IDENTIFYING INDIVIDUAL EURASIAN OTTERS (*LUTRA LUTRA*) BASED ON MEASUREMENTS OF THEIR FOOTPRINTS – STANDARDIZATION OF THE METHOD AND ITS POTENTIAL FOR CENSUSING AND MONITORING WILD OTTER POPULATIONS

by

JITKA VĚTROVCOVÁ

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ABSTRACT

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Jitka Větrovcová, M.S.

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Supervising Professor: Daniel R. Formanowicz, Jr.

A method for identifying individual Eurasian otters (*Lutra lutra* L.) from measurements of their footprints was first tested by performing experiments with captive animals. Digital photographs were taken of imprints in mud of all paws of 12 known individuals. Using customized software, 131 distance, angle, and proportion measurements were generated and subjected to discriminant and canonical analyses. The stepwise procedures of discrimination were the principal statistical analyses used. Analyzing each foot separately while using all available imprints yielded 86-93%

correct classification and 59-77% Jacknife cross-validation. The efficiency of these analyses was not significantly affected by randomly reducing the number of imprints per otter. However, better results were achieved when only four best-quality imprints per animal were included in analyses (96-100% correct classification, 70-87% correct Jacknife cross-validation). The method was further improved by combining measurements of both left, both right, or all four feet together in statistical analyses, and was standardized by employing only the best discriminating parameters, identified as such in the previous "preliminary" analyses. The combined analyses all yielded 100% correct classification and 79-93% accuracy of Jacknife cross-validation.

In addition, a short field study was done in order to assess the method's potential for practical application. Four out of seven documented tracks could be analyzed statistically. Both analyses of each foot separately and of left front and left hind feet combined indicated with high certainty that the 4 tracks were made by 4 different otters (100% correct classification and 93-100% efficiency by Jacknife cross-validation). These promising results suggest that the proposed method might prove useful in otter population monitoring and censusing in the future, providing the advantage of being non-invasive and relatively inexpensive.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Ecology of the Eurasian otter

Otters are carnivores of the family Mustelidae, which also includes weasels, martens, mink, or badgers. Most members of this family have elongated bodies with short legs and a flattened head in common. Otters are amphibious and their life is associated with the presence of freshwater or marine biotopes: streams, rivers, lakes, ponds, swamps, marshes, or the coastline. Their adaptations to an aquatic environment include small ears (leveled with eyes and nose for swimming), long tail with thick base, webbed feet, waterproof fur, and whiskers that help them hunt in murky waters (Mason and Macdonald 1986).

Eurasian otters (*Lutra lutra* L.) exhibit sexual dimorphism with males being bigger and heavier. However, the dimorphism is not as pronounced as in other mustelid species (Bateman 1984, Mason and Macdonald 1986). Males usually reach a mass of approximately 9 kg, females approximately 6 kg; the overall length range is 1020 – 1370 mm (Kučerová and Roche 1999). Otters are active hunters and capture most of their prey in water. Up to 95% of their diet is composed of fish, but they have been reported to consume amphibians, birds, small mammals, mollusks, crustaceans, reptiles, or insects as well. The percentage of prey types in the otter diet varies and depends mostly on availability (Chanin 1985). Otters have a very rapid metabolism, therefore an adult individual eats approximately 0.9 to 1.5 kg of food every day, which equals about 12% of its body weight (Erlinge 1968, Mason and Macdonald 1986). Eurasian otters are nocturnal and typically show two peaks of activity, around dusk and dawn. They may travel quite far overnight, even across land. Distances of up to 9 km have been reported (Mason and Macdonald 1986).

Eurasian otters are solitary and form pairs only during the breeding season, which may be almost any time of the year, depending on environmental conditions (Bateman 1984). Gestation takes 61-65 days and there are typically 2-3 cubs in a litter. Males do not take part in raising the cubs. The female stays with her young until they are approximately 13 months old and sexual maturity is reached at 2-3 years (Chanin 1985, Mason and Macdonald 1986). The average life span of a wild river otter is only 3-4 years (Kruuk 1995), but in captivity, they can live for up to 15 and sometimes even 20 years (Chanin 1985).

Each otter has a home range, defined by Mason and Macdonald (1986) as "an area that the animal learns thoroughly and habitually patrols." The size of home ranges can vary depending primarily on quality of biotope and prey availability. For example, radiotracking of otters from the south of the Czech Republic found home ranges of 2.6-27.3 km^2 (Poledník 2005). Home ranges are typically marked with the otters' faeces or anal gland excretions (= sprainting). These marks are often left at very conspicuous places, such as on stones, rocks or tree trunks, or in other predictable places (e.g. under bridges, at junctions of rivers, in basins; Ruiz-Olmo et al. 2001). The scent marks are believed to communicate information on the individual's dominance, sex, breeding status, and perhaps relatedness, as well as to delineate the territorial boundaries (Bateman 1984, Chanin 1985, Mason and Macdonald 1986).

Although very difficult to spot, otters are highly vocal and use a large repertoire of calls, which may differ among the individual otter species (Bateman 1984). As can be concluded from the use of scent marks, otters have a good sense of smell. Their hearing is also very good, but for hunting, the most important senses are touch and sight (Chanin 1985).

Distribution and status of Eurasian otters worldwide, in Europe and in the Czech Republic

There are 13 species of otters classified in 6 genera. All of them are listed by CITES (Convention on International Trade in Endangered Species) and 4 species are threatened. The Eurasian otter (*L. lutra*) is the most widespread one of all 13 species. Its original range extended from Ireland to Japan and from the Arctic Circle to North Africa and Sri Lanka (Kučerová and Roche 1999; Fig. 1).

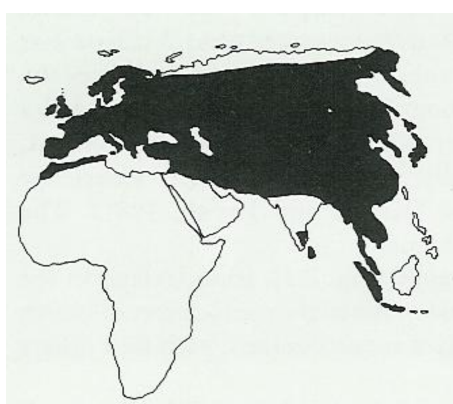

Figure 1. The original worldwide range of *L. lutra* at the beginning of 19th century (from Macdonald and Mason 1986).

Otter populations in Europe started decreasing in the second half of the 19th century, and the trend accelerated in the first half of the 20th century due to habitat destruction, persecution, pollution, and increasing numbers of road kills (Mason and Macdonald 1986). In many European countries, otters are considered critically threatened species today and occur only in isolated areas in relatively small populations (Foster-Turley et al. 1990, Kučerová and Roche 1999). Fig. 2 shows the approximate current distribution in Europe.

Figure 2. Distribution of *L. lutra* in Europe. Dark grey – areas with permanent occurrence, light grey – areas with rare occurrence. The territory of the Czech Republic also outlined (from the Czech Otter Foundation Fund website).

Otter populations in the Czech Republic went through a similar history but seem to be recovering in the last 15 years (Zemanová 2006). In the 90's, three discrete populations were reported in the Czech Republic, covering 25-30% of the country's area (Kučerová and Roche 1999). These populations persist and seem to be expanding (Fig. 3). Four population estimates were done for the entire Czech Republic in the last

30 years. Baruš and Zejda (1981) estimated 174 individual river otters based on data from questionaries for the years 1976-78. In 1989-92, 300-400 individuals were estimated based on presence or absence of otter signs in 11x12 km quadrants (Toman 1992). Using the same method, 800 individuals were estimated for 1997-2003 (Kučerová et al. 2001). Recently, Poledník (2005) has estimated as many as 1600-2200 adult individuals based on a mathematical model relating otter numbers to certain landscape factors, namely the length of ponds' banks.

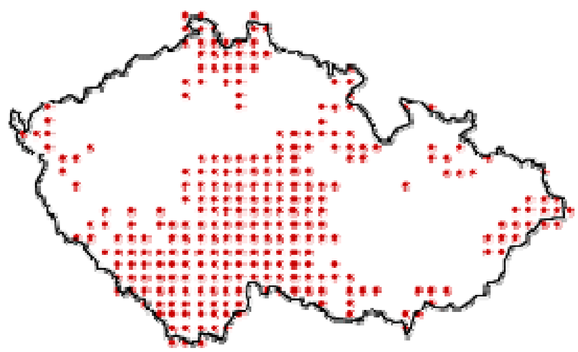

Figure 3. Distribution of Eurasian otters in the Czech republic in 2001. Dots represent positive quadrates (= otter occurrence) (from the Czech Otter Foundation Fund website).

Eurasian otter has been protected by the law in the Czech Republic since 1956. Currently, it is classified as a strongly threatened species and is protected in all their developmental stages, along with their biotopes, by the "Law of protection of the environment." Internationally, the Czech Republic has enforced CITES since 1993, where *L. lutra* is listed in appendix II. Czech Republic also signed the Bern convention, under which Eurasian otter is classified as a strictly protected species (Appendix II). The recent increase of otter populations apparent from the above estimates now brings more conflicts between conservationists and fishermen, especially in the fishpond area in the south of the country, where otter densities are highest and sometimes mean extensive economic losses for the fishermen (Poledník 2005).

Survey and censusing methods

The very first methods of survey of Eurasian otter populations involved questionnaires that were circulated to hunters, fishermen, game wardens or naturalists, or analyses of hunting returns (Mason and Macdonald 1986). For obvious reasons, these methods are not very reliable and could only point to population trends.

The first standardized methods of surveying distribution were developed at the end of the 1970's and beginning of the 1980's (Mason and Macdonald 1986, Ruiz-Olmo et al. 2001). Such surveys were based on the identification of indirect otter signs (tracks and spraints) in selected sites that were part of a map grid. In this way, positive or negative sites were identified and corresponding distribution maps were developed (Ruiz-Olmo et al. 2001). The method was discussed and updated in 1999 at a workshop named "How to better standardize the 'standard' (IUCN/SSC Otter Specialist Group) method for otter surveys?" during the 3rd European Congress of Mammalogy in Jyväskylä, Finland. Details about this standardized survey method are described in Reuther et al. (2000). The authors of this report conclude that even though the method cannot show complete distribution of otters over the entire chosen area and does not demonstrate otter numbers, it does allow comparisons between different areas or comparisons of distribution in the same area over time, and it is probably the most appropriate way for studying otter distribution over large areas.

Estimating otter numbers and establishing their population density is even more problematic. There are certain difficulties associated with any one technique used to achieve these goals (Reuther et al. 2000, Ruiz-Olmo et al. 2001, Poledník 2005). The most common methods are:

1. visual census (Kruuk 1995, Ruiz-Olmo et al. 2001)
2. main holt (=otter den) use or holt census (Kruuk et al. 1989)
3. spraint counting (Macdonald and Mason 1987, Kruuk 1992)
4. snow tracking (Erlinge 1967, Reid et al. 1987, Roche and Roche 2004)
5. radioisotope marking (Mitchell-Jones et al. 1984)
6. DNA typing from spraints (Dallas and Piertney 1998, Effenberger et al. 1999, Dallas et al. 2000, Jansman et al. 2001, Zemanová 2006)
7. measuring footprints (Hertweck et al. 2002)

The main problem with visual census is that otters are nocturnal and very secretive, and are therefore difficult to spot. However, Ruiz-Olmo et al. (2001) reported that both visual and footprint censuses compared well with data obtained by radiotracking in the same area, and therefore concluded that these methods can provide population numbers close to the real number of otters. Also on Shetlands, where otters are active during daylight, visual census was successfully used (Kruuk 1995). Otter holts (= their dens) are very difficult to find (Hobza 2005) and their use depends on many different factors. Spraint counting is problematic because sprainting seems to be seasonal (Macdonald and Mason 1987), the role of spraints in communication is not properly known (Erlinge 1968, Kruuk 1992), and the persistence of spraints depends strongly on weather and site of deposition (Kranz 1996). Radioisotope marking can provide very good data, but is very expensive, requires capturing the animals, and is usually only used for smaller focal areas (Ruiz-Olmo et al. 2001, Poledník 2005). The relatively new genetic studies seem to be promising noninvasive methods, but are also highly costly, time-consuming, and still deal with certain issues regarding the amounts and quality of DNA obtained from otter spraints (Zemanová 2006). In general, standardized snow tracking is considered to be the most cost effective method for estimating otter numbers (Poledník 2005) and is used both in local areas (e.g. in the Czech Republic; Poledník 2005) and in larger areas in Central Europe (Kranz and Knollseisen 1998). The main disadvantage of this method is clearly the dependency on weather conditions (few suitable days in northern countries, no snow in southern countries).

Individual identification by footprint measurements

The above problems with population censuses are true not only for the Eurasian otter, but also for many other species of mammals, especially larger carnivores that are cryptic or nocturnal, often solitary, and occupy large geographic areas (Grigione et al. 1999). It is difficult to observe these species in the field, to obtain reliable demographic data, to make population size estimates, and determine viability of their populations.

Using measurements of animal tracks on digitized photographs therefore seems to be a possible non-invasive and cost-effective method for monitoring carnivore populations, potentially providing clues about sex, age, approximate size, behavior, or foraging strategy (Miller 2001). For this method to be useful in population estimates, individuals of the population have to be recognizable by their tracks. This technique, along with the use of discriminant analysis to distinguish individuals, has been tested with promising results on a number of different species, including pumas, tigers, snow leopards, jaguars, black rhino, mountain tapir, pine marten (Gore et al. 1993, Riordan 1998, Grigione et al. 1999, Zalewski 1999, Miller 2001, Jewell et al. 2001, Hertweck et al. 2002).

Recently, analysis of otter tracks as a potential non-invasive method of estimating population size and density was done in Upper Lusatia, Germany (Hertweck et al. 2002). Tracks were photographed on frozen fish ponds with light snow cover, measured using computer software, and analyzed with discriminant analysis. For 13 tracks (track is considered to be a set of imprints clearly made by the same individual), using only data from right front paws, results showed that 83.3% of the cases (imprints) could be correctly classified (Hertweck et al. 2002). However, the authors note that to further improve the method, the number of parameters needed for successful classification should be reduced, and more studies need to be conducted, preferably on different substrates (soil, mud, sand) to explore other options. The above is essentially the purpose of this study. I tried to verify the methods used in Hertweck et al. (2002), using the same software for footprint measurements, but conducting the experiments with known captive animals, and on a different substrate, river mud. This was meant to ensure enough good quality imprints for each animal, and also provided the advantage of knowing the identity of the imprints. Imprints of all 4 paws were photographed for comparison and possible combinations in analyses. In addition, a short-term field study was done in the south of the Czech Republic, in which several tracks of unknown wild otters were obtained, and based on the track measurements and the techniques standardized in the captive experiments the number of otters responsible for these tracks was estimated.

In summary, the main goals of this study were:

1) to verify the method of individual otter identification by footprint measurements on captive animals
2) to improve the percentage of correct classification by combining measurements for different feet in statistical analysis, and by reducing the number of parameters needed for the identification
3) to identify a set of best discriminating parameters for analysis and to overall standardize the method as much as possible for future needs
4) to explore the method's potential for application in the field as a tool to census and monitor wild otter populations.

CHAPTER 2

METHODS

Experiments with captive otters

Data collection

All data were collected during the summer of 2005 and summer of 2006 at three different facilities in the Czech Republic that house captive Eurasian otters. These facilities were: Otter Station in Pavlov near Ledeč nad Sázavou, Zoo Ohrada and Zoo Jihlava. Table 1 contains more information on all individual otters involved in the experiments.

Table 1. Details on all the captive otters involved in the experiments.

otter	sex	facility	age at data collection (years)	time of data collection
Beskydka (B)	F	Otter Station Pavlov	7	summer 2005
Čibák (C)	M	Otter Station Pavlov	14	summer 2005
Fousek (F)	M	ZOO Jihlava	1	summer 2006
Gesa (G)	F	Otter Station Pavlov	13	summer 2005
Lucka (L)	F	Otter Station Pavlov	1	summer 2006
Matýsek (M)	M	Otter Station Pavlov	5	summer 2005
Neznámý (N)	M	Otter Station Pavlov	Not known*	summer 2005
Polka (P)	F	Otter Station Pavlov	2	summer 2006
Styx (S)	M	Otter Station Pavlov	9	summer 2005
Sylvestr (Y)	M	ZOO Ohrada	6	summer 2006
Vydrýsek (V)	M	Otter Station Pavlov	4	summer 2005
Žanetka (Z)	F	ZOO Ohrada	4	summer 2006

* this male was brought to the Otter Station as an adult in spring 2005 and was released in the Fall of that year

First, preliminary tests were run to assess the quality of imprints made on different substrates. Sand of different coarseness and moisture, two types of soil, and mud from creek/stream banks were tested. The mud clearly provided the best quality tracks (= with clear edges and all five toes visible), and was therefore chosen to be used. The local "stream mud" available in the areas around the three facilities was of similar structure and consistency, so that results from these different locations should be comparable.

For the purpose of my study, each otter was placed in a separate enclosure. Suitable flat areas approximately 1m x 2m large were chosen inside each otter enclosure (Fig. 4). These areas were cleared of vegetation and covered with a layer of mud brought from a nearby source, each layer was about 3 cm thick. This was done every evening (5 – 8 p.m.), while most otters were still asleep, and to ensure the substrate would not dry out in the sun. Occasionally, a food item or a spraint of another animal was placed on or near the mud layer, to attract otters to the experimental area. Experimental areas were checked the next morning (at 7:00 h) and imprints were photographed, making sure to note which otter the prints belonged to. A digital camera (Canon Power Shot A 520) fixed at a standard focal distance on a "tetrapod" (Fig. 5) was used. The tetrapod was equipped with a centimeter scale at the bottom to calibrate the photograph. The scale extended on the left and bottom of the view of the camera. The tetrapod was also helpful to ensure photographs were taken perpendicular to the surface (to the imprint). A camera flash was not used, as it is not recommended for this purpose (Miller 2001). Occasionally, pictures were taken in the evening, as the otters were already active while I was preparing the experimental areas, and made a couple of fresh imprints. This should not have a negative impact on results, since both morning and evening (with sun casting angled light over the footprints) were reported by Miller (2001) as the best times of the day to take photographs. Weather was sometimes an obstacle, as rainfall at times washed the tracks away or severely affected their quality. In such cases, no photographs were taken and the experimental areas were prepared for the next day. This evening preparation typically consisted of mixing all the dried mud with enough water to achieve proper consistency, spreading it over the experimental area again, and adding a very thin layer of "fresh mud" taken from the creek/other source. A few animals were very well tamed and habituated to the presence of humans, which allowed me to take many imprint photographs in just one session with these animals.

Figure 4. Example of a prepared experimental area in one of the otter enclosures.

Figure 5. Photograph of the "tetrapod" fitted with a centimeter scale that was used in this study. Camera was attached close to the top of the tetrapod, at a fixed focal distance. The camera display was visible through a square hole in the top part of the tetrapod.

Data analysis

All photographs of imprints were loaded onto a computer and evaluated as to what foot they belonged to (left/right, front/rear, Fig. 6). As previously stated, the identity of imprints of individual animals was known and was indicated on each photograph. Several photographs were eliminated because the distinction between left/right or front/rear paw was not clear enough. After that, eight good quality photographs of each foot for each otter (where available) were selected for further analyses. Each selected photograph was adjusted using PT Lens software, which corrects lens distortion, and was cropped in Photoshop to provide a larger image. A software program designed by Mönkemeyer specifically for measuring otter imprints was used. After selecting seven particular points (Fig. 7) on each imprint and calibrating the measurements by selecting 5 cm on the scale in the photograph, the program measures 131 parameters (distances, angles and proportions). Each photograph was measured 9 times to correct for measurement error, and the means of these values were used in analysis, resulting in 8 mean measurements for each animal and foot (in most cases but not all).

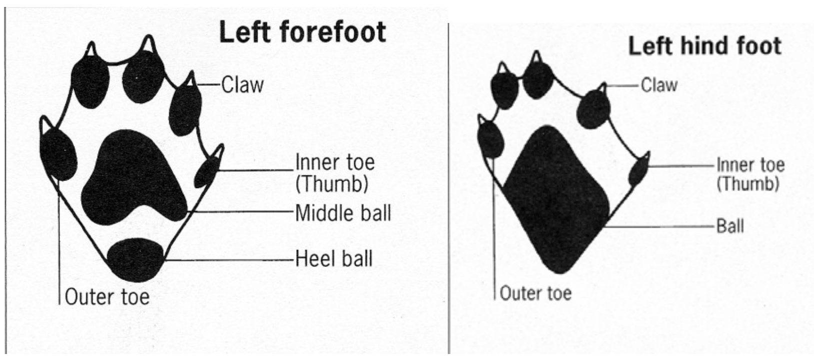

Figure 6. Left front and hind otter footprints. Note the presence of middle ball on the front foot and its absence on hind foot. Since the heel ball is rarely seen on a front foot imprint, the bottom of the middle ball was taken as point FB on all front feet, whereas the bottom of the heel ball was taken as point FB on all hind feet (refer to Fig. 7 to locate the points for measurements). From Reuther et al. 2000.

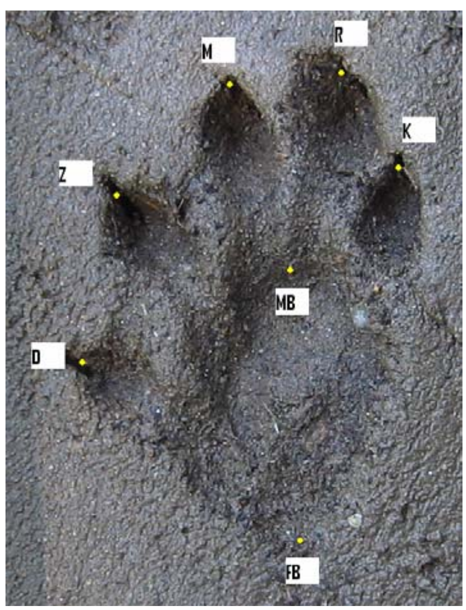

Figure 7. Photograph of a right hind otter imprint, showing the 7 points used to generate 131 different measurements (distances, angles and proportions). Points D, Z, M, R, and K were placed in tops of the toes, excluding the claws, if visible. Measurements will be indicated later in this paper by listing the corresponding 2 or 3 points that make the appropriate line, angle or proportion. D = “thumb”, K = “small finger”. From Mönkemeyer.

Statistical methods

Analyses were done using SYSTAT, SAS, and PC ORD 4 software. The principal statistical method used to evaluate the data was discriminant analysis (DA). It is a multivariate statistical technique with two main objectives (as described by McGarigal et al. 2000): 1) to explain the differences among groups (= descriptive DA, using linear, canonical functions), and 2) to predict group membership for an entity of unknown origin based on its measured values on the discriminating variables (= predictive DA, using classification functions). In this study, the descriptive DA approach was taken to assign individual otter imprints to pre-determined groups (individual otters) on the basis of their predicted identities, given several measurements for each group. Since the identity of all imprints was known in the captive study, it was possible to test the efficiency of the DA method by subjecting it to Jacknife cross-validation. This procedure can be run simultaneously with the DA analysis in SYSTAT and consists of the following: each imprint is in turn excluded from the pre-assigned means and subsequently input as an “unknown” to test accuracy by seeing which otter it is predicted to belong to (Jewell et al. 2001). This removes the problem of using the same observations to define the discriminant rule and to judge its accuracy, present in other resubstitution methods (Mardia et al. 1979). In addition, canonical centroid plots with values plotted on different combinations of the best canonical variables formed from the test space and with ellipses representing 95% confidence intervals were generated. This method provides a visual representation of the results and is useful because it allows classification of unknown imprints which may not belong to a pre-assigned group (Jewell et al. 2001). This is not possible with the DA technique described above.

Different statistical approaches were employed and their results compared. First, data for each foot were analyzed separately, using all 8 selected photographs per animal and foot (where available).

Next, the number of photographs/imprints per animal and foot was randomly reduced one at a time, until only 4 photographs per animal and foot remained, and 4 different analyses (numbered 1, 2, 7, and 8 in Table 2) were run on the 20 resulting data sets, along with the associated Jacknife cross-validation procedures. The main purpose of this was to see whether the accuracy of discrimination was negatively affected by subsequently smaller sample sizes (number of photographs, or number of imprints per track/animal).

It was also of interest to see whether the quality of the individual imprints influences the resulting accuracy of discrimination. Therefore, the 4 best quality photographs/imprints per animal and foot were selected and subjected to the same statistical analyses. The quality of photographs was evaluated based on several criteria, including clear visibility of all 5 toes, as well as the middle or heel ball (as these guide the placement of the 7 landmark points on which all measurements are based), and sharp edges, not distorted by either the animal sliding, or the substrate being too soft. Figures 8 and 9 illustrate some examples of good versus bad quality photographs of imprints.

a) 

b) 

Figure 8. Examples of a good (**a**) and worse (**b**) quality left front paw imprints. One can see edges are much sharper in the photograph on left, and it is also quite clear it is a left paw imprint, whereas in the photograph on right, this distinction is harder to make.

a) 

b) 

Figure 9. Examples of good (**a**) and worse (**b**) quality photographs of right hind paw imprints. Again, edges are much sharper in the first picture and fifth toe is clearly visible (fifth toe not showing is unfortunately a common imperfection in hind otter imprints in general).

Since the anatomy of front and hind feet differs quite a bit in Eurasian otters, it was thought that combining measurements of either both left or both right feet in the DA would add more variation among the individual animals, and could therefore discriminate among them better. This was tested using the data sets with 4 best quality photographs per animal and foot, employing 10 measurements indicated as best discriminating variables for the front foot, and 10 measurements indicated as best discriminating variables for the hind foot, by the respective DA's done earlier on each foot separately and using all available photographs. To test this idea even further, all 4 feet were combined in one analysis, using 5 measurements for each foot that were indicated as the best discriminating variables in the earlier respective analyses.

In discriminant analysis, the total number of cases has to exceed the number of variables by more than two (Klecka 1980). The data sets used here included 40-92 cases and 131 variables (with the exception of the combined analyses, where only 20 variables were employed). Therefore, the number of variables in the first analyses had to be reduced to meet the above condition. This was achieved in three different ways. First, 50 (or 30 in the case of smaller data sets with only 4 photographs per animal) variables with the highest F-ratios were selected, as suggested by Jewell et al. (2001). This selection can be achieved by using a stepwise procedure of DA and examining the F-to-enter and F-to-remove values for each variable. Stepwise DA is a procedure that should produce an optimal set of discriminating variables – even though the superiority of the end product to all others is not guaranteed (Klecka 1980). In this study, both forward (selecting the best discriminating variables one at a time) and backward (removing the least discriminating variables one at a time from the original set) analyses were performed. Second, principal component analysis (PCA) was run on the original variables, and the ones best correlated with each of the identified axes of variance were selected for subsequent DA. Third, 20 variables were selected visually (or geometrically), based on my subjective judgment of what combination of available parameters was likely to yield the best overall description of the footprint without the parameters correlating with one another (Fig. 10).

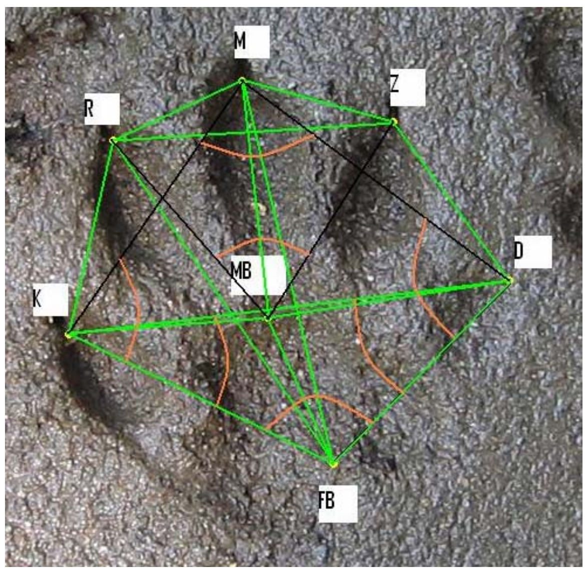

Figure 10. Twenty visually chosen variables used in some of the analyses. Distances are in green, angles in orange.

Overall, there were 9 statistical approaches used in the analyses of each foot separately. These are listed in Table 2. The combined data sets were only analyzed using the backward and forward stepwise DA.

The assumptions of DA (equality of variance-covariance matrices, multivariate normality, no singularities and outliers, identifiable prior probabilities) were not completely satisfied in all data sets. An attempt to remove the problems by log transformation was not successful. At the end, the few violations in the data were ignored, mostly because of the following:

1) many extremely large data sets

2) lack of information on how to deal with such data sets, especially when violations occur (e.g. assumptions of DA and their violations are not even mentioned in many articles employing DA)

3) DA is generally believed to be “robust” to violations of assumptions (Klecka 1980)

4) results of the slightly different statistical approaches taken were quite consistent

5) most importantly – the nature of the study (specifically the captive part) was mainly exploratory, with focus on discovering useful discriminating variables and comparing the different approaches taken (the difference between confirmatory and exploratory analyses is explained, and the use of DA on even “messy” data is encouraged by Williams 1983).

Table 2. Summary of the 9 statistical procedures used in this study. (DA = discriminant analysis)

1	stepwise backward DA on all 131 variables
2	stepwise forward DA on all 131 variables
3	stepwise backward DA on 50 variables with highest F-ratios
4	stepwise forward DA on 50 variables with highest F-ratios
5	complete DA on 50 variables with highest F-ratios
6	complete DA on 6 variables that best correlate with the first 6 eigenvectors from PCA
7	stepwise backward DA on 20 visually chosen variables
8	stepwise forward DA on 20 visually chosen variables
9	complete DA on 20 visually chosen variables

Experiments with wild otters

Study area and study sites

The field experiments were done in the southern part of the Czech Republic referred to as Dačicko (area with the main town of Dačice), close to the Austrian border (Fig. 11). The area is characterized by being highlands (450–650 m) with a moderate continental climate, and relatively low density of human settlements. The countryside is a balanced mix of forests and agricultural lands and includes many small streams and rivers, as well as ponds. Otters have been repeatedly reported as permanent inhabitants in this area since the national otter survey by Toman in 1992 (Poledník 2005).

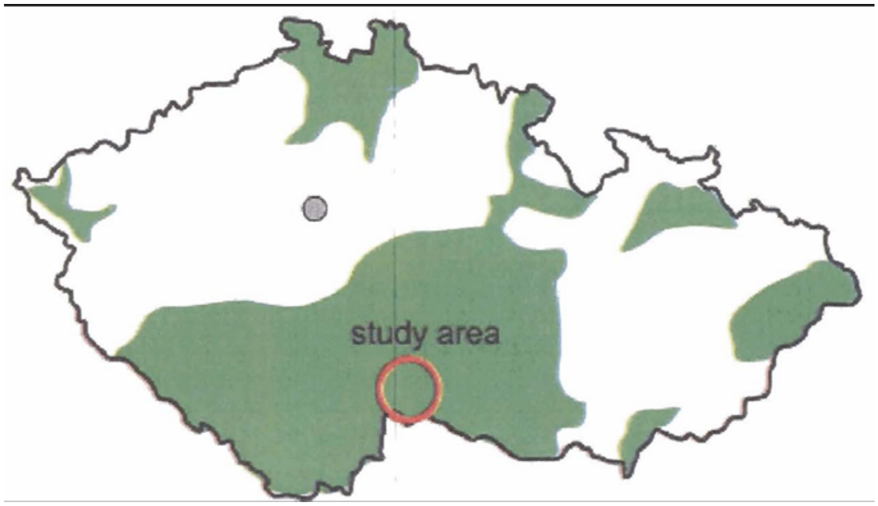

Figure 11. Location of the study area within the territory of the Czech Republic. Areas of otter distribution also shown. (from Poledník 2005)

Four different streams/rivers were selected for monitoring: Volfířovský stream, Bolíkovský stream, Pstruhovec stream, and Moravská Dyje river. Two bridges (on Volfířovský stream and Bolíkovský stream), and two suitable flat banks with alluviums (on Pstruhovec stream and on Moravská Dyje river) were selected as the sites to establish experimental mud areas (Fig. 12). The bank sites were selected based on the presence of a few otter imprints observed in natural substrate before installation of the experimental areas. Mud was usually found in the near proximity of each site or was brought from Bolíkovský stream, where a large quantity was available under the bridge.

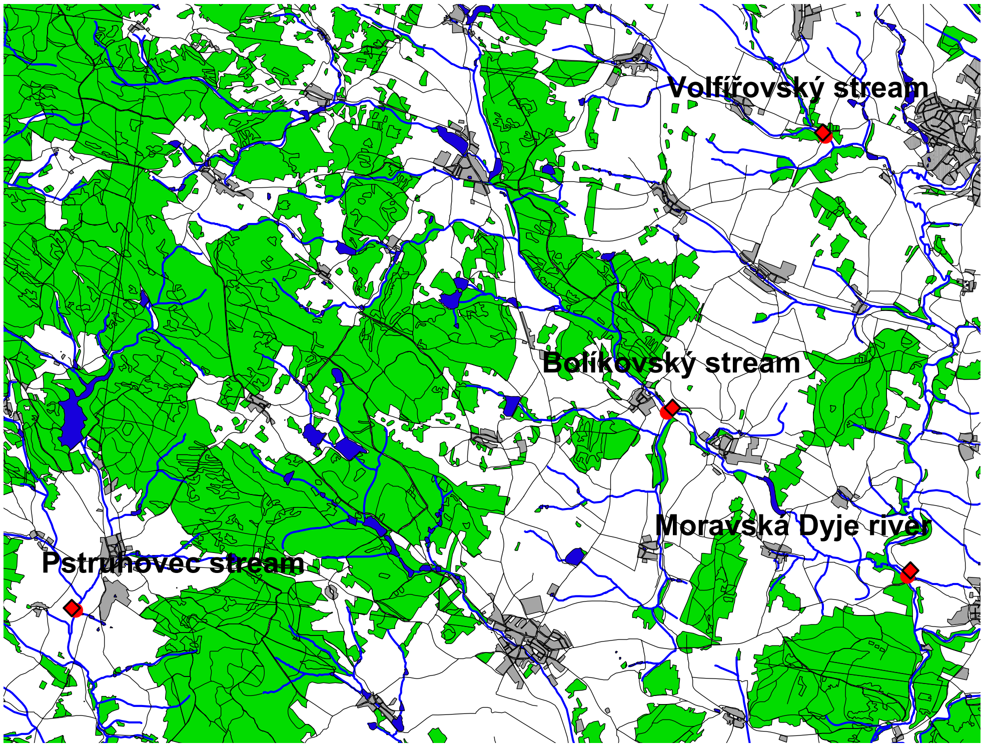

Figure 12. Local map of the study area showing the 4 selected monitoring sites.

Bridges were originally the first choice for selecting suitable sites but unfortunately during the time of the study the water level was very high, covering the bridge ledges where otters usually walk through. This substantially limited the study. Ten bridges in the experimental area were checked but only 2 were suitable – the rest were either not of the appropriate construction type or the water level was too high. Figures 13-16 show the four experimental mud areas that were used and Table 3 lists their approximate dimensions.

Figure 13. Experimental mud layer under the bridge on Volfířovský stream.

Figure 14. Experimental mud layer under the bridge on Bolíkovský stream.

Figure 15. Experimental mud layer on the bank of Pstruhovec stream.

Figure 16. Experimental mud layer on the bank of Moravská Dyje river.

Table 3. Approximate dimensions of the four experimental mud layers used in the field experiments.

Experimental site:	length	width
Volfířovský stream	550 cm	80 cm
Bolíkovský stream	270 cm	80 cm
Pstruhovec stream	140 cm	80 cm
Moravská Dyje river	200 cm	60 cm

Data collection and analysis

Data were collected over a period of 10 days in a similar manner as in the captive experiments. After experimental areas were established, they were regularly visited every evening to make sure they were well prepared (e.g. without imprints of other animals, mud not too dry, etc.) and every morning to photograph any otter tracks present. Otter spraints collected in the Otter Station in Pavlov were used to attract wild otters to the experimental areas. As before, a Power Shot A 520 digital camera was used, fixed to a "tetrapod" with a centimeter scale at the bottom (as described in section 2.1.1.). All imprints forming a visible track (= imprints made by a single individual) were labeled as belonging to that track.

On a computer, imprints were then sorted by foot, grouped by the appropriate track, and photographs selected for analysis were adjusted by PT Lens software to correct lens distortion and cropped in Photoshop. Again, the software program by Mönkemeyer was used to generate all measurements. Each imprint was measured 9 times to correct for measurement error, mean values were then used for statistical analysis.

Statistical methods

As in the captive experiments, SYSTAT and SAS software packages were used to analyze the data. Discriminant analysis along with the associated Jacknife cross-validation technique was run on all data sets, and canonical centroid plots were constructed. In contrast to the captive study, the identity of collected imprints was not known. Because of that, “tracks” were used here in place of “individual otters”, where track is defined as a single trail of imprints clearly made by one individual animal. Tracks observed and photographed on different days or at different study sites may therefore represent different otters, but may as well be made by the same animal (especially if study area is small, as was the case here).

Again, different statistical approaches were taken and their results compared. The principal statistical method used was stepwise DA (both backward and forward). First, every foot (where large enough sample size available) was analyzed separately using all available imprints of that foot per track. These analyses were done using different sets of variables: 1) all variables available from the measuring software, 2) twenty visually chosen variables (as in 2.1.3.), 3) two sets of variables selected as the best discriminating ones by the previous analyses of the appropriate foot in the captive experiments (either by analysis of all available imprints or of the four best quality imprints).

To test the separation of tracks even further, data for left front and left hind feet of each track were combined and subjected to DA, employing again sets of best discriminating variables as indicated earlier in the captive study.

CHAPTER 3

RESULTS

Experiments with captive otters

All analyses described in the Methods section were performed and yielded similar results. However, only the techniques that resulted in the best group separation are reported here.

Analyzing each foot separately using 8 imprints/photographs per animal

Stepwise backward DA turned out to be the best predictive technique when analyzing each foot separately and using all available photographs.

For left front foot, the data set included 92 imprints. Table 4 shows that using a stepwise backward DA on 50 variables with the highest F-ratios, 10 of the 92 imprints were misclassified, giving an accuracy of classification of 89%. Testing the efficiency of the method by Jacknife technique yielded a 71% accuracy (Table 5). Fig. 17 shows the separation of groups (otters) resulting from this procedure, plotted on different combinations of the canonical variables in the test space, and shows 95% confidence ellipses. One can see that several pairs of ellipses are not overlapping, suggesting a significant separation between these pairs of animals. The stepwise backward DA procedure selected 17 variables as the optimal set for separation (Table 6).

Table 4. Results for stepwise backward discriminant analysis separating among 12 individual otters based on measurements of their left front paw imprints. Correct or incorrect classification of each imprint to each individual otter is shown. 50 parameters were used and 17 were selected as the optimum for best separation.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	7	0	0	0	0	1	0	0	0	0	0	0	88
otter C	0	7	0	0	0	1	0	0	0	0	0	0	88
otter F	0	0	7	0	0	1	0	0	0	0	0	0	88
otter G	0	0	0	8	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	8	0	0	0	0	0	0	0	100
otter M	0	0	0	0	0	8	0	0	0	0	0	0	100
otter N	0	0	0	0	0	2	6	0	0	0	0	0	75
otter P	0	0	0	0	1	0	0	7	0	0	0	0	88
otter S	0	0	0	0	0	0	0	0	8	0	0	0	100
otter V	0	0	0	0	0	0	0	0	0	7	0	0	88
otter Y	0	0	0	0	0	0	1	0	0	1	6	0	75
otter Z	0	0	0	1	0	0	0	0	0	0	0	3	75
total	7	7	7	9	9	13	7	7	8	8	6	3	**89**

Table 5. Jacknifed classification matrix testing the efficiency of separation among individual otters based on measurements of their left front paw imprints. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 4.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	7	0	0	0	0	0	0	0	0	0	1	0	88
otter C	0	5	1	0	0	1	0	0	1	0	0	0	63
otter F	0	1	2	0	0	1	0	1	1	2	0	0	25
otter G	0	0	0	7	0	0	0	0	0	0	1	0	88
otter L	0	0	0	0	8	0	0	0	0	0	0	0	100
otter M	0	0	0	0	0	7	0	0	0	1	0	0	88
otter N	0	0	0	0	0	2	6	0	0	0	0	0	75
otter P	0	0	0	0	1	0	0	7	0	0	0	0	88
otter S	0	1	1	0	0	1	1	0	4	0	0	0	50
otter V	0	0	1	0	0	2	0	1	0	4	0	0	50
otter Y	0	0	0	0	0	0	2	0	0	1	5	0	63
otter Z	0	0	0	1	0	0	0	0	0	0	0	3	75
total	7	7	5	8	9	14	9	9	6	8	7	3	**71**

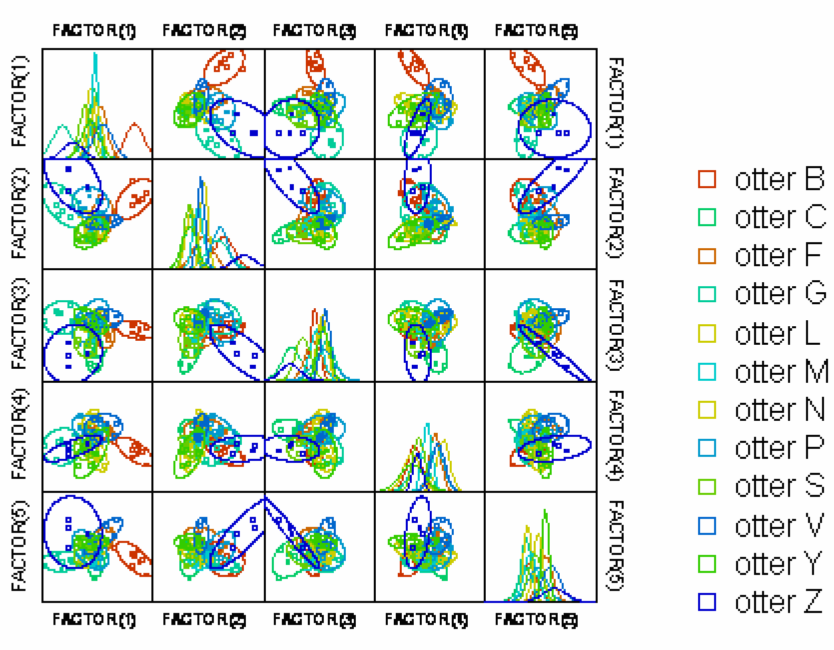

Figure 17. Canonical scores plot showing the results associated with the method applied in Table 4. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses. Each ellipse repserensts a set of 8 left front paw imprints (where available) for the particular otter.

For left hind foot, the data set included 91 imprints, of which 6 were misclassified by a stepwise backward DA run on all variables (Table 7), giving an overall 93% correct classification. The Jacknife cross-validation technique for this proceduregave a 77% accuracy (Table 8). Figure 18 shows the group separation resulting from this analysis using 95% confidence ellipses. This stepwise backward DA procedure selected 17 variables as the optimal combination (Table 6).

Table 6. Variables selected as the optimal discriminating set by the best separating procedures when analyzing each foot separately, using all available photographs/imprints. Refer to Fig. 7 for definition of the measurements (measurement abbreviations with no underscores = distances, with one underscore = proportions, with two underscores = angles).

	Left front foot: stepwise backward DA on 50 variables with highest F-ratios	**Left hind foot: stepwise backward DA on all variables**	**Right front foot: stepwise backward DA on 50 variables with highest F-ratios**	**Right hind foot: stepwise forward DA on all variables**
measurements:	FBR	MBR	MBM	MBD
	KZ	MBK	FBR	MBM
	Z_FB_K	MBFB	MBFB	MBR
	KM	FBR	MBR	RD
	FB_K_Z	ZD	MZ	RZ
	MBM	MBD_FBD	FBD	MD_K_FB
	FBZ	MBZ_FBZ	D_R_M	MD_M_FB
	MBFB	MD_Z_FB	Z_D_K	K_M_FB
	KD	FB_K_Z	MB_R_M	R_M_MB
	MBZ	R_M_FB		MB_R_D
	M_FB_K	FB_R_Z		D_MB_R
	RZ	FB_R_D		
	FBK	FB_Z_D		
	FBD	K_R_MB		
	Z_MB_R	MB_K_D		
	MBK_FBK	K_D_MB		
	R_FB_MD	R_M_D		

Table 7. Results for stepwise backward discriminant analysis separating among 12 individual otters based on measurements of their left hind paw imprints. Correct or incorrect classification of each imprint to each individual otter is shown. All 131 parameters were used and 17 were selected as the optimal set for best separation.

	otter B	**otter C**	**otter F**	**otter G**	**otter L**	**otter M**	**otter N**	**otter P**	**otter S**	**otter V**	**otter Y**	**otter Z**	**% correct**
otter B	8	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	8	0	0	0	0	0	0	0	0	0	0	100
otter F	0	0	5	0	0	0	0	0	0	0	0	0	100
otter G	0	0	0	8	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	7	0	1	0	0	0	0	0	88
otter M	0	0	0	0	1	6	1	0	0	0	0	0	75
otter N	0	0	0	0	0	1	7	0	0	0	0	0	88
otter P	0	0	0	0	0	0	0	8	0	0	0	0	100
otter S	0	0	0	0	0	0	0	0	8	0	0	0	100
otter V	0	0	0	0	0	0	0	0	0	8	0	0	100
otter Y	0	0	0	0	0	0	0	0	0	0	6	0	100
otter Z	0	0	0	0	1	1	0	0	0	0	0	6	75
Total	8	8	5	8	9	8	9	8	8	8	6	6	**93**

Table 8. Jacknifed classification matrix testing the efficiency of separation among individual otters based on measurements of their left hind paw imprints. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 7.

	otter B	**otter C**	**otter F**	**otter G**	**otter L**	**otter M**	**otter N**	**otter P**	**otter S**	**otter V**	**otter Y**	**otter Z**	**% correct**
otter B	8	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	5	0	0	0	0	1	0	2	0	0	0	63
otter F	0	0	1	0	0	1	2	0	0	0	1	0	20
otter G	0	0	0	7	1	0	0	0	0	0	0	0	88
otter L	0	0	0	0	7	0	1	0	0	0	0	0	88
otter M	0	0	0	0	1	6	1	0	0	0	0	0	75
otter N	0	0	0	0	0	3	5	0	0	0	0	0	63
otter P	0	0	0	0	0	0	0	8	0	0	0	0	100
otter S	0	2	1	0	0	0	1	0	4	0	0	0	50
otter V	0	0	0	0	0	1	0	0	0	7	0	0	88
otter Y	0	0	0	0	0	0	0	0	0	0	6	0	100
otter Z	0	0	0	0	1	1	0	0	0	0	0	6	75
total	8	7	2	7	10	12	11	8	6	7	7	6	**77**

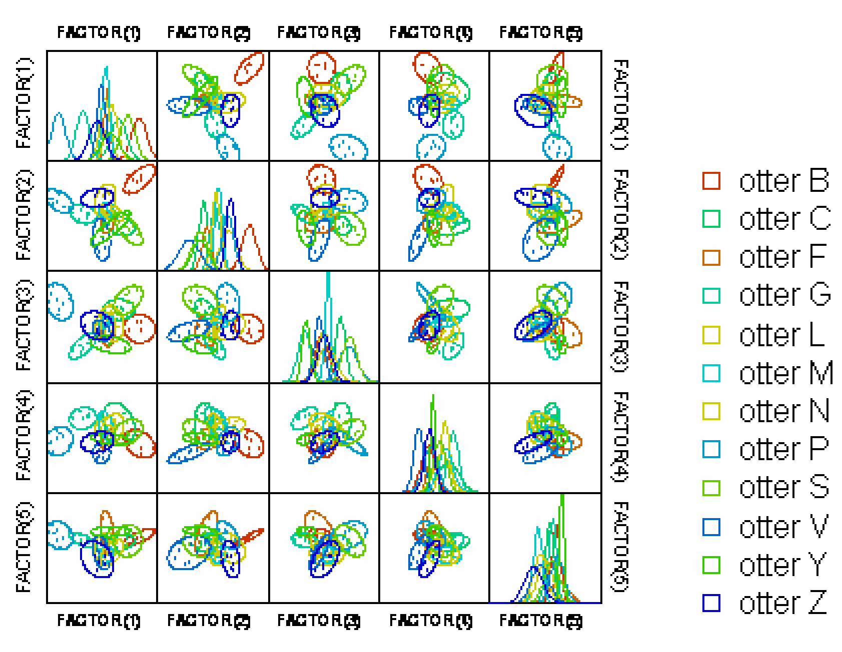

Figure 18. Canonical scores plot showing the results associated with the method applied in Table 7. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses. Each ellipse represents a set of 8 left hind paw imprints (where available) for the particular otter.

For right front foot, the data set consisted of 88 imprints, and a stepwise backward DA on 50 variables with the highest ratios yielded an accuracy of 86% for the test of assigned against predicted classification (Table 9), and an accuracy of 70% for the Jacknife cross-validation technique (Table 10). Fig. 19 is the graphical representation of these results. Only 9 variables were indicated as the optimal separating set by this procedure (Table 6). A few of the other statistical approaches actually yielded a better accuracy of classification for right front feet, but their associated tests of efficiency by the Jacknife technique were lower, which was considered a more important criterium

Table 9. Results for stepwise backward discriminant analysis separating among 12 individual otters based on measurements of their right front paw imprints. Correct or incorrect classification of each imprint to each individual otter is shown. 50 parameters were used and 9 were selected as the optimal set for best separation.

	otter B	**otter C**	**otter F**	**otter G**	**otter L**	**otter M**	**otter N**	**otter P**	**otter S**	**otter V**	**otter Y**	**otter Z**	**% correct**
otter B	7	0	0	0	0	0	0	1	0	0	0	0	88
otter C	0	5	0	0	0	0	0	0	0	0	0	0	100
otter F	0	0	6	0	1	0	0	0	1	0	0	0	75
otter G	0	0	0	8	0	0	0	0	0	0	0	0	100
otter L	1	0	0	0	6	0	0	1	0	0	0	0	75
otter M	0	0	0	0	0	6	0	0	0	1	1	0	75
otter N	0	0	0	0	0	0	8	0	0	0	0	0	100
otter P	0	0	0	0	1	0	0	7	0	0	0	0	88
otter S	0	0	0	0	0	0	0	0	5	0	0	0	100
otter V	0	0	0	0	0	1	0	0	0	6	1	0	75
otter Y	0	0	0	0	0	0	0	0	0	1	7	0	88
otter Z	0	0	0	0	1	0	0	0	0	0	0	5	83
total	8	5	6	8	9	7	8	9	6	8	9	5	**86**

Table 10. Jacknifed classification matrix testing the efficiency of separation among individual otters based on measurements of their right front paw imprints. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 9.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	7	0	0	0	0	0	0	1	0	0	0	0	88
otter C	0	4	0	0	0	1	0	0	0	0	0	0	80
otter F	1	0	4	0	2	0	0	0	1	0	0	0	50
otter G	1	0	0	6	1	0	0	0	0	0	0	0	75
otter L	1	0	0	0	5	0	0	1	0	1	0	0	63
otter M	0	0	0	0	0	4	1	0	0	1	2	0	50
otter N	0	0	0	0	1	0	7	0	0	0	0	0	88
otter P	0	0	0	0	2	0	0	6	0	0	0	0	75
otter S	0	0	0	1	0	0	0	0	3	0	1	0	60
otter V	0	0	0	0	0	1	0	0	0	6	1	0	75
otter Y	0	0	0	0	0	2	0	0	0	1	5	0	63
otter Z	0	0	0	0	1	0	0	0	0	0	0	5	83
total	10	4	4	7	12	8	8	8	4	9	9	5	**70**

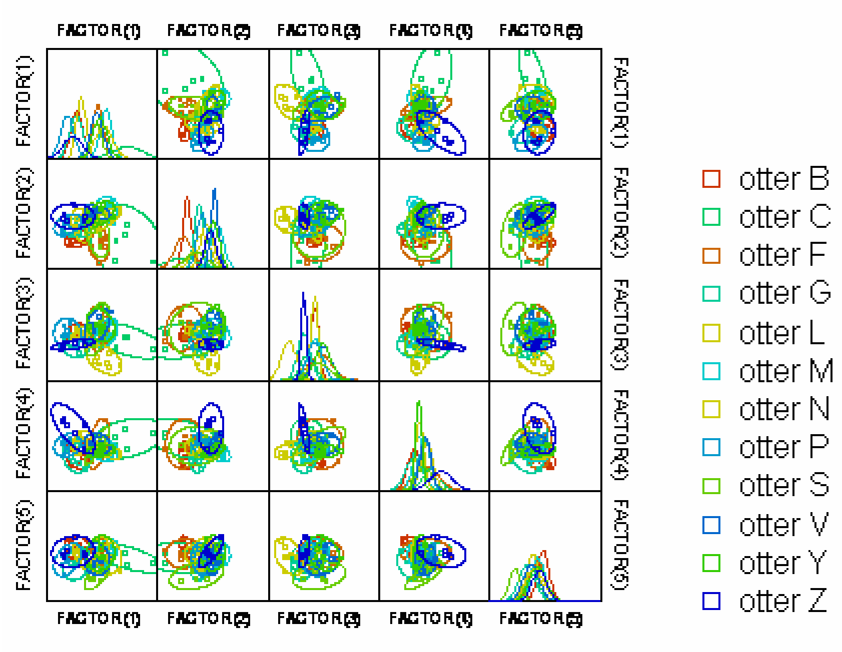

Figure 19. Canonical scores plot showing results associated with the method applied in Table 9. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses. Each ellipse represents a set of 8 right front paw imprints (where available) for the particular otter.

Analyzing right hind feet, the data set included 79 imprints and the best performing procedure was a stepwise forward DA run on all variables. It yielded a 91% accuracy of classification (Table 11), but only 59% accuracy for the Jacknife cross-validation technique (Table 12). Fig. 20 again shows the group separation using canonical variables and 95% confidence ellipses. The stepwise forward DA procedure selected 11 variables as the optimum for separation (Table 6).

Table 11. Results for stepwise forward discriminant analysis separating among 12 individual otters based on measurements of their right hind paw imprints. Correct or incorrect classification of each imprint to each individual otter is shown. All 131 parameters were used and 11 were selected as the optimal set for best separation.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	2	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	7	1	0	0	0	0	0	0	0	0	0	88
otter F	0	0	7	0	0	0	1	0	0	0	0	0	88
otter G	0	0	0	6	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	7	0	0	1	0	0	0	0	88
otter M	0	0	0	0	0	8	0	0	0	0	0	0	100
otter N	0	0	0	0	0	0	7	0	1	0	0	0	88
otter P	0	0	0	0	0	0	0	3	0	0	0	0	100
otter S	0	0	0	0	0	0	1	0	6	1	0	0	75
otter V	0	0	0	0	0	0	0	1	0	7	0	0	88
otter Y	0	0	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	0	0	8	100
total	2	7	8	6	7	8	9	5	7	8	4	8	**91**

Table 12. Jacknifed classification matrix testing the efficiency of separation among individual otters based on measurements of their right hind paw imprints. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 11.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	0	0	0	0	0	0	0	1	0	0	0	1	0
otter C	0	5	1	0	0	0	1	0	1	0	0	0	63
otter F	0	1	1	0	1	1	1	1	2	0	0	0	13
otter G	0	0	0	6	0	0	0	0	0	0	0	0	100
otter L	1	0	0	0	5	0	0	1	0	0	0	1	63
otter M	0	0	0	0	0	7	0	0	0	0	0	1	88
otter N	0	0	1	0	0	0	5	0	2	0	0	0	63
otter P	0	0	1	0	0	0	1	1	0	0	0	0	33
otter S	0	1	2	0	0	0	3	0	1	1	0	0	13
otter V	0	0	0	0	0	0	0	1	1	6	0	0	75
otter Y	0	0	0	0	0	0	0	0	0	0	4	0	100
otter Z	1	0	0	0	0	1	0	0	0	0	0	6	75
total	2	7	6	6	6	9	11	5	7	7	4	9	**59**

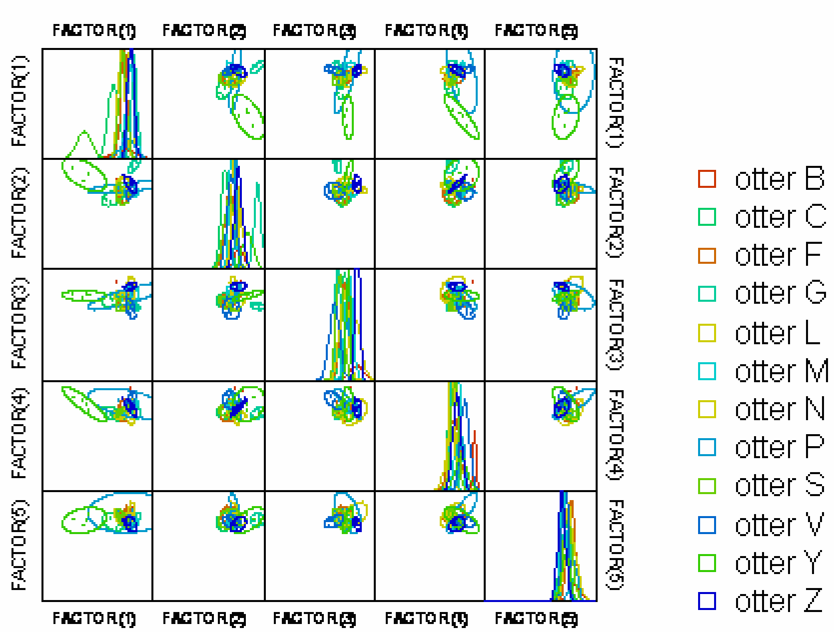

Figure 20. Canonical scores plot showing the results associated with the method applied in Table 11. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses. Each ellipse represents a set of 8 right hind paw imprints (where available) for the particular otter.

All the above results are summarized in Table 13.

Table 13. Summary of results analyzing each foot separately and using 8 imprints per animal (where available). LF = left front, RF = right front, LH = left hind, RH = right hind foot.

	LF	RF	LH	RH
% correct classification in DA	89	86	93	91
% correct cross-validation by Jacknife method	71	70	77	59

Analyzing each foot separately and randomly reducing the number of imprints/photographs per animal

These analyses were done to test the effects of reducing sample size (= fewer imprints per animal) on the accuracy of discrimination.

Figures 21-24 represent the performance of the four applied statistical techniques in the test of assigned against predicted classification for left front, left hind, right front, and right hind foot, respectively. Figures 25-28 show the efficiency tests of these techniques by the Jacknife procedure. It is evident from the graphs that reducing the sample size did not have a clear negative effect on the percentage of correctly classified footprints, as might have been expected. The accuracy of the cross-validating Jacknife procedure did go down slightly with the successively fewer imprints used, even though the trend was not so strong.

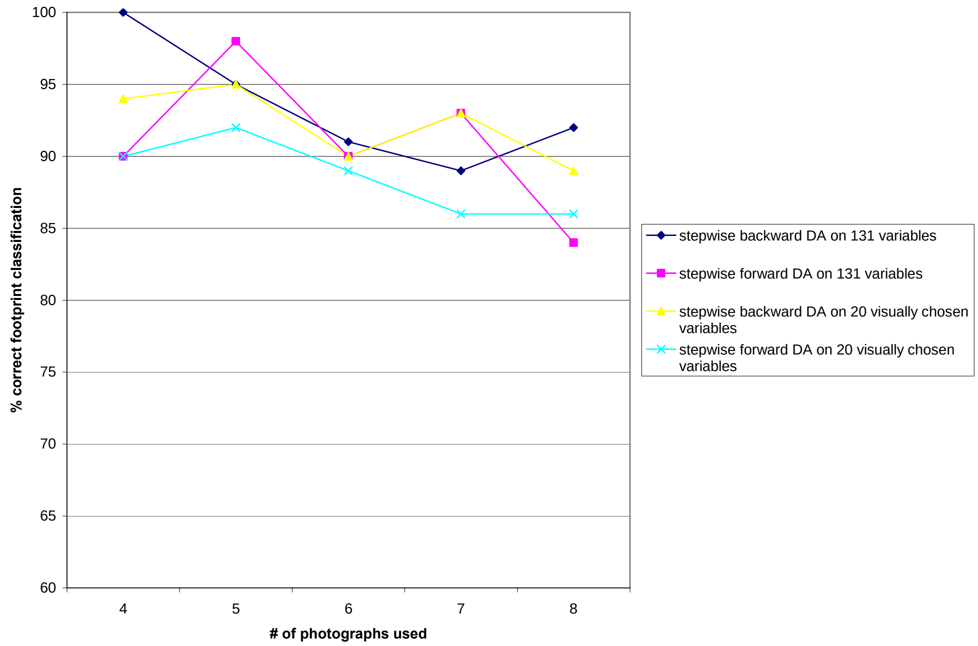

Figure 21. Performance of four different statistical methods when different numbers of imprints/photographs were used for analysis (left front foot analyzed separately).

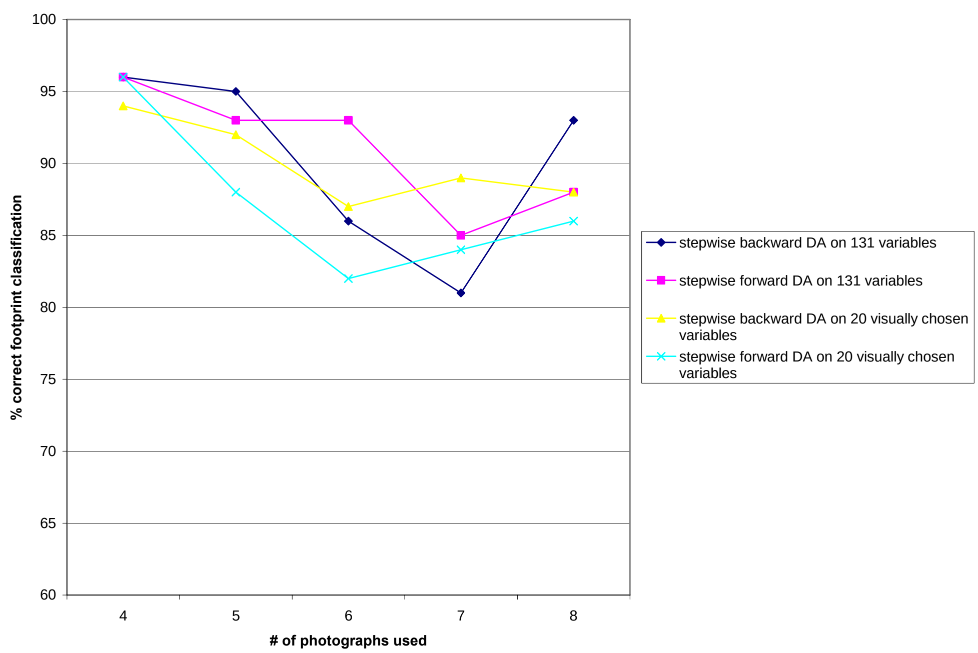

Figure 22. Performance of four different statistical methods when different numbers of imprints/photographs were used in analysis (left hind foot analyzed separately).

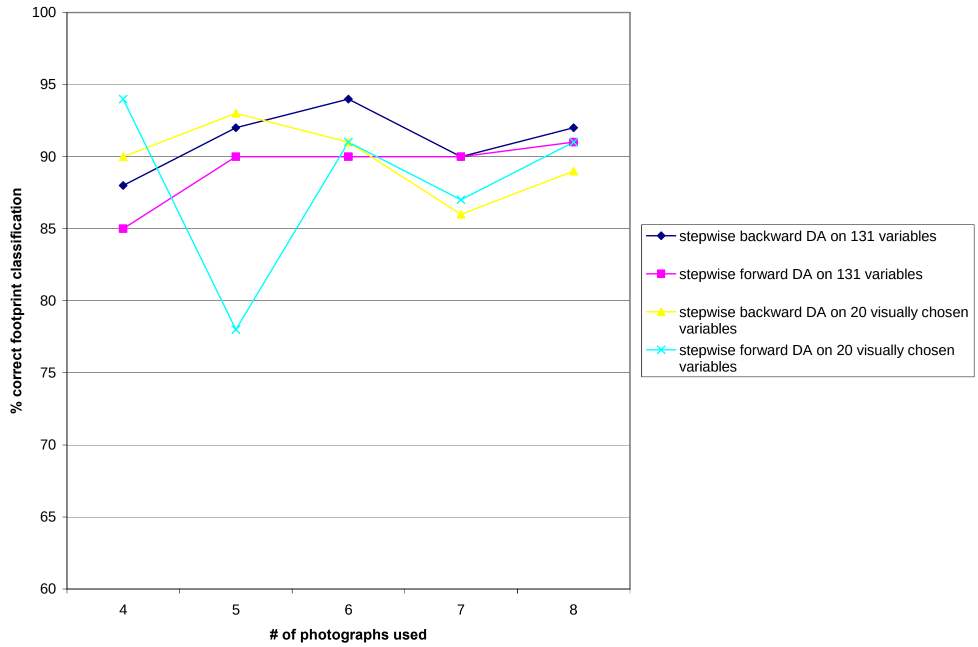

Figure 23. Performance of four different statistical methods when different numbers of imprints/photographs were used in analysis (right front foot analyzed separately).

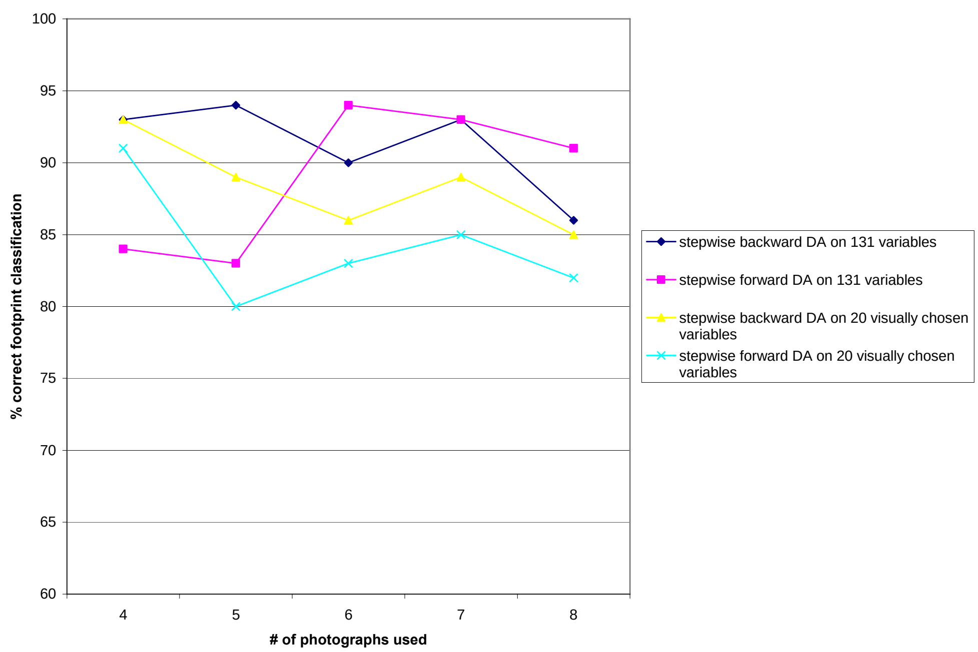

Figure 24. Performance of four different statistical methods when different numbers of imprints/photographs were used in analysis (right hind foot analyzed separately).

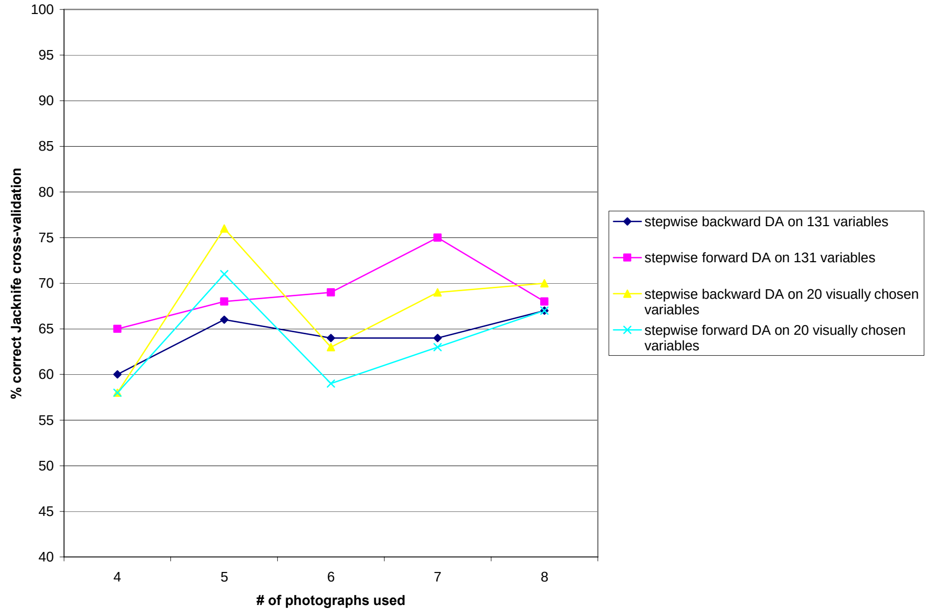

Figure 25. Effect of reducing sample size (number of imprints per otter) on the efficiency of four different statistical methods, as shown by the Jacknife cross-validation procedure. Left front feet analyzed here.

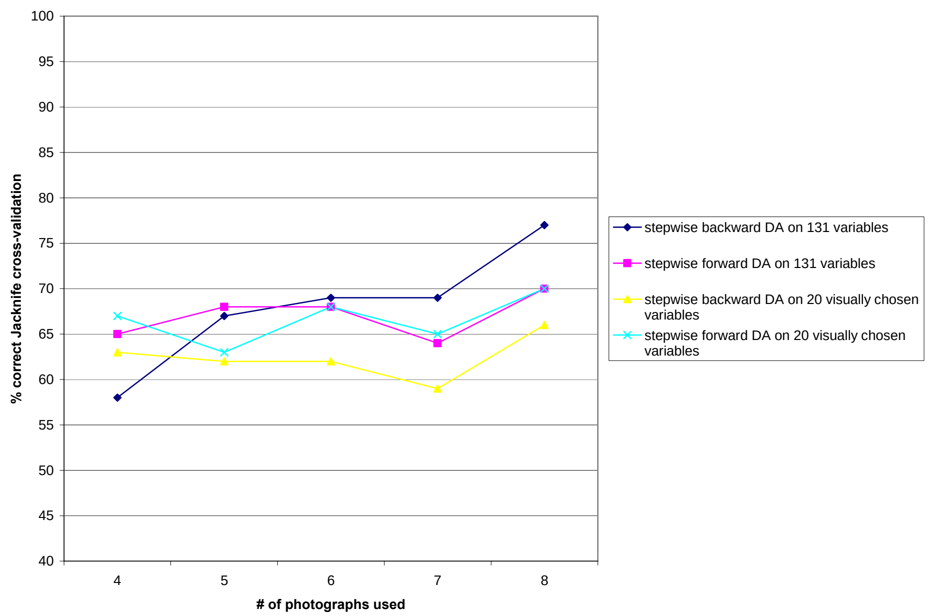

Figure 26. Effect of reducing sample size (number of imprints per otter) on the efficiency of four different statistical methods, as shown by the Jacknife cross-validation procedure. Left hind feet analyzed here.

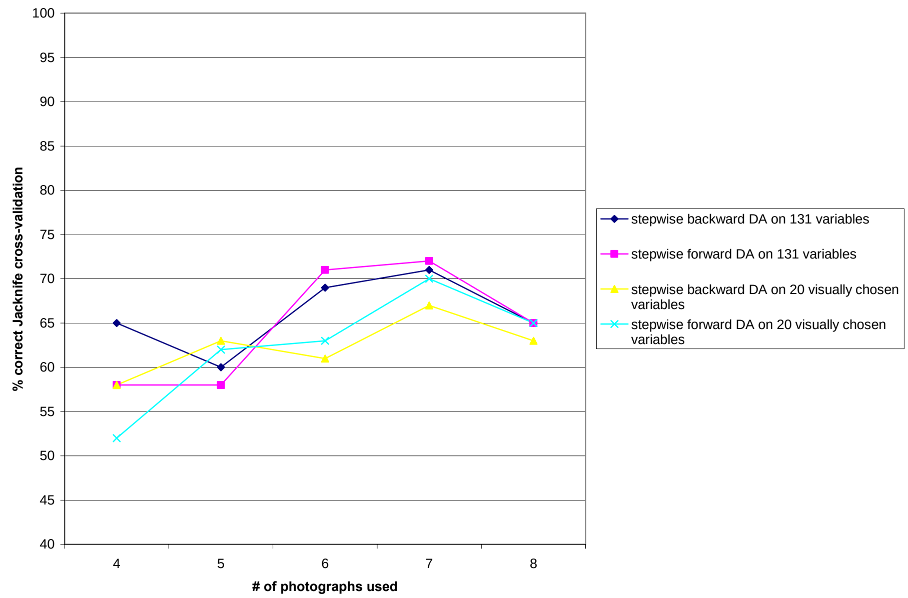

Figure 27. Effect of reducing sample size (number of imprints per otter) on the efficiency of four different statistical methods, as shown by the Jacknife cross-validation procedure. Right front feet analyzed here.

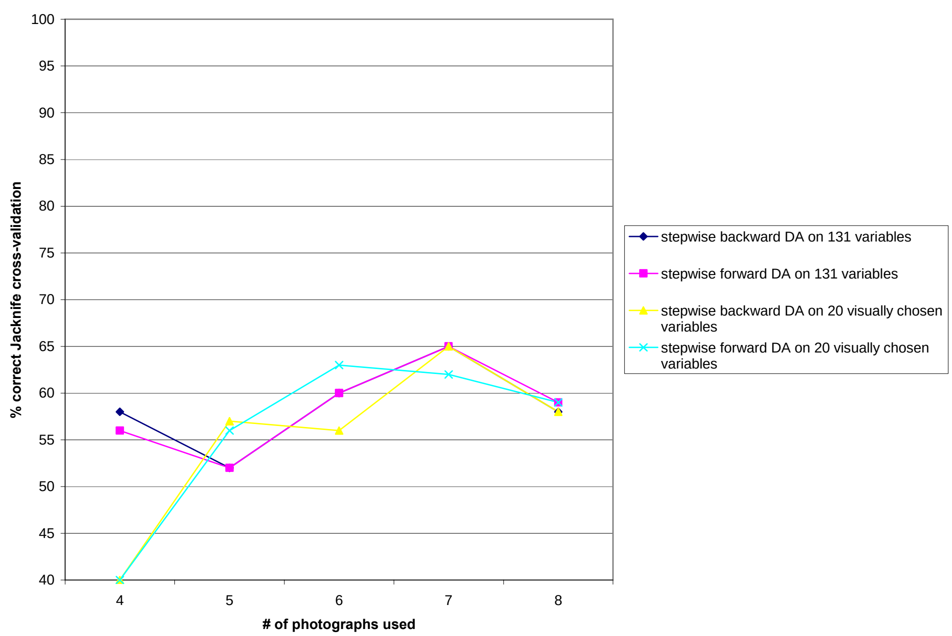

Figure 28. Effect of reducing sample size (number of imprints per otter) on the efficiency of four different statistical methods, as shown by the Jacknife cross-validation procedure. Right hind feet analyzed here.

Analyzing each foot separately using only four selected best quality imprints/photographs per animal

As in section 3.1.1., all analyses described in the Methods section were performed on these data sets and yielded fairly similar results, but only the best predictive techniques are reported here. Overall, stepwise forward DA proved to be the most efficient procedure in these analyses.

For left front foot, the data set included 47 imprints and a stepwise forward DA on all variables resulted in 100% correct footprint classification (Table 14), and 70% (accuracy in the Jacknife cross-validation procedure (Table 15). Fig. 29 shows the separation among individual otters in canonical space achieved by this method. Fifteen variables were selected as the optimal discriminating set in this case (Table 16).

Table 14. Results for stepwise forward discriminant analysis separating among 12 individual otters based on measurements from the four best quality left front paw imprints per animal (in most cases). Correct or incorrect classification of each imprint to each individual otter is shown. All parameters were used and 15 were selected as the optimal set for best separation.

	otter B	**otter C**	**otter F**	**otter G**	**otter L**	**otter M**	**otter N**	**otter P**	**otter S**	**otter V**	**otter Y**	**otter Z**	**% correct**
otter B	4	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	4	0	0	0	0	0	0	0	0	0	0	100
otter F	0	0	4	0	0	0	0	0	0	0	0	0	100
otter G	0	0	0	4	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	4	0	0	0	0	0	0	0	100
otter M	0	0	0	0	0	4	0	0	0	0	0	0	100
otter N	0	0	0	0	0	0	4	0	0	0	0	0	100
otter P	0	0	0	0	0	0	0	4	0	0	0	0	100
otter S	0	0	0	0	0	0	0	0	4	0	0	0	100
otter V	0	0	0	0	0	0	0	0	0	4	0	0	100
otter Y	0	0	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	0	0	3	100
total	4	4	4	4	4	4	4	4	4	4	4	3	**100**

Table 15. Jacknifed classification matrix testing the efficiency of separation among individual otters based on measurements from the four best quality imprints of left front paws. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 14.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	3	0	0	0	0	1	0	0	0	0	0	0	75
otter C	0	4	0	0	0	0	0	0	0	0	0	0	100
otter F	0	1	0	0	0	0	0	2	0	1	0	0	0
otter G	0	0	0	4	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	4	0	0	0	0	0	0	0	100
otter M	0	0	0	0	0	2	1	1	0	0	0	0	50
otter N	0	0	0	0	0	1	1	0	0	1	1	0	25
otter P	0	0	0	0	0	1	0	3	0	0	0	0	75
otter S	0	0	0	0	0	0	1	0	3	0	0	0	75
otter V	0	0	0	0	0	1	0	0	0	3	0	0	75
otter Y	0	0	0	0	0	0	0	0	0	1	3	0	75
otter Z	0	0	0	0	0	0	0	0	0	0	0	3	100
total	3	5	0	4	4	6	3	6	3	6	4	3	**70**

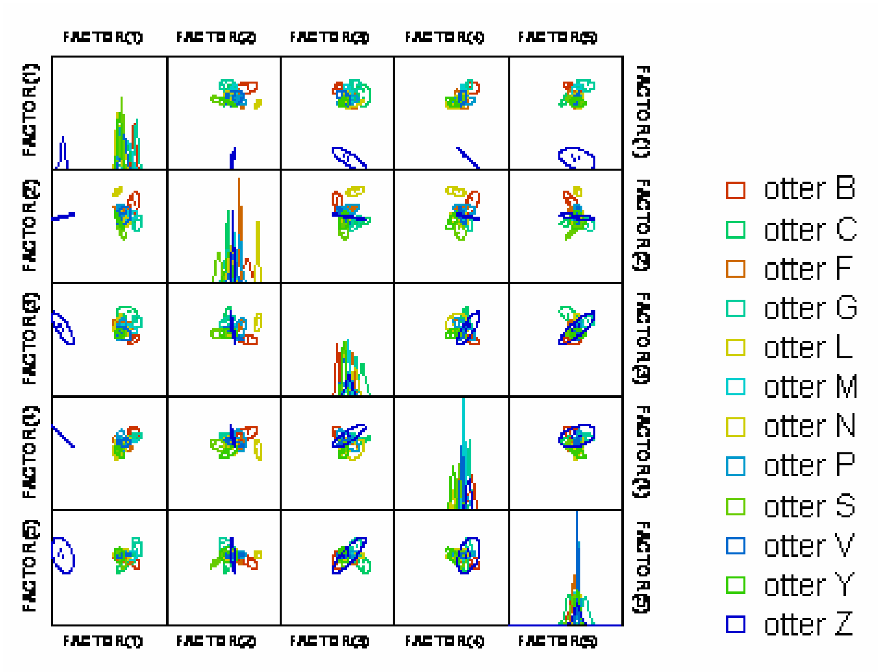

Figure 29. Canonical scores plot showing the results associated with the method applied in Table 14. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses. Each ellipse represents a set of four best quality left front paw imprints (in most cases) for the particular otter.

Table 16. Variables selected as the optimal discriminating set by the best predictive procedures when analyzing each foot separately, using best four imprints/photographs per otter. Refer to Fig. 7 for definition of the measurements (measurement abbreviations with no underscore = distances, with one underscore = proportions, with two underscores = angles).

	Left front foot: stepwise forward DA on all variables	**Left hind foot:** stepwise forward DA on all variables	**Right front foot:** stepwise forward DA on all variables	**Right hind foot:** stepwise backward DA on 30 variables with highest F-ratios
measurements:	MBFB	FBR	FBM	MBR
	FBZ	KR	FB_MB_R	RD
	KD	RD	MD_Z_FB	R_Z_MB
	KZ	RZ	FB_K_Z	RM
	MBD_FBD	MD	FB_R_Z	MBD
	MBK_FBK	FB_R_M	MB_R_D	R_M_MB
	K_FB_MD	Z_FB_R	M_D_K	MB_K_R
	MD_M_FB	D_FB_M	D_R_M	MBM
	Z_FB_MD	R_MB_K		M_FB_R
	MB_K_Z	MB_K_D		MB_K_M
	K_Z_MB	K_D_MB		D_MB_R
	MB_K_D	MB_R_Z		D_FB_M
	M_MB_R	Z_D_MB		MB_K_D
	D_MB_R	K_M_D		
	R_Z_D			

The data set for left hind foot was composed of 46 imprints. Using a stepwise forward DA on all variables, no imprints were misclassified, giving a 100% classification accuracy (Table 17). Testing the efficiency of the method by Jacknife procedure provided 87% accuracy (Table 18). Fig. 30 is a graphical representation of these results and shows 95% confidence ellipses (representing individual otters) plotted on different pair combinations of the canonical variables. This stepwise forward DA indicated fourteen variables to be the optimal separation set (Table 16).

Table 17. Results for stepwise forward discriminant analysis separating among 12 individual otters based on measurements from the four best quality left hind paw imprints per animal (in most cases). Correct or incorrect classification of each imprint to each individual otter is shown. All parameters were used and 14 were selected as the optimal set for best separation.

	otter B	**otter C**	**otter F**	**otter G**	**otter L**	**otter M**	**otter N**	**otter P**	**otter S**	**otter V**	**otter Y**	**otter Z**	**% correct**
otter B	4	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	4	0	0	0	0	0	0	0	0	0	0	100
otter F	0	0	2	0	0	0	0	0	0	0	0	0	100
otter G	0	0	0	4	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	4	0	0	0	0	0	0	0	100
otter M	0	0	0	0	0	4	0	0	0	0	0	0	100
otter N	0	0	0	0	0	0	4	0	0	0	0	0	100
otter P	0	0	0	0	0	0	0	4	0	0	0	0	100
otter S	0	0	0	0	0	0	0	0	4	0	0	0	100
otter V	0	0	0	0	0	0	0	0	0	4	0	0	100
otter Y	0	0	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	0	0	3	100
total	4	4	2	4	4	4	4	4	4	4	4	3	**100**

Table 18. Jacknifed classification matrix testing the efficiency of separation among individual otters based on measurements from the four best quality imprints of left hind paws. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 17.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	4	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	3	0	0	0	0	1	0	0	0	0	0	75
otter F	0	0	0	0	0	0	1	0	0	0	1	0	0
otter G	0	0	0	4	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	3	1	0	0	0	0	0	0	75
otter M	0	0	0	0	0	4	0	0	0	0	0	0	100
otter N	0	0	0	0	0	1	3	0	0	0	0	0	75
otter P	0	0	0	0	0	0	0	4	0	0	0	0	100
otter S	0	1	0	0	0	0	0	0	3	0	0	0	75
otter V	0	0	0	0	0	0	0	0	0	4	0	0	100
otter Y	0	0	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	0	0	4	100
total	4	4	0	4	3	6	5	4	3	4	5	4	**87**

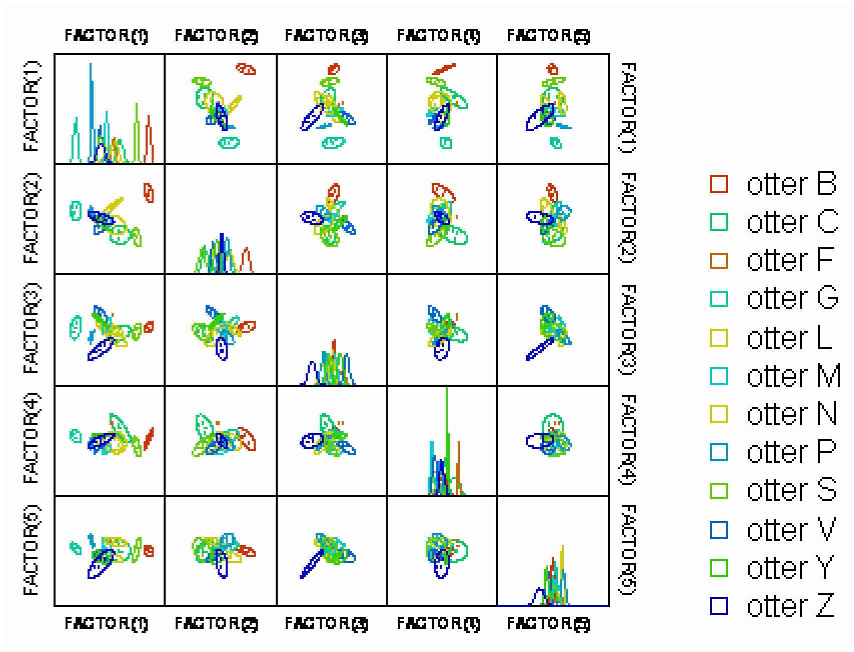

Figure 30. Canonical scores plot showing the results associated with the method applied in Table 17. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses. Each ellipse represents a set of four best quality left hind paw imprints (in most cases) for the particular otter.

For right front foot, the data set included 48 imprints, and again, the best performing technique was a stepwise forward DA run on all available variables. It resulted in 96% correct footprint classification (Table 19), and 71% accuracy of the Jacknife cross-validation (Table 20). Fig. 31 shows the group separation visually in canonical space. The procedure selected eight variables as the discriminating optimum (Table 16).

Table 19. Results for stepwise forward discriminant analysis separating among 12 individual otters based on measurements from the four best quality right front paw imprints per animal. Correct or incorrect classification of each imprint to each individual otter is shown. All parameters were used and 8 were selected as the optimal set for best separation.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	4	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	4	0	0	0	0	0	0	0	0	0	0	100
otter F	0	0	2	0	0	0	0	0	0	0	0	0	100
otter G	0	0	0	4	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	3	0	0	1	0	0	0	0	75
otter M	0	0	0	0	0	4	0	0	0	0	0	0	100
otter N	0	0	0	0	0	0	4	0	0	0	0	0	100
otter P	0	0	0	0	0	0	0	4	0	0	0	0	100
otter S	0	0	0	0	0	0	0	0	4	0	0	0	100
otter V	0	0	0	0	0	0	0	0	0	3	1	0	75
otter Y	0	0	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	0	0	3	100
total	4	4	2	4	3	4	4	5	4	3	5	3	**96**

Table 20. Jacknifed classification matrix testing the efficiency of separation among individual otters based on measurements from the four best quality imprints of right front paws. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 19.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	4	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	3	0	0	0	1	0	0	0	0	0	0	75
otter F	0	0	3	0	0	0	0	0	1	0	0	0	75
otter G	0	0	0	3	1	0	0	0	0	0	0	0	75
otter L	0	0	0	0	2	0	0	2	0	0	0	0	50
otter M	1	0	0	0	0	2	0	0	0	1	0	0	50
otter N	0	0	0	0	0	0	3	0	0	0	1	0	75
otter P	0	0	0	1	1	0	0	1	0	0	1	0	25
otter S	0	0	0	0	0	0	0	0	4	0	0	0	100
otter V	0	0	0	0	0	1	0	0	0	2	1	0	50
otter Y	0	0	0	0	0	0	0	0	1	0	3	0	75
otter Z	0	0	0	0	0	0	0	0	0	0	0	4	100
total	5	3	3	4	4	4	3	3	6	3	6	4	**71**

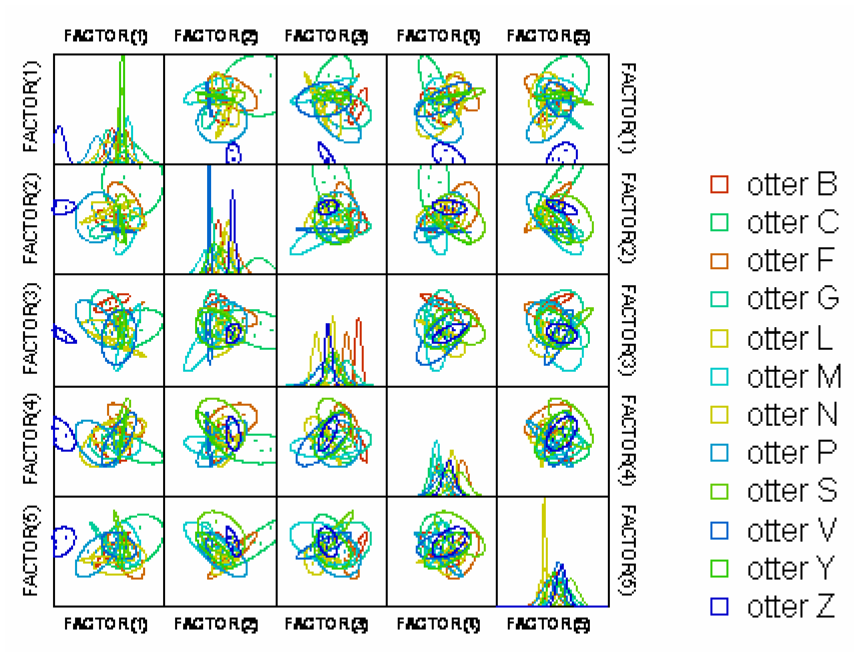

Figure 31. Canonical scores plot showing the results associated with the method applied in Table 19. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses. Each ellipse represents a set of four best quality right front paw imprints for the particular otter.

For right hind foot, the data set included 45 imprints and the procedure that performed best was a stepwise backward DA on 30 variables with the highest F-ratios. It gave a 100% correct footprint classification (Table 21), and 82% accuracy of the jackknife cross-validation (Table 22). Fig. 32 shows the resulting group separation visually. Thirteen variables were selected to be the optimal separation set (Table 16).

Table 21. Results for stepwise backward discriminant analysis separating among 12 individual otters based on measurements from the four best quality right hind paw imprints per animal (in most cases). Correct or incorrect classification of each imprint to each individual otter is shown. All parameters were used and 13 were selected as the optimal set for best separation.

	otter B	**otter C**	**otter F**	**otter G**	**otter L**	**otter M**	**otter N**	**otter P**	**otter S**	**otter V**	**otter Y**	**otter Z**	**% correct**
otter B	2	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	4	0	0	0	0	0	0	0	0	0	0	100
otter F	0	0	2	0	0	0	0	0	0	0	0	0	100
otter G	0	0	0	4	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	3	0	0	0	0	0	0	0	100
otter M	0	0	0	0	0	4	0	0	0	0	0	0	100
otter N	0	0	0	0	0	0	4	0	0	0	0	0	100
otter P	0	0	0	0	0	0	0	3	0	0	0	0	100
otter S	0	0	0	0	0	0	0	0	4	0	0	0	100
otter V	0	0	0	0	0	0	0	0	0	4	0	0	100
otter Y	0	0	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	0	0	3	100
total	2	4	2	4	3	4	4	3	4	4	4	3	**100**

Table 22. Jacknifed classification matrix testing the efficiency of separation among individual otters based on measurements from the four best quality imprints of right hind paws. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 21.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	2	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	4	0	0	0	0	0	0	0	0	0	0	100
otter F	0	0	3	0	0	1	0	0	0	0	0	0	75
otter G	0	0	0	3	0	1	0	0	0	0	0	0	75
otter L	0	0	0	0	4	0	0	0	0	0	0	0	100
otter M	0	0	0	0	0	2	0	0	0	1	0	1	50
otter N	0	0	1	0	0	0	3	0	0	0	0	0	75
otter P	0	0	0	0	0	1	0	2	0	0	0	0	67
otter S	0	0	0	0	0	0	0	0	3	1	0	0	75
otter V	0	0	0	0	0	0	0	0	1	3	0	0	75
otter Y	0	0	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	0	0	4	100
total	2	4	4	3	4	5	3	2	4	5	4	5	**82**

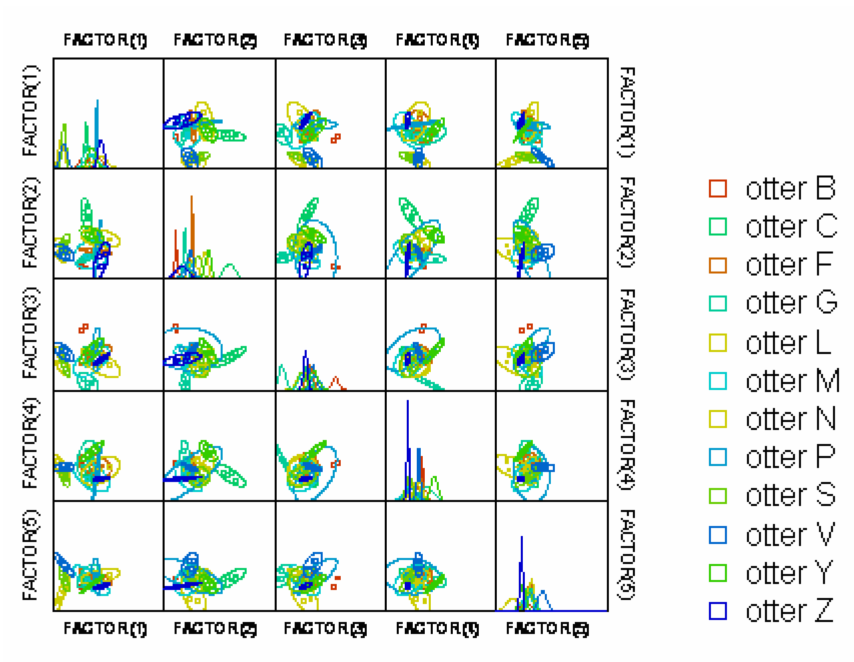

Figure 32. Canonical scores plot showing the results associated with the method applied in Table 21. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses. Each ellipse represents a set of four best quality right hind paw imprints (in most cases) for the particular otter.

Table 23 summarizes the results of the classification and cross-validation analyses.

Table 23. Summary of results analyzing each foot separately and using four best quality imprints per animal (where available). LF = left front, RF = right front, LH = left hind, RH = right hind foot.

	LF	RF	LH	RH
% correct classification in DA	100	96	100	100
% correct cross-validation by Jacknife method	70	71	87	82

Combined analyses of both left and both right feet

These analyses were done using the data sets with four best quality imprints per animal (as these gave better results), and employing only twenty variables that were selected based on the results of the previous discriminant analyses. From these previous analyses, the ten variables repeatedly chosen as the best discriminating variables for the corresponding front foot were combined with the ten variables repeatedly chosen as the best discriminating variables for the corresponding hind foot (Table 24), and then both backward and forward DA's were performed on these data sets.

Table 24. List of variables used in the combined analyses of left feet together and right feet together (identified as best discriminating ones in the previous analyses of each foot separately, using four best quality imprints per otter). Refer to Figure 7 for definition of these measurements (measurement abbreviations with no underscores = distances, with one underscore = proportions, with two underscores = angles).

Left front foot	Left hind foot	Right front foot	Right hind foot
KZ	MD	M_D_K	MBR
FBM	RD	MBM	RD
FBZ	FBR	FBM	MB_R_D
KM	Z_FB_R	M_Z_K	R_Z_MB
MBZ	RZ	R_M_D	RM
FBR	MZ	K_M_Z	M_D_R
R_D_FB	M_FB_R	MBR	MBD
MBM	MBR	MBFB	R_M_MB
RM	KD	KM	M_MB_R
M_D_FB	Z_MB_R	FBR	MB_K_R

For left front and hind feet combined, both backward and forward stepwise procedures yielded a 100% correct footprint classification. The backward procedure was a little more efficient, giving 81% accuracy of jackknife cross-validation, while the forward procedure resulted in 79% accuracy for this measure. Tables 25 and 26 and Fig.

33 show the results of the stepwise backward procedure, which selected 11 out of the twenty employed variables as the discriminating optimum (Table 27).

Table 25. Results for stepwise backward discriminant analysis separating among 12 individual otters based on combined measurements of front and hind left paw imprints. Correct or incorrect classification of each imprint to each individual otter is shown. Twenty variables were used and 11 were selected as the optimal set for best separation.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	4	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	4	0	0	0	0	0	0	0	0	0	0	100
otter F	0	0	4	0	0	0	0	0	0	0	0	0	100
otter G	0	0	0	4	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	4	0	0	0	0	0	0	0	100
otter M	0	0	0	0	0	4	0	0	0	0	0	0	100
otter N	0	0	0	0	0	0	4	0	0	0	0	0	100
otter P	0	0	0	0	0	0	0	4	0	0	0	0	100
otter S	0	0	0	0	0	0	0	0	4	0	0	0	100
otter V	0	0	0	0	0	0	0	0	0	4	0	0	100
otter Y	0	0	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	0	0	4	100
total	4	4	4	4	4	4	4	4	4	4	4	4	**100**

Table 26. Jacknife classification matrix testing the efficiency of separation among individual otters based on combined measurements of their left front and left hind paw imprints. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 25.

	otter B	otter C	otter F	otter G	otter L	otter M	otter N	otter P	otter S	otter V	otter Y	otter Z	% correct
otter B	4	0	0	0	0	0	0	0	0	0	0	0	100
otter C	0	4	0	0	0	0	0	0	0	0	0	0	100
otter F	0	0	0	0	0	1	1	0	0	1	1	0	0
otter G	0	0	0	4	0	0	0	0	0	0	0	0	100
otter L	0	0	0	0	4	0	0	0	0	0	0	0	100
otter M	0	0	0	0	0	2	0	0	0	2	0	0	50
otter N	0	0	0	0	0	0	4	0	0	0	0	0	100
otter P	0	0	0	0	0	0	0	4	0	0	0	0	100
otter S	0	1	0	0	0	0	0	0	3	0	0	0	75
otter V	0	0	0	0	0	0	0	0	0	4	0	0	100
otter Y	0	0	1	0	0	0	0	0	0	0	3	0	75
otter Z	0	0	0	1	0	0	0	0	0	0	0	3	75
total	4	5	1	5	4	3	5	4	3	7	4	3	**81**

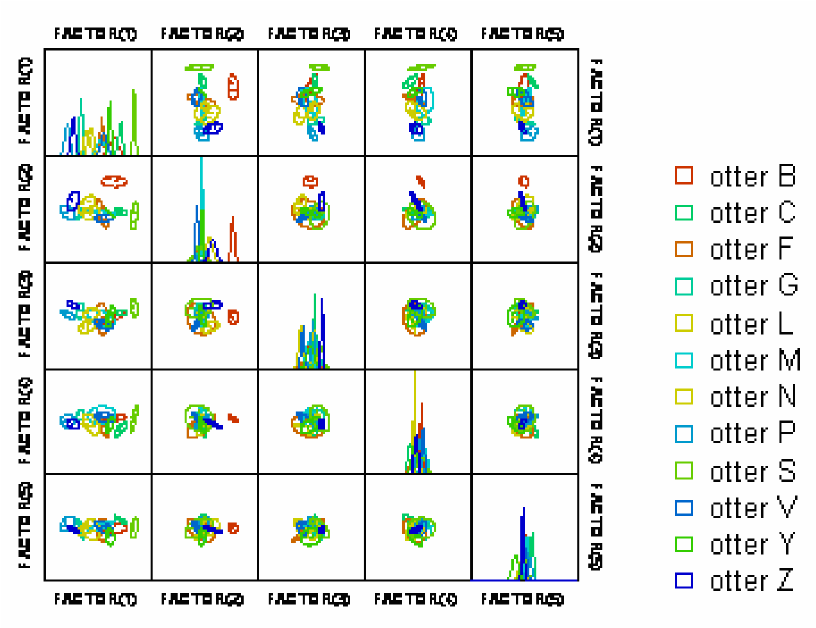

Figure 33. Canonical scores plot showing the results associated with the method applied in Table 25. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses, representing the individual otters.

Table 27. Summary of sets of measurements indicated as the optimal sets for separation by slightly different statistical procedures in the combined analyses (section I. 4. and 5.) Letters in parentheses show which paw is the measurement associated with: LF = left front, LH = left hind, RF = right front, RH = right hind. Refer to Figure 7 for definition of the measurements (measurement abbreviations with no underscores = distances, with one underscore = proportions, with two underscores = angles).

	Combined analysis of left feet (stepwise backward DA)	**Combined analysis of right feet (stepwise forward DA)**	**combined analysis of all feet**	
			stepwise backward DA	**stepwise forward DA**
Measurements	FBR (LF)	M_D_K (RF)	FB_K_M (LF)	Z_FB_K (LF)
	R_D_FB (LF)	FBM (RF)	KZ (LF)	FB_K_M (LF)
	MBM (LF)	R_M_D (RF)	Z_FB_R (LH)	KZ (LF)
	RM (LF)	FBR (RF)	FBR (LH)	Z_FB_R (LH)
	M_D_FB (LF)	MBR (RH)	RZ (LH)	FBR (LH)
	RD (LH)	MB_R_D (RH)	MBM LH)	FBM (RF)
	FBR (LH)	RM (RH)	MBR (LH)	FBR (RF)
	Z_FB_R (LH)	M_D_R (RH)	FBM (RF)	MBR (RH)
	RZ (LH)	R_M_MB (RH)	MZ (RF)	RZ (RH)
	MZ (LH)	M_MB_R (RH)	RZ (RF)	MB_R_D (RH)
	KD (LH)	MB_K_R (RH)	MBM (RF)	
			FBR (RF)	
			MBR (RH)	
			MB_R_D (RH)	

For right front and hind feet combined, both of the stepwise procedures yielded exactly the same results: 100% correct footprint classification and 90% accuracy of Jacknife cross-validation. Tables 28 and 29 and Fig. 34 show results of the stepwise forward DA. Out of the 20 variables used, this procedure eliminated 9, leaving 11 to be the optimal set (Table 27). This is two variables less than what the stepwise backward procedure indicated as the optimal set for separation.

Table 28. Results for stepwise forward discriminant analysis separating among 10 individual otters based on combined measurements of front and hind right paw imprints. Correct or incorrect classification of each imprint to each individual otter is shown. 20 variables were used and 11 were selected as the optimal set for best separation. Two otters were excluded from this analysis due to small number of either of the needed imprints.

	otter C	otter F	otter G	otter L	otter M	otter N	otter S	otter V	otter Y	otter Z	% correct
otter C	4	0	0	0	0	0	0	0	0	0	100
otter F	0	4	0	0	0	0	0	0	0	0	100
otter G	0	0	4	0	0	0	0	0	0	0	100
otter L	0	0	0	4	0	0	0	0	0	0	100
otter M	0	0	0	0	4	0	0	0	0	0	100
otter N	0	0	0	0	0	4	0	0	0	0	100
otter S	0	0	0	0	0	0	4	0	0	0	100
otter V	0	0	0	0	0	0	0	4	0	0	100
otter Y	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	4	100
total	4	4	4	4	4	4	4	4	4	4	**100**

Table 29. Jacknife classification matrix testing the efficiency of separation among individual otters based on combined measurements of their right front and right hind paw imprints. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 28. Two otters were excluded from analysis due to small sample (imprint) size.

	otter C	**otter F**	**otter G**	**otter L**	**otter M**	**otter N**	**otter S**	**otter V**	**otter Y**	**otter Z**	**% correct**
otter C	4	0	0	0	0	0	0	0	0	0	100
otter F	0	4	0	0	0	0	0	0	0	0	100
otter G	0	0	4	0	0	0	0	0	0	0	100
otter L	0	0	0	4	0	0	0	0	0	0	100
otter M	0	1	0	0	2	0	0	1	0	0	50
otter N	0	0	0	1	1	2	0	0	0	0	50
otter S	0	0	0	0	0	0	4	0	0	0	100
otter V	0	0	0	0	0	0	0	4	0	0	100
otter Y	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	4	100
total	4	5	4	5	3	2	4	5	4	4	**90**

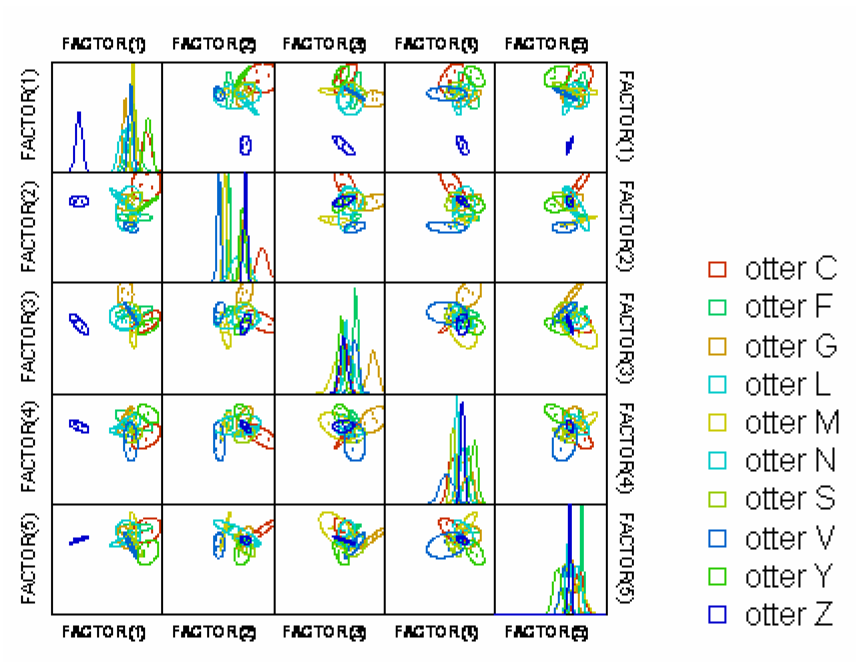

Figure 34. Canonical scores plot showing the results associated with the method applied in Table 28. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses, representing the individual otters.

The results of the classification and cross-validation analyses are summarized in Table 30.

Table 30. Summary of results for analyses that combined measurements of left front and hind feet together, and measurements of right front and hind feet together. Note that in the analysis of right feet 2 otters had to be excluded.

	left feet		**right feet**	
Type of analysis	stepwise backward DA	stepwise forward DA	stepwise backward DA	stepwise forward DA
% correct classification in DA	100	100	100	100
% correct cross-validation by Jacknife method	81	79	90	90

Combined analysis of all feet together

This analysis was again based on the data sets of four best quality imprints and employed twenty variables: five variables for each foot that were indicated as the best discriminating ones by the various statistical techniques performed in section I. (Table 31). This data set was again subjected to both backward and forward stepwise DA. The backward procedure performed slightly better, giving 100% correct footprint classification (Table 32), and 93% accuracy of Jacknife cross-validation (Table 33). Fig. 35 is the graphical representation of these results. The forward procedure was not far behind, resulting also in 100% correct footprint classification, but "only" 90% accuracy of Jacknife cross-validation. The backward and forward procedures selected fourteen and ten variables as the optimal discriminating set, respectively (Table 27).

Table 31. Variables associated with each paw that were indicated as best discriminating variables in previous analyses (of each foot separately, using all available imprints), and were used here in the combined analysis of all feet together. Refer to Figure 7 for definition of the measurements (measurement abbreviations with no underscores = distances, with one underscore = proportions, with two underscores = angles).

	Left front foot	**Left hind foot**	**Right front foot**	**Right hind foot**
Measurements:	Z_FB_K	Z_FB_R	FBM	MBR
	KM	FBR	MZ	RZ
	FB_K_M	RZ	RZ	MB_R_D
	FBR	MBM	MBM	KD
	KZ	MBR	FBR	R_Z_MB

Table 32. Results for stepwise backward discriminant analysis separating among 10 individual otters based on combined measurements of all four paw imprints. Correct or incorrect classification of each imprint to each individual otter is shown. 20 variables were used and 14 were selected as the optimal set for best separation. Two otters were excluded from this analysis due to small sample size of right hind feet imprints.

	otter C	otter F	otter G	otter L	otter M	otter N	otter S	otter V	otter Y	otter Z	% correct
otter C	4	0	0	0	0	0	0	0	0	0	100
otter F	0	4	0	0	0	0	0	0	0	0	100
otter G	0	0	4	0	0	0	0	0	0	0	100
otter L	0	0	0	4	0	0	0	0	0	0	100
otter M	0	0	0	0	4	0	0	0	0	0	100
otter N	0	0	0	0	0	4	0	0	0	0	100
otter S	0	0	0	0	0	0	4	0	0	0	100
otter V	0	0	0	0	0	0	0	4	0	0	100
otter Y	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	4	100
total	4	4	4	4	4	4	4	4	4	4	**100**

Table 33. Jacknife classification matrix testing the efficiency of separation among individual otters based on combined measurements of all paw imprints. Each imprint is input into the data set as an unknown and classified either correctly or incorrectly to an individual otter. This matrix represents a test of the method from Table 32. Two otters were excluded from this analysis due to small sample (imprint) size for right hind foot.

	otter C	otter F	otter G	otter L	otter M	otter N	otter S	otter V	otter Y	otter Z	% correct
otter C	4	0	0	0	0	0	0	0	0	0	100
otter F	0	3	0	0	1	0	0	0	0	0	75
otter G	0	0	4	0	0	0	0	0	0	0	100
otter L	0	0	0	4	0	0	0	0	0	0	100
otter M	0	1	0	0	3	0	0	0	0	0	75
otter N	0	0	0	0	1	3	0	0	0	0	75
otter S	0	0	0	0	0	0	4	0	0	0	100
otter V	0	0	0	0	0	0	0	4	0	0	100
otter Y	0	0	0	0	0	0	0	0	4	0	100
otter Z	0	0	0	0	0	0	0	0	0	4	100
total	4	4	4	4	5	3	4	4	4	4	**93**

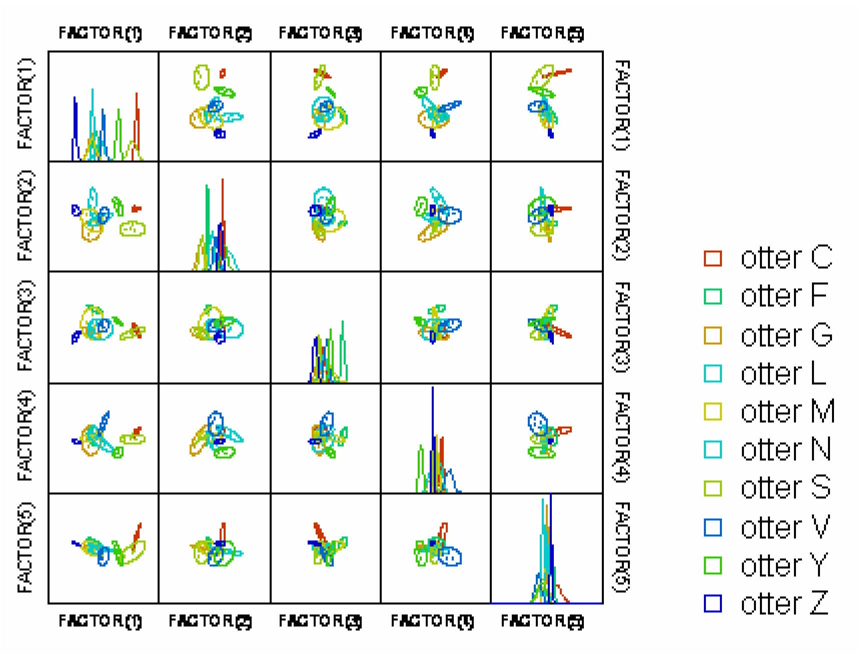

Figure 35. Canonical scores plot showing the results associated with the method applied in Table 32. All bivariate combinations possible for the first 5 canonical variables are shown, along with 95% confidence ellipses, representing the individual otters.

In summary, the analyses of each foot separately using all available imprints yielded 86-93% correct classification and 59-77% correct Jacknife cross-validation. The accuracy of classification was not significantly affected by reducing the number of imprints per otter. Analyses that used only the four best quality imprints per animal and foot resulted in 96-100% correct classification and 70-87% correct Jacknife cross-validation. Combining either both left or both right feet measurements in the DA gave even better results, with 100% correct classification and 79-90% correct Jacknife cross-validation. When measurements of all four feet were combined together for analysis, all imprints were correctly classified and the accuracy of Jacknife cross-validation was 90-

93%. The combined analyses were standardized by selecting and employing sets of 20 best discriminating variables.

Field study

During the field study in August 2006, a total of 7 otter tracks were documented when regularly checking four experimental sites (Table 34). However, only four of the tracks had enough good quality imprints and could be included in statistical analyses. Moreover, track 3 did not yield any suitable imprints of hind paws and was therefore excluded from analysis of left hind feet and from the combined analysis of left front and left hind feet. There were very few suitable imprints of right hind paws overall, which made the analysis of right hind feet separately and of combined right front and hind feet impossible.

Table 34. A day by day record of the four experimental sites.

	Volfířovský stream (bridge)	**Bolíkovský stream (bridge)**	**Pstruhovec stream (bank)**	**Moravská Dyje river (bank)**
8-Aug	installed			
9-Aug	no tracks			
10-Aug	3 tracks	installed	installed	
11-Aug	1 tracks	no tracks	no tracks	installed
12-Aug	1 track	1 track beside the experimental area in sand (low quality)	few imprints outside the experimental area	no tracks
13-Aug	no tracks	no tracks	no tracks	no tracks
14-Aug	1 track	no tracks	no tracks	no tracks
15-Aug	no tracks	1 track under another pillar of the bridge (too wet, low quality)	no tracks	1 imprint and feces
16-Aug	no tracks	1 track beside the experimental area in sand (low quality)	no tracks	no tracks
17-Aug	1 track	no tracks	no tracks	destroyed by people previous evening

Analyzing each foot separately using all available imprints per track

Stepwise forward DA run on all available variables proved to be the best predictive technique in all of these analyses. Looking at left front foot, it separated the four tracks as coming from four different individual otters, with 100% classification accuracy (Table 35) and 95% accuracy of the corresponding Jacknife efficiency test (Table 36). Fig. 36 shows the separation of tracks visually in canonical space.

The first two discriminant functions (factors 1 and 2 in Figure 36) together explained 98% of the variation between tracks. These two functions had highest correspondence with two angle and two proportion measurements: angle between first toe, heel, and fourth toe; angle between heel, middle ball, and fourth toe; distance between middle ball and second toe to the distance between heel and second toe; distance between middle ball and first toe to the distance between heel and first toe.

Table 35. Results for stepwise forward DA of 4 otter tracks based on analysis of left front paw imprints. All available variables were used and seven were selected as the optimal set for separation.

	track 1	**track 2**	**track 3**	**track 4**	**% correct**
track 1	3	0	0	0	100
track 2	0	6	0	0	100
track 3	0	0	5	0	100
track 4	0	0	0	6	100
total	3	6	5	6	**100**

Table 36. Jacknife classification matrix testing the efficiency of separation among otter tracks based on measurements of left front paw imprints. This matrix represents a test of the method from Table 35.

	track 1	**track 2**	**track 3**	**track 4**	**% correct**
track 1	3	0	0	0	100
track 2	0	5	0	1	83
track 3	0	0	5	0	100
track 4	0	0	0	6	100
total	3	5	5	7	**95**

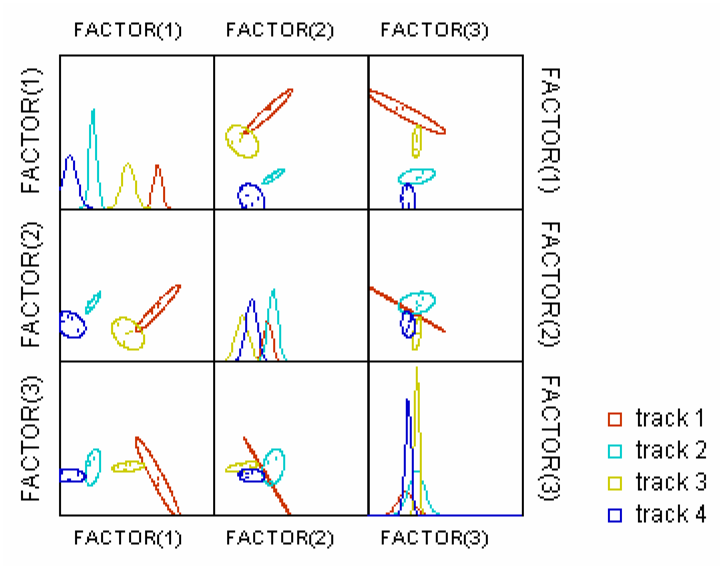

Figure 36. Canonical scores plot showing results of track separation by stepwise forward DA based on measurements of left front paw imprints. All bivariate combinations possible for the first 3 canonical variables are shown, along with 95% confidence ellipses, representing individual tracks.

Analysis of right front foot further supported the result, also showing that the four tracks represent four different otters. Again, all imprints of each track were classified to that respective track (Table 37), accuracy of the Jacknife cross-validation technique was 94% (Table 38). Fig. 37 is a graphical representation of these results, showing 95% confidence ellipses that represent the individual tracks.

The first two discriminant functions (factors 1 and 2 in Fig. 37) explained approximately 98% of the variation between tracks. Four angle measurements contributed the most to these two factors: angle between heel, fifth toe, and first toe; angle between second toe, middle ball, and third toe; angle between first toe, heel, and fourth toe; angle between fifth, fourth, and second toe.

Table 37. Results for stepwise forward DA of 4 otter tracks based on analysis of right front paw imprints. All available variables were used and four were selected as the optimal set for separation.

	track 1	track 2	track 3	track 4	% correct
track 1	5	0	0	0	100
track 2	0	2	0	0	100
track 3	0	0	3	0	100
track 4	0	0	0	6	100
total	5	2	3	6	**100**

Table 38. Jacknife classification matrix testing the efficiency of separation among otter tracks based on measurements of right front paw imprints. This matrix represents a test of the method from Table 37.

	track 1	track 2	track 3	track 4	% correct
track 1	4	0	1	0	80
track 2	0	2	0	0	100
track 3	0	0	3	0	100
track 4	0	0	0	6	100
total	4	2	4	6	**94**

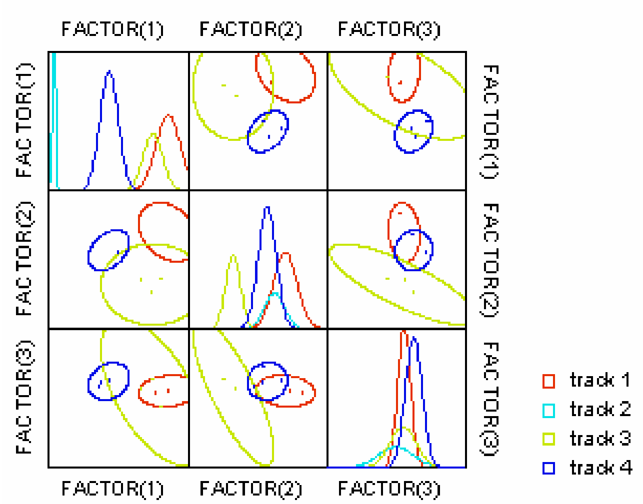

Figure 37. Canonical scores plot showing results of track separation by stepwise forward DA based on measurements of right front paw imprints. All bivariate combinations possible for the first 3 canonical variables are shown, along with 95% confidence ellipses, representing individual tracks. Note that track 2 only had two imprints (light blue dots), resulting in the confidence ellipse for that track missing, but they both seem to be well separated from all of the other tracks based on the first two combinations of canonical variables.

Track 3 was excluded from the analysis of left hind foot due to lack of suitable left hind paw imprints. Stepwise forward DA still indicated that the three remaining tracks were made by three different individuals. No imprints were classified to another track (Table 39) and the Jacknife cross-validation procedure yielded 93% efficiency (Table 40). Separation among the three tracks is shown visually in Fig. 38.

The two generated discriminant functions were dominated by two distance and two angle measurements: distance between heel and fourth toe; distance between third and first toe; angle between first toe, heel, and fourth toe; angle between first toe, middle ball, and third toe.

Table 39. Results for stepwise forward DA of 3 otter tracks based on analysis of left hind paw imprints. All available variables were used and six were selected as the optimal set for separation.

	track 1	**track 2**	**track 4**	**% correct**
track 1	6	0	0	100
track 2	0	5	0	100
track 4	0	0	4	100
total	6	5	4	**100**

Table 40. Jacknife classification matrix testing the efficiency of separation among otter tracks based on measurements of left hind paw imprints. This matrix represents a test of the method from Table 39.

	track 1	**track 2**	**track 4**	**% corect**
track 1	6	0	0	100
track 2	0	4	1	80
track 4	0	0	4	100
total	6	4	5	**93**

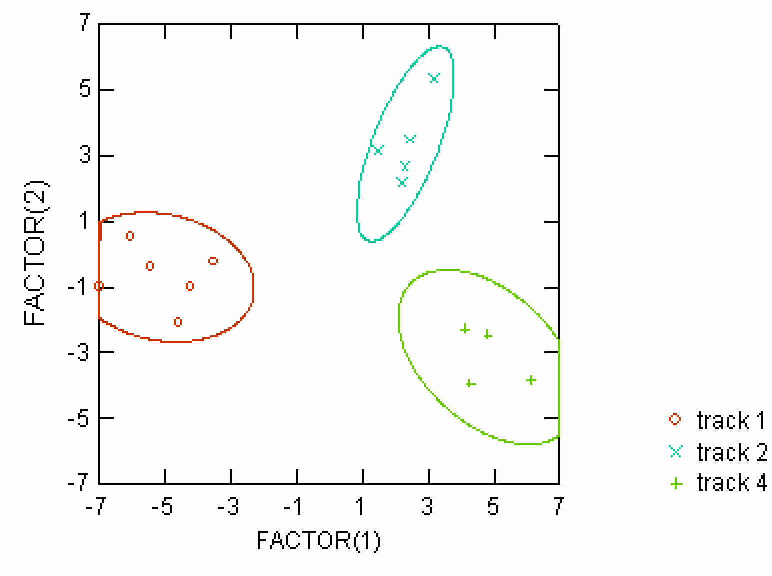

Figure 38. Canonical scores plot showing the results of track separation based on measurements of left hind paw imprints. 95% confidence ellipses representing individual tracks are shown in canonical space, using the first two canonical variables.

Combined analysis of left front and left hind feet

These analyses were performed using sets of variables identified as the best discriminating ones in the previous captive experiments (specifically in section 3.1.3., the analyses of each foot separately using 4 selected best quality imprints). Both types of the stepwise DA, as well as a complete DA were run on: 1) 20 variables (10 best discriminating ones for each foot), and 2) 10 variables (5 best discriminating ones for each foot) – all these variables are listed in Table 24 (first two columns). The third set of variables used was the one identified as the discriminating optimum by the best performing procedure of the combined analysis of both left feet in the captive experiments (Table 27, first column).

Again, stepwise forward DA gave the best results, identifying the three tracks as coming from three different otters with 100% accuracy (Table 41), and also giving a 100% efficiency of the Jacknife cross-validation test (Table 42). These same results were generated employing both 20 and 10 variables in the DA. Fig. 39 shows the visual separation among the three tracks achieved by stepwise forward DA run on 10 selected variables (9 distances, 1 angle).

In Fig. 39, most of the variation between tracks (99%) is explained by the first discriminant function itself, where the function expresses several distance measurements (distances between tips of toes or between the heel and tips of different toes). This suggests that the imprints of the different tracks vary mostly in their size. However, when the same analysis was done employing 20 measurements (15 distances, 5 angles), the same results were achieved, but the first discriminant function explained "only" 87% of the variation between tracks, and was dominated by two distance and one angle measurements. This points to the fact that angular measurements are also important for successful discrimination.

Table 41. Results for stepwise forward DA of 3 otter tracks based on combined analysis of measurements of left front and left hind paw imprints.

	track 1	**track 2**	**track 4**	**% correct**
track 1	3	0	0	100
track 2	0	5	0	100
track 4	0	0	4	100
total	3	5	4	**100**

Table 42. Jacknife classification matrix testing the efficiency of separation among otter tracks based on measurements of both left front and left hind paw imprints. This matrix represents a test of the method from Table 41.

	track 1	track 2	track 4	% correct
track 1	3	0	0	100
track 2	0	5	0	100
track 4	0	0	4	100
total	3	5	4	**100**

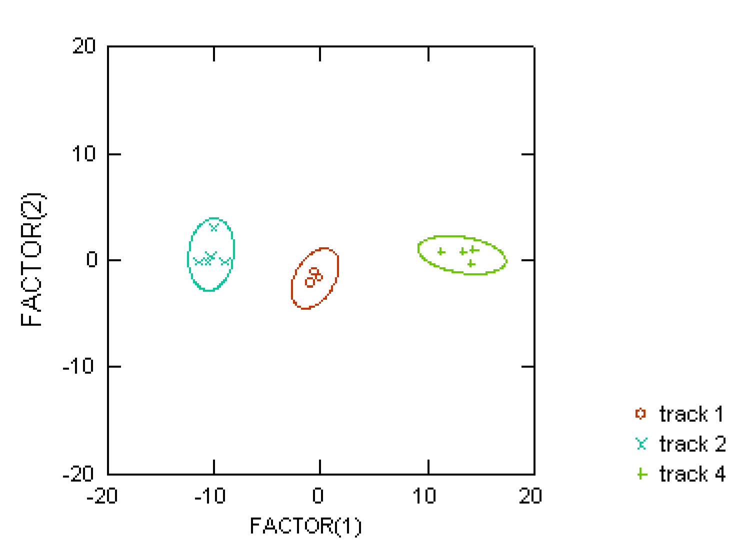

Figure 39. Canonical scores plot showing the results of track separation based on measurements of both left front and left hind paw imprints. 95% confidence ellipses representing individual tracks are shown in canonical space, using the first two canonical variables.

In summary, the four tracks documented during the field study were separated as tracks of four different otters. Separation based on data for each foot separately yielded 100% classification accuracy and 93-95% efficiency of the corresponding Jacknife test. Separation based on data for both left feet (only 3 tracks included) yielded 100% classification accuracy and 100% efficiency of the Jacknife cross-validation test. The combined analyses were performed using only 10 or 20 best discriminating variables, as indicated by the captive experiments.

CHAPTER 4

DISCUSSION

Captive experiments

Overall, the results of this study were comparable to those of other authors where identification of individuals of the same species by their tracks was the aim (Grigione et al. 1999, Jewell et al. 2001, Miller 2001, Hertweck et al. 2002).

When analyzing imprints of each foot separately, 86-93% of the imprints were classified correctly (to the correct otter). However, these values are probably overly optimistic because the classification rule in DA is evaluated using the same cases used to compute it. The more realistic accuracy of classification is suggested by the Jacknife classification matrix, which tests the group assignment by using functions computed from all of the data except the case being classified. The highest value of total correct assignment resulting from the Jacknife classification matrices associated with these analyses was 77%, the lowest only 59%. These lower values for the Jacknife cross-validation were not surprising, since it is typical of situations when there are still too many predictors in the discriminant model. This was a problem in this study as well – the number of original measurements/parameters used in discrimination was extensive and none of the parameters selected by the stepwise procedures explained considerably large amount of variance. Moreover, different sets of parameters were identified as good predictors by the various statistical approaches. However, compared to Hertweck et al. (2002), who also focused on distinguishing individual river otters by their tracks, the number of parameters was slightly reduced. Hertweck et al. (2002) used 33 parameters and subsequently eliminated 10 by a stepwise method of discrimination. Here, 9-17 parameters performed quite well in the stepwise procedures. In general, stepwise backward DA proved to be the best performing method when separating otters based on imprints of each foot separately. It should also be mentioned that sizes of the 95% confidence ellipses in the canonical plots are proportional to the variances of the individual groups, which may potentially affect the accuracy of their pairwise comparisons.

Interestingly, the accuracy of classification in this study was not significantly affected by reducing the number of imprints per otter. Results for analyses of data sets with the numbers of imprints randomly reduced by one at a time (going from 8 imprints /otter to 4 imprints/otter) were fairly similar. The accuracy of Jacknife cross-validation did show a slight decrease with the smaller sample sizes being employed, but the trend was not clear enough to be considered important. This finding is not in agreement with other similar studies (Jewell et al. 2001, Miller 2001), where 8 or 10 imprints per animal/track, respectively, were considered the minimum for good separation.

Moreover, analyses of each foot separately using only 4 best quality imprints per animal yielded better results (96-100% correct classification, 70-87% correct Jacknife cross-validation) than analyses that employed all available imprints (above). This fact further supports the finding regarding sample sizes, and suggests that the quality of imprints (or of their photographs) is more important than their number. It can be easily explained by the “better quality data set” having smaller within group variances (as was the case here), which then in turn helps reveal the between group variances (= separation among otters). For the analyses of 4 best imprints per animal only, stepwise forward DA was in general the most successful method. The number of parameters sufficient for separation was reduced to 8-15.

Even better results were achieved when measurements of either both left or both right feet were combined in one analysis. All stepwise procedures run on such data sets gave a 100% correct classification of imprints, the associated Jacknife cross-validation accuracies ranged from 79% to 90%. This success is likely to be explained by increased variation among individual otters added by combining measurements of two different feet. Furthermore, only 20 parameters identified in the earlier analyses as good discriminators were used in the stepwise DA procedures, significantly reducing the original input and likely also the correlations among the parameters. Both stepwise backward and forward DA performed equally well with these data sets. The combined analyses of either both left or both right feet seem to be best suited for application in the field – they give very high percentages of correct classification while relatively small number of imprints per otter/track are required, and only 20 measurements seem to be generally sufficient for use in the DA procedure. That already represents a fairly standardized method.

Finally, the combined analyses of measurements of all 4 feet together yielded the best results – 100% correct imprint classification and 90-93% accuracy of Jacknife cross-validation. As in the previous combined analyses, only 20 parameters indicated in previous analyses as good discriminators were used as the input for DA, and both backward and forward stepwise procedures performed almost equally well. However, this type of analysis is not likely to be very useful in the field, since obtaining enough high quality imprints of all 4 paws from one track is almost impossible.

In summary, the captive experiments showed that reasonable separation among individual otters is achieved through analysis of measurements of only one foot. Results are further improved by combining measurements of both front and hind feet together and by using only selected best discriminating variables in the stepwise DA procedure. Sets of 20 parameters were identified here to perform well in the combined analyses, but may require additional testing to prove their consistency. These sets of parameters consisted of 15/10 distance measurements, and 5/10 angle measurements (Table 24), including the length and width of the imprint, as well as some distances between tips of toes. The combined analysis of front and hind feet measurements standardized in this way is believed to be potentially useful in analyzing wild otter data.

Field study

The field study showed that collection of wild otter data is possible. In only about 2 weeks, 7 otter tracks were documented and 4 of them were suitable for analysis. Data collection in the field even seems to have certain advantages when compared to the captive experiments. Since Eurasian otters are solitary, typically only one track at a time is found of the animal passing through (Fig. 40) – unless it is a female with cubs. Leaving just one clear track also makes the sorting of imprints (as to right/left and front/hind feet) easier, visible from the walking pattern (Fig. 41). This was a particular problem in the captive experiments. As the otter was active all night in one enclosure and often left “a mess of imprints” on the experimental area with no clear track visible, which then made sorting of the imprints extremely difficult.

Figure 40. One of the tracks documented during the field study (only part is shown).

The field study also identified bridges as the best suited sites for data collection of this type. Otters commonly pass under bridges, using the ledges on the sides. The bridge typically provides a long enough stretch of flat area for long tracks to be obtained. Moreover, the bridge protects the applied mud layer from rain and sun, reducing the possible negative weather effects and making the everyday preparation of the site easier.

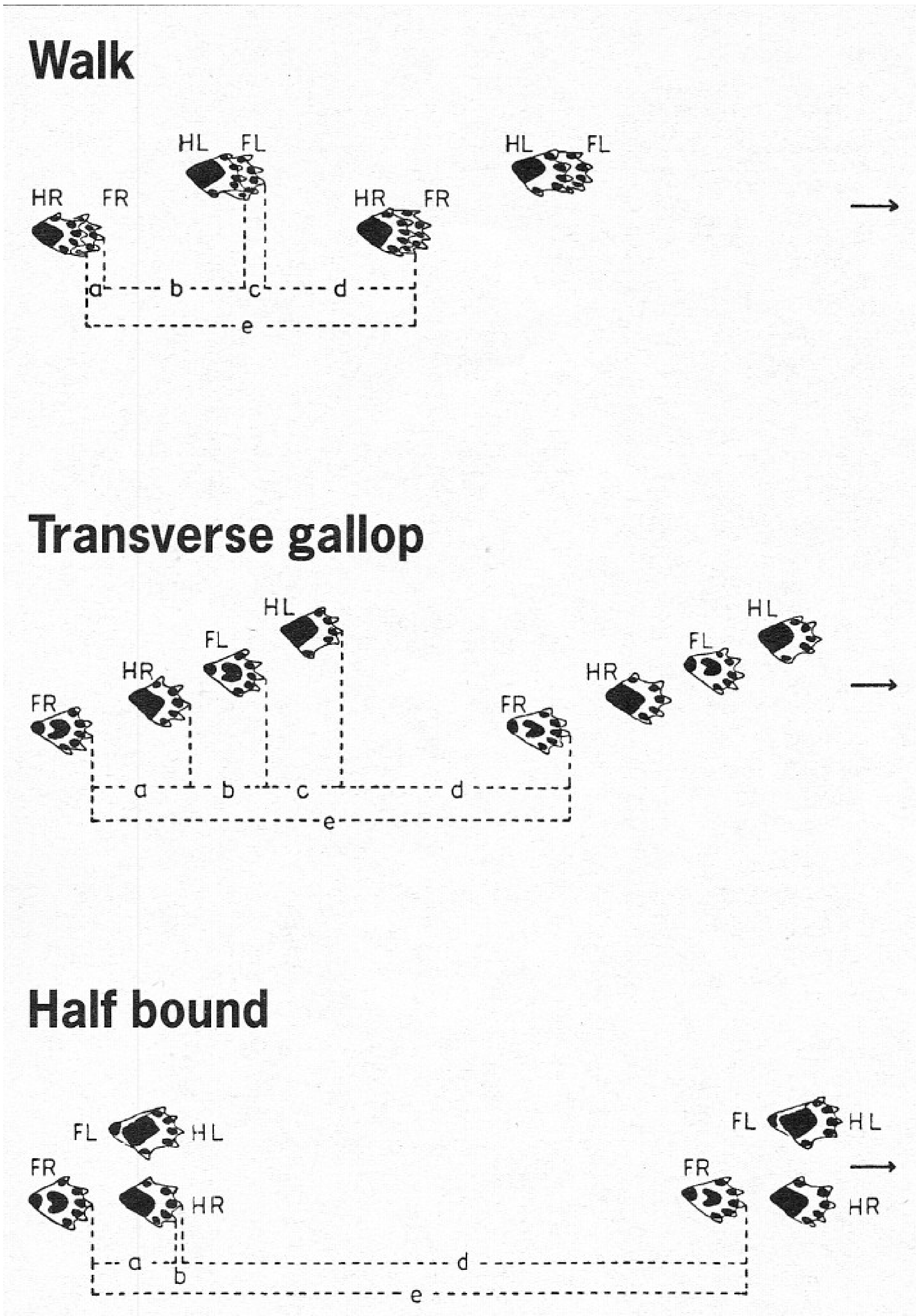

Figure 41. The most common gaits of otters. F = front foot, H = hind foot, L = left, R = right (from Reuther et al. 2000).

The 4 tracks analyzed were identified as belonging to 4 different individuals by both separate analyses of one foot at a time and combined analyses of left front and left hind feet together with high levels of accuracy. Analyzing left front feet, right front feet, and left hind feet separately yielded a 100% correct classification of imprints to their respective tracks in all cases. The accuracy of the associated Jacknife cross-validations ranged from 93% to 95%. These are very good results, considering that the number of variables in these analyses exceeded the number of cases many times. However, the stepwise procedure of DA seemed to take care of this problem and successfully eliminated the unnecessary parameters. In particular, the stepwise forward DA was the one yielding best results for all the field data analyses.

Combined analyses could only be performed on measurements of left front and left hind feet, leaving out one of the tracks due to inadequate sample size. The 3 remaining tracks were separated as belonging to 3 different individual otters with 100% classification accuracy and a 100% Jacknife cross-validation test. Again, stepwise forward DA was the most successful method and more importantly – this perfect separation was achieved while employing only 10, 11, or 20 variables selected previously in the DA. This shows that the sets of variables selected in the captive experiments may really be universally well performing sets of discriminators. Angle measurements proved to be important in discrimination, especially when using front feet imprints.

Overall, the above results support one another in the conclusion that the 4 analyzed tracks were made by 4 different otters. This is an interesting result, considering that all the tracks were collected at the same site (Volfířovský stream). Tracks 1 and 2 were documented on the same day (08/10/06), when a total of 3 tracks were observed, possibly made by a female and her one or two almost adult cubs (there was no visible size difference between the tracks). Tracks 3 and 4 were documented at the same site on 08/11/06 and 08/17/06, respectively. This suggests that many otters visit the site, and therefore their ranges possibly overlap. Roche and Roche (2004) made the same observation, noting that in the Třeboň Biosphere Reserve, males had larger home ranges than females, overlapping or completely covering home ranges of one or two females, and the female home ranges overlapped with those of up to 4 other females.

Although more field experiments are needed to further improve and standardize the method, I believe it has a great potential in censusing wild otter populations, as well as monitoring otter movements and providing information on home ranges, their sizes and overlaps. The combined stepwise forward DA of only 10 or 20 measurements of left front and left hind paw imprints proved to be highly efficient here using only a sample size of 3-6 imprints per track. Of course, this field study analyzed only 4 tracks, and the efficiency can be expected to go down with greater numbers of tracks, or possibly more measurements might be needed as input in the DA to achieve sufficient discrimination (as noted by Miller 2001). However, this problem could be solved by taking a ”pairwise-comparison approach” similar to the one employed by Jewell et al. (2001). Tracks with as many imprints as possible could be collected and then compared to one another in a pairwise manner using the canonical centroid plots, possibly also incorporating a "reference centroid value" (RCV) – as described by Jewell et al. (2001). In this way, a database of otter tracks could be developed, into which direct observations or information (sex, age, etc.) on any known animals of the population could be entered as well. Assuming that (over time) tracks from most otters in the population are obtained, population size estimates could be made. Hopefully, once the database is well established, only a few or even a single imprint might be needed to determine the presence of a given otter at a particular place, which could provide information about their ranges.

In order to achieve the above goals, several things need to be considered:

1. Areas to be surveyed should be examined for suitable bridges and monitoring stations should be established under the bridges, with an effort to cover most water systems in the area. If needed, additional monitoring stations can be established on river or pond banks.
2. The monitoring stations need to be surveyed frequently (preferably every day) to obtain fresh footprints and to maintain good quality of the mud layer. Some newly developed substances could be tested to see whether they can keep the substrate moist (e.g. OASIS® foam submerged in water underneath the substrate as described by Reynolds et al. 2004).
3. The standardized method of data collection and manipulation needs to be kept and carefully followed. Note of the location of each photographed track should also be included in the database, along with any other additional information.

The software used in this study to generate all imprint measurements proved to be very useful. Even though the original number of obtained parameters is extensive, the software is easy to work with and the unnecessary parameters can be eliminated before performing the standardized discriminant procedure. Enabling the software to generate area measurements as well may prove benefitial, as area or shape measurements were reported as very useful by Lewison et al. (2001) in distinguishing individual mountain lions by their tracks.

Other advantages of the proposed censusing and monitoring technique are its low cost and non-invasive nature. No special or expensive equipment is required except for a digital camera and a "tetrapod" (or similar device). Personnel requirements may vary depending on the size of the area being studied, but local-level surveys can be carried out by just one experienced person (although quite time consuming that way). In addition, the proposed method is better suited to be carried out during the summer, possibly also spring or fall. Since the currently most common method for estimating otter numbers is standardized snow tracking (Poledník 2005), and since there are certain seasonal differences or trends in the life history of otters that probably affect their abundance and distribution during different seasons (Roche and Roche 2004), it would be interesting to compare winter and summer surveys from the same area. Of course, the method may have a great potential in southern countries (e.g. Spain, Portugal), where snow almost never occurs and snow tracking is therefore not an option. Furthermore, the above proposed method could be fitted for winter conditions as well given snow quality is good (as done in Hertweck et al. 2002). However, it should be noted that combining tracks made on different substrates in one analysis is not recommended (Grigione et al. 1999).

Finally, I believe that combining this “tracking technique” with a genetic markers method may prove beneficial in the future. The genetic markers method also enables the identification of individuals of the same population (Jones et al. 2004). In addition, it allows the determination of sex ratio in the population and provides information on the population’s genetic structure (heterozygosity, relatedness, etc.; Zemanová 2006). Therefore, the cheaper “tracking method” could be used to monitor otter movements, and the genetic method would aid in obtaining more detailed information on the population under study.

In conclusion, identification of individual otters by their footprints is possible. Using digital camera to take the photographs, software programs to extract the measurements, and discriminant/canonical statistics to analyze the data gives reasonable accuracies of classification, both in captivity and in the field. Although further experiments may be needed, the method was improved here by combining measurements for front and hind feet and by identifying the best discriminating variables. The method may prove useful in population monitoring and censusing in the future, mostly because it is non-invasive, cheap, and at least for local level studies does not require too many personnel.

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BIOGRAPHICAL INFORMATION

Jitka Větrovcová was born in Prague, Czech Republic, on March 3, 1981, to Marie Větrovcová and Oldřich Větrovec. She attended elementary and secondary schools in Prague and graduated from Gymnázium Nad Alejí high school in Prague in 1999. Year later, she received a full athletic scholarship at the University of Texas at Arlington and played tennis for the university for four years, while pursuing a Bachelor of Science degree, majoring in Environmental Biology. She received this degree in May 2004, and entered the Graduate School of the University of Texas at Arlington the following fall semester. She worked as a graduate teaching assistant while pursuing her Master of Science degree. She is interested in the ecology of the weasel family and would like to do more research on these species in the Czech Republic and Central Europe.