* Molina, 1782) nella Riserva Naturale Speciale Biologica 'Macchia Foresta del Fiume Irminio' (Ragusa). *Tecnica Agricola* **1/3**, 87–97.

Pollock, J.F. (2006). Detecting population declines over large areas with presence-absence, time-to-encounter, and count survey methods. *Conserv. Biol.* **20**, 882–892.

Pollock, K.H., Nicholls, J.D., Brownie, C. & Hines, J.E. (1990). Statistical inference for capture-recapture experiments. *Wildl. Monogr.* **107**, 3–97.

Prigioni, C., Remonti, L. & Balestrieri, A. (2005). Control of the coypu (*Myocastor coypus*) by cage-trapping in the cultivated plain of northern Italy. *Hystrix* **16**, 159–167.

Prigioni, C., Balestrieri, A., Fontana, E., Casagni, L. & Fusar Poli, F. (2013). *Progetto* Myocastor coypus*: monitoraggio e raccolta dati nell'ambito del PO 'Osservatorio faunistico regionale'*. Technical report. University of Pavia, Italy.

Reggiani, G., Boitani, L. & Destefano, R. (1995). Population-dynamics and regulation in the coypu *Myocastor coypus* in Central Italy. *Ecography* **18**, 138–146.

Reid, V.H., Hansen, R.M. & Ward, R.L. (1966). Counting mounds and earth plugs to census mountain pocket gophers. *J. Wildl. Mgmt.* **30**, 327–334.

Rooney, T.P., Rogers, D.A., Wiegmann, S.M. & Waller, D.M. (2004). Monitoring nonnative plant invasions over fifty years in Wisconsin forests. *Weed Technol.* **18**, 1266–1268.

Sadlier, L.M.J., Webbon, C.C., Baker, P.J. & Harris, S. (2004). Methods of monitoring red foxes *Vulpes vulpes* and badgers *Meles meles*: are field signs the answer? *Mamm. Rev.* **34**, 75–98.

Santini, L. (1978). La Nutria (*Myocastor coypus* Molina) allo stato selvatico in Toscana. *Frustula entomologica* (XVI) **I**, 273–288.

Sargeant, G.A., Johnson, D.H. & Berg, W.E. (1998). Interpreting carnivore scent-station surveys. *J. Wildl. Mgmt.* **62**, 1235–1245.

Scaravelli, D. (2002). Problema *Myocastor*: considerazioni dell'esperienza ravennate. In *Atti del convegno nazionale 'La gestione delle specie alloctone in Italia: il caso della nutria e del gambero rosso della Louisiana'*: 25–28. Pedrini, R. (Ed.). Firenze: Quaderni del Padule di Fucecchio 2. Centro di Ricerca, Documentazione e Promozione del Padule di Fucecchio.

Silvy, N.J., Lopez, R.R. & Peterson, M.J. (2012). Techniques for marking wildlife. In *Wildlife techniques manual*: 230–257. Silvy, N.J. (Ed.). Baltimore: The Johns Hopkins University Press.

Stander, P.E. (1998). Spoor counts as indices of large carnivore populations: the relationship between spoor frequency, sampling effort and true density. *J. Appl. Ecol.* **35**, 378–385.

United Nations. (1992). Convention on Biological Diversity . 28 pp. Available at www.cbd.int/doc/legal/cbd-en.pdf (accessed 13 November 2014).

Vezza, P., Comoglio, C., Rosso, M. & Viglione, A. (2010). Low flows regionalization in North-Western Italy. *Water Resour. Manage.* **24**, 4049–4074.

Vilà, M. & García-Berthou, E. (2010). Monitoring biological invasions in freshwater habitats. In *Conservation monitoring in freshwater habitats – a practical guide and case studies*: 91–100. Hurford, C., Schneider, M. & Cowx, I.G. (Eds). Dordrecht: Springer.

Vilà, M., Basnou, C., Pysek, P., Josefsson, M., Genovesi, P., Gollasch, S., Nentwig, W., Olenin, S., Roques, A., Roy, D., Hulme, P.E. & DAISIE partners (2010). How well do we understand the impacts of alien species on ecosystem services? A Pan-European, cross-taxa assessment. *Front. Ecol. Environ.* **8**, 135–144.

Wilson, G.J. & Delahay, R.J. (2001). A review of methods to estimate the abundance of terrestrial carnivores using field signs and observations. *Wildl. Res.* **28**, 151–164.

Zalba, S.M., Politi, N. & De La Fuente, G. (2001). Habitat quality for coypu (*Myocastor coypus*) in a southern Argentinean agroecosystem. *Vida Silv Neotrop*. **10**, 50–55.