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Day resting site use and fidelity of Alpine otters (*Lutra lutra*) on southeast Austria

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MESTRADO EM BIOLOGIA DA CONSERVAÇÃO
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To Stefan, who was (is!) behind my motivations to travel to Austria, for all the love, care and understanding (especially when I was in "dragon" mode).

ABSTRACT

The availability of resting sites is known to influence the distribution and density of animals and they are key resources to nocturnal mammals that need to spend daylight in safe refuges. This is the reason why these structures have to be taken in account in the efforts of mammal species conservation. Aside from being simply a place to rest, these structures also provide natural protection against predators and thermal isolation in harsh environments. In the 1960's Eurasian otters (*Lutra lutra*) crashed in many parts of its European distribution, this being the case of Austria. With the species re-colonization, favored by an interconnection with the Czech and the Hungarian populations, a need emerged for a better understanding on how animals are using such resources in the Alpine landscape as the species is still considered endangered.

The main goals of this study were to evaluate (assess) if: (1) there is a structural pattern of daytime resting site according to gender and age; (2) human disturbance affects the choice of resting site type; (3) snow cover has any influence on the type of resting site used; (4) rest-site choice can be predicted based on environmental variables and (5) there is a geographic pattern in resting site choice.

Four otters (two adult females, one adult male and one juvenile female) were captured and radio-tagged to locate resting structures which were characterized according to a set of physical and environmental parameters. Daily climate data was obtained from local weather stations.

In spite of sampling size constrains results revealed that underground structures were used more often than surface lairs and suggest a different use of resting site type between females and breeding females. No relation was found between rest-site type and human density. Snow cover influence could not be asserted due to environmental conditions during the sampling period, but temperature, vegetation cover and river width seem to play a significant role in predicting resting site choice. Concerning the geographical pattern, the results, framed by previous studies, imply its existence but it was not possible to identify the factors behind it.

Keywords: Eurasian otter, Resting ecology, Resting sites use pattern, Radio-telemetry

Resumo

Pertencendo à família dos mustelídeos, a lontra Euroasiática (*Lutra lutra* L. 1758) é a espécie de lontra cuja distribuição é mais alargada. Pode ser encontrada na Europa, na maior parte da Ásia e ainda no Norte de África. É uma espécie exclusivamente aquática que utiliza tanto sistemas lênticos como sistemas lóticos e ainda zonas costeiras. São animais solitários ao longo da sua vida, alimentando-se na sua maioria de peixes, embora possam alimentar-se de vários outros grupos animais. Esta espécie, à semelhança de outros mamíferos, passa uma grande parte do dia no interior de abrigos. Estas estruturas conferem-lhes protecção contra predadores e isolamento térmico, além de um local para dormir. Os refúgios desenpenham um papel fundamental no ciclo de vida dos animais, podendo em situações de escassez, determinar a sua densidade e abundância.

Na década de 1960 as populações de lontra sofreram um declínio acentuado em grande parte da sua distribuição original. Este sentiu-se principalmente a nível Europeu, embora a situação seja maioritariamente desconhecida para outros locais da sua distribuição. Alguns países, como a Suíça, chegaram mesmo a uma situação de extinção local, da qual apenas recentemente começam a recuperar. Apesar das causas deste declínio serem variadas, todas têm em comum a sua origem humana. As quatro ameaças principais devem-se à perseguição directa, destruição de habitat, poluição por metais pesados e rarefacção dos recursos alimentares.

Com o aumento do número de lontras a re-colonizarem a parte Este dos Alpes, torna-se fundamental compreender melhor determinados aspectos da sua ecologia nos sistemas fluviais Alpinos.

O presente estudo teve por objectivo a caracterização e identificação dos padrões de utilização de abrigos diurnos por parte da lontra no Sudoeste Austríaco. Foram testadas as seguintes hipóteses: (1) existe um tipo estrutural de abrigo mais usado consoante a idade e sexo dos indivíduos; (2) abrigos mais expostos serão utilizados em áreas menos perturbadas e vice versa; (3) após a chegada da neve, locais que beneficiem de isolamento térmico serão mais usados; (4) é possível prever o tipo de estrutura escolhido com base em variáveis ambientais e (5) existe um padrão de utilização geográfico dos vários tipos de abrigo.

Para responder às questões colocadas, recorreu-se à utilização de radio-marcação e de rádio-telemetria. Foram efectuadas 2 sessões de armadilhagem (Maio 2010 e Novembro 2010), que resultaram na captura de 4 indivíduos, dos quais duas fêmeas adultas, um macho e uma fêmea juvenil. Os animais foram localizados 47 a 187 vezes ao longo de 9 meses. Sempre que um indivíduo era localizado e considerado inactivo, registaram-se as características do abrigo e factores do ambiente envolvente tais como variáveis relacionadas com o curso de água, como largura, profundidade e tipo de margem; e outras relacionadas com a presença humana, como distância a estradas e povoações. Além destas caracterísitcas, registou-se ainda o tipo de habitat em que os abrigos se inseriam e a sua constituição estrutural, resultando em cinco tipos diferentes de abrigo. Posteriormente, foram obtidos dados relativos às temperaturas diárias minímas e máximas, bem como à precipitação diária. Estes dados foram obtidos em duas estações meteorológicas localizadas na área de estudo recorrendo-se aos dados da estação mais próxima ao território de cada animal.

Por os dados não cumprirem os pressupostos da estatística paramétrica na análise dos dados foi utilizada estatística não-paramétrica. De modo a facilitar o tratamento de dados, os animais foram agrupados em três classes: fêmeas, macho e fêmeas com crias. Para analisar a fidelidade dos indivíduos aos abrigos, calculou-se o índice de reutilização correspondente. O mesmo índice, foi também comparado para cada tipo de abrigo antes e depois da cobertura de neve se instalar. Foi ainda calculado para cada abrigo a sua frequência de uso relativa, de modo a determinar a existência de uma preferência por um ou mais tipos de abrigo. De modo a evidenciar padrões estruturais dos vários tipos de abrigo, foi realizada uma análise de ordenação. Os resultados desta ordenação indicaram a separação de dois tipos de abrigos: de superfície ou no subsolo. Seguidamente foi efectuada uma análise de regressão logística testando-se a existência ou não de correlação entre as variáveis originais. Foram introduzidas dez variáveis no modelo inicial, sendo reduzidas a quatro após um procedimento de *backward stepwise*. Após a verificação de que o modelo final e o modelo inicial não eram significativamente diferentes, testou-se a adequabilidade e precisão do modelo final.

Constatou-se que não existem diferenças estatísticas entre a taxa de reutilização das três classes de indivíduos, embora os dados sugiram uma diferença ao nível das fêmeas reprodutoras e não reprodutoras. Não existiu também uma diferença ao nível da utilização de abrigos consoante a presença/ausência de cobertura de neve. Este resultado estará provavelmente relacionado com a época em que três dos indivíduos

foram capturados, já que a temperatura do ar já estava a decrescer antes dos animais começarem a ser seguidos.

Embora não tivesse sido possível concluir àcerca de uma relação entre áreas muito ou pouco humanizadas e o tipo de abrigo, confirmou-se a reacção desta espécie à presença humana, em que apenas pertubações de cariz directo, causaram a fuga do animal. Na maioria das situações, o indivíduo permaneceu no abrigo, apesar de alguma pertubação bastante próxima.

As variáveis estatisticamente relevantes obtidas no modelo de associação abrigo-habitat foram a temperatura, a cobertura vegetal e a largura do rio. Segundo os coeficientes obtidos, quanto mais elevada for a temperatura, maior é a probabilidade de um abrigo à superfície ser escolhido. O contrário acontece quando a temperatuda desce. Em relação à vegetação, esta tem um papel muito importante na existência de abrigos, já que é responsável (directa ou indirectamente) por muitos dos abrigos utilizados. Uma menor largura do rio está relacionada, embora não tão claramente como as variáveis anteriores, com a escolha de abrigos à superfície.

Embora existam indícios de que existirá um padrão geográfico, não foi possível discernir que factores estarão na origem desse padrão.

Demonstrou-se a importância de algumas estruturas resultantes de actividade humana; os abrigos constituídos de pilhas de ramos ou troncos foram bastante utilizados. Assim, recomenda-se que a sua gestão se mantenha como até então, de modo a permitir a sua utilização contínua.

Tendo em conta o tamanho da amostra, alguns resultados não significativos podem estar a ser mascarados. De modo a confirmar estas situações, seria necessário analisar uma amostra maior. A nível da existência de um padrão geográfico, será necessário efectuar estudos mais concretos e direccionados a essa temática, uma vez que os dados existentes são bastante heterogéneos.

A informação recolhida permitirá decisões mais ponderadas a nível da ecologia do repouso da lontra, i.e. à cerca de factores que possam ser limitantes. Este estudo permitirá também analisar que factores ambientais são favoráveis à re-colonização e expansão da espécie, beneficiando a sua conservação.

Palavras-chave: Lontra euroasiática, ecologia do repouso, padrão de utilização de abrigos, rádio-telemetria.

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Chapter 3

HAPTER 1

General Introduction

Identifying the essential resources of a species is crucial to an area like conservation biology (Weber 1989). Mustelids, as many other mammals, are mostly nocturnal and spend the majority of the day concealed inside resting structures (e.g. Beja 1996, Kowalczyk et al. 2004; Baghli & Verhagen 2005). While offering thermal isolation (López-Martín et al. 2005), these structures also provide natural protection against predators (Buskirk 1984; Lesmeister et al. 2008) aside from being simply a place to rest. Avoiding predation has obvious advantages, whilst thermoregulation is costly for this animal group (Weber 1989) due to their elongated body shape. Besides these factors, there are others shaping the rest-site choice, like their location regarding a food source (Lutermann et al. 2010) or the presence of an escape route (Ruggiero et al. 1998). For these reasons, the location of these sites is rarely arbitrary. For example, American pine martens (*Martes americana*) use mainly other animal's nests (Buskirk 1984) and resting site choice is associated with thermal benefits (Spencer 1987). Polecats' (*Mustela putorius*) choice seems to be dependent both on food availability and sufficient warmth (Weber 1989). In the end, site preferences depend on different factors, because individuals tend to select habitat characteristics that maximize their fitness in conformity with their current biological needs (Szor et al. 2008). As a result, the occurrence of fitting resting sites is determinant for considering a habitat as suitable (Fernández & Palomares, 2000).

For mustelids, lower numbers of suitable resting sites can diminish their densities and increase intraspecific competition for this resource (Zalewski 1997a; Purcell et al. 2009). In these conditions, rest-site availability may shape the territory size (Zalewski 1997b) as the use of certain sites "can influence an animal's time- and energy-budget" (Weber, 1989).

Eurasian otters

Otters (*Lutra lutra*) are one of the few amphibian groups of mustelids, being represented by 13 species belonging to the Lutrinae subfamily (Mason & Macdonald 1986). Of all otters, merely one species of river otter can be found throughout Europe: the Eurasian river otter (*Lutra lutra,* L. 1758). Eurasian otters' distribution includes, as the name says, Europe and Asia but it can also be found in a small region on the North of Africa (Conroy & Chanin 2002). This otter occupies a variety of habitats, being found in freshwater systems, either lotic (rivers and streams) or lentic (lakes, pounds, swamps and, recently, dam's reservoirs) (Mason & Macdonald 1986; Kruuk 2006). Surprisingly, it can also dwell in coastal systems, providing a freshwater source is near (Beja 1989). Feeding mostly on fish, this mustelid also takes in its diet a diversity of other species like amphibians, crustaceans (Mason & Macdonald 1986; Kruuk 2006), reptiles (Remonti et al. 2009), small birds and even some small mammals like rabbits (Carss 1995). Otters, as the majority of mustelids, are solitary single-living species (Erlinge 1968).

Due to their secretive and largely nocturnal habits and little individual distinctiveness, otters are extremely difficult to study (Macdonald & Mason 1994; Kalz et al. 2006). For these reasons, this animal has been mainly studied through the use of signs of presence, like spraints and footprints (White et al. 2002). Other methods using infrared technology are also being tested and developed (Garcia de Leandiz et al. 2006) in order to monitor elusive riparian mammals.

Melquist & Hornocker (1983) were the first to use radio-telemetry in otters (*Lutra canadensis*) in Idaho. Green and his team (1984) published an extensive article about radio-tracked otters (*Lutra lutra*) in northern Scotland. Both Green et al. (1984) and Rosoux & Libois (1996) used radio-collars modified into harnesses. However, this methodology was abandoned and intra-peritoneal implanted transmitters were used instead (e.g. Kruuk et al. 1998). Most radio-telemetry efforts focus on reintroduction studies (e.g. Sjöåsen 1996; Saavedra 2002) or habitat selection (e.g. Ruiz-Olmo et al. 2005) and just a few on resting sites (Beja 1996; Jimenéz & Palomo 1998). Nevertheless, rest structures began to be studied before the radio-tagging of individuals. Erlinge (1967) described underground rest structures with two entrances, being one of them underwater, as a typical otter resting site. With the arrival of radio-telemetry, it was discovered that rest sites with these precise characteristics are scarcely found, and rest structures are frequently undetectable without this technique (Green et al. 1984). Moreover, otters seem to use resting sites opportunistically and do not usually build them, profiting from other animals' burrows (Erlinge 1967).

<u>European background and present situation</u>

Central European countries (Germany, Italy, Switzerland and Austria) were largely affected by the *Lutra lutra* population crash in 1960's (Macdonald & Mason

1994). The same is true for other countries were Eurasian otter is found, but since the present project is being developed in the Alps region, emphases will be made on this area. Otter situation is mostly unknown in Asian countries, though researchers like Cho et al. (2009) are now beginning to fill this gap. There are many combined causes related to the general decline in otter numbers, felt in Europe in the second half of the XX century. In one way or another, all of them are exclusively related to human activity (Mason & Macdonald 1986). These causes can be included in four major threath categories: direct persecution, habitat destruction, habitat degradation by pollution and simply the lack of food supplies (Saavedra 2002; Bedford 2009).

While vulnerable to the threats mentioned above, otters benefit now from a good conservation management, allowing them to slowly recover from the decline felt decades earlier. There is now evidence from several European countries of an increase in otter numbers (e.g. Kranz et al. 2001; Conroy & Chanin 2002). This is mainly due to three reasons: i) the fact that otters are protected in almost every European country (Saavedra 2002), ii) a heavier control of pollutants like PCBs through EU legislation (Simpson et al. 2000) and iii) the development of pisciculture activities, providing new habitat opportunities particularly in terms of prey (Kranz et al. 1996).

Austrian context

The status of this species varies significantly between different parts of Austria (Gutleb 1992). Despite the fact that 80% of the country can be deemed “otter-free” (Bodner 1995), *Lutra lutra* can be found at least in four counties: Lower Austria, Upper Austria, Styria and Burgenland (Gutleb 1992). There has been recent evidence of an increase in otter numbers (Kranz 1994), as they are favored by an interconnection with two other populations (Czech Republic’s and Hungary’s) (Kranz et al. 2001). However, the species is still regarded as endangered in Austria (Conroy & Chanin 2002). While there have been evidences of illegal hunting actions, several carcasses with old fragments of bullets having being found in the Lower Austria county (Gutleb et al. 1995), it is forbidden to hunt otters in Austria since 1947 (Bodner 1994). On the origin of these human-otter conflicts is probably the increase of fish-farms in the same region.

Project Lutra alpina, in the frame of which this study was conducted, is being financed by two Swiss organizations (ProLutra & Zurich Animal Protection Society) in collaboration with the University of Zurich. This project aims to ensure the natural return of the Eurasian otter to Switzerland's subalpine and Alpine valleys. In order to achieve this, the project was created as a mean to learn the essential requirements for this species survival in the Alps, and ultimately to improve habitat in the Swiss river systems. PCBs contamination in fishes was appointed as the main factor for the otter populations' decline but this does not quite explain why the species disappeared from pristine river systems in the Swiss Alps region (Weber 1990).

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HAPTER 2

Sleeping like an otter: patterns of day resting site use on an Alpine landscape.

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Abstract

Otters can spend up to 70% of the day inside a resting site. These structures can have key effects on the abundance and distribution of the species and are especially important in certain periods of their biological cycle (e.g. resting and breeding). The following study took place in the Austrian Alps from May 2010 to January 2011. Four radio-tracked otters (3 females and 1 male) were followed and data concerning resting site choice was registered. During the study, underground structures were used more frequently (62%) than above ground sites. Females and females with cubs seem to differ in their use pattern of the different types of resting site. A relation between temperature, vegetation cover and width of the river, with the resting site relative positioning (i.e. above or below ground) was discovered. Data from previous articles concerning otter resting sites were analyzed in order to find if there is a geographical influence on rest site choice. Conservation implications for otter re-colonization and expansion are discussed.

Mammals are known to spend a large amount of time being inactive (Elgar et al. 1988), within resting site structures. These structures are known to provide thermoregulatory advantages and protection from predators (e.g. Buskirk 1984; Weber 1989; Bakker & Hastings 2002; Lesmeister et al. 2008). Proximity to food resources is also often shaping the choice of a resting site (Lutermann et al. 2010). Furthermore, resting site preferences can change with the biological cycle of the animals: for example a breeding female has to select suitable structures which provide safety but also food in its vicinity, rearing new born cubs while minimizing their mortality (Ruiz-Olmo et al. 2005). It is already known that these structures play a central role in mustelid populations (Birks et al. 2005).

For otters (*Lutra lutra* L. 1758), one of the few amphibian mustelids, resting can go up to 70% of the day (Green et al. 1984; Beja 1996; Saavedra 2002). Otter resting sites' have been studied through the use of radio-telemetry, few with harnesses bearing transmitters (Green et al. 1984; Rosoux & Libois 1996) and most with intra-peritoneally implanted transmitters (e.g. Melquist & Hornocker 1983; Beja 1996; Saavedra 2002). The number of animals followed was never more than four and more females were trapped than males (e.g. Kruuk et al. 1998). There is almost no agreement in the categories used to classify resting sites, except that all authors grouped them into two major groups: underground dens and surface lairs/couches.

It was already observed for other species of mustelids that resting site choice is influenced by environmental features. Zalewski (1997) discovered that not only temperature influences pine martens rest site choice, but also it reflects a geographical pattern. A similar result was obtained for fishers (*Martes pennanti*), in which the individuals selected subnivean sites when temperatures where below -14ºC (Weir et al. 2000). Concerning otter rest site choice, Rosoux & Libois (1996) noticed a relation between type of resting site and populated areas. Couches were associated with less humanized sites, while dens were located in places where human disturbance was stronger. Aside from this, intraspecific pressure can also alter resting site selection; for example, Mason & Macdonald (1986) discovered that breeding females may have temporary territory dominance over both males and non-breeding females.

This kind of information on resting sites would not be possible to obtain without radio-tracking; in fact, chances are that less than 10% of the rest sites were recognized as such (Green et al. 1984). These structures seem to influence otter distribution and abundance (Mason & Macdonald 1986), particularly since they play a limiting role in some areas of this animal's geographical range (e.g. Beja 1996; Ruiz-Olmo et al. 2005). Since after a generalized European decline the otter is slowly returning to its previous distribution (Macdonald & Mason 1994), knowing which factors facilitate this re-colonization is a major contribution for conservation in regions where these animals went extinct (like Switzerland – Weber, 1990; or Netherlands – Kruuk 2006).

In Austria, where otters are still considered endangered (Conroy & Chanin 2002), population trends indicate a clear increase of numbers (Kranz 1994) as they are favored by an interconnection with two other populations (Czech Republic's and Hungary's) (Kranz et al. 2001). With the increase of the otter numbers in Austria, mostly in the eastern part of the Alps, the need for ecological studies arises, especially considering that such studies were never carried out on Alpine river systems. This research was carried out under the project Lutra alpina.

The aim of this study is to characterize otter resting sites on an Alpine environment and their use patterns. Five main hypotheses are tested in this study: 1) Rest-site use pattern differ with sex and breeding status; 2) Above ground structures are favored in isolated places, while underground lairs are used in more human disturbed areas; 3) Snow cover influences the choice of resting site type, with thermal isolated structures being used more often; 4) The choice of resting structure can be predicted using environmental parameters as surrogates 5) Geographic location shapes the use of resting site type differently. The contribution of the results obtained will be discussed in the light of the successful re-colonization of alpine environments by otters and their long-term conservation.

METHODS

Study site

This study took place in two areas in the southeast of Austria, in the state of Styria. The study areas were basically delineated by the home range of the otters being tracked at each location. This included a valley dominated by the presence of the 24km long river Laming which encompassed the range of two adult females and the area connecting the city of Kapfenberg, the village of Innerzwain the small valley called Hinterberg, holding the territories of an adult male and a sub-adult female.

Landscape is dominated by Alpine and subalpine valleys with altitudes ranging from 468 to 1629m (http://www.bruckmur.at/die-stadt/geographie accessed on 29/09/2011). The area, as most of Austria, is characterized by an Alpine climate, typically with short summers and long winters. Habitat includes coniferous forests (strong presence of spruce species), mountain mixed forests (in which broadleaf trees are dominant), grassland and pastures. Human settlements are present throughout the study area and main roads are often quite near the water bodies used by the radio-tracked otters. In all study regions, recreational fishponds are present.

Otter trapping and handling

Four otters were captured during two trapping sessions (May and November 2010), one male and three females (Table 1). Animals were trapped with soft-catch foot-hold traps

(#2 Victor® Long–Spring–Woodstream Corporation, Lititz, Pennsylvania, USA), fitted with trap transmitters. Captured individuals were sedated with Ketamine with midazolam, as recommended by previous literature (Spellman et al. 1993 in Ó Néill et al. 2008), being subsequently placed in a transport box and taken to the local veterinary. There, after light surgery anaesthesia was induced, the animals underwent surgery in order to implant the radio transmitter intra-peritoneally. The mortality-sensitive radio transmitters (Telonics® Inc., Mesa, Arizona, USA) use a VHF system, transmitting frequencies around 150 mHz. Their size was decided upon capture after weighting the otter so that a maximum of 5% of its body weigh was not exceeded as recommended (American Society of Mammalogists 1998). A few hours following surgery and after fully recovering from anaesthesia, animals were released in the surroundings of the capture site.

Animals were classified into three classes according to both age and sex for comparison purposes: females (Alena & Baujke), females with cubs (Cleo) and males (Dan). One of the adult females (Alena) showed signs of breeding during the tracking period, but she lost the offspring shortly after they were born. It is common, in mammal species, for young females to fail to rear their first litter (Kruuk 2006) and presumably that was the case with Alena. Because of this, the breeding period could not be used, preventing her inclusion in the "female with cubs" category. The sub-adult Cleo had an estimated age of four months, which was based on tooth development and wear, allowing for a comparison between breeding and non-breeding females. This is possible since cubs are known to stay with the mother until their independency, which happens usually in about one year (Kruuk 2006).

Table 1 - Number of resting sites (RS) and diurnal locations for each radio-tracked animal

Otter	Sex	Age	Tracking period	Resting sites	Daily Locations
Alena	Female	Adult	7.05.2010-30.01.2011*	31	187
Baukje	Female	Adult	6.11.2010-30.01.2011*	5	56
Cleo	Female	Sub-adult	09.11.2010-30.01.2011*	13	47
Dan	Male	Adult	10.10.2010-30.01.2011*	9	54
			Total	**58**	**344**

* Period referred concerns data used in this study; animals continue to be tracked in the frame of the project.

Otter resting sites

Individuals were regularly positioned by the homing-in method (White & Garrot 1990) with a Sika receiver from Biotrack Ltd. (Dorset, UK) and a hand-held H-type aerial. Animals were found resting both during night and daytime. However, resting sites exclusively used during the night were hard to recognize without disturbing the animal and daylight excursions to the places often did not reveal an obvious structure. For these reasons, no nocturnal resting sites were used in this approach even though a few were used.

Whenever an animal was located during daytime and deemed inactive, this place was considered as a diurnal resting site. Tagged individuals were located resting during daylight between 47 and 187 days (Table 1). For all animals, the detection rate (number of days an animal was found by the number of days an animal was searched) of the resting structures was 92%. For each location the correspondent UTM (Universal Transverse Mercator) coordinates were taken by a portable GPS device (eTrex H, Garmin) with less than 10 meters of error. All sites were categorized according to their structure and environmental parameters: water-related variables (distance to water, width, depth, current speed and margin type) and surroundings (habitat, distance to roads and villages, altitude and vegetation height class).

Meteorological data

Climate data was obtained from two weather stations, one located in Kapfenberg and the other in Aflenz (Fig. 1). The first was used to gather meteorological data for the two adult females, since their territories were closer to this weather station. The cub and the male were usually closer to Aflenz and so, the data from the former station will be used for their resting sites. The data obtained included minimum and maximum daily temperatures, and also daily precipitation. During this study, temperatures ranged from 34,1 to -16,5 °C in Kapfenberg and from 31,3 to -20,1 °C in Aflenz (since May 2010). Precipitation was almost non-existent throughout the duration of this study.

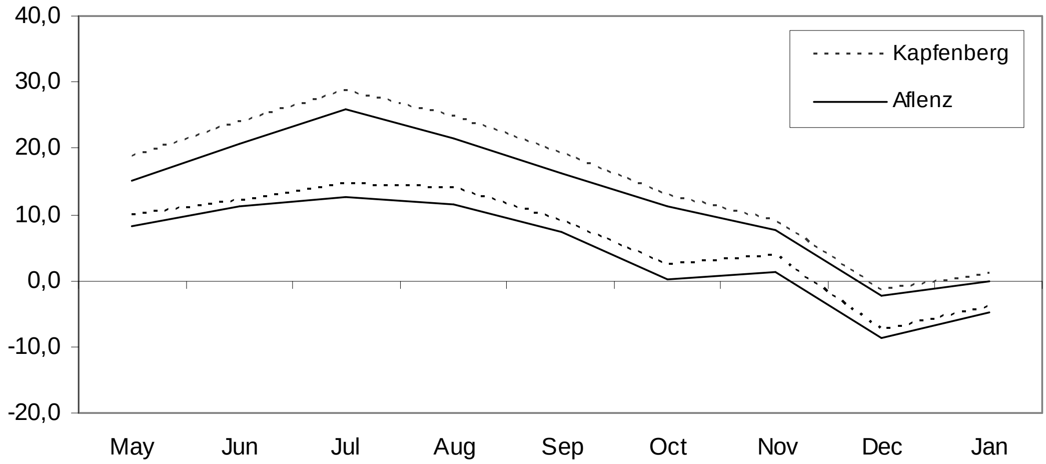

Figure 1 – Average daily minimum and maximum temperatures per month and per weather station.

Data analysis

In order to assess if the sampling effort was appropriated to determine all the resting sites used during the tracking period, a resting site discovery cumulative curve was constructed for each individual. Preference of the different types of resting sites was determined by the amount of days individuals spent inside each type. Values were modified as to represent the proportional use for all individuals (since they differ in the total number of days located) and then compared. Individual rest-site fidelity was assessed through the re-use index (Zalewski 1997; Baghli & Verhagen 2005), where the number of resting sites found is divided by the number of total diurnal locations.

In most cases, the raw data collected did not meet the basic assumptions to allow the use of parametric tests. Values transformation did not help to achieve normal distribution; as such, only nonparametric tests were used. The software STATISTICA version 8.0 (StatSoft Inc. 2007) was used for all non-parametric tests: Wilcoxon matched-pairs, Friedman ANOVA, Mann-Whitney and Kruskal-Wallis (Zar 1999).

In order to identify patterns in the structure of the resting sites, an ordination method (non-metric multidimensional scaling – NMDS) was used. Two types of daytime resting sites ("Reed" and "Artificial structures") were excluded from the analyses, since they lacked sufficient numbers. Hence, only the types "Wood pile", "Root system" and "Underground den" were used in the NDMS. This was repeated for four dimensions (2 to 5) where stress and R^2 values were compared in order to select the best ordination.

A logistic regression was carried out in order to determine which of the environmental parameters surrogates best predicted resting site use. Prior to this, non-parametrical correlations were used to assess relationships within two groups of variables: meteorological variables and variables corresponding to rest sites physical attributes. The significance threshold considered for the Spearman correlations was of $\alpha = 0,01$ for both analysis. Resultantly, ten variables, three continuous and the rest discrete, were initially included in the regression. The final model's accuracy in resting site type classification was assessed based on a logistic cut point of 0,5. This value was deemed appropriate after a ROC curve analysis (Quinn & Keough 2002).

R version 2.13.0 (R Development Core Team 2011) was used for the ordination technique – package vegan (Oksanen et al. 2011) –, the logistic regression – package MASS (Vernables & Ripley 2002), and the ROC curve procedure – package Epi (Carstensen et al. 2011). Every statistical test was carried out with $\alpha = 0,05$ unless indicated otherwise. This value was corrected with a Bonferroni adjustment (Quinn & Keough 2002) when multiple comparisons were needed.

RESULTS

Sample size

Analyzing the resting site cumulative curve (Fig. 2), it can be assumed that all of the resting sites used during the study period were identified. This is also supported by a rest site detection rate of nearly 100%. While there are noticeable oscillations in the total number of new sites at a certain time, these changes are not constant and without these oscillations, the curve reaches an asymptote. Since the assumption that all rest sites used were discovered is met, sample size problems are somewhat reduced; even with a small sample and a tracking period that is not ideally long.

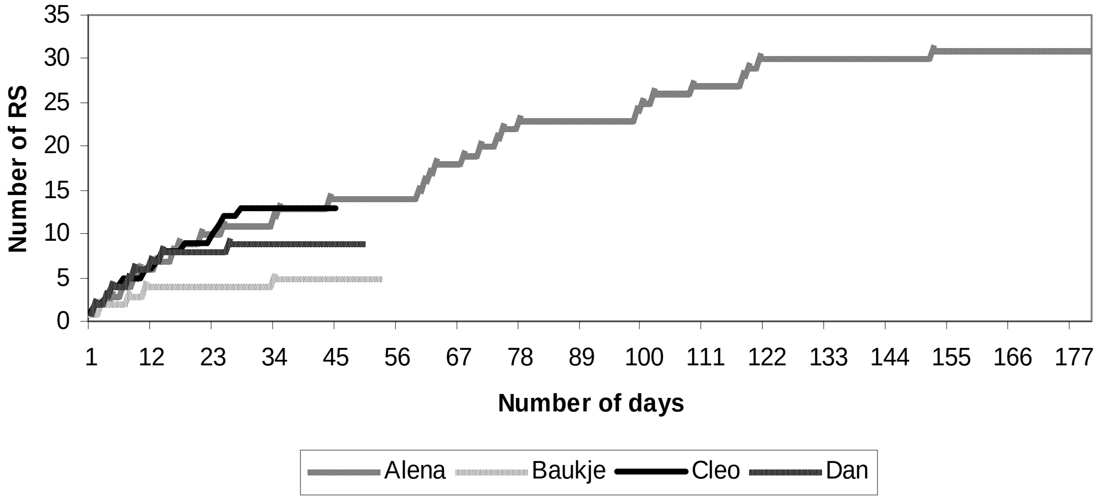

Figure 2 - Cumulative relationship between number of new resting sites and days of radio location

Resting site description

A total of 58 resting sites were located during the nine months of this preliminary study (Table 2). Eight of these could not be unequivocally identified and described, and another could not be further used in the analysis since it was destroyed. As expected, the majority of the daytime rest sites found were not built by the animals, but were natural or man-made structures. Wood piles comprised for the largest group of resting site type (23 of 50), including both stick and log piles. The second type included root systems with 15 sites recorded (Table 2). Other types consisted of underground dens, artificial structures (an old weir wall and tunnels inside of an industrial complex) and a reed bed (*Phragmites sp.*). Resting structures were found in an average of 275 meters (range: 538-813m) of altitude. Most of them (96%) were not located more than five meters away from a water body. Of all considered resting sites, 31 were located in riverside woodland, while only 20% (n = 10) were in the forest. There was no relationship (correlation) between the use of the main types of the resting sites and distance to roads and villages.

Table 2 - Number and use (locations) of the different types of daytime resting sites per individual

Animal	Type and use of daytime resting site				
	Root system	**Underground den**	**Artificial structures**	**Wood pile**	**Reed**
Alena	4 (27)	2 (32)	1 (4)	19 (80)	1 (12)
Baukje	1 (46)	2 (7)	0	1 (2)	0
Cleo	7 (36)	3 (5)	0	2 (2)	0
Dan	3 (28)	2 (12)	1 (4)	1 (4)	0
Total	**15 (137)**	**9 (56)**	**2 (8)**	**23 (88)**	**1 (12)**

Rest site use

Of all the rest sites, 30% were only used once and from these, 57% correspond to underground dens. In fact, the majority of all sites (60%) were not occupied more than four days (Fig. 3). No differences (Kruskal-Wallis: H = 5,25; P = 0,629) were found between the frequencies of use for the different types of resting sites. For this reason, it does not make sense to compare the characteristics of the reused sites against the sites only used once. The male otter used the same resting site for 5 consecutive days, but it was one of the females that spent the maximum number of consecutive days (n = 10) inside the same resting structure. However, consecutive use was rare, as the animals moved daily from a resting site to another. Sometimes, this change was just a few meters up or down stream.

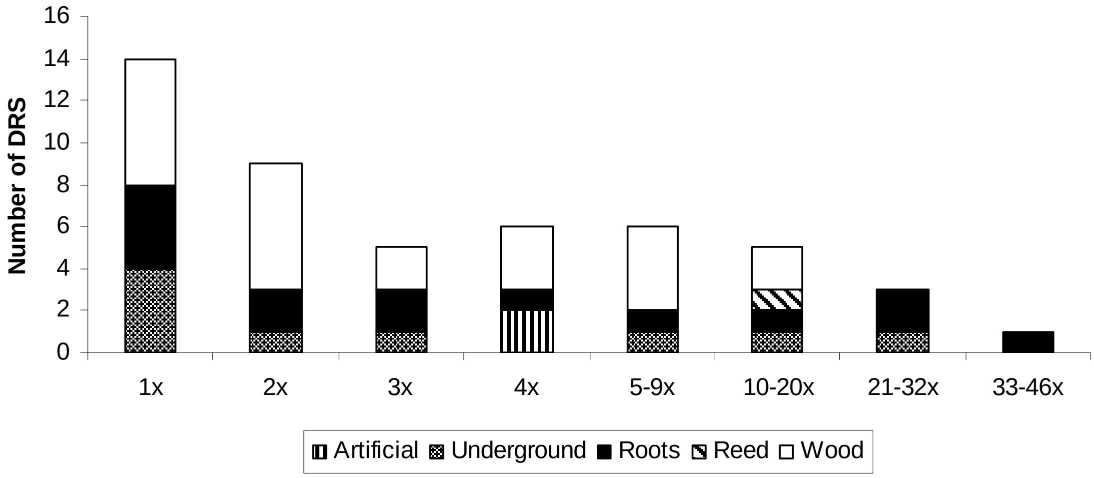

Figure 3 - Total number of times each resting site type was used.

Reuse index differed between sex/age classes (Kruskal-Wallis test: H = 6,46; P = 0,039). A post-hoc test using Mann-Whitney tests showed significant differences between females and females with cubs (Z = 2,22; P = 0,026). However, the threshold for significance with a Bonferroni adjustment is slightly lower (α = 0,016) altering the p-value obtained to non significant. The reuse index for the male did not show differences from both classes of females. Moreover, presence of snow cover does not appear to influence the reuse of the rest sites differently (Wilcoxon matched pairs test: Z = 0,18; P = 0,859), although, underground rest sites seemed to be used more often than above ground ones when snow cover was present.

Resting site choice

There was a difference found for the site choice (Friedman ANOVA: χ_4^4= 12,52; P = 0,013). However, upon the use of post-hoc tests, no significant values were found, even before Bonferroni adjustments (with P = 0,067). While it was not possible to statistically assess this, root systems were used considerably more than the rest of the resting site types (recall Table 2). Reed beds and artificial structures comprise for the less used rest sites suggesting these types as the least preferred.

Structural patterns and predictive power of environmental surrogates

A good adjustment quality of 0,06, according to Krukal's scale (Kruskal 1964 in Pereira 1999) was obtained for the NMDS. Also, two dimensions proved sufficient to explain 99% of the variation found. While the grouping of the three resting site types was not explicit, it was possible to distinguish two main clusters: above-ground and below-ground structures (see Appendix II). This is supported by the high significance of the variable concerning resting site type. Additionally, the significant variables responsible for the variation were: type of resting site, altitude, vegetation class, distance to village, habitat, width of the river and margin type.

When From the first group, maximum temperatures were significantly correlated with precipitation, and since there was almost no rain during this study, rainfall was removed from further analysis. In the second group, two variables were significantly correlated with two other (distance to water with flow velocity and distance to villages with margin types). Distance to water was included, since otters depend on water availability (Prenda et al. 2001) and so, flow velocity was rejected for being less relevant, although that in some conditions it can be related to hunting success. In agreement with Rosoux & Libois (1996), the distance to villages is a more appropriate parameter to determine rest site type and as such, margin type was discarded.

The model was shortened by six variables after a backward stepwise procedure (Table 3). Wald's chi-square statistics calculated the contribution each of the four variables to the final model. No significant differences were found between the ten variable model and the four variable one. Vegetation height class, width of the water course and maximum temperature were the variables contributing significantly to explain rest-site selection (Table 3). Since distance to water was included in the final model but it was not significant, a model without this variable and the four variable one were compared and as the result was highly significant ($P < 0{,}00001$) it was decided to maintain this variable. Maximum temperature seems to weight more on the choice of resting site type; higher temperatures will increase the probability of choosing a resting site above ground. In a similar way, the existence of trees (the highest class of vegetation) increases the chance of an individual choosing an underground structure. The overall accuracy of the model was about 93,47%, enhanced by the AUC (area under the curve) of 0,953 obtained in the ROC curve analysis. According to Swets (1988 in Manel et al. 2001), a AUC value above 0,9 denotes high accuracy.

Table 3 - Environmental parameters contributing to the prediction of resting site type as determined by logistic regression

Parameter	β	SE	Z	Sig
Intercept	-9,5816	371,855	-0,026	0,9794
Veget	0,887	0,3595	2,467	0,0136*
Tmax	-0,2175	0,074	-2,938	0,0033**
Water	-2,6207	371,8036	-0,007	0,9944
Width	1,2817	0,5912	2,168	0,0302*

"Sig" values are Wald's chi-square statistics.

* $P < 0,05$; ** $P < 0,01$

DISCUSSION

Data limitations

Generalization is always risky when few animals are sampled (Spencer, 1987). This is the case of this study where only four otters were tracked for a limited and variable time period. All the four otters are still being followed in the frame of the project and results gathered will complement these preliminary data and allow more robust analyses and data interpretations.

Otters are really hard animals to trap, which can be perceived by the low numbers of animals used in radio-tracking studies (e.g. Rosoux & Libois 1996; Kruuk et al. 1998), this study included. Three of the animals were tracked for relatively small periods of time, comparing to the female followed since May 2010. This might cause a misrepresentation of the relations between the animals and their resting site use/choice. Since the animals were not searched for every day, some resting sites may have been more consecutively used than what was observed. However, given the amount of daily changes of resting site, this should not change much the overall scenario. Moreover, as small as the sample is, daytime resting site discovery curve along with a nearly 100% detection rate, informs us that approximately all the rest sites used during the tracking period were discovered.

Relative to statistical constraints, aside from the ones deriving from sample size, there is always the small possibility that some correlations will be due to chance and not due to a real association linking variables. This could be the case in the correlation between flow velocity and distance to water, for example. It was decided to use Bonferroni adjustments in order to obtain solid statistical results, even in the presence of a small sample.

Does the rest site use pattern differ with sex and breeding status?

Daily change of resting site was observed when consecutive radio locations took place. This tendency is consistent with findings from literature concerning *Lutra lutra* sleeping behavior (e.g. Green et al. 1984; Rosoux & Libois 1996; Saavedra 2002) where frequent changes of resting site occur. While this pattern has been noted repeatedly, no hypotheses have been brought forward for otters. It has been proposed for other species that this kind of behavior could be an avoiding parasites strategy (Kowalewski & Zunino 2005). This hypothesis can be put aside as otters may return to the same resting site with only one day interval. Zabala et al. (2003) suggests that animals that do not spend energy building their resting structures, tend to use a higher number of such places and in addition, to be less faithful to them. This seems more suitable considering otter behavior. As it was said previously, otters tend to occupy already existing structures rather than building their own. This is even more evident in habitats where natural/artificial rest site structures are highly available. A larger range of resting site options, i.e. higher availability, will lead to a lower fidelity to those same sites. On the other hand, habitats where resting sites are a limited resource, as in coastal areas (Beja 1996), the reuse index will clearly be higher. This is when food availability is high, otherwise, even if there are many accessible rest sites, animals will tend to select those in close proximity with food resources, increasing the fidelity of those same sites.

Re-use index differed between females and breeding females. The presence of a litter has already been described to alter habitat use (Ruiz-Olmo et al. 2005) in comparison with single otters. However, these associations lost significance after a Bonferroni adjustment, inferring a relationship that may be validated by a larger study, considering that this result was obtained for a small sample already divided into sex and age classes.

The results obtained for rest site choice were statistically inconclusive; the Friedman ANOVA found the global set to be significantly different, but a closer analysis with post-hoc tests showed no relevant results. While this could be explained by the lower statistical power of the non-parametric tests, it is probably also be due to the effects of a small sample size.

Is human presence an influence on daytime resting site choice?

In contrast with the findings of Rosoux & Libois 1996, where couches were associated with areas less accessible to people and dens with humanized areas, no such relation was found in the present study. In fact, one of the animals was located sleeping just opposite to a parking lot quite busy with hikers talking loudly. The same animal only moved to another resting site upon the close presence of a dog in one time and of a bulldozer in another. With these exceptions, when one animal went to sleep, it did not change its location until the next sunset. This is not new (e.g. Green et al. 1984; Mason & Macdonald 1986; López-Martín et al. 1998; Durbin et al. 1998; Ruiz-Olmo et al. 2005): otters do not seem to be bothered by indirect human disturbance when they are resting, even when this disturbance is close. Such behavior is not exclusive of this otter species, it has been observed also in *Lutra perspicillata* (Anoop & Hussain 2004) and in *Lutra canadensis* (Melquist & Hornocker 1983). Some authors suggest this response is associated with a higher availability of rest sites (Prenda et al. 2001) and of food (Melquist & Hornocker 1983). Bedford (2009) relates this behavior with urban expansion, which might have forced otters to become more tolerant. The choice of resting site seems to be independent of the distance to both roads and villages, thus rejecting the initial hypothesis concerning the choice of above or below ground structures in relation to disturbance proximity. However, the population density in the study area might not show enough variation to affect rest site location or maybe these highly populated areas are simply less used. Both these reasons can account for the inexistence of a relation between rest site location and human presence.

Does snow cover differently favor the use of certain resting sites?

There was no distinction found between the re-use index of the different types of resting site without and with snow cover. Whilst this could be related to the highly efficient thermal insulation of otter fur (Weisel et al. 2005), it is more likely associated to the period when three of the animals were captured. These three only began to be followed in the first days of November, while snow cover settled by the 26th of the same month. Air temperature was already dropping since October, which may have resulted in an apparent lack of difference when snow cover was present. Consequently, it can not be considered that snow influences or not the choice of certain types of resting sites.

Which environmental parameters surrogates best predict rest site choice?

The logistic regression results suggest that vegetation cover, width of the river system and maximum temperature (Table 3) are the most relevant characteristics associated with a specific type of structure. Although some authors say vegetation cover is not associated with otter distribution (Kruuk et al. 1998), many authors classified this feature as an essential requirement in otter habitat (e.g. Ottino & Giller 2004; Bedford 2009). In fact, it has been shown that otters avoid areas without vegetation cover (Mason 1995; Cho et al. 2009). The coefficient obtained for this variable in the logistic regression point to a relation between the absence of trees and the use of above ground structures. Since resting sites made of root systems are more common than underground sites without any trees, this result is also understandable concerning the role of resting site provider that trees have.

Variation in use patterns caused by weather conditions has already been described for mustelids (e.g. Wilbert *et al.*, 2000; Weir *et al.*, 2004; Kowalczyk et al. 2004). The results obtained in the logistic regression, suggest temperature is related to rest site location, i.e. above or below ground, as it is the case with pine martens (Zalewski 1997). When temperatures decrease, there is a higher probability that otters will use resting structures below ground and the same is true for the opposite. This tendency makes sense when thermoregulation needs are taken into account, especially when considering the isolation properties of otter fur.

River width plays an important role concerning feeding and cub rearing (Ruiz-Olmo et al. 2005) and Cho et al. (2009) found that otters tend to occupy low-order streams. It is not clear why a larger river width is associated with underground rest sites, unless it is a result of width relation to depth. In general, wider rivers tend to be also deeper and it is more functional to build an underground den with an underwater entrance when rivers have more depth. In summary, above ground structures seem to be chosen more often with higher temperatures, lower vegetation height (fewer trees) and smaller river widths.

Distance to the nearest water body was included in the final model even though its lack of statistical significance. Moreover, when a model without this variable was compared to the final model, there was a highly significant result, which justifies its inclusion in the final model. This might be due to an interaction between variables, as in distance to water not being related more to above or below ground structures but being related to both instead.

Is there a geographical-related resting site use pattern in Europe?

In spite of the high number of otter studies in Europe, only a small fraction (n=5) considered resting site use. Time-span of such studies was highly variable (38 to 179 mean locations per individual) and there seems to be an arbitrary bias towards females (Table 4). As a result, the data gathered from available literature concerns only 5 males out of 18 otters.

Costa Vicentina (Portugal) was the region with a lower mean number of resting sites per individual (i.e. number of resting sites divided by the number of animals tracked), counting only 3,67 rest sites per animal. However, this study (Beja 1996), analyzed exclusively coastal otters and as such, it can not be directly comparable with the remaining, which were located in freshwater systems. The otters in Bohemia (Austria/Czech Republic) had a higher number of resting sites per individual (n=82), compared to the other studies. Kranz (1995) relates this with the migratory behavior of one of the otters.

Table 4 - Rest site use frequency of use, total locations, number of resting sites, number of animals tracked, mean number of resting sites per animal and mean locations per animal in 6 localities in Europe.

Localities	Den		Surface lair		Total locations	Number of RS	Animals Tracked	RS per animal	Locations per animal
	n	%	n	%					
Costa Vicentina, Portugal[1]	0	**0**	116	**100**	116	11	**3**♀*	**3,67**	**38**
Marais Poitevin, France[2]	99	**67,8**	47	**32,3**	146	59	**2**♀	**29,5**	**73**
Perthshire, Scotland[3]	99	**49**	101	**51**	200	45	**3** (2♀,1♂)	**15**	**66**
Deeside, Scotland[4]	282	**42**	387	**58**	669	38	**4** (1♀,3♂)	**9,5**	**167**
Bohemia, Austria & Czech Republic[5]	81	**22,6**	277	**77,4**	358	164	**2**♀	**82**	**179**
Steiermark, Austria[6]	203	**67,4**	98	**32,6**	301	50	**4** (3♀.1♂)	**12,5**	**75**

*Beja (1996) only shows data for the coastal otters, even though he caught a forth animal from an estuarine environment.

[1] Beja 1996; [2] Rosoux & Libois 1996; [3] Green et al. 1984; [4] Kruuk et al. 1998; [5] Kranz 1995 and [6] the present study.

Frequency of use is perhaps the best way to analyze this data, since it is possible to transform and compare it in the same unit (percentage) for all studies. Percentage of use of both main types of rest site is quite similar between the two radio-telemetry studies in Scotland and this suggests an existing geographically related pattern, given that proximity implies a somewhat identical habitat. However, rest site use does not seem to be related to any factors gradually changing with geographical coordinates. If it was related to longitude, there should be a visible difference between the study in France and the present study (latitude is similar between both sites). On the other hand, a latitudinal pattern can also be excluded, because the transitional stage (each type being used similarly) only happens in the northernmost study area, whereas it should happen in between. An altitude related pattern can also be excluded since two study areas (Marais Poitevin and the present study) with fairly different altitudes, present similar results. In fact, altitudinal gradient has been described as “poor indicator of otter presence” (Robitaille & Laurence 2002).

While temperature can indeed shape the rest site choice, as Zalewski (1997), for pine martens, and this study demonstrated, other factor(s) must be acting as well. It could be related to the dominant type of vegetation, which provides resources for both main types of rest-structures (wood and roots). However, all of this is of subjective and hard interpretation, since there is a large spatial dispersion and heterogeneity between data. This is especially true when considering that males and females might have different use patterns as Green et al. (1984) observed in their study. More studies are needed if the geographical use pattern is to be better understood.

Conservation implications

Being root systems used more often than any other type of resting site, it can be considered that these structures can only be a limiting factor, in Alpine river systems, when tree density is very low. This was not the case, since Styria is the most forested district of Austria. As long as thermoregulation needs are met and vegetation cover is present, there seems to be no obstacle to otter re-colonization of Styria, considering that food is available. However it must be investigated if there are different limiting factors in the remaining areas of Austria that might hinder otter return. As evidences for expansion and re-colonization accumulate in other countries, hope is now renewed for the same to happen in countries where the otter went extinct, like Switzerland.

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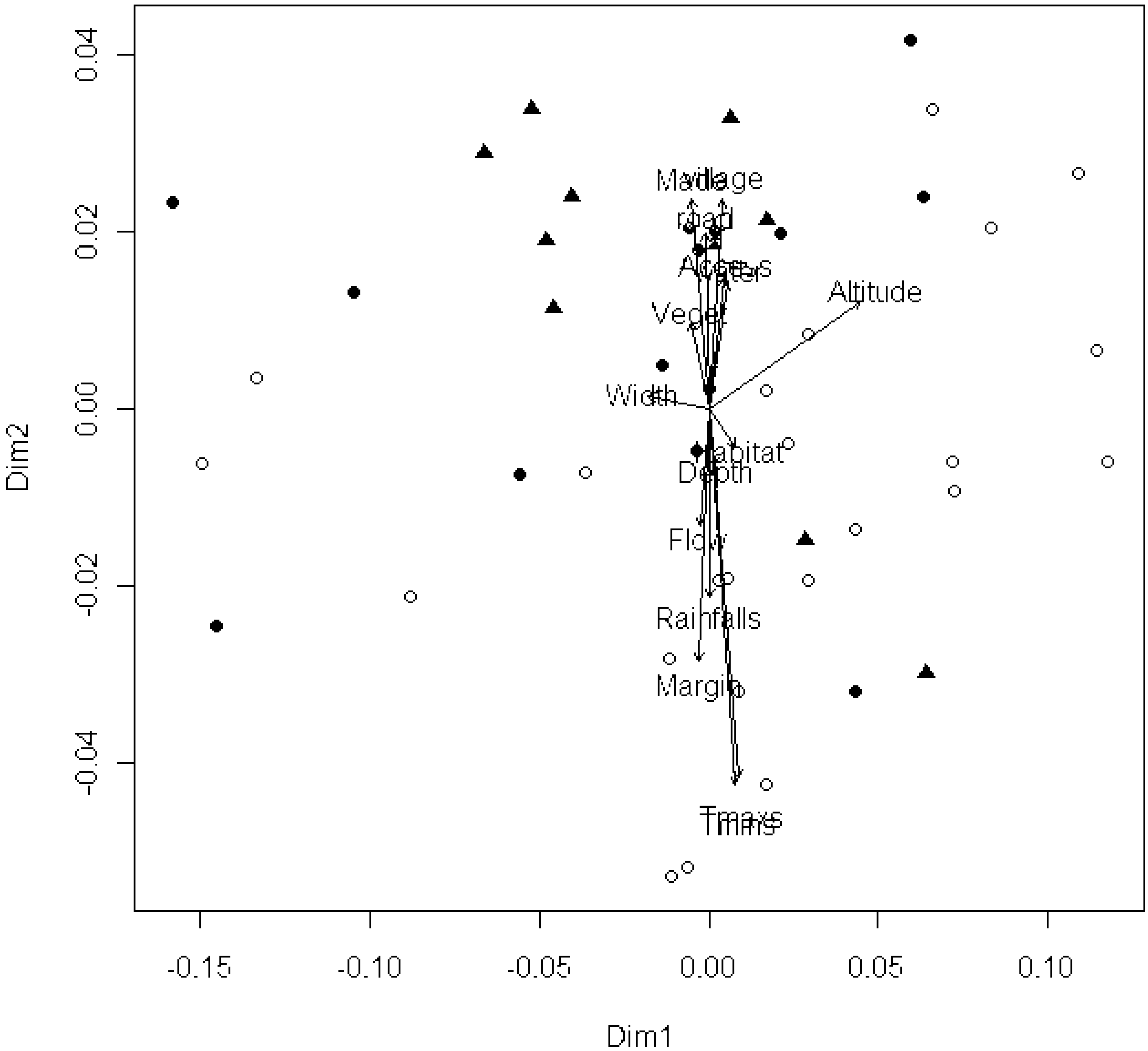
HAPTER 3

FINAL REMARKS

There are still many questions to be answered concerning resting ecology of otters. In Europe, it is exceptionally hard to carry out any kind of research that requires the capture of individuals necessary for radio-telemetry. Because of its conservation status, otters are fully protected; often, legislations associated with this causes difficulties to researchers, slowing scientific progress.

For future studies, it would be interesting to further explore the geographical pattern of resting site use. Several European areas are lacking this kind of data, especially northern (e.g. Scandinavian Peninsula) and southern zones (e.g. Italy). Data from these regions might contribute enormously to discover which features are responsible for variation in use patterns. Also, the way this topic is approached, needs to be reassessed. Generally, there is not an interconnection of areas; either it is diet or habitat related questions. Food availability can change the way otters use their available resting sites. Furthermore, it can be altering or enhancing other environmental variables, masking a geographical pattern, e.g. areas with higher food availability might have *versus* areas with less food resources. This kind of knowledge would bring insight on the specific needs that this carnivore has, concerning resting and denning behavior. This is essential in order to better understand species-habitat relationship, ultimately strengthening conservation decisions.

APPENDIX I - NMDS Results



Variables considered:
Made – Main type of resting site;
Water – Distance to nearest water-body;
Altitude – Altitude;
Veget – Class of vegetation height;
Access – Accessibility of a resting site;
road – Distance to nearest road;
village – Distance to nearest village;
Habitat – Main habitat type in which the resting site was situated;
Width – Width of the nearest river stretch;
Flow – Current speed;
Depth – Depth of the nearest river stretch;
Margin – Level of margin alteration.