Universitat de Girona

REINTRODUCTION OF THE EURASIAN OTTER (LUTRA LUTRA) IN MUGA AND FLUVIÀ BASINS (NORTH-EASTERN SPAIN): VIABILITY, DEVELOPMENT, MONITORING AND TRENDS OF THE NEW POPULATION

Deli SAAVEDRA BENDITO

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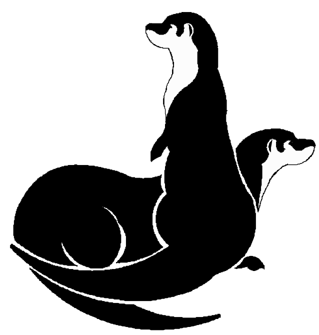

Deli Saavedra
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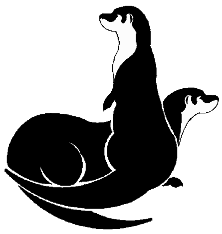

REINTRODUCTION OF THE EURASIAN OTTER *(Lutra lutra)* IN MUGA AND FLUVIÀ BASINS (NORTH-EASTERN SPAIN): VIABILITY, DEVELOPMENT, MONITORING AND TRENDS OF THE NEW POPULATION

Memòria redactada per a optar al grau de Doctor en
Ciències Biològiques presentada pel Llicenciat Deli Saavedra

Conformes, els directors:

Dr. Ramon Moreno-Amich, Catedràtic d'Ecologia del Dept. de Ciències Ambientals de la Universitat de Girona

Dr. Jordi Ruiz-Olmo, Cap de Servei de Protecció de la Fauna, de la Flora i dels Animals de Companyia del Dept. de Medi Ambient de la Generalitat de Catalunya.

Girona, 1 d'octubre de 2002

Per l'Alba i en Pol

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0. Acknowledgements

Acknowledgements are obviously the most emotional chapter of any publication and perhaps for that reason, their translation is most difficult. I have considered that the contents of the acknowledgements are of interest for the people that are mentioned in them, mostly from Catalonia. And whilst I understand that English is in our global time a main vehicle of communication, I want to pay tribute to the diversity of languages and to my mother language by writing the following paragraphs in Catalan.

És ben sabut que els projectes de conservació i gestió d'espècies són (o haurien de ser) multidisciplinaris, i que necessiten d'un equip de persones amb coneixements i experiències variades, sovint depassant l'àmbit de les ciències biològiques i entrant en de les ciències socials i polítiques. El Projecte Llúdriga, sens dubte, ha estat exitós gràcies a la convergència d'un gran nombre de persones en un objectiu comú. Un engrescador objectiu comú en forma d'espècie-bandera el que, lògicament, ha facilitat les coses. La meva feina, en tant que coordinador del projecte, ha estat liderar el conjunt d'esforços i aguantar ben fort el timó per tal de no errar el rumb desitjat.

La present tesi, com a filla natural del Projecte Llúdriga, ha estat concebuda i executada de la mateixa forma i era de preveure que el resultat fos un recull de temàtica diversa on l'autoria i la coordinació s'entrellaça i barreja.

D'aquesta forma, és inevitable que el capítol d'agraïments sigui ben extens i que, ben segur, oblidi a moltes persones que també hi van posar el seu gra de sorra perquè la tornada de la llúdriga fos una realitat. Vagi aquest primer agraïment a totes elles.

Jordi Sargatal, director de la Fundació Territori i Paisatge, ex-director del Parc Natural dels Aiguamolls de l'Empordà i gran amic, ha estat l'autèntic pare del Projecte Llúdriga. A en Jordi dec la meva implicació en la defensa de la natura, la meva formació als Aiguamolls i els meus inicis professionals com a biòleg. Quan, el 1993, em va proposar que iniciéssim aquesta incerta aventura, no m'ho vaig pensar ni un sol moment i – com amb les altres "aventures incertes" en les que ens hem embarcat – mai he hagut de penedir-me.

Els primers diners pel Projecte Llúdriga van ser generosament aportats per Jorge de Pallejà el 1993. A l'any següent, gràcies al suport de l'Albert Vilalta, en aquells moments conseller de Medi Ambient i de Cristòfol Jordà, llavors director general de Patrimoni Natural, la Junta de Sanejament va finançar l'estudi de viabilitat i els primers alliberaments de llúdrigues. A partir de 1997, l'Obra Social de Caixa Catalunya primer, i la Fundació Territori i Paisatge, pertanyent a la mateixa entitat, d'ençà la seva creació, es van fer càrrec del patrocini del projecte. També es va obtenir una subvenció del Parc Natural dels Aiguamolls de l'Empordà, que durant alguns anys va cedir a més l'ús dels vehicles propis.

Estic segur que el Projecte Llúdriga no hagués pogut tirar mai endavant sense el suport incondicional de l'equip tècnic del Zoo de Barcelona, a qui vam demanar que portessin tots els aspectes veterinaris del projecte. Els mitjans humans i materials que van posar a la nostra disposició van ser tants i de tanta qualitat que mai els haguéssim pogut pagar. En Jesús Fernández, veterinari del Zoo, ha estat un gran amic i un gran professional, sempre disposat a dedicar un munt d'hores extres a les llúdrigues. La seva activitat *científica en aquest projecte ha estat ingent, com ho demostren els articles publicats sobre els aspectes veterinaris de la reintroducció de la llúdriga. També vull agrair especialment la col·laboració de Ferran Costa, Conrad Ensenyat, Salvador Filella, Justo Rodríguez i Esteve Tomàs.*

El Dr. Jordi Ruiz-Olmo, Cap de Servei de Protecció de la Fauna, la Flora i els Animals de Companyia del Departament de Medi Ambient i un dels més reconeguts investigadors de la llúdriga a nivell europeu, em va introduir (fins llavors jo era ornitòleg) en l'apassionant món d'aquesta espècie. El seu consell va ser valuós en moltes ocasions i, allà per l'any 1996, em va incitar a començar una tesi doctoral, la que ara teniu a les mans.Òbviament, el seu paper de codirector d'aquest treball ha estat bàsic perquè finalment hagi sortit a la llum.

L'altre baluard en la direcció d'aquesta tesi doctoral ha estat el Dr. Ramon Moreno-Amich, catedràtic d'ecologia, vicerector primer de la Universitat de Girona i ex-professor meu, a qui haig d'agrair en primer lloc haver-me acollit en el seu equip i, en segon lloc, l'assessorament donat ja des de la preparació de l'estudi de viabilitat i després, en totes les fases de l'execució d'aquest treball.

En l'estudi de viabilitat vaig poder comptar amb l'ajut inestimable de Rafa Mateo, del Departament de Toxicologia de la UAB, en la monitorització de la contaminació dels peixos i de Lluís Zamora, del Departament de Ciències Ambientals de la UdG, en l'avaluació de les poblacions íctiques. En la realització del treball de camp (sobretot les inoblidables pesques elèctriques) van participar Lluís Colomer, Bernat Garrigós i Sergi Romero.

Haig d'agrair d'una forma molt especial la col·laboració de l'amic Ponç Feliu. Amb la seva activitat frenètica i imparable ha estat sempre disposat a donar un cop de mà i va participar en les captures de llúdrigues, en els radioseguiments i, un cop llicenciat, va portar a terme la campanya de sensibilització ambiental del Projecte Llúdriga, juntament amb Toni Batet i Toni Llobet.

El Projecte Llúdriga es va iniciar des del Parc Natural dels Aiguamolls de l'Empordà. Vull creure que tot el personal va viure amb il·lusió el projecte i moltes d'aquestes persones van ajudar en les feines més diverses. Vull esmentar a Elisabet Brunsó, Maria Carbó, Alejo Ceballos, Carlos Ceballos, Anna Colomer, D62, Josep Espigulé, Ramon Font, Pep Garcia, Rosa Llinàs, Núria Palomares, Gabriel Pujades, Sergi Romero, Teresa Rosell i Ernest Sagué. Després el projecte es va executar des de l'Associació d'Amics del Parc Natural dels Aiguamolls de l'Empordà (APNAE) i mereix un agraïment especial el suport constant de la Marta Julià, gerent, així com el de la Junta Directiva.

La translocació de les llúdrigues va poder ser portada a terme gràcies als acords de col·laboració que la Generalitat de Catalunya va signar amb la Junta de Extremadura, el Principado de Asturies i el Governo de Portugal. Darrera aquests acords polítics s'amaguen magnífics tècnics, amb autèntic interès en la conservació de la llúdriga, que van ajudar a aconseguir els permisos i a fer més fàcil la nostra feina. Em refereixo a Angel Sánchez, Juan Carlos del Campo i Anabela Trindade.

El treball de camp a les zones de captura sempre es va veure facilitat per les orientacions de guardes i investigadors locals, entre els que vull destacar especialment a Carlos Dávila (Junta de Extremadura), Ginés Armando González. (Principado de Asturias) i Teresa Sales i Nuno Pedroso (Universidade de Lisboa).

Una constant en el Projecte Llúdriga és la participació de nombrosos voluntaris en molts treballs. Si totes aquestes feines s'haguessin hagut de pagar, la reintroducció de la llúdriga mai hagués estat possible. Les captures no van ser una excepció, i per Extremadura, Astúries i Portugal van passar moltes persones: Maria Àngels Argelés, Laura Arjona, Alba Argerich, Emili Bassols, Toni Batet, Mayte Blasco, Enric Capdevila, Francesc Capdevila, Teresa Colomer, Xavi Díaz, Ponç Feliu, Pablo Garcia, Àstrid Geis, Marta Julià, Toni Llobet, Sisco Mañas, Raimon Mariné, Ferran Martí, Berto Minobis, Santi Palazón, Alejo de Pallejà, Alejandro Pascual, Maria Josep Pérez Rati, Jaume Piera, Simon Pinder, Elena Rafart, Sergi Romero, Carme Rosell, Joan Ramon Sanz J, Jaume Solé, Marian Vila i Josep Vilanova (Nino).

El radioseguiment de les llúdrigues reintroduïdes va comportar centenars d'hores de feina i la gran majoria de les vegades vaig comptar amb l'ajuda de voluntaris, gràcies als quals es van poder completar molts seguiments intensius de 24 hores (i les fredes nits d'hivern es van fer més divertides). De les moltes persones que van participar vull esmentar especialment a Laura Arjona, Munsa Badia, David Caselles (Kape), Martí Cortey, Toni Costa, Ponç Feliu, Pablo Garcia, Àstrid Geis, Maribel Pérez, Sergi Romero, Maria Sàbat i Jaume Targa. Tots ells van somniar alguna nit amb el monòton bip-bip del receptor...

Pels radioseguiments periòdics realitzats des de l'aire en avioneta vaig poder comptar amb l'entusiasme i professionalitat de dos grups magnífics de pilots, Forestals 01, del Cos d'Agents Rurals, i Jaume Comas i el seu equip de l'Aeròdrom d'Empúria-brava.

El Projecte Llúdriga ha gaudit d'una gran cobertura mediàtica, la qual cosa ha estat un important recolzament. Encara que vull agrair la feina de tots els periodistes, alguns ens van tractar especialment bé, esforçant-se perquè el projecte tingués un bon ressò. Tinc un especial i molt grat record per Albert Arean, Santi Coll, Marga Pereda i Magda Valls.

Els meus pares no han participat en el Projecte Llúdriga ni en aquesta tesi, però hi tenen una gran responsabilitat. Ells sempre em van envoltar de llibres i del gust per aprendre coses noves i no es van cansar mai de treure'm al camp, la meva màxima passió. Responsabilitat compartida amb el meu cosí Carlos, que em va ensenyar les meravelles de la natura a les estades estiuenques familiars en un poblet de Galícia. A la Marta, les llúdrigues i jo li devem massa coses com per explicar-les en un paràgraf.

Per últim, moltíssimes més persones han col·laborat amb el Projecte Llúdriga, sigui amb els censos visuals (que han mobilitzat molta gent) sigui amb d'altres gestions, potser petites i sense importància, però que, sumades, formen aquesta tesi i són les responsables que la llúdriga torni a viure a les comarques gironines. Aquí hi són (espero) quasi totes: Maria Teresa Abelló, Ferran Aguilar, Doris Albert, Xavi Almanza, Neus Andreu, Anna Aragay, Núria Asensio, Rosa Astor, Núria Baldric, Teresa Ballesta, Anna Banet, Manolo Barcells, Íngrid Barceló, Manel Barrios, Agnès Batlle, Joan Batlle, Jordi Baucells, Brais Benítez, Ibrahim Benítez, David Berenguer, Joan Besson, Núria

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Les llúdrigues, evidentment, han estat en tot moment les muses d'aquesta tesi.

1. Introduction

This thesis deals with an otter reintroduction program carried out in the Muga and Fluvià basins in North-eastern Spain during the second half of the 1990s. Before proceeding to detailed discussion, some generalities concerning otters and the role of re-introductions in species management are in order. Thesis objectives are listed at the end of this chapter, along with an exposition of structure and organisation.

1.1. Otters

Otters are included within the Order Carnivora and belong to the Subfamily Lutrinae, of the Family Mustelidae (Kruuk, 1995). Mustelidae include badgers, weasels, pine martens and polecats. Otters are animals with cylindrical body, short extremities, webbed feet and long, wide tails. Fur is dense with exceptional thermal insulation and water resistance. The head is flat, with eyes, ears and nose placed on the upper part. At the muzzle and forearms the vibrissae (whiskers with a tactile function) are placed. Otters have a great natatorial and diving ability (Mason & MacDonald, 1986; Ruiz-Olmo, 2001).

All these characteristics are adaptations to moving and feeding in and around water . These animals are called semiaquatic mammals because they spend most of their activity period in the water but other basic activities, such as reproduction and resting, happen outside the water as with other terrestrial mammals (Mason & MacDonald, 1986).

1.2. Otters in the World

Currently accepted taxonomy lists 13 species of otters worldwide, , grouped into four different genus (Wozencraft, 1989; Van Zyll de Jongh, 1991; Koepfli & Wayne, 1998). Otters are present in all continents except Australia and Antarctica. Weight varies from 4 to 40 kg. All species have similar morphology, comparable semiaquatic behaviour and feeding habits based on fish and crayfish, except the sea otter, which eats, sleeps, mates and gives birth in the sea (Foster-Turley et al., 1990).

Table 1.1. Otter species of the world (from Foster-Turley et al., 1990).

Latin name	English name	Distribution	Total length(m)	Concern
Aonyx capensis	Cape Clawless Otter	Africa	1.17-1.62	LCC
Aonyx congica	Congo Clawless Otter	Western&Central Africa	1.17-1.62	GCC
Aonyx cinerea	Asian Small-clawed Otter	Asia	0.65-0.94	LCC
Enhydra lutris	Sea Otter	NE Asia, NW America	0.67-1.63	RAC
Pteronura brassiliensis	Giant Otter	S America	1.45-2.00	GCC
Lontra Canadensis	North American River O.	N America	1.00-1.53	RAC
Lutra felina	Marine Otter	SW America	0.87-1.15	GCC
Lutra longicaudis	Neotropical Otter	America	0.90-1.36	LCC
Lutra provocax	Southern River Otter	S America	1.00-1.16	GCC
Lutra maculicollis	Spotted-necked Otter	Africa	0.95-1.17	LCC
Lutra perspicillata	Smooth Otter	SE Asia	1.07-1.30	LCC
Lutra sumatrana	Hairy-nosed Otter	SE Asia	1.05-1.33	GCC
Lutra lutra	Eurasian Otter	Palearctic	0.95-1.36	LCC

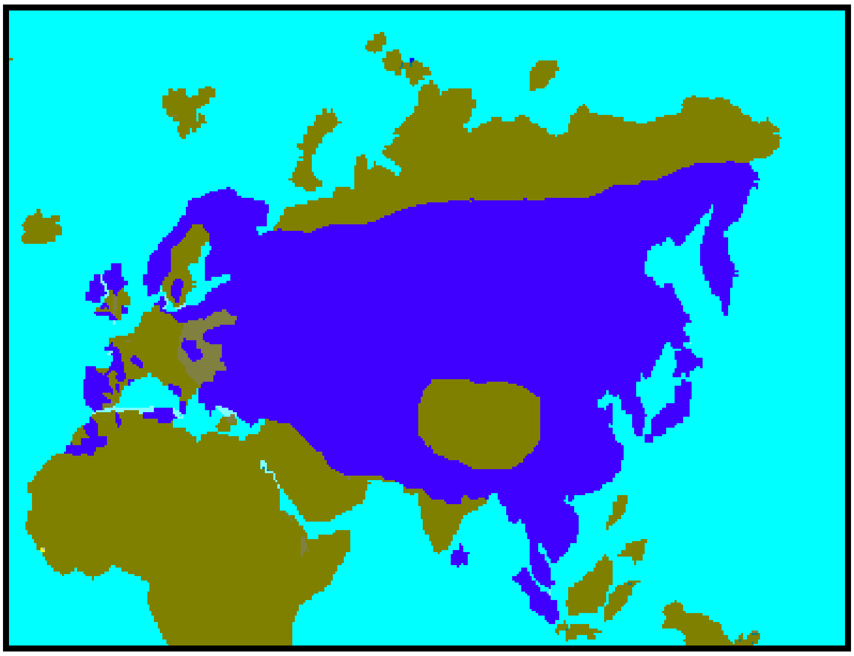
GCC (Global Conservation Concern); LCC (Local Conservation Concern); RAC (Receiving Adequate Conservation).

The denomination of *Lutra nippon* has recently been proposed for the Japanese Eurasian otter (Suzuki et al., 1996), but this new species is still not accepted.

1.3. The Eurasian otter

The species studied here is the Eurasian otter (*Lutra lutra*). Its geographical range is enormous, larger than the remaining other 12 species. It inhabits Europe, North Africa, Russia, China, Japan, Indonesia, Malaysia and part of India (Mason & MacDonald, 1986; Foster-Turley et al., 1990), although it has disappeared from several areas of its historical range due to human causes (see 1.3).

Figure 1.1. Distribution map of the Eurasian otter (Lutra lutra) according to Foster-Turley et al. (1990).



With populations living in such a huge geographical area, differences in size occur. . Iberian otters are smaller than those in central and North Europe (Ruiz-Olmo et al., 1998). As mentioned in this study, British males weighed on average 2.6 kg more than Iberian ones, one third of total weight. The maximum weight found in northern Europe is 14 kg, while the Iberian peninsula never surpassed 10 kg (Ruiz-Olmo, 2001). There are also differences between sexes. In Iberian otters, adult females measure between 95 and 110 cm from the muzzle to the tip of the tail and weigh between 4.5 and 7 kg. Males measure between 105 and 120 cm and weigh between 6.5 and 10 kg (Ruiz-Olmo, 2001).

Eurasian otters reach sexual maturity at two or three years old (Mason & MacDonald, 1986). Females give birth to one to four cubs after two months of gestation (Ruiz-Olmo, 1995). Cubs live with the mother until they can be independent, usually less than one year (Mason & MacDonald, 1986).

The base of the diet of the Eurasian otter in the Iberian Peninsula is fish, but American crayfish (*Procambarus clarkii)* have become important too, especially where this introduced species has become plentiful (Delibes & Adrián, 1987; Ruiz-Olmo & Palazón, 1997; Ruiz-Olmo & Delibes, 1998). Eurasian otters can also prey on water snakes, frogs, water rats, birds, mammals or insects.

The Eurasian otter lives in a great variety of aquatic habitats: rivers, streams, wetlands and even coastal areas (Ruiz-Olmo, 2001), although then only if fresh water is permanently available. (Beja, 1992; Kruuk, 1995).

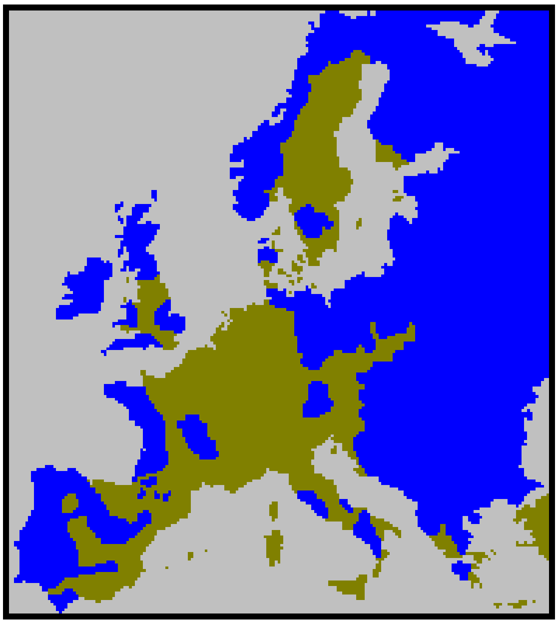
Although the typical image of the otter habitat is a mountain river with plentiful riparian cover, different studies showed that in Mediterranean areas prey availability is more important than riparian floristic composition (Prenda & Granado-Lorencio, 1996; López-Martín et al., 1998; Prenda & López, 1999; Ruiz-Olmo et al., 2001). Thus, the preferred habitat of the otters coincides with the middle and lower river stretches, more productive but usually also more degraded.

L. lutra is a fully protected species. At the international level, it is classified as V*ulnerable* in the IUCN (The World Conservation Union) Red Data Book, included in Annex I of CITES (maximum trade prohibition from the Washington Convention) and included in Annex II (strict protection) of the Bern Convention. At the European level, the Eurasian otter is included in Annexes II (designation of special areas of conservation is needed) and IV (strict protection) of Habitats Directive (92/43/CEE). At the national level, it is protected by Catalonian (Law 3/1988) and Spanish (Law 4/1989) legislation.

1.4. Decline and present situation

If at the beginning of the XXth century the otter was present across Europe, by the 1970s and 80s it was endangered or already extinct in Belgium, Luxembourg, Switzerland, France, England, Austria, The Netherlands, Germany and Italy (Mason & MacDonald, 1986; Foster-Turley et al, 1990). On the other hand, in countries like Portugal, Greece, Norway, Scotland or Ireland healthy populations still existed.

Figure 1.2. Distribution of the Eurasian otter (Lutra lutra) in Europe during the 1980s according to Ruiz-Olmo (2001).



Before otter reintroduction in Girona, the Iberian population showed two distinct parts: to the west, thriving populations were present almost continuously from Galicia to Extremadura, including all Portugal (Delibes, 1990; Ruiz-Olmo & Delibes, 1998; Trindade et al., 1998). To the east, there were huge areas where the otter was extinct, above all bordering the Mediterranean, where rivers are short and pass through arid areas but, at the same time, most of the Spanish human population is concentrated.

Figure 1.3. Approximative distribution of the Eurasian otter (Lutra lutra) in Iberian Peninsula towards 1994 (modified from Ruiz-Olmo & Delibes, 1998 and Trindade et al., 1998).

In Catalonia, as in the rest of Europe, the otter inhabited all rivers, streams and marshes from sea level to Pyrenean areas above 2000 meters (Ruiz-Olmo & Gosálbez, 1988; Ruiz-Olmo & Oró, 1993; Saavedra & Sargatal, 1993; Ruiz-Olmo, 1995 and 2001). The decline started during the 1950s and when the first otter survey was conducted (1984-85) the otter was then only found in sparsely-populated (<25 hab/km^2) unindustrialised mountainous areas (Ruiz-Olmo, 1985 and 2001; Delibes, 1990).

In the 15 years since the first Catalonian otter survey the population has showed a recovery (Ruiz-Olmo & Delibes, 1998), both naturally and through the reintroduction studied in this work (Saavedra & Sargatal, 1998; Ruiz-Olmo, 2001). Currently (otter survey of 1999-2000) the otter inhabits the pre-Pyrenean basins of Noguera Ribagorçana, Noguera Pallaresa and Segre, as well as the Matarranya and Algars rivers, in the south of Catalonia (Ruiz-Olmo, 2001). As a consequence of reintroduction, the otter returned to the Aiguamolls de l'Empordà, Muga and Fluvià basins, and also to the Ter basin, through colonization from individuals coming from the reintroduction program.

Figure 1.4. Distribution of the Eurasian otter (Lutra lutra) in Catalonia towards 2001 (from Ruiz-Olmo & Delibes, 1998; Saavedra & Sargatal, 1998; Ruiz-Olmo, 2001).

1.5. Causes of decline (1950-1990)

The causes of disappearance or depopulation of otters in Europe are varied, but relate exclusively to human activity (Mason & MacDonald, 1986; MacDonald & Mason, 1994):

1.5.1. **Direct persecution**.

It appeared to be an important cause of decline in many European regions until the 1970s (Green, 1991). Otters were hunted for their fur value or because they were considered a competitor for fishermen and fishfarmers. They were killed with shotguns, traps, poison or dogs. In Spain, the government paid a bounty for each dead animal and it wasn't until 1973 that it was declared a protected species (Blas-Aritio, 1970; Delibes, 1990; Ruiz-Olmo & Delibes, 1998).

1.5.2. **Habitat destruction.**

Otters require safe places in river banks to rest and breed. Riparian forest is the best habitat, but decades of channelling, gravel extraction and dam construction have taken toll (Ruiz-Olmo & Delibes, 1998). Well conserved river banks are necessary not only for otters but also for fish populations. Wetlands, another essential habitat for the otter, were dried out or polluted in many areas (Saavedra & Sargatal, 1993). Water resources are scarce in Mediterranean habitats, and human use and dams have further reduced them in Catalonia (Jiménez & Lacomba, 1991; Ruiz-Olmo et al., 1991; Ruiz-Olmo, 2001; Ruiz-Olmo et al., 2002).

1.5.3. **Pollution.**

Two kinds of pollution affected otters. Organic pollution from urban and industrial wastewater, detergents and fertilisers killed fish through anoxia, depriving otters of their principal food source(Ruiz-Olmo & Delibes, 1998); secondly, and in all probability responsible for the Europe-wide decline, chemicals built up following ingestion through food. The effects on animals can be lethal (rapid death) or sub-lethal, reducing the ability to reproduce or survive. The bioaccumulating substances considered most dangerous are organochlorines (PCBs & DDTs) and heavy metals, especially mercury (Mason & MacDonald, 1986; Mason, 1989; Foster-Turley et al., 1990; MacDonald & Mason, 1994; Smith et al., 1994; Kruuk, 1995; Sjöåsen et al., 1997; Ruiz-Olmo et al., in press), but there is controversy about the main substance responsible for the decline (for the role of PCBs see Kruuk, 1997 and Mason, 1997).

1.5.4. **Food supply reduction**.

This factor derived from the last two: pollution, habitat destruction and overfishing. Otters are food-limited so that availability affects both breeding and mortality (Kruuk, 1995; Kruuk & Carss, 1996; Ruiz-Olmo et al., 2001). In the Iberian peninsula, food supply reduction was also influenced by the disappearance of migratory species with the construction of dams (Sostoa, 1990), especially the eel (*Anguilla anguilla*), and also by the decline of European crayfish (*Austropotamobius pallipes*) due to afanomicosis disease (Ruiz-Olmo & Delibes, 1998). But reduction in numbers could affect most of the species, mainly after the 1960s.

1.6. Natural recovery

Reduction and even removal of the causes of decline have brought the spontaneous recovery of the otter in some European regions, confirmed through surveys carried out periodically in several countries including Spain (Delibes, 1990; Ruiz-Olmo & Delibes, 1998) and Portugal (Trindade et al., 1998), and also through specific regional studies (Ruiz-Olmo, 1995 and 2001).

Natural recovery due to habitat improvement and reduction of the causes of decline is cheaper and of bigger scale than the artificial recovery through management tools like reintroduction (see 1.7 and 1.8). But natural recovery is not always possible, because there are basins where the existence of barriers (highly polluted basins acting as barriers, high mountain ranges without rivers nearby, dams difficuly or impossible to cross because situated in canyons, etc.) makes it extremely difficult. Ruiz-Olmo & Delibes (1998) argue that in Spain there are only two areas in good conditions to accept otters but where natural recovery is unlikely because of barriers. One of these zones is the proposed reintroduction area of the Muga and Fluvià basins, in Girona province.

1.7. Reintroductions: a tool for species conservation

A reintroduction is an attempt to establish a species in an area which was once part of its historical range, but from which it has been extirpated or become extinct (IUCN, 1995). The final objective of reintroduction is to re-establish a viable population of the species, which long-term will not require management. (Beck, 1992; Kleiman & Beck, 1994; Caughley & Gunn, 1996).

Table 1.2. Definitions of reintroduction and translocation terms by IUCN – The World Conservation Union (IUCN, 1995).

***Reintroduction:** an attempt to establish a species in an area which was once part of its historical range, but from which it has been extirpated or become extinct.*
***Translocation:** deliberated and mediated movement of wild individuals to an existing population of conspecifics.*

The artificial restoration of a population is a quick and easy objective to propose, surely because managers usually underestimate the difficulty of the task (Ruiz-Olmo & Delibes, 1998). However, some studies show that only a small percentage of such programs with endangered species ends in success. Griffith et. al (1989) surveyed the results of almost 200 translocations and only 46% of the endangered species programs were completely successful. Translocations of more and exclusively wild-caught animals with a larger founded population into better habitats were significantly more successful.

Breitenmoser & Breitenmoser (2002) surveyed the results of 24 reintroductions of carnivores in Europe. 29% of those projects were successful and 33% failed, but 37% were still on course. It is interesting to mention that they did not find any correlation between the following of IUCN guidelines (see this section) and the success of the reintroduction. The two factors that in their opinion are responsible for this fact are demographic stochasticity and the importance of conflicts with humans, both very important aspects in carnivore reintroductions.

Of 145 reintroduction projects using captive-born animals studied by Beck et al. (1994), only 11% were successful, although not all the rest were complete failures, as in some projects there was progress towards a self-sustaining population and in other cases other indirect benefits from reintroduction were achieved, such public support for conservation, professional training, enhanced habitat protection and increased scientific knowledge. Several authors have argued that reintroduction programs are very long, expensive and complex (Campbell, 1980; Wemmer and Derrickson, 1987) but that they can serve also to support less attractive aspects of a conservation program, like improving habitat protection and management (Kleiman, 1989), agreeing with objective 2 of the reintroduction Project (see 1.9). Different points of view in the case of otter reintroductions can be found in Reuther (1998) and de Jongh (1998).

Effectively, the benefits of reintroduction go beyond the simple return of a species, because it can influence human attitudes and have an impact on the local economy and the quality of natural areas (Beck, 1992). Thus, the return of an emblematic species can be used as the flag for an educational campaign to conserve an entire ecosystem (Dietz et al., 1994). The reintroduction of certain species for hunting could increase local incomes. Finally, habitat conservation and restoration done to allow the return of one species can positively rebound on the entire ecosystem (in those cases the species is called *umbrella-species*).

The increasing number of reintroductions led to the establishment of the IUCN Reintroductions Specialist Group. In 1987, the group produced "IUCN position statement on the translocation of living organisms" (IUCN, 1987). In 1995, as this form of management became increasingly common and because some succeeded but many failed, the statement was updated to produce the "Guidelines for reintroductions" (IUCN, 1995).

In this guidelines, the importance of a multidisciplinary approach for the success of a reintroduction is well stressed. There are also a list of the activities a reintroduction project must follow which are summarized in this section.

- **Pre-project activities**
  - *Biological*
    + Feasibility study and background research.
    + Research into similar previous reintroductions.
    + Choice of release site and type.
    + Evaluation of reintroduction site.
    + Availability of suitable release stock.
  - *Socio-economic and legal requirements*
    + Long-term financial and political support.
    + Impact assessment of the reintroduction to local human populations.
    + Acceptation and support by local communities.
    + Permission and involvement of all relevant government agencies.
    + Adequate provisions for compensations, in case of risk to property.

- **Planning, preparation and release stages**
  + Construction of a multidisciplinary team.
  + Identification of short- and long-term success indicators.
  + Secure adequate funding for all the program phases.
  + Design of pre- and post-release monitoring program.
  + Appropriate health and genetic screening of release stock.
  + Development of transports plans for delivery of stock.
  + Determination of release strategy.
  + Development of conservation education for long-term support.
  + Public relations through the mass media and in local community.
  + Involvement where possible of local people in the program.

- **Post-release activities**
  + Post-release monitoring of all (or a sample of) individuals.
  + Demographic, ecological and behavioural studies of released stock.
  + Studies on long-term adaptation by individuals and the population.
  + Collection and investigation of mortalities.
  + Interventions (supplemental feeding, veterinary aid) when necessary.
  + Habitat protection or restoration to continue when necessary.
  + Continuing public relations activities.
  + Evaluation of cost and success of reintroduction techniques.
  + Regular publication in scientific and popular literature.

1.8. Otter reintroductions

Otter reintroduction techniques began with the Sea Otter (*Enhydra lutris*) in the USA, in 1965 (Jameson et al., 1982). During the 1980s and 90s, several states (Arizona, Colorado, Indiana, Iowa, Kansas, Kentucky, Minnesota, Missouri, Nebraska, New York, Oklahoma, Ohio, Pennsylvania, Tennessee or West Virginia) translocated river otters (*Lontra canadensis*) in an attempt to reestablish breeding populations (Tango et al., 1991; Summer, unpublished). In many programs only limited follow-up information was available and it was difficult to determine the fates of translocated otters (Erickson & McCullough, 1987), while in other cases, complete pre and post-release studies were conducted and published in scientific literature (Serfass & Rymon, 1985; Serfass et al., 1993; Serfass et al., 1996).

Nowadays (2001) more than 4000 otters have been reintroduced in 21 different states (Tom Serfass, pers. comm.). The mean number of animals translocated per program is 123, although with big differences. For example, in Missouri 845 otters have been translocated (the biggest number of animals used in a single reintroduction program until now) and in New York state 281 have released (Romeo Spinola, pers. comm.). Most of the otters (some 2000) came from the populations of south Louisiana (Tom Serfass, pers. comm.).

In Europe, the first reintroduction programs were developed in England and Sweden. In England, The Otter Trust maintained a breeding center and from 1983 periodically released small groups (1-3 captive-bred animals) into lowland English rivers. By

1996, a total of 80 otters had been released (Jefferies et al., 1987; Wayre, 1992; Strachan & Jefferies, 1996). Although only a small part of the released animals were closely monitored by radiotracking, and then only for short periods, many breeding records were obtained and it appeared that the population was spreading; but also in coincidence with a natural recovery of the species in the country (Jessop & Cheyne, 1992).

In Sweden, the World Wildlife Fund for Nature (WWF), the Swedish Environmental Protection Agency and the Swedish Hunters Association started a reintroduction program that combined the releasing of wild and captive-bred animals (Sjöåsen, 1996; 1997). A breeding center was built in central Sweden and stocked by otters from Norway. During 1987 and 1988, the first 11 otters were released without radiotransmitters in the southern part of Sweden, in an area of 5200 km^2. Signs of otter expansion were found in the area during 1988. Between 1989 and 1992, 36 otters were released fitted with radio-transmitters, 11 wild-caught (from Norway) and 25 captive-bred. The population appears to be well established in the area and signs of otter are found throughout the area (Sjöåsen, 1997, pers. comm.).

Other reintroduction proposals (or starting projects) exist in Austria, Czech Republic, France, Germany and Italy (International Otter Colloquium, Chile, 2001). In Alsace (France), the *Centre des Loutres de Hunawihr* released two otters bred in captivity and radiotracked them for several months, as a preparation to the probable reintroduction of 20 otters in four years (www.cigogne-loutre.com/html/reintroloutre.html). In Abruzzo (Italy), a couple of otters were released in summer 2001, also provided with transmitters, and two more were ready to be released at the end of the year (A. Antonucci, pers. comm.). In the Rhône basin, a project is in preparation, managed by Compagnie Nationale du Rhône, FRAPNA and CORA (www.cnr.tm.fr/cnr/fr/7451.asp).

1.9. The Girona Otter Reintroduction Project

The *Girona Otter Reintroduction Project (GORP)* is a shorter name for the Reintroduction of the Eurasian otter (*Lutra lutra*) in Aiguamolls de l'Empordà Natural Park and Muga and Fluvià basins (see fig. 1.6). The main objectives were:

1. The restoration of an eradicated population.
2. The promotion of river and wetland conservation through an emblematic species.

In order to achieve the first objective and to accomplish the IUCN guidelines (1.7), a viability study was undertaken, a pre and post-release monitoring protocol was prepared which would track most of the released animals by telemetry, the causes of death were studied and scientific method was used to approximate all aspects of reintroduction.

1.9.1. Chronology of otter disappearance in Muga and Fluvià basins

Eurasian otters inhabited the Fluvià and Muga basins and Empordà wetlands until the middle of this century when, due to factors explained in 1.5, the population began to decrease until it became completely extinguished in the 80's (Ruiz-Olmo & Gosálbez, 1988; Saavedra & Sargatal, 1993; Gosálbez et al., 1994; Ruiz-Olmo, 1995 and 2001).

From the available bibliography and through inquiries to naturalists, hunters and fishermen, it was possible to reconstruct the evolution of the otter population in the area from the 1950s until the 1980s, when extinction occurred (Ruiz-Olmo & Oró, 1993; Saavedra & Sargatal, 1993).

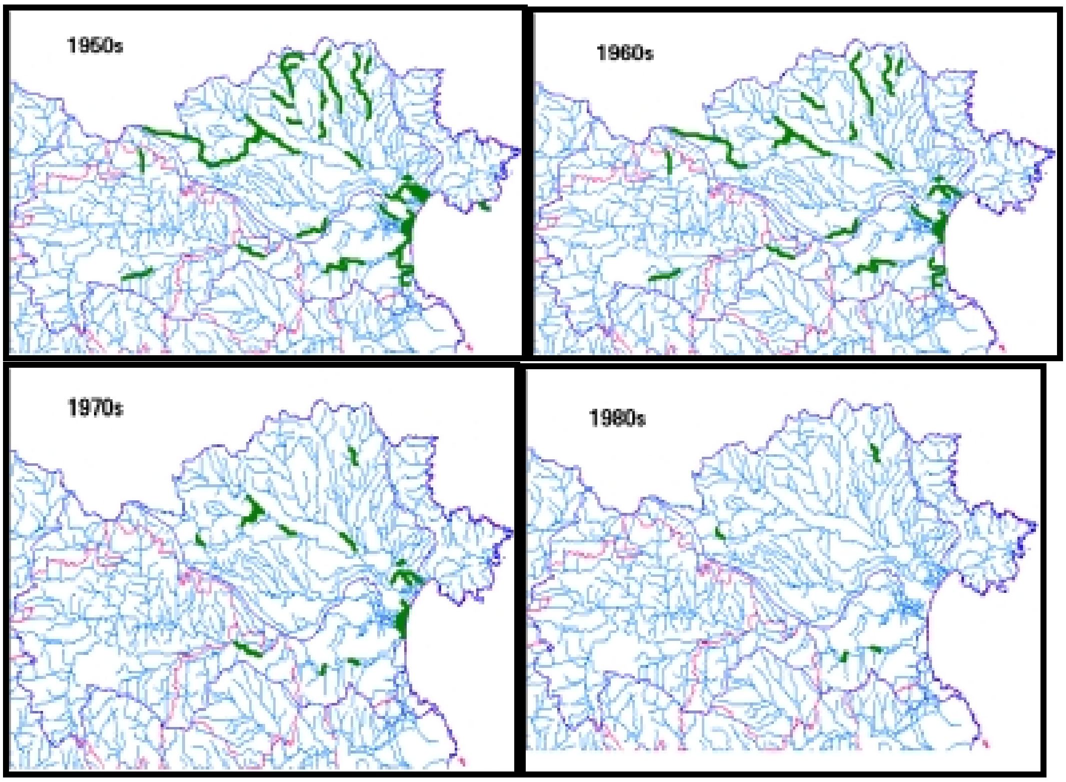
In the 1950s, the otter could be found in almost all the rivers and streams of the Muga and Fluvià basins, as well as in the channels, lagoons and marshes of the Empordà wetlands. Of particular interest are the references to otters living in small mountain streams with little water, perhaps indicating that "better" stretches would have high densities of individuals. Many otters were hunted, using firearms, traps and even pitchforks. A sighting at Cap de Creus may even indicate some individuals living on the coast.

During the 1960s the otter was still abundant, but fewer individuals were killed, which could indicate a reduction in population. The references from the Albera range diminished, but in the Empordà wetlands otters were still very common, for example in ricefields.

The most spectacular reduction occurred at the beginning of the 1970s, when the otter disappeared from many areas. Only a few references from the Albera range were found and in the Fluvià basin, more industrialized, the same probably occurred. In the Empordà wetlands some references were still found, although it disappeared from many sectors. In the Muga river only a handful of observations in the upper stretch existed, above the Boadella dam.

Finally, the 1980s saw the extinction of the otter in the study area, with only four isolated observations, the last one in 1984 (Ruiz-Olmo, 1985).

Figure 1.5. Distribution of otter references in Muga and Fluvià basins during the decades of 1950s, 60s, 70s and 80s.



1.9.2. Habitat protection and restoration

From the 1980s, habitat conservation began with the establishment of natural parks in the upper stretches of the Fluvià basin (Zona Volcànica de la Garrotxa), the upper course of Muga tributaries (Serra de l'Albera) and in the lower part of the Muga and Fluvià rivers (Aiguamolls de l'Empordà). Here the restoration of the old wetlands brought more suitable habitat for the otter. Moreover, the declaration of three Natural Reserves specially designed along stretches of river to protect otter habitat represented an opportunity to preserve the riparian forest and to maintain a corridor for animals among the different Natural Parks.

1.9.3. Reintroduction

Reintroduction started in 1993 because the possibilities of recolonization from other populations were extremely low (Ruiz-Olmo & Delibes, 1998; Saavedra & Sargatal, 1998) and because the reintroduction of otters has been successful in other countries (see 1.8).

Following IUCN guidelines and other interpretations (Illana & Paniagua, 1993) a list of requisites was produced:

1. Causes of extinction in the study area must be identified.
2. These causes must be eliminated.
3. The probabilities of natural recolonization are scarce or nil.
4. The proposed reintroduction area should have sufficient carrying capacity to support a viable population.
5. Removal of individuals for reintroduction must not endanger donor population.
6. The source population should ideally be closely related genetically to the original native population.
7. The reintroduction program must be fully understood, accepted and supported by local communities.

Reintroduction through translocation of wild animals was chosen because it is desirable (Griffith et al. 1989; IUCN, 1995; Sjöåsen, 1996) and there are healthy populations in certain parts of the Iberian Peninsula (Delibes, 1990; Delibes and Ruiz-Olmo, 1998).

Between 1995 and 2000, 41 wild otters from SW (Extremadura) and N (Asturias) Spain and SE (Alentejo) Portugal were captured and released, 93% of them fitted with an implanted radiotransmitter. The reintroduction program finished in 2001, although indirect monitoring through tracks and spraints will continue. Another long-term kind of monitoring is now starting using molecular scatology methodology, or DNA typing of spraints (Jansman et al., 2001). With this technique it would be possible to know the number of individuals present in the area several years after the beginning of reintroduction and also some years beyond the life of transmitter batteries.

Great efforts went into the second *GORP* objective, the promotion of river and wetland conservation using an emblematic species, using different educational materials (Saavedra et al., 1995) and an environmental education campaign (Saavedra and Sargatal, 2001). No details are given because it is not the aim of this study.

1.9.4. Geographical area of the project

The reintroduction project was carried out in the Muga and Fluvià river basins, a territory which includes approximately 2,000 km². The area is situated in the North-eastern Iberian Peninsula and administratively belongs to three counties (Alt Empordà, Pla de l'Estany and Garrotxa), of the Autonomous Community of Catalonia (Spain) and borders with France to the North.

Muga and Fluvià are both low headwaters Mediterranean rivers with irregular water regime, fed by surface waters (rainfall) and lower flow typified by a low absolute volume. The Muga basin presents a surface of 854 km² and an average flow of 2.44 m³/seg; the total length of its main branch is 65 km. The most important tributary is the Llobregat d'Empordà, because it carries more water than the Muga main branch and together with secondary tributaries drains the Albera range. The Fluvià river is 97 km long, with a mean flow of 1.27 m³/seg and a basin surface of 1124 km². The most important tributaries arrive in Garrotxa county (Ser, Ridaura, Llierca) while in the

Empordà plain it receives only the contribution of some minor torrents (Bach, 1989; Brusi, 1992). Both rivers flow into Aiguamolls de l'Empordà wetlands, with 4,800 ha protected as Natural Park and 800 ha as Strict Reserve (Fig 1.6).

The Empordà plain is drained by channels that collect water from rivers and from the basins of old lakes which are now dry (Bach, 1989). Some of the most important (Madral, Mugueta and Salins) drain the enormous Castelló lake. Between the Muga and Fluvià rivers, two long channels (Corredor and Sirvent) cross the plain from west to east. Dozens of kilometers of smaller channels connect and traverse most of the Aiguamolls Natural Park.

But the main characteristic of Aiguamolls de l'Empordà is the presence of temporary or permanent flooded areas. It is possible to distinguish two different types of flooded areas: those associated with old lakes, such as the great Castelló lake, and the coastal lagoons, formed by the interaction between surface waters in search of an exit to the sea and marine processes (coastal bars) obstructing passage. The lakes contain fresh water and the coastal lagoons brackish water, with composition depending on situation and the influence of the sea.

The riparian forests include *Populus alba, Fraxinus angustifolia* and *Alnus glutinosa*, but in many places have been replaced with plantations of *Populus nigra* and *Platanus x hybrida*. Helophytic and halophil vegetation (*Phragmites sp., Typha sp., Arthrocnemum sp.*) occurs on the wetlands and coastal zones (Folch & Franquesa, 1984; Gosálbez et al., 1994).

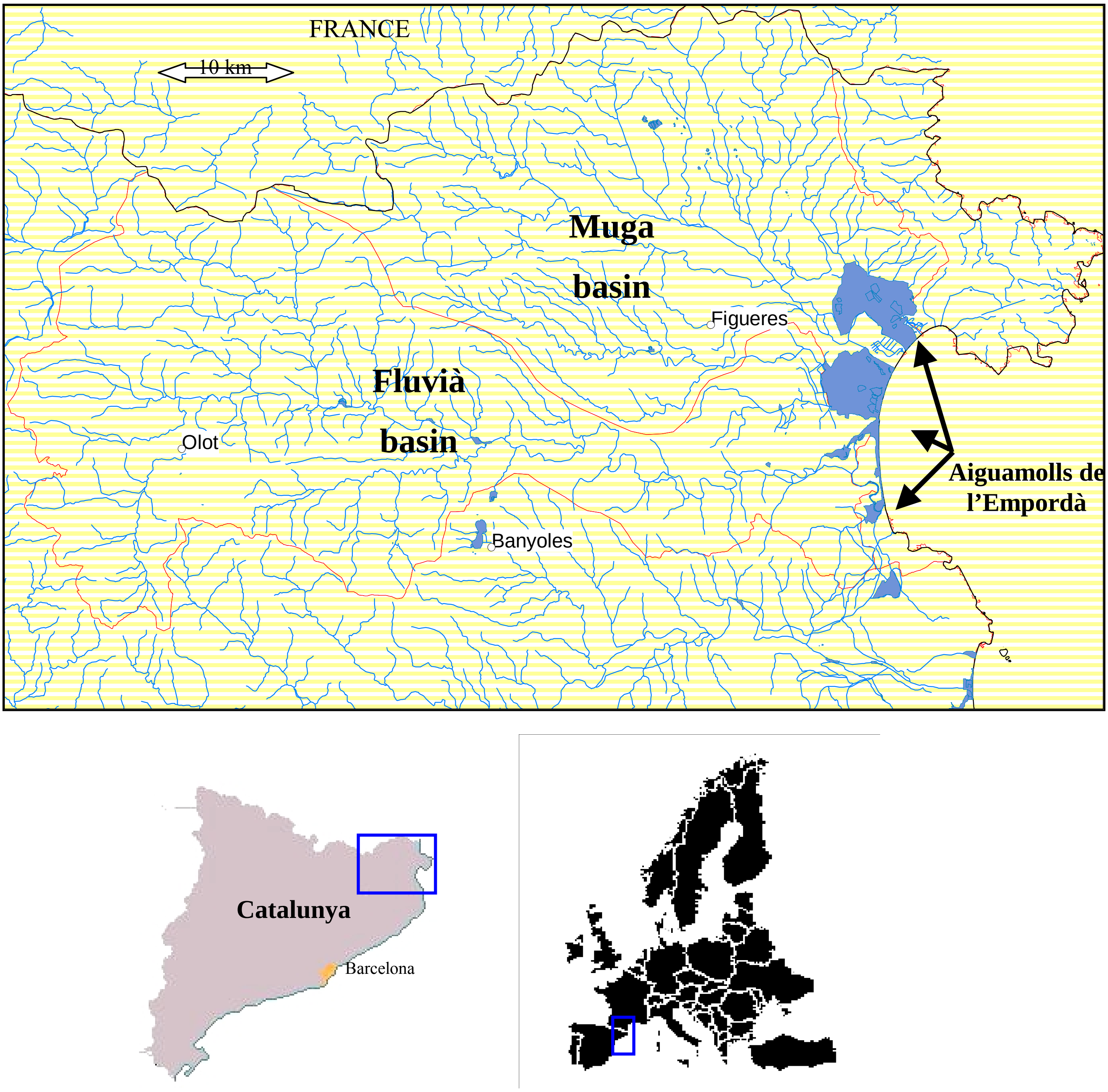
The fish community is mainly represented by Ciprinidae, with many introduced species (Carp, *Cyprinus carpio*; Roach, *Rutilus rutilus*; Rudd, *Scardinius erythrophtalmus*) and Anguillidae (Eel, *Anguilla anguilla*). In the upper course Brown Trout (*Salmo trutta)* are found, and near the mouth of both rivers and in the wetlands some marine species (Mullet, *Liza sp., Mugil cephalus, Chelon labrosus*; Sea Bass, *Dicentrarchus labrax*) are common (Sostoa, 1990; Zamora et al. 1996).

Population is c.60 habitants/km², but increases on the coast during summer. The main activities in the study area are agriculture and tourism, with more than 400,000 tourist places. The main roads pass through the area from North to South, connecting Spain with the rest of Europe. (Cals, 1987; El País, 1993).

The only semi-aquatic mustelid present in the area is the polecat (*Mustela putorius*) though there are some observations of American mink (*Mustela vison*), coming from a nearby population (Ruiz-Olmo & Aguilar, 1995; Ruiz-Olmo et al., 1997).

The Muga irrigates a farming area, and only in the middle-low stretches receives some urban sewage (from Figueres). The Fluvià river crosses an industrial area in Olot near its source, and flows through arable land to the sea.

Figure 1.6. Geographical area of the reintroduction.



1.10. Objectives and organization of the work

An important part from the much information obtained during the *GORP* is included in this thesis, which has the following objectives:

1. To demonstrate the viability of otter reintroduction (before execution).
2. To demonstrate the success of otter reintroduction (after completion).
3. To go deeply into the study of ecological and behavioural aspects that have in the reintroduction an unique opportunity to dispose of a "designed" population.
4. Conduct ecological and behavioural research under unique controlled conditions
5. To determine survival rates for the reintroduced population, and factors affecting it.
6. To help in the preparation of standard methodologies, from both veterinary science and population ecology.

The *GORP* and its follow-up monitoring involved several biological and veterinary disciplines and specialists therein; the result is a thesis perhaps slightly unorthodox in its heterogenous approach.

Chaptering is according to objectives, as follows:

Chapter 1. Introduction.
Contains background and necessary basic information. Otter generalities, the rarefaction process and the causes of sharp decline are reviewed. Reintroduction as a tool for species conservation is discussed, and previous otter reintroductions discussed. Finally, general aspects of the *GORP* are included, such as project steps and geographical area.

Chapter 2. Viability of reintroduction.
This chapter includes the main studies carried out in order to prove that habitat was in good conditions to receive the otter. Specifically, the studies presented are the organochlorates and heavy metals levels in fishes and water and the fish biomass and production assessment.

Chapter 3. Trapping, handling and medical management of reintroduced otters.
Presents results of management and handling from capture to release. Includes capture and anesthesia methodologies, veterinary checks and transmitter implants.

Chapter 4. Colonization, density and post-release mortality.
The results of monitoring ecology and behaviour are presented in three chapters. Chapter 4 covers colonisation patterns, distribution over time, comparative density and principal causes of death after releasing.

Chapter 5. Spatial and temporal ecology.
Ch. 5 presents most of the telemetry-based findings, basically periodic radiolocations and 24-hour tracking sessions. Studies of range, activity patterns, movement parameters in an activity period, resting sites and the effect of water availability on otter movements are also included.

Chapter 6. Post-parturition movements.
Reproductive behaviour in the wild of secretive, nocturnal mammals like the otter is difficult to study. For this reason, the data presented here were considered sufficient to warrant a chapter of their own. They include the results of radiotracking two females exhibiting breeding behaviour, with activity and movement patterns.

Chapter 7. Testing different survey and census methods for Eurasian otter.
The reintroduction program in Catalonia has been a magnificent opportunity to study an artificially designed population. With these data and other data from the Ebro basin, the different methodologies used to count otters (otter surveys, track censuses and visual censuses) are compared and reliability discussed.

Chapter 8. Modeling the viability of the reintroduced otter population.
A PVA (Population Viability Analysis) of the reintroduced population was developed using the Vortex program, with the objective of determining which parameters most affect survival, with a view to establishing priorities in the present and future management of the reintroduced population. The study presents results for a projection time of 100 years under 48 different scenarios, produced by the combination of selected parameters.

Chapter 9. Conclusions.
Conclusions from previous chapters are summarised, emphasising those of interest for otter management.

2. Viability of reintroduction

2.1. Introduction

Monitoring reintroduced otters yielded much ecological and behavioural data, examined over three chapters. The first looks at colonization by the new population, how distribution develops, density compared to other well-studied otter populations, and the causes of mortality after release. In chapter 5, most of the data obtained through 24 hours tracking sessions is presented, together with studies of range, patterns of activity, movement parameters in an activity period, resting sites and the effect of water availability on otter movements. Chapter 6 studies the movements of two females that presented breeding behaviour.

Of the aspects studied in this chapter, only post-release mortality has been well studied in previous reintroduction programs, as data essential to evaluating the success of reintroduction (Hoover et al., 1984; Serfass & Rymon, 1985; Erickson & McCullough, 1987; Jessop & Cheyne, 1992; Sjöansen, 1997; Johnson & Berkley, 1999; Spinola et al., 2001).

Other demographic aspects such as density of population, colonisation and dispersal were not specifically monitored; efforts were concentrated on radiomarked individuals rather than the overall population.

In the *GORP*, two other tools were also used besides radiotracking: regular checking of a "standard otter survey" (Reuther et al., 2000) station network to determine colonization over time, and use of visual censuses to obtain data on otter densities in rivers and channels for comparison with other nearby well-documented populations (Ruiz-Olmo, 1995, 2001). Both tools were also used to test different methodologies in the study of otter numbers (see chapter 7).

2.2. Assessment of water pollution

2.2.1. Introduction

The study of the levels of pollutants in water gives only information about the concentrations contained in the moment of sampling and can present an important variation through time. This is a difficulty to know the toxic compounds levels hold up by the riparian ecosystem in long-term level, but the advantage of the study of water pollution consists in the availability of numerous studies and monitorings which allows to establish comparisons with many parts of the world.

Although the organochlorine compounds (OCs) (Mason, 1989; Smith et al., 1994) and methyl mercury (Kruuk & Conroy, 1991; Gutleb, 1995; Kruuk, 1995) are considered the main substances implied in the otter decline, the oppotunity has been taken to analize the levels of many other substances which can affect the fauna of riparian ecosystems, as organofosforates, triazines and some heavy metals other than mercury.

2.2.2. Materials and methods

Aiguamolls de l'Empordà (Empordà marshes) surface water was analyzed (figure 2.1). All the sampling stations correspond to rivers, channels and lagoons inside the Natural Park or in its influence area: Muga and Fluvià rivers, Mugueta, Madral, Corredor and Sirvent channels and Rogera coastal lagoon.

Samples collection

A monthly (from May to October) water sample was collected at each station. The Muga river sample corresponding to October broke and was not analyzed. Also, an methodologic error made impossible the heavy metals analysis of all samples corresponding to June. Thus, 41 samples for pesticides and 34 for heavy metals were analyzed.

A 2 liter pirex bottle was filled with surface water of every sample station. Containers were kept in a portable refrigerator and arrived to Agbar laboratory in less than 24 hours, were contents were analyzed.

Substances analyzed

All the analyzed substances are listed below, with the lower detection limit between brackets.

Pesticides: (ng/l)
Organochlorines: HCH's (2), Lindane (2), Heptaclor (4), Epoxid heptaclor (2), Aldrin (2), Dieldrin (2), Endrin (5), Endrin aldehid (5), op'-DDT (5), pp'-DDT (5), op'-DDE (3), pp'-DDE (3), op'-DDD (3), pp'-DDD (5), Endosulfan I (5), Endosulfan II (5), Sulfate endosulfan (5), PCBs (5).

Organofosforates: Dichlorvos (1), Methamidophos (2), Mevinphos (1), Naled (1), Phorate (1), Diazinon (1), Disulfoton (1), Dichlorfenthion (1), Dimethoate (1), Fenchlorphos (2), Metil paration (1), Fenithrotion (1), Chlorpyriphos (1), Etil paration (1), Malation (1), Metil bromophos (1), Etil bromophos (1), Chlorfenvinphos (1), Tetrachlorvinphos (1), Methidation (2), Ethion (1), Phosalone (1), Methil azinphos (2), Coumaphos (1).

Triazines: Propazine (10), Atrazine (10), Terbutilazine (10), Simazine (10), Prometrin (10), Ametrin (10), Terbutrin (10).

Others: Propachlor, Azyprotrine, Alachlor, Desmetryne, Metolachlor, Fluazifop-butil, Bitertanol.

Heavy metals : (mg/l)
Aluminium (5), Zinc (0.5), Lead (1), Mercury (0.2).

Analytic methodology

Pesticides:
Liquid-liquid Extraction was done with dicloromethane to the appropriated volume concentration. The liquid was injected in gas chromatographs equipped with ECD (organoclorates) and NPD (phosphorates i triazines) detectors.

Chromatographic conditions were the following:
ECD: column 60 m x 0.32 mm DB-5. 70°C to 150°C at 20°C/min, until 250°C at 4°C/min. Carrier gas: hydrogen. Injection of 1ml "splitless".
NPD: 30 m x 0.32 mm DB-17. Same temperature programming than ECD. Injection of 1ml "on column".

Pesticides confirmation was done through gas chromatography and mass spectrometer (CG/EM) joining in SIR mode, with most representative fragments of different substances.

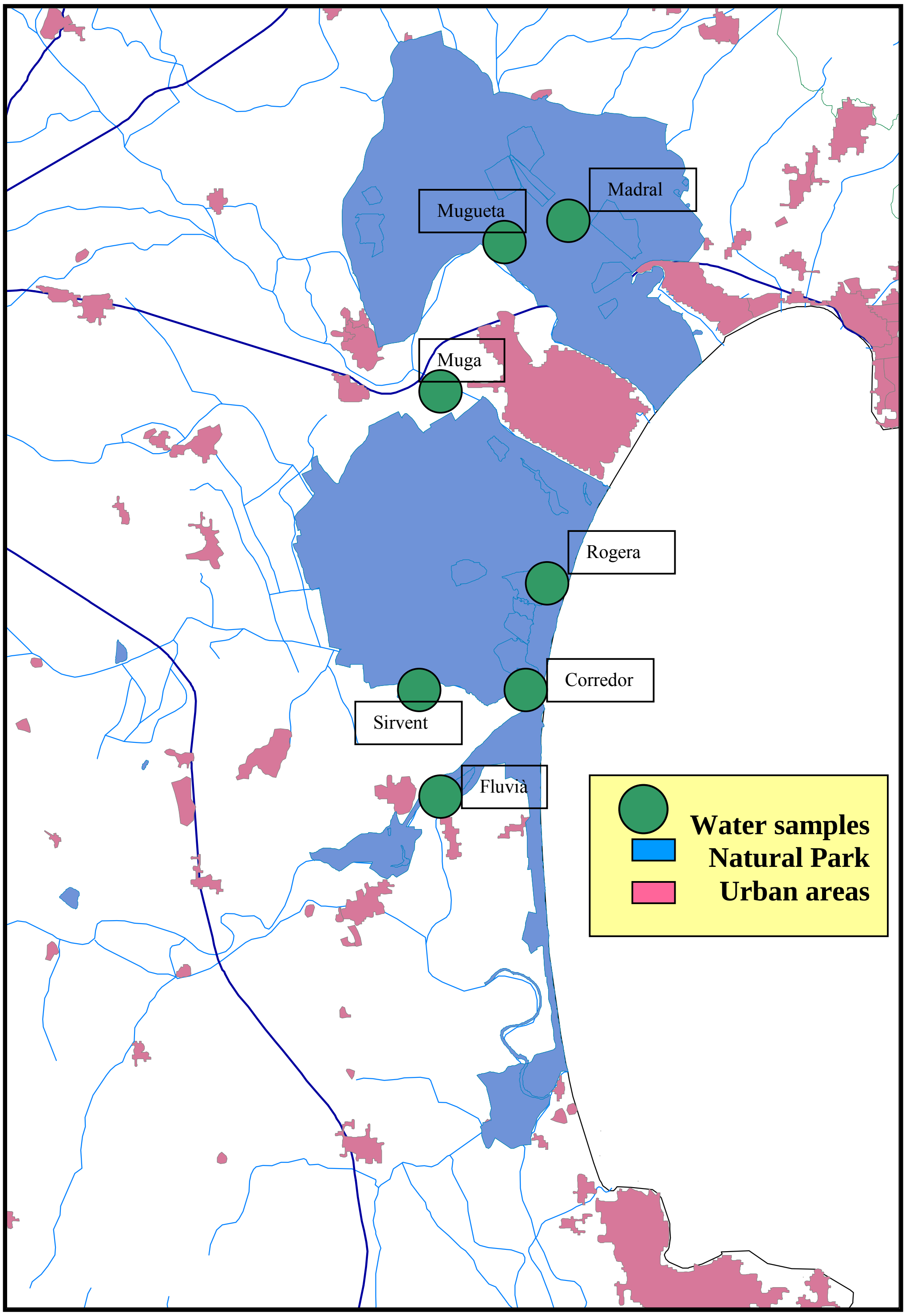
Only confirmed pesticides by CG/EM joining are shown. Other substances which gave signal at same retention time of a patron pesticide but weren't unequivocal identified through CG/EM joining were discarded.

Heavy metals:
Heavy metal determination was done by atomic absorption spectrometry.

2.2.3. Results

Some of the results from water analysis are presented below (tables 2.1 to 2.4). Only a small percentage of pesticides analyzed was found (7%), showing that Empordà marshes surface water is, at first glance, quite clean.

Figure 2.1. Water sampling stations at Empordà marshes.



The substances considered responsibles of otter population decline in North America and Europe are PCBs, DDTs, Dieldrin and Mercury. Thus, the absence of these substances in water samples is a positive caracteristic of the results.

Table 2.1. Levels of Lindane in surface water of Empordà marshes, May-October 1994.
NA = no analyzed ND = below detection level (<2 ng/l)

(ng/l)	may	june	july	august	september	october	Mean	SD
MUGUETA	11	56	39	12	ND	ND	**19.7**	**20.8**
MADRAL	6	ND	ND	ND	ND	ND	**1.0**	**2.2**
MUGA	26	19	18	ND	ND	NA	**12.6**	**10.7**
ROGERA	ND	ND	10	ND	ND	ND	**1.7**	**3.7**
CORREDOR	9	ND	15	ND	ND	ND	**4.0**	**5.9**
SIRVENT	37	ND	ND	ND	ND	ND	**6.2**	**13.8**
FLUVIÀ	ND	ND	ND	ND	ND	ND	**--**	**--**
Mean	**13**	**11**	**12**	**2**	**--**	**--**	**6.3**	**12.6**

Table 2.2. Total levels of Triazines and their metabolites in surface water of Empordà marshes, May-October 1994. NA = no analyzed ND = below detection level (<10 ng/l)

(ng/l)	may	june	july	august	setember	october	Mean	SD
MUGUETA	100	1373	313	ND	ND	ND	**298**	**494**
MADRAL	ND	ND	186	ND	ND	202	**65**	**92**
MUGA	1420	774	721	135	9	NA	**612**	**506**
ROGERA	30	150	62	ND	ND	45	**48**	**51**
CORREDOR	113	ND	148	ND	ND	130	**44**	**62**
SIRVENT	90	220	339	ND	ND	ND	**108**	**130**
FLUVIÀ	66	ND	ND	ND	ND	ND	**11**	**25**
Mean	**260**	**360**	**253**	**19**	**1**	**63**	**162**	**328**

Table 2.3. Levels of Mercury (Hg) in surface waters of Empordà marshes, May-October 1994. NA = no analyzed ND = below detection level (<0.2 mg/l)

(mg/l)	may	june	july	august	setember	october	Mean	SD
MUGUETA	ND	NA	ND	ND	ND	ND	**--**	**--**
MADRAL	ND	NA	ND	ND	ND	ND	**--**	**--**
MUGA	ND	NA	ND	ND	ND	NA	**--**	**--**
ROGERA	ND	NA	ND	ND	ND	ND	**--**	**--**
CORREDOR	ND	NA	ND	ND	ND	ND	**--**	**--**
SIRVENT	ND	NA	ND	ND	ND	ND	**--**	**--**
FLUVIÀ	ND	NA	ND	ND	ND	ND	**--**	**--**
Mean	**--**	**--**	**--**	**--**	**--**	**--**	**--**	**--**

Table 2.4. Levels of Lead (Pb) in surface waters of Empordà marshes, May-October 1994. NA = no analyzed ND = below detection level (<1 mg/l)

(mg/l)	may	june	july	august	setember	october	Mean	SD
MUGUETA	3	NA	2	ND	1	1	**1.4**	**1.0**
MADRAL	2	NA	2	ND	5	1	**2.0**	**1.7**
MUGA	1	NA	3	ND	1	NA	**1.3**	**1.1**
ROGERA	4	NA	5	ND	4	1	**2.8**	**1.9**
CORREDOR	1	NA	4	ND	3	8	**3.2**	**2.8**
SIRVENT	2	NA	2	ND	4	1	**1.8**	**1.3**
FLUVIÀ	4	NA	7	ND	5	1	**3.4**	**2.6**
Mean	**2.4**	**--**	**3.6**	**--**	**3.3**	**2.2**	**2.3**	**2.1**

2.2.4. Discussion

2.2.4.1. **Organochlorines**

The only detected organochlorine was lindane still widely used in agriculture.

Geographical differences

Important differences were detected between stations (table 2.1). Muga and Mugueta obtained highest lindane concentration in Empordà marshes. The same geographical origin of water from both stations is reflected in these results.

The channels sampled contained lower levels of lindane, descending from Sirvent to Corredor and finally Madral.

At Rogera lindane was detected only in July. The low levels were predictable because this lagoon is quite isolated during spring and summer from surface water contributions.

Finally, at Fluvià river lindane was always below detection level.

These results seems to indicate that channels, with a shorter course, collecte a lower quantity of pesticides. But another reason is needed to explain differences between main rivers, because both traverse cultivation areas. A possible explanation is strong development of Muga river irrigation scheme. Instead, Fluvià sampling station was situated close to the mouth, where water body is enormous and sea water enter periodically.

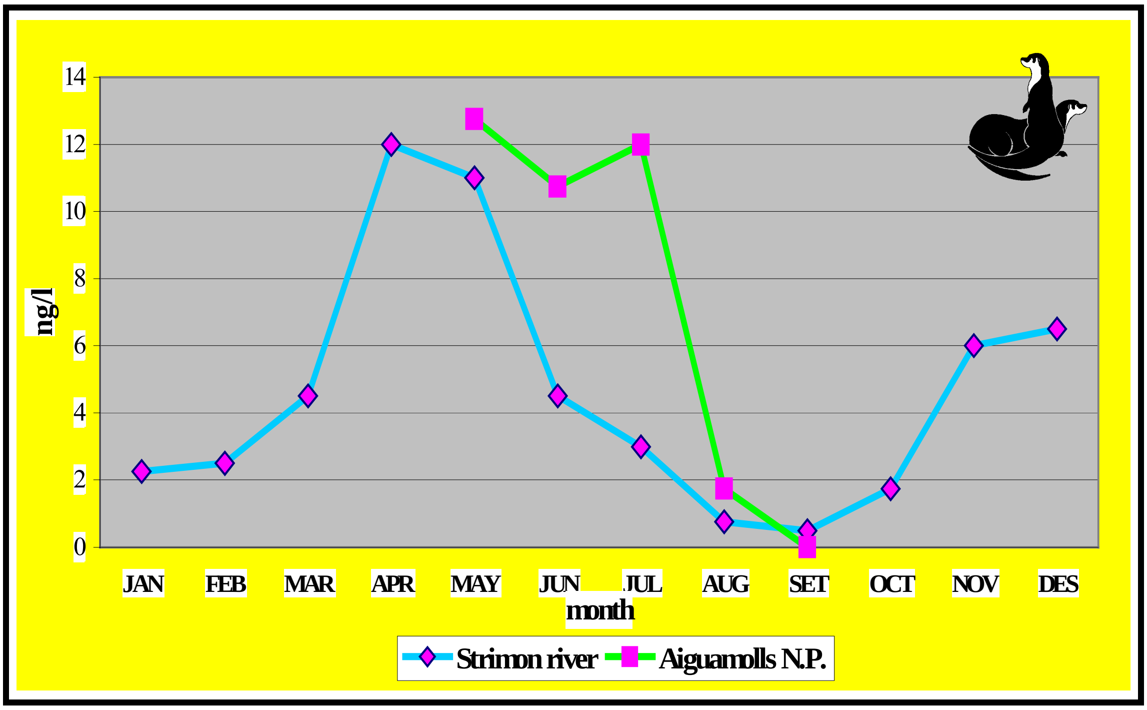
Seasonal differences

The farming origin of lindane pollution was confirmed with the increasing of its levels during the period of cultivation maxim activity. Thus, highest levels were observed from May to July, when crops are growing and also plagues that affect them.

Lindane levels evolution throughout the year between Empordà marshes and Strimon river were compared. Strimon is a Mediterranean river situated in Greece at a similar latitude. Figure 2.2 show low levels during all year, with a small peak in november-desember and an espectacular increasing at the end of spring (Kilikidis *et al*., 1992).

Following Strimon river pattern, it is possible to assume that lindane levels at Empordà marshes must be indetectables during non-prospected months, diminishing mean level from 6.3 to probably 3.1 ng/l.

Figure 2.2. Lindane levels throughout the year in Strimon river and Aiguamolls de l'Empordà Natural Park.



2.2.4.2. Triazines

Triazines were detected in high levels (table 2.2). Triazines are widely used as herbicides due to its (comparatively with organochlorines) low toxicity. Their high solubility in water cause a quick apparition in water coming from farmlands. The levels found in this study, in most cases above EU recommended limit for water destinated to human consumption, are generalized in many regions of Europe (Croll, 1990; Albanis, 1992).

Triazines detected in present study analysis were atrazine, simazine and terbutilazine.

Geographical differences

The geographical pattern observed was the same than in lindane. Muga and Mugueta obtained highest triazines levels, followed by Sirvent, Corredor and Madral channels. Rogera obtained very low levels but, as with lindane, Fluvià station obtained lowest triazines level.

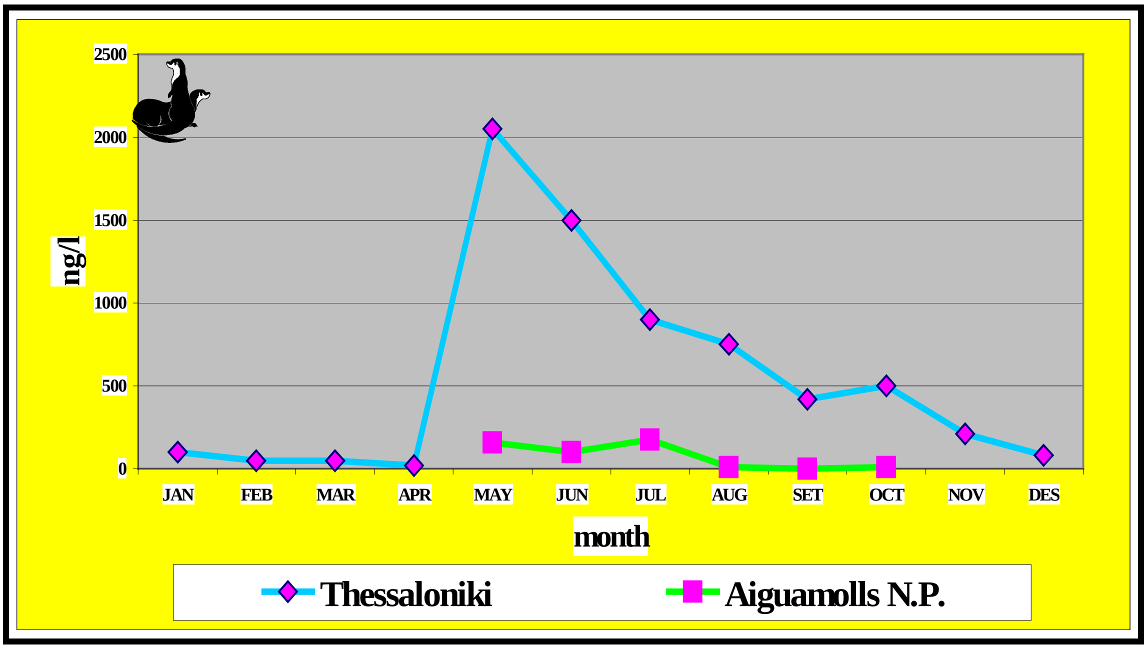
Seasonal differences

A seasonal variation on triazine levels was observed. At figure 2.3 a comparation between levels obtained at Thessaloniki, Greece (Albanis, 1992) throughtout the year and Empordà marshes levels (six months) was established, using in both cases the mean of all sampling stations (eight at Thessaloniki and seven at Empordà marshes).

The highest levels were obtained in May and June, both at Thessaloniki and Empordà marshes. After July levels decrease and remained very low the rest of the year.

As was described for lindane, atrazine (and other triazines) levels at Empordà marshes decrease during the months without data and the year mean must be much lower, probably around 50 ng/l.

Figure 2.3. Atrazine levels throughout the year in Thessaloniki farmland and Aiguamolls de l'Empordà Natural Park.



2.2.4.3. **Heavy Metals**

All the mercury samples were below detection level and this is the most interesting finding (table 2.3). Lead levels were very low and below any danger threshold (table 2.4). No geographical or seasonal differences can be found with presented data.

2.2.4.4. **Comparison with other world areas**

In order to obtain an overall view of surface water pollution levels at Empordà marshes, a review of similar studies carried out in other parts of the world was done. Results are presented at tables 2.5 and 2.6.

Lindane levels are similar to levels found in Greece and Croatia, and perhaps similar as well to levels found in England and Scotland, although means for these two last countries are lacking. Instead, levels found in Empordà marshes are lower than levels of other Spanish rivers and wetlands, as Guadalquivir river or Doñana National Park. In some developing countries lindane levels are much higher.

DDT was not detected (any isomer) in Empordà marshes, but it was detected in Strimon river (Greece). In most Mediterranean rivers and wetlands DDTs levels are much higher (Baluja *et al*., 1985; Hernandez *et al*, 1992).

Dieldrin, substance claimed as responsible for decline of several otter populations (Mason & Macdonald, 1986) was not detected. The same result is found in other consulted European studies (Hernández *et al*., 1992; Miliadis, 1993) while in Africa levels well above 200 ng/l are found (Mhlanga & Madziva, 1990; Nwankwoala & Osibanjo, 1992).

PCBs, probably the most dangerous substances for otter populations viability, were not found in Empordà marshes (and in Strimon river either), while in Doñana National Park levels are far more high and concerning (Baluja *et al*., 1985).

Triazines levels are high but well below levels found in Thessaloniki farmland (seven times higher) or England (Croll, 1990; Albanis, 1992).

Mercury was not found and detection level (<0,2 mg/l) is 15 times lower than levels found in surface water from Doñana National Park (Baluja *et al*., 1985).

Lead levels were the lowest found in all the sources consulted, below the mean for USA surface waters and much lower than Doñana National Park levels (Baluja *et al*., 1985; Albaiges *et al.*, 1987). However, Empordà marshes levels are higher than natural (0.006-0.05 mg/l), considering measured levels in remote US rivers (Trefry *et al*., 1985).

Finally, pesticide and heavy metal levels found in Empordà marshes surface water are better than most other levels found in similar studies of other parts of the world. From consulted bibliography, the area with most similar characteristics was Strimon river in Greece. This river hold an important otter population (Macdonald & Mason, 1985, 1992).

*Table 2.5. Pesticide levels found in water of other parts of the world (ng/l). (First number is mean, second number -between brackets- is positive stations percentage and last two numbers are the range. n= number of samples, SD= standard deviation, *= total HCH's)*

Locality /n	Lindane	DDT	Dieldrin	PCBs	Atrazine	Simazine	Reference
Aiguamolls (Catalonia) /41	6.3 (29) <2-56	ND	ND	ND	79 (44) <10-700	29 (24) <10-429	present study
Strimon river (Greece) /48	4.6(100) 0.3-12.6	ND	ND	ND			Kilikidis et al. 1992
Thessaloniki (Greece) /272					550 (71) <100,5900		Albanis 1992
rivers and lakes (Greece) /14	<3 (10) <3-15		<10 (0)				Miliadis 1993
Kupa river (Croatia) /36	6 (100) 2-20			3 (100) 1-8			Fingler et al. 1992
Doñana (Spain) /800	30* SD=30	210 SD=110		1030 SD=240			Baluja et al. 1985
Doñana (Spain)		197 90-540		1035 10-2530			Albaiges et al. 1987
Guadalquivir (Spain)	54*(100) 9-137	7 (90) 1-33	ND	141(100) 85-222			Hernández et al. 1992
Nile Delta (Egypt) /8	129.6(88) <1-255.2	58.0(100) 0.3-102.7		161.5(100) 8.3-652.9			El-Gendy et al. 1991
Port-Said; sea (Egypt) /20	0.9 0.1-7.6	57.6 0.2-284.8		64.9 12.6-189.0			El-Dib & Badawy 1985
England /700	(16) <10-55				(58)<20-9000	(42)<20-7100	Croll 1990
Grampian (Scotland)	<10-349						Littlejohn & Melvin1991
Lake Mcllwaine (Zimbabwe)	100* 20-270	400 30-700	200 0-530				Mhlanga & Madziva1990
Ibadan (Nigeria) /10	100 (100) 7-297	310 (70) ND-1266	250(100) 17.8-657				Nwankwoala & Osibanjo 1992

*Table 2.6. Heavy metal levels found in waters of other parts of the world (mg/l). (First number is mean, second –between brackets- positive stations percentatge and last two numbers are the range. n= number of samples, SD= standard devistion, *= total HCH's)*

Locality /n	Hg	Pb	Reference
Aiguamolls (Catalunya) /34	<0.2 (0)	2.3 (79) <1-8	present study
Doñana (Spain) /800	3 (SD=0.7)	11 (SD=2)	Baluja et al. 1985
Doñana (Spain)		11 (SD=3)	Albaiges et al. 1987
Piaseczno lake (Poland) /3		18.0	Radwan et al. 1990
USA	0.2	4	Smith et al. 1987
mean world rivers		3	Turekian 1969
drinking water (USA)		3.7	Durfor & Becker 1964
drinking water (Norway) /384		<35 (<35-680)	Flaten 1991

2.2.4.5. Comparison with drinking water levels

To verify that Empordà marshes water quality is adequate for human beings does not assure that it is adequate for otter because pollutant bioaccumulation is produced through the food intake (Mason, 1989). But toxic substances accumulating in fish come firstly from water and an excellent quality in otter habitats affect enormously to the bioaccumulation rate of the aquatic ecosystem organisms.

Thus pesticides and heavy metals levels were compared with maximum allowed levels in different countries and international organizations legislation (tables 2.7 and 2.8), nevertheless having in mind that this is not a definitive proof of water quality suitability for otter reintroduction.

Mean lindane concentration found in Aiguamolls de l'Empordà marshes is under all legislated levels presented in table 2.7 (with the exception of Croatia). From 41 analyzed samples only one exceeded Spanish (MOPTMA) recommended level and no samples exceeded EU recommended level.

The rest of organoclorates were not detected so they are obviously under legislated levels.

Triazines were found in much higher concentrations. Thus, while levels were some magnitude orders under legislated maximum levels in US, sometimes were higher than the 100 ng/l recommended by EU for any pesticide (CE, 1980). Exactly, triazine exceded this level in 22% of the samples, simazine in 10% and terbutilazine in 5%.

Regarding heavy metals, mean lead levels detected in Aiguamolls de l’Empordà water would classify it as drinking water following norwegian legislation (SIFF, 1987). Specifically, from 34 analyzed samples, 85% woud be classified as good and 15% as acceptables.

From the exposed data Aiguamolls de l’Empordà Natural Park water can be considered drinkable regarding the studied compounds. Nevertheless, some places where it is necessary to diminish lindane and triazine levels have also been found.

Table 2.7. Average concentration of some compounds in the Aiguamolls de l'Empordà Natural Park versus maximum pesticide recommended levels in drinking water (ng/l). (= total HCH's)*

Country	Lindane	DDT	Dieldrin	PCBs	Atrazine	Simazine	Reference
Aiguamolls	6.3 (<2-56)	ND	ND	ND	79 (<10-700)	29 (<10-429)	present study
European Union	100	100	100	100	100	100	CE 1980
Spain	50*	25000					Hernández et al. 1992
United Nations	3000						WHO 1984
Great Britain	3000				2000	10000	DoE 1989
USA	200				150000	1505000	EPA 1985
Canada	4000						Canadian Gov. 1978
Croatia	10			1			Regulatory Act 1978

Note: Two european directives (*CEE, 1986, 1988*) determined the following objectives for surface water quality: total DDT 25000 ng/l, dieldrin 10 ng/l.

Table 2.8. Average concentration of some compounds in the Aiguamolls de l'Empordà Natural Park versus quality criteria of drinking water concerning heavy metals established by norwegian government (SIFF, 1987) and European Union (CE, 1980), in mg/l.

Country	Criteria	Hg	Pb
Aiguamolls		<0.2	2 (<1-8)
CE	Maximum	1000000	50000000
Noruega	Good		<5
Noruega	Acceptable		5-20

2.2.5. **Conclusions**

A small proportion of analyzed pesticides were found (a 7% aproximately). The main compounds considered responsibles from otter's decline in vasts areas of Europe and North America (PCBs, DDT, dieldrin and mercury) were absents - or presents below level detection - from Aiguamolls de l'Empordà waters. Variable concentrations of lindane (the only organoclorate still allowed for agriculture purposes) were found. Highest levels were found in may, june and july, when crops are growing and also the plagues that affect them. Triazines are widely used due to its low toxicity and have also high solubility in the water. Both reasons explain the high levels found in Aiguamolls Natural Park waters. Muga and Mugueta rivers presented the pesticides highests concentrations while Fluvià river obtained the lowest levels. Lead levels were very low and mercury was not detected.

From consulted bibliography, the area with most similar characteristics was Strimon river in Greece. This river hold an important otter population (Macdonald & Mason, 1985, 1992).

Aiguamolls de l'Empordà Natural Park water is suitable for human consumption regarding the compounds analyzed.

Pollution levels which could affect an otter population were not found. Nervertheless, it is necessary to notice that the majority of compounds are bioaccumulatives. Thus, more important than know its concentration in surface water in a given moment, it is necessary to know the bioaccumulation suffered by aquatic organisms in the long-term.

2.3. Assessment of organochlorine levels in fish

2.3.1. Introduction

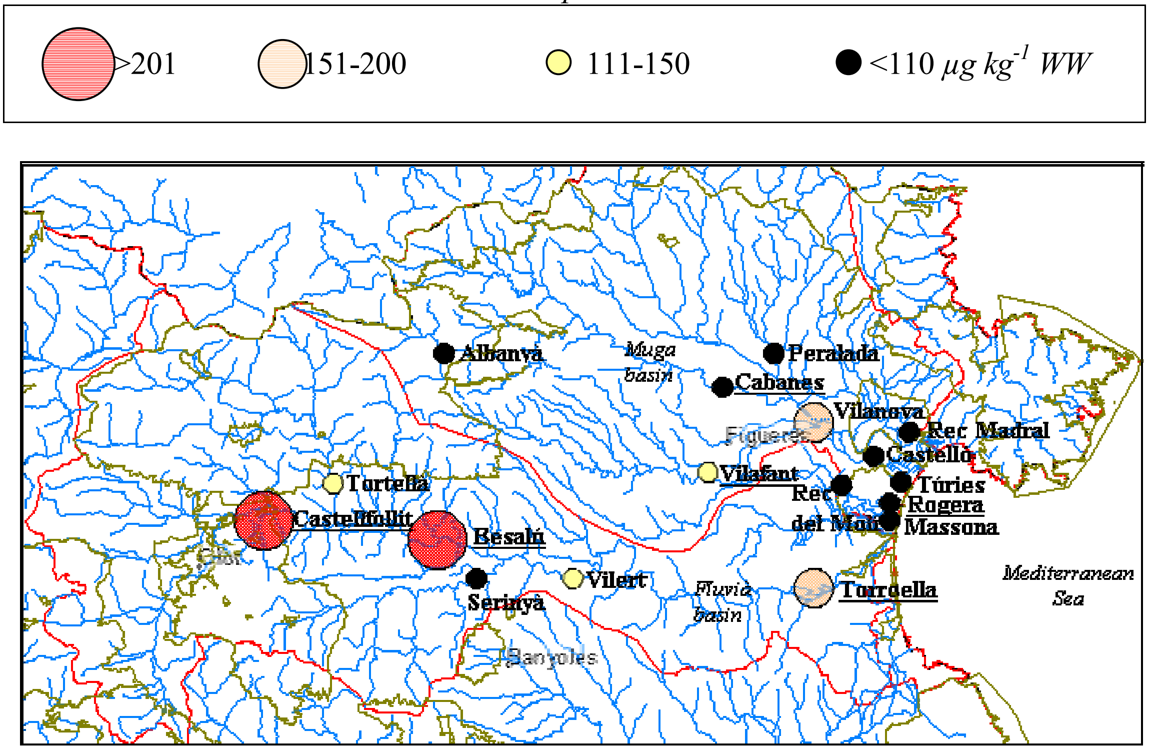
In the declining of otter *Lutra lutra* in Europe for the last forty years (Mason & Macdonald, 1994), several causes have been involved, such as hunting, habitat destruction or human disturbances. But pollution from organochlorine compounds (OCs) and methyl mercury (Mason, 1989; Kruuk & Conroy, 1991; Smith et al., 1994; Gutleb, 1995; Kruuk, 1995; Ruiz-Olmo et al., 1994; 1998; in press) seems to have been played an important role in this decline.

For this reason one of the parts of the feasibility of otter reintroduction implied the assessment of the levels of known pollutants responsibles for otter decline (Macdonald & Mason, 1994). The levels of OCs in freshwater fish were only available for single points of the Muga and Fluvià rivers (López-Martín *et al.*, 1995) and further analysis were necessary. This chapter describes the results of the analysis of OCs in the potential preys for the otters in the Muga and Fluvià basins.

2.3.2. Materials and methods

Seventeen sampling points were distributed along the rivers Muga, Fluvià (including tributaries) and the Empordà marshes, between the mouths of both rivers (Figure 2.4). To study fluctuations in OCs levels during the year, four samples (one per season) were collected in 7 points, while the rest were sampled in winter. However, summer samples from Vilafant and Rogera lagoon could not be collected as a cause of the drought. A spring sample of crayfish *Procambarus clarki* from Vilafant and a summer sample of fish from Serinyà were collected instead.

Figure 2.4. Study area with indication of concentration of ΣPCBs in μg kg^{-1} WW of fish in the sampling sites. Those sites sampled several times during the year are shown underlined. Green surrounded areas correspond to Natural Parks.



Sampling was carried out by electrofishing and the first 10 (6-12) fishes were captured to obtain a representation of the potential diet of otters in each point, thus one sample could be composed by several species and sizes (table 2.9). Fishes from each sample were weighted, ground whole, pooled and an aliquot of 50 g was stored at -20ºC until analysis.

Table 2.9. Fishes and crustacea included in the study.

		Weight (g)			
Species	***n***	**Mean**	**S.D.**	**Median**	**Range**
CRUSTACEA					
Procambarus clarki	12	14	12	11	3-46
ANGUILLIDAE					
Anguilla anguilla	105	73	76	53	8-435
MUGILIDAE					
Mugil cephalus	25	225	259	36	23-918
Chelon labrosus	3	404	383	378	35-800
Liza aurata	9	464	450	379	23-1,190
Liza ramada	14	238	268	62	21-805
CIPRINIDAE					
Barbus meridionalis	126	57	49	44	4-320
Leuciscus cephalus	15	257	147	282	15-493
Rutilus rutilus	19	55	35	52	16-143
Scardinius erythrophthalmus	8	79	101	53	15-322
Cyprinus carpio	39	712	618	658	66-3,418

Solvent and reagents were pesticide residue grade or similar, and were purchased from Merck (Darmstad, Germany) and Panreac (Montcada i Reixac, Spain). Quantitative solutions of Aroclor 1254, Aroclor 1260 and pesticide mixture were obtained from Alltech (Deerfield, IL, USA), and pure standards of several individual polychlorinated biphenyls (PCBs) congeners and pesticides were purchased from Promochem (Wesel, Germany).

Analyses were done following the method described by Guitart *et al.* (1990) with some modifications. Briefly a portion of 2 g of sample (wet weight) was homogenized with 15 g of anhydrous sodium sulphate. Extraction was done with 15 ml of n-hexane by mechanical shaking (10 min) and ultrasonic treatment (5 min). This procedure was repeated with 10 additional ml of hexane. PCBs #1 and #209 were used as internal standards. An aliquot (5 ml) of each extract was removed to calculate the percent of extractable fat by gravimetry. Clean-up was repeated two times by adding 3 ml of concentrated sulphuric acid (Murphy, 1972; Veierov & Aharonson, 1980). Clean extract was dried on rotary evaporator and rediluted in 100 ml of hexane.

A RTX-5 (crossbonded 95% dimethyl-5% diphenyl polysiloxane) fused silica column of 30 m, 0.53 mm ID and 0.5 mm film thickness (Restek, Bellefonte, PA, USA), coupled to an electron capture detector (ECD), was used for the analysis. The temperature program was: 145^{o}C initial, then run to 297^{o}C at a rate of 2.5^{o}C/min. Carrier gas (He) was set at a linear velocity of 25 cm/s. Make-up gas (N_2) was adjusted at a flow of 60 ml/min; injector and detector temperatures were 320 and 330^{o}C, respectively.

Gas chromatographic analyses were made on a Perkin-Elmer Autosystem, connected to a 1020 Perkin-Elmer/Nelson computer. Peak heights were automatically integrated and the report files in ASCII were reanalyzed on a special computer-assisted program (on BASIC language) created in our laboratory (Guitart *et al.*, 1996). The experimental retention times of PCBs #1 and #209, and pp'-DDE, were used to localize all the other OC peaks, and the height (Guitart *et al.*, 1996) of PCB #209 for quantitative purposes. In the case of PCBs, the corrected Ballschmiter and Zell nomenclature system (Guitart *et al.* 1993) has been utilized throughout this paper. For calibration purposes a mixture (1:1) of Aroclor 1254 and Aroclor 1260 with the composition reported by Schulz *et al.* (1989) was used to quantify PCBs (IUPAC numbers): 66+95, 90+101, 82+151, 135, 118, 123+149, 153, 141, 179, 138+160+158, 187, 128, 174, 177, 156+171+202, 180+193, 196+203, 195+208 and 194. Ruiz-Olmo & López-Martín (1994), in basis to field observations, established in 110 μg/kg WW of these PCBs congeners in the diet the upper limit to sustain stable otter populations in Catalonia and it was taken to evaluate the viability of reintroduction.

Calibration for pesticides and their metabolites has been carried out with a mixture of 14 compounds. Recoveries with fortified samples showed values that ranged from 70 to 100% for all residues except for endrin and dieldrin (0%). Corrections were not introduced in quantification in basis to recovery data. Variability, in terms of coefficient of variation of repeated analysis (n=4) was between 1 and 8%. Blanks were processed between samples to check the absence of contamination.

Concentrations were normalized by logarithm transformation (ln[OC]+1) and parametric tests were used. Differences in OCs concentrations between areas (Muga, Fluvià and Empordà marshes) and seasonality were studied by two way ANOVA without interactions. Ratio ΣDDTs/ΣPCBs (indicative of the agriculture/industry origin of pollution) not showed a lognormal distribution and were analyzed by Kruskal-Wallis test. Linear correlations between OCs levels and characteristics of the sample (weight % of cyprinidae, mugilidae and anguillidae, and % of extractable lipid) were studied. The sample of crayfish was not included in the statistical analysis.

2.3.3. Results

Organochlorine residues in fish samples (table 2.10) were mainly ΣPCBs, followed by ΣDDTs (sum of pp'DDE, ppDDD and pp'DDT) and ΣHCHs (sum of hexachlorocyclohexane isomers). Levels of HCB (hexachlorobenzene), ΣHCHs, ΣDDTs and ΣPCBs were significantly correlated between them except in the case of ΣHCHs with ΣDDTs. The highest correlations were obtained between ΣPCBs and HCB levels (r=0.67, p<0.001).

Table 2.10. Organochlorines levels (µg/kg WW) in the whole samples.

	Fish (n=37)		
Compounds	Geometric mean	95% C.I.	Range
HCB	2.0	1.6-2.4	0.6-8.6
a-HCH	0.9	0.7-1.1	0.2-3.1
b-HCH	3.0	2.7-3.4	0.6-6.5
g-HCH	2.5	1.8-3.5	0.5-20.0
d-HCH	0.4	0.3-0.5	n.d.-1.0
Heptachlor	0.6	0.4-0.8	n.d.-2.1
Heptach. epoxide	0.2	0.0-0.3	n.d.-6.5
Aldrin	0.5	0.4-0.6	n.d.-1.5
a-Endosulfan	0.7	0.4-1.1	n.d.-8.4
Endosulf.sulphate	1.0	0.8-1.3	n.d.-4.2
pp'DDE	16.6	12.3-22.5	1.2-80.8
pp'DDD	4.0	2.9-5.3	n.d.-16.9
pp'DDT	4.7	3.5-6.2	0.7-34.8
PCB #66+95	12.6	8.7-18.0	n.d.-92.3
#90+101	12.0	8.9-15.9	1.9-70.4
#82+151	2.9	2.0-4.1	n.d.-14.8
#135	5.3	3.9-7.1	0.4-45.8
#123+149	1.5	0.7-2.8	n.d.-20.0
#118	4.1	2.7-6.2	n.d.-39.9
#153	20.0	15.0-26.6	3.1-86.7
#141	2.1	1.5-2.9	n.d.-8.8
#179	0.5	0.3-0.7	n.d.-2.9
#160+138+158	11.1	8.4-14.7	2.4-50.1
#187	5.1	3.9-6.6	0.5-17.2
#128	3.1	2.4-3.9	1.0-13.6
#174	2.1	1.6-2.7	n.d.-8.0
#177	1.8	1.4-2.4	n.d.-6.5
#202+171+156	4.2	3.2-5.5	n.d.-18.4
#180+193	7.0	5.2-9.3	0.9-32.7
#203+196	1.1	0.9-1.4	0.2-3.4
#208+196	0.5	0.5-0.6	0.2-1.3
#194	0.8	0.6-1.0	n.d.-2.4
ΣPCBs	107.2	81.0-136.4	18.6-426.5

Some differences between areas for OCs levels could be established (table 2.11). Levels of ΣDDTs were higher in Muga and Fluvià river samples than in Empordà marshes, and the difference was significant for pp'DDT (F=6.0, p<0.01, 2 and 31 d.f.). The area with higher levels of ΣPCBs was the Fluvià river (F=10.7, p<0.001, 2 and 31 d.f.). Thus, the ratio ΣDDTs/ΣPCBs was higher in this most industrialized area (c^2=10.7, p<0.01, 2 d.f.). The highest levels of ΣPCBs have been located just downstream of the two most populated cities of both basins, Olot in the Fluvià basin and Figueres in the Muga basin.

Table 2.11. Geometric mean with 95% C.I. of organochlorines levels (µg/kg WW) in fish samples from the main studied zones.

Zone	n	pp'DDT	ΣDDT_S	ΣPCB_S	$\Sigma DDT_S/\Sigma PCB_S$[a]
Muga river	14	7.1**A**[b] (4.3-11.3)	32.5 (19.4-54)	81.5**B** (57.5-115.3)	0.53**B** (0.16-1.55)
Empordà marshes	7	1.7**B** (1.1-2.4)	12.4 (7.2-20.9)	51.5**B** (36.5-72.6)	0.27**AB** (0.12-0.61)
Fluvià river	16	4.8**A** (3.2-6.9)	27.9 (17.3-44.6)	179.1**A** (123-260.6)	0.17**A** (0.05-0.27)

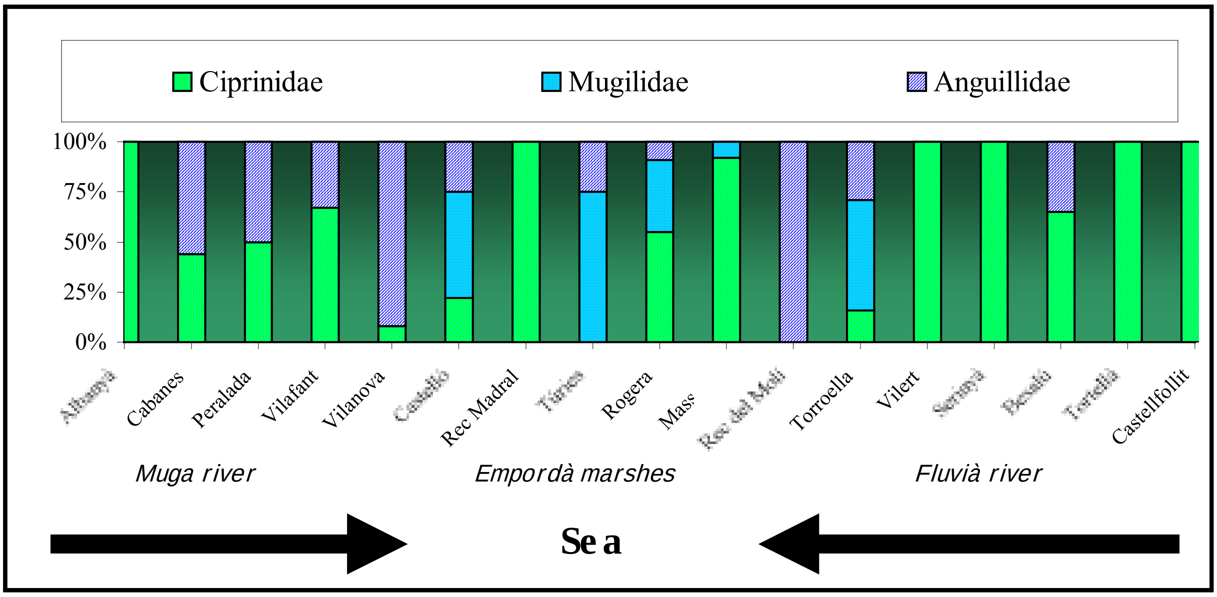
[a]*Arithmetic mean (range).*
[b]*Two means sharing a letter do not differ significantly (Tukey test and Kruskal-Wallis test).*

Seasonality was assessed for g-HCH (lindane) level. It was higher in spring (geometric mean=6.6; 95% C.I.=2.0-18.2) than in winter (1.7; 1.0-2.6; F=5.1, p<0.01, 3 and 31 d.f.).

The potential diet of otters in the studied areas has less than 110 mg ΣPCBs/kg WW in 67% sampling points in the Muga basin, 17% in the Fluvià basin and 100% in the Empordà marshes (figure 2.3). Accounting for the number of samples, percents were 57, 6.25 and 100% respectively.

The presence of anguillidae in the pooled fish was correlated positively with the OCs contents, mainly of pp'DDT (r=0.44, p<0.01) and pp'DDD (r=0.35, p<0.05) levels, but this effect could be explained by the high lipid content of anguillidae (the presence of anguillidae in the samples was correlated positively with lipid contents; r=0.53, p<0.001). If concentrations were referred to lipid weight, the OCs levels, in special ΣPCBs, were correlated positively with the % in weight of cyprinidae in the pooled sample (r=0.44, p<0.01). It could be explained because cyprinidae are the predominant species in the samples taken in the most polluted areas of the Fluvià river (figures 2.4 and 2.5).

Figure 2.5. Composition of the samples analyzed throughout the study area (% of a family by weight).



2.3.4. Discussion

The levels of OCs found in the analyzed samples are in the order of those described in fish captured between 1990 and 1992 in rivers of Catalonia, with arithmetic means in WW of 21 µg/kg of ΣHCHs, 81 µg/kg of ΣDDTs and 181 µg/kg ΣPCBs (López-Martín *et al.*, 1995). Fluvià and Muga rivers were sampled too by López-Martín *et al.* (1995) and their results were similar for ΣDDTs, but slightly higher for ΣHCHs and lower for ΣPCBs.

The higher level observed for g-HCH in spring have been previously described in the Strimon river water in Greece (Kilikidis *et al.*, 1992) and otter faeces from SW England (Mason & Macdonald, 1994), and it must represent the moment of its use in agriculture and livestock.

The OCs levels have also been found to be related to fish species pooled in each sample. It could be due to the differences between species by two factors, the lipid contents and the migratory behaviour. In relation to the lipid contents, Ruiz & Llorente (1991) have detected higher levels of DDTs and PCBs in eels than in carps from the Ebro delta (Catalonia, Spain), and it was mainly due to differences in extractable lipids (20% in eels and 0.5% in carps). On the other hand, the migratory behaviour to the less polluted sea waters of mugilidae could explain the lower OCs levels detected in the Empordà marshes or in the low stretches of Fluvià and Muga rivers (Cullen & Connell, 1992). In general, marine fish species contains lower OCs levels than those from freshwater or estuaries (Albaigés *et al.*, 1987). Another reason for the lower levels detected into the Empordà marshes could be the retention of OCs along a complex system of vegetated channels as occurs in eels from reedbeds compared to eels from rivers (Mason, 1993).

The role of OCs on the feasibility of otter reintroduction must be focused on PCBs, thus pesticides other than dieldrin (not analyzed by us) have not been associated with otter extinction (Mason, 1989, Macdonald & Mason, 1994). High levels of ΣPCBs have been detected along the Fluvià river, specially in the upper course, and in some points of the Muga basin. However, several points of the coastal marshes and the greatest part of the Muga basin showed PCBs levels below those observed in Catalonian rivers where otters are still present (Ruiz-Olmo & López-Martín, 1994). Thus, it can be expected that lagoons and channels of Aiguamolls de l'Empordà Natural Park could be a good place to begin the reintroduction in the zone. Saavedra and Ruiz-Olmo (in Saavedra, 1995) estimated in basis to trophic resources the number of otters supported in this area around 37-44 otters. It seems possible to establish here a breeding population able to expand gradually waters above. This process have been observed in SW England, with colonizations of polluted areas by otters expanded from consolidated populations (Mason & Macdonald, 1994).

This movement of the otters between Empordà marshes and both rivers could increase the exposure to PCBs above the critical level of 110 µg/kg WW. However, if we assume the bioconcentration factor of PCBs from fishes to otter tissues (x2.9) obtained in Catalonian rivers (López-Martín & Ruiz-Olmo, 1996), then it can be predicted a mean (95% C.I.) level of PCBs in tissues of reintroduced otters of 22.6 (17.4-29.0) µg/g LW (lipid weight) (table 2.12). This value is below the critical level of 50 µg/g LW associated to reproductive failures in laboratory mink (Jenssen *et al.*, 1977), a figure assumed also for otters (Mason, 1989). Anyway, it must be taken into account that 18.9% of collected samples can produce ΣPCBs accumulation levels in otters above this critical level.

Table 2.12. Estimated geometric means (95% C.I.) of ΣPCBs levels (µg/g LW) in otter tissues in basis to the bioconcentration factor (x2.9) determined by López-Martín & Ruiz-Olmo (1996) and our levels in fish from the same areas.

Zone	n	% LW Mean±S.D.	Fish	Otter	% Fish samples >14.2 µg/g LW[a]
Muga river	14	1.79±0.78	5.1 (3.6-7.3)	14.8 (10.4-21.2)	0.0
Empordà marshes	7	0.77±0.48	7.9 (4.5-13.7)	22.9 (13.0-39.7)	14.3
Fluvià river	16	1.87±1.00	11.1 (7.3-16.9)	32.2 (21.2-49.0)	37.5

[a] ΣPCBs level in fish producing 50 µg/g LW in otter tissues.

The main question of this study was the choice of the concentration of ΣPCBs in the diet considered as incompatible with otter reintroduction. The level of concern we used of 110 µg/kg WW (Ruiz-Olmo & López-Martín, 1994) is similar to 80 µg/kg WW described in eels from rivers with the presence of otters (Mason, 1989) or to 72 µg/kg WW in diet found as NOAEC (no adverse effect concentration) in mink (Giesy *et al.*, 1994). However, Macdonald & Mason (1994) have established the maximum level of PCBs in the diet for thriving otter populations in 26 µg/kg WW. If we take this last value, otter reintroduction in the Empordà marshes could not be carried out in a short term, but a recent review of these critical levels of PCBs (Kruuk & Conroy, 1996) encouraged us to take the level obtained by Ruiz-Olmo & López-Martín (1994) in similar conditions to our study area.

2.4. Stock assessment, biomass and fish production

2.4.1. Introduction

As it has been showed in previous chapters, the level of pollutants in water and fishes is low. Now, it is necessary to assess the population of the principal prey: fish.

The aim of the work contained in this chapter is to estimate population sizes, biomasses and production of fish communities in order to establish a framework to guarantee the success of the otter reintroduction with regard to the principal prey source.

2.4.2. Material and methods

In November 1995, several sets of data were collected by the Natural Park biologists in successive catches of fish. 12 sites were selected in order to survey the main flows of both basins. The general characterization of each site was obtained (table 2.13). Lengths of these sampling sites varied within the range of 50 and 150 meters.

Figure 2.6. Geographic locations of the study sites.

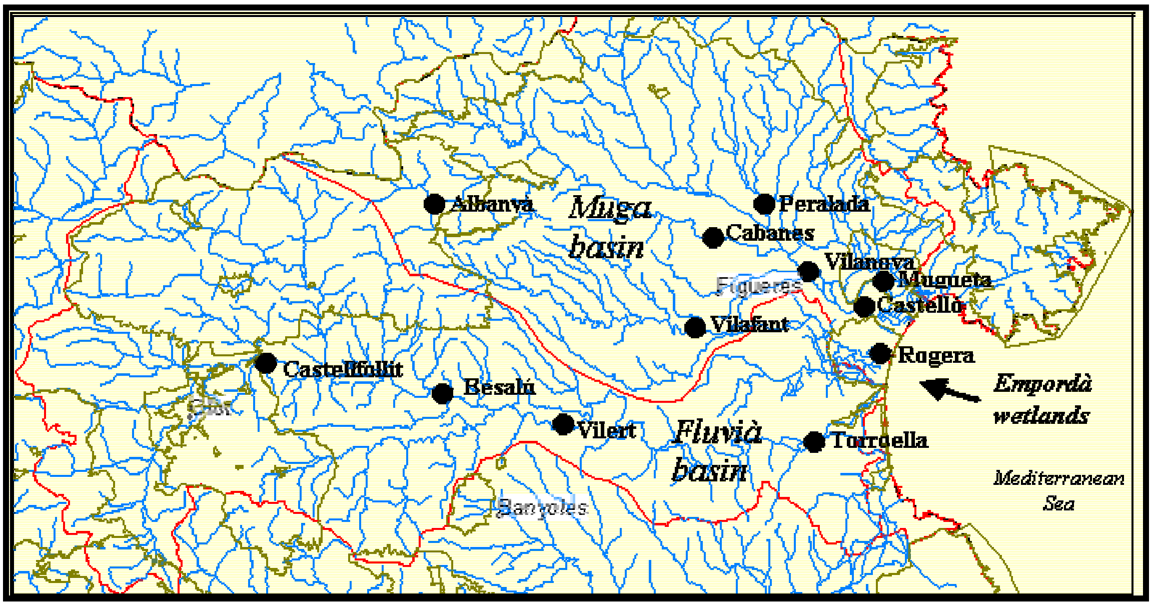


Table 2.13. Physical characteristics of the study sites in the Empordà wetlands and Muga and Fluvià basins at the time of samplings. [(a)] s-sand, st-stone, sp-submerged plants and m-mud.

Study site	Date	Area (m^2)	Min. width (m)	Max. width (m)	Mean depth (m)	Length (m)	Bottom structure[a]
Fluvià basin							
Castellfollit	30.11.94	640	13	14	1-1.5	50	st – s
Besalú	29.11.94	4100	35	38	0.4-1.3	110	st
Vilert	29.11.94	1340	13	40	0.5-1.5	65	st
Torroella	24.11.94	3550	15	45	0.3-1	150	s –sp
Muga basin							
Albanyà	23.11.94	1760	14	24	0.5	90	st
Peralada	18.11.94	680	4.5	8	0.5	120	st – sp
Cabanes	25.11.94	450	4	13	0.5	50	s – sp
Vilanova	18.11.94	1290	10	22	1-1.5	90	s
Vilafant	23.11.94	540	7	22	0.4	60	sp
Castelló	28.11.94	1020	10	30	1-1.2	80	s – sp
Empordà wetlands							
Mugueta	28.11.94	300	5	5	1	60	m
Rogera	21.11.94	750	-	-	0.5-1.5	-	m

At all study sites, electrofishing surveys were conducted using the following procedure: sections were blocked off with barrier nets and upon 3 successive electrofishings, performed from the downstream net up to the upstream net. Electric fishing was conducted on foot by one diver in shallow areas using a generator-powered unit (ERREKA model SEINA) that provides fully-rectified triphasic AC (between 50-500 V). The working voltage was generally 200-350 V, 2-3 A. The triplicate fishings were carried out trying to keep the effort constant in order to respect the assumptions of catch-effort methods (Seber, 1982).

All fish was identified, counted, sampled and retained in screened cages until the survey was completed and released thereafter. Sampling included measuring fork lengths (mm) and wet weight (g) (Bohlin, 1990).

Densities of fish were calculated using catch-depletion data and the removal method (Zippin, 1956 and Seber, 1982) where maximum-likelihood estimates of N (population size) and P (probability of capture) were made for each species separately. Removal is a catch-effort method for closed populations when a constant sampling effort is applied. The basic assumptions of this method are that: (1) the population is closed during the experiment, (2) the probability of capture in a sample is the same for each individual exposed to capture and, (3) the probability of capture p remains constant from sample to sample. The second and third assumptions are more problematic to be easily assured (Mahon, 1980; Schnute, 1983; Bohlin *et al.*,1989) and typically verified by goodness-of-fit statistics (Zippin, 1956). Density estimates were made using the computer program REMOVAL (García-Berthou, 1993), developed to compute all the calculations of the removal method for a population size estimation. The program follows the maximum likelihood methodology, checks the failure conditions, applies the appropriated formula, and displays the estimates of population size and catchability, with their standard deviations and coefficients of variation, and two goodness-of-fit statistics with their significance levels. Where catchability varied significantly between sweeps, density was estimated as the ratio of the total catch to the average catchability of the species (Lobón-Cerviá, 1990).

Estimated standing crops (SC) were calculated as (BtCt)+Bc(N-Ct), where Bt is the total weight of fish caught, Ct is the total number of fish caught and Bc(N-Ct) represents the standing crop of the uncaptured fish, estimated by multiplying its number by the mean weight of fish caught in the last electrofishing, as has been applied by different authors (Penczak *et al.,* 1986; Lobón-Cervià & Utrilla, 1992).

Production was estimated indirectly by multiplying the average biomass by the mean P/B ratio (Bagenal, 1978). This information has been compiled from published sources (Mann & Penczak, 1986 and Jorgensen, 1978), which provide a revision of turnover ratio for non-salmonid fishes in European rivers. We applied the following values: AAN 1,15; ELU 0,7; BME 1,87; CCA 0,5; RRU 1,12; SER 1,12; DLA 0,4; LGI 0,95; CHL 0,5; LRA 0,4 and MCE 0,4.

Diversity among species was calculated, using the H' Shannon-Weaver index, derived from their information measure, as $H' = - \sum p_i \log p_i$ where p_i has been estimated by the ratio n_i / N.

Size structure was examined due to its direct usefulness in assessing the community and population structure, and because of the fact that any size selectivity imposed by electrofishing can influence other estimates such as density and biomass (Mahon, 1980).

2.4.3. Results

2.4.3.1. **Fish assemblage**

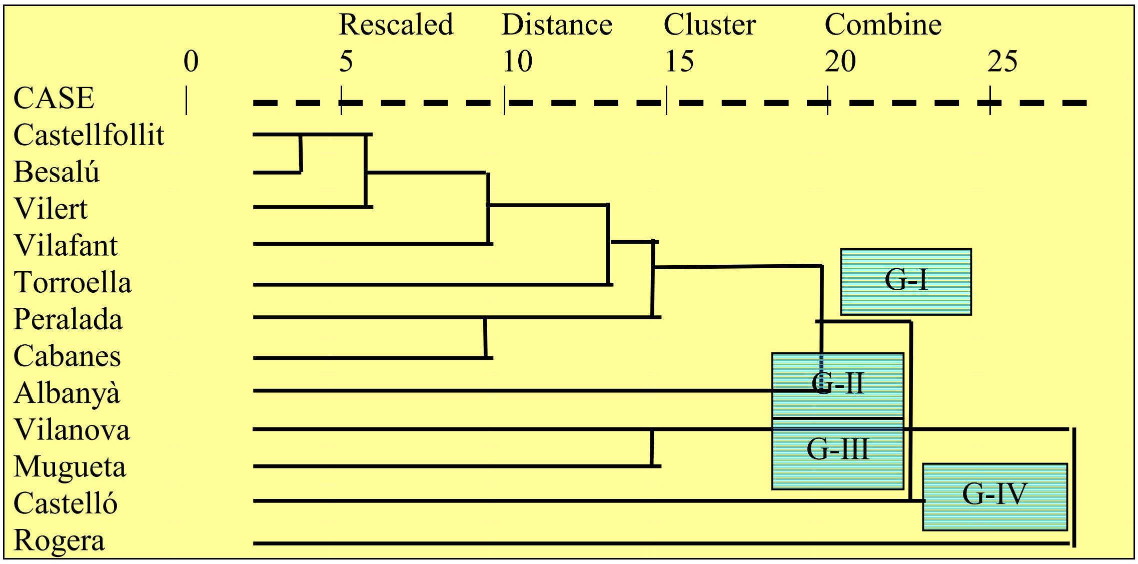
Species recorded at 12 study sites on the Muga and Fluvià rivers, by electrofishing catches, are shown in table 2.14. Size selectivity of electrofishing gear may induce a lack of species with small total size, like *Blennius fluviatilis* (Asso), and *Gasterosteus aculeatus* (L.) at some stations. A code for each species based on its scientific name is used in order to simplify the presentation of different results.

Table 2.14. Fish assemblage of Muga (1) and Fluvià (2) basins and (3) Empordà wetlands.

Code	Fish species	Common names	Area
AAN	Anguilla anguilla	European eel	1,2,3
ELU	Essox lucius	Pike	1
BME	Barbus meridionalis	Mediterranean barbel	1,2
CCA	Cyprinus carpio	Common carp	1,2,3
LCE	Leuciscus cephalus	Chub	1,2
PPH	Phoxinus phoxinus	Minnow	1
RRU	Rutilus rutilus	Roach	1
SER	Scardinius erythrophthalmus	Rudd	1
GAC	Gasterosteus aculeatus	Three-spined stickleback	1
DLA	Dicentrarchus labrax	Sea bass	1
LGI	Lepomis gibbosus	Bluegill sunfish	1
CHL	Chelon labrosus	Thicklipped mullet	1,2
LRA	Liza ramada	Striped mullet	1,3
MCE	Mugil cephalus	Grey mullet	1
BFL	Blennius fluviatilis	Freshwater blenny	1

The Muga basin presents a fish assemblage of 15 detected species, in contrast with the 3 fish species of the Empordà wetlands and the 5 species of the Fluvià Basin. In order to compare the different study sites in terms of fish species richness a Cluster Analysis has been performed, obtaining the classification presented in dendrogram of figure 2.7.

Figure 2.7. Dendogram using Average Linkage between groups (study sites) showing the relations between sites and groups of sites using the similarity ratio.



2.4.3.2. Populations size, biomass and production

Stock density, standing crop and production estimated at the sites after three catches are presented in table 2.15 for the Muga basin, in table 2.16 for the Empordà marshes and table 2.17 for the Fluvià basin. Out of 46 density assessments, the method of Moran and Zippin modified in 3 samples by Junge & Libosvarkî (1965), could be applied in 17 cases. The factor limiting the application of the removal method (in 29 cases) was the low number of fish of a given species at a particular site, and the failure of the method because the biggest amount of catches occurred in C2 or C3 (second and third sweeps).

Table 2.18 presents a summary of REMOVAL program output results with a classification of the size population estimate for each site and those fish species in which the removal method could be applied, those in which the estimate was statistically significant and those in which there was a failure.

Table 2.15. Population size, biomass and production obtained for sites and their fish species for ***Muga basin****. N are the estimated number of fish by station; p: catchability; s(N): Standard deviation of N; s(p): Standard error of p. Total biomass, Standing Crop, Biomass and Production as fresh weight (g).*

Study site	Species	Total catch	N	p	s(N)	S(p)	Total biomass (g)	Standing Crop (g)	Biomass ($g.m^{-2}$)	Production ($g.m^{-2}y^{-1}$)
Albanyà	BME	60	60,1	0,89	0,28	0,04	1045	1045	0,594	1,11
	LCE	3	3,1	0,71	0,36	0,28	40	40	0,023	0,03
		63	**63,1**				**1085**	**1085**	**0,616**	**1,1**
Cabanes	AAN	105	120,9	0,49	8,50	0,07	1780	8005,5	17,8	20,5
	BME	2	2	1	-	-	90	90	0,2	0,4
	ELU	2	2	1	-	-	645	645	1,4	1
	LCE	5	7	0,72	-	-	70	98	0,2	0,2
	RRU	208	214,3	0,69	-	-	8010	8253	18,3	20,5
		322	**346,3**				**10595**	**17091,5**	**37,9**	**42,6**
Peralada	AAN	21	22,7	0,58	-	-	2020	2077,5	3,1	3,5
	BME	25	48,4	0,21	34,42	0,20	425	822,5	1,2	2,3
	CCA	2	2,1	0,64	-	-	1315	1315	1,9	1
	LCE	25	25,7	0,7	-	-	960	986,5	1,5	1,7
	RRU	8	12,8	0,62	-	-	400	640	0,9	1,1
		81	**111,6**				**5120**	**5841,5**	**8,6**	**9,5**
Vilanova	AAN	67	72,4	0,58	-	-	9495	10254,6	7,9	9,1
	CCA	45	47,2	0,64	-	-	66780	70044,8	54,3	27,1
	LGI	4	4,6	0,5	-	-	40	40	0,03	0,03
		116	**124,1**				**76315**	**80339,4**	**62,3**	**36,3**
Vilafant	AAN	14	50,8	0,10	132,29	0,29	1690	6137,1	11,4	13,1
	BME	1	2	0,7	-	-	35	70	0,1	0,2
	CCA	76	122,9	0,27	-	-	52200	84399,1	156,3	78,1
	LGI	4	5,3	0,5	-	-	35	46,6	0,1	0,1
	LCE	2	2,7	0,7	-	-	10	13,5	0,02	0,03
	RRU	1	1,6	0,62	-	-	70	112	0,20	0,2
		98	**185,3**				**54040**	**90778,4**	**168,1**	**91,8**
Castelló	AAN	46	47,6	0,68	-	-	6565	6787,6	6,6	7,6
	CHL	17	17	0,94	0,05	0,05	12295	12295	12,1	6,03
	CCA	49	49,4	0,80	0,71	0,06	82090	82090	80,5	40,2
	DLA	27	28,6	0,61	2,00	0,11	24560	26046,2	25,5	10,2
	LGI	1	2	0,5	-	-	5	10	0,01	0,009
	LCE	28	29,5	0,63	1,87	0,11	365	384,5	0,4	0,4
	LRA	199	203,1	0,72	-	-	140425	143290	140,5	56,2
	MCE	18	18,7	0,66	-	-	11825	12291,4	12,05	4,8
	RRU	2	2,2	0,56	0,74	0,44	8	43,4	0,04	0,048
		387	**398**				**278138**	**283238,2**	**277,7**	**125,6**

Table 2.16.Population size, biomass and production obtained from each site and its fish species in the ***Empordà wetlands,*** *N is the estimated number of fish per station; p: catchability; s(N): Standard deviation of N; s(p):Standard error of p. Total biomass, Standing Crop, Biomass and Production as fresh weight (g).*

Study site	Species	Total catch	N	p	s(N)	S(p)	Total biomass (g)	Standing Crop (g)	Biomass ($g.m^{-2}$)	Production ($g.m^{-2}y^{-1}$)
Mugueta	AAN	4	4	1	-	-	410	410	1,4	1,6
	CCA	2	2	0,64	-	-	1315	1315	4,4	2,2
		6	**6**				**1725**	**1725**	**5,7**	**3,8**
Rogera	CCA	39	40,9	0,64	-	-	62735	65791,3	87,7	43,9
	LRA	41	47,4	0,49	-	-	13535	15647,7	20,9	8,3
		80	**88,3**				**76270**	**81439,02**	**108,6**	**52,2**

Table 2.17. Population size, biomass and production obtained from each site and its fish species in the ***Fluvià Basin****. N is the estimated number of fish per station; p: catchability; s(N): Standard deviation of N; s(p): Standard error of p. Total biomass, Standing Crop, Biomass and Production as fresh weight (g).*

Study site	Species	Total catch	N	p	s(N)	S(p)	Total biomass (g)	Standing Crop (g)	Biomass ($g.m^{-2}$)	Production ($g.m^{-2}y^{-1}$)
Castellf.	AAN	2	4	0,5	-	-	690	1380	2,1	2,5
	BME	65	81,6	0,4	11,1	0,09	2575	3064,6	4,8	8,9
	CCA	108	169	0,3	-	-	108160	169240,3	264,4	132,2
	LCE	41	64,6	0,3	1,9	0,1	8215	10946,1	17,1	19,7
		216	**319,2**				**119640**	**184631**	**288,5**	**163,3**
Besalú	AAN	38	42	0,5	3,7	0,1	9760	10787,4	2,6	3,02
	BME	67	72,1	0,6	3,8	0,07	1745	1878,3	0,5	0,8
	CCA	106	110,4	0,6	-	-	75645	78785	19,2	9,6
	LCE	53	54	0,7	-	-	6925	7053	1,7	2,0
		264	**278,5**				**94075**	**98503,7**	**24,02**	**15,5**
Vilert	AAN	116	116	1	-	-	13920	13920	10,4	11,9
	BME	1045	1045	1	-	-	81635	81635	60,9	113,9
	CCA	25	25	1	-	-	25025	25025	18,7	9,4
	LCE	3965	3965	1	-	-	334687	334687	249,8	287,2
	SER	568	568	1	-	-	16294	16294	12,2	13,6
		5719	**5719**				**471561**	**471561**	**351,9**	**436**
Torroella	AAN	23	23,03	0,9	0,2	0,06	570	570	0,16	0,18
	CHL	11	11,715	0,6	1,3	0,1	9270	9270	2,6	1,3
	CCA	16	16,02	0,9	0,1	0,08	21345	21345	6,01	3,006
	LCE	3	4,1	0,7	-	-	5	6,8	0,002	0,002
		53	**54,9**				**31190**	**31192**	**8,8**	**5**

Table 2.18. Summary of REMOVAL program output results: classification of sites and their fish species acording to goodness-of-fit statistics of population size estimate. ***Estimation of population size not significant*** *.- 1: Method of Moran (1951) and Zippin (1956) modified for 2 samples by Seber-Le Cren (1967); 2: Method of Moran (1951) and Zippin (1956) modified for 3 samples by Jungè & Libosvêrsky (1965);* ***Estimation of population size significant****.- 5%: Significant at the 5% level ; 1%: Significant at the 1% level ; 0,1%: Significant at the 0.1% level;* ***Failures.-*** *F1: N(1)=N(3) or q=0 (Lelek 1974); F2: SUM(I->N) (s+1-(2*I))*N(I)<0 (Seber & Whale 1970); F3: s=3 and X=Y or ((Y**2)+(6*X*Y)-(3*(X**2))<0 where X=2*N(1)+N(2) and Y=2*N(1)-N(3). Population size not estimated (small sample): Blennius fluviatilis, Phoxinus phoxinus, Gasterosteus aculeatus.*

Sample sites	not significant.		significant at the level			Failures		
	Method 1	Method 2	5%	1%	0,1%	F1	F2	F3
Albanyà		BME LCE						
Peralada		BME					AAN CCA RRU	LCE
Cabanes		AAN	RRU		LCE	BME ELU		
Vilanova							AAN CCA	LGI
Vilafant		AAN	CCA			LCE	LGI	BME
Castelló		CHL CCA DLA LCE RRU	AAN MCE	LRA				
Mugueta						AAN CCA		
Rogera			LRA			AAN CCA		CCA
Castellfollit		BME LCE	CCA					AAN
Besalú		AAN BME			CCA LCE			
Vilert	AAN BME CCA LCE SER							
Torroella		AAN CHL CCA				LCE		

The density assessment at sites which belong to both significant and failure status, has been obtained by multiplying total catches by catchability. Confidence intervals for the population density are not provided due to high values of standard error.
Due to heterogeneity among study sites, mean standing crops and productions have not been calculated. Muga river presents a range of standing crop variability of 1,085-283,238 kg, 0,616-277,6 $g.m^{-2}$ biomass, and 1,136-125,637 $g.m^{-2}.y^{-1}$ production. Fluvià river presents standing crop ranges between 31,191 and 471,561, 8,786-351,911 $g.m^{-2}$ biomass, and 4,49-436,05 $g.m^{-2}.y^{-1}$ production. These ranges correspond to a gradient between upstream waters and currents flowing into the wetlands.

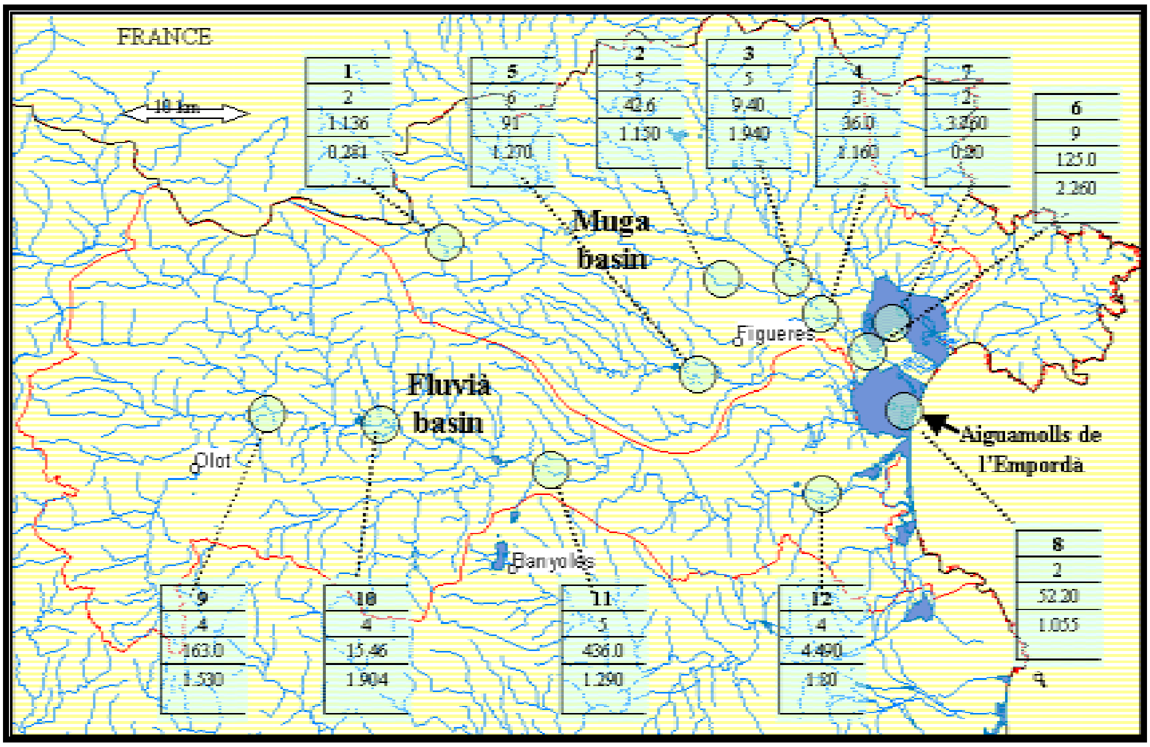
2.4.3.3. **Density**

Species richness as total number of species, fish density expressed as ind.ha^{-1} and diversity values for study sites are given in figure 2.8. Diversity was calculated according to the *Shannon-Weaver measure of information*. Muga river presents a higher richness due to the presence of the Boadella reservoir, with many introduced species and the influx of sea fish. Values increase along the river as it becomes wider and it finds a higher diversity of habitats. Fluvià river presents a more homogeneous pattern with an important change in Vilert, where fish density is very high and this involves the maximum biomass and production.

Figure 2.8. Species richness as total number of species, fish density expressed as ind.ha^{-1} and diversity values for study sites.

study site
species richness
fish density
diversity

Study sites	
1. Albanyà	7. Mugueta
2. Cabanes	8. La Rogera
3. Peralada	9. Castellfollit
4. Vilanova	10. Besalú
5. Vilafant	11. Vilert
6. Castelló	12. Torroella



2.4.3.4. **Length-frequency comparisons**

Regarding size structure, we have observed a size selection with a lack of young fish. Despite this evidence, we can not use this representation to determine the minimum size caught or size selection of larger fish since there is also an effect on population structure.

A summary of the major variables measured and its values is shown in table 2.19 for each fish species detected.

Table 2.19. Descriptive statistics for fish species detected: mean (minimum-maximum) of weight (g) and lenght (cm), with standard deviation (s) of mean. Total numb.: Number of valid observations (listwise).

Fish specie	Total Number	WEIGHT Mean	s	LENGTH Mean	s
Anguilla anguilla (L.)	320	36,8 (15-71)	11,19	119,9 (5-750)	133,2
Essox lucius (L.)	2	35 (33-37)	2,83	322,5 (280-365)	60,10
Barbus meridionalis (Risso)	220	11,55 (6-22)	3,03	26,75 (5-160)	25,48
Cyprinus carpio (L.)	443	35,68 (6-57)	10,21	1071 (5-3575)	696,6
Leuciscus cephalus (L.)	160	17,59 (6-35)	7,56	108,1 (0-520)	131,9
Phoxinus phoxinus (L.)	4	4 (1-9)	4,36	-	-
Rutilus rutilus (L.)	219	13,56 (7-22)	3,15	39,09 (5-180)	30,17
Scardinius erythrophthalmus (L.)	1	31	-	-	-
Gasterosteus aculeatus (L.)	1	4	-	-	-
Dicentrarchus labrax (L.)	27	38,37 (26-60)	9,5	909,6 (200-3100)	809,5
Lepomis gibbosus (L.)	9	7,67 (4-9)	1,73	8,89 (0-20)	6,01
Chelon labrosus (Risso)	28	36,79 (27-47)	4,04	770,2 (400-1630)	262,8
Liza ramada (Risso)	240	35,75 (9-49)	8,49	641,5 (10-1390)	275,6
Mugil cephalus (L.)	18	36,28 (23-45)	4,32	656,9 (145-1310)	235,3
Blennius fluviatilis (Asso)	1		-	-	-

2.4.4. Discussion

Two are the limitations which can be born in mind when the results are examinated. The first, that the study area is so extensive that it would imply a larger sampling effort. This has been restricted to 12 sites assigned to the Muga and Fluvià basins and the Empordà marshes with the assumption that each site represents the stretch. The traits of the study sites are the same up to the middle of the distance from the follow-up point, both downstream and upstream. On the other hand, electrofishing surveys were conducted all at a time, in November, so that production will be estimated indirectly from the P/B ratio of each fish species. This ratio takes different values in accordance with the environment where the fish species is found.

Early surveys carried out in the study area (Sostoa, 1990) show a fish species richness that coincides with our own results, with a maximum diversity (Shannon-Weaver index) never higher than 2 bits. In a basin, in general, complexity increases (as for species richness and diversity) from head to mouth, in correlation with altitude, slope and river order (Sostoa, 1990). The fish species composition in both rivers differs slightly, being the European eel, the mediterranean barbel and the chub the clear dominant ones at headwaters, and common carps, rudds, the bluegill sunfish and the

European eel downstream; at some locations near the mouth, marine fish species have been detected (sea bass, thicklipped mullet, striped mulled and grey mullet). The following are accompanying species, characterized by a low occurrence: pike, minnow, roach and freshwater blenny.

Dendrogram of figure 2.6 shows the relations between sites where hierarchical structure is indicated by the branching pattern using Average Linkage between groups (study sites). The dice (or Czekanowski or Sorenson) similarity measure has been used because it is a matching coefficient measure in which joint absences are excluded in both the numerator and the denominator and double weight is given to matches. Joint absences are not suitable when removals are not exhaustive, because the absence of species can be due to a low efficiency of caughts. Moreover, double weight of joint presences increases similarity values when complete matching can not be expected due to a low number of species and an inefficient sampling.

This analysis classifies sites in 4 groups distinguished, respectively, from top to bottom, by basins and sites located at the same distance from the sea (GI), sites next to the mouth with a low number of species (GII), presence of a large number of species (GIII, Case 6-Castelló) and la Rogera (GIII) as the most different with only two species and no joint presence.

After having applied the Removal method over 46 density assessments, 17 estimates come out non-significant. In 29 cases we obtained bad estimations as a consequence of small samplings and method failures. In these cases the model for tested populations is not valid by a violation of basic assumptions.

Three possible sources of low efficiency or error can be identified: (1) differences between species, which are called biotic factors, (2) locality characteristics such as visibility, overhanging vegetation, substrates, called abiotic factors, and (3) the experience of electrofishers. It is evident that there is a large proportion of unexplained variability in error as well. The accuracy of the removal method applied in electric fishing has been assessed by different authors (Bohlin, 1990) in order to improve the efficiency of catches.

Failures occur in all species and places (except Albanyà) when fishes were caught in a single removal. Low density probably produced this result in those places in which the habitat was the least suitable to determinate fish species (Mediterranean barbels in Vilafant or European eels in Castellfollit, for instance).

Differences among species with regard to behavioral, physiological and morphological features (biotic factors) could affect capture. Significant estimations or failures were obtained for fish species such as common carp (Vilafant, Besalú), striped mullet (Castelló) or chub (Besalú), which form shoals.

Abiotic factors which can affect the electrofishing efficiency are river width and presence of submerged plants. Efficiency of electrofishing was found to decrease with an increasing river width (Kennedy *et al.*, 1981); this occurs in Torroella and Castelló or Besalú, where the river-bed bottom presented an large area occupied by vegetation.

Large fishes are always assumed to be more susceptible to the electric current and therefore more catchable than the small ones. It is generally expected that selectivity will manifest itself as a decrease in mean size in successive catches. Whether large or small fishes are more vulnerable and removed first, decreasing catchability and consequent underestimation will result (Zalewski, 1983). To determine the extent to which size-selectivity occurred, correlations between mean weight and catch number were calculated. Only five cases show a correlation: the European eel in Cabanes and Peralada, the mediterranean barbel in Besalú and Castellfollit and the chub in Castellfollit.

In any case, the most important factor that has determined electrofishing efficiency has been the variability of the fishing effort. After catchability among removal were examined, we have noticed that it takes different values when we would have expected it to be constant if the fishing effort had been unchanged. Consequences of this variability are a low absolute catchability, getting small samples and an irregular decline in the population removed. This is the case of the bad estimations from Castelló (European eel, grey mullet), Vilafant (common carp), Castellfollit (common carp), Besalú (common carp, chub) or Cabanes (roach).

The density of fish at the surveyed locations varies from 1,13 to 163 ind.ha^{-1}. If these results are referred to the components of the ichthyofauna, it can be stated that the highest density of fish and biomass states were recorded from locations in which the common carp and the chub were present.

Estimation of production is usually measured by computation between times at which estimates of population abundance and average fish size are available. In our case, since data were collected in a single time, annual production as fresh weight was estimated indirectly. Considering all the fish present in the population over a year time, based on the effects of size selectivity on population estimates with electrical fishing gear, we have calculated the production using turnover ratio, estimates for fish species (systematic compilation of P/B values from literature). Although it involves a rough estimation of production with an associated error, we use this result as an approach to the measure of production to compare our study sites with other rivers inhabited by otters.

As to the aim of this work, which was to evaluate the viability of the reintroduction of the Eurasian otter, the results show that the study area can sustain a stable density of otter population, similar to other densities present in otter rivers of the Iberian Peninsula (Saavedra, 1995). Using the foot-print and visual censuses (Ruiz-Olmo et al., 2001) and comparing with fish biomass studies the carrying capacity depending on fish biomass can be established in 0,4 to 0,9 otters.km^{-1} in stretches with 30-80 g.m^{-2} and 0,1-0,3 otters.km^{-1} when fish biomass is 10-30 g.m^{-2} (Ruiz-Olmo, 1995). Biomass and fish production present ranges which are able to carry, in the whole area, an otter density of 130-160 individuals.

2.5. Conclusions: viability of reintroduction

The viability study of otter reintroduction in Aiguamolls Natural Park and Muga and Fluvià basins was carried out (Saavedra, 1995) in order to determine if the project observed the guidelines for reintroductions proposed by the International Union for the Conservation of Nature (IUCN, 1987; 1995). The parts of the viability study contained in this thesis are the assessment of water pollution, the assessment of organochlorine levels in fish and the assessment of stock, biomass and fish production.

Regarding the aspects studied, fish biomass varied between 0.6 and 351.9 gr/m^2 (n=12), with only two sites (17%) with a biomass lower than 8 gr/m^2, the minimum value calculated to support permanently an otter population (Kruuk, 1995; Ruiz-Olmo, 1995). The levels of organochlorine compounds in fish of the area are in the order of those calculated in rivers of Catalonia (López-Martín et al., 1995). The levels of PCBs, the main compounds associated with the decline of the otter, ranged between 81 and 136.4 µg/kg wet weight (95% CI, n=37), with 41% of the sites with values higher than 110 µg/kg ww (Mateo et al., 1995). The fishes with levels lower than this 'level of concern' live in catalonian rivers with healthy otter populations (Ruiz-Olmo and López-Martín, 1994; Ruiz-Olmo, 1995; Ruiz-Olmo et al., in press).

In the viability study, other data were presented. Some are presented in chapter 1 and some are not included in this thesis, but these aspects are important to understand the consideration of the project as viable and for this reason are briefly explained here.

The Eurasian otter is classified as Vulnerable for its total distribution (IUCN, 1990) and for Spain (Blanco & González, 1992). The reintroduction was proposed in Aiguamolls de l'Empordà Natural Park and Muga and Fluvià basins, which are part of the original area of distribution (Delibes, 1990; Ruiz-Olmo & Aguilar, 1995; Ruiz-Olmo, 1995; Ruiz-Olmo & Delibes, 1998). The causes of extinction were persecution (hunting, trapping), pollution and habitat destruction (Ruiz-Olmo, 1995; Saavedra, 1995). Extinction in the study area was complete, with the last citation in 1984 (Delibes, 1990). Between Muga and Fluvià basins and the nearest otter populations there were several basins without otters, so spontaneous recolonization was difficult to occur (Ruiz-Olmo & Delibes, 1998). The proposed donor populations (from Extremadura, Galicia, Asturias and Portugal) are healthy and increasing in number (Ruiz-Olmo & Delibes, 1998; Trindade et al, 1998). Though there are no specific studies based on genetics, there are no significant morphological differences between the Iberian otters (Ruiz-Olmo, 1995). The proposed donor populations are the nearest with enough individuals and live in comparable mediterranean habitats. The extension of the study area is 200,000 ha, with more than 600 km of river and 3,000 ha of wetlands. Considering the densities found in Catalonia (0,1-1,2 otters/km; Ruiz-Olmo, 1995), there is enough habitat for a population with a range between 60 and 720 animals (Saavedra, unpublished data). As in other mammal reintroduction projects, the objective is to release 50 otters, because a number smaller disposes a population to inbreeding depression (Gilpin, 1987). Nevertheless, this number implies a isolated population that has been decreasing until 50 individuals who are genetically closely related. In our case, the 50 otters will come from different populations and will have a much higher genetic variance. The human local population is mainly in favour of the reintroduction, because the otter does not negatively affect the interests of any economic group in the area (Saavedra, 1995). In Spain it's a fully protected species from 1973.

All this aspects brought to the conclusion that the Girona Reintroduction Otter Project was viable and was initiated in 1995 with the permission and support of the government agencies.

3. Trapping, handling and medical management of reintroduced otters

3.1. Introduction

The presentation and approval of the viability study gave way to the beginning of the execution of reintroduction project.

The study of various reintroductions carried out in different places of the world confirmed that success is higher in wild animal translocations than in the release of captive bred animals (see chapter 1). Instead, in the western part of Iberian Peninsula important otter populations exists (see chapter 1). Thus, it was decided to carry out the reintroduction project through the translocation of Iberian otters.

It was judged also very important that all or most of the released otters were fitted with a radiotransmitter, that would allow field biologists to monitor the movements and the adaptation of the otters following their release into the wild.

A reintroduction protocol was prepared to organize otters capture, transport, revision, radiotransmitter implantation and releasing.

Veterinary assistance was requested to the Barcelona Zoo (BZ) to evaluate the health of live-trapped otters, to rule out any infectious or parasitic disease of the reintroduced otters, treat trap-related injuries, and perform the intra-abdominal surgical implantation of the radiotransmitter.

This chapter describes the trapping, handling, and medical management protocols used during this project.

3.2. Materials and methods

3.2.1. Capture

Fifty five otters were live-trapped with padded leg hold traps (#1-1.5 Soft Catch, Woodstream Corp., Lititz, Pennsylvania 17543, USA) by Girona Reintroduction Otter Project (*GORP)* personnel throughout the project from Southwestern (Extremadura), Northern Spain (Asturias) and Portugal. As described in Serfass *et al*. (1996), one factory spring from each trap was replaced by a #2 spring. Traps were set in water, preferentially in shallow passages between rocks or in river beaches. These places were found only in small tributaries or in main rivers during the summer drought. Traps were bound to one meter chains, that were tied to trunks or secured to big rocks, using climbing bolts, hammered manually. Traps were set in groups of two or three units. No baits or lures were used. Trapping occurred throughout the year depending mostly on permits obtained from the different governments involved and weather conditions, attempting to avoid spring and summer months. Potential captures were defined as the sum of captures and escapes. Capture rate was defined as the number of otters captured divided by potential captures (Serfass et al., 1996).

As otters are mainly nocturnal in capture areas, traps were examined daily between 0500 and 0800. To reduce the risk of injury, otters were chemically immobilized at trap sites with a combination of 5 mg/kg ketamine hydrochloride (Imalgene 1.000, 100 mg/ml, Rhône Merieux, Lyon 69002, France) with 50 µg/kg medetomidine (Domtor, 1 mg/ml, Pfizer, S.A., Madrid 28002, Spain) administered intramuscularly (i.m.) by a blow pipe using plastic darts (Dan-inject, International GmbH, Gelsenkirchen 45889, Germany), following the protocol described in Fernández et al. (2001). Once completely immobilized, each otter was carefully released from the trap and was subjected to physical examination, including weighing, and sexing.

Otters showing signs of chronic illness or injuries, as well as pregnant or lactating females were not included in the reintroduction project and were immediately released. Young or subadult animals were preferred over older animals. The age of the animals was estimated according to their dentition (Heggberget, 1984). Nineteen animals received a dose of the long acting neuroleptic (LAN) perphenazine enantathe (Trilafon enantat, Sheering-Plough S.A.,Madrid 28046, Spain; 100 mg/ml; 20-30 mg, i.m.) to decrease the stress level during handling, transport and management while in captivity. After examination, immobilized otters were placed in transport kennels and were let to recover in a cold and dark room. In the afternoon, they were transported to the Barcelona Zoo (BZ) by van or commercial airline.

3.2.2. Housing and care

Upon arrival at the BZ, otters were individually housed indoors in wire- mesh cages (2.44 m long x 1.22 m. wide x 1.22 m. high), with attached wooden nest boxes (0.91 m long x 0.61 m wide x 0.51 m high) and suspended above the ground. Room temperature was kept between 18- 24 C. Alfalfa hay was used in the wooden nests to serve as bedding material.

Food and water were offered *ad libitum*. During the first few days and until the animals ate normally, the diet consisted of a mixture of fresh or thawed trout, chicks, fresh eels, and fresh crayfish. Afterwards, the diet consisted mostly of trout. Daily food intake was recorded for every animal while in captivity. Fresh drinking water was provided in a plastic dishpan (0.6 m long x 0.4 m wide x 0.1 m high) containing 20 l of water. Feces and urine samples were collected daily from each animal by using a plastic dish located on the floor under the cages. Once an animal left the BZ, its cage was thoroughly disinfected (CR-36, Laboratorios Collado, S.A., Barcelona 08027, Spain).

When otters started to eat, which usually occurred two days after their arrival at the BZ, they were immobilized again using the technique described below to carry out a complete medical evaluation that included radiographs, blood collection, physiological monitoring, identification by a microchip (Trovan, EID Ibérica S.L., Madrid 20546, Spain), and treatment if necessary of trap related injuries. Also, every animal received subcutaneous (s.c.) 0.4 mg/kg of ivermectin (Ivomec 1%, MSD Agvet. Madrid 28027, Spain) for the treatment of endo and ectoparasites.

The anesthetic agents were administered intramuscularly (i.m.) in the hind limb by means of a 2 ml plastic dart equipped with a 1.1 x 38 mm needle and delivered by blow pipe (Dan-inject International, Gelsekirchen, Germany). A mixture of approximately 5 mg/kg of ketamine hydrochloride (100 mg/ml, Imalgene 1000, Rhône Mérieux, Lyon, France) and 50 µg/kg of medetomidine hydrochloride (1 mg/ml, Domtor, Orion Corporation, Turku, Finland) was delivered. For reversal atipamezole hydrochloride (5mg/ml, Antisedan, Orion Corporation) was administered i.m. at 5 mg per mg medetomidine hydrochloride. When needed, a complementary dose of ketamine was administered at 2.5 mg/kg.

Trap related injuries were classified according to the following categories: I- no lesions or puncture wounds, lacerations, missing nails, and swelling; II- closed luxations of the interphalangeal joints of one or more digits; III- open luxation of one or more digits; IV- as for III but with exposed or missing phalanges. We also looked carefully for oral problems such as teeth damage or wounds produced during capture or transport.

3.2.3. Surgery and release

Different radiotransmitter devices were used during this project: Advanced Telemetry Systems (470 First Av, Isanti, Minnesota 55040, USA; 32-40 gm; 30 animals), Telonics (932E, Impala Av, Mesa, Arizona 85204, USA; 30 gm; 4 animals) and Wagener (Herwarthstr 22, Köln 50672, Germany; 30 gm; 2 animals). Overall, radiotransmitters were thus implanted intraperitoneally in 36 otters for monitoring of post release movements and survival. Otters were considered to be ready for surgery after being 5-10 days in captivity and only if severe infections or capture-related diseases had being properly treated or ruled out, and animals had eaten regularly. Surgery was delayed in otters with low food ingestion or signs of disease. Otters were fasted at least 5 hr prior to surgery, but were allowed to water.

The same combination of ketamine and medetomidine as indicated above was injected i.m. by a blow pipe while the otters were inside the wooden nest box, at day time. Immobilized otters were intubated by a 2.5-3 cuffed endotracheal tube to establish and maintain inhalation anesthesia of isoflorane (Forane, Abbot Laboratories, Madrid 28027, Spain) and oxygen. Inhalation anesthetics were delivered by a precision vaporizer in an open-circuit system. Otters showing heart rate bellow 100 beats/min were given atropine (Atropina, 1 mg/ml, Braun Medical, Barcelona 08191, Spain; 0.04 mg/kg). An area of 5 to 6 by 4 cm was shaved along the ventral midline over the umbilicus and aseptically prepared with povidone-iodine scrub and 70% ethyl alcohol washes. Each radiotelemetry device was sterilized with ethylene oxide gas and prewarmed (38 C) before being placed ventrally in the abdomen following the surgical incision (7 to 8 cm) through the *linea alba*. Three layer closure were done by use of 2-0 polyglycolic acid suture material (Dexon, Braun-Dexon, Barcelona 08191, Spain). Suture in a single interrupted pattern was used for the *linea alba* and peritoneum, and subcutaneous tissues. Skin closure was accomplished with 2-0 polyglicolic acid in a discontinuous horizontal mattress pattern. Following surgery, otters were given a penicilin-estreptomicin combination (Dipenisol Retard, Bayer, Bayer, S.A. Barcelona, 08029, Spain; 0.5 ml s.c.) and were placed back into the wooden nest boxes of the cages and allowed to recover slowly. Fifteen minutes after extubation a dose of 250 µg/kg Atipamezole (Antisedan, 5 mg/ml; Pfizer, S.A. Madrid, 28002, Spain; 250 mg/kg i.m.) was manually injected for recovery. Although drinking water was provided, the plastic dishpans were removed from the cages temporarily for 3-5 days to keep incisions dry during initial healing. Food was provided within 3-5 hr after otters returned to the cage.

Ten to twelve days post surgery, otters were chemically immobilized again, with the same combination of medetomidine and ketamine as indicated before and were clinically evaluated and weighed before being released. Also, abdominal radiographs were performed to determine the exact location of the radiotransmitter. Overall, otters remained at the BZ during a period between 20-30 days.

All otters were transported over a 2 hr trip by car to the release area. When a post release death occurred, the animal was taken to the BZ in order to establish the cause of the death and to determine the location of the radiotransmitter.

3.3. Results

3.3.1. Capture

A total of 8,773 night traps were placed and 55 animals were captured (159 night traps/ otter), with 36 animals escaping (potential captures = 91), resulting in a capture rate of 0.60. None of the trapped otters died during capture. A total of 111 individuals belonging to 15 different species were accidentally trapped (table 3.1), the commonest being striped-necked terrapin (*Mauremys leprosa*), 32%; mallard (*Anas platyrhynchos*), 14%; moorhen (*Gallinula chloropus*), 14%; brown rat (*Rattus norvegicus*), 12%, and white stork (*Ciconia ciconia*), 10%.

Table 3.1. Species accidentally trapped during otter captures with leg-hold traps.

Latin name	**English name**	**N**	**%**
Mauremys leprosa	Striped-necked Terrapin	**35**	**32**
Emys orbicularis	European Pond Terrapin	**2**	**2**
Ciconia ciconia	White Stork	**11**	**10**
Ciconia nigra	Black Stork	**1**	**1**
Ardea cinerea	Grey Heron	**2**	**2**
Egretta garzetta	Little Egret	**2**	**2**
Anas platyrhynchos	Mallard	**16**	**14**
Gallinula chloropus	Moorhen	**15**	**14**
Turdus merula	Blackbird	**1**	**1**
Rattus norvegicus	Brown Rat	**13**	**12**
Arvicola sapidus	Water Vole	**1**	**1**
Herpestes ichneumon	Mongoose	**1**	**1**
Genetta genetta	Genet	**3**	**3**
Martes foina	Beech Marten	**3**	**3**
Vulpes vulpes	Red Fox	**5**	**5**

Accidental trapping of species different than the otter were occurred more often in spring than in other seasons, due probably to an increase in the activity (Terrapins) and an increase in the utilization of small streams to feed (White Stork) or breed (Mallard).

Table 3.2. Seasonal differences in accidental trapping of animals other than the otter.

	number of traps placed	%	number of accidental captures	%	nº animals/nº traps placed
Spring	2458	25	72	65	0.0293
Summer	1505	15	8	7	0.0053
Autumn	4704	48	21	19	0.0045
Winter	1046	11	10	9	0.0096

Among the 43 otters captured with modified foot hold traps and transported to BZ, 79% had category I injuries, 7% category II, 12% category III, and 2.% category IV. No significant oral cavity injuries occurred in most of our animals and only 19% (8) showed lesions associated to biting or chewing during capture or confinement. 37% of the captured animals were male and 63% were female (see table 3.3). Except for one case, all injuries responded well to treatment and all digit wound had resolved or were close to complete healing by the time otters were implanted the radiotransmiter. Otters having an infected wound showed body weight decrease even when eating normally. Wound management included cleansing, debridement, suturing and oral antibiotics and nonsteroideal anti-inflammatory drugs.

During transport most otters remained calm, although some tried to escape by biting the steel door or grasping with the front legs. Animals treated with perphanazine appeared to be calmer and more relaxed.

3.3.2. Housing and care

Most otters drank water immediately after being released into the wire cage and shortly afterwards disappeared into the wooden box nest. Otters spent most of their time inside the wooden nests, except during nighttime. Otters appeared to adjust rapidly to captivity and most animals eat within the first 48 hr post arrival. Most otters ate fresh or thawed trout but exceptionally, some animals showed a clear preference for crayfish, chicks or eels.

Some otters tried to escape during the first 2-3 nights on captivity. Three males escaped destroying the joint between wire cage and wood nest, but were recaptured shortly afterwards.

Table 3.3. Summary of data on 43 Eurasian otters (Lutra lutra) (16 males, 27 females) handled by the GORP.

Animal	Year	Trap Injury Category[a]	Captured	Released	Days in Captivity	Sex[b]	BWV[c]	Observations
1	1996	I	07/02/96	22/03/96	43 d	F	U	
2	1996	I	21/09/96	21/10/96	30 d	F	1,2	
3	1996	III	02/10/96	21/10/96	19 d	M	0,7	
4	1996	II	02/10/96	03/10/96	1 d	F	U	Died in Captivity
5	1996	I	10/10/96	11/10/96	1 d	F	U	Died in Captivity
6	1996	I	09/10/96	28/10/96	19 d	F	0,6	
7	1996	I	18/10/96	04/11/96	17 d	M	1,5	
8	1996	I	23/10/96	11/11/96	19 d	M	0,3	
9	1996	I	26/10/96	11/11/96	16 d	F	1,5	
10	1996	I	22/11/96	19/03/97	117 d	M	2,7	
11	1996	I	01/11/96	02/12/96	31 d	F	0,6	
12	1996	I	01/11/96	02/12/96	31 d	F	0,5	
13	1996	I	03/11/96	26/11/96	23 d	M	0,5	
14	1996	I	01/11/96	02/12/96	31 d	F	-0,4	
15	1996	I	16/11/96	05/12/96	19 d	M	U	Died in Captivity
16	1996	II	06/03/96	21/03/96	15 d	F	U	
17	1996	I	20/11/96	11/12/96	21 d	M	0,3	
18	1996	I	25/11/96	23/12/96	28 d	F	U	
19	1996	I	25/11/96	13/12/96	18 d	M	-0,4	
20	1997	I	19/03/97	08/05/97	50 d	F	0,9	
21	1997	III	04/11/97	10/12/97	36 d	M	0,8	
22	1997	I	16/11/97	15/12/97	29 d	F	U	
23	1997	III	16/05/97	21/06/97	36 d	M	1,2	
24	1998	I	13/05/98	08/06/98	26 d	M	0,3	
25	1998	II	31/08/98	18/09/98	19 d	F	0,5	
26	1998	I	31/08/98	18/09/98	19 d	F	0,5	
27	1998	III	03/09/98	02/10/98	29 d	F	0,5	
28	1998	I	03/09/98	18/09/98	15 d	F	0,7	
29	1998	I	29/09/98	15/10/98	16 d	F	0,2	
30	1998	I	29/09/98	15/10/98	16 d	F	-0,1	
31	1998	I	30/09/98	15/10/98	16 d	F	0,1	
32	1998	III	27/09/98	27/10/98	30 d	F	U	
33	2000	I	19/03/00	11/04/00	23 d	F	0,8	
34	2000	I	07/03/00	24/03/00	17 d	M	0,3	
35	2000	I	13/03/00	27/03/00	14 d	F	1	
36	2000	I	14/03/00	27/03/00	13 d	M	-0,3	
37	2000	I	14/03/00	19/03/00	5 d	M	U	Died in Captivity
38	2000	I	26/03/00	14/04/00	19 d	F	-0,2	
39	2000	I	29/03/00	19/04/00	21 d	F	0,3	
40	2000	I	24/03/00	19/04/00	26 d	F	0	
41	2000	I	26/07/00	10/08/00	14 d	M	-0,2	
42	2000	IV	25/09/00	15/10/00	20 d	F	0.9	
43	2000	I	28/09/00	12/10/00	14 d	M	U	Died in Captivity

[a] Trap injury category. I: puncture wound, lacerations, missing nails or swelling only; II: closed luxations of the interphalangeal joints of one or more digits; III: open luxations of one or more digits; IV: as for III but with exposed or missing phalanges; V: distal limb fracture.
[b] Sex; M: male; F: female[c] BWV: Body weight (kg) variation during captive period. U: unkown.

3.3.3. Surgery and release

Polyglycolic acid sutures used proved to be adequate for the surgeries performed. Erythema, swelling, or drainage were not observed in any of the 36 animals subjected to surgery; healing of the surgical incisions was rapid and without complications.

Nine animals died during the first year after being released. On necropsy, the radiotelemetry devices were found to be free within the ventral part of the abdomen and in craniocaudal orientation in all cases. There were no mesenteric or omental adhesions, and the surgical incisions had healed completely.

3.3.4. Mortality during captivity

During captivity, 5 animals (3 males and 2 females) died. Otter #4 died just upon arrival at BZ. On necropsy, a bronchiolar obliteration caused by a piece of herb possibly inspired during capture was found. This animal died showing signs of respiratory distress. Another animal (#37) never eat, possibly because it was still very young and was severely stressed by the capture process. A third animal (#5) died 48 hr post arrival due to severe capture miopathy according to clinical and physiological studies. Another animal (#43) was injured while trying to escape and suffered a severe infection caused by a pre-existing pneumonia; this animal never eat. Finally, one otter (#15) died after surgery and before being released. On necropsy, a chronic purulent myocarditis caused presumably by a capture-related infected wound on a digit was found. This systemic infection was not detected as neither the animal behavior or its blood biochemical values showed any change. Overall mortality rate of otters during captivity was thus 11.2%.

3.4. Discussion

3.4.1. Capture

The breeding cycle of the North American river otter is well known and includes spring breeding, immediate development of the egg(s) to blastocysts, a delay of 9-10 months, intrauterine implantation, and a 61-63 day development period of the embryo-fetus to term (Reid *et al.* 1986). In contrast, the Eurasian otter is not seasonal and cubs may be born at any time of the year, although births are more common in spring and summer (Ruiz-Olmo & Delibes, 1998). Consequently, even though most animals were captured avoiding these months, juveniles and lactating females were occasionally captured. Although these animals were immediately released, one of the animals that died was probably a juvenile, but was still included in the project due to its big size and general aspect. This animal never adapted to captivity and rejected food. Care must be taken to avoid translocation of too young individuals.

Although the capture method used was the same as that described by other authors (Serfass *et al.*, 1996), the number of traps per captured otter was significantly higher in our project (60 *versus* 159) as compared with theirs, although the capture rate was similar (0.57 *versus* 0.60). In the North Carolina translocation program (Spelman, 1998), the trapping success was even higher with only 26 trap night per otter. These differences could be due to variances in the capture areas of each study, as even within our project, important differences in the trapping success were observed between areas.

The use of a blow pipe as a method to deliver the anesthetic combination worked well and this technique is strongly recommend to avoid injuries or worsen those produced by trapping, e.g., luxations, fractures, etc. Also, avoiding physical contact with conscious animals, darting them from a distance, and waiting longer than 3 min proved to be safe in our animals, although other authors have recommend the use of net and manual restraint by trained personnel for anesthesia administration (Serfass *et al.*, 1993; Spelman, 1998). The use of a modified squeeze cage for otter restraint has been reported by others (Williams *et al.*, 1990).

The animals captured suffered very few severe injuries. Indeed, no animal suffered leg fractures, one animal (2%) had type IV lesions, five animals (12%) had type III injuries and only nine animals (21%) suffered some kind of digit luxation. Although comparison with other studies is difficult due to the variety of capture methods used, our results show that soft catch traps can be used to humanely capture wild otters (Serfass *et al.*, 1993). Minor abrasions to foot pads and warn toe-nails on untrapped feet during attempts to pull free of the trap have been reported before (Serfass *et al.*, 1996) and were observed in this project also.

In contrast with other studies, very few dental injuries were detected in our study. Only eight animals (19%) had these type of lesions, a much lower percentage than that reported by other authors (Serfass *et al.*, 1996). Different escape behavior while in traps may explain this finding; the escape behavior of our trapped otters consisted primarily of pulling out the trap and digging and destroying surrounding vegetation rather than biting the trap as referred by Serfass *et al.* (1996).

As documented in Serfass *et al.* (1993), the leukocyte count and level of enzymes indicative of muscle necrosis were specially valuable for monitoring clinical improvements of otters suffering from contaminated wounds.

3.4.2. Housing and care

In general captive otters adjusted well to cages and accepted food readily. Live fish (eels and trout) and chicks seemed to act as environmental enrichment tools and elicited eating in some individuals that initially rejected food.

The anesthetic protocol used in this project (Fernández et al., 2001) showed to be effective and safe for the type of procedures described in this paper.

3.4.3. Surgery and release

The fact that all the otters eat normally the same day of the surgery shows that this procedure has minor effects on the animals: the otters did not seem to care about the incision sites and there were no exposed skin sutures that could be damaged or become irritated or infected. As referred by others (Hoover, 1984; Arnemo, 1991), the radiotelemetry devices used in our study appeared to be somewhat too large for intraabdominal use in Eurasian otters. Although our devices were considerably smaller (30-40 g) compared with those used before (110-120 g), it has to be consider the smaller size of the Eurasian otter compared with the North American river otter.

The idea of immediate surgery and early release has been advocated by some authors (Arnemo, 1991) in order to reduce stress. However, the fact that our animals seemed to adjust well to captivity as well as the risk of incomplete healing after surgery (Arnemo, 1991), suggests that a period in captivity after surgery is convenient, and 10 days seemed to be adequate for this purpose. In our case the main objective was reintroduction in a new area, so otters did not have a pressure to come back to their home range being lost day by day.

Different surgical approaches have been advocated for radiotransmitter implantation in otters. Some authors (Melquist & Hornocker, 1979; Serfass *et al.*, 1993) did not recommend the ventral midline approach because otters rub their ventrum during grooming. Although our otters were occasionally observed rubbing their ventrum, no adverse effect on the healing process was recorded. Further, the subcutaneous and intraperitoneal approach used by other authors (Williams & Siniff, 1983; Ruiz-Olmo, 1995) may cause serious concern. Based on the 36 surgical procedures done in our study without any problem, the ventral midline approach is recommended, although the lateral approach recommended by other authors (Melquist & Hornocker, 1979; Serfass *et al.*, 1993) may be another interesting option.

Concerns has been expressed on the potential for heat loss after shaving the skin on the surgical area (Hoover, 1984). In our animals, this did not seem to be a problem, perhaps due to the benign climatic conditions in our release area. Further, in the final clinical examination we confirmed the rapid growth of the inner fur.

The few references about the use of the radiotransmitters in Eurasian otter refers to small number of animals and their results are inconclusive (Arnemo, 1991; Sjöåsen, 1996, 1997). Therefore, the data presented here with 35 animals implanted and released provide useful information. The effects of the intraperitoneal transmitters on reproduction was tested in North American river otters and found no detrimental (Reid *et al.*, 1986). Our results are in agreement with theirs, as at least 3 radioimplanted otters have bred successfully in the releasing area (Saavedra & Sargatal, 1998). The radiotransmitter have been essential to gather information about the released animal's movements and mortality.

Our approach to capturing, handling, and translocating otters has succeeded in restoring an eradicated otter population in Catalonia (Spain) and can provide useful information for similar programs.

4. Colonization process, density and post-release mortality

4.1. Introduction

Monitoring reintroduced otters yielded much ecological and behavioural data, examined over three chapters. The first looks at colonization by the new population, how distribution develops, density compared to other well-studied otter populations, and the causes of mortality after release. In chapter 5, most of the data obtained through 24 hours tracking sessions is presented, together with studies of range, patterns of activity, movement parameters in an activity period, resting sites and the effect of water availability on otter movements. Chapter 6 studies the movements of two females that presented breeding behaviour.

Of the aspects studied in this chapter, only post-release mortality has been well studied in previous reintroduction programs, as data essential to evaluating the success of reintroduction (Hoover et al., 1984; Serfass & Rymon, 1985; Erickson & McCullough, 1987; Jessop & Cheyne, 1992; Sjöansen, 1997; Johnson & Berkley, 1999; Spinola et al., 2001).

Other demographic aspects such as density of population, colonisation and dispersal were not specifically monitored; efforts were concentrated on radiomarked individuals rather than the overall population.

In the *GORP*, two other tools were also used besides radiotracking: regular checking of a "standard otter survey" (Reuther et al., 2000) station network to determine colonization over time, and use of visual censuses to obtain data on otter densities in rivers and channels for comparison with other nearby well-documented populations (Ruiz-Olmo, 1995, 2001). Both tools were also used to test different methodologies in the study of otter numbers (see chapter 7).

4.2. Materials and methods

Information was obtained from different methods applied during the reintroduction project:

4.2.1. Radiolocations

Due to the number of animals with radioimplants (table 4.1), it was very difficult to obtain a large amount of radiolocations for every one. Efforts were made to locate all animals at least once a week, using 4-wheel drive vehicles and a small Cessna plane. Some individuals were tracked more closely, with many more radiolocations.. A total of 1843 radiolocations were obtained from 38 different otters.

Table 4.1. Period of radiotracking for every implanted otter.

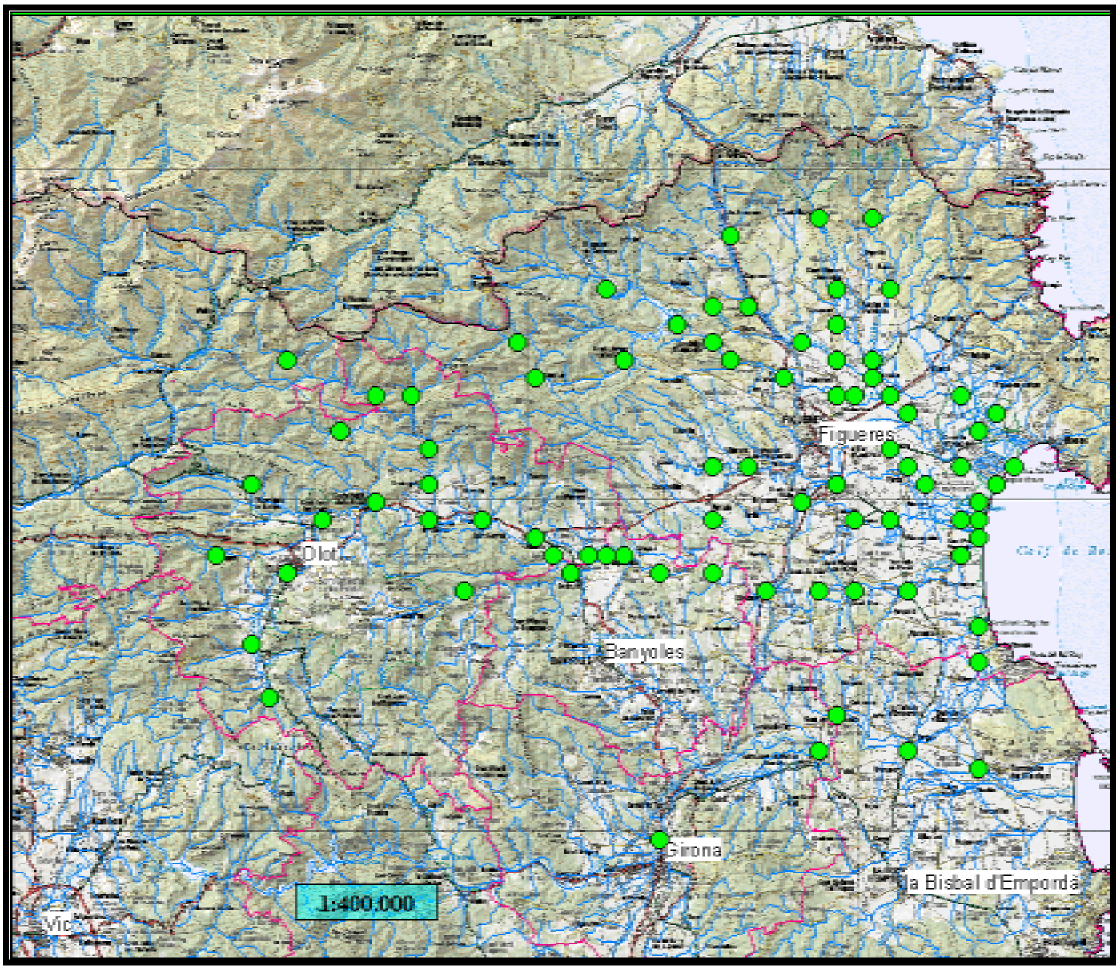
Code	**Name**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**
F01	Cuanti						
M01	Roc						
M02	Meu						
F02	Derek						
F03	Xurra						
F04	Seca						
M03	Victor						
F05	Vega						
M04	Cano						
M05	Salor						
F06	Candela						
F07	Lupe						
F08	Berta						
F09	Llobeta						
M06	Ros						
M07	Esquerp						
M08	Trau						
F10	Dolça						
F11	Montse						
M09	Jonc						
M10	Secem						
M11	Mosquit						
F12	Esva						
F13	Xana						
F14	Rati						
F15	Berna						
F16	Mercè						
F17	Llum						
F18	Celta						
F19	Jroñe						
M12	Nano						
F20	Teresa						
F21	Keta						
F22	Lauda						
F23	Ribeira						
F24	Fera						
M13	Garelu						
F25	Júlia						

4.2.2. Otter surveys

A network of 80 otter sites was surveyed once a year in 1995 and 1996 and every season from 1997 to 2001, totalling 20 surveys (figure 4.1). The Otter Survey methodology is widely used in the distribution area of the Eurasian otter (see Lenton et al., 1980; Ruiz-Olmo, 1985; Delibes et al., 1991; Mason & MacDonald, 1991 and Reuther et al., 2000). In our case, the sites were surveyed to determine only the presence or absence of otters and it was halted as soon as traces of the species were found, when the station was classified positive. The percentage of the 80 sites where tracks or spraints were found was called "% of positive stations" and used in 4.3.2, together with the "percentage of surveyed stations" that gave an idea of the effort carried out every season.

The Otter Survey network initially comprised the Aiguamolls Natural Park and Muga and Fluvià basins. Since 1999, five sites from the Ter basin have been added.

Figure 4.1. Otter survey sites map.



4.2.3. Visual censuses

The visual censuses were carried out in accordance with the method described in Ruiz-Olmo (1995a), based on the positioning of a number of observers (13 – 32) at a distance of 500 m apart along a variable stretch of rivers or channels (6.5 – 16 km). Each observer was stationed so they could observe the maximum length of the stretch and, at some points, both banks. The censuses were carried out between 15th May and 15th July (table 4.2), coinciding with maximum daylight, when otters exhibit significant crepuscular activity in the study area. A census involves counts being carried out at dawn (5.30 to 7.00 am) and dusk (8.00 to 10.00 pm).

Table 4.2. Visual censuses effort.

Date	Area	number observers	hours observation	covered length (km)
30/05/97	Muga	13	45.5	6.5
13/06/97	Fluvià	16	56.0	8.5
14/06/97	Aiguamolls1	23	80.5	11.5
19/06/98	Aiguamolls1	23	80.5	11.5
20/06/98	Aiguamolls2	20	70.0	10.0
26/06/98	Muga	22	77.0	11.0
27/06/98	Fluvià	23	80.5	11.5
21/05/99	Aiguamolls1	24	84.0	12.0
22/05/99	Aiguamolls2	32	112.0	16.0
28/05/99	Muga	19	66.5	9.5
29/05/99	Fluvià	18	63.0	9.0
25/06/99	Aiguamolls1	19	66.5	9.5
26/06/99	Fluvià	23	80.5	11.5
01/07/00	Aiguamolls1	22	77.0	11.0
07/07/00	Muga	22	77.0	11.0
08/07/00	Aiguamolls2	22	77.0	11.0
22/07/00	Fluvià	18	63.0	9.0
	TOTAL	**359**	**1256.5**	**180**

The result of the census is obviously the number of otters detected. When it was impossible to determine whether an otter observed by more than one observer was or was not the same animal, a range was used (i.e. 1-2 otters). To avoid such results in density calculation, the mean was used (i.e. 1.5 otters).

The theoretical density of otters in the reintroduction area was obtained from the number of released otters and the area with otters present according to the census. Wetlands were quantified by the channel kilometer, in order to be able to compare them directly with river habitats.

4.2.4. Other available data

Data from sightings without radiotracking devices, and from traces found outside the OS network, were used to study colonisation (Section 4.3.2) and to construct Fig. 4.2. A distinction was made between linear and bidimensional ecosystems (that is, between rivers and wetlands) for purposes of comparison: for rivers, the unit used was kilometres, for wetlands, hectares. As Fig. 4.2 depicts dispersion tendency, the numbers for any year are a sum of that year itself plus all previous years. Summation is similar for the number of otters released - for example, the number of otters released is the same for 1998 and 1999, because none was released in 1999.

4.3. Results

4.3.1. Radiolocations

Radiotracking data was obtained from 38 different otters, 25 females and 13 males, tracked between 1995 and 2000. The mean tracking period was 151 days , although it varied between one and 631 days, depending on the number of transmitters active in a given period, the geographical area where the individual was situated, the special interest of intensive radiolocation (i.e. a breeding female) and how difficult it was to locate each animal. Thus the radiolocation/tracking period index was in most cases lower than zero, or less than one radiolocation per day. The mean for all 38 otters was 0.78 (0.04-1.80).

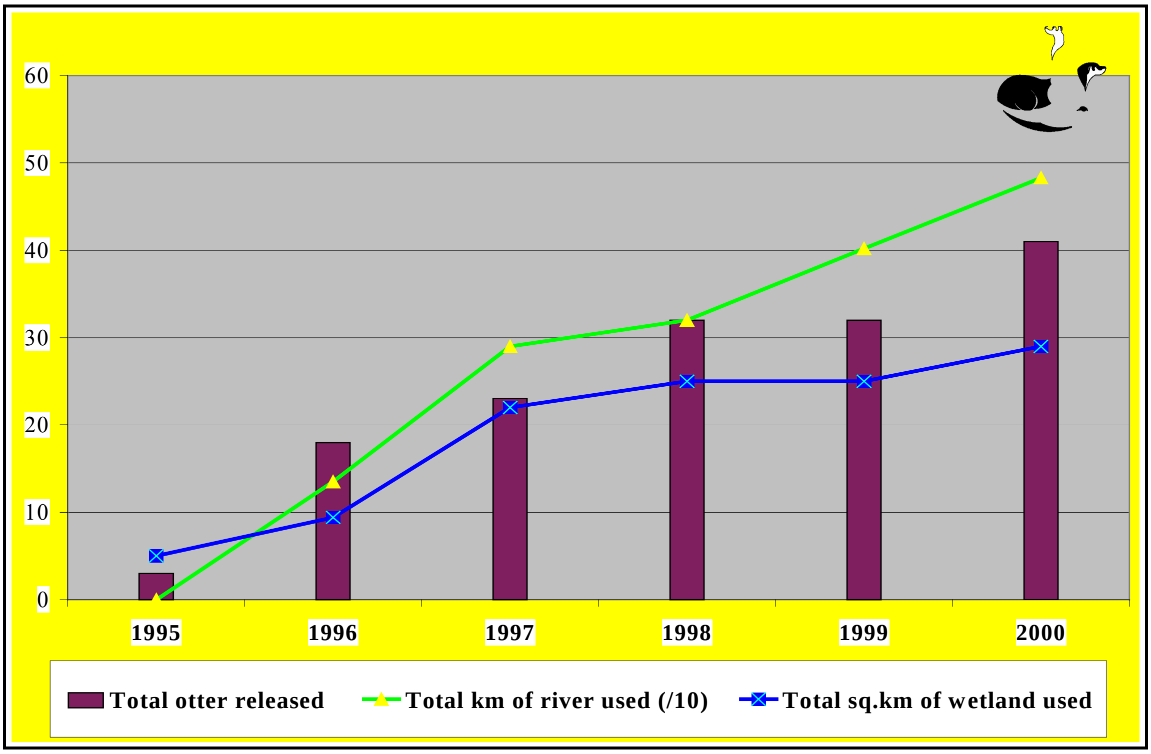
Table 4.3. Radiotracking period and effort data.

Code	Animal	Sex	Tracking dates (month/yr)	Tracking period (days)	number radiolocations	number 24h tracking sessions	radiolocations/ tracking period
F01	Cuanti	F	11/95-12/95	35	46		1.31
M01	Roc	M	12/95-07/97	587	100		0.17
M02	Meu	M	12/95-06/96	210	79		0.38
F02	Derek	F	03/96-10/96	233	106		0.45
F03	Xurra	F	03/96-10/97	597	139	3	0.23
F04	Seca	F	10/96-12/96	53	10		0.19
M03	Victor	M	10/96-07/97	279	73	3	0.26
F05	Vega	F	10/96-06/97	229	147	5	0.64
M04	Cano	M	11/96-05/97	194	25		0.13
M05	Salor	M	11/96-04/97	170	50		0.29
F06	Candela	F	11/96-12/96	37	8		0.22
F07	Lupe	F	12/96-01/97	55	2		0.04
F08	Berta	F	12/96-12/96	12	4		0.33
F09	Llobeta	F	12/96-12/96	9	2		0.22
M06	Ros	M	11/96-12/96	22	6		0.27
M07	Esquerp	M	12/96-12/96	2	2		1.00
M08	Trau	M	12/96-05/97	155	16		0.10
F10	Dolça	F	12/96-01/97	18	3		0.17
F11	Montse	F	05/97-06/97	53	12		0.23
M09	Jonc	M	12/97-01/98	30	4		0.13
M10	Secem	M	12/97-02/98	63	60	2	0.95
M11	Mosquit	M	06/98-06/99	379	332	12	0.86
F12	Esva	F	09/98-09/98	12	2		0.17
F13	Xana	F	09/98-09/98	11	7		0.64
F14	Rati	F	09/98-12/99	468	122	3	0.26
F15	Berna	F	10/98-04/99	211	85	3	0.40
F16	Mercè	F	10/98-11/98	25	3		0.12
F17	Llum	F	10/98-03/99	139	18		0.13
F18	Celta	F	10/98-07/00	631	155	5	0.24
F19	Jroñe	F	10/98-11/98	15	27	1	1.80
M12	Nano	M	03/00-09/00	175	144	5	0.82
F20	Teresa	F	03/00-07/00	122	8		0.07
F21	Keta	F	04/00-07/00	82	5		0.06
F22	Lauda	F	04/00-05/00	48	4		0.08
F23	Ribeira	F	04/00-11/00	227	8		0.03
F24	Fera	F	04/00-04/00	1	1		1.00
M13	Garelu	M	08/00-11/00	109	37	2	0.34
F25	Júlia	F	10/00-11/00	41	4		0.10
	TOTAL				**1843**	**44**	

4.3.2. Colonization by the new population

To study the colonization process of the reintroduced population of otters as a whole, we combined all data obtained about otter presence (radiotracking, otter surveys, visual censuses and other available data; see section 4.2) in the area for every year. In figure 4.2, these data were separated into linear and bidimensional ecosystems (rivers and wetlands).

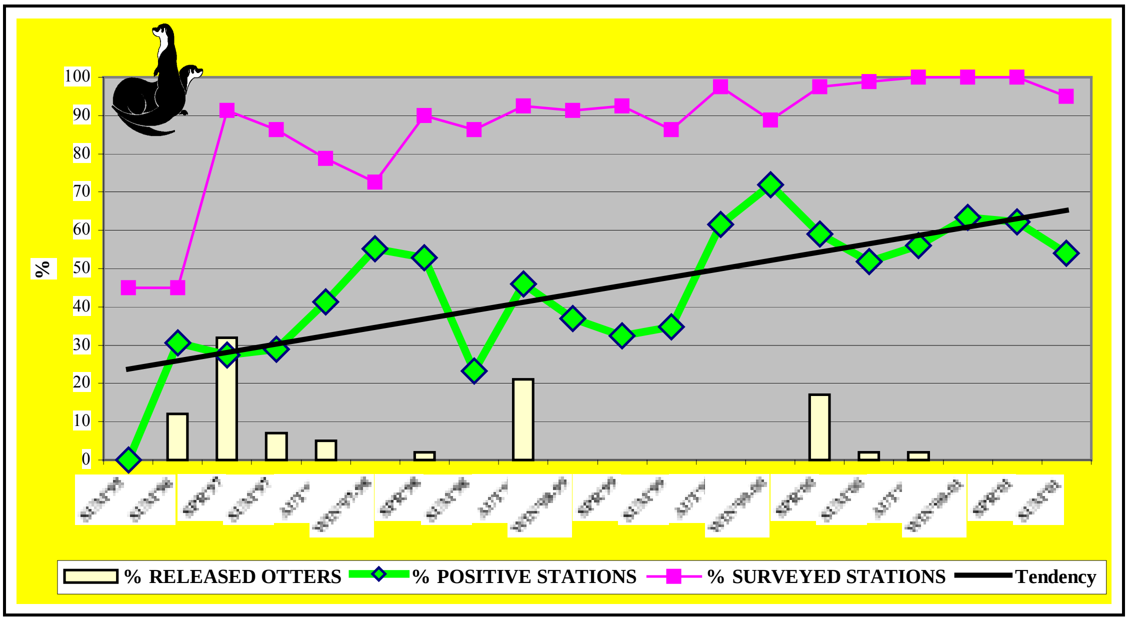
Figure 4.2. Colonization process of the new otter population using all available data.



The length of rivers and surface of wetland covered by the otter population grew constantly, with most growth occurring during the first two years of the reintroduction project (from 1995 to 1997). The next year (or two in the case of wetlands), colonization slowed: otters were already present in most of the reintroduction area (Aiguamolls de l'Empordà Natural Park and Muga and Fluvià basins). Values increased again in 1999 and 2000, with colonization of the neighbouring Ter and Tec basins, to the South and North respectively.

But the summation of all available data does not give us enough information about the effective use of colonized areas, as some stretches of river, for example, could have been used in one year but abandoned the next. To achieve seasonal detail, only otter survey data was used. In figure 4.3, the percentage of positive otter survey stations is shown, and also the percentage of surveyed stations (for effort comparison) and the percentage of released otters for each season.

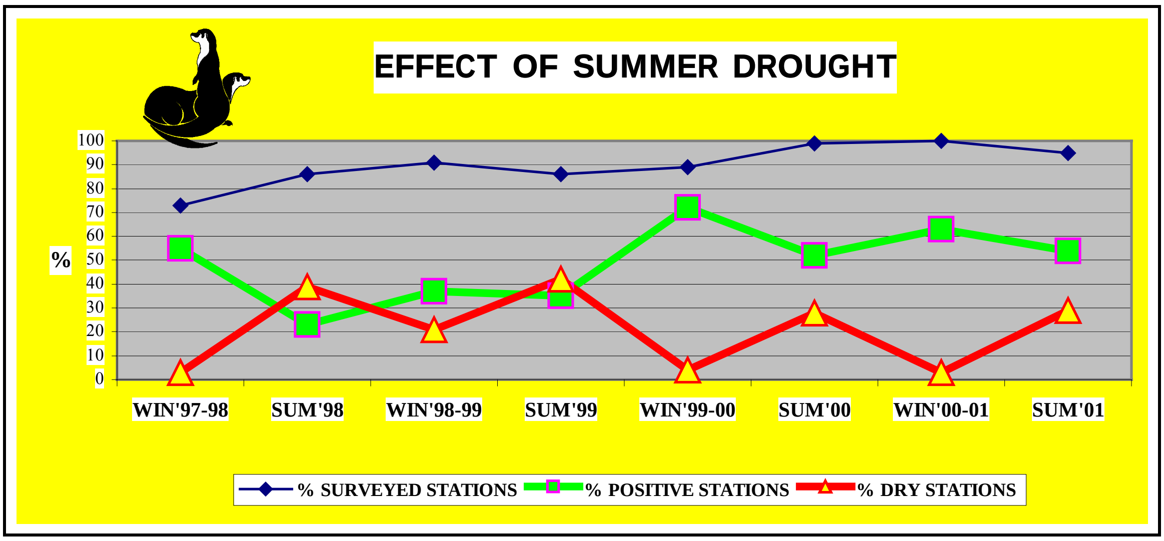
Figure 4.3. Colonization of otter population through time using otter survey data.



Again, the dispersal tendency was positive. The percentage of positive otter surveys increased from 0% in Summer 1995 (just prior to first releases) to 27% in Spring 1997 and 72% in Winter 1999-2000.

Since 1998 a pattern has been found with lower percentages in summer and higher in winter (figure 4.4). A negative correlation ($r = -0.81$; $p<0.05$) between dry and positive stations has been found, showing that the population concentrates in the smaller area with water during the summer drought. The effect of water availability on otters' use of space has been discussed in chapter 5 (section 5.4.2).

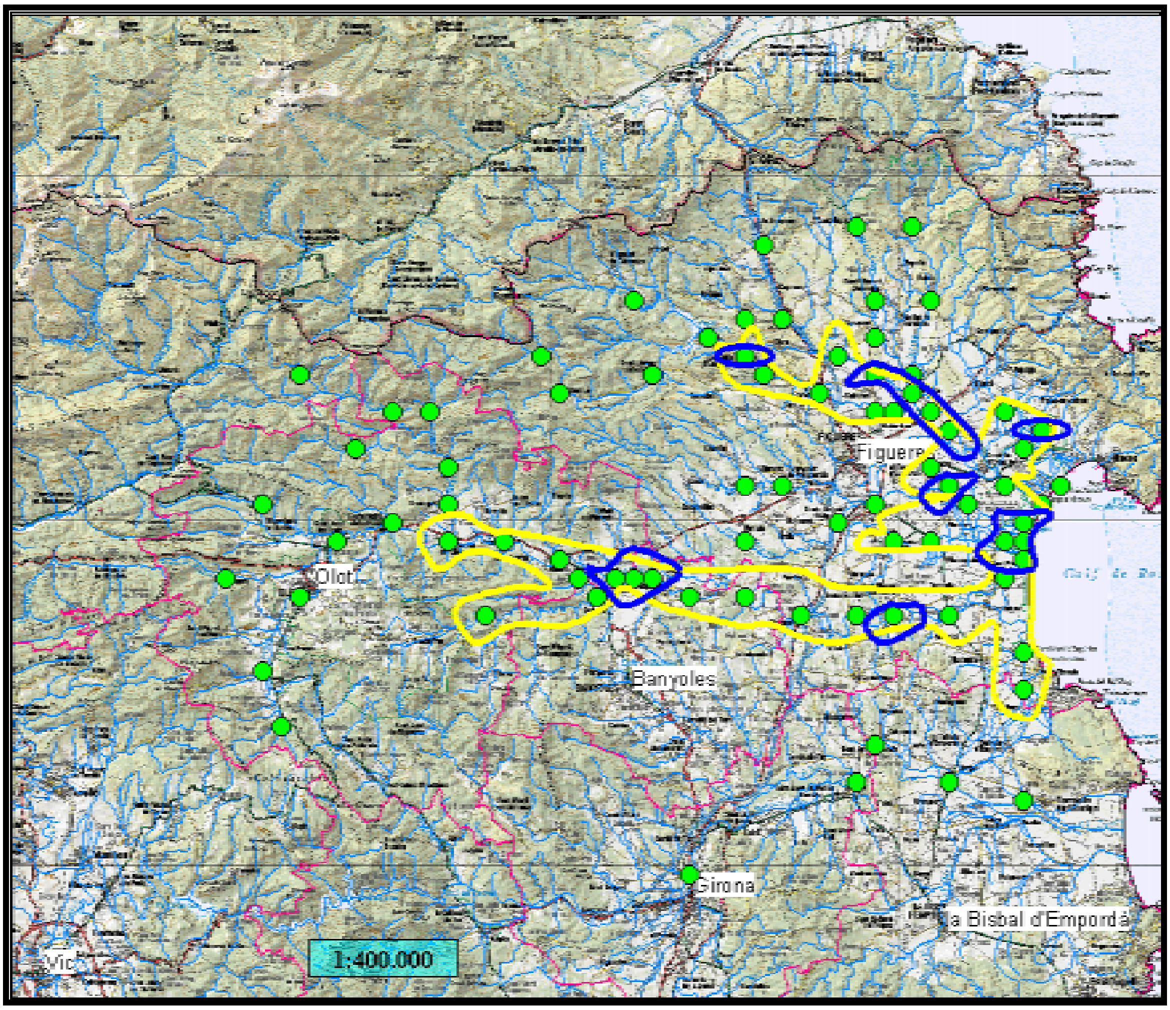
Figure 4.4.Comparison between percentage of positive stations and dry stations found in otter survey.



Obviously, the stations we surveyed did not show positive results the same number of times: great differences were found. Figure 4.5 shows the most positive stations.

The low and middle courses of both main rivers, and the lagoons and channels of the Aiguamolls were positive in more than 50% of surveys. Maximum results (>75%) were found at two stretches of the Fluvià, two stretches of the Muga and three areas of Aiguamolls Natural Park. These areas coincided with the places where most of the otters were released.

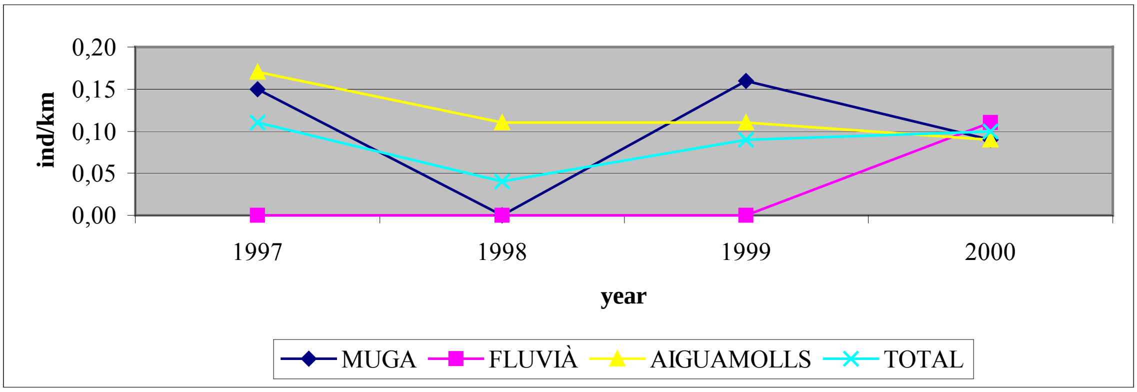
Figure 4.5. Areas with a higher percentage of positive results in the Otter Survey.
>50% positive surveys *>75% positive surveys*



4.3.3. Density

Visual censuses were used to give data about densities of otters per kilometer of river or channel and were executed between 1997 and 2000. Four visual censuses were organized each year: one in the Muga, river, one in the Fluvià and two in Aiguamolls marshes (see 4.2.4).

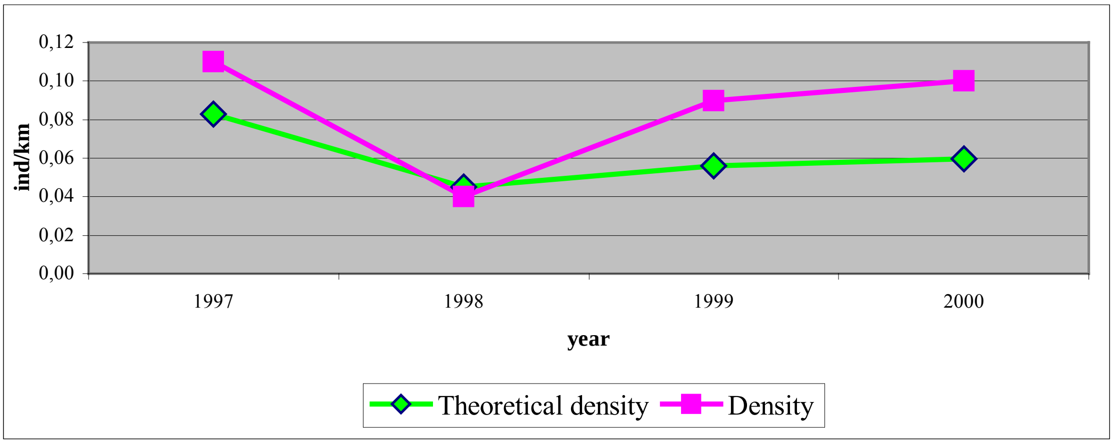
Figure 4.6. Density of otters extracted from visual censuses data.



Densities extracted from visual censuses were low and in some cases the result was zero, although visual censuses are less effective in medium to wide rivers like the Fluvià (see chapter 7). Total density ranged between 0.04 and 0.11 otters/km.

It was possible to obtain a theoretical density for the reintroduction area using the data of length of river with otter presence and the number of otters released at the moment of the visual census, assuming that some otters died before the census but some females bred (figure 4.7).

Figure 4.7. Theoretical density of otters in the reintroduction area.



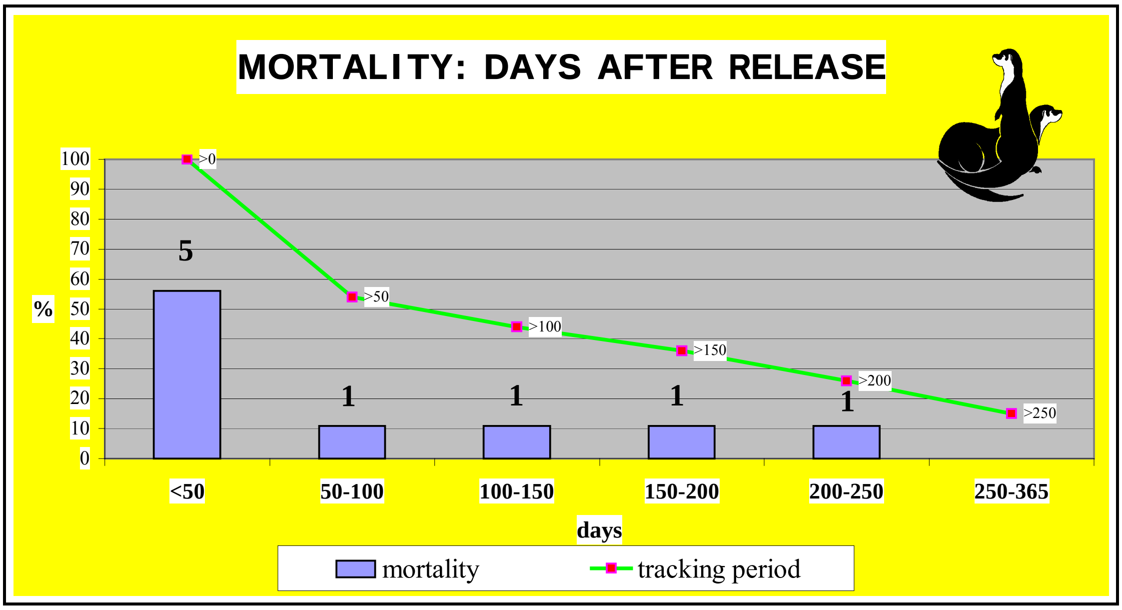
The evolution of both densities (theoretical and calculated through visual censuses) is similar, but with higher numbers for visual censuses.

4.3.4. Post-release mortality

Nine animals died during the first year after being released (22%, n=41). Mortality was bigger in females (67%) than in males (33%), although 61% of reintroduced otters were females. Mortality was bigger in juveniles and subadults (55%) than in adults (44%), although 44% of reintroduced otters were adults. Mortality was due to car crashes (five ind., 56%), fyke nets (one ind., 11%), channel siphons (one ind., 11%), carbofuran poisoning (one ind., 11%) and unknown causes (one ind., 11%).

Two-thirds of the mortality occurred within the first two months after releasing.

Figure 4.8. Time occurred between releasing and death in reintroduced otters
(mortality = percentage of otters dead after the given period; tracking period = percentage of otters radiotracked at least the given period) .



4.4. Discussion

4.4.1. Colonization process of the new population

The reintroduction of the otter in the Muga and Fluvià basins succeeded, if we look at the evolution of the distribution of the new population. The length of rivers and surface of wetlands covered by the otter followed an upward progression (figure 4.2) and the geographical area effectively occupied (figure 4.3) also showed a tendency to increase. The degree of effective colonization and the persistence through time is essential in the assessment of the success of a reintroduction, because radiotracking, even with the total number of released animals, does not allow us to know the fate after the end of transmitter life and, besides, there is no available information about the cubs possibly born in the area. With the monitoring of an "otter survey" stations network it is possible to know the general situation of the new population and also the pattern of colonization.

And the colonization followed some general guidelines, some of them mentioned elsewhere (Saavedra et al., 1997; Ruiz-Olmo, 2001).

Otters seemed firstly to occupy the most appropriate places, with more food and more riverbank vegetation as was indicated by Ruiz-Olmo (2001) in the Pyrenees. As these places were in fact chosen for the releases, the first individuals became established in the releasing area during the first weeks or months.

When an otter was released in a stretch with no intraspecific competitors, the movements tended to be very big, so that a single animal could mark and use a long stretch of river (see chapter 7).

On the other hand, otters released into areas already occupied by former released animals tended to disperse more or to produce changes in the territories of neighbouring otters (Saavedra et al., 1997; Ruiz-Olmo et al., 2001). Serfass & Rymon (1985) stated too that the dispersal of released otters appeared related to the presence of previously released otters and Sjöansen (1997) showed that otters released in areas occupied by conspecifics usually moved further before they became established than individuals released into unoccupied areas. This author even suggested that there should be c. 10 km between the release sites and areas occupied by other otters in order to avoid aggression.

Gaps left by casualties or disappearances of individuals are quickly occupied, especially in the areas with the most appropriate habitat.

Colonization occurs fast, mainly because, as mentioned before, the first individuals tend to visit very long stretches of the available geographical area. Thus, in 1997, two years after the beginning of the releases and with only 23 reintroduced animals, radiotracking data, tracks or spraints were collected from more than 300 km of rivers and streams, which means almost the totality of the Muga and Fluvià basins. And more than 50% of "otter surveys" were already positive. This process stopped momentarily during 1998, in fact due to the inexistence of available habitat to visit (although it must be repeated that this does not mean that it is occupied permanently). And in 1999 an acceleration in colonization, reaching 400 km of rivers and streams and with a peak of 72% of positive "otter survey" stations. What is most notable is that the increase occurred as a consequence of dispersal to the neighbouring basins (the Ter in the South and the Tec to the North) and that it occurred precisely the only year with no new releases, so that the dispersal must be at least partly due to the juveniles or subadults already born in the reintroduction area. Actually, 1999 was the first year when an important number of cub tracks (five) were found.

The range of the reintroduced population suffered constant changes, not only due to the supplementation of new otters, but to the seasonal variation of stretches with water (see 5.4.3). Thus, although only 19% of the "otter survey" stations were positive in more than 75% of the surveys, 84% were positive in more than 10% of the surveys.

There is a low-frequency rate of otters crossing passes from one basin to another. It is easier and more frequent in the lowlands, especially when basins are connected by irrigation channels, as happens between the Fluvià and Ter basins, where it was confirmed that a minimum of five individuals passed in three years. Basin changes occurred a long time before the population became saturated and such crossings happened even with very low density levels (4.3.2). Generally, the adults were the protagonists of this kind of long and usually more dangerous displacement and these individuals were run over in a higher proportion than the mean of released animals.

4.4.2. Density

The values found are low compared with the densities found in other Iberian regions, but they approach what can be expected in the current stage of the reintroduced population, which is still small but extended over a wide area.

In populations with such low densities, a visual census can easily give the result of zero. For example, a stretch with a density of 0.05 ind/km means that there is an otter every 20 km. A visual census habitually reaches some 10 km., thus, if the otter is absent that day from the stretch being checked, it will not be detected and the census result will be 0, when the real density is 0.05 ind/km (see chapter 7).

The development of total densities both from visual censuses and from theoretical calculation is similar, but the values are higher for visual censuses. One explanation is that visual censuses were conducted in stretches with a more regular and continued otter presence through Otter Survey (see figure 4.7), which could indicated a higher preference for these stretches and perhaps a bigger density of individuals as well.

Generally, the results show that the density of the reintroduced population is still low, similar to the density found in oligotrophic rivers from the Pyrenees (Ruiz-Olmo, 2001).

4.4.3. Post-release mortality

The main problem when analyzing mortality data is that not all causes of death are equally likely to be detected (Kruuk, 1995). An otter dead on the road can be found easily, but not if she dies inside the den. The situation changes if all otters are equipped with radiotransmitters, because during battery life the individuals can be found whatever the cause of death (Ruiz-Olmo et al., 1997).

Post-release mortality in the reintroduction project was 22% n=41 (one year after release). This percentage is similar to the New York (18% n=28, Spinola et al., 2001) and Missouri *Lontra canadensis* translocation programs (19% n=31, Erickson & McCullough, 1987) but lower than in other reintroduction programs, as in Indiana (29% n=15, Johnson & Berkley, 1999), Oklahoma (40% n=10, Hoover et al., 1984) or Sweden (38% n=36, Sjöansen, 1997), the last with *Lutra lutra*.

More than half of the deaths (56%, n=41) were due to road kill. This percentage is consistent with the levels in all developed European regions or countries, such as Denmark (45.4% n=145, Madsen et al., 1999), Shetland (42% n=119, Conroy, 1992), England (83% n=77, Simpson, 1997), all United Kingdom (60% n=643, Green, 1991), Ireland (70%, Simpson, 1997) or Spain (90% n=147, Ruiz-Olmo et al., 1997). From five deaths registered on the 28 otters monitored in the New York River Otter translocation program (Spinola et al., 2001), 60% were due to collisions with vehicles and exactly the same percentage (60% n=5) was found in the Indiana reintroduction program (Johnson & Berkley, 1999). It is interesting to point out that although road kills are the most likely deaths to be recorded (Conroy, 1992) in our radiotracked otters (results not slant) road kills continue to be the main cause of mortality.

Mortality caused by illegal (in reintroduction area) fyke nets is not so important (11%) as in other countries like Denmark (32.5% n=145, Madsen et al., 1999) or the United Kingdom (27% n=643, Green, 1991). Other risks, like poisoning or drowning in irrigation infrastructures, should also be considered. Nevertheless, post-release mortality has been due to accidents and not human persecution, and this is positive to the success of the reintroduction project.

More than fifty percent of otters found dead died before two months after releasing, exactly the same percentage reported in Swedish otter reintroduction (Sjöansen, 1996). Our radiotracking studies suggest that animals which dispersed very fast (and usually far) from release areas experienced higher mortality than those that remained in the vicinities or dispersed slowly, thus giving more importance to the speed than to the extent of dispersion (only this last factor is considered by Erickson & McCullough, 1987).

The "fast dispersion" theory can be exemplified using the five otters run over. Three individuals died in roads far away from rivers or even channels, after 1, 25 and 48 days after release. Another died close to a bridge over a river after 55 days, but in another basin at more than 100 km from the release site. The fifth otter, though, died after 127 days in a very dangerous area where a busy road crosses rice fields (two more otters, already born in reintroduction area, were run over in the same area).

While Erickson & McCullough (1987) sustain that "hazards were greater at more distant locations …. because dispersing otters were less familiar with habitat and consequently at greater risk in their constantly changing surroundings", our data suggest that unknown habitat is a problem faced by all the released animals that can be solved if they explore it at slow pace even if after some months they have dispersed far away from the release site.

The four otters that were run over shortly after release and in unexpected places were adults, which could suggest that these animals were well established in the capture area and tried to reach it after the releasing.

5. Spatial and temporal ecology

5.1. Introduction

Telemetry is a basic tool in the research of ecological and ethological aspects of the Eurasian otter, as a nocturnal mammal that inhabits aquatic habitats is very difficult to survey.
Although otter telemetry studies have been carried out in north and central Europe since the eighties (beginning in Scotland; Green et al., 1984) this technique has not been used until recently in Mediterranean habitats. Firstly, two otters were radiotracked in the Pyrenees for less than a month (Ruiz-Olmo et al., 1995). Then, at the same time as our reintroduction project, an otter study was initiated at the Bergantes river (Jiménez et al., 1998) radiotracking until now a total of ten animals.

The *GORP* has as a novelty the implantation of radiotracking devices to a large number of otters (38 animals), only surpassed by some American reintroduction projects with *Lontra canadensis* (Tom Serfass & Romeo Spinola, pers. comm.).

Most of the information obtained through telemetry is set out in this chapter (but see also 4.2.1, 4.3.1 and 6.3), including the patterns of activity, the home ranges, the movement parameters on a 24 hour basis and the typology of resting sites used.

5.2. Materials and methods

5.2.1. Home ranges

Total home range and core area were calculated for otters radiotracked more than 20 days (n=25). While all the radiolocations were used to calculate total range, core area was defined as minimum linear distance that cover 50% of radiolocations.

Some of the tracked otters used marshes, thus resulting a part of the range in a bidimensional scale (hectares). As this information is no comparable with range in rivers or channels it has been omitted in the present study.

An ANOVA was used to test differences in total range and core area between males and females and between juveniles and adults.

5.2.2. 24 hours tracking sessions

A total of 50 “24 hour tracking sessions” were done, but eight were partial, due to the loss of the signal during the tracking session. Thus, the total amount of time following the otters was 1152 hours. During the tracking sessions radiolocations were taken at least every hour, but usually more frequently, especially when animals changed their direction of travel or their activity. The transmitter’s signal fluctuation helped to differentiate between activity and inactivity and even between water or land displacement, but the last was not used in this study.

For each tracking session, some movement parameters were calculated, following the methodology used by Durbin (1996):

- Range (R). Length of waterway used by an otter during the 24 hour tracking session, expressed in kilometers.
- Distance (D). Total distance travelled by the animal, including revisited areas, expressed in kilometers. Example: if an otter cover a length of 1 km of river and then changes direction and returns to the original point, the range is 1 km but the distance is 2 km.
- Time (T). Amount of time spent active, expressed in hours. The fluctuations in the transmitter signal (activity sensitive) were used to determine whether the otter was active, even when it was eating or cleaning itself on the shore.
- Rate of range use (R/T). Defined as the length of waterway used (R) divided by the time spent active (T), expressed in kilometers per hour.
- Rate of travel (D/T). Defined as the distance traveled (D) divided by the time spent active (T), expressed in kilometers per hour.
- Revisit index (D/R). Defined as the distance traveled (D) divided by the length of waterway used (R). It gives information about the proportion of stretches visited more than once in a tracking period and has no units.
- Speed (S). Distance travelled in a period of time (km/h). It is used for short periods of time, because the mean speed for the 24 hours tracking period is the rate of travel (D/T).

The activity data obtained in the 24h tracking sessions were summarised in a single discrete value for every hour: active (more than 30 minutes showing activity) or inactive (less than 30 minutes showing activity). Thus, 1008 activity data (42 tracking sessions x 24 hours) were obtained with positive (activity) or negative (inactivity) results.

The 24h tracking sessions were grouped according to the season, and the percentage of activity for each of the four seasons and 24 hours was calculated. The hours were grouped into three groups (always diurnal, always nocturnal and variable depending on the time of the year) and the percentage of activity for every hour was calculated (figure 5.1). An ANOVA was used to test the differences in the activity according to the groups and the seasons.

The moment of emergence and retirement was also studied. The amount of time between the moment of emergence and sunset and between the moment of retirement and sunrise was calculated. An ANOVA was used to test the seasonal differences in this behaviour. All tests were significant using $p < 0.05$.

The differences between sex and time of year in the movement parameters of the radiotracked otters were studied. ANOVA and Post Hoc Test (Tukey) were used to test these differences.

5.2.3. Resting sites

During 1999 and 2000, 39 resting sites used by tracked otters were found, used in 50 different resting periods. All these resting sites were located with precision (< 2 m) and the following characteristics were annotated:

- Location: in UTM grid of 100 x 100 meters.
- DTS: distance to the shore.
- HAS: height above shore.
- River width.
- Distance to the nearest house and to the nearest road.
- Typology (to choose between forested riverbank, shrubby riverbank, marshy riverbank, rocky riverbank, sandy riverbank or other)
- Commonest plant species: annotating the 3 most abundant species.
- Dates of use.

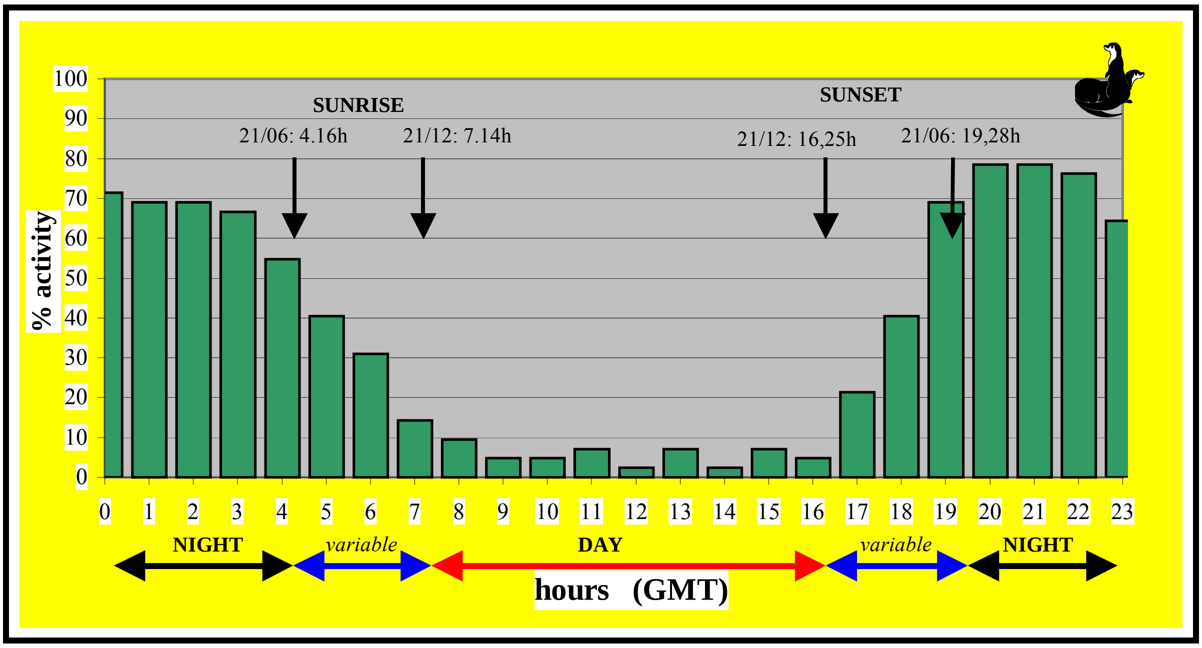
Due to the difficulty of entering compact masses of vegetation, to not disturb the otters and to the big number of radiotracked individuals, we did not "look" into the resting site and therefore we did not differentiate between holts (underground dens) and couches (above ground couches).

5.3. Results

5.3.1. Patterns of activity

Translocated otters showed nocturnal and crepuscular activity (figure 5.1), including otters from northern Spain, often diurnal in origin.

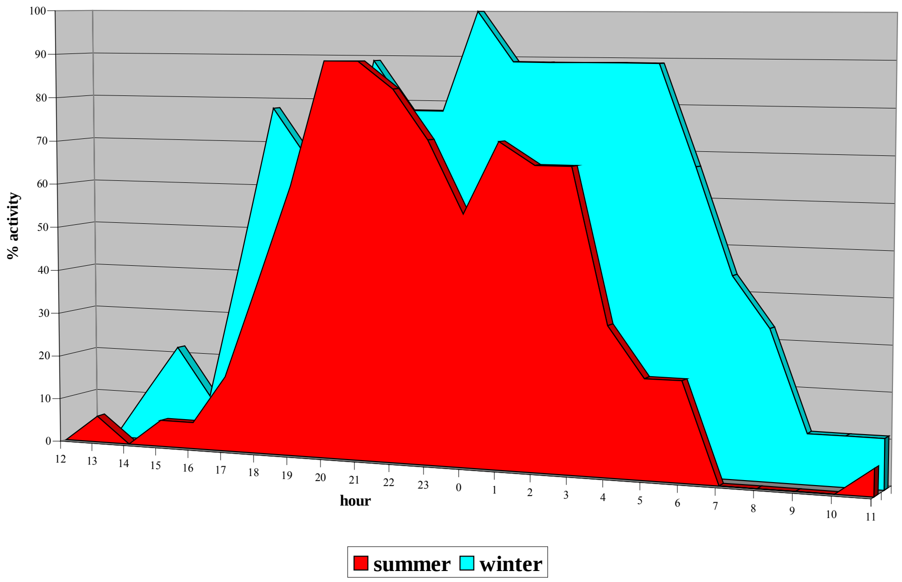
Figure 5.1. Total activity pattern, calculated from 42 '24 hours' tracking sessions (activity data = 1008).



From the 1008 activity data 376 were positive (37%), which means that otters were active approximately a third of the time. Diurnal activity comprised only the 3.7% of the total activity recorded.

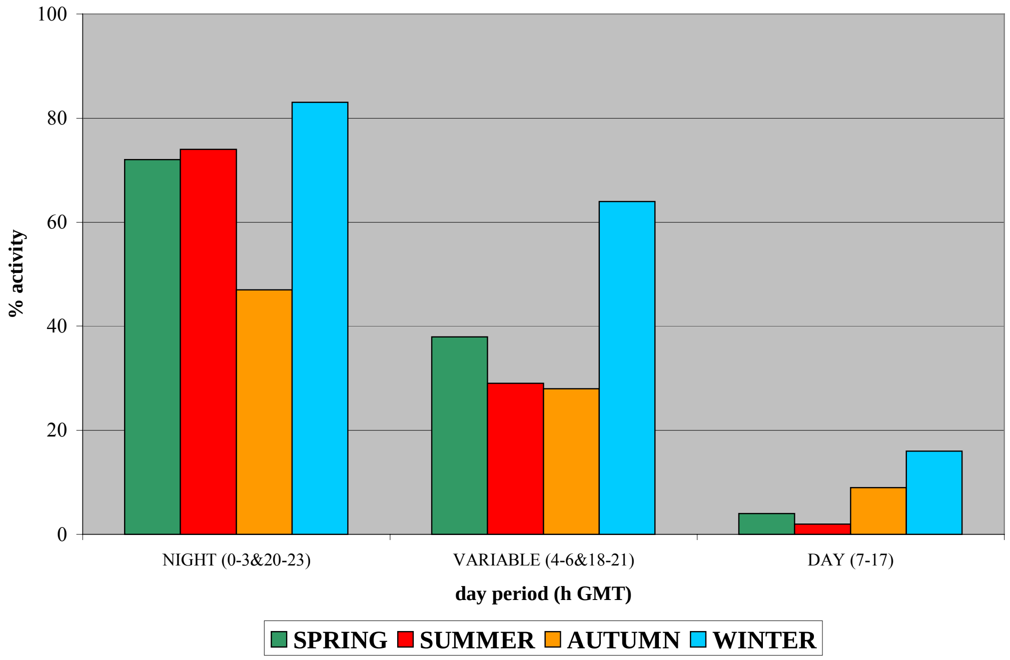
The percentage of activity varied significantly depending on the season (X^2 = 8139.583, d.f. = 3, $p < 0.001$), bigger in winter (52%) than in summer (34%). Our otters appeared to have greater early morning activity in winter than in summer, coincident with longer nights in winter (figure 5.2).

Figure 5.2. Percentage of activity in two different seasons: summer (n = 18) and winter (n = 9).



When grouping the activity percentages into time groups, the differences are maintained (figure 5.3). Winter values are higher in the three groups, but especially in the diurnal and variable groups. The activity percentages found in autumn could be unrepresentative, because the low sampling size (n = 9) and the fact that three of the tracking sessions correspond to a female from Asturies that presented strange behaviour during the first months, with abnormally short activity periods, which meant almost all periods of one hour were considered negative (inactivity).

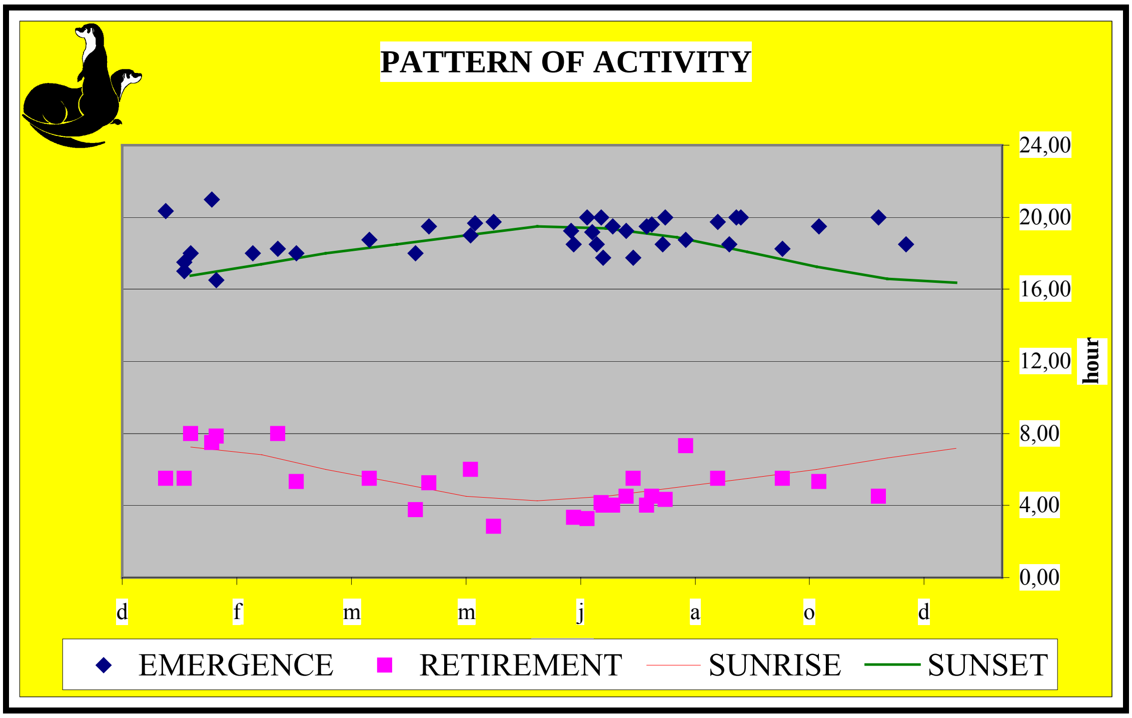
Figure 5.3. Average percentage of activity in the four seasons for the three time groups considered.



The moment of emergence and retirement was also studied. The amount of time between the moment of emergence and the sunset and between the moment of retirement and the sunrise was calculated and an ANOVA was used to test the seasonal differences in this behaviour.

The pattern in emergence and retirement behaviour was different in each case. For emergence, the value sunset – emergence was significantly different between the seasons (X^2 = 22.995, d.f. = 3, p = 0.002). Concretely, in the multiple comparisons (Scheffé) the differences were significant between summer and autumn and summer and winter. Figure 5.4 shows that emergence in summer happens generally before sunset and the opposite situation is produced during autumn and winter. In spring the situation is more variable. For retirement, the sunrise – retirement value has no significant differences between the seasons (X^2 = 1.936, d.f. = 3, p = 0.664) and the multiple comparisons did not present any significant difference.

Figure 5.4. Emergence and retirement pattern in relation to sunset and sunrise times.



5.3.2. Home ranges

Home range and core area was calculated for 25 otters tracked more than 20 days with a mean tracking period of more than six months. For home range calculation the individuals could be separated by sex (13 females and 12 males) and age (11 juveniles and 14 adults) (table 5.1).

Table 5.1. Sizes of home ranges and core areas.

number of otters	mean tracking period (days)	average total range (TR) (km)	average core area (CA) (km)	CA/TR
total (25)	208	34.2 (3-90)	5.1 (0.25-21)	0.15
males (12)	198	39.1 (3-90)	6.1 (1–21)	0.16
females (13)	218	29.7 (3-85)	4.4 (0.25-12.5)	0.15
juveniles (11)	241	33.6 (3-90)	3.5 (0.25-12.5)	0.10
adults (14)	183	34.6 (12-85)	6.8 (1.5-21)	0.20

Total ranges did not present significant differences between males and females (X^2 = 581.397, d.f. = 1, p = 0.361) nor between juveniles and adults (X^2 = 27.892, d.f. = 1, p = 0.840). Core areas were either significant according to either sex (X^2 = 27.267, d.f. = 1, p = 0.237) or age (X^2 = 71.208, d.f. = 1, p = 0.063).

5.3.3. Movement parameters

Twenty-four hour tracking sessions gave data for complete activity periods, from emergence at dusk to retirement at dawn, and also including occasional diurnal movements.

The chosen movement parameters were analyzed for each animal (table 5.2) but the number of sessions was too small to establish individual differences. So data was processed in groups of tracking sessions, depending on biological (sex) or seasonal characters (table 5.3).

Table 5.2. Mean movement parameters of '24 hours' tracked otters.

Animal		24h sessions	Range (kms)	Distance (kms)	Time (hrs)
Vega	female	4	8.0 (2.5-13.5)	8.1 (2.7-13.5)	7.8 (2.0-10.8)
Xurra	female	3	7.5 (1.2-15.0)	9.2 (2.2-16.0)	6.4 (1.7-10.1)
Berna	female	3	0.0 (0.02-0.2)	0.2 (0.04-0.4)	6.9 (5.7-7.5)
Jroñe	female	1	0.5	1.0	9.4
Rati	female	3	2.8 (0.2-6.3)	3.0 (0.6-6.3)	5.8 (3.2-9.7)
Celta	female	5	3.2 (1.7-6.5)	10.2 (3.5-11.2)	5.1 (3.7-7.0)
Victor	male	3	14.2 (5.0-23.0)	15.6 (7.2-25.0)	5.8 (4.7-7.7)
Secem	male	2	2.2 (1.0-3.5)	4.5 (2.0-7.0)	9.4 (8.2-10.5)
Mosquit	male	12	3.7 (0.1-16.0)	6.2 (0.4-17.6)	10.3 (6.1-16.1)
Nano	male	3	1.8 (0.4-4.2)	2.0 (0.9-4.2)	6.6 (6.0-7.0)
Garelu	male	2	0.8 (0.1-1.5)	1.1 (0.2-2.2)	5.9 (3.5-8.2)
Animal		**D/T (km/h)**	**L/T (km/h)**	**Revisit index**	**Speed (km/h)**
Vega	female	1.1 (0.7-1.7)	1.1 (0.7-1.7)	1.0 (1.0-1.1)	1.5 (0.5-3.0)
Xurra	female	1.4 (1.3-1.6)	1.0 (0.7-1.5)	1.5 (1.1-1.8)	2.3 (1.2-4.0)
Berna	female	0.03 (0.01-0.1)	0.02 (0.01-0.03)	2.0 (2.0-2.0)	-
Jroñe	female	0.1	0.1	2.0	-
Rati	female	0.4 (0.2-0.6)	0.4 (0.1-0.7)	1.7 (1.0-3.0)	
Celta	female	1.2 (0.7-1.9)	0.6 (0.4-1.1)	1.9 (1.7-2.0)	-
Victor	male	2.5 (1.5-3.3)	2.3 (1.1-3.0)	1.2 (1.0-1.4)	2.6 (1.2-3.7)
Secem	male	0.5 (0.2-0.8)	0.3 (0.1-0.4)	2.0 (2.0-2.0)	-
Mosquit	male	0.5 (0.04-1.4)	0.3 (0.01-1.2)	2.2 (1.0-4.0)	-
Nano	male	0.3 (0.1-0.7)	0.3 (0.1-0.7)	1.4 (1.0-2.0)	-
Garelu	male	0.1 (0.1-0.2)	0.1 (0.03-0.2)	1.7 (1.3-2.0)	-

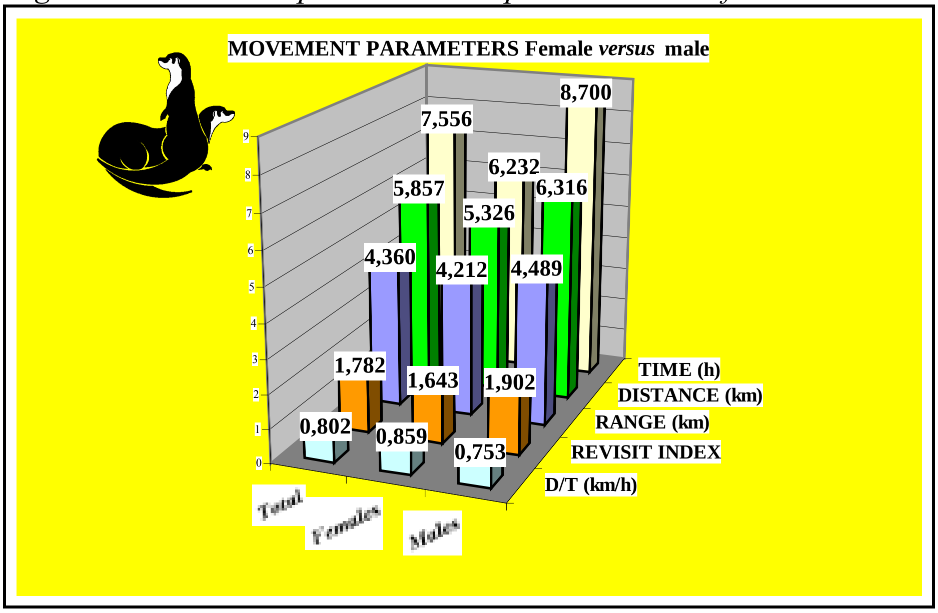
Table 5.3. Mean movement parameters of '24 hours' tracked otters (per group).

Group	24h sessions	Range (kms)	Distance (kms)	Time (hrs)
All	41	4.4 (0.02-23.0)	5.9 (0.04-25.0)	7.6 (1.7-16.1)
Females	19	4.2 (0.02-15.0)	5.3 (0.04-16.0)	6.2 (1.7-10.8)
Males	22	4.5 (0.1-23.0)	6.3 (0.2-25.0)	8.7 (3.5-16.1)
Winter (November-April)	15	5.3 (0.02-16.0)	7.6 (0.04-17.6)	8.2 (1.7-16.1)
Summer (May-October)	26	3.8 (0.1-23.0)	4.8 (0.1-25.0)	7.2 (3.5-11.7)
Group	**D/T (km/h)**	**L/T (km/h)**	**Revisit index**	**Speed (km/h)**
All	0.8 (0.01-3.3)	0.6 (0.01-3.0)	1.8 (1.0-4.0)	2.1 (0.5-4.0)
Females	0.9 (0.01-1.9)	0.6 (0.01-1.7)	1.6 (1.0-3.0)	1.8 (0.5-4.0)
Males	0.7 (0.04-3.3)	0.6 (0.01-3.0)	1.9 (1.0-4.0)	2.6 (1.2-3.7)
Winter (November-April)	0.9 (0.02-1.9)	0.6 (0.01-1.7)	1.8 (1.0-3.0)	1.7 (0.8-2.6)
Summer (May-October)	0.7 (0.01-3.3)	0.6 (0.01-3.0)	1.8 (1.0-4.0)	2.5 (2.0-3.0)

5.3.3.1. Differences between males and females

The movement parameters of the males were generally bigger than those of the females. Nevertheless, the application of an ANOVA showed than only activity time in the 24 hour period was significantly bigger in males than in females (p = 0.013).

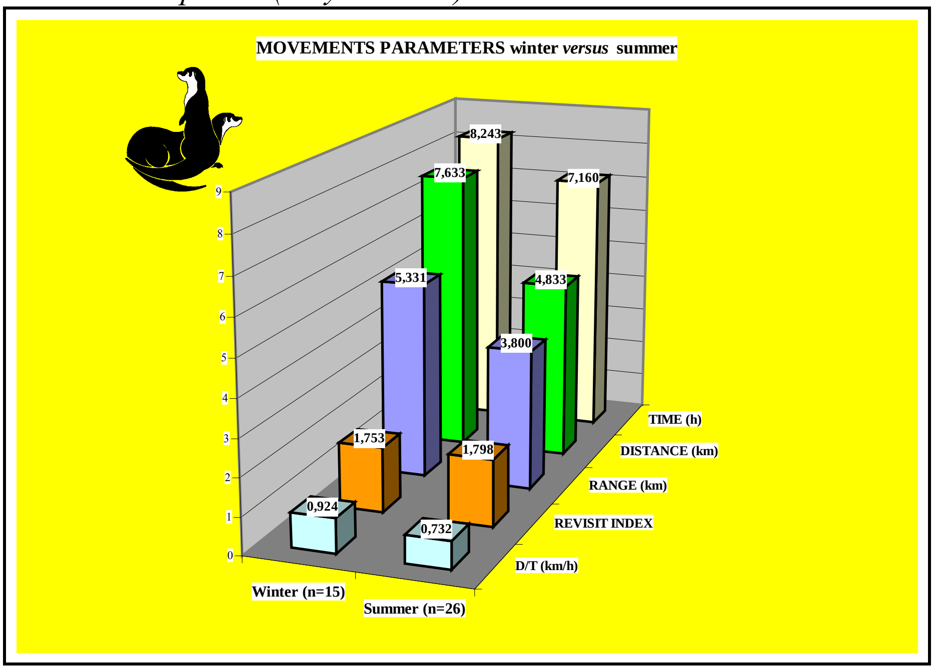
Figure 5.5. Movement parameters comparison between female and male.



5.3.3.2. Differences in period of year

Movement parameters of the radiotracked otters were bigger in "November-April" than "May-October", as shown in figure 5.6. However, the differences were not significant.

Figure 5.6. Movement parameter comparison between 'winter' period (November-April) and 'summer' period (May-October).



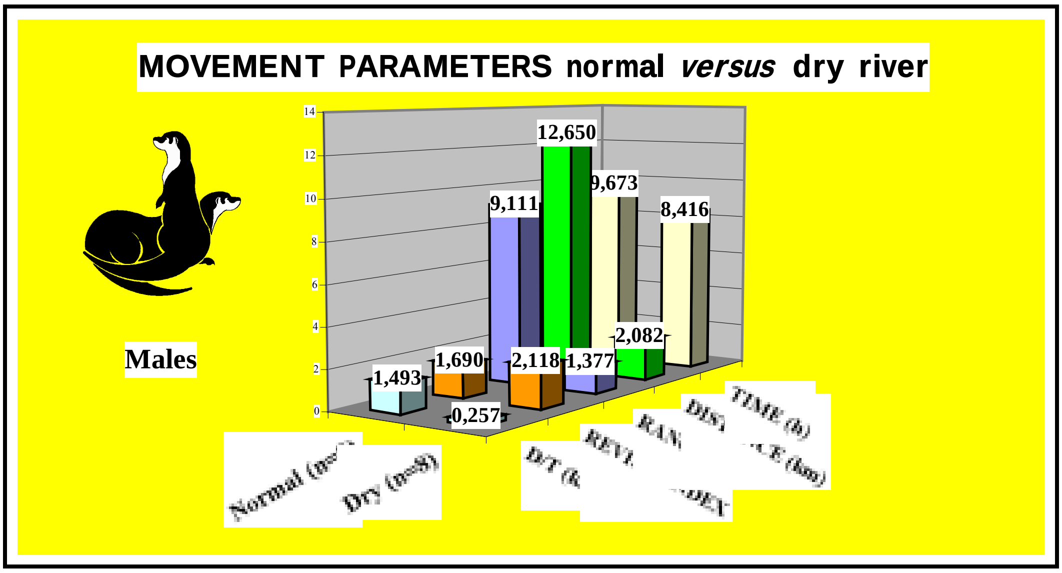
Analyzing the movement parameters by period, but applying ANOVAs separating the results by sex, the differences were not significant for the females, but they were for the males (p = 0.009) (figure 5.7). In the Post Hoc Tests (Hukey), the activity time (p = 0.004) and the distance (p = 0.042) were significantly lower for "summer" period than for "winter" period (although this does not happen for other parameters).

5.3.3.3. Differences between dry and flowing watercourses

Taking into account that 72% of the "summer" (May-October) "24 hour tracking sessions" following males were done in conditions of drought (dry river with scattered pools), while no tracking sessions were done with females in those conditions, it seemed plausible that differences in period were mostly due to a different use of the space depending on the existence or not of flowing water in the river.

Thus, a comparison was made between 24 tracking sessions done with male otters living in flowing watercourses (n = 9) and with males living in dry rivers (n = 8) with scattered pools.

Figure 5.7. Movement parameter comparison between flowing and dry watercourse.



The ANOVA used to test showed that the summary of the effects of the five movement parameters were significantly different (p = 0.010) between flowing and dry rivers. In the post hoc tests (Tukey) range (p = 0.003), distance (p = 0.0003) and D/T (p = 0.001) parameters were significantly different, while time (p = 0.357) and revisit index (p = 0.304) were not. Thus, the movements of the otters inhabiting dry rivers are significantly smaller than the ones realized by otters inhabiting flowing watercourses. These data are discussed in section 5.4.3.

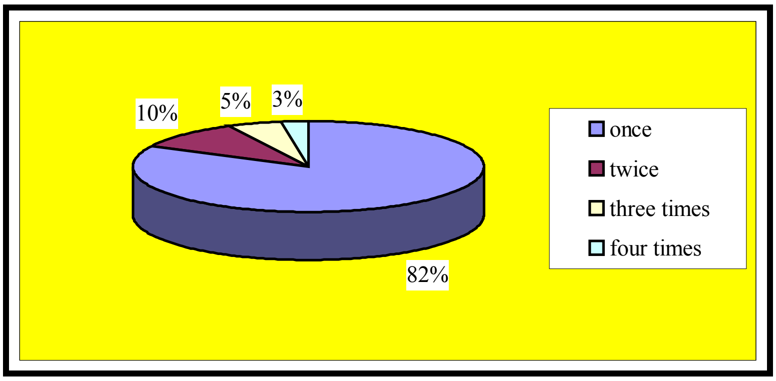
5.3.4. Resting-sites

Between 1999 and 2000, 39 otter resting sites were identified corresponding to 10 different individuals. Locations of sites were not consecutive in most cases.
32 (82%) resting sites were found occupied by an otter once, 4 (10%) twice, 2 (5%) three times and in only one case four times (2.5%). None of the resting sites were later found occupied by a different otter.

Table 5.4. Number of resting sites found for every tracked otter.

Code	Name	Sex	Number of resting sites	Number of resting periods
M11	Mosquit	M	10	12
F14	Rati	F	11	12
F18	Celta	F	3	3
F17	Llum	F	3	3
F15	Berna	F	1	1
M12	Nano	M	6	11
F21	Keta	F	2	3
F22	Lauda	F	1	1
F23	Bella	F	1	1
M13	Garelu	M	1	3
	TOTAL		**39**	**50**

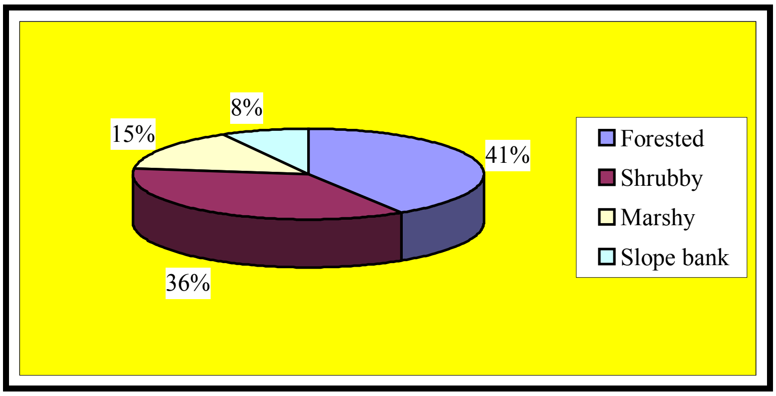
Figure 5.8. Number of occupations for every resting site found.



According to the situation, distance to the shore (DTS) and height above shore (HAS) were calculated for every resting site (see 5.2.3).
DTS mean was 2.1 (0.5-10) meters, but in 59% of the cases was closer or equal to one meter. No resting sites studied were more than 10 meters from the shore.
HAS mean was 1.4 (0-10) meters, but in 51% of the cases it was lower or equal to one meter. The highest resting site was found 10 meters above shore.

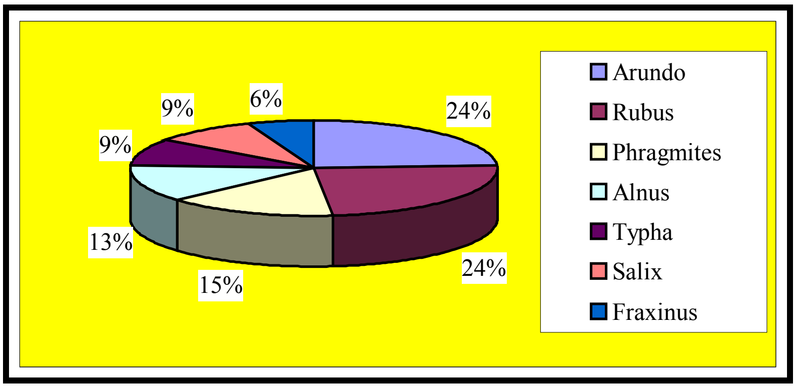
River width at the resting site was also calculated. Mean was 16.9 meters (1-100) but 54% were narrower or equal to 10 meters.
Distance to the nearest house and road was calculated too. In the first case, the mean fell between 100-200 meters and in the second case in the 40-50 meters class.
Resting sites were situated mostly in vegetated shores: 41% in forested riverbank, 36% in shrubby riverbank and 15% in marshy riverbank. 8% were situated in sandy banks or slopes, although usually with some reed or cattail on the base.

Figure 5.9. Typology of resting sites studied.



The commonest plant species around the resting sites were cane (*Arundo donax*), blackberry (*Rubus ulmifolius*) and reed (*Phragmites communis*).

Figure 5.10. Commonest plant species around studied resting sites.



No resting sites were found in rocky riverbank, but some apparently shrubby riverbank borders were situated in old channelling works, with boulders beneath the shrubs which otters can use as a refuge, but which are extremely difficult to reach without disturbing the animal.

5.4. Discussion

5.4.1. Pattern of activity

The activity pattern of the reintroduced otters proved mainly nocturnal and crepuscular, with little diurnal activity. These data agree with other studies carried out in similar latitudes (Rosoux, 1995 ; Ruiz-Olmo, 1995).

The radiotracked otter showed greater activity in winter than summer, as mentioned in the study of Green et al. (1984) in Scotland. Comparing the activity by time bands, activity was clearly higher during diurnal and variable (daylight or dark depending on the season) periods (figure 5.1). In the first case, diurnal activity in winter has been observed by other authors (Ruiz-Olmo, 1995), in coincidence with very low temperatures. Thus, the otter could complete its nocturnal activity, perhaps shortened by the low temperatures, although these temperatures are usual in other latitudes where the species lives. During the summer, the shorter night could favour this behaviour if the otter sought to maintain the number of hours of activity (Ruiz-Olmo, 1995), but this does not appear to happen in our case, so that the diurnal activity is residual and the total percentage of activity is smaller than in winter. In the second case, during the variable time group, activity is significantly greater in winter, because these time bands are in complete darkness then.

The period of quiescence during the middle of the night was observed only on some occasions, but it was not a general pattern and was not reflected when grouping the results (figures 5.2 and 5.3). The randon nature and variability of this resting period have been disclosed by other authors (Green et al., 1984 ; Rosoux, 1995; Ruiz-Olmo, 1995).

The emergence or beginning of activity is quite constant (figure 5.4), so that seasonal significant differences are found when comparing this moment with sunset. Obviously, if emergence is constant, during the summer it occurs in daylight, but during the winter activity begins when it is already dark. These results agreed with Ruiz-Olmo (1995), although almost all data are estival and with Rosoux (1995). In contrast, Green et al. (1984) found a close correlation with the solar cycle, with emergence just after sunset during the periods studied (February-March; July-October).

Retirement or the end of activity was much more variable and no significant seasonal differences were found, as mentioned in other studies (Green et al., 1984; Rosoux, 1995).

5.4.2. Use of space

The ranges found for the 25 otters radiotracked for more than 30 days were of the same order as those found in other studies in European and North-American rivers (with *Lontra canadensis* in North America) (Melquist and Hornocker, 1979; Green et al., 1984; Kruuk et al., 1993; Kruuk, 1995; Durbin, 1996). However, this range was bigger than in studies made in similar places, such as the Pyrenees and the Bergantes river (Ruiz-Olmo et al., 1995; Jiménez et al., 1998). In both cases there is probably an influence caused by a short radiotracking period (26 and 20 days for Pyrenees and 120, 90 and 120 days for Bergantes river) comparing with a mean tracking period of 208 days (30-631) in *GORP*.

As in the majority of the studies consulted (table 5.5), males' home range is clearly bigger (32%) than females, but the differences between sexes are not significant, perhaps due to a low sample size. Nevertheless, Johnson & Berkley (1999) recently found no significant differences in total home range and core area between sexes, although this was with a different species (*Lontra canadensis*).

Table 5.5. Comparison between the home ranges of the studied otters in reintroduction area and other in the literature (adapted from Jiménez et al., 1998).

Area [+]	**Sex**	**Range (km)**	**Source**
Girona province	M	39.1	present study
	F	29.7	
Bergantes	M	30.0±8.7	Jiménez et al., 1998
	F	20.3	
Pyrenees	M	20.7	Ruiz-Olmo et al., 1995
	F	11.8	
Scotland	M	39.1	Green et al., 1984
	F	26.3±4.5	
Scotland	M	39.4±27.6	Kruuk et al., 1993
	F	20.0±19.5	
Scotland	M	67.3±24.4	Durbin, 1996
	F	24.0	
North America*	M	33.2±20.0	Melquist and Hornocker, 1979
	F	27.9±8.6	

[+] *Only riparian habitats (no marshes and wetlands)*
* *Lontra canadensis*

Core areas, defined as minimum linear distance covering 50% of radiolocations, occupy 15% of total range.

No significative differences were found between reintroduced and wild otters, as already stated in a previous study combining data from Girona province (*GORP*) and Bergantes river (Saavedra et al., 1997).

Movement parameters have been studied following Durbin (1996) methodology. Mean range was 4.2 km for females (n=19) and 7.6 km for males (n=11; excluding males in almost dry rivers; see 4.4.4), smaller than in Scotland (the female tracked by Durbin presented a range of 5.9 km and the two males 6.5 and 9.9 km respectively) and also smaller than in the river Bergantes (female range of 5.8, males 6.2 and 11.8 km).

In all three cases males presented significantly bigger ranges than females, probably because of male patrolling behaviour in search of females to mate with (Durbin, 1996). In this sense, the mean range of an adult male (n=3) is 14.2 km, much higher than the mean of 6.6 km (n=6) of three subadult otters.

On the other hand, the night activity period is much longer in our otters (mean of 7.6 hours) than in Durbin's study in Scotland (mean of 4.7 hours). These results appear to have no logical explanation, as Mediterranean rivers are more productive and reintroduced otters would need less time to satisfy their trophic requirements, added to the fact that Iberian otters are smaller than Scottish ones. The cause may be methodological differences in the attribution of activity or non-activity based on telemetry signals.

Again, these differences in the calculation of the activity period would appear to cause the big differences found in travel rate (D/T) (0.8 versus 2.4 in Scotland) and in range use rate (L/T) (0.6 versus 2.0 in Scotland).

Nevertheless, reintroduced otters had a higher tendency to revisit the same stretches during an activity period (1.8) comparing with the otters studied in Scotland (1.3). In fact, in the 41 activity periods studied, on 29 occasions (70%) the tracked otter changed direction and revisited river stretches, on 6 occasions it (15%) traveled upriver only, and on 6 occasions (15%) traveled only downriver.

Speed in uninterrupted activity periods where the otter maintains the same direction (generally between 45 and 90 minutes) ranged between 0.5 and 4 km/h, with a mean of 2.1 km/h. The speed was higher downriver (2.45 km/h n=28) than upriver (1.42 km/h n=32). All these data are similar to other studies (Green et al., 1984; Jefferies et al., 1986; Ruiz-Olmo et al., 1995; Jiménez et al., 1998).

5.4.3. Effect of water availability

In the Mediterranean regions, water availability varies greatly over the year. Every summer some watercourses dry out. Some years, more severe droughts dry most of the watercourses with the exception of the biggest rivers and reservoirs.

Water availability is crucial because it determines the otters' food supply directly (Prenda et al., 2001; Ruiz-Olmo et al., 2001). Two approximations were used to study the otters' response to this environmental factor: changes in the distribution of the reintroduced population and the daily movements of specific individuals.

Study of the dispersion of the reintroduced population shows a pattern, from 1998 on, of an increase in positive survey stations in winter and a decrease in summer, the latter marked by a severe drought in 1998 and 1999 (see figure 4.4 in chapter 4).

Comparison of positive stations and dry stations (figure 4.4 in chapter 4) shows a population concentrated into a smaller area during the summer drought, due to the increase of stretches uninhabitable because of lack of food. These periodical expansions and contractions in otter population ranges, coinciding with greater or less availability of water in their habitat, has also been described in other studies carried out in Mediterranean habitats, such as Castelló (Ruiz-Olmo et al., 2001), Málaga (Carpena et al., 1997) or Córdoba provinces (López et al., 1998; Prenda et al., 2001).

Population movement in response to drought is probably linked to density. Where low (as here), alternative stretches of water are easily found by these refugees from hydric stress (Prenda et al., 2001). But in saturated areas like Extremadura (a donor zone for the *GORP*) the Spanish Otter Survey found otter presence in 45.2% of dry rivers surveyed (Ruiz-Olmo & Delibes, 1998), indicating that some individuals are obliged to live in zones without water because the "good ones" are already occupied. Otter mortality and territorial behaviour during severe droughts are important aspects to be studied in the future.

We also had the opportunity to compare the daily movements (24 hour tracking sessions) of different individuals inhabiting flowing watercourses and dry rivers with scattered pools (see figure 5.7). The mean range occupied in an activity period was reduced seven times (9.1 to 1.4 km) in the individuals that inhabited dry rivers. Similarly, the total distance traveled was six times lower (12.6 to 2.1 km). Also, the revisit index (distance/range, see 4.2.2) was higher in dry rivers (2.1 versus 1.7). Finally, scouting speed was a sixth of that registered in running water rivers. In another otter study held in a Mediterranean river (Bergantes, Valencia region) (Jiménez et al., 1998) findings included a reduction of home range and movements for the same individual and the concentration of otter activity around pools (J. Jiménez, pers. comm.).

Thus, these data give a clear picture of the behaviour of individuals living in dry rivers with scattered pools. These individuals "fixed" to one or several pools where they feed and pass most of their activity period (Ruiz-Olmo et al., 2001). They move quickly by the dry river between the pools (due to dangerous exposure to predators when running outside the water) and usually they visit the same pools several times in an activity period, increasing the revisit index.

Durbin (1996) points out that the unpredictability of resource availability may necessitate large nightly and total range. During water shortage conditions, the unpredictability disappears and the otter knows where prey are to be found, because all are concentrated in the scattered pools. Therefore, it is not necessary for the otter to patrol long stretches of habitat; instead it can stay close to the concentrated food sources.

In the cases studied in our project when the pools dried completely the individuals abandoned the river stretch.

5.4.4. Resting sites

The use of resting sites by the reintroduced otters followed the same pattern as found in a similar study carried out in Bergantes river (Jiménez & Palomo, 1998). The different individuals mostly used a different resting site every day, repeating on very few occasions; this behaviour has been pointed out by other authors (Green et al., 1984; Rosoux, 1995; Ruiz-Olmo, 1995).

From the 44 “24 hour tracking sessions” done, on only six occasions (14%) did the otter return to the same resting site at the end of the activity period. And of the six times this happened, four corresponded to a male in a dry river – where movements are very small (see 4.4.4) – and the other two to a breeding female that obviously must return every day to the natal holt. Thus, it can be noted that the consecutive reutilization of a resting site in a female is a quite clear sign of breeding.

The resting sites selected by the otter were forested in almost half the cases, suggesting a dependence on riparian forest. However, the plants that surrounded the resting site (and that formed it in many cases) were plants which grow fast and in compact masses down to the water’s edge, constituting a secure refugee for the otters, such as the cane (*Arundo donax*), the blackberry-bush (*Rubus ulmifolius*) and the reed (*Phragmites sp.*).

The otters did not appear to select resting sites very far away from infrastructures or human constructions and were found often during the inactivity period resting a few meters from busy roads, inhabited houses, gravel extraction areas or river stretches with heavy boat traffic. These data are consistent with Durbin (1998) who found that some radiotracked otters used river stretches close to roads and houses more than expected and that on average resting sites were found no further away than other parts of the range. Thus, otters would be tolerant to indirect forms of disturbance.

The natal den of the two females that bred with the transmitter still active were localized in low frequented areas, far from the main river in one case and in a channel completely covered with reed and bulrush in another. The location of the natal holt may be related to the distance from the main otter activity centers observed in the Shetland Islands (Taylor & Kruuk, 1990), and/or safety from floods (Jenkins, 1980; Green et al., 1984).

Most of the resting sites were placed close to the shore (approximately half of them at less than one meter of distance and height) so that the resting sites may be effectively protected by protecting the first meters of the river bank (Jiménez & Palomo, 1998).

6. Post-parturition movements

6.1. Introduction

Knowledge of breeding behaviour is considered of great significance to conservation, and it is difficult and rare to have the opportunity to obtain such data. Radiotracking of females was used to study the sites selected by otters for breeding, the change of cubs from the natal den to another and the pre-parturition and post-parturition movements (Taylor & Kruuk, 1990; Rosoux, 1995; Durbin, 1996b).

During telemetry studies of the reintroduced otters, two radio-tagged females gave birth, so their post-parturition movements could be followed in detail.

6.2. Material and methods

The data used for the study of post-parturition movements came from radiotracking, mostly from random radiolocations but also from the "24 hour tracking periods" (see section 5.2).
Monitoring of the F18 breeding den was conducted with the help of a data collector (DCC II; Advanced Telemetry Systems, 470 First Av, Isanti, Minnesota, USA) which allowed us to record her presence or absence in the natal holt. Data was recorded continuously for 36 days (section 6.3.2).

6.3. Results

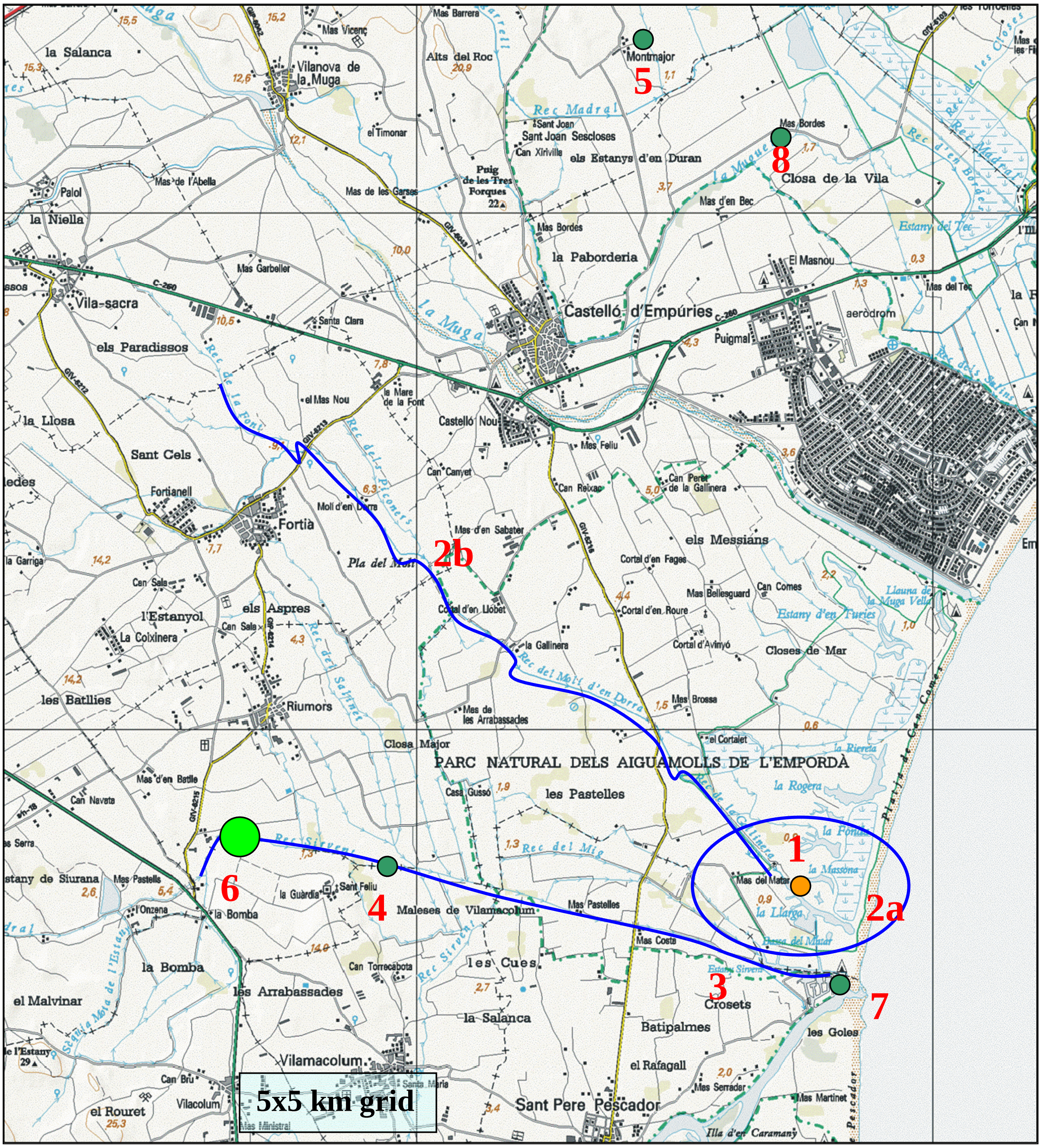
While the translocated otters were subject to radio tracking, two females changed suddenly their behaviour, using the same resting area and restricting their movements for two months, in what is considered to be typical breeding behaviour (Taylor & Kruuk, 1990; Durbin, 1996b). In one case (F02) one cub was seen in the area after this period, but in the other case (F18) no cub observations were made and it was assumed that any cubs died.

6.3.1. F02 (Derek)

Derek was captured in Extremadura in February 1996 and released on 1st of March. The releasing point was Les Llaunes marshes (1), in Aiguamolls de l'Empordà Natural Park (figure 6.1). There she moved around the lagoons (2a) and the Corredor channel (2b) until May, when she moved to the Sirvent channel (3). In July she made unexpected long displacements. On 11 she was in Sirvent channel (4), on the 18 she appeared in Pedret channel (5) some 20 km away after crossing through different watercourses and infrastructures, and on the 30 she moved again to the Sirvent channel (6). She remained there until 24 September and it was assumed that she gave birth between the 19th and 30th of July.
During the two months in the Sirvent area she used two dens, 150 meters apart (6), the first from 30 July to 12 August, and from 30 August to 24 September. The second was used from 13 to 29 August. Both sites were situated on the channel bank, inside

enormous and impenetrable blackberry bushes, so it was impossible to record details of the holts nature and dimensions. The Sirvent channel in this stretch was completely covered with reedbed.

Figure 6.1. Movements of F02 (Derek) during radio-tracking period. Numbers represent location in chronological order (see 6.3.1).

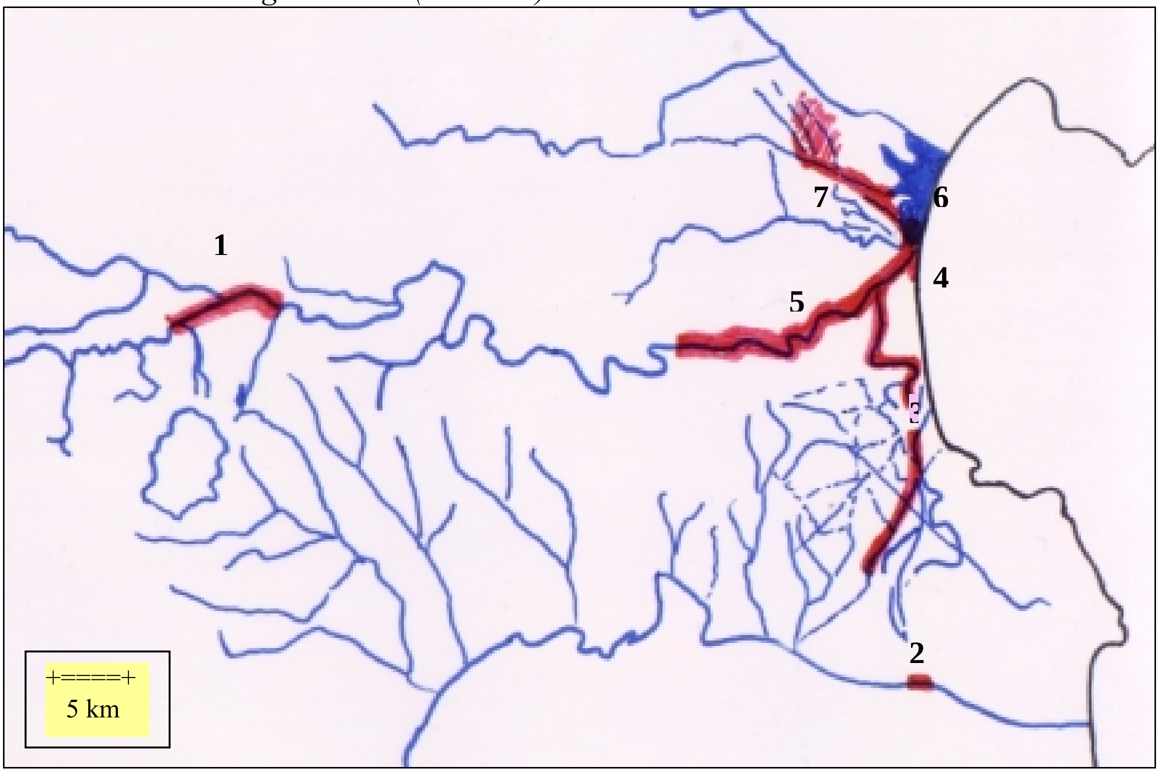


The last localization in the breeding area was on 24th of September and she wasn't found again until 2 October at the mouth of Sirvent channel (7). In this interval, a farmer saw an otter cub in the Sirvent channel, some 400 meters from the den area. Derek disappeared again until 16th of October, when it was located in the Mugueta river (8), some 15 kilometers from the last site. This river was again covered by reed and cattail so it was not possible to visually confirm presence on 18/19 October. After this day, the signal was definitively lost, probably due to battery failure.

6.3.2. F18 (Celta)

Celta was captured in Extremadura in September 1998 and released on 15 October. The area she travelled is illustrated in figure 6.2. The release point was in the intersection between the Ser and Fluvià rivers (1). She moved around a stretch of 5 km for a month and on the 4th of November she disappeared from the area.

Figure 6.2. Movements of F18 (Celta) during radio-tracking period. Numbers represent location in chronological order (see 6.3.2).



After 6 months, she was located again in an unexpected place (2), in a basin outside the reintroduction area. She was found in the Ter river, near the weir at Ullà, on 24 May (1999). In the middle of June she was radio-tracked around l'Escala channels (3), which are part of the corridor between the Fluvià and Ter basins, and lost again until August. During a flight survey on the 27th of August, she was located in the Fluvià river, near Sant Pere Pescador (4). On 29 August she established in the old meander river of Sant Pere Pescador. She used the same resting site for two months and used
1 no more than 5 km of river, so it was assumed that she gave birth. On the 20th of October she left the old meander and moved to the Fluvià river (between Sant Pere Pescador and Torroella de Fluvià) (5) and around l'Escala channels (3), but no cubs

could be seen accompanying her. She moved around areas 3,4,5 and 6 (Les Llaunes marshes) until March (2000), covering in this last zone some 200 ha of wetland. On 12 April she was located again in the Fluvià river, but soon moved to Corredor channel (7). At the same time a new female was released in the Sant Pere Pescador area. From April to July Celta was located in the Corredor channel, which she shared with another female (Ribeira - F23) for the first week of July. The last radiolocation was obtained on 7th of July.

Celta was radio-tracked for a period of 631 days in which she went over 85 km of river and 200 hectares of wetland.

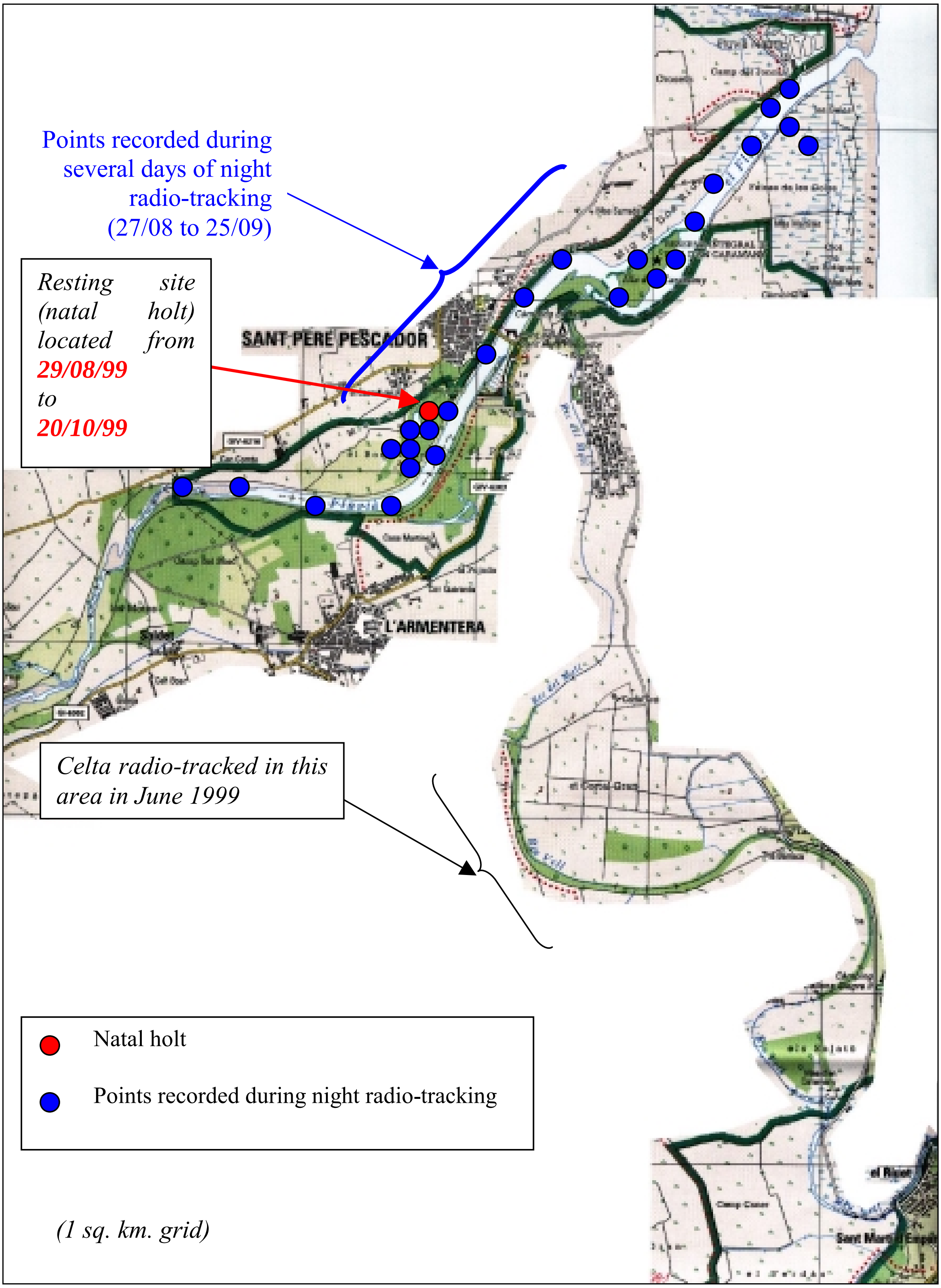
The resting site (natal den) Celta used during two months was situated in an old meander of the Fluvià river (figure 6.3), dredged in part and converted into a wide lagoon. Small channels remained at both ends, completely covered with a reedbed. Bank vegetation consisted of huge blackberry bushes surrounded by old poplar plantations and riparian forest. The natal holt was situated inside one 5x4x3 meter bush, so it was not possible to take details of the holt's nature and dimensions.

During the post-parturition period (two months for *L. lutra*, from parturition to leaving the natal holt) her home range was greatly reduced, using only a short stretch of the Fluvià river (figure 6.3). The revisit index was higher (2.0) because she returned to the same holt every night using the same stretch of river twice.

Table 6.1. Ranging parameters of F18 during post-parturition period, comparing with other period for the same female and all radio-tracked females mean (see table 5.2)

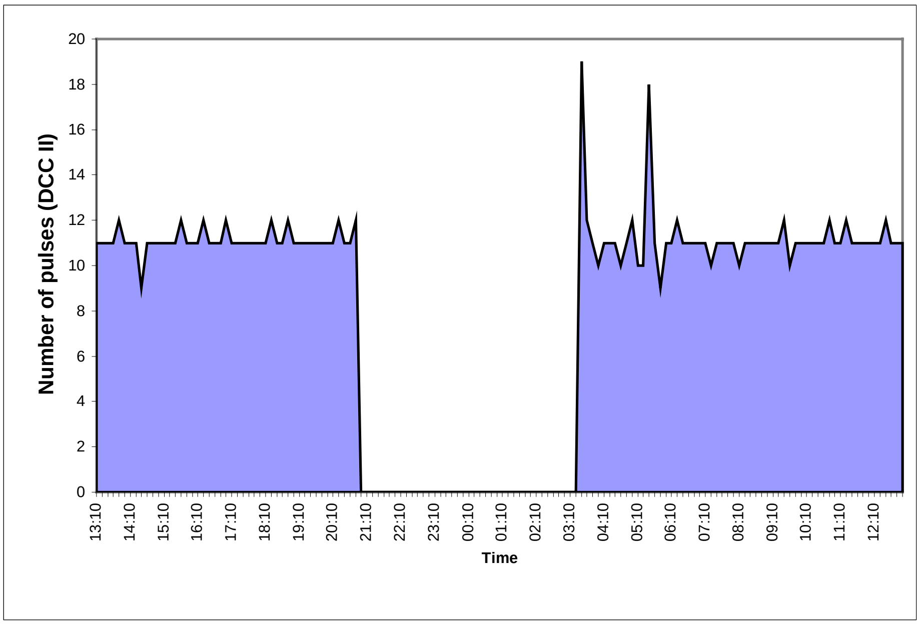
	total range (km)	**travel rate (km)**	**revisit index**	**time active (h)**
Post-parturition period	2.4	1.0	2.0	4.9
Other period	6.5	1.9	1.7	6.0
Females mean	4.2	0.9	1.6	6.2

Figure 6.3. Home range of Celta during the period in the old meander.



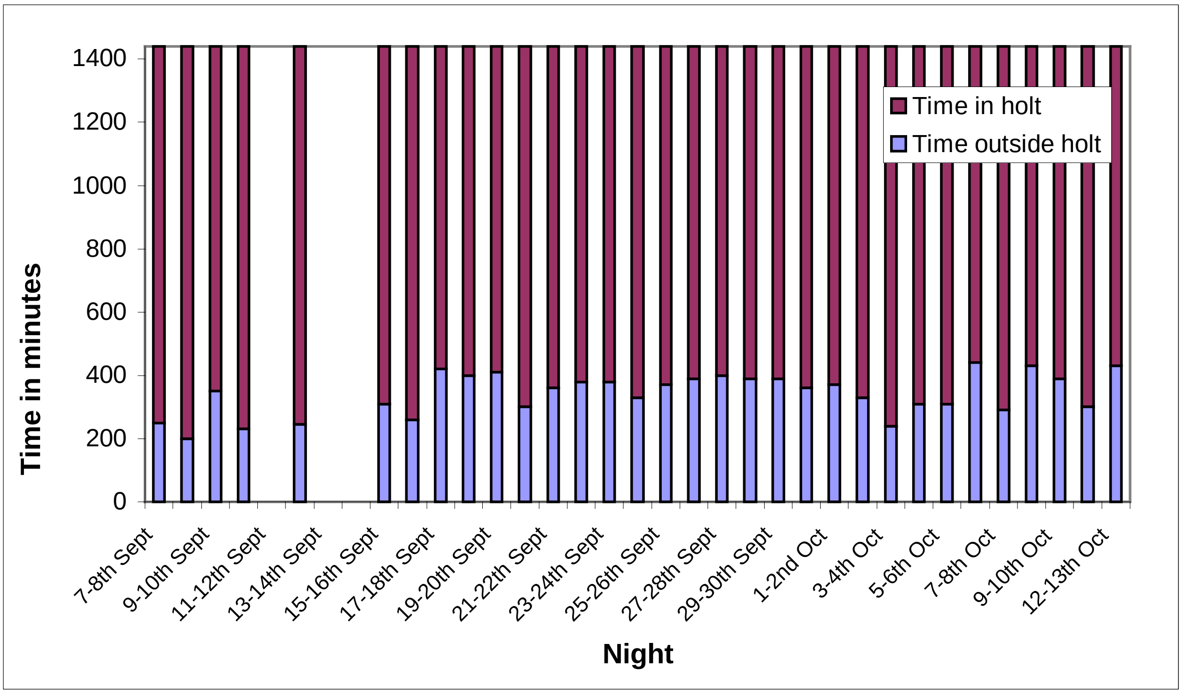
During the period between 7 September and 13 October, Celta was radio-tracked 24 hour a day using a DCC II data collector.

Figure 6.4. Example of the data collected by the DCCII for a 24 hour period, showing resting site activity and absence for the night between the 18th and 19th of September.



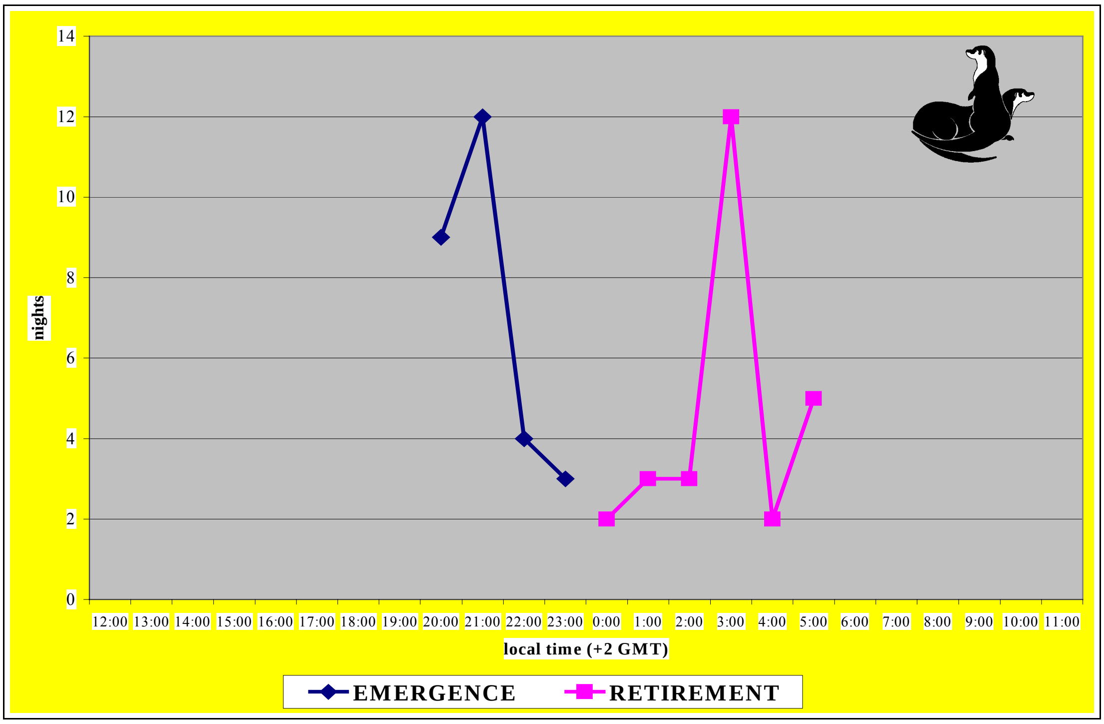
During this period, Celta spent an average of 5 hours and 42 minutes (30.63% of the day), outside the resting site, spending most of the time in the holt. During the first week, the amount of time spent active (not recorded because the data collector was installed on 7 September) was lower (3 hours average) compared to the amount spent in the later records.

Figure 6.5. Twenty four hour activity of Celta showing time in and out of the holt.



Emergence and retirement patterns were similar throughout the post-parturition period, with the otter leaving the holt around 9 pm and returning between 3 and 5 am. (local summer time; GMT+2).

Figure 6.6. Emergence and retirement pattern of Celta female during post-parturition period (number of activity periods considered = 28).



6.4. Discussion

The study of two implanted breeding females yielded some data that may be useful for conservation purposes:

- Female otters make large movements just prior to give birth.
As in the case of a female radiotracked by Taylor & Kruuk (1990), Derek made an unexpected, dangerous and long displacement of 20 km., crossing through different watercourses and infrastructures, only to be at the initial site ten days later, where she finally gave birth.

- Female otters greatly reduce their activity after giving birth.
During the first week after parturition, the amount of time spent active was around 3 hours on average, increasing the following weeks but reaching pre-birth levels only in the last days of presence in the natal den. This activity pattern is the same as those found by Durbin (1996b) and Taylor & Kruuk (1990) in Scotland.

- Female otters alter their daily movements after cubs are born.
In the case of Celta the movements changed after parturition, as compared with other life periods and with the mean of all studied females (see table 6.1). Night range and time active were (significantly) smaller, while the revisit index was (significantly) bigger due to the necessity to return every night to the same resting site (the natal den). These results agree with Durbin's (1996) only in revisit index data.

- Female otters diminished total range significantly after parturition. Celta's total range during the two months post-parturition period was 5 km, 6% of the total range for the tracking period (631 days). These results are coincident with Rosoux's (1995), who found that the post-parturition range of a female was 10% of the pre-parturition range.

7. Testing different survey and census methods for Eurasian otter

7.1. Introduction

Otters (*Lutrinae*) are aquatic and semi-aquatic animals whose populations have undergone marked declines during the last century as a result of persecution, the destruction of habitats, sensitivity to contamination and in the availability of their prey (Foster-Turley *et al.*, 1990). To ensure the successful management and conservation of otters, further studies are required. Determining the distribution of otter species and their abundance is an essential first step in this process. However, as otters live at low densities and are often nocturnal or crepuscular their study is not straightforward.

At the end of the 1970s and beginning of the 1980s, the first standardized method of survey was developed, based on reliable data.. The method was rapidly put into practice throughout Europe and the north of Africa (Crawford *et al.*,1979; Green & Green, 1980; Lenton *et al.*, 1980; Chapman & Chapman, 1982; Mason & Macdonald, 1986). It was first applied to the Eurasian otter (*Lutra lutra*), providing information on the western range of its distribution (see also Macdonald & Mason, 1994). The surveys were based on the identification of indirect but indisputable signs (mainly tracks and spraints) of the species. The sites were situated throughout a territory, running along 600 to 1000 m of riverbanks and waterways. The fact that otters leave spraints in visible spots (stones, rocks, trunks ,etc.) and in predictable places (under bridges, at junctions of rivers, in basins, etc.) facilitates survey work. In this way, it is possible to differentiate between positive and negative sites (presence detected or not) and to count the number of signs.

The method was quickly adopted and was expanded to include other species of otter, such as the southern river otter (*Lontra provocax*) (Chehébar, 1985), *Lutra maculicollis* and *Aonyx capensis* (Rowe-Rowe, 1992) and the smooth-coated otter (*Lutra perspicillata*) (S.A. Hussain, pers. comm..).

The initial enthusiasm led to an attempt at the exhaustive mapping of otter distribution and an estimate of relative abundance and habitat selection (Macdonald & Mason, 1983, 1985). There followed a debate between those who were in favour of using the method in this way (Jefferies, 1986; Macdonald & Mason, 1987: Mason & Macdonald, 1991) and those who questioned the validity and precision of the results obtained (Kruuk *et al.*, 1986; Conroy & French, 1987, 1991; Kruuk & Conroy, 1987), because of temporal, spatial and individual variation apparent in otter sprainting behaviour (see also Macdonald & Mason, 1987; Jahrl, 1995; Kruuk, 1995; Kranz, 1996; Ruiz-Olmo & Gosálbez, 1997). Some findings can be pointed out:

a) The number of spraints does not enable us to estimate easily the number of otters (although some findings tend to show that, on the whole, the more excrements that are found, the more otters there are in an area; Mason & Macdonald, 1993; Strachan & Jefferies, 1996).
(b) The absence of signs does not necessarily imply absence of otters. There are "false negatives", that can be a consequence of the ability of surveyors to locate them or of the otter behaviour.
(c) The number of signs in any one place does not necessarily correlate with the intensity of use, and therefore it does not seem to be a good method for studying habitat selection (Kruuk *et al.*, 1986; Kruuk, 1995).

(d) The otter surveys are differently applied in different countries, and must be designed to suit particular circumstances (Mason & Macdonald, 1986; O'Sullivan, 1993; Romanowski *et al.*, 1996; Romanowski & Brzezinski, 1997).

Recently, otter surveys have detected recolonization of some regions in Europe (Green & Green, 1987; Andrews *et al.*, 1993; Brzezinski *et al.*, 1996; Rosoux *et al.*, 1996; Strackan & Jefferies, 1996; Ruiz-Olmo & Delibes, 1998), but there remains a degree of uncertainty as to the significance of the positive and negative sites identified in otter surveys and as to how precise the findings are.

The difficulties encountered in establishing otter population density and estimating numbers by any technique are great. The techniques most frequently used are:

(a) Footprints in snow or mud, and measuring their lengths (Reid *et al.*, 1987; Sidorovich, 1991, 1992).
(b) Visual censuses, with groups of observers working in places where the otters are diurnal (Lejeune & Franks, 1990; Estes, 1991; Kruuk, 1995; Parera, 1996) or are crepuscular (Ruiz-Olmo, 1995).
(c) Holt censuses in marine environments in the north of Europe (Kruuk *et al.*, 1989).
(d) Intensity of use of certain stretches of river by counting the number of nights spent on them, as revealed by radio-tracking (Kruuk *et al.*, 1993).
(e) Marking of captured individuals with radio-isotopes (Kruuk *et al.*, 1980).
(f) DNA fingerprint studies of hair, faeces or other remains (being developed in otters in different countries such Great Britain, Spain, Denmark, etc.).

Techniques (b) and (c) are only useful in those places where otters display diurnal or crepuscular behaviour, (c) in marine environments, and (d) and (e) for focal areas. Methods (a) and (f), and in part (b) may have more general use. However, none of these has been shown to be entirely reliable: Are all the animals detected?; Are some repeat observations?; Is abundance correctly estimated?; Are the census units (generally 10km) correct ?

Here, we present data in order to evaluate the reliability of otter surveys and otter censuses by means of tracks and crepuscular observations.

7.2. Material and methods

7.2.1. Study areas

The results presented here are drawn from three programs (Fig. 7.1):

- The capture of wild otters was carried out in Extremadura, in SW Spain (Saavedra & Sargatal, 1998). Otters living in this area are included in the great Western and Central Spanish and Portuguese metapopulation (Ruiz-Olmo & Delibes, 1998; Trindade *et al.*, 1998), and their translocation as part of a reintroduction program to the Muga and Fluvià river basins in the north-east of Spain and control via radiotracking.

- Radiotracking of wild otters in the basin of the Bergantes river in the east of Spain as part of an ecological and behavioural study (Jiménez *et al.,* 1998; López-Martín *et al.*, 1998).
- A census program conducted in the two areas mentioned above, and in the rivers Noguera Ribagorçana and Noguera Pallaresa (Pyrenees) (Ruiz-Olmo, 1995*a*).

a) Muga and Fluvià River Basins.- These are found in the extreme NE of the Iberian Peninsula (Girona province). They are both short Mediterranean rivers (the principal branch of the river Muga measures 65 km, the Fluvià, 97 km), with irregular hydrological regimes. They are fed mainly by rainfall and have an average volume of 2.4 m^3/sec in the Muga and 1.3 m^3/sec in the Fluvià. Riverine woodland dominates with *Fraxinus angustifolia* and *Populus alba*, although in many stretches this has been substituted by plantations of *Populus nigra x canadensis* and *Platanus orientalis*. The Aiguamolls de l'Emporda marshes, which occupy more than 5,000 ha, are situated between the two river mouths. Here, the dominant habitats are fresh water canals and lagoons, where reeds (*Phragmites* sp.) and bulrushes (*Typha* sp.) grow in abundance.
b) Rivers Bergantes, Noguera Ribagorçana and Noguera Pallaresa.- River Bergantes is a tributary of the Guadalope, which in turn flows into the River Ebro. The principal watercourse is nearly 50 km long and it runs through an arid mountainous area. The volume in the lower part varies between 0.5 and 3 m^3/sec, and in the upper half there is almost no flow of water during the summer. Rivers Noguera Ribagorçana and Noguera Pallaresa lie in the Northern Ebro Basin in the Spanish Pyrenees, province of Lleida with 1-3 m^3/sec (in most of the study area) and 8-12 m^3/sec, respectively. The riverbed consists principally of mud and earth, stones and rocks, and contains many pools. In all three rivers, the vegetation on the banks (dominated by *Salix purpurea* with *Populus nigra*, alternating with reeds *Typho-Schoenoplectetum glauci*) is poor due to the effects of grazing. In the lower stretches of the Noguera Pallaresa the vegetation is however, exuberant, and mainly formed by white poplar *Populus alba, with P. nigra, Fraxinus angustifolia, Ulmus minor* and *Salix purpurea*, with sections of reeds (*Typho-Schoenoplectetum glauci* and *Phragmition australis*). In mid and higher stretches of this river, the vegetation, which is scarce, is *Alnus glutinosa*, with *Populus nigra* and *Salix purpurea.*

7.2.2. Otter surveys

The objective of the study was to compare radiotracking data in areas occupied by otters with the conventional results derived from an otter survey conducted in the same area. The otter survey methods are summarized in Mason & Macdonald (1986), Lenton *et al.* (1989) and Ruiz-Olmo & Delibes (1998).

In Spain, sites had been surveyed to determine only the presence (positive) or absence (negative) of otters, a survey being halted as soon as traces of the species were found (Ruiz-Olmo & Delibes, 1998). For the present study, a network of 83 sites was inspected in the Muga and Fluvià Basins. Sites (600 m long) were inspected on five occasions (spring 1996, summer 1996, winter 1996-97, spring 1997 and summer, 1997).

For comparison, data from the otter surveys carried out in 1984 by Ruiz-Olmo & Gosálbez (1987) (n = 30 sites), in 1989 by Ruiz-Olmo (1995*b*) (n = 34), and in 1993 by D. Saavedra (pers. comm.) (n = 144 sites) were used.

7.2.3. Radiotracking

During the first part of the reintroduction project, five otters were captured and released into the north-east of Girona between November 1995 and March 1996 and were radiotracked until September 1996. An attempt was made to radiolocate the otters daily from the ground, using four-wheel drive vehicles. When the signal was lost, small Cessna planes were used. Up until the end of summer 1997, when the present study was concluded, a further 21 otters had been captured, 14 being released in the reintroduction basins.

When captured, using padded leg hold traps, otters were anaesthetized with ketamine (0.05 mg/kg) and metedomidine (0.05 mg/kg) for evaluation in the field. Sex, length, and weigh were determined, and also potential wounds where inspected. With these data and antibiotic injection was calculated and administrated. Also the body condition index *K* (Kruuk *et al.*, 1987), calculated for Iberian otters (Ruiz-Olmo, *et al.*, 1998), was determinate, collecting only the otters with $K > 0.90$; the rest of animals were released on the capture site. Otters for reintroduction were injected of neuroleptics (Haloperidol and Trilafon) for the captivity period, and calmed in quiet rooms before the transport. After it, were reversal by Antisedans (0.05 ml/kg) and transported in a safe plastic box until the Barcelona Zoo (Barcelona), were otters were isolated in individual boxes. Otters were in the veterinary services for a two-four weeks period, being evaluated regularly and provided of transmitters (TELONICS, Mesa, Arizona, USA; ATS, Isanti, Minnesota, USA; and WAGENER, Köln, Germany), weighing between 36 and 40 g (0.5-1.6 % of the otters weigh), using the same anesthetics.

In the River Bergantes two more wild otters were captured, tagged and released, following the same protocol, the otters being transported to the Wildlife Recovery Centre of El Saler (Valencia).

Table 7.1 shows the otters radiotracked, the number of radiolocations and days when radiotracking was carried out.

A number of animals from both areas, whose transmitters emitted between the 1st June and 15th of July 1996 (the period in which the visual censuses were carried out) were located during 24 h radiotracking periods (at least one radiolocation every thirty minutes). Special attention was paid to observing the moment at which they abandoned and returned to the rest sites.

Table 7.1.- Radiotracked otters in the current study. (M): Males; (F): Females.

Area	Animal	Weight (kg)	Period	Nº of days of radio contact (until summer'97)	Cause of loss
Muga and Fluvià river basins (reintroduction)	F1	5.1	14.11.95 / 18.12.95	35	killed by fish net
	M1	3.8	01.12.95 / 09.07.97	588	lost signal
	M2	4.0	01.12.95 / 27.06.96	210	unknown
	F2	6.0	01.03.96 / 19.10.96	233	lost signal
	F3	4.0	21.03.96 / 20.08.97	519	lost signal
	F4	4.2	21.10.96 / 13.12.96	53	lost signal
	M3	7.6	21.10.96 / 25.07.97	279	lost signal
	F5	5.1	28.10.96 / 13.06.97	229	lost signal
	M4	6.7	04.11.96 / 19.05.97	198	lost signal
	M5	6.7	11.11.96 / 30.04.97	170	killed by car
	F6	4.7	11.11.96 / 17.12.96	37	unknown
	F7	4.8	02.12.96 / 25.01.97	55	killed by car
	F8 (*)	2.6	02.12.96 / 13.12.96	12	lost signal
	F9 (*)	2.3	02.12.96 / 10.12.96	9	lost signal
	M6	7.5	26.11.96 / 17.12.96	22	lost signal
	M7	7.6	10.12.96 / 11.12.96	2	killed by car
	M8	7.8	27.12.96 / 30.05.97	155	lost signal
	F10	4.8	27.12.96 / 13.01.97	18	found in siphon
	F11	5.2	08.05.97 / 13.06.97	38	lost signal
River Bergantes	M9	8.0	23.11.96/14.03.97	26	lost signal
	F12	4.4	22.12.96/3.05.97	110	killed by car
	M10	7.8	7.05.97/31.08.97	111	lost signal

(*) Daugthers of F7

7.2.4. Track and Visual Censuses

The visual censuses were carried out in accordance with the method described in Ruiz-Olmo (1995*a*), based on the positioning of 18-24 observers along a 9-12 km long river stretch, at a distance of 500 m (±100 m). Each observer was stationed in such a place as to allow them to observe the maximum length of the stretch and at some points, both banks. The censuses were carried out between the 1st June and the 15th July, coinciding with the period of maximum daylight, the time when otters present significant crepuscular activity in the study area. A census involves counts being carried out at dawn and dusk, lasting for some two hours each.

The track censuses were carried out following the method described in Sidorovich (1992). Several footprints were measured in each of the otter tracks found. The maximum length of the forefoot print was used (both, without nail and nail included) and the maximum length of the heel pad. Given the low densities of animals during the censuses distinguishing between individuals caused no difficulty. Recent footprints (less than one day old, sometimes two) were differentiated from old prints. To differentiate them, (a) a foot-print census was carried out before the visual census in some cases, (b) it was determined whether prints were in the new mud after the rain or on a wet morning, and (c) foot-prints were examined to determine whether they were dry or not. In the study area, in summer, foot-prints dry after one or two days, and it is, therefore, relatively simple to distinguish fresh prints.

Eight visual and track censuses were carried out (both in the same day) in Muga and Fluvià basins and in the Bergantes in 1997 and 1998, in stretches where the presence of at least one individual the preceding day had been detected via radiotracking. Furthermore, 16 visual and track censuses were carried out in the Noguera Ribagorçana and Noguera Pallaresa rivers (with no tagged otters at that point) between 1995 and 1998. In total, 24 censuses were carried out, a total of 602 individual vigils over 1067 h.

7.2.5. Statistics

Chi-square (X^2) for contingence tables was used to compare the number of positive and negative sites from the network of 83, after the otter surveys and radio-tracking findings (Siegel, 1956). We used the conditioned probability (Alonso *et al.*, 1979) to estimate the probability of finding otters in a number of surveyed sites (Fig. 7.5); for each new surveyed site the result of the previous sites was take in account.

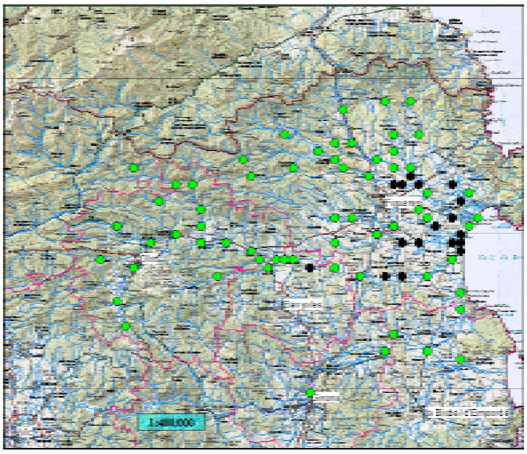
The results of the visual and track censuses were compared by means of a regression and Pearson correlation analysis resulting in a linear function as the best adjustment.

7.3. Results

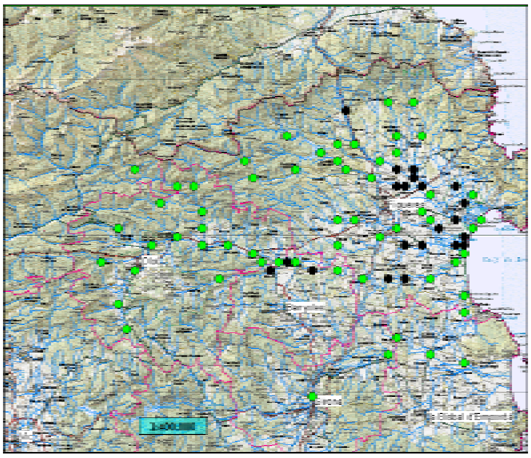
7.3.1. Distribution of Otters in Muga and Fluvià basins

Figures 7.2 and 7.3 show the increase in the percentage of positive sites in the Muga and Fluvià river basins, from 0 in the three previous otter surveys to the reintroduction of the otter in autumn 1995, to near 50% of the sites in summer 1997.

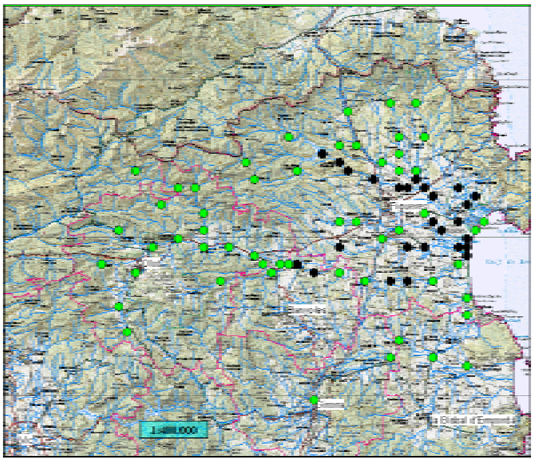
Figure 7.2. Results of the five otter surveys carried out between spring 1996 and summer 1997 in the reintroduction area of north-eastern Girona.



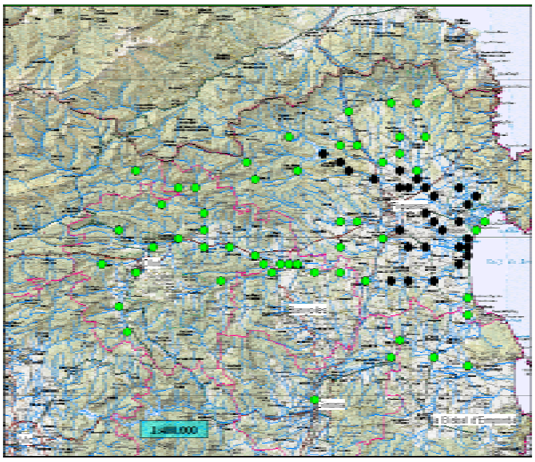
Spring 1996 *3 otters*



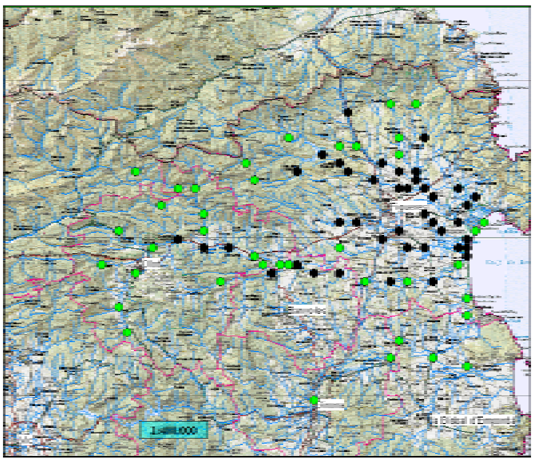
Summer 1996 *4 otters*



Winter 1996-97 *4 otters*



Spring 1997 *>9 otters*



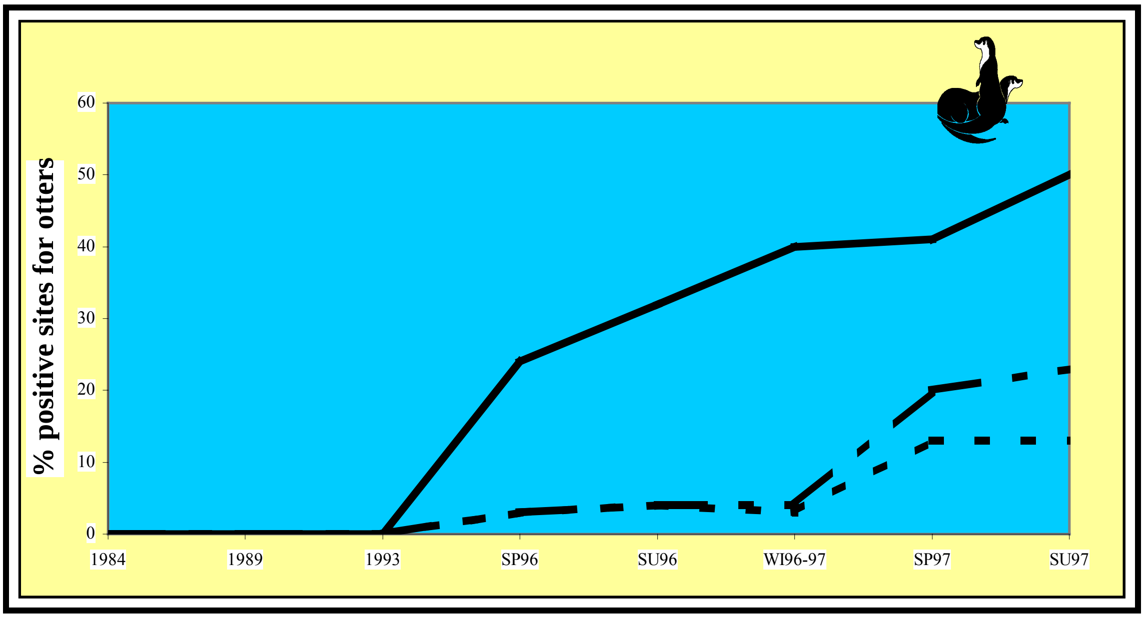
Summer 1997 *>9 otters*

● Black circles, positives sites (presence of otters).

● Green circles, negative sites.

Between November 1995 and March 1996 only five otters were released, one of which died shortly after release (drowned in a fyke net in December) while another died in June 1996. With only four otters surviving, 23.2% of the sites were positive in early 1996, occupying about 80 km of rivers and 940 ha of marshes. With a similar number of animals (four at the beginning) in winter 1996, the percentage rose to 39.0% (X^2 = 4.65; 1 d.F., p = 0.030) occupying 135 km and 940 ha. In spring 1997 remained at 39.2%, not statistically different from the previous period (X^2 = 0,0055; 1 dF; p = 0,955), despite the release of a further 19 individuals (with transmitters running).

Figure 7.3. Changes in the percentage of positive sites (presence of otter) (——) in the reintroduction area of north-eastern Girona. Minimum number of otters (......) and number estimated (▬ ▪ ▪) in the same area.



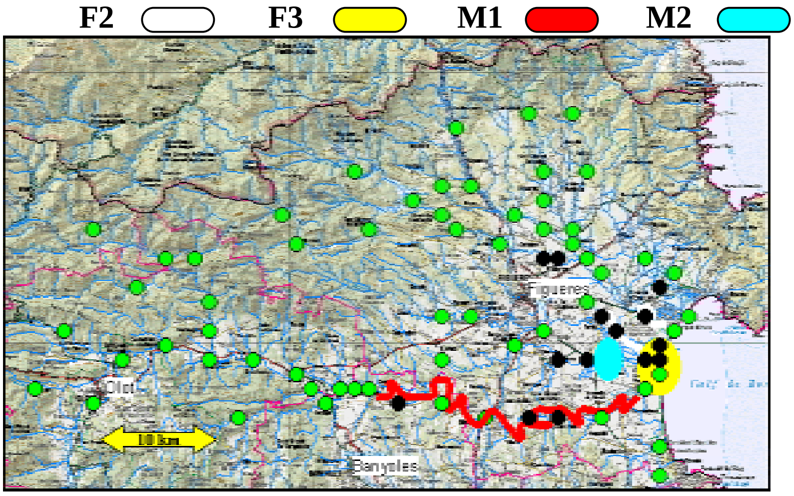
From April to May 1996 the four otters living in the whole reintroduction study area, each tended to use a different stream during a five-to six-month period (Table 7.2 and Figure 7.4), and were still tagged with their transmitters in full working order. The female F2 reproduced in July 1996 (with one single young) and the transmitter stopped functioning in October 1996. From this moment onwards the female and her young occupied a separate area from the rest of the otters, detected because, otter spraints and adult and cub footprints were found in a new area not used by the remaining two radiotracked otters.

In this way it was possible to determine the exact number of otters living in given stretches between April and September 1996. Subsequently, as the transmitters ceased to function and new otters were released in October 1996, it became impossible to know how many individuals were present in each area.

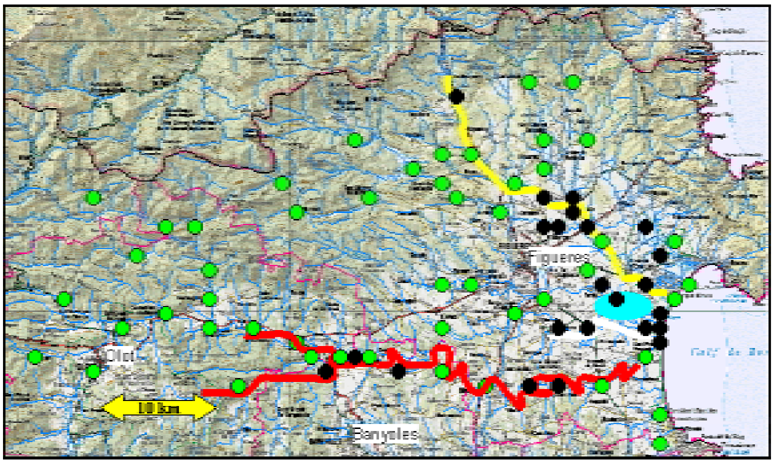
Table 7.2.- Rivers use of the first four released otters in the basins of the rivers Muga and Fluvià, the littoral marshes and other small rivers (see figure 7.1), during the first seven months. Otters living alone in a river or body mass are show in bold.

	March				*April*				*May*				*June*				*July- September*		
	F2	F3	M1	M2	F2	F3	M1	M2	F2	F3	M1	M2	F2	F3	M1	M2	F2	F3	M1
Muga-Mugueta-Llobregat										**X**				**X**				**X**	
Reserva Natural Integral 2	X	X		X	X	X		X				**X**							
Rec del Molí	**X**				**X**											**X**			
Rec Sirvent			**X**			**X**			**X**				**X**				**X**		
Fluvià						**X**					**X**				**X**				**X**

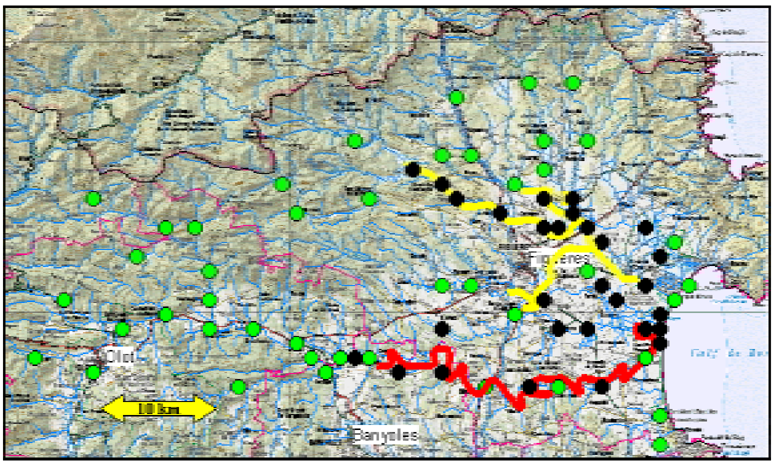
Figure 7.4. Changes in home ranges of the first reintroduced otters in north-eastern Girona, and position of the positive (black circles) and negative (green circles) sites during the otter surveys carried out in the same season.



Spring 1996



Summer 1996



Winter 1996-97

7.3.2. Comparison between otter survey and radiotracking data

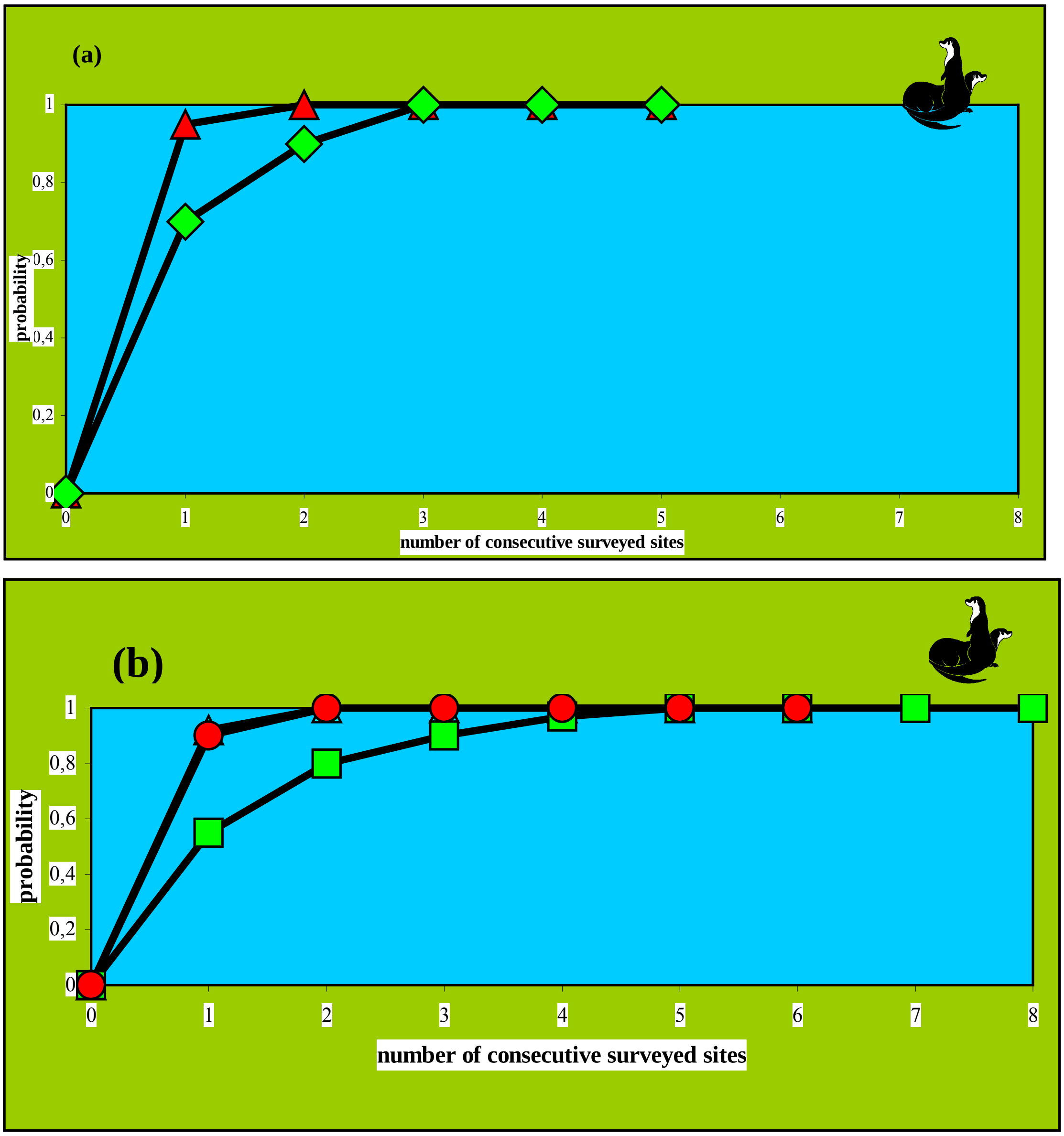
Comparison of the home ranges of tagged otters with the results from the otter surveys carried out in the same areas (Fig. 4) showed very similar distributions ($X^2 = 110.9$; 1 dF; $p = 0.0001$), but were not exact; otter signs (footprints and spraints) were found in sites outside the home ranges as estimated by radiotracking: 37.5% of the sites proved positive (n = 144). The fact that otters were not found on all the days can explain this finding. Similarly, 24.5% of survey sites, which proved negative for otters, were inside the home ranges of otters (n = 119).

70.9% of the sites that were examined in which only one otter lived were positive (n = 86), 100% in those where two otters existed (n = 19) and 92.3% in those where there were three or more otters (n = 13). Taken together, those sites examined in areas with two or more otters turned out to be positive on 96.9% occasions (n = 32), a significant difference with those in which there was only one otter ($X^2 = 3.51$; 1dF; $p = 0.006$).

No significant differences were found between young and older otters. However, the differences were significant between medium-size watercourses (15-40 m average width) and small watercourses (5-15 m) ($X^2 = 52.51$; 1 dF; $p < 0.0001$), and between medium watercourses and lakes and marshes ($X^2 = 6.63$; 1dF; $p = 0.010$). The lowest values were recorded in the medium rivers where the otters were detected in 55.5% of the inspected sites inside the home ranges (n = 54). However, in the small rivers (93.3% of positive sites; n = 106) and lakes and marshes (90.5%; n = 21) the differences were not significant.

Our results show that the survey of two or three sites is sufficient to find otters with an efficiency of 100% in most of cases (figure 7.5); only in medium rivers a total of seven sites need be surveyed to find the otter with 100% of certainty (however, with three sites otters will be detected in 92% of cases, and with four in 97%).

Figure 7.5. Conditioned probability of finding otters in consecutive surveyed sites in the same stream: (a) comparing rivers with a single otter (triangle) or with two or more otters living together (squares); (b) comparing small size (<15 m wide) rivers (triangles), medium size (15-40 m wide) rivers (squares) and lakes and marshes (circles).



The length of watercourse occupied by otters released during the first part of the reintroduction project (discounting the marshes which are measured in units of area) were 3 km in 35 days (F1), 90 km in 588 days (M1), 3 km in 210 days (M2), 35.5 km in 233 days (F2) and 64 km in 519 days (F3). In all the cases, otters left spraints and prints over large distances (dozens of km), enabling easy detection of the species.

The average number of spraints per latrine (1.25 - 2.5) did not vary significantly throughout the five surveyed periods (Mann-Witney U; $p > 0.05$).

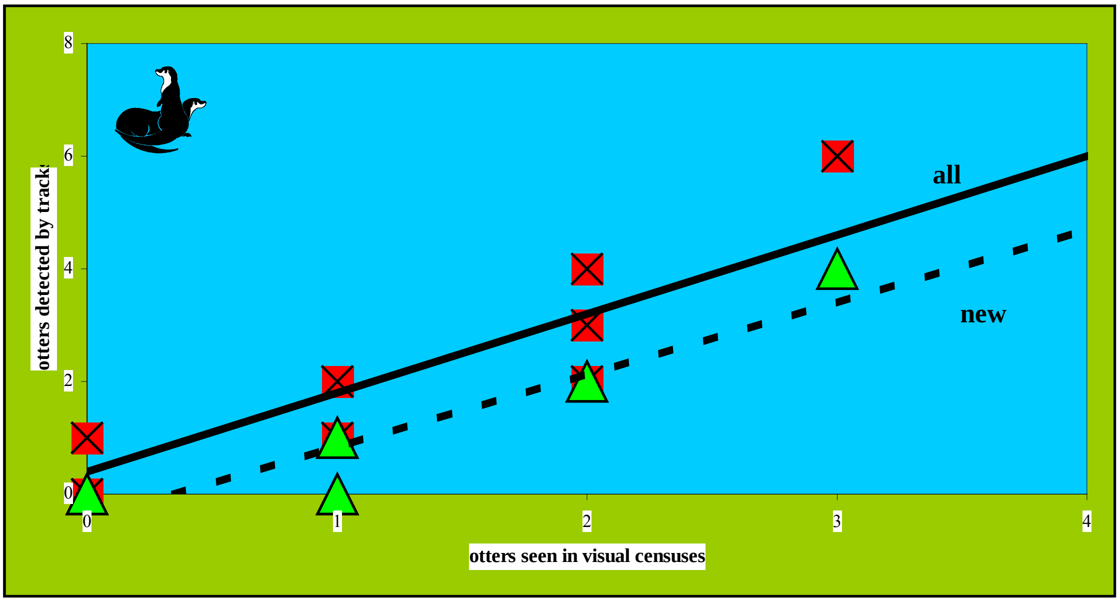
7.3.3. Detection of the Otters During the Visual Censuses.

All the otters tagged with transmitters and present in the stretches where censuses were carried out (n = 7) were seen during the standard visual censuses. The otters were active outside the rest sites during the standard otter visual census period in 76.2% of cases during dawn (n = 21) and 95.5% during dusk (n = 22). In a standard census which includes consecutive dawn and dusk watches, 100% of the otters would have been seen if they had been in the field of vision of a census taker.

7.3.4. Comparison of Visual and Footprint Censuses

Figure 7.6 shows that a high correlation exists between the number of otters seen during the visual censuses and the number of otters detected by means of the lengths of their footprints. If only recent footprints are used (those from the previous night) a virtually identical density is obtained (otters/km surveyed). If all otter foot-prints are used (old and new) the number of otters seen is overestimated by approximately twice. In a few cases otters were not seen but were detected by their footprints became an individual was not active that day, or appeared later and returned before daybreak, or was simply not spotted by an observer. However, other otters were sometimes seen and no detected by their prints. The average value of the censuses carried out using both methods (when using only recent prints) produced very similar results.

Figure 7.6. Linear function relationship and correlation between the numbers of otters detected by new tracks (1 or 2 days old)(■) and all tracks (new and old) (▲), and the number of otters seen during visual censuses in the same stretches and day (n=16).
y=1.27x – 0.348, r=0.921, n=16, P<0.001 for new tracks.
y=1.39x + 0.44; r=0.863, n=16, P<0.001.



7.4. Discussion

7.4.1. Usefulness of the Otter Surveys

In the introduction, the use of otter surveys and their limitations was discussed. Until now, we have not known how to interpret indirect signs indicating the presence or absence of otters. The reintroduction program of the Eurasian otter to NE Spain has enabled us to study an artificially designed population. During the early months we knew the exact number of otters constituting the population and the number of otters which lived in the different waterbodies. One of the most striking results is that in waterways of small or average width a single otter is capable of marking many km so that it can be detected in most of the sites (71% of sites monitored by conventional surveys). Thus, if one site is negative, it is highly probable that the otter will be detected at the next site several km away, if the area actually is occupied by this individual. The survey of several consecutive sites without otters must be interpreted to mean that they are absent in the area. This result is of special importance on the margins of the species' distribution, where a single individual can be easily detected in such waterways. An example is provided by the decrease in number of otter signs with altitude (Green & Green, 1980; Chapman & Chapman, 1982; Ruiz-Olmo & Delibes, 1998), which is actually attributable to a decrease in the number of individuals (Ruiz-Olmo, 1998). This means that the non-detection of otters in one area during a period of time suggests they are absent altogether.

Frequently we talk about isolated otters in small mountain streams or in areas far away from human activity. Otters have large home ranges, normally consisting of 5-100 km and will often move up to 20 km a day (Green *et al.*, 1984; Jefferies *et al.*, 1986; Kranz, 1995; Kruuk, 1995; Ruiz-Olmo *et al.*, 1995; Jiménez *et al.*, 1998). A single otter is therefore capable of marking many km. Thus the traditional image of the single otter (or "a pair") living in a short stretch (often in hills or mountains), and difficult to locate, is not realistic.

The results for wetlands are similar. However, in large rivers of widths greater than 20 m, the rate of detection decreases. Even so, we found signs of otters in approximately half of the sites along a river 15-40 m wide. In large rivers, otter surveys in areas with only one otter are less efficient.

Larger rivers, nevertheless, provide a wider habitat, and greater availability of places for depositing spraints. Thus, it seems that an increase in the density or the number of otters in these big rivers would lead to greater detection. Our results tend to confirm this. In the stretches in which two or three otters are present, the probability of detecting the otter in a conventional surveyed site is nearly one. Two or three animals alone are capable of providing sufficient signs for otters to be found in almost all the places that they occupy. The densities of otters in freshwaters are frequently low, about 0.05 - 0.6 otters/km (Ruiz-Olmo, 1995*b*; Sidorovich, 1991, 1992). In other words, the presence of between one and four otters in one fluvial stretch can be considered standard, and otters must be detected after very few surveyed sites.

A further factor needs to be considered: the otters' use of the stretches. Kruuk *et al.* (1993) demonstrated a differential use according to food availability while López-Martín *et al.* (1998) the importance of availability of food, river pools and cover. Our results refer to sedentary otters occupying one stretch for months. However, there are otters which do not have clearly defined home ranges, or which use determined stretches only for dispersion. The fact that otters were not detected in a given area does not imply they were absent from that area. One of our animals was detected mainly in those sites which constituted its core area. The sites in the Fluvià river in which it was not detected actually corresponded to stretches which it used very little and then only for moving between core areas.

Areas of frequent use and sedentary presence must be distinguished from others in which otters move quickly over large distances across unsuitable watercourses, without food, water or cover, until they reach the next core area, frequently coinciding with an area of good quality habitat (Jiménez *et al.*, 1998; López-Martín *et al.,* 1998). These areas of sporadic presence where, for example an otter which has been run over might sometimes be found, far from the habitual areas of detection, are not well defined by the otter surveys. On the other hand, areas with stable otter populations tend to be detected with greater frequency. Thus, if an isolated individual, wandering or a newcomer to an area, leaves a spraint in an atypical place as it passes through one day, it should be interpreted with caution before further data can be found. If the report is not repeated, the diagnosis is then clear: the otter is just moving.

Four otters, those released during the first autumn-winter, were detected at 40% of the surveyed sites in the basins where they were reintroduced, along 80 km of fluvial basin and some 940 ha of wetlands. However, in summer 1997, when a total of 19 otters has been reintroduced, and with a minimum of nine individuals remaining in the population (eleven estimated by tracks), failed to increase notably the percentage of positive sites and the length of the occupied basin (135 km and 940 ha of wetlands). This indicates that a linear relationship does not exist between the number of otters and the number of positive sites, since a few individuals spread their signs over large areas and those which are being released tend to occupy the same areas, presumably those that offer the most favourable conditions. Ruiz-Olmo (1995*b*) reported that in the recolonization of a Pyrenean basin, the otters initiated this process in those stretches with the greatest availability of food and lowest altitude.

The comparison of the rate of increase in positive sites found in our study area, and those from other studies from Britain (Mason & Macdonald, 1993; Strachan & Jefferies, 1997) and Spain (Ruiz-Olmo & Delibes, 1998), show some interesting findings. When a river is recolonized by otters (both, naturally or after reintroduction), the presence of the species in most of waterbodies of the basin is reached in very few years, often 1-4, explained by their great individual home ranges.

7.4.2. Estimation of Abundance

It is not easy to establish how many otters live in one specific sector of a habitat. The large distances covered, the use of home ranges according to the availability of food over time, the otters' particular social structure and their chiefly linear habitat (Kruuk, 1995), make any decisions concerning the area or length of the census, the best time to carry out the census and the otters that should be counted, particularly difficult to make. For this reason, use of the time spent by the otter (number of nights radiotracking, Kruuk *et al.*, 1993; number of radiolocations, López-Martín *et al.*, 1998) is certainly more precise and in closer agreement with the ecological reality of the species. But, this involves methods that are difficult to apply in management and which eventually tell us very little about the numbers and density of otter populations.

We found that when there were several otters in an area, a greater number of otter signs were detected. Thus, the number of spraints could provide a rough idea of otter abundance. However, in the NE Spain, Ruiz-Olmo & Gosálbez (1997) found that the number of spraints per latrine was mostly between 1 and 3, which is consistent with the data recorded in the present study for only one otter living at each stretch. Another parameter to be considered is the density of signs (not quantified in our study), which according to the study cited above is narrowly correlated with the number of latrines. Therefore, our results, which require further confirmation, indicate that even if the greater the presence of otters, the greater the number of latrines and the greater the number of spraints, there is a rapid saturation of spraints, without linearity.

Most studies consider 10-km long stretches when conducting otter censuses (Ruiz-Olmo, 1991; Sidorovich, 1991, 1992). In *Lontra canadensis*, studies were carried out on stretches greater than 100 km (Reid *et al.*, 1987). Considering an otter's need for space, these 10 km stretches appear to be short, since a single otter can cover more than 20 km a day and even a 100 km on some days. Thus, foot-prints can be found in many 10 km stretches. In line with our results, if we count all these stretches, with foot-prints more than a few days old, we might count up to ten otters when only a single otter is living in an area. In contrast if we only consider one day-old tracks, or the otters seen in one activity period (with daily movements on average of 4-7 km, range 0-20; Green *et al.*, 1984; Jefferies *et al.*, 1986; Ruiz-Olmo *et al.*, 1995; Dülfer *et al.*, 1998; Jiménez *et al.*, 1998; in *Lontra canadensis* with a maximum of 42 km in a single night, Melquist & Hornocker 1983), the use of 10 km stretches as a census unit seems to be sufficient, but perhaps 20 or 30 km stretches may bring us more accurate results. It must be assumed that the same otter might be counted in two units, but also that a resident otter can wander outside this unit. The estimated density is similar to the results of the census. Precision can be improved by consecutive census and the subsequent calculation of mean and standard error. The fact that both recent print and visual censuses give similar results (and show a high level of correlation) gives greater consistency to the results. However, the data obtained by radiotracking also indicate that practically all the otters can be seen by experienced observers, which confirms the validity of the visual censuses. Ruiz-Olmo (1995*b*) demonstrates that significant differences exist in the capacity to see otters during censuses, with the possibility of observation almost doubling with an experienced census taker. The visual censuses also allow us to apply different indices to the estimates of the abundance (otters observed/km, otters/ha, otters observed/hour, etc.), all of which are highly correlated

(Ruiz-Olmo, 1995*a*). Indices which use the number of observations per unit of time constitute an interesting form of estimation of the use of the different stretches (see the index proposed by Kruuk *et al.*, 1993).

In conclusion, we can affirm that fresh foot-print and visual censuses (at least in our study area) provide information which is very close to the real number of otters. Several studies using visual censuses conducted in the center of Spain (Bravo *et al* , 1998) and in the Czech Republic (R. Dülfer, pers. com.) present good results, though they have always to be used in the standard form by experienced census takers, according to established periods and procedures.

However, in those areas or periods of high otter density (>0.6 otters/km) we run the risk of underestimating the real number of individuals if we use foot-print censuses, for where there are two individuals of the same sex and similar in size, they may be confused. This will not normally occur with visual censuses. In contrast, in areas of low otter density (<0.1/km) individuals are not easily detected in visual censuses, because they can go outside the census stretch.

8. Modeling the viability of the reintroduced otter population

8.1. Introduction

Population viability analysis (PVA) is a procedure for estimating the viability of small populations of animals and plants (Shaffer, 1990; Lacy & Clark, 1993). This analysis uses mathematical models to simulate the demographic behaviour and the persistence of a population in a given period (Lacy & Clark, 1990). The predictions of these models in quantitative terms have been considered unreliable due to the poor quality of demographic data used, difficulties in estimating variance in demographic rates and lack of information on dispersal (Caughley, 1994; Beissinger & Westphal, 1998). In fact, most of the published PVAs come from intensively studied populations(Lacy & Clark, 1993; Lindenmayer & Lacy, 1995; Bustamante, 1996; Gaona et al., 1998; Naves et al., 1999). However, PVAs could become a very useful tool if the analysis is made in relative terms, that is, to identify the processes that threaten a population and to help in the optimization of management decision making (Lindenmayer et al., 1993; Bustamante, 1996; Beissinger & Westphal, 1998).

Current guidelines for reintroductions (UICN, 1995) state that population should be modeled in order to assist in the assessment of project viability (de Jong et al., 1997; van Ewik et al., 1997; South et al., 2000) and afterwards, in the study of long-term adaptation by the population (Bustamante, 1996; Novellie et al., 1996).

During the viability study of *GORP* (Saavedra, 1995) the possibility of undertaking a PVA prior to reintroduction was not considered. Instead, this technique has been used to provide a prediction of long-term adaptation.

Although we work with a “designed” population, in which the number of founder animals is perfectly known, the demographic pattern is not well known, due to the limitation of monitoring methods (i.e. radiotracking) and periods in respect to the life cycle of the species. Thus, data from the reintroduced population have been mixed with other data coming from other intensively studied Catalan populations where PVAs were used in order to determine the viability of isolated wild populations (Ruiz-Olmo, 1995), or from PVAs done to assess the viability of future otter reintroduction in The Netherlands (van Ewijk et al., 1997; Klop et al., 1998; F.J.J. Niewold, unpublished data). This study is a case, as mentioned above, where poor data invalidate the results in quantitative terms, but where data can be used in qualitative terms to test the main threats to the new population, using the PVA as a sensitivity analysis (Gaona et al., 1998; Jiménez, 2000).

In this way, the PVA has been applied with the objective of determining which parameters could have a major impact on the survival of the reintroduced population and thus to establish priorities in the present and future management of the population.

8.2. The Population Viability Analysis model

The program VORTEX (Lacy, 1993) version 8 (Miller & Lacy, 1999) was selected, because it is powerful, user friendly, easily available, tried and tested and continuously updated. VORTEX models the population processes as discrete and sequential events, with probabilistic outcomes. The program simulates birth and death processes and the transmission of genes through the generations by generating random numbers to determine whether each animal lives or dies and whether each female produces litter of each size during each year (Lacy & Clark, 1993). Thus VORTEX simulates the survival and reproduction of every individual of the population through successive years using the Monte Carlo method, including the effects of deterministic and stochastic (environmental, demographic and genetic) forces in the history of the population (Lacy, 1993). It is especially designed for small populations of vertebrates.

As discussed above, the information on the parameters included in the PVA did not come mainly from the reintroduced population, but from other Mediterranean populations studied for a long period near our study area (Ruiz-Olmo, 1994; 1995; in press), from the PVAs carried out prior to an otter reintroduction in The Netherlands (van Ewijk et al., 1997; Klop et al., 1998; F.J.J. Niewold, unpublished data) and other sources of general data for the species (Mason & Macdonald, 1986; Kruuk, 1995; Ruiz-Olmo & Delibes, 1998; Ruiz-Olmo, 2001). Parameters included in the VORTEX basic model are summarized in table 8.1 and explained later.

The projection time was 100 years, although shorter-time periods for making the projections have been recommended, in order to minimize error propagation (Bessinger and Westphal, 1998). Each set of parameters was repeated 100 times. One single population was considered as all the reintroduced otters released in places without barriers for otters in between, as could be checked with the aid of telemetry studies.

Many authors have warned about the effect of small population size on the loss of genetic variability (Soulé, 1986, 1987), which could produce inbreeding depression. The first mithochondrial DNA studies on Iberian otters showed low genetic variation (Pérez et al., 2001) but cytogenetic studies on the reintroduced population found no G-band pattern differences between individuals or population origin, no numerical or structural chromosomal anomalies and no chromosomal reorganization that could decrease the viability of the descendants or the fitness of the reintroduced population (Garrabou et al., in press). Thus, inbreeding depression was not incorporated in any scenario, and it was assumed that this factor does not affect the population.

The fluctuation of birth and death rates, known as environmental variation, (EV) is one of the most difficult parameters to obtain, because it is necessary to make demographic measurements over many years to sample the range of this variation. The use of data from short-term studies usually underestimates EV (Bessinger and Westphal, 1998). Assuming that the reintroduced population is at first well below its carrying capacity and that otter food and habitat requirements are wide enough to accept this annual environmental variation as “normal”, arbitrary and low values are used. This will not occur with bigger changes, but these are what we will call catastrophes.

We considered that environmental variation was concordant with reproduction and mortality (good years in reproduction are good years in survival as well) (Ruiz-Olmo, in press).

The Eurasian otter is a polygamous species. The age of first reproduction is 2 or more years (Mason & MacDonald, 1986). The maximum breeding age is 12 years (Ruiz-Olmo, 1995). Only 50% of males are included in the reproductive pool, reflecting the effect of male competition for females (Ruiz-Olmo, in press).

For the basic scenario (see table 8.1) the mean litter size was assumed to be 2.5 cubs per female (with a percentage of occurrence of 20%, 30%, 30% and 20% for 1,2,3 and 4 cubs per female, respectively) corresponding to a population in expansion (Ruiz-Olmo, in press). The "sex-ratio" at birth was assumed to be 1:1 (Ruiz-Olmo, 1995). Reproduction was assumed to be density independent during the first years due to the fact that otters were released in a high food availability area. Only 75% of the females breed each year. Mortality was divided into age categories, following the survival curve in Iberian otters developed by Ruiz-Olmo (1995). The only catastrophe modeled (a severe drought) had a probability of occurrence of 5% each year of the simulation and was assumed to kill 5% of the animals and to reduce reproduction by 50% during that year. Finally, we modeled the reintroduction process by initiating it with only the first 3 otters translocated in 1995 and supplementing them over 5 years with the real number of individuals of different ages and sexes released each year, until the total of 41 otters released was reached. This approach looks more realistic than initiating the analysis with a population of 41 individuals.

Table 8.1. Summary of parameter values used in Vortex basic (baseline) model for reintroduced otter population.

Simulation repeated 100 times.
Simulation run 100 years.
Extinction defined as only one sex left.
One population included in the model.
Inbreeding depression not incorporated.
Environmental variation (EV) concordant in reproduction and mortality.
Carrying capacity (k): 300 ind. (SD due to k EV = 10).
Breeding system: polygamous.
Percent of the adult males in breeding pool: 50%.
Breeding age in females: 2 years.
Breeding age in males: 2 years.
Maximum breeding age: 12 years.
Sex ratio at birth: 0.5
Maximum number of young per year: 4.
Reproduction assumed as density independent.
Adult females breeding each year: 75% (SD=10).

Distribution of reproductive success

% of breeding females producing:

1 offspring	20%
2 offspring	30%
3 offspring	30%
4 offspring	20%

Annual mortalities of females (% and SD due to EV):

0-1 yr old	31.7% (1.72)
1-2 yr old	22.0% (2.20)
>2 yr old (adults)	17.2% (4.63)

Annual mortalities of males (% and SD due to EV):

0-1 yr old	31.7% (3.17)
1-2 yr old	22.0% (2.2)
>2 yr old (adults)	17.2% (1.72)

Catastrophes

One type of catastrophe modeled (severe drought).
Probability: 5%.
Severity with respect to reproduction: 0.50
Severity with respect to survival: 0.95

Age distribution

Initial population size: 3 ind. (1 females age 1 and 2 males age 1).
Supplementation:
First year of supplementation: 1
Last year of supplementation: 5
Number of animals supplemented per year:

Age/Sex	F	M
1	**3**	**1**
2	**2**	**2**

8.3. The sensitivity analysis

Starting from the basic model and making variations in some of the parameters, other scenarios were established to try to identify the effect of these parameters on the persistence of the reintroduced population. A total of 48 different scenarios were simulated, as summarised in tables 8.2 and 8.3.

Table 8.2. Scenarios analyzed for reintroduced otter population including variations in some chosen parameters.

Scenario	Conditions
1	**Baseline (table 8.1)**
2	As 1, but mortality rates following Ewijk et al. (1997).
3	As 1, but mortality rates following reintroduction results (see below).
4-6	As 1- 3, but catastrophes = 10%; 50% reproduction and 50% survival.
7-12	As 1 - 6, but carrying capacity, k = 600.
13-24	As 1 - 12, but supplementing the same number of otters in only 2 years.
25-48	As 1 - 24, but a % of occurrence of 44.4/44.4/5.6/5.6 for 1,2,3,4 cubs per female.

Each scenario was repeated 100 times.
The parameters and variation are justified as follows:

*** Catastrophes:** The occurrence of more severe and recurrent droughts is simulated. This is an improbable but possible scenario that could happen in the north Mediterranean as a consequence of climate change. In the model, the corresponding probability has been assumed as 10%, affecting 50% of reproduction and 50% of individuals of the population.

*** Mortality:** Two different scenarios are simulated with the mortality rates proposed by Ewijk et al. (1997) and a pattern calculated after the mortality rates found in the reintroduction project itself.

a/ Ewijk et al. (1997)

EW	Females	Males
Age 0-1	31.5%	31.5%
Age 1-2	22%	33%
Adults (>2)	27.5%	33.6%

b/ Saavedra (this study)
Mortality rates were calculated using the observed mortality of reintroduced otters on a yearly basis for every age class. Differences in mortality between sexes were corrected as follows: Male Age 0-1 = Female Age 0-1, Male Age 1-2 = Female Age 1-2 x 1.5 and Male Adult = Female Adult x 1,22 (data from van Ewijk et al., 1997 and Niewold, unpublished data). Further, all results were increased by 20% to compensate for hidden mortality not detected by radiotracking.

SA	Females	Males
Age 0-1	26.4%	26.4%
Age 1-2	25.2%	37.8%
Adults (>2)	26.4%	32.2%

* **Carrying capacity:** The carrying capacity used in the basic model (k=300) is the capacity calculated in the viability study for the Muga and Fluvià basins (Saavedra, 1995), the original reintroduction area. Since otters have colonized two other basins (Ter and Tec) and there is much more habitat available than expected in the study, we tested the use of a much bigger carrying capacity, up to 600 individuals.

* **Supplementation:** The pace in otter translocation was slower than wished due to administrative problems. A scenario where it would be possible to supplement the same number of otters in only 2 years was simulated.

* **Reproductive success:** Although at the beginning the population will colonize an empty area with great carrying capacity, other factors (e.g. pollution) could have an impact by decreasing reproductive success. Thus, more conservative data are used, obtained from breeding and group observations in Northern Spain (Ruiz-Olmo, 1994). A mean litter size of 1.72 cubs per female is assumed, with a percentage of occurrence of 44.4%, 44.4%, 5.6% and 5.6% for litters of 1,2,3 and 4, respectively.

Table 8.3. Parameters changed for each scenario.
(BAS = mortality rates used in basic scenario, following Ruiz-Olmo (1995), EWI = van Ewijk et al. (1997) and SAA = mortality rates adapted from reintroduction data)

Scenario	Mortality	Catastrophe	K	Supplementation	Reproduction
1	BAS	5%(50-5)	300	5	20/30/30/20
2	EWI	5%(50-5)	300	5	20/30/30/20
3	SAA	5%(50-5)	300	5	20/30/30/20
4	BAS	10%(50-50)	300	5	20/30/30/20
5	EWI	10%(50-50)	300	5	20/30/30/20
6	SAA	10%(50-50)	300	5	20/30/30/20
7	BAS	5%(50-5)	600	5	20/30/30/20
8	EWI	5%(50-5)	600	5	20/30/30/20
9	SAA	5%(50-5)	600	5	20/30/30/20
10	BAS	10%(50-50)	600	5	20/30/30/20
11	EWI	10%(50-50)	600	5	20/30/30/20
12	SAA	10%(50-50)	600	5	20/30/30/20
13	BAS	5%(50-5)	300	2	20/30/30/20
14	EWI	5%(50-5)	300	2	20/30/30/20
15	SAA	5%(50-5)	300	2	20/30/30/20
16	BAS	10%(50-50)	300	2	20/30/30/20
17	EWI	10%(50-50)	300	2	20/30/30/20
18	SAA	10%(50-50)	300	2	20/30/30/20
19	BAS	5%(50-5)	600	2	20/30/30/20
20	EWI	5%(50-5)	600	2	20/30/30/20
21	SAA	5%(50-5)	600	2	20/30/30/20
22	BAS	10%(50-50)	600	2	20/30/30/20
23	EWI	10%(50-50)	600	2	20/30/30/20
24	SAA	10%(50-50)	600	2	20/30/30/20
25	BAS	5%(50-5)	300	5	44.4/44.4/5.6/5.6
26	EWI	5%(50-5)	300	5	44.4/44.4/5.6/5.6
27	SAA	5%(50-5)	300	5	44.4/44.4/5.6/5.6
28	BAS	10%(50-50)	300	5	44.4/44.4/5.6/5.6
29	EWI	10%(50-50)	300	5	44.4/44.4/5.6/5.6
30	SAA	10%(50-50)	300	5	44.4/44.4/5.6/5.6
31	BAS	5%(50-5)	600	5	44.4/44.4/5.6/5.6
32	EWI	5%(50-5)	600	5	44.4/44.4/5.6/5.6
33	SAA	5%(50-5)	600	5	44.4/44.4/5.6/5.6
34	BAS	10%(50-50)	600	5	44.4/44.4/5.6/5.6
35	EWI	10%(50-50)	600	5	44.4/44.4/5.6/5.6
36	SAA	10%(50-50)	600	5	44.4/44.4/5.6/5.6
37	BAS	5%(50-5)	300	2	44.4/44.4/5.6/5.6
38	EWI	5%(50-5)	300	2	44.4/44.4/5.6/5.6
39	SAA	5%(50-5)	300	2	44.4/44.4/5.6/5.6
40	BAS	10%(50-50)	300	2	44.4/44.4/5.6/5.6
41	EWI	10%(50-50)	300	2	44.4/44.4/5.6/5.6
42	SAA	10%(50-50)	300	2	44.4/44.4/5.6/5.6
43	BAS	5%(50-5)	600	2	44.4/44.4/5.6/5.6
44	EWI	5%(50-5)	600	2	44.4/44.4/5.6/5.6
45	SAA	5%(50-5)	600	2	44.4/44.4/5.6/5.6
46	BAS	10%(50-50)	600	2	44.4/44.4/5.6/5.6
47	EWI	10%(50-50)	600	2	44.4/44.4/5.6/5.6
48	SAA	10%(50-50)	600	2	44.4/44.4/5.6/5.6

8.4. Comparison with other population estimations

In order to compare Vortex simulations of the number of individuals with other estimations obtained in this thesis through other methods, the basic model (table 8.1) was run, but only over 10 years, with reports every two years. The estimated mean population size was represented in a graphic where we also added the estimated population obtained from the multiplication of density found during the visual censuses of 1998 and 2000 (see 4.3.3) by the number of kilometers of rivers and hectares of wetlands (assuming 10 ha = 1 km) occupied by the reintroduced population (Saavedra & Sargatal, 1998) during those years.

8.5. Results

Results of 48 simulations with the parameters calculated by Vortex program are shown in table 8.4.

Table 8.4. Summary results of the 24 different scenarios.

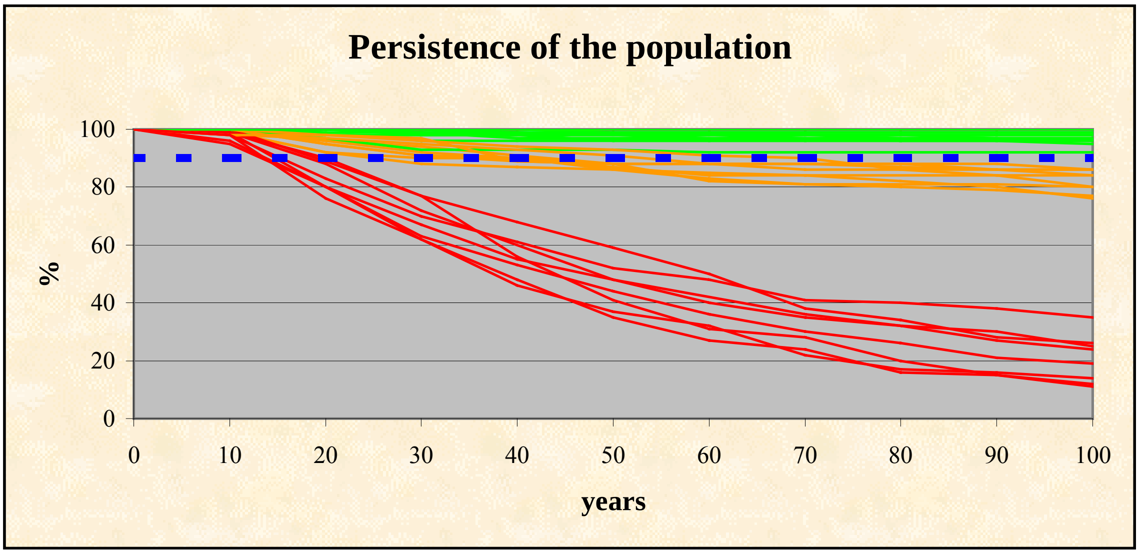
SCE	det.r	stoc.r	SD(r)	PE	N-extant	SD(Next)	N-all	SD(Nall)	Het	SD(Het)	Med.TE	MeanTE
1	0.197	0.217	0.158	0.000	299.08	13.22	299.08	13.22	0.8794	0.0345	0	0.0
2	0.135	0.153	0.179	0.000	293.48	15.07	293.48	15.07	0.8545	0.0400	0	0.0
3	0.150	0.171	0.174	0.000	296.84	12.27	296.84	12.27	0.8479	0.0496	0	0.0
4	0.142	0.147	0.307	0.010	249.18	77.95	246.69	81.46	0.8347	0.0647	0	18.0
5	0.079	0.085	0.331	0.140	206.23	93.71	177.39	112.70	0.7076	0.1732	0	39.7
6	0.094	0.096	0.327	0.050	225.21	90.30	213.96	100.85	0.7247	0.1453	0	38.6
7	0.197	0.217	0.154	0.000	600.14	13.73	600.14	13.73	0.9204	0.0185	0	0.0
8	0.135	0.154	0.175	0.000	590.37	32.44	590.37	32.44	0.8887	0.0307	0	0.0
9	0.150	0.168	0.171	0.000	592.43	23.46	592.43	23.46	0.8942	0.0298	0	0.0
10	0.142	0.144	0.308	0.020	517.80	134.56	507.44	151.82	0.8747	0.0588	0	20.5
11	0.079	0.081	0.333	0.160	404.89	193.89	340.16	231.82	0.7610	0.1747	0	40.3
12	0.094	0.092	0.336	0.080	400.16	205.11	368.15	224.89	0.7822	0.1402	0	28.0
13	0.197	0.218	0.212	0.000	298.69	11.37	298.69	11.37	0.8852	0.0254	0	0.0
14	0.135	0.156	0.230	0.000	292.91	14.49	292.91	14.49	0.8516	0.0489	0	0.0
15	0.150	0.170	0.227	0.000	296.57	13.87	296.57	13.87	0.8494	0.0471	0	0.0
16	0.142	0.144	0.343	0.030	253.46	77.07	245.86	87.46	0.8383	0.0669	0	37.0
17	0.079	0.081	0.374	0.240	215.41	91.70	163.74	122.10	0.7098	0.1793	0	49.3
18	0.094	0.096	0.371	0.140	228.93	92.40	196.88	117.07	0.7118	0.1904	0	33.7
19	0.197	0.218	0.210	0.000	600.22	16.73	600.22	16.73	0.9252	0.0176	0	0.0
20	0.135	0.156	0.229	0.000	589.57	24.56	589.57	24.56	0.8905	0.0351	0	0.0
21	0.150	0.170	0.223	0.000	589.95	28.43	589.95	28.43	0.8881	0.0347	0	0.0
22	0.142	0.145	0.344	0.020	512.71	128.57	502.46	146.29	0.8818	0.0464	0	23.5
23	0.079	0.082	0.369	0.160	411.39	196.51	345.57	235.27	0.7611	0.1729	0	27.6
24	0.094	0.101	0.356	0.030	442.85	184.82	429.56	197.20	0.8022	0.1087	0	36.7
25	0.109	0.131	0.156	0.000	296.95	13.03	296.95	13.03	0.8934	0.0313	0	0.0
26	0.039	0.060	0.187	0.040	259.83	49.48	249.44	70.48	0.7829	0.1055	0	55.5
27	0.054	0.075	0.178	0.000	279.46	24.75	279.46	24.75	0.8047	0.0959	0	0.0
28	0.054	0.059	0.312	0.200	167.54	97.40	134.05	110.00	0.7726	0.1461	0	61.0
29	-0.016	0.017	0.368	0.810	74.26	90.65	14.11	48.49	0.4943	0.2638	44	40.1
30	-0.001	0.017	0.365	0.760	59.54	62.65	14.30	39.56	0.4998	0.2927	48	43.1
31	0.109	0.130	0.156	0.000	594.12	20.86	594.12	20.86	0.9203	0.0190	0	0.0
32	0.039	0.060	0.187	0.030	483.08	151.18	468.59	170.36	0.7869	0.1288	0	50.3
33	0.054	0.075	0.176	0.010	558.89	62.50	553.30	83.61	0.8351	0.0818	0	43.0
34	0.054	0.060	0.307	0.140	310.81	220.52	267.30	231.30	0.7780	0.1524	0	39.9
35	-0.016	0.013	0.368	0.880	103.25	134.82	12.43	56.18	0.5089	0.1961	43	45.3
36	-0.001	0.018	0.359	0.740	84.85	116.95	22.06	69.66	0.4953	0.2892	59	47.5
37	0.109	0.132	0.215	0.000	295.64	14.35	295.64	14.35	0.8917	0.0263	0	0.0
38	0.039	0.061	0.243	0.030	260.34	51.82	252.53	67.80	0.7586	0.1271	0	30.7
39	0.054	0.074	0.233	0.020	280.02	22.66	274.42	45.34	0.8164	0.0677	0	38.5
40	0.054	0.062	0.352	0.230	188.91	100.66	145.48	118.98	0.7704	0.1019	0	45.2
41	-0.016	0.015	0.440	0.890	59.73	85.09	6.60	32.92	0.5566	0.1123	37	39.2
42	-0.001	0.022	0.417	0.750	107.16	93.84	26.84	65.63	0.5879	0.1492	45	40.4
43	0.109	0.132	0.213	0.000	592.65	20.62	592.65	20.62	0.9238	0.0160	0	0.0
44	0.039	0.062	0.240	0.040	518.46	108.95	497.72	147.70	0.7799	0.1312	0	38.5
45	0.054	0.075	0.231	0.010	559.92	69.38	554.32	88.89	0.8186	0.0869	0	34.0
46	0.054	0.063	0.347	0.200	362.44	218.54	289.95	243.60	0.7860	0.1838	0	42.8
47	-0.016	0.017	0.440	0.860	119.57	135.95	16.75	64.54	0.5581	0.1565	36	38.4
48	-0.001	0.021	0.408	0.650	88.97	93.64	31.17	69.49	0.5568	0.2378	58	39.0

det.r = deterministic growth rate calculated from the input life table.
stoc.r = mean stochastic growth rate resulting from the simulation.
SD(r) = mean stochastic growth standard deviation.
PE = probability of extinction over the duration of the simulation.
N-extant = mean population size for simulated populations that survived.
SD(Next) = mean surviving population size standard deviation.
N-all = mean population size for all populations.
SD(Nall) = mean population size standard deviation.
Het = mean expected heterozygosity for extant populations.
SD(Het) = mean heterozygosity standard deviation.
MedTE = median time to extinction (only in simulations in which the populations became extinct in at least 50% of the simulations)
Mean.TE = mean time to extinction of those simulated populations that became extinct.

In the basic scenario the population growth is positive and the probability of extinction after 100 years is 0%. Thus, the probability of survival in the reintroduced population would be 100%, well above the criterion of a minimum of 90% probability of survival cited in other studies as a viable population and a successful reintroduction (van Ewijk et al., 1997). The mean expected heterozygosity is 88% and the population does not become extinct in any simulation.

In 8 scenarios (16.7%), the population shows negative deterministic growth and the population becomes extinct in a minimum of 50% of simulations. But in all other scenarios the population persists after 100 years (83.3%) and in a wide majority (64.6%) the population has a probability of survival higher than 90%. In 52.1% of the scenarios the population retains mean heterozygosity above 80%.

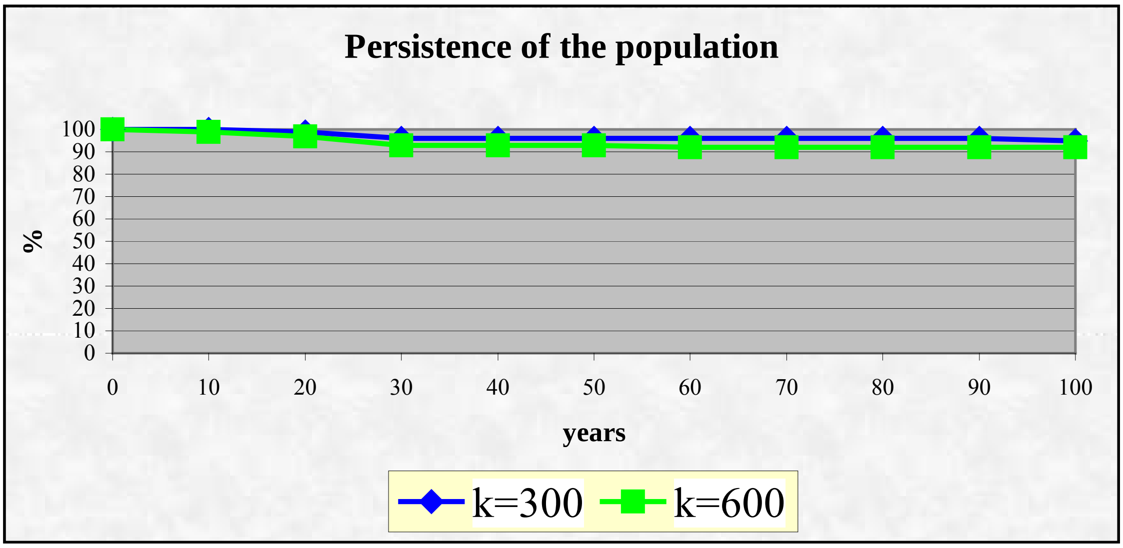
Figure 8.1. Vortex results of 48 different scenarios for the reintroduced population.
(blue line = criterion of a minimum of 90% probability of survival; green = scenarios which meet 90% criterion; yellow = scenarios which do not meet 90% criterion; red = scenarios which do not meet 90% criterion and in which the population became extinct in more than 50% of the simulations)



The sensitivity analysis was based on the study of variations for four different factors: mortality rates, catastrophes, carrying capacity, and reproductive success and supplementation.

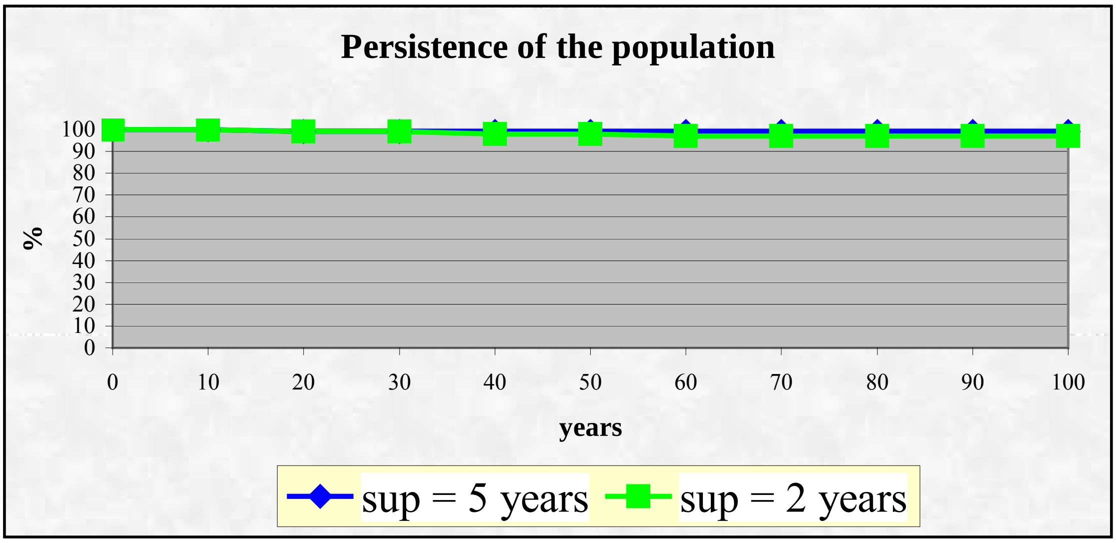
The model was not specially sensible to increases in carrying capacity. To double the k value (from 300 to 600) did not produce an important increase in the probability of survival of the reintroduced population.

Figure 8.2. Vortex results of two scenarios (6 and 12) that differ only in the carrying capacity.



To shorten the supplementation period from 5 to 2 years did not appear to influence the probability of survival of the new population, as shown in figure 8.3.

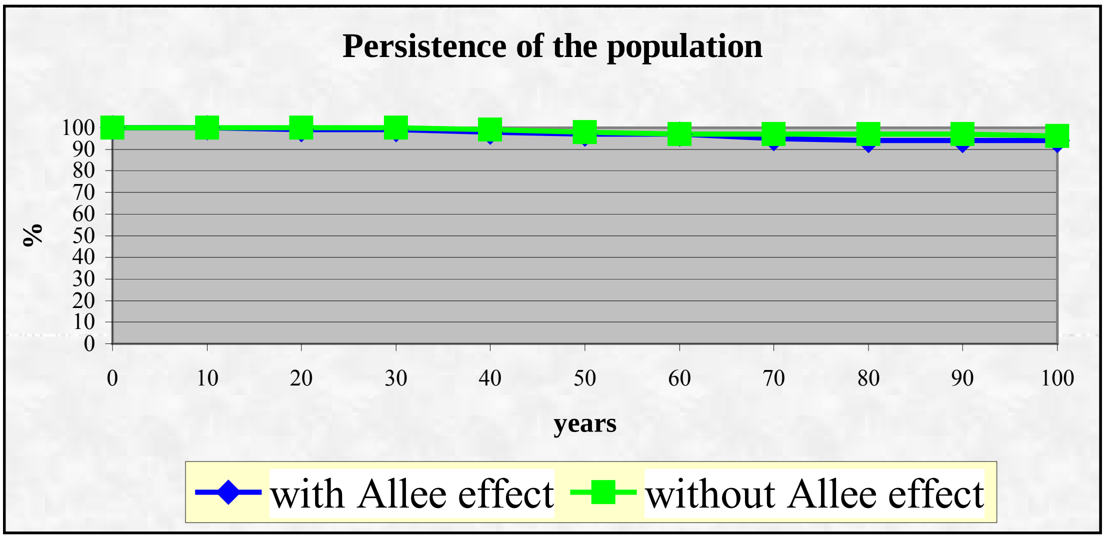
Figure 8.3. Vortex results of two scenarios (4 and 16) that differ only in the supplementation rate.



Nevertheless, in species with long dispersion patterns such as the otter, the supplementation rate could have an influence on reproduction due to the difficulty in finding mates, which would produce an Allee effect (Miller & Lacy, 1999) in the first stages of the new population, that is, a decrease in the proportion of females breeding at low densities.

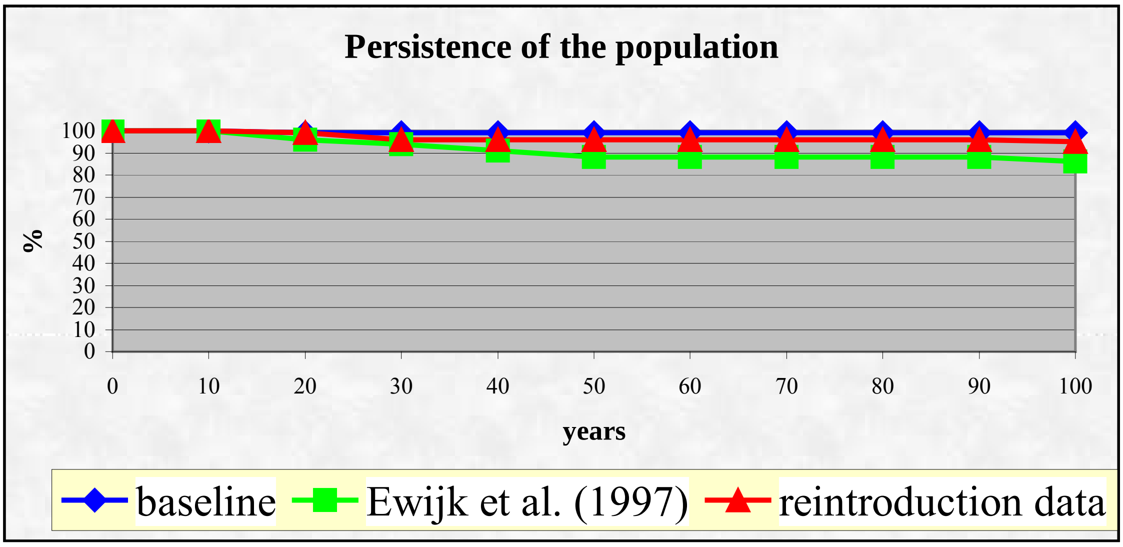
An Allee effect was simulated for the reintroduced population in figure 8.4, but only a small influence on the probability of survival was observed.

Figure 8.4. Vortex results for scenario 29 and a variation introducing an Allee effect (breeding females = 75%; Allee effect parameter = 1)



Likewise, the use of mortality data extracted from three different sources (see 8.3) did not appear to have a strong influence on the probability of survival of the new population, as shown in figure 8.5.

Figure 8.5. Vortex results of three scenarios (4, 5 and 6) that differ only in the mortality rates.



Variation in reproductive success affected the probability of survival unequally. In the scenarios where survival was high (>90%) there was no important difference in the probability of survival between the two distributions of breeding success (figure 8.6), but when an increase in catastrophes was already producing an increase in the probability of extinction, the sensitivity of the model to changes in reproductive success was high, in some cases producing extinction in populations with intermediate probabilities of survival under the former parameters (figure 8.7).

Figure 8.6. Vortex results of two scenarios (1 and 25) that differ only in the reproductive success.

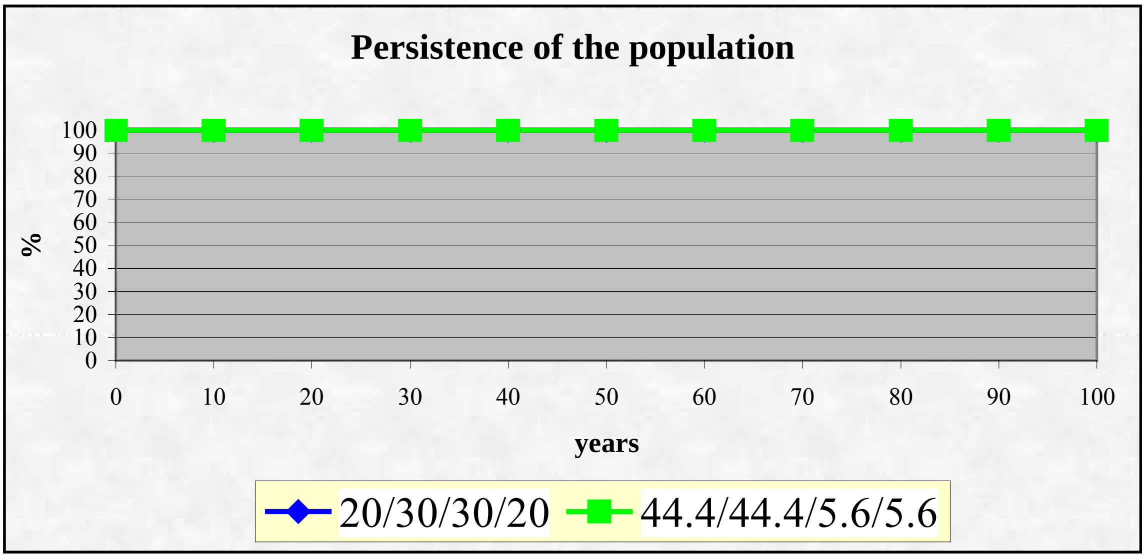
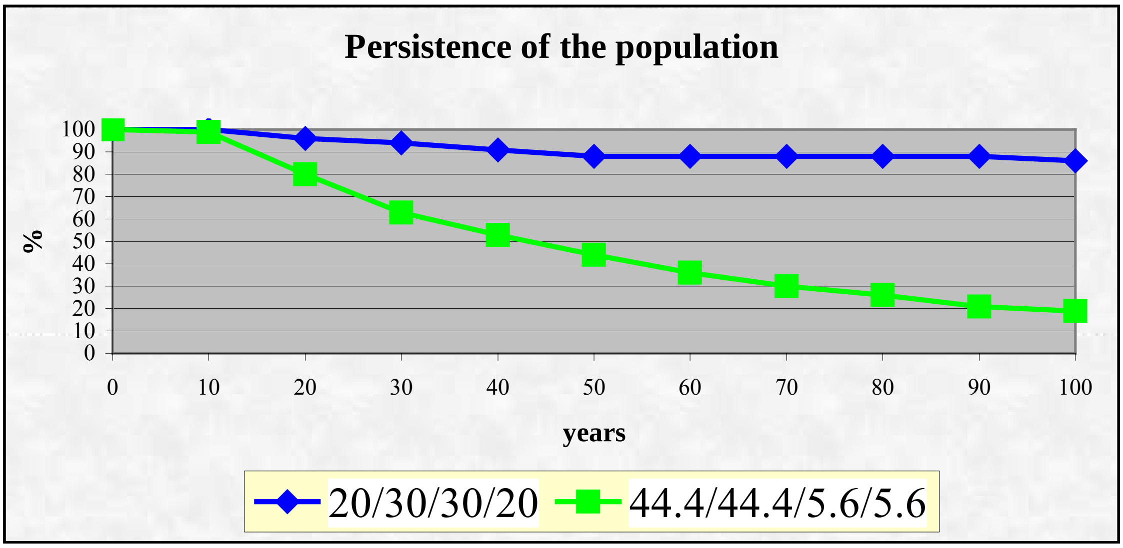


Figure 8.7. Vortex results of two scenarios (5 and 29) that differ only in the reproductive success.



Finally, variation in the probability and severity of catastrophes was important when associated with a decrease in reproductive success. Thus, in the eight scenarios where population growth was negative and when extinction occurred in at least 50% of simulations, lower breeding success (1.72 cubs/female) worked together with higher catastrophe probability (10%) and severity (decreasing reproduction to 50% and killing 50% of the individuals). In contrast, when breeding success was higher (2.5 cubs/female) there was no important difference in the probability of survival between the two catastrophe patterns (figure 8.9).

Figure 8.8. Vortex results of two scenarios (26 and 29) that differ only in the catastrophe pattern.

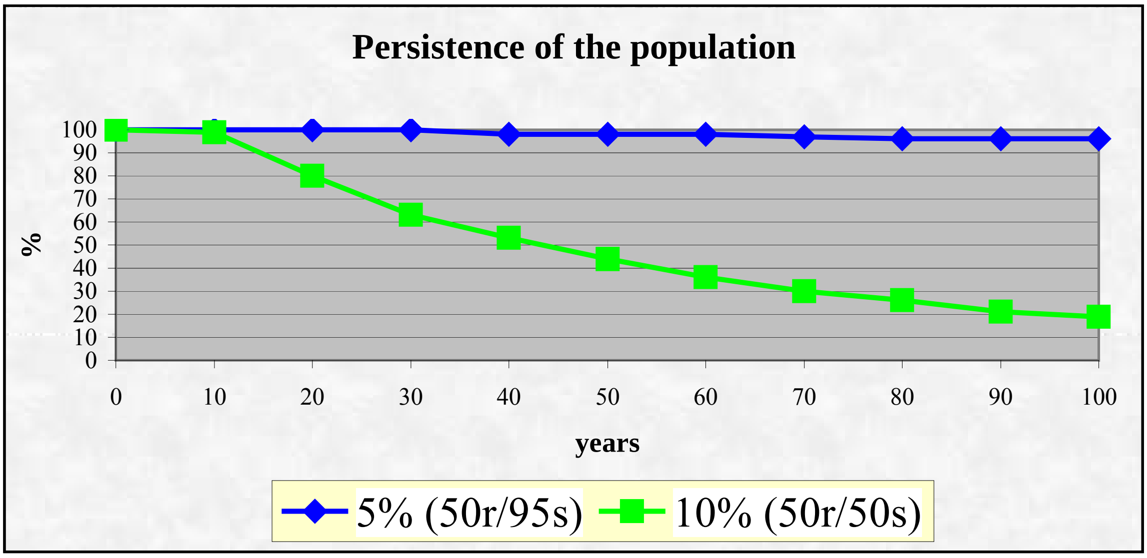
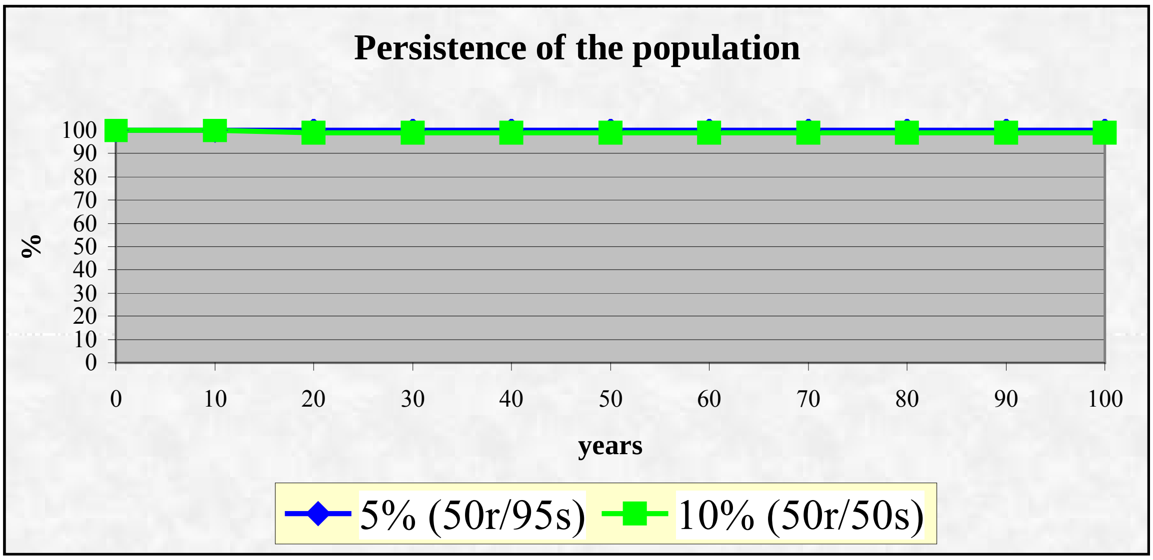


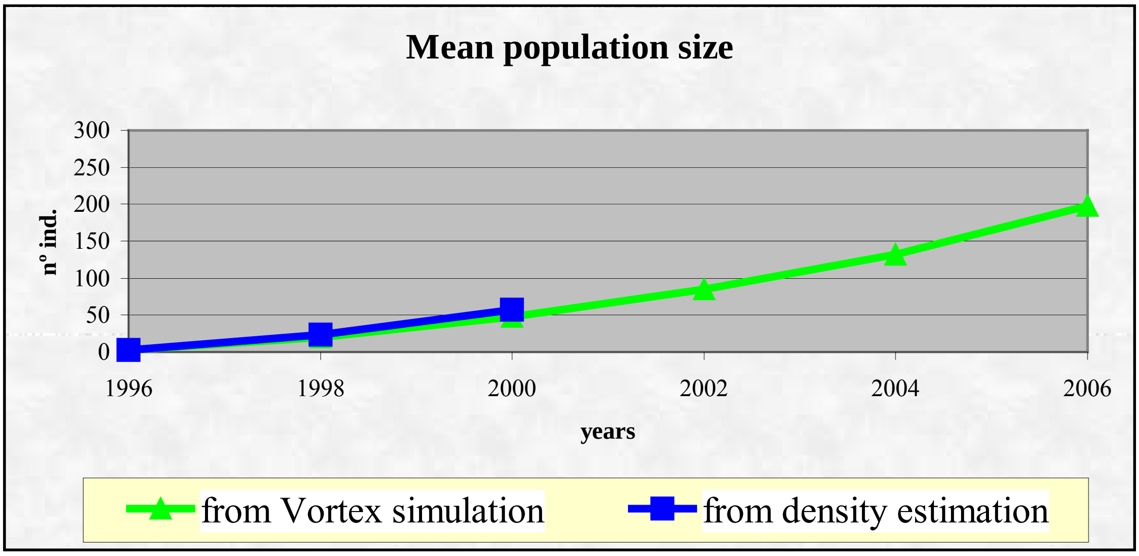
Figure 8.9. Vortex results of two scenarios (1 and 4) that differ only in the catastrophe pattern.



<u>Comparison between Vortex simulation and density obtained through visual censuses</u>:

The results of the comparison proposed in 8.4 appear in figure 8.10. Although only results for 1998 and 2000 can be compared, there are no important differences between the values found.

Figure 8.10. Comparison between Vortex results for basic model run 10 years and estimated number of individuals obtained from density calculated through visual censuses.



8.6. Discussion

The otter population reintroduced in the Muga and Fluvià basins has a low risk of extinction in the next 100 years. Most of the scenarios (64.6%) met the criterion of a minimum 90% probability of survival stressed in the PVA for Dutch otter reintroduction (van Ewijk et al., 1997). Extinction of the population in more than 50% of simulations occurred in only eight scenarios (16.7%) where the two most negative parameters coincided: low reproductive success (1.72 cubs/female) together with devastating (killing the 50% of the population) and recurrent (every 10 years on average) catastrophes.

Both parameters are probably not adjusted to our reality, for several reasons:

- In the reintroduction area there are several stretches of river and wetlands that never dry out, even in the most persistent droughts (Sargatal & Félix, 1989; Saavedra, 1995). These stretches are of the greatest importance in Mediterranean otter survival (Ruiz-Olmo et al., 2002).

- The otter is able to feed on aquatic species that resist dry periods much better than fish because of their ability to survive out of the water, species such as frogs, crayfish and insects, and it can even feed on non-aquatic species as birds (Ruiz-Olmo & Delibes, 1998; Ruiz-Olmo, 2001). All these species are abundant in the reintroduction area, due to its combination of lowlands and wetlands.

- During at least the first years otter densities will be low, but fish biomass is very high (Saavedra, 1995) in most of the reintroduction area, which would permit good reproductive success, close to the 2.5 cubs per female expected for a Mediterranean population in expansion (Ruiz-Olmo, in press) and to the 2.3, 2.4 and 2.8 cubs per female used respectively in three PVAs developed to assess an otter reintroduction program in The Netherlands (Ewijk et al., 1997; Klop et al., 1998 and F.J.J. Niewold, unpublished data).

In any case, modeling of the reintroduced population has been built up using several assumptions and data which do not come, in most cases, from the study of the reintroduced population itself. It seems reasonable, then, to put limited confidence in the extinction probabilities and other data generated by the models (Beissinger and Westphal, 1998). However, the results for each scenario do provide a basis with which to determine and prioritize actions for species conservation and management (Lacy & Clark, 1993).

Population modeling shows that the main threats for the reintroduced population come from factors which are difficult to manage at a local level, factors such as climate change (that could bring more recurrent and severe droughts) or the general state of habitat (that influences in breeding success). But another key factor to monitor in order to ensure the viability of the reintroduced population is mortality, an aspect stressed in similar models done in The Netherlands (van Ewijk et al., 1997; Klop et al., 1998). An important increase in these parameter rates would involve a spectacular increase in the probability of extinction. In Girona reintroduction area, road kills cause more than 50% of otter mortality, often concentrated in a few “black spots”. Thus, the construction of fauna passages, the fencing of some dangerous road stretches and speed restrictions on some roads are basic actions to be taken in order to maintain or reduce a parameter which is crucial for the viability of the new population, and which can be managed through technical and administrative measures.

On the other hand, other management actions such as increasing the carrying capacity of the reintroduction area, are not so decisive in increasing the probability of survival of the population. That does not mean that carrying capacity is not decisive in the survival of species in our study area, but only that it is so when k is small, which also usually means we are working with an isolated population in a small area (Ruiz-Olmo, 1995) or in a habitat unsuited to the persistence of a minimum viable population.

The model had low sensitivity to supplementation rates of new otters, suggesting that it is not particularly important to carry out translocation as quickly as possible. But in our data we only took into account the demographic component, not the spatial one (measuring dispersion) as other authors propose (South et al., 2000). As noted in chapter 4, the dispersion of the reintroduced otters is particularly fast, which could cause difficulties in finding a mate when the number of individuals is very small, that is, as explained above, an Allee effect. This effect, in the case of the population studied, does not greatly affect the survival of the population (figure 8.4). The opposite effect, when the proportion of females breeding would decrease as population size becomes large, has been recommended for a reintroduction otter PVA in The Netherlands (F.J.J. Niewold, pers. comm..), but always in small isolated areas where the carrying capacity is much lower than the numbers found in the present study.

Finally, it can be concluded that a reintroduction of only some tens of individuals could be perfectly viable, whenever the area would be big enough and the habitat in good enough condition (giving high carrying capacity) and mortality rates would be maintained at low levels. These results agree with those of Ruiz-Olmo (1995), who found that small isolated populations could recover from some tens of individuals, when no limits in carrying capacity occur or when carrying capacity is high (hundreds or thousands of animals). In our case, no important barriers for otter recovery are found.

As noted elsewhere, the model presented here was based on the use of assumptions and data from other populations for simulation of the different scenarios. As has been suggested (South et al., 2000), monitoring the reintroduced population could allow the simulation of new models based on the information collected, which could then be used to predict population viability more precisely and to prioritize management actions.

To some degree, the incorporation of results during monitoring of the reintroduced otter population was an attempt to compare outcomes from the Vortex simulation with results using density and dispersion found in field studies (figure 8.10). And it is interesting to observe that both results are similar and comparable, although the error associated with the estimate of population through density could be high. In any case, these results show that the theoretical increase of the number of individuals predicted by Vortex is in fact occurring. Although it is impossible to know the real number of births and deaths in the reintroduced population, there are at least two sources of evidence for the hypothetical increase. Firstly, the population continues to disperse and has already colonized two basins outside the reintroduction area: the Ter basin to the South and the Tec basin to the North (in France). Secondly, the three otters found dead (all run over) during 2000 and 2001 were adults (two males and one female) which had been born in the reintroduction area.

9. Conclusions

1. The Girona Otter Reintroduction Project (*GORP*) observed the guidelines for reintroductions produced by the International Union for the Conservation of Nature (IUCN). The viability study focused mainly on the levels of PCBs in fish from the area, which were of the order (81-136.4 µg/kg wet weight) of those calculated in rivers of Catalonia with otter populations, and on fish biomass in the proposed reintroduction area (0.6-351.9 gr/m^2), which could carry an otter population of more than one hundred individuals.

2. Reintroduction of the otter in the Muga and Fluvià basins succeeded, as regards the developing distribution of the new population. The length of rivers and surface of wetlands visited by the otter followed an upward progression (483 km of river and 2900 ha of wetland just after the completion of the translocations) and the geographical area effectively occupied increased to 64% of positive otter stations in winter 2001-02.

3. The finding of three adult otters born in the reintroduction area (without transponder or transmitter) is further proof to support the success of the reintroduction program.

4. Density values found through visual censuses were low (0.04-0.11 otters/km), but they approached what could be expected in the first stages of a reintroduced population, still small but extended over a wide area. At this moment (2002) it is similar to the density found in oligotrophic rivers in the Pyrenees.

5. Post-release mortality was 22% one year after release, similar to or lower than successful otter reintroduction programs. Mortality was due mainly to traffic (56%) and more than 50% of otters found dead were found less than two months after release, suggesting that animals which dispersed very fast from release areas experienced higher mortality than those that remained in the vicinities or dispersed slowly.

6. The activity pattern of the reintroduced otters proved mainly nocturnal and crepuscular (84-98%), with scarce diurnal activity (2-16%). The radiotracked otter showed longer activity in winter than in summer. The emergence or beginning of activity was quite constant while retirement or the end of activity was much more variable.

7. The ranges of the reintroduced otters were of the same order (34.2 km) as those found in other studies in Europe. Male home ranges were clearly bigger (32%) than females, but the differences between sexes were not significant, perhaps due to a low sample size. Core areas, defined as the minimum linear distance covering 50% of radiolocations, occupied 15% of total range.

8. Daily movement parameters were also bigger in males. Mean range (length of waterway used by an otter during the 24 hour tracking session), was 4.2 km for females and 7.6 km for males, probably because of male patrolling behaviour surveying for females to mate with.

9. During radiotracking two females bred, making large movements just prior to giving birth, then greatly reducing activity after giving birth, increasing it progressively the following weeks and reaching pre-birth levels only in the last days of presence in the natal den. Breeding female otters changed their daily movements after the cubs were born, with lower ranges and lower amounts of time with activity.

10. The response of the reintroduced otter population to high seasonal fluctuations in water availability in Mediterranean regions consisted of concentration in a smaller area during summer droughts, due to the increase of dry stretches which were uninhabitable for the otter because of lack of food, and so caused periodical expansions and contractions in the otter population's range. During water shortage conditions the otters did not patrol long stretches of habitat but stayed close to the pools, which the only food available was concentrated.

11. The resting sites selected by the otter were mostly surrounded by plants which grow fast and in compact masses down to the water's edge, constituting secure refuges for the otters. The different individuals mostly used (82%) a different resting site every day.

12. Long-term persistence of the reintroduced population was studied through a Population Viability Analysis (PVA). The result was low risk of extinction in the next 100 years, with most scenarios (65%) meeting the criterion of a minimum of 90% probability of survival.

13. Population modelling highlighted the importance of preventing fatal accidents if the population were to remain viable. A big increase in this parameter rate would involve a spectacular increase in the probability of extinction. In the reintroduction area, road kills cause more than 50% of otter mortality, and they can be reduced through the construction of fauna passages, the fencing of some dangerous road stretches and the use of speed restrictions.

14. The Girona Reintroduction Otter Project tuned a protocol for trapping, handling and releasing wild otters that can provide useful information for similar programs.

15. The otter reintroduction represented a unique opportunity to study an artificially designed population and to compare several methods of estimating otter distribution and density. The main conclusions were the usefulness of otter surveys in detecting otter presence, and the validity of visual censuses in estimating the abundance of otters in a stretch of river.

16. Finally, the reintroduction carried out in the Muga and Fluvià basins has achieved the creation of a new otter population, that persists over time, reproduces regularly and is gradually dispersing, even to new river basins.

10. Summary

The preparation and execution of the Girona Otter Reintroduction Project (*GORP*) used accurate monitoring combining several fields in biology and veterinary science, and permitted completion of this thesis. In previous chapters, the aspects that meet the objectives of the present work have been extensively developed and in this chapter a comprehensive and briefer summary is offered.

Objective 1. To demonstrate the viability of the otter reintroduction.

As set out in chapter 1, the International Union for the Conservation of Nature produced the guidelines for reintroductions (IUCN, 1987; 1995) which must be observed prior to authorisation of any reintroduction or reinforcement project. The *GORP* produced a viability study (Saavedra, 1995) in order to determine if the project met such guidelines.

Before studying habitat quality, it was possible to assess some of the IUCN's considerations for reintroductions. Although still common in some parts of its area of distribution, the Eurasian otter was classified as Vulnerable (IUCN, 1990). The species had originally been present in the proposed area of reintroduction, Aiguamolls de l'Empordà Natural Park and the Muga and Fluvià basins (Delibes, 1990; Ruiz-Olmo & Aguilar, 1995; Ruiz-Olmo, 1995; Ruiz-Olmo & Delibes, 1998). The causes of extinction were well known: persecution (hunting, trapping), pollution and habitat destruction (Ruiz-Olmo, 1995; Saavedra, 1995). Extinction in the study area was complete at the moment of the beginning of the project (Delibes, 1990). Muga and Fluvià were two of the few basins without otters where spontaneous recolonization was unlikely to occur (Ruiz-Olmo & Delibes, 1998) in the short or middle term. The proposed donor populations (from Extremadura, Galicia, Asturias and Portugal) were healthy and increasing in number (Ruiz-Olmo & Delibes, 1998; Trindade et al, 1998). The Iberian otters did not present significant morphological (Ruiz-Olmo, 1995) differences. The proposed donor populations were the nearest with enough individuals and lived in comparable Mediterranean habitats. The extension of the study area was 200,000 ha, with more than 600 km of river and 3,000 ha of wetlands. Considering the densities found in Catalonia (0,1-1,2 otters/km; Ruiz-Olmo, 1995), the existence of enough habitat for a population with a range between 60 and 720 animals was calculated (Saavedra, unpublished data). The local human population was mainly in favour of the reintroduction, because the otter did not negatively affect the interests of any economic group in the area (Saavedra, 1995) and, finally, the species was a fully protected species in Spain from 1973.

The habitat conservation studies tried mainly to answer two questions: is the habitat too polluted for the establishment and persistence of an otter population, and is there enough food for an otter population?

lLevels of organochlorine compounds in fish from the area were of the order of those calculated in rivers of Catalonia (López-Martín et al., 1995), with arithmetic means in wet weight of 21 μg/kg of ΣHCHs, 81 μg/kg of ΣDDTs and 181 μg/kg ΣPCBs. The role of organochlorines in the feasibility of otter reintroduction was focused on PCBs, because these substances were considered the principal causes of otter extinction in large areas (Mason, 1989, Macdonald & Mason, 1994), although there is still controversy about this point (see Kruuk, 1997 and Mason, 1997). Levels of PCBs ranged between 81 and 136.4 µg/kg wet weight, with 41% of sites with values higher than 110 µg/kg wet weight (Mateo et al., 1995). Fish with levels lower than this 'level of concern' lived in Catalonian rivers with healthy otter populations (Ruiz-Olmo and López-Martín, 1994; Ruiz-Olmo, 1995).

High levels of ΣPCBs were detected along the Fluvià river, especially in the upper course, and in some points of the Muga basin. However, several points of the coastal marshes and the greater part of the Muga basin showed PCB levels below those observed in Catalonian rivers where otters are still present (Ruiz-Olmo & López-Martín, 1994). Thus, it was expected that lagoons and channels of the Aiguamolls de l'Empordà Natural Park would be a good place to begin reintroduction in the zone.

Fish biomass in the proposed reintroduction area varied between 0.6 and 351.9 gr/m^2 (n=12), with only two sites (17%) with biomass lower than 8 gr/m^2, the minimum value calculated to support an otter population in Mediterranean rivers (Ruiz-Olmo, 1995). These results showed that the area could sustain a stable density of otter population, similar to other densities present in otter rivers of the Iberian Peninsula (Saavedra, 1995). The carrying capacity depending on fish biomass could be established at between 0.4 to 0.9 otters/km in stretches with 30-80 g/m^2 and 0.1-0.3 otters/km when fish biomass is 10-30 g/m^2 (Ruiz-Olmo, 1995). From these data it was established that the area studied (which did not comprise the whole Muga and Fluvià basins, but only the main branches) were able to carry an otter population of 130-160 individuals.

Objective 2. To demonstrate the success of the otter reintroduction.

The main monitoring schemes were directed to verification of the success or failure of the otter reintroduction. Studies of the persistence of the new population were carried out through direct (visuals censuses and radiotracking) and indirect ("otter survey" stations network) procedures.

Reintroduction of the otter in the Muga and Fluvià basins succeeded, as regards the development in distribution of the new population. The length of rivers and surface of wetlands visited by the otter followed an upward progression, from 0 km of river and 500 ha of wetland at the end of 1995 (47 days after the first release) to 483 km of river and 2900 ha of wetland at the end of 2000 (just after the completion of the 41 translocations). The geographical area effectively occupied (results from otter survey) ranged from 0% in summer 1995 (before the first release) to 64% in winter 2001-02, with a tendency to increase also apparent.

Colonization occurred fast, mainly because the first individuals tended to visit very long stretches of the available geographical area. Thus, in 1997, two years after the beginning of the releases and with only 23 reintroduced animals, radiotracking data, tracks or spraints were collected from more than 300 km of rivers and streams, which means almost all the Muga and Fluvià basins. And more than 50% of “otter surveys” were already positives. This process halted momentarily during 1998, due to the lack of available habitat to visit (although it must be repeated that this does not mean that it was permanently occupied). And in 1999 an acceleration in colonization was found, reaching 400 km of rivers and streams and with a peak of 72% of positive “otter survey” stations. What was most notable was that the increase in kilometers of river covered occurred as a consequence of dispersal to the neighbouring basins (the Ter in the South and the Tec to the North) and that that occurred in the only year with no new releases, so that it is possible that juveniles or subadults born in the reintroduction area were at least partly responsible. In fact, 1999 was the first year when an important number of different cub tracks (five) were found.

Density values found through visual censuses were low (0.04 - 0.11 otters/km), but they approached what would be expected in the first stages of a reintroduced population, still small but extended over a wide area. At this moment it is similar to the density found in oligotrophic rivers of the Pyrenees (Ruiz-Olmo, 2001). The evolution of total densities obtained from visual censuses and calculated theoretically from numbers released was similar, but the values were higher for visual censuses, perhaps because visual censuses were conducted in stretches with a more regular and continued otter presence.

Research into mortality of the reintroduced population served also to confirm the persistence of the population. Post-release mortality was 22% (one year after release), similar to or lower than other successful otter reintroduction programs. All mortality was due to accidents (mainly car crashes with 56%) and not human persecution, and this was an important point for the success of the program. Also, more than fifty percent of otters found dead were found less than two months after release, suggesting that animals which dispersed very fast (and usually far) from release areas experienced higher mortality than those that remained in the vicinities or dispersed slowly.

During 2000 and 2001 three otter were found dead, all run over. All three otters, a female and two males, were adults without any transponder or transmitter, which means that they were born in the reintroduction area, offspring of the translocated otters. Finding these animals is further proof of the success of the reintroduction program, and the individuals found were already mature enough (and perhaps had already done so).

Objective 3. To examine certain ecological and behavioural questions in depth, making use of a unique opportunity to study a "designed" population.

Only on rare occasions is it possible to study the behaviour of almost all the components of a new and artificially created carnivore population, in a geographical area without any other individuals of the species. This is the case of the *GORP*, where the release of 41 otters, almost all equipped with transmitters, into an area where the species had become extinct, could be used to study several aspects of the use of space and time by the Eurasian otter in Mediterranean habitats.

The activity pattern of the reintroduced otters proved to be mainly nocturnal and crepuscular, with little diurnal activity. The radiotracked otter showed longer activity in winter than in summer, and diurnal activity during the winter was also longer than in summer, as observed by other authors (Ruiz-Olmo, 1995) in coincidence with very low temperatures. The period of quiescence during the middle of the night was observed only on some occasions, but it was not a general pattern. The emergence or beginning of activity was quite constant and significant seasonal differences were found when comparing this moment with sunset. On the other hand, retirement or the end of activity was much more variable and no significant seasonal differences were found.

The ranges of the reintroduced otters (radiotracked more than 30 days) were of the same order (34.2 km) as those found in other studies in European and North-American rivers (with *Lontra canadensis* in America), although they were bigger than in studies made in more similar places, such as the Pyrenees and the Bergantes river (Ruiz-Olmo et al., 1995; Jiménez et al., 1998). In both cases there was probably an influence of a shorter radiotracking period (26 and 20 days for Pyrenees and 120, 90 and 120 days for Bergantes river) compared with a mean tracking period of 208 days (30-631) in *GORP*. As in the majority of the studies consulted, male home ranges were clearly bigger (32%) than females', but differences between sexes proved not significant, perhaps due to a low sample size. Core areas, defined as the minimum linear distance covering 50% of radiolocations, occupied 15% of total range. No significant differences were found between reintroduced and wild otters (studied in Bergantes river, Spain).

Movement parameters were studied following Durbin (1996) methodology. Mean range (length of waterway used by an otter during the 24 hour tracking session), was 4.2 km for females (n=19) and 7.6 km for males (n=11; excluding males in almost dry rivers), smaller than in Scotland and Bergantes river studies. In all three cases males presented significantly bigger ranges than females, probably because of male patrolling behaviour surveying for females to mate (Durbin, 1996). On the other hand, the night activity period was much longer in our otters (mean of 7.6 hours) than in Durbin's study in Scotland (mean of 4.7 hours), perhaps caused by methodological differences in the attribution of activity or non-activity from the telemetry device signals. The speed in uninterrupted activity periods where the otter maintains the same direction of progression (generally the periods taken fluctuated between 45 and 90 minutes) ranged between 0.5 and 4 km/h, with a mean of 2.1 km/h. The speed was higher downriver (2.45 km/h, n=28) than upriver (1.42 km/h, n=32).

A major environmental factor acting in the Mediterranean regions is water availability and its fluctuations during the year. The otter response to this environmental factor was studied through two different approximations: changes in the distribution of the reintroduced population, and daily movements on the part of specific individuals. Distribution was studied through the otter survey stations network and the ensuing maps showed that the population concentrated in a smaller area during summer droughts, due to the increase of dry stretches, uninhabitable for the otter because of lack of food. These periodical expansions and contractions in otter population ranges, coincidental with greater or less availability of water in their habitat, was also described in other studies carried out in Mediterranean habitats (Carpena et al., 1997; López et al., 1998; Prenda et al., 2001; Ruiz-Olmo et al., 2002).

Water availability affected daily movements (24 hour tracking sessions) mainly by reducing the stretch of river travelled by otters living in dry rivers with scattered pools. These individuals “fixed” to one or several pools where they fed and spent most of their activity period. They moved quickly by the dry river between the pools (due to dangerous exposure to predators when running outside the water) and usually they visited the same pools several times in an activity period. Thus, during water shortage conditions, the otters did not patrol long stretches of habitat but stayed close to the concentrated food sources, the only ones available.

The use of resting sites by the reintroduced otters was also studied. The different individuals mostly used a different resting site every day, repeating on a very few occasions, for example when living in a dry river – where movements are very small – or in the case of a breeding female which obviously must return every day to the natal holt. Thus, it may be noted that consecutive reutilization of a resting site by a female is a clear sign of breeding. The resting sites selected by the otter were in most cases surrounded by plants which grow fast and in compact masses down to the water’s edge, constituting secure refuges for the otters; plants such as the cane (*Arundo donax*), the blackberry-bush (*Rubus ulmifolius*) and the reed (*Phragmites sp.*). The otters did not appear to select resting sites very far away from infrastructures or human constructions and were often found during the inactivity period resting only a few meters from busy roads, inhabited houses, gravel extraction areas or river stretches with many boats. These data showed that otters are tolerant to indirect forms of disturbance.

The study of two implanted breeding females gave interesting data that could be useful for conservation purposes. Female otters made large movements just prior to give birth. They greatly reduced their activity after giving birth, increasing it progressively in the following weeks and reaching pre-birth levels only in the last days of presence in the natal den. Breeding female otters changed their daily movements after the cubs were born, with lower ranges and also lower amounts of time with activity. Both significantly reduced their total range after parturition. And finally, the natal dens of breeding females were located in little- frequented areas, far from the main river or in a channel completely covered with reed and bulrush.

Objective 4. To determine the probabilities of survival of the reintroduced population and the factors that affect it.

Previously, the persistence of the reintroduced population has been shown. Nevertheless, although short-term persistence (5 years) has been verified, it is not known if the new population will persist after some decades or after a century. This aspect was studied through a Population Viability Analysis (PVA), a procedure for estimating the viability of small populations of animals and plants.

Although we worked with a “designed” population, in which the number of founder animals was perfectly known, the demographic pattern was not well known, due to the limitation of monitoring methods (i.e. radiotracking) and periods in respect to the life cycle of the species. Thus, data from the reintroduced population had to be mixed with other data coming from other Catalan populations that had been intensively studied and where PVAs were used in order to ascertain the viability of isolated wild populations (Ruiz-Olmo, 1995), and data from PVAs carried out to assess the viability of a future otter reintroduction in The Netherlands (van Ewijk et al., 1997; Klop et al., 1998). Although the impossibility of using accurate data could invalidate the results in quantitative terms, they could be used in qualitative terms to determine the main threats to the new population, using the PVA as a sensitivity analysis.

The otter population reintroduced in the Muga and Fluvià basins has a low risk of extinction in the next 100 years. Most of the scenarios (65%) met the criterion of a minimum of 90% probability of survival stressed in the PVA for Dutch otter reintroduction (van Ewijk et al., 1997). The extinction of the population in more than 50% of the simulations occurred in only eight scenarios (17%) where the two most negative parameters occurred together: low reproductive success (1.72 cubs/female) together with a most devastating (that would kill the 50% of the population) and recurrent (every 10 years on average) catastrophe. But both parameters were probably unrealistic in our case, because in the reintroduction area there are several stretches of river and also wetlands that never dry out, even in the most persistent droughts, because the otter is able to feed on aquatic species that resist dry periods much better than fish (frogs, crayfish and insects) and even on non-aquatic species as birds and, finally, because during at least the first years, otter densities will be low but fish biomass is very high in most of the reintroduction area (Zamora et al., 1996), which would involve higher reproductive success, close to the 2.5 cubs per female expected for a Mediterranean population in expansion (Ruiz-Olmo, in press) and to the 2.3, 2.4 and 2.8 cubs per female used respectively in three PVAs developed to assess an otter reintroduction program in The Netherlands (Ewijk et al., 1997; Klop et al., 1998 and F.J.J. Niewold, unpublished data).

From the population modeling it can be deduced that the main threats to the reintroduced population come from factors which are difficult to manage from a local perspective, such as climate change (that could bring more recurrent and severe droughts) or the general state of the habitat (which influences breeding success). But another key factor to control to assure the viability of the reintroduced population is mortality. A big increase in this parameter would involve a spectacular increase in the probability of extinction. In the reintroduction area, road kills cause more than 50% of otter mortality, often concentrated in a few “black spots”. Thus, the construction of fauna passages, the fencing of some dangerous road stretches and speed restrictions on some roads are basic actions which can be taken to maintain or reduce a parameter which can have a major effect on the viability of the new population and which can be managed with technical and administrative measures.

From the results of the PVA it can be concluded that a reintroduction made with only some tens of individuals could be perfectly viable, whenever the area would be big enough and the habitat in good enough conditions (thus giving high carrying capacity) and if mortality rates could be kept low.

Objective 5. To help in the preparation of standard methodologies, both in veterinary science and in population ecology.

Methods for trapping and handling Eurasian otters

The Girona Reintroduction Otter Project tuned a protocol for trapping and handling the wild otters, from capture until release, taking as a basis other reintroduction projects, mainly done with *Lontra canadensis* in North America.

Capture.

Fifty five otters were live-trapped with padded leg hold traps (Soft Catch), modified with one factory spring in each trap replaced by a #2 spring. Traps were set in water, preferentially in shallow passages between rocks or in river beaches. Traps were bound to one meter chains, which were tied to trunks or secured to big rocks, using climbing bolts, hammered in manually. Traps were set in groups of two or three units. No baits or lures were used.

The use of a blow pipe as a method to deliver the anesthetic combination worked well and this technique is strongly recommended to avoid injuries or worsen those produced by trapping, e.g., luxations, fractures, etc. Also, avoiding physical contact with conscious animals, darting them from a distance, and waiting longer than 3 min proved to be safe in our animals.

The animals captured suffered very few severe injuries. Indeed, no animal suffered leg fractures, 6 animals (14%) had open luxation injuries and only 9 animals (21%) suffered some kind of digit luxation. Very few dental injuries (19%) were detected in our study. These results show that soft catch traps can be used to humanely capture wild otters.

Housing and care.

After examination in the field, immobilized otters were placed in transport kennels and were let to recover in a cold dark room. In the afternoon, they were transported to Barcelona Zoo (Barcelona, Spain) by van or commercial airline.

In Barcelona Zoo the otters were individually housed indoors in wire-mesh cages with attached wooden nest boxes and suspended above the ground. In general, captive otters adjusted well to these cages and accepted food readily. Live fish (eels and trout) and chicks seemed to act as environmental enrichment tools and elicited eating in some individuals that initially rejected food.

The anaesthesia used during capture and handling was a mixture of ketamine hydrochloride (Imalgene) and medetomidine hydrochloride (Domtor). For reversal atipamezole hydrochloride (Antisedan) was administered. The complete anesthetic protocol used in this project (explained elsewhere, Fernández et al., 2001) proved to be effective and safe for the type of procedures described in this paper.

Nineteen animals received a dose of the long acting neuroleptic (LAN) perphenazine enantathe (Trilafon enantat) to decrease the stress level during handling, transport and management while in captivity. The possible benefits of using LAN during translocation programs of wild caught otters deserves further research. Our observations suggest that LAN can be beneficial. Indeed, although there was a large individual variation in behavior, most otters that had received LAN appeared much calmer during 5-7 days post-administration than those that had not, while feeding normally. These animals were less aggressive when the wooden box was opened for inspection purposes and they seemed to react less to external stimuli. Interestingly, treated animals did not show predatory behavior when offered live chicks, whereas control animals readily killed them.

Surgery and release.

The fact that all the otters ate normally the same day of the surgery shows that this procedure has minor effects on the animals: the otters did not seem to care about the incision sites and there were no exposed skin sutures that could be damaged or become irritated or infected. However, it seemed convenient to allow a period of ten days in captivity after surgery for complete healing. The idea of immediate surgery and early release advocated by some authors (Arnemo, 1991) in order to reduce stress did not seem convenient. The radiotelemetry devices used in our study (30-40 g) could be somewhat large for intraabdominal use in Eurasian otters, considering the smaller size of the Eurasian otter compared with the North American river otter.

The surgical approach used for radiotransmitter implantation in *GORP* was the ventral midline approach that is clearly to be recommended, based on the 36 surgical procedures done in our study without any problem. Heat loss after shaving the skin in the surgical area did not seem to be a problem in our animals, perhaps due to the benign climatic conditions in our release area. Furthermore, in the final clinical examination we confirmed the rapid regrowth of the inner fur.

The effects of the intraperitoneal transmitters on reproduction were found non-detrimental, as at least three radioimplanted otters have bred successfully in the releasing area (Saavedra & Sargatal, 1998).

The same day of the last examination otters were transported on a two hour trip by car to the reintroduction geographical area and released a few hours later.

The *GORP* also served for research into other different veterinary aspects of Eurasian otters not mentioned in this work but published elsewhere (Fernández et al., 2001a; 2001b; 2001c; 2002).

Methods of estimating Eurasian otter distribution and density

The *GORP* enabled us to study an artificially designed population. During the early months, thanks to radiotracking studies, we knew the exact number of otters constituting the population and the number of otters which lived in the different waterbodies. This fact gave us a unique oportunity to compare several methods of estimating otter distribution and density. The methods compared were the otter survey, based on the identification of signs (mainly tracks and spraints) of the species, track censuses, based on the finding of footprints in snow or mud and measuring their lengths, and visual censuses, based on the placing of groups of observers working in places where the otters are crepuscular.

Usefulness of the otter surveys.

One of the most striking results was that in waterways of small or average width a single otter was capable of marking many km so that it could be detected in most of the sites (>70%). Thus, if one site is negative, it is highly probable that the otter will be detected at the next site several km away, if the area actually is occupied by this individual. The survey of several consecutive sites without otters must be interpreted to mean that they are absent in the area. This result is of special importance on the margins of the species' distribution, where a single individual can be easily detected in such waterways. This means that the non-detection of otters in one area during a period of time suggests they are absent altogether. And we can note that the traditional image of the single otter (or "a pair") living in a short stretch (often in hills or mountains), and difficult to locate, is not realistic. The results for wetlands were similar while in large rivers (>20 m) the rate of detection decreased (50%).

But these were the detection results for a single otter living in a stretch. The densities of otters in fresh water are about 0.05 - 0.6 otters/km (Ruiz-Olmo, 1995b; Sidorovich, 1991, 1992), which means that the presence of between one and four otters in one fluvial stretch can be considered standard. And in the stretches in which two or three otters were present, the probability of detecting the otter in a conventionally surveyed site was nearly 100%. So two or three animals alone are capable of providing sufficient signs for otters to be found in almost all the places that they occupy, and otters must be detected after very few surveyed sites.

The results of the study also indicated that a linear relationship does not exist between the number of otters and the number of positive sites, since a few individuals spread their signs over large areas and those which are being released tend to occupy the same areas, presumably those that offer the most favourable conditions. Thus, when a river is recolonized by otters (both naturally or after reintroduction), the presence of the species in most waterbodies of the basin is reached in a very few years, often 1-4.

Estimation of abundance.

It is not easy to establish how many otters live in one specific sector of a habitat due to the large distances covered, the use of home ranges according to the availability of food over time, the otters' particular social structure and their chiefly linear habitat (Kruuk, 1995). The use of radio-tracking would certainly be more precise and in closer agreement with the ecological reality of the species, but this method is difficult to apply in management and tells us very little about the numbers and density of otter populations.

In this study it was found that the most reliable methods of estimating the abundance of an otter population are track censuses (considering only one day-old tracks) and visual censuses (otters seen in one activity period). With both methods, the use of 10 km stretches as a census unit seem to be sufficient, but perhaps 20 or 30 km stretches may bring more accurate results. It must be assumed that the same otter might be counted in two units, but also that a resident otter can wander outside this unit. The fact that both recent track and visual censuses give similar results (and show a high level of correlation) gives greater consistency to the results. The data obtained by radiotracking also indicate that practically all the otters can be seen by experienced observers, which confirms the validity of the visual censuses. The visual censuses also allow us to apply different indices to estimates of abundance (otters observed/km, otters/ha, otters observed/hour, etc.), all of which are highly correlated (Ruiz-Olmo, 1995a).

However, in those areas or periods of high otter density (>0.6 otters/km) we run the risk of underestimating the real number of individuals if we use track censuses, for where there are two individuals of the same sex and similar in size, they may be confused. This will not normally occur with visual censuses. In contrast, in areas of low otter density (<0.1 otters/km) individuals are not easily detected in visual censuses, because they can go outside the census stretch.

In conclusion, we can affirm that fresh tracks and visual censuses (at least in our study area) provide information which is very close to the real number of otters.

11. References

Albaiges, J.; Algaba, J.; Arambarri, P.; Cabrera, F.; Baluja, G.; Hernández, L.M. & Castroviejo, J. (1987). Budget of organic and inorganic pollutants in the Doñana National Park (Spain). *The Science of the Total Environment*, 63: 13-28.

Albaigés, J.; Farrán, A., Soler, M. & Gallifa, A. (1987) Accumulation and distribution of biogenic and pollutant hydrocarbons, PCBs and DDT in tissues of Western Mediterranean fishes. *Marine Environmental Research,* 22: 1-18.

Albanis, T.A. (1992). Herbicide losses in runoff from the agricultural area of Thessaloniki in Thermaikos Gulf, N. Greece. *The Science of the Total Environment*, 114: 59-71.

Alonso, G., Ocaña, J. & Cuadras, C.M. (1979). *Fundamentos de probabilidad en Bioestadística.* Eunibar, S.A., Barcelona.

Andrews, E.M., Howell, P. & Johnson, P. (1993). *Otter Survey of Wales 1991.* The Vincent Wildlife Trust, London.

Arnemo, J.M. (1990). Chemical immobilization of European river otter (*Lutra lutra*). *Norsk Veterinaertidsskrift*, 102: 767-770.

Armeno, J.M. (1991). Surgical implantation of intraperitoneal radiotelemetry devices in European river otters (*Lutra lutra*). *Habitat*, 6: 48-55.

Bach, J. (1989). Les aigües. In: Sargatal, J. & Félix, J. (eds.). *Els Aiguamolls de l'Empordà : aspectes ecològics, històrics i socials.* Carles Vallès editor. Figueres.

Bagenal, T. (1978). *Methods for Assessment of Fish Production in Fresh Waters*. Ed. Blackwell Scientific Publications, Oxford. 365 pp.

Baluja, G.; González, J.; Rico, C. & Hernández, L.M. (1985). Sources and transport of organochlorine compounds and heavy metals into waters of the National Park of Doñana. *Bulletin of Environmental Contamination and Toxicology*, 35: 482-489.

Beck, B.B. (1992). Guidelines for reintroduction of animals born or held in captivity. AZA reintroduction advisory group (3 June 1992). American Zoo and Aquarium Association. 7 pp.

Beissinger, S.R. & Westphal, M.I. (1998). On the use of demographic models of population viability in endangered species management. *Journal of Wildlife Management*, 62(3): 821-841.

Blanco, J.C. & González, J.L. (1992). Libro rojo de los vertebrados de España. ICONA. Madrid.

Blas, L. (1970). *Vida y costumbres de los Mustélidos españoles*. ICONA. Madrid.

Bohlin, T., Hamrin, S., Heggberget, T.G., Rasmussen, G. & Saltveit, S.J. (1989). Electrofishing - Theory and practice with special emphasis on salmonids. *Hydrobiologia*, 173: 9-43.

Bohlin, T. (1990). Estimation of population parameters using electric fishing: aspects of the sampling design with emphasis on salmonids in streams. In: Cowx, I.G. ed. *Developments in Electric Fishing.* Fishing News Books. Oxford. Chapter 23: 156-173.

Bohlin, T. & Cowx, I.G. (1990). Implications of unequal probability of capture by electric fishing on the estimation of population size. In: Cowx, I.G. ed. *Developments in Electric Fishing.* Fishing News Books. Oxford. Chapter 22: 145-155.

Bravo, C., Bueno, F. & Sánchez-Aguado, F. (1997). Censo visual de Nutria (*Lutra lutra*) y Visón Americano (*Mustela vison*) en el Parque Natural de las Hoces del río Duratón (Segovia). *Galemys*, 10: 89-96.

Breitenmoser, U. & Breitenmoser, C. (2002). Un bilan des réintroductions de carnivores en Europe. *Actes du XVIII Colloque Francophone de Mammalogie*: 17-19.

Brusi, D. (1992). Les aigües. In: Pallí, L. & Brusi, D. ed. El medi natural. Diputació de Girona - Universitat de Girona. Girona.

Brzezinski, M., Romanowski, J., Cygan, J.P. & Pabin, B. (1996). Otter *Lutra lutra* (L.) distribution in Poland. *Acta Theriologica*, 41: 112-126.

Bustamante, J. (1996). Population Viability Análisis of captive and released bearded vulture populations. *Conservation Biology*, 10: 822-831.

Cals, J. (1987). *L'Alt Empordà: recursos i estructura econòmica*. Caixa d'Estalvis de Catalunya. Barcelona.

Campbell, S. (1980). Is reintroduction a realistic goal? In: Soulé & Wilcox (eds). *Conservation biology*: 263-269.

Canadian Government Publishing Centre, Supply and Services Canada (1978). *Guidelines for Canadian Drinking Water Quality.*

Carpena, J.M.; Palomo, L.J. & Román, A. (1997). Distribución de la nutria (*Lutra lutra*) en la provincia de Málaga. Comparación entre dos períodos con climatología muy diferente. *Actas de las III Jornadas Españolas de Conservación y Estudio de Mamíferos*: 96. SECEM.

Caughley, G. (1994). Directions in conservation biology. *Journal of Animal Ecology*, 63: 215-244.

Caughley, G. & Gunn, A. (1996). *Conservation biology in theory and practice*. Blackwell Science. Cambridge. 459 pp.

Chapman, P.J. & Chapman, L.L. (1982). *Otter Survey of Ireland, 1981-82.* The Vincent Wildlife Trust, London.

Chehébar, C.E. (1985). A Survey of the Southern River Otter *Lutra provocax* (Thomas) in Nahuel Huapi National Park, Argentina. *Biological Conservation*, 32: 299-307.

Comunidad Europea (1980). Directiva del Consejo de 15 de julio de 1980 relativa a la calidad de las aguas destinadas al consumo humano (80/778/CEE). *Diario Oficial de las Comunidades Europeas*, L229/11: 174-192.

Comunidad Europea (1986). Directiva del Consejo de 12 de junio de 1986 relativa a los valores límite y los objetivos de calidad para los residuos de determinadas sustancias peligrosas comprendidas en la lista I del Anexo de la Directiva 76/464/CEE (86/280/CEE). *Diario Oficial de las Comunidades Europeas*, L181: 16-27.

Comunidad Europea (1988). Directiva del Consejo de 16 de junio de 1988 por la que se modifica el Anexo II de la Directiva 86/280/CEE relativa a los valores límite y los objetivos de calidad para los residuos de determinadas sustancias peligrosas comprendidas en la lista I del Anexo de la Directiva 76/464/CEE (88/347/CEE). *Diario Oficial de las Comunidades Europeas*, L158: 35-41.

Conroy, J.W.H. & French,D.D. (1987). The use of spraints to monitor populations of Otters (*Lutra lutra* L.). *Symp. Zool. Soc. Lond.*, 58: 247-262.

Conroy, J.W.H. & French,D.D. (1991). Seasonal patterns in the sprainting behaviour of Otters (*Lutra lutra* L.) in Shetland. *Habitat*, 6: 159-166.

Courtijn, E.; Vandecasteele, C. & Dams, R. (1990). Speciation of aluminium in surface water. *The Science of the Total Environment*, 90: 191-202.

Crawford, A.K., Evans, D., Jones, A. & McNulty, J. (1979). *Otter Survey of Wales.* Soc. Prom. Nature Conserv., Lincoln.

Croll, B.T. (1990). Pesticides in surface and underground waters. *Symposium on pesticides in soil and water*, 11-12 april 1990, London: 15 pp.

Cullen, M.C. & Connell, D.W. (1992) Bioaccumulation of chlorohydrocarbon pesticides by fish in the natural environment. *Chemosphere*, 25: 1579-1587.

de Jongh, A. (1998). Reintroduction of otters: support or risk for otter conservation? *IUCN Otter Spec. Group Bull.*, 15(2): 80-86.

de Jongh, Y.A.; van Olst, P.R. & de Jong, R.C.C.M. (1997). Feasibility of reintroduction of the Eurasian lynx (*Lynx lynx*) on “De Veluw”, The Netherlands, by using the stochastic simulation programme VORTEX. *Z. Saugetierkd.*, 62 (S2): 44-51.

Delibes, M. (1990) *La nutria en España*. ICONA. Madrid.

Delibes, M. & Adrián, I. (1987). Effects of crayfish introduction on otter *Lutra lutra* food in the Doñana National Park, SW Spain. *Biological Conservation*, 42: 153-159.

Delibes, M.; MacDonald, S.M. & Mason, C.F. (1991). Seasonal marking, habitat and organochlorine contamination in otters (*Lutra lutra*): a comparison between catchments in Andalucia and Wales. *Mammalia*, 55(4): 567-578.

Department of Environment (1989). The Water Supply (Water Quality) Regulations 1989. Guidance on Safeguarding the Quality of Public Water Supplies. September 1989.

Dietz, J.M.; Dietz, L.A. & Nagagata, E.Y. (1994). The effective use of flagship species for conservation of biodiversity: the example of lion tamarins in Brazil. In: Olney, P.S.J.; Mace, G.M. & Feistner, A.T.C. (eds). *Creative Conservation: Interactive management of wild and captive animals.* Chapman & Hall, London.

Dülfer, R., Foerster, K. & Röche, K. (1997). First phase report of the Treborn otter project, Council of Europe: Habitat use, home range and behavior. *Nature and environment,* 93: 31-46.

Durbin, L.S. (1996). Individual differences in spatial utilization of a river-system by otters *Lutra lutra*. *Acta Theriologica*, 41(2): 137-147.

Durbin, L.S. (1996). Some changes in the habitat use of a free-ranging female otter *Lutra lutra* during breeding. *J. Zool., Lond.*, 240: 761-764.

Durbin, L.S. (1998). Habitat selection by five otters *Lutra lutra* in rivers of northern Scotland. *J. Zool., Lond.*, 245: 85-92.

Durfor, C.N. & Becker, E. (1964). Public water supplies of the 100 largest cities in the United States, 1962 US Geol. Surv.; Water Supply Pap. nº 1812, United States Government Printing Office. Washington, DC.

Edebes, H. (1993). Game farming in South Africa. In: Fowler, M.E. ed.. *Zoo and Wild Animal Medicine: Current Therapy 3.* W.B. Saunders Co., Philadelphia, Pennsylvania: 1112-1222.

El-Dib, M.A. & Badawy, M.I. (1985). Organochlorine insecticides and PCBs in water, sediment and fish from the Mediterranean Sea. *Bulletin of Environmental Contamination and Toxicology*, 34: 216-227.

El-Gendy, K.S.; Abdalla, A.A.; Aly, H.A.; Tantawy, G. & El-Sebae, A.H. (1991). Residue levels of chlorinated hydrocarbon compounds in water and sediment samples from Nile branches in the Delta, Egypt. *Journal of Environmental Science and Health*, B26(1): 15-36.

El País (1993). *Atlas de España*. Diario El País. Barcelona.

Erickson, D.W. & McCullough, C.R. (1987). Fates of translocated river otters in Missouri. *Wild. Soc. Bull.*, 15: 511-517.

Estes, J.A. (1991). Status of Sea Otter (*Enhydra lutris*) populations. *Habitat*, 6: 27-35.

Farinha, N., Florencio, E. and Trindade, A. (1997). Distribuiçao da lontra em Portugal continental. *Actas de las III Jornadas Españolas de Conservación y Estudio de Mamíferos*: 96. SECEM.

Fernández, J., Saavedra, D. and Ruiz-Olmo, J. (1997). Aspectos veterinarios en el programa de reintroducción de la nutria en los Aiguamolls de l'Empordà (NE de Girona). *Ponencias y comunicaciones del I Congreso Internacional de Medio Ambiente y Veterinaria*: 401-406. Colección Monografías. Junta de Extremadura. Mérida.

Fernández-Morán, J.; Molina, L.; Flamme, G.; Saavedra, D. and Manteca-Vilanova, X. (2001). Hematological and biochemical reference intervals for wild caught Eurasian otter from Spain. *Journal of Wildlife Diseases*, 37 (1): 159-163.

Fernández-Morán, J.; Pérez, E.; Sanmartin, M.; Saavedra, D. and Manteca-Vilanova, X. (2001). Reversible immobilization of Eurasian otters (*Lutra lutra*) with a combination of ketamine and medetomidine. *Journal of Wildlife Diseases*, 37 (3): 561-565.

Fernández-Morán, J.; Saavedra, D. & Manteca, X. (in press). Reintroduction of Eurasian Otter (Lutra lutra) in North-eastern Spain: trapping, handling and medical management.

Fingler, S.; Drevenkar, V.; Tkalcevic, B. & Smit, Z. (1992). Levels of polychlorinated biphenyls, organochlorine pesticides and chlorophenols in the Kupa River water and in drinking waters from differents areas in Croatia. *Bulletin of Environmental Contamination and Toxicology*, 49: 805-812.

Flaten, T.P. (1991). A nation-wide survey of the chemical composition of drinking water in Norway. *The Science of the Total Environment*, 102: 35-73.

Folch, R. & Franquesa, T. (1984). *Història Natural dels Països Catalans, vol. 7 Vegetació*. Enciclopèdia Catalana. Barcelona.

Foster-Turley, P.; Macdonald, S.M. & Mason, C.F. (eds). (1990). *Otters: An action plan for their conservation*. SSC Otter Specialist Group. IUCN.

Gaona, P.; Ferreras, P. & Delibes, M. (1998). Dynamics and viability of a metapopulation of the endangered Iberian lynx (*Lynx pardinus*). *Ecological Monographs*, 68(3): 349-370.

García-Berthou, E. (1993). A computer program for estimating population size by the removal method. *Butlletí de la Institució Catalana d'Història Natural,* 61: 13-18.

Garrabou, G.; Roig, I.; Saavedra, D.; Fernández, J. & Ponsà, M. (in press). Estudios citogenéticos en la población de nutria eurasiática (*Lutra lutra*) reintroducida en el Parc Natural dels Aiguamolls de l'Empordà y comparación cariotípica con otros mustélidos. *Galemys*.

Giesy, J.P., Verbrugge, D.A., Othout, R.A., Bowerman, W.W., Mora, M.A., Jones, P.D., Newsted, J.L., Vandervoort, C., Heaton, S.N., Aulerich, R.J., Bursian, S.J., Ludwig, J.P., Dawson, G.A., Kubiak, T.J., Best, D.A. & Tillit, D.E. (1994) Contaminants in fishes from Great Lakes-Influenced sections and above dams of three Michigan rivers. II: Implications for health of mink. *Archives of Environmental Contamination and Toxicology*, 27: 213-223.

Gilpin, E. (1987). Spatial structure and population vulnerability. In: Soulé, M.E. ed.. *Viable populations for conservation*. Cambridge University Press. UK.

Gosálbez, J.; Serra, J. & Velasco, E. eds. (1994). *Els sistemes naturals dels Aiguamolls de l'Empordà*. Treballs de la Institució Catalana d'Història Natural, 13. Barcelona.

Green, J. & Green, R. (1980). *Otter Survey of Scotland 1977-79.* The Vincent Wildlife Trust, London.

Green, J. & Green,R. (1987). *Otter Survey of Scotland 1984-85.* The Vincent Wildlife Trust, London, 40 pp.

Green, J., Green, R. & Jefferies, D.J. (1984). A radio-tracking survey of otters *Lutra lutra* on a Perthshire river system. *Lutra*, 27: 85-145.

Green, R. (1991). The impact of hunting, poaching and accidents on otter survival and measures to protect individual animals. *Habitat,* 6: 171-190.

Griffith, B.; Scott, J.M.; Carpenter, J.W. & Reed, C. (1989). Translocation as a species conservation tool: status and strategy. *Science*, 245: 477-480.

Guitart, R., Guerrero, X., Silvestre, A.M., Gutiérrez, J.M. & Mateo R. (1996). Organochlorine residues in tissues of striped dolphins affected by the 1990 Mediterranean epizootic: Relationship with the fatty acid composition. *Archives of Environmental Contamination and Toxicology*, 30: 79-83.

Guitart, R., Puig, P. & Gómez-Catalán, J. (1993) Requirement for a standardized nomenclature criterium for PCBs: computer assisted assignment of correct congener denomination and numbering. *Chemosphere*, 27: 1451-1459.

Guitart, R., Riu, J.L., Puigdemont, A. & Arboix, M. (1990) Organochlorine residues in adipose tissue of chamois from the Catalan Pyrenees, Spain. *Bulletin of Environmental Contamination and Toxicology* , 44: 555-560.

Gutleb, A.C. (1995). Umweltkontaminanten und Fischotter in Österreich eine Risikoabschätzung für *Lutra lutra*. Ph. D. Thesis. University of Wien. 219 pp.

Heggberget, T.M. (1984). Age determination in the European otter *Lutra lutra*. *Zeitschrift für Säugetierkunde*, 49: 299-305.

Hernández, L.M.; Fernández, M.A. & González, M.J. (1992). Organochlorine pollutants in water, soils and earthworms in the Guadalquivir River, Spain. *Bulletin of Environmental Contamination and Toxicology*, 49: 192-198.

Holmes, A.A. (1974). Immobilon in the otter. *Veterinay Record*, 95: 574.

Hoover, J.P. (1984). Clinical evaluation of American river otters in a reintroduction study. *Journal of American Veerinary Medical Association,* 185: 1317-1319.

Hoover, J.P. (1984). Surgical implantation of radiotelemetry devices in American river otters. *Journal of American Veterinary Medical Association,* 184: 1317-1320.

Illana,A. and Paniagua,D. (1993). La nutria (*Lutra lutra*). Análisis de la situación en la Comunidad Autónoma Vasca y directrices para un plan de recuperación. 360 pp. unpublished.

International Union For Conservation of Nature, IUCN (1987). The IUCN position statement on translocation of living organisms. Gland, Switzerland.

International Union For Conservation of Nature, IUCN (1990). 1990 IUCN Red List of Threatened Animals. IUCN, Gland, Switzerland and Cambridge, UK.

International Union For Conservation of Nature, IUCN / Species Survival Commission, SSC (1995). Guidelines for re-introductions. Gland, Switzerland.

Jahrl, J. (1995). Marking behaviour of *Lutra lutra* in Austria. *Habitat*, 11**:** 131-134.

Jalanka, H.H. (1989). Medetomidine-and ketamine induced immobilization of snow leopards (*Panthera uncia*): doses, evaluation, and reversal by atipemazole. *Journal of Zoo and wildlife Medicine*, 20: 154-162.

Jalanka, H.H. and Roeken, B.O. (1990). The use of medetomidine, medetomidine-ketamine combinations, and atipemazole in non domestic mammals. *Journal of Zoo and Wildlife Medicine*, 21: 259-282.

Jameson, R.J.; Kenyon, K.W.; Johnson, A.M. & Wight, H.M. (1982). History and status of translocated Sea otter populations in North America. *Wild. Soc. Bull,* 10(2): 100-107.

Jansman, H.A.H.; Chanin, P.R.F. & Dallas, J.F. (2001). Monitoring otter populations by DNA typing of spraints. *IUCN Otter Specialist Group Bulletin*, 18: 11-18.

Jefferies, D.J. (1986). The value of otter (*Lutra lutra*) surveying using spraints: an analysis of its success and problems in Britain. *J. Otter Trust*, 1985: 25-32.

Jefferies, D.J., Wayre, P., Jessop, R.M.; Mitchell-Jones, A.J. & Medd, R. (1987). The composition, age, size and pre-release treatment of the groups of otters *Lutra lutra* used in the first releases of captive-bred stock in England. *J. Otter Trust*, 1986: 11-16.

Jefferies, D.J., Wayre, P., Jessop, R.M. & Mitchell-Jones, A.J. (1986). Reinforcing the native otter *Lutra lutra* population in East Anglia: an analysis of the behaviour and range development of the first release group. *Mammal Rev.*, 16 (2): 65-79.

Jenkins, D. & Gorman, M.L. (1981). Anaesthesia of the European otter (*Lutra lutra*) using ketamine hydrochloride. *Journal of Zoology*, 194: 265-267.

Jensen, S., Kihlström, J.E., Olsson, M., Lundberg, C. & Örberg, J. (1977). Effects of PCB and DDT on mink (*Mustela vison*) during the reproductive season. *Ambio*, 6: 65-79.

Jessop, R.M. & Cheyne, D.L. (1992). The reintroduction of European otter into Lowland England carried out by the Otter Trust 1983-1992: a progress report. *Otters, Journal of the Otter Trust*, 6: 11-16.

Jiménez, I. (2000). *Los manatíes del río San Juan y los canales de Tortuguero*. Amigos de la Tierra. Cosa Rica. 111 pp.

Jiménez, J. & Lacomba, I. (1991). The influence of water demands on otter (*Lutra lutra*) distribution in Mediterranean Spain. *Habitat*, 6: 249-255.

Jiménez, J. & Palomo, J.J. (1998). Utilización de refugios por la nutria en el río Bergantes (cuenca del Ebro). *Galemys,* 10: 167-173.

Jiménez, J., Ruiz-Olmo, J. & Pascual, A. (1998). Uso del espacio por la nutria en un río mediterráneo (Bergantes, cuenca del Ebro). *Galemys,* 10: 201-209.

Jorgensen, L.A. (1978). *Handbook of Environmental Data and Ecological Parameters*. Editorial board: M.B. Friis, J. Henriksen. Norway.

Johnson, S.A. & Berkley, K.A. (1999). Restoring river otters in Indiana. *Wildlife Society Bulletin*, 27(2): 419-427.

Junge, C.O. & Libosvarskî, J. (1965). Effects of size selectivity on population estimates based on successive removals with electrical fishing gear. *Zool. listy.* 14, 2: 171-178.

Kennedy, G.J.A. & Strange C.D. (1981). Efficiency of Electric Fishing for Salmonids in Relation to River Width. *Fish. Mgmt* 12, No.2: 55-60.

Kilikidis, S.D., Kamarianos, A.P. & Karamanlis, X.N. (1992). Seasonal fluctuations of organochlorine compounds in the water of the Strimon river (N. Greece). *Bulletin of Environmental Contamination and Toxicology*, 49: 375-380.

Kleiman, D.G. (1989). Reintroducing of captive mammals for conservation. *BioScience*, 39(3): 152-161.

Kleiman, D.G. (1994). Criteria for reintroductions. In: Olney, P.S.J.; Mace, G.M. & Feistner, A.T.C. (eds). *Creative Conservation: Interactive management of wild and captive animals.* Chapman & Hall, London.

Klop, E.; Lodewikus, C.; Schonck, S. & Weterings, M. (1998). The reintroduction of the Eurasian otter *(Lutra lutra*) in The Netherlands: a population viability analysis, using the stochastic simulation programs Alex and Vortex. unpublished.

Koepfli, K.P. & Wayne, R.K. (1998). Phylogenetic relationships of otters (Carnivora: Mustelidae) based on mitochondrial cytochrome B sequences. *Journal of Zoology*, 246: 401-416.

Kranz, A. (1995). *On the Ecology of Otters (Lutra lutra) in Central Europe*. Ph. D. Thesis. University of Agriculture of Vienna, Vienna.

Kranz, A. (1996). Variability and seasonality in sprainting behaviour of otters *Lutra lutra* on a highland river in Central Europe. *Lutra*, 38: 33-44.

Kreeger, T.J., Callahan, M. & Beckel, M. (1996). Use of medetomidine for chemical restraint of captive gray wolves (*Canis lupus*). *Journal of Zoo and Wildlife Medicine*, 27: 507-512.

Kruuk, H. (1995). *Wild Otters. Predation and Populations.* Oxford University Press. Oxford. 304 pp.

Kruuk, H. (1997). The significance of PCBs in otters: a reply. *IUCN Otter Spec. Group Bull.*, 14: 54-56.

Kruuk, H., Carss, D.N., Conroy, J.W.H. & Durbin, L. (1993). Otter (*Lutra lutra* L.) numbers and fish productivity in rivers in north-east Scotland. *Symp. Zool. Soc. Lond.*, 65: 171-191.

Kruuk, H. & Carss, D.N. (1996). Costs and benefits of fishing by a semi-aquatic carnivore. The otter *Lutra lutra*. In: Greenstreet, S.P.R. & Tasker, M.L. (eds). *Aquatic predators and they prey*: 10-16. Fishing New Books.

Kruuk, H. & Conroy, J.W.H. (1987). Surveying Otters *Lutra lutra* Populations: A discussion of Problems With Spraints. *Biological Conservation*, 41: 179-183.

Kruuk, H., Conroy, J.W.H., Glimmerveen, U. & Ouwererk, E.J. (1986). The Use of Spraints to Survey Populations of Otters *Lutra lutra.* *Biological Conservation*, 35: 187-194.

Kruuk, H., Groman, M. & Parish, T. (1980). The use of 65-Zn for estimating populations of carnivores. *Oikos*, 34: 206-208.

Kruuk, H., Moorhouse, A., Conroy, J.W.H., Durbin, L. & Frears, S. (1989). An Estimate of Numbers and Habitat Preferences of Otters *Lutra lutra* in Shetland, UK. *Biological Conservation*, 49: 241-254.

Kruuk, H. & Conroy, J.W.H. (1991). Mortality of otters (*Lutra lutra*) in Shetland. *Journal of Applied Ecology*, 28: 83-94.

Kruuk, H. & Conroy, J.W.H. (1996) Concentrations of some organochlorines in otters (*Lutra lutra* L.) in Scotland: Implications for populations. *Environmental Pollution*, 92: 165-171.

Kuiken, T. (1988). Anaesthesia in the European otter (*Lutra lutra*). *Veterinary Record,* 123: 59.

Lacy, R.C. (1993). VORTEX: a computer simulation model for population viability analysis. *Wildlife Research*, 20: 45-65.

Lacy, R.C. & Clark, T.W. (1990). Population viability assessment of the eastern barred bandicoot in Victoria. In: Clark, T.W. & Seebeck, J.H. (eds). *The management and conservation of small populations.* Chicago Zoological Society, Brookfields, Illinois.

Lacy, R.C. & Clark, T.W. (1993). Simulation modelling of American marten (*Martes americana*) populations: vulnerability to extinction. *Great Basin Naturalist*, 53(3): 282-292.

Lejeune, A. & Frank, V. (1990). Distribution of *Lutra macullicolis* in Rwanda. Ecological constraints. *IUCN Otter Spec. Group Bull.*, 5: 8-16.

Lenton, E.J., Chanin, P.R.F. & Jefferies, D.J. (1980). *Otter Survey of England 1977-79.* Nature Conservance Council, London.

Lenvik, K.; Steinnes, E. & Pappas, A.C. (1978). Contents of some heavy metals in Norwegian rivers. *Nordic Hydrol.*, 9: 197-206.

Lewis, J.C.M. (1991). Reversible immobilisation of Asian small-clawed otters with medetomidine and ketamine. *Veterinary Record*, 128: 86-87.

Lindenmayer, D.B.; Clark, T.W.; Lacy, R.C. & Thomas, V.C. (1993). Population Viability Analysis as a tool in wildlife management: a review with reference to Australia. *Environmental Management*, 17(6): 745-758.

Lindenmayer, D.B. & Lacy, R.C. (1995). Metapopulation viability of Leadbeater's Possum, *Gymnobelideus leadbeateri*, in fragmented old-growth forests. *Ecological Applications*, 5(1): 164-182.

Littlejohn, J.W. & Melvin, M.A.L. (1991). Sheep-dips as a source of pollution of freshwaters. A study in Grampian Region. *Journal IWEM*, 5: 21-27.

Lobón-Cervià, J., Bernat, Y. & Rincón, P.A. (1990). Effects of eel (*Anguilla anguilla* L.) removals from selected sites of a stream on its subsequent densities. *Hydrobiologia* 206: 207-216.

Lobón-Cervià, J. & Utrilla, C.G. (1992). A simple model to determine stream trout (*Salmo trutta* L.) densities based on one removal with electrofishing. *Fisheries Research*, 15: 369-378.

López-Martín, J.M., Ruiz-Olmo, J.; Borrell, A. & Nadal. J. (1993). Nivells de compostos organoclorats als peixos d'aigües continentals de Catalunya. Unpublished report. DARP. Generalitat de Catalunya.

López-Martín, J.M. & Ruiz-Olmo, J. (1996). Organochlorine residue levels and bioconcentration factors in otters (*Lutra lutra* L.) from Northern Spain. *Bulletin of Environmental Contamination and Toxicology*, 57: 532-535.

López-Martín, J.M., Ruiz-Olmo, J. & Borrell, A. (1995). Levels of organochlorine compounds in freshwater fish from Catalonia, NE Spain. *Chemosphere*, 31(6): 3523-3535.

López-Martín, J.M., Jiménez, J. & Ruiz-Olmo, J. (1998). Caracterización y uso del hábitat por parte de la nutria (*Lutra lutra*) en un río de tipo Mediterráneo. *Galemys*, 10: 175-190.

López-Nieves, P.; Prenda, J. & Bravo, R. (1998). Distribución espacial de la nutria (*Lutra lutra*) en la provincia de Córdoba en el período 1992-96: el uso de hábitat en relación con la disponibilidad de agua. *Cuzna*, 1: 137-147.

Macdonald, S.M. & Mason, C.F. (1983). Some factors influencing the distribution of the otters (*Lutra lutra*). *Mammal Rev.*, 13: 1-10.

Macdonald, S.M. & Mason, C.F. (1985). Otters, their habitat and conservation in Northeast Greece. *Biological Conservation*, 31: 191-210.

Macdonald, S.M. & Mason, C.F. (1987). Seasonal Marking in an Otter Population. *Acta Theriol.*, 32(2): 449-462.

Macdonald, S.M. & Mason, C.F. (1994) *Status et besoins de conservation de la loutre (Lutra lutra) dans le Paléarctic occidental.* Sauvegarde de la nature, nº 67. Conseil de l'Europe, Strasbourg.

Madsen, A.B.; Dietz, H.H.; Henriksen, P. & Clausen, B. (1999). Survey of Danish free living otters *Lutra lutra.* A consecutive collection and necropsy of dead bodies. *IUCN Otter Spec. Group Bull.*, 16: 65-75.

Mahon, R. (1980). Accuracy of catch-effort methods for estimating fish density and biomass in streams. *Env. Biol. Fish.* vol. 5, 4: 343-360.

Mann, R.H.K. & Penczak, T. (1986). Fish production in rivers: A review. *Pol. Arch. Hydrobiol.* 33: 233-247.

Mason, C.F. (1989) Water pollution and otter distribution: A review. *Ultra*, 32: 97-131.

Mason, C.F. (1993) Organochlorine pesticide residues and PCBs in eels (*Anguilla anguilla*) from some British freshwaters reedbeds. *Chemosphere*, 26: 2289-2292.

Mason, C.F. (1997). The significance of PCBs in otters at national and regional scales. *IUCN Otter Spec. Group Bull.*, 14: 3-12.

Mason, C.F. & Macdonald, S.M. (1986). *Otters: ecology and conservation. Cambridge University Press*. Cambridge.

Mason, C.F. & Macdonald, S.M. (1987). The Use of Spraints for Surveying Otter *Lutra lutra* Populations: An Evaluation. *Biological Conservation*, 41: 167-177.

Mason, C.F. & Macdonald, S.M. (1991). Assessment of Otter (*Lutra lutra*) Survey Methods Using Spraints. *Habitat*, 6: 167-170.

Mason, C.F. & Macdonald, S.M. (1993). Impact of organochlorine pesticide residues and PCBs on otters (*Lutra lutra*): a study from western Britain. *Science of Total Environment*, 138: 127-145.

Mason, C.F. & Macdonald, S.M. (1994) PCBs and organochlorine pesticide residues in otters (*Lutra lutra*) and otter spraints from SW England and their likely impact on populations. *The Science of the Total Environment*, 144: 305-312.

Mateo, R., Saavedra, D. & Guitart, R. (1999). Reintroduction of the otters *(Lutra lutra)* into Catalan rivers, Spain: Assessing organochlorine residue exposure through diet. *Bulletin of Environmental Contamination and Toxicology*, 63: 248-255.

Meffe, G.K. (1984). Effects of abiotic disturbance on coexistence of predator-prey fish species. *Ecology*, 65(5): 1525-1534.

Melquist, W.E. & Hornocker, M.G. (1979). Methods and techniques for studying and censusing River Otter populations. *Forest, Wildlife, and Range Technical Reports*, 8. Univ. Idaho. Moscow. Idaho.

Melquist, W.E. & Hornocker, M.G. (1983). Ecology of River Otters in West Central Idaho. *Wildlife Monographs*, 83**:** 1-60.

Mhalanga, A.T. & Madziva, T.J. (1990). Pesticide residues in Lake Mcllwaine, Zimbabwe. *Ambio*, 19(8): 368-372.

Miliadis, G.E. (1993). Gas chromatographic determination of pesticides in natural waters of Greece. *Bulletin of Environmental Contamination and Toxicology*, 50: 247-252.

Miller, P.S. & Lacy, R.C. (1999). VORTEX: *A stochastic simulation of the extinction process. Version 8 user's manual*. Apple Valley, MN: Conservation Breeding Specialist Group (SSC/IUCN).

Murphy, P.G. (1972) Sulfuric acid for the cleanup of animal tissues for analysis of acid-stable chlorinated hydrocarbon residues. *Journal of the Association of Official Analytical Chemists*, 55: 1360-1362.

Naves, J.; Wiegand, T.; Fernández, A. & Stephan, T. (1999). *Riesgo de extinción del oso pardo cantábrico: la población occidental.* Fundación Oso de Asturias. 284 pp.

Niewold, F. (2000). Population viability analysis of the reintroduction of the Eurasian otter *(Lutra lutra*) in The Netherlands using the simulation programs Vortex. Unpublished.

Novellie, P.A.; Millar, P.S.; & Lloyd, P.H. (1996). The use of VORTEX simulation models in a long term programme of re-introduction of an endangered large mammal, the Cape mountain zebra (*Equus zebra zebra*). *Acta Oecologica*, 17: 657-671.

Nwankwoala, A.U. & Osibanjo, O. (1992). Baseline levels of selected organochlorine pesticide residues in surface waters in Ibadan (Nigeria) by electron capture gas chromatography. *The Science of the Total Environment*, 119: 179-190.

Parera, A. (1996). Estimating River Otter *Lutra longicaudis* Population in Iberá Lagoon Using Direct Sightings Methodology. *IUCN Otter Spec. Group Bull.*, 13(2): 77-83.

Penczak, T., Lobón-Cervià, J., O'Hara, J. & Jakubowski, H. (1986). Production and Food Consumption by Fish Populations in the Pilawa and Dobrzyca Rivers, North Poland. *Pol. Arch. Hydrobiol.* 33, 3/4: 345-372.

Pérez, M.; Viñas, J.; Mañas, F.; Batet, A.; Ruiz-Olmo, J. & Pla, C. (2001). Caracterización genética de la nutria (*Lutra lutra*) en la Península Ibérica. *Actas de las V Jornadas de la Sociedad Española de Conservación y Estudio de Mamíferos*: 110-111.

Prenda, J. & Granado-Lorencio, C. (1996). The relative influence of riparian habitat structure and fish availability on otter (*Lutra lutra*) sprainting activity in a small mediterranean catchment. *Biological Conservation*, 76: 9-15.

Prenda, J. & López-Nieves, P. (1999). La nutria, un indicador ecológico de la calidad de los ríos de la península ibérica. *Quercus,* 161: 10-16.

Prenda, J.; López-Nieves, P. & Bravo, R. (2001). Conservation of otter *Lutra lutra* in a Mediterranean area: the importance of habitat quality and temporal variation in water availability. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 11: 343-355.

Radwan, S.; Kowalik, W. & Kowalczyk, C. (1990). Occurrence of heavy metals in water, phytoplankton and zooplankton of a mesotrophic lake in eastern Poland. *The Science of the Total Environment*, 96: 115-120.

Ramsden, R.O., Coppin, P.F. & Johnston, D.H. (1976). Clinical observation on the use of ketamine hydrochloride in wild carnivores. *Journal of Wildlife Diseases*, 12: 221-225.

Regulatory Act (1984). Maximum permissible concentrations of hazardous substances in fresh waters and coastal sea-waters. *Narodne novine* 2: 7-11.

Reid, D.G., Melquist, W.E., Woolington, J.D. & Noll, J.M. (1986). Reproductive effects of intraperitoneal transmitter implants in river otters. *Journal of Wildlife Management*, 50: 92-94.

Reid, D.G., Bayer, M.B., Code, T.E. & McLean, B. (1987). A possible Method for Estimating River Otter, *Lutra canadensis*, Populations Using Snow Tracks. *Canadian Field-Naturalist*, 101(4): 576-580.

Reuther, V.C. & Brandes, B. (1984). Occurrence of hyperthermia during immobilization of European otter (*Lutra lutra*) with ketamine hydrochloride. *Deutsche Tieräztliche Wochenschrift,* 91: 66-68.

Reuther, C. (1998). Reintroduction of otters: support or risk for otter conservation? *IUCN Otter Spec. Group Bulletin*, 15(2): 71-79.

Reuther, C.; Dolch, D.; Green, R.; Jahrl, J.; Jefferies, D.J.; Krekemeyer, A.; Kucerova, M.; Madsen, A.B.; Romanowski, J.; Roche, K.; Ruiz-Olmo, J.; Teubner, J. & Trindade, A. (2000). Surveying and monitoring distribution and population trends of the Eurasian otter *(Lutra lutra*). Guidelines and evaluation of the standard method for surveys as recommended by the European section of the IUCN/SSC Otter Specialist Group. *Habitat,* 12: 1-148.

Romanowski, J. & Brzezinski, M. (1997). How standard is the satandard technique of the Otter Survey?. *IUCN Otter Spec. Group Bull.*, 14(2): 57-61.

Romanowski, J., Brezezinski, M. & Cygan, J.P. (1996). Notes on the technique of the otter field survey. *Acta Theriologica*, 41: 199-204.

Rosoux, R. (1995). Cycle journalier d'activités et utilisation des domaines vitaux chez la loutre d'Europe (*Lutra lutra*) dans le Marais Poitevin (France). *Cahiers d'Ethologie*, 15: 283-306.

Rosoux, R., Tournebize, T. & Maurin, H. (1996). Etude de la répartition de la loutre d'Europe (*Lutra lutra*) en France. Actualisation 1993. *Cahiers d'Ethologie*, 15 (2-3-4): 195-206.

Rowe-Rowe, D.T. (1992). Survey of South African otters in a freshwater habitat, using sign. *South African Journal of Wildlife Research*, 22(2): 49-55.

Ruiz-Olmo, J. (1985). *Distribución, requerimientos ecológicos y alimentación de la nutria (Lutra lutra) en el N.E. de la Península Ibérica*. Tesis de licenciatura. Universitat de Barcelona.

Ruiz-Olmo, J. (1994). Reproducción y observación de grupos de nutria (*Lutra lutra*) en el norte de España. *Miscel·lània Zoológica*, 17: 225-229.

Ruiz-Olmo, J. (1995*a*). Visual Eurasian otter census in freshwaters: a new method. *Habitat*, 11: 125-130.

Ruiz-Olmo, J. (1995*b*). *Estudio bionómico sobre la nutria (Lutra lutra L., 1758) en aguas continentales de la Península Ibérica.* Ph.D. Thesis. University of Barcelona.

Ruiz-Olmo, J. (1998). Influence of altitude on the distribution, abundance and ecology of the otter (*Lutra lutra*). In: Dunstone, N. & Gorman, M. (eds). *Behaviour and Ecology of Riparian Mammals:* 114-116. Cambridge Univ. Press, Cambridge.

Ruiz-Olmo, J. (in press). Modelling otter population viability in Mediterranean rivers. *The Journal of Wildlife Research*.

Ruiz-Olmo,J. (2001). *Pla de conservació de la llúdriga a Catalunya: biologia i conservació*. Documents dels Quaderns de Medi Ambient, 6. 145 pp.

Ruiz-Olmo, J. & Aguilar, A. (1995). *Els grans mamífers de Catalunya*. Lynx edicions. Barcelona.

Ruiz-Olmo, J. & Delibes, M. (1995) Recherches sur la loutre (*Lutra lutra*) et son statut en Espagne. *Cahiers d'Ethologie*, 15: 169-180.

Ruiz-Olmo, J. & Delibes, M. (1998). *La nutria (Lutra lutra L.) en España ante el horizonte del año 2000.* SECEM, Málaga.

Ruiz-Olmo, J., Delibes, M., Saavedra, D. & Zapata, S.C. (1997). Morfometría externa, demografía y causas de mortalidad de la nutria en la Península Ibérica. *Actas de las III Jornadas Españolas de Conservación y Estudio de Mamíferos*: 109.

Ruiz-Olmo, J. & Gosálbez, J. (1988). Distribution of the Otter (*Lutra lutra* L., 1758) in the N.E. of the Iberian Peninsula. *Publ. Dept. Zool. Barcelona*, 14: 121-132.

Ruiz-Olmo, J. & Gosálbez, J. (1997). Observations on the sprainting behaviour of the otter (*Lutra lutra* L. 1758) in the NE Spain. *Acta Theriologica*, 42: 259-270.

Ruiz-Olmo, J., Jiménez, J. & Lacomba, I. (1991). Lenght of hydrographic basins and population viability of the otters in rivers in eastern Spain. *Habitat*, 6: 255-258.

Ruiz-Olmo, J., Jiménez, J. & López-Martín, J.M. (1995). Radio-tracking of otters *Lutra lutra* in North-Eastern Spain. *Lutra*, 37: 1-6.

Ruiz-Olmo, J.; Jiménez, J.; Palazón, S. & López-Martín, J.M. (2002). Ecologie et conservation de la loutre (*Lutra lutra*) et du vison d'Europe (*Mustela lutreola*) aux milieux mediterranées. *Actes du XVIII Colloque Francophone de Mammalogie.*

Ruiz-Olmo, J.; Lafontaine, L.; Prignioni, C.; López-Martín, J.M. & Santos-Reis, M. (in press). The pollution and their effects on otter populations in Southwestern Europe. *Proceedings of the Toxicology Otter Conference, Skye, September 2000.*

Ruiz-Olmo, J. & López-Martín, J.M. (1994) Otters and pollution in North-East Spain. In *Seminar on the conservation of the European Otter (Lutra lutra)*, pp. 144-146. Council of Europe, Strasbourg.

Ruiz-Olmo, J.; López-Martín, J.M. & Delibes, M. (1998). Otters and pollution in Spain. In: Dunston, N. & Gorman, M., eds. *Behaviour and ecology of riparian mammals.* Cambridge University Press. Cambridge: 325-338.

Ruiz-Olmo, J.; López-Martín, J.M. & Palazón, S. (2001). The influence of fish abundance on the otter (*Lutra lutra*) populations in Iberian Mediterranean habitats. *Journal of Zoology*, 254: 325-336.

Ruiz-Olmo, J. & Oró, D. (1993). La nutria (*Lutra lutra*) en las zonas húmedas litorales de Catalunya, N.E. Ibérico. *Revista del Parc Nat. Delta de l'Ebre*, 8: 36-41.

Ruiz-Olmo, J. & Palazón, S. (1997). The diet of the European otter (*Lutra lutra*) in Mediterranean freshwater habitats. *Journal of Wildlife Research*, 2(2): 171-181.

Ruiz-Olmo, J., Palazón, S., Bueno, F., Bravo, C., Munilla, I. & Romero, R. (1997). Distribution, status and colonization of the American mink *Mustela vison* in Spain. *Journal of Wildlife Research*, 2(1): 30-36.

Ruiz-Olmo, J.; Saavedra, D. & Jiménez, J. (2001). Testing the surveys and visual and track censuses of Eurasian otters (*Lutra lutra*). *Journal of Zoology*, 253: 359-369.

Ruiz, X. & Llorente, G.A. (1991) Seasonal variation of DDT and PCB accumulation in muscle of carp (*Cyprinus carpio*) and eels (*Anguilla anguilla*) from the Ebro delta, Spain. *Vie et Milieu*, 41: 133-140.

Saavedra, D. & Sargatal, J. (1993). El Projecte Llúdriga: Aiguamolls de l'Empordà i conques de la Muga i del Fluvià. 180 pp. Unpublished.

Saavedra, D. (1995). Estudi de viabilitat de la reintroducció de la llúdriga al Parc Natural dels Aiguamolls de l'Empordà i conques de la Muga i del Fluvià. Informe Projecte Llúdriga. 144 pp. Unpublished.

Saavedra, D., Ruiz-Olmo, J. & Jiménez, J. (1997). Areas de campeo y relaciones sociales de la nutria (*Lutra lutra*) en aguas continentales del NE de la Península Ibérica. *Actas de las III Jornadas Españolas de Conservación y Estudio de Mamíferos*: 112.

Saavedra, D., Sargatal, J. & Llobet, T. (1995). *El retorn de la llúdriga*. Department of Environment, Government of Catalonia. Barcelona.

Saavedra, D. & Sargatal, J. (1996). Memoria de la reintroducción piloto de nutrias (*Lutra lutra*) en los Aiguamolls de l'Empordà. Aiguamolls de l'Empordà Natural Park. Unpublished.

Saavedra, D. & Sargatal, J. (1997). Reintroducción de la nutria en la provincia de Girona. *Actas de las III Jornadas Españolas de Conservación y Estudio de Mamíferos*: 113.

Saavedra, D. & Sargatal, J. (1998). Reintroduction of the otter *(Lutra lutra)* in Northeast Spain (Girona province). *Galemys* 10: 191-199.

Saavedra, D. & Sargatal, J. (2001). Història del Projecte Llúdriga. *El Bruel*, 36: 4-10.

Salbu, B.; Pappas, A.C. & Steinnes, E. (1979). Elemental composition of Norwegian rivers. *Nord. Hydrol.*, 10: 115-140.

Sargatal, J. & Félix, J. eds. (1989). *Els Aiguamolls de l'Empordà : aspectes ecològics, històrics i socials.* Carles Vallès editor. Figueres.

Schmitt, C.J. & Brumbaugh, W.G. (1990). National Contaminant Biomonitoring Program: Concentrations of Arsenic, Cadmium, Copper, Lead, Mercury, Selenium, i Zinc in US Freshwater Fish, 1976-1984. *Archives of Environmental Contamination and Toxicology*, 19: 731-747.

Schnute, J. (1983). A new approach to estimating populations by the removal method. *Can. J. Fish. Aquar. Sci.*, 40(12): 2153-2169.

Schulz, D.E., Petrick, G. & Duinker, J.C. (1989). Complete characterisation of polychlorinated biphenyl congeners in commercial Aroclor and Clophhen mixtures by multidimensional gas chromatography-electron capture detection. *Environmental Science and Technology*, 23: 852-859.

Seber, G.A.F. (1982). *The estimation of animal abundance and related parameters.* Griffin. London.

Serfass, T.L. & Rymon, L.M. (1985). Success of river otter reintroduced in Pine Creek drainage in northcentral Pennsylvania. *Transactions of the Northeast Section of the Wildlife Society (4th Annual Northeast Fish and Wildlife Conference)*: 138-148.

Serfass, T.L.; Brooks, R.P. & Rymon, L.M. (1993). Evidence of long-term survival and reproduction by translocated river otters, *Lutra candensis*. *The Canadian Field-Naturalist*, 107: 59-63.

Serfass, T.L., Peper, R.L., Whary, M.T. and Brooks, R.P. (1993). River otter (*Lutra canadensis*) reintroduction in Pennsylvania: prerelease care and clinical evaluation. *Journal of Zoo and Wildlife Medicine*, 28: 28-40.

Serfass, T.L., Brooks, R.P., Swimley, T.J., Rymon, L.M. & Hayden, A.H. (1996). Considerations for capturing, handling and translocating river otters. *Wildlife Society Bulletin*, 24(1): 25-31.

Shaffer, M.L. (1990). Population Viability Analysis. *Conservation Biology*, 4: 39-40.

Sidorovich, V.E. (1991). Structure, reproductive status and dynamics of the otter population in Byelorussia. *Acta Theriologica*, 36 (1-2): 153-161.

Sidorovich, V.E. (1992). Numbers of otters and approach to population estimation in Byelorussia. *IUCN Otter Spec. Group Bull.*, 7: 13-16.

Siegel, S. (1956). *Nonparametric statistic for the behavioural Sciences*. MacGraw-Hill, New York.

SIFF. (1987). *Kvalitestnormer for drikkevann*. Drikkevann Rep., G2. National Institute of Public Health. Oslo.

Sih, A., Crowley, P., McPeek, M., Petranka, J. & Strommeirer, K. (1985). Predation, competition and prey communities. A review of field experiments. *Ann. Rev. Ecol. Syst.*, 16: 269-311.

Simpson, V.R. (1997). Health status of otters (*Luta lutra*) in south-west England based on postmortem findings. *Veterinary Record*, 141: 191-197.

Sjöåsen, T. (1996). Survivorship of captive-bred and wild-caught reintroduced European otters (*Lutra lutra*) in Sweden. *Biological Conservation*, 76: 161-165.

Sjöåsen, T. (1997). Movements and establishment of reintroduced European otters *Lutra lutra*. *Journal of Applied Ecology*, 34: 1070-1080.

Sjöåsen, T.; Ozolins, J.; Greyerz, E. & Olsson, M. (1997). The otter (*Lutra lutra*) situation in Latvia and Sweden related to PCB and DDT levels. *Ambio*, 26(4): 196-201.

Smith, R.A.; Alexander, R.B. & Wolman, M.G. (1987). Water-quality trends in the Nation's Rivers. *Science*, 235: 1607-1615.

Smith, M.D.; Leonards, P.E.G.; van Hattum, B. & de Jongh, A.W.J.J. (1994). PCBs on European otter (*Lutra lutra*) populations. Institut voor Milieu-vraagstukken. Vrije Universiteit Amsterdam, Amsterdam. 45 pp.

Sostoa, A. (1990). *Història Natural dels Països Catalans. Volum 11, Peixos*. Enciclopèdia Catalana, Barcelona.

Soulé, M.E. ed. (1986). *Conservation biology : the science of scarcity and diversity*. Sinauer Associates. Sunderland, Massachusetts, USA.

Soulé, M.E. ed. (1987). *Viable populations for conservation*. Cambridge University Press. Cambridge (United Kingdom). 189 pp.

South, A.; Rushton, S. & Macdonald, D. (2000). Simulating the proposed reintroduction of the European beaver (*Castor fiber*) to Scotland. *Biological Conservation*, 93: 103-116.

van Ewik, K.Y.; Knol, A.P. & de Jongh, R.C.C.M. (1997). An otter PVA as a preparation of a reintroduction experiment in The Netherlands. *Z. Saugetierkd.*, 62(S2): 238-242.

Spelman, L.H. (1998). North American river otter (*Lutra canadensis*) translocation in North Carolina 1989-1996. *Proceedings of EAZWV and BVZS II Scientific Meeting*, Chester, U.K., May 21-24, 1998: 461-465.

Spelman, L.H., Summer, P.W., Levine, J.F. & Stoskopf, M.K. (1993). Field anesthesia in the North American river otter (*Lutra canadensis*). *Journal of Zoo and Wildlife Medicine,* 24: 19-27.

Spelman, L.H., Summer, P.W., Levine, J.F. & Stoskopf, M.K. (1994). Medetomidine-ketamine anesthesia in the North American river otter (*Lutra canadensis*) and reversal by atipemazole. *Journal of Zoo and Wildlife Medicine*, 25: 214-223.

Spelman, L.H., Summer, P.W., Karesh, W.B. & Stoskopf, M.K. (1997). Tiletamine-zolazepam anesthesia in North American river otters (*Lutra canadensis*) and partial antagonism with flumazemil. *Journal of Zoo and Wildlife Medicine*, 28: 418-425.

Spelman, L.H. (1999). Otter anesthesia. In: Fowler, M. & Miller, E. (eds). *Zoo and Wild Animal Medicine, Current Therapy 4th*. W.B. Saunders Company, Philadelphia, USA: 436-443.

Spinola, R.M.; Serfass, T.L. & Brooks, R.P. (2001). River otter reintroduction in a human-dominated landscape. *Proceedings of the VIII International Otter Colloquium.* Valdivia (Chile).

Strachan, R. & Jefferies, D.J. (1996). *The Otter Survey of England 1991-1994.* The Vincent Wildlife Trust, London. 223 pp.

Summer, P.W. (1995). *Techniques utilized and developed for the restoration of river otters in North Carolina.* North Carolina Wildlife Resources Comission rapport. 43 pp.

Suzuki, T.; Yuasa, H. & Machida, Y. (1996). Phylogenetic position of the Japanese river otter *Lutra nippon* inferred from the nucleotide sequence of 224b of the mitochondrial cytochrome b gene. *Zool. Sci.*, 13: 44-55.

Tango, P.J.; Michael, E.D. & Cromer, J.I. (1991). Mating and first-season births in interstate transplanted river otters, *Lutra canadensis*. *Brimleyana*, 17: 53-55.

Targa, J. (2000). *Reintroduction of otters in north-east Catalonia (Spain). A practical review.* Unpublished project. Oxford Brookes University.

Taylor, P.S. & Kruuk, H. (1990). A record of an otter (*Lutra lutra*) natal den. *J. Zool., Lond.,* 222: 689-692.

Trefry, J.H. (1985). A decline in lead transport by the Mississippi River. *Science*, 280: 439-441.

Trindade, A., Farinha, N. & Florêncio, E. (1998). *A distribuçao da lontra Lutra lutra em Portugal. Situaçao em 1995.* Istituto da Conservaçao do Ambiente, Lisboa.

Turekian, K.K. (1969). The oceans, stream and atmosphere. In: K.H. Wedepohl ed.. *Handbook of Geochemistry, vol. 1.* Springer-Verlag, Berlin: 297-323.

United States Federal Register (1985). Part IV. Environmental Protection Agency. National Primary Drinking Water Regulation; Synthetic Organic Chemicals, Inorganic Chemicals and Micro-organisms; Proposed Rule. 40 CFR Part 141. Federal Register vol. 50 n. 219, November 3rd 1985: 46936-47022.

Van Zyll de Jongh, C.G. (1991). A brief review of the systematics and classification of the Lutrinae. *Habitat*, 6: 79-84.

Veiereov, D. & Aharonson, N. (1980). Improved cleanup of large lipid samples for electron capture gas chromatographic quantitation and gas chromatographic-mass spectrometric confirmation of organochlorine residues. *Journal of the Association of Official Analytical Chemists*, 63: 202-207.

Vogt, P. (1994). *Husbandry Guidelines for Lutra lutra*. European Endangered Species Program, EEP, (Studbook for *Lutra lutra*). 17 pp.

Wayre, P. (1992). The first twenty-one years of the Otter Trust and its re-introduction programme. *Otters, The Journal of The Otter Trust*, 6: 17-22.

Wemmer, C. & Derrickson, S. (1987). Reintroduction: the zoobiologists dream. Respects and problems of reintroducing captive bred wildlife. *AAZPA 1987 Annual Proceedings*: 48-65.

Williams, T.D. & Siniff, D.B. (1983). Surgical implantation of telemetry devices in the sea otter. *Journal of American Veterinary Medical Association*, 183: 1290-1291.

Williams, T.D., Baylis, D.M., Downey, S.H. & Clark, R.O. (1990). A physical restraint device for sea otters. *Journal of Zoo and Wildlife Medicine*, 21: 105-107.

World Health Organisation (1984). *Guidelines for Drinking Water Quality, vol. 1*. Geneva.

Wozencraft, W.C. (1989). Classification of the recent Carnivora. In: Gittleman, J.L. ed. *Carnivore Behaviour, Ecology and Evolution.* Chapman & Hall. London: 563-569.

Zalewski, M. (1983). The Influence of Fish Community Structure on the Efficiency of Electrofishing. *Fish. Mgmt.*, 14(4): 177-186.

Zamora, L., Saavedra, D. & Moreno, R. (1996). Stock assesment, biomass and fish production in two mediterranean basins (NE Spain). Limnética, 12(1): 47-58.

12. Addenda

12.1. Conclusions (*in catalan)*

1. El projecte de reintroducció de la llúdriga a les conques dels rius Muga i Fluvià ha complert les directrius per a les reintroduccions de la Unió Internacional per a la Conservació de la Natura (UICN). L'estudi de viabilitat es va centrar principalment en els nivells d'organoclorats als peixos de la zona (81-136.4 µg/kg de pes fresc), que van resultar de l'ordre dels calculats a rius de Catalunya amb poblacions de llúdriga, i en la biomassa íctica existent a l'àrea de reintroducció (0.6-351.9 g/m^2), prou elevada com per mantenir una població de llúdrigues superior als cent individus.

2. La reintroducció de la llúdriga a les conques dels rius Muga i Fluvià va reeixir, si observem l'evolució de la distribució de la nova població. La longitud de riu i la superfície d'aiguamolls visitats per la llúdriga va presentar una progressió creixent (483 km de riu i 2900 ha d'aiguamoll just després de completar les translocacions) i l'àrea geogràfica ocupada efectivament també es va incrementar fins a un 64% d'estacions positives a l'hivern 2001-02.

3. La troballa de tres exemplars adults nascuts a l'àrea de reintroducció (sense "transponder" o emissor) és una altra prova que valida l'èxit del programa de reintroducció.

4. La densitat d'exemplars calculada a través dels censos visuals ha resultat baixa (0.04-0.11 llúdrigues/km), però s'aproxima al que hom pot esperar en els primers estadis d'una població reintroduïda, encara poc nombrosa però distribuïda en una gran àrea. En aquest moment (2002), és similar a les densitats trobades en rius oligotròfics dels Pirineus.

5. La mortalitat post-alliberament va ser del 22% un any després de l'alliberament, similar o inferior a la d'altres programes de reintroducció de llúdrigues reeixits. La mortalitat va ser deguda principalment a atropellaments (56%) i més del 50% de les llúdrigues trobades mortes havien estat alliberades feia menys de dos mesos, el que suggereix que els animals que es dispersen molt ràpidament de les zones d'alliberament pateixen una major mortalitat que aquells que romanen a la zona d'alliberament o es dispersen lentament.

6. El patró d'activitat de les llúdrigues reintroduïdes va esdevenir principalment nocturn i crepuscular (84-98%), amb una escassa activitat diürna (2-16%). Les llúdrigues radioseguides van presentar un major període d'activitat a l'hivern que a l'estiu. L'inici diari de l'activitat va ser força constant, mentre que el final de l'activitat va ser molt més variable.

7. Les àrees vitals de les llúdrigues reintroduïdes van ser del mateix ordre (34,2 km) que les calculades en d'altres estudis realitzats a Europa. Les àrees vitals dels mascles van ser clarament més extenses (32%) que la de les femelles, però les diferències entre sexes no van resultar significatives, potser a causa de la petita mida mostral. Les "àrees nucli", definides com la mínima distància lineal que cobreix el 50% de les radiolocalitzacions, van ocupar un 15% de l'àrea vital total.

8. Els paràmetres diaris de moviment també van ser majors en el cas dels mascles. La longitud mitjana de riu recorreguda per una llúdriga durant 24 hores va ser de 4,2 km per les femelles i 7,6 km pels mascles, probablement a causa del comportament de "patrullar" a la recerca de femelles amb qui aparellar-se.

9. Durant el període de radioseguiment dues femelles van criar. Van presentar llargs moviments just abans de parir, reduint considerablement la seva activitat després del naixement de les cries, incrementant-la progressivament durant les setmanes següents i assolint els nivells d'activitat pre-naixement poc abans d'abandonar el cau de cria. Aquestes femelles van canviar els seus moviments diaris després del naixement de les cries, amb àrees vitals i períodes d'activitat menors.

10. La resposta de la població de llúdrigues reintroduïda a les fluctuacions estacionals en la disponibilitat d'aigua, habitual a les regions mediterrànies, va consistir en la concentració en una àrea menor durant el període de sequera estival, a causa de l'increment de trams secs, inhabitables per la llúdriga per la manca d'aliment, fet que va provocar expansions i contraccions periòdiques en l'àrea de distribució. Durant aquestes condicions de manca d'aigua, les llúdrigues no van recórrer llargs trams de riu, sinó que es van situar a prop dels tolls, les úniques i concentrades fonts d'aliment.

11. Els amagatalls seleccionats per la llúdriga van estar envoltats, en la majoria dels casos, per plantes de creixement ràpid que formen masses compactes que baixen fins a la vora de l'aigua, constituint refugis segurs per a les llúdrigues. Aquestes van usar gairebé sempre (82%) un amagatall diferent cada dia.

12. La persistència a llarg termini de la població reintroduïda va ser estudiada mitjançant una Anàlisi de Viabilitat Poblacional (PVA). El resultat va ser un baix risc d'extinció de la població en els propers 100 anys i la majoria dels escenaris simulats (65%) van assolir el criteri d'un mínim de 90% de probabilitat de supervivència.

13. Del model poblacional construït es dedueix que un punt clau per assegurar la viabilitat de la població reintroduïda és la reducció de la mortalitat accidental. Un important increment en aquest paràmetre comporta un espectacular increment de les probabilitats d'extinció de la població. A l'àrea de reintroducció, els atropellaments causen més del 50% de la mortalitat i aquesta pot ser reduïda mitjançant la construcció de passos de fauna, el tancament lateral d'alguns trams de carretera perillosos i el control de la velocitat en algunes vies.

14. El projecte de reintroducció a les conques dels rius Muga i Fluvià ha posat a punt un protocol per a la captura, maneig i alliberament de llúdrigues salvatges, que pot contenir informació útil per a programes similars.

15. La reintroducció de la llúdriga a les conques dels rius Muga i Fluvià ha suposat una oportunitat única d'estudiar una població dissenyada artificialment i poder comparar diversos mètodes per estimar la distribució i la densitat de poblacions de llúdrigues. Les conclusions principals són la utilitat dels "otter survey" en la detecció de la presència de la llúdriga i la validesa dels censos visuals en l'estimació de l'abundància de llúdrigues en un tram de riu.

16. Finalment, la reintroducció portada a terme a les conques dels rius Muga i Fluvià ha aconseguit crear una nova població de llúdrigues, que persisteix en el temps, que es reprodueix regularment i que es dispersa progressivament, fins i tot a noves conques fluvials.

12.2. Sumari (*in catalan)*

La preparació i execució del projecte de reintroducció de la llúdriga a les conques dels rius Muga i Fluvià (abreujat com a *GORP* en el text en anglès i com a *Projecte Llúdriga* en el text en català) ha comportat un acurat seguiment de la nova població, que ha involucrat diversos camps de la biologia i la veterinària i que ha provocat la redacció d'aquesta tesi. En els capítols principals de la tesi s'han desenvolupat extensament els aspectes que donen resposta als objectius d'aquest treball i, en aquest apartat final, es resumeixen en un complet però breu sumari.

Objectiu 1. Demostrar la viabilitat de la reintroducció.

Com ja s'ha exposat al capítol 1, la Unió Internacional per a la Conservació de la Natura va publicar unes directrius per a les reintroduccions (IUCN, 1987; 1995) que han de ser observades abans d'autoritzar cap projecte de reintroducció o reforçament. El *Projecte Llúdriga* va redactar un estudi de viabilitat (Saavedra, 1995) per tal de determinar si el projecte complia les esmentades directrius internacionals.

Abans d'entrar en els estudis de la qualitat de l'hàbitat, algunes de les consideracions de la UICN quant a les reintroduccions van ser valorades. Tot i que encara comuna en algunes parts de la seva àrea de distribució, la llúdriga euroasiàtica estava classificada com a Vulnerable (IUCN, 1990). Aquesta espècie habitava l'àrea de reintroducció proposada, és a dir, el Parc Natural dels Aiguamolls de l'Empordà i les conques dels rius Muga i Fluvià (Delibes, 1990; Ruiz-Olmo & Aguilar, 1995; Ruiz-Olmo, 1995; Ruiz-Olmo & Delibes, 1998). Les causes de l'extinció eren ben conegudes: persecució (caça, trampeig), contaminació i destrucció de l'hàbitat (Ruiz-Olmo, 1995; Saavedra, 1995). L'extinció a l'àrea d'estudi havia estat completa en el moment d'iniciar el projecte (Delibes, 1990). Muga i Fluvià eren dues de les conques sense llúdrigues on es considerava que la recolonització espontània de l'espècie era improbable (Ruiz-Olmo & Delibes, 1998) a curt o mig termini. Les poblacions donants proposades (d'Extremadura, Galícia, Astúries i Portugal) es trobaven en bon estat i amb un creixement progressiu dels seus efectius (Ruiz-Olmo & Delibes, 1998; Trindade et al., 1998). Les llúdrigues ibèriques no presentaven diferències morfològiques significatives (Ruiz-Olmo, 1995). Les poblacions donants proposades eren les més properes amb un nombre suficient d'individus i vivien en hàbitats mediterranis comparables. L'extensió de l'àrea proposada era de 200.000 ha, amb més de 600 km de rius i 3.000 ha d'aiguamolls. Considerant les densitats trobades a Catalunya (0,1-1,2 llúdrigues/km; Ruiz-Olmo, 1995), es va calcular l'existència d'hàbitat suficient per una població amb un rang d'efectius d'entre 60 i 720 animals (Saavedra, dades inèdites). La població local humana estava en general a favor de la reintroducció, ja que la llúdriga no lesionava els interessos de cap sector econòmic de la zona (Saavedra, 1995) i, finalment, l'espècie estava totalment protegida a Espanya des del 1973.

Els estudis sobre l'estat de conservació de l'hàbitat van intentar respondre a dues qüestions principals: està el medi massa contaminat com per permetre l'establiment i persistència d'una població de llúdrigues? Hi ha suficients recursos tròfics per a una població de llúdrigues?

Els nivells de substàncies organoclorades als peixos de la zona es van situar dins del rang dels trobats als rius de Catalunya (López-Martín et al., 1995), amb mitjanes aritmètiques en pes fresc de 21 µg/kg de ΣHCHs, 81 µg/kg de ΣDDTs i 181 µg/kg de ΣPCBs. El paper dels organoclorats en la viabilitat de la reintroducció de la llúdriga es va centrar en els PCBs, perquè aquestes substàncies han estat considerades les responsables de l'extinció de la llúdriga en grans àrees (Mason, 1989, Macdonald & Mason, 1994), tot i que encara persisteix la controvèrsia respecte a aquest punt (veure Kruuk, 1997 i Mason, 1997). Els nivells de PCBs es van situar entre els 81 i els 136,4 µg/kg de pes fresc, amb el 41% de les estacions de mostratge amb valors superiors als 110 µg/kg de pes fresc (Mateo et al., 1995). Els peixos amb nivells inferiors a aquest "llindar de perillositat" viuen en rius catalans amb bones poblacions de llúdrigues (Ruiz-Olmo & López-Martín, 1994; Ruiz-Olmo, 1995). Els nivells elevats de ΣPCBs van ser detectats al llarg del riu Fluvià, especialment en el tram alt, així com en alguns punts de la conca de la Muga. Tanmateix, els punts de les llacunes litorals i la major part de la conca de la Muga presentaven nivells de PCBs per sota dels observats als rius catalans on les llúdrigues són presents (Ruiz-Olmo & López-Martín, 1994). Així, es va preveure que els recs i les llacunes dels Aiguamolls de l'Empordà podrien ser un bon lloc on iniciar la reintroducció.

La biomassa íctica a l'àrea de reintroducció proposada va variar entre 0,6 i 351.9 g/m² (n=12), amb només dos trams (17%) amb una biomassa menor de 8 g/m², el mínim valor necessari per suportar una població de llúdrigues als rius mediterranis (Ruiz-Olmo, 1995). Aquests resultats van mostrar que la zona podia mantenir una població de llúdrigues amb una densitat estable, similar a les densitats trobades a d'altres rius de la Península Ibèrica (Saavedra, 1995). La capacitat de càrrega, depenent de la biomassa de peixos, es va establir en 0,4 a 0,9 llúdrigues/km a trams amb 30-80 g/m² i 0,1-0,3 llúdrigues/km amb biomasses de 10-30 g/m² (Ruiz-Olmo, 1995). Amb aquestes dades, es va establir que l'àrea d'estudi (que no comprenia la totalitat d'ambdues conques, sinó tan sols els rius principals) podia sostenir una població de llúdrigues de 130-160 individus.

Objectiu 2. Demostrar l'èxit de la reintroducció.

Els principals treballs de seguiment van ser dirigits a la verificació de l'èxit o el fracàs de la reintroducció. Els estudis de la persistència de la nova població van ser realitzats mitjançant procediments directes (censos visuals i radioseguiment) i indirectes (xarxa d'estacions de "otter survey").

La reintroducció de la llúdriga a les conques dels rius Muga i Fluvià va reeixir, a jutjar per l'evolució de la distribució de la nova població. La longitud de rius i la superfície d'aiguamolls visitada per la llúdriga va seguir una progressió ascendent, des de 0 km de riu i 500 ha d'aiguamoll a finals de 1995 (47 dies després del primer alliberament) fins a 483 km de riu i 2.900 ha d'aiguamoll a finals del 2000 (moment de finalització de les 41 translocacions). L'àrea geogràfica ocupada efectivament (resultats del "otter survey") va evolucionar des del 0% a l'estiu del 1995 (just abans del primer alliberament) fins al 64% a l'hivern 2001-02, presentant una clara tendència a l'increment.

La colonització es va produir amb celeritat, principalment perquè els primers individus van tendir a visitar llargs trams de l'àrea geogràfica disponible. Així, al 1997, dos anys després de l'inici de la reintroducció i amb només 23 animals alliberats, ja es recollien dades de radioseguiment, excrements o petjades a més de 300 km de rius i rierols, el que significa la pràctica totalitat de les conques Muga i Fluvià. I més del 50% de les estacions de "otter survey" eren ja positives. Aquest procés es va detenir momentàniament al 1998, degut realment a la inexistència d'hàbitat disponible per visitar (encara que cal repetir que això no significa que estigués permanentment ocupat). I al 1999 es va produir una acceleració en la colonització, assolint els 400 km de rius i rierols amb un pic del 72% de les estacions de "otter survey" positives. El més notable és que aquest increment en el nombre de quilòmetres colonitzats va ocórrer com a conseqüència de la dispersió a les conques veïnes (Ter al sud i Tec al nord) i que va succeir precisament l'únic any sense nous alliberaments, de forma que els responsables parcials de la dispersió van poder ser els joves o subadults nascuts ja a l'àrea de reintroducció. De fet, el 1999 va ser el primer any amb un nombre important de rastres de cries localitzats (cinc).

Les densitats trobades mitjançant els censos visuals van ser baixes (0.04 - 0.11 llúdrigues/km), però es van aproximar al que es pot esperar en les primeres fases d'una població reintroduïda, encara petita però estesa per una gran àrea. En aquest moment, són similars a les densitats trobades a rius oligotròfics dels Pirineus (Ruiz-Olmo, 2001). L'evolució de les densitats totals segons els censos visuals i segons el càlcul teòric dels exemplars alliberats és similar, però els valors són més alts pels calculats mitjançant els censos visuals, potser perquè els censos visuals han estat realitzats en els trams de presència més regular i continuada de llúdrigues.

La recerca realitzada sobre la mortalitat de la població reintroduïda ha servit també per verificar la persistència de la població. La mortalitat post-alliberament va ser del 22% (després d'un any de l'alliberament), similar o menor a d'altres programes de reintroducció de llúdrigues reeixits. Tota la mortalitat va ser a causa d'accidents (principalment atropellaments, amb un 56%) i no es va detectar persecució humana, aspecte molt important per a l'èxit del projecte. A més, més del 50% de les llúdrigues es van trobar mortes abans dels dos mesos de l'alliberament, el que suggereix que els animals que es dispersen molt ràpidament (i habitualment molt lluny) de les zones d'alliberament experimenten una mortalitat més elevada que aquells que romanen als voltants del punt d'alliberament o que es dispersen lentament.

Durant els anys 2000 i 2001 es van trobar tres llúdrigues mortes, totes elles atropellades. Els tres exemplars, una femella i dos mascles, van resultar ser animals adults sense transponder o emissor, el que significa que aquests animals van néixer a l'àrea de reintroducció, com a fills de les llúdrigues translocades. La localització d'aquests exemplars és una altra prova de l'èxit de la reintroducció, ja que aquestes llúdrigues estaven ja en disposició de reproduir-se (i potser ja ho havien fet).

Objectiu 3. Profunditzar en l'estudi d'aspectes ecològics i etològics que tenen en la reintroducció una oportunitat única de disposar d'una població "de disseny".

Només en rares ocasions és possible estudiar individualitzadament a quasi tots els components d'una població nova i artificialment creada de carnívors, en una àrea geogràfica sense cap altre individu de la mateixa espècie. Aquest ha estat el cas del *Projecte Llúdriga*, on l'alliberament de 41 llúdrigues, quasi totes equipades amb emissors, en una àrea on l'espècie s'havia extingit, ha pogut ser emprat per estudiar diversos aspectes de l'ús de l'espai i del temps per part de la llúdriga euroasiàtica en hàbitats mediterranis.

El patró d'activitat de les llúdrigues reintroduïdes va resultar principalment nocturn i crepuscular, amb una escassa activitat diürna. Les llúdrigues radioseguides van presentar un període d'activitat més prolongat a l'hivern que a l'estiu, i l'activitat diürna durant l'hivern va ser també més llarga que a l'estiu, aquest darrer aspecte observat per d'altres autors (Ruiz-Olmo, 1995) coincidint amb temperatures ambientals molt baixes. El període d'inactivitat al mig de la nit va ser observat en algunes ocasions, però no va constituir un patró general. L'emergència o començament de l'activitat va ser força constant i es van trobar diferències estacionals en comparar aquest moment amb la posta de sol. En canvi, la retirada o final de l'activitat va ser molt més variable i no es van trobar diferències estacionals significatives.

Les àrees vitals de les llúdrigues reintroduïdes (radioseguides més de 30 dies) van resultar del mateix ordre (34,2 km) que les trobades a d'altres estudis realitzats a Europa i Amèrica del Nord (en aquest darrer continent amb *Lontra canadensis*) (Melquist & Hornocker, 1979; Green et al., 1984; Kruuk et al., 1993; Kruuk, 1995; Durbin, 1996), però més grans que als estudis realitzats als llocs precisament més similars, com els Pirineus i el riu Bergantes (Ruiz-Olmo et al., 1995; Jiménez et al., 1998). En ambdós casos, la diferència potser va ser deguda al període de radioseguiment, més curt (26 i 20 dies pels Pirineus i 120, 90 i 120 dies pel riu Bergantes) que el del *Projecte Llúdriga*, amb una mitjana de 208 dies (30-631). Com a la majoria dels estudis consultats, les àrees vitals dels mascles van ser clarament majors (32%) que les de les femelles, però les diferències entre sexes no van resultar significatives, potser a causa de la petita mida mostral. Les àrees nucli, definides com la distància lineal mínima que cobreix el 50% de les localitzacions, van ocupar de mitjana un 15% de l'àrea total vital. No es van trobar diferències significatives entre les llúdrigues reintroduïdes i les estudiades a les seves àrees d'origen (estudiades al riu Bergantes, Castelló de la Plana).

Els paràmetres de moviment van ser estudiats seguint la metodologia de Durbin (1996). El "rang" mitjà (tram de riu patrullat per una llúdriga durant el període de seguiment de 24 hores) va ser de 4,2 km per les femelles (n=19) i 7,6 pels mascles (n=11, excloent mascles en rius gairebé secs), resultats menors que els trobats als estudis d'Escòcia i el riu Bergantes. En tots tres casos, els mascles van presentar "rangs" significativament superiors als de les femelles, probablement degut al comportament consistent a patrullar a la recerca de femelles amb qui aparellar-se (Durbin, 1996). D'altra banda, el període d'activitat nocturna va ser molt més prolongat a les nostres llúdrigues (mitjana de 7,6 hores) que a les de l'estudi de Durbin a Escòcia (mitjana de 4,7 hores), potser a causa de diferències metodològiques en l'atribució d'activitat o inactivitat, segons els senyals del receptor de radioseguiment. La velocitat a períodes d'activitat ininterromputs on la llúdriga mantenia la mateixa direcció de progressió (generalment els períodes calculats oscil·laven entre 45 i 90 minuts) va variar entre 0,5 i 4 km/h, amb una mitjana de 2,1 km/h. La velocitat va ser superior riu avall (2,45 km/h, n=28) que riu amunt (1,42 km/h, n=32).

Un dels principals factors ambientals que actuen a les regions mediterrànies és la disponibilitat d'aigua i les grans fluctuacions que pateix al llarg de l'any. La resposta de la llúdriga a aquest factor va ser estudiada mitjançant dues aproximacions diferents: els canvis en la distribució de la població reintroduïda i els moviments diaris per part d'individus específics. La distribució va ser estudiada a través de la xarxa d'estacions de "otter survey" i als mapes elaborats amb aquestes dades es va poder observar com la població es concentrava en una àrea menor durant el període de sequera estival, a causa de l'increment en el nombre de trams secs, inhabitables per la llúdriga degut a la manca d'aliment. Aquestes expansions i contraccions periòdiques en l'àrea de distribució de la població de llúdrigues, coincident amb una major o menor disponibilitat d'aigua al seu hàbitat, ha estat també assenyalada en d'altres estudis portats a terme en hàbitats mediterranis (Carpena et al., 1997; López et al., 1998; Prenda et al., 2001; Ruiz-Olmo et al., 2002).

La disponibilitat d'aigua va actuar sobre els moviments diaris (sessions de radioseguiment de 24 hores) reduint el tram de riu prospectat per les llúdrigues que habitaven a rius secs amb tolls o basses disperses. Aquests individus es "fixaven" a una o vàries basses, on s'alimentaven i passaven la major part del seu període d'activitat. Aquests exemplars es desplaçaven ràpidament pel riu sec entre bassa i bassa (a causa d' una perillosa exposició als depredadors fora de l'aigua) i habitualment visitaven les mateixes basses diverses vegades en un mateix període d'activitat. Així, durant les condicions d'escassetat d'aigua, les llúdrigues no patrullaven llargs trams de riu, sinó que restaven properes a les úniques i concentrades fonts de recursos.

També es va estudiar l'ús d'amagatalls o indrets de descans per part de les llúdrigues reintroduïdes. Els diferents individus van emprar més freqüentment un amagatall diferent cada dia, repetint en ben poques ocasions, per exemple, mentre van habitar en un riu sec – on els moviments eren ben petits – o en el cas de les femelles que havien criat les quals, òbviament, havien de retornar al cau de reproducció després de cada període d'activitat. Així, es pot afirmar que la utilització consecutiva d'un amagatall per part d'una femella és un signe clar de cria. Els amagatalls seleccionats per la llúdriga van estar envoltats, en la majoria dels casos, per plantes de creixement ràpid que formen masses compactes que baixen fins a la vora de l'aigua, constituint refugis segurs per les llúdrigues, com la canya (*Arundo donax*), l'esbarzer (*Rubus ulmifolius*) o el canyís (*Phragmites sp.*). Les llúdrigues no van seleccionar amagatalls molt allunyats d'infraestructures o construccions humanes i sovint van ser trobades, durant el període d'inactivitat, a pocs metres de carreteres transitades, cases habitades, plantes d'extracció d'àrids i trams de riu amb una elevada circulació d'embarcacions. Aquestes dades mostren que les llúdrigues són tolerants respecte a formes indirectes de pertorbació.

L'estudi de dues femelles implantades amb emissor que van criar va aportar interessants dades, que poden ser d'utilitat per la conservació. Ambdues femelles van realitzar grans moviments abans de parir. Després de donar a llum, van reduir enormement la seva activitat, incrementant-la durant les següents setmanes i recuperant els nivells previs a la reproducció només durant els darrers dies de la seva presència al cau de reproducció. Aquestes femelles van canviar els seus moviments diaris després de parir, amb "rangs" i temps d'activitat menors. Ambdues van disminuir significativament la seva àrea vital després del part. I finalment, el cau de reproducció es va localitzar en zones poc freqüentades, lluny del riu principal o en un rec completament cobert de vegetació helofítica.

Objectiu 4. Conèixer les probabilitats de supervivència de la població reintroduïda i els factors que la poden afectar.

En apartats anteriors, s'ha provat la persistència de la població de llúdrigues reintroduïda. Però, tot i que la persistència a curt termini (cinc anys) ha estat verificada, no se sap si la nova població persistirà en les properes dècades o el proper segle. Aquest aspecte va ser estudiat mitjançant una Anàlisi de Viabilitat Poblacional (PVA, sigles en anglès), un procediment per estimar la viabilitat de petites poblacions d'animals i plantes.

Encara que podem afirmar que la població reintroduïda era "de disseny", en la qual el nombre d'exemplars fundadors es coneixia perfectament, el patró demogràfic no es va esbrinar adequadament, a causa de la limitació dels mètodes (per exemple, radioseguiment) i dels períodes de seguiment respecte al cicle vital de l'espècie. Així, les dades procedents de la població reintroduïda s'han hagut de barrejar amb altres dades procedents d'altres poblacions catalanes estudiades intensivament i on es van emprar PVAs per tal de conèixer la viabilitat de poblacions salvatges aïllades (Ruiz-Olmo, 1995), així com dades de PVAs realitzats per determinar la viabilitat de futures reintroduccions de llúdrigues a Holanda (van Ewijk et al., 1997; Klop et al., 1998). Tot i que la impossibilitat d'emprar dades prou acurades podria invalidar els resultats en termes quantitatius, aquests poden ser perfectament usats en termes qualitatius per testar les principals amenaces per la nova població, usant el PVA en forma d'anàlisi de sensibilitat.

La població de llúdrigues reintroduïda a les conques dels rius Muga i Fluvià va presentar un baix risc d'extinció de la població en els propers 100 anys. La majoria dels escenaris simulats (65%) van assolir el criteri d'un mínim de 90% de probabilitat de supervivència, utilitzat en el PVA de la reintroducció de la llúdriga a Holanda (van Ewijk et al., 1997). L'extinció de la població en més del 50% de les simulacions es va donar només en vuit escenaris (17%), on es van donar plegats els dos paràmetres modelats més negatius: un èxit reproductor baix (1,72 cries/femella) juntament amb una catàstrofe molt devastadora (que eliminés el 50% de la població) i recurrent (que succeís de mitjana cada 10 anys). Però, probablement, cap dels dos paràmetres s'ajusta a la nostra realitat ja que: 1) a l'àrea de reintroducció existeixen molts trams de riu i d'aiguamoll que mai no queden secs, fins i tot en les sequeres més persistents, 2) la llúdriga és capaç d'alimentar-se d'espècies aquàtiques que resisteixen els períodes de sequera millor que els peixos (granotes, crancs i insectes) i també pot alimentar-se d'espècies terrestres, com els ocells i, finalment, 3) la densitat de llúdrigues serà baixa, almenys durant els primers anys, mentre que la biomassa íctica és molt alta en gran part de l'àrea de reintroducció (Zamora et al., 1996), el que provocaria un èxit reproductor major que el modelat, proper a les 2,5 cries per femella esperat per una població mediterrània en expansió (Ruiz-Olmo, in press) i proper també a les 2,3, 2,4 i 2,8 cries per femella assumides respectivament en els tres PVAs desenvolupats per assessorar el programa de reintroducció de llúdrigues a Holanda (Ewijk et al., 1997; Klop et al., 1998 i F.J.J. Niewold, dades no publicades).

Del model poblacional construït es pot deduir que les principals amenaces per la població reintroduïda provenen de factors que són de difícil gestió des d'una perspectiva local, com el canvi climàtic (que podria comportar sequeres més severes i recurrents) o l'estat general de l'hàbitat (que influeix en l'èxit reproductor). Però un altre factor clau que cal controlar per assegurar la viabilitat de la població reintroduïda és la mortalitat. Un important increment en aquest paràmetre comporta un espectacular increment de les probabilitats d'extinció de la població. A l'àrea de reintroducció, els atropellaments causen més del 50% de la mortalitat, sovint concentrada en uns pocs "punts negres". Així, la construcció de passos de fauna, el tancament lateral d'alguns trams de carretera perillosos i el control de la velocitat en algunes vies són actuacions bàsiques per mantenir o reduir un paràmetre que pot afectar de forma important la viabilitat de la nova població i que pot ser gestionat mitjançant mesures tècniques i administratives.

Amb els resultats del PVA es pot concloure que una reintroducció de llúdrigues realitzada amb només algunes desenes d'individus pot ser perfectament viable, sempre que l'àrea sigui suficientment extensa, l'hàbitat estigui en bones condicions (el que comportarà una elevada capacitat de càrrega) i els índexs de mortalitat es mantinguin a nivells baixos.

Objectiu 5. Col·laborar en la preparació de metodologies estàndard, tant des de la ciència veterinària com des de l'ecologia de poblacions.

Mètodes per capturar i manipular llúdrigues euroasiàtiques.

El *Projecte Llúdriga* ha posat a punt un protocol per capturar i manipular llúdrigues salvatges, des de la captura fins a l'alliberament, prenent com a base altres projectes de reintroducció, principalment portats a terme amb *Lontra canadensis* a Amèrica del Nord.

Captura.

Cinquanta-cinc llúdrigues van ser capturades amb paranys acotxats (Soft Catch), modificats de forma que una de les dues molles del mecanisme era reemplaçada per una un xic més potent (n.2). Els paranys es col·locaven dins l'aigua, preferentment en passatges poc profunds entre roques o en platges del riu. Als paranys se'ls afegia una cadena d'un metre de longitud, que es lligava a troncs o s'assegurava a grans roques usant "spits" d'escalada clavats manualment. Els paranys se situaven en grups de dues o tres unitats. No es va emprar cap tipus d'esquer o atraient.

L'ús de la sarbatana com a mètode per injectar la combinació anestèsica va funcionar correctament i aquesta tècnica es recomana especialment amb la finalitat d'evitar ferides o empitjorar les produïdes pel parany (luxacions, fractures, etc.). Així, evitar el contacte físic amb animals conscients, injectant-los l'anestèsic a distància i esperant més de tres minuts, va resultar segur per als animals tractats.

Els animals capturats van patir molt poques ferides severes. De fet, cap animal va patir una fractura d'extremitat, sis animals (14%) van patir luxacions obertes i només nou animals (21%) van patir algun tipus de luxació als dits. A més, es va detectar un nombre molt reduït (19%) de ferides dentals. Aquests resultats mostren que els paranys acotxats poden ser emprats per a la captura de llúdrigues salvatges.

Allotjament i assistència veterinària.
Després de l'examen en el camp, les llúdrigues (anestesiades) eren col·locades en gàbies de transport i es deixava que es recuperessin en una habitació fresca i fosca. A la tarda o vespre, eren transportades al Zoo de Barcelona amb furgoneta o línia aèria comercial.

Al Zoo de Barcelona, les llúdrigues eren allotjades de forma individual en gàbies de filferro amb caixes-cau de fusta, sospeses sobre el terra i situades dins d'unes dependències interiors habilitades exclusivament per a aquest ús. En general, les llúdrigues captives es van habituar bé a aquestes gàbies i acceptaven el menjar ràpidament. Els peixos vius (anguila i truita) i els pollets van semblar actuar com a eines d'enriquiment ambiental i van animar a menjar a alguns individus que, inicialment, el van refusar.

L'anestèsia emprada durant la captura i la manipulació va consistir en una barreja d'hidroclorit de ketamina (Imalgene) i hidroclorit de medetomidina (Dormtor). Com a antagonista, es va administrar hidroclorit d'atipamezol (Antisedan). El protocol anestèsic complet emprat en aquest projecte (explicat a Fernández et al., 2001) va resultar ser efectiu i segur per al tipus de procediments descrits en aquesta tesi.

Cirurgia i alliberament.
El fet que quasi totes les llúdrigues mengessin normalment el mateix dia de la cirurgia mostra que aquest procediment té efectes menors en els animals. Les llúdrigues no van semblar preocupar-se pels punts d'incisió i no es van deixar sutures a la pell exposades que poguessin fer-se malbé, irritar-se o infectar-se. No obstant, va semblar convenient mantenir-les un mínim de 10 dies en captivitat després de la cirurgia per assegurar una completa cicatrització. La idea d'una cirurgia immediata i un ràpid alliberament defensat per alguns autors (Arnemo, 1991) amb l'objectiu de reduir l'estrès no sembla convenient. Els emissors utilitzats en el nostre estudi (30-40 g) podrien ser una mica massa grans per a l'ús intrabdominal en llúdrigues ibèriques, considerant la seva petita mida si la comparem amb *Lontra canadensis*.

L'aproximació quirúrgica emprada al *Projecte Llúdriga* en l'implant dels emissors va ser la central-ventral, que és clarament recomanada en base a les 36 intervencions realitzades sense problemes al llarg del nostre estudi. La pèrdua de calor en afaitar l'àrea d'intervenció quirúrgica no va semblar un problema en els nostres animals, potser per les benignes condicions climàtiques de l'àrea de reintroducció. A més, en l'examinació clínica final es va poder confirmar el ràpid creixement de la capa interna de pèl.

No s'han trobat efectes nocius dels emissors intraperitoneals en la reproducció i almenys tres llúdrigues implantades han criat a l'àrea de reintroducció (Saavedra & Sargatal, 1998).

El mateix dia de l'examinació final, les llúdrigues eren transportades en furgoneta durant dues hores a l'àrea de reintroducció i alliberades unes hores més tard.

El *Projecte Llúdriga* ha servit també per investigar altres aspectes veterinaris de la llúdriga euroasiàtica, no esmentats en aquesta tesi però publicats a d'altres llocs (Fernández et al., 2001a; 2001b; 2001c; 2002).

Mètodes per estimar la distribució i densitat de la llúdriga euroasiàtica.

El *Projecte Llúdriga* va possibilitar l'estudi d'una població dissenyada artificialment. Durant els primers mesos, gràcies als estudis de radioseguiment, es va poder conèixer el nombre exacte de llúdrigues que constituïen la població i el nombre d'animals que vivien en les diferents masses d'aigua. Aquest fet va suposar una oportunitat única per a comparar diversos mètodes d'estimació de la distribució i densitat de la llúdriga. Els mètodes comparats van ser el "otter survey", basat en la identificació de rastres (principalment excrements i petjades) de l'espècie, censos de petjades, basat en la troballa i mesura de petjades a la neu o fang, i censos visuals, basats en la col·locació de grups d'observadors treballant en zones on les llúdrigues són crepusculars.

Utilitat dels "otter survey".

Un dels resultats més sorprenents va ser que, als cursos d'aigua de petita o mitjana amplada, una sola llúdriga era capaç de marcar molts quilòmetres, de forma que podia ser detectada a la majoria dels punts (>70%). Així, si un punt era negatiu, era bastant probable que la llúdriga fos detectada al següent punt alguns quilòmetres més enllà, si el tram estava ocupat per aquest individu. La prospecció de diversos punts consecutius sense rastres de llúdrigues ha de ser interpretat com que l'espècie és absent del tram. Aquest resultat és d'especial importància en els extrems de la distribució de l'espècie, on un sol individu pot ser fàcilment detectat en aquests cursos d'aigua. Això significa que la no detecció de llúdrigues en una zona durant un període de temps suggereix que realment aquestes hi són absents. I podem notar que la imatge tradicional d'una sola llúdriga (o "una parella") vivint en un tram curt (sovint entre muntanyes) i difícil de localitzar no és realista. Els resultats als aiguamolls van ser similars, mentre que als rius més amples (>20 m) la taxa de detecció va disminuir (50%).

Però aquests van ser els resultats per a una sola llúdriga vivint en un tram. Les densitats de llúdrigues en aigües dolces varien entre 0,05 i 0,6 llúdrigues/km (Ruiz-Olmo, 1995b; Sidorovich, 1991, 1992), el que significa que la presència d'entre una i quatre llúdrigues en un tram fluvial pot ser considerada estàndard. I als trams on dues o tres llúdrigues van ser presents, la probabilitat de detectar la llúdriga en un punt de prospecció convencional va ser pràcticament del 100%. De forma que només dos o tres exemplars eren capaços de produir suficients rastres de llúdrigues per ser trobats en gairebé tots els llocs que ocupaven i, per tant, les llúdrigues havien de ser detectades després de prospectar ben pocs punts.

Els resultats de l'estudi van indicar també que no existeix una relació lineal entre el nombre de llúdrigues i el nombre de punts positius, ja que uns pocs individus estenien els seus rastres sobre grans àrees i els següents que eren alliberats tendien a ocupar les mateixes zones, presumiblement aquelles que oferien les condicions més favorables. Així, quan un riu és colonitzat per la llúdriga (naturalment o mitjançant una reintroducció), la presència de l'espècie en la majoria dels cursos d'aigua de la conca és assolida en pocs anys, sovint 1-4.

Estimació de l'abundància.
No és fàcil establir quantes llúdrigues viuen en un sector o hàbitat específic, a causa de les llargues distàncies que recorren, de l'ús d'àrees vitals que varien segons la disponibilitat d'aliment al llarg del temps, de la seva particular estructura social i del seu hàbitat principalment lineal (Kruuk, 1995). L'ús del radioseguiment podria ser certament un mètode més precís i més proper a la realitat ecològica de l'espècie, però aquests mètodes són difícils d'aplicar en la gestió i ens expliquen ben poc sobre el nombre i densitat de les poblacions de llúdrigues.

En aquest estudi es va trobar que els mètodes més fiables per estimar l'abundància d'una població de llúdrigues són els censos de petjades (considerant només les petjades del darrer dia) i els censos visuals (llúdrigues observades en un període d'activitat). Amb els dos mètodes, l'ús de trams de 10 km com a unitat de cens sembla suficient, però potser trams de 20 o 30 km ens donarien resultats més acurats. Cal assumir que una mateixa llúdriga pot ser comptada en dues unitats, però també que una llúdriga resident pot divagar fora de la unitat. El fet que tant el cens de petjades noves com el cens visual donin resultats similars (i presentin un alt nivell de correlació) dóna una major consistència als resultats. Les dades obtingudes a través del radioseguiment indiquen, a més, que pràcticament la totalitat de les llúdrigues poden ser vistes per observadors experimentats, el que confirma la validesa dels censos visuals. Els censos visuals també ens permeten aplicar diversos índexs a les estimacions d'abundància (llúdrigues observades/km, llúdrigues/ha, llúdrigues observades/hora, etc.), totes elles ben correlacionades (Ruiz-Olmo, 1995a).

Tanmateix, en aquelles àrees o períodes d'elevada densitat de llúdrigues (>0,6 llúdrigues/km) correm el risc de subestimar el nombre real d'individus si emprem el cens de petjades, ja que dos individus del mateix sexe i de mida similar podran ser confosos. Això no succeirà normalment amb el cens visual. En canvi, en àrees amb una densitat de llúdrigues molt baixa (<0,1 llúdrigues/km), els individus són difícilment detectats als censos visuals, ja que poden trobar-se fora del tram censat.

Com a conclusió, podem afirmar que els censos de petjades fresques i els censos visuals (com a mínim a la nostra àrea d'estudi) aporten informació molt propera al nombre real de llúdrigues.