A R T I C L E

A NON-INVASIVE GENETIC SURVEY OF OTTERS (*Lutra lutra*) IN AN URBAN ENVIRONMENT: A PILOT STUDY WITH CITIZEN SCIENTISTS

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Abstract: Acquiring reliable estimates for an elusive species' distribution and population size can be problematic. For cryptic species such as the Eurasian otter (*Lutra lutra*), traditional monitoring approaches rely heavily on identifying field signs that may under or overestimate population sizes. Increasingly, non-invasive genetic sampling is effectively applied to assess the abundance and population structure of otters by genotyping faeces (spraints). Here we present the results of a non-invasive survey conducted in Cork City, Ireland, which aimed to estimate otter population size, sex ratio and genetic diversity. We incorporated a citizen science approach by training members of the public in sprint collection, thus increasing our search effort and sample detection rate. From October 2011 to May 2012, 199 sprints were collected and 187 (94%) were genetically identified as otter. Of these positive otter samples, 13 sprints (7%) yielded genetic information identifying 11 individuals (5 female and 6 male) using nine microsatellite loci. The results indicate that the urban environment does not prevent otters from using the area and we consider the implications based upon contemporary knowledge on otter spatial behaviour. This study demonstrates that non-invasive survey techniques combined with a citizen science approach can effectively reveal otter population parameters and increase urban otter awareness within the community.

Keywords: Urban ecology, population size, non-invasive genetic sampling, sex ratio, faecal DNA, Ireland

INTRODUCTION

The world is becoming increasingly urbanised, and wildlife are adapting to this frequently, albeit with some difficulties (McKinney, 2002). Numerous carnivore species have demonstrated a capability to adapt to urbanisation, such as red foxes (*Vulpes vulpes)* and stone martens (*Martes foina*) which are now common in many European cities (Gloor et al., 2001; Ní Lamhna, 2008; Herr et al., 2009). In North America, coyotes (*Canis latrans*) and raccoons (*Procyon lotor*) also occur in highly urbanised areas (Ordeñana et al., 2010). Sleeman and Moore (2005) and Park et al. (2011) suggest otters may also be a familiar, though rarely observed, urban species. Throughout Europe, otter populations declined in the last century due to habitat loss, persecution and bio-accumulative pollutants in the food chain (Ruiz-Olmo et al., 2008). However, in the past few decades otter populations have recovered across

much of their range (Conroy and Chanin, 2000; Mason and Macdonald, 2004) and otters today are still common throughout Ireland (Marnell et al., 2011; Reid, 2012), including in urban areas (Chapman and Chapman, 1982; Scott, 2004; Sleeman and Moore, 2005; Ní Lamhna, 2008).

Although otters are a widespread and well-studied species (Ruiz-Olmo et al., 2008), knowledge regarding fundamental aspects of their resource selection and population dynamics are still limited (Kruuk, 2006). This is especially true in urban areas that are often assumed to be barriers or poor otter habitat (Lundy and Montgomery, 2010; Hobbs et al., 2011; Park et al., 2011). This lack of information suggests a need for improved survey techniques to better understand otter populations. Non-invasive genetic sampling (NGS), which entails the extraction of DNA from hair, scat and other sources (Taberlet et al., 1999; Broquet et al., 2007), is a key element to improve surveys (Kelly et al., 2012) and has proven to be an effective method for monitoring cryptic and rare species (Palomares et al., 2002; Bellemain et al., 2005). NGS has previously been effective for otters (Park et al. 2011, Hájková et al., 2009). Analysis of DNA from otter spraints (faeces) can provide information on this species distribution, abundance, sex ratio and genetic diversity (Park et al., 2011). Regular wildlife sampling methods such as direct sightings and radio telemetry are not always appropriate for otters (Kruuk, 2006), therefore these indirect NGS methods offer a more reliable approach (Arrendal et al., 2007; Hájková et al., 2009; Park et al., 2011). Constraints with NGS do exist however, specifically with the quantity and quality of DNA extracted from spraints (Hájková et al., 2006; Bonesi et al., 2012).

NGS can be further complemented by the incorporation of citizen science. This approach involves the use of volunteers that are trained to participate in the collection of data for scientific studies. Citizen science has proven to be a valuable tool in providing information for research (Silverton, 2009). For instance, the OPAL (Open Air Laboratories) project in Britain has encouraged the public to participate in environmental surveys throughout the country generating large amounts of data (http://www.opalexplorenature.org, Tweddle et al., 2012). The National Biodiversity Data Centre in Ireland also encourages online submissions by citizen scientists to record mammal sightings to help build the "Atlas of Irish Mammals" (http://mammals.biodiversityireland.ie/). This approach to wildlife monitoring has also proven effective for otter research. For example, Black (2009) built a network of sightings of the river otter (*Lontra canadensis*) in North America involving citizen scientists, as have researchers (Okes, 2013) in South Africa's Cape Peninsula studying urban Cape clawless otters *(Aonyx capensis*) and Park et al. (2011) recorded the collection of Eurasian otter spraints in Daegu City, Korea for a TV show, a process that engaged viewers in the survey method. One of the first studies in Britain that relied on volunteers for the collection of spraints for genetic analysis was conducted by Coxon et al. (1999). Similarly Cardiff University's otter project (e.g. Stanton et al., 2009) avails of social networking and the use of volunteers.

In this study, NGS complemented with citizen science was used to survey a population of urban otters in Cork City. Spraints were collected by trained volunteers to reveal otter presence, numbers, and genetic diversity. Our specific aims were to (1) test the efficacy of NGS from spraints collected by citizen scientist volunteers, (2) determine the sex ratio of otters in Cork City, (3) determine the minimum number of individual otters and (4) estimate the genetic diversity of otters present in the city.

METHODS
Study area

Cork City in the south of Ireland is located on the edge of Cork Harbour with an urban area incorporating roughly 37 km^2. The River Lee, with various channels and tributaries, flows through the city (Fig 1).
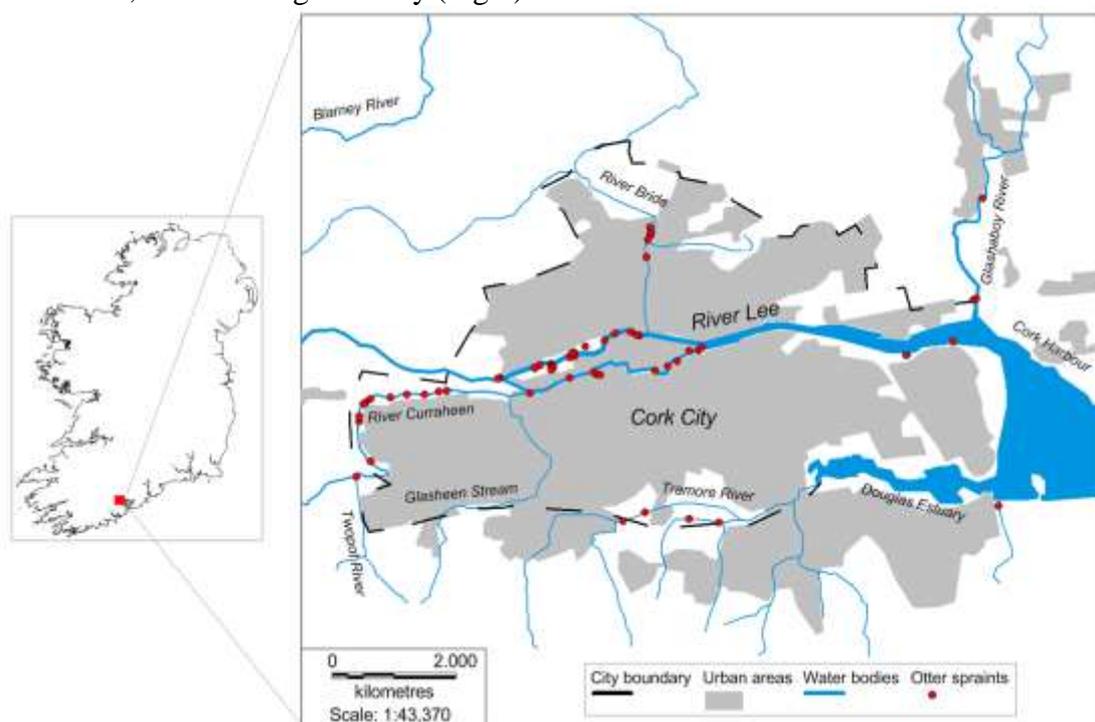
Figure 1. Map of the study area and distribution of genetically verified otter spraints collected during the survey.

Sampling and Analysis

Data for this project was acquired using citizen science monitoring, and volunteers were recruited through the Cork branch of the Irish Wildlife Trust (IWT). Social media was also used to generate public interest. Training events took place before each survey where volunteers were presented with short talks about general otter ecology and guided through standardised otter survey methods (Reuther, 2000).

Spraint collection began in 2011 with 22 trained volunteers and surveying was conducted over 14-21 days across three survey periods (Table 1). The city was divided into five areas that could be comfortably surveyed by teams of 2-4 volunteers. An experienced team leader was assigned to each group who was responsible for submitting the team's spraint collection and documentation to the survey organisers. Surveys were not performed during or immediately after rain when spraints would likely be washed away. River banks were surveyed for otter signs (tracks, holts, resting sites and slides) and spraints were collected in plastic tubes labelled with the location, date and surveyor. Spraints were stored at -20 °C prior to DNA extraction. DNA extraction, molecular species and sex identification of spraints by real-time TaqMan® polymerase chain reaction (PCR) assays and individual identification by microsatellite genotyping (Table 2) were used according to O'Neill et al. (2013). For microsatellite genotyping, primer sets Lut435, Lut833, Lut701, Lut818 (Dallas and Piertney 1998), Lut457 (Dallas et al., 2002), 04OT05, 04OT14 and 04OT22 (Huang et al., 2005) and 04OT17mini-r (O'Neill et al., 2013) were used. Samples were assayed in duplicate, independent PCRs and only those with allele replicates at a minimum of eight of the nine loci were used for data analysis.

Data analysis

The software GIMLET, version 1.3.4 (Valière, 2002) was used to assess the replicated data for the presence of errors including the presence of allelic drop out and false alleles. GIMLET was also used to calculate the percentage of positive PCRs in the overall dataset and the final sample set. The software program GENEPOP, version 4.0.10 (Rousset, 2009) was used to assess gametic phase linkage disequilibria by Fisher's method (1000 dememorizations and 5000 iterations) and deviations from Hardy–Weinberg equilibrium (default settings, exact tests). Expected (H_E) and observed (H_O) heterozygosities, the number of alleles and the probability of identity (PI) were calculated using GENALEX (Peakall and Smouse, 2006). Allelic richness (R_S) and the allele size range (a_s) were assessed using FSTAT, version 2.9.3.2 (Goudet, 2002).

RESULTS

From October 2011 to May 2012, 199 spraints were collected for DNA analysis throughout the five urban study areas. In 2011 and 2012, 53 and 146 samples were collected, respectively. In total, 187 samples (94%) were genetically identified as otter spraint. Three mink (*Neovison vison*) scats were identified by DNA sequence analysis and the remaining 9 samples could not be identified to species, most likely due to degraded DNA. Of the positively identified otter spraints, 42 were female, 87 were male, 47 were undetermined and 11 were not tested due to insufficient quantities of DNA. All genetically identified otter spraints from Cork City were mapped onto Ordnance Survey Ireland (OSI) maps using MapInfo 11.0 ™ GIS software (Fig.1). Of the positively identified otter spraints, thirteen (7%) were successfully identified to the individual at 9 microsatellite loci, providing 11 unique genotypes (male n=6, female n=5) (Table 1). The probability of identity ($PI = 3.2 \times 10^{-3}$) indicated that a minimum of four loci were needed to identify unique individuals.

A total of 71 samples were genotyped. Across this entire dataset, PCR success averaged 0.47, ranging from 0.24 at Lut818 to 0.64 at Lut435. Allelic Dropout across all loci was 0.059, ranging from 0.019 at Lut701 to 0.2 at Lut701. Two loci remained error free, 04Ot17 and 04Ot22. To ensure that the data was accurate and to avoid errors, especially identifying spurious individuals, we only used samples that amplified at a minimum of 8 loci, and when the data replicated exactly matched. This reduced the final dataset to 13 samples. Within this dataset, PCR success rates ranged from 0.80 at Lut457 and Lut701 to 1.0 at Lut833, 04OT14, 04OT17 and 04OT22, with an overall average of 0.92 across all loci. There was no evidence of allelic dropout or false alleles in the final dataset used to identify individual otters. All otters identified were unique to each study period with the number of otters detected during each survey ranging from 2-5. One male was detected three times on the same day approximately 300 metres apart. No other individuals were recaptured during surveys.

Table 1. Numbers and sex of otters identified

Survey period	No. of Otters	Male	Female	Recapture
Oct/Nov 2011	4	1	3	0
Jan/Feb 2012	2	1	1	0
May 2012	5	4	1	2
Total:	**11**	**6**	**5**	**2**

Genetic variability

The number of alleles per locus per sample ranged from two at *04OT14* and *04OT17* to four at *Lut435, Lut701* and *Lut833* (Table 2). Low levels of allelic richness were also observed (R_s = 2.0) at *04OT14* and *04OT17*, with the highest level observed

at *Lut701* (R_s = 4.0) (Table 2). H_E ranged from 0.236 at *04OT17* to 0.710 at *Lut701* and H_O ranged from 0.091 at *04OT14* to 1.00 at *Lut701* (Table 2). The following loci exhibited significant deviations from Hardy-Weinberg expectations Lut435, Lut701, Lut818, 04OT14 and 04OT22 (P = 0.05), although the sample size used for this analysis was small.

Table 2. Summary statistics

	Locus									Mean across loci
	Lut 435	*Lut 457*	*Lut 701*	*Lut 818*	*Lut 833*	*04 OT05*	*04 OT14*	*04 OT17*	*04 OT22*	
N	10	9	9	10	11	10	11	11	11	10.2
a	4	3	4	3	4	3	2	2	3	3.1
R_s	3.8	3.0	4.0	3.0	3.8	3.0	2.0	2.0	2.8	3.0
a_s	122-134	138-142	194-206	180-188	157-169	168-176	122-142	124-128	148-160	
H_E	0.545	0.426	0.710	0.620	0.690	0.555	0.434	0.236	0.368	0.509
H_O	0.200	0.444	1.000	0.600	0.727	0.700	0.091	0.273	0.182	0.469
HW	**0.005**	0.238	**0.025**	**0.011**	0.111	1.000	**0.017**	1.000	**0.033**	

N = number of individuals; a = number of alleles, R_s = allelic richness, a_s = allele size range; H_E = Expected heterozygosity; H_O = observed heterozygosity, HW = Probability values of Hardy-Weinberg expectations.

DISCUSSION

At least 11 otters, five females and six males, used the city during the survey period. Considering the small study area (~37 km²) and current knowledge of otter spatial ecology (Kruuk, 2006; Koelewijn et al., 2010), a relatively high number of otters were found in the city centre. Previous studies relying on the identification of otter tracks speculated that there were between 5-6 otters using Cork City (Sleeman and Moore, 2005). As Cork City is connected to a number of fresh water systems (Fig. 1) and borders a large natural marine harbour, otters may be using Cork City as an important corridor between marine and freshwater habitats to increase resources and foraging opportunities. This may explain why recaptures were low as otters may not reside long term in the area. Our low success rate at identifying individual otters may have also contributed to this. Alternatively, a source-sink dynamic may exist between rural and urban otter habitats and populations (Sulkava et al., 2007). The high number of otters in Cork City, along with similar findings by Park et al. (2011), challenges the assumption that cities are poor habitats for otters. To address these questions, there is a need for a more comprehensive study into the wider otter population of County Cork, applying recommendations for improving spraint collection methods. In addition, dietary studies could be used to establish the food resources available in the local environment.

Although there did not appear to be a sex bias in the genetically identified individuals, 67% of the samples identified to sex were male. Bonesi et al. (2012) described a similar problem on the River Thames where males were more likely to be territorial and spraint more frequently than females, introducing a possible sex bias in the sampling strategy. Since little is known about the sex ratio of the urban population, it is not possible to infer if the results presented in this study are indeed biased due to the small number of genetically identified individuals, or if the result is representative of the true population. A low to moderate level of genetic diversity was observed among otters in Cork City, but the sample size was small. Three loci used in

this study are comparable with the urban otter study by Park et al. (2011): *Lut435, Lut457, Lut701,* which resulted in similar levels of expected heterozygosity ($H_E =$ ~0.6). Future studies should increase the sampling region outside the city to get a wider estimate of the genetic diversity of the population in the area, as this study has not been able to ascertain if the otters sampled are resident individuals.

Only 7% of sprants yielded genotypes that were adequate to identify individuals, a low success rate compared with published studies (Arrendal et al., 2007; Bonesi et al., 2012; Hájková et al., 2009; Koelewijn et al., 2010; Park et al., 2011). Our findings therefore represent a minimum population size. A number of reasons may have contributed to the low genotyping success rate observed in this study. Many of the sprants collected were old and possibly of low DNA quality. The use of DNA extracted from anal jelly that occurs with sprants can increase the genotyping success rate (Lampa et al., 2008), but this was not frequently encountered by the survey teams. Sprants were sometimes not submitted by volunteers up to one week post survey. DNA in sprants degrades faster when exposed to warm temperatures (Hájková et al., 2006), and as sprants were typically not frozen by volunteers prior to submission, reducing the time between sample collection and freezer storage could improve the success rate. Surveyors could also be encouraged to collect only fresh sprants and to freeze them in their homes. An alternative may be to provide volunteers with 96% ethanol, but the feasibility of using this with volunteers has not been investigated.

Citizen scientists aided this study by increasing the sampling effort and area surveyed. An additional benefit to the wider community by involving volunteers in such research is that it helps to increase their knowledge of urban ecology. During the training events that were held for each survey, volunteers were presented with the results of the previous survey and were kept informed of the study progress. Local schools were also involved through talks and school children were encouraged to name the genetically identified otters. An education resource pack was developed by the IWT to disseminate the findings of this survey to local schools. Citizen science requires a commitment to continually engage with volunteers and ensure that adequate feedback is provided to participants. This can be time consuming and the constraints of such an approach should be considered when developing similar projects. The combination of NGS and citizen science will be valuable in Britain where citizen science is already well established and the otter population seems to be recovering (Hobbs et al., 2011; Harris, 2013).

Despite a small sample size, this study demonstrates the first use of non-invasive genetic survey techniques on urban otter ecology in Ireland and Britain, and provides baseline estimates of the distribution, genetic diversity, sex and number of otters in an urbanized area. The genetic sampling of otter faecal DNA may complement and improve existing traditional protocols and yield more accurate information into their spatial habits, population parameters and behaviours, thus ensuring that their conservation needs are met.

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RÉSUMÉ

ETUDE GÉNÉTIQUE NON INVASIVE DES LOUTRES (*Lutra lutra*) DANS UN ENVIRONNEMENT URBAIN: PROGRAMME PILOTE AVEC DES CITOYENS SCIETIFIQUES.

Obtenir des données fiables afin d'estimer la distribution et la taille de population d'une espèce à faible probabilité de détection peut être problématique. Pour des

espèces cryptiques telles que la loutre Eurasienne (*Lutra lutra*), les approches traditionnelles de suivi reposent majoritairement sur l'identification de signes présents sur le terrain, et pouvant entrainer la sous ou surestimation de la taille de population. De plus en plus souvent, une méthode d'échantillonnage génétique non invasive est utilisée efficacement afin d'évaluer l'abondance et la structure de population des loutres en génotypant les fèces (épreintes). Dans cette étude, on présente les résultats d'un suivi non invasif réalisé à Cork City (Irlande), dont le but est d'estimer la taille de population, le sexe ratio ainsi que la diversité génétique de la loutre. Le suivi intègre une approche scientifique civile qui consiste à entrainer les membres du public à collecter les épreintes, permettant d'augmenter l'effort de recherche et le taux de détection d'un échantillon. D'Octobre 2011 à Mai 2012, 199 épreintes ont été collectées et 187 (94%) ont été génétiquement identifiées comme loutre. Parmi ces échantillons positifs de loutres, 13 épreintes (7%) ont révélées, en utilisant neuf loci microsatellites, l'information génétique de 11 individus (5 femelles et 6 males). Les résultats indiquent que l'environnement urbain ne restreint pas les loutres pour l'utilisation de la zone, aussi, on considère les implications basées sur les connaissances contemporaines du comportement spatial de loutre. Cette étude révèle que la combinaison des techniques de suivi non invasives et de l'approche scientifique civile peut de façon efficace révéler les paramètres de population de loutre et augmenter la conscience collective urbaine en ce qui concerne les loutres.

RESUMEN
ANÁLISIS GENÉTICO NO INVASIVO DE NUTRIAS (*Lutra lutra*) EN UN MEDIO URBANO: ESTUDIO PILOTO USANDO CIENCIA CIUDADANA.
La adquisición de estimaciones fiables sobre la distribución y el tamaño poblacional de una especie esquiva puede acarrear ciertos problemas. En el caso de especies crípticas como la nutria europea o paleártica (Lutra lutra), el monitoreo tradicional se basa en la robusta confianza en la identificación de restos en la zona de estudio, lo que puede generar una infra o sobre-estimación del tamaño de la población. Además, muestreos genéticos no invasivos como la genotipificación de heces, están siendo aplicados, positivamente, para la evaluación de la abundancia y la estructura de la población de nutria. En el presente articulo se facilitan los resultados de un muestreo no invasivo llevado a cabo en la ciudad de Cork, Irlanda, que tenía por objetivo la estimación del tamaño de la población, la proporción de sexos y la diversidad genética. La incorporación de un enfoque científico-ciudadano mediante el entrenamiento de personas del público general para la recogida de excrementos, incrementó el esfuerzo de muestreo y el rango de detección de muestras. Desde octubre del 2011 hasta mayo de 2012 fueron recogidas 199 muestras de excrementos donde 187 (94%) fueron identificadas genéticamente como de nutria. Del conjunto de estas últimas, 13 de ellas (7%) dieron una información genética, que mediante la localización de 9 microsatélites, permitieron identificar 11 individuos (5 hembras y 6 machos). Los resultados indican que el medio urbano no previene a las nutrias en el uso del espacio y consideramos las implicaciones sobre la base del conocimiento contemporáneo en el comportamiento espacial de la nutria. Este estudio demuestra que las técnicas de estudio no invasivo combinadas con un enfoque de ciencia ciudadana pueden revelar con eficacia los parámetros de población de nutria y aumentar la conciencia de los urbanitas acerca de la nutria dentro de la comunidad.