Noninvasive Methods for the Identification of Carnivores

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Abstract
The study of food chains and their influence on the environment helps in understanding the different processes involved in functioning of the ecosystem. As far as carnivores are concerned they are shy, charismatic, dangerous, wide ranging, elusive and nocturnal, hence it is almost impossible to precisely evaluate their abundance, distribution, diet and behaviour by direct observations in the field. Capture and handling are highly stressful and potentially dangerous to both humans and animals, especially with large carnivores. The scientists have solved this problem by applying various indirect or noninvasive sampling methods. This idea of collection of samples without actually seeing or touching an animal appeal many as carnivores are dangerous and extremely difficult to capture and handle. Carnivores are animals of great attention and concern because many of its species are declining at a very fast rate and the efforts are being continuously made for their conservation and management.
Keywords: Noninvasive, carnivores, abundance, distribution

Introduction
The Order Carnivora is made up of variety of species that differ in various aspects. Noninvasive techniques have found many applications in wildlife ecology and conservation biology. Noninvasive method is a set of field, laboratory and diagnostic techniques that allow studying the ecological processes without having even to observe individuals. It has been recommended as a reliable method for investigating the biology of mysterious, rare or endangered species. Noninvasive methods open a new way of sampling that was never imagined before 30 years. These techniques are non-destructive and the ease with which the samples are collected, large data can be retrieved. Non-invasive techniques are indirect methods to determine ecology of carnivores. These methods also provide practical as well as ethical benefits by increasing well-being and safety to animals (Pauli et al., 2010). Moreover, handling and capturing of animals particularly carnivores become highly stressful and is unsafe to both animals and humans.

Material and Methods
Non-invasive sampling: Sign investigations
Ecologists have examined carnivores non-invasively for years. Expert identification of scats, tracks, hairs, hooves, kills, bones and scratch marks provide great information regarding the distribution and habitat of carnivores. Identification of coyote (*Canis latrans*) scats shows greater than 90% accuracy despite the presence of other carnivores in the Alaska region (Prugh and Ritland 2005). However in many cases morphological identification of the scats leads to inaccuracy like the scats of martem (*Martes martes*) which show only 18% accuracy (Davison *et al*., 2002). Track studies are very effective as well as economical for the studies like distribution and occurrence. Tracking surveys during winter has been widely used in Canada and Scandinavia to monitor the distribution of

carnivores (Hellstedt *et al*., 2006). The presence of the carnivores can be determined from hair by applying both macroscopic as well as microscopic examination of the hairs (Raphael *et al*., 1994). This can be aided by signs like scrape or scratches and pug marks in the field through sign survey.

Genetic sampling

Fecal DNA

Carnivores are identified by the genomic analysis of fecal matter. Fecal matter contains DNA due to shredding of intestinal lining and this DNA is extracted from the scat samples (Gorman and Trowbridge 1989; Barja *et al*., 2005). Carnivores walk through definite trails and by identifying those tracks, scats are effectively collected even during snow, dust or mud (McKelvey *et al*., 2006; Ulizio *et al*., 2006; Marucco *et al*., 2008). Researchers can increase the rate of scat collection by using scent-detecting dogs (Wasser *et al*., 2004; Long *et al*., 2007). The scats must be stored properly in order to avoid degradation of DNA by enzymes and also the quality of the DNA depends on the region and location of the scat collected.

Hairs

Snags and rub devices are often used to obtain hairs of animals. Hair snag devices are very economical and are easy to fix. Researchers have used barbed wire around bait for collection of the left over hairs of the bears (Woods *et al*., 1999; Kendall *et al*., 2009). Glued tips are used to collect hairs from small carnivores (Zielinski *et al*. 2006). Hair snag devices are very economical and are easy to fix. Higher quality DNA is extracted from the hairs with follicles and usually multiple hairs are pooled together to extract DNA for the carnivore species detection. However researchers must develop a hair snag that allows only one animal to rub it (Beier *et al*., 2005; Bremner-Harrison *et al*., 2006).

Camera-trap method

Camera trapping technique in wildlife appeared in 1877 (Guggisberg 1977) and was used very less until the invention of infrared automatic cameras. Later in the mid- 1990s use of lightweight cameras were used with capture and recapture method to estimate the abundance and distribution of animals (Karanth 1995). The invention of digital camera and its widespread use has resulted in an increase in carnivore inventories as it can facilitate photography of multiple species at a time (Barea-Azcon *et al*., 2007; Datta *et al*., 2008; Tobler *et al*., 2008; Pettorelli *et al*., 2009).

Hormone sampling

Hormones are a class of signaling molecules that regulate behaviour and physiology of an organism needed and helps to survive in the ever-changing environment. Conservation biology helps us to understand the various physiological processes and the responses of animals to the changing environment. To monitor wildlife population, different indirect endocrine tools are used (Berger *et al*., 1999; Foley *et al*., 2001; Garnier *et al*., 2002; Sands and Creel 2004; Cockrem 2005). Scats, hair, urine and saliva samples are immunoassayed and the concentration of the particular or selected hormones and their metabolites are measured (Wasser *et al*., 1998, Creel *et al*., 1997, Barja *et al*., 2008). Adrenal and gonadal hormones are considered most important hormones for non-invasive studies of wild animals. Adrenal hormones includes glucocorticoids which indicates physiology of an organism and gonadal hormones like estrogens, androgens reveal information about puberty, ovulation, estrous, pregnancy and sex (Brown and Wildt 1997; Morato *et al*., 2004; Sanson *et al*., 2005; Graham *et al*., 2006; Dehnhard *et al*., 2008; Herrick *et al*., 2010). Immunoassays and spectrometric techniques are used in conservational biology, however, wildlife endocrinology is not widely used non-invasive method as it is error prone.

References

Barea-Azcon, J.M., Virgos, E., Ballesteros-Duperon, E., Moleon, M., and Chirosa, M. 2007. Surveying carnivores at large spatial scales: a comparison of four broad-applied methods. ***Biodiversity and Conservation.*** **16**: 1213–1230.

Barja, I., Silván, G., Rosellini, S., Piñeiro, A., Illera, M.J. and Illera, J.C. 2008. Quantification of sexual steroid hormones in faeces of Iberian wolf (*Canis lupus signatus*): a non-invasive sex typing method. ***Reprod. Domest. Anim*. 43:** 701–7.

Beier, L.R., Lewis, S.B., Flynn, R.W., Pendleton, G., and Schumacher, T.V. 2005. From the Field: A single-catch snare to collect brown bear hair for genetic mark-recapture studies. ***Wildlife Society Bulletin.*** **33**(2): 766-773.

Berger, J., Testa, J., Roffe, T. and Monfort, S.L. 1999. Conservation Endocrinology: a Noninvasive Tool to Understand Relationships between Carnivore Colonization and Ecological Carrying Capacity. ***Conserv. Biol*. 13:** 980–989.

Bremner-Harrison, S., Harrison, S.W.R., Cypher B.L., Murdoch J.D., Maldonado, and Darden S.K. 2006. Development of a single-sampling noninvasive hair snare. ***Wildlife Society Bulletin*. 34**(2): 456–461.

Brown, J.L., and Wildt, D. E. 1997. Assessing reproductive status in wild felids by noninvasive faecal steroid monitoring. ***International Zoo Yearbook*.** **35**(1): 173-191.

Cockrem, J.F., 2005. Conservation and behavioral neuroendocrinology. ***Horm. Behav*. 48:** 492-501.

Creel, S., Creel, N., Mills, M. and Monfort, S.L., 1997. Rank and reproduction in cooperatively breeding African wild dogs: behavioral and endocrine correlates. ***Behav. Ecol*. 8:** 298–306.

Datta, S., Costantino, N., and Zhou, X. 2008. Identification and analysis of recombineering functions from Gram-negative and Gram-positive bacteria and their phages. ***Proceedings of the National Academy of Sciences.*** **105**(5): 1626-1631.

Davison, A., Birks, J.D., Brookes, R.C., Braithwaite, T.C., and Messenger, J.E. 2002. On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. ***Journal of Zoology*. 257**(2): 141-143.

Dehnhard, M., Naidenko, S., Frank, A., Braun, B., Göritz, F. and Jewgenow, K., 2008. Non-invasive monitoring of hormones: a tool to improve reproduction in captive breeding of the Eurasian lynx. ***Reprod. Domest. Anim*. 43:** 74–82.

Foley, C.H., Papageorge, S., Wasser, S.K., 2001. Noninvasive Stress and Reproductive Measures of Social and Ecological Pressures in Free-Ranging African Elephants. ***Conserv.Biol*. 15**: 1134–1142.

Garnier, J.N., Holt, W.V, Watson, P.F. 2002. Non-invasive assessment of oestrous cycles andevaluation of reproductive seasonality in the female wild black rhinoceros (*Diceros bicornis minor*). ***Reproduction*** **123:** 877–889.

Gorman, M. L., and Towbridge, B. J. 1989. The role of odor in the social lives of carnivores. In: **Carnivore Behaviour, Ecology, and Evolution**. (Ed. J. L. Gittleman, Cornell University Press: New York) **pp. 57–86.**

Graham, L.H., Byers, A.P., Armstrong, D.L., Loskutoff, N.M., Swanson, W.F., Wildt, D.E., Brown, J.L. 2006. Natural and gonadotropin-induced ovarian activity in tigers (*Panthera tigris*) assessed by fecal steroid analyses. ***Gen. Comp. Endocrinol*. 147:** 362–70.

Guggisberg, C.A.W. 1977. *Early wildlife photographers*. Taplinger Publishing Company.

Hellstedt, P., Sundell, J., Helle, P., and Henttonen, H. 2006. Large-scale spatial and temporal patterns in population dynamics of the stoat, *Mustela erminea,* and the least weasel, *M. nivalis*, in Finland. ***Oikos.*** **115**(2): 286-298.

Herrick, J.R., Bond, J.B., Campbell, M., Levens, G., Moore, T., Benson, K., D'Agostino, J., West, G., Okeson, D.M., Coke, R., Portacio, S.C., Leiske, K., Kreider, C., Polumbo, P.J. and Swanson, W.F. 2010. Fecal endocrine profiles and ejaculate traits in black-footed cats (*Felis nigripes*) and sand cats (*Felis margarita*). ***Gen. Comp. Endocrinol*. 165:** 204–214.

Karanth, K.U. and Sunquist, M.E. 1995. Prey selection by tiger, leopard and dhole in tropical forests. ***Journal of Animal Ecology*. 64**: 439-450.

Kendall, K.C., Stetz, J.B., Boulanger, J.A., Macleod, C., Paetkau, D. and White, G.C. 2009. Demography and genetic structure of a recovering grizzly bear population. ***Journal of Wildlife Management.* 73**:3–17.

Long, R.A., Donovan, T.M., MacKay, P., Zeilinski, W.J. and Buzas, J.S. 2007. Comparing scat detection dogs, cameras, and hair snares for surveying carnivores. ***Journal of Wildlife Management.* 71** (6): 2018-2025.

Marucco, F., Pletscher, D. H., and Boitani, L. 2008. Accuracy of scat sampling for carnivore diet analysis: wolves in the Alps as a case study. ***Journal of Mammalogy*. 89 (**3): 665-673.

McKelvey, K. S., von Kienast, J., Aubry, K. B., Koehler, G. M., Maletzke, B., Squires, J., Lindquist, E., Loch, S., and Schwartz, M. K. 2006. DNA analysis of hair and scat collected alolng snow tracks to document presence of Canada lynx. ***Wildlife Society Bulletin*. 34**:451–455.

Morato, R.G., Bueno, M.G., Malmheister, P., Verreschi, I.T.N., Barnabe, R.C. 2004. Changes in the fecal concentrations of cortisol and androgen metabolites in captive male jaguars (Panthera onca) in response to stress. ***Braz. J. Med. Biol. Res.* 37:** 1903–7.

Pauli, J.N., Whiteman, J.P., Riley, M.D., and Middleton, A.D. 2010. Defining noninvasive approaches for sampling of vertebrates. ***Conservation Biology*. 24** (1): 349-352.

Pellikka, J., Rita, H. and Lindon, H. 2005. Monitoring wildlife richness- Finnish applications based on wildlife triangle censuses. **Annales Zoologici Fennici 42**: 123-1

Pettorelli, N., Bro-Jørgensen, J., Durant, S. M., Blackburn, T., and Carbone, C. 2009. Energy availability and density estimates in African ungulates. ***The American Naturalist*. 173** (5): 698-704.

Prugh, L.R., and Ritland, C.E. 2005. Molecular testing of observer identification of carnivore feces in the field. ***Wildlife Society Bulletin*. 33** (1): 189-194.

Raphael, Y., Athey, B.D., Yu, W., Lee, M.K., and Altschuler, R.A. 1994. F-actin, tubulin and spectrin in the organ of Corti: comparative distribution in different cell types and mammalian species**. *Hearing Research*. 76** (1-2): 173-187.

Sands, J., and Creel, S. 2004. Social dominance, aggression and faecal glucocorticoid levels in a wild population of wolves, Canis lupus. ***Animal Behaviour*. 67**(3): 387-396.

Sanson, G., Brown, J.L., Farstad, W. 2005. Non-invasive faecal steroid monitoring of ovarian and adrenal activity in farmed blue fox (*Alopex lagopus*) females during late pregnancy, parturition and lactation onset. ***Anim. Reprod*. Sci. 87:** 309–19.

Tobler, M.W., Carrillo-Percastegui, S.E., Pitman, R.L., Mares, R., and Powell, G. 2008. An evaluation of camera traps for inventorying large-and medium-sized terrestrial rainforest mammals. ***Animal Conservation.* 11** (3): 169-178.

Ulizio, T.J., Squires, J.R., Pletscher, D.H., Schwartz, M.K., Claar, J.J., and Ruggiero, L.F. 2006. The efficacy of obtaining genetic-based identifications from putative wolverine snow tracks. ***Wildlife Society Bulletin*. 34** (5): 1326-1332.

Wasser, S.K., Davenport, B., Ramage, E.R., Hunt, K.E., Parker, M., Clarke, C. and Stenhouse, G. 2004. Scat detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. ***Canadian Journal of Zoology.* 82**: 475-492.

Wasser, S.K., Norton, G., Rhine, R. and Kleindorfer. S. 1998. Aging and social rank effects on the reproductive system of free-ranging yellow baboons (*Papio cynocephalus*) at Mikumi National Park, Tanzania. ***Hum Reprod J*. 55**:501–516

Wasser, S.K., Risler, L., Steiner, R. 1988. Excreted steroids in primate feces over the menstrual cycle and pregnancy. ***Biol. Reprod*. 39:** 862–872.

Woods, J.G., Paetkau, D., Lewis, D., McLellan, B.N., Proctor, M., and Strobeck, C. 1999. Genetic tagging of free-ranging black and brown bears. ***Wildlife Society Bulletin*. 27**(3): 616-627.

Zielinski, W.J., Schlexer, F.V., Pilgrim, K.L. and Schwartz, M.K. 2006. The efficacy of wire and glue hair snares in identifying mesocarnivores. ***Wildlife Society Bulletin*. 34** (4): 1152-1161.