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Brown rats (*Rattus norvegicus*) in urban ecosystems: are the constraints related to fieldwork a limit to their study?

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Abstract

Nowadays, the majority of human beings live in urban ecosystems, with this proportion expected to continue increasing in the future. With the growing importance of urban rat-associated issues (e.g. damages to urban infrastructures, costs of rat-control programs, rat-associated health risks), it is becoming indispensable to fill the identified gaps in knowledge on the urban brown rat regarding, among others, its density, home range, genetic structure, and infectious status. In this context, live-trapping is a crucial prerequisite to any scientific investigation. This paper assesses the main constraints and challenges regarding the urban field and describes the major steps to be considered when planning research on urban rats. The primary challenges are i) the characterization of the urban experimental unit; ii) the choice of a trapping design: the use of live-trapping in capture-mark-recapture design, in association with modern statistics, is highly recommended to answer ecological questions (although these methods, mostly developed in natural ecosystems, need to be implemented for the urban field); iii) the potential ethical considerations with regard to animal welfare and field-worker safety; iv) the building of mutually-beneficial collaborations with city stakeholders, pest control professionals, and citizens. Emphasis must be put on communication to the public and education of field-workers. One major need of modern urban rat research is a peer-validated field methodology allowing reproducibility, repeatability, and inference from urban field studies and enabling researchers to answer long-standing key questions about urban rat ecology.

Keywords *Rattus norvegicus* · Urban ecosystem · Trapping · Fieldwork · Guidelines

Introduction

Across the world urban ecosystems are growing incredibly quickly while rural areas are transformed into peri-urban landscape (Elmqvist et al. 2013; Seto et al. 2011). Urbanization drives dramatic changes in the abiotic and biotic environment including habitat fragmentation, increased impervious surface cover, higher temperatures and noise, and light pollution (Alberti et al. 2017; Johnson and Munshi-South 2017). These urban disturbances impact behavior, morphology, and physiology of wildlife species (Costantini et al. 2014; French et al. 2008; Meillère et al. 2015; Partecke et al. 2006), animal geographical ranges and densities (McKinney 2008; Prange et al. 2003), animal communities (Aronson et al. 2016; Cavia et al. 2009; Horsák et al. 2013), genetic population structure (Ruell et al. 2012), interspecies interactions (Faeth et al. 2005; Thomas et al. 2012), species evolution (Johnson and Munshi-South 2017), epidemiology of infectious diseases (Bradley and Altizer 2007; Himsworth et al. 2013b; Neiderud 2015), and environmental contamination with pathogens (Giraudeau et al. 2014; Rothenburger et al. 2017).

Without doubt, urban ecosystems present physical, environmental, social, and societal constraints to the study of commensal brown rats (*Rattus norvegicus*), particularly with regard to their capture. With the increasing importance of urban rat-associated issues (e.g. damages to urban infrastructures, costs induced by the rat-control programs, rat-associated health risks, stress to citizens), it is becoming indispensable to fill the identified gaps in knowledge on urban rats regarding, among others, their density, ecology, home range, genetic structure, and infectious status (Feng and Himsworth 2014; Himsworth et al. 2013b; Parsons et al. 2017). In this context, live-trapping of individuals is a crucial prerequisite to any scientific investigation.

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The study of urban *R. norvegicus* remains a challenge. This might be due to i) the field constraints inherent to the urban environment. Indeed, the high rate of environmental change of the urban habitat can narrow the spatial and temporal window within which researchers can comfortably and effectively mount field operations which is a critical condition for independent serial experiments able to support inference; ii) the relative difficulty of trapping wild brown rats, which likely depends on weather, food abundance, presence of urban predators, animal experience/age, and probably other unknown parameters (Clapperton 2006; Hancke and Suárez 2017; McGuire et al. 2006; Parsons et al. 2017), and iii) the social taboo (Parsons et al. 2017) that limits the study of urban rats. Indeed rats are associated with poverty and poor hygiene, and most citizens do not report rat sightings around their neighbourhood (Zink and Walter 2016).

Nowadays, urban research in rat ecology needs, as a priority, to answer the key question of rat population density and the factors influencing it: how many rats are there? Where are they? Does their density fluctuate seasonally? Some authors have attempted to evaluate the intensity of urban rat infestation using trapping methods, e.g. in Baltimore, USA, where the authors estimated the total number of rats living within the city (Brown et al. 1952; Davis and Fales 1950; Easterbrook et al. 2005), or in Salzburg, Austria, where the authors assessed the rat density in different urban habitats (Traweger et al. 2006). However, these works suffer from several shortcomings, e.g. the study is hardly reproducible, does not explore seasonality (Easterbrook et al. 2005), has insufficient data (Traweger et al. 2006), and results from spatially-limited investigations were extrapolated to the entire city (Easterbrook et al. 2005).

Fieldwork is time consuming and labor intensive. As a result, it is crucial to optimize the time spent preparing for and working in the field. Papers raising the practical issues of trapping rats in urban habitats are scarce (Mathee et al. 2010; Parsons et al. 2017, 2016). Mathee et al. (2010) presented an interesting review of the fieldwork challenges of urban health research in Africa. The authors noted the difficulty of obtaining informal settlement maps, discussed the issue of language, highlighted the complexity of getting access to selected sites (suburbs, habitations) and people, and addressed the issue of personal safety. Parsons et al. (2016) published a five-step protocol providing inspiring and practical advices on materials and procedures to be used in the studies of urban *R. norvegicus*.

Based on field experience and literature review, this paper provides a realistic and practical guidance to researchers investigating one of the most important urban species, the brown rat. We present the main urban-specific constraints to fieldwork and propose keys and clues to overcome them. The major steps include (i) the choice and description of the urban experimental unit(s), (ii) the choice of an appropriate trapping design, (iii) compliance with high standard concerning animal welfare and fieldwork, and (iv) communication to the public and partnership building. We restricted the scope of this paper to urban ecosystems within the so-called "developed regions", as defined by the United Nations (i.e. North America, Europe, Australia, New Zealand, and Japan) (United Nations 2016), because urban habitats in developed countries present a common general framework (Johnson and Munshi-South 2017), which makes the approach to urban fieldwork relatively similar in these regions.

The urban experimental unit

Choice of the urban experimental unit

The choice of the delimited geographical area in which to conduct the study, hereafter called *experimental unit*, is one of the most challenging element of urban research (Parsons et al. 2017). Each experimental unit must be spatially and qualitatively defined before trapping commences. To facilitate fieldwork, we recommend the experimental unit be accessible and safe for the field-workers and close to a parking area (with regard to transport of traps and materials). Parking fees may have to be added to the project budget.

The fragmentation of the urban habitat into patches delimited by streets (Fig. 1) divides a typical spatial pattern of the urban rat populations into several spatial (sub)populations (or units) corresponding to city blocks (Davis et al. 1948; Emlen et al. 1949) or alleys (Easterbrook et al. 2005) which are totally or partially isolated (Combs et al. 2018). These spatial units enable experimental protocols where some blocks can be used as control areas while others can undergo experimental manipulations (Emlen et al. 1948). For example, the effect of rats on the environment could be evaluated by comparing results from a unit with a high rat density versus a unit considered as rat-free (or with a lower density). In one of their founding works, Emlen et al. (1948) studied the natural rate of recovery of block populations of brown rats after poisoning and/or trapping campaigns by comparing treated blocks with undisturbed populations (blocks without intervention). In their recent work, Lee et al. (2018) evaluated the effect of culling on *Leptospira* carriage using a well-designed study including a control area: some study sites were used as intervention sites where kill-trapping occurred, whereas others were considered as control sites where capture-release was performed without killing.

By evaluating the spatial distance over which rats are related, genetic data can facilitate delineating the appropriate geographic scale of the study, i.e. whether a single or several independent rat colonies have to be investigated. Rats show strong site fidelity (Gardner-Santana et al. 2009) and the typical home range of an urban colony falls between 40 and 150 m (Davis 1953; Davis et al. 1948; Gardner-Santana et

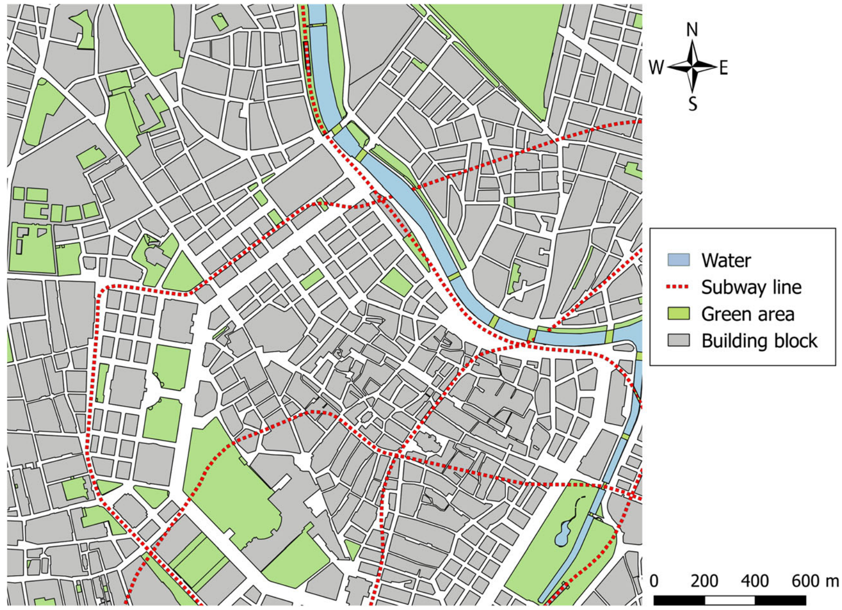

Fig. 1 Map of Vienna city centre, Austria, illustrating the fragmentation of the urban habitat into patches delimited by streets, and the geometric urban structure, mostly composed of lines and polygons. Map created using QGIS software (QGIS Development Team 2016)

al. 2009; Kajdacsi et al. 2013). In Salvador, Brazil, a large degree of genetic divergence has been recently identified between rat populations located <50 m apart (Richardson et al. 2017). However in their study of urban rat populations in New York City, Combs et al. (2018) evaluated the boundaries of a single colony to a range of 1400 m (Combs et al. 2018) which is greater than the average range previously described (Davis 1953; Davis et al. 1948; Gardner-Santana et al. 2009; Kajdacsi et al. 2013; Recht 1988; Richardson et al. 2017). Pattern of isolation and home range of urban rat populations are mostly attributable to physical environmental barriers (Davis 1953; Gardner-Santana et al. 2009; Richardson et al. 2017). These parameters appear highly variable and must be addressed locally (Rothenburger et al. 2017).

Description of the urban experimental unit

One of the most striking contrasts between urban and non-urban environments is the speed with which city frameworks change, whereas agricultural or natural landscapes are more stable (Hulme-Beaman et al. 2016). Common changes in urban land-use include the constant modification of road and public transport networks, the construction of new residences, the renovation of buildings, the renovation or enlargement of the sewer system, the enlargement of green areas, the rapid development of commercial zones, the construction of underground parking space, etc. Updated remote sensing resources and tools, (e.g. maps, geographic information system (GIS) files, satellite and aerial pictures) are ideal aids in planning fieldwork (Banzhaf and Netzband 2011; Yuan et al. 2005).

Once spatially delimited, the experimental unit has to be qualitatively characterized. Landscape attributes must be urban-specific, of a limited number, relevant for the study of *R. norvegicus* (Childs et al. 1998; de Masi et al. 2010; Himsworth et al. 2014b; Johnson et al. 2016; Langton et al. 2001; Traweger et al. 2006; van Adrichem et al. 2013; Walsh 2014), and reproducible. For some urban centres very detailed land-use GIS datasets are available, such as the "real land-use dataset 2007/2008" for Vienna (Realnutzungskartierung ab 2007/08 – Wien, freely available at https://www.data.gv.at/), which includes up to 32 primary land-use structure types.

Fine-scale evaluation of the experimental unit can only be performed in the field and must be conducted in a systematic manner. Himsworth et al. (2014b), in their study on urban *Rattus* sp., proposed an interesting "Environmental observation tool" to help in qualitatively characterizing the urban experimental units. This tool presents 58 measurements arranged in six major categories: (1) land use characteristics, (2) property condition, (3) green space characteristics, (4) alley surface characteristics, (5) presence of waste, and (6) alley use. Moreover, the authors provided an excellent data sheet and photo library illustrating the different items.

Several days and nights should be dedicated to the reconnaissance of the experimental unit to ground-truth the geographic data while also observing human usage. Field reconnaissance is essential to check for the presence of rats or rat signs (Fig. 2), the feasibility of fieldwork, ongoing potential rodenticide treatment in the area which could interfere with the study, the presence of other wildlife species which could reduce the trap success, or human presence which could impact fieldwork (e.g. gardeners, homeless persons, proximity of a children playground). In cities, human activity and density are widely variable between days (i.e. week day versus week-end) and within the day (rush-hours versus off-hours) and can disturb fieldworkers and decrease trap success. Being familiarized with the experimental unit before starting the captures

Fig. 2 Examples of rat signs that can be observed in urban habitats. **a**, **b**. Burrow entrances. **c**, **d**. Rat faeces. **e**. Dead rat, possibly resulting from anticoagulant poisoning (Pictures A. Desvars-Larrive)

will considerably reduce the chance of failure during fieldwork.

Trapping design

Live-traps *versus* kill-traps

Snap-traps are cheap, can be acquired almost everywhere, are easy to handle, small, and light. However, snap-traps can have detrimental effects on non-target species and do not allow for the collection of complete sets of biological data (Barnett and Dutton 1995), because tissue characteristics change rapidly in dead animals, blood is coagulated, bacteriological samples are biased by post mortem leakage and colonizing bacteria, while virus detection is generally compromised (Mills et al. 1995). Additionally, in about 7–14% of all cases (Mason and Littin 2003), kill-traps fail to kill rats while causing sub-lethal injuries. In these cases, the researcher needs a back-up plan for humanely killing the animal in the field (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016).

Only live-trapping allows capture-mark-recapture (CMR) design which, associated with modern statistics, e.g. spatial capture-recapture models, allows for estimation of animal density (Borchers and Efford 2008; Efford 2004, 2011; Efford et al. 2004; Efford and Hunter 2017; Wilson et al. 2007), population dynamics (Efford et al. 2006), home range (Wilson et al. 2007), survival (Horváth and Wagner 2003), and behavior (Parsons et al. 2015). CMR methods also allow for monitoring of individuals in order to produce high-quality records on biological status and samples (Efford et al. 2006). Live-trapping facilitates adherence to ethical standards regarding animal welfare and the choice of the best (humane) killing strategy if terminal biological samples are needed or if captures are conducted for pest management purposes (Mason and Littin 2003; Meerburg et al. 2008).

Closed box-type traps (e.g. Sherman) or cage-traps, made of open material such as wire mesh (e.g. Tomahawk), are the two main types of live-traps available. They are easy to maintain, handily transportable, relatively light, and most of them exist in space-saving collapsible formats. However, in winter, aluminium box traps are cold, and mesh traps do not protect the captured animals from environmental conditions (Barnett and Dutton 1995; Hoffmann et al. 2010), introducing the risk of hypothermia. This can be mitigated by placing the trap into "waxed cardboard" containers saved from milk or juice products or adding some bedding material (cotton or shredded paper) (Wilson et al. 2007) into the live-traps, as long as it does not block the treadle mechanism (Hoffmann et al. 2010). Whichever trap is chosen, it must meet animal welfare requirements (Barnett and Dutton 1995; Powell and Proulx 2003; Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016). Description and performances of some commonly used traps can be found in the literature (Anthony et al. 2005; Dizney et al. 2008; Hoffmann et al. 2010; Jones et al. 1996; Powell and Proulx 2003); although, to our knowledge, no study has yet compared the efficacy of these different traps on *Rattus* trap success.

Bait

Food is commonly used as bait, most often peanut butter, but also bacon fat, cat food, fresh-water fish, oats, pieces of chicken, balls of bird seed, cucumber, or apple (Desvars-Larrive et al. 2017; Easterbrook et al. 2007; Frye et al. 2015; Gardner-Santana et al. 2009; Glass et al. 2016; Heiberg et al. 2012; Himsworth et al. 2014a; Kataranovski et al. 2011; Schroder and Hulse 1979). Commercial formulations of non-toxic attractive baits can also be an option (Sacchi et al. 2008). They have the advantage of being covered by a thick layer of paraffin that makes them resistant to moisture. Parsons et al. (2015) recommended fresh scent (obtained from soiled rat bedding with sebum, dander, faeces, and urine) to increase the trap success of urban rats. One shortcoming is the potential role of fresh scent as source of pathogen contamination for captured rats, e.g. via urines (Costa et al. 2015; Kariwa et al. 1998) or faeces (Kilonzo et al. 2013; Widén et al. 2014). For more details on taste preferences and lures of *R. norvegicus,* refer to the review of Clapperton (2006).

Commensal brown rats show neophobic responses to unfamiliar objects (Barnett 1976; Clapperton 2006; Feng and Himsworth 2014; Inglis et al. 1996) and towards new food (Barnett 1976; Clapperton 2006; Inglis et al. 1996), which can make trapping in urban habitat remarkably hard. One option to overcome this difficulty is to pre-bait the traps (i.e. baiting for several days prior to setting the traps). Pre-baiting is often used in rodent studies (Gurnell 1980; Himsworth et al. 2014a; Krojgaard et al. 2009; Taylor et al. 1974). However, several recently published researches did not employ it (Desvars-Larrive et al. 2017; Kajdacsi et al. 2013; Panti-May et al. 2016, 2017; Rouffaer et al. 2017). Trap success (and/or the assumed neophobic behavior) may also reflect the general availability of food in the vicinity of the traps and low success rate is expected when abundant food is available in or close to the experimental unit (Clapperton 2006). Therefore, the stability and abundance of food resource may constrain the trap success.

Trapping strategy

Traps are usually set up at dusk and checked at dawn, as rats are usually nocturnal (Feng and Himsworth 2014), although diurnal trapping is conceivable in areas with high rat density or rat-sighting reports during daylight hours (Desvars-Larrive et al. 2017). Traps must be checked at least twice a day and once a night, depending on weather conditions, because non-target species must be released as quickly as possible to avoid death or injuries and because stress caused to the target species by the capture must be reduced (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016). It is strongly recommended not to leave a captured rat for more than 4 −6 h in the trap. Automated sensor traps that automatically transmit a signal to a receiving device (pager, computer, mobile phone) can be used to alert fieldworkers that an individual has been captured (Notz et al. 2017; Parsons et al. 2016).

There are three main methods of arraying traps: grid, line (or transect), and sign method. Grid and line methods have been previously described (Hoffmann et al. 2010; Jones et al. 1996; Weihong et al. 1999). The sign method places traps at locations most likely to catch animals based on animal signs and microhabitat characteristics. It usually provides the greatest trap success but does not allow density estimation. The landscape features of urban areas are mostly delineated by lines and blocks (Fig. 1). As a consequence, the line method could be preferred when working along roads, urban paths, tramway lines, blocks. A grid method can be used in urban parks for example, while the sign method may be preferred in private properties.

Answering the fundamental questions about rat density (how many, where, when) can only be achieved by longitudinal (temporally replicated) studies that compare spatial replicates of several urban habitats or cities (Parsons et al. 2017) and use a CMR method that gives information on space used by individuals as well as modern statistics to analyze the data. By accounting for individual heterogeneity, modern field and statistical methods allow for improvement upon inferences from capture-recapture techniques and evaluation of animal density with negligible bias (Burnham and Overton 1978; Cubaynes et al. 2012; Gimenez and Choquet 2010; Pledger et al. 2003). For example, the “hollow grid” method combined with inverse prediction from capture-recapture data proposed by Efford et al. (2005), uses the edges of a “hollow” grid in the design and thus eliminates bias from edge effect (the method requires some intertrap distance data on the scale of animal home range). The spatially explicit capture-recapture (SECR) method, adapted for grid and linear transect designs, uses polygon searches and can integrate binary data (animal detected or not detected on each polygon) or count data (multiple detections of an individual or signs from one individual per polygon) to calculate animal density (Efford 2011). For example, with the SECR method, rat faeces or hair samples identified by DNA might be used to model rat density in different urban patches. Statistical approaches have also been used to infer *Rattus rattus* population dynamics from trapping records in a forest ecosystem (Efford et al. 2006). Once implemented for urban field studies, these methods could help to improve urban rat monitoring.

To answer ecological or behavioral research questions, camera traps (CTs) present an effective, low impact, and safe (i.e. without handling of animals) alternative to live-trapping (Parsons et al. 2015; Rendall et al. 2014). CTs also give insights into occurrence, density, abundance, distribution, and activity pattern of the studied species (O'Connell et al. 2014; Rendall et al. 2014). Radio-frequency identification of rats through microchips enhances appreciation of individual variations in behavior and activity pattern (Parsons et al. 2015). Used in concordance with live-trapping design, CTs can help to quantify the rate of "neophobic" behavior of rats. There is a variety of CT models available, each has advantages and drawbacks (Silvy 2012), but all have the same basic principle: a photo (and/or video) camera, which relies on a physical triggering (e.g. pressure pads) or which is triggered automatically when an animal moves in front of it (Sollmann et al. 2013). Better quality images can be obtained with white light flash cameras. However, infrared flashed cameras minimize disturbance to the animals (Sollmann et al. 2013) and are less prone to be noticed by the public, decreasing the risk of theft or vandalism (see below). Because the material is expensive and data recovery depends on the retrieval of the camera, these must be, as far as possible, hidden from the public, affixed and secured. Factors to consider in the schedule of CT studies include survey timing (time of year, month), single versus repeated surveys (e.g. investigation of a seasonal trends), duration of the survey, and frequency of camera checking (i.e. data download).

Factors interfering with urban trap success

To reduce theft, vandalism, or persons closing the traps, it is recommended to clearly label each trap with the name of the institution leading the project (name, website, address, phone number), to add a small description of the aim of the trap, and to clearly state that people must not manipulate it. This should be written in the local language. Traps can be covered with materials (leaves, branches, grass) to camouflage them (which also provides insulation to the captured animal and protection from potential predators). Traps could also be locked to a stationary objects with a chain (Himsworth et al. 2014a). One particularly useful strategy in urban environments might be placing traps inside plastic bait boxes (some are designed as "tamper-resistant") which are so ubiquitous in cities that people tend to pay little attention to them. To avoid any delay in the fieldwork schedule, it is recommended to order more traps and/or CTs than needed in the study design due to the high probability of theft and vandalism.

By playing with the traps, moving them and/or damaging them, domestic dogs and cats, crows, and medium sized urban wild mammals, which have successfully colonized cities (Bateman and Fleming 2012; Hadidian 2015; Plumer et al. 2014; Scott et al. 2014; Zink and Walter 2016), can have a negative impact on the trap success. In North America, grey squirrels (*Sciurus carolinensis*) are commonly trapped instead of urban rats, limiting the trap success.

Ethics

Ethics committee approval

Working in the field, researchers have to comply with institutional and national regulations regarding research and must adhere to high standards concerning animal welfare (Costello et al. 2016; Wallace and Curzer 2013), regardless the species (i.e. including invasive and pest species) (Guidelines for the treatment of animals in behavioural research and teaching 2018; Mason and Littin 2003; Meerburg et al. 2008). One of the most important stages of fieldwork preparation is obtaining the necessary animal experimentation permits. Additional permits pertaining to environmental and wildlife protection may also be needed.

Protection levels, legal requirements, and ethical standards for the use of animals in research vary between countries. The following section gives a brief overview of the legal framework and regulatory committees/organizations in some developed countries.

North America In Canada, use of animals in connection with research, teaching, testing and production is strictly regulated by the Canadian Council on Animal Care (CCAC), a national, peer-review organisation that supervises the use of animals under the Animals for Research Act (1971) (Canadian Council on Animal Care 2018).

In the USA the Animal Welfare Act (AWA), sets the minimum standards of care and treatment for animals used in research since 1966 (e.g., Public Law 99–158 "Animals in Research", Public Law 103–43 "Plans for Use of Animals in Research"). The use of animals for research purposes is monitored by the Institutional Animal Care and Use Committee (IACUC) (Institutional Animal Care and Use Committee (IACUC) 2018). The Animal and Plant Health Inspection Service (APHIS) within the United States Department of Agriculture (USDA) unit has amended the AWA to oversee field studies (United States Department of Agriculture 2018). The American Society of Mammalogists (ASM) publishes Guidelines for the Use of Animals in Research (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016). The American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals cover the principles of euthanasia, the agents that can be used, species and the environment in which they are to be euthanized (AVMA Panel on Euthanasia 2013).

Europe The European Union (EU) has a specific legislation covering the use of animals for scientific purposes since 1986. On 22 September 2010, the EU adopted Directive 2010/63/EU, which updated and replaced the 1986 Directive 86/609/EEC on the protection of animals used for scientific purposes and took full effect on 1 January 2013 (The European

Parliament and the Council of the European Union 2010). This directive aims to harmonise national measures covering the welfare of animals used in research. Member states have to comply with these general principles. For example, in Sweden this is recorded in the Animal Welfare Act (1988:534) (AWA) and Animal Welfare Ordinance (1988:539) (AWO) (Lundmark et al. 2016), in the UK in the Animal (Scientific Procedures) Act 1986 (A(SP)A) (The National Archives 2018), and in France in the articles R214–87 to R214–137 of the "Code Rural" (Legifrance 2018).

Oceania In Australia, the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, was established in 1969 and is revised periodically (last update in 2013) (National Health and Medical Research Council 2013). It sets out the common framework of guiding principles to promote the ethical, humane, and responsible care and use of animals for scientific purposes.

In New Zealand, animal use in research, testing, and teaching is controlled under the Animal Welfare Act 1999 (New Zealand Parliamentary Counsel Office/Te Tari Tohutohu Pāremata 2018).

Japan In Japan, animals used in research are protected by the Law Concerning the Protection and Control of Animals enacted in 1973, then amended in 1999 and entitled "the Law for the Humane Treatment and Management of Animals". This Law provides laws, standards relating to the care and management of experimental animals, and a guide for the disposal of animals (National Research Council 2004).

When the purpose of the study is to monitor living animals and involves biological samples (e.g. blood, biopsy such as ear or tail punches) or technical equipment (i.e. internal or external biologgers), the principles of the 3Rs (Replacement, Reduction and Refinement), originally proposed by Russell and Burch (1959), should be followed. These principles are being increasingly incorporated into legislations, guidelines, and practice of animal experiments in order to protect animal welfare (Fenwick et al. 2009). They refer to measures aiming at reducing the number of animals used in experiments and the stress caused to these animals: "replacement" includes measures aiming at avoiding or replacing the use of animals in experiments, "refinement" refers to experimental approaches that minimize the suffering of animals used, and "reduction" leads to measures that minimize the number of animal used in experiments through statistical optimisation and clever experimental design (more information on the webpage of the European Commission (2018)).

Safety of field-workers

In addition to the inherent hazards linked to working with rats (i.e. risk of zoonotic disease transmission and bites), working in cities poses other threats to field-workers. Ensuring the safety of workers in the field is a preeminent issue. The level of safety varies greatly between cities (International Committee of the Red Cross and European Institute for Security Studies 2012; The World Bank 2011), but working in the late evening (trap setting) and early morning (trap checking) increases the likelihood of encounters involving offensive behavior, verbal abuse, physical aggression, or even violent crime (Glaeser and Sacerdote 1999; Matthews et al. 2010; The World Bank 2011). Single workers are particularly vulnerable, and whenever possible, fieldwork should be performed in teams of at least two people. Field-workers must have a mobile phone that allows workers to be reached or to call for help when necessary.

Communication and partnerships

Communication to the public

Working discreetly in order not to attract public attention to the ongoing research can be difficult and even counterproductive for the study. Indeed, in a highly populated area concealment is very hard, if not impossible. Field-workers will necessarily meet observers and must be advised beforehand on how to handle them while working. In our opinion, actively engaging with the public will limit rumours and hostile behaviors. It is part of the pre-fieldwork stage to decide which information should be shared with the public. Informing the community, stimulating interest, and raising awareness about the project can be done through local newspapers, local radio programs, a webpage, and/or social media. As a good example, a great communication strategy has been implemented by the Vancouver Rat Project (http://vancouverratproject.com/). Additionally, it can also be useful to have flyers or brochures available for distribution to inquisitive on-lookers, which introduce the institution supporting the research and provide information about the ongoing study. This information must be formulated comprehensibly for the general public and in the local language. See Fig. 3 for an example of informative flyer that has been used in a Viennese study on urban rats to inform the public while working in the field.

Partnerships with stakeholders

Integration of the local government and community stakeholders must be implemented at the project outset. Obtaining the support of "gatekeepers", including local councillors, urban worker managers, or private owners may constitute a major challenge but is also potentially key to successful project implementation (Parsons et al. 2017). If the study design includes a control area (see

Fig. 3 Example of informative flyer intended for the public in a study on urban rats in Vienna, Austria. **a**: original version, in German; **b**: translated version, in English

above), support from city stakeholders can help to ensure that the area will be protected from human disturbance. A protected experimental unit ensures secure trapping and safety of animals, facilitates field-workers tasks, and enables daytime trapping (Desvars-Larrive et al. 2017). Moreover, engaging local animal welfare associations in the experiment/trapping set-up (prior fieldwork starts) might lead to a better public acceptance of the project (Simberloff 2013) and may contribute to decrease potential human disturbance. Working within a city may require additional permitting by the local authorities, e.g. access to specific zones (for example, in Vienna, Austria, urban parks are administered by an independent entity) or driving permit for some specific areas (e.g. pedestrian zones, protected green areas). CTs induce a risk of incidentally and accidentally capturing images of humans who wander into the area under surveillance. As a consequence, privacy protection legislation must also be considered, and permission of use may be required. Important investments in time and diplomacy are often required to gain approvals and supports. Taking into account the stakeholders and partners´ expectations (Parsons et al. 2017) and providing feed-back regarding the results are appreciated and can expedite research opportunities for future researchers.

Notification of or collaboration with private pest control firms or municipal pest control professionals can also be important for the smooth running of the research project (Parsons et al. 2017). Informed pest control operators will be less likely to remove traps, and more importantly their knowledge of and experience with the state of the rat population and control program in the city will provide valuable adjunct information to the researchers in the field. If the trapping proves unsuccessful, a back-up approach has to be developed as early as possible, i.e. other methods of obtaining rats have to be considered. One advantage of working in cities is the possibility to acquire *R. norvegicus* individuals (dead or alive) through pest management professionals (Franssen et al. 2016; Grandemange et al. 2010; Himsworth et al. 2013a; Meerburg et al. 2014; Parsons et al. 2017; Purcell et al. 2011). This method may help to increase sample size, or the study can be designed solely on this method.

Citizen science

The acquisition of records via citizen science (i.e. public participation in scientific research) (Shirk et al. 2012) has proven to be a valuable tool that contributes, for example, to biogeography studies (Lepczyk 2005; Roelfsema et al. 2016), conservation actions (Roy et al. 2015), and health research (Den Broeder et al. 2016). Involving citizen scientists can enhance the quantity of data collected in the field (while it may also negatively influence data quality due to observer bias) (Gura 2013). Urban rats represent a good subject for citizen science research projects because "*people (…) have strong feelings towards the rats, which makes them an interesting topic of citizen science*" (University of Helsinki 2018). For example, the Helsinki Urban Rat Project uses a citizen science approach to understand rat population dynamics in the city of Helsinki (University of Helsinki 2018). However, based on reports from the Viennese citizen science project StadtWildTiere, which aims at collecting wild animal sightings in the city of Vienna (StadtWildTiere Österreich 2018; Zink and Walter 2016), few rat sightings are reported. One hypothesis is that citizens tend to report spotting animals that they find "cute", while seeing rats often stresses people (German and Latkin 2016) and rats are sometimes not even considered as wildlife species, but rather as pest, by common opinion (Sullivan 2012; Zink and Walter 2016). Accordingly, using citizen science methods to collect data requires an effective public relations toolkit to motivate and inform citizens.

Education and training of field-workers

Field-workers (e.g. researchers, technicians, students, pest control professionals, citizens) play a major role in the success of a project, their instruction and training are essential (Molyneux et al. 2013). Field-workers must be educated in the recognition of signs associated with the presence of rats (Fig. 2) (Centers for Disease Control and Prevention 2006) and in the characterization of the experimental unit (see for example the "Environmental observation tool" photo library of Himsworth et al. (2014b)). To ensure high-quality data as well as reproducibility and repeatability of the experiment, standardized methods of data collection must be taught (e.g. systematic completion of the datasheet, systematic spatial and temporal characterization of observations). It is important to emphasize that the absence of rat sighting/rat signs is also a result worth reporting. To guarantee a good trap success and state-of-the-art sampling, urban field-workers must also be trained on specific protocols (e.g. how to set up the traps, how and what to sample, how to store samples). Potential safety issues in the urban environment should also be part of the field-workers´ training program. Importantly, they have to be informed on the zoonoses potentially carried by rats (Mills et al. 1995) and on how to prevent contamination (see the list of personal protective equipment provided by Parsons et al. (2016)). Instruction and training of field-workers must be regularly updated, firstly to obtain highly skilled scientists and to ensure standardized and comparable datasets, but also to maintain high motivation (Gura 2013; Momanyi et al. 2016).

Conclusion

Research on brown rats has historically concentrated on perceived natural (i.e. non-urbanized) landscapes, while cities (or slums) were largely neglected. Cities provide a relatively diverse and stable environment for urban brown rats, since food and structural resources are consistently available. Working in the complex urban environment that is potentially inhospitable and unaccommodating with regard to biological investigations may constitute a scientific and social challenge. This paper addresses the major constraints to be considered when studying urban *R. norvegicus* and suggests practical guidance to overcome some limitations related to the urban field. The principal challenges identified include, i) the experimental unit has to be clearly (quantitatively and qualitatively) characterized, ii) the use of live-trapping in CMR design, combined with powerful statistical methods of field data analyses, is highly recommended to answer ecological questions and make inference (although most methods have not been developed for the urban field and modifications may be needed), iii) potential ethical issues must be considered with regard to animal welfare and safety of field-workers, iv) urban studies should be conducted employing greater mutually-beneficial collaboration with city gatekeepers, pest control professional, and citizens. In order to collect high-quality data, emphasis must be put on communication with the public and education of scientist and non-scientist field-workers. In spite of urban-specific limitations, cities also offer advantages in rat-focused research, for example, an urban context is especially amenable to a citizen science approach, which can facilitate large data collection via engagement of interested volunteers and/or access to private properties.

Methods are needed to estimate the density of brown rat populations in different urban habitats/patches, and to describe, model, and predict their population dynamics. One of the prime objectives facing urban rat research is probably to define a common, peer-validated, and peer-accepted methodology that could empower reproducibility, repeatability, and inference of urban field studies and offer answers to long-standing key questions about urban rat ecology. To achieve this objective, world specialists must engage in dialogue regarding the technical and societal issues met in different urban environments, and reach a collegial agreement about the best protocol to be proposed. Once the investigation methods are clearly defined and the crucial question of rat density answered, other scientific points of interest can be investigated, e.g. the quantitative evaluation of the role of urban rats in the epidemiology of some zoonotic diseases, the assessment of the appropriate methods of control or management of urban rats, or the determination of the role of brown rats in urban ecosystems as ecological engineers.

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Compliance with ethical standards

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