UNIVERSIDADE DE LISBOA
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OTTERS AND DAMS IN MEDITERRANEAN HABITATS: A CONSERVATION ECOLOGY APPROACH

Nuno Miguel Peres Sampaio Pedroso

DOUTORAMENTO EM BIOLOGIA
(Ecologia)

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Tese co-orientada por
Professora Doutora Margarida Santos-Reis
Professor Doutor Hans Kruuk,
especialmente elaborada para a obtenção
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Na elaboração desta dissertação foram usados artigos já publicados, ou submetidos para publicação, em revistas científicas indexadas ou em livros. De acordo com o previsto no nº 1 do artigo 45º do Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, publicado no Diário da República, 2.ª série, n.º 65, de 30 de Março de 2012, o candidato esclarece que participou na concepção, obtenção dos dados, análise e discussão dos resultados de todos os trabalhos, bem como na redacção dos respectivos manuscritos.

A dissertação, por ser uma compilação de publicações internacionais, está redigida em Inglês.

Apesar de alguns dos artigos científicos integrados na dissertação já terem sido publicados a sua formatação foi alterada para uniformizar o texto.

Lisboa, Agosto de 2012

Nuno M. Pedroso

PRELIMINARY NOTE

According to Article 45.nr.1 of the Post-graduate Studies Regulation (Diário da República, 2ª série, nº 265, 30 March 2012) this dissertation includes papers published or submitted for publication and the candidate, as co-author, was involved in the scientific planning, sampling design, data collection, statistical analyses and writing of all manuscripts.

Papers format was made uniform to improve text flow.

The dissertation, being composed of a series of international publications, is written in English.

Lisbon, August 2012

Nuno M. Pedroso

DEDICATÓRIA

À minha avó e ao meu pai,

que cobrem o meu coração de saudade!

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É significativo que na maioria das teses seja este o último texto a ser escrito. Talvez porque se pense que seja o mais fácil, *errado*, ou porque simplesmente há sempre alguém que nos ajuda mesmo até ao último minuto da elaboração da tese. Mas também é significativo que seja o primeiro texto que surge na dissertação. Sem ajuda, uma tese seria muito diferente, mais demorada, mais difícil, mais triste, ou simplesmente, não seria! Uma tese de doutoramento, como outro processo longo e difícil na nossa vida, ajuda-nos a crescer e a criar e fortalecer contactos e amizades, e no fim saímos a ganhar, muito!

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Estudar lontras é um desafio, temos de estar preparados para estudar uma espécie que alguns dizem que não existe, outros que confundem com as lontras do oceanário, ou que a nossa própria família não conhece. Temos de dizer que estudamos os seus “indícios”, forma politicamente correcta para dejectos, porque o raio do bicho não se vê! Mas quando o vemos, nas raras vezes, percebe-se imediatamente porque lhes tenho de agradecer. Porque sem elas não existiria esta tese!

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RESUMO

As barragens têm sido vistas como solução para satisfazer as exigências humanas de energia e água e como um investimento que, a longo prazo, pode proporcionar vários benefícios. No entanto, principalmente nas últimas décadas, os impactes sociais e ambientais das grandes barragens também se tornaram evidentes, especialmente ao nível ambiental.

Entre os factores que levam à destruição dos ecossistemas ribeirinhos, as barragens são a ameaça física mais drástica, fragmentando e alterando ecossistemas aquáticos e terrestres. As barragens reduzem a conectividade dos rios, impedindo os movimentos naturais e as migrações dos peixes e de outras espécies aquáticas e semi-aquáticas, promovem a perturbação do habitat a larga escala e a diminuição do fluxo e da qualidade da água, e criam condições para o estabelecimento de espécies não-nativas, com consequências negativas sobre a diversidade autóctone. Adicionalmente, e especialmente em regiões quentes com uma forte componente agrícola, os reservatórios criados por barragens comportam-se como grandes lagos cuja matéria orgânica e outros nutrientes sedimentam levando ao aparecimento de algas, como cianobactérias, que são tóxicas e podem causar mortalidade nos peixes e ser um risco para a saúde pública.

Esta temática é especialmente relevante na região do Mediterrâneo, considerada uma das regiões que enfrentam as maiores mudanças no clima em todo o mundo e onde a gestão da água é feita principalmente através da construção de barragens e da regularização dos rios. Os habitats mediterrânicos registam variações sazonais extremas no fluxo de água. Um período de stress, quando o nível e fluxo de água são reduzidos ou nulos, ocorre geralmente no verão quando se registam frequentes e longos períodos de seca. As barragens podem agravar esta situação influenciando os regimes de escoamento de água. Este aspecto é tanto mais relevante quanto a região do Mediterrâneo apresenta elevados níveis de biodiversidade que levaram à sua inclusão na lista mundial de "*hotspots*" de biodiversidade definidos para estabelecimento de prioridades de conservação e identificação das principais regiões a proteger.

A adaptação dos animais à perda, fragmentação e mudança de habitat é um aspecto fundamental da conservação das espécies. As actividades humanas, por outro lado, são importantes componentes dos ecossistemas, e compreender como os valores naturais persistem dado o extenso uso humano é relevante.

A lontra Eurasiática (*Lutra lutra* Linnaeus, 1758) é um animal semi-aquático cujas populações sofreram um declínio acentuado durante o século passado como resultado de perseguição, destruição de habitat, sensibilidade à contaminação e escassez na disponibilidade de presas.

Todos os aspectos da biologia da lontra são condicionados pelo facto de esta espécie passar a maior parte do seu tempo na água. A lontra, além de viver num ambiente naturalmente instável, é também influenciada pela presença humana nos ambientes aquáticos. A destruição do leito do rio, a alteração da vegetação ribeirinha, a poluição da água, a extracção de água e sedimentos, a perturbação e exploração das presas, a perturbação humana e as alterações climáticas são os principais factores de perturbação para as lontras. Dadas estas características, a lontra é um modelo adequado para abordar a adaptação animal à perda de habitat e à mudança causada pela implementação de uma barragem.

A lontra está presente em rios, ribeiras, lagoas, reservatórios, estuários e habitats costeiros e preda principalmente na água. A densidade de lontras depende, entre outros factores, da capacidade de carga do habitat. A lontra é um predador maioritariamente piscívoro, mas tem um comportamento oportunista, tirando proveito das espécies de peixe mais abundantes, mas também dos picos sazonais de outras classes de presas como crustáceos e anfíbios. A lontra, devido ao historial recente de declínio na globalidade da sua distribuição beneficia do estatuto de "Quase Ameaçada" atribuído pela UICN - União Internacional para a Conservação da Natureza. Além disso, está listada em várias convenções internacionais sendo uma espécie estritamente protegida a nível europeu. A nível nacional, onde a população de lontras é aparentemente estável e abundante, a espécie está classificada como "Pouco Preocupante".

As barragens têm sido consideradas como tendo uma influência negativa na distribuição da lontra e são sugeridas como um factor co-responsável pelo declínio passado desta espécie na Europa. A montante, as barragens criam reservatórios de água de grande dimensão e profundidade, muitas vezes com margens íngremes, não sendo assim ideais para a lontra caçar, o que geralmente ocorre em águas pouco profundas. Além disso, a flutuação rápida e frequente do nível de água faz com que a vegetação nas margens seja escassa e não ofereça o refúgio e segurança adequados para a espécie. As barragens fragmentam o habitat e, dependendo das condições orográficas e hidrológicas locais, também a população de lontras. Outro efeito causado pela presença de barragens é a redução do fluxo de água nos rios a jusante, durante o período mais quente do ano. Perturbações adicionais associadas à construção de barragens incluem desmatações na área de inundação, plantação de árvores de produção na envolvente, bem como actividades recreativas, como desportos aquáticos e pesca, na área dos reservatórios. Independentemente destes aspectos negativos há indicações de que os reservatórios são utilizados por lontras em habitats mediterrânicos mas estas evidências advêm de estudos limitados no tempo e/ou no espaço. A falta de informação leva à necessidade de recolha de dados ecológicos adicionais sobre as lontras em barragens, especialmente num contexto da política de gestão da água como a implementada nas últimas décadas na Península Ibérica, com várias centenas de barragens já construídas e muitas outras previstas para os próximos anos.

Assim, esta tese teve como principais objectivos avaliar a presença e o grau de uso pela lontra em barragens, e linhas de água adjacentes, no sul de Portugal, e determinar as alterações induzidas pela construção de uma grande barragem na disponibilidade dos principais requisitos ecológicos da lontra.

A metodologia geral incluiu a pesquisa de indicíos de presença da espécie em reservatórios e linhas de água, a correspondente avaliação da dieta através da análise laboratorial de dejectos e a avaliação da disponibilidade de presas através de pesca eléctrica (linhas de água) e da colocação de redes de pesca (reservatórios). Adicionalmente caracterizaram-se os locais de amostragem através de um conjunto de variáveis ecológicas e de outras relativas aos sistemas aquáticos, seleccionadas de acordo com a sua relevância para a lontra. Estas variáveis foram medidas, estimadas e/ou categorizadas, sendo posteriormente usadas em processos de modelação (e.g. modelos lineares generalizados) e relacionadas com a presença/ausência e/ou intensidade de marcação de lontra.

Para averiguar se existe uma utilização generalizada dos reservatórios resultantes de grandes barragens pela lontra, num contexto mediterrânico, foram estudados 12 reservatórios e linhas de água adjacentes em diferentes estações do ano e com condições climáticas diversas (época seca de 2002, época extraordinariamente seca de 2005 e época húmida de 2006). Quatro destas barragens (Caia, Vigia, Monte Novo e Lucefecit) estão localizadas na bacia do rio Guadiana e oito (Alvito, Odivelas, Pego do Altar, Vale do Gaio, Fonte Cerne, Campilhas, Roxo e Monte da Rocha) na bacia do Sado. Além disso, em Pego do Altar e Monte Novo foram ainda recolhidos dados sobre a microbiota intestinal de lontra e a resistência antimicrobiana das bactérias e seus determinantes, através da recolha de dejectos e sua posterior análise laboratorial.

Para perceber se as lontras usam diferencialmente as grandes barragens e os reservatórios de pequeno-médio porte, 30 destes reservatórios foram estudados na Serra de Monfurado, (PTCON0031 - Sitio de Importância Comunitária - Rede Natura 2000).

As alterações na presença, e consequente distribuição, da lontra ao longo do tempo, em resposta às alterações nos requisitos principais da espécie impostas pela construção de uma grande barragem, foram abordadas através do acompanhamento da implementação da Barragem do Alqueva (bacia do Guadiana), em todas as suas fases (2000 a 2006).

Todas as áreas acima descritas estão incluídas na região do Alentejo, no sul de Portugal (região do Mediterrâneo).

Com base na experiência alcançada neste estudo, e noutros anteriores sobre lontras em grandes barragens, foi proposta uma adaptação ao método de amostragem padrão recomendado pelo '*IUCN Otter Specialist Group*' para monitorização de lontra em sistemas lóticos (ribeiras e rios) de forma a melhorar a monitorização da espécie em sistemas lênticos, nomeadamente grandes barragens. Esta adaptação inclui considerações acerca da dimensão espacial da amostragem, do número e localização dos pontos de amostragem, entre outras, tornando mais eficaz a recolha de informação quando se amostram barragens, seja o objectivo apenas detectar presença/ausência de lontra ou recolher dejectos frescos para análise molecular.

Esta tese demostrou que os grandes reservatórios são regularmente usados pela lontra no sul de Portugal e que estes elementos do habitat podem ser adequados para a espécie em cenários particulares. É o caso, por exemplo, de áreas em que os sistemas ribeirinhos sofrem alterações sazonais marcadas na disponibilidade de água e as populações de lontra são estáveis e relativamente abundantes. Contudo, estes reservatórios são menos adequados para a lontra do que as ribeiras e rios pré-existentes à implementação da barragem.

A disponibilidade de presas, independentemente do tamanho do reservatório, demostraram ser o factor chave para a utilização destes pelas lontras, e a sua disponibilidade influencia de forma significativa a presença da espécie. Independentemente das diferenças sazonais observadas na composição e estrutura das comunidades de presas, registou-se uma aparente estabilidade a nível da sua disponibilidade nos grandes reservatórios. Tal tem um papel relevante para a subsistência da lontra em determinadas áreas porque, durante a estação quente, mais de metade das linhas de água adjacentes às barragens estudadas secam ou ficam restritas a pequenos pêgos. Inversamente, os grandes reservatórios oferecem alimento à lontra durante todo o ano, sugerindo que as presas são os elementos chave para a utilização dos reservatórios por esta espécie em zonas Mediterrânicas ou noutras zonas áridas ou semi-áridas onde haja uma marcada sazonalidade de recursos (água e presas). As presas dominantes da lontra foram peixe e lagostim-americano *Procambarus clarkii*, quer nas ribeiras quer nos reservatórios.

Outro factor determinante para a presença e uso de grandes reservatórios pela lontra é a proximidade às linhas de água. Tal sugere que nem todo o perímetro do reservatório é igualmente adequado para a lontra e que as linhas de água que desaguam no reservatório têm um papel determinante. O elemento chave relacionado com as linhas de água é a presença de vegetação ripícola que proporciona abrigo e possibilita a reprodução, e que é um recurso limitado ao longo das margens dos reservatórios.

A tipologia das margens dos reservatórios surgiu como outro factor limitante: águas pouco profundas e margens complexas oferecem à lontra melhor sucesso de captura do que águas profundas uma vez que estas últimas limitam a capacidade dos indivíduos de apanhar presas ao aumentar as possibilidades de fuga das presas.

Os resultados relativos ao uso pela lontra de reservatórios de pequena ou média dimensão foram, em geral, concordantes com os obtidos para grandes reservatórios: i) as lontras estão presentes e utilizaram a grande maioria dos reservatórios, ii) observou-se uma variação sazonal na intensidade de marcação, revelando a maior importância dos reservatórios na época seca; iii) os reservatórios são habitats sub-óptimos para a lontra em termos de abrigo e pressão humana quando comparados com as linhas de água, mas funcionaram como importantes áreas de alimentação, especialmente quando se localizavam perto de linhas de água com boas condições de abrigo, mas escassez de presas; iv) a dieta das lontras que utilizaram os reservatórios reflectiu o comportamento oportunista desta espécie nomeadamente através da selecção de presas sazonalmente mais disponíveis, particularmente o lagostim-americano. Contudo, ao contrário das grandes barragens, os reservatórios de pequena-média dimensão não mostraram diferenças a nível dos padrões de ocupação (presença/ausência) nas estações seca e húmida. A associação negativa encontrada entre o uso pela lontra dos reservatórios de pequena-média dimensão e a extensão de linhas de água com galeria ripícola desenvolvida na proximidade dos mesmos reflectiu a preferência da lontra por ribeiras e rios melhor preservados, em detrimento dos reservatórios artificiais sem oportunidades de abrigo; como consequência, quando ocorrem habitats de elevada qualidade na proximidade, a necessidade utilização de recursos dos reservatórios é reduzida. Outro factor diferenciante é a pressão de gado que demonstrou afectar negativamente o uso pela lontra de reservatórios pequenos, mas não os de grandes dimensões.

O impacte das várias fases de construção, em particular de grandes barragens, é pouco conhecido. O caso de estudo usado como exemplo, a barragem do Alqueva (a maior barragem na Europa), foi acompanhado nas várias fases da sua implementação: pré-construção, desmatação, enchimento, pós-enchimento. Os dados recolhidos demonstraram que a desmatação e enchimento afectaram significativamente a lontra resultando num decréscimo marcado da sua presença na área de inundação. Embora a espécie tenha recolonizado a área após o enchimento da barragem, e a sua presença se tenha tornado relativamente constante quando o nível de água estabilizou, esta não alcançou o nível anterior à construção da barragem.

A análise da dieta mostrou que a resposta da lontra às alterações criadas pela implementação da barragem foi clara, reflectindo as grandes mudanças na composição e abundância da comunidade de presas, o que é provavelmente a alteração mais visível na ecologia da lontra. Verificou-se uma alteração significativa no consumo de peixes nativos com o aumento das espécies não-nativas de peixes e crustáceos (lagostim-americano), que passaram a dominar a dieta. Além disso, a generalidade das presas tornaram-se menos disponíveis para a lontra dada a maior dificuldade de captura em águas profundas e o efeito de dispersão dos peixes no grande reservatório, pelo menos nos anos imediatamente após a implementação da barragem quando a colonização, nomeadamente por espécies não-nativas, é um processo em curso. Em paralelo com as alterações no uso dos recursos alimentares, os resultados ilustram uma alteração noutros requisitos ecológicos da lontra ao longo do tempo: conectividade de habitats (corredores ecológicos com elevada qualidade de habitat e abundância de presas), cobertura de vegetação nas margens, abrigo e tocas, e zonas de alimentação. Com a excepção da disponibilidade de água, todos os outros principais requisitos ecológicos da lontra ficaram menos disponíveis após a construção e implementação da barragem do Alqueva.

A construção da barragem cria impactes que se estendem muito para além do espaço inicial (área de enchimento) e tempo (calendário de construção) considerados na proposta de acompanhamento da infra-estrutura. Os resultados obtidos nesta tese enfatizam a importância dos estudos de monitorização a longo prazo que incluam todas as fases da construção e pós-construção, para verdadeiramente avaliar a resposta das espécies aos impactes. Este facto é relevante uma vez que nem todos os Estudos de Impacte Ambiental incluem fases de monitorização pós-inundação ou consideram a lontra como uma das espécies-alvo. O verdadeiro impacte na lontra apenas pode ser avaliado depois do final da fase de impacte (desmatação e enchimento), e depois da estabilização das condições do reservatório (nível de água, vegetação nas margens, comunidades de presas).

Os dados, conhecimento e experiência que resultaram desta tese foram utilizados na elaboração, em conjunto com outros membros do '*IUCN Otter Specialist Group*', de recomendações que pretendem guiar os promotores e consultores quando da preparação de estudos de impacte ambiental (EIA) de grandes barragens, bem como ONGs e consultores de EIA, e avaliadores que têm que verificar se a lontra foi devidamente considerada no decurso do EIA (ANEXO).

Um resultado importante dos estudos efectuados no decurso desta tese diz respeito à primeira evidência de resistência antimicrobiana na microbiota de lontras que utilizam barragens e ribeiras. Assim, foi detectada a presença de bactérias resistentes a compostos antimicrobianos em amostras de dejectos de lontra recolhidos na barragem de Pego do Altar, de Monte Novo, e ribeiras adjacentes. Considerando a localização dos pontos de amostragem, estas lontras terão sido provavelmente expostas a compostos antimicrobianos presentes na água ou no solo por contaminação através de dejectos de animais de criação (e.g. gado bovino) ou de actividades agrícolas. Esta inferência é especialmente relevante, e com possíveis consequências para a saúde pública, em locais onde decorrem actividades humanas recreativas, como banhos, prática de desportos de água ou campismo; estas actividades ocorrem com elevada frequência em barragens, como observado nas várias barragens amostradas neste estudo.

A estratégia de conservação para a lontra a longo prazo, em zonas mediterrânicas, como o sul de Portugal, deve ser centrada na manutenção de uma população saudável de lontra, melhorando as suas condições de habitat e a densidade de presas naturais, em coexistência com as actividades humanas. Especificamente, o sistema conjunto de reservatórios e linhas de água adjacentes aparenta ter um papel relevante na permanência da lontra em determinadas zonas mediterrânicas. Esta relevância pode ser assegurada através da aplicação de medidas específicas de conservação e acções de gestão como as que são referidas de seguida:

i) Promover a existência de refúgio e cobertura para a lontra nos sistemas ribeirinhos e nas margens dos reservatórios. As grandes barragens podem sustentar mais lontras, se as linhas de água adjacentes tiverem boas condições de habitat e refúgio, que é normalmente escasso nas margens das barragens. Controlar o acesso de gado, o corte de vegetação ripícola, e a extracção de água para fins agrícolas, tudo práticas comuns no sul de Portugal, são bons exemplos de acções para a manutenção de habitat ribeirinho. Especial atenção deverá ser dada às áreas de interface entre reservatórios e linhas de água.

ii) Proteger as ilhas que se criam nos reservatórios das barragens após o enchimento à cota máxima. Estas ilhas podem constituir novas oportunidades de habitat para as lontras, desde que não sujeitas a perturbação humana, podendo ser especialmente importantes para permitir à população de lontra recuperar parcialmente dos impactes da desmatação e enchimento.

iii) Promover a existência de pequenas baías e a complexidade de habitat nas margens nos reservatórios, pois tanto a tipologia das margens (diferentes estratos e substratos) como a alternância de baías e penínsulas oferecerem melhores oportunidades à lontra para capturar presas, ao criarem áreas de reduzida profundidade e condições de emboscada. Além disso, a manutenção de alguma vegetação aquática pode funcionar como refúgio para peixes, lagostim americano e anfíbios aumentando a sua disponibilidade nessas baías.

iv) Controlar a perturbação causada pelo gado e actividades agrícolas nas imediações dos reservatórios e nos sistemas ribeirinhos adjacentes de forma a evitar a degradação da vegetação ripicola, reduzir a poluição orgânica da água e diminuir o potencial de transferência de bactérias resistentes e compostos antimicrobianos, contribuindo assim para melhorar a adequação de habitat para a lontra, bem como para outra fauna aquática.

v) Promover o uso eficiente da água através de uma gestão responsável da água, que requer sensibilidade dos gestores para uma ampla gama de questões. Um ponto de partida é entender os impactes dos actuais e futuros sistemas de gestão de água (incluindo infra-estruturas). O cenário actual das alterações climáticas na Europa prevê impactes nos sistemas ribeirinhos na região do Mediterrâneo, principalmente, através da extensão do período de seca. Este aspecto deve ser considerado no planeamento da conservação da lontra em ambientes mediterrânicos.

vi) Gerir as barragens e as descargas de água dos seus reservatórios de tal forma que minimizem os efeitos sobre a lontra e suas populações de presas. A libertação de água deve ser progressiva, para que o caudal dos sistemas ribeirinhos a jusante siga um regime de fluxo mais natural com a manutenção de caudais ecológicos.

vii) A utilização de peixes não-nativos como presa pela lontra não deve ser considerada uma ferramenta para a conservação. Além de competirem com espécies de peixes nativas presentemente com problemas de conservação, há evidências de que os estes últimos, quando em abundância, são presas preferidas pela lontra. Devem ser assim protegidos os sistemas ribeirinhos que ainda têm populações de presas nativas, especialmente durante a estação seca. Deve ser ainda controlada a introdução ilegal de espécies invasoras nos reservatórios.

viii) O actual estatuto da lontra em Portugal pode resultar não só num desinvestimento na investigação sobre a espécie, mas também na desvalorização do seu interesse de conservação, especialmente no âmbito dos estudos de impacte ambiental (EIA). No entanto, a lontra é uma espécie bandeira, eficaz na conservação dos sistemas aquáticos, e a preservação da lontra ainda é uma questão vital na Europa e em Portugal, sendo a sua conservação obrigatória de acordo com a Directiva Habitats. Devido a este facto, a lontra deve ser adequadamente considerada no decurso de uma EIA. Especialmente importante é que a estrutura e monitorização dos EIAs de grandes barragens incluam não só os períodos de construção mas igualmente a fase pós-implementação. As medidas de mitigação e compensação para lontra devem ser proporcionais à escala dos impactes produzidos.

Está comprovado que barragens têm efeitos negativos sobre a ecologia da lontra, embora estes efeitos sejam menos visíveis em áreas de ocorrência de populações amplamente distribuídas e aparentemente abundantes, tal como a observada em Portugal. Também está confirmado que constituem um complemento de habitat aos sistemas ribeirinhos naturais sujeitos a secas, pressão climática e humana, cuja relevância pode ser promovida através da aplicação de medidas de conservação e gestão aqui propostas. No entanto, a destruição de sistemas ribeirinhos adequados à ocorrência da lontra que decorre da construção de barragens, em

particular as de grandes dimensões, deve ser motivo de preocupação, especialmente em áreas de instabilidade e fragilidade de população de lontra.

Palavras-chave: lontra Euroasiática, *Lutra lutra*, barragens, reservatórios, requisitos ecológicos, impactes ambientais, monitorização

SUMMARY

Human activities are important drivers of ecosystems change and understanding how natural values persist given extensive use of the landscape is of conservation importance. Dams, particularly large-sized, have been described as negatively influencing the distribution and ecology of Eurasian otters (*Lutra lutra*) in Europe but, although data is still scarce, evidences exist that Mediterranean otters use these new habitat elements.

This thesis focus mainly on assessing otters' presence and use of dam reservoirs and adjacent streams in the south of Portugal, and determining the changes in the availability of otter ecological requirements imposed by the a large dam construction. Signs of presence were the basis of the otter-related fieldwork and an adaptation of the standard otter river survey method was proposed and implemented to survey dams more efficiently. Results showed a generalized use of large reservoirs by otters, although these habitats were less suitable than pre-existent streams and rivers. Reservoirs acquired special importance during the dry season when water and aquatic prey availability are limiting resources in streams. Prey abundance was one of the main factors promoting otter use of reservoirs. Throughout the construction of a large dam, otter presence decreased during the impact phases but recovered although not to levels prior to dam construction. After the construction of the dam otter diet became based on non-native prey species and monitoring revealed a decrease in habitat connectivity, bankside vegetation cover, breeding and foraging grounds, throughout the reservoir. These results emphasize the importance of long-term monitoring studies that include post-impact phases. Evidence of antimicrobial resistance in otter fecal bacteria was detected in reservoirs and adjacent streams, most probably promoted by high levels of cattle density, with unknown consequences for otters' fitness and human health.

In widely distributed and healthy populations, such as the one occurring in Portugal, dams are less concerning. In areas affected by Mediterraneity reservoirs may even constitute a habitat complement to natural riverine systems under climate and human pressures, and can be enhanced by conservation measures and management actions. Nevertheless, the destruction of riverine systems is a matter of concern, especially in areas of otter population fragility and/or instability.

Keywords: Eurasian otter, *Lutra lutra*, dams, reservoirs, ecological requirements, environmental impacts, monitoring

PART I – INTRODUCTION

I.1. Water management and the role of large dams

Freshwater represents less than 3% of the total water volume on Earth and a large part of it is located in the Antarctic and the Artic regions, in the form of glaciers and permanent snow. Another part is located in inaccessible underground aquifers, so that only a small fraction is left for the global rivers and lakes water reserves (0.26%), which constitute the main source of water for human consumption (Gonçalves, 2001).

In developed/first world countries, water has been taken for granted, seen as a natural renewable resource that is inexhaustible, and with which no concerns have to be taken regarding its eventual limits. It has also been largely assumed that economic progress requires ever-increasing amounts of resources, namely water resources. As a result, the difference between a constantly growing population water needs and a decrease in the supply of available water resources increases day by day, with current human needs largely exceeding water availability all around the globe (ESA, 2001; Gonçalves, 2001).

Over time, decisions aiming to ease water shortage have focused in improving the uptake of available resources, through pollution control, transfer of water resources and their storage. As such, the solution was to build large structures that allowed water storage and transfer (Biswas and Tortajada, 2001). The increasing number of water infrastructures has been determined by the three most influential factors of quantitative and qualitative use of water resources: population growth, economic development and the expansion of agricultural irrigation (Gleick, 1998). Thus, the planning and management of water resources is associated with a policy of dams construction.

The World Comission on Dams (WCD), in its final report on Dams and Development (WCD, 2000), foresees an increasing competition for water resources so that: *i*) competition among/for the three main water usages will globally increase - agriculture (67%), industry (19%) and municipal/residential use (9%); *ii*) evaporation in water reservoirs can represent an important factor of water shortage in dry climates (5% of total water); *iii*) irrigation may demand an increase of 15% to 20% in water volume by 2025; *iv*) 3.5 billions of people will live in countries having water needs/shortage by 2025; *v*) the demand for electricity in developing economies is on the rise as two million people still do not have electricity; *vi*) a large percentage of the world floodplain areas has already disappeared; *vii*) freshwater species, particularly fish, are increasingly threatened; and *viii*) the ability of aquatic ecosystems to produce products and services on which societies depend is rapidly declining.

Throughout the 20th century, many countries have seen dam construction as the way to meet the ever-increasing demand for water. In fact, between the 30s and 70s, the construction of large dams has become synonymous of economic development and progress. By being icons of modernization and of the ability of humans to use and control natural resources, dam construction dramatically increased. This trend reached its maximum in the 70s when, on average, each day two or three new large dams were approved for construction worldwide (WCD, 2000).

Nowadays, almost half of the world's rivers have at least one large dam (dam wall height ≥ 15m or height 5-15m and reservoir volume > $3x10^6$ m^3) (WCD, 2000). According to the International Commission on Large Dams – ICOLD, there are nowadays more than 45 000 large dams all around the world. These type of dams produce 19% of the world’s electricity. In addition, a third of the world countries depend on hydroelectric dams to produce over half of their electricity. Half of those dams have been built exclusively or primarily for irrigation purposes and about 30 to 40% of the 271 million hectares of irrigated land worldwide rely on dams (WCD, 2000). The volume of confined water in dams quadrupled since 1960, and is three to six times greater than in natural rivers. The extraction of water from rivers and lakes has doubled since 1960; much of the water used goes to agriculture (Millennium Ecosystem Assessment, 2005).

Large dams have been seen as good solutions to fulfill energy and water requirements and as a long-term investment that can provide multiple benefits. These consist in deep transformations in local societies, increased employment, higher purchasing power, tourism, allocation of agricultural wealth, improved land use and new activities, and are often cited as additional reasons for the construction of such infrastructures (Biswas and Tortajada, 2001; Schelle et al., 2004).

This issue is, currently, of primary concern. The revenues from investments put into the construction of dams have been highly questioned and the balance between costs and benefits has become of serious public concern as more knowledge is gathered about the performance and impacts of dams. Based on several studies and on information on the impacts of dams on both people and ecosystems, as well as on their economic performance, resistance to the construction of dams has strongly increased. A decrease in the construction of dams occurred, especially in North America and in Europe, since the best places for dam construction had already been used and also because, by then, a greater concern with the environment started to arise (Grant, 2001). In the beginning, controversy was focused on some specific dams and their local impacts but, with time, this evolved to a broad and general discussion which has nowadays global proportions. The two main sides of the debate reflect points of view based on the experience gained by the construction of large dams in the past: one of them points out the discrepancy between the supposed benefits and their effective outcomes; the other one examines the challenges of water and energy development considering national construction and allocation of resources. At this point, the debate stopped being a local scale process of cost-benefit evaluation to become a process in which dams elicited global discussions on strategies and development plans. This is clearly perceptible in the Iberian Peninsula: Portugal and Spain share four river basins, which downstream parts are located in Portugal, meaning that the decisions concerning dams taken in the Spain will surely affect our ecosystems.

Dams can surely have a decisive role in helping meet people's needs and they have several positive outcomes. For instance, it is clear how hydraulic projects have contributed to the development of civilizations, by allowing large populations to colonize inhospitable regions, and becoming symbols of modernity's quest to conquer and urbanize nature (Kaika, 2006). However, in the last 50 years, social and environmental impacts of large dams have also become evident. Some of the major impacts are listed below.

Over 400 000 km^2 of land in the world were submerse representing 0.3% of the world's terrestrial area (WCD, 2000). At first sight, this value of habitat loss may appear of little significance, but its importance rises when realizing that it is exactly in the river valley areas that the most fertile land can be found, along with the most important forest ecosystems and wetlands. Not only the ecological component is affected, sometimes with drastic fauna and flora population reductions and even extinction, but the social part is also highly affected (WCD, 2000). Countries with a strong agricultural component turn agricultural land to water reservoir, which renders them unusable to plant production. In other cases, pastures are sacrificed, reducing livestock production. Among other factors leading to riparian ecosystem depletion, dams are the main physical treat, fragmenting and changing aquatic and terrestrial ecosystems. In the last years, at least 20% of over 9 000 species of fresh water fish were extinct or severely endangered due to dams' construction (WCD, 2000).

Another major impact is the change in water quality. Especially in warm regions with a strong agricultural component, reservoirs created by dams behave like large lakes whose organic matter and other nutrients (like phosphorus and nitrogen) sedimentation leads to algae appearance, like cyanobacteria which are toxic and can lead to fish death and be a risk to public health if the dam is used for public consumption (WCD, 2000).

One of the impacts that a dam imposes on the physical environment is the interruption of the solid particle flow. The sediment deposition occurs above the dam, in the reservoir. The sediment accumulation leads to depletion of the reservoir, whose volume decreases with time. There can be a moment when the decrease of the reservoir's volume stops its useful lifetime and the dam loses its purpose (WCD, 2000). The most drastic effects are felt below the dam, with the interruption of the natural flow of river sediments, loss of agricultural and forest fertility due to loss of natural fertilizers that can affect the estuary region causing salt intrusion and disturbance of the faunal communities which live and breed there (like fish). Velocity in rivers is particularly important because it determines rates of nutrient and oxygen replenishment and relates to the lift and drag force on aquatic species (McDonnell, 2000; WCD, 2000). The elimination of the benefits of seasonal flooding downstream of dams may be the single most ecologically damaging impact of dam construction (Maingi and Marsh, 2002). It is important to mention that the streams below the dam need water to maintain the ecological flow, especially during the dry season which is exactly when the dam retains higher volumes of water. This leads to extensive ecological degradation and loss of biologic diversity (e.g. Jansson et al., 2000).

Dams reduce connectivity of rivers resulting in negative effects on stream biota above and below the impoundment (Tiemann et al., 2004). The construction of dams is considered a significant environmental issue, especially because of the impact it has riparian habitats and fish populations. The construction of a dam also impacts riparian vegetation to a great extent. For example, downstream, invading species, being more resistant to floods, can grow undisturbed and thus accelerate the narrowing process (Tealdi et al., 2011). Regarding fauna, the barriers created prevent natural movements and migration of fish (Holmquist et al., 1996, Collares-Pereira et al., 2000) and, especially in the case of large dams, lotic systems are transformed into extensive lentic systems, promoting large-scale habitat disturbance (Alam et al., 1995; Vié, 1999), and the decrease in water quality and flow create new conditions for the establishment of non-native species with negative consequences on autochthonous diversity (Collares-Pereira et al., 2000; Clavero and Hermoso, 2010).

The World Resources Institute assessed 227 of the major river basins in the world and showed that 37% of the large rivers are strongly affected by dam-related fragmentation and altered flows, 23% are moderately affected, and 40% are unaffected (Revenga et al., 2000). This, and the fact that it is estimated that 1 500 or so dams are currently under construction, nearly 400 of which are over 60 m high (IJHD, 2004) makes the issue on dam construction and its ecological impacts a top priority.

This context of concern is especially relevant in the Mediterranean region, defined by the Mediterranean Sea basin. The Mediterranean basin is considered to be one of the regions that will face the largest changes in climate worldwide (Giorgi, 2006) and where water management is mainly conducted through river regulation (dams) (Collares-Pereira et al., 2000). Water shortage has always been a vital issue in the history of Mediterranean people (Blondel, 2006). Mediterranean habitats experience extreme seasonal variation in water flow. A stress period usually occurs in summer when water flow and level are low to null, following frequently long periods of drought. Reservoirs can affect this situation by further influencing water flow regimes and acting as species movement barriers (e.g. Collares-Pereira et al., 2000; Ruiz-Olmo et al., 2001). The Mediterranean region’s current biodiversity also comprises species whose core distribution is located in no other biogeographical region, which led to its inclusion in the list of the world’s biodiversity hotspots for conservation priorities and main regions to protect (Myers et al., 2000; Brooks et al., 2006). This rich species diversity with a high number of endemisms are the result of the conjunction of three factors: biogeography, geology and history (Blondel and Aronson, 1999). The region hosts about 25,000 plant species (50% of which are endemic), more than 150 000 insect species (on average 15–20% of endemics; up to 90% of endemics in cave systems) and more than 1,100 terrestrial vertebrates (endemism rates range from 17% for breeding birds up to 64% for amphibians (Maiorano et al., 2011).

Like in other Mediterranean countries, the situation of the water resources in Portugal is less favorable when compared with the European context. The negative circumstances found in Portugal result from the high irregularity of the flow distribution in time and space (seasonal and inter-annual), that are not felt in other more northern European countries. The climatic conditions in great part of Portugal also lead to high water consumption in agriculture irrigation and seasonal consumption in touristic activities, occurring manly in periods of water shortage (Cunha, 1996).

The water management is a relatively long-standing process in Portugal and always linked to a hydraulic vision. It is important to mention that the water politics in Portugal result from a political, economic, social and scientific reality developed in the 50s and 60s, dedicated to hydroelectric enterprises and irrigation systems (INAG/MAOT, 2004). According to the Portuguese National Commission for Large Dams (CNPGB), managing water in Portugal means, above all, to invest in hydraulic infra-structures (CNPGB, 1995). This notion and action has always been the main driver of the Portuguese politics and society regarding water management.

Being a country whose landscape is scarce in natural lentic aquatic systems (lakes and lagoons), Portugal has registered, in the last decades, a high increase in lentic water volume. According to the CNPGB, there are 168 large dams in mainland. The north of the country has the higher percentage (57.7%) with 10 large dams located in the Douro river. This higher concentration is due to the fact that these dams are destined to hydroelectric production and find better conditions for this (permanent flow and higher altitude) in the rivers in the North of the country. The South of the country has completely different characteristics, with vast plains and irregular water flow, which do not promote hydroelectric use. The southern dams are therefore mainly for irrigation (the largest agriculture irrigation areas are found in the South) and public supply.

The importance and location of agricultural production, the industrial concentration, the few fossil energetic resources and the population distribution in Portugal, gave a major importance to the proper use of the hydraulic resources. In this scenario, it was decided that, whenever possible, the higher number of uses to each infra-structure should be considered, resulting in a multipurpose scheme, decreasing the specific costs while decreasing the operational costs; as a consequence the majority of our large dams are multipurpose (CNPGB, 1995).

Several irrigation schemes were created in the South of the country associated to large dams: Campilhas, Odivelas, Vigia, Vale do Sado, among others. The larger irrigation area is now being implemented to be supported by the Alqueva dam (110 000 ha). Besides all the irrigation schemes already made, 2 000 individual irrigation schemes were created, spread across Alentejo, based on small and medium sized reservoirs (Godinho and Castro, 1996). The primary use, resulting from the creation of a reservoir, depend on the initial purpose (irrigation, supply, energy production, flow regularization, etc.). However, in practice, there are several secondary uses that can be explored. Among the recreation activities linked to reservoirs, sport fishing arises as the main activity to develop, as some species (e.g. largemouth bass *Micropterus salmoides*) are attractive and important enough to the increase tourism in regions nearby the best fishing locations (Godinho and Castro, 1996).

For three decades, the central focus of energy policy in Portugal has been the promotion of new energy sources, including new electric power plants, to satisfy a growing energy demand. The National Program for Dams with High Hydroelectric Potential (PNBEPH - INAG/DGEG/REN, 2007), was approved by the Portuguese Government in 2007, with the intent to reduce energy dependency and greenhouse gas emissions, improve renewable share of energy production and complement wind power with hydroelectric pumping.

According to the Portuguese Government, Portugal is one of the European Union countries with the highest unexplored water potential and with the higher energetic dependence from the outside. Due to this situation, the government defined goals to the water energy that translate in a clear increase in the current hydroelectric potency. To achieve that goal, which will mean a decrease from 54% to 33% in unexplored water potential until 2020, a series of investments in hydroelectric uses are programmed and described in the PNBEPH (INAG/DGEG/REN, 2007). Up until now, the Government has approved 7 out of the 10 dams predicted for implementation in the PNBEPH as there were no private investors interest in the exploration in two of the dams and one was flunked during the Environmental Impact Assessment due to recognized ecological impacts especially on the freshwater pearl mussel *Margaritifera margaritifera* an endangered species).

I.2. Otters and dams

Animal adaptation to habitat loss, fragmentation and change is a key aspect of species conservation. Human activities are important components of ecosystems, and understanding how natural values persist given extensive human use is important (Palmer et al., 2004).

Otters (Class *Mammalia*; Order *Carnivora*; Family *Mustelida;* Sub-família *Lutrinae*) are semi-aquatic animals whose populations have undergone marked declines during the last century as a result of persecution, destruction of habitats, sensitivity to contamination and changes on the availability of their prey (Foster-Turley et al., 1990). All aspects of otter biology including their shape, metabolism, locomotion, food needs, foraging behavior, social organization, survival and mortality are conditioned by the fact that otters spend most their time in water (e.g. Kruuk, 2006). Otters, besides living in a naturally fluctuating environment, with floods and droughts, are also influenced by the human presence in the aquatic environments. Water pollution, river destruction, water and sediment extraction, prey disturbance and exploitation, bank side vegetation alteration, human disturbance and climate change are major disrupting factors for otters (Mason and MacDonald, 1986; Kruuk, 2006).

Given these characteristics, the otter is a suitable model species to address animal adaptation to habitat loss and change caused by dam implementation.

The Eurasian otter

The Eurasian otter (*Lutra lutra* Linnaeus, 1758) is, of the 13 otter species existing worldwide, the only one existing in Europe. The species is always associated with rivers, streams, ponds, reservoirs, estuaries, or coastal habitats and preys mostly in the water (Chanin, 1985; Kruuk, 1995; Mason and Macdonald, 1986). Iberian otters are smaller than North and Central European ones, with males reaching, on average, 1.20 m and 8 kg and females 1 m and 5 kg (Kruuk, 1995; Ruiz-Olmo, 1995; Ruiz-Olmo, 2007). About one third of this length is tail. Otters are solitary, intra-gender territorial animals, as males will tolerate females within their territory but not other males and vice-versa. Nevertheless, males and females usually avoid each other except for the breading season (Erlinge, 1968). Cubs stay with the mother until 10-12 months and are then driven away.

Otter density is dependent, among other things, on the carrying capacity of the habitat. In Spain otter density in fresh water streams habitats varies between one and seven otters per 10 km stretch of river (Bravo et al., 1998; Ruiz-Olmo, 2007; López-Martín and Jiménez, 2008).

Nevertheless, males may have territories of several dozen kilometers (Erlinge, 1967, 1968; Ruiz-Olmo, 1995; Beja, 1996a; Saavedra, 2002). In lake and extensive fish farm areas densities may reach 16 individuals in 100 km² (Gossow and Kranz, 1998; Sales-Luís et al., 2009).

Otters are generally active during dusk and dawn, although in marine habitats, like Shetland, otters are active during day time (Kruuk and Hewson, 1978; Kruuk, 2006). Dens (holts) are often holes under bank side tree root systems, rocks or piles of flood debris (e.g. branches) (Harper, 1981). There may be several entrances, including an underwater one. Otters often use above ground resting places that can be identified by flattened vegetation such as reed (Hewson, 1969).

The otter is mostly a piscivorous predator but has an opportunistic behavior, taking advantage of the most abundant fish prey but also of seasonal peaks of other classes of prey like amphibians and crustaceans. Reptiles, birds and mammals are also occasionally consumed (Kruuk, 1995; Clavero et al., 2008). The introduction of the American crayfish (*Procambarus clarkii*) in the Iberian Peninsula, altered the diet of the otter as this prey became important to the otters, especially in the south of the peninsula. Nevertheless, crayfish generally does not replace fishes as main prey. In fact otter populations in the Iberian Peninsula are considered to be restricted by fish abundance (Beja, 1996b; Ruiz-Olmo et al., 2001; Clavero et al, 2008; López-Martín and Jiménez, 2008).

In 1999, due to approximately a 20% population decline across Europe over three generations, the Eurasian otter was listed as Vulnerable (VU) by the IUCN (International Union for Conservation of Nature) Red List of Threatened Species (Hilton-Taylor, 2000). Since then, an overall European population recovery has been recorded namely in Spain (Ruiz-Olmo and Delibes, 1998; López-Martín and Jiménez, 2008), Germany (Reuther, 1995), United Kingdom (White et al., 2003) and Denmark (Madsen and Gaarmand, 2000), although in some countries like Italy the population is recovering rather slowly and is still considered at risk (Prigioni et al., 2006; Marcelli and Fusillo, 2009; Loy et al., 2009). Although recovering, otters are still considered “Near Threatened” throughout their range (IUCN, 2011). Also, the otter is still listed in several International Conventions being a strictly protected species European wide: Annex II of the Bern Convention, Annexes B-II e B-IV of the Habitats Directive and Annex I-A of the Washington Convention (CITES).

Otters are known in Portugal from historical times (Santos-Reis et al., 1995). However in 1990, given the scarcity of scientifically data of the species in Portugal, the conservation status of otters was listed as Insufficiently Known in the Portuguese Red Data Book of Terrestrial Vertebrates (SNPRCN, 1990). After this, several studies where performed, resulting in a more comprehensive analysis of the species status. One of the most important studies, organized by the Portuguese Institute for Nature Conservation (ICN), included a nation-wide survey that demonstrated the broad distribution of otters across Portugal, and allowed the mapping of the species distribution (Trindade et al., 1998). Other short-term research projects, which resulted in a some papers and several unpublished reports (thesis), were since carried out in different aquatic environments: rivers (Trindade, 1990; Florêncio, 1994; Afonso, 1997; Chambel, 1997a,b; Freitas, 1999; Lopes, 1999; Bernardo, 2008; Sales-Luís et al., 2012), intermittent streams (Matos, 1999; Salgueiro, 2009; Marques, 2010; Sales-Luís et al., 2012), rice fields (Trindade, 2002) high altitude lagoons (Sousa, 1995), large dams (Pedroso, 1997; Sales-Luís, 1998), estuaries (Campos, 1993; Trigo, 1994; Trindade, 1996; Freitas et al., 2007; Sales-Luís et al., 2009) and sea coast environments (Beja, 1992; Gomes, 1998; Pedrosa, 2000; Cerqueira, 2005). The overall result of these studies demonstrated the existence of a healthy population of otters in Portugal. Consequently, given its broad distribution and inferred high abundance, otters were downgraded in Portugal to the “Least Concern” category (Cabral et al., 2005).

The main threats for otter in Portugal are mostly related with habitat change or destruction mainly thought human intervention, or a direct consequence of human actions. The **destruction of the riparian vegetation** commonly associated with agricultural fields’ maintenance and expansion, gravel and sand extraction and opening of cattle accesses, reduces drastically the shelter and prey availability (ICN, 2006) and thus overall habitat carrying capacity. **Human development and the attraction for riverine, costal and wetland areas** also poses a threat to otter populations (Beja, 1995; ICN, 2006). The **mortality by road kill**, although not expected to seriously affect otters as these are semi-aquatic mammals, as more roads are built and upgraded to sustain more traffic the number of road kills increases, and otters are no exception, although having danger hotspots and being particularly impacted when roads are near lakes or reservoirs or cross over water lines (Grilo et al., 2009). **Direct persecution and hunting** of otters still happens (Trindade, 1991). For example Santos-Reis et al. (2007) showed that otters frequently used fish farming areas and fish farmers perceived them as a problem, and use different methods of deterrence (e.g. fencing, and dogs) or direct persecution (trapping, shooting or even poisoning) to reduce the predator’s impact. **Accidental death by drowning in fike nets** is overall not a very significant threat but it can be important locally (e.g. Castro Marim area - ICN, 2006). The **introduction and invasion of non-native species** commonly has impacts on local prey and competitors and are therefore considered a threat to biodiversity and a conservation issue. The American crayfish and mink (*Neovison vison*) introductions and invasion in Portugal have effects on otters, as the first has become a common prey and possibly is acting as a contributing factor for the spreading of the second which might be viewed as a competitor (Rodrigues et al., 2011). The **water pollution** by toxic compounds, aggravated by the bioaccumulation through the aquatic food chain, affects the otter reproduction ability and cub survival (Olsson and Sandegren, 1991; Roos et al., 2001). Although many toxic compounds have been banned (European Council Directive 79/117/EEC; EC Regulation No 850/2004) contamination from heavy metals and other sources of pollution (industrial and agricultural) still occur namely in Portuguese basins (e.g. Sado basin; MAOT/ARH_Alentejo, 2011). The lack of knowledge about the effects of drastic otter diet changes and bioaccumulation through the aquatic food chain (e.g., metal accumulation in American crayfish in river Sado – Henriques, 2010) needs to be further investigated. Somehow related to pollution issues is the growing public concern for wildlife welfare, the human medical interest in zoonoses, the biologists interests in wild animals potential role as environmental pollution monitors, and the veterinary interest in wildlife potential role as reservoir of infection and antimicrobial resistant bacteria (Simpson, 2000; Oliveira et al., 2011). Nevertheless, little is known about the role of free-ranging wildlife animals as **potential vectors of pathogenic bacteria and antimicrobial resistance determinants** to the environment and vice-versa, as well as the role of antimicrobial resistant pathogens in wildlife health.

Otters in dams

There are several indications that otter species use dams. Sheldon and Toll (1964) found river otter *Lutra canadensis* in a reservoir in Massachusetts (USA) and Passamani and Camargo (1995) confirmed Neotropical otter *Lutra longicaudis* feeding in Furnas reservoir (Brazil). Cape clawless otters *Aonyx capensis*, a species widely distributed in sub-Saharan Africa, occur mainly in freshwater habitats such as rivers, marshes, lakes but also in dams (Somers and Nel, 2004). Anoop and Hussain (2004, 2005) in a study on smooth-coated otter *Lutra perspicillata* along the Periyar Dam (India) noticed habitat use and otter feeding in that reservoir.

Nevertheless, dams have been inferred to adversely influence the distribution of Eurasian otters and are suggested as a contributing factor for the past decline of this species in Europe. According to Macdonald and Mason (1994), habitat destruction and loss through river alteration, such as the creation of dams and reservoirs, together with large-scale wetland drainage, have been severe throughout the range of Eurasian otters. However, this impression has been fostered largely by inference from casual surveys of reservoirs and nearby river stretches and not from dedicated studies. Macdonald and Mason (1982) surveyed several types of habitat in Portugal, including six sites in dams, all negative for otter presence. Elliot (1983) also surveyed 20 dams in Spain and only four had otter presence. Delibes (1990) in a census of otter in Spain mentioned only a small number of positive surveys in dams. Michelot and Bendelé (1995) surveyed isolated river stretches in the river Rhône, France, and found no signs of otter presence.

Optimal habitats for otters are usually defined as areas with good bankside vegetative cover, presence of potential dens providing shelter (e.g. Macdonald and Mason, 1982; Bas et al., 1984; Macdonald and Mason, 1984; Lunnon and Reynolds, 1991; Ruiz-Olmo et al., 2005), high prey availability (e.g. Kruuk et al., 1993; Prenda and Granado-Lorencio, 1995; Beja, 1996b) and low water pollution and human disturbance (e.g. Lunnon and Reynolds, 1991, Prenda and Granado-Lorencio, 1995; Robitaille and Laurence, 2002; Ruiz-Olmo et al., 2005). Upstream, dams create large and deep reservoirs, often with steep shorelines, that are not ideal for otter foraging, which usually occurs in more shallow waters of lotic systems (Houston and McNamara, 1994; Kruuk, 1995; Macdonald and Mason, 1994). Cape clawless otters do not use large surface areas for foraging, but mostly the margins of dams and rivers, being the central part of dams avoided due to depth (Somers and Nel, 2004). The smooth-coated otter in the Periyar Dam selected less rocky, less slanting, shallower and narrower areas of the reservoir for foraging (Anoop and Hussain, 2004, 2005). In addition, the rapid and frequent fluctuation of water level results in scarce riparian vegetation that does not offer enough refuge and security for otters.

As a result, reservoirs presumably are less suitable for otters (e.g. Macdonald and Mason, 1982; Lunnon and Reynolds, 1991; Prenda and Granado-Lorencio, 1995), and dams fragment the habitat and possibly the otter population (Michelot and Bendelé, 1995). This can lead to local extinction of otters and reduce populations below sustainable levels (Macdonald and Mason, 1982). Bouchardy (1986) noted that in drainage systems with multiple dams, otter populations become fragmented and that individuals were constrained to unaffected river stretches up and downstream or other streams in the vicinity. Gutleb (1992) quoting unpublished work by A. Kranz, stated that on the River Kamp, a tributary of the Danube in Austria, many otter signs were found on the upper river which still flows its natural course. However, on the following 35 km, comprising deep reservoirs, very few signs were found. If the lengths of suitable habitat that remain are too short to support viable populations, then the species can be locally lost.

Another effect caused by the presence of dams, especially in North Africa and Southern Europe, is the reduction, or even elimination, of water flow during the warmest period of the year in the rivers downstream. Jiménez and Lacomba (1991) described the extirpation of the otter population on the Palancia River, Spain, which dried following the construction of dams. The problem of reduced flow is exacerbated by use of water for irrigation. Additional disturbances commonly associated with construction of dams include tree-cutting followed by the massive plantation of exotic trees and recreational activities such as water sports and fishing.

Regardless of these aspects, and specifically for Eurasian otters, there are also some indications that reservoirs are used by this species. Gourvelou et al. (2000) confirmed feeding of Eurasian otters in a reservoir in northern Greece. But this reservoir (Lake Kerkini) has high diversity and adundance of fish, meadows and lower deepness and therefore cannot be compared with common reservoirs. Georgiev and Stoycheva (2006) on a study of otter habitats, distribution and population density in the Western Rhodopes Mountains (Southern Bulgaria) surveyed large dams using track measuring in the snow. The population density on reservoir bank sides was very low and the otter presence was always associated with headstream inflows. When all the bankside of Golyam Beglik Dam (21.8 km) was searched, only one possible resident female was found. On Batak Dam (30.0 km), there were three possible resident females and one adult male. The otter was additionally found at Dospat Dam but with no information on the population density.

For otters living in semiarid or Mediterranean environments of Spain and northern Africa, permanently staying in places where vegetation or water is very scarce or non-existent (Kruuk, 1995; Ruiz-Olmo and Delibes, 1998). Ruiz-Olmo et al. (2005) noted that oters were able to survive in such areas by using reservoirs and man-made irrigation channels. In Mediterranean areas, and in population favorable conditions such as the one existing in Portugal, it is proven that otters use these altered habitats (Trindade et al., 1998; Pedroso et al., 2004, 2007). Portugal is one of the countries where more scientific work relating to otters and dams has been conducted but again these are studies restricted in number of dam or seasons. The nation-wide survey that demonstrated the broad distribution of otters across Portugal, included the survey of 28 dams (one survey site per dam) and all were positive for otter presence (Trindade et al., 1998). A four year study on use, prey availability and diet of the Eurasian otter in the Aguieira Dam and associated tributaries (central Portugal) demonstrated that otters regularly use the reservoir, which provide a good prey base, and the associated tributaries, which provide shelter (Pedroso et al., 2007; Sales-Luís et al., 2007). Finally Pedroso (2003) and Pedroso and Santos-Reis (2006) studied the summer diet of otters in 12 large lams of South Portugal and found that all dams had evidence of otter presence.

Reservoir use by otters may depend however on their size, the regularity of their water level and whether they act as a barrier for the otter or not (Ruiz-Olmo, 1995; Ruiz-Olmo, 2001). Prenda et al. (2001) sampled one time 24 small and medium-sized reservoirs in Córdoba province (Southern Spain). These authors stated that areas of large streams and reservoirs that contain water even in the driest months may act as otter refuges during stressful periods and none of the reservoirs (small and medium-sized ones) seemed to act as barriers impeding otter dispersal, both upstream and downstream.

I.3. Thesis rational, structure and aims

The above review of the literature highlights the need for more specific studies on otters in dams. The influence of these structures on otter's life cycle and how they can constitute a disturbance factor is not yet clear. In Europe, as shown in the previous section, the few published data are commonly inconclusive and even contradictory. There are indications that reservoirs are used by Eurasian otters in Mediterranean habitats, but all are limited in time or space (one dam or one season). The lack of information suggests an urgent need for collecting further ecological data on otters and dams, especially when the sound otter population conditions, still existing in Portugal, may be affected by water management policy as implemented over recent decades, with the construction of 168 large dams since 1920, two in currently in construction process and at least another seven planned for the forthcoming years.

The rationale of the thesis affected the choice of study area, the South of Portugal (see Part II - Study Area), included in the Mediterranean area. In the Mediterranean basin, water ecosystems inherently suffer seasonal events of summer drying and wet season floods, which vary markedly on a multi-annual scale contributing to a high natural variability of flow conditions (Magalhães, 2007). Native species are historically resistant to these harsh and highly variable systems, but general trends towards reduced overall precipitation and increased inter-annual variability, which are consistent with scenarios of future climate changes, exacerbated by growing demands for water by agricultural, industrial and tourist activities (Rodríguez-Diáz et al, 2010), will probably have extensive impacts on Mediterranean freshwater ecosystems (Magalhães, 2007). In the southern Portuguese river basins, where rivers commonly have an intermittent water regime and form water pools in summer, aquatic species seem to be living already at the edge of their tolerance limits and water and river management and man uses surely influence the viable maintenance of many water species, including the otter.

Scat or spraint (term used specifically for otter scats) deposition is associated with territoriality and resource defense, and it is a powerful intraspecific means of communication (Kruuk, 1992). Otters are generally active during dusk and dawn, although in marine habitats, like Shetland, otters are active during day time (Kruuk and Hewson, 1978). Therefore, direct observation studies are quite difficult and so otter field signs (spraints, tracks and jellies – musky anal secretions) have been traditionally used in ecological studies (Kruuk et al., 1986; Mason and Macdonald, 1987). Spraint surveys have been widely used over the years as a surrogate variable to assess the otter distribution, identify habitat features considered of importance to otters, and to indicate population status. Nevertheless the relationship between the number of spraints found and the number of otters in that particular site has always been a controversial issue (Jefferies, 1986; Kruuk et al,. 1986; Mason and Macdonald, 1987; Kruuk, 1995; Kranz, 1996). Moreover, most authors agree that sprainting activity varies with sex, season, and with social and reproductive status (Macdonald, 1983; Kruuk, 1992; Kranz, 1996). These observations lead to the conclusion that the number of spraints cannot be used to assess otter densities, although it is a useful tool for comparing defence of resources (Kruuk, 1992) and intensity of use between sites.

A survey method based on otter signs detection was developed for the Eurasian otter (Macdonald, 1983), and first adopted on a large scale during the national surveys of Britain and Ireland. This method uses surveys of stretches of 600 m of river banks for searching evidence of otter presence. Results are usually expressed in terms of percentage of positive sites, whether describing the data for a country, a region, a catchment, or some artificial unit such as a 10x10 km square (Chanin, 2003). This standard method for otter surveys was recognised as such by the IUCN Otter Specialist Group (OSG) after a major review of surveying methods carried out by Reuther et al. (2002). Nevertheless, this is standard methodology is more directed for monitoring otter in lotic systems (streams, rivers) and may gain to be adjusted when applied to different types of systems, like lentic systems (large dams). This is especially relevant when addressing otter presence and distribution in dry areas, such as the Mediterranean region, where different habitats or systems, such as dams, appear to have a role to play. This will be the first issue to be addressed in the framework of this thesis **– PART III**.

PART IV will focus mainly on assessing the degree of use and presence of otter in reservoirs and adjacent streams, and measuring the changes in otter habitat and ecological requirements (e.g. refuge, foraging) with two main aims:

1) To understand if there is a generalized use of the reservoirs resulting from large dams by otter in a Mediterranean region, by addressing as main questions: Can large dams be suitable habitat elements for otters? Is that use variable according to season, characteristics of the reservoir, prey abundance, shelter quality? Do otters use reservoirs of small-medium sized and large dams differentially? This will be addressed by studying, several reservoirs in the Alentejo provinces, South of Portugal throughout different seasons on a wider scale;

2) To understand how otter distribution changes over time in response to the changes in the species main requirements imposed by the construction of a large dam, by addressing as main questions: How does the habitat (e.g. available cover, vegetation) change during and after the construction of a dam and how does that affect the otter? How does the otter prey community and suitable feeding habitats change after dam construction and does the otter adjusts to this

change? This will be addressed by studying one particular dam throughout all phases of its implementation.

Additionally, due to the importance of water pollution and contamination issues, and the fact that reservoir waters are frequently affected by upstream agriculture activities and nearby cattle raising, this thesis aims to collect data about the antimicrobial resistance of bacteria of otter enteric microbiota and the role of otter as a potential vectors of pathogenic bacteria and antimicrobial resistance determinants to the environment and vice-versa. This is especially relevant in sympatric areas of human activities (recreational activities in water and economic – agriculture, cattle raising) and otter occurrence, such as in dams in the Mediterranean areas - **PART V**.

Data integration of the different components of this thesis will contribute to a better understanding of use of dam reservoirs by the otter, especially of the overall impact of large dams in otter populations, and of the implications for the species conservation strategy and for conservation management in dams in Mediterranean areas **– PART VI**.

Furthermore, the data, knowledge and experience that resulted from the several studies implemented in the context of this thesis helped to write, along with other members of the IUCN Otter Specialist Group, recommendations intended to guide developers and consultants preparing environmental impact assessments (EIAs), as well as NGOs and EIA advisors (biologists and lawyers) in administrations, who have to check that the otter has been properly considered in the course of an EIA according to the amended Council Directive 85/337/EEC **– APPENDIX**.

This Ph.D. dissertation translates into two papers published in scientific journals, one book chapter, and two submitted manuscripts, all peer-reviewed publications. The publications, by Parts, composing this dissertation are:

Part III – Monitoring otters in dam reservoirs

1) Pedroso, N.M., Santos-Rei, M., 2009. Assessing otter presence in dams: a methodological proposal. IUCN/SCC Otter Specialist Group Bulletin 26, 97–109. (Paper 1)

Part IV – Ecology of Iberian Otters in Dams

2) Pedroso, N.M., Santos-Rei, M., (submitted). Can large reservoirs be suitable habitats elements for otters? A multi-dam approach in a Mediterranean region. Biodiversity and Conservation. (Paper 2)

3) Basto, M.P., Pedroso, N.M., Mira, A., Santos-Reis, M., 2011. Use of small and medium-sized water reservoirs by otters in a Mediterranean ecosystem. Animal Biology 60, 75–94. (Paper 3)

4) Pedroso, N.M., Marques, T.A., Santos-Reis, M., (submitted). Otter response to environmental changes imposed by large dam construction. Aquatic Conservation: Marine and Freshwater Ecosystems (Paper 4)

Part V – Otter as potential vectors of pathogenic bacteria

5) Oliveira, M., Pedroso, N.M., Sales-Luís. T, Santos-Reis, M., Tavares, L., Vilela, CL., 2009. Evidence of antimicrobial resistance in Eurasian otter (*Lutra lutra* Linnaeus, 1758) fecal bacteria in Portugal. In: Wildlife: Destruction, Conservation and Biodiversity. J.D. Harris and P. L. Brown (Ed.). Nova Science Publishers, Inc. pp. 201-221. (Paper 5)

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PART II – STUDY AREAS

II.1. Studied dams

The geography of continental Portugal is a mixture of Atlantic and Mediterranean influences, with the former dominating the north of the country and the second the south. The Mediterranean influence is felt primarily in the south and east regions: Alentejo and Algarve. The Alentejo is subdivided into four major river basins: Tejo, Sado, Guadiana and Mira, and several small basins draining the western slopes of the Grândola and Cercal mountain range. In Alentejo, with plain areas and irregular water flow, large dams were built mainly for irrigation (the largest agriculture irrigation areas are found in the South) and public supply (CNPBG, 2012).

The use of large dam reservoirs by otters in a Mediterranean region was addressed in this dissertation by studying 12 dams (reservoirs and associated river stretches) from the Guadiana and Sado river basins throughout different seasons. Four of these dams (Caia, Vigia, Monte Novo and Lucefécit) are located in the Guadiana river basin and eight (Alvito, Odivelas, Pego do Altar, Vale do Gaio, Fonte Cerne, Campilhas, Roxo and Monte da Rocha) in the Sado river basin (Figure II.1.1.). Additionally, Pego do Altar and Monte Novo dams and associated river stretches were targeted to further collect data about the antimicrobial resistance of bacteria of otter enteric microbiota and antimicrobial resistance determinants (Figure II.1.1.).

To understand if otters differentially use small-medium size reservoirs and large dams, 30 small and medium sized reservoirs were studied in “Serra de Monfurado”, a Natura 2000 Site (PTCON0031), located in the Sado river Basin (Figure II.1.1.).

To comprehend how otter distribution changes over time in response to the changes in the species main requirements imposed by the construction of a large dam, one particular dam was studied throughout all phases of its implementation, the Alqueva Dam, in the Guadiana river basin (Figure II.1.1.).

Detailed description of each study area is available in PART IV and V.

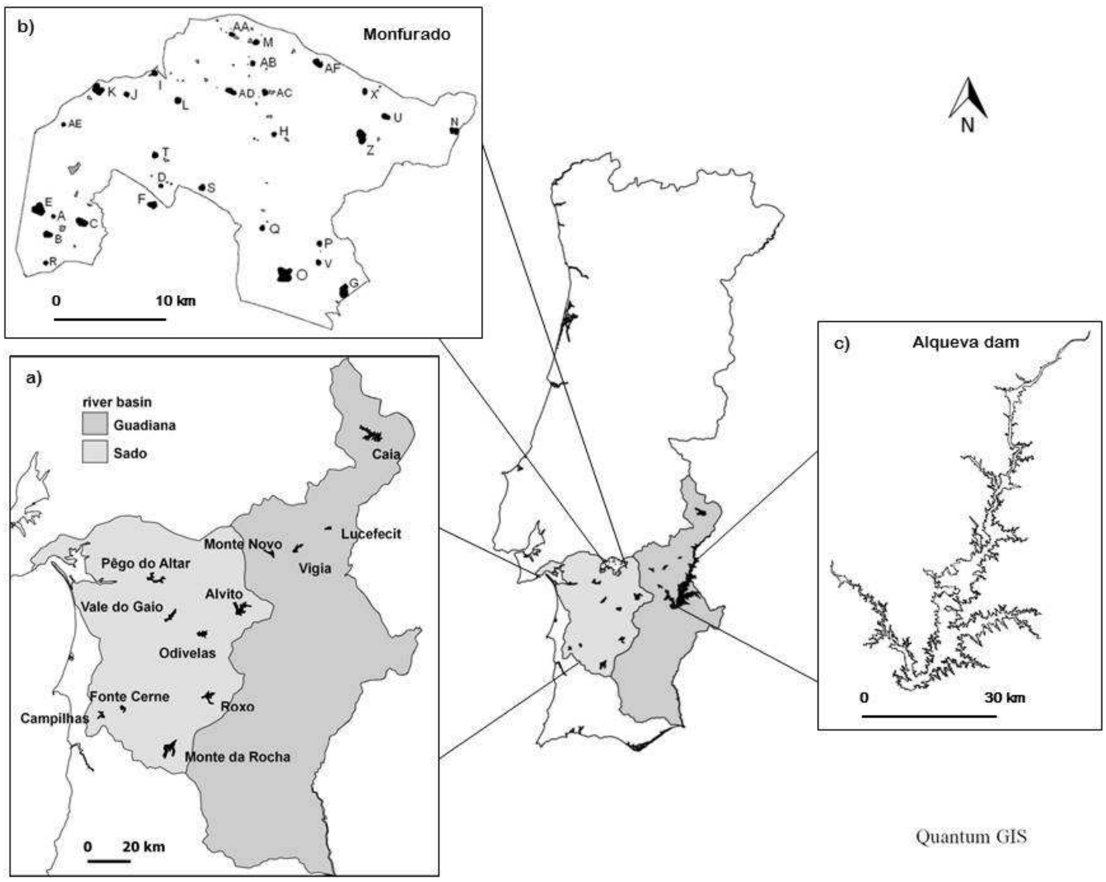

Figure II.1.1 – Location of study areas in Portugal and in Sado and Guadiana river basins: a) Caia, Vigia, Monte Novo, Lucefécit, Alvito, Odivelas, Pego do Altar, Vale do Gaio, Fonte Cerne, Campilhas, Roxo and Monte da Rocha dams; b) “Serra de Monfurado”, Natura 2000 Site PTCON0031; c) Alqueva dam.

II.2. Guadiana and Sado river basins

River flow variability along the year, as well as in a year to year basis, is influenced by precipitation and greater variation occurs in dryer regions. Portuguese northern rivers, located in higher precipitation areas, have a more permanent and stronger flow rate while rivers in the south tend to dry partially or completely in summer. In Alentejo, the yearly average precipitation varies from 500 to 800 mm. Precipitation in Portugal, besides being irregularly distributed in space, shows also a great variability within and among years (http://www.inag.pt). Nevertheless, precipitation is concentrated mostly during the October to March period. Generally, the months of December and January have the highest rainfall values with the lowest occurring in July and August.

Guadiana river is amongst the four most important rivers flowing in Portugal – the Minho, Douro, Tejo and Guadiana – that are all international basins originating in Spain. Sado river basin is the largest watershed entirely located in Portuguese territory (INAG/MAOT, 2004). Both basins are located in a typically Mediterranean setting. Here, water ecosystems inherently suffer seasonal events of summer drying and wet season flooding, which vary markedly on a multi-annual scale and contribute to a high natural variability of flow conditions (Magalhães et al., 2007). Many of the small streams in these basins are ephemeral drying partially or even completely during summer. The surrounding landscape is mostly covered by Mediterranean cork (*Quercus suber* Linnaeus, 1753) and holm (*Quercus ilex* Linnaeus, 1753) oak woodlands but there are also considerable areas of agriculture fields, and plantations of olive trees (*Olea europaea* Linnaeus, 1753), of maritime pine (*Pinus pinaster* Aiton, 1789) and eucalyptus (*Eucaliptus globulus* Labill, 1800). In this dry climate the effects of water shortage has led to an increasing intervention on watercourses and most water management is based in hydraulic infrastructures, particularly the construction of water reserves for public supply and agricultural purposes. As a result, aquatic ecosystems have undergone changes that undermine their aquatic fauna, including the otter, but particularly native fish fauna, with the loss of longitudinal river continuity and the destruction of natural habitats. In fact, a large number of fish species currently present conservation status including the categories of Vulnerable, Rare or Endangered (Cabral et al., 2005). The fresh water fish fauna of southern Portugal has a high interest in evolutionary and ecological terms. Many species are Iberian endemism, increasing their number from north to south, and presenting its highest expression in the Guadiana River (Almaça 1978, Collares-Pereira, 1985).

Guadiana river basin

The Guadiana river basin covers an area of 66 960 km^2 of which only 17% (11 700 km^2) are located in national territory. The Guadiana river travels 150 km in Portugal and about 110 km in the border with Spain being bound to the north by the Tagus river basin, to the east by the river Odiel Jucar (Spain) and on the west by Sado, Mira and Arade river basins. In Portugal, the main tributaries on the right bank are the rivers Caia and Degebe and the streams Cobres, Vasco, Foupana, Oeiras and Odeleite, and on the left bank the river Ardila, and the streams Alcarrache and Chança. The natural regime annual runoff of the Guadiana river averaged 6 700 hm^3 of which 1 820 hm^3 were from its national part and 4 900 hm^3 from the Spanish side (INAG, 2004). Flows from Spain, due to increased water retention and use, have however been reduced severely, with expected values in 2012 that do not exceed 2 135 hm^3 / year; that is 44% of the mean annual runoff under natural regime (INAG/MAOT, 2001). The existence of such a high consumption and retention in Spain lead to a significant reduction in the influx to Portugal, besides an increased irregularity of the flow. Water retention through hydraulic infrastructures is also the chosen management approach in Portugal. The Guadiana river basin has 25 large reservoirs (Figure II.1.1.) and several smaller reservoirs. The Alqueva dam alone allowed an additional water retrieval source of 1500 hm^3 / year (INAG/MAOT, 2001). From the global water uses in the basin, agriculture (irrigation and livestock) is by far the largest user sector, representing about 84.5% of the total consumption value. Public supply corresponds to 10,8%, industrial consumption to 1,9%, services to 1.4% and the remainder, just over 1.4% of the needs of the basin, refers to tourism, including water used in golf fields maintenance (ARH_Alentejo 2011). The expected water balance for the Guadiana basin, after the full implementation of the Alqueva dam and surrounding agriculture fields, presents a scenario of water scarcity in dry or average rain years, water needs being far superior then availability (ARH_Alentejo 2011).

According to the application of Water Framework Directive (WFD – Directive 2000/60/EC) classification, approximately 41% of the 260 surface waters of Guadiana river basin are ranked as “Good”, 36% as “Reasonable”, 20% as “Mediocre” and 0,4% as “Bad”, being Alqueva included in this last category (inferred from RH7 data - ARH_Alentejo 2011). The aim is to achieve the status “Good” for most water lines.

The Guadiana river high temperature is a parameter that conditions water quality classification. In fact, the reduced flow rates available in the hydrographic network of the Guadiana basin during summer, associated with the high temperatures felt, lead to an increased vulnerability to pollution of the river system. It is also observed that the values of physico-chemical parameters characterizing water quality in the river Guadiana have large spatial variability (values quite different along the water line) and a very irregular temporal distribution. The high variability of the state of the water quality is due to a combination of various factors, amongst which, high temporal distribution irregularity of the circulating water volumes and pollution loads. Regarding eutrophication, the stretches of the river Guadiana are usually meso-eutrophic. The reservoirs in general are in a mesotrophic or meso / eutrophic state, some being even oligotrophic (INAG/MAOT, 2001).

Due to geographical barriers and the particular environmental constraints, the Guadiana river basin has the highest number of endemic fish species of the Portuguese mainland basins therefore, requiring more attention in conservation terms (Collares-Pereira, 1985). Apparently, the fish communities are well adapted to the temporary flow regime, highlighting ecological strategies in face of such systems. However, in recent decades, there has been growing interventions in the Guadiana basin, in particular impoundments and the use of water for multiple purposes. As a result, many fish stocks are currently in an apparent state of depletion, and some have been classified by the Portuguese Red Book (Cabral et al., 2005) as Threatened or Endangered species such as the saramugo (*Anaecypris hispanica*). Also to be noted is the presence of some species, such as the Iberian straight-mouth nase (*Pseudochondrostoma willkommii),* classified as Vulnerable (Cabral et al., 2005) and the Iberian small-head barbel (*Barbus microcephalus*), Southern Iberian barbel (*Barbus sclateri*) and Iberian long-snout barbel (*Barbus comizo*) classified as Endangered (Cabral et al., 2005) species that can only be found in this basin, and also anadromous migratory species such as the sea lamprey (*Petromyzon marinus*), the allis shad (*Alosa alosa*) and the twaite shad (*Alosa fallax*), all also Endangered. The ell (*Anguilla anguilla*), a catadromous migratory species that completes its life cycle in fresh waters, was considered commercially threatened due to fishing pressure over larval states. Overall, there are 16 native freshwater species and 11 non-native species in the Guadiana river basin (Ribeiro et al., 2007). The common carp (*Cyprinus carpio*) and the goldfish (*Carassius auratus*) are old introductions in Iberian rivers (probably from the early XVIIIth century), while other, like pumpkinseed sunfish (*Lepomis gibossus*), mosquito fish (*Gambusia holbrooki*) or largemouth bass (*Micropterus salmoides*) among others, result from more recent introduction events (last decades) (Almaça, 1995; Ribeiro et al., 2008). Most of these non-native species have a preference for lentic habitats for they are largely lentic in their native range (Filipe et al., 2004; Ribeiro et al., 2008).

Sado river basin

The river Sado rises in the Serra da Vigia, about 230 meters above sea level, and after a path of 175 kilometers flows into the Atlantic ocean near the city of Setúbal through a broad estuary with about 100 km2 (INAG/MAOT, 2004). This river features an unusual orientation in Portugal, running nearly south to north. It is considered a lowland river, since more than half of its course falls below 50 meters in altitude. The entire basin covers an area of about 7 692 km^2, making it the largest of the exclusively Portuguese rivers (INAG/MAOT, 2004). The annual runoff of the river Sado averages 1 000 hm^3. The Sado river basin is the Portuguese watershed with fewer mean annual water surface resources per unit area (INAG/MAOT, 2004). Even so, due to the extensive catchments of the river Sado, the main river usually maintains flow. The main tributaries and sub-tributaries of the river Sado are: in the right margin the Marateca, São Martinho, Alcáçovas, Xarrama, Odivelas and Roxo streams; and in the left margin Grândola, Corona and Campilhas streams. The basin has 14 large dams and several smaller reservoirs (Figure. II.2.1). Such as in the Guadiana basin, in all reservoirs of the Sado basin, with the exception of the Alqueva dam, the existence of one or two years of drought, causes the immediate drop in water levels in the reservoir implying consequent restrictions on the supply of water.

Global water needs in the Sado River Basin is distributed as follows: 80.4% for agriculture and livestock; 9.5% for urban supply; 7.6% to industry; and 2.5% for other uses. Most of the demand is covered with surface water as only 23% represents abstractions from ground water. The available surface water, based also on the adjustment capacity of reservoirs, varies immensely from dry, to average to wet years, and the satisfaction of all consumptive uses exceeds the amount of surface water available every dry year (CCDR_Alentejo 2001).

According to the application of Water Framework Directive (WFD – Directive 2000/60/EC) classification, approximately 42% of the surface waters of Sado basin are ranked as “Good”, 41% as “Reasonable”, 13% as “Mediocre” and 3% as “Bad” (inferred from RH6 data - MAOT/ARH_Alentejo 2011).

Water quality in Sado watershed is strongly conditioned by the seasonal character of its flow and the regional variation of its climatic conditions. The river Sado, mainly due to receiving "artificially" generated flows from waste water and irrigation runoff, combined with high temperatures and degree of insulation in summer, provide a high primary production and the frequent occurrence of blooms of microalgae (cyanobacteria). These blooms may negatively impact water quality, especially with regard to the aquatic fauna. This situation is also observed in other water bodies, like coastal lagoons (e.g., Sancha, Melides, and St. André) and reservoirs. This intrinsic condition, favouring water quality degradation and inducing a high vulnerability to other pollution sources, may aggravate this situation. Particularly critical in this context are the significant additional loads of organic matter and nutrients, especially nitrogen and phosphorus, the main drivers of algal growth (a situation that may occur with increased pollutant loads from irrigation), enhancing eutrophication. Identified sources of nitrogen and phosphorus belong mainly to the agricultural and livestock sector (> 95%) (CCDR_Alentejo 2001)

Overall the river Sado has significant problems of water quality degradation, associated essentially with eutrophication, but triggered by natural and anthropogenic factors. It is possible to recognize a large temporal and spatial variability associated mainly with the seasonal pattern of river flow, the strong irregularity of the basin physiography and an irregular distribution of pollution sources (Sales-Luís, 2011). As in other systems, Sado river basin has undergone multiple and successive interventions, mainly motivated by the need for the use of water resources.

The Sado river basin has a less rich fish community (lower species richness and endemism) than the Guadiana basin. There are 10 native freshwater species (Ribeiro et al., 2007). Of the six Iberian endemic species present, five are cyprinids and only the Portuguese arched-mouth nase (*Iberochondrostoma lusitanicum*) is considered Critically Endangered (Cabral et al., 2005). Some of the endemisms, Iberian barbel (*Barbo bocagei*), southern straight-mouth nase (*Pseudochondrostoma polylepis*) are included in annexes II and V of the Habitats Directive (92/43/CEE). In what concerns non-native species, Sado basin presents 8 non-native species (Ribeiro et al., 2007).

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PART III – MONITORING OTTERS IN DAMS

III.1. Assessing otter presence in dams: a methodological proposal

PAPER 1

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Assessing otter presence in dams: a methodological proposal

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Abstract

Standard otter survey methodology proposed by the IUCN Otter Specialist Group enables comparisons in present/absence data in different countries or in different regions. However, otter presence and distribution assessment in dry areas, such as the Mediterranean region, which are characterized by highly marked seasonal climate with intermittent water flow coupled with different types of habitats or systems such as dams, may gain from adjustment to the methodology. Pressure for dam building still occurs in these regions and the need for studies on ecological communities and species protection is increasing. Dams are very different from usual otter riverine habitat and we need to understand their influence on otter populations. Variation of dam location, reservoir characteristics and season will all influence spraint detectability. Environmental Impact Assessment Studies and ecological studies are frequently limited by both budget and time requiring field researchers to apply more efficient methodologies. Based on experience from studies conducted in Portugal we propose adjustments to the standard survey methodology (using spraints) surveying otter presence in dams to be applied specifically to Mediterranean-type ecosystems. We define aspects to be considered regarding survey season, survey length and width, number and location of survey sites, among others. This paper will allow researchers to plan more effective field surveys based on standard otter survey methodology for the purpose of dam surveys, be that to detect otter presence/absence, a more in-depth comparative studies of otter presence, or simply improving the collecting of fresh spraints for molecular spraint analysis.

Keywords: Eurasian otters, Mediterranean, reservoirs, survey methodology.

Introduction

One of the five species evaluation criteria in the IUCN Red List Categories and Criteria is the population trend (IUCN, 2001), and this requires observing changes in species distribution over time and space. For Eurasian otters (*Lutra lutra*) this translates mostly in presence/absence data resulting from spraint surveys. This occurs because otters are difficult to capture (particularly since the prohibition of use of leg-hold-traps – EEC 1991 - Council Regulation No 3254/91) and consequently to radio-track, and non-invasive molecular methods are still expensive and with a low rate of success due to the degraded condition of DNA from faecal samples.

In the 80's a survey method was developed for the Eurasian otter (Macdonald, 1983), and first adopted on a large scale during the national surveys of Britain and Ireland. This method uses surveys of stretches of 600 m of river banks for searching evidence of otter presence. Results are usually expressed in terms of percentage of positive sites, whether describing the data for a country, a region, a catchment, or some artificial unit such as a 10x10 km square (Chanin, 2003). This standard method for otter surveys was recognised as such by the IUCN Otter Specialist Group (OSG) after a major review of surveying methods carried out by Reuther et al., (2002). Most importantly the OSG standardised methodology enabled comparisons in presence/absence data in different countries whereas before it was difficult to assure that data were comparable (due to different surveys efforts, etc). This standard methodology for monitoring purposes in lotic systems (streams, rivers) may however, be complemented when applied to different types of systems, like lentic systems (large dams). This is especially relevant when we address otter presence and distribution in dry areas, such as the Mediterranean region, considered one of the biodiversity hotspots for conservation priorities (Myers et al., 2000), where different habitats or systems, such as dams, appear to have a role to play.

Dams, and specifically large ones (defined as having a dam wall with ≥ 15 m of height, or a wall height between 5-15 m and a reservoir volume greater than three million m^3 - WCD, 2000), are very different from the usual otter riverine habitats and we need to understand the influence of these "man-made" habitats on otter populations to be able to act on its protection. This context is especially relevant in Mediterranean areas or other similar regions, where water policy is largely based on such infrastructures, and streams suffer several other pressures (climatic and human). A reduction in dam building has occurred in North America and Europe in the last years, due to the facts that most of the technically appealing places for dam implementation having already been occupied, and there is now a greater concern for the environment. Nevertheless, dam building, especially large dams still continued in the last years in several countries (e.g. in Mediterranean countries like Portugal or Spain, or in developing Asian countries like India or China). As a consequence, case studies of otters and large dams appeared and, in the near future and with the uprising of environmental concerns, more studies are expected to occur, most of them linked with minimization and compensation environmental measures regarding dam implementation. The current scenario of climate change in Europe affects the riverine systems in the Mediterranean regions, mostly by extending the drought period. This is relevant, since increasing demand and management of water can influence otter distribution and affect long-term viability (Barbosa et al., 2003).

Portugal, located in Western Mediterranean Europe, has always been one of the strongholds of otter in Europe, considered to be one of the most viable and widespread populations in Europe. Nation-wide surveys conducted in 1995 and 1998 at the coarse grid resolution of 10x10 km, as advised by the OSG, resulted in a broad otter presence across all of the country with the exception of the two main cities (Lisbon and Oporto). As a result, otters were downgraded in Portugal from “Insufficiently Known” to “Least Concern” (Cabral et al., 2005), a result that contrasts with its European status - “Near Threatened” category (IUCN, 2006).

The objective of this paper is to propose adjustments to the OSG standard otter survey methodology, to focus on otter presence in dams, which can be applied specifically in the Mediterranean region or Mediterranean-type ecosystems, characterized by a highly marked seasonal climate with intermittent river flow. We will identify concerns of applying the OSG standard methodology to dam surveys and use the experience gained in studies conducted in Portugal to address these adjustments.

Otters in dams

Few studies have addressed the use of dams by otters. Most of these complement the use of spraints with other methods, either because the authors were able to capture and radio-track otters (Somers and Nel, 2004), or because they could watch the individuals (Annop and Hussain, 2004; 2005; Rosas et al., 2007). Somers and Nel (2004) caught seven Cape clawless otters *Aonyx capensis* in wire-cage traps in two rivers of South Africa and all included the Clanwilliam and Bulshoek dams in their home ranges. Anoop and Hussain (2004, 2005) in a study on smooth-coated otters *Lutra perspicillata* along the Periyar Dam (India) detected resting and foraging activities in that reservoir; they used both otter signs and direct observations of the species because smooth-coated otters are social carnivores that forage in groups and use communal sites for defecation (Hussain, 1996; Hussain and Choudhury, 1997). Rosas et al. (2007) collected data on the occurrence and habitat use of giant otters *Pteronura brasiliensis* in the Balbina hydroelectric lake in central Amazonia (Brazil), using motor boats to observe the otters.

Regarding Eurasian otters, studies involving lentic systems are mostly on diet analysis on the basis of spraints (Gourvelou et al., 2000; Rhodes, 2004, Sales-Luís et al., 2007), and those that address habitat use mainly use snow-tracking (from early studies - e.g. Erlinge 1967, 1968; to other more recent: e.g Rimov reservoir, Czech Republic – Rhodes, 2004; central Finland lakes - Sulkava, 2006). Georgiev and Stoycheva (2006) in a study of otter in the Rhodopes Mountain (Bulgaria) that included two large dams, used both tracking in mud and wet soil and spraints. However, neither snow-tracking nor telemetry can be easily applied in Mediterranean countries, such as Portugal, since captures are difficult (particularly since the prohibition of use of leg-hold-traps – EEC 1991 - Council Regulation No 3254/91) and there are very few areas with snow due to the average high temperatures. Furthermore, studies relying on direct observations are difficult to implement.

Research on the importance of lentic systems in the ecology of Eurasian otters in Portugal started in 1996 and is still ongoing. In this country, the authors showed that Eurasian otters use the reservoirs of large dams (Santos et al., 2007; Pedroso et al., 2007) and feed in them (Pedroso and Santos-Reis, 2006; Sales-Luis et al., 2007). Reservoirs seem to constitute an “attraction point” for otters particularly in drought periods when rivers and streams dry up (Prenda et al., 2001; Pedroso and Santos-Reis, 2006).

During these studies, that were sign survey-based, some methodological difficulties were encountered and adaptations were implemented. Of the 13 studied large dams, one is the Aguieira Dam, located in central Portugal, on the middle section of the River Mondego, which has a permanent water flow. The other 12 dams are all located in the South of Portugal, along the Guadiana and Sado river basins, that suffer high water level variation as in dry seasons most of the tributaries dry up (Table III.1.1) (for further dam characterization and information see Pedroso and Santos-Reis, 2006; Pedroso et al., 2007; Sales-Luis et al., 2007).

Table III.1.1- Characteristics of surveyed dams in Portugal.

Dam	Alvito	Odivelas	P. Altar	M. Rocha	F. Cerne	Campilhas	Aguieira
area (ha)	1 480	973	876	1 100	105	333	2 000
wall (m)	49	55	63	55	18	35	89
perimeter (km)	90 296	58 639	85 397	82 594	19 960	32 979	39 310
Dam	**Roxo**	**V. Gaio**	**Caia**	**Lucefecit**	**Vigia**	**M. Novo**	
area (ha)	1 378	550	1 970	169	262	277	
wall (m)	49	51	52	23	30	30	
perimeter (km)	95 829	41 215	107 457	19 001	20 000	24 000	

Parameters of concern

Usually standard OSG method is applied to lotic systems, however there are obvious differences when we are dealing with large lentic systems (large reservoirs). From our studies we can point out several specific parameters of the lentic systems that can influence spraint detectability and otter marking behaviour, and should therefore be taken in consideration when surveying this type of habitats.

1 – Water level variation

All of the reservoirs have fluctuation of the water level that result in a bank flooding area with scarce riparian vegetation. This fluctuation may be rapid and frequent, when we are dealing with dams used for electricity production, and water level may change very quickly over some meters in few days (e.g. dams of North and Center of Portugal, like the Aguieira Dam), or be slower, occurring over several weeks or months, as in dams used for irrigation or water consumption (e.g. dams of South of Portugal, like the 12 studied dams in the Sado and Guadiana basins). When reservoirs fill up to maximum or near maximum capability, the bank is flooded up to the vegetation line. This happens especially during wet seasons when the reservoirs fill up due to rainfall, and maintain the water level high, if possible up to the end of spring acting as a water reserve for irrigation and public consumption during summer and autumn. In the Aguieira Dam study (Pedroso et al., 2007), the water level was very high from March to July, sometimes submerging all the bank flooding area and thereby reducing spraint detectability. In this study, the decrease in numbers of signs in some of the months was associated with high water level in the reservoir.

To avoid loss of information, particularly in the drier months when water was at its lower level, surveys in all of the 13 dams were extended to the entire bank flooding area, easily recognisable by the absence of vegetation due to water level variation. Signs found were distributed throughout the entire width of the bank flooding area, although around 80% were concentrated in the first five meters from the water edge. (Pedroso et al., 2007; Pedroso, unpublished data). Similar results were found by Anoop and Hussain (2004), in a study with smooth-coated otters (*Lutra perspicillata*) in the Periyar Lake Dam (Kerala, India), who found that if otters are present in an area their signs were most likely to be encountered within the 10 m perpendicular to the shoreline, and these authors used surveys of 250x10 m along the water's edge.

2 – Rainfall

Rainfall influences spraints durability in the field. Otter surveys should not be carried out during periods when there is heavy rain since this may lead to a decline in the proportion of positive sites (Chanin, 2003). In reservoirs, bank flooding areas usually lack riparian vegetation and frequently have steep margins. These promote the washing out of spraints when subjected to rain. In the Aguieira Dam study, the decrease in number of signs between months (up to 260%) was associated with heavy rain, mainly from November to January (Pedroso et al., 2007).

3 – Presence of marking sites

Most signs found in the studied reservoirs comprised spraints – 90.2%; 3.3% were prey remains, 3.0% were scent marks and 1.7% were footprints/tracks. The marking sites were consistent with those described for the species: isolated or small groups of rocks (55.0%), large rocky boulders (17.0%), soil surface (14.3%) and logs/branches/tree roots (8.0%); vegetation and small sand hills (<5.0% each) were also used (Figure III.1.1).

Figure III.1.1 - Otter marking sites at Monte Novo Dam, South Portugal.

Several papers state the use of these substrates as common marking places for otter (e.g. Georgiev 2007, in a study in South-East Bulgaria that included two small artificial lakes – 2.7 ha and 4.7 ha - refer that stones were the most marked substrate).

Spraints at a specific marking site (e.g conspicuous rock) were a common feature of most surveyed sites. This site fidelity by *Lutra lutra* was already reported by several authors (e.g. Kruuk et al., 1986; Mason and Macdonald, 1986). Kruuk (2006) also states that spraints are left on prominent aspects of landscape (e.g. rocks, logs) that may be permanent spraint sites. This author reports that, in the Shetlands, the same sprainting sites are often used for many years. A similar observation was made by Anoop and Hussain (2004) in the Periyar Lake Dam where grooming and sprainting sites were regularly visited by smooth-coated otters. In our studied dams the number of spraints found was correlated with the number of marking sites (Pedroso et al., 2007; Basto et al., in prep.), which is a relation common in streams also (Sales-Luís et al., 2007). Relations between marking intensity and otter use in certain survey sites must take this in consideration.

4 - Bank side characteristics

One aspect that may influence spraint detectability is bank steepness. Although no statistical significance was detected in the studied dams, generally banks with greater steepness had fewer signs than those less steep. Less steep stretches have a wider surface of bank flooding, corresponding to a larger potential marking area in months of low water level and, when flooded, a better chance of prey capture (e.g. Kruuk, 2006). To what extent otters use the deeper water in the studied reservoirs it is still unclear but it is likely that the otter is mainly using the shoreline of the reservoir, restricting its activity, especially foraging, to the littoral zones. This is supported by the fact that the main prey consumed in the reservoirs studied are present mainly around the shoreline (Sales-Luís et al., 2007) (Figure III.1.2).

Figure III.1.2 - Reservoir of Aguieira Dam, central Portugal.

Other literature also addresses this aspect. Anoop and Hussain (2004) found that smooth-coated otters in the Periyar Lake Dam were confined to the shallower and narrower regions of the lake, where the bank was gradually sloping mud, avoiding deeper parts of the lake, with steeper rocky banks. The preferred areas for otters in the Periyar Lake were characterized by a slope of <5◦ and low water depth that extended over a few metres, and offered excellent foraging ground. Duplaix (1980) in Surinam made a similar observation, with giant otters *Pteronura brasiliensis* preferring low sloping banks with good cover and easy access to abundant prey. In the Shetlands, Kruuk (1995) also made similar observations for the eurasian otter. For example, otters foraged mainly in water usually less then 8 meters deep, with short dives. Georgiev and Stoycheva (2006) also did not found evidence of otter in the deep margins of two large reservoirs, and only in the areas close to the river inflow.

Riparian vegetation, which plays an important role for otters in river tributaries (Macdonald and Mason, 1982; Bas et al., 1984; Lunnon and Reynolds, 1991), is expected to be also important in lentic systems. Kruuk and Goudswaard (1990), while investigating the reasons for the declining number of otters in Lake Victoria (Tanzania), described the virtual absence of otters from a section of the lake where the bank-side vegetation was poor. Anoop and Hussain (2004) made similar observations in Periyar Lake and suggest that vegetation cover on the bank may be important to otters throughout their distribution range. Results of the 12 dams in the South of Portugal confirm the importance of this resource, as vegetation availability was positively correlated with the number of spraints found. So it was expected that positive sites for otter presence would mostly be found in areas with good cover. However, in the Aguieira Dam, the number of otter signs was negatively correlated to cover (Pedroso et al., 2007). This, however, may have been a consequence of the fact that the reservoir of the dam was full for several months of the year, diminishing the bank flooding area; the flooding area available for survey was most of the times small or absent and the area next to it was difficult to survey since it was occupied by dense vegetation. Also, Kruuk (2006) stated that radio-tracked otters in Shetland did not show preference for particular vegetation and just happen to spraint near trees or shrubs.

5 – Survey length and width

In the Aguieira Dam, 16 sites (six 600 m and ten 200 m transects) were surveyed monthly for one year (Pedroso et al., 2007). All 600 m transects were visited by otters in the 12 months of the survey, with the exception of one where no otter signs were found on one survey. Similarly, all the shorter transects (200 m) were visited by otters and the visiting rate (number of occasions a given transect was found positive for otter presence over the total number of surveys of that transect) varied from 0.42 to 0.95, with 4 showing values above 0.75. In the other 12 dams, which were surveyed only once (during summer), a series of 200m transects were regularly spaced along the water edge (Pedroso and Santos-Reis, 2006). Whenever no otter signs were found, the transect was extended to 600m but this was necessary just in 16% of the occasions. This result suggests that 200m transects may be suitable for heavily marked dams (like the ones in dry regions during dry seasons). Although, Anoop and Hussain (2004) used 94 survey sites of 250m and only 69% were positive for otters, indicating that in less marked dams 200m might not be enough.

6 - Location of surveys

As in streams, location of surveys in reservoirs is important for spraint detectability. When present in a reservoir, otters may not use the entire reservoir so we have to bear this in mind when choosing survey sites. In each of the 12 surveyed reservoirs in southern Portugal, a set of survey sites was implemented around the entire perimeter and 14.5% of these proved negative for otter presence (Pedroso and Santos-Reis, 2006). The criterion for selection of survey sites was that these should be approximately 5 km apart. According to Erlinge (1967) most home ranges of Eurasian otter family groups that included lakes within their boundaries were found to extend over distances greater than 5 km, and those on rivers were larger. However, this data concerns the snow tracking method and a northern European country (Sweden). For Mediterranean streams, Saavedra (2002) found a mean of around 30 km of average total range and around 6 km of core area, (Catalonian Region - Spain) and Jiménez et al. (1998) found home ranges of around 30 km for males and 20 km for females (Castellón and Teruel - Spain).

Therefore, the chance of a home range lying entirely between two survey points is low thus minimizing false negatives.

Also, proximity to tributaries seems to be important, especially in reservoirs in the drier regions of southern Portugal. Here confluences of tributaries in reservoirs were heavily marked (Sales-Luís et al., 2007). The otters seemed to divide their time and movements between the dam, which provided nourishment, and the associated tributaries, which provided shelter (Sales-Luís et al., 2007; Pedroso et al., 2007; Pedroso, unpublished data). Georgiev (2009), in a study with eurasian otters in Southern Bulgaria, also addressed the question of where to place the monitoring zones, although this was in relation to small dams. In that study, the author stated that most of otter spraints were found in the areas close to the river inflow, followed by those near the wall of the dam. Anoop and Hussain (2004) also found that smooth-coated otters in the Periyar Lake Dam were usually found at the mouths of small streams that join the lake. The number of streams joining the lake, which influences the congregation of fish and the vegetation density, was interpreted to be the most important factor in determining habitat selection by otters around the Periyar Lake.

One last aspect of survey location is the wall of the dam. All of the studied dam walls that allowed otter passage from and to the downstream tributary (small wall steepness, short distance from the wall to the downstream river) were marked in the surroundings, most of them on and around the wall itself and on small dirt pedestrian tracks leading from the wall to the reservoir and the tributary (Figure III.1.3). Georgiev (2009) found that after the areas close to the river inflow, it was in the areas near the wall of the dam where most of otter spraints were found (although it must be repeated that this study took place in small dams).

Figure III.1.3 - Location of otter spraints found around Alvito Dam wall: ✦– otter spraint; 1 - downstream; 2 – wall road; 3 – upstream.

7 – Human disturbance

Human disturbance influences otter presence in several ways and may even limit otter populations (e.g. Beja, 1992; Robitaille and Laurence, 2002; Kruuk, 2006). That being so, one would expect marking behaviour and the corresponding amount of otter signs detected would also be influenced by human disturbance (i.e. fishing and aquatic sports). However, in the studied dams, no such relation was found. This may be because during most of the year disturbance in reservoirs is quite low, except during summer time when sporting and camping activities take place, or because there is a certain degree of adaptation to human presence and encounters are avoided with the usual secretive otter behaviour (nocturnal and discrete). This tolerance to human presence was also observed in the Periyar Lake, India (Anoop and Hussain, 2004, 2005).

Proposal for a methodological approach

Portugal and other Mediterranean countries have a very distinct seasonality in water availability, with an almost complete absence of water from many streams in the dry season. The favourable status of otter populations in Portugal may lead otters to occupy, especially during dry seasons, habitats which are suboptimal in terms of refuge but offer profitable prey (Pedroso et al., 2004; Pedroso et al., 2007; Sales-Luís et al., 2007). Pressure for dam building and water management issues relating to it are more intense in drier regions, hence the necessity for studies on ecological communities and species protection, especially in Environmental Impact Assessments. The following methodological proposal, although clearly directed at dry regions, can also apply to regions with different characteristics and otter status.

The suggested methodological approach to survey otter presence in dams is based on the standard survey methodology (OSG), which surveys 600 m of riverbank for evidence of otter presence. Transects should be located along the bank of the reservoir (one bank instead of two as in streams). Further adjustments relate to:

Surveying season

Consideration of season is important in rivers with intermittent water regimes. As the effect of water level and rain can mask the influence of other variables in otter sprainting activity, it is important to survey in months that minimize these effects. In Mediterranean countries that may principally be in the summer and autumn. When performing single surveys these should not be implemented during the days after heavy rain or high water level (according to Chanin, 2003, there should be a period of at least five days without rain before surveying). Also, when dealing with sprainting, one should take into account seasonal variations in otter sprainting behaviour, which maybe up to tenfold between seasons (e.g. Kruuk, 2006). So, annual monitoring schemes should be conducted each year in the same season to diminish otter sprainting behavioural differences.

Survey length and width

Similarly to river surveys, 600 m transects are a reasonable compromise for otter regular monitoring, either only to detect otter presence/absence or for more in depth comparative studies. The survey should be concentrated in the first five metres from shore of the entire width of the bank flooding area. Nevertheless, for monitoring otter presence/absence in dams in dry regions, 200 m transects are adequate as this minimises survey time and thus the number of sites to be surveyed per day. However, this survey length should only be used when more than one site is to be surveyed in the same reservoir. Alternatively, surveys may be planned to begin with 200m transects, moving up to a maximum of 600 m transects in case no otter signs are found. Similarly to river surveys, this has the effect of avoiding false negatives.

Number of survey sites

To make it possible to say that otters are present in all of a reservoir, a series of regularly spaced survey sites, approximately 5 km apart, should be established. This degree of surveying in depth would give an insight into the otter's specific habitat use, such as whether they are present at the point where tributaries run into the reservoir, or if they use the overall perimeter of the reservoir.

Location of the survey sites

When performing presence/absence surveys, and if proving otter presence in the entire reservoir is unimportant, the most crucial aspect is choosing the right sites for surveying. Suitable sites are usually selected by ease of access. Our data suggest that surveys located in the main inflow of the tributaries into the reservoir (Pedroso, unpublished data; Sales-Luís et al., 2007) are ideal places to survey. However, it must be considered that this is not necessarily representative of otter use in the totality of the reservoir perimeter, particularly when dealing with large reservoirs. To improve the probability of finding otter signs, surveys should be located on gently sloping banks, with many suitable potential marking places, near shallow water and beside or under any existing bridge. The dam wall itself can also be a good place to consider surveying, providing the wall is not steep and is a short distance from the downstream river, allowing otters to cross (Figure III.1.4). Furthermore, if surveys are replicated seasonally, special attention must be given to traditional otter marking places in order to promote efficiency in detecting spraints.

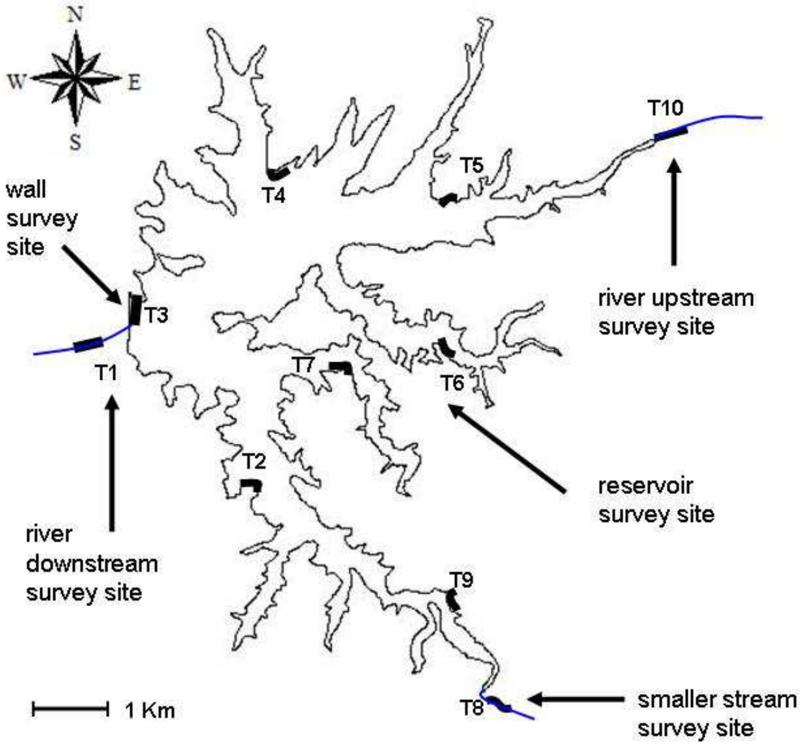

Figure III.1.4 - Location of otter survey sites in Roxo Dam, South Portugal.

Vegetation

Survey sites with good vegetation cover above the bank flooding areas may reveal a high degree of marking behaviour and otter presence. However, the same sites during high water level may yield a very small surveying area due to the fact that the water reaches the dense vegetation (Pedroso et al., 2007). If surveys are to be repeated in time, looking for good marking substrates above the flooding area (if the vegetation allows it) is advisable before choosing survey sites.

Type of dam

The function of the dam is an important aspect, since it implies different water management policies. Hydroelectric dams have larger and faster shifts in water level, while agriculture/water reserve dams do not suffer sudden fluctuations. Water discharge downstream is also very different, and surveys in tributaries below hydroelectric dam may be compromised by the sudden water discharge that occurs for electricity production. Field surveys should therefore take place in the shortest time frame, in order to guarantee comparable conditions. The same applies to comparisons of presence/absence surveys in different years, such as in nation-wide surveys.

Concluding remarks

The above suggestions may assist in a more efficient design of methodology for assessing otter presence/absence, and they contain some adjustments for applying standard survey methodology to large dams for Mediterranean or Mediterranean-type ecosystems which are characterized by highly marked seasonal climate with intermittent water flow. Generally Environmental Impact Assessment Studies are limited by both budget and time, and field researchers and biologists are forced to apply efficient and cheap methodologies. Good distribution data on otters is a first step to assess the effects of dam construction. The Eurasian otter is a target species under the Habitat Directive, requiring regular monitoring. Surveys in reservoirs, particularly when combined with surveys in associated tributaries, will help to understand the impact of dam building on otter populations. Further understanding of marking behaviour for making inferences on habitat use and time spent by otters in these man-made habitats must take into consideration not only that otters often use certain areas without leaving any detectable signs, but that there is also variation in the marking behaviour of otters of different age, gender and reproductive status. Seasonality and prey availability may also bring variation to marking behaviour and should be taken into account. Radio-tracking data or molecular spraint analysis of otters in dams would therefore complement these data, giving a better understanding of how the otters use dams. This paper gives researchers some basic guidelines in preparing dam surveys, to allow more effective surveys to be conducted, whether surveying simply for otter presence, for regular monitoring or for improving the collecting of fresh spraints for molecular spraint analysis.

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PART IV – ECOLOGY OF IBERIAN OTTERS IN DAMS

IV.1. Can large reservoirs be suitable habitat elements for otters? A multi-dam approach in a Mediterranean region.

PAPER 2

Pedroso, N.M., Santos-Reis, M., (submitted). Can large reservoirs be suitable habitat elements for otters? A multi-dam approach in a mediterranean region. Biodiversity and Conservation.

Can large reservoirs be suitable habitat elements for otters? A multi-dam approach in a Mediterranean region

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ABSTRACT

Large dams have been described as negatively influencing the distribution of Eurasian otters (*Lutra lutra*) and suggested as a contributing factor for the past decline of the species in Europe. However, growing evidence suggest that otters use large water reservoirs but available data are still limited. Otter presence was documented and studied use of food resources in twelve reservoirs and adjoining streams in Portugal. Otter use was inferred from signs, and diet studied through spraint analyses and prey availability. Results indicated that otters used both reservoirs and streams, but intensity of use differed seasonally. Conspicuous marking site availability, number of nearby streams/rivers flowing to and from the reservoir, bank shape, and vegetation cover influenced otter presence and the use of reservoirs. The fish community in the reservoirs was strongly dominated by non-native species and represented the bulk of otter diet. Established reservoirs with abundant fish populations are a complementary habitat for otters, whose primary habitats are natural streams. Results showed that reservoirs are a suboptimal habitat when compared to streams but do not constitute a setback to otter conservation in areas of the Mediterranean where otter populations thrive, like in Southern Portugal. However, the destruction of streams and rivers by the construction of large dams should be a matter of concern especially in areas of otter population fragility and/or instability.

Keywords: dams, Lutra lutra, diet, Mediterranean, seasonality, streams

Introduction

Foster-Turley et al. (1990) and Ruiz-Olmo (2001) have argued that the physical characteristics of dams including the barrier represented by the dam wall and the ecological constrains imposed by the length and depth of the water reservoir, have influenced the distribution of Eurasian otters (*Lutra lutra* Linnaeus, 1758) negatively. Further, it is suggested that through habitat loss and destruction, river alteration, and fragmentation of otter populations (e.g. Macdonald and Mason, 1994; Santos et al., 2008) dams have been a contributing factor in past declines of otters in Europe. Optimal habitats for otters include dense bankside vegetation cover, with potential shelters providing refuge and breeding dens, prey abundance and easy-to-find and use foraging grounds (e.g., Macdonald and Mason, 1982; Ruiz-Olmo et al., 2005). These features are usually absent in reservoirs where fluctuating water level impede vegetation growth in the margins. In large and deep reservoirs, often with steep shorelines, otter foraging is difficult (e.g., Kruuk, 2006). In large dams these negative aspects are even more pronounced. As a result, dams presumably provide sub-optimal conditions for otters (e.g., Macdonald and Mason, 1982; Prenda and Granado-Lorêncio, 1995). Ecological and conservation concerns are especially relevant in Mediterranean areas, or other dry regions, where water management is largely based on reservoirs. This is the case of the Iberian Peninsula where dam building, especially large dams, is still ongoing. Portugal has 168 large dams (wall height ≥ 15 m or wall height between 5-15 m and a reservoir volume ≥ 3 000 000 m^3; WCD 2000) with at least 8 more scheduled for construction in the forthcoming years. However, growing evidence exists that reservoirs are used by Eurasian otters in Mediterranean regions, but these studies are limited (Prenda et al,. 2001; Pedroso and Santos-Reis, 2006; Santos et al., 2008). Given this context, important questions addressed in this study are: i) Is the use of reservoirs by otters related to particular ecological variables or reservoir features that facilitate its use?; ii) Are prey resources the key driver for otter use of the reservoirs? iii) Can large reservoirs be suitable habitat elements for otters?

Material and methods

Study area

The study was conducted in 12 large dams and adjoining streams included in two of the most important river basins in Portugal - Sado and Guadiana, located in the Alentejo region (South Portugal - Figure IV.1.1). All dams were built for irrigation and water reserve purposes and their main features are indicated in Table IV.1.1.

The Alentejo region is characterized by vast plains and a relatively uniform dry climate although, due to the sea influence, aridity increases towards the southeast, with higher temperatures and lower air humidity. Annual precipitation ranges from 800mm to less than 500mm. The landscape, typically Mediterranean, is characterized mainly by large cereal fields and oak woods, with cork oak (*Quercus suber*) dominating in the west and holm oak (*Quercus rotundifolia*) in the east. The dry season is pronounced and includes four to five months with low to no precipitation, and high temperatures resulting in droughts. Droughts are seasonally predictable events in Mediterranean streams, occurring each year in summer-early fall (June to September/October), but varying markedly in intensity among years (Gasith and Resh, 1999). Occasionally dry seasons are extremely dry as a result of the duration and extension of drought in streams. The wet season is from late October/November to May.

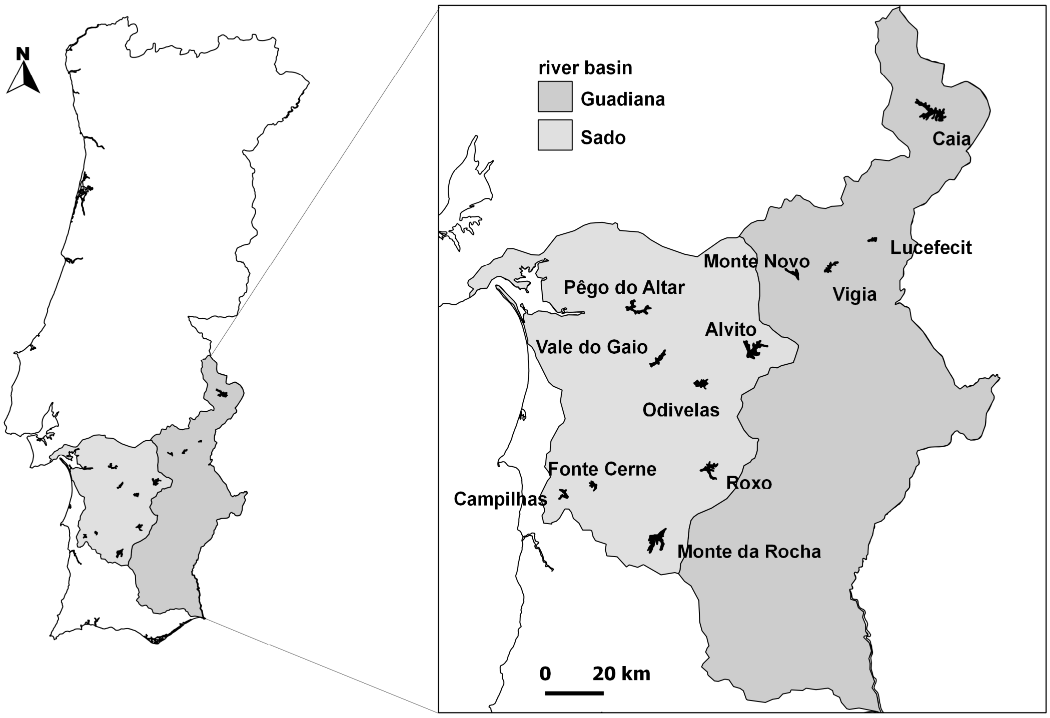

Figure IV.1.1 – Surveyed Large Dams in the Alentejo region (Southern Portugal).

Table IV.1.1 – Features of surveyed Large Dams in Southern Portugal.

Dam	Basin	River/ Stream	End of construction	Volume (hm^3)	Wall (m)	Perimeter (km)
Alvito	Sado	Odivelas	1977	132	49	90
Monte da Rocha	Sado	Sado	1972	105	55	83
Odivelas	Sado	Odivelas	1972	96	55	59
Roxo	Sado	Roxo	1967	96	49	96
Pego do Altar	Sado	Santa Catarina	1949	94	63	85
Vale do Gaio	Sado	Xarrama	1949	63	51	41
Campilhas	Sado	Campilhas	1954	27	35	33
Fonte Cerne	Sado	Vale Diogo	1976	5	18	20
Caia	Guadiana	Caia	1967	203	52	107
Vigia	Guadiana	Vale do Vasco	1981	17	30	20
Monte Novo	Guadiana	Degebe	1982	15	30	24
Lucefécit	Guadiana	Lucefécit	1982	10	23	19

Otter use of reservoir and adjoining streams

Three surveys were conducted in each dam, covering different climatic conditions: dry season (September 2002), extreme dry season (September 2005) and wet season (May 2006). In 2002, dry season mean precipitation (21.48mm) and temperature (21.3ºC) was as expected for this season and region, but in 2005 the dry season reached extreme values (2.4mm of mean precipitation and 22.5ºC mean temperature) which resulted in an unusual low or completely absent water flow in some of the streams. In the 2006 wet season mean precipitation was 45.93mm and mean temperature 14.2ºC. Climatic conditions during the three sampling periods thus represented contrasting climatic and ecological conditions.

Otter presence/absence was assessed by otter sign surveys (spraints, scent marks, prey remains, and footprints) using 200 m transects spaced approximately 5 km apart around all the reservoir perimeter of each dam and one per stream in all of the streams/rivers flowing to and from the reservoir of each dam. If otter signs were not found in the first 200 m, the survey was extended to a maximum of 600 m (Reuther et al., 2000). Overall, 102 sites (76 along the reservoirs and 26 along the streams) were surveyed per field campaign. *Marking intensity* (number of signs/km) was used as a surrogate of intensity of use and an indication of resources defence (sites with more spraints may indicate the site is important to the otter in terms of the habitat and/or resources) (e.g., Kruuk, 2006, Sulkava, 2006; Guter et al., 2008). Since strong seasonality in sprainting behaviour has been previously described (e.g., Kruuk and Conroy, 1987; Mason and Macdonald, 1987; Kruuk, 2006) inferences from marking intensity were therefore used only as a complement to otter presence/absence and no deduction on otter density or time spent in a section of reservoir or river were made. Marking intensity was applied only to reservoirs since direct comparison to streams is not advised due to different detectability factors in each environment. As an effort to a better understanding of true intensity of use of reservoirs, otter *daily visiting rates* were studied in two dams and adjoining tributaries: Pego do Altar Dam (September 2007) and Monte Novo Dam (March 2008). Visiting rate (VR) is a measure of the frequency with which animals visit specific locations (Gruber et al., 2008). VR was defined as:

$$VR = \frac{\text{number of positive surveys for otter presence}}{\text{total number of surveys*}}$$

*600m surveys for 7 consecutive days to identify fresh spraints from the previous night

Diet and prey availability

To assess otter diet, spraints were collected in May (end of wet season) and September (end of dry season) 2006 in five dams (Alvito, Pego do Altar, Vigia, Vale do Gaio, and Monte Novo) and adjoining streams; in each system we selected three survey sites in the dam reservoir and two in the adjoining streams. Otter diet results, presented for total reservoirs and streams, based on prey remains found in all spraints collected, were expressed as percentage of occurrence:

$$PO_{(\text{item A})} = \frac{\text{total number of individuals of prey item A consumed}}{\text{total number of individuals consumed}} \times 100$$

For further details in spraint and diet analysis methods see Pedroso and Santos-Reis (2006). Concurrently, abundance of the most common otter prey (fish and American crayfish *Procambarus clarkii*) was assessed both in the reservoirs and streams in the 5 reservoirs/streams systems, also in May and September 2006. At each dam survey site, and because otters prefer shallow waters for foraging (Kruuk, 2006), a sequence of two fyke-nets were placed near and parallel to shore for fish capture, combined with three carboys, adapted and baited for capturing *P. clarkii;* both sets were left overnight. In the stream survey sites, prey species availability (fish and *P. clarkii*) was assessed by electro-fishing in 50 m stretches for 30 minutes (see Sales-Luís et al., 2007 for further details on methods). The chosen watercourses were streams (or rivers) of the highest possible river order to assure water and prey communities in all seasons (Filipe et al., 2002). All prey captured were identified, counted, weighed, measured, and then released into the water. Data was converted into number of individuals of each species per capture effort.

Statistical analysis

Chi-square tests were used to evaluate significance of observed differences between otter presence/absence and marking intensity in sampling seasons. Variables influencing the presence of otters in reservoirs were described using generalised linear models with a binomial error distribution and a logit link function, after testing spatial autocorrelation using Moran's Index. Ecological and reservoir related variables were selected for analysis according to their expected relevance for otter ecology (Pedroso et al., 2007; Basto et al., 2011) and measured or estimated and categorised at each survey site using quantitative scores (Table IV.1.2). Because otters are more attracted to reservoirs during the dry season, (e.g., Pedroso and Santos-Reis, 2006), only data from wet season was used in the models in order to better understand what features may influence otter presence in reservoirs, excluding the influence of season.

Table IV.1.2 – Variables used to describe otter presence in reservoirs of Southern Portugal Large Dams.

Acronyms	Variable description	Data categories or units	Source
Prey			
AMPHIBIAN	Amphibian observations	0 – absent or 1-5 ind., 1 – 6- 20 ind., 2 – 21-40 ind., 3 – > 40 ind.	Field work
CRAYFISH	American crayfish observations	0 – absent or 1-5 ind., 1 – 6- 20 ind., 2 – 21-40 ind., 3 – > 40 ind.	Field work
FISH	Fish observations	0 – absent or 1-5 ind., 1 – 6- 20 ind., 2 – 21-40 ind., 3 – > 40 ind.	Field work
PREY (TOTAL)	Amphibians + crayfish + fish	0 – absent / 1 – scarce / 2 – moderate / 3 – dense	Field work
Refuge			
REFUGES_ABFA	Refuges availability (above bank flooding area): presence of large rocks, logs and other type of refuge structures	0 – absent / 1 – scarce / 2 – moderate / 3 – high / 4 – very high	Field work
REFUGES_BFA	Refuges availability (bank flooding area) – presence of large rocks, logs and other type of refuge structures	0 – absent / 1 – scarce / 2 – moderate / 3 – high / 4 – very high	Field work
VEGETATION_ABFA	Vegetation availability (above bank flooding area)	1 – absent or offering no suitable cover for otters, 2 – present in patches, offering scarce cover for otters, 3 – present in large patches offering suitable cover for otters, 4 - continuous dense vegetation, providing excellent cover for otters	Field work
VEGETATION_BFA	Vegetation availability (bank flooding area)	1 – absent or offering no suitable cover for otters, 2 – present in small patches, offering scarce cover for otters, 3 – present in large patches offering suitable cover for otters, 4 - continuous dense vegetation, providing excellent cover for otters	Field work

Acronyms	Variable description	Data categories or units	Source
REFUGES_TOTAL	Refuges/vegetation availability (total area)	1–absent or present but offering no suitable cover for otters, 2–present in small patches, offering some cover for otters, 3–present in large patches offering suitable cover for otters, 4-continuous dense vegetation, providing excellent cover for otters	Field work
Reservoir			
BF_AREA	Bank flooding area	Meters	Field work
B_STEEPNESS	Bank steepness	1 – flat / 2 – slightly steep / 3 – moderately steep / 4 – steep / 5 – very steep	Field work
DEEPNESS	Water deepness	Meters	Field work and maps
MARK_SITES	Potential marking sites (e.g. stones, tree roots)	0 – absent / 1 – scarce / 2 – moderate / 3 – abundant	Field work
PERIMETER	Perimeter of each reservoir	square meters	Maps
PN_STREAMS	Nearby streams/rivers flowing to and from the reservoir	presence/absence	Field work
SUBSTRACTE	Type of substract (from mud to rock)	1 – mud / 2 – sand-dirt / 3 – mix / 4 – mostly boulders and rocks / 5 – very rocky	Field work
TYPOLOGY	Bank typology	1 – deep valley/ 2 – bay / 3 – exposed bank / 4 – peninsula	Field work
WATER_QUALITY	Water quality (High quality corresponding to low eutrophication)	0 – very poor / 1 – poor / 2 – moderate / 3 – high	Field work
WIDTH	Distance to nearest opposite bank	1 – 0 -150m / 2 – 150-300m / 3 – 300-450m / 4 – 450-600m / 5 - > 600m	Maps
Disturbance			
CATTLE	Presence of cattle settlements	0 – absent / 1 – few / 2 – moderate / 3 – many	Field work
HUMAN_IMPACT	Human activities	1 – none / 2 – few / 3 – moderate 4 – many	Field work and maps

In the modelling procedure each pair of variables were first tested for independence and were considered strongly correlated when rs > 0.7; this being the case only the one with a higher correlation with the dependent variable was used henceforward. In total, 128 a priori models of otter presence in reservoirs were generated accounting for one of the following combination of variables: 1) otter ecological requirements (prey and refuge related); 2) reservoir (including disturbance) related variables; and 3) a combination of both. The Akaike Information Criterion (AIC) was used to rank the models according to their capacity to parsimoniously describe the data and top models were selected using the criteria of delta AIC < 2. Model inference was obtained using the Multi-Model Inference package from R software as well as the Relative Variable Importance procedure for predictor variables (Burnham and Anderson, 2002). Jacobs' index of preference (*D*) (Jacobs, 1974) was used to illustrate the degree of preference shown by otters for prey categories and Student's *t* tests were used to assess differences between the mean value of *D* obtained for each prey category and *D*=0 (no preference). Chi-square tests were used to compare consumption of prey categories.

Statistical calculations were performed using R software (version 2.10.1), SPSS® version 19.0 for Windows®, and Microsoft® Office Excel® 2003 with a probability level of $\alpha = 0.05$.

Results

Otter Presence/Absence

The number of positive survey sites was always higher than the number of negative sites, regardless of season, both in reservoirs and streams; difference were always significant except for the 2005 dry season survey in streams – $\chi^2 = 0.1024$, P = 0.749 where otter were absent in almost half of the streams (Figure IV.1.2).

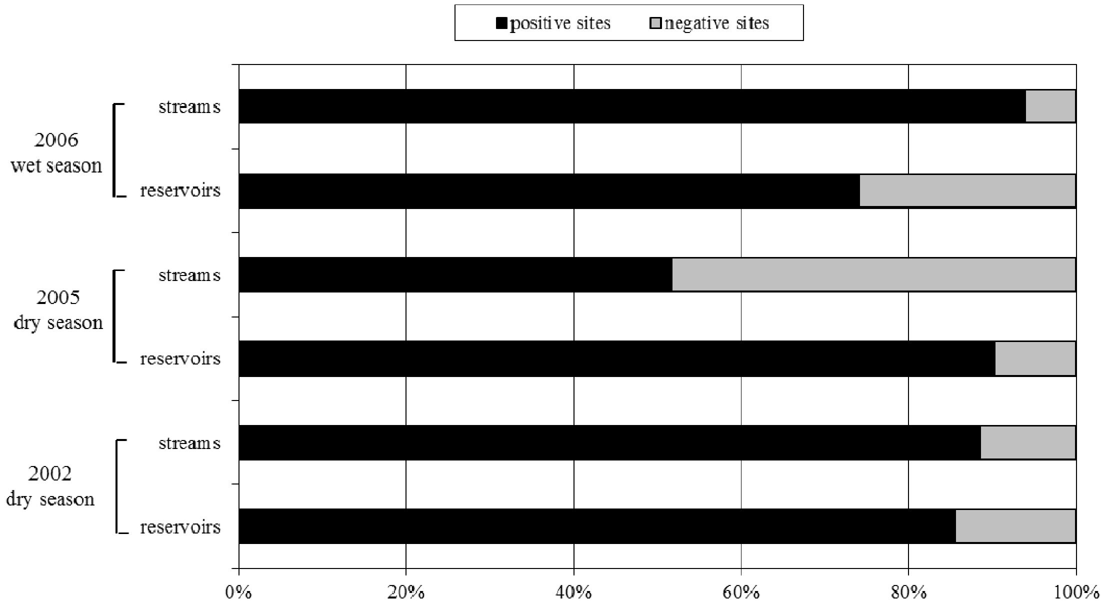

Figure IV.1.2 – Percentage of positive and negative sites for otter in reservoirs (reservoirs site = 76) and streams (stream sites = 26) in 2002, 2005 and 2006 survey seasons in 12 Southern Portuguese large dams.

Marking intensity

Marking intensity in reservoirs was significantly higher during the 2005 dry season (51.8 signs/km in average) than in the other two sampling occasions (22.5 signs/km during the 2002 dry season vs. 14.8 signs/km in 2006 wet season) (χ^2 = 25.68; $p < 0.005$). No significant difference was found between the 2002 dry season and the 2006 wet season.

Visiting rates

Otter daily visiting rates at the two reservoirs and streams chosen are illustrated in Figure IV.1.3.

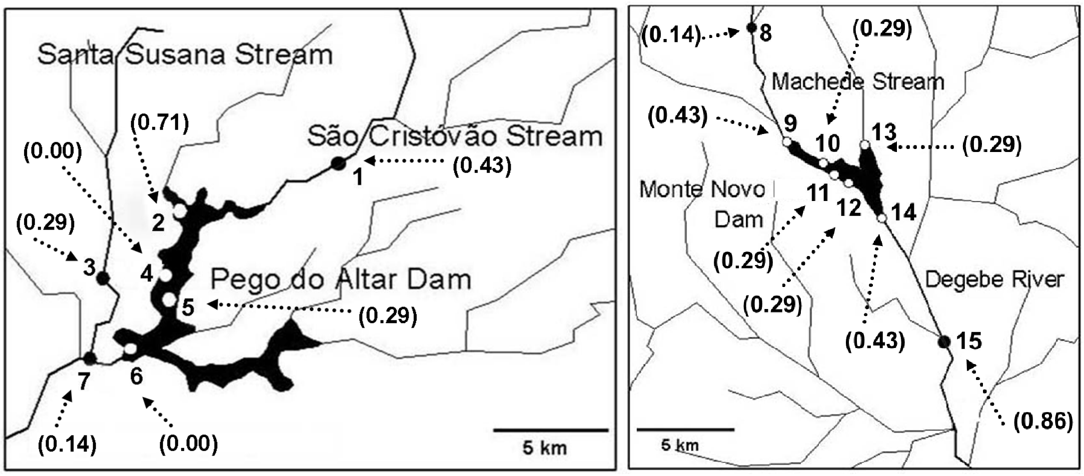

Figure IV.1.3 - Visiting Rates ( ) and location of the survey sites in Pego do Altar (1-7) and b) Monte Novo (8-15) systems.

In Pego do Altar Dam, from the seven survey stations, five were visited by otters at least once during the survey period. Visiting rate varied from 0.00 to 0.71 (five visits out of seven days). The most regularly visited site (#2) was located in the reservoir but so were the two sites not used by otters (#4 and #6). All three stream sites were visited by otters with 0.43, the highest rate, found at site 1. The streams were not totally dry but had very low water levels, particularly at site #7 that also showed a lower visiting rate (0.14). In Monte Novo Dam, all survey stations were visited by otters at least once and visiting rate varied from 0.14 (one visit) to 0.86 (six visits out of the seven days). The Degebe River (#15), a large and wide river holding an abundant prey community and margins with good riparian vegetation providing suitable refuge for the otter, was the most regularly visited site. In contrast the Machede Stream, characterized by a reduced water flow, even in wet season, and low prey abundance and refuge availability was the less visited site. Most of the Monte Novo reservoir sites presented a low (0.29) visiting rate (#10 to #13) and the two reservoir sites with the highest (0.43) visiting rate were located in the vicinity of streams (#9 and #14).

Ecological and reservoir-related variables influencing otter presence

No spatial autocorrelation was found for otter data (Moran’s I = -0.099, p = 0.40). From the top 12 models, seven include both ecological (prey and refuge) and reservoir-related variables and five only contained reservoir related variables (Table IV.1.3).

Table IV.1.3 - Summary of the best models describing otter presence in reservoirs. AIC - Akaike Information Criterion; wAIC - Akaike weights. See Table IV.1.2 for a description of the variables.

Models	**Model code**	**Deviance**	**AIC**	***w*AIC**
Reservoir variables				
MARK_SITES + PN_STREAMS + TYPOLOGY	50	41.68	49.68	0.039
MARK_SITES	3	46.25	50.25	0.038
MARK_SITES + PERIMETER	15	44.48	50.48	0.031
MARK_SITES + PERIMETER + PN_STREAMS	45	42.19	50.19	0.030
MARK_SITES + PN_STREAMS	16	44.55	50.55	0.030
Combination of ecological and reservoir variables				
MARK_SITES + PN_STREAMS + TYPOLOGY + VEGETATION_BFA	119	35.57	47.57	0.072
MARK_SITES + PERIMETER + PN_STREAMS + TYPOLOGY + PREY + VEGETATION_BFA + WATER_QUALITY	93	38.26	48.26	0.065
MARK_SITES + PN_STREAMS + TYPOLOGY + PREY + VEGETATION _BFA	108	36.61	48.61	0.043
MARK_SITES + PERIMETER + PN_STREAMS + TYPOLOGY + PREY	86	39.46	49.46	0.036
MARK_SITES + PERIMETER + TYPOLOGY + PREY + VEGETATION_BFA + WATER_QUALITY	125	34.38	48.38	0.036
MARK_SITES + PERIMETER + PN_STREAMS + TYPOLOGY + VEGETATION_BFA	117	37.31	49.31	0.030
MARK_SITES + PN_STREAMS + VEGETATION_BFA + WATER_QUALITY	92	40.00	50.00	0.027

The best model describing the presence of otter in reservoirs explained 48% of deviance and included marking sites availability, presence of nearby streams, bank typology and vegetation cover in the bank flooding area (Table IV.1.4).

Table IV.1.4 – Variables included in the best model for otter presence in reservoirs with the highest support (higher wAIC) for standardized parameter estimates. Variable codes as in Table IV.1.2.

Variables	**Estimate**	**S.E.**	**Z**	**P**
Intercept	-14.3175	5.2833	-2.710	0.00673**
MARK_SITES	2.9018	0.8980	3.232	0.00123**
PN_ STREAMS	3.2157	1.4231	2.260	0.02385*
WATER_QUALITY	1.6846	1.0916	1.543	0.12276
TYPOLOGY	1.2925	0.6827	1.893	0.05832
VEGETATION _BFA	0.6105	0.7152	0.854	0.39332

The most important predictor is the availability of marking sites, followed by the presence of nearby streams. Table IV.1.5 describes the rank of the Relative Importance of the Variables of the top models.

Table IV.1.5 – Relative importance of variables for the top 12 models explaining otter presence in reservoirs.

Variables	**Rank**
MARK_SITES	1.00
PN_STREAMS	0.86
TYPOLOGY	0.67
VEGETATION _BFA	0.57
WATER_QUALITY	0.28
PERIMETER	0.27
PREY	0.16

Otter Diet

A total of 429 spraints (reservoirs: wet season n= 94, dry season n = 195; stream: wet season n = 69, dry season n = 71) were analysed.

Diet in reservoirs

Otter diet in the reservoirs consisted mostly of fish and *P. clarkii*, but relative importance varied with season (Figure IV.1.4). Fish dominated otter diet during dry season (58.1%, χ^2 = 3.84; P < 0.05). Eight fish species were eaten; three dominated the diet and were all non-native: *Lepomis gibbosus* pumpkinseed sunfish, *Gambusia holbrooki* eastern mosquitofish, and *Cyprinus carpio* common carp (percentage of occurrence: PO > 10.0%, Figure IV.1.5). The number of prey fish species per reservoir ranged from four to six (0-2 native species and 3-4 non-native species). With the exception of crustaceans, all other prey classes were of marginal importance (< 2.0%). In the wet season, crustaceans and fish had more similar PO (46.1% and 44.0% respectively) and although fish dominated in three of the five studied reservoirs (Figure IV.1.5), just four fish species (all non-native) were preyed in wet season: *L. gibbosus* (27.2%), *C. carpio, M. salmoides* largemouth bass and *G. holbrooki*, all with PO<10.0% . Amphibians and reptiles were consumed much less (6.3% and 3.1%, respectively) but still comparably important. Insects and birds were occasionally eaten.

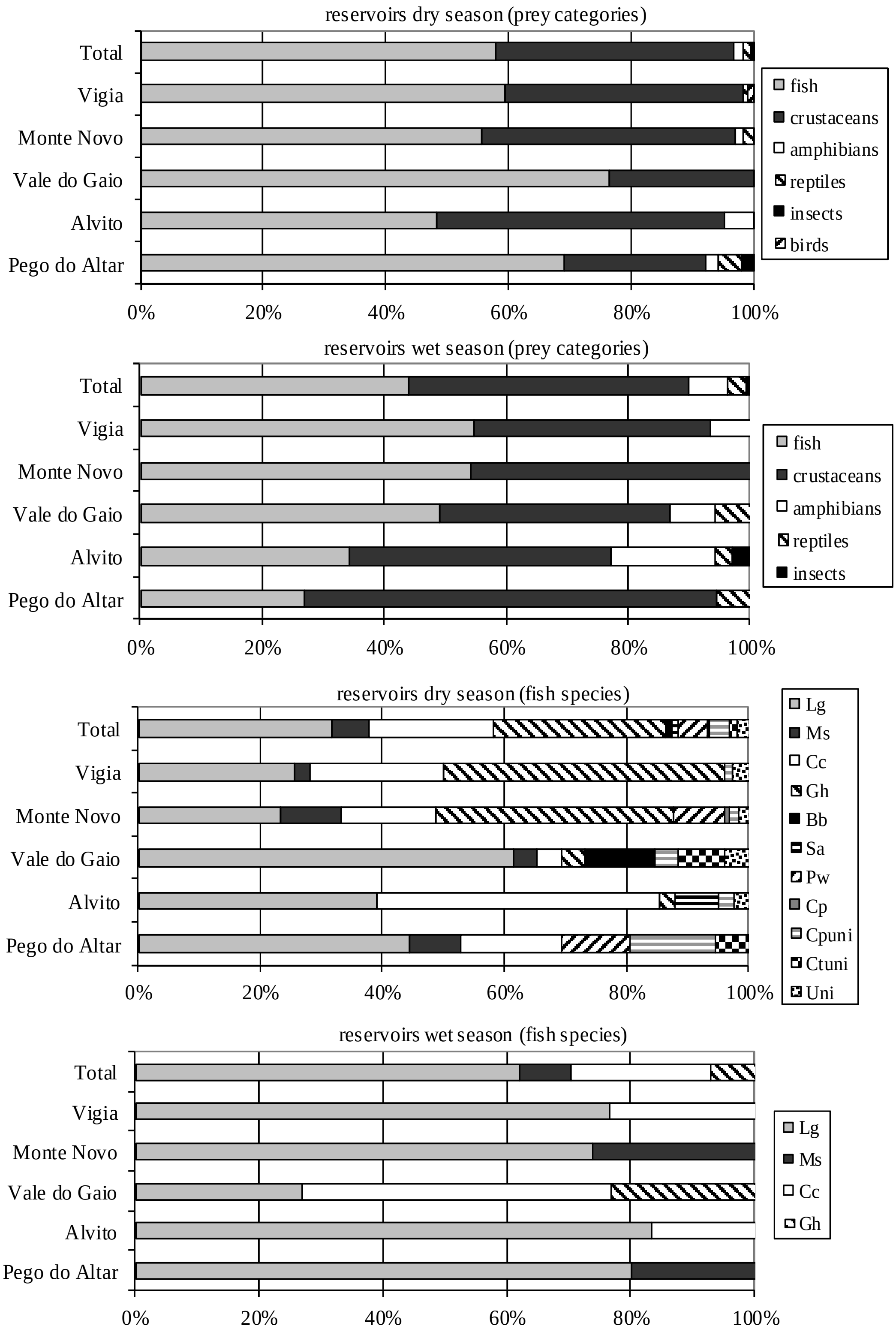

Figure IV.1.4 – Percentages of occurrence of prey categories in otter diet in Vigia, Monte Novo, Vale do Gaio, Alvito, and Pego do Altar reservoirs in dry and wet season (Lg: *Lepomis gibbosus*; Ms: *Micropterus salmoides*; Cc: *Cyprinus carpio*; Gh: *Gambusia holbrooki*; Bb: *Barbus bocagei;* Sa: *Squalius alburnoides*; Pp: *Pseudochondrostoma wilkommi*; Cp: *Cobitis paludica*; Cpuni – Unidentified Cyprinids; Ctuni – Unidentified Centrarchids; Uni – Unidentified fish).

Diet in streams

During the dry season, fish prevailed in the otter diet (51.3%) although consumption was not significantly different from that of crustaceans (46.6%, χ^2 = 0.049; P = 0.82). All other prey classes were of marginal importance (< 2.0%, Figure IV.1.6). *G. holbrooki* dominated the diet (27.6%), followed by important contributions from *L. gibbosus* and *C. carpio* (PO > 8.0%). These three non-native species were preyed in all stream systems. The native *Barbus bocagei* Iberian barbel and *Anguilla anguilla* ell were consumed much less frequently (PO < 0.5%), and each in just one stream system (Figure IV.1.6). Inversely, during the wet season, crustaceans were the most consumed prey (53.8%) although not significantly different from fish (40.4%, χ^2 = 1.90; P = 0.1674) in two of the five studied streams. Also, more fish species were preyed during wet season (nine) then dry season (six). In wet season amphibians had some importance (3.8%) while reptiles and insects were occasionally eaten (Figure IV.1.5).

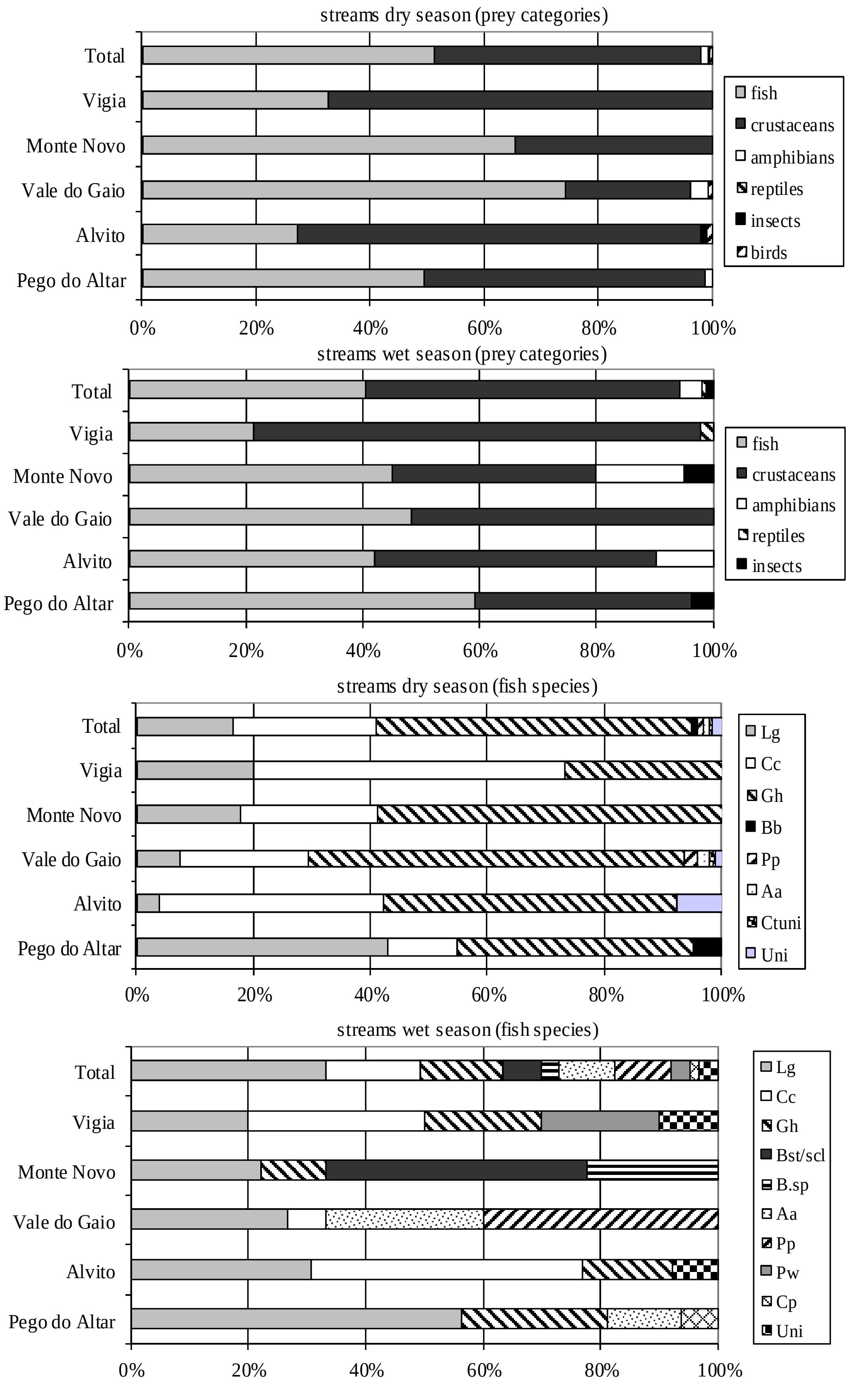

Figure IV.1.5 – Percentage of occurrence of prey categories in otter diet in adjacent streams of Vigia, Monte Novo, Vale do Gaio, Alvito, and Pego do Altar dams in dry and wet season (Lg: *Lepomis gibbosus*; Ms: *Micropterus salmoides*; Cc: *Cyprinus carpio*; Gh: *Gambusia holbrooki*; Bb: *Barbus bocagei;* Bst/scl - *Barbus steindachneri/sclateri*; Bsp: *Barbus* sp.; Aa: *Anguilla anguilla*; Pp: *Pseudochondrostoma wilkommi*; Cp: *Cobitis paludica*; Cpuni – Unidentified Cyprinids; Ctuni – Unidentified Centrarchids; Uni – Unidentified fish).

Prey availability

Prey availability in reservoirs

Besides *P. clarkii,* eight fish species were sampled in the reservoirs. *Mauremys leprosa* (Mediterranean turtle) and *Natrix* sp. (water snakes) were occasionally captured but not considered in the analyses as the sampling methods did not target these species. No significant differences were found between the total prey captured (both individuals and biomass) in the wet and dry seasons, nor between seasons for any species. Captures were more diverse in the dry season (Table IV.1.6).

Table IV.1.6 – Main otter prey captured by fyke-nets and carboy traps in the reservoirs expressed as catch (number of individuals (Ind.) and biomass (Biom.) (kg)) per unit effort (h) per trap.

Prey categories		**Wet season**		**Dry season**	
		Ind.	Biom.	Ind.	Biom.
Crustacean					
Procambarus clarkii	American crayfish	0.165	2.007	0.022	0.268
Fish					
Cyprinus carpio	common carp	-	-	0.060	11.453
Lepomis gibossus	pumpkinseed sunfish	0.595	10.957	0.407	3.980
Squalius sp.	chub	0.017	0.659	0.025	0.465
Micropterus salmoides	largemouth bass	0.013	0.547	0.027	0.253
Pseudochondrostoma polylepis	southern straight-mouth nase	-	-	0.003	0.245
Barbus bocagei	north Barbel	-	-	0.005	0.153
Pseudochondrostoma willkomii	Iberian straight-mouth nase	-	-	0.014	0.030
Sander lucioperca	pike-perch	0.002	0.265	-	-
Total		0.792	14.434	0.563	16.847

The distribution of catch per reservoir was different depending upon if individuals (with higher values for wet season) or biomass (with higher values for dry season) were considered (Figure IV.1.6).

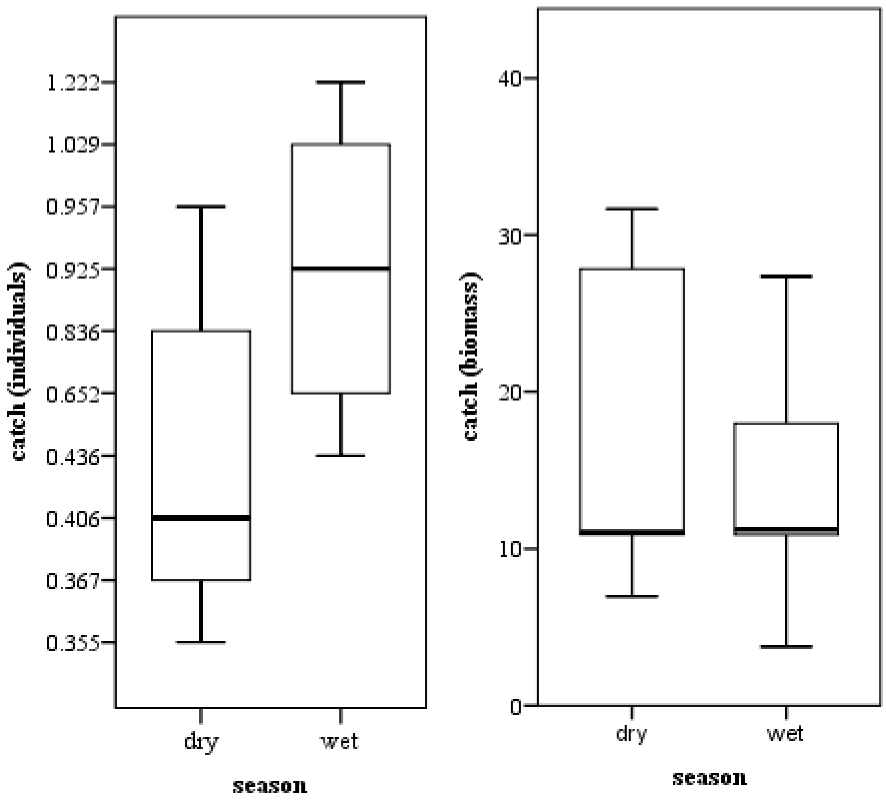

Figure IV.1.6 - Distribution (minimum, first quartile, median, third quartile, and maximum) of main otter prey captured by fyke-nets and carboy traps in the five reservoirs expressed as catch (left - number of individuals; right - biomass) per unit effort (h) per trap in dry and wet season.

Prey availability in streams

Besides *P. clarkii*, 14 fish species were sampled in the streams. Although total number of individuals captured was higher during the dry season, total biomass capture was higher during the wet season. Captures were similar in number of species per season (n = 12), but the ratio of non-native/native species was different (wet season = 0.25; dry season = 0.53) favouring non-native species in the dry season (Table IV.1.7).

Table IV.1.7 – Otter prey captured in streams using electrofishing. Expressed as catch (number of individuals and biomass (kg)) per unit effort (h).

Prey categories		**Wet season**		**Dry season**	
		Ind.	Biom.	Ind.	Biom.
Crustacean					
Procambarus clarkii	American crayfish	43.80*	359.16*	7.11	86.76
Fish					
Cyprinus carpio	common carp	-	-	23.78	1244.87
Barbus bocagei	north Barbel	6.20	1163.24*	4.00	155.87
Cobitis paludica	southern Iberian-mouth nase	6.00	461.49*	22.67*	35.97
Lepomis gibossus	pumpkinseed sunfish	8.40	55.40	34.00*	418.48*
Anguilla anguilla	eel	3.00	288.90*	2.67	52.18
Barbus steindachneri	Steindachner barbel	0.40	199.70	-	-
Gambusia holbrooki	eastern mosquitofish	68.00	23.54	165.56*	62.67*
Micropterus salmoides	largemouth bass	-	-	0.89	41.69
Australoheros facetus	chameleon cichlid	-	-	1.33	41.16
Barbus comizo	Iberian long-snout barbel	1.60	39.40	-	-
Squalius pyrenaicus	southern Iberian chub	0.80	16.92	0.67	22.71
Pseudochondrostoma polylepis	Iberian straight-mouth nase	0.80	10.32	0.44	4.84
Barbus sclateri	southern Iberian barbel	0.40	5.68	-	-
Squalius alburnoides	bordalo	0.60	1.40	0.67	3.13
Total		140.00	2625.16*	263.78*	2170.31

*significant difference (position of symbol indicates higher value season)

Prey selection

In reservoirs, otters seemed to select *C. carpio* and *G. holbrooki* independently of season, and *P. clarkii* and *C. paludica* during the dry season (D ≠ 0; Figure IV.1.7). *S. pyrenaicus*/*S. alburnoides* and *L. gibbosus* during both seasons were consumed less than expected (Figure IV.1.7). In streams, *C. carpio, P. willkommi* and *P. polylepis* were selected in the wet season and *P. clarkii* in dry season (D ≠ 0; Figure IV.1.7). There seems to be a significant avoidance of *S. pyrenaicus*/*alburnoides* in both seasons, of *C. paludica* in dry season and of *G. holbrooki* in wet season (Figure IV.1.7).

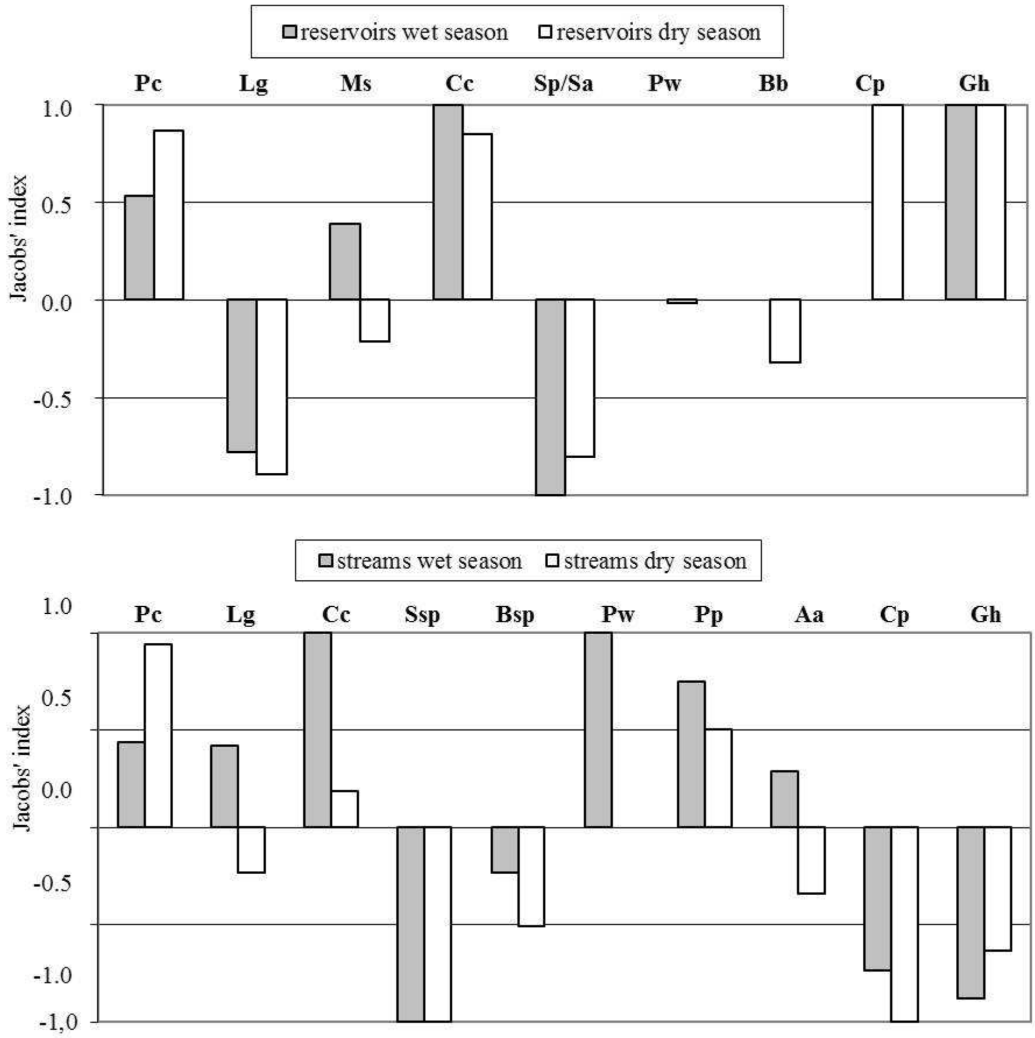

Figure IV.1.7 – Jacobs' index of preference relative to the number of individuals consumed and captured by otter vs. numbers sampled in streams with eluectrofishing and in reservoirs with traps. (Pc: *P. clarkii*; Lg: *L. gibbosus*; Ms: *M. salmoides*; Cc: *C. carpio*; Ssp: *S. pyrenaicus*/*S. alburnoides*; Bsp: *Barbus* sp.; Aa: *A. anguilla*; Pw: *P. wilkommi*; Pp: *P. polylepis*; Cp: *C. paludica*; Gh: *G. holbrooki).* Prey that had very low values of consumption and abundance were not considered.

Discussion

Is the use of reservoirs by otters related to particular ecological variables or reservoir features that facilitate its use?

Results showed that otters made use of reservoirs in Southern Portugal, particularly during the dry season when individuals were frequently absent from the streams that were dry or reduced to a few pools. Although if otters are not detected in a given area this does not always imply that they are truly absent from that area, at least, one can state that absence of signs mean these are more unsuitable areas (Jiménez et al., 1998; Ruiz-Olmo et al., 2001).

Otter presence and marking behaviour showed clearly that they made more use of the reservoirs in the dry seasons, especially in extreme weather conditions. Marking intensity was significantly higher in the extreme dry season, and has already been observed in streams in the Iberian Peninsula, both in Portugal (e.g., Magalhães et al., 2002) and in Spain (e.g. Ruiz-Olmo et al., 2007). In both these studies, the number of spraints and latrines was higher during the dry periods and in pools with more prey. Basto et al. (2011) showed similar patterns of occupancy by otters in small and medium-sized reservoirs in Portugal. These results support the hypothesis of increased marking behaviour as a sign of resource importance and defence, especially when resources are scarce (Kruuk, 2006).

This study showed that certain ecological variables and reservoir features may facilitate the reservoir use by otters. The availability of marking sites was present in all of the best models describing otter presence in reservoirs. Marking behaviour is an important aspect of otter ecology and the availability of marking sites in an otter territory is consequently important. The presence of large rocks, a frequent otter marking site, may additionally provide potential otter shelters. Nearby streams was one of the key drivers for otter presence and use of large reservoirs. According to otter visiting rates, the sites most regularly visited in the reservoirs were located near the confluence with streams. Sites far from streams had lower visiting rates or no visits at all. This suggests that the use of reservoirs by otters is conditioned by the presence of streams and therefore, not all the reservoir perimeter is equally suitable for otters. A similar situation was described by Beja (1992) for the coastal area of Alentejo where otter distribution was conditioned by the presence of freshwater streams flowing into the sea. Freshwater represents a scarce resource needed to clean the fur from salt acquired while foraging in the sea. In the present study, the stream-related key driver is the riparian vegetation that provides shelter and enhances breeding, a resource that is scarce along reservoirs margins. The maintenance of suitable conditions for otter in streams surrounding a reservoir (e.g., preventing water extraction for agriculture purposes, or cutting riparian vegetation, common actions in South Portugal) may help to promote otter use of reservoirs.

Bank typology emerged as important to otters. The presence of small bays with shallow waters offer better otter foraging opportunities vs. open areas and steep margins where it is more difficult for otter to pursue and trap prey. Large and deep water bodies are not ideal for otter foraging. Fishing at greater depths is energetically more demanding with longer subsequent recovery on land from the dives (Nolet and Kruuk, 1989; Nolet et al., 1993; Houston and McNamara, 1994; Kruuk, 2006). Also, vegetation cover usually occurring in small bays bank flooding area, although scarce and not enough to provide refuge to otter, is important because it can attract more prey (submerged vegetation acts as refuge for fish, crayfish and even amphibians). The maintenance of small bays of shallow waters with some vegetation both below and above water level may be another action for promoting otter use of reservoirs.

Apart of the ecological relevance of the variables selected in the modelling procedure, there was variance not explained by the resulting models. This either means that there are additional factors explaining the presence of otter in reservoirs that were not considered in the analysis, or may be a result of the low variation of some variables that although selected are less relevant in terms of contribution. This may be the case with water quality, which was rather uniform in the studied reservoirs. Some sites (especially in small bays) had poor water quality because of increased eutrophication. The presence of cattle also appeared to contribute to organic water pollution thus reducing habitat suitability for otters (Macdonald and Mason, 1983; Trindade et al., 1998; Kruuk, 2006). Perimeter size is correlated with otter use probably because the larger the dam, the higher number of streams. Prey abundance is a key driver for otter occupation of reservoirs but not significant in the models because prey abundance was similar in all reservoirs.

One can conclude that, although reservoirs are not ideal habitats for otters there are some ecological variables and reservoir features that facilitate the use of reservoirs by the species and if present, support otter occupancy.

Are prey resources the key driver for otter use of the reservoirs?

Despite the different compositions in prey communities in dry and wet season there was an apparent stability in terms of number and biomass prey availability in reservoirs. This is important for otters because during the dry season more than half of adjacent streams to the studied dams were dry or had very small pools, and although data prey availability from sampled streams showed higher number of captured individuals in dry season, the overall captured biomass was nevertheless lower. This occurred because non-native species (dominant in dry season) like *L. gibosus* and *G. holbroki* native species have lower biomass then native species (dominant in wet season). Also, fish recruitment occurs from April onwards leading to the capture of lower biomass individuals early in the dry season (e.g., *B. bocagei*, *C. paludica*). Nevertheless during droughts, fish were confined to small areas and usually at considerable higher densities (Pires et al., 1999) making large pools important areas for otter feeding during dry season (Magalhães et al., 2002). So, during dry seasons, prey availability in the reservoirs constitutes a feeding alternative to the adjacent dryer streams.

Otter presence is highly influenced by prey availability, as confirmed in this study. As expected, fish and *P. clarkii* were the dominant prey resources for otters both in streams and in reservoirs. Other groups, especially amphibians, contributed to a more diverse diet and although a less consistent food source because of seasonal changes in abundance, may constitute an important alternative prey for otters in some streams (e.g., the Monte Novo stream system). This is consistent with the review of otter diets in the Iberian Peninsula compiled by Clavero et al. (2008 - more than 200 locations, including data from present work). Both prey consumption and prey availability revealed *P. clarkii* as a key species in the diet.

Captures of *P. clarkii* were higher in wet season in both systems (reservoirs and streams). These results are related to the date of prey availability survey. The May survey (end of wet season) reflects a higher availability since in April-May this crustacean is more active (reproduction period). The September survey (end of dry season) reflects the higher temperatures felt in the Alentejo region which make this crustacean more inactive due to burrowing habits when exposed to high temperatures (Oliveira and Fabião, 1998). Nevertheless, *P. clarkii* availability is expected to be higher in the dry season months prior to September and therefore in most of the dry season. Otters take seasonal advantage of higher crayfish activity and therefore detectability in this season (Almeida et al., 2012).

Besides *P. clarkii*, otter diets in reservoirs were dominated by non-native fish species, which is consistent with the fish community patterns observed in this study and others (Godinho et al., 1998). Clavero and Hermoso (2010), analysing patterns of distribution and abundance of freshwater fish in the Guadiana river basin, also found that the dominance of non-native species, especially *L. gibbosus*, *M. salmoides* and *C. carpio*, tended to be associated with the presence of reservoirs. Otter diets were less diverse in reservoirs during the wet season. This pattern of consumption again is driven by prey availability. During spring, *C. carp*, *Barbus* spp. *Pseudochondrostoma* spp. migrate upstream from the reservoirs into the streams to reproduce and so are less abundant in the reservoirs (Rodriguez-Ruiz and Granado-Lorêncio, 1992). Species behaviour also explains why, during prey surveys, captures of *L. gibbosus* were higher in May (end of wet season) in the reservoirs: during the reproduction period (peak April-July, Ribeiro and Collares-Pereira, 2010), this species defends nests in shallow waters and thus is more available and vulnerable to predation. During the dry season, fish species with higher biomass become more available since the level of oxygen in the reservoir water is lower and fish concentrate in the thermocline, making it easier for otter to capture them, as opposed to periods of colder water when fish are inactive and located in deeper waters.

Low gear selectivity for small sized species, particularly evident in reservoirs, probably contributed to the positive selection of *C. paludica* and *G. holbrooki* which were too small to be captured in the fyke-nets. Similar, fyke-nets in littoral areas are less effective to larger species once these species occupy mostly the middle of the reservoirs (and are less available for otters foraging near the margins, see Sales-Luís et al., 2007). This means that fyke-nets most be complemented by other fishing methods (trammel nets, electrofishing) to properly access all fish community in reservoirs (see Godinho et al., 1998). Clavero and Hermoso (2010), using a more complete combination of passive capture techniques and fishing methods, found that, although total species richness was not different between rivers and reservoirs in the Guadiana river, the latter had more invasive species then native ones. The present study confirmed that the otter is an opportunist predator not only choosing more abundant prey but also avoiding the ones with small biomass. Studied reservoirs have a non-native prey community that has, with the exception of *C. carp*, species of lower biomass (mean weight of four most consumed species: *G. holbrooki* = 0.3g, *L. gibbosus* = 10.7g, *C. carpio* = 179.7g; *P. clarkii* = 12.2g - Pedroso and Santos-Reis, 2006). This suggests that the prey community in streams are more appealing to otter in normal situations. Nevertheless, it was confirmed that otters using the streams around dams in Alentejo region relied on a very low abundance prey community during the dry season, sometimes only eating *P. clarkii*. Also habitat features (cover, breeding) in reservoirs did not appear to promote otter use of these man-made habitats. Finally, where prey was plentiful (e.g., the coasts of the Shetland Islands), otters occurred in large numbers despite the scarcity of cover (Kruuk 2006). All of the above, associated with the fact that the reservoirs offer food abundance both in dry and wet seasons, suggests that prey resources are key drivers for otter use of the reservoirs in areas such as the one described here, Mediterranean areas or other dry regions where there is a marked seasonality in resources (water and prey).

Can large reservoirs be suitable habitat elements for otters?

The information collected in this study is relevant to understand otter use of highly modified environments (dams) especially in Mediterranean climate regions where most rivers are impounded and/or diverted. It is clear that under certain circumstances large reservoirs may be suitable habitat elements for otter. First, the otter population in Portugal is considered stable and relatively dense, with optimal habitats like high quality streams already completely occupied by territorial individuals. In this case, otter population pressure may drive the use of suboptimal habitats like large reservoirs. Conjointly the features of the studied area, where streams are characterized by strong yearly changes in water flow, reservoirs constitute a complementary habitat to streams. In other, less dry, areas of Europe, otters may not use as regularly the reservoirs. Even in Portugal, reservoirs may be used differently in the north (non-Mediterranean) where rivers and streams have more stable water and prey conditions (Godinho, et al. 1998).

One might think that the presence of reservoirs may be beneficial for sustaining otter populations in Mediterranean areas. However, ecological and conservation consequences are not as simple. Large dams are always located in rivers or large streams. These large streams or rivers, before impounded, are able to sustain otter populations (Trindade et al., 1998). In large reservoirs, the entire perimeter is seldom used regularly and the areas of highest use are those near streams suggesting a complementary use. The use of both systems may not increase the carrying capacity for otters linearly as one might think. Larger dams may sustain more otter individuals if the number of surrounding streams is higher and have suitable conditions for otter (water, prey and cover availability). However, the larger the dam the, larger the flooding area and usually the disappearance of otter stream habitats. Conservation of otter relies also on the recognition of the potential impact of increasing human development within river corridors (Lundy and Montgomery, 2010) and these large dams with theirs reservoir and usually unsurpassable walls contribute to the fragmentation of these corridors. Estimates of otter abundance and space use are essential to fully clarify otter use of non-optimal habitats such as dam reservoirs. Also, the substitution of large areas of river by reservoirs means a trade-off between the gain of more permanent water source but a loss of refuge, less suitable foraging areas, and less areas for reproduction. Breeding, feeding, and resting areas are critical for a species (e.g., Fernández and Palomares, 2000; Kruuk, 2006). Because the otter has a considerably higher metabolism than would be expected for its body mass (McNaab, 1989), breeding is a time of high energy requirements (e.g. Ruiz-Olmo et al., 2005). Natal dens are still a limiting resource in some areas (e.g., in southwestern Portugal – Beja, 1996a; in marine environments in Northern Europe - Kruuk et al., 1989; Yoxon, 2000). Also, the substitution of rivers and streams by reservoirs means a shift from a more balanced native/non-native species diet in streams and rivers to an almost totally non-native species based-diet in reservoirs. In otter foraging habitats in the Mediterranean region, non-native species occur in higher abundance and are easier to capture (Almeida et al., 2012) but represent in most cases low biomass and energetic contribution, long handling times (e.g *P. clarkii* has an high percentage of hard parts) and otters may be still limited by native prey populations, and strategies aimed at the conservation of otters in Iberian streams should include the conservation of prey species native (Beja, 1996b).

These aspects contribute to the reservoirs being a suboptimal habitat when compared to streams but do not constitute a setback to otter conservation in areas of the Mediterranean where otter populations thrive, like in Southern Portugal. However Marcelli and Fusillo (2009) on a study in Italy assessing range re-expansion and recolonization of human-impacted landscapes found evidence, although weak, of a negative effect in otter expansion of the proximity of dam reservoirs. So the destruction of streams and rivers by the construction of large dams should be a matter of concern especially in areas of otter population fragility and/or instability (low numbers, recovering or expanding populations in border distribution areas).

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IV.2. Use of small and medium-sized water reservoirs by otters in a Mediterranean ecosystem

PAPER 3

Basto, M.P., Pedroso, N.M., Mira, A., Santos-Reis, M., 2011. Use of small and medium-sized water reservoirs by otters in a Mediterranean ecosystem. Animal Biology 60, 75–94.

Use of small and medium-sized water reservoirs by otters in a Mediterranean ecosystem

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Abstract

Water is a limiting factor in Mediterranean regions, being especially important to aquatic species such as the Eurasian otter. The seasonal fluctuation of this resource is often addressed by constructing small and medium-sized water reservoirs. However, their role in the ecology and conservation of Eurasian otters is largely unknown. Our main goals were to assess the level of use of these reservoirs by otters and determine the main factors that may explain the observed levels of use. Intensity of use was determined by signs of otter presence and related to environmental variables using generalized linear models and variation partitioning techniques. Otters were present in the majority of reservoirs, both in the wet and dry seasons. Otter marking intensity was higher during the dry season, and positively associated with abundance of fish and American crayfish, existence of refuges and marking sites, number of watercourses and area of reservoir. In contrast, cattle settlements, annual crops and length of watercourses in the surrounding area negatively affected the use of the reservoirs. Otter diet reflected their opportunistic behaviour through the selection of seasonally available prey and corroborated the importance of American crayfish as a food item.

Our results confirm that otters use the majority of small and medium-sized reservoirs in the study area. Despite this, reservoirs may be considered suboptimal habitats and seem to be specially relevant in the dry season, increasing water availability and acting as important feeding areas mainly when close to watercourses with good refuge conditions. Management implications are discussed.

Keywords: *Lutra lutra*; habitat selection; diet; water reservoirs; Mediterranean region.

Introduction

The Eurasian otter (*Lutra lutra* L., 1758) is an aquatic carnivore associated with riverine-type habitat. The preservation of such habitat has been a major priority for otter conservation because it is rich in prey, optimal for shelter and facilitates animal movement (Foster-Turley et al., 1990). Anthropogenic changes in aquatic habitats such as construction of water reservoirs have been identified as one of the causes contributing to riparian habitat destruction and consequent decline of European otter populations in the past (e.g. Macdonald and Mason, 1983; Foster-Turley et al., 1990).

Recent conservation efforts resulted in a recovery of the otter in several European countries (e.g. Robitaille and Laurence, 2002) and today the species has been documented to occur in natural, altered and man-made aquatic systems (e.g. Kranz and Toman, 2000). This is particularly evident in Mediterranean regions, where use of reservoirs by otters has been documented (Prenda et al., 2001; Pedroso and Santos-Reis, 2006) as a response to multiple threats such as destruction of riparian vegetation, reduction of stream flows and increased drought frequency (Jiménez and Lacomba, 1991; Prenda et al., 2001) due to climate change scenario.

Large reservoirs affect water flow by acting as a barrier (Ruiz-Olmo et al., 2001), induce changes in prey communities such as fish (becoming dominated by exotic species), constrain the fishing ability of otters due to their steep margins and deep waters (Kruuk, 2006), and do not offer good refuge conditions due to frequent and unpredictable water level fluctuations that result in lack of bank vegetation (e.g. Prenda and Granado-Lorencio, 1995). However, a study in large reservoirs in southern Portugal (Pedroso and Santos-Reis, 2006) showed a somewhat otter occupancy of reservoirs, and similar results were obtained in southern Spain, where Prenda et al. (2001) showed that otters, although favouring streams, used reservoirs according to their availability.

The effect of reservoirs on otters may differ according to their size with small and medium-sized reservoirs (perimeter of less than 3500 m) inducing different responses than larger ones. Further studies are needed to clarify the importance of the reservoir-like aquatic systems as water and food sources, especially when these resources are limited in summer, as in southern Portugal (Beja, 1992; Kruuk, 2006).

The main goal of our study is to assess reservoir use by otters in southern Portugal and discuss their role in promoting persistence of otter populations in Mediterranean-type ecosystems. Specifically, we aim to identify factors like shelter, food availability and disturbance, which are most likely influencing the use of such reservoirs.

Materials and methods

Study area

The study area (Figure IV.2.1) is located in “Serra de Monfurado”, a Natura 2000 Site (MN2000), in southern Portugal (Alentejo region). Survey area covered 25 163 ha (23 946 ha are inside the Site) with altitudes ranging between 150 and 420 m. The climate is predominantly Mediterranean, with long summer droughts and irregular river flows. The average annual rainfall, in the study year (2004), was c. 700-800 mm with 91% falling between October and April (www.cge.uevora.pt).

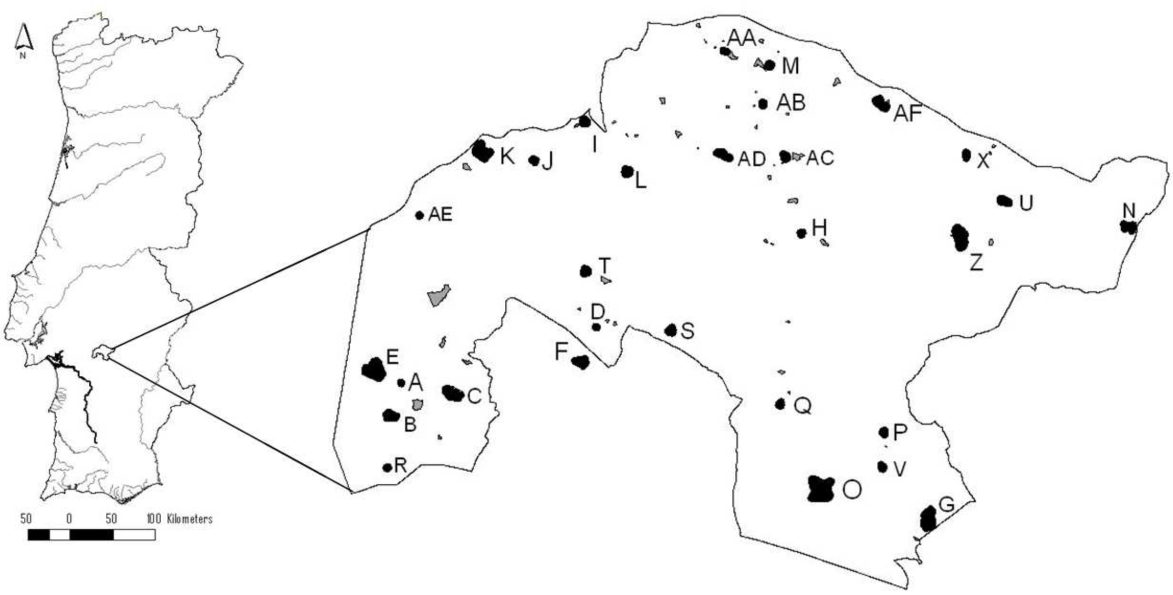

Figure IV.2.1 - Study Area – Monfurado Natura 2000 Site (“Sítio de Monfurado”). The black-filled reservoirs are the ones surveyed in the study. In the map of Portugal, basins’ limits and main River Sado are indicated

The MN2000 Site is included in the Mediterranean Basin, a world biodiversity hotspot (Mittermeier et al., 2004). It is dominated by old-growth woodlands of cork *Quercus suber* and holm *Quercus rotundifolia* oaks (“Montado”), covering approximately 70% of the study area. The remaining area is covered either by pastures (20%) or by small patches of annual crops and olive groves (10%). About 250 ha (1%) are occupied by small and medium-sized water reservoirs (n ≈ 100), dispersed all over the area though clustered in small groups (Figure IV.2.1).

Located within the Sado River hydrological basin (Figure IV.2.1), most of the watercourses in the study area are seasonal, drying up or having severely reduced water flow during the dry season. Water quality is considered moderate but the increased use of pesticides, manure, or fertilizers in the surrounding agricultural lands has lead to contamination in some areas (Trindade et al., 1998).

Fish inventories recorded nine species in 31 watercourses and seven species in 13 reservoirs within the MN2000 Site, several of which registered in both systems. These included native Iberian barbel (*Barbus bocagei*) and loach (*Cobitis paludica*), and exotic mosquito fish (*Gambusia holbrooki*), pumpkinseed (*Lepomis gibbosus*), and largemouth bass (*Micropterus salmoides*). Eels (*Anguilla anguilla*), arched-mouth Portuguese nases (*Chondrostoma lusitanicum*), Iberian nases (*Chondrostoma polylepis*) and Iberian chubs (*Squalius pyrenaicus*) are autochthonous species detected only in watercourses while other species such as *B. bocagei* and *C. paludica* are scarce in the reservoirs. Redfish (*Carassius auratus*) and carp (*Cyprinus carpio*) were found only in reservoirs where the fish community is dominated by exotic species and where higher abundances of *L. gibbosus* occur (Almeida et al., 2005; P. Raposo, personal communication). The American crayfish, *Procambarus clarkii*, another exotic species, was recorded in all the surveyed reservoirs. Small mammals, water fowl, aquatic reptiles, amphibians, large diving beetles were also commonly found, particularly in riverine systems.

Otter surveys

Otter surveys were based on detection of otter signs such as faeces (here termed spraints), scent marks, prey remains, and footprints in 30 small and medium sized-reservoirs scattered across the study area. These reservoirs (n=30) were randomly selected within three reservoir perimeter (boundary of water area of each reservoir) classes: 1- < 500 m (n = 13); 2 - between 500 m - 1500m (n = 11); 3 - > 1500 m (n = 6).

Although some authors reject the idea of a clear association between the number of spraints and the number of otters or the amount of time spent by them in a certain place, (e.g. Kruuk and Conroy, 1987; Mason and Macdonald, 1987) others advocate that the number of signs left by otters can be used to assess habitat preferences (e.g. Prenda and Granado-Lorencio, 1995; Hutchings and White, 2000; Clavero et al., 2006) and higher sprainting/marking activity may indicate defence of a scarce resource, especially prey (e.g. Clavero et al., 2006; Sulkava, 2006; Ruiz-Olmo et al., 2007). Following the last, we used otter signs (marking intensity) as a relative measure to infer intensity of use of reservoirs.

Sign surveys were carried out between January and August 2004 along the perimeter of each reservoir, ranging from 100 m to about 3 000 m. Each reservoir was visited thrice: first, to remove existing otter signs (January-February 2004), second, to sample the wet season (March-April 2004) and third, to sample the dry season (July-August 2004).

The intensity of otter use of each reservoir was estimated using the number of signs per kilometre (e.g. Maillard et al., 2001; Pedroso et al., 2004) and values obtained for the dry and wet seasons were compared using the Wilcoxon signed-rank test. Spatial autocorrelation was tested using Moran's Index (e.g. Premo, 2004) with the software Statistical Analysis with ArcView (Arcview® 3.2 ESRI, 1992-1999). Prior to analysis, data were transformed (log [x+1] or arcsine [√x] for proportions) to stabilize error variance (Tabachnick and Fidell, 2001).

Eco-geographical descriptors

Eco-geographical descriptors were selected on the basis of their relevance for otter ecology and behaviour and were classified in three variable sets defined as landscape attributes, reservoir attributes and availability of marking sites (Table IV.2.1). Some descriptors were categorised using quantitative scores in order to soften measurement or estimation errors.

Table IV.2.1– Eco-geographical descriptors used to describe otter's marking intensity of the reservoirs.

Acronyms	Variable description	Type	Data categories and units	Source
LENGHT_WATER_COURSE	Length of watercourses	L	Meters	Maps
LENGTH_RIPARIA	Length of watercourses with develop riparian vegetation	L	Meters	Maps
NEAR_WATER_COURSE	Distance from the reservoir to the nearest water course	L	Meters	Maps
NEAR_RESERVOIR	Distance from one reservoir to the nearest reservoir	L	Meters	Maps
SCRUBLAND	Proportion of Scrubland patches	L	Meters (proportion)	Maps
"MONTADO"	Proportion of Montado (*Quercus* sp.) patches	L	Meters (proportion)	Maps
ANNUAL_CROPS	Proportion of annual crops patches	L	Meters (proportion)	Maps
CATTLE	Area occupied by cattle settlements	L	Meters (proportion)	Maps
NEAR_ROADS	Distance from the reservoir to the nearest paved roads	L	Meters	Maps
AMPHIBIA	Amphibian observations	R	0– Absence or 1-5 ind., 1– < 20 ind., 2– 20-40 ind., 3– > 40 ind.	Field work
CRAYFISH	American Crayfish observations	R	0– Absence or 1-5 ind., 1– < 20 ind., 2– 20-40 ind., 3– > 40 ind.	Field work
FISH	Fish observations	R	0– Absence or 1-5 ind., 1– < 20 ind., 2– 20-40 ind., 3– > 40 ind.	Field work
REFUGES	Refuges in the flooding area	R	1–cover absent or present, but offering no suitable cover for otters, 2–present in patches, offering some cover for otters, 3–large area of suitable cover for otters, 4-continuous dense vegetation, providing excellent cover for otters	Field work
N_WATER_COURSE	Number of watercourses flowing to the reservoir	R	Number	Field work
AREA	Area of each reservoir	R	Square Meters	Maps
PHOSPHATE	Levels of phosphate-total in a sample of water	R	mgl^{-1} (Kit Lovibond – range 0,07-3 mgl-1)	Laboratory
NITROGEN	Levels of nitrogen-total in a sample of water	R	mgl^{-1}(Kit Lovibond – range 0,5-14 mgl-1)	Laboratory
MARK_SITES	Potential marking sites (e.g. stones, tree roots)	MS	0 – absence / 1 – scarce / 2 – medium / 3 - high	Field work

Notes: Source: Field Work – all the variables were visually estimated; Maps – Information obtained by the Military cartography (1: 25000) and by digital data inserted in all the studies carried out in the study area (reports of CCDR); Laboratory: data collected from field sampling and analysed in laboratory. Type: L – Landscape attributes; R – Reservoir attributes; MS – Availability of Marking Sites. Ind. - Individuals.

Landscape attributes included: a) length of the adjacent watercourses (LENGTH_WATER_COURSE); b) length of riparian vegetation (LENGTH_RIPARIA); c) minimum distance to water sources (NEAR_WATER_COURSE; NEAR_RESERVOIR); d) area occupied by different land uses (SCRUBLAND; "MONTADO" (cork/holm-oak forests); ANNUAL_CROPS); e) area occupied by cattle grazing (CATTLE) and f) minimum distance to paved roads (NEAR_ROADS) (Table IV.2.1). Proportions of land uses and length of watercourses were assessed in a 1 km buffer around each reservoir and were obtained from maps assimilating military charts data (1: 25 000), 2001 aerial photography and field surveys (Table IV.2.1).

Reservoirs were characterised using eight descriptors: a) food availability considering the main prey categories (AMPHIBIA; CRAYFISH; FISH) estimated according to the number of observations of each prey type during otter survey transects and categorised following Ruiz-Olmo et al. (2005) criteria; b) the abundance of refuges near the reservoirs (REFUGES), following Beja (1992) attributes; c) the number of watercourses flowing to the reservoir (N_WATER_COURSE); d) the area of each reservoir (AREA) and e) water quality, only evaluated in the dry season, by calculating the concentration of total phosphates (PHOSPHATE) and nitrogen (NITROGEN) (Table IV.2.1).

The availability of otter scent marking sites (MARK_SITES), categorised in classes (Table IV.2.1), was determined during otter surveys and inferred from the presence of stones, logs, tree roots, bridges, sand bars or stream confluences (Foster-Turley et al., 1990).

The relationship between the number of signs per kilometre and the eco-geographical descriptors was assessed through a Generalized Linear Model (GLM) with the unity link (Gaussian regression). A preliminary univariate regression analysis (Hosmer and Lemeshow, 2000) was performed to evaluate the significance of individual descriptors on otters' use of reservoirs. Unimodal responses for each variable were checked by introducing the corresponding quadratic term into the univariate model. Variables ascertained as significant ($P < 0.25$) at this stage (Hosmer and Lemeshow, 2000) were evaluated for collinearity through the Spearman correlation coefficient. Redundant information was eliminated, when coefficients were higher than 0.7 (Tabachnick and Fidell, 2001). In each correlated pair, the variable that had the lower correlation with the number of signs per kilometre was excluded. The Spearman correlation was also used to compare the number of available marking sites in both seasons.

Then multiple linear models were built separately for each set of variables. Selection of the best models was based on combinations of variables that minimized the Aikaike Information Criterion value (Zuur et al., 2007).

All variables selected in the three best multivariate models were used in a variation partitioning procedure, following the methodology proposed by Borcard et al. (1992) with an extension to three sets of variables (e.g. Reino et al., 2006; Galantinho and Mira, 2009). We built several GLMs to obtain the following components of variation: i) pure effect of landscape, ii) pure effect of reservoirs, iii) pure effect of marking sites, iv) mixed effect of landscape-reservoirs, v) mixed effect of reservoirs-marking sites, vi) mixed effect of landscape-marking sites, vii) mixed effect of landscape-reservoirs-marking sites and, viii) unexplained variation. The R^2 (coefficient of determination), was used as a measure of the explained variation by each model and the area under the curve (AUC) to assess the models' performance. Statistical tests were considered significant at a 0.05 significance level except where stated otherwise. Analyses were performed using the Statistical Software Brodgar (version 2.5.2).

This analysis allows isolating the pure effect of each set, evaluating its relative importance on marking intensity of otters and understanding the specific contribution of the availability of marking sites.

Otter Diet

Otter diet was analysed in a subsample of 12 reservoirs using a total of 318 spraints (168 dry season, 150 wet season). Methods for spraint analysis followed Sales-Luís et al. (2007). Whenever possible, prey remains were identified to the species level. Results were expressed in percentage of occurrence [PO(item A) = total number of individuals of prey item A consumed / total number of individuals consumed in all spraints * 100].

Chi-square statistics with Yate's correction for continuity were used (e.g. Sokal and Rohlf, 1995) to detect seasonal differences in percentage of occurrence of prey items in otters' diet. Statistical calculations were performed using SPSS for Windows ® version 11.0 (SPSS Inc., Chicago, Illinois, USA) and Microsoft® Office Excel® 2003.

Results

Otter use of reservoirs

Otters used a majority of the reservoirs in both seasons: 23 reservoirs (77%) in the wet and 21 (70%) in the dry season. Six reservoirs did not show signs of otter presence in both seasons; one was used only in the dry season and three only in the wet season.

A total of 2 331 otter signs were identified during the study period, 71% (n = 1664) of which were counted in the dry season (Figure IV.2.2) and showed a significant seasonal variation in the number of signs per kilometre (Z = -2.000; P = 0.046). No spatial autocorrelation was found for otter data in both seasons (Moran's I = -0.047, Z = -0,101, $p < 0.05$ – dry season; Moran's I = -0.025, Z = 0.074, $p < 0.05$ – wet season).

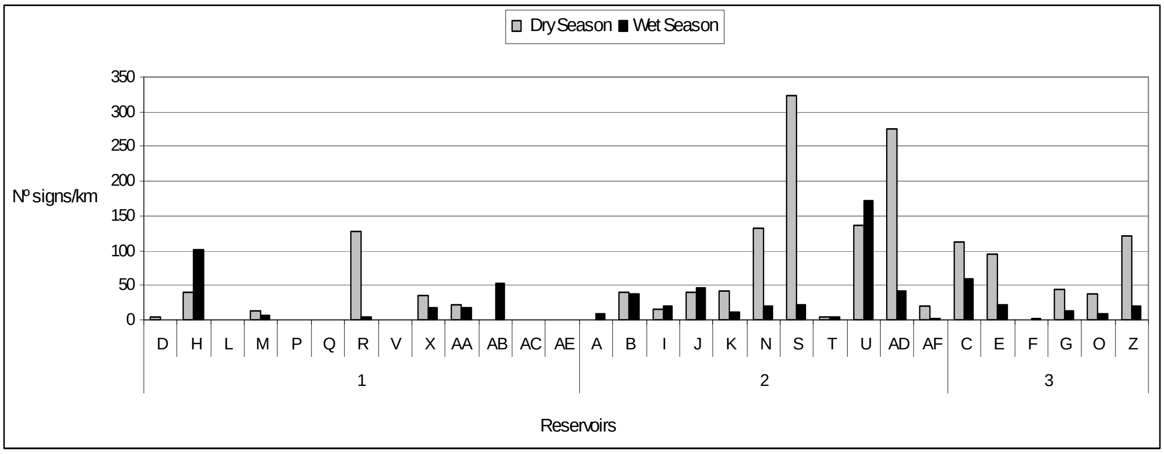

Figure IV.2.2 - Otter signs per kilometre in each reservoir in both seasons. The reservoirs are categorised by perimeter classes (1- < 500m; 2 - between 500m - 1500m and 3- > 1500m).

When considering landscape effects, all significant or nearly significant variables were negatively associated with otter marking intensity: ANNUAL_CROPS in dry season; CATTLE in wet season; and LENGTH_RIPARIA in both seasons. ANNUAL_CROPS and the LENGTH_RIPARIA variables were the most significant in the dry and wet season, respectively. Reservoirs descriptors such as CRAYFISH, FISH, REFUGES, N_WATER_COURSE, and AREA, were significantly and positively related with the number of signs per kilometre in the dry season, while in the wet season, only CRAYFISH showed a positive association. The number of available marking sites (MARK_SITES) was positively related with the number of signs per kilometre in both seasons (Table IV.2.2 and 3). A significant correlation (Spearman's rho = 0.876; $p < 0.001$) in marking sites availability between seasons indicates that water level fluctuation had little effect on the number/quantity of marking sites, allowing an unbiased comparison.

Table IV.2.2 – Univariate linear regressions of landscape, reservoir and marking sites variables. Variables in bold had significant results. Significance levels are given in brackets. The amount of explained variation (R^2) is given for each model / n.s. – non significant; ***** - not applicable; (Ln) – applied transformation (Log (x+1)). The number of reservoirs was 30 in both seasons.

Variables	**Dry Season – Univariate**				**Wet Season - Univariate**			
	Mean	SE	Linear regression	R^2	Mean	SE	Linear regression	R^2
LANDSCAPE								
LENGHT_WATER_COURSE	54,200	3,117	+ (n.s.)	0,011	54,200	3,117	- (n.s.)	0,000
LENGTH_RIPARIA	**5,767**	**0,583**	**- (0.092)**	**0,098**	**5,767**	**0,583**	**- (0.009)**	**0,219**
NEAR_WATER_COURSE	20828,200	5012,735	+ (n.s.)	0,001	20828,200	5012,735	-(n.s.)	0,024
NEAR_RESERVOIR	90587,567	11006,654	+(n.s.)	0,082	90587,567	11006,654	+ (n.s.)	0,006
SCRUBLAND	17,233	3,707	+ (n.s.)	0,066	17,233	3,707	+ (n.s.)	0,001
"MONTADO"	28,300	3,212	- (n.s.)	0,019	28,300	3,212	+(n.s.)	0,001
ANNUAL_CROPS	**37,500**	**3,463**	**- (0.051)**	**0,129**	37,500	3,463	- (n.s.)	0,088
CATTLE	1663,700	407,117	- (n.s.)	0,048	**1663,700**	**407,117**	**- (0.089)**	**0,100**
NEAR_ROADS	102518,600	16974,062	+ (n.s.)	0,000	102518,600	16974,062	0.001(n.s.)	0,083
RESERVOIR								
AMPHIBIA	2,067	0,143	+ (n.s.)	0,003	1,967	0,176	- (n.s.)	0,020
CRAYFISH	**0,900**	**0,169**	**+ (<0.001)**	**0,373**	**0,567**	**0,157**	**+ (0.011)**	**0,208**
FISH	**1,433**	**0,202**	**+ (0.002)**	**0,294**	1,133	0,202	+(n.s.)	0,009
REFUGES	**2,400**	**0,149**	**+ (0.001)**	**0,331**	2,333	0,088	+ (n.s.)	0,004
N_WATER_COURSE	**1,333**	**0,138**	**+(0.023)**	**0,172**	1,333	0,138	+(n.s.)	0,011
AREA (Ln)	**4,213**	**0,120**	**+ (0.039)**	**0,143**	4,213	0,120	+(n.s.)	0,040
NITROGEN (Ln)	1,357	0,078	+ (n.s.)	0,032	*****	******	*****	******
PHOSPHATE (Ln)	0,985	0,146	- (n.s.)	0,001	*****	******	*****	******
MARKING SITES								
MARK_SITES	**2,133**	**0,142**	**+(<0.001)**	**0,497**	**1,967**	**0,112**	**+ (0.085)**	**0,102**

Table IV.2.3 **–** Summary of the relationship between otter's use of reservoirs and eco-geographical variables as assessed from multivariate linear regression for landscape, reservoir and marking sites models on dry and wet seasons. Directions of association, positive (+) or negative (-), are given for each model showing significant ($P < 0.05$) or nearly significant ($P < 0.10$) relationships. Variables in bold are those selected for the multivariate models while the other variables show only significant univariate relationships. The amount of explained variation (R^2) and overall significance (P) is given for each best model.

VARIABLES	DRY SEASON		WET SEASON	
	Direction of Association	P	Direction of Association	P
LANDSCAPE				
LENGTH_RIPARIA	-	0.092	-	**0.009**
ANNUAL_CROPS	**-**	**0.051**		
CATTLE			-	**0.089**
R^2 (%)	9.5	0.097	21.9	0.009
RESERVOIR				
AREA	+	0.039		
N_WATER_COURSE	+	0.023		
REFUGES	**+**	**0.001**		
FISH	+	0.002		
CRAYFISH	**+**	**<0.001**	+	**0.011**
R^2 (%)	56.7	<0.001	20.8	0.011
MARKING SITES				
MARK_SITES	**+**	**<0.001**	+	**0.085**
R^2 (%)	49.7	<0.001	10.2	0.085

GLM's showed that 62% of the variation in otter marking intensity in the dry season is explained by the selected variables (see Table IV.2.4), but only about 33% of the variation is accounted for in the wet season.

Table 4 - Partitioning of variation of the number of signs per kilometre on dry and wet seasons explained by best models incorporating landscape, reservoir and availability of marking sites effects. For pure effects, significance levels are given in brackets.

Components	DRY SEASON	WET SEASON
	Variance explained (%)	Variance explained (%)
Pure Landscape	0.06 (0.917)	10.04 (0.060)
Pure Reservoir	17.84 (0.002)	8.15 (0.088)
Pure Marking Sites (MS)	5.26 (0.034)	0.26 (0.754)
Landscape * Reservoir	0.62	4.32
Landscape * MS	-0.06	1.62
Reservoir * MS	29.30	2.42
Landscape * Reservoir * MS	8.93	5.90
Unexplained	38.05	67.29

Regarding the pure effect of each set of variables, in the dry season, reservoir descriptors were the most important, explaining a significant (P=0.002) amount of variation (18%) followed by marking sites that *per se* explain about five percent of variance; in this season landscape descriptors were not significant. In the wet season, the pure effect of the landscape was the most important, explaining about 10% of the response variable variance. In this season, the pure effect of reservoir characteristics explained about eight percent of the variation (Table IV.2.4).

The largest fraction of the explained variation in the dry season (29%) was due to the combined effect of reservoir characteristics and availability of marking sites (Table IV.2.4).

Otter Diet

A total of 798 occurrences of 27 different prey items were identified. Crustaceans (represented only by *Procambarus clarkii*) constituted 48.6% of all occurrences. Fish was the next most frequent class (35.0%), while insects (6.6%), amphibians (6.1%), fruits (represented by only one species – blackberry *Rubus* sp., 2.9%), reptiles (0.5%), birds (0.1%) and mammals (0.1%) were of minor importance in otter's diet.

Otter diet varied seasonally (Figure IV.2.3), with amphibians being consumed more in the wet season, and fruits being consumed only in the dry season. Crustaceans were the most consumed class in both seasons, although slightly more in the dry (55.1%) than in the wet (42.6%) season; fish were more important during the wet season (38.5%).

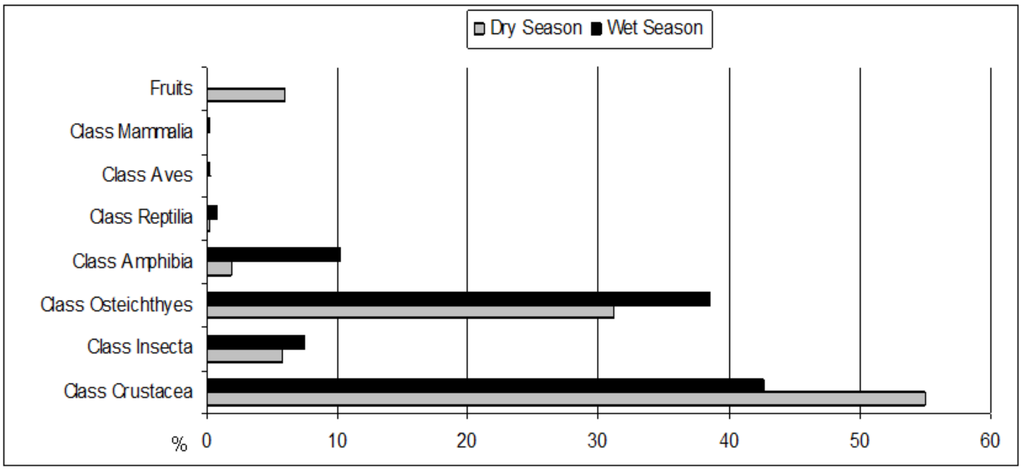

Figure IV.2.3 - Percentage of occurrence of prey classes categories in otter diet per season in the 12 reservoirs.

Lepomis gibbosus was the most frequently consumed fish species in both seasons (wet – 30.3%; dry – 15.6 %), followed by *Gambusia halbrooki*, specially in the dry season (9.4% *vs*. 0.5% in the wet) and *Micropterus salmoides*, almost equally consumed in both seasons (dry - 3.6% and wet - 3.1%) (Figure IV.2.4)

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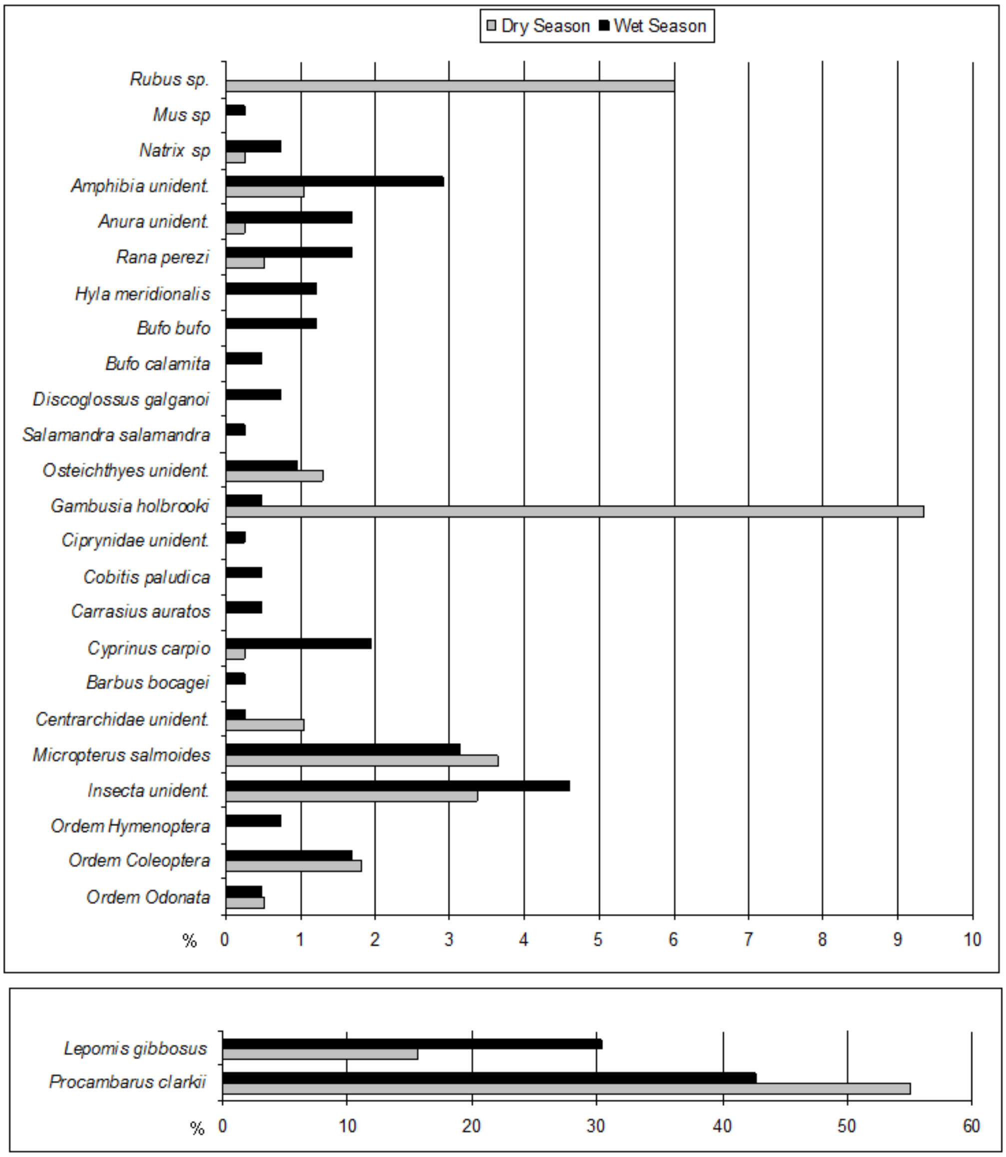

Figure IV.2.4 - Percentage of occurrence of prey categories in otter diet per season in the 12 reservoirs

Chi-square tests showed differences in occurrence of prey items in otter diet between seasons. These differences were highly significant for amphibians (χ^2 = 24.11, p < 0.001), fishes (χ^2 = 4.71, p = 0.03), crustaceans (χ^2 = 12.36, p < 0.001) and fruits (χ^2 = 25.40, p < 0.001).

Discussion

Patterns of occupancy by otters of small and medium-sized reservoirs in the study area were in accordance with the findings of other studies, both in watercourses (e.g. Ottino and Giller, 2004) and in reservoirs (e.g. Prenda et al., 2001; Pedroso et al., 2004), with a higher number of signs being found in the dry season. Elliot (1983) also described higher sprainting activity on rivers which almost dry up compared to those maintaining a reasonable flow during summer. As prior referred seasonality of sprainting behaviour may occur (e.g. during breeding time, females hide more often the spraints - Kruuk, 2006 - although this breeding time can occur all year long in continental Europe - Kranz, 1996). Usually in Mediterranean areas, more spraints are found in summer than winter (Palomares et al., 1989; Ruiz-Olmo and Gosálbez, 1997). However, it is also acknowledged that high marking activity may indicate defence of a scarce resource (e.g. Macdonald and Mason, 1982; Sulkava, 2006). In our case, similar to other Mediterranean areas (Prenda et al., 2001), we suspect that aquatic prey availability and water (as a consequence) are the limiting resources. Kruuk (2006) states that not all habitat preferences are habitat requirements e.g. where prey is plentiful (such as along the coasts of Shetland), otters occur in large numbers despite the scarcity of cover.

Seasonal differences found in the marking intensity at the same reservoir suggest the higher importance of reservoirs, as a resource in the dry rather than the wet season. Drought may be the leading cause since in the study area most watercourses have no water in the dry season due to the great number of reservoirs and few large streams and rivers. This concentration of reservoirs may promote a change in otter's behaviour by making them allocate more time to systems with higher water availability, that are probably also richer in aquatic prey (Prenda et al., 2001). Effectively, in these circumstances, the territories of many otters have been shown to alter dramatically with season (Macdonald and Mason, 1982; Kruuk, 2006). This was also reported in a study in the Mediterranean where otter activities during the dry season tended to concentrate around remaining running waters, pools, and small reservoirs, effectively reducing territory size and scent marking boundaries (Ruiz-Olmo et al., 2007).

The negative association found between the use of reservoirs and the length of watercourses with developed riparian vegetation in the surrounding areas may reflect the otter preference for better-preserved streams and rivers, instead of man-made artificial reservoirs lacking in refuge opportunities. Riparian woodlands and shrubs represent suitable habitats for otters (e.g. Ruiz-Olmo et al., 2005) by favouring water retention in dry periods and consequently, prey occurrence. Thus, when this vegetation type is present, the need for reservoir resources is lower.

The negative influence of cattle observed in the wet season may be an important result of our study in view of the cattle grazing intensification observed throughout the study area in the last few years. Higher densities of cattle promote strong disturbance around the reservoir. Although the direct relationship between otters and cattle is not well documented, grazing inhibits woody vegetation recovery (Carmel and Kadmon, 1999) and also contributes to organic water pollution reducing habitat suitability for otters (Macdonald and Mason 1983; Trindade et al., 1998; Kruuk, 2006). Although the evaluation of the effect of seasonality is based on only one sampling year, it seems that the absence of any cattle effect in the dry season may reflect the higher importance of water as a limiting resource in the dry season.

In contrast, the negative effect of the area occupied by annual crops on the otters' use of reservoirs is higher in the dry season. In this case, it is possible that the human disturbance associated with crop harvesting specifically during this season and the consequent lower vegetation cover negatively impact otters (Mason and Macdonald, 1986; Ottino and Giller, 2004).

Our study showed that the abundance of American crayfish was one of the most important variables positively associated with otter markings in the reservoirs. Other studies in Mediterranean areas revealed the importance of crayfish as prey for otters (Magalhães et al., 2002; Clavero et al., 2004; Pedroso and Santos-Reis, 2006). In fact, American crayfish represents a new food resource, particularly in stressful periods of severe drought, and is likely to have increased the carrying capacity of the environment for aquatic predators such as the otter. The importance of American crayfish in otter diet, corroborated by the diet results of this study, can be interpreted as a response to the high abundance of this species in the reservoirs. This is relevant in view of the low diversity and density of fish found in the rivers and streams of the study area (Almeida et al., 2005) especially in dry periods.

The positive association between otter use and the availability of refuges (represented here by the number of watercourses flowing into the reservoirs) has also been observed elsewhere (e.g. Elliot, 1983; Prenda et al., 2001) and must reflect the fact that they provide shelter and safe resting and breeding places (e.g. Foster-Turley et al., 1990; Jimenez and Lacomba, 1991). Furthermore, a high cover allows otters to move between food and refuge areas with less susceptibility to disturbance factors such as human harassment.

The significant relevance of the reservoir area in the drought period may reflect the shortage of water in smaller reservoirs and surrounding watercourses. In fact, very small reservoirs are unlikely to be able to sustain prey communities during all of the dry season (Almeida et al., 2005; Magalhães et al., 2007; Sales-Luís et al., 2007) and thus may be less important for the otter or sustain lower number of otters.

The influence of reservoirs' characteristics, independently or together with other descriptors, on use of these man-made water bodies by otters, mainly in dry season, is emphasized by our variance partioning results. The summer drought that usually characterizes Mediterranean regions, resulting in water shortage in watercourses, may turn reservoirs into valuable alternatives as foraging grounds for otters during dry periods. During this season a larger area in the reservoir vicinity is not covered by water and otter marking activity may be focused in specific marking sites along the reservoirs closer to the water and therefore food. In the wet season, however, food resources and water are readily available, and otter choices seem to be mostly influenced by surrounding landscape features.

Otters using small and medium-sized reservoirs feed mainly on abundant prey in the study area (*P. clarkii* and *L. gibbosus* - Almeida et al., 2005) in each season, confirming the largely known opportunistic character of the otter (for a review see e.g. Kruuk, 2006). More interesting is the fact that no fish species existing only in watercourses were found in the analysed spraints. Although the very fast digestive system of otters does not allow confirmation that otters do not feed along the nearby rivers, we expected some of the species to be present in at least some spraints due to close proximity to the rivers. This result suggests that the use of the reservoir by the otter may be linked to higher food availability, a scenario also found in other studies (Pedroso and Santos-Reis, 2006; Sales-Luís et al., 2007). In fact, the smaller Mediterranean watercourses, such as the ones occurring in the study area, may have lower prey availability (especially fish) during summer months (Almeida et al. 2005; Magalhães et al., 2007; Sales-Luís et al., 2007). In hot and dry summers, characteristic of Mediterranean climates, there is a shortage of surface waters with fish becoming confined to pool refugia and to small reaches maintaining flowing waters, where they are at high risk of mortality from desiccation, predation or anoxia (Magalhães et al., 2002). This may explain the higher dependence on *P. clarkii* consumption in dry periods, a species that better survives large periods of water shortage.

To summarize, otters use the small and medium-sized water reservoirs both in dry and wet seasons. Although this work addresses only one dry and one wet sampling season and conclusions on seasonality are limited by this, the results suggest a more intense use of reservoirs during the dry season, corroborating findings of other studies (e.g. Pedroso et al., 2007; Sales-Luís et al., 2007).

These reservoirs may be suboptimal habitats for otters in terms of refuge and human pressure when compared with rivers and streams, but seem to act as important feeding areas especially when close to watercourses with good refuge conditions and scarcity of prey. A co-use of close-by watercourses and large reservoirs was identified by Pedroso et al. (2004, 2007) and Sales-Luís et al. (2007), suggesting that otters inhabiting watercourses may spend a large amount of their time feeding in the reservoir, and this may well apply to the study area in spite of not being addressed in this study. Additionally, smaller reservoirs, as the ones studied here, may have a lower negative impact on otters than larger ones as they do not represent such a loss of natural habitat, have less effect on water flow regimes, induce fewer changes in prey communities, and do not constrain otter fishing ability due to their smoother margins and shallow waters.

Effective protection of otters depends mainly on safeguarding of large areas of suitable habitat, due to their large spatial requirements (Foster-Turley et al., 1990). Hence otter conservation and the management of freshwater systems at the Monfurado Natura 2000 site should be viewed in its regional context. In this area, many watercourses are already altered from their pristine state and water is a scarce resource mainly in summer; yet, the number of reservoirs is high and seems to be contributing to the still widespread distribution of otters in the area without significant threats to their persistence. However, distribution range does not necessarily mean higher population numbers (Prenda et al., 2001).

Thus, the best long-term conservation strategy is to maintain this presumably healthy otter population by improving its natural prey and habitat conditions while sustaining human activities. To do so, reservoirs must be kept in place but degradation of surrounding areas through grazing and agricultural intensification must be controlled (Collares-Pereira et al., 2000). Due to general conservation concerns and goals, fishery activities also require management for exotic and autochthonous fish populations. This includes the exotic American crayfish, which, although playing a relevant role in otter conservation in Iberian streams as prey (e.g. Beja, 1996; Clavero et al., 2004), negatively impacts freshwater autochthonous fish and amphibian species through competition and predation (e.g. Gil-Sánchez and Alba-Tercedor, 2002; Cruz and Rebelo, 2005). Consequently, the control of the American crayfish and exotic fishes should take place simultaneously with a program aiming to recover native prey species to population levels which could represent a true alternative for the otters, when exotics become less available.

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IV.3. Otter response to environmental changes imposed by large dams construction

PAPER 4

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Otter response to environmental changes imposed by large dams construction

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Abstract

There are few examples of long term monitoring studies involving otters and dams, although the number of these infrastructures is progressively increasing. Our objectives were to assess how Eurasian otter *Lutra lutra* distribution changed over time in response to the changes in the species main ecological requirements with the construction of a large dam. We monitored otter responses to the construction of the Alqueva dam (250 km^2, SE Portugal) from 2000 to 2006. We surveyed the area to be flooded and its surroundings throughout the pre-deforestation/flooding, deforestation, flooding and post flooding phases at two different resolutions: 25km^2 and 1km^2. In each phase and in all selected cells, we seasonally surveyed 600m transects for otter presence and recorded information on its ecological requirements. In eight survey sites, distributed throughout the study area, we collected otter spraints to assess diet and to compare it to prey availability. Within the flooding area, otter presence was widespread prior to dam construction (always above 82.4%), decreasing during deforestation (68.5%), and particularly during the flooding phase (33.3%). A recovery was observed in the post flooding phase, although not to the level prior to dam construction (61.5% up to 83.3%). Otter diet was dominated by fish and American crayfish *Procambarus clarkii* both in pre-deforestation/flooding phase (56.7% and 35.3% of occurrences, respectively; n = 1921). This dominance was maintained at the end of the post-flooding phase (60.7% and 33.2%; n = 658), but species richness of preyed fish decreased with flooding (16 to 8), and so did the ratio of native/non-native preyed fish species (1.7 to 0.3). Changes in the suitability of otter main ecological requirements were similar to the pattern of otter distribution in the flooding area throughout the impact phases and decreased during deforestation and flooding with some recovery being observed in the post flooding phase. Our results emphasize the importance of long-term monitoring studies that include several post-impact phases, to truly evaluate species response to impacts, beyond the mandatory framework of Environmental Impact Assessment. This will allow better planning of mitigation and compensation measures.

Keywords: Lutra lutra, reservoir, ecological requirements, monitoring, impacts, conservation.

Introduction

Adaptation to habitat change, loss and fragmentation is a key aspect of species conservation, especially when resulting from the establishment of infrastructures that pose new challenges for their survival. Monitoring species responses to habitat change is crucial therefore to predict species trends in human-altered environments. Climate changes and increase in water demands by humans contribute to conservation pressure for aquatic species. This is especially so in the Mediterranean basin, which is considered to be one of the regions that will face the largest changes in climate worldwide (Giorgi, 2006), and where water management is conducted through river regulation (dams) (Collares-Pereira et al., 2000). Dams cause large-scale habitat disturbance with major impacts on fish and other riparian populations and habitats (Collares-Pereira et al., 2000; WCD, 2000). Mediterranean habitats experience extreme seasonal variation in water flow. A stress period usually occurs in summer, when water flow and level are low to zero, following frequently long periods of drought. Reservoirs can affect this situation by further influencing water flow regimes and acting as species movement barrier (Ruiz-Olmo et al., 2001).

The Eurasian otter (*Lutra lutra*) is a semi-aquatic mammalian carnivore that occupies an apex position in the food web of European fresh waters, preying on a wide range of prey living in water with emphasis on fishes (Almeida et al., 2012). One of the main threats for otters living in inland waters of the Mediterranean is the reduction of a naturally unstable water flow in rivers and streams (Jimenez and Lacomba, 1991). Flow reduction is often a consequence of river damming and of increased water demands, particularly for irrigation (Ruiz-Olmo et al., 2001). It is also very likely that climate change will contribute to the decrease of highly suitable otter habitats, in particular in the Iberian Peninsula. This is linked to a potential increase in drought frequency, extent and intensity as the climate warms, which can lead in some cases to the disappearance of shallow water bodies, including small rivers (Cianfrani et al., 2011), causing major declines in prey abundance. So, the preservation of riverine habitats is, and always has been a major priority for otter conservation because it is prey-rich, it offers adequate conditions for sheltering, and facilitates animal movement (e.g. Foster-Turley et al., 1990).

Given these characteristics, the otter is an ideal model species to address animal adaptation to habitat loss and change as caused by dam implementation in Mediterranean regions.

Dams, especially large ones, have been described as having a negative effect on the distribution of Eurasian otters, and are suggested as being a contributing factor in the past decline of the species in Europe (Foster-Turley et al., 1990; Macdonald and Mason, 1994). Some of the major impacts caused by dam construction on otters include direct habitat loss and destruction, and fragmentation of otter populations, as well as lack of adequate foraging grounds due to the creation of large and deep reservoirs (e.g. Foster-Turley et al., 1990; Macdonald and Mason, 1994; Kruuk, 2006; Santos et al., 2008). In Mediterranean regions, despite available evidence of otter presence and use of established large dams (Prenda et al., 2001; Pedroso and Santos-Reis, 2006), with a few exceptions (e.g. Santos et al., 2008) there is a lack of information on otter response in all dam implementation phases (pre-dam, flooding, post-dam). Consequently evaluation of impacts is currently incomplete. This is especially relevant since for many Environmental Impact Assessments of large dams, evidence of otter presence in the reservoirs is used to state that either (1) no impacts occurred or (2) that there were no significant consequences for otter populations after the change from a previous existent riverine system to a reservoir system. Long term monitoring studies are therefore required, covering all phases of dam implementation. This is important because of the increasing number of these infrastructures, especially so in Mediterranean countries where water management is largely based on dam building, and under the current scenario of climate change that affects riverine systems mostly by extending the drought period.

The aims of the present study were to assess: (1) if and how otter distribution changed along the different phases of a large dam construction, (2) if and how otter diet changes after dam implementation, and (3) whether there were changes in the otter's main ecological requirements during and after construction of the dam.

Methods

Study Area

The Alqueva dam was built in the valley of Guadiana River, in south-eastern Portugal. Its construction started in 1998 and in late 2003 created Europe's largest artificial lake, flooding an area of 25 000 ha (Figure IV.3.1). In addition, the Alqueva dam project includes a massive irrigation system affecting 120 600 ha, with profound changes in the surrounding landscape structure, land cover and use. Furthermore, the Alqueva dam was implemented in the

Mediterranean region, which is considered to be one of the biodiversity hotspots for conservation priorities (Brooks et al., 2006). The region is characterized by a multi-patch landscape, dominated by holm oak (*Quercus ilex*) and cork oak (*Quercus suber*) woodlands, interspersed with agricultural fields (cereals, vegetables and olive-yards) and forest plantations (eucalyptus and pine). The climate is characterized by mild winters and hot summers, with average temperatures of <18ºC in the coldest months and >22ºC in the warmest months. Precipitation varies between 400-600mm per year (Chícharo et al., 2001). Most rivers and streams in this region have a temporary character.

Field methods

The otter response to the construction of the Alqueva dam was monitored during four periods (monitoring phases): pre-deforestation/flooding (2000), deforestation (2001), flooding (2002 and 2003) and post-flooding (2004 to 2006).The first four years of monitoring (2000-2003) were performed in the frame of the project Monitoring of Threatened Carnivores, mandatorily included in the Monitoring Program of the Alqueva Dam Project.This Program defined the study area: 11 (1:25 000) military maps (441, 452,463, 474, 481, 482, 483, 490, 491, 492 and 501). These included the flooding area of the artificial reservoir created by the construction of the Alqueva dam (impacted area) and the surrounding area (non-impacted area). We monitored an additional three years period to include the post-flooding phase. During the dam implementation, vast areas of vegetation were removed (deforestation) in order to reduce the biomass and potential eutrophication of the reservoir, resulting in a large area of removed trees and shrubs covering the predicted maximum water level (152 m asl). The dam-wall was closed in February 2002, leading to the start of the flooding. From 2002 until the end of 2003, water level and flooding area increased considerably. The post-flooding phase corresponds to the slow final flooding of the area (from 140 m to the 152 m asl), and represents therefore a more stable water level. The parameters monitored were otter distribution, otter ecological requirements and otter diet.

Otter distribution was studied at two resolutions, 25 and 1 km^2. First the study area was divided into a grid of 25 km^2 cells, resulting in a total of 76 survey cells (Figure IV.3.1). Otter presence/absence in all these cells was assessed following the World Conservation Union **(**IUCN) Otter Specialist Group (OSG) survey guidelines (Foster-Turley et al., 1990; Reuther et al., 2000) by using a maximum transect length of 600 m per site (Macdonald, 1983). Otter signs (spraints, scent marks, prey remains, and footprints) were searched for along the transects.

Surveys were performed twice each year (wet and dry seasons). A cell was considered positive for the year if at least one seasonal survey was positive. To evaluate in detail the evolution of otter response in the directly impacted area, 39 1km^2 cells were randomly selected within the flooding area and, following the same procedure, each cell was surveyed every three months for otter signs. The transect location was adjusted following the rise of the water level, as "stream transects" became "reservoir transects" in the perimeter of the dam.

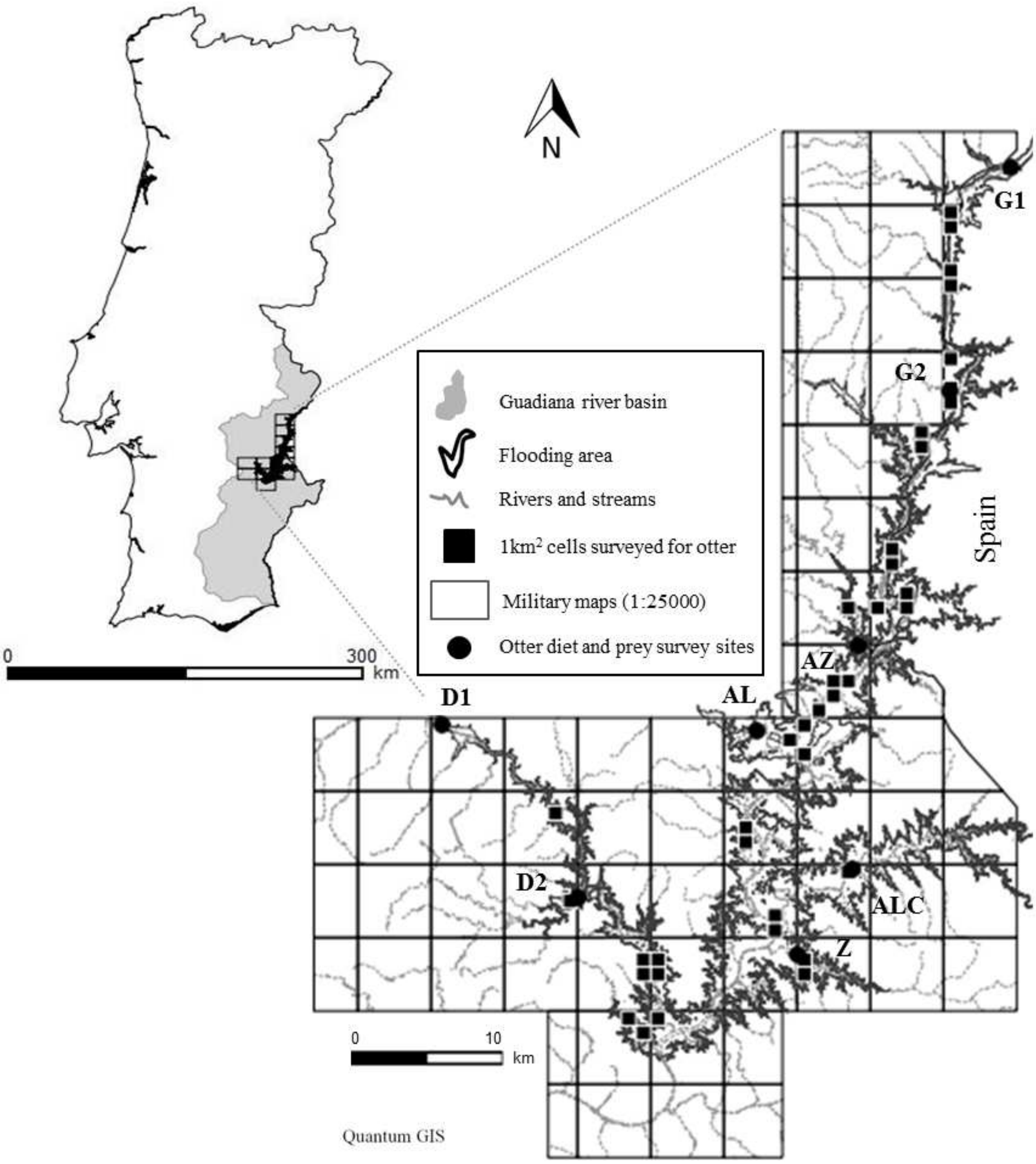

Figure IV.3.1 - Study area and Alqueva reservoir location in southern Portugal, showing the 25km^2 and the 1km^2 (black squares) otter survey grid cells and the sites (black circles) where otter diet and prey were assessed. Two sites (G1 and G2) were located in the main River Guadiana. Other sites were located in tributaries of the Guadiana River: the Azevel stream (AZ), Álamo Stream (AL), Degebe Stream (D1 and D2), Alcarrache Stream (ALC) and Zebro Stream (Z).

Otter diet and prey availability were assessed at sites defined by the Monitoring Fish for the Environmental Impact Assessment of the Alqueva Dam Project team (M.J. Collares-Pereira et al., unp. data). Otter spraints were collected at eight fish survey sites to assess otter diet (Figure IV.3.1).

Both otter diet and prey availability were assessed in the pre-deforestation/flooding phase (2000, five sampling periods: February, April, June, August and October) and at the end of post flooding phase (2006, three sampling periods: April, August and December). Otter diet was based on prey remains found in spraints expressed as percentage of occurrence (PO(item A) = total number of individuals of prey item A consumed / total number of individuals consumed x 100). Food items were identified to the lowest possible taxonomic level following a standard approach (Sales-Luís et al., 2007) and using a reference collection of fishes and other vertebrates, as well as published literature (Prenda and Granado-Lorencio, 1992; Conroy et al., 1993; Prenda et al., 1997, 2002).

Data on fish availability for the stream survey sites was gathered in 2000 in the frame of another study (M.J. Collares-Pereira et al., unp. data), and collected thought electro-fishing in 50m stretches for 30 minutes. In 2006, as these sites became ‘lentic’, we used several complementary methods, to avoid fishing gear selectivity when assessing fish assemblages in reservoirs (Godinho et al., 1998; Clavero and Hermoso, 2010). Because otters prefer shallow waters for foraging (Kruuk, 2006), a sequence of two fyke-nets were placed near and parallel to shore for fish capture, combined with three carboy, adapted and baited for capturing American crayfish *P. clarkii*. Also, one trammel net (15 m x 2 m; inner mesh: 25 mm; outer mesh: 100 mm) was set at a depth of 1.5 m at a minimum distance of 150 m from the margin. All sets were left overnight. All captured individuals were identified, counted, weighed, measured, and then released into the water. Data was converted into percentage of occurrence (as defined in Ribeiro et al., 2006).

Otter ecological requirements, as determined by IUCN OSG (IUCN Otter Specialists Group, 2009) were characterised in each surveyed 1km^2 cell in the flooding area of the artificial lake. These were: (1) availability of prey and feeding areas; (2) availability of resting sites; (3) suitability for breeding areas; (4) availability of corridors for movement and dispersal and (5) accessibility to fresh water. Each variable was categorised using a 1 (minimum) to 5 (maximum) scale and ranked from the worst to the best available environment for the otter – suitability index (Table IV.3.1). The value associated with each requirement was the mean of the corresponding variables for that requirement. Otter ecological requirements and variables were selected and classified according to observers' experience and available literature (Beja, 1992; Ruiz-Olmo et al., 2005; Kruuk, 2006; Pedroso and Santos-Reis, 2006; Ribeiro et al., 2006; Pedroso and Sales-Luís, 2007; Sales-Luís et al., 2007; IUCN Otter Specialists Group, 2009; Basto et al., 2011). Results were then related to otter presence and marking intensity (number of detected otter signs per km).

Table IV.3.1 – Otter requirements, and variables related to each requirement, used in flooding area of the Alqueva reservoir.

Requirement / variable	Variable description	Data categories or units (suitability index)	Source
FOOD	**Availability of prey and feeding areas**	**1 - minimum to 5 - maximum**	
Prey	Abundance of amphibians, crayfish and fish	1–absent / 2–scarce / 3–moderate / 4–high / 5–very high	Field work and maps
Steepness	Bank steepness	1– very steep / 2-steep/ 3– moderately steep / 4–slightly steep / 5–flat	Field work and maps
Typology	Bank typology	1–peninsula / 2–exposed bank / 3-exposed bank with irregular perimeter/4–bay / 5–narrow bay or deep valley	Field work and maps
REST	**Availability of resting sites**	**1 - minimum to 5 - maximum**	
Bank refuges (above flooding area)	Refuge availability: presence of large rocks, logs and other type of refuge structures	1–absent / 2–scarce / 3–moderate / 4–high / 5–very high	Field work
Bank refuges (in flooding area)	Refuge availability : presence of large rocks, logs and other type of refuge structures	1–absent / 2–scarce / 3–moderate / 4–high / 5–very high	Field work
Bank vegetation (above flooding area)	Vegetation availability for otters refuge	1–absent / 2–present but offering no suitable cover / 3 – present in patches, offering scarce cover / 4–present in large patches offering suitable cover / 5-continuous dense vegetation, providing excellent cover	Field work
Bank vegetation (in flooding area)	Vegetation availability for otter refuge	1–absent / 2–present but offering no suitable cover / 3–present in small patches, offering scarce cover / 4–present in large patches offering suitable cover / 5-continuous dense vegetation, providing excellent cover	Field work
BREED	**Suitability for breeding**	**1 - minimum to 5 - maximum**	
Breeding watercourses	Number of watercourses with breeding conditions	1–absent / 2–one / 3–two / 4–three /5–more than three	Field work and maps
Natal holts	Potential for natal holts (rock formations, deep tree roots with holes, thick vegetation systems)	1–absent / 2–scarce / 3–moderate / 4–high / 5–very high	Field work and maps

Requirement / variable	Variable description	Data categories or units (suitability index)	Source
Rearing areas	Potential for rearing areas (protected areas with lower current, very dense vegetation and rich food supply, stable availability of water)	1–absent / 2–scarce / 3–moderate / 4–high / 5–very high	Field workand maps
CORRIDORS	**Availability of corridors for movement and dispersal**	**1 - minimum to 5 - maximum**	
Corridor number s	Number of potential corridors (watercourses)	1–absent / 2–one / 3–two / 4–three /5–more than three	Field work and maps
Corridor type	Type of corridors	1–small stream without water / 2-small stream / 3–stream / 4–river / 5–large river	Field work and maps
Refuge	Refuges/vegetation availability for otters in potential corridors	1–absent / 2- present but offering no suitable cover / 3–present in small patches, offering some cover / 4–present in large patches offering suitable cover / 5-continuous dense vegetation, providing excellent cover	Field work
WATER	**Accessibility to fresh water**	**1 - minimum to 5 - maximum**	
Watercourse number	Number of watercourses	1–absent / 2–one / 3– two / 4–three /5–more than three	Field work and maps
Watercourse type	Type of watercourses (given resistance to drought and carrying capacity)	1–none / 2-small stream / 3–stream / 4–river / 5–large river or reservoir	Field work and maps

Statistical analyses

Differences in otter distribution in the 25 km^2 grid over the years and between seasons were analysed using chi-square tests. To analyse the influence/impact of the different phases on the presence-absence of otter over time, and to account for multiple surveys of the same location, a generalized additive mixed model (GAMM) regression framework was used (Wood, 2006). Presence/absence of otter signs was modelled as a smooth of trimester values, with the smoothness chosen using the default generalized cross validation procedure available in R’s (R Development Core Team, 2011) library mgcv (Wood, 2006). Grid was included as a random effect and the residuals within sites were assumed to follow a 1st order autoregressive model. Spearman correlations were calculated between the five otter requirements rankings and otter presence as well as with marking intensity in each 1 km^2 cells in the flooding area in each sampled trimester. Marking intensity was used as a surrogate of intensity of use and an indication of resources’ defence where sites with more spraints may indicate the site is important to the otter in terms of the habitat and/or resources (e.g. Kruuk, 2006; Sulkava, 2006; Guter et al., 2008). Chi-square tests were further used to compare consumption of prey categories between 2000 and 2006. Since fishing methods used in each year are not directly comparable, results on prey availability will be limited to comparing species presence and relative abundance within each year. Statistical calculations were performed using R software (version 2.14).

Results

Otter distribution

Temporal dynamics of otter presence/absence over the different phases and years of monitoring at the 25 km^2 grid resolution is shown in Figure IV.3.2.

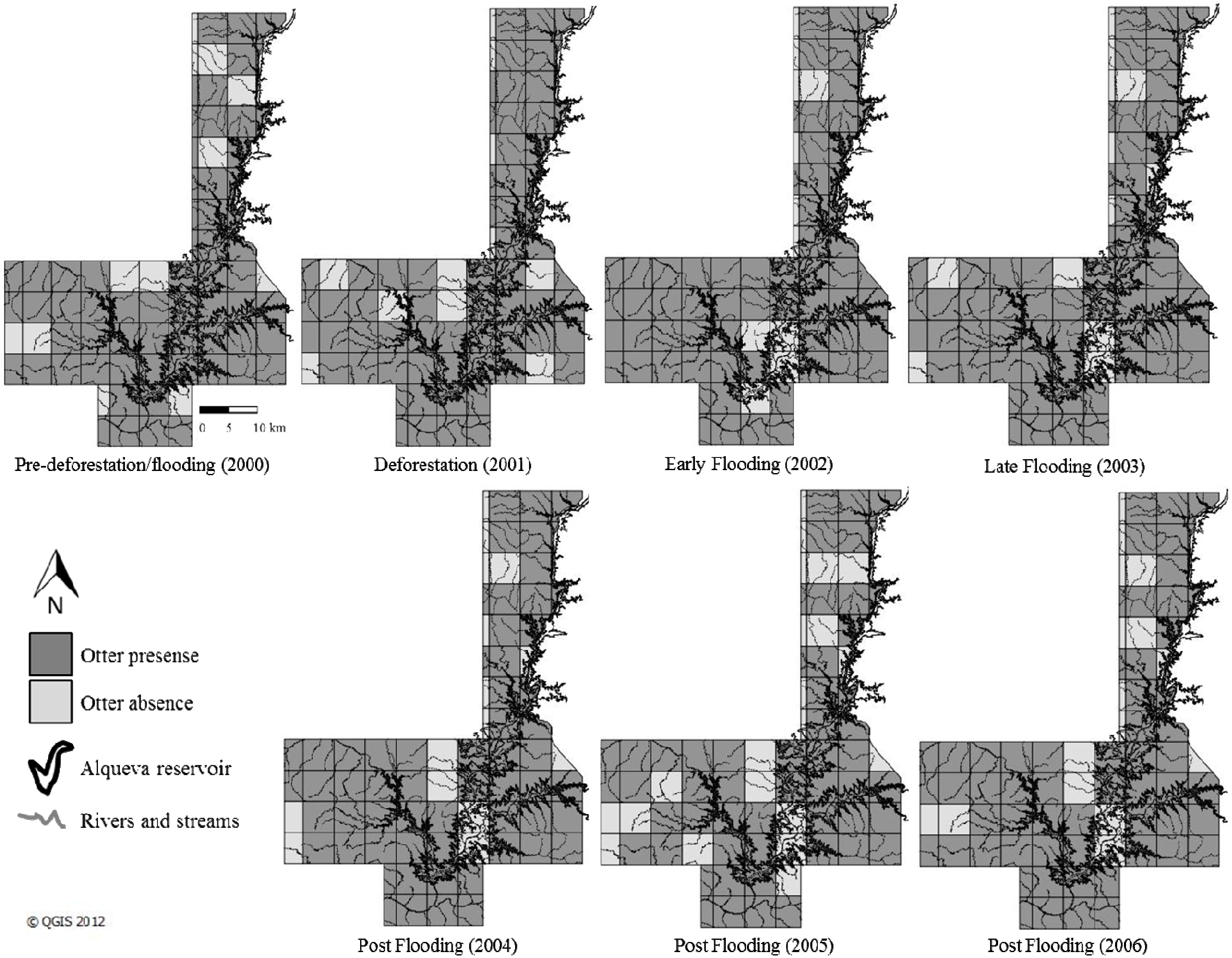

Figure IV.3.2 – Dynamics of otter distribution in the study area in a 25km^2 grid along the different monitoring phases and years.

The percentage of area occupied by the species was fairly stable across seasons within years, with only subtle changes for the last 2 years (and respective seasons) surveyed (Table IV.3.2), all non-significantly different.

Table IV.3.2– Percentage of positive25 km^2 grid cells per season (dry and wet) per year

Year	Dry season (%)	Wet season (%)
2000	84.2	84.2
2001	85.5	85.5
2002	85.5	85.5
2003	84.2	84.2
2004	80.3	80.3
2005	68.4	72.4
2006	76.3	78.9

During all monitoring phases, otter presented a widespread distribution in rivers, streams, small reservoirs and the Alqueva reservoir, always with more than 70% of positive cells. Most negative cells correspond to areas with few or small streams that dry out for long seasonal periods. From 2000 to 2003 otter presence patterns were stable. Otter distribution was continuous throughout the study area but there were local changes from presence to absence and vice-versa. Additionally, in the deforestation phase (2001), three cells were negative despite having apparently adequate streams and/or small reservoirs that could be used by otters. During early flooding (2002) there was a considerable change in the location of negative cells. With the exception of smaller cells and one new negative cell in the north of the study area (also less suitable for otter), all 2001 negative cells were positive in 2002. Also in 2003, there were shifts in negative cells, most of them being, again, less suitable areas for otter. Additionally, two flooding core cells were also negative, a situation maintained throughout the rest of the monitoring. In the post flooding phase (2004 to 2006), although not significantly different, the otter distribution was more restricted than in other phases. Contributing to this are cells with considerable flooded area. Within this phase, 2005 was the year with lower records of otter presence.

Figure IV.3.3 represents the pattern of otter distribution (% of presences) in the 39 1km^2 cells. Otter presence was widespread prior to dam construction (with levels always higher than 82.0%), decreasing during deforestation and flooding phase. Recovery occurred in the post flooding period. The evolution of trimester surveys show major impacts in early deforestation (first trimester of deforestation – 68.5%), with some signs of recovery afterwards, and another major decrease in otter presence in early flooding (first two trimesters after the beginning of flooding – 33.3%). Otter presence showed a recovery in the post flooding phase, although not to the level prior to dam construction (61.5% up to 83.3%). Modelling of presence/absence showed that the smooth term is highly significant (smooth term p-value<10^{-6}) emphasizing that there is a non-linear relation between otter presence and time.

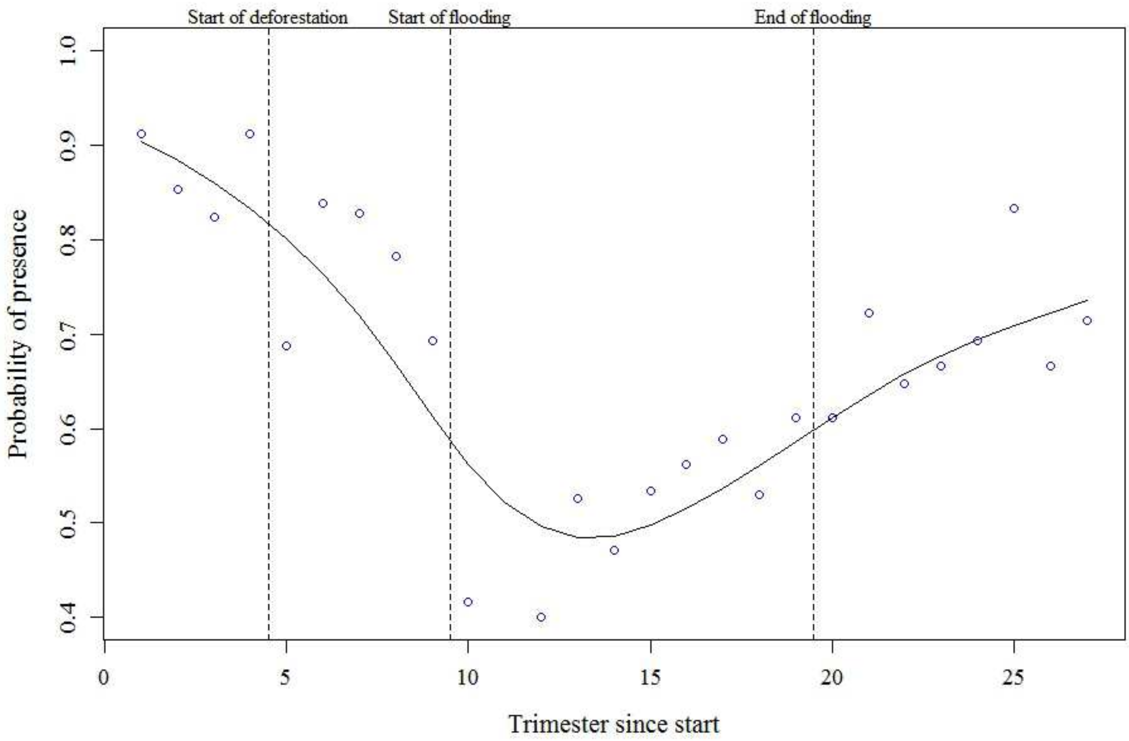

Figure IV.3.3- Probability of otter presence as a function of trimester in the flooding area of the Alqueva reservoir. Data are represented by points and fitted model by a black line.

Otter diet and prey availability

Out of a total of 2579 otter spraints collected (pre-deforestation/flooding phase, year 2000, n=1921; end of post-flooding phase, year 2006, n=658), 858 (2000: n = 675; 2006: n = 183) were analysed resulting in 2579 prey items. Otter diet was dominated by fish and crustaceans (*P. clarkii*) both in pre-deforestation/flooding phase (56.7% and 35.3% of occurrences) and at the end of post-flooding phase (60.7% and 33.2% of occurrences) (Figure IV.3.4). Insects and mammals were only consumed in the streams/rivers. No significant difference was found in the consumption of fish (χ^2 = 0.1363, P = 0.712), crustaceans (χ^2 = 0.0644, P = 0.799), amphibians (χ^2 = 0.142, P = 0.706), reptiles (χ^2 = 0.288, P = 0.591) nor birds (χ^2 = 2.017, P = 0.156) between both phases.

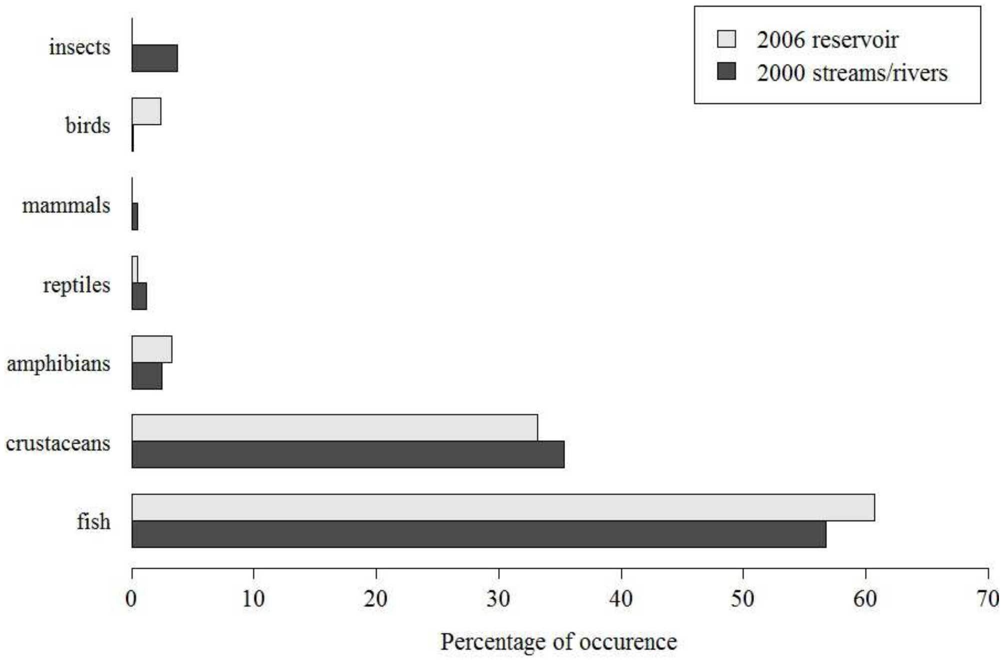

Figure IV.3.4 – Percentage of occurrence (PO) of prey classes in otter diet in the flooding area of the Alqueva reservoir (year 2000 = pre-deforestation/flooding phase; year 2006 = end of post-flooding phase).

Concerning prey availability, in 2000, 17 fish categories were captured in the sampled sites, five of them non-native. *Lepomis gibbosus*, *G. holbrooki* and *S. alburnoides* dominated (total of 77.0% of relative abundance) (Fig. IV.3.5). In 2006, main otter prey captured by fyke-nets and carboy traps in the Alqueva reservoir were fish (89.4%), reptiles (Mediterranean turtle *Mauremys leprosa* – 7.5%) and crustaceans (*P. clarkii* – 3.1%). Regarding fish species, *L. gibbosus* dominated captures in the reservoirs margins and in the reservoir itself. Only four fish species were captured in the margins (three of them non-native), when comparing with the species captured in the reservoir (where at least two barbels species and one nase were captured – all native) (Figure IV.3.5A). Richness of preyed fish decreased with flooding (16 to 8) as well as the ratio of native/non-native fish species (1.7 to 0.3). In 2000, otter diet was dominated by pumpkinseed *Lepomis gibbosus*, barbells *Barbus* sp. and chubs *Squalius* sp. By 2006 otter fish diet was dominated by *L. gibbosus*, mosquito fish *Gambusia holbrooki* and largemouth bass *Micropterus salmoides*, all non-native species. Calandino *Squalius alburnoides*, southern Iberian chub *Squalius pyrenaicus*, Iberian long-snout barbel *Barbus comizo*, Iberian small-head barbel *Barbus microcephalus*, Iberian arched-mouth nase *Iberochondrostoma lemming*, Iberian straight-mouth nase *Pseudochondrostoma willkommii*, Southern Iberian spined-loach *Cobitis paludica*, chameleon cichlid *Australoheros facetus* and freshwater blenny *Salaria fluviatilis* were not consumed in the reservoir. With the post flooding appearance of the black bullhead

Ameiurus melas it also became important in otter diet (Figure IV.3.5.B). Significant differences were found in consumption of *Barbus sclateri/steindachneri* (χ^2 = 5.9, P = 0.015) and *G. holbrooki* (χ^2 = 9.3, P = 0.002).

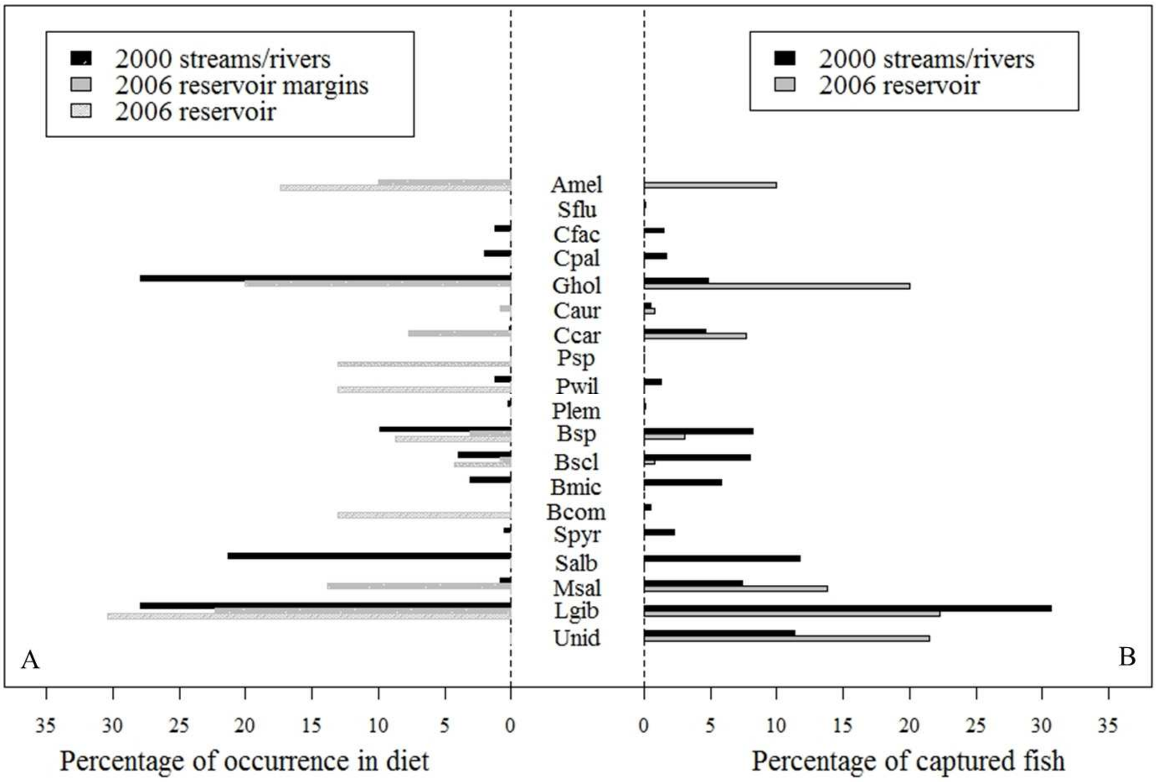

Figure IV.3.5.A) Percentage of occurrence (PO) of fish species in otter diet in the flooding area of the Alqueva reservoir (year 2000 = pre-deforestation/flooding phase; year 2006 = end of post-flooding phase). Non-native (nn) species indicated. B) Fish species captured in 2000 by electro-fishing in streams and rivers of the future flooding area of the Alqueva reservoir; in 2006 by fyke-nets and carboy traps in the Alqueva reservoir margins, by trammel nets in the Alqueva reservoir (relative abundance); Amel – *Ameiuras melas* ; Sflu – *Salaria fluviatilis*; Afac – *Australoheros facetus*; Cpal – *Cobitis paludica*; Ghol - *Gambusia holbrooki*; Caur – *Carassius auratus*; Ccar - *Cyprinus carpio*; Psp - *Pseudochondrostoma* sp.; Pwil – *Pseudochondrostoma willkommii*; Ilem – *Iberochondrostoma lemmingi*; Bsp - *Barbus* sp.; Bscl – *Barbus sclateri/steindachneri*; Bmic – *Barbus microcephalus*; Bcom – *Barbus comizo*; Spyr - *Squalius pyrenaicus*; Salb – *Squalius alburnoides*; Msal – *Micropterus salmoides*; Lgig – *Lepomis gibbosus*; Unid - Unidentified;

Otter ecological requirements

The evolution of suitability indexes of otter ecological requirements in the flooding area showed a clear decrease after deforestation and flooding and a slight recovery in the post-dam situation (Figure IV.3.6).

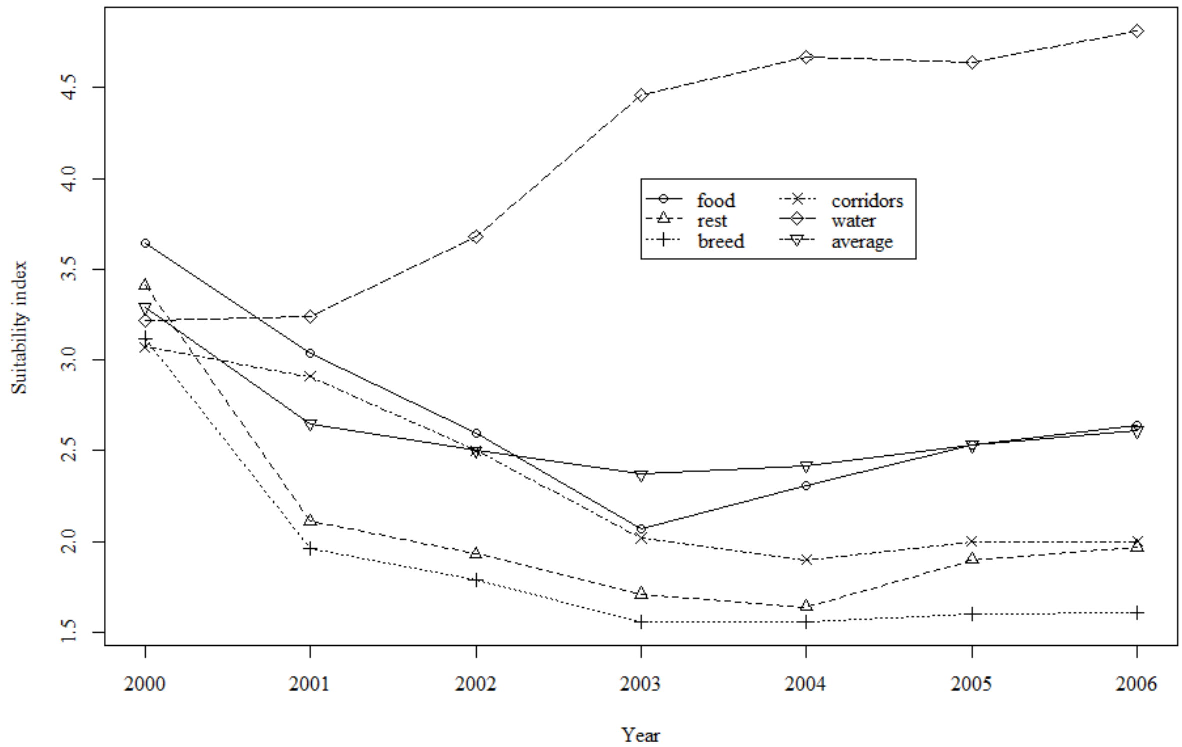

Figure IV.3.6 – Yearly average of suitability index of otter ecological requirements from 2000 (pre-deforestation/flooding) to 2006 (post-flooding) in the flooding area of the Alqueva reservoir.

The only otter requirement that increased was the availability to fresh water, hardly a surprise given the presence of the new reservoir itself. The average value of the other requirements decreased from 2000 to 2006.

Table IV.3.3 shows the correlations between both otter presence and marking intensity, with otter requirements. Marking behaviour was consistently correlated with several otter requirements through time, although there were changes in the degree of correlation and the type of requirement. Significant negative correlations were only found between water availability and both otter presence and marking intensity.

Table IV.3.3 - Correlations between otter presence and marking intensity in the flooding area of the Alqueva reservoir and otter requirements throughout the dam implementation phases (cc – Spearman correlation coefficient; P – significance).

Monitoring phase		**Otter requirements**				
Pre-deforestation/flooding – 2000		**food**	**rest**	**breed**	**corridors**	**water**
Otter presence	Cc	0.109	0.106	0.033	0.014	0.072
	P	0.205	0.220	0.701	0.868	0.403
Marking behaviour	Cc	0.708**	0.753**	0.736**	0.874**	0.483**
	P	0.000	0.000	0.000	0.000	0.009
Deforestation - 2001		**food**	**rest**	**breed**	**corridors**	**water**
Otter presence	Cc	0.056	0.288	0.225	0.277	0.096
	P	0.017	0.715	0.055	0.136	0.065
Marking behaviour	Cc	0.373*	0.488**	0.351*	0.562**	0.190
	P	0.013	0.001	0.019	0.000	0.216
Early flooding - 2002		**food**	**rest**	**breed**	**corridors**	**water**
Otter presence	Cc	-0.166	0.034	-0.009	-0.051	-0.278**
	P	0.069	0.715	0.920	0.582	0.002
Marking behaviour	Cc	0.673**	0.451**	0.535**	0.570**	-0.153
	P	0.000	0.000	0.000	0.000	0.094
Late flooding - 2003		**food**	**rest**	**breed**	**corridors**	**water**
Otter presence	Cc	0.283*	0.165	0.016	0.048	0.009
	P	0.010	0.139	0.889	0.667	0.937
Marking behaviour	Cc	0.387**	0.201	0.262*	0.297**	-0.220*
	P	0.000	0.070	0.017	0.007	0.047
Post flooding – 2004		**food**	**Rest**	**breed**	**corridors**	**water**
Otter presence	Cc	0.032	-0.019	0.016	-0.136	-0.037
	P	0.772	0.864	0.886	0.218	0.735
Marking behaviour	Cc	0.206	0.407**	0.364**	0.458**	-0.348**
	P	0.061	0.000	0.001	0.000	0.001
Post flooding – 2005		**food**	**rest**	**breed**	**corridors**	**water**
Otter presence	Cc	0.036	0.242*	0.115	0.243*	-0.227*
	P	0.759	0.034	0.321	0.032	0.047
Marking behaviour	Cc	0.243*	0.234*	0.345**	0.372**	-0.373**
	P	0.034	0.041	0.002	0.001	0.001
Post flooding – 2006		**food**	**rest**	**breed**	**corridors**	**water**
Otter presence	Cc	-0.029	0.221	0.067	0.072	0.027
	P	0.820	0.080	0.599	0.574	0.830
Marking behaviour	Cc	-0.085	0.365**	0.354**	0.376**	-0.141
	P	0.505	0.003	0.004	0.002	0.265

Requirements: food - availability of prey and feeding areas ; rest - availability of resting sites; breed - suitability for breeding areas; corridors - availability of corridors for movement and dispersal; water - accessibility to fresh water

**highly significant (P < 0.001); *significant (P < 0.05)

Discussion

Our study showed that during dam construction and implementation, the otter had a widespread distribution during all the monitoring phases. This was consistent at both scales of analysis. At the $25km^2$ cell grid scale the percentage of positive cells for otter presence in the surrounding area of the flooding area was always similar but the location of negative cells shifted in the different years/phases. Most absences reflected areas of less suitable conditions for otters. Otter distribution also reflected the disturbance cause in the deforestation phase, since cells that included large areas of deforestation activities were also negative. The results showed that deforestation, and specially flooding, forced otters to look for territories outside the main river and streams. However otters did not settle there permanently. In the post-flooding phase, 2005 was the year with the highest absence of otters, reflecting the extreme drought of that year. Results at the $1km^2$ cell scale showed that the first months of deforestation caused a major decrease in otter presence in the flooding area. This corresponds to the period of high degree of disturbance, caused by the machinery and manpower used to cut the vegetation from the watercourses. Although otter presence has recovered somewhat immediately after, the complete lack of vegetation in the watercourses probably hindered otter resettlement. The effect of flooding, the abrupt loss of rivers and streams, was felt very strongly in the first six months. Otter presence was fairly stable after the water level stabilised, with some signs of recovery in the surveyed sites, although not reaching the level prior to dam construction.

Our analysis showed that the otter’s response to changes created by the dam implementation was even clearer in their diet, reflecting major shifts both in abundance and composition of prey communities. There was a significant prey switch from a native to a non-native fish and crustacean (*P. clarkii*) dominated diet. Most native fish species were more, or in most cases only, caught and preyed in the streams/rivers system (2000). These are species more adapted to a lotic environment and, when occurring in lentic systems, occur in very low abundances and/or mostly the middle of the reservoirs. This makes them less available for otters, which usually forage in more shallow waters near the reservoir margins (Sales-Luís et al., 2007). Reservoirs provide stable lentic habitats in which non-native species can develop thriving populations (Clavero et al., 2004), for they are largely lentic in their native range (Filipe et al., 2004; Ribeiro et al., 2008). A previous study by Ribeiro et al. (2006), also in the Alqueva dam, found that 95.0% of the captured fish were non-native. These authors also found an impressive increase in abundance and distribution of *A. melas* after the flooding. Our data similarly show an increased importance of *A. melas* after the flooding, both in the fish community and as otter prey. The otter is an opportunistic forager and its diet normally reflects the most abundant prey available (Kruuk et al., 1993; Copp and Roche, 2003).

The change in the composition of the fish community is perhaps the most substantial impact of dam construction on otter ecology. Non-native species generally have a lower biomass then native species, as is the case of *L. gibbosus* and *G. holbrooki* (the most consumed fish in 2006). This means that the otter must capture a larger number of individuals to meet the same biomass requirements. Sales-Luís et al. (2007), in a study in a large reservoir in central Portugal, found a preference for larger length classes of *L. gibbosus*, reflecting greater energetic profits. Also in the reservoir the otter diet becomes less diverse in terms of fish species, therefore more dependent on fewer species.

P. clarkii was the single most consumed species by the otter. Basto et al. (2011) showed that the abundance of this crayfish was one of the most important variables positively associated with otter use of small and medium-sized reservoirs. Other studies in Mediterranean areas also showed the importance of *P. clarkii* as prey for otters (Magalhães et al., 2002; Clavero et al., 2004; Pedroso and Santos-Reis, 2006). *P. clarkii* represents an important food resource especially in stressful periods of severe drought, and is likely to have increased the carrying capacity of the environment for aquatic predators such as the otter.

In parallel to changes in diet, our results also illustrate a change in otter requirements over time, consistently correlated with otter marking behaviour. Otter usually forage in shallow waters (e.g. Kruuk, 2006), and in the reservoir, therefore, prey capture efficiency can be expected to decrease. This can affect not only the size of home ranges but also lead to the absence of otter in less adequate foraging areas. This is confirmed by the highly significant correlation between availability of prey and feeding areas, and otter marking behaviour in the years of the flooding, and a significant correlation with otter presence in the late flooding. In the post-flooding phase, this relation does not exist or is less important, reflecting the increase in prey availability.

Otters are less selective regarding resting sites than for breeding areas, and they may use several in their home range (Jiménez et al., 1998). However, the availability of both became restricted in the Alqueva reservoir. Refuges like large rocks, logs and other type of refuge structures are few. Substantial vegetation cover is present almost only in small bays of the reservoir, whilst most of the banks are dominated mostly by new aquatic vegetation (helophyte species, e.g. *Juncus* spp) which do not offer refuge for otters. Both marking behaviour and otter presence in the post flooding phase reflected that otter post-impact colonization of the reservoir is significantly based on areas with more refuge and cover. Melquist and Hornocker (1983) suggested that the availability of adequate escape cover and shelter were reasons for Canadian otters preferring streams over lakes, reservoirs and ponds.

Prior to dam implementation, evidence of otter breeding was found in the Guadiana river and adjacent streams (Saavedra, 2002). After dam implementation no indication of cub or juvenile presence was found in the reservoir margins. Otter dens, particularly natal ones, are difficult to find without radio-tracking and often there is no evidence of their presence, such as spraints (Moorhouse, 1988). Some studies in the Iberian Peninsula showed that den locations were restricted to rock formations, deep tree roots with holes, bankside vegetation, tangled vegetation carried downstream by floods, and helophytic vegetation systems (Ruiz-Olmo, 1995; Jiménez and Palomo, 1998; Ruiz-Olmo et al., 2005). The flooding caused the disappearance of previously existent valleys of rivers and streams, along with potential natal holts. In the reservoir margins, refuges or potential dens are now restricted to small rocky agglomerates, all quite exposed. Rearing areas where cubs stay after having moved from the breeding areas are also important. Permanent access to stable water levels and undisturbed areas play an important role in determining good rearing areas were small cubs can safely learn to swim (Kruuk, 2006). Ruiz-Olmo et al. (2005) also found female otters rearing small cubs in selected stretches with high food availability, with suitable dens and deeper and calm water. Although there are several undisturbed areas in the Alqueva reservoir, these do not sustain high concentration of prey populations, or suitable refuges. Conditions for breeding were correlated with marking behaviour in all of the years, reflecting a lack of appropriate areas. Breeding may be the otter requirement most affected after dam implementation.

Foster-Turley et al. (1990) and Ruiz-Olmo (2001) have argued that the physical characteristics of dams have negatively influenced the distribution of otters in Europe. Alqueva's dam wall is 96m high and its insertion in the Guadiana river valley effectively cuts movements of otter individuals, isolating upstream and downstream otter populations. After flooding, otter movements were limited within the reservoir and the adjacent watercourses. Smaller streams constitute movement corridors between suitable habitats such as larger rivers, or they are themselves suitable habitats. Pedroso et al (submitted) demonstrated that streams flowing in and out of reservoirs were one of the key drivers for otter presence and use of large reservoirs. A similar conjoint system of reservoir-streams was found in the centre of Portugal (Aguieira dam), with streams already identified as important otter refuge areas and even possible (and main) breeding areas (Pedroso et al., 2007; Sales-Luís et al., 2007). We found significant correlations between the presence of corridor structures and otter marking behaviour, and also otter presence in a late phase of the monitoring. This shows that these corridors are a determinant aspect for otter presence and colonization of the reservoir.

Finally, permanent accessibility to fresh water was the only otter requirement that increased after dam implementation. This is one of the most important factors influencing the occurrence of otters in dry areas in summer (e.g. Beja, 1992; Prenda et al., 2001). With the construction of the dam, a permanent water source is available for the otter all year long. However it is clear that the flooding also had a negative impact (negative correlation both marking behaviour, in the late flooding and post-flooding phases, and otter presence in post-flooding, when the impact of the flooding was more evident).

Implications for otter conservation

Suitable habitats for otters are connected aquatic environments with high prey abundance, dense bankside vegetation cover, shelters and breeding dens, and foraging grounds that are easy-to-find and use (e.g. Macdonald and Mason, 1982; Ruiz-Olmo et al., 2005). With the exception of the availability of fresh water, all those other otter ecological requirements are less suitable after the construction and implementation of the Alqueva dam. Breeding, feeding, and resting areas are critical for a species (e.g., Fernández and Palomares, 2000; Kruuk, 2006). Specifically in recently constructed reservoirs, when the vegetation is non-existent and fish populations still have not had the time to colonize, reproduction and breeding may be limited or not existent, as breeding is a time of high energy requirements for otters.

Nevertheless, this reflects an impact on otter distribution that may not be a serious setback for otter conservation in areas where otter populations thrive. The favourable otter population situation found in the Guadiana river basin in Southern Portugal (Saavedra, 2002) may have been the key to the otter colonization of less adequate habitat areas like the Alqueva large reservoir, since the streams surrounding it are well-occupied. This also means that in areas were the otter is present at low density this scenario may be quite different.

We should keep in mind that otter presence in reservoirs of already existing dams is not sufficient to conclude that such infrastructures offer equal opportunities to otter populations compared with the previous network of rivers and streams. The Alqueva Dam was implemented along a stretch of a large river (Guadiana River) in a confluence area with two other large streams (Degebe and Alcarrache). These, before impounded, were able to sustain high-density otter populations. In large reservoirs, the entire perimeter is seldom used regularly, and the areas of highest use are those near streams, suggesting a complementary use (Sales-Luís et al., 2007; Pedroso et al., submitted). We suggest that the carrying capacity for otters in the Alqueva reservoir is now lower than that in the area previous to its construction.

Dam construction and water development projects create wide-ranging social and environmental consequences, with impacts extending well beyond the initial planning area.

Ecologists and environmentalists are often challenged by the complex interaction of forces at work in these environments, making prediction of overall effects difficult (Maingi and Marsh, 2002). Our results emphasize how important are long term monitoring studies that include several phases of construction, post construction and flooding, to truly evaluate species response to impacts, sometimes beyond the mandatory framework of Environmental Impact Assessment. Such studies will enable better planning of mitigation and compensation measures, such as protection of riparian vegetation in streams near to the reservoir, promotion of vegetation in the margins of the reservoir, protection of islands in the reservoir as refuges of the otter, promotion of inlets/bays to improve foraging efficiency, and the protection of rocky formations and possible rearing areas for otter.

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PART V – OTTERS AS POTENTIAL VECTORS OF PATHOGENIC BACTERIA

V.1. Evidence of antimicrobial resistance in Eurasian otter (*Lutra lutra* Linnaeus, 1758) fecal bacteria in Portugal

PAPER 5

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Evidence of antimicrobial resistance in Eurasian otter (*Lutra Lutra* Linnaeus, 1758) fecal bacteria in Portugal

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Abstract

Bacterial antimicrobial resistance is a well recognized problem for human and animal health. Evaluation of antimicrobial resistance in enteric microbiota is a powerful tool to monitor selective pressure from drugs used for treating animal and human infectious diseases, or from compounds used in farming practices for prophylaxis. Screening antibiotic susceptibility patterns of wildlife animal isolates provides useful information on environmental contamination by resistant strains, deriving from contaminated effluents or from free-ranging animals that are potential vectors of resistance determinants.

Recently, we examined the antimicrobial resistance of *Escherichia coli* (n=7) and *Enterococcus* spp. (n=26) isolates obtained from 35 fecal samples from Eurasian otter (*Lutra lutra* Linnaeus, 1758) free-living in Pego do Altar and Monte Novo reservoirs and associated river stretches in Alentejo region, South Portugal. The 12 antimicrobials, tested as recommended in the Clinical and Laboratory Standards Institute guidelines, belonged to different antimicrobial drug classes, acting through inhibition of transduction (n = 6), cell wall synthesis (n = 4), DNA gyrase (n = 1) and folate synthesis (n = 1).

Levels of resistance were different for the two bacterial genera considered. All *E. coli* isolates were susceptible to 5 of the antimicrobials tested, while none of *Enterococcus* spp. isolates was susceptible to all compounds. All enterococci were resistant to cephalexin, cefotaxime, enrofloxacin and streptomicin. With exception of one *E. coli* isolate, all bacteria presented a multiresistant profile, being resistant to more than one antimicrobial drug class.

The animals sampled were not likely to have been subject to antibiotherapy. Therefore, low resistance levels were expected, since antimicrobial treatment exposure is still considered the major reason for emergence, selection, and dissemination of resistant bacteria. The multiresistant profile found in most isolates supports the hypothesis that environmental exposure of intestinal microbiota to antimicrobial agents may select for resistant bacterial

strains, but the occurrence of point mutations or acquisition of transmissible mobile DNA elements responsible for antimicrobial resistance must also be taken into consideration.

The antimicrobial resistance profile of *E. coli* and *Enterococcus* spp. isolates from otters' fecal samples may provide useful information to assess the potential of antimicrobial resistance transmission from the environment contaminated by humans and domestic and wild animals, being particularly relevant in dams and rivers where human activities, such as farming or outdoor recreational activities, occur near or in the water. Resistance profiles should be taken upon consideration in future plans regarding management and conservation of otters, particularly in environments where cattle density near aquatic systems is high.

Introduction

Eurasian otters (*Lutra lutra* Linnaeus, 1758) are medium size mustelids, with approximately 60 to 90 cm long, 6 to10 kg weight, short legs and a fusiform body ending in a long tail, usually 35 to 47 cm long. These free-range otters have interdigital membranes that allow them to actively swim and forage in water. They are territorial, solitary and nocturnal animals. They breed all year, although births of one to five cubs are most common in spring, after a two months gestation. They may live up to 9 years in the wild and up to 12 years in captivity. They eat mainly fish, but their diet may also include crustaceans, amphibians, birds and small rodents (Carnivora, 2008).

Eurasian otters are classified as "Near Threatened" mammals. In Europe, they are one of the main animal species under protection, and their capture and habitat destruction are interdicted by the Berna and Washington Conventions (IUCN, 2006). Their distribution extended across Europe and Asia, but after the 1950s it declined in Western Europe, and otters became absent from large areas of their former range (Mason and MacDonald, 1986; Foster-Turley et al., 1990). There has been a species recovery in most of Europe due to natural conditions, conservation projects and reintroduction programs (Cortés et al., 1998; Kranz and Toman, 2000; IUCN, 2006).

Otters can be found in a wide variety of aquatic habitats, including highland and lowland lakes, rivers, streams, marshes, swamp forests and coastal areas. They are easily adaptable, being able to live in salt and freshwater habitats and in sewerage systems from urban areas. Generally, otter distribution is correlated with the presence of freshwater and bank side vegetation (Reuther and Hilton-Taylor, 2004), and their habitats are extremely vulnerable to man-made changes. Populations' densities are affected by rivers canalisation, removal of bank side vegetation, dam construction, wetlands draining, aquaculture activities and associated man-made impacts on aquatic systems. Pollution is a major threat to otters in western and central Europe, where the main pollutants are the organochlorines dieldrin and DDT/DDE, polychlorinated biphenyls and mercury, since rivers and lakes acidification results in the decline of fish biomass, reducing the otter's food resources. This reduction can also be due to organic pollution from nitrate fertilisers, untreated sewage, or farm slurry (Reuther and Hilton-Taylor, 2004). Other major causes of otters' mortality are drowning, due to the presence of fyke nets set for eels or fish as well as creels set for marine crustaceans, road kills, and poaching.

Contrasting the apparent pattern of decline in Eurasian otter populations until the 1990s (Mason and MacDonald, 1986; Foster-Turley et al., 1990; MacDonald and Mason, 1994; Romanowski et al., 1997; Cortés et al., 1998; Ruiz-Olmo and Delibes, 1998; Kranz and Toman, 2000; Conroy and Chanin, 2002), otters in Portugal have always been considered one of the most viable populations from Europe (Macdonald and Mason, 1982; Santos-Reis, 1983; Mason and MacDonald, 1986; Santos-Reis et al., 1995; Trindade et al., 1998). Although not well known, otter densities are suspected to be high in Portugal (Santos-Reis et al., 2003). They are distributed throughout the country, inhabiting all types of river and wetland systems, including coastal areas (Beja, 1995; Santos-Reis et al., 1995; Trindade et al., 1998), which may result from the large diversity of preys' and favourable habitat found (MacDonald and Mason, 1982; Farinha and Trindade, 1994; Santos-Reis et al., 1995). Some populations cross the boundary between Portugal and Spain.

In Portugal, otters' status was recently changed from "Insufficiently Known" to "Least Concerned" (Cabral et al., 2005). Otters' conservation in this country is a vital issue and their conservation is mandatory by the "Habitats" Directive and Natura 2000 Network, since populations are stable, representing a biodiversity "hotspot".

Dams can adversely influence Eurasian otters' distribution and are frequently suggested as a contributing factor to the decline of this species in Europe (MacDonald and Mason, 1984; Ruiz-Olmo, 2001). However, otters can use these man-made habitats under some circumstances, such as the ones occurring in Portugal. The favorable status of otter populations in Portugal may lead them to occupy, especially during the dry season, habitats like dams, which are suboptimal in terms of refuge but offer profitable prey (Pedroso and Santos Reis, 2006). Sales-Luís et al. (2007) suggested that otters using tributaries near dams feed predominantly in the dam, and results by these authors indicate that otter populations make use of these conjoint systems to ensure their survival. These infra-structures offer plenty of water and suitable preys, attracting otters during the recurrent periods of dryness suffered by Mediterranean water systems (Prenda et al., 2001; Pedroso and Santos Reis, 2006). In addition, dams are used by humans for recreational activities, like bathing, camping and water sports.

The accurate determination of otter populations is extremely difficult, and capture, telemetry and other invasive methods are being questioned. Studies can rely on direct observation, but this method only allows to determine otters numbers in specific habitats and regions where the animals are diurnal (Kruuk, 1995). Direct observation requires experienced observers and special habitat features (Ruiz-Olmo et al., 2001), which is also true for quantification methods based on footprints identification. The urge for information regarding genetic, demographic and life-history data from this species made the development of molecular identification methods essential (Kohn and Wayne, 1997; Kruuk, 2006). Individual identification can now be performed by fecal DNA analysis obtained from fresh scats collected from surveys conducted at sunrise in consecutive days, which allows the determination of otter's abundance and territory range (Dallas et al., 2003; Arrendall et al., 2004; Selkoe and Toonen, 2006).

Regarding river-otters health status, these animals can be suffer from viral, bacterial, fungal and parasitic diseases (Kimber and Kollias II, 2000a,b), but valid microflora characterization studies are scarce and little is known about the clinical significance of isolates and environmental contaminant-related diseases, or about their potential role as reservoirs of antimicrobial resistant bacteria.

There are several studies that describe the difficulties experienced in the treatment of human-related infections promoted by antimicrobial resistant bacteria (Monroe and Polk, 2000). It has also been observed that human and animal infections are increasingly related, making studies on identification of animal and environmental reservoirs of antimicrobial resistant bacteria a matter of public health importance (Cole et al., 2005; Sayah et al., 2005).

Until recently, it was assumed that the emergence, selection, and dissemination of antimicrobial resistant bacteria were mainly caused by selective pressure related to antibiotic misuse and abuse (Monroe and Polk, 2000; Sayah et al., 2005). Antimicrobial resistance characteristics may be due to intrinsic mechanisms that promote the inactivation or modification of antimicrobial compounds, the decrease of bacterial cell wall or membrane permeability, the activation of efflux pump mechanisms that actively expel antimicrobials, or the modification of target receptors (Sayah et al., 2005). Resistance characteristics may also be explained by the ability of expression of acquired resistance mechanisms that some bacterial strains demonstrate. Examples of these mechanisms comprise the occurrence of chromosomal gene mutations or the expression of resistance genes present in plasmids, transposons, bacteriophages or other mobile elements aquired from other bacteria. These elements are known to be responsible for the transfer of multiresistant profiles, characterized by the phenotypic resistance to more than one antimicrobial drug class (Sayah et al., 2005).

Antimicrobial resistance characteristics presented by the normal enteric microbiota can be used to infer on the extent of selective pressure caused by antimicrobial use in human and animal populations (SVRAM, 2007). Commensal intestinal bacteria, such as *Escherichia coli* and *Enterococcus* spp., may act as reservoirs for resistance genes and are able to disseminate bacterial resistant determinants to animal or human pathogens (Skurnik et al., 2006; SVRAM, 2007). Therefore, the evaluation of antimicrobial resistance profiles of bacteria belonging to the normal enteric microbiota of healthy animals can be used as an indicator of antimicrobial resistance dispersion in the environment (SVRAM, 2007).

E. coli is a facultative anaerobic non-spore forming Gram-negative bacteria. Their pleomorphic rods are often mobile by peritrichous flagella (Bettelheim, 1992; ICMSF, 1996). This commensal bacterium is commonly found in the digestive tract of humans and warm blood animals, being isolated from feces from humans and domestic and wild animals (Bettelheim, 1992; ICMSF, 1996). *E. coli* is mainly transmitted by fecal-oral route, but dissemination through contaminated water has also been described (Bettelheim, 1992; Blackburn and McCarthy, 2000; Bertin et al., 2001). In fact, since *E. coli* represents 1% of the total fecal microbiota of humans and warm blood animals, it is likely for sewers to always contain high levels of this microorganism.

E. coli may present stable plasmids that can harbor antimicrobial resistance genes or other virulence factors (Bettelheim, 1992; Kimura et al., 2000). The ability of this microorganism to survive in adverse conditions facilitates the potential transmission of antimicrobial resistance genes or other virulence factors (Paton and Paton, 1998; Blackburn and McCarthy, 2000; Natvig et al., 2002).

Enterococci are facultative anaerobes and catalase-negative Gram-positive bacteria, with a coccoid shape, and presenting the Lancefield D group antigen (Sneath et al., 1986; Giraffa, 2002, 2003; Klein, 2003). They are also commensal microrganisms, representing a major percentage of these bacteria associated with the gastrointestinal tract of mammals (Aarestrup et al., 2000; Giraffa, 2002, 2003; Klein, 2003). After fecal elimination to the environment, they may colonize several ecosystems due to their exceptional capacity to resist and multiply in hostile environments. Consequently, enterococci are not only associated to warm blood animals, but also to soils, water and vegetables (Giraffa, 2002, 2003; Klein, 2003). Nevertheless, it should be noticed that enterococci distribution varies according to the species, due to host specificity (Vancanneyt et al., 2002). E. fecalis usually occurs in human intestines, although it can also be found in other mammals feces. On the other hand, *E. faecium* and *E. durans* are more common in swine feces, and the last one can also be found in birds (Sneath et al., 1986; Giraffa, 2002; Klein, 2003).

Generally these microorganisms present low virulence, but the emergence of *E. faecium* and *E. fecalis* strains resistant to antimicrobial compounds is becoming a serious problem. This resistance can be intrinsic or acquired, by mutation or by transfer of mobile elements such as plasmids or transposons (Sneath et al., 1986; Van den Boggard et al., 1997; Jensen, 1998; Jensen et al., 1998; Aarestrup et al., 2000; Borgen et al., 2000; Aarestrup et al., 2002; Vancanneyt et al., 2002; Van den Bogaard et al., 2002; Domig et al., 2003). Epidemiologic studies indicate that *E. faecium* constitutes a major threat in terms of antimicrobial resistance (Knudtson and Hartman, 1992; Giraffa, 2002).

These are several studies that describe the occurrence and distribution of antimicrobial resistant bacteria isolated from domestic and farm animals, but little is known about the presence of antimicrobial resistant determinants in the microbiota of wild animals (Caprioli et al., 1991; Sayah et al., 2005). This information is extremely relevant to establish the accurate prevalence and distribution of antimicrobial resistant determinants, and to evaluate the potential role of wild animals in environmental contamination with resistant bacterial strains (Cole et al., 2005).

In this work we have shown the presence of resistant *Enterococcus* spp. and *E. coli* in fecal samples of free-ranging Portuguese otters. Bacteria were isolated from stool swabs collected in September 2007 and March 2008, from the river and reservoir stretches from Pego do Altar and Monte Novo, two dams located in Alentejo region, South Portugal. Bacterial isolates were identified on the basis of their morphology and metabolic pathways, and their antimicrobial resistance profile regarding 12 antimicrobial drugs was evaluated by the standard disk diffusion method, according to the CLSI guidelines.

Material and Methods

Study Area

The study was conducted in two large dams and associated river stretches, located in Alentejo region, in the South of Portugal. Pego do Altar Dam is located in the Sado River Basin and Monte Novo Dam is located in the Guadiana River Basin (Figure V.1.1).

Guadiana and Sado rivers are two of the most important rivers in Portugal. Alentejo region is characterized by its vast plains and a relatively uniform climate. However, because of the sea influence, aridity increases towards the southeast, with higher temperatures and lower air humidity. Annual precipitation ranges from 800mm in the north and west to less than 500mm in the southeast. The landscape, typically Mediterranean, is characterized by large cereal fields and holm/cork oak woods, with cork oaks (*Quercus suber*) dominating in the west and holm oaks (*Quercus faginea*) in the east. Considerable areas of pine (*Pinus* spp.) and eucalyptus (*Eucalyptus globules*) trees can also be found. Many of the small streams in Alentejo do not have a permanent water regime, drying up partially or completely in summer.

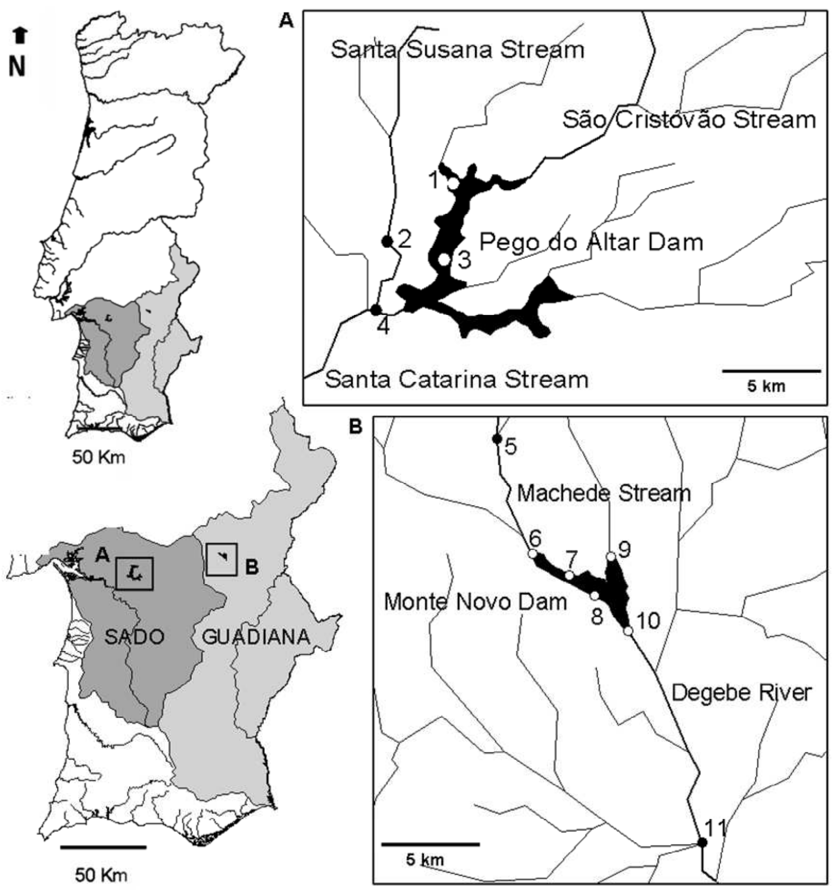

Figure V.1.1– Location of the sampled stretches in Sado (A) and Guadiana (B) Basins in South Portugal: 1 - Pego do Altar Dam (north) 2 - Santa Susana Stream (north); 3 - Pego do Altar Dam (center); 4 - Santa Susana Stream (south); 5 – Machede Stream (upstream); 6 - Machede Stream (near dam); 7 - Monte Novo Dam (west); 8 - Monte Novo Dam (center); 9 - Monte Novo Dam (North); 10 - Monte Novo Dam (dam wall); 11- Degebe River (Downstream).

In general, Alentejo basins are located in semi-dry regions were the lack of water availability promoted an increased human intervention in the water courses, namely with construction of water reserves for human and agriculture consumption. As a result, aquatic ecosystems have been suffering changes that endanger fauna and flora, specially the aquatic species, such as autochthonous fish species. Pego do Altar Dam is the oldest dam (1949), with a volume of 37100 m^3 and Monte Novo Dam the more recent one (1982), with a volume of 31 000 m^3. Both dams were essentially created for irrigation and water reserve. In general, water quality in both basins in degraded, but it hasn't been established yet if this characteristic is associated with intense local pollution of urban and agro-industrial origin or with diffuse pollution sources, mainly caused by agricultural practices (Ferreira et al., 2004; INAG, 2007).

Scat Sampling

Otters are free-living and mostly nocturnal. To assure the collection of fresh scats that were deposited by the otter within less than 15h, surveys were conducted at sunrise, for seven consecutive days. Scat collection in Pego do Altar Dam and adjacent river-stretches was conducted in September 2007, and in Monte Novo Dam and adjacent river-stretches was performed in March 2008. Otter scats are easily recognizable to the trained eye and are usually deposited by the otter in prominent places as marking activity for territory defense. The surveyed streams included common otter deposition sites, in order to increase the probability of collecting fresh scats. Survey length in each sampling site was 600 m (Pedroso et al., 2007). Scats collected at one sampling site may be deposited by the same individual, but scats from different sampling sites probably are not, since sampling sites are distant.

In Pego do Altar Dam were selected for survey seven transects in streams adjacent to the dam (São Cristovão, Santa Catarina and Santa Susana streams) and four dam transects. In Monte Novo Dam were surveyed six transects in streams adjacent to the dam (three in Machede Stream and three in Degebe River) and four dam transects.

Thirty-five scats were collected and processed for microflora analysis and antimicrobial resistance determination (Table V.1.1). For each scat sample analysed, an AMIES swab (FL medical) was immediately performed for bacteria identification, and kept refrigerated until further processing.

Table V.1.1 **-** Fecal otter (*Lutra lutra*) samples collected at surveyed sampling sites of Pego do Altar and Monte Novo Dams and adjacent streams.

Sample Nº	**Sample Code**	**Sample identification**	**Location**
		Dam – Pego do Altar	
1	L3/07	DF3T2APA	Santa Susana Stream (North)
2	L4/07	DF4T3PA	Pego do Altar Dam (North)
3	L5/07	DF5T3PA	Pego do Altar Dam (North)
4	L6/07	DF6T3PA	Pego do Altar Dam (North)
5	L7/07	DF7T3PA	Pego do Altar Dam (North)
6	L8/07	DF85aPA	Santa Susana Stream (South)
7	L9/07	DF9T3PA	Santa Susana Stream (South)
8	L10/07	DF10T3PA	Santa Susana Stream (South)
9	L11/07	DF11T3PA	Santa Susana Stream (South)
10	L12/07	DF12T3PA	Santa Susana Stream (South)
11	L13/07	DF13T4PA	Pego do Altar Dam (center)
12	L14/07	DF15T3PA	Pego do Altar Dam (North)
14	L16/07	DF14T3PA	Pego do Altar Dam (North)
15	L17/07	DF16T3PA	Pego do Altar Dam (North)
		Dam – Monte Novo	
16	L1/08	DF16RMM3	Machede Stream (near dam)
17	L2/08	DF24MN5	Monte Novo Dam (North)
18	L3/08	DF23RD2	Degebe River (Downstream)
19	L4/08	DF13RD2	Degebe River (Downstream)
20	L5/08	DF20MN2	Monte Novo Dam (dam wall)
21	L6/08	DF21RMM2	Machede Stream (upstream)
22	L7/08	DF8MN4	Monte Novo Dam (center)
23	L8/08	VF18RMM3	Machede Stream (near dam)
24	L9/08	DF11MN2	Monte Novo Dam (dam wall)
25	L10/08	DEIOMNRD3	Degebe River (Downstream)
26	L11/08	DF22MN7	Monte Novo Dam (West)
27	L12/08	DF15MN2	Monte Novo Dam (dam wall)
28	L13/08	DFMN3T4	Monte Novo Dam (center)
29	L14/08	DF4RD2	Degebe River (Downstream)
30	L15/08	DFMN2T4	Monte Novo Dam (center)
31	L16/08	DF12RD4	Degebe River (Downstream)
32	L17/08	DFMN1T4	Monte Novo Dam (center)
33	L18/08	DF13MN7	Monte Novo Dam (West)
34	L19/08	DF6MNT2	Monte Novo Dam (dam wall)
35	L20/08	DF5RD2	Degebe River (Downstream)

Bacteria Identification and Antimicrobial Resistance Determination

The isolation of *E. coli* and *Enterococcus* spp. from the otters fecal AMIES swab samples was performed using specific bacteriological protocols.

E. coli isolation was carried out in MacConkey agar plates (Oxoid), incubated at 37ºC for 24h. This selective medium includes bile salts and crystal violet to inhibit the growth of Gram-positive bacteria. It also includes lactose and neutral red, and lactose positive *E. coli* colonies are easily recognized due to their bright pink coloration (Biokar Diagnostics, 2008; Quinn et al., 1994).

Enterococcus spp. isolation was performed using Slanetz and Barley agar (Oxoid), incubated at 37ºC for 24 h. This selective medium includes sodium azide that inhibits the growth of Gram-negative bacteriam, and triphenyltetrazolium chloride, an indicator that it is reduced to an insoluble formazan inside the cells, turning enterococci colonies red to maroon (Biokar Diagnostics, 2008).

Identification of isolates was performed through their macro and microscopic morphology, staining characteristics and biochemical profiles using the identification systems API 20E for *E. coli* identification and API Strep for *Enterococcus* spp., according to manufacturer's instructions (bioMérieux).

Antimicrobial susceptibility testing was performed by the disk diffusion method recommended in the Clinical and Laboratory Standards Institute (CLSI) guidelines, using Mueller-Hinton agar plates (Biokar Diagnostics). Antimicrobial drugs tested were as follows: amoxycillin/clavulanate (AMC, 30 μg), ampicillin (AMP, 10 μg), chloramphenicol (C, 30 μg), cephalexin (CL, 30 μg), gentamicin (CN, 10 μg), cephotaxim (CTX, 30 μg), enrofloxacin (ENR, 5 μg), nalidixic acid (NA, 30 μg), penicillin G (P, 10 units), streptomycin (S, 10 μg), sulphamethoxazole/trimethoprim (SXT, 19:1, 25 μg) and tetracycline (TE, 30 μg). Antimicrobial disks were purchased from Oxoid.

Results

Thirty-five fresh scats (Table V.1.1) were collected in 13 of the 21 surveyed sites (Figure V.1.1 and Table V.1.1) and a total of 26 *Enterococcus* spp. isolates (Table V.1.2), and seven *E. coli* isolates were obtained (Table V.1.3).

The antimicrobial resistance profile of the isolates under study is shown in Table V.1.2 and Table V.1.3. For analysis purpose, isolates with intermediate susceptibility were considered as resistant. None of the 33 isolates tested was susceptible to all antimicrobial agents tested.

All the 26 enterococci isolates were resistant to CL, CTX, ENR and S. Five isolates of these were resistant to all antimicrobials tested. High levels of resistance were observed for CN (n =

21, 80.77%) and NA (n = 25, 96.15%). Intermediate levels of resistance were observed for AMP (n = 12, 46.15%), SXT (n = 13, 50.0%) and TE (n = 18, 69.23%). Lower levels of resistance were observed for AMC (n = 9, 34.62%), and C and P (n = 10, 38.46%). All enterococci isolates presented a multiresistant profile. Similar antimicrobial resistance patterns were observed in enterococci isolates from fecal samples L3 and L4/2007, from samples L11 and L16/2007, from samples L4, L6 and L9/2008, and from samples L3 and L19/2008 (Table V.1.2 and Table V.1.3).

E. coli isolates presented lower levels of resistance. All seven isolates were susceptible to C, CN, CTX, ENR and SXT. None of the isolates was resistant to all antimicrobials testes. High levels of resistance were observed for TE (n = 8, 71.43%) and P (n = 6, 85.71%). Intermediate levels of resistance were observed for AMP (n = 4, 57.143%) and NA and S (n = 3, 42.86%). Lower levels of resistance were observed for AMC and CL (n = 2, 28.57%). Only one *E. coli* isolate did not present a multiresistant profile, and similar patterns of resistance were observed in *E. coli* isolates from fecal samples 14/2007 and 16/2007 (Table V.1.2 and Table V.1.4).

Table V.1.2 - Levels of resistance observed for *Enterococcus* spp. and *E. coli* isolates from otter (*Lutra lutra*) scat samples.

Antimicrobial compounds	*Enterococcus* spp. (N=26)	*E. coli* (N=7)
	Resistant bacterial isolates	
AMC	9 (34.62%)	2 (28.57%)
AMP	12 (46.15%)	4 (57.14%)
C	10 (38.46%)	0
CL	26 (100%)	2 (28.57%)
CN	21 (80.77%)	0
CTX	26 (100%)	0
ENR	26 (100%)	0
NA	25 (96.15%)	3 (42.86%)
P	10 (38.46%)	6 (85.71%)
S	26 (100%)	3 (42.86%)
SXT	13 (50.0%)	0
TE	18 (69.23%)	5 (71.43%)

Table V.1.3 - Antimicrobial resistance profile of enterococci isolated from otter (*Lutra lutra*) scat samples.

Isolate Nº	Sample code	Isolate identification	Antimicrobial resistance profile
1	L3 / 2007	*E. faecium*	AMC-AMP-C-CL-CN-CTX-ENR-NA-P-S-SXT-TE
2	L3 / 2007	*E. durans*	CL-CTX-ENR-NA-S
3	L4 / 2007	*E. fecalis*	CL-CTX-ENR-NA-S

Isolate Nº	Sample code	Isolate identification	Antimicrobial resistance profile
4	L5 / 2007	*E. durans*	CL-CTX-ENR-NA-S-SXT-TE
5	L7 / 2007	*E. fecalis*	CL-CTX-ENR-NA-S-TE
6	L8 / 2007	*E. faecium*	AMP-CL-CTX-ENR-NA-S-SXT
7	L9 / 2007	*E. faecium*	AMC-AMP-CL-CN-CTX-ENR-NA-S
8	L10 / 2007	*E. faecium*	AMC-AMP-CL-CN-CTX-ENR-NA-S-SXT-TE
9	L11 / 2007	*E. fecalis*	C-CL-CN-CTX-ENR-NA-S-SXT-TE
12	L16 / 2007	*E. fecalis*	C-CL-CN-CTX-ENR-NA-S-SXT-TE
14	L1 / 2008	*E. faecium*	CL-CN-CTX-ENR-NA-P-S-TE
15	L3 / 2008	*E. faecium*	CL-CN-CTX-ENR-NA-S-TE
18	L4 / 2008	*E. faecium*	CL-CN-CTX-ENR-NA-S
19	L6 / 2008	*E. faecium*	CL-CN-CTX-ENR-NA-S
20	L8 / 2008	*E. faecium*	AMP-C-CL-CN-CTX-ENR-NA-S-TE
21	L9 / 2008	*E. faecium*	CL-CN-CTX-ENR-NA-S
22	L10 / 2008	*E. faecium*	AMC-AMP-C-CL-CN-CTX-ENR-NA-P-S-SXT-TE
24	L11 / 2008	*Ent.* sp.	AMC-AMP-CL-CN-CTX-ENR-P-S-SXT
25	L12 / 2008	*E. faecium*	AMC-AMP-CL-CN-CTX-ENR-NA-P-S-TE
26	L13 / 2008	*E. faecium*	AMC-AMP-C-CL-CN-CTX-ENR-NA-P-S-SXT-TE
27	L15 / 2008	*E. faecium*	AMC-AMP-C-CL-CN-CTX-ENR-NA-P-S-SXT-TE
28	L16 / 2008	*E. faecium*	AMC-AMP-C-CL-CN-CTX-ENR-NA-P-S-SXT-TE
30	L18 / 2008	*E. faecium*	AMP-C-CL-CN-CTX-ENR-NA-P-S-SXT-TE
31	L18 / 2008	*E. fecalis*	C-CL-CN-CTX-ENR-NA-P-S-SXT-TE
32	L19 / 2008	*E. durans*	CL-CN-CTX-ENR-NA-S-TE
33	L19 / 2008	*E. durans*	CL-CN-CTX-ENR-NA-S-TE

Table V.1.4 - Antimicrobial resistance profile of *E. coli* isolated from otter (*Lutra lutra*) scat samples.

Isolate Nº	Sample code	Antimicrobial resistance profile
10	L14 / 2007	AMC-AMP-CL-NA-P-TE
11	L16 / 2007	AMC-AMP-CL-NA-P-TE
13	L17 / 2007	NA-P-TE
16	L4/2008	S-TE
17	L4/2008	AMP-P-TE
23	L11 / 2008	P-S
29	L17 / 2008	AMP-P-S

Discussion

Public interest in wildlife animals' welfare and conservation has grown in the last years. Studies have focused on aspects related wildlife health status, diseases and causes of mortality, since investigators have realized that these animals can be used as potential environmental pollution monitors, while being, simultaneously, potential vehicles of zoonoses, diseases transmission to farm and companion animals, and antimicrobial resistant bacteria (Simpson, 2000; Salyers and Shoemaker, 2006). In fact, Cole et al. (2005) already established the role of wild birds in pathogenic bacteria and antimicrobial resistance gene transmission to humans, through the contamination of agricultural fields and water used for human consumption.

Free-range river otters are well spread in Portugal, in a wide variety of aquatic environments, therefore being ideal vectors from the transmission of fecal bacteria and antimicrobial resistance factors.

The evaluation of antimicrobial resistant *E. coli* and *Enterococcus* spp. present in fecal samples from free-ranging river otters is an important tool for monitoring the spread of antimicrobial resistance. These bacteria belong to the normal enteric microbiota of healthy animals, and may be responsible for spreading transferable resistance genes located in mobile elements to pathogenic or commensal human and animal bacteria (SVARM, 2007).

Cultured-based methods for *E. coli* and *Enterococcus* spp. isolation from the fecal samples may present several limitations for fecal microflora characterization (Leser et al., 2002). In fact, although some authors suggested the use of molecular biology methods for the characterization of fecal bacteria (Kohn and Wayne, 1997), these methods can also be insufficient (Harmsen et al, 1999), as they provide no information on viability of microorganisms, while culture-based methods have proven adequate to verify if viable *E. coli* and *Enterococcus* spp. isolated from the fecal samples carried antimicrobial resistance determinants, since these bacteria are extremely resistant and can survive in adverse conditions for long periods of time (Waterman and Small, 1998). The use of appropriated medium to transport the samples to the laboratory and the bacterial survival ability are a guarantee of a successful recovery by culture-based methods. The use of selective procedures for f the target bacteria isolation, as recommended by Nikolova et al.¸ 2001, helps eliminating the background microflora that sometimes is observed during bacteriological examinations.

From the 35 fecal samples tested in this study, it was possible to identify seven *E. coli* isolates and 26 *Enterococcus* spp isolates. The low frequency of *E. coli* isolates was not expected, since *E. coli* strains usually correspond to 1% of the total number of intestinal bacteria of warm blood animals (Bettelheim, 1992). *E. coli* low frequency suggests the occurrence of variations in fecal mammals' microbiota, and that otters' intestine could have been colonized during evolution by other *Enterobacteriaceae* and related bacteria, such as *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Morganella morganii, Pantoea* spp., *Salmonella* spp., *Serratia* spp., *Shigella* sp., and *Enterobacter* spp. Another hypothesis is that these others diet may have changed, since it is known that sudden diet changes may affect the bacteria ration in intestinal microbiota (Bettelheim, 1992).

Regarding *E. coli* antimicrobial resistance, none of the isolates was susceptible to all the antimicrobial agents tested, and all except one isolate presented a multiresistant profile. No obvious differences on resistance to antimicrobials with different action mechanisms were observed. All *E. coli* isolates were susceptible to antimicrobials that inhibit translation (C, CN), cell wall synthesis (CTX), folate synthesis (SXT) and replication, by impairing DNA gyrase action (ENR). Lower levels of resistance were observed for antimicrobials that inhibit translation (AMC), and also cell wall synthesis (CL). Intermediate levels of resistance were observed for drugs that impair cell wall synthesis (AMP) and translation by ribosomes (NA, S). Higher levels of resistance were observed for antimicrobials that affect cell wall synthesis (P) and translation (TE) (Koolman and Roehm, 2005).

In spite of the recognized geno and phenotypic differences between *E. coli* and *Enterococcus* spp., analogous results were obtained for enterococci isolates. None of the isolates was susceptible to all antimicrobial agents tested, all presenting a multiresistant profile, with no evident differences in resistance to antimicrobials with different action mechanisms. Lower levels of resistance were observed for antimicrobials that inhibit translation (AMC, C), and cell wall synthesis (P). Intermediate levels of resistance were observed for drugs that impair cell wall synthesis (AMP), translation by ribosomes (TE) and folate synthesis (SXT). Higher levels of resistance were observed for antimicrobials that affect cell translation (CN, NA) (Koolman and Roehm, 2005).

The major difference between the antimicrobial resistance characteristics of the two bacterial species was related to the number of antimicrobial compounds to which each specie was resistant to. While *E. coli* isolates were resistant to a maximum of five antimicrobial drugs, a profile presented by only two isolates, all enterococci tested were resistant to a minimum of five compounds, with 5 isolates (19.23%) resistant to all antimicrobials tested. These data are supported by the fact that enterococci are able to present not only acquired resistance mechanisms but also intrinsic resistance to antimicrobial drugs, and suggest that *Enterococcus* spp. might be a more serious problem regarding antimicrobial resistance transmission than *E. coli*.

Antimicrobial resistant bacteria have already been isolated from wild animals (Caprioli et al., 1991; Cole et al., 2005; Skurnik et al., 2006). It is generally believed that the main reason for the emergence, selection, and dissemination of antimicrobial resistant determinants is excessive exposure to antimicrobial drugs. In fact, Sayah et al. (2005) stated that wildlife that has not been deliberately exposed to high concentrations of antimicrobial compounds shows low antimicrobial resistance. However, our results contradict this hypothesis. The fecal samples analyzed in this study were obtained free-ranging otters that are extremely unlikely to have been submitted to antibiotherapy. The isolation of high antimicrobial resistant bacteria isolated from animals that had never been exposed to drugs has also been described in a previous study on Timorese river buffalos (Oliveira et al., 2007), suggesting that environmental exposure of commensal microorganisms to antimicrobial agents may contribute for the selection for resistant bacterial strains. Considering the location of the sampling sites, these otters were probably exposed to antimicrobial compounds present in ground water contaminated by animal and human wastes (Cole et al., 2005; Sayah et al., 2005; Salyers and Shoemaker, 2006; Skurnik et al., 2006). The indirect contact with livestock fecal material that may bear antimicrobial resistant bacteria could be involved in the transmissiom of resistant determinants from commensal microorganisms to free-ranging wildlife, turning them into new environmental reservoirs of resistant microorganisms (Cole et al., 2005). River Sado Basin pollution is mainly due to agriculture practices (Ferreira et al., 2004). No water pollution sampling stations are present near the surveyed sites and so it is not possible to correlate the presence of pathogenic and antimicrobial resistant bacteria with specific pollution sources (Ferreira et al., 2004). However, no intensive agriculture is present neither in Pego do Altar Dam nor in its adjacent streams and contaminations are only likely to occur upstream. Regarding Monte Novo Dam and its surroundings, the water quality is considered bad, probably due to direct discharges from small villages (INAG, 2007). It is also important to point out that some sampling sites, such as Santa Susana and Machede Streams, are located near small villages and present bad quality eutrophic water probably due to lack of stream current and the presence of cattle. In other parts of the reservoir, the water is also eutrophic due to the presence of cattle and human activities. The presence of cattle is continuous in all sampling sites, sometimes very high, not only in number of individuals but also in the occurrence of cattle droppings in the dam and stream margins.

Non-antibiotic selection pressure due to the presence of heavy metals or disinfectants used in agricultural practices may also select for antibiotic resistance genes (Harbottle et al., 2006). This hypothesis must also be taken into consideration. Although the sampling sites near the Sado Basin are far from agricultural fields, the agriculture practices along this river basin may originate chemical compounds that reach the sampled area, and therefore may contribute for the occurrence of non-antibiotic selection pressure. On the contrary, agricultural levels in the Guadiana Basin are low. However, some level of agricultural practices is observed near the

Guadiana sampling sites, which may explain the similarity in the antimicrobial resistance levels of the bacteria isolated from scats obtained in the two river Basins.

Chloramphenicol resistance was observed in enterococci isolates, suggesting that another mechanism of resistance transmission has occurred, since the administration of this drug in cattle therapeutic is legally forbidden, and its use for the treatment of human infection is not frequent. Resistance to quinolones and cephalosporins has been attributed to the occurrence of point mutations in *Enterococcus* spp. and *E. coli* (Sayah et al., 2005; Harbottle et al., 2006), and these stable mutations can persist in bacteria, even in the absence of selective pressure (Sayah et al., 2005). These two bacterial species can also acquire antimicrobial resistance determinants from environmental bacteria via genome integration of mobile DNA elements, as described by Harbottle et al. (2006), Rice (2006) and Sayah et al. (2005). These authors have described the association of the acquisition of cassette-associated genes present in integrons to aminoglycosides, phenicols and sulfonamides resstance, and the relation of plasmids integration with β-lactams, tetracycline, cephalosporines and sulphonamides resistance. The multiresistance profiles could be due to co-selection or co-transfer phenomena that may originate the simultaneous development of resistance traits to antimicrobial drugs with different modes of action (Sayah et al., 2005).

Antimicrobial resistance is not always related to antimicrobial administration, since the isolation of antimicrobial resistant bacteria and the detection of resistance genes and transference mechanisms are previous to the application of modern antimicrobial drugs in human and animal therapeutics (Harbottle et al., 2006). This hypothesis is supported by the fact that resistant bacteria were found in historic culture collections. Therefore, these intrinsic resistance mechanisms may have evolved in antibiotic-producing microorganisms presenting characteristic physiological or biochemical features, such as soil bacteria, to protect them against their own antimicrobial compounds. Antibiotic-producing microorganisms have been extensively used in antibiotic preparations, which might have influenced the transmission and dissemination of resistant determinants and the establishment of multiple resistant profiles (Harbottle et al., 2006).

Antimicrobial resistance profiles of multirresistant bacteria can be used to differentiate between distinct resistant strain populations, and to identify the resistance source (Kaszanyitzky et al., 2003; Sayah et al., 2005). In this study, similar patterns of resistance were found in two *E. durans* isolates from the one sample (L19/2008) obtained in Monte Novo Dam, suggesting that they are probably the same strain. Similar patterns of resistance were also found in two *E. coli* isolates from fecal samples L14/2007 and L16/2007 obtained in the same sampling site (Pego do Altar dam), suggesting that these fecal samples may be originated from the same individual. However, similar patterns were observed in *E. fecalis* from samples L11/2007 and L16/2007, and in *E. faecium* from samples L4/2008, L6/2008 and L9/2008, collected from different sampling sites. As the probability of scats collected in distant sampling sites belonging to the same individual is low, these results point out to the probability of dissemination of specific antimicrobial resistance patterns between different animals and/or to the probability that these patterns are originated from a common source. This probability is further enhanced by the fact that the same antimicrobial profile is observed in scats from the two sampling sites, and also in different bacterial species. In this study, an *E. durans* isolate from sample L3/2007 and an *E. fecalis* isolate from sample L4/2007 showed the same antimicrobial resistance profile, as well as an *E. faecium* isolate from L3/2008 and an *E. durans* isolate from L19/2008. For the accurate identification of the contamination source, fingerprinting of these isolates is suggested. These new data would also contribute for the development of effective management plans to minimize environmental contaminations (Smith et al., 2002).

The present study presents strong evidence of antimicrobial resistance occurrence in *Lutra lutra* fecal bacteria in Portugal. Characterization of the antimicrobial resistance profile of otters' fecal bacteria can be useful to evaluate the potential of these animals to act as reservoirs for resistance genetic determinants and to contribute for environmental contamination, which could be responsible for the transmission of resistant bacteria from humans and animals to autochthon wildlife and vice-versa. It could also contribute for the geographic localization of the contamination source, and for the strategic management and conservation of otters'. This is especially relevant in sites near water where cattle density is high or where human recreational activities such as bathing, water sport practice or camping occur with high frequency, as observed for the dams sampled in this study.

The collection a wider set of otter fecal samples will allow to perform genetic characterization and clonality studies on the bacterial isolates, which may elucidate on the origin of the high antimicrobial resistance frequency observed, on the environmental routes of antibiotic resistance dissemination and also on the potential role of wildlife as vectors of antimicrobial resistance.

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PART VI – DAMS AND OTTER CONSERVATION IN MEDITERRANEAN AREAS

VI.1. Main findings

The ecological information collected in the course of this thesis contributed for understanding otters' use of highly changed environments (dams reservoirs), especially in Mediterranean climate regions where many rivers are impounded and/or diverted in the frame of water management policies. The main findings may be summarized as follows:

How to efficiently survey otters in dams

Based on the experience gathered in this and in previous studies of otters in large dams (Pedroso and Santos-Reis, 2006; Pedroso et al., 2007), an adaptation of the standard survey method adopted by the IUCN Otter Specialist Group (OSG) for monitoring otters in lotic systems (streams, rivers) (Reuther et al., 2002), was proposed to improve monitoring of the species in lentic systems, especially in large dams (Paper 1). This includes considerations regarding survey season, survey length and width, number and location of survey sites, among other criteria. This adaptation of the survey method will allow researchers to be more effective when surveying dams either when the aim is simply to detect otter presence/absence, or to collect fresh spraints for molecular analysis. These oriented surveys are especially relevant when addressing otter's presence, distribution or intensity of use in dry areas, such as the Mediterranean region, where dams have a role to play, and were the basis of the field work conducted along the thesis.

Large dams as suitable habitat elements for otters

Although it has been suggested that dams adversely influence the distribution of Eurasian otters and are a contributing factor for the past decline of the species in Europe, this study show that large reservoirs are regularly used by the otter in southern Portugal and these habitat elements can be seen as suitable for the species in some scenarios, i.e. when otter populations are stable and relatively dense, and in areas where streams suffer marked seasonal changes in water flow. However, reservoirs are still less suitable for otters then the undisturbed pre-existent streams and rivers (Paper 2).

Season, prey abundance and reservoir characteristics influence otter presence and use of dams

Prey resources, irrespectively of reservoir size, proved to be key drivers of otters' use of the reservoirs and their availability highly influenced the species presence. Moreover, being an opportunist predator, the otter not only preys on the more abundant prey species, but also avoids those representing smaller biomass (Papers 2, 3 and 4).

Despite the observed seasonal differences in the composition and structure of prey communities, there was an apparent stability in prey availability (both in numbers and biomass) in large reservoirs (Papers 2, 3 and 4). This is vital for otters' survival because during the dry season more than half of the dam's adjoining streams dried or were reduced to very small pools with fewer prey species and individuals. Reservoirs, though, offer abundant food, both in dry and wet seasons, suggesting that prey resources are key drivers for otter's use of reservoirs in Mediterranean areas or in other dry regions, where there is a marked seasonality in resources (water and prey). Fish and American crayfish were the dominant prey resources used by otters, both in streams and in reservoirs.

Another determinant for otter presence and use of large reservoirs is the presence of nearby streams. The sites most regularly visited in the reservoirs were located near the confluence with streams, in contrast to sites far from streams, which had lower visiting rates or no visits at all. This suggests that not all the reservoir perimeter is equally suitable for otters. The stream-related key driver is the riparian vegetation that provides shelter and enhances breeding, which in turn is a scarce resource along the reservoirs margins (Papers 2 and 4). It is also know that the reservoirs' tails are actively chosen by female otters with developed cubs/juveniles (Ruiz-Olmo et al., 2005a), probably because those sites are where fish species (e.g. cyprinids) gather during annual dispersal movements.

Bank typology emerged as another major constraining factor; shallow waters and complex margins offer to otters better foraging opportunities than deep waters that limit the species ability to catch fast swimming prey (Nolet and Kruuk, 1989; Nolet et al., 1993; Houston and McNamara, 1994; Kruuk, 2006) (Paper 2).

Commonalities and dissimilarities in otter use of differently-sized dams

Otter use of small and medium-sized reservoirs was, in general terms, in accordance with the findings obtained on large reservoirs: i) otters were present and used the majority of the reservoirs; ii) there were seasonal differences in marking intensity, suggesting a higher importance of reservoirs in the dry season; iii) reservoirs were suboptimal habitats for otters in terms of refuge and human pressure when compared with rivers and streams, but acted as important feeding areas, especially when close to watercourses with good refuge conditions and scarcity of prey; iv) diet of otters using the reservoirs reflected the species opportunistic behavior, through the selection of the most seasonally available prey, in particular the American crayfish (Paper 3).

However, in contrast to large dams, small-medium sized reservoirs showed no differences in the patterns of occupancy (presence/absence) in the wet and in dry seasons. They either had otters that were present throughout the year, or otters were never present; the latter was especially true for very small reservoirs that could not sustain prey communities. The negative association found between the use of small-medium reservoirs and the length of watercourses with developed riparian vegetation in the surrounding areas, reflected the otter preference for better-preserved streams and rivers over artificial reservoirs with no refuge opportunities; as a consequence, when good habitat is present close-by, the need for reservoir resources is lower (Paper 3). Another influencing factor was the effect of cattle pressure, which had a negative influence over otter use of the smaller reservoirs, but not in larger reservoirs (Paper 2 and 4).

Dam construction affects otter main ecological requirements

As previously shown in the first chapters of this thesis, otters in the South of Portugal make use of the resources provided by dam reservoirs, irrespectively of size (Papers 2 and 3), but true impacts of the construction, in particular of large dams, were poorly known. Using Alqueva dam (the largest dam in Europe) as an example, data collected demonstrated that deforestation and flooding significantly impacted the otter population resulting in a marked decrease of otter presence in the flooding area. Although otters returned after flooding, the complete lack of vegetation in the watercourses probably hindered the population resettlement, and the species presence became fairly constant when the water level stabilized but did not reach the use level prior to dam construction.

The analyses showed that the otter's response to changes created by the dam implementation was even clearer in their diet, reflecting major shifts both in composition and abundance of prey communities, which is perhaps the most substantial effect of dam construction on otters. There was a significant prey switch from a native to a non-native fish and crustacean (American crayfish) dominated diet; moreover, prey are less available for otters due to increased difficulty in foraging in deep waters, as well as a dispersion effect of fish in the large reservoir, at least in the immediate years after the dam implementation when colonisation by mainly non-native species is still an ongoing process. In parallel to changes in use of food resources, the results also illustrate a change in other otter ecological requirements over time: habitat connectivity (ecological corridors with high habitat quality and high prey abundance), dense bankside vegetation cover, shelters and breeding dens, and foraging grounds that are easy-to-find and use (e.g. Macdonald and Mason, 1982; Ruiz-Olmo et al., 2005a). With the exception of the availability of fresh water, all those other otter ecological requirements are less available after the construction and implementation of the Alqueva dam. The lack of breeding, feeding, and resting areas are particular important, since these are critical the species (e.g., Fernández and Palomares, 2000; Kruuk, 2006).

Long term monitoring studies as a requirement to address species response to impacts

Dam construction and water development projects create impacts extending well beyond the initial space (flooded area) and time (construction timeline) considered in the infrastructure proposal. Results obtained in this thesis emphasize how important are long-term monitoring studies, that include all phases of construction (post construction to flooding and beyond), to truly evaluate species response to impacts. This is of relevance, because not all Environmental Impact Assessments include post-flooding monitoring phases, or they may not even consider the otter as a target species (Paper 4). The real impact on otters can only be evaluated after the end of the impacting phases (deforestation and flooding), and after the stabilization of the reservoir conditions (flooding, vegetation, prey communities).

Otters and environmental impact assessments

The data, knowledge and experience that resulted from the context of this thesis helped us to elaborate, along with other members of the IUCN Otter Specialist Group, recommendations intended to guide developers and consultants when preparing environmental impact assessments (EIAs), as well as NGOs and EIA advisors (biologists and lawyers) in administrations, who have to check that the otter has been properly considered in the course of an EIA (APPENDIX). These recommendations call the attention to the main species requirements potentially affected by development of such infrastructures (food species and feeding areas, including the movements and migrations of prey; resting sites; breeding areas including natal holts; corridors for movement and dispersal; permanent accessibility to fresh water)., an approach that was followed in Paper 4. They also include suggestions regarding mitigation/compensation measures (considering scaling issues and approaches to meet the impact) and monitoring actions (e.g. need to evaluate the effectiveness of proposed mitigation measures).

Evidence for antimicrobial resistance in otter fecal bacteria

An important asset to the main findings regarding otter ecological response to dam's presence, was the first evidence of antimicrobial resistance in otters using dams and streams. It is generally believed that the main reason for the emergence, selection, and dissemination of antimicrobial resistant determinants is the misuse and abuse of antimicrobial drugs administration. In fact, Sayah et al. (2005) stated that wildlife that has not been deliberately exposed to high concentrations of antimicrobial compounds shows low antimicrobial resistance. However, the results of Paper 5 contradict this hypothesis, since the presence of antimicrobial resistant bacteria in otter faecal samples collected in Pego do Altar and Monte Novo, and adjacent streams, was detected and following studies also confirmed these results (Oliveira et al., 2010, 2011). The individuals producing such spraints were not likely to have been subject to antibiotherapy. Considering the location of the sampling sites, these otters were probably exposed to antimicrobial compounds present in ground water and this was contaminated by animal and human wastes, as cattle was present in all sampling sites. This is especially relevant in sites where human recreational activities such as bathing, water sport practice or camping occur with high frequency, as observed for the dams sampled in this study. Characterization of the antimicrobial resistance profile of otters' fecal bacteria can be useful to evaluate the potential of these animals as reservoirs for resistance determinants, and their contribution to environmental contamination, which could be responsible for the transmission of resistant bacteria from humans and animals to local wildlife and vice-versa. It could also contribute to the geographic localization of the contamination source, and for the strategic management and conservation of otters.

VI.2. Implications for Iberian otter conservation in a changing scenario

The integration of the output of the different components of this thesis aims to contribute to a better understanding of dam and reservoir use by the otter. Of particular importance are the implications for a species conservation strategy and for dam-management in Mediterranean areas

Specific challenges in the Mediterranean and Iberian Peninsula

The Mediterranean characteristics carry extra challenges for otter conservation. This is particularly true for Iberian riverine systems, due to the combination of intermittent presence of water and the long lasting human colonization of the area. Native wildlife has evolved and/or adapted to limited availability of water. One example is the Mediterranean native fish species, adapted and thus resistant to the highly variable water flow. For example, present-day droughts have been causing relatively small and transient changes to these stream fish assemblages (Magalhães et al., 2007). But human induced changes must not go beyond the thresholds of recovery and resilience that characterize arid Mediterranean ecosystems (Blondel, 2006). In the particular case of the Iberian Peninsula, which is poor in natural lakes, over 1 200 large reservoirs have created a huge amount of new lentic freshwater habitat (Clavero and Hermoso, 2010). There is a cumulative effect of such a large number of infra-structures on decreasing river flow downstream, population fragmentation caused by consecutive dam walls, or water quality degradation. This can cause significant impacts on otter populations in those areas of Mediterranean were this cumulative effect occurs.

In addition, present tendencies show that the earth’s climate is warming and this has further implications for water availability (Hall et al., 2008). Climate change scenarios predict an increased inter-annual variability and a change in total precipitation (Santos et al., 2002; Santos and Miranda, 2006; Hall et al., 2008; Kundzewicz et al., 2008; Bloschl and Montanari, 2010). Depending on location, precipitation is predicted to either increase (e.g., in tropical and high latitude areas) or decrease (e.g., in the Mediterranean basin and western and southwestern USA), and may also fluctuate more in time or occur with more frequent extreme events (Santos et al. 2002, Hall et al. 2008, Kundzewicz et al 2008). Potential implications for water availability will be very important, with huge impacts on freshwater ecosystems (Santos et al., 2002; Hall et al., 2008; Kundzewicz et al., 2008). These changes will affect river flow (Hall et al., 2008; Kundzewicz et al., 2008; Bloschl and Montanari, 2010) even further in already highly variable water flow areas like the Mediterranean basin.

Cianfrani et al. (2011) assessed climate change threats to the European otter using two climate change scenarios and several forecasting approaches and climate models. Results for the Mediterranean bioregion suggest that there will be a decrease in otter habitat suitability in the Iberian Peninsula, probably linked to a potential increase in droughts as the climate warms (Cianfrani et al., 2011). Effectively, in these circumstances, the territories of many otters have shown to alter dramatically with season (Macdonald and Mason, 1982; Kruuk, 2006). This was also reported in a study in the Mediterranean where otter activities during the dry season tended to concentrate around remaining running waters, pools, and small reservoirs, effectively reducing territory size and scent marking boundaries (Ruiz-Olmo et al., 2007).

Human development and attraction for riverine, costal and wetland areas also poses threats to otter populations. The growing demand for inland waters (reservoirs or lakes) to establish tourist settlements and infrastructures, increases human disturbance in these habitats (e.g. boats, camping and water sports). The same applies to coastal areas. For example, the escalation of the tourism pressure in the Southwest coast of Portugal (*Costa Vicentina*) can be especially deleterious to the otter population living in the area and using the sea and several sea flowing small streams (Beja, 1996). Predicted decreased in river flow due to climate constraints will further be aggravated by growing demands of water, mostly for agriculture (Bloschl and Montanari, 2010; Rodríguez-Diáz and Topcu, 2010). In Alentejo, the total area under irrigation has been increasing and replacing traditional extensive cereal steppes (Caetano et al., 2009; INE, 2011). This is projected to continue as irrigation plans for this region predicts the development of a set of hydraulic works for inter-basin water transfer between Sado and Guadiana rivers, with the Alqueva dam (Guadiana basin) as the main water provider. The final inter-basin diversion will be made to the Alvito dam (Sado basin) and from there the water will be distributed by a channels' system that extends west and south to other Sado basin dams (CCDR_Alentejo, 2001). These developments are predicted to increase the irrigation area from 40 000 ha to 60 000 ha.

Increased irrigation imposes significant water demand problems because, unlike other water withdrawals that return most of the water to the watershed, water withdrawn for irrigation returns less to the aquifer (Hall et al., 2008). Even so, agricultural intensification will also result in non-negligible risk of simultaneous degradation of surface and ground water reserves due to additional loads of organic matter and nutrients, pollution and eutrophication. Water quality is strongly conditioned by the seasonal nature of its flow (CCDR Alentejo, 2001;

Moreira et al., 2004). Aspects like waste water and irrigation runoff (INAG/MAOT, 2004) induce blooms of cyanobacteria that may have an extremely negative impact on water quality and the aquatic fauna (CCDR Alentejo, 2001). Major anthropogenic sources causing freshwater eutrophication are animal farming, urban and agricultural runoff, industrial wastes, and sewage effluents. As a result, significant increases in the concentration of nutrients (e.g., nitrogen, phosphorus) and the abundance of primary producers (e.g., phytoplankton, benthic algae, macrophytes) have occurred in many freshwater ecosystems around the world (Wetzel, 2000). Water quality degradation increases vulnerability to other pollution sources, resulting in a higher probability of eutrophication (MAOT/ARH Alentejo, 2011) and reduction of overall prey availability. This is aggravated in reservoirs of large dams, especially in warm regions with a strong agricultural component. Reservoirs are like large lakes whose organic matter and other nutrients (like phosphorus and nitrogen) sedimentation leads to algae appearance, like cyanobacteria. The establishment of more stable environmental conditions (e.g. low water turbulence, longer water retention time) and the increase of irradiance during the dry period favor this growth of cyanobacteria (Geraldes and Boavida, 2004). These are toxic and can lead to fish death and be a risk to public health if the dam water is used for public consumption (WCD, 2000). It is not only in reservoirs that this eutrophication can occur as a result of a presence of a dam. Upper reaches of rivers and streams can also experience this phenomenon as consequence of bottom releases from dams (Camargo et al., 2005).

Besides contributing to this eutrophication, the presence of cattle in almost all the studied reservoirs (large, medium and small sized), sometimes in large numbers (mostly near medium-small sized reservoirs that are often used for the supply of water to livestock), along with agriculture run-off, may also contribute to exposure to antimicrobial compounds present in contaminated ground water (Paper 5). Little attention has been devoted to microbial communities in dam reservoirs but Simek et al. (2001), in a study in highly eutrophic Sau Reservoir (NE Spain) found that organically polluted rivers have considerable bacterial abundance and biomass, even with higher concentrations than in reservoirs, and both higher in dry seasons. They suggest that possibly the different composition of the microbiota community at the inflow of the stream was associated with organic substrates of allochthonous origin (e.g. agriculture), whereas the community in the reservoir areas was largely associated with autochthonous organic substrates produced in the processes of primary production (e.g. eutrophication). The composition of bacterial communities in the dams surveyed in these studies, are probably influenced by the presence of wastewater from agriculture and cattle farming. Not only this is a matter for otter conservation, since it can affect species fitness, but it is also a public health problem, since some of the studied dams have human recreational activities, such as bathing, water sport practice or camping, not only in southern Portugal, but all over the Iberian Peninsula.

On the one hand, there is evidence of the adaptability of Eurasian otters to more extreme environments: marine environments in northern Europe or in semiarid or Mediterranean environments of Spain or northern Africa, in places where vegetation or water is very scarce or non-existent, in reservoirs or man-made irrigation channels, in urban areas, and even close to industrial complexes (Mason and Macdonald, 1986; Kruuk, 1995; Strachan and Jefferies, 1996; Kruuk et al., 1998; Ruiz-Olmo and Delibes, 1998; Kranz and Toman, 2000). So, otters can be found living in most type of aquatic environment, whether marine or fresh- water, natural, man-made or altered, especially when population saturation occurs.

On the other hand: climate and agricultural changes are expected to enhance the demand of already limited water resources, resulting in declines or species local extinctions , including the most sensitive fish species (Magalhães et al., 2007). As a result, in the southern Portuguese river basins, such as Sado and Guadiana river Basins, the viable maintenance of many water related species, including the otter, may became particularly challenging in the future (Barbosa et al 2003; Cianfrani et al., 2011).

Otter prey and non-native species

We have shown that prey availability during the dry season is one of the key factors affecting otter presence in reservoirs (Paper 2, 3 and 4). A similar pattern was detected in other areas, such as along the coasts of Shetland, where prey is easily available and otters occur in large numbers despite the scarcity of cover (Kruuk, 2006). Paper 2, 3 and 4 also contributed to better characterize this predator feeding behavior in the Mediterranean. Other studies also showed the importance of the American crayfish as prey for otters in Mediterranean areas riverine systems (Magalhães et al., 2002a; Clavero et al., 2004). It represents an important food resource especially in stressful periods of severe drought, and its presence is likely to have increased the carrying capacity of the environment for aquatic predators, such as the otter. The recovery of the otter in Spain, after a species decline in the 1970s, was apparently related to the introduction of this crayfish (Ruiz-Olmo and Delibes, 1998). The anticipated impoverishment of native fish fauna, associated to the increase of non-native species abundance, may be seen as a disadvantage for native ecosystems, but introduced species can be more abundant and easier to capture than native species, potentially allowing otters to re-colonize parts of their former native range (Almeida et al., 2012).

The American crayfish was the most preyed upon species in reservoirs. However, our knowledge of the effects of drastic diet changes induced by dam implementation (e.g., the dominance of American crayfish over fish; the shift from a native to a non-native dominated prey community) is limited. Non-native species have had significant effects on native species and ecosystems through predation, competition and the alteration of habitat conditions; however, predicting the impacts of exotic species is difficult because of the wide variation in the characteristics of invaders and invaded ecosystems (Levine et al., 2003; Lake and Leishman, 2004; Moyle and Marchetti, 2006; Gerhardt and Collinge, 2007). The change in the composition of the fish community is perhaps the most visible and detectable impact of dam construction on otter ecology. With the exception of reservoirs dominated by common carp, non-native species dominating reservoir fish communities generally have a lower biomass (pumpkinseed sunfish, eastern mosquitofish) than native species. It is clear that otters choose more abundant prey, but studies also show that species smaller in length and biomass are not “attractive” for the otter (Sales-Luís et al., 2007; Blanco-Garrido et al., 2008). This known preference reflects the greater energetic profits of larger species compared with the smaller ones.

This concern on how and if otter responds to major shifts in prey communities is justified by the recent dramatic decrease of the Shetland otter population, one of the strongholds of otters during the population crash of the 1950-80’s. Preliminary estimates of the populations occurring along the entire coastline suggest that less than one-third of those populations remain, and with little reproduction (Kruuk, 2006). Currently, Shetland otters eat mostly crabs, a prey previously largely avoided. Most likely, there have been significant changes in the fish populations around the islands (Kruuk, 2006). In the case of the American crayfish, an extra concern arises. Although many toxic compounds have been banned, contamination from heavy metals and other sources of pollution still occours, for example, in the Sado river basin (Moreira et al. 2004, MAOT/ARH Alentejo 2011). Henriques (2010) studied the presence of cadmium and mercury in otter spraints in the river Sado basin and reported that cadmium contamination levels seem to follow a spatial pattern while the mercury results indicate a time pattern related to shifts in the diet. Metal accumulation in American crayfish suggests a potential relationship between predator and dominant-prey and so otters may be highly vulnerable to the contamination of this crustacean (Henriques, 2010). Bioaccumulation through the aquatic food chain affects otter breeding success and cub survival (Olsson and Sandegren, 1991; Roos et al., 2001) and influences otter population persistence and viability.

Size and type of dam

It is clear that in some cases, the presence of reservoirs may be beneficial in sustaining otter populations in areas subjected to drought periods, especially where riverine systems dry out in dry seasons. This is particularly true for small and medium sized reservoirs with medium-high prey availability. Furthermore, in such situations the positive effect of reservoirs may be further enhanced by an adequate riparian vegetation cover in the reservoirs.

However, ecological and conservation consequences are not as simple when dealing with large reservoirs. Large dams are always located in rivers or large streams. These large streams or rivers, before impounded, are able to sustain healthy otter populations in countries like Portugal (Trindade et al., 1998). There are serious habitat setbacks when substituting a river with a large dam (Paper 4). In large reservoirs, the entire perimeter is seldom used regularly by the otters and the areas of highest use, as shown, are those near the confluence with streams. The larger the dam, the larger the flooding area a fact that promotes the disappearance of otter stream habitats. Reservoirs represent less refuge, fewer suitable foraging areas, and fewer areas for reproduction (Paper 4). Breeding is a time of high energy requirements for otter (e.g. Ruiz-Olmo et al., 2005a) and natal dens are still a limiting resource in Mediterranean areas (e.g., in southwestern Portugal – Beja, 1996). Thus, dam construction in previously suitable riverine systems aggravates this problem.

Large dams with their reservoirs and usually unsurpassable walls contribute to the fragmentation of otter populations. The type of dam, dependent on its use, constrains the size of the wall, with higher walls associated with hydroelectric use. This type of dam is mostly located in the north of Portugal, in areas of higher altitude and deeper valleys of rivers. These contrast with dams used mostly for irrigation purposes, located in the south of the country, were the main agriculture areas are found and where the flatness of the regions do not allow substantial electricity production. These dams have smaller walls and softer insertion in the landscape. Thus, dams in the south of Mediterranean Europe may have less impact on fragmentation. The Alqueva dam nevertheless, although located in the south, can be compared to the northern type of dams, since it is of multipurpose use (primarily for irrigation, and secondly for electricity production and tourism). Alqueva's dam wall is 96m high and its insertion in the Guadiana river valley does not allow movements of otters, isolating upstream and downstream otter populations. In Spain, this barrier effect has been shown for otter populations south of the Pyrenees. Upstream populations took several years to recolonize river stretches downstream of a dam (at just 10-20 m distance). This recolonization movement was much more slowly than those in other populations much more distant but with no intermediate barriers (Ruiz-Olmo, 2001). Ruiz-Olmo and Jiménez (2008) also found that since the otter is Spain is recovering, the effect of such a barrier goes unnoticed since there are otter populations in both sides of the dams, although with no connection between one another.

The function of the dam is also an important aspect, since it implies different water management policies. Hydroelectric dams have larger and faster shifts in water level, while agriculture/water reserve dams do not suffer sudden fluctuations. Water discharge downstream is also very different, and tributaries below a hydroelectric dam suffer sudden water discharge with consequent impacts on freshwater communities, including otters (e.g. den flooding and disturbance of prey communities). On the other hand, most Iberian running water ecosystems are in Mediterranean climatic conditions, having a summer dry period during which surface flow is low or non-existent and unpredictable floods occur between autumn and spring. This temporal and spatial variability in environmental conditions is the main force structuring Mediterranean freshwater communities (Gasith and Resh, 1999; Magalhães et al., 2002a). Dams soften, eliminate or even reverse the natural variability of freshwater systems, both in the reservoir itself and in downstream water courses, through the contention of floods and the artificial maintenance of water flows in summer months (Poff et al., 2007).

Reservoir use by otters, however depends on their size, the regularity of their water level and whether or not they act as a barrier (Ruiz-Olmo, 1995; Ruiz-Olmo, 2001). It is clear that, in Mediterranean habitats, areas of large streams and reservoirs that contain water even in the driest months act as otter refuges during stressful periods. Medium or smaller reservoirs may also provide this without the impacts that larger dams cause. Smaller reservoirs have a lower negative impact on otters than larger ones as they do not represent such a loss of natural habitat, have less effect on water flow regimes, induce fewer changes in prey communities, and do not constrain otter fishing ability due to their smoother margins and shallower waters (Paper 3 and 4). Larger dams may sustain more otter individuals if the number of streams going into and coming from the reservoir is also higher. However, these streams must have adequate conditions for the otter (water, prey and cover availability). Trindade et al. (1998) draws attention to the controversy of the dams' construction but states that it is often the existence of small and medium-sized reservoirs that allows the survival of the species in certain locations in Portugal. Small and medium-sized reservoirs, as opposed to larger ones, do not act as barriers impeding otter dispersal, both upstream and downstream.

Otters and dams in the framework of Environmental Impact Studies

The Mediterranean area, as described previously (see PART I – I.1) is considered to be one of the regions that will face the largest climate changes worldwide (Giorgi, 2006) and where water management is mainly conducted through river regulation (dams) (Collares-Pereira et al., 2000). In the Iberian Peninsula there has been an increase in dam construction in the last decades and it is still on-going, although the recent financial crisis has put on hold some of the projected investments in water infra-structures. There are several Environmental Impact Assessments (EIA) produced in Portugal that deal with otter presence in future dams areas. In these studies, generally the otter is not considered an important species, since: i) its status was changed from "Insufficiently Known" to "Least Concern" (Cabral et al., 2005) as a reflex of the increasing knowledge about the species distribution and ecology; ii) is present in many of the existent dams in Portugal and so, adapted to this man-made habitat. Both points are debatable. First, otter preservation is still a vital issue and their conservation is mandatory in accordance with the Habitats Directive. This is especially relevant in the Mediterranean region, considered one of the biodiversity hotspots for conservation priorities (Brooks et al., 2006). Also, although we know a great deal about otter occurrence and diet, few attempts have been made to quantify population density and valid abundance assessments are also scarce and limited. Studies on reproduction, fitness, breeding, among other basic ecological parameters are still needed. Second, one must keep in mind that otter presence in reservoirs of already existing dams is not sufficient to conclude that such infrastructures offer equal opportunities to otter populations compared with the previous network of rivers and streams (Paper 4). In high-density otter populations, carrying capacity for otters in the reservoir is expected to be lower than that previously existing in the area prior to its construction.

Does otter status matter?

Data presented illustrates how reservoirs are used by otters but also that these are suboptimal habitats for the species. However, it is important to stress that they do not constitute a setback to otter conservation in areas of the Mediterranean region where otter populations thrive, like in Southern Portugal. They can be suitable habitat elements for otters under certain circumstances, namely in areas where streams are characterized by strong yearly changes in water flow, and reservoirs provide a co-joint system to already occupied streams. In other, less dry, areas of Europe, otters may not use as regularly the reservoirs. Even in Mediterranean areas where the otter is present at low density this scenario may be quite different. Marcelli and Fusillo (2009) on a study in Italy, assessing range expansion and recolonization of human-impacted landscapes, found evidence, although weak, of a negative effect in otter expansion in the vicinity of dam reservoirs. So the destruction of streams and rivers by the construction of large dams should be a matter of concern especially in areas of otter population fragility and/or instability (low numbers, recovering or expanding populations in edge distribution areas). The effect of otter population weakness and expansion on dam occupancy was also detected in Spain. After the decline suffered by the species in the 1970's, otters in Spain recovered and progressively colonized the Ebro river tributaries (Cataluña and Aragón regions, Spain), from the 1980's to present day. Reservoirs were colonized more slowly than "natural" streams and rivers, and this colonization only happened in a remarkable way after 2000 (Ruiz-Olmo and Jiménez, 2008).

The starting point of this thesis was mostly based in the 1970-90's state of the art. European otter populations had been declining and dams were by then viewed as having a clear negative effect on existent fragile populations in spite of already existing evidences that otters used dams in Mediterranean areas. Nowadays, improved knowledge of the otter's ecology in the Mediterranean and the population recovery observed in several European countries, add complexity to the otter-dam interactions with implications for the species conservation. Dams still have clear negative effects on otter populations, mainly masked by widely distributed and healthy populations, but also constitute a habitat complement to natural riverine systems subjected to climate and human pressures, that seasonally may not be sufficient to supply all needed otter requirements.

VI.3. Conservation and management actions

Long-term otter conservation strategies in Mediterranean areas, such as southern Portugal, should focus on maintaining healthy otter population by improving its natural prey and habitat conditions, while sustaining human activities. Specifically the joint system of reservoirs and adjacent streams can be a significant player in otter conservation in Mediterranean areas, the importance of which can be enhanced by the application of specific conservation measures and management actions.

Promoting the existence of refuge and cover in streams and in the margins of the reservoirs

Large dams may sustain more otter individuals if the number of surrounding streams is high and have suitable habitat conditions. The maintenance of suitable stream conditions for otters should be maintained as a priority, for example, by preventing cattle access or controlling the cutting of riparian vegetation and water extraction for agriculture purposes, common practices in South Portugal. Actions that sustain riparian vegetation in streams will favor water retention in dry periods and, consequently, otter prey occurrence. Minimizing already occurring drought effects is important since droughts can also decrease otter population density, cause change or even temporary abandonment of home ranges, and even hinder breeding (Delibes et al., 2000; Ruiz-Olmo, 2001). As otters breed more frequently in complex and stable habitats (Ruiz-Olmo and Jiménez, 2009), reservoirs, with lack of refuge, and smaller streams, characterized by periods of water scarcity, are usually not adequate habitats for breeding. Thus, maintaining stream habitat structure in reservoir’s adjacent streams is vital for otters, allowing them to profit from this combined system. Additionally, interface areas between reservoirs and adjacent streams are also determinant for the use of reservoirs by otters, due to higher cover availability and prey accumulation. These should be managed to prevent human disturbance associated with, for example fishing, water sports, or cattle raising.

In recently constructed reservoirs, when the vegetation was cleared due deforestation actions and water level variation, and before fish populations had time to decrease in numbers, otter use may be limited (e.g. presence but without reproduction) or non-existent. Promoting higher cover, and consequently of refuge, in the margins of the reservoir will diminish these effects. This can be done by accumulating logs, shrubs and leftover wood from the deforestations actions, but assuring its location above bank level to minimize the risks of eutrophication and navigation hazard. The presence of large rocks or rock agglomerations may provide potential shelters for otter. In the Rimov reservoir (Czech Republic), stone rip-rap, used for strengthening the banks of the reservoir, provided habitat for both the otter and young fish, which were otherwise absent (K. Roche, pers.com).

Dam-islands preservation

When dams are flooded islands may appear in areas with higher altitude than the dam's mean water level. This was the case of Alqueva dam that resulted in around 200 islands, most of which maintain a dense vegetation cover, and have been left undisturbed as they have been protected from human presence and intervention, offering otters a new habitat opportunity (e.g. for breeding). This can be especially important to allow otter populations to partially recover from the earlier impacts of deforestation and flooding. From a total of 27 islands surveyed (own unpublished data) 96% were positive for otter presence. Bernardo (2008), in a telemetry study, also found two otters resting on small islands in small reservoirs in the Alentejo region. It is clear that otters respond positively to the presence of these islands, which may act as safe heaven, offering refuge that is almost absent in the reservoir's margins. It is therefore important to protect reservoir islands, not only in Alqueva Dam, but in other reservoirs with similar characteristics.

Promoting small bays and margin complexity in reservoirs

Both perimeter complexity (intricacy and alternation of different margin type – bays and peninsulas) and the presence of small bays offer better otter foraging opportunities to otters by promoting a greater area of shallow waters and ambush opportunity sites. Additionally, the maintenance of some vegetation (e.g. helophytic vegetation systems), both below and above water level, that acts as refuge for fish, crayfish and amphibians in these bays promotes otter use of the reservoirs.

Controlling livestock and agricultural disturbance in reservoirs and streams

Degradation of surrounding reservoir areas through grazing and agricultural intensification is a key factor in densely populated areas such as the Mediterranean. Cattle assess to reservoirs and streams should be controlled to prevent riparian vegetation degradation, allow vegetation recovery and reduce water organic pollution and diminish potential transfer of antimicrobial resistant bacteria, thus contributing to improve habitat suitability for otters as well as for other aquatic fauna.

Efficient water management

Responsible water management requires managers' sensitivity to a wide range of issues. A starting point is the understanding of the impacts of present and future water development systems (Maingi and Marsh 2002). The current scenario of climate change in Europe predicts high impacts in the riverine systems in the Mediterranean region, mostly by extending the drought period. This aspect must be considered in otter conservation planning. Efficient water management processes are determinant critical not only for otters, but to also for other water dependent fauna and flora. The National Water Use Efficiency Program ("Programa Nacional para o Uso Eficiente da Água") (PNUEA, 2012) is a key management plan that is presently in public discussion. PNUEA has as main objective the promotion of the efficient use of water in Portugal, especially in urban, agricultural and industrial sectors. This strategy will help to minimize the risk of water shortage and to improve environmental conditions in water resources, without jeopardizing the vital needs and the quality of life of human populations, as well as the socio-economic development of the country. The levels of efficiency have been improving in the urban sector, but in agriculture they are still quite modest. The present PNUEA intends to reduce water use by 35% in this sector by 2020. Investments in reduction of water loss in irrigation and water transport infra-structures are urgent but may be postponed because of the economic crises, resulting also in the postponement of the obvious benefits of such actions: less pressure on the ecosystems, economic gains and reduction in the price of water.

Managing reservoirs and rivers ecological flow

Reservoirs regulate and halt river floods that are frequent in Mediterranean areas. However, the effect of retention and sudden release (either for electricity production or because reservoirs reached maximum level), during the major floods can be worse for riparian communities (e.g. fish, otter) than natural flood events (Ruiz-Olmo et al., 2001). If an adequate ecological flow is maintained, it will promote otter regular presence downstream, and even recolonization of previously dry streams in specific periods. On the other end, if badly managed, dams can reduce the annual run-off and modify the temporal flow patterns, as well as the duration, timing, frequency, magnitude and the rate recession of floods. Consequently, the dry season may begin earlier and may last for almost 8 months, frequently with a critical decrease in water quality (Bernardo and Alves, 1999). Downstream water discharges of reservoirs should be managed to minimize the effects on otter prey populations. The release of water should be progressive, avoiding the sudden opening and closing of channels, so that resulting floods follow a more natural flow regime (Ruiz-Olmo et al., 2001).

Native vs non-native species as prey

The apparent importance of non-native species as prey for otter constitutes a dilemma regarding biodiversity conservation. Their importance as otter prey, as shown in this thesis, should not be considered as a tool for otter conservation, since, besides putting native species in jeopardy (especially fish), there are indications that native freshwater fishes when in abundance are still the preferred prey of otters (Prenda and Granado-Lorencio, 1995; Beja, 1996). Magalhães et al. (2002b) found that the increased frequency and severity of droughts, predicted for the Mediterranean region by current climate change models, may result in significant modifications of stream fish assemblages. This will induce population declines or even local extinctions of at least the species most sensitive to summer droughts, and their potential replacement by more resistant species. One conservation measure that can be adopted is the conservation of large streams and rivers flows, which still have more native prey populations, by avoiding water extraction (for human proposes) from these waterlines, especially during dry season. Creating artificial pool-refugia, helping populations of fish and fish predators to survive the dry season, is another possible measure. However, the suitability of this strategy still needs to be tested (Beja, 1995) and there is indirect evidence to suggest that large prey items, when confined in these pools, are in fact prone for depletion by otters (Marques et al., 2010). Adequate management of such pools should avoid total fish depletion by fish predators like the otter, by managing the pools' size (e.g. not too small) and numbers (more foraging sites imply less foraging pressure per site), assuring underwater habitat complexity (e.g. increased fish refugia) and riparian cover. Such actions need to be taken into consideration in management plans. This would at least partly prevent otter prey shortages in dry seasons, while ensuring an improvement in native fish survival opportunities.

The homogenizing effect of reservoirs as regards fish species, and especially the influence on adjacent riverine systems, could be softened by reducing the number of illegal introduction of invasive species in reservoirs (Clavero and Hermoso, 2010), through increased regulation and control and public awareness.

Otter monitoring and Environmental Impact Assessments

The current status of the otter in Portugal (Least Concern - Cabral et al., 2005) may have the immediate effect of reducing the importance of otter research, especially in the scope of environmental impact assessments – (EIA) studies. However, otters represent an effective flagship species in river conservation and otter preservation is still a vital issue in Europe and Portugal, being mandatory in the Habitats Directive. The otter is listed in Annex II and IV of the Habitat Directive 92/43/EEC which has implications and obligations for developers within the EU. Portugal is considered to have one of the most viable otter populations in Europe (Foster-Turley et al. 1990, Trindade et al. 1998), suggesting greater responsibilities and obligations in conservation of the species. The recent crash of the Shetland population (Kruuk, 2006), one of European otter strongholds, shows how important it is to regularly monitor otter populations and their prey and to try to forecast possible threats. The Habitat Directive implies that the species and its habitats, including corridors connecting them, must be considered in EIAs throughout the EU territory, and not only in protected areas. Due to this, developers and consultants promoting EIAs, as well as NGOs, EIA advisors and administration commissions responsible for supervising results of EIAs, should check if the otter has been properly considered in the course of an EIA (APPENDIX). The enforcement of legislation and environmental impact assessment regulations is necessary for all water infrastructure constructions (Ruiz-Olmo, 2001), especially large dams. Enforcement of environmental impact assessment procedures should involve the whole area affected by flooding operations (Ruiz-Olmo, 2001). Especially important is that the framework of EIAs and monitoring of large dams include all pre- and post-impact phases. Mitigation and compensation measures for otter, like habitat improvement, prey management, minimization of fragmentation, must be proportionate in scale and approach to meet the impact. These should include, amongst other actions already mentioned above, appropriate measures to ensure the subsistence and survival of the largest number of fish (Ruiz-Olmo, 2001), including, if necessary, fish passage systems that also contribute to otter presence in dams.

Passage systems implemented for aquatic fauna

Reservoirs should be equipped with structures that allow otters and fish to pass through the dam allowing up and down-river movements, in order to avoid the isolation effect. This should be obligatory in new constructions (Ruiz-Olmo, 2001).

Despite the scarce literature available on this subject, it is likely that otters use certain passage systems for other aquatic fauna, such as fish (Santo, 2004). Such systems, although not specifically directed to the otter may occasionally be used by the species. Therefore, these structures contribute to the maintenance of the aquatic habitats continuity between the upstream and downstream sections of the reservoir, and they facilitate the movements of individuals between sections.

The technical, environmental feasibility and potential adaptability of installed fish passes to be used by otters should always be confirmed. Main fish passes are fish ladders and bypass channels. A fish ladder is a structure that facilitates fishes' natural migration by transforming one high water leap in a series of smaller, surmountable obstacles. A bypass channel is designed to circumvent the stream barrier (Wildman et al., 2002). If the slope allows, it can consist of a small water line, or a series of pools (step-pools fishways).

Eurasian otters occasionally use fish passes to move between reservoirs sections. In all known cases these were installed in small hydropower passages. Examples include a step-pool fishway in river Zêzere Portugal called Janeiro de Cima, (AFN, 2003) and a fishway, in river Monnow, Monmouthshire, Country of Wales (EAW, 2010). Also, in Segre river (Cataluña, Spain), a 10m wall was hindering the otter recolonization downstream. The construction of a fish ladder allowed otters to bypass the dam and the population to expand beyond this obstacle (Ruiz-Olmo and Delibes, 1998). In the Massíf Central, an elevated region in south-central France consisting of mountains and plateaus, an otter population could not move through a small 3m dam wall in a narrow valley (Ch. Bouchardy, pers. com. in Ruiz-Olmo and Delibes, 1998). The problem was solved by installing a small passage for the otter, and the population colonized the entire region downstream. The otter response to this type of measures is usually quite immediate.

Bypass channels can also be constructed to reduce the dam barrier effects for species other than fishes, like the Iberian Desman *Galemys pyrenaicus*. For the otter to use such a structure, these channels should maintain the natural features, including form and building materials, connecting an up stream stretch of river to the downstream river via a channel internally lined by rocks. These passages, by allowing the colonization of plant species, provide shelter and tend to form a continuum with the natural habitats. However, they are more functional and efficient in small hydroelectric power plants and small dams. There are also examples of the efficiency of such structure for other otter species that demonstrated the use of artificial passes; *Lutra canadensis* was reported to use a fish ladder in the United States (e.g. fish ladder at Fairmount Dam; FWWIC Fish Ladder, 2005).

However, high dam heights involve the construction of very long fish passes, consisting of a large number of basins/pools, which derails their construction, and limit its feasibility and efficiency. Moreover, their construction can be extremely costly, often with low cost-effectiveness rates. For higher walls, the only efficient fish passes are fish-elevators and this is not a solution for otters.

The otter is also known to use some paths with terrestrial vegetation surrounding the smaller (and even larger) dams, usually aimed at agricultural uses and not electricity production, and most common in southern Portugal. In four of the dams studied in this thesis (Lucefécit, Roxo, Campilhas and Alvito), whose walls had gradual slopes and parallel paths allowing terrestrial movements between the wall and the tributary downstream (Pedroso, unpublished data), otter cross-barrier movements were detected (Fig. V.1.1).

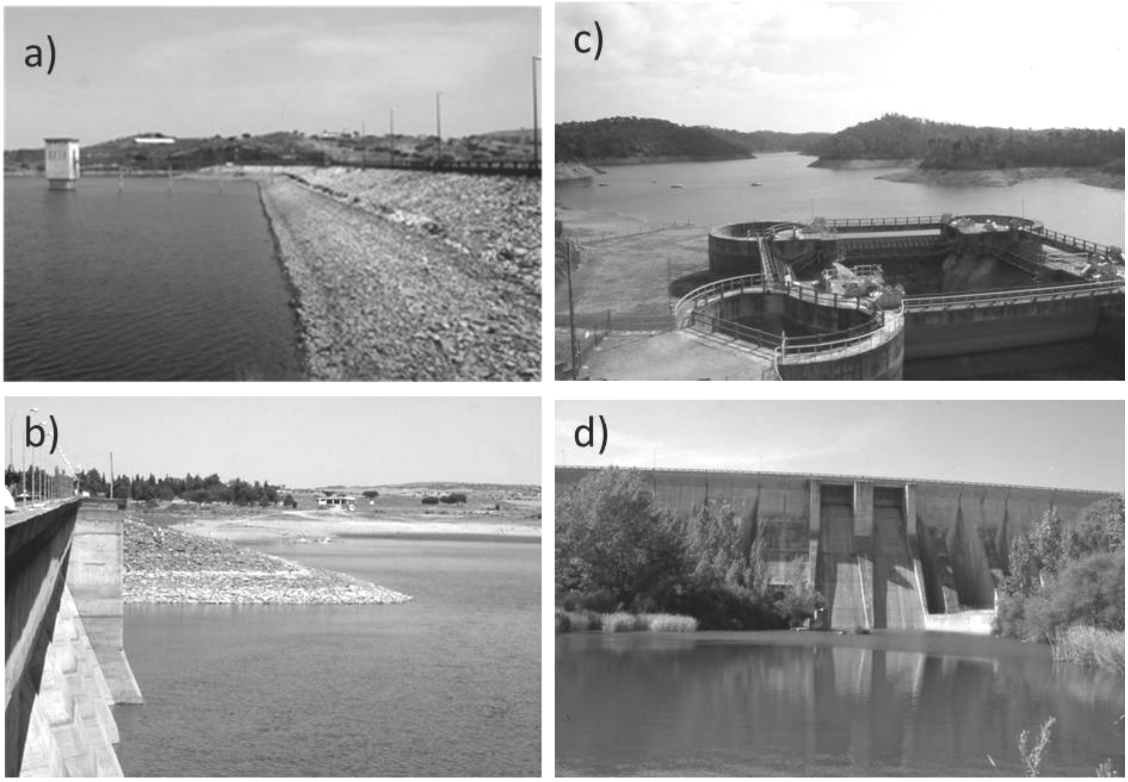

Figure V.1.1 – Wall of a) Lucefecit dam, b) Roxo dam (crossable by otter), c) Pego do Altar dam and d) Caia dam (uncrossable by otter)

The otter, though mostly aquatic, has the ability to move inland, and may travel considerable distances during dispersal or to surpass obstacles that disrupt the river continuum. However, data show that these paths are used only over fairly small distances, in areas of minimum disturbance, to minimize the probability of otter being detected outside their natural environment, where they are more vulnerable (unpublished data).

This scenario was also described in Spain, in the Ordesa National Park. There, the mountainous terrain produces steep waterfalls along narrow, often serial long stretches. These obstacles did not prevent otter movement through terrestrial routes used by people around the waterfalls, as confirmed by several otter tracks and the observation of one individual (Ruiz-Olmo et al., 2005b).

VI.4. Future needs of research

The ecological information collected throughout this thesis is expected to represent a strong contribution to otter ecology and their use of highly changed environments (dams). However, several specific questions, some of which deriving from the data collected, were left unanswered and should be addressed in future studies.

Based on all the information already available for Mediterranean areas (e.g. diet, habitat use, distribution – see PART I – I.2) and the present data on otter presence and use of reservoirs, future studies and conservation actions should concentrate on otter breeding, feeding and fitness. These should include population density estimates, in order to assess whether the number of individuals using a reservoir changed, when compared to the pre-dam situation. Furthermore, analysis of spatial and landscape use is also a fundamental issue to understand the otters' response to disturbance and their use of non-optimal habitats, such as dam reservoirs. Live trapping and radio-tagging of otters is the usual way to assess population abundance, and home range size and shape. Nevertheless, the methods needed to capture otters (leg-hold or foot-traps) were not considered legal in Portugal at the time of the conduted field work in the present thesis. The Portuguese Institute for Nature Conservation and Biodiversity (ICNB) was not issuing capture permits, in spite of the fact that studies showed successful results with low injury rates (Saavedra, 2002). Other approaches are needed to compensate for this missing abundance/density data. Examples of such approaches are direct observations, multi-dimensional characterization of footprints and molecular scat analyses.

Direct observations have been used to assess otter number in a given river/coastal stretch, but they are limited to a few habitat types and regions (e.g., Shetland – where otters are diurnal; Kruuk, 1995), or the method involves a considerable number of experienced observers and special habitat features (Ruiz-Olmo et al., 2001). On the other hand, footprints are not detectable in all substrate types, causing identification problems. More recently one the most widespread non-invasive technique is to genetically identify individuals through molecular scat analyses. This has been shown to yield unbiased estimates of population composition and sex ratio (Dallas et al., 2003). However, DNA analyses of scats or hair samples are still quite laborious and expensive. This approach was only been recently used in Portugal, but results are site-specific (Sales-Luís et al., 2009).

Molecular analyses would also allow addressing possible fragmentation effects caused by dam walls. For the present thesis field and lab work was implemented to address this problem, but results were not conclusive, as DNA extraction success was low and limited funds constrained analyses.

Ruiz-Olmo at al. (2005a) showed that stream sectors where otters breed and rear cubs are often subject to human disturbance (e.g. forest fires, gravel extraction, roads, reservoirs) and to drastic changes due to natural agents (e.g. catastrophic flooding, heavy snowfall). This means that special attention needs to be devoted to these sites if otter populations are to be conserved and correctly managed. Future studies should determine if and where otters breed in reservoirs, aimed at mapping rearing sectors within the dam. Special attention should be given to islands and adjacent streams as possible breeding sites.

Further research is also required on the effect of the reservoir environment on otter fitness. Reservoirs are characterized by different prey communities (with direct effects on otter diet), more challenging conditions for catching prey (with possible longer and deeper dives), human disturbance (e.g. water sports, fishing) and greater exposure to water pollution (e.g. cattle scats, eutrophication, agriculture run-off, boat fuel and oil spills). All these features may alter otter's fitness and should be investigated to assess the potential for populations to exist and reproduce in such disturbed areas. Specific studies on the consequences to otters of the major shifts in prey communities (e.g. increase of non-native species abundance, especially in the case of the American crayfish) are also crucial to assess the impacts of dam on otter fitness. Furthermore, there is contamination from heavy metals and other sources of pollution, for example, in the Sado river basin (Moreira et al., 2004; MAOT/ARH Alentejo, 2011) and Henriques (2010) reported metal accumulation in American crayfish. How this affects otter breeding success and cub survival in Mediterranean areas is, *per se*, a matter of conservation priority.

Water pollution, environmental contamination and transfer of antimicrobial resistant bacteria are also a potential problem to wildlife and public health. Resistant bacteria may reach new hosts via several pathways: via surface water resources, which are used as drinking water by humans and animals, for irrigating agriculture fields and for recreational activities; or via fertilizers application to farmland soils, which may be responsible for the migration of resistant bacteria to ground and surface waters. Available information (including data presented in this thesis), suggests that current treatment processes applied to the dam water and to the wastewater from farming and agricultural activities may be unable to prevent the dissemination of antimicrobial resistant bacteria into aquatic environments, as they are unable to completely remove or inactivate all potential pathogenic bacteria. Bacteria clonality and resistance traits should be taken in consideration in risk assessment and decision support for intervention, management and conservation of wildlife, particularly in environments with high cattle density near aquatic systems. The collection of a larger set of otter fecal samples will allow genetic characterization and clonality studies on the bacterial isolates. This may elucidate the origin of the high antimicrobial resistance, the environmental routes of antibiotic resistance dissemination, and also the potential role of wildlife as vectors of antimicrobial resistance.

Large dams are still being built in several countries (e.g. in Mediterranean countries such as Portugal or Spain, or in Asian countries such as India or China). In the near future and with increasing environmental concerns, more studies of otters and large dams are expected to be developed, most of them linked to EIAs of dam implementation, and the resulting knowledge will allow better adjustment measures for otter conservation. Adaptive management of the dams should take into account the ecological impact of these infra-structures, in order to effectively marry nature conservation with the economic viability of dams and the energy and water needs of each country.

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APPENDIX

IUCN Otter Specialist Group: Otters in Environmental Impact Assessments Recommendations

Target audience

These recommendations are intended to guide developers and consultants preparing environmental impact assessments (EIAs), as well as NGOs and EIA advisors (biologists and lawyers) in administrations, who have to check that the otter has been properly considered in the course of an EIA for a development according to the amended Council Directive 85/337/EEC.

Background

The otter (*Lutra lutra*) is listed in annex II and IV of the Habitat Directive 92/43/EEC which has implications and obligations for developers within the EU. The Habitat Directive implies that the species and its habitats, including corridors connecting them, must be considered in EIAs throughout the EU territory, and not only in SACs. The future needs of otters should also be considered in areas which have yet to be recolonised, for instance to ensure that the routes they use along water ways are not obstructed and so impede movements**.** EIAs for otters should always be carried out by suitably qualified ecologists acquainted with otter ecology and relevant field work. An integral part of these recommendations is an appendix giving a brief description of the biology of the species and methods used in surveys, including details of any assumptions made and limitations of the methodology used.

Otter habitat

Otter habitats cover all wetlands and aquatic ecosystems, both fresh water and coastal. They comprise the water body plus a strip of bank or coast at least a 100 m wide. For natal holts, this distance, especially in coastal areas, can be much greater.

Habitat assessment

Features potentially affected by development are 1) food species and feeding areas, including the movements and migrations of the food species; 2) resting sites; 3) breeding areas including natal holts, i.e. dens where cubs are born; 4) corridors for movement and dispersal, 5) permanent accessibility to fresh water.

For food and resting sites, the sensitivity of an area affected by a development depends on its (the development's) extent in the context of otter home range sizes in that area. Independent of home range size, breeding sites, movement corridors and areas of permanent access to water are always important for otters.

Each of these five habitat features must be assessed in any EIA. Where they are not relevant, this should be explicitly stated. In most cases assessment should be based on field surveys, although it may be possible to obtain information from other sources.

To properly assess the importance of habitats for otters, field studies should take place during at least two different seasons. In case of major developments, the monitoring must cover all four seasons to determine the *status quo* before the evaluation takes place and mitigation or compensation measures are formulated. Where it is likely that the timing of a development will have significant effects, for example, where natal or breeding areas are known to exist, these areas should be surveyed again immediately prior to work commencing.

Otter status, distribution and population trend

Consideration of status, distribution and population trends are essential, because they influence the impact of a development. The conservation status should be considered at a regional as well as a local level. The regional approach sets the local situation in the context of the larger population. For example, does the development take place in the core area of a population or on the edge in isolated populations, or in an area where otters may be expected to occur in near future?

Information on status and population trends is often available through published and unpublished reports (e.g. Article 17 reports under the Habitat Directive). If such information is not available, appropriate surveys must be carried out. In addition, the actual status of the otter in the area directly affected by the development must be determined. When practical, discussions should take place with local people, familiar with the area and with an understanding of the distribution of the species.

Impact of the development

The assessment of the impact on ecological functionality of aquatic habitats, adjacent areas and otter populations must take into account 1) conservation status, 2) food supply, 3) resting sites, 4) breeding areas, 5) corridors.

The EIA should use this information as the basis on which to assess the potential for changes to the population, its conservation status and viability.

The methods to be used in an assessment should be determined by an otter specialist and should take into account the particular situation of a development.

Mitigation / Compensation measures

Mitigation and compensation measures must be proportionate in scale and approach to meet the impact.

Monitoring

During the construction phase an environmental clerk of works should be appointed to oversee and monitor the quality of work carried out and that person must seek advice from otter experts.

Where approval is given for a development to proceed, subject to the inclusion of mitigation work for otters, it should be a requirement that monitoring of the effectiveness of the mitigation is undertaken after completion. Where appropriate, this should include reference studies undertaken before work begins to provide baseline data with which to compare the results of surveys during and post-construction.

Assessment review

Where an EIA is being reviewed and the reviewer is not familiar with otters or has concerns about what has been written, advice should be sought from appropriate experienced ecologists acquainted with otter ecology and relevant field work.

Appendix

Biology and ecology of the Eurasian otter (*Lutra lutra*)

Otters are highly specialised carnivores preying mainly on fish, but also on amphibians, crustaceans, insects etc. Otter habitat comprises not only rivers, tributaries, lakes, estuaries and coastlines, but also small trickles, springs, bogs, swamps, ditches, artificial channels and all kind of man made water bodies such as reservoirs and fish ponds. In addition, otter habitat includes suitable corridors, sometimes over dry land, between adjacent water bodies, through which otters move. Bank and river/sea bed structures and water depth are important parameters for the availability of prey. Deep water bodies and those with no or few structures in the water, where prey can hide, characterise suboptimal habitats. In contrast visibility of the water is not a prerequisite; in most areas, the species being nocturnal. Otters are not limited to pristine habitats; they may be found anywhere, including cities and industrial complexes as long as food and other key habitat factors are available. During the 21st century, recovering otter populations have been be observed in many regions of Europe. Increased population pressure is usually the reason for the re-colonization of abandoned areas. This may lead to the occurrence of otters in sub-optimal habitats.

There are places in Europe where otter are absent at present, but are likely to colonise in the future. The needs of the species should also be taken into account here. It is of particular importance that roads and other developments are designed to ensure that when otters do re-colonise these areas, they are able to move freely and safely through their habitats.

Since otters spend a considerable time in the water and have rather poor fat reserves, they are highly vulnerable to starvation. The permanent availability of food is therefore crucial. The availability of prey may undergo significant seasonal changes (ice cover, droughts, etc.). The size of home range depends on the availability of food and other key habitat factors, such as fresh water in marine habitats (in a marine environment, the Eurasian otter needs fresh water to get rid of the salt in the fur in order to maintain body temperature), holts and breeding areas. In order to give an idea of the areas involved, home ranges of females can cover 5 -20 km of river length plus the adjacent tributaries, while males can be twice as large. In marine habitats, estuaries and cultural landscape with artificial food supply (fish farming), home ranges may be smaller.

Otters can give birth at any time of the year. Females take care of their cubs at least for one year. Natal dens may be located relatively far from water. Disturbance of the rearing female during the first year, especially when cubs are not yet able to search for food, may result in their abandonment by their mother and subsequent death by starvation. Sub-adults, freshly independent of their mother, often depend on readily available prey such as amphibians, crustacean, insects and certain slow moving fish species. Thus it is not only the overall availability of food, but specific prey items (buffer food), which may have a significant effect on the wellbeing of otters.

Due to their adaptations for a semi-aquatic life style, otters are less mobile on land and this makes them more prone than many other small to medium sized carnivores to be killed by cars.

Methods and approaches

General

Developments may affect otter habitat at scales from a few tens of metres to several tens of kilometres. We cannot be prescriptive here but would anticipate that the level of survey work involved in an EIA for otters would be proportionate to the scale and potential effects.

Otter presence, status, densities

Otters produce spraints (scats, faeces) which are characteristic of the species. Their presence is a simple and reliable indicator for otter presence, but on a small scale the absence of such signs does not necessarily mean that there are no otters in the area. Spraint numbers cannot be used to determine otter numbers. However, spraint sites with several spraints of different age indicate the use by otters over a period of time; in contrast single or a few very old spraints may originate from transient animals, possibly in sub-optimal areas or those not yet colonised (special caution is necessary since breeding females tend to leave no signs of their presence until the cubs are two months old). The durability of spraint is affected by weather conditions (rain, snow, vegetation growth, falling of leaves, tide). Seasonal changes in marking behaviour by the otter can also influence what is found during a survey. This has to be taken into account particularly when undertaking consecutive surveys, where results are compared in order to indicate use of otters / the success of a mitigation or compensation measure. Questionnaires and discussions with local people (fishermen, hunters, foresters, land owners) can be unreliable and should only be used in combination with other methods.

Status, densities and population trend may be available through published and unpublished reports (e.g. also article 17 reports according FFH-Directive of the EU). The question of status and trend in most instances, however, refers to relatively large areas. If such information is not available, surveys may need to be carried out covering at least several hundred square kilometres. In each 10 x 10 km square at least four sites must be checked for signs of otter presence. Such a site can be a stretch of up to 600 m of bank length or a suitable bridge depending on the survey method adopted.

In addition, an assessment of the density of signs may be made in a study area by calculating the number (spraints, spraint sites, dens, tracks etc.) per kilometre of bank searched. Variations in this may be detected by carrying out repeated standardised searches. Interpretation of these data should be made with caution since they are likely to be highly influenced by seasonal aspects such as snow or ice cover as well as the extent of vegetation cover and the sprainting behaviour of the individual otters when comparing different seasons and by the nature of the habitat when comparing sub-areas at a given time.

Females with dependent cubs may be identified by searching for tracks in appropriate substrate, by direct observations (visual and audible) and the use of remote cameras. Indications for absolute otter numbers may be derived from genetic analysis of scats, by snow tracking and under special habitat conditions (e.g. Iberian Peninsula) by direct observations.

Habitat

The habitat functions (food, day resting sites, breeding areas and corridors) must be investigated in the field by searching the bank or shore line for otter signs (tracks, spraints, rolling places, trails, food remains) and structures under water as well as the bank itself (potential for above and below ground resting sites). Depending on the area affected, the availability of food may be estimated by otter spraint analysis, electric fishing or reference to existing, recent reports. Consideration should be given to the potential presence of natal holts (i.e. dens where cubs are born and where they can remain for up to ten weeks). Such sites are often found some distance from water, may have few, if any, evidence of otter presence and are consequently difficult to identify. Similarly important are rearing areas, where cubs stay after having moved from the breeding areas. They are found closer to the water, frequently amongst very dense vegetation and are usually near areas with a rich food supply. Both, natal holts and rearing areas are key determinants of the status of otters with long-term implications at local and regional level if they are adversely affected.