Linking quarries and its surroundings by restoration ecology for semi-aquatic mammals

Final Project Report

1. Contestant profile

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2. Project overview

Title:	Linking quarries and its surrounding by restoration ecology for semi-aquatic mammals.
Contest:	The Quarry Life Award, 3rd Edition 2016.
Quarry name:	Áridos Sanz gravel quarry. La Cistérniga, Valladolid, España.
Prize category:	☐ Education and Raising Awareness ☐ Habitat and Species Research ☐ Biodiversity Management ☐ Student Project ☒ Beyond Quarry Borders

ABSTRACT

The extraction of materials in gravel quarries can produce the emergence of aquatic habitats which represent an opportunity for biodiversity enhancement. The ecological connectivity of these habitats with its surroundings is key for its stability and sustainability. In this context, the main goal of the project was to understand and enhance connectivity and biodiversity of gravel quarries lagoon systems and its surroundings. We have used the Eurasian otter (*Lutra lutra*), that were to our knowledge evidence of presence in Áridos Sanz gravel quarry, as indicator species to address the analysis of the state and connectivity of the quarry lagoon system. Aiming to develop a broad perspective, the project consisted of three interrelated scientific studies of the lagoon system and its surroundings in an area of around 700km^2:

1. Otter population study, which include three complimentary analysis: a) analysis of camera-traps pictures, b) analysis of spraint marking intensity, and c) genetic analysis of spraints to identify individuals. We set 19 sampling transects, 600-metres length each, where we took into account all types of water bodies: artificial lagoons, ditches, Duero River, and irrigation channels.
2. Habitat suitability analysis for otters using multivariate statistical methods which integrate the environmental features from sampled areas with the results of the otter population study.
3. Ecological connectivity analysis using Geographical Information Systems.

Our project proves that the extraction activity of Áridos Sanz gravel quarry is compatible with high local biodiversity in the lagoon system. The establishment of an otter family in the lagoons confirms that this type of habitat can be suitable for breeding and the subsequent dispersion into the surroundings, reinforcing the source-sink dynamic of the territory. In addition, the genetic analysis of spraints provides information regarding the otter population structure and otters home range in the study area, revealing that the lagoon systems is an essential area for the species. However, the high presence of the invasive red swamp crayfish (*Procambarus clarkii*) in the otter diet might be reflecting a low ecosystem stability which should be monitored. Regarding habitat suitability, our models show that forested buffer areas, and vegetation cover of water sides, are key to reduce the negative impact of human disturbances on otter activity.

We have verified that habitat connectivity is vital for the integration of restored ecosystems into its surroundings, and that artificial water channels are used by otter as corridors between core areas of their territory. Consequently, artificial channels should be included and managed in restoration projects. Finally, we stress that Geographical Information Systems is an ideal tool to analyze processes at landscape scale, due to facilitation of the selection and prioritization of optimum areas for the development of corridors and implementation of measures that enhance connectivity, this way contributes to the integration of restored areas into its surroundings areas.

Finally, this project stresses that the use of scientific tools can build bridges of understanding between environmental management, science and economic activities, which ultimately creates synergies among scientific, technical, social and business sectors.

FINAL REPORT

1. INTRODUCTION AND OBJECTIVES

The extraction of materials in gravel quarries can produce the emergence of artificial water bodies which well-managed could be colonized by several species, many of them with some degree of threat. The occurrence of these aquatic-terrestrial habitats are scarce and vulnerable in Mediterranean ecosystems, and provides an opportunity for enhancing biodiversity and conservation. A key aspect of aquatic ecosystems in general, and specifically of human-created gravel quarry habitats, is its ecological connectivity with the surrounding habitats. This connectivity ensures migration, reproduction and genetic interchange processes, which are vital for ecosystems stability and sustainability.

The main goal of the project was to improve the understanding of the integrated functioning of this type of systems through the understanding and enhancement of connectivity and biodiversity of gravel quarries lagoons systems and its surroundings.

In this context, due to their habitat requirements, semi-aquatic mammals are good umbrella species for semi-aquatic ecosystems, and therefore are a sensitive indicator of the ecosystems state. For this reason, we took advantage that in Áridos Sanz gravel quarry there were evidences of presence of Eurasian otter (*Lutra lutra*) to use this species as an indicator for analyzing the state, conservation, and connectivity of the gravel lagoon ecosystem and its surroundings.

2. PROJECT APPROACH AND METHODOLOGY

The project uses a comprehensive approach to understand the functioning of a gravel quarry lagoon system in a landscape context. For this purpose, we analyzed if the lagoons integrate in the surroundings (through a connectivity analysis), and if they generate opportunities for biodiversity conservation. The project have followed a **restoration ecology approach**. Different analysis were used to monitor and evaluate the state of the restored ecosystem, which allowed us to make applied recommendations for the adaptive management of the gravel lagoon system. Because we aimed to develop a **wide perspective**, we used **several approaches and techniques** that are grouped in the **three interrelated studies** that compose this project (*Figure 1*):

1) Population analysis of the otters living in the lagoon system and its surroundings. This analysis is divided in **three complementary analysis**:

a) **Analysis of camera-trap images**, which allowed us to analyze otter behavioral patterns and habitat selection within the lagoon system, as well as to provide information about the macro-fauna biodiversity in the gravel quarry.

b) **Spraints marking intensity analysis**, within, near, and far from the lagoon system, which estimated the otter's intensity use of the different available habitats and complemented the territory connectivity analysis.

c) **Genetic analysis of spraints**, which identified otter individuals and their sex, as well as allowed us to estimate population size and structure, and to investigate current connectivity of the otter territory.

2) **Otter habitat suitability analysis** in the gravel quarry system and its surroundings, which integrated the environmental features of the sampled areas and the results from the otter population analysis. The information gathered of the habitats characteristics selected by otters, allowed us to propose specific applied measured to improve the quality of restored ecosystems.

3) **Connectivity analysis** of the lagoons system and its surroundings, analyzed by means of Geographical Information Systems, revealed the contribution of lagoons to the territory connectivity at different spatial scales, and helped to identify barriers between water bodies (e.g. due to the lack of permeability of the territory).

2.1. Sampling design

Transects. We performed 19 transects 600-metres long in our study area of around 700km^2. These transects were used for the analysis of spraints marking intensity, genetic analysis of spraints and habitat suitability analysis. Transects were located both within and outside the gravel quarry lagoon system, taking into account all the exiting water bodies in the area: artificial lagoons, ditches, Duero River and irrigation channels. **Inside the gravel quarry we defined 11 transects** (*Figure 2*): six in the lagoons, two in the Duero River at the South edge of the gravel quarry, two in the river irrigation channel at the North edge of the gravel quarry, and one in the ditch which connects the irrigation channel with the Duero River along the West edge of the gravel quarry. **In the gravel quarry surroundings we defined 8 transects** at two different distances from the gravel quarry, which were defined based on the mean otter home range in Mediterranean ecosystems (i.e. 20-40 river km) (*Figure 3*). We sampled four transects both in the Duero River and in the irrigation channel: 5 and 15 km East and West from the gravel quarry.

Sampling campaigns. Between April and July, we carried out **four sampling campaigns for 3 consecutive-days per month**. In each campaign, the same two observers walked every transect searching for otter spraints (*Figure 4*). Spraint locations were geographically referenced with a GPS device. We recorded the spraint condition: fresh, semi-fresh, or dry (*Figure 5*). Fresh spraints were collected for genetic analysis. We also visually identified the spraint prey content and classified it as: mainly fish, mainly crayfish, or crayfish and fish. Finally, we characterized the habitat features of each transect for the habitat suitability analysis.

3. POPULATION ANALYSIS

3.1. Analysis of camera traps images

Specific Methodology. The camera trap survey focused in the gravel quarry lagoon system and its surroundings, where we set **10 camera traps** in sites with evidences of otter presence (i.e. spraints, paths and resting sites,

Figure 6). The **Middle Lagoon** was intensively monitored due to its particularly high presence of otter signs. Camera traps were checked every sampling campaign, and those without otter pictures were moved to a new site. We carried out two analysis; firstly, we analyzed the intensity of otter presence in the different sites, and secondly, we analyzed the temporal distribution of otter activity. In addition, we identified all the individuals captured by the cameras and listed all species identified in the gravel quarry.

Results and discussion. In total, **3199 pictures and 417 videos** of animals were taken. Otters were located in 75% of the sites, although the intensity of use varied greatly among lagoons. In particular, the highest intensity of otter captured by camera-traps were in the Middle Lagoon and in the end of the ditch where it joins with the Duero River.

One of the main findings of the camera-trap survey was the confirmation that **otters are breeding in the lagoon system of the gravel quarry** (*Figure 7*). Furthermore, we observed that the otter family used the lagoon system when the cubs are still small. During this phase females select quiet areas with abundance of food and shelter. Later on, when the cubs gain a larger size, they extended their home range, exploring the surroundings, and they were sight often in the joint of the ditch with the Duero River (*Figures 7, 8, and 9*). The videos we have managed to record provided a valuable information about otter behavior during breeding season, including the recorded of images never seen until now in Mediterranean ecosystems (https://www.youtube.com/watch?v=MmH4hsgDGOM). The **selection of the gravel quarry lagoon system by otters for breeding reveals its good ecological state and the success of its restoration**. Another finding of particular importance for the design of lagoon systems in gravel quarries is the confirmation that **otters are using the ditch**, **even** the days **when it is dry** (*Figure 10*).

Finally, the camera trap study allowed us to detect the presence of many other animal species in the gravel quarry. We were able to identify 35 animal species, including otters (*Figure 11*). Particular remarkable is the **identification of the water vole (*Arvicola sapidus*)**, which is currently endangered (UICN Red List, 2016), and which presence in the gravel quarry has been confirmed as a results of this project.

3.2. Sprains marking intensity analysis

Specific methodology. We developed **2 indicators of the intensity of use of the territory by otters** based on the spraints collected in the 19 transects: a) **Total spraint intensity** (total number of spraint /100m), and b) **Fresh spraint intensity** (number of fresh or semi-fresh spraints/100m)

Results and discussion. We found otter spraints in all the sampling transects within and outside the gravel quarry, except one, although the **spraint intensity varied greatly among locations and sampling campaigns** (*Figure 12*). The gravel quarry locations, especially the **Middle Lagoon and the ditch**, were the locations with the **highest marking intensity** (*Figures 12, 13 and 14*), possibly due to the presence of the otter family observed in the camera traps survey. River Duero transects had an intermediate marking intensity, except for those transects located in, or very close to town centers (15km West and 5km East). The transects in the irrigation channel had the lowest marking intensity. Otters possibly visit the irrigation channel occasionally, using it as a corridor between territory core areas, because the channel might not be an optimum habitat due to water side degradation and lack of sheltered sites. Finally, the discrepancy of marking intensity between sampling campaigns implies that **repeated samplings** in population studies of animal species, especially in restored ecosystems, are needed in order to have a robust estimate of the use of the territory by the species.

The analysis of spraint content showed that the red swamp crayfish (*Procambarus clarkii*) is very important for the otter diet in the area, in particular in the lagoon system where 99% contained mainly crayfish (*Figure 15*).

3.3. Genetic analysis of spraints

Specific methodology. The genetic analysis of otter spraints was implemented following the methodology developed by Vergara et al. (2014)[1], which consisted of four stages: a) DNA extraction, which was carried out using the DNA purification Stool kit 4015-02 of Omega Biotek, b) otter DNA replication using a set of 11 microsatellites grouped in four multiplex, c) sex identification by DNA typification, and d) DNA fragments analysis, which is the electrophoretic analysis of PCR results. DNA extraction phase was implemented by the project members in the laboratories of the departments of Genetics and Zoology of the Biological Sciences Faculty of the Complutense University of Madrid, and the rest of stages were conducted by the Genomic-Proteomic Unit of the same University (*Figure 16*).

Results and discussion. A total of 55 fresh spraints were collected and analyzed. We got microsatellite profile with enough quality for individual identification in 28 samples (54% of total samples), which is within the normal success rate of this type of studies in Spain.

Overall, we identified 12 otter individuals, 7 females and 5 males (*Figure 17*). Five of these individuals were located only in the lagoon system and its close surroundings (i.e. ditch and irrigation channel and Duero River going through the gravel). These five individuals are likely to be the members of the otter family detected in the camera trap survey. Another six individuals were located only outside the gravel quarry: four individuals in the irrigation channel at different distances to the east and west of the gravel quarry. And finally, two individuals in the Duero River east of the gravel.

Finally, we want to highlight the location of a male otter (identified with a red triangle in *Figure 17*) found both within the lagoon system and its close surroundings, and in the Duero River as far as 15km East from the gravel quarry. The great mobility of this individual could have been because is a territorial alfa male. Nevertheless, this finding reveals the connectivity for otters of the gravel quarry lagoon systems and its close and far surroundings.

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[1] Vergara, M., Ruiz-González, A., de Luzuriaga, J. L., & Gómez-Moliner, B. J. (2014). Individual identification and distribution assessment of otters (*Lutra lutra*) through non-invasive genetic sampling: recovery of an endangered species in the Basque Country (Northern Spain). *Mammalian Biology-Zeitschrift für Säugetierkunde*, 79, 259-267.

4. HABITAT SUITABILITY ANALYSIS

Specific methodology. We explored the effect of 13 habitat variables on the spraints marking intensity as an indicator of otter territory use. In particular we used "total spraint intensity" as an indicator of the **total territory** of otters, and "fresh spraint intensity" as an indicator of **core areas of the territory** (see Section 3.2). **Habitat variables** were related with; *i*) **land use** at different distances from the gravel quarry, *ii*) **vegetation cover of water sides**, *iii*) **human disturbances**, and *iv*) **distance to roads** (*Figures 18 and 19*).

We carried out two types of analysis. Firstly, an exploratory analysis of the relationships between marking intensity and habitat variables, and between habitat variable to each other, using **correlation matrices** and **Principal Component Analysis** (PCA). And secondly, a **multiple linear regression models** to estimate the effect of habitat variables on marking intensity, taking into account the relationships between habitat variables to each other.

Results and discussion. Overall, PCA shows that the habitats with the highest use were those with high vegetation cover, surrounded by forested areas and far from roads (*Figure 20*). Total spraint intensity was closely related to forest areas while Fresh marking intensity was closely related with bush cover of water sides. Human disturbances, bare soil in water sides, and urban and agricultural land use, affected negatively to the otters use of the territory.

Multiple linear regression models identified the specific habitat variables with the highest effect on the territory use by otters. The habitat variables that showed positively related with the habitat quality of otter **core areas** were **bush cover of water sides**, and **forested buffer areas of 300m** wide around water bodies (*Figure 21*). Conversely, **human disturbances was negative related** with habitat quality of core areas. However, otters seemed to tolerate moderate disturbance levels if habitat quality is good enough (*Figure 22*). Regarding the influence of habitat variables on total otter territory, forested buffer areas and human disturbances were still key factors of habitat quality, however bush cover of water sides did not have a significant effect (*Figure 23*). This is possibly due to the fact that total territory includes fauna passage areas, which are used by otter to move between core areas, and therefore the state of its water side would not be determinant.

5. CONNECTIVITY ANALYSIS

Specific methodology. We analyzed two aspects of the ecological connectivity of the gravel quarry lagoon system with its surroundings by using **Geographical Information Systems** (GIS): a) the **functional connectivity** of the territory, which is the degree to which the landscape facilitates or impedes movement of species among aquatic habitats, and b) the **permeability of the matrix to the presence and dispersion of otters**, which is how easy is for otters to move through the territory among water bodies.

To model the effect of the lagoon system on the territory functional connectivity, we used **two connectivity indexes** using the graph theory (Conefor 2.6 software): the Equivalent Connected Area (ECA) and the Probability of Connectivity (PC). In particular, we calculated the connectivity of the territory before and after the existence of the lagoon system at **two geographic scales**: 104 km^2 and 724 km^2 (*Figures 24 and 25*).

Regarding the matrix permeability analysis, we developed a **spatial permeability model** composed of **three components**, which were selected based on the results of the habitat suitability analysis: **a) land uses, b) distances to roads, and c) distances to water bodies**. In GIS terms the model consisted of three raster layers with a 10x10m pixel size resolution. The three layers were developed using a six-category model in which pixels were assigned to a 1-to-6 value, from less to more favorability to the movement and presence of otters. The categories of the land use layer were: water bodies (6), forests (5), pastures (4), recreational (3), agricultural (2) and urban and industrial (1) (*Figure 26*). Categories for the layers of distance to human disturbances and water bodies were calculated based on the Euclidean distances to infrastructures (buildings and roads) and water bodies, respectively (*Figures 27 and 28*).

Results and discussion. The analysis of the territory functional connectivity showed that the creation of the **gravel quarry lagoon system increased remarkably the territory connectivity**. Specifically, the lagoon system increased ECA by 17.4% and PC by 27% at the minor scale, and by 4.4% (ECA) and 8% (PC) at the large scale (724km^2) (*Figures 29 and 30*). The effect of the lagoon system on the territory connectivity was very similar regardless of the otter dispersion distance used (5, 7, and 15km). This result implies that, in this case, connectivity is independent of dispersion distances, which for otters varied with sex and season.

The results of the **final matrix permeability model** was a map which facilitates, not only the evaluation of the territory permeability for otters, but also the identification of the best habitat for this species, becoming a **sensible and useful tool for action planning aiming to enhance connectivity between optimum areas** (*Figure 31*). For the specific case of Áridos Sanz gravel quarry, this final map shows that permeability is highest to the South, and once in the Duero River, the best habitats are found eastwards. Therefore, restoration actions with the highest potential to enhance the lagoon system connectivity with its surrounding, should be carried out in this direction.

6. GENERAL DISCUSSION

The use of a variety of techniques and the integration of the findings of the population analysis, habitat suitability analysis and connectivity analysis, has allowed us to understand ecosystem processes and functions of the restored lagoon system, from the local to the landscape scale. Based on this comprehensive approach and the scientific information generated here, we are in a position to suggest recommendations, following ecological restoration criteria, for the enhancement of biodiversity and connectivity of gravel quarries lagoon ecosystems and its surroundings.

Firstly, our project shows that Áridos Sanz gravel quarry current extractive activity is compatible with high local species biodiversity in the lagoon system. We have listed 35 macro-fauna species, two of which are autochthonous semi-aquatic mammals with certain degree of endangerment (UICN Red List, 2016): the water vole and the Eurasian otter. These findings are of particular importance, since they show up that **successfully restored gravel quarries can become biodiversity hot-spots and a wildlife refuge for indigenous species**.

The otter has proven to be a **suitable indicator species** to evaluate the ecological state of the restored gravel quarry and its surroundings. We have found that otters are using the gravel **lagoon system as a breeding area**, which therefore provides a suitable habitat for otter reproduction, **modifying the source-sink dynamic** of wildlife in the territory. In total, we have identified **12 otter individuals** in the studied area which gives a linear abundance of 0.18 otters/km. Five of these individuals are located only in the gravel quarry and its close surroundings, another six were found only in the far surroundings, and one both within the gravel and in its close and far surroundings. This last individual is likely to be a territorial alfa male moving throughout a connected territory. The **otter diet analysis** revealed the great importance of the **red swamp crayfish** in the area, especially in the lagoon system. The presence of this invasive species generates threats and opportunities for biodiversity that should be evaluated in future studies.

Regarding the habitat suitability models, our results indicate that the most influential factors for otter habitats are: 1) high **bush cover of water sides**, 2) presence of **forested buffer areas** around the water bodies of at least 300-metres wide, and 3) low **human disturbances.** However, **otters tolerate disturbances to a certain degree**, therefore, high quality habitat can minimize the negative impact of disturbances, and even become core areas of otter territory, as it happens in the gravel quarry lagoon system studied here. In the lagoon system, otter used lagoons, even very close ones, with different intensity, which might be due to differences in initial lagoon design and it later management. As an example, the Middle Lagoon might be the most favorable habitat due to its irregular shape with a sharp peninsula, the abundance of red swamp cray fish, the high quality water and a wide forested buffer area. Finally, we have shown that areas with low quality habitats, as the irrigation channel and the Duero River crossing through urban areas, are used by otters as passages areas among more suitable habitats. We interpret all these findings into **specific measures related to the design and enhancement of high quality habitats, which are presented in the following section**.

We should highlight that restored ecosystems in entropized areas are sensitive systems prone to suffer sudden short and mid-term changes on habitat quality, which could be substantial, even becoming **trap habitats** for wildlife, and specifically for semi-aquatic mammals. Therefore, it is essential to evaluate and monitor the stability of restored ecosystems to avoid biodiversity losses due to unexpected changes in environmental conditions.

Throughout this project we have shown that the **ecological restoration of gravel quarries lagoon systems significantly enhance territory connectivity** for semi-aquatic species. We have proved that the **water supply channel network** (irrigation channels, ditches, etc.) can be used as **ecological corridors** among restored lagoon systems and its surroundings, especially when water sides are naturalized and human disturbances intensity are low. For this reason, this type of infrastructures should be taken into account and managed in restoration ecology projects to improve connectivity of gravel quarries lagoons. Finally, we want to highlight that **spatial analysis of connectivity using GIS are a suitable decision-making tool** to integrate restored areas with its surroundings by informing the **selection and prioritization of best areas** to establish corridors and implement actions to enhance connectivity of the whole territory.

7. PRACTICAL IMPLICATIONS FOR ECOLOGICAL RESTORATION OF GRAVEL QUARRIES LAGOON SYSTEMS TO FAVOUR SEMIACUATIC MAMMALS

1) The ecological restoration of gravel quarries lagoon systems requires an integrated management of the lagoons, water sides and buffer areas to protect core habitats. The restoration process has to ensure the existence of water side vegetation cover (≥5m), and the establishment of forested buffer areas (≥300m wide), to minimize the negative effect of human disturbances on semi-aquatic mammals and other wildlife species.

2) The enhancement of connectivity increases the chances of success of local ecological restoration actions. Restoration projects have to consider the establishment and management of artificial water channels, including small ditches, due to its positive effect on connectivity and on ecosystems functionality. Projects have to include evaluations of the territory connectivity at a large spatial scale, which have to consist at least on the identification of the best habitats of a given area, together with a permeability analysis, with the final aim of planning actions and measures to facilitate wildlife movements.

Geographical Information Systems are a suitable decision-making tool that facilitate actions-decisions to integrate restored lagoon systems with its surrounding, and to identify best areas to establish ecological corridors.

3) An adaptive management approach: systematic and periodic monitoring and evaluation of restored ecosystems are essential to properly manage, if necessary, the state of restored ecosystems with a stable aquatic mammal populations. Some sensible indicators to evaluate lagoon ecosystems trajectories are: sudden changes of water quality/quantity or of environmental conditions, rate of habitat destruction, evolution of wildlife populations, and presence and evolution of invasive species. Monitoring studies should include habitat characterization to allow carrying out habitat suitability analysis for the species of interest, which in turn should inform actions to improve habitat quality.

Semi-aquatic mammals, especially otters, due to its habitat requirements, are suitable umbrella species and indicators of ecosystems condition. There are three validated non-invasive and complementary methods for monitoring semi-aquatic mammal populations: camera-trap surveys, feces marking intensity surveys, and genetic analysis of feces. Genetic analysis are of particular scientific and technical interest because it allows analyzing landscape connectivity, being the definitive proof of the movements of animal individuals in and out of restored areas.

The presence of invasive species, such as the red swamp crayfish generates both threats and opportunities for other wildlife species. Therefore, populations of invasive species should be systematically and periodically monitored and managed to ensure a proper functioning of ecosystems.

8. PROJECT ADDED VALUE AND POTENTIAL EXTENSIONS

We have designed and developed the project using a **scientific applied approach**: techniques and methodologies are scientific, however the final aim of the project was to provide **relevant information and management guidelines** for the ecological restoration of aquatic-terrestrial habitat in gravel quarries. To this end, we have produced two documents as the outcome of this project: **a) a detailed scientific report**, and **b) a technical handbook on restoration ecology for semi-aquatic mammals in gravel quarries and their surroundings**. Therefore, we believe that the **project outcomes are of interest to both the scientific community and the extractive and mining business sector**. Also, our project brings to light that the use of scientific tools can build bridges of understanding between environmental management, science and economic activities which benefits to all sides.

The extension of this project, or one of a similar approach, **to a greater number of gravel quarries** would be of great technical and scientific interest. By considering a greater heterogeneity of cases, such a project extension would generate results and **practical recommendations solid enough to be extrapolated to a wider variety of situations** (climates, water basin types, countries, etc.). The costs of this type of project would be around 18-20,000 €/gravel quarry and, at least, one year of duration.

The methodology and analysis approach used in this project could be applied as a whole or separately in other gravel quarries considering the establishment of lagoon ecosystem for its restoration. The methodology could be easily adapted to the use of other water-bodies-dependent animal or plant species as indicators.

Finally, based on the project results, the following actions and studies could be implemented in Áridos Sanz gravel quarry:

- **Monitoring the evolution of the gravel quarry otter population and of the lagoons habitat suitability** (6-7,000€/year).
- **Monitoring the red swamp crayfish** in the gravel quarry and evaluation of its impact on biodiversity and in the lagoon ecosystem stability (6-7,000€/year, 2-3 years).
- **Action plan to improve water sides and buffer areas of the lagoon system** focusing in the areas with the lowest otter use intensity. Establishment of an ecological corridor towards the Duero River to the South of the gravel quarry, improving the state of water sides in the key areas for otter connectivity. Costs of these actions are variable depending on the scale of the intervention.

Project tags (select all appropriate): This will be use to classify your project in the project archive (that is also available online)	
Project focus: ☒Biodiversity management ☐Cooperation programmes ☐Education and Raising awareness ☐Endangered and protected species ☐Invasive species ☒Landscape management - rehabilitation ☒Rehabilitation ☒Scientific research ☐Soil management ☐Urban ecology ☒Water management Flora: ☐Conifers and cycads ☐Ferns ☐Flowering plants ☐Fungi ☐Mosses and liverworts Fauna: ☐Amphibians ☐Birds ☐Dragonflies & Butterflies ☐Fish ☒Mammals ☐Reptiles ☐Spiders ☐Other insects ☐Other species	Habitat: ☐Cave ☐Cliffs ☐Fields - crops/culture ☐Forest ☐Grassland ☐Human settlement ☐Open areas of rocky grounds ☐Recreational areas ☐Screes ☐Shrubs & groves ☐Soil ☐Wander biotopes ☒Water bodies (flowing, standing) ☐Wetland Stakeholders: ☐Authorities ☒Local community ☐NGOs ☐Schools ☒Universities

Appendix. Tables y figures

Tables, figures and photos included in this Appendix section complement the information contained in the main body of the final project report.

Figure 1. Conceptual map of project analysis: data used in each analysis and areas were data were collected.

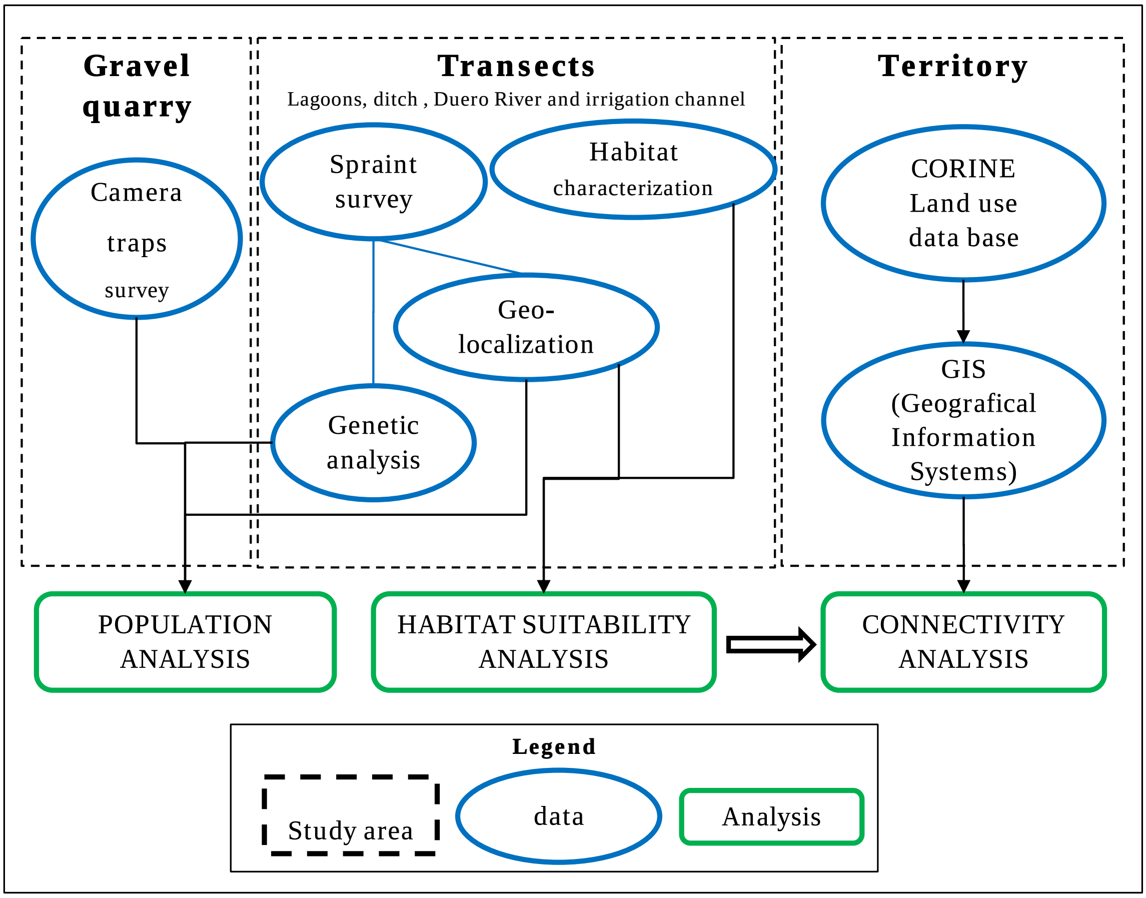

Figure 2. Location of sampling transects in the gravel quarry: lagoon system, ditch, irrigation channel and Duero River.

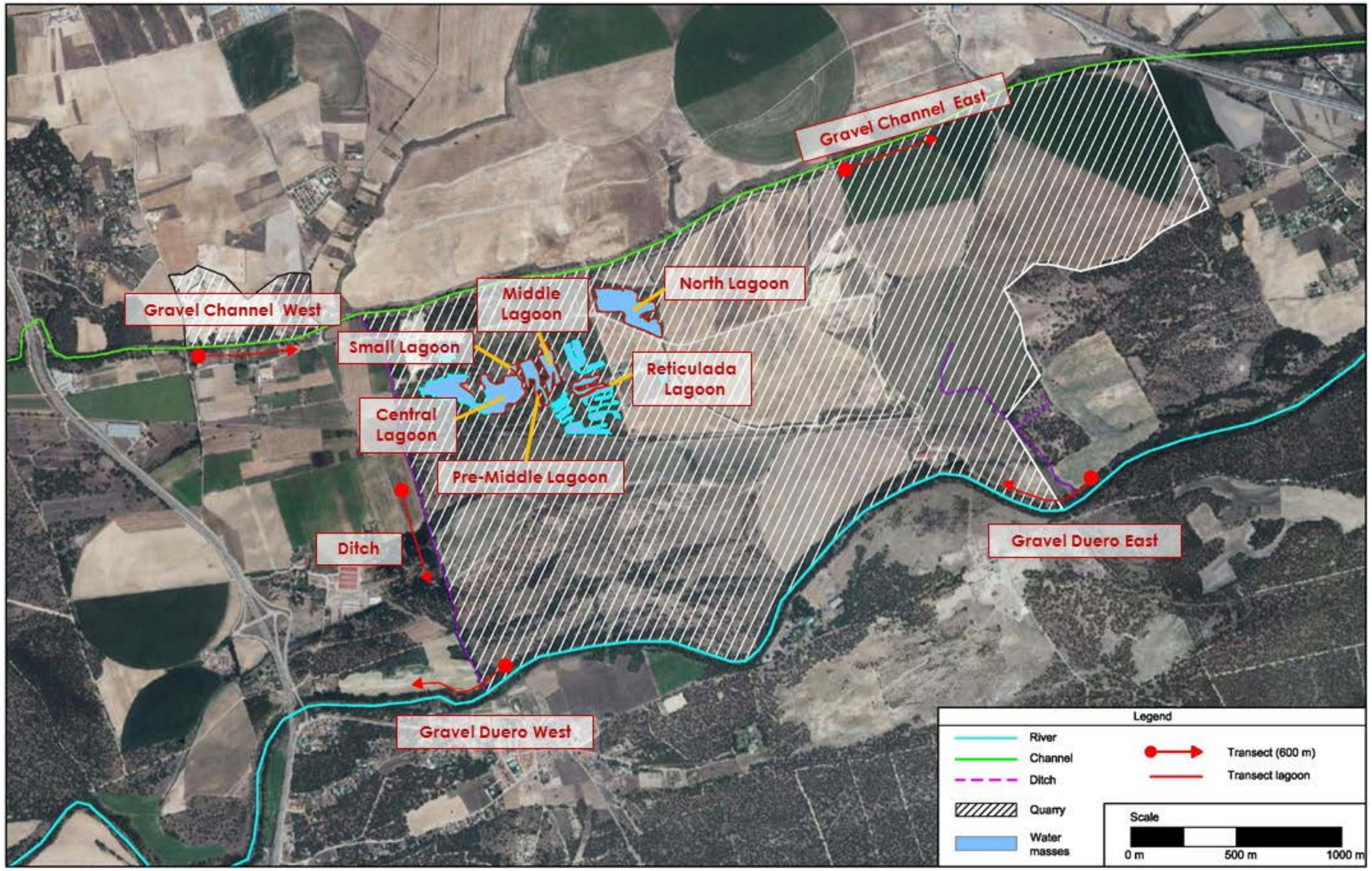

Figure 3. Location of sampling transects in the gravel quarry surroundings: irrigation channel and Duero River.

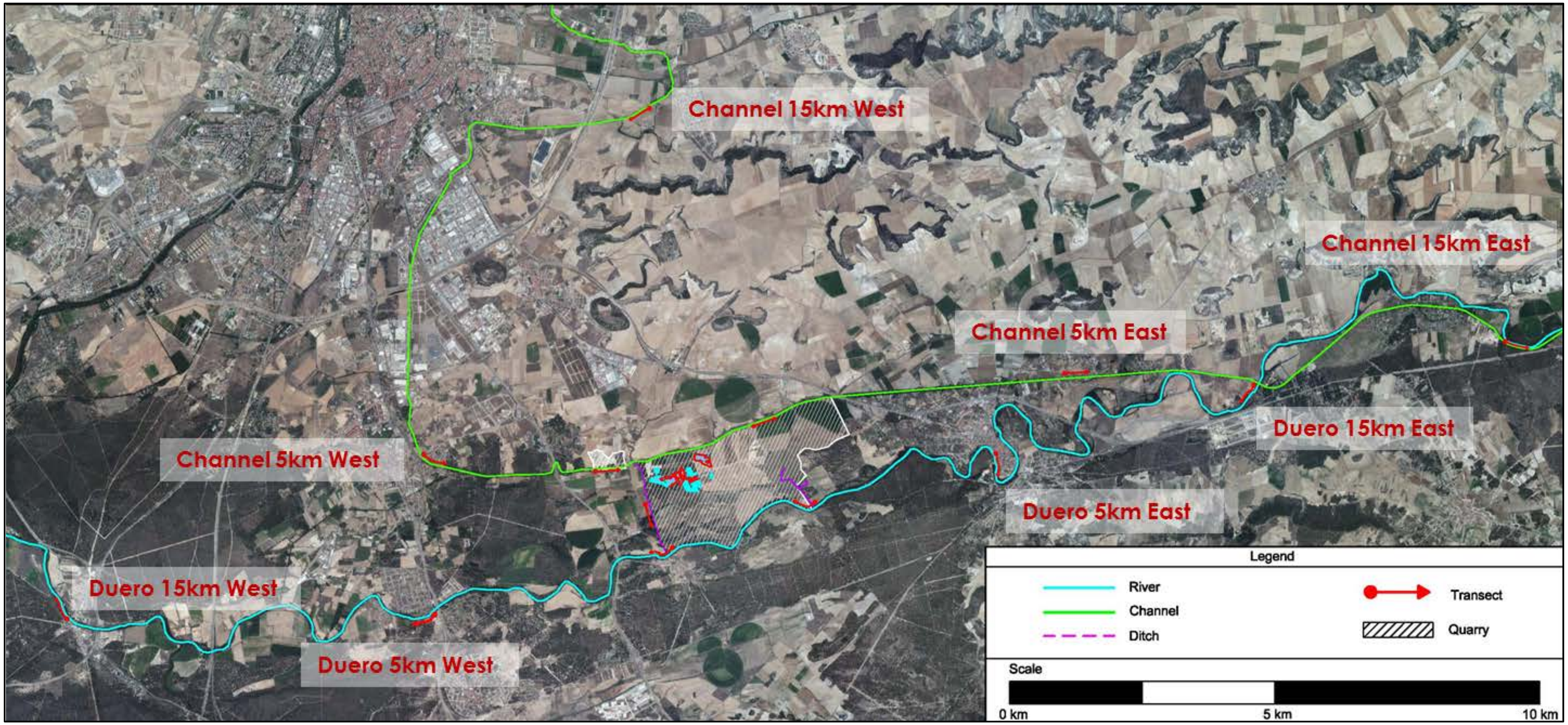

Figure 4. Team members during sampling transect walks at different locations: a) Duero 15km West, b) Channel 5km East, c) ditch, d) Central Lagoon.

Figure 5. Otter spraints; a) fresh spraints (mainly crayfish), b) fresh spraints (mainly fish), c) semi-fresh spraints, d) dry spraints.

Figure 6. Location of the 10 camera traps in each survey campaign.

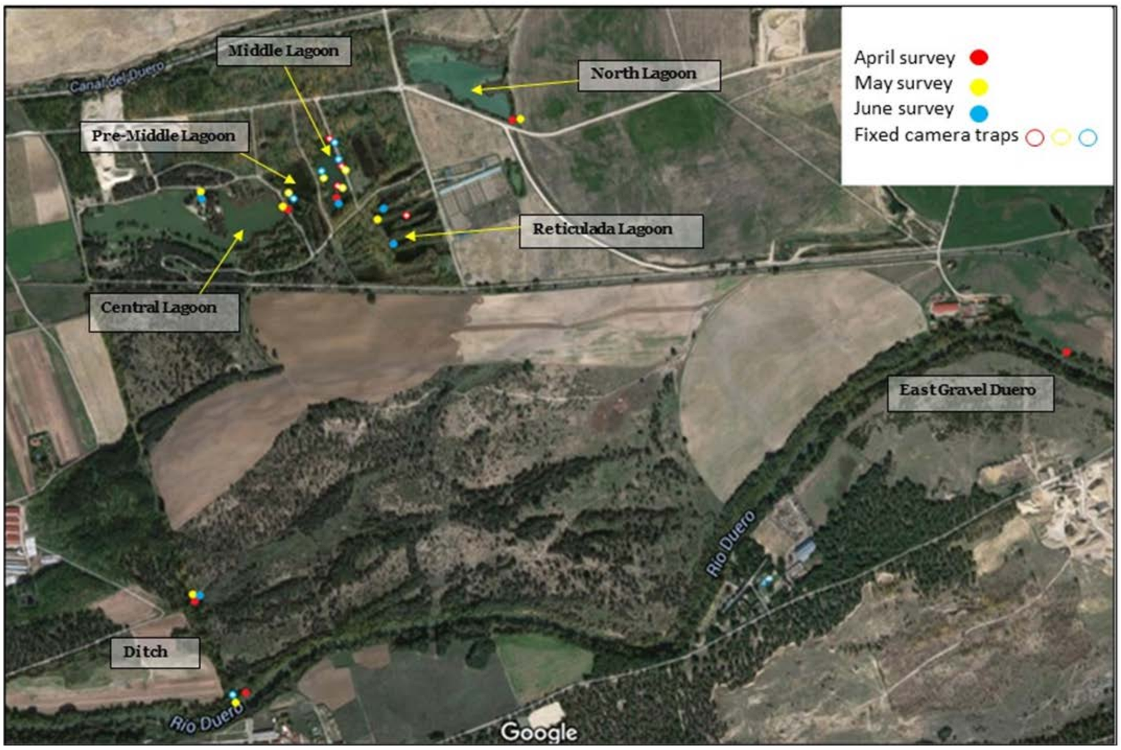

Figure 7. Otter family with small cubs in the waterside of the Middle Lagoon at the end of April.

Figure 8. Temporal pattern of otter presence in the gravel quarry lagoon system from April to July.

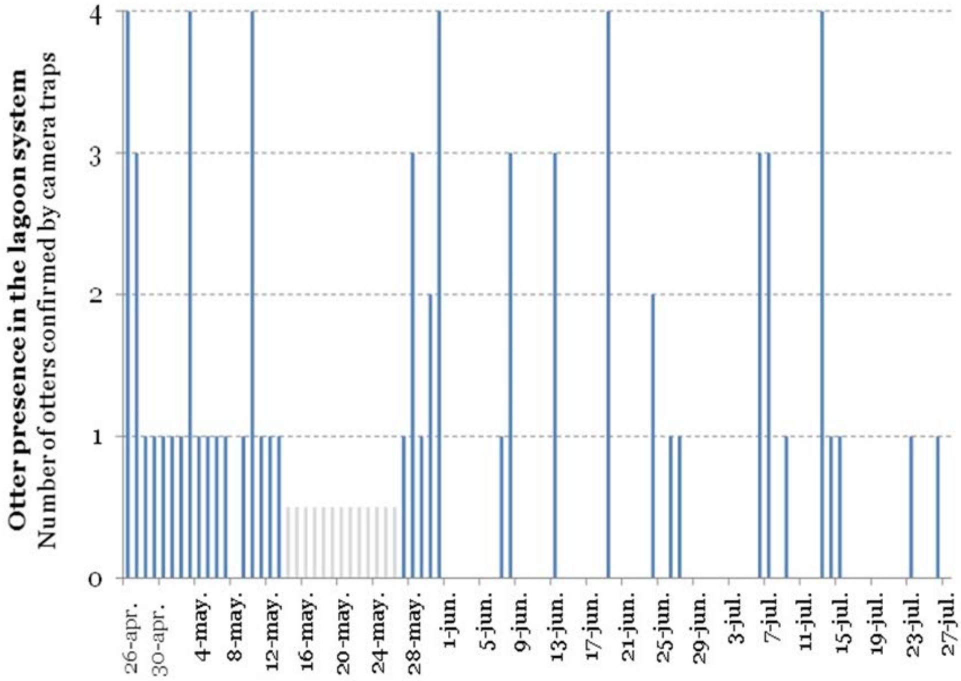

Figure 9. Otter family in the confluence of the ditch witn the Duero River at the end of July.

Figure 10. Otter family using the dry ditch.

Figure 11. List of fauna identified using camera traps at different locations of the gravel quarry and its surroundings.

Species	Lagoons					Ditch	Duero oeste gravera-Fin de acequia
	Middle	Pre-middle	Reticu _lada	North	Central		
Otter (*Lutra lutra*)	x	x	x	x	x	x	x
Badger (*Meles meles*)	x	-	x	-	-	x	x
Genet (*Genetta genetta*)	-	-	-	-	-	x	x
Stone marten (*Martes foina*)	-	-	-	-	-	x	x
Fox (*Vulpes vulpes*)	x	-	x	x	-	-	-
Roe deer (*Capreolus capreolus*)	x	-	-	-	-	-	x
Wild boar (*Sus scrofa*)	-	-	-	-	-	x	x
Water vole (*Arvicola sapidus*)	x	-	-	-		-	-
House rat (*Rattus rattus*)	x	-	-	-	x	-	-
Brown rat (*Rattus norvegicus*)	x	-	-	x	x	-	-
Long-tailed field mouse *(Apodemus sylvaticus)*	x	-	-	-	x	-	-
Red-eared slider (*Trachemys scripta elegans*)	x	-	-	-	-	-	-
Purple heron (*Ardea purpurea*)	x	x	x	x	x	-	-
Grey heron(*Ardea cinerea*)	x	-	-	-	-	-	-
White stork (*Ciconia ciconia*)	x	-	-	-	-	-	-
Northern goshawk (*Accipiter gentilis*)	-	-	-	-	-	x	-
Western marsh-harrier (*Circus aeruginosus*)	x	-	-	-	-	-	-
Water rail (*Rallus aquiaticus*)	x	-	-	-	-	-	x
Common nightingale (*Luscinia megarhynchos)*	x	x	-	-	x	-	x
European goldfinch (*Carduelis carduelis*)	x	-	-	-	-	-	-
Common chiffchaff (*Phylloscopus collybita*)	-	-	-	-	-	-	x
European robin (*Erithacus rubecula*)	-	-	-	-	-	-	x
Blackcap (*Sylvia atricapilla*)	-	-	-	-	-	-	x
Eurasian jay (*Garrulus glandarius*)	-	-	-	-	-	-	x
European serin (*Sermnus serinus*)	-	-	-	-	-	-	x
Great tit (*Parus major*)	-	-	-	-	-	-	x
Eurasian chaffinch (*Fringilla coelebs*)	-	-	-	-	-	-	x
Eurasian greenfinch (*Chloris chloris*)	-	-	-	-	-	-	x
Eurasian tree sparrow (*Passer montanus*)	x	-	-	-	x	-	x
Grey Wagtail (*Motacilla cinerea*)	-	-	-	-	-	-	x

In addition to the species indluded in the table we have to add the omnipresent mallards (*Anas platyrhynchos*), common moorhen (*Gallinula chloropus*), eurasian blackbird (*Turdus merula*), song thrush (*Turdus philomelos*) and european rabbits (*Oryctolagus cuniculus*), which were found in almost all the locations.

Figure 12. Spraint marking intensity at each transect in the four survey campaigns. Pale colours indicate dry spraints and intense colours indicate fresh and semi-fresh spraints. *The Pre-Middle lagoon was not included in the first sampling campaign.

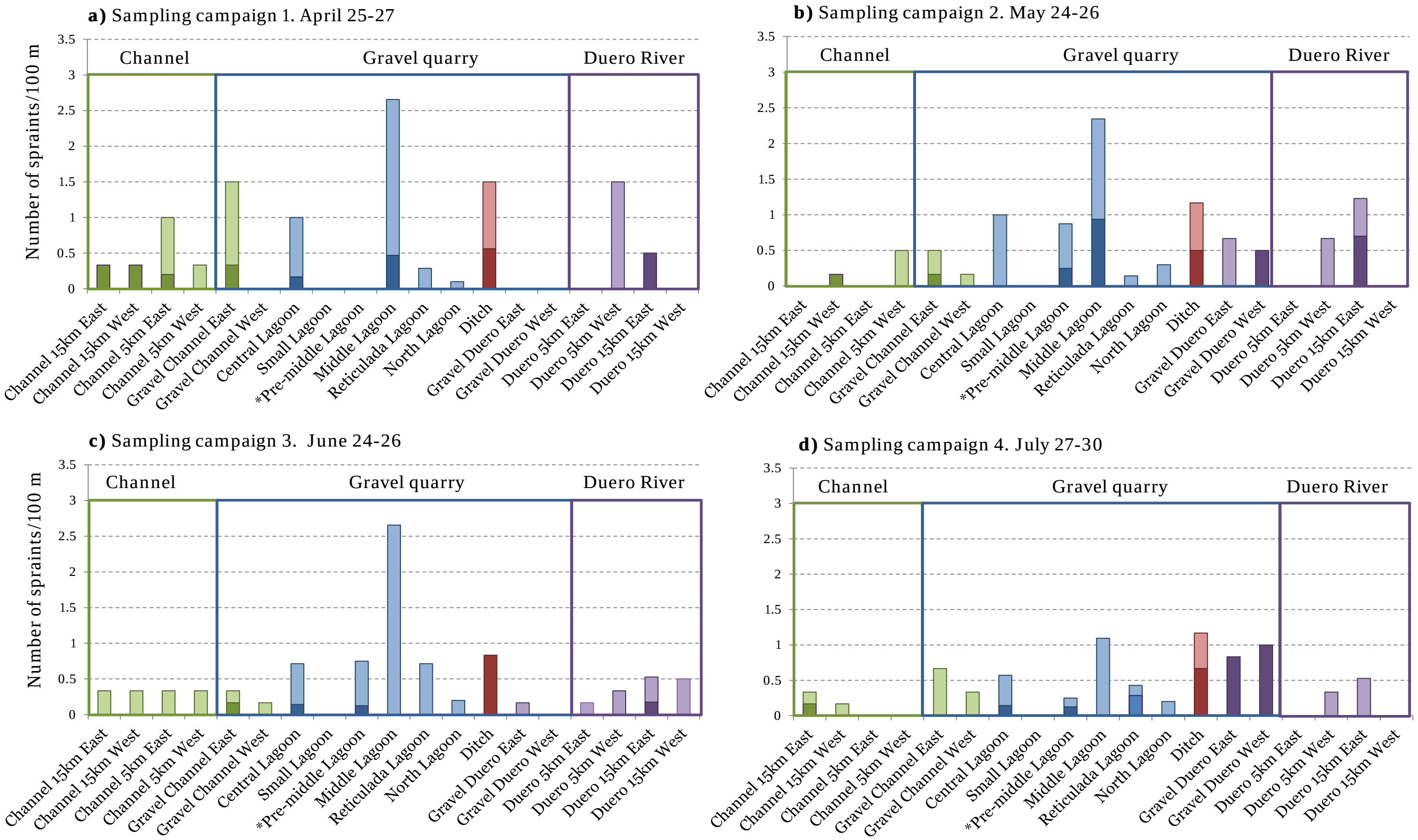

Figure 13. Mean total spraint marking intensity across survey campaigns (*total number of spraints/100m*) in the Duero River, the irrigation channel and the ditch transects.

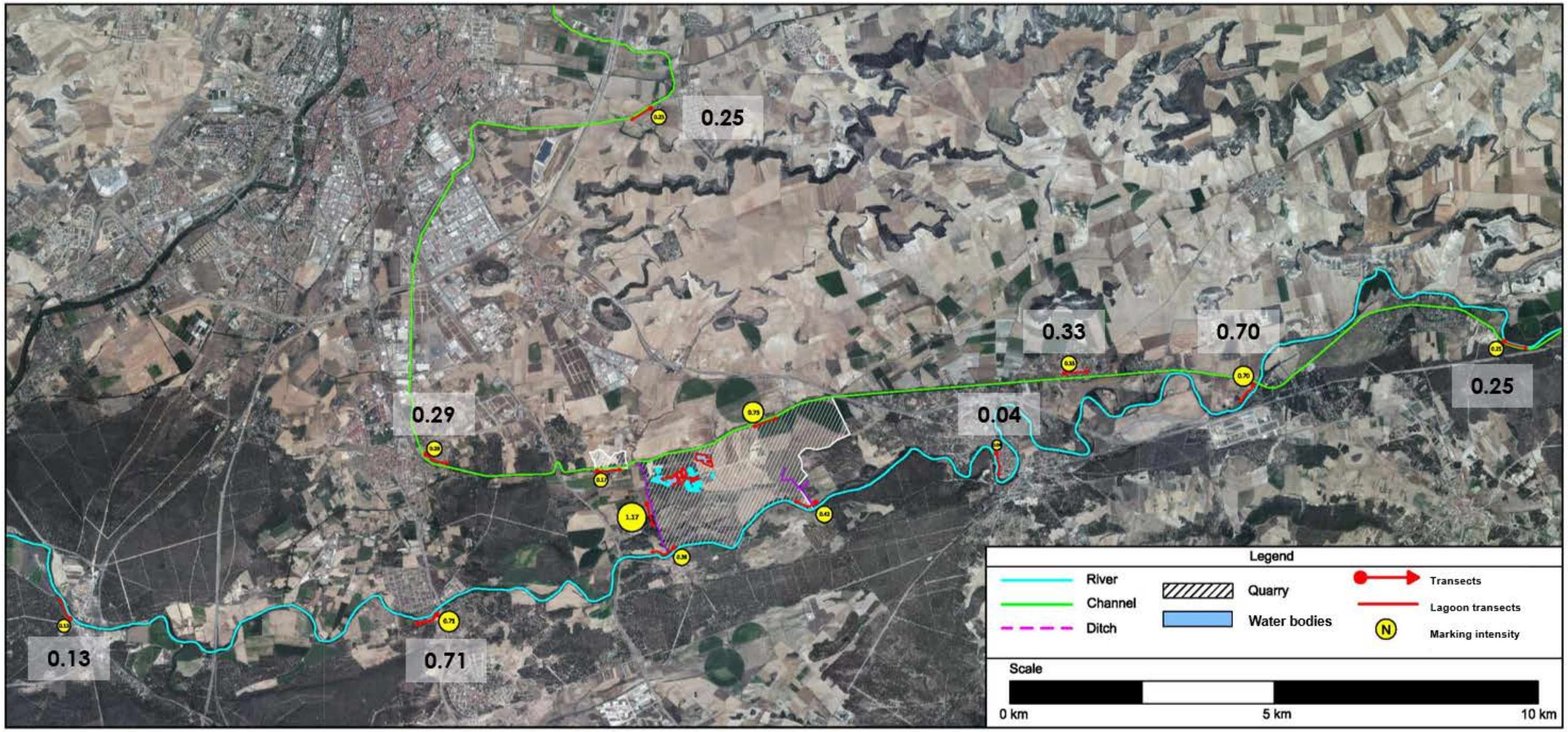

Figure 14. Mean total spraint marking intensity across survey campaigns (*total number of spraints/100m*) in the gravel quarry transects.

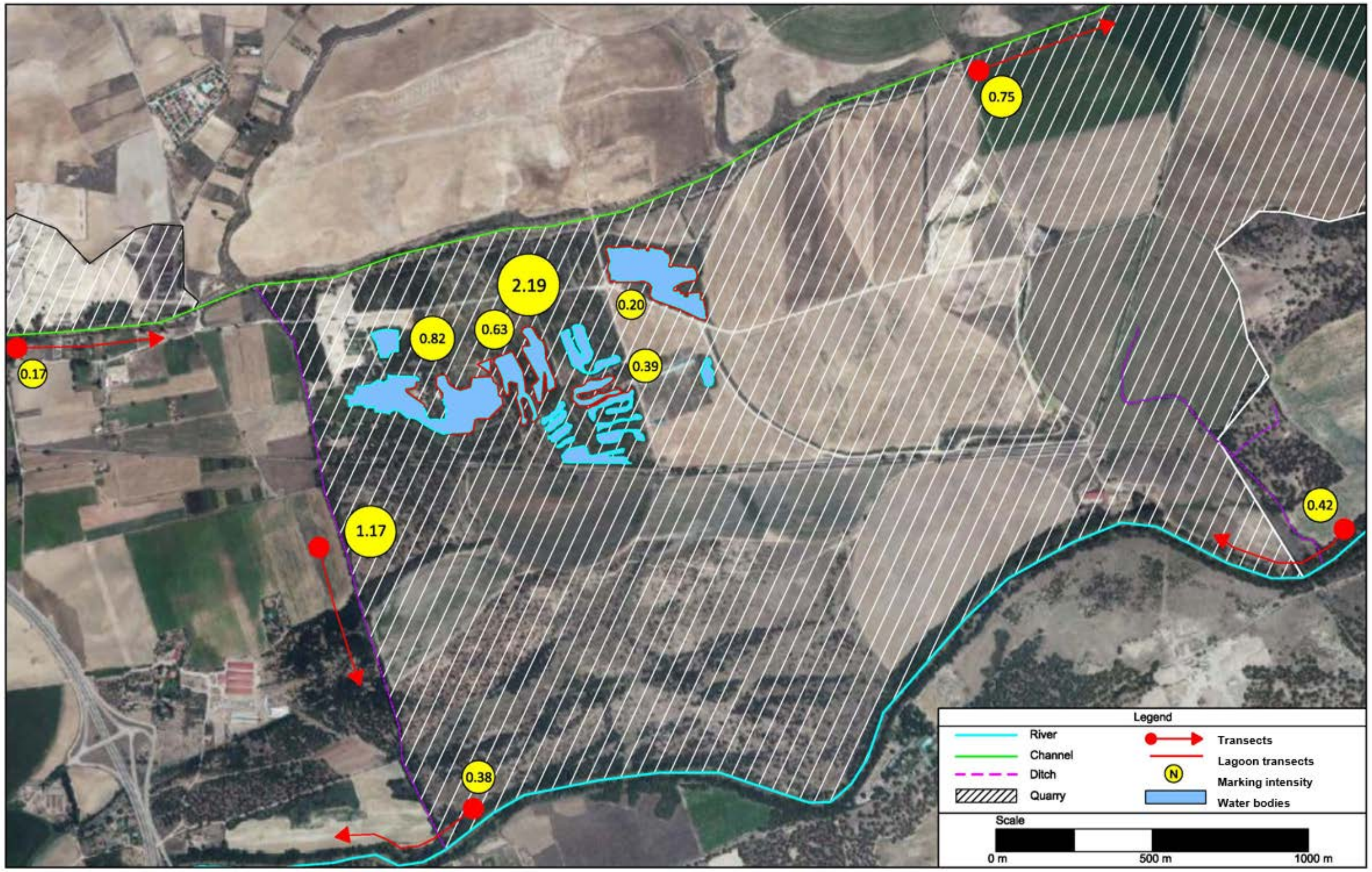

Figure 15. Characterization of otter diet by visual identification of sprants prey content at different water bodies.

Spraint prey content	n	Irrigation channel	Lagoons	Ditch	Duero River
Mainly crayfish	178	74%	99%	75%	62%
Crayfish and fish	22	21%	1%	7%	27%
Mainly fish	11	5%	0%	18%	11%
n	211	44	104	28	37

*Chi-square p-value is < 0.00001

Figure 16. Team members during the process of DNA extraction s from otter spraints; a) laboratory of Zoology Department of Complutense University of Madrid, b) DNA extracted from otter spraint samples.

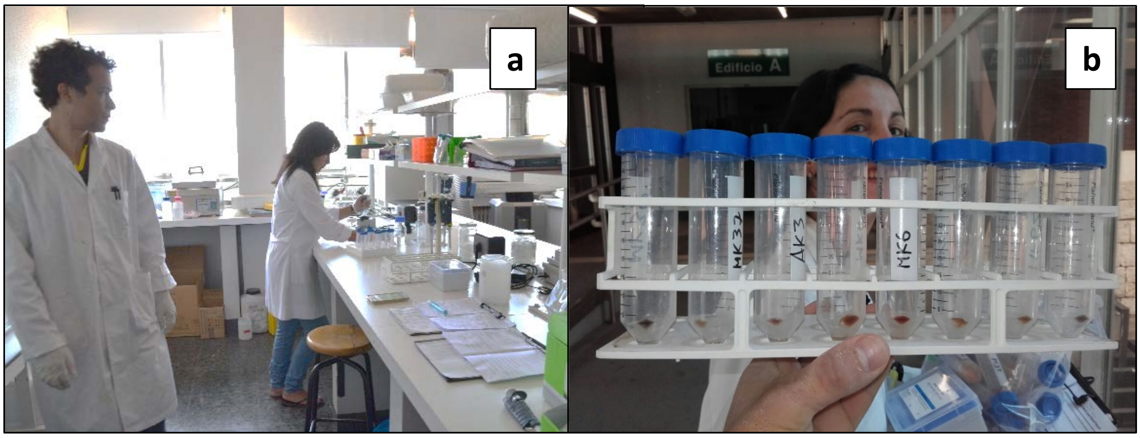

Figure 17. Spatial distribution of otters indentified in the studied area by genetic analysis of spraints using microsatellites.

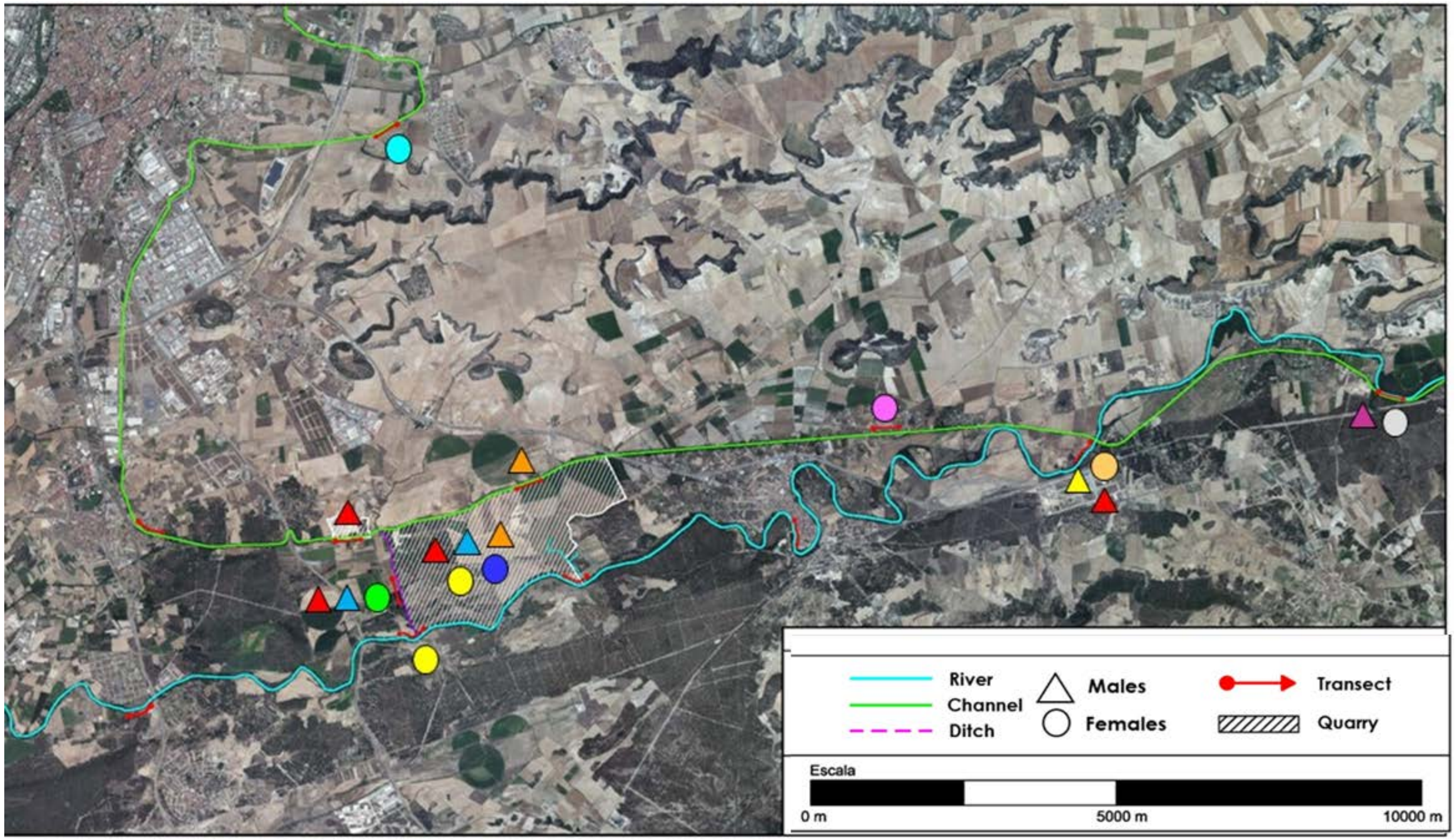

Figure 18. Habitat variables used in the habitat suitability analysis.

Waterside vegetation (up to 5 metres to water bodies)	Herbaceous cover (%)
	Bush cover (%)
	Tree cover (%)
	Bare soil (%)
Water emerging vegetation coverage (up to 5 meters from waterside) (%)	
Impacts	*Distance to road (m)
	Recreational use area (Yes/No)
Human disturbances	Intensive disturbances (Yes/No)
	Common disturbances (paths, low density urban areas) (Yes/No)
	Occasional disturbances (farmers, fishermen) (Yes/No)
	Very low disturbances; difficult access areas (Yes/No)
* Land use in the water body surroundings (up to 100, 300, 500, 1000 meters from water side)	Recreational (%)
	Water bodies (%)
	Urban and industrial (%)
	Forest (%)
	Agriculture (%)
	Pastures (%)

* Calculated by Geographical Information Systems (Arc Gis software) based on CORINE Land Cover 2006 land use date base (Spanish National Geography Institute, Ministry of Development)

Figure 19. Influence areas defined using Geographical Information Systems to calculate land uses up to 100, 300, 500 and 1000 metres from the sampling transects; examples for Middle Lagoon and Gravel Duero East transects.

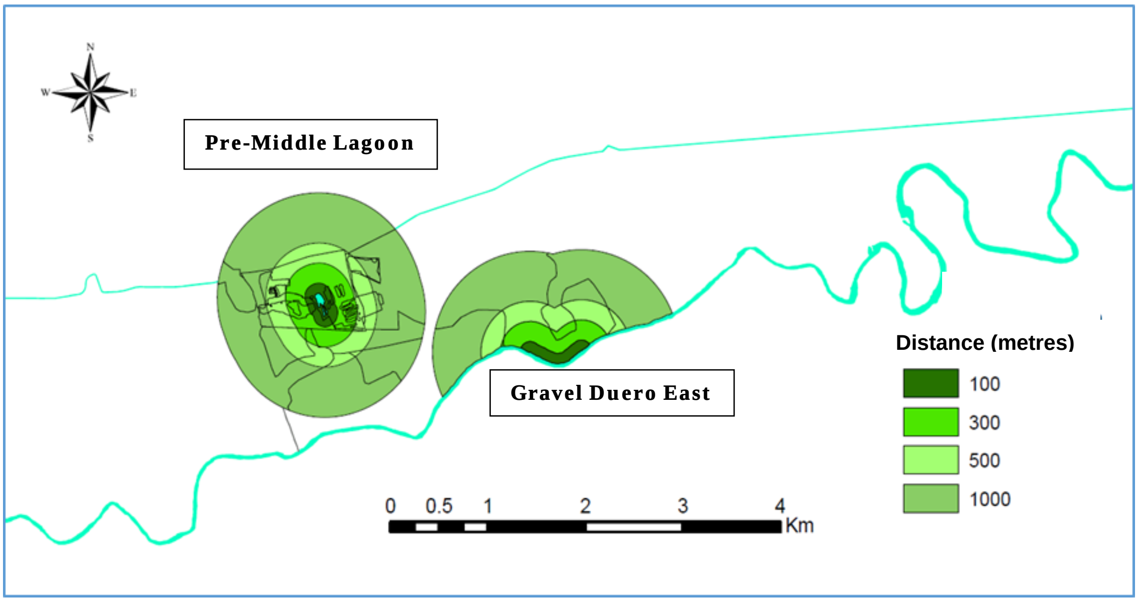

Figure 20. Habitat variable weights on the first 2 principal components (PC) and its relation to spraint marking intensity. We only considered land use variables at 300m due to their high correlation with the land use variables for the rest of distances.

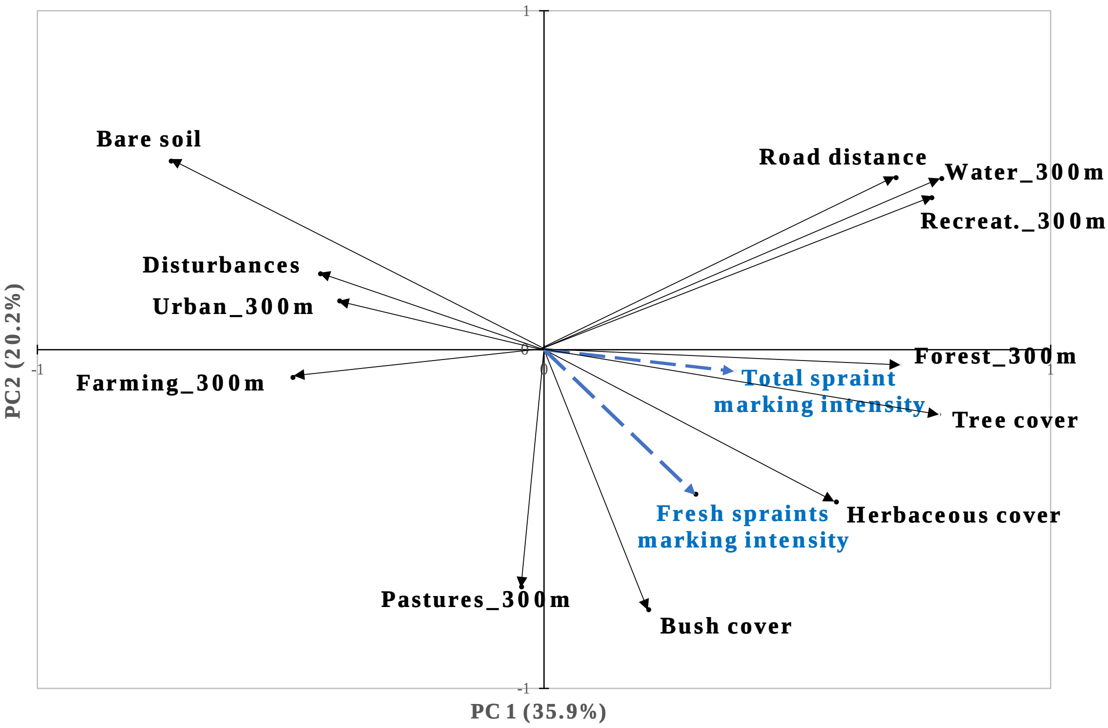

Figure 21. Estimates of habitat variables effect on fresh spraints marking intensity. Multiple regression model coefficients for significant habitat variables.

Coefficients	Mean ± Standard error	t	p	
Intercept	0.087 ± 0.096	0.906	0.381	
Bush coverage of waterside	0.014 ± 0.005	2.816	0.015	*
Human disturbances	-0.013 ± 0.048	-0.257	0.801	
Forest_Land Use_300m	0.006 ± 0.003	1.991	0.068	.
Disturbances * Forest_300m	-0.003 ± 0.002	-2.12	0.046	.
Disturbances * Bush coverage	-0.005 ± 0.003	-1.821	0.097	*
Residual standard error: 0.08698 in 13 freedom degrees				
Multiple R^2: 0.82 , adjusted R^2: 0.75				
F= 11.92, p-value < 0.001				

Significance codes: "***" <0.001; "**" <0.01; "*" <0.05; "." <0.1; " " non significative

Figure 22. Multiple regression model´s estimates for the effects of habitat degradation and human disturbance on fresh spraint marking intensity.

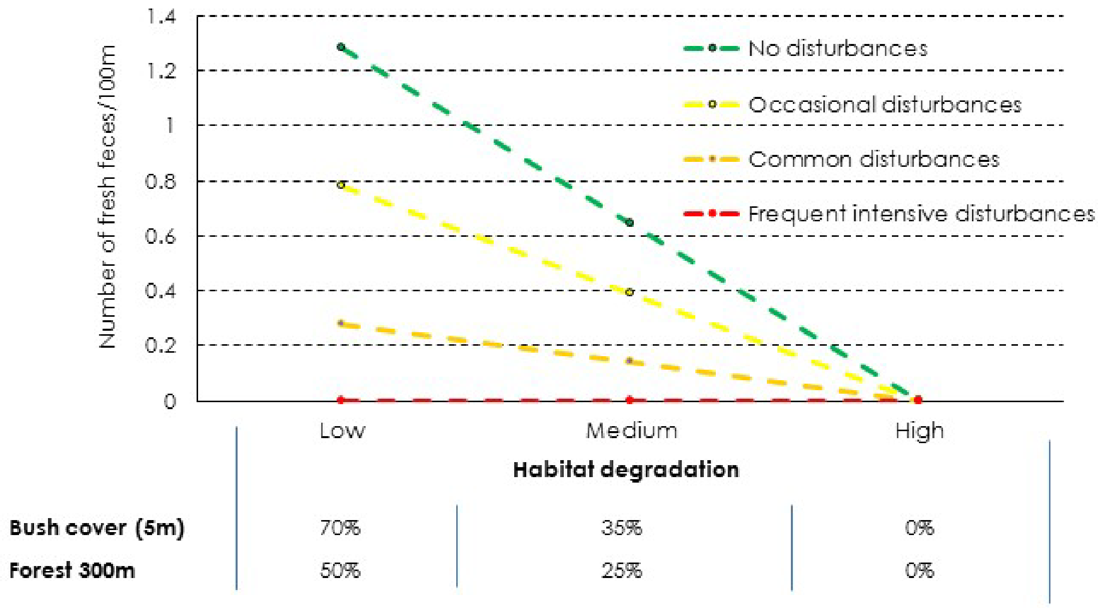

Figure 23. Estimates of habitat variables effect on total spraints marking intensity. Multiple regression model coefficients for significant habitat variables.

Coefficients	Mean ± Standard error	t	p	
Intercept	0.031 ± 0.3621	0.087	0.932	
Human disturbances	0.157± 0.1755	0.895	0.385	
Forest_Land Use_300m	0.042 ± 0.0117	3.553	0.003	**
Disturbances * Forest_300m	-0.020 ± 0.0063	-3.246	0.005	**
Residual standard error: = 0.3735 in 13 freedom degrees				
Multiple R^2 = 0.54 , ajusted R^2 = 0.45				
F = 5.97, p-value < 0.01				

Significance codes: “***” <0.001; “**” <0.01; “*” <0.05; “.” <0.1; “ ” non significative

Figure 24. Map of the studied area before the existence of the gravel quarry lagoon system, used for the analysis of the lagoon system influence on the functional connectivity of the territory. Aerial photographs taken in 1986. The small rectangle corresponds with the small scale analysis area (104 km^2) and the whole picture corresponds with the large scale analysis area (724 km^2).

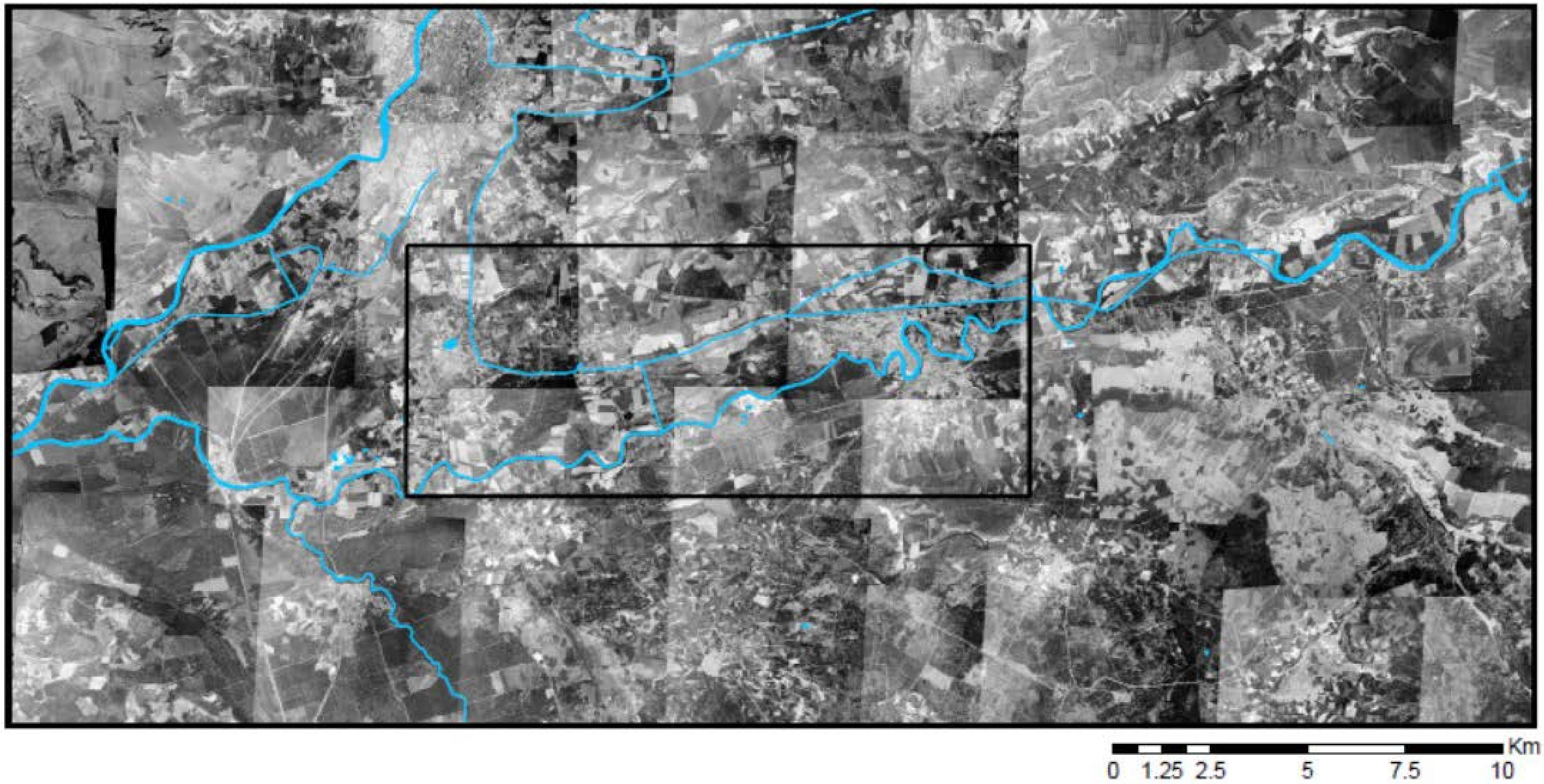

Figure 25. Map of the studied area with the gravel quarry lagoon system, used for the analysis of the lagoon system influence on the functional connectivity of the territory. Aerial photograph taken in 2014. The small rectangle corresponds with the small scale analysis area (104 km^2) and the whole picture corresponds with the large scale analysis area (724 km^2).

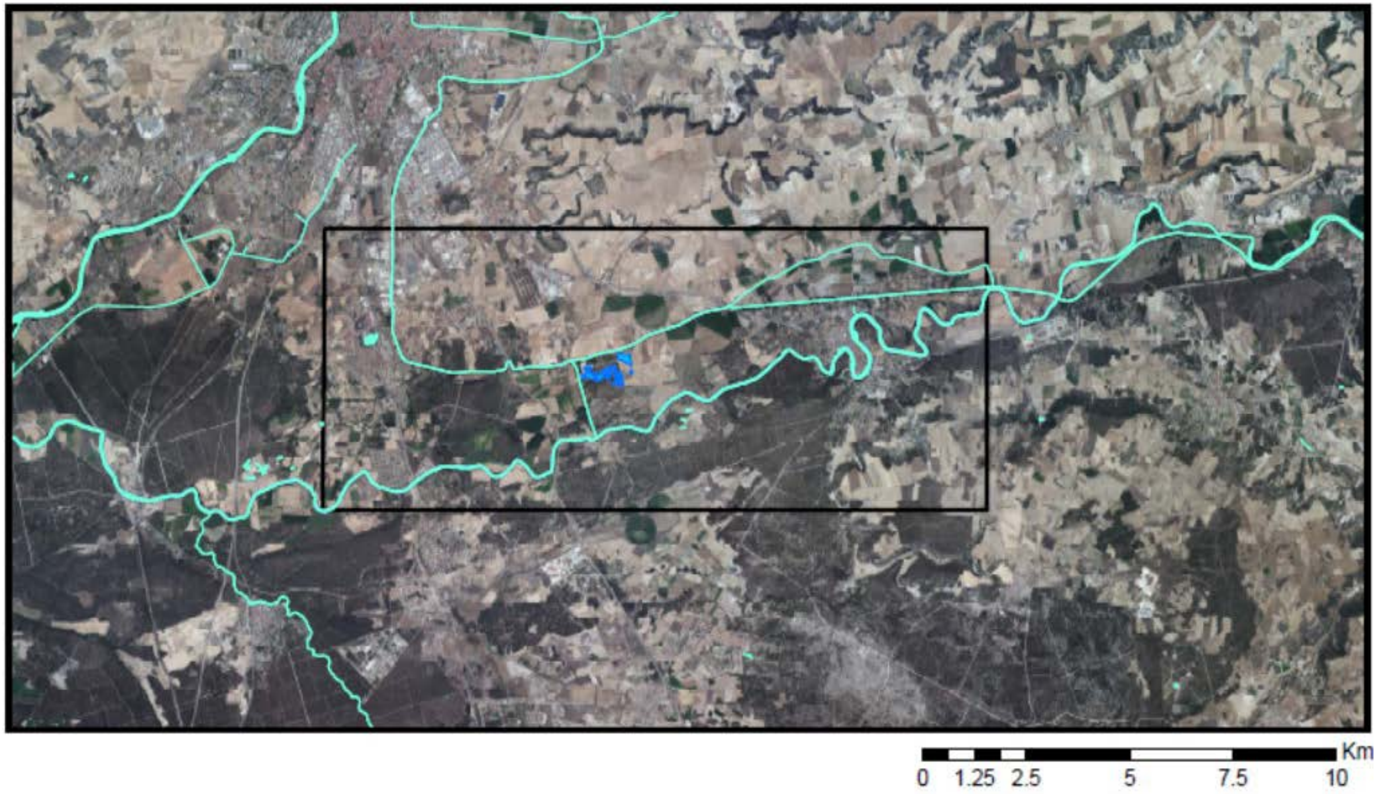

Figure 26. Land use map generated by Geographical Information Systems (ArcGis software) for the matrix permeability (connectivity) analysis.

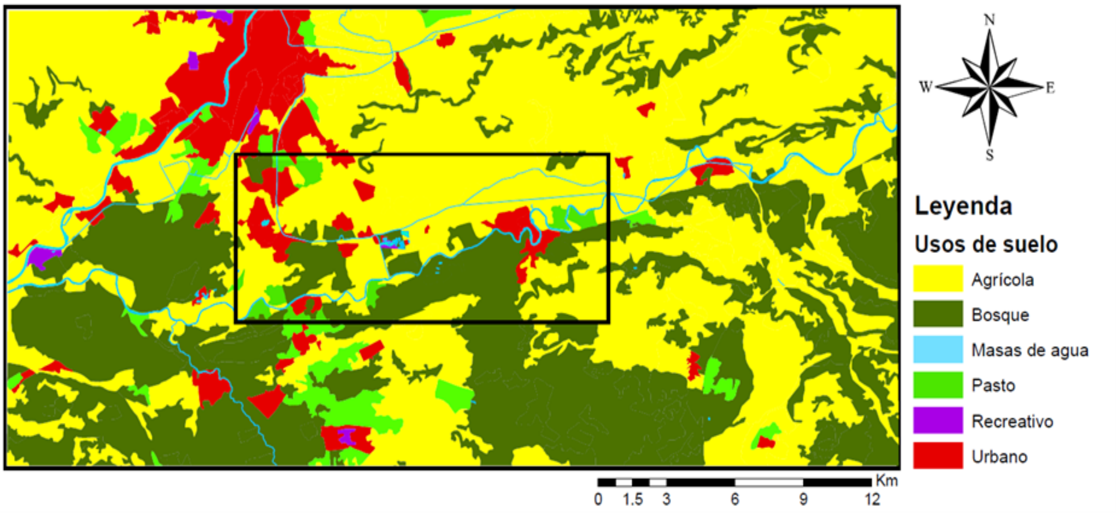

Figure 27. Map of distances to infrastructures (human disturbances) generated by Geographical Information Systems (ArcGis software) for the matrix permeability (connectivity) analysis.

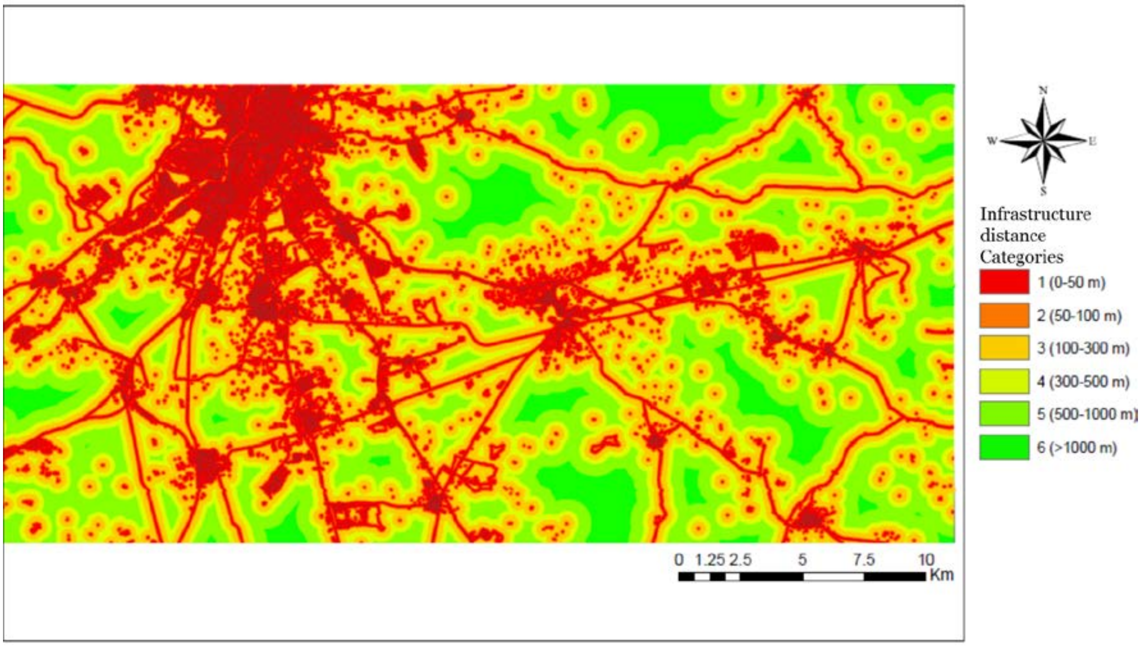

Figure 28. Map of distances to water bodies generated by Geographical Information Systems (ArcGis software) for the matrix permeability (connectivity) analysis.

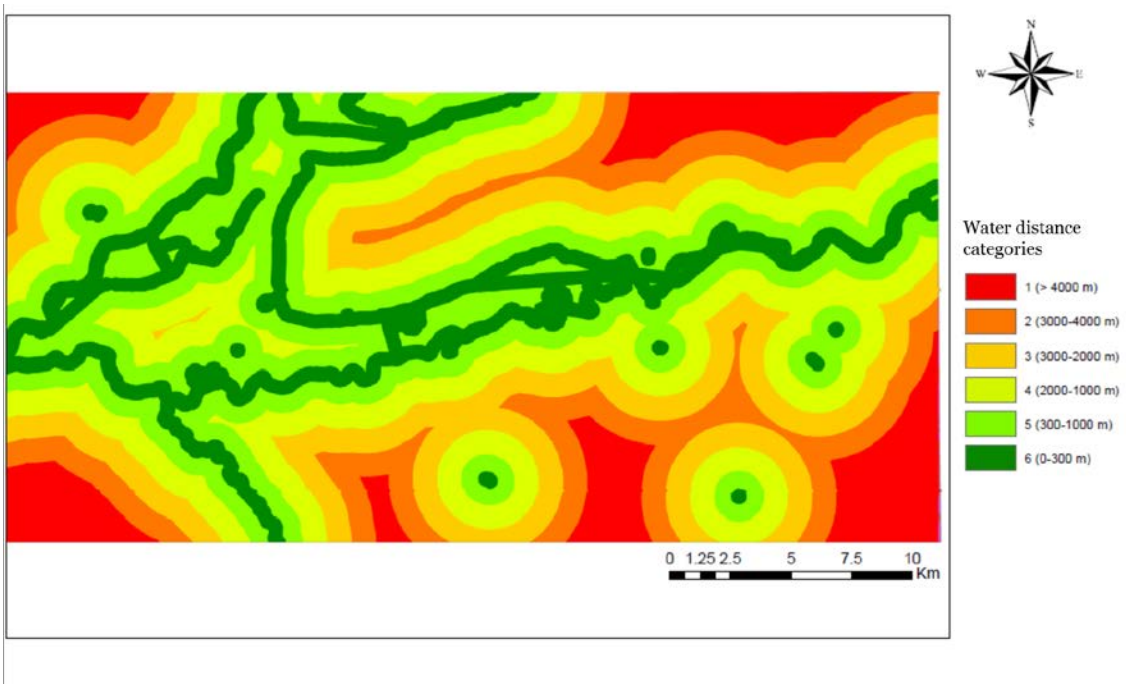

Figure 29. Effect of the gravel quarry lagoon system on the functional conectivity of the landscape. Table of connectivity gain based on the Equivalent Connected Area index (ECA) value at different analysis scales and dispersal distances.

Dispersal distance	Scenario	Equivalent Connectivity Area (ECA)	
		Small analysis scale (104 km^2)	**Large analysis scale** (724 km^2)
5 km	Without lagoons	1.01	3.96
	With lagoons	1.19	4.13
	***Connectivity gain** (%)	14.8	4.2
7 km	Without lagoons	1.01	3.97
	With lagoons	1.19	4.15
	***Connectivity gain** (%)	14.8	4.2
15 km	Without lagoons	1.01	3.99
	With lagoons	1.19	4.17
	***Connectivity gain** (%)	14.9	4.2

*Connectivity gain (%)=(ECA with lagoons - ECA without lagoons)/(ECA with lagoons)*100 (Saura & Torne, 2009)

Figure 30. Effect of the gravel quarry lagoon system on the functional conectivity of the landscape. Table of connectivity gain based on the Probability of Connectivity index (PC) value at different analysis scales and dispersal distances.

Dispersal distance	Scenario	Probability of Connectivity (PC)	
		Small analysis scale (104 km^2)	**Large analysis scale** (724 km^2)
5 km	Without lagoons	1.02	1.84
	With lagoons	1.41	1.99
	***Connectivity gain** (%)	27.7	7.5
7 km	Without lagoons	1.02	1.88
	With lagoons	1.41	2.04
	***Connectivity gain** (%)	27.7	7.8
15 km	Without lagoons	1.03	1.94
	With lagoons	1.41	2.10
	***Connectivity gain** (%)	27.0	7.6

*Connectivity gain (%)=(PC with lagoons - PC without lagoons)/(PC with lagoons)*100 (Saura & Torne, 2009)

Figure 31. Final result of the matrix permeability model. Map of territory permeability for otter movement, in Áridos Sanz gravel quarry and its surroundings, as a function of land use, distance to infrastructures and buildings, and distance to water bodies. The location of the lagoon system is marked with a grey rectangle.