Sampling techniques for studying non-volant terrestrial mammals

ECM – T5

2021/2022 – Miguel Rosalino

Ciências ULisboa

Sampling strategies

Core research questions in Ecology and Conservation

- How animals occur throughout the landscape?
- How many individuals are there?
- Which is the population trend?
- Why populations evidence those trends?

Sampling strategies

Different resolution levels:

1. Where animals occur?

Distribution

2.

3.

4.

Sampling strategies

Different resolution levels:

1. Where animals occur? **Distribution**
2. How many individuals are there? **Abundance**

Relative
It uses abundance indexes (e.g., number of signs of presence, visitation rates) that can be compared as a function of time or between areas

Absolut
It requires the use of counting methods (censuses) that allow estimating the number or density of individuals in the population

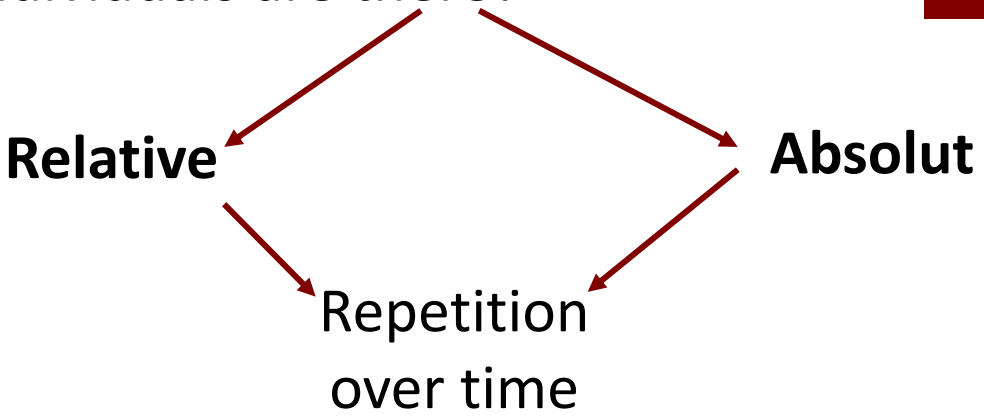
3.

4.

Sampling strategies

Different resolution levels:

1. Where animals occur? **Distribution**
2. How many individuals are there? **Abundance**

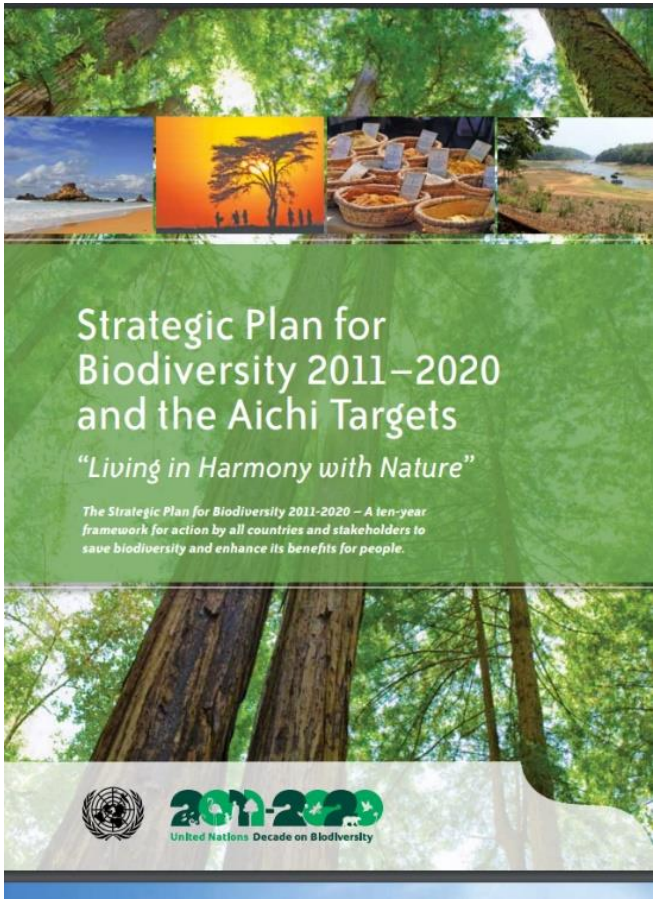


3. Which is the population trend? **Monitoring**
4.

Sampling strategies

Why monitoring animal populations is important?

- Only way to evaluate the effect of **impacts** and the effectiveness of **conservation programs**
- Needed to provide knowledge to conservation strategies at regional and national or global scales (e.g., United Nations Biodiversity Convention, Sustainable Development Objectives, Ecosystem Millenium Assessment)



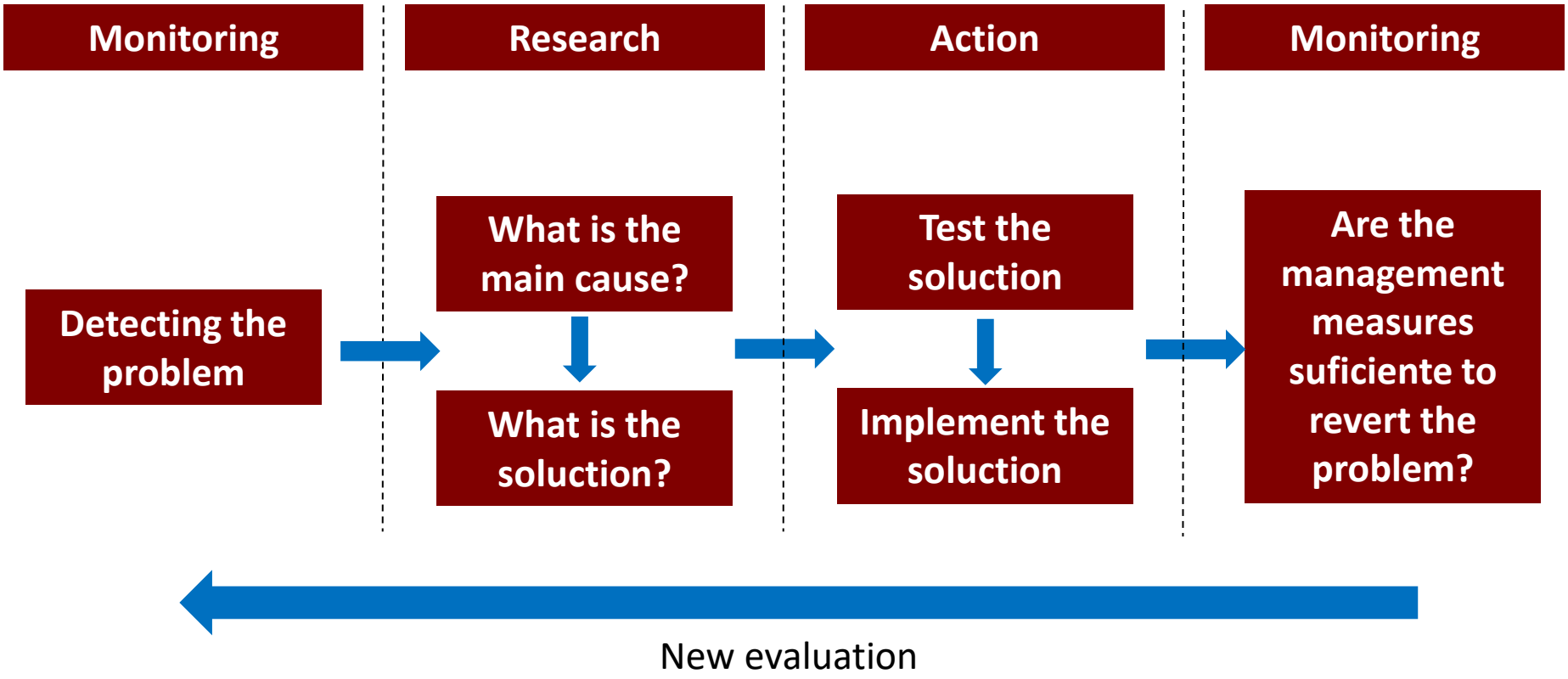
Sampling strategies

Aichi Biodiversity Targets

Strategic Goal A: Address the underlying causes of biodiversity loss by mainstreaming biodiversity across government and society	4
Strategic Goal B: Reduce the direct pressures on biodiversity and promote sustainable use	6
Strategic Goal C: To improve the status of biodiversity by safeguarding ecosystems, species and genetic diversity	3
Strategic Goal D: Enhance the benefits to all from biodiversity and ecosystem services	3
Strategic Goal E: Enhance implementation through participatory planning, knowledge management and capacity building	4

Sampling strategies

Conservations needs monitoring, and monitoring needs research

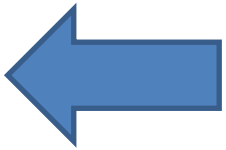


Sampling strategies

Different resolution levels:

1. Where animals occur? — **Distribution**
2. How many individuals are there? — **Abundance**
3. Which is the population trend? — **Monitoring**

Population trend	Objective
Increase	Control
Stable	Sustainable exploitation
Decrease/ regression	Conservation



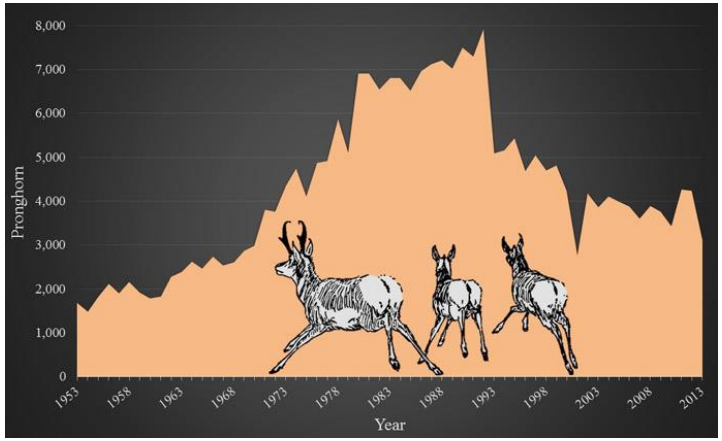
Management strategies

4.

Sampling strategies

Different resolution levels:

1. Where animals occur? — **Distribution**
2. How many individuals are there? — **Abundance**
3. Which is the population trend? — **Monitoring**
4. Why populations evidence those trends, i.e. why the stability or the change? (demographic processes) — **Research**

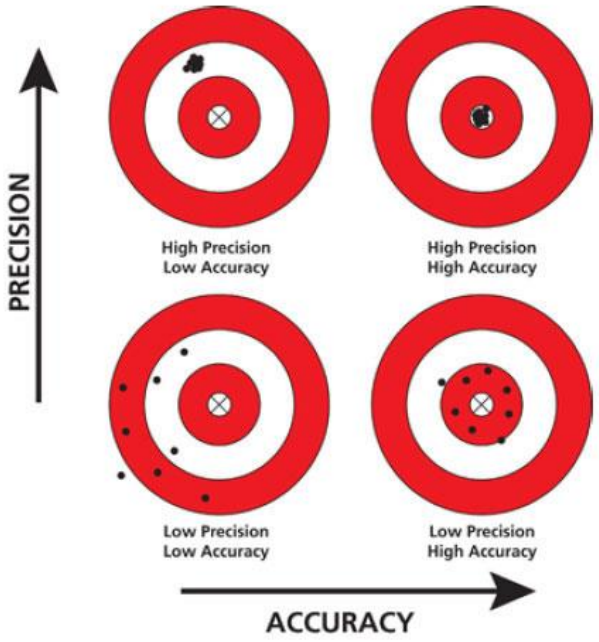


Sampling methods

Sampling methods may be direct or indirect and vary according to a gradient of:

- **Precision** (how similar are the measured values, e.g. SD values)
- **Accuracy** (how close is the estimate of the actual value)
- **Cost**

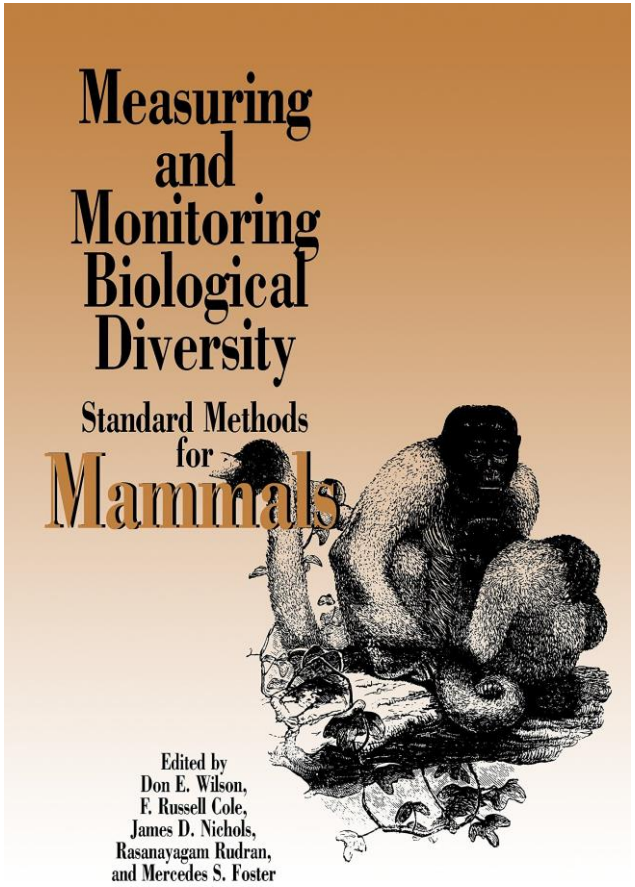
The selection of the method depends on the issue under analysis and the cost-benefit relationship



Sampling methods

Three fundamental questions in method's selection:

- Probability of observation or capture
- Size of the study area (time and money investment are constraints)
- Available human resources



Sampling methods

Mammals can be difficult to study because they often:

- Evidence secretive behaviors
- Show nocturnal habits
- Occupy vast areas
- Prefer areas with high vegetation cover
- Live in low density

Complex census and monitoring approaches

Sampling methods - Indirect

Questionnaires – face-to-face/oral, written, reports of observations

Advantages: non-invasive method, applicable to different scales (including broad scale), low cost

Disadvantages: misidentification of species, reduced response rates, concentration of observations - e.g. along roads, in proximity to houses or areas of concentrated activity

Figure 1. Spotted skunk wanted poster.

Sampling methods - Indirect

Questionnaires

Inquérito à População

Muitos fatores têm contribuído para que o lobo-ibérico (*Canis lupus signatus*) seja admirado por muitas pessoas, mas odiado por outras. A falta de esclarecimento, informação e proteção das pessoas tem contribuído para que este problema continue, colocando em risco, ao longo dos anos, a sobrevivência de um ícone da fauna portuguesa.
Um estudo mais elaborado sobre o conhecimento e atitudes do homem face ao lobo constitui assim um meio indispensável para a proteção e esclarecimento das populações, bem como para uma melhor gestão e conservação do lobo-ibérico e seu habitat.
Posto isto, este questionário, no âmbito da dissertação de mestrado em Ecologia Aplicada da aluna Diana Lopes, da Universidade de Aveiro, é uma ferramenta indispensável para o cumprimento destes objetivos.

Solicita-se assim a colaboração de todos para o seu preenchimento.

Idade: ____ Sexo: M☐ F☐ Localidade: _____
Profissão: _____ Freguesia: _____
Habilitações Académicas: _____ Concelho: _____
Tem gado doméstico? Sim☐ Não☐
(se respondeu 'não' avance para o **Grupo I**)
Tem cães de guarda/gado? Sim☐ Não☐

Grupo I - As seguintes perguntas são sobre experiências pessoais e conhecimento sobre o lobo. Por favor, assinale a resposta que melhor descreve a sua.

1) Já avistou lobos na sua zona?	Sim	Não
2) Tem conhecimento de ataques de lobos a animais doméstico?	Sim	Não
3) Já sofreu perdas de animais domésticos por ataque de lobos?	Sim	Não
4) Tem conhecimento de ataques de lobos a humanos?	Sim	Não
5) Quando o lobo ataca um animal domestico, o proprietário é sempre compensado?	Sim	Não
6) Já houve reintroduções de lobos em Portugal?	Sim	Não
7) O lobo alimenta-se principalmente de animais de caça maior	Sim	Não
8) A população de lobos na sua zona tem aumentado	Sim	Não
9) O número de ataques de lobos a gado tem aumentado	Sim	Não
10) A presença do lobo na sua região pode implicar prejuízos financeiros?	Sim	Não

Sampling methods - Indirect

Questionnaires

Sampling methods - Indirect

Signs of presence – scent stations, track-plates, hair-traps, transects for scat collection and DNA fecal analysis

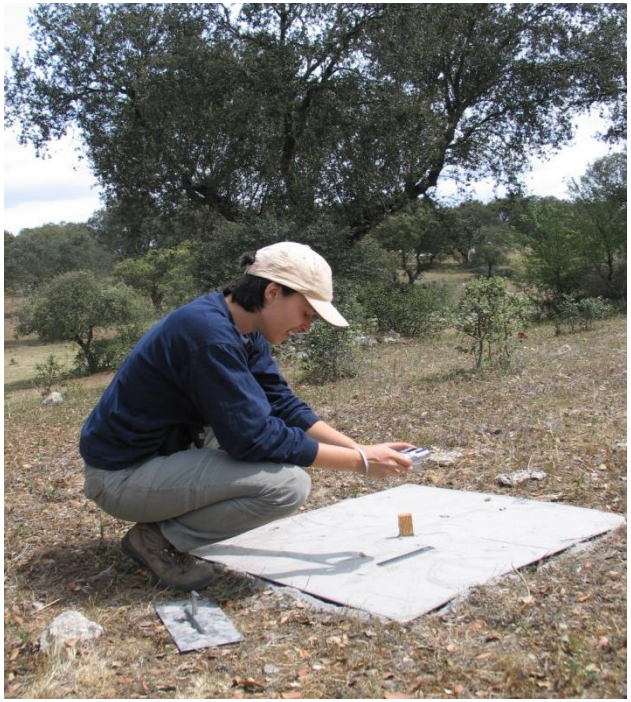
Advantages: non-invasive method, often high accuracy

Disadvantages: complexity, high cost (DNA analysis), and applicability only at lower spatial scales

Sampling methods - Indirect

Signs of presence

Scent stations



Sampling methods - Indirect

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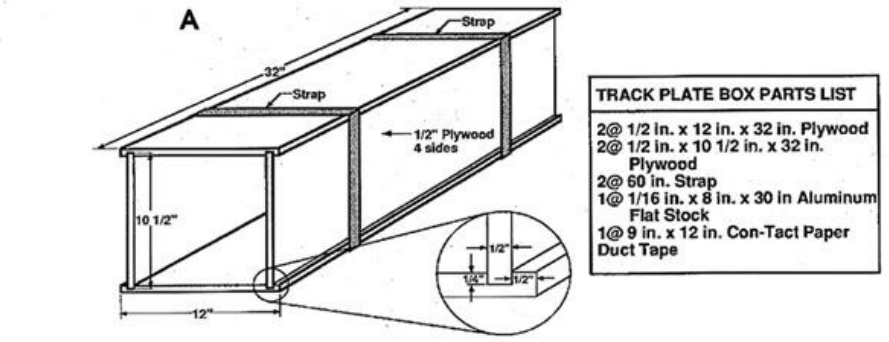
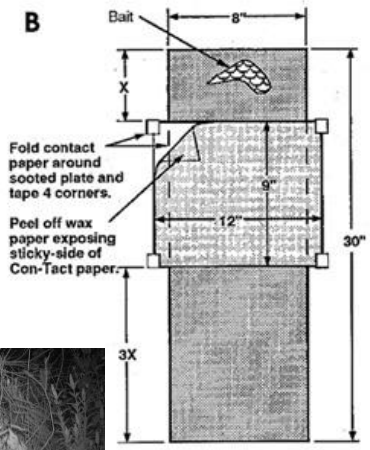
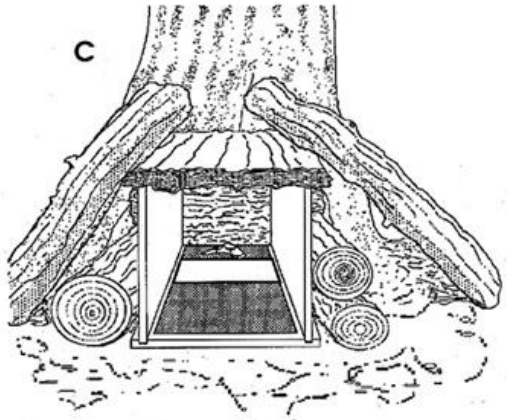
Signs of presence

Track-plates





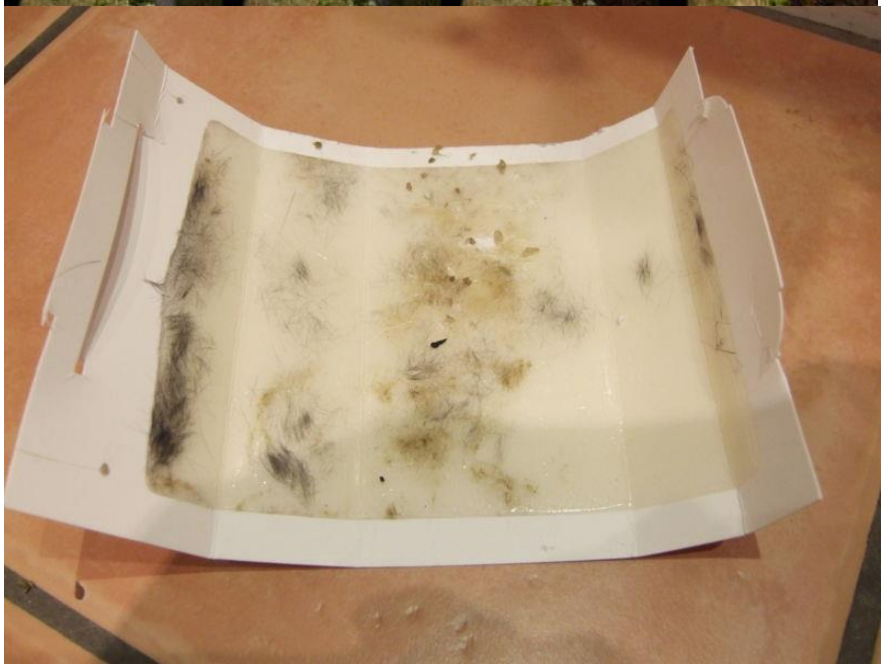


Sampling methods - Indirect

ECM

Signs of presence

Hair-traps



Sampling methods - Indirect

Signs of presence

Hair-traps

Sampling methods - Indirect

Signs of presence

Footprints and trails

Sampling methods - Indirect

Signs of presence

Criteria for identifying footprints

- Profile
- Size
- Shape
- Location

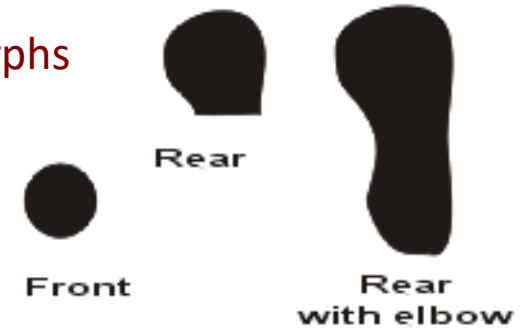
Sampling methods - Indirect

Signs of presence

Criteria for identifying footprints

- Overall Profile

Lagomorphs



Rodents and
Eulipotyphla

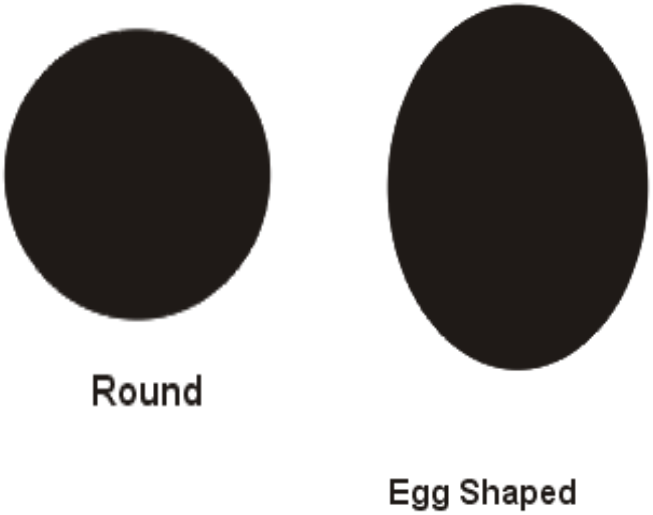
Canids

Felids

Mustelids (others)

Viverrids

Herpestids



Cervids

Suids

Mustelids

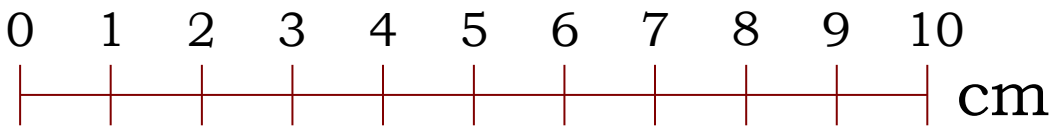
(Otter, badger)

Sampling methods - Indirect

Signs of presence

Criteria for identifying footprints

- Size



Eulipotyphla

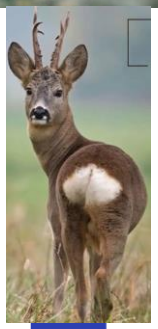
Rodentia

Lagomorpha

Carnivora

Cervidae

Suidae

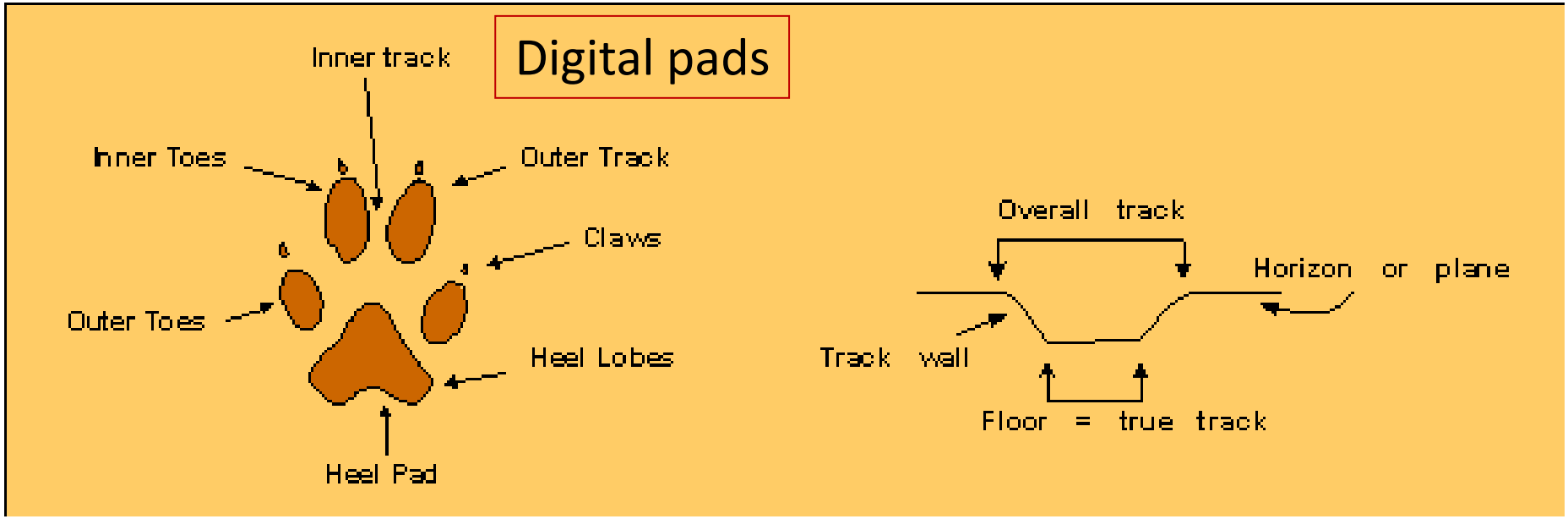


Sampling methods - Indirect

Signs of presence

Criteria for identifying footprints

- Shape – Digital and palmar pads



Palmar pads

Sampling methods - Indirect

Signs of presence

- There are 3 types of footprints:

I – Without any clear distinction between digital and palmar pads
RODENTS, EULIPOTYPHLA

II – With a clear distinction between digital and palmar pads
CARNIVORES, LAGOMORPHS

III – Hoof marks
CERVIDS, SUIDS

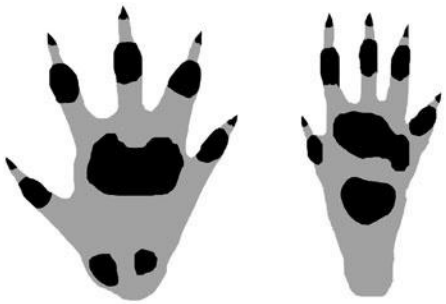
Sampling methods - Indirect

Signs of presence

RODENTS and EULIPOTYPHLA

5 finger in front and hind foot

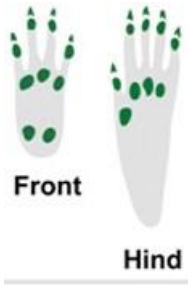
Shrews (Soricidae)
Moles (Talpidae)
Hedgehogs (Erinacidae)



4 finger in front and 5 in hind foot

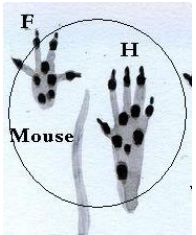
Front and hind foot of same or similar size

Squirrels (Sciuridae)



Front and hind foot of different size

Mice and rats (Muridae)
Voles (Cricetidae)
Garden dormouse (Gliridae)



Sampling methods - Indirect

Signs of presence

CARNIVORES and LAGOMORPHS

5 finger in front and hind foot

With visible claws

With digital membrane

Otters (Mustelidae)

Without digital membrane

Weasel, stoat, polecat, mink, Stone and Pine marten, badger (Mustelidae)

Without visible claws

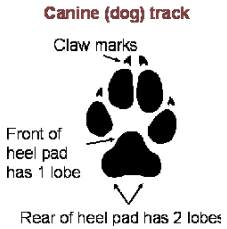
Genet (Viverridae)

4 finger in front and hind foot

With visible claws

Well defined pad print

Wolf, fox and dog (Canidae)

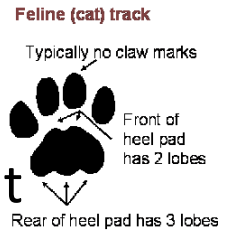


Poorly or undefined pad print

Hare and rabbit (Leporidae)

Without visible claws

Cat and Lynx (Felidae)



Sampling methods - Indirect

Signs of presence

UNGULATES

Footprints with an even number of hoofs

Footprints with a odd number of hoofs

With 4 elements

With 2 elements

Horse (Equidae)

Wild boar (Suidae)

Rectangular silhouette

Heart silhouette

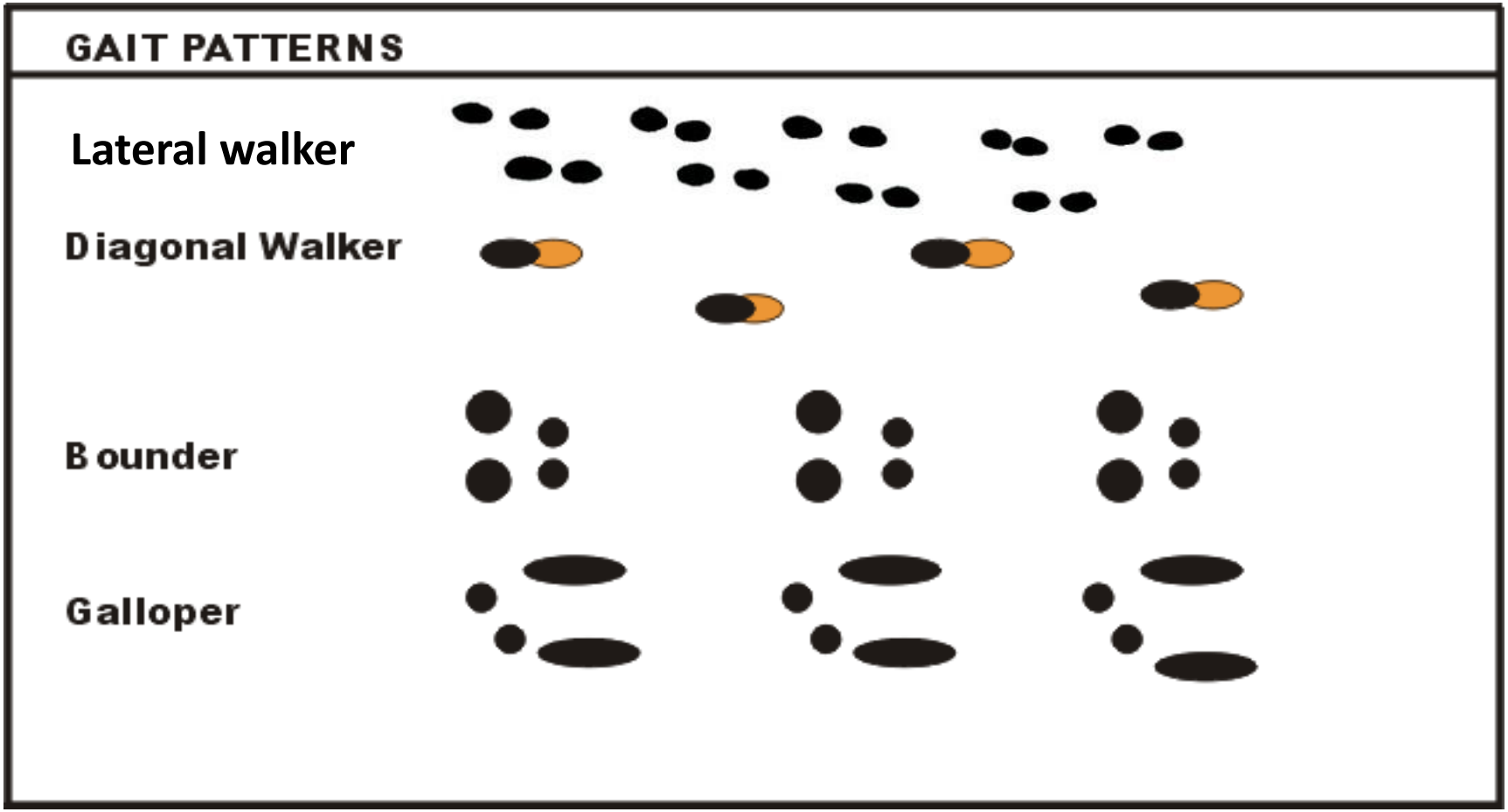
Red and fallow deer (Cervidae)

Roe deer (Cervidae)

Sampling methods - Indirect

Signs of presence

TYPES OF MOVEMENTS



Sampling methods - Indirect

Signs of presence TYPES OF MOVEMENTS

Lateral walkers

- Move the same side of the body at the same time (e.g. RF & RR)
- These animals have wide, rotund bodies.
- Most of the time these animals use this pattern. As speed increases, they change their pattern.

- e.g. badgers, skunk, porcupine opossum, raccoon, bear

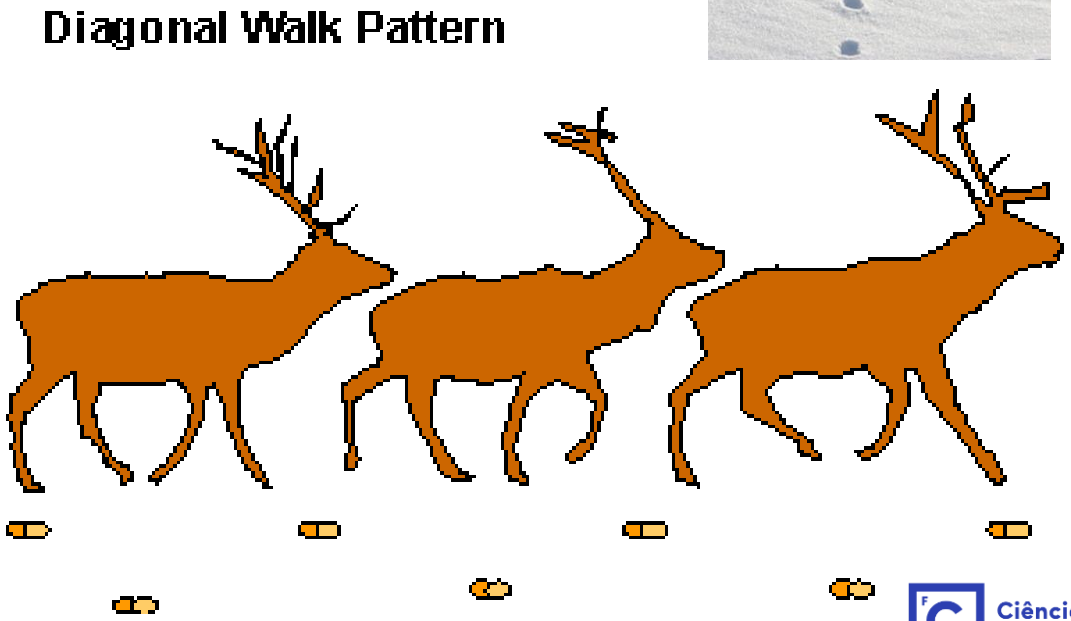
Sampling methods - Indirect

Signs of presence

TYPES OF MOVEMENTS

Diagonal walkers

- The animal moves the opposite sides of the body at the same time (e.g. RF & LR move simultaneously).
- e.g. Ungulates, canids, felids



Sampling methods - Indirect

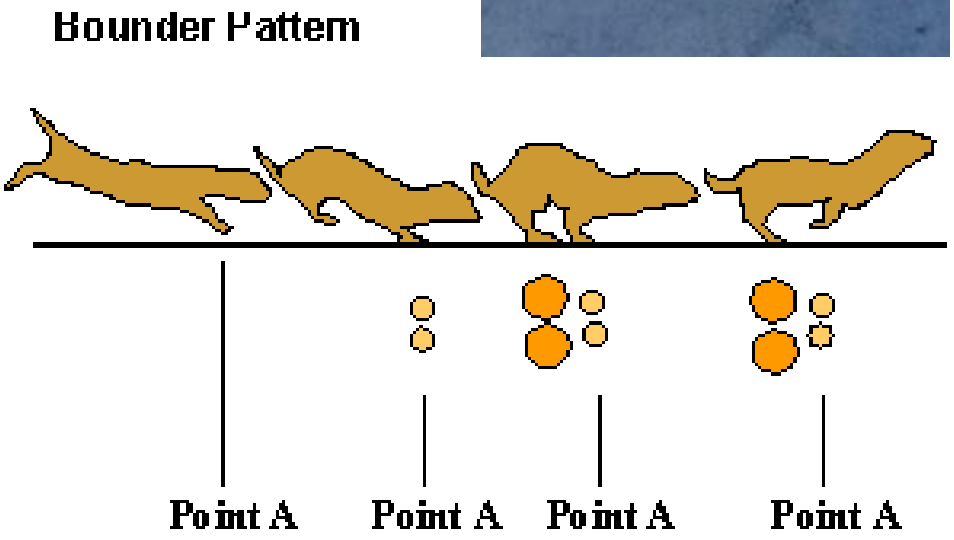
Signs of presence

TYPES OF MOVEMENTS

Bound Walkers ("salto")

- The front feet land together, then the rear feet behind
- Most of the time these animals use this pattern even when moving slow or fast.

e.g. Mustelids - All members except skunks & badgers



Sampling methods - Indirect

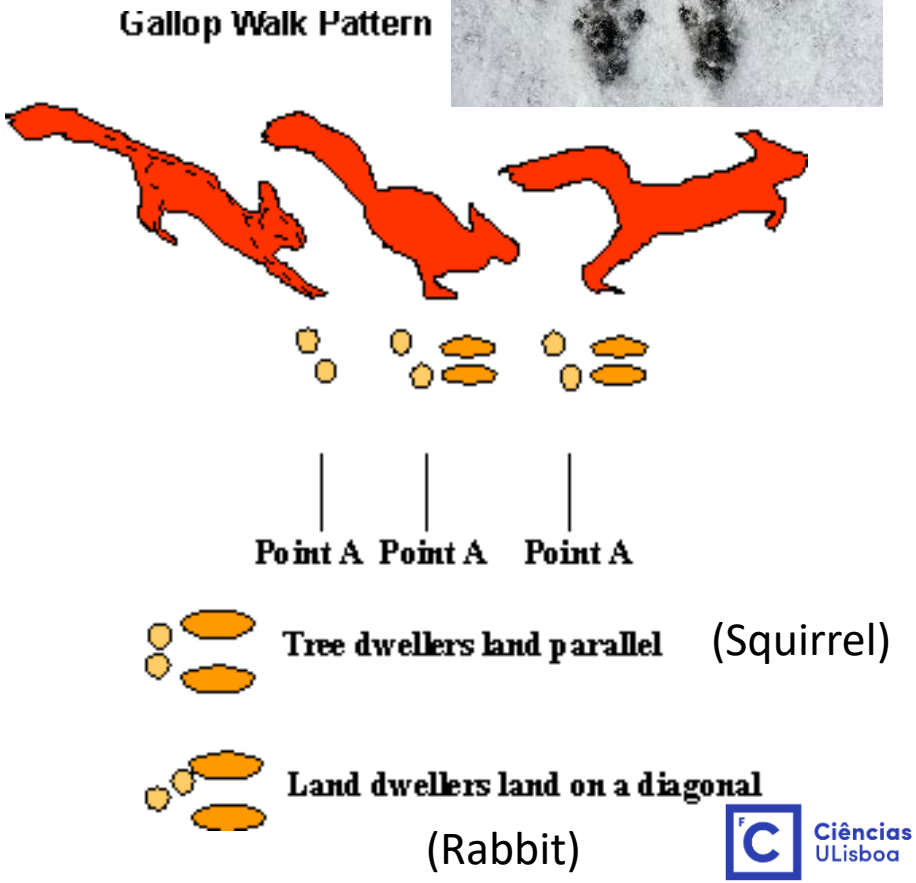
Signs of presence

TYPES OF MOVEMENTS

Gallop Walkers

- The front feet land first, then the rear feet come on the outside of the front feet and land ahead.
- Most of the time these animals use this pattern even when moving slow or fast. The pattern doesn't change with speed.
- The distance between sets of tracks increases with speed.

e.g. Lagomorphs, most Rodents



Sampling methods - Indirect

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Research Article

Can footprints of small and medium sized felids be distinguished in the field? Evidences from Brazil's Atlantic Forest

William Douglas de Carvalho[1,2,3*], Luís Miguel Rosalino[3], Júlio Cesar Dalponte[4], Bárbara Santos[1], Cristina Harumi Adania[1] and Carlos Eduardo Lustosa Esbérard[2]

Ocelot, *Leopardus pardalis* (8,5-16kg)

Abstract

Carnivores, particularly felids, face threats in many regions of the world. They are a crucial component of biodiversity with a functional role in the top of the food chain. Therefore, they have been the target of surveys and monitoring and ecological studies, most of which are based on footprint identifications, an efficient and low-cost method compared to other approaches. In these cases, species identifications may suffer from a high degree of bias due to the overlap in the size and shape of footprints among species. We experimented with small to medium captive wild felids of five species: ocelot, *Leopardus pardalis*, margay *L. wiedii*, oncilla, *L. guttulus*, domestic cat, *Felis catus*, and jaguarondi, *Puma yagouaroundi*).We tested for differences in footprint measurements, including main pad and toe pad sizes. We used humid sand as substrate and took measurements from several front and hind footprints of seven animals per species (except jaguarondi, for which only four animals were available). Our results showed that ocelot is the only species for which it is possible to obtain 100%-accurate footprint identifications, mainly because of its footprint area (*i.e.*, length x width). The remaining species presented a wide variation in measurements, making them almost impossible to distinguish based solely on footprint

Margay *Leopardus wiedii* (2,6-3,9kg)

Jaguarundi, *Puma yagouaroundi* (4,5-9kg)

Oncilla, *Leopardus guttulus* (1,8-3,5kg)

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Sampling methods - Indirect

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Signs of presence

Scats






Sampling methods - Indirect

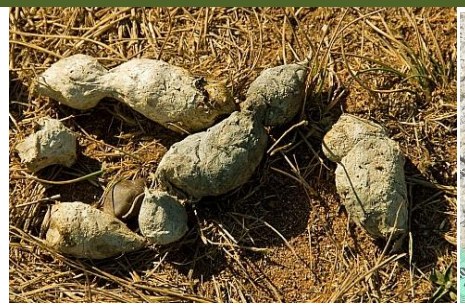
Signs of presence

Criteria for identifying scats

- Size
- Shape
- Location
- Odour

Sampling methods - Indirect

Signs of presence




Criteria for identifying scats

- **Shape**
  - Tubular – Canidae, Ursidae
  - "Teardrop" – Felidae
  - "Rolled ribbon" – Mustelidae
  - "M&M" – Lagomorpha
  - Oblong (may have a tip at the end) - Cervidae
  - "Pencil lead" - Rodentia




(**Fox** - Tubular & tapered at both ends - between dog and cat)

Sampling methods - Indirect

Signs of presence

Criteria for identifying scats

- **Location**
  - Deposition site – soil, tuft of vegetation, tree branch, roof, near water, etc.)
- **Number**
  - Latrines or isolated scats
- **Type of habitat**
- **Positioning on the trail** - crossroads of paths, sett/den entrance, pit in the ground etc.)

Sampling methods - Indirect

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Signs of presence

Criteria for identifying scats

Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

Factors affecting the (in)accuracy of mammalian mesocarnivore scat identification in South-western Europe

P. Monterroso[1,2,3], D. Castro[1], T. L. Silva[1,2], P. Ferreras[3], R. Godinho[1] & P. C. Alves[1,2,4]

Table 1 Red fox *Vulpes vulpes*, stone marten *Martes foina* and European wildcat *Felis silvestris* relative abundances and genetic results for the scats morphologically identified, collected at Cabañeros National Park (CNP) and Guadiana Valley Natural Park (GVNP), during the summer 2009 and winter 2010

						Proportion (%) of samples genetically identified as:				
Putative species	Season	Study area	TS	*n*	SGI (%)	Red fox	Stone marten	European wildcat	Polecat	Dog
Red fox	Summer/autumn	CNP	22.08 ± 22.04	26	64.00	**82.35**	17.65	0.00	0.00	0.00
		GVNP	4.16 ± 6.46	39	79.49	**93.55**	0.00	0.00	0.00	6.45
	Winter/spring	CNP	34.19 ± 34.68	54	77.78	**83.33**	11.90	2.38	0.00	2.38
		GVNP	2.27 ± 4.96	38	71.05	**85.19**	3.70	3.70	0.00	7.41
	Overall		16.78 ± 25.28	157	75.52	**86.32**	7.69	1.71	0.00	4.27
Stone marten	Summer/autumn	CNP	3.53 ± 5.72	30	90.00	7.41	**92.59**	0.00	0.00	0.00
		GVNP	1.63 ± 3.58	19	94.74	16.67	**72.22**	0.00	11.11	0.00
	Winter/spring	CNP	2.14 ± 3.83	32	75.00	45.83	**54.17**	0.00	0.00	0.00
		GVNP	6.26 ± 7.96	45	86.67	15.38	**84.62**	0.00	0.00	0.00
	Overall		3.34 ± 5.71	126	85.71	20.37	**77.78**	0.00	1.85	0.00
European wildcat	Summer/autumn	CNP	0.33 ± 0.99	1	100.00	100.00	0.00	**0.00**	0.00	0.00
		GVNP	2.56 ± 3.50	19	84.21	80.00	6.67	**13.33**	0.00	0.00
	Winter/spring	CNP	0.74 ± 1.95	0	–	–	–	–	–	–
		GVNP	1.89 ± 3.71	17	69.23	90.00	0.00	**10.00**	0.00	0.00
	Overall		1.29 ± 2.80	37	78.78%	84.62	3.85	**11.54**	0.00	0.00

Monterroso et al (2012) Factors affecting the (in)accuracy of mammalian mesocarnivore scat identification in South-western Europe. *Journal of Zoology*, **289**, 243-250.

Sampling methods - Indirect

Signs of presence

Other signs of presence (e.g. dens, setts)





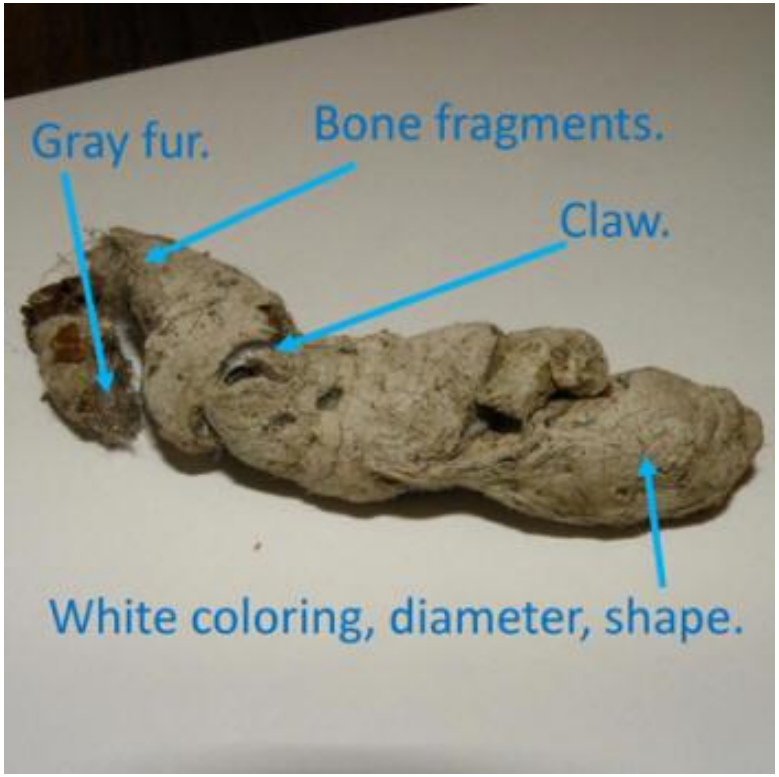
Sampling methods - Indirect

Signs of presence

Other signs of presence (e.g. marks, tree scratches)

Sampling methods - Indirect

Scat and pellet analysis
(e.g. carnivores, owl)



COMPLEMENTAR METHOD

Sampling methods - Indirect

ECM

Scat and pellet analysis
(e.g. carnivores, owl)

Advantages: non-invasive method, moderate accuracy (difficulty in locating the capture site), low cost, applicable to large scale studies

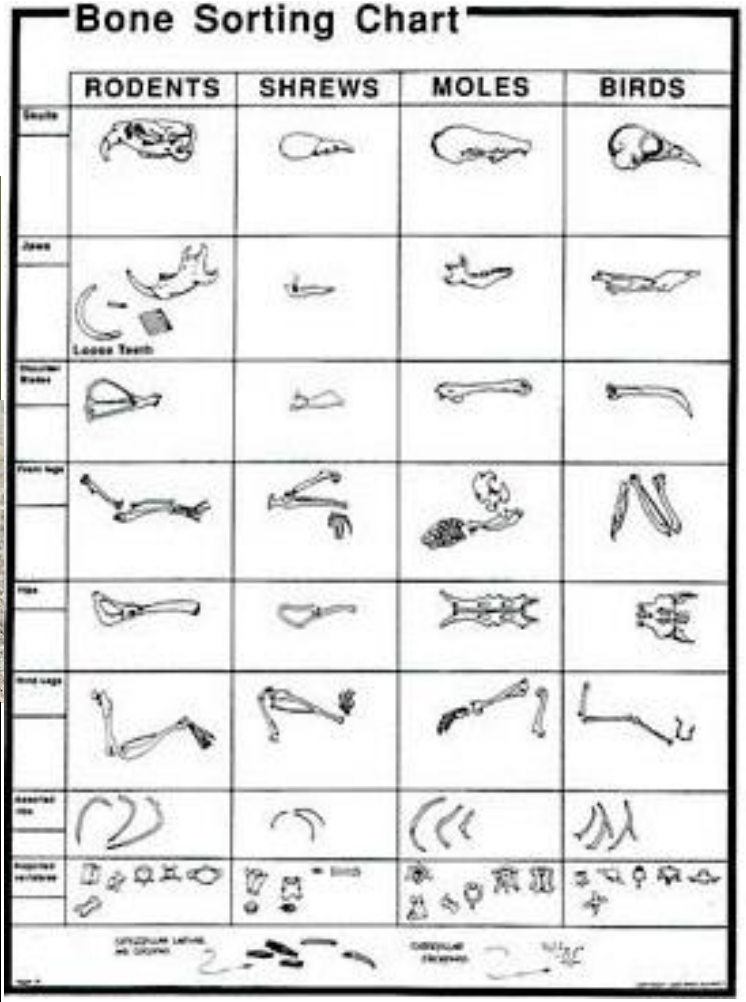
Disadvantages: time demanding, knowledge about the size of the predator's home range

COMPLEMENTAR METHOD

Sampling methods - Indirect

Scat and pellet analysis

(e.g. carnivores, owl)



Sampling methods

Direct observation, live captures

e.g.:

- Direct observation
- Spotlighting
- Live trapping
- Camera-trapping
- Video surveillance
- Drones

Advantages: high accuracy

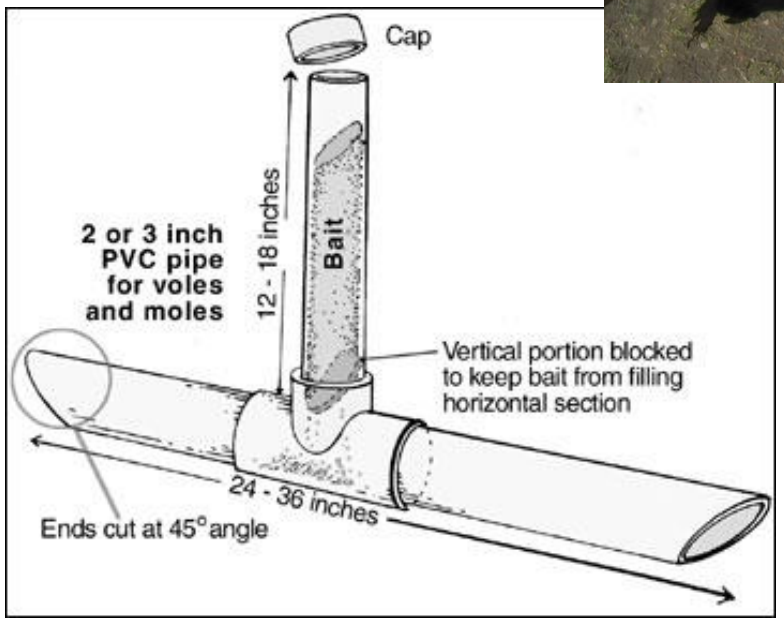
Disadvantages: some invasive method (disturbance - e.g. headlamp, or handling), complexity, high cost and only applicable in small scale studies

Sampling methods

ECM

Direct observation, live captures

(Live) trapping



Sampling methods

Direct observation, live captures

(Live) trapping

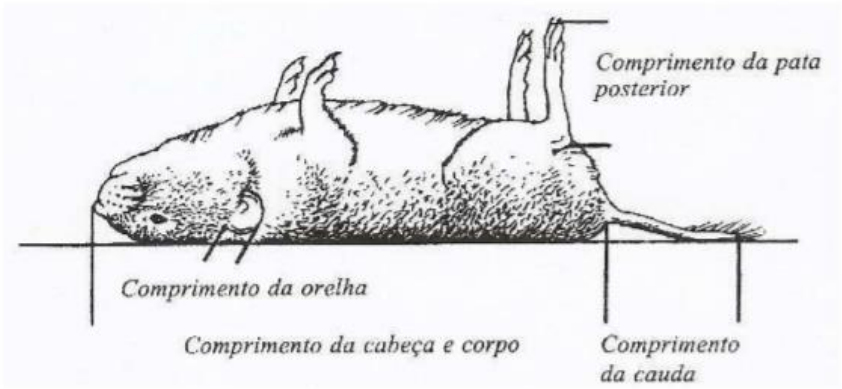
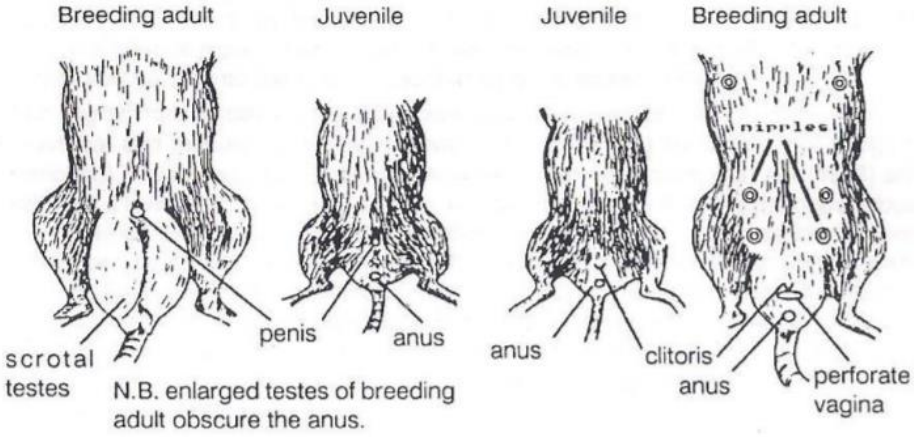


Fig. 9- Biometrias gerais de um mamífero (Fonte: Macdonald &



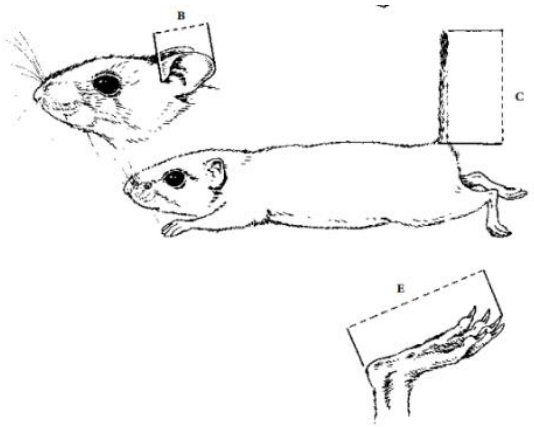
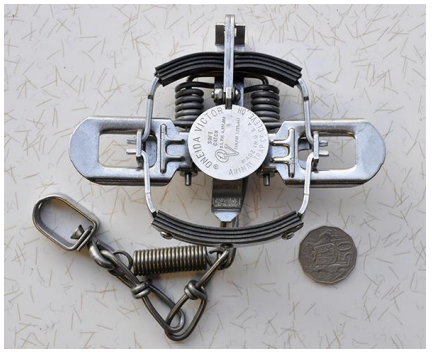


Fig.10- Características sexuais de machos (à esquerda) e fêmeas (à direita) (Fonte: Gurnell & Flowerdew, 1990).

Sampling methods

Direct observation, live captures

Live trapping



Sampling methods

Live trapping

Sampling methods

Direct observation, live captures

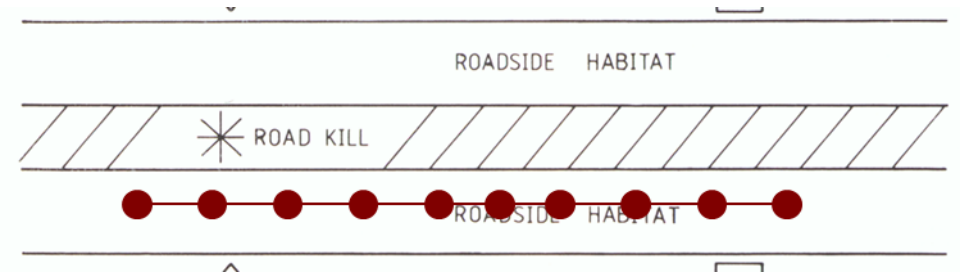
Live trapping

Sampling methods

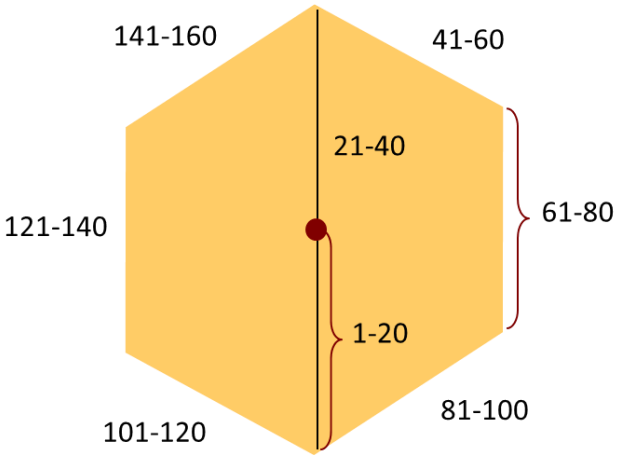
Direct observation, live captures

Live trapping

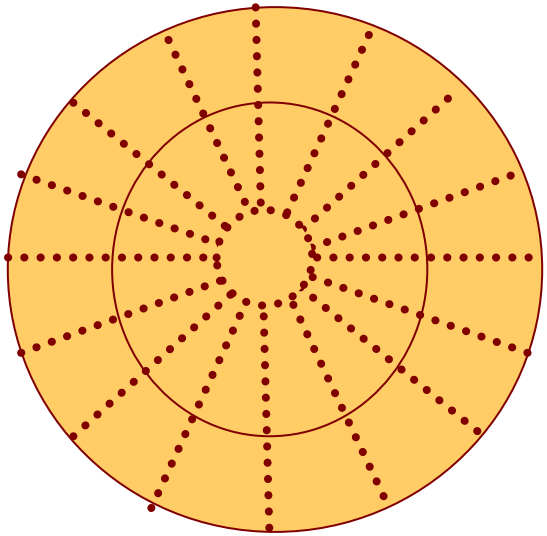
Trapping transect **Abundance**



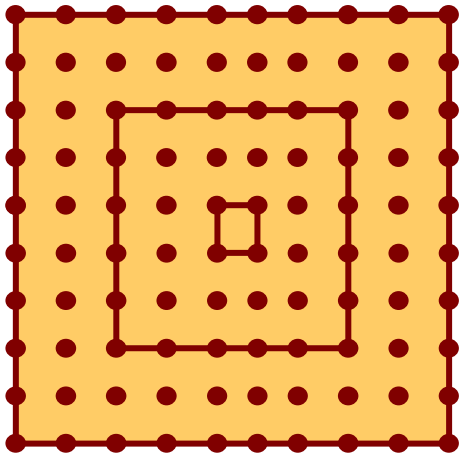
Trapping hexagon **Density**



Trapping web **Density**



Trapping grid **Density**



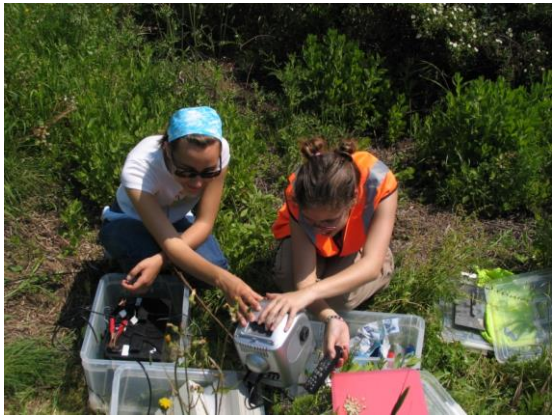
Sampling methods

Direct observation, live captures

Camera-trapping



Video surveillance



Sampling methods

ECM

Direct observation, live captures

Video surveillance

Sampling methods

ECM

Sampling methods

ECM

Direct observation, live captures

Video surveillance

Sampling methods

ECM

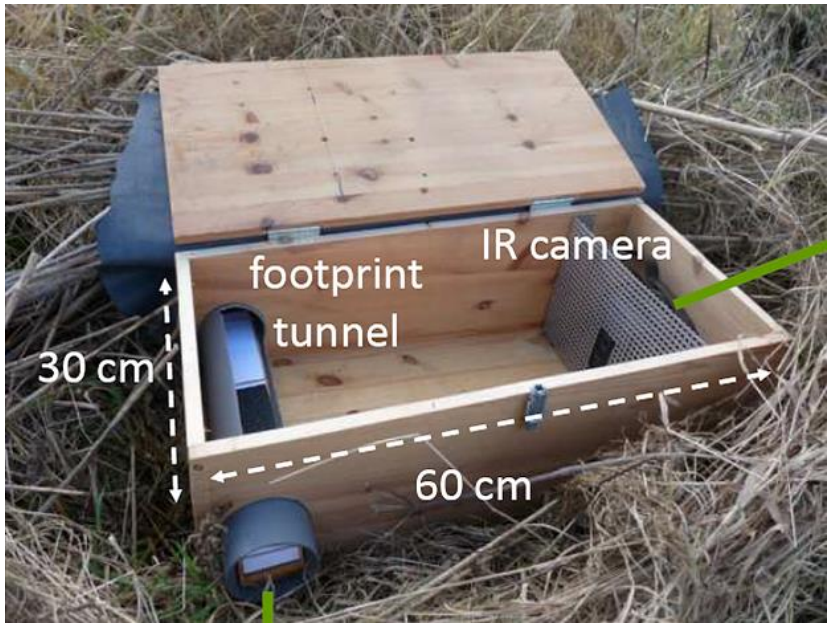
Direct observation, live captures

Camera-trapping

Sampling methods

Direct observation, live captures

Camera-trapping – Mostela-trap



Sampling methods

ECM

Camera-trapping – Mostela-trap

Sampling methods

Spotlighting sampling

Advantages: Less-invasive method, moderate accuracy (difficulty in locating due to distance and lighting conditions), low cost, applicable to local scale

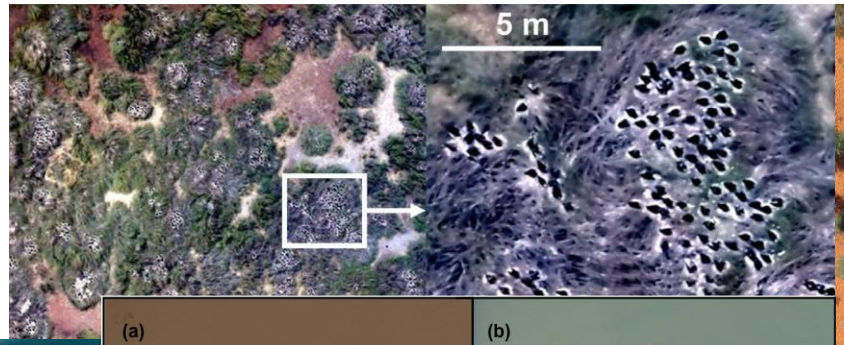
Disadvantages: need for human resources, knowledge about species behavior, good visual acuity

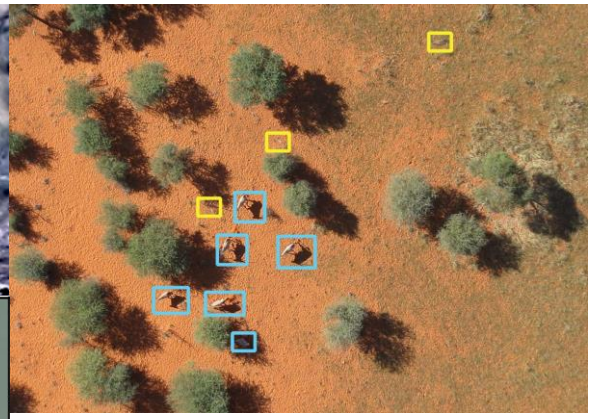
Sampling methods

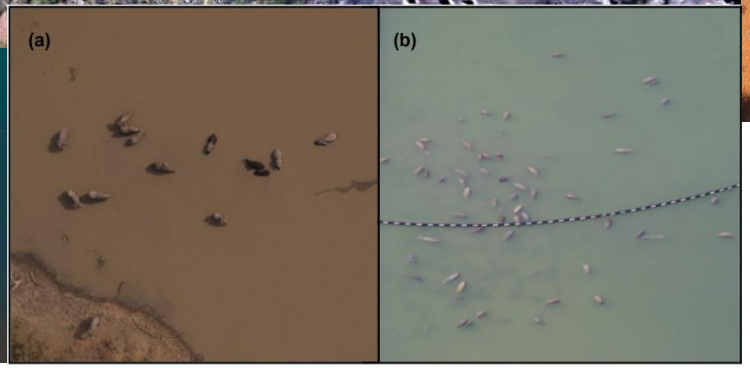
Sampling methods

ECM

Drones







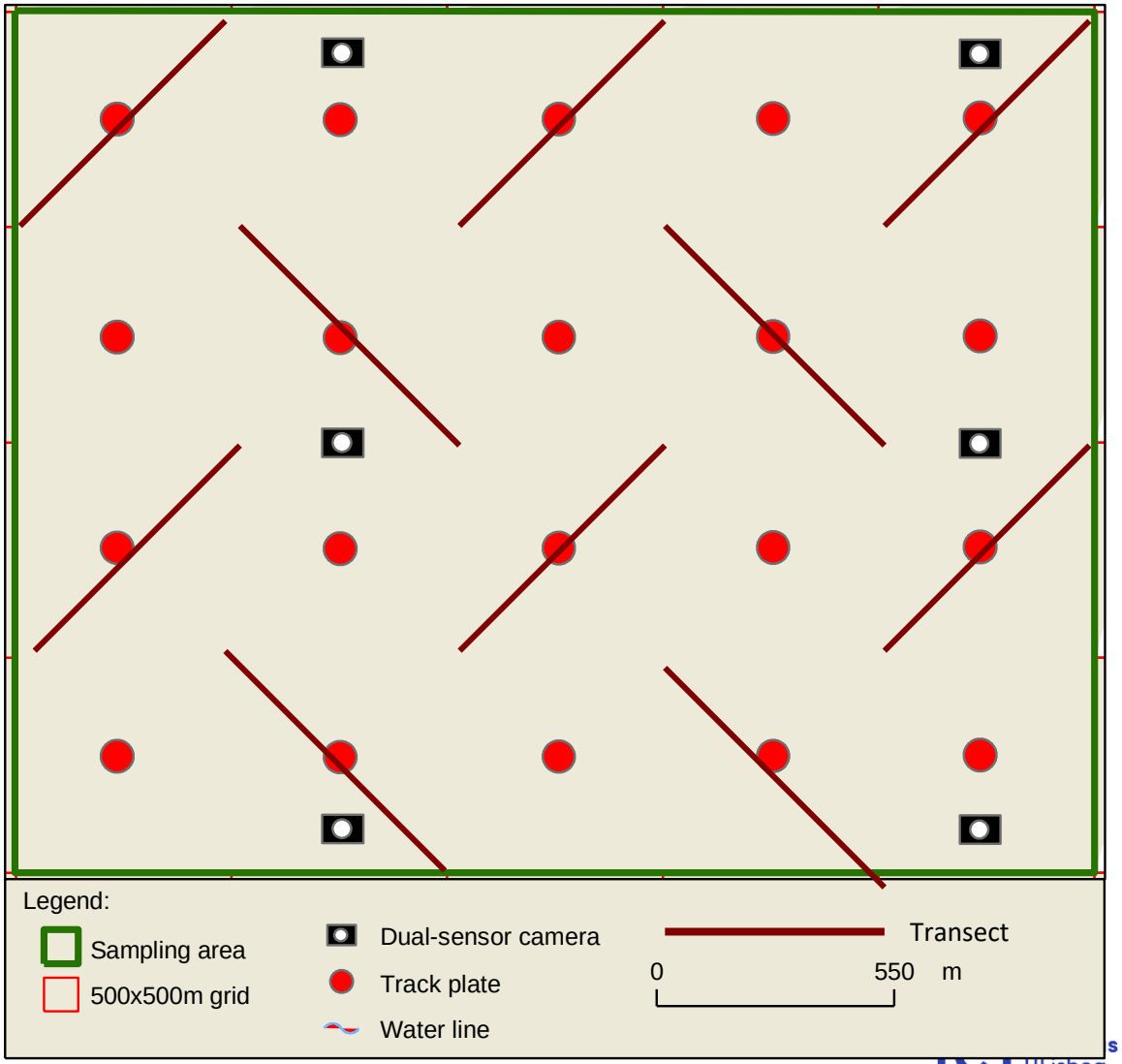
Advantages: moderate accuracy (more efficient for large sized mammals), able to cover wide areas with lower effort

Disadvantages: cost of the drones, need for specialized human resources to maneuver the drones, only applicable to open areas

Sampling methods

Combined strategies

They produce better results especially in situations of low density



Sampling methods

Combined strategies

Sampling methods

Radio-tracking

- Movements
- Estimation of home-ranges
- Behavior (e.g. circadian rhythms)
- Patterns of resource use (e. g. habitat preferences)

Advantages: moderate to high accuracy

Disadvantages: invasive method (envolves animal's capture and handling), complexity, high cost and mostly applicable to small scale studies

Sampling methods

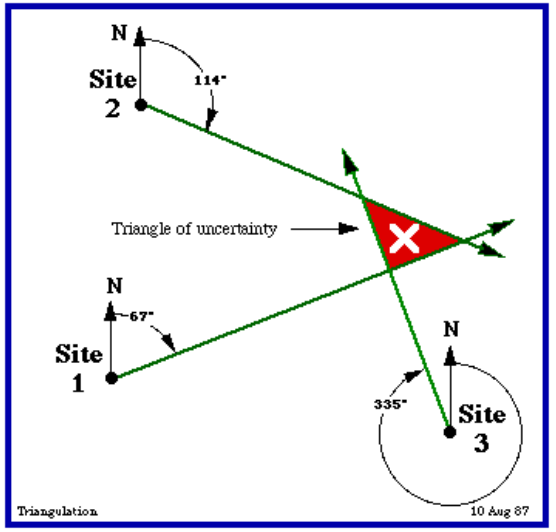
Radio-tracking

- Transmitters emitting on a single frequency, placed on a collar, harness or intraperitoneally through a surgical intervention
- Each location has an associated vector (x, y, t), where x and y are the spatial coordinates and t the time coordinate.
- Attributes associated with the vector, e.g., weather conditions, signal intensity, location description, habitat, and behavior (active / inactive) of the animal at location time, etc.

Sampling methods

Radio-tracking

- Triangulation

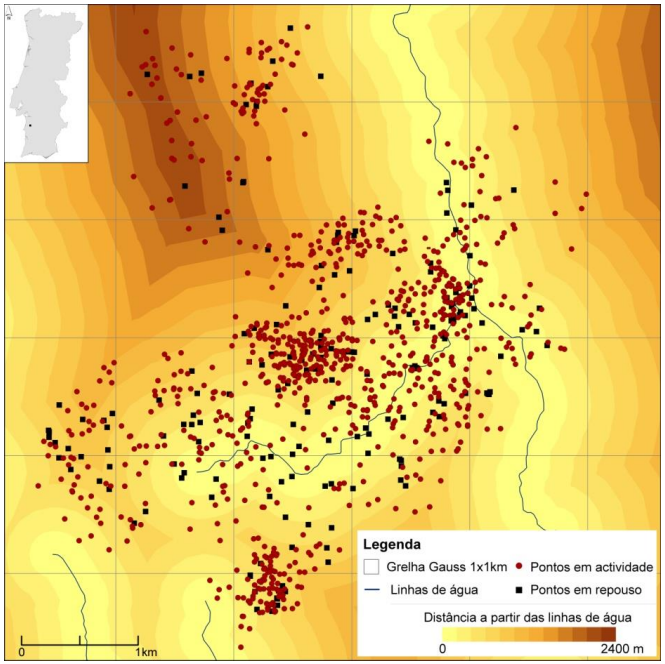


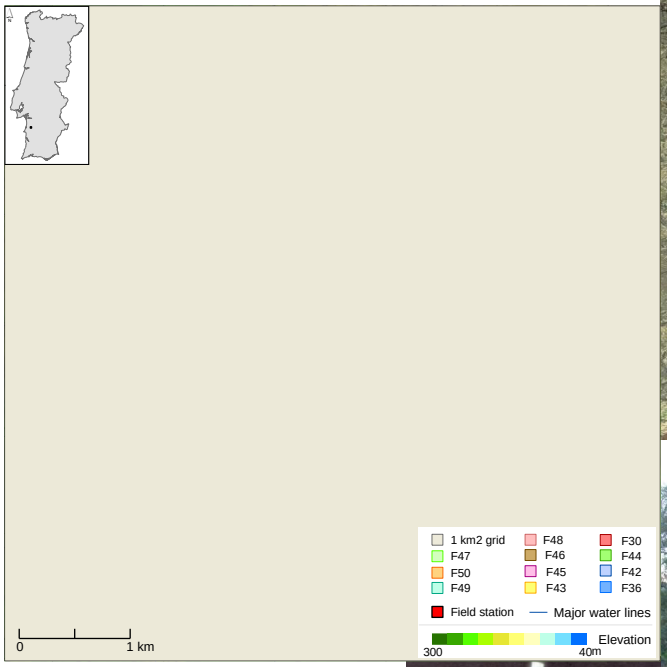
- Homing
  - Location of the animal on foot and within walking distance.

Sampling methods

ECM

Radio-tracking





Sampling methods

Radio-tracking

Sampling methods

Radio-tracking

Sampling methods

Radio-tracking – Effects on Mammals

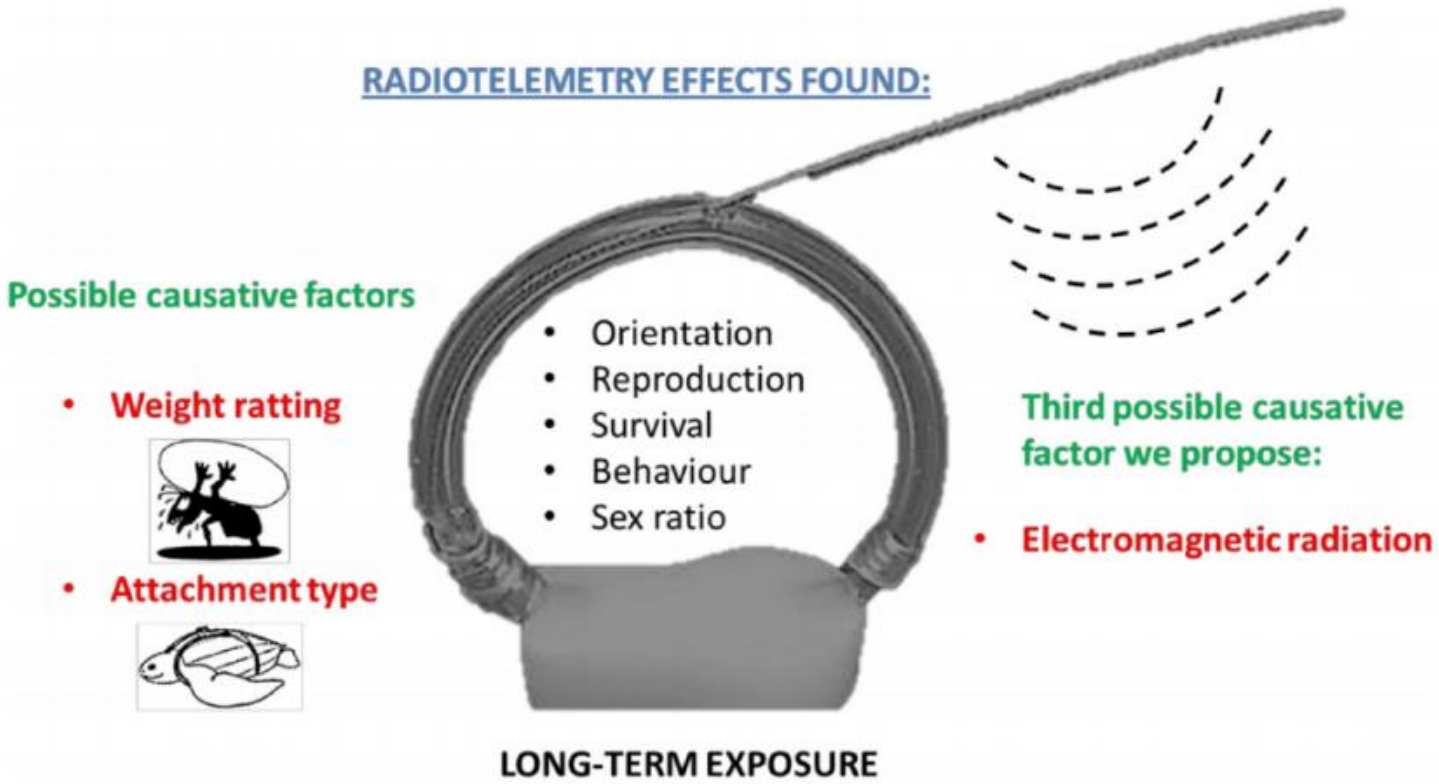


Fig. 1. Radiotelemetry effects found and possible causative factors.

Balmori A. (2016). Radiotelemetry and wildlife: Highlighting a gap in the knowledge on radiofrequency. Sci Total Environ 543: 662-669.

Sampling methods

Radio-tracking – Effects on Mammals

Weight rating

- It is recommended that the total radio transmitter and associated devices should not exceed 2–5% of the body weight
- Can affect:
  - Behaviour
  - Movements
  - Reproduction
  - Survival

Sampling methods

Radio-tracking – Effects on Mammals

Type of attachment

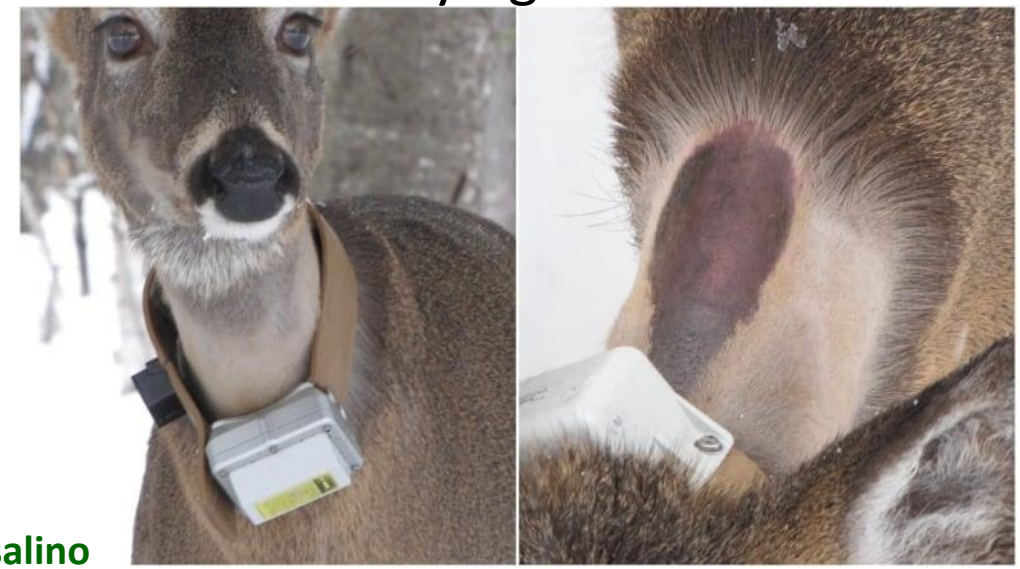
- various types of attachments might have severe effects such as:
  - impaired survival
  - altered behavior
  - lower reproductive rate
- back-mounted or harness-attached transmitters may cause pathological lesions
- Mortality is more common in implants, harnesses, collars; with no mortality (or rare) reported in studies using tail mounts and glue

Sampling methods

Radio-tracking – Effects on Mammals

The importance of considering time

- Studies that found no adverse effects - ran for a few weeks/year
- No studies assessed the cumulative/long-term effects
- Generally, the damage is long-term, and the presence of pathological lesions was significantly associated with the length of time animals had been carrying their radio transmitters



Sampling methods

Radio-tracking – Effects on Mammals

Non-ionising electromagnetic radiation, i.e. radiofrequency radiation, RFR, from transmitters emitting the signals necessary for tracking

- RFR can cause sublethal physiological disruptions
  - Increase in stress proteins synthesis
  - Calcium channels - increased flow calcium into the brain (Physiology impacts)
  - Immune system
  - Nervous system and behavioural effects (e.g. cognitive function, sleep and electrical brain (EEG) response)
  - Genotoxic effects and potential carcinogenicity
  - Fertility, reproduction, offspring viability and sex ratio (e.g. oxidative stress and free-radical might affect fertility and reproduction)
  - Navigational disruption

How to design the sampling strategy

Factors to consider:

- What is the question we want to answer?
- The study spatial scale
- The study temporal scale
- Which is(are) the object(s) of study (physical characteristics and prior knowledge of its biology and ecology)
- What are the characteristics of the environment to be sampled (e.g., homogeneous vs. heterogeneous, terrestrial vs. aquatic)
- Which is(are) the most appropriate study method(s)
- Which is the most appropriate sample design?