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Inland Wetlands in the Lower Tagus: land uses, habitat condition and fish communities

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Abstract

Inland wetlands are widely recognized as biodiversity hotspots, and among the most threatened ecosystems worldwide. Conservation management and restauration actions are thus urgently needed in inland wetlands, especially in areas harbouring endangered endemic species. The role of inland wetlands in Lower Tagus in supporting endangered endemic fish remains unclear. This work assessed the status of inland wetlands in the region and its local fish communities.

Inland wetlands were identified using the Normalized Difference Water Index with *Sentinel-2* imagery. Land use and change were derived from soil use and occupation charts. Fish communities were surveyed using multiple techniques and the Gear Mean Standardization approach, and related to habitat, landscape, and land use and change. In total, 409 water bodies were identified in the Lower Tagus, 30 of which with potential to host fish. Seven out of 11 wetlands assessed were heavily disturbed, though intensive agriculture tended to reduce and be replaced by extensive agriculture and natural areas in the last years.

Fish communities included five native species, but were dominated by eight non-native species. Setil and Sto Estevão were directly connected to the Tagus River and harboured Iberian barbel and Thinlip grey mullet, while the deep and isolated Golegã and Gouxa harboured Largemouth bass and Pumpkin-seed sunfish and the shallow Caniceiras included threatened European eel, Southern Iberian Spined loach and Lisbon arched-mouthed nase.

These results indicate that some wetlands in the Lower Tagus may act as refuge habitats for threatened fish, despite non-native fish prevalence. Efforts should focus on preserving and restoring these wetlands and in controlling non-native species spread in the region.

Keywords: Land use change, remote sensing, freshwater habitats, endangered fish, biodiversity loss.

Resumo

O aumento da população mundial e a consequente conversão de terras para agricultura e ocupação urbanística, têm vindo a acentuar a perda e degradação de habitats e o colapso da biodiversidade. As zonas húmidas de água doce são um dos ecossistemas mais ameaçados do mundo e importantes "*hotspots*" de biodiversidade. Constituem refúgios para diversas espécies em declínio, e fornecem diversos Serviços de Ecossistema, e múltiplos benefícios para o bem-estar humano. Estes aspetos têm vindo a ser crescentemente reconhecidos, e a conservação e restauro das zonas húmidas de água doce assume cada vez mais relevância nos planos para a inverter a perda de biodiversidade a nível nacional e regional. Esta tendência é notória nos planos da Convenção sobre Diversidade Biológica (CBD), Estratégia de Biodiversidade para 2030 da União Europeia e na Estratégia Nacional de Conservação da Natureza e Biodiversidade para 2030, que figuram como o maior desafio da política ambiental do século XXI.

As zonas húmidas de água doce são particularmente importantes na região do Mediterrâneo, devido à elevada taxa de espécies endémicas ameaçadas nesta região, e à sua acrescida suscetibilidade a pressões humanas e às alterações climáticas. Identificar e monitorizar as comunidades biológicas nestes ambientes e avaliar as pressões que os afetam é por isso da maior relevância, particularmente em zonas com menor informação. A identificação e monitorização de zonas húmidas tem vindo a melhorar substancialmente nos últimos anos, em associação com o desenvolvimento de técnicas como a deteção remota, e a disponibilização de informação pelas missões de satélite *Sentinel*. A utilização desta informação, com elevada precisão e cobertura temporal, facilita a monitorização das alterações nos ecossistemas e permite atuar de forma mais informada nos locais com maior necessidade de conservação.

O Tejo é o rio mais longo da Península Ibérica, e no seu segmento terminal, desde a Golegã até Lisboa, drena uma extensa planície aluvial. A região do Baixo Tejo, é historicamente caracterizada por inundações anuais, que transforma toda a paisagem numa imensa zona húmida, podendo inclusive isolar algumas localidades. Atualmente estas situações são cada vez menos frequentes devido à construção de barragens e regularização dos caudais do rio, as quais permitiram também a atual expansão da agricultura intensiva, mas que também terão contribuído também para o desaparecimento de várias zonas húmidas na região. Este trabalho pretendeu assim identificar as zonas húmidas de água doce que persistem atualmente no Baixo Tejo, quantificar os usos atuais do solo e as alterações que ocorreram nos últimos 10 anos nestas zonas, e caracterizar as comunidades de peixes dulciaquícolas locais. Assim, a área de estudo abrange apenas zonas de baixa altitude (≤ 50 m), sendo esta mais suscetibilidade à ocorrência de inundações, e onde existe uma maior probabilidade de ocorrência de zonas húmidas de água doce. Com base em deteção remota, e na combinação de imagens captadas pelo satélite *Sentinel-2* através do Índice de Água de Diferença Normalizada (NDWI), foram identificadas 409 massas de água na região do Baixo Tejo, entre a Golegã e Lisboa. Destas, 30 apresentaram capacidade de manutenção de água durante o período seco, tendo desta forma, sido consideradas como possuidoras de potencial para albergar comunidades de peixes. Após análise *in loco*, foram selecionadas para amostragem 11 zonas húmidas, globalmente representativas da variabilidade de condições ambientais observadas na região do Baixo Tejo. O uso do solo entre 2007 e 2018 foi quantificado a partir das Cartas de Uso e Ocupação do Solo da Direção Geral do Território. Para facilidade de interpretação e comparação, os usos do solo foram reclassificados e agrupados em quatro classes abrangentes, designadamente, Urbano/Industrial (UI), Agricultura Intensiva (IA), Agricultura Extensiva (EA) e Áreas Naturais (NA). Especificamente, a alteração do uso do solo foi determinada a partir da diferença da representação das classes EA e NA de dois períodos distintos, em 2018 e a média do período correspondente aos anos de 2007, 2010 e 2015. Complementarmente, com base na representação da UI e IA em 2018, as zonas húmidas foram classificadas em cinco categorias pressão, de Excelente a Severamente Perturbado.

Os usos e ocupação do solo nas áreas adjacentes às zonas húmidas apresentaram uma grande heterogeneidade. O uso mais representado e transversal às zonas húmidas foi a IA (em média 42.9 %), sendo muito relevante na Golegã, Santana e Setil (> 70 %). Foram também observadas percentagens elevadas (> 60 %) de NA em Gouxa, Granho e Sto Estevão, e de UI em Manique Intendente e Caniceiras, não ocorrendo esta última em Muge, Granho e Sto Estevão. Entre os períodos analisados, verificou-se um decréscimo da UI e IA nas áreas adjacentes a todas as zonas húmidas, excetuando no Setil, Caniceiras e Pinhal Novo onde a IA tem vindo a aumentar. As zonas húmidas com maior perturbação foram Golegã, Manique do Intendente e Caniceiras, com UI elevada, e Santana, Muge, Setil e Pinhal Novo, com IA elevada. As zonas húmidas classificadas em bom estado, foram Gouxa, Granho, Ota e Sto Estevão, onde tem vindo a ocorrer uma diminuição da agricultura intensiva em favor da agricultura extensiva e de áreas naturais nas áreas adjacentes.

As comunidades piscícolas das 11 zonas húmidas selecionadas foram amostradas entre 6 de maio e 11 de junho de 2021, com recurso a armadilhas, redinha e pesca-elétrica. As comunidades foram caracterizadas em termos de capturas por unidade de esforço (CPUE), com base no método *Multi Gear Mean Standardization*, que tem vindo a revelar-se particularmente adequado no estudo da estrutura de comunidades. No total foram capturados 8 272 peixes, de cinco espécies nativas e de oito espécies não-nativas. Embora a generalidade das comunidades piscícolas locais tenham sido dominadas por espécies não-nativas, foram encontradas duas espécies classificadas como criticamente ameaçadas (CR) a nível global, nomeadamente, a Enguia europeia (*Anguilla anguilla*) e a Boga-de-boca-arqueada-de-lisboa (*Iberochondrostoma olisiponense*), em cinco e duas zonas húmidas, respetivamente. As comunidades piscícolas de Setil e Sto Estevão que mantêm conectividade direta com o curso principal do Rio Tejo, foram as únicas que incluíram uma espécie migradora e uma espécie potamódroma, respetivamente a Taínha fataça *Chelon ramada* e o Barbo-ibérico *Luciobarbus bocagei*. Por sua vez, Golegã and Gouxa que são isolados e profundos apresentaram a maior abundância de Achigã *Micropterus salmoides* e de Perca-sol *Lepomis gibbosus*. Pelo contrário, Caniceiras que é pouco profundo e mantém alguma conectividade com a rede hidrográfica em eventos de cheia, apresentou o maior número de espécies nativas, incluindo não só a Enguia-europeia e a Boga-de-boca-arqueada-de-lisboa, mas ainda o Verdemã-comum (*Cobitis paludica*). A Boga-de-boca-arqueada-de-lisboa foi ainda encontrada no Granho, sendo esta uma nova localização para a sua área de distribuição no baixo Tejo.

Apesar da prevalência de espécies não-nativas nas comunidades locais, os resultados obtidos indicam que algumas zonas húmidas do Baixo Tejo poderão ainda servir como zonas de refúgio para espécies de peixes ameaçadas. No entanto, a colonização de espécies não-nativas nas zonas húmidas é muito preocupante e pode representar uma pressão significativa para a fauna nativa. A expansão de áreas naturais e agricultura extensiva, abrem oportunidades para implementação de melhores práticas agrícolas na vizinhança das zonas húmidas, as quais, em associação com processos de conservação e restauro, podem contribuir para inverter a tendência de expansão de espécies não-nativas e de perda de biodiversidade.

Palavras-chave: Alteração de uso do solo, Deteção remota, Zonas húmidas de água doce, Peixes ameaçados, Perda de biodiversidade.

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1. Introduction

1.1 Biodiversity loss

Global drivers of environmental change continue to increase being responsible for biodiversity collapse across natural ecosystems, with habitat loss and degradation featuring as the main driver of population declines and species losses (MEA, 2005; Ceballos *et al.*, 2015; Reid *et al.*, 2019). The conversion of natural ecosystems for human use in association with economic drivers and demographic pressures (i.e. land use change), such as land conversion into cropland and urban settlement, have been the main contributors for habitat loss worldwide (Foley *et al.*, 2005). Human footprint increasingly affects key aspects of Earth System functioning, due to alteration of the land cover (i.e. biophysical attributes of earth's surface) and land use (i.e. human purpose or intend applied to these attributes) (Lambin *et al.*, 2001; Venter *et al.*, 2016).

Ambitious plans have been designed in the last years by governments and non-state actors to reverse biodiversity loss, combining national and international co-operations to put biodiversity on the path to recovery by 2030. Commitments advanced on the post-2020 global biodiversity framework (CBD, 2021) and EU Biodiversity Strategy for 2030 (EC, 2020) to tackle biodiversity loss, include widening and improving the protected areas network to provide additional protection and nature restoration. EU Biodiversity Strategy for 2030, intents to protect at least 30% of the land and 30% of the sea in European countries by 2030, implicating the protection of additional 4% for land and 19% for sea (EC, 2020). Portugal commitment on addressing biodiversity loss is highlighted in the Portuguese National Strategy for Nature Conservation and Biodiversity for 2030 (ENCNB 2030). However, there is a long way to achieve the proposed targets with many challenges to surpass. Several recommendations and actions needed to enhance biodiversity recovery and achieve the commitments pinpointed are identified on the extensive report *"Biodiversidade 2030"* (Araújo *et al.*, 2022). This report stress out that biodiversity loss is a major challenge for environmental policy in the 21st century and the need to address this problem, trough the expansion of protected areas, and the reinforcement of current management policies to achieve 10 % of total protection, also the restoration of populations and ecosystems degraded to define future perspectives for addressing biodiversity conservation policies according to the commitments for 2030 (Araújo *et al.*, 2022).

Datasets covering long term population changes across species distribution ranges are crucial to capture variability in processes behind biodiversity loss and to enable precise targeting of any necessary remedial conservation actions (Čížková *et al.*, 2011). However, problems in data availability, continuous datasets (e.g., monitoring), and open access are a major issue susceptible to constrain the better use and delimitation of new protected areas for the Portuguese territory (Araújo *et al.*, 2022). There is a need to identify gaps in environmental and biological data, and to produce standardized and continuous datasets in order to improve biodiversity assessments. Reliable data are fundamental to identify and establish areas with high natural value and prioritize and improve conservation management in areas with extreme importance for endemic and endangered species. Designing co-management models for conservation areas with local councils and intermunicipal entities, for active management of the natural capital is also important (Araújo *et al.*, 2022). Moreover, the integration of current natural areas at local and regional levels into the Portuguese Protected Areas Network (Rede Nacional de Áreas Protegidas, RNAP) and the definition of targets for ecosystem restoration are also key for biodiversity conservation.

1.2 Wetlands importance and assessment

There is an increasingly recognition of the importance of wetlands for the conservation of biodiversity, human well-being, and ecosystem services (MEA, 2005; De Groot *et al.*, 2012; Cimon-Morin *et al.*, 2016). Wetlands are considered to play a crucial role on achieving global commitments under the post-2020 global biodiversity framework (CBD, 2021) and EU Biodiversity Strategy for 2030 (EC, 2020) to halt biodiversity loss (Thorslund *et al.*, 2017, GWO, 2021). Wetlands are major biodiversity hotspots, harbouring many wetland-dependent species, several of which are currently endangered and undergo severe decline (Gibbs, 2000; Finlayson, 2012). Wetland loss and degradation is expected to increase due to ongoing climate change and associated increases in drought severity and sea level rise, affecting coastal and inland wetlands, and also to land use intensification and population growth (Leberger *et al.*, 2020).

Wetlands are transition zones providing several important ecological interactions between terrestrial and aquatic ecosystems (Cherry, 2011). The definition of wetland by the Ramsar Convention is broad and captures different environments: "areas of marsh, fen, peat land, or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish, or salty, including areas of marine water the depth of which at low tide does not exceed six meters" (Ramsar, 1998). Considering this definition, inland wetlands can be permanently or temporary flooded, and include bogs, peatlands, marshes, swamps, fens and other environments (Davidson, 2018).

Inland wetlands present the fastest declining rate among freshwater ecosystems in Europe and are being lost also at alarming pace elsewhere (Malak *et al.*, 2019). Recent estimates, indicate that about 64-71% of inland wetlands have already been lost worldwide, largely surpassing losses in other ecosystems, including coastal wetlands (Gardner & Finlayson, 2018; Davidson, 2018). However, most inland wetlands (89%) remain unprotected (Reis *et al*., 2017).

Wetland assessment improved considerably in recent years, largely benefitting from top-down approaches, involving the replacement of aerial photo-interpretation by more recent techniques, and the use of remote sensing and satellite imagery, high resolution multispectral imagery, LiDAR, and Geographic Information Systems (GIS), combined with automated or semi-automated identification processes (Kloiber *et al.*, 2015; Hu *et al.*, 2017; Lefebvre *et al.*, 2019). Identification and long-term monitoring of wetlands of international importance in the Mediterranean region have benefit significantly from programs like RAMSAR, GWO, MWO and SWOS (e.g., Geijzendorffer *et al.*, 2018; Malak *et al.*, 2019; GWO, 2021; Galewski *et al.*, 2021). However, information on a large proportion of wetlands of international importance is still out of date or being updated (Davidson *et al.*, 2019).

1.3 Inland wetlands in the Mediterranean region

Inland wetlands play a particularly important role in biodiversity conservation in the Mediterranean region hotspot, though face significant historical and contemporary threats than can be significantly exacerbate under future climates (Geijzendorffer *et al.*, 2018; Leberger *et al.*, 2020)

Mediterranean inland wetlands have a long history of conversion for other purposes and loss, as they have been erroneously considered as diseases hotbeds (e.g., malaria vector) or putrefy environments, source of unpleasant smell. Besides, they have been widely recognized as nutrient rich areas, and extraordinary locations for agriculture practices, and thus have been extensively dried and transformed for expanding agriculture and dammed for water supply (Horwitz, & Finlayson, 2011; Reyes, 2023). This, combined with other pressures, such as increasing water demand and overexploitation, urban and tourism development, and urban and industrial pollution has led to significant and permanent wetland degradation and loss (Smith & Darwall, 2006, Balbo *et al.*, 2017). Indeed, according to the Mediterranean Wetlands Outlook 2, wetlands in the Mediterranean region are estimated to have declined in 50% since 1970 (Geijzendorffer *et al.*, 2018).

Currently, the rate of loss of Mediterranean inland wetlands appears to show signs of stabilizing, but still face strong pressures and threats, such as pollution, water abstraction and ongoing climate change, that may lead to their collapse (Davidson, 2018; Malak *et al.*, 2019). The identification and monitoring of the remnant Mediterranean inland wetlands is thus a priority, and assessments of their current condition and biodiversity are urgently needed (Taylor *et al.*, 2021). Although, some vertebrates in the Mediterranean region, have shown positive trends in the last years benefiting from protected inland wetlands, such as birds, other wetland dependent vertebrates, show continuous declines, such as amphibians and fish (Balbo *et al.*, 2017; Galewski *et al.*, 2021).

1.4 Freshwater fish in the Mediterranean region

Freshwater ecosystems harbour a high number of species relative to the area they occupy worldwide (Revenga & Mock 2000). This is also the case, in the Mediterranean region which is a hotspot of biodiversity (Myers *et al.*, 2000; Cuttelod *et al.*, 2009), and particularly important for freshwater fish (Freyhof *et al.*, 2020).

Freshwater fish in the Mediterranean region include a high proportion of endemic species, some of which are among the most threatened biota worldwide (Abell *et al.*, 2008; Leprieur *et al.*, 2008; Ribeiro & Leunda, 2012). Indeed, endemic fish are three times more threatened than other animal groups in the region, with 70 % listing as Critically Endangered (CR), Endangered (EN) and vulnerable (VU) or as extinct (Smith & Darwall, 2006; Hermoso & Clavero, 2011). Prevalent threats to Mediterranean freshwater fish are the increasing water demand for agriculture, that combined with the effects of climate change can lead to population declines and local extinctions (Cuttelod *et al.*, 2009; Jarić *et al.*, 2019). The continued habitat degradation can also favour the proliferation of non-native fish species, that increasingly threat native fish fauna and causing species losses in the region (Ribeiro & Leunda 2012; Clavero *et al.*, 2013; Marr *et al.*; 2013). The status of Mediterranean freshwater fish is particularly worrisome but still not gained global importance and recognition (Darwall *et al.*, 2014; Máiz-Tomé *et al.*, 2017). In fact, there is a lack of long-term monitoring of species diversity, population size and trend, and also of habitat quality, which hampers the understanding, that hampers understanding of fish responses to current and future threats, and would be critical for conservation management, namely of inland wetlands (van Rees *et al.*, 2021).

Freshwater fish fauna in Portugal is rich, includes 42 native species, 10 of which are endemic to the region and 16 to the Iberian Peninsula (Collares-Pereira *et al.*, 2021). However, there are also 19 non-native fishes in the region which represent nearly 30% of total fish fauna (Collares-Pereira *et al.*, 2021), and new introductions are expected to occur at the rate of one additional species every two years (Anastácio *et al.*, 2019). Moreover, non-natives generally spread rapidly, with dispersion being particularly fast in top predatory fish (Martelo *et al.*, 2021). Not surprisingly, freshwater fish in Portugal are currently already severely threatened, with 63 % of the species listing as Critically Endangered, Endangered and Vulnerable (Cabral *et al.* 2005).

1.4 Tagus wetlands and fish fauna

Protected areas in Portugal occupied by inland wetlands remained practically the same from the 80's to 2010, demonstrating the weak conservation importance attributed to these areas during the establishment of the Portuguese Protected Areas Network, according to Araújo *et al.* (2022).

The most important protected inland wetland in the Lower Tagus is the Paul do Boquilobo, located in Golegã with a flooded area of ~180 hectares (ha), has been classified at national level as Natural Reserve since 1980 and also internationally by UNESCO Biosphere Reserve and Ramsar Convention (Decreto-Lei nº 198/80; http://icnf.pt/portal/ap/r-nat/rnpb). Additionally, other inland wetlands of relevant importance have been referenced in the region and classified as Local Protected Landscapes in the 80's (Decreto-Lei nº 197/80) by the Institute for Nature Conservation and Forests (ICNF), namely the Açude Monte da Barca and Açude Agolada, but are mostly artificialized water bodies, particularly reservoirs for irrigation purposes (Farinha & Trindade, 1994; Farinha *et al.*, 2001). More recently, two other inland wetlands have been georeferenced (Carta de Uso e Ocupação do Solo, COS 2018), namely Granja (Trancão sub-basin) and the riverine section of Gouxa (Ulme sub-basin), but there is still little or no information on local biodiversity (DGT, 2019). The last one is currently Paul da Gouxa getting classified as Natural Reserve, based on peatland (priority to EU Habitats Directive) present in their riverine section (personal observation). Nevertheless, other inland waters in the region remain poorly know, and unprotected.

Paul do Boquilobo, together with the Tagus estuary, also listed under Ramsar Convention, present the most important wetlands in the Lower Tagus, although these wetlands recent biodiversity assessments have been predominantly based on bird fauna (e.g., Catry *et al.*, 2011; Alonso *et al.*, 2019; Alonso *et al.*, 2022). The presence of other important inland wetlands in Lower Tagus have long been recognized and its classification as important sites for conservation is mainly based on the occurrence of priority habitats, flora, and birds (e.g., Decreto-Lei nº 198/80) and there is little information about other taxonomic groups, particularly freshwater fish.

The Tagus River Basin harbours the most important regional pool of freshwater fish in Portugal, including ~65 % of the species currently recognized in the country, and a total of 42 native species,10 of which are Portuguese endemics, and two endemic to this basin, Lisbon arched-mouth nase

(*Iberochondrostoma olisiponense*) (Gante, Santos & Alves, 2007) and Lampreia-do-Nabão (*Lampetra auremensis*) Mateus, Alves, Quintella & Almeida, 2013. Data on fish communities in the Lower Tagus are scarce, and mainly restrict to Paul do Boquilobo (Collares-Pereira *et al.*, 1994; Correia & Teixeira, 2003) and Paul das Caniceiras (Veríssimo *et al.*, 2018). To date, 19 fish species have been recorded in the region, 13 of which are native. In particular, inland wetlands harbour threatened endemics, the regional Lisbon arched-mouth nase (*Iberochondrostoma olisiponense*), and the Portuguese, Arched-mouth nase (*Iberochondrostoma lusitanicum*) (Collares-Pereira, 1980), both classified as Critical Endangered (CR) and Ruivaco (*Achondrostoma oligolepis*) (Robalo, Doadrio, Almada & Kottelat, 2005) with Least Concern (LC) classification, but also other species with elevated threatened status, including European eel (*Anguilla Anguilla*) (Linnaeus, 1758) (CR), Three-spined stickleback (*Gasterosteus aculeatus*) Linnaeus, 1758, national Endangered (EN), Comizo (*Luciobarbus comizo*) (Steindachner, 1864), Vulnerable (VU), and Southern chub (*Squalius pyrenaicus*) (Günther, 1868), (EN) (Cabral *et al.*¸2005; IUCN, 2022). However, the extent to which inland wetlands may still serve as refuge, nursery and feeding areas for native fish remains largely unknow, thought this has already been previously acknowledge (see Veríssimo *et al.*, 2018; Collares-Pereira et al 2021).

Inland wetlands in the Lower Tagus face significant degradation and loss. The Lower Tagus drains a large alluvial floodplain, by a low altitude and low gradient (~0.24 m/km), strongly shaped by annual floods occurring in late winter and early spring (Azevedo *et al.*, 2004; Vis *et al.*, 2010). This results in a mosaic of inland wetlands, some of which temporary or annual, many of which may have been lost over the centuries (Gibbs, 2000; Fernandes *et al.*, 2020). Indeed, the loss of inland wetlands in the Lower Tagus is expected to be high, due to mainstem channel transformation for flood protection and expansion of agricultural lands since the 16th century, and later (middle of 20th century) due to water regulation by dam construction, that is estimated to have increased the irrigation lands 25-fold (see Fernandes *et al.*, 2020 and references therein). Moreover, wetland loss may have been accelerated since the 50's in association with manmade modifications in natural flow regimes and flood timing, due to dam construction, artificial canalization, land use change, and water diversion for intensive agriculture (Fernandes *et al.*, 2020). The area has been historically used for agriculture, at least since 1855, but intensive agriculture has expended since 1940, with irrigated crops dominating 89 % of the area by 2000 (Fernandes *et al.*, 2020). This agricultural expansion most likely has contributed to the drainage of wetlands, similar to what has happened throughout other European countries (Finlayson *et al.*, 2005). Currently, there is a lack of historical and contemporary information about small wetlands, most of which are included in particular lands and private landholdings. Moreover, inland wetlands are currently threatened by, agriculture development, water withdrawal, water pollution and species invasions, among other pressures (Cordovil *et al.*, 2018; Fernandes *et al.*, 2020). This may contribute to wetland permanent loss without previous assessment of their natural values.

Remnant inland wetlands in the Lower Tagus may still be unique rare environments harbouring a rich fish species diversity and providing important ecological steppingstone corridors, and together with Tagus mainstem play an important role in maintaining the regional aquatic biodiversity (Gibbs, 2000). However, it is also possible that inland wetlands are used by at least some of the 15 non-native fish currently occurring in the Tagus basin. To date, no regional assessment of inland wetlands has been conducted, and there is little or no information about local fish communities, which represents a major knowledge gap and largely constraints conservation management.

1.5 Objectives

The primary aim of this study was to characterize inland wetlands in the Lower Tagus River, by assessing current land uses, habitat conditions and local fish communities. To achieve this, a multi-disciplinary approach was developed, combining remote sensing, geographic information techniques and fish and habitat monitoring. Firstly, inland wetlands across the region were identified using remote sensing and image interpretation techniques. Secondly, land use and change in the surroundings of selected wetlands were evaluated during Period 2 (2018) and Period 1 (2007, 2010, 2015), and their impacts were categorized. Third, local habitats were surveyed, and fish communities were sampled using multiple techniques and characterized in terms of species composition and abundance. Finally, all the gathered information was integrated and analysed for their implications in freshwater fish and wetland conservation management and restoration in the Lower Tagus.

2. Study area

The study was conducted in the freshwater realm of the Tagus River Basin, particularly in its Lower section, between Golegã and Lisbon, in Portugal (Figure 2.1). Tagus is the longest river in the Iberian Peninsula with a catchment area of 80 630 km^2, of which ~30% in Portugal. It flows for ~1 100 km, from the Sierra de Albarracín (Spain) at ~1 600 m above sea level (a.s.l.), into the Atlantic Ocean in Lisbon (Portugal) (Azevêdo *et al.*, 2004; Vis *et al.*, 2009; Feio & Ferreira, 2019). The climate in the Tagus Basin is temperate Mediterranean, with a north-south gradient in precipitation, ranging from Per-humid (A) to Dry sub-humid (C1), following the Thornthwaite classification system (APA, 2015). The average annual precipitation is ~870 mm but ranges north-south from 2 000 to 600 mm. Precipitation is highly seasonal, with 75% occurring between October to April.

This has a major influence on flow regimes, with discharges averaging 600 m^3/s and total volume *per* year 19 km^3, of which 66% originate in Spain (APA, 2015; Cordovil *et al.*, 2018). In Portugal, the Upper and Middle sections of the Tagus in Vila Velha de Rodão (V.V. Rodão) and Serra da Estrela (Figure 2.1) present a relevant orography, from 170 to 1900 m, while the Lower section includes an alluvial plain, ~85 km long and 2 to 10km wide, with low gradient (~24 cm *per* km), slow flow and side channels along the mainstem (Azevêdo *et al.*, 2004; Vis *et al.*, 2009; Fernandes *et al.*, 2020).

The region is characterized by a strong water demand for irrigation purposes, particularly for intensive rice paddies, orchards and arable crops (~1 173 hm3 per year) and for drinking water supply (~392 hm3 *per* year) (Cordovil *et al.*, 2018 and references therein).

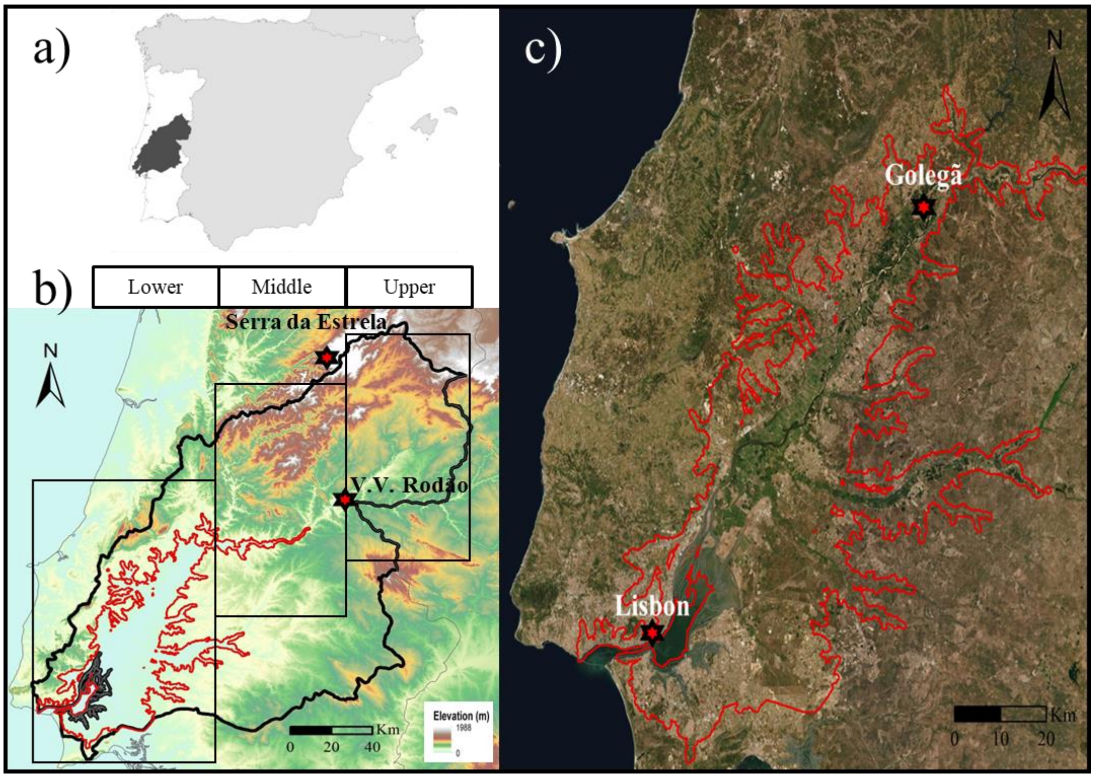

Figure 2.1 – Study area, showing the location of the Portuguese Tagus River Basin (dark) in the Iberian Peninsula (a), its Upper, Middle and Lower sections (b), and the limits of the study area (red) in the Lower Tagus (c) (Source: ArcGIS Base map).

This study focused on the alluvial floodplain in the Lower section of the Tagus basin henceforth designated as Lower Tagus (Figure 2.1). This area, with ~3 927 km^2, encompass 11 sub-basins draining into the Tagus mainstem, including from North to South: River Almonda (213 km^2), River Alviela (483 km^2), River Maior (923 km^2), River Alenquer (287 km^2), River Grande da Pipa (118 km^2) and River Trancão (279 km^2) on the right margin and Alpiarça Ditch & Ulme Stream (457 km^2), Muge Stream (703 km^2), Magos Stream (200 km^2), River Sorraia (7 611 km^2) and Estuário Streams (1 227 km^2) on the left margin. River Sorraia is the main contributor to annual discharge into the Lower Tagus (APA, 2015).

Intensive and extensive agriculture occupy ~51 % of the Lower Tagus, intensifying towards the mainstem. Specifically, intensive agriculture encompasses ~35% of the area, and includes mainly arable lands (i.e., irrigated and non-irrigated arable land, permanently irrigated fields, and rice fields) and permanent crops (i.e., vineyards, fruit and berry plantations, and olive groves), while extensive agriculture occupies ~16 %, and includes heterogeneous areas such as complex cultivation patterns and agroforestry. Urban and industrial uses occupy ~11% of the area, and the remnant natural uses ~38%, include grasslands, scrubs and forests (see classes selection criteria in Table 3.2) (DGT, 2019).

3. Methods

The methodological approach followed in this study included four main steps (Figure 3.1). First, wetlands throughout the Lower Tagus were identified using remote sensing techniques and image interpretation. Second, wetlands for sampling were characterized for habitat and landscape features. Third the selected wetlands were assessed for current land use, land use change, and anthropogenic pressures. Finally, fish assemblages were surveyed for composition and structure (Figure 3.1).

Figure 3.1 – Workflow of the methodological approach used in the study, including the main steps.

3.1. Wetlands inventory and selection

Study sites were selected after an inventory of wetlands in areas with elevations ≤ 50 m a.s.l. across the Lower Tagus, covering 3 927.20 km^2. Wetlands were assessed as locations maintaining water, based on Remote Sensing (RS) and Geographic Information System (GIS) techniques, using the software ArcGIS (Esri® ArcGIS Desktop 10.7.1).

Using a Digital Elevation Model (DEM) with 25 m × 25 m resolution, derived from the Shuttle Radar Topography Mission (STRM) for the Portuguese section of the Tagus basin, a 500 meters *buffer* was added to the elevation limits, to reduce highly reticulated and isolated sections and create a continuous inventory area. Wetlands were identified using a set of three *Sentinel-2* images, 10 m × 10 m resolution, acquired in the *Copernicus Open Access Hub* website (https://scihub.copernicus.eu/). Images selection was contingent on hydrological balance and satellite images availability for periods of low water levels to reduce the biases associated with the inclusion on rain flooded locations in the analysis (Perennou *et al.*, 2018). Specifically, the images selected were from 8th of August to 10th of October 2019, when rainfall in the area was virtually null (data consulted at https://snirh.apambiente.pt). Locations maintaining water, were identified using the Normalized Difference Water Index (NDWI), which is widely recognized as adequate to delineate open water features in remotely sensed digital imagery (McFeeters, 1996). NDWI makes use of reflected near-infrared radiation and visible green light to enhance water features while eliminating soil and terrestrial vegetation features and is defined as:

$$NDWI = \frac{\rho green - \rho NIR}{\rho green + \rho NIR}$$

where *ρgreen* is the reflectance of the green band (Band 3 of *Sentinel-2* images), and *ρNIR* is the reflectance of the near-infrared band (Band 8 of *Sentinel-2* images). The NDWI is negative (or zero) for soil and terrestrial vegetation and positive for water features, with values < 0.2 indicating areas with levels of humidity and values ≥ 0.2 indicating water surfaces. The function "*cloud mask*" was used in image treatment to avoid cloud associated biases, and input data were normalized to ensure NDWI ranged between 0 and 1. Satellite images were thoroughly interpreted, and locations with NDWI ≥ 0.2 were manually georeferenced using GoogleEarth® (Google Inc., Mountain View, CA, USA), and further investigated using the viewing feature, Historical Imagery® (Memphis, TN, USA). Pending on Google Earth images availability (generally from 2006 to 2021), environmental characteristics and hydrological regime at each location were assessed, and its potential to withstand fish communities over summer during the past two available years was evaluated. Specifically, locations were considered unsuitable in case they met one of the following Exclusion Criteria (E.C.): E.C. 1 – subjected to complete drought; E.C. 2 – highly artificialized (e.g., dammed); E.C. 3 – completely isolated (e.g., by weirs or irrigation peats); E.C. 4 – inadequately sized (< 0.5 hectares (ha) or > 50.0 ha) (Table S1 - Annex II). From a total 409 locations with water surfaces, 30 were considered as having the potential to support fish (see chapter 4.1 Wetlands distribution and size).

The 30 wetlands potentially supporting fish were visited from 27th to 29th of April of 2021, and evaluated for accessibility (public or private holdings), sampling feasibility (e.g., landing areas for boat) and safety for the material (e.g., vehicle and gear) and personnel (e.g., absence/presence of wild cattle) (Table S1 - Annex II). These factors were considered together as another exclusion criteria (E.C. 5). Whenever one of these aspects was not observed the wetland was excluded from sampling design. In total, 11 wetlands gathered adequate conditions for sampling (Figure 3.2; Table 3.1). These wetlands were distributed across the study area, six in the right margin and five on the left margin of the Tagus River and ranged between 0.5 and 9.0 ha (Table 3.1). For sampling design and in order to assure efficiency in habitat and fish assessments, selected wetlands were categorized according to their area into small (< 2.5 ha), medium (< 2.5 – 5.0 ha) and large (> 5.0 ha).

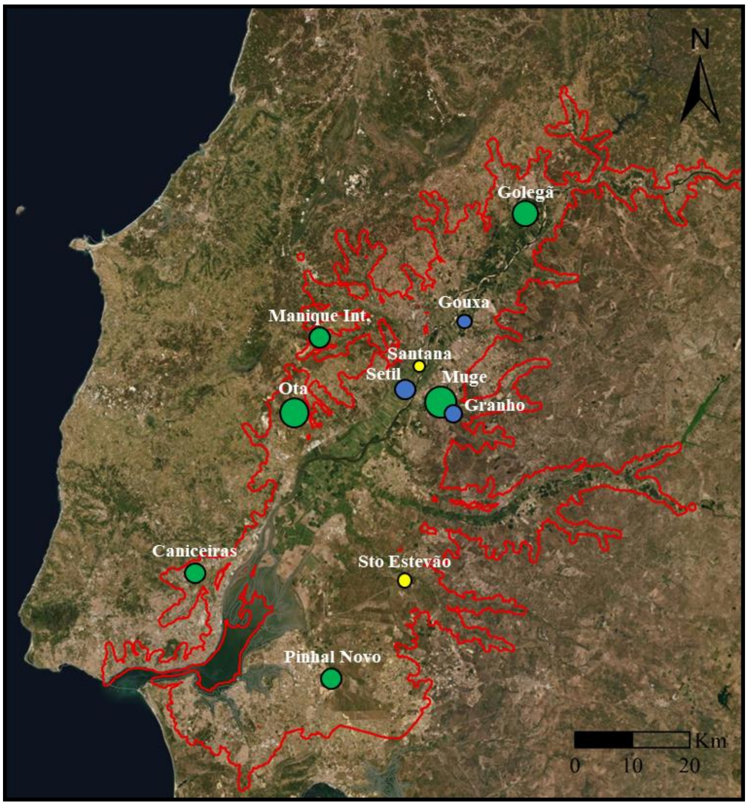

Figure 3.2 - Distribution of wetlands sampled for fish, habitat and land use and change across the study area (red). Wetlands are categorized into small < 2.5 ha (yellow), medium < 2.5 – 5.0 ha (blue) and large > 5.0 ha (green) and circle sizes are proportional to their area (Source: ArcGIS Base map).

Table 3.1 – Characteristics of the selected wetlands, with indication of coordinates (latitude and longitude), river margin (Right or Left), area in hectares (ha) and size category. * Henceforth coded as Manique Int.

Wetland	Latitude	Longitude	Margin	Area	Size category
Golegã	39.397814	-8.481033	Right	6.4	Large
Gouxa	39.235637	-8.585251	Left	2.5	Medium
Manique Intendente*	39.217467	-8.883387	Right	5.6	Large
Santana	39.141594	-8.755085	Right	0.5	Small
Muge	39.110913	-8.666394	Left	9.0	Large
Granho	39.094573	-8.638887	Left	2.8	Medium
Setil	39.113543	-8.767990	Right	3.8	Medium
Ota	39.101042	-8.947929	Right	7.5	Large
Sto Estevão	38.866444	-8.768979	Left	2.4	Small
Caniceiras	38.849556	-9.147281	Right	5.5	Large
Pinhal Novo	38.652897	-8.878746	Left	5.2	Large

3.2 Habitat and Landscape characterization

Habitat conditions at each wetland were characterized based upon 9 variables, between 6th of May to 11th of June 2021. Habitat was characterized at 5, 6 or 9 points randomly distributed across small, medium and large wetlands respectively. Conductivity (mS/cm), Temperature (ºC), Oxygen (O_2 %) and (O_2 mg/L), pH and Chlorophyll a (mSPU) were assessed using a multiparametric probe (YSI EXO2 Multiparameter Water Quality Probe). Turbidity was quantified using a Secchi Disk (cm), and Depth (cm) using a portable water depth sounder gauge (Vexilar LPS-1). The presence of cover (i.e., aquatic vegetation, branches or roots) was visually assessed and quantified in percentage according to their frequency of occurrence, and measurements for the remaining variables were averaged.

Each wetland was also characterized for landscape context based on Area (ha), Elevation (m), Strahler stream order, Hydrological distance to the Tagus River (km) and Connectivity to the nearest watercourse. Wetland area determined from its limits, elevation, and Strahler stream order (Strahler, 1957), determined from the nearest watercourse in the drainage network, were derived from the Digital Elevation Model (DEM) (see chapter 3.1 Wetlands inventory and selection). Hydrological distance was determined along the watercourse from the wetland limit to the Tagus River using Google Earth measurement tool. Connectivity to the nearest watercourse was evaluated *in loco* and wetlands were categorized as 1 – isolated from the watercourse (closed), 2 – Susceptible to watercourse flooding (partial), 3 – directly connected to the watercourse (open).

3.3 Land Use, land change and anthropogenic pressure characterization

The Land Use / Land Cover (LULC), LULC Change (LULCC) and anthropogenic pressures (Pressure Class) were also characterized for each wetland and will henceforth be designated together as land use variables. LULC was assessed from the Soil Use and Occupancy Charts (Carta de Uso e Ocupação do Solo, hereafter COS) for 2007, 2010, 2015 and 2018, provided by the Portuguese General Directorate for Territorial Development (Direção Geral do Território, hereafter DGT). COS were chosen instead of CORINE Land Cover, since COS are the official cartography for LULC and present a higher spatial resolution (Caetano & Marcelino, 2017; Caetano *et al.*, 2017).

For easy of interpretation and comparability, the land use classes considered in the multiple COS (Caetano *et al.*, 2017; DGT, 2019) were reclassified and categorized into four broad categories: 1 - Urban/Industrial (UI); 2 - Intensive Agriculture (IA); 3 - Extensive Agriculture (EA); 4 - Natural (NA).

The UI category encompasses classes from level 1 previously included in "Artificial areas"; IA encompasses level 1 classes from "Agricultural areas" to level 5 class 2.3 "Complex cultivation patterns"; EA encompasses the remnant level 5 classes from "Agricultural areas"; and NA all the classes from level 1 "Forest and semi-natural areas" to "Water bodies" (see Table 3.2). The LULC were assessed in an external 500 m *Buffer* surrounding the wetland limits. Specifically, the percentage of area occupied by each land use category in each wetland was determined from LULC homogenous patches using ArcGIS.

The LULC Change (LULCC) over years 2007, 2010, 2015 and 2018, was evaluated from the percentage areas of land use aggregated categories Extensive Agriculture (EA) and Natural (NA), which putatively represent areas with less impacts and highest biodiversity in opposition to Urban/Industrial (UI) and Intensive Agriculture (IA) categories that have been acknowledged to drive wetlands conversion and present a higher perversive impact on aquatic resources and biodiversity (Foley *et al.*, 2005; Asselen *et al.*, 2013). Specifically, the LULCC gain/loss in area for EA and NA was determined by its averaged area for 2007, 2010 and 2015 (LULC Period 1) minus its area in 2018 (LULC Period 2).

Anthropogenic pressures in each wetland were assessed using an adaptation of the Impact index by Hermoso *et al.* (2009). In its original form, the index classifies pressures into five categories, from excellent (1) to heavily disturbed (5) based on a hierarchical categorization of the percentage area occupied by Urban/Industrial (UI) and Intensive Agriculture (IA), with the benchmark for UI set at 1 %. Because this value will allow no discrimination of wetlands in the Lowland Tagus, with most falling in the heavily disturbed class, in the current study the benchmark for UI was set at 5 % (Table 3.3), and the index was calculated using data derived from COS 2018.

Table 3.2 – Aggregated categories of Land Use /Land Cover (LULC), and LULC Change (LULCC) used in this study, obtained from Land Use classes (Level 5) in Carta de Uso e Ocupação de Solos (COS) obtained from Portuguese General Directorate of Territorial Development (DGT). 1 – Urban/Industrial, 2 – Intensive Agriculture, 3 – Extensive Agriculture, 4 – Natural.

Aggregated classification	Level 5	Level 1
1 - Urban/Industrial (UI)	1.1.1.2 Continuous urban fabric predominantly horizontal; 1.1.2.1 Discontinuous urban fabric; 1.1.2.2 Sparse discontinuous urban fabric; 1.2.1.1 Industrial units; 1.2.2.1 Commercial units; 1.2.3.1 Agricultural facilities; 1.3.2.1 Water supply infrastructure; 1.3.2.2 Waste and wastewater treatment infrastructure; 1.4.1.1 Road network and associated land; 1.4.1.2 Rail network and associated land; 1.6.2.1 Campsites; 1.6.3.1 Cultural facilities and historic zones; 1.6.5.1 Other leisure facilities; 1.7.1.1 Parks and gardens	Artificial areas
2 – Intensive Agriculture (IA)	2.1.1.1 Non-irrigated arable land; 2.1.1.2 Rice fields; 2.2.1.1 Vineyards; 2.2.2.1 Orchards; 2.2.3.1 Olive groves	Agricultural areas
3 – Extensive Agriculture (EA)	2.3.2.1 Complex cultivation patterns; 2.3.3.1 Agriculture with natural and semi-natural vegetation; 2.4.1.1 Agricultural nurseries and greenhouses; 3.1.1.1 Improved pastures; 3.1.2.1 Spontaneous pastures; 4.1.1.1 AFS of cork oak with permanent crops; 4.1.1.7 AFS of other mixtures with permanent crops	
4 - Natural (NA)	5.1.1.1 Cork oak open forests; 5.1.1.2 Holm oak open forests; 5.1.1.5 Eucalyptus open forests; 5.1.1.7 Open forests of other broadleaved species; 5.1.2.1 Maritime pine open forests; 5.1.2.2 Stone pine open forests; 5.1.2.3 Open forests of other coniferous species; 6.1.1.1 Open shrublands	Forest and semi-natural areas
	9.1.1.1 Natural water courses; 9.1.2.2 Natural inland lakes and lagoons; 9.1.2.4 Small dam reservoirs; 9.1.2.5 Artificial ponds	Water bodies

Table 3.3 – Hierarchical categorization of anthropogenic pressure in inland wetlands in the Lower Tagus, according to percent areas occupied by Urban/Industrial (UI) and Intensive Agriculture (IA) (adapted from Hermoso *et al.* (2009)).

Pressure variables		**Pressure class**
UI	IA	
	> 30 %	5 (Heavily disturbed)
≥ 5 %	10 - 30 %	4 (Disturbed)
	< 10 %	3 (Weakly disturbed)
	> 30 %	4 (Disturbed)
< 5 %	10 – 30 %	2 (Good)
	< 10 %	1 (Excellent)

3.4 Fish community survey

A combination of multiple techniques was used to assess community composition and structure, including electrofishing, seine net and minnow traps. Given the efficiency limitations of each individual technique in association with habitat variability, this approach was the most adequate to increase species detectability and catchability and thus maximize fish richness and captures (Gibson-Reinemer *et al.*, 2017).

Sampling was conducted between 6th of May until 11th of June 2021 with sampling effort adjusted to wetland size and habitat heterogeneity (Table 3.4). In each wetland, three electrofishing transects were performed using a standard gear (EL62 II, Hans Grassl, Germany; discharging 300-600V, 2-5 A, DC), each lasting for 10, 20 and 30 minutes in small, medium and large wetlands, respectively. A seine net with 15 m long, 1.20 m height and 2.5 mm mesh size, was used at one, two or three locations, in small, medium and large wetlands, respectively. Finally, baited minnow traps, one large (60x25 cm with 15.0 mm mesh) and one small (45x25 cm with 2.0 mm mesh) were set in pairs, and 3, 6 and 9 pairs of traps were distributed across small, medium and large wetlands respectively. Traps bait consisted in 24.0 mm commercial Wellmix Boilies® (monster crab flavour), set between 10:00 a.m. and 1:30 p.m., and maintained for 180 to 510 min.

All captured fish were identified to species level and counted. After record, native species were returned to the wetland, and non-native species were euthanised, using an overdose of clove oil (BIOVERT®), complying with the National and European regulations on handling wild animals (Decreto-Lei n.º 92/2019; Directive 2010/63/EU).

Table 3.4 - Fishing techniques used in Small, Medium and Large wetlands, with indication of area covered (m^2) and time (min) of use. NA – Not applied due to abundant vegetation.

		Electrofishing		Seine net	Minnow traps
Wetland	**Category**	Area (m^2)	Time (min)	Area (m^2)	Time submerged (min)
Golegã	Large	2 100	90	800	4 590
Gouxa	Medium	800	60	80	2 520
Manique Int.	Large	1 850	90	1 450	3 780
Santana	Small	600	30	150	1 500
Muge	Large	900	90	450	3 240
Granho	Medium	1 700	60	400	2 520
Setil	Medium	900	60	300	2 400
Ota	Large	1 250	90	600	3 600
Sto Estevão	Small	800	30	450	2 160
Caniceiras	Large	1 300	90	750	3 510
Pinhal Novo	Large	2 300	90	NA	1 080

3.5 Data analysis

Geographic information analysis was performed in ArcGIS Desktop (version 10.8.1), data projected in the coordinate system PT- TM06/ETRS89.

Data on habitat, landscape, land use, and fish communities were described using basic statistics (i.e. mean, standard deviation, minimum and maximum) and further analysed in R Studio software (version 1.4.1717) using *vegan* package (Oksanen *et al.*, 2007).

Significance of statistical testing was assessed at $p<0.05$ (Zar, 1999).

3.5.1 Environmental gradients

Principal Component Analysis (PCA) was used to summarize environmental gradients among wetlands (McCune *et al.*, 2002). Because the number of wetlands was low relative to the number of environmental variables assessed, three separate PCAs were carried, for habitat, landscape and land use. Prior to analysis, variables were inspected for normality and outliers and transformed as Log10 or arcsin squared root for percentages (see Table 4.1; Zar, 1999). To avoid multicollinearity issues, Pearson correlations between variables were determined and highly correlated ($R> |0.70|$) pairs were eliminated, retaining for analysis the variable from each pair most relevant to the interpretation of fish patterns as derived from the literature (Zuur *et al.*, 2010).

3.5.2 Fish community structure and composition

For the analysis of fish community structure and composition, the catch per unit effort data from different sampling gears were combined using the Multigear Mean Standardization (MGMS) method developed by Gibson-Reinemer *et al.* (2017). Data from each gear were expressed as catch *per* unit effort (CPUE), differentiating each technique by catchability and effort. Specifically, different values of catchability were attributed to electrofishing (1), seine net (0.5) and minnow traps (0.1) based on the total numbers of fish captured. Electrofishing accounted for 5 270 fish, seine net for 2 428 fish and minnow traps for 574 fish (~10 %). The "*per* unit effort" was defined, as the time submerged (minutes) *per* pair of minnow traps, the total time (minutes) for electrofishing, and the total area (m^2) covered by the seine net (Table 3.4). The CPUE data for each gear were standardized using a form of mean centring, and only after combined, by adding centred data from each gear together for each species from each wetland. This method preserves the species structure within and among wetlands, since values of catchability for each gear are kept constant, and maintains species abundance, because rare and abundant species weight equally in the data transformation, providing a more robust basis for multivariate analysis (Gibson-Reinemer *et al.*, 2017). Hereafter, the fish data standardization will be referred as MGMS data.

Fish communities data was standardized prior being assessed in terms of richness (number of species; S), and diversity (Shannon-Wiener diversity index; H') (Spellerberg, 2008). Also, variation in fish community structure among wetlands was analysed using Detrended Correspondence Analysis (DCA) (Legendre & Legendre, 1998). Prior to analysis, MGMS data were Hellinger transformed to dampen the influence of zero's in the analysis matrix (Borcard *et al.*, 2011).

3.5.3 Fish-environment relationships

Canonical Correspondence Analysis (CCA) was used to explore fish-environment relationships. A unimodal model of ordination was performed, because preliminary DCA (see chapter 4.5) showed turnovers along the first axes over to 2 SD, which is the recommended criterion for choosing unimodal and linear ordination models (ter Braak & Verdonschot, 1995). Because preliminary use of PC axis as explanatory variables produced a non-significant ordination model (not shown here), that CCA was carried on MGMS species data, and the main variables retained from the first two PC axes of the habitat (DEP, CVR), landscape (CONN, SORD) and land use (UA, LULCC) were used as explanatory variables (see Table 4.4; Legendre & Legendre, 1998). This way, explanatory variables reduced to six. Prior to analysis, MGMS data were Hellinger transformed to dampen the influence of zero's in the matrix, as suggested in Borcard *et al.* (2011). Model building used a forward selected procedure, based on Akaike Information Criterion (AIC), and was conducted using the *"step"*. Statistical significance in CCA was determined by permutations tests (999 permutations), as recommended by Borcard *et al.* (2011).

4. Results

4.1 Wetland distribution and size

From the 409 locations that presented NDWI > 0.2 across the study area, 199 were completely isolated, 94 were inadequate in size (< 0.5 ha), 54 were heavily artificialized and 32 exhibited periods of complete desiccation and were considered to have no potential to sustain fish. Also 19 locations were visited and excluded due to lack of access and conditions for sampling (Table S1 – Annex II). The 11 wetlands selected for sampling showed NDWI ≥ 0.5 (Figure 4.1), ranged from 0.5 to 9.0 ha. And covered a total of 51.2 ha. Specifically, two wetlands were small < 2.5 ha (Santana and Sto Estevão), three medium 2.5 – 5.0 ha (Gouxa, Granho and Setil) and six of large size > 5.0 ha (Golegã, Manique Intendente, Muge, Ota, Caniceiras and Pinhal Novo).

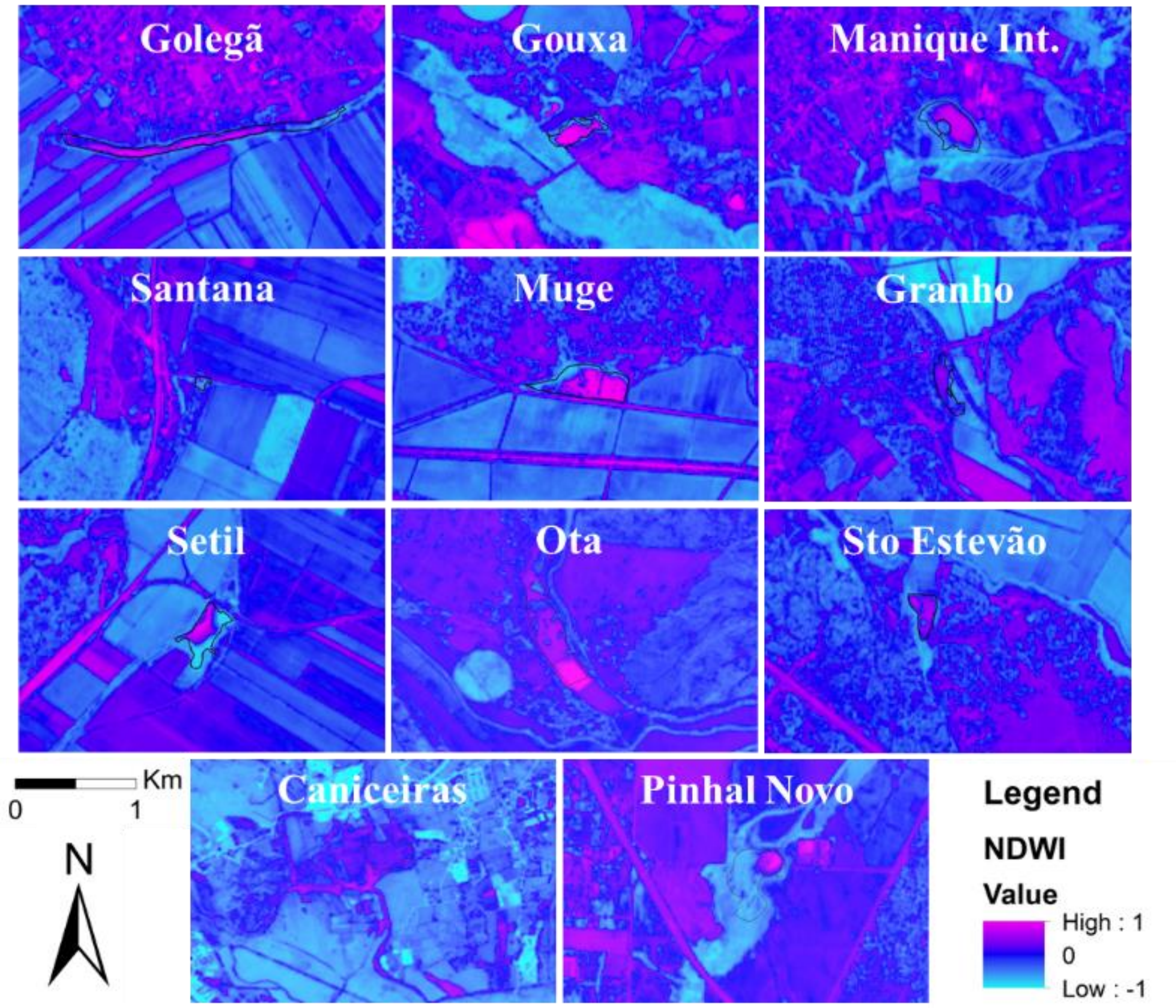

Figure 4.1 – Variation in NDWI among the 11 selected wetlands identified in the Lower Tagus.

4.2 Habitat and landscape contexts

Wetlands habitat, landscape and land use characteristics are summarized in Table 4.1. Temperature varied from 17.4ºC to 22.1ºC in Santana and Caniceiras, respectively. Conductivity varied from 0.261 µs/cm to 1.023 µs/cm in Granho and Santana. The lower and higher levels of pH were registered in Granho (6.7) and Sto Estevão (9.3). The wetland with lowest depth was Muge (40 cm) and the deepest was Golegã (263 cm). Maximum percentage of cover was registered in Pinhal Novo (100 %).

Most wetlands were located at less than 10 m a.s.l., and Santana was the lowest (2 m). Wetlands at higher elevations, were Golegã (18 m), Gouxa (10 m), Pinhal Novo (21 m) and Manique Intendente (35 m). Golegã, Muge and Pinhal Novo were not connected to watercourse, and Granho, Setil, Ota and Sto Estevão directly connected to the water course. The highest stream order occurred in Setil (5), followed by Muge and Sto Estevão (4) and the lowest order was observed for Golegã, Santana and Caniceiras (1). The wetlands more distant of Tagus mainstem were Manique do Intendente (46.8 km) and Sto Estevão (28.9 km), and the closest was Golegã (2.5 km).

Table 4.1 – Habitat, landscape and land use for studied wetlands in the Lower Tagus, between May and June of 2021. For each variable, is indicated the units, type of transformation, method of extraction (*in situ*, GIS, Google Earth) and variable code. Values are the mean ± standard deviation and the range (minimum-maximum) in the original units. Note: Variables included in the multivariate analysis in bold and variables excluded with *.

Variables (units, transformation)	Method	Code	Mean ± Std Dev	Range (min – max)
Habitat				
Temperature (ºC, log10)	*in situ*	TEMP	20.7 ± 1.4	(16.3 – 22.9)
Conductivity (µs/cm, log10)	*in situ*	COND	0.570 ± 0.229	(0.255 – 1.095)
Dissolved oxygen (%, arcsin√p) *	*in situ*	DO %	84.3 ± 37.5	(3.9 – 158.4)
Dissolved oxygen (mg/L, log10) *	*in situ*	DO (mg/L)	7.5 ± 3.3	(0.4 – 14.5)
pH (log10)	*in situ*	PH	7.7 ± 0.6	(6.5 – 9.5)
Chlorophyll a (mSPU, log10) *	*in situ*	CHA	28.7 ± 20.8	(3.2 – 75.0)
Secchi (cm, log10) *	*in situ*	SCH	46.4 ± 48.2	(5.0 – 180.0)
Depth (cm, log10)	*in situ*	DEP	98.9 ± 93.2	(20.0 – 528.0
Cover (%, arcsin√p)	*in situ*	CVR	78.5 ± 11.2	(66.7 – 100.0)
Landscape				
Area (ha, log10)	GIS	AREA	4.7 ± 2.5	(0.5 – 9.0)
Elevation (m, log10)	GIS	ELEV	10.4 ± 10.3	(2.0 – 35.0)
Connectivity (log10)	*in situ*	CONN	2.1 ± 0.8	(1 – 3)
Stream order (log10)	GIS	SORD	2.5 ± 1.4	(1 – 5)
River distance (Km, log10)	Google Earth	RD	17.0 ± 11.9	(2.5 – 46.8)
Land use				
Urban/Industrial (%, arcsin√p)	GIS	UI	7.7 ± 8.8	(0.0 – 24.7)
Intensive Agriculture (%, arcsin√p)	GIS	IA	46.8 ± 23.6	(10.2 – 83.1)
Extensive Agriculture (%, arcsin√p)	GIS	EA	12.9 ± 10.4	(0.0 – 38.4)
Natural (%, arcsin√p) *	GIS	NA	32.6 ± 22.3	(2.3 – 72.8)
LULC Change (%, arcsin√p)	GIS	LULCC	3.8 ± 6.5	(-5.4 – 12.6)
Pressure Class (log10) *	GIS	PC	3.6 ± 1.4	(2.0 – 5.0)

4.3 Land use and change and anthropogenic pressures

There were a high heterogeneity of land uses across the wetlands in the Lower Tagus over 2007, 2010, 2015 and 2018 (Figure 4.2).

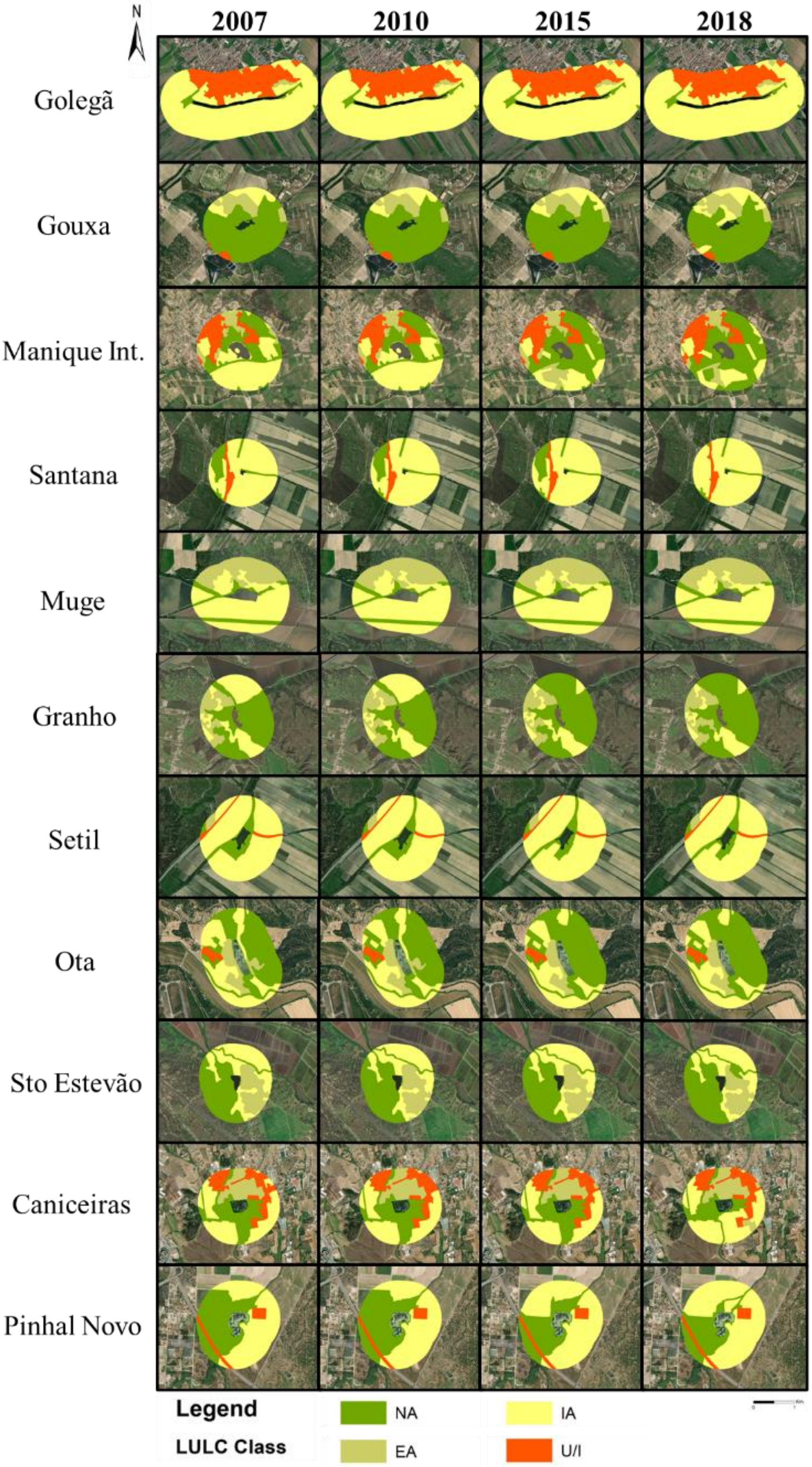

Figure 4.2 – Land Use Land Cover (LULC) percentage over years 2007, 2010, 2015 and 2018 *per* studied wetland in the Lower Tagus (Source: ArcGIS Base map).

In 2018, the percentage of Urban/Industrial area (UI) in wetlands surroundings was on average 7.8 %, with high values in Golegã (24.7 %), Caniceiras (20.9 %) and Manique Intendente (19.1 %). Conversely, Muge, Granho and Sto Estevão surroundings were not occupied by UI areas. Intensive Agriculture (IA) was the most represented use on average (42.9 %), reaching a maximum of 83.1 % in Setil, percentages over 30 % in Golegã, Santana, Muge, Caniceiras and Pinhal Novo, and never less than 10 % in the remaining wetlands (10.2 % - 26.3 %). Extensive Agriculture (EA) averaged 18.1 % of area, being less represented in Golegã (1.5 %) and most represented in Muge (38.4 %). Natural area (NA) occupied on average 31.2 % of the wetlands surroundings being less represented also in Golegã (2.3 %) and reaching over 50.0 % in Gouxa (66.5 %), Granho (65.9 %) and Sto Estevão (64.5 %) (Table 4.2).

Table 4.2 – Percentage (%) of Land Use Land Cover (LULC) in Period 2 (2018) and mean Period 1 LULC obtained from years (2007 – 2010 – 2015) for each wetland in the Lower Tagus.

Wetland	**Period 2 LULC (%)**				**Mean Period 1 LULC (%)**			
	UI	**IA**	**EA**	**NA**	**UI**	**IA**	**EA**	**NA**
Golegã	24.7	71.4	1.5	2.3	24.7	71.6	1.4	2.3
Gouxa	1.7	10.2	21.6	66.5	1.7	12.0	14.0	72.3
Manique Int,	19.1	18.6	37.8	24.5	18.2	36.3	12.5	33.0
Santana	6.3	77.1	11.8	4.8	6.3	80.0	0.0	13.8
Muge	0.0	49.0	38.4	12.7	0.0	59.4	27.9	12.7
Granho	0.0	11.8	22.3	65.9	0.0	28.2	15.3	56.5
Setil	3.8	83.1	2.0	11.1	3.8	81.9	2.0	12.4
Ota	2.9	26.3	23.0	47.8	2.9	32.8	11.5	52.8
Sto Estevão	0.0	21.5	14.0	64.5	0.0	36.8	20.8	42.5
Caniceiras	20.9	41.1	19.5	18.5	21.0	35.2	18.0	25.8
Pinhal Novo	6.3	61.7	7.2	24.8	6.3	54.5	0.0	39.2
Mean ± Std Dev	7.8 ± 9.2	42.9 ± 27.1	18.1 ± 12.5	31.2 ± 25.2	7.7 ± 9.1	48.0 ± 22.9	11.2 ± 9.4	33.0 ± 22.0
Range (min – max)	(0.0 – 24.7)	(10.2 – 83.1)	(1.5 – 38.4)	(2.3 – 66.5)	(0.0 – 24.7)	(12.0 – 81.9)	(0.0 – 27.9)	(2.3 – 72.3)

Between the Period 1 (2007-2010-2015) to Period 2 (2018) there were considerable increase in the EA percentage due to the loss of area in IA and NA. However, these tendencies were not observed, in Setil, Caniceiras and Pinhal Novo where IA increased, while in Granho and Sto Estevão NA increased area due to IA decline. Overall, eight wetlands (Golegã, Gouxa, Manique Int., Santana, Muge, Granho, Ota and Sto Estevão) lost percentage area of IA over time while three gained (Setil, Caniceiras and Pinhal Novo) (Table 4.2).

UI practically maintained its percentage areas in all wetlands, with exception of Manique Intendente where it increased by 1.0 %. The major differences were observed at IA, with an average loss of -5.1 %, and higher decline in Manique Intendente (-17.7 %), Muge (-10.4 %), Granho (-16.4 %) and Sto Estevão (-15.2 %). However, some wetlands gained percentage of IA, in particular Setil (1.2 %), Caniceiras (5.9 %) and Pinhal Novo (7.2 %). Also, EA increased on average 6.9 % in area, with major gains in Manique Intendente (25.3 %), Santana (11.8 %) Muge (10.5 %) and Ota (11.5 %). Conversely, Sto Estevão lost -6.8 % area of EA (Table 4.2).

NA areas showed a low overall decrease of -1.8 %, with declines in Setil (-1.2 %), Caniceiras (-7.3 %) and Pinhal Novo (-14.4 %) associated with IA increases, while in Gouxa (-5.8 %), Manique Intendente

(-8.6 %), Santana (-9.0 %) and Ota (-5.0%) with increases in EA. Still, NA increased in Granho and Sto Estevão, with gains reaching 9.5 % and 22.0 %, respectively. Golegã and Muge maintained NA areas (Table S1 – Annex I).

In overall, the Land Use Land Cover Change (LULCC) presented a gain of EA and NA areas in the surroundings of the majority of wetlands (70.2 %). The total gain was 70.2 %, and the wetlands with major gains were Manique Intendente (16.7 %), Granho (16.5 %) and Sto Estevão (15.2 %). Conversely, loss of EA and NA in detriment to IU and IA areas occurred in Setil (-1.2 %), Caniceiras (-5.8 %) and Pinhal Novo (-7.2 %) (Figure 4.3).

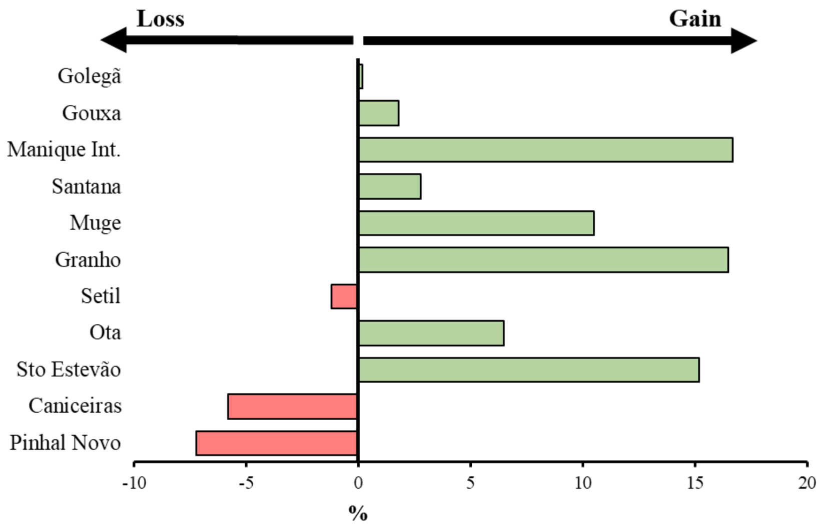

Figure 4.3 – Gain/loss (%) of Land Use Land Cover Change (LULCC) for EA + NA areas between Period 1 (2007, 2010, 2015) and Period 2 (2018) for each studied wetland in the Lower Tagus.

In 2018, most wetlands were classified in high classes of anthropogenic pressure, namely three as Disturbed (Manique Int., Muge and Setil) and four as Heavily disturbed (Golegã, Santana, Caniceiras and Pinhal Novo). The remaining four wetlands (Gouxa, Granho, Ota and Sto Estevão) were classified Good in terms of anthropogenic pressures (Table 4.3).

Table 4.3 – Anthropogenic pressure classification for each studied wetland in the Lower Tagus, according to LULC from 2018.

Wetland	**Pressure class**	**Classification**
Golegã	5	Heavily disturbed
Gouxa	2	Good
Manique Int.	4	Disturbed
Santana	5	Heavily disturbed
Muge	4	Disturbed
Granho	2	Good
Setil	4	Disturbed
Ota	2	Good
Sto Estevão	2	Good
Caniceiras	5	Heavily disturbed
Pinhal Novo	5	Heavily disturbed

4.4 Main environmental gradients

High correlations (*R*> |0.70|) were observed between habitat variables and also between land use variables, but not between landscape variables (< |0.40|) (Figure S1 – Annex I). Variables retained for ordination analysis are presented in Table 4.1.

The results of the PCAs of habitat, landscape and land use variables are summarized in Figure 4.4 and shown in detail in Tables S2, S3, S4, S5, S6, S7, in Annex I. Only the first two PC in each of analysis were interpreted (Table 4.4), since together they explained most of the total variation in the data (> 50%).

The first two PC accounted for 55.2 % of the total variation in habitat context (Figure 4.4a), with PC1 (31.7 %) describing an increase Cover (0.590) and pH (0.520), while PC2 (23.5 %) was reflected a decreased in Depth (-0.630) and an increase in Temperature (0.599). Caniceiras, Granho and Golegã differentiated from the remaining wetlands along PC1, while there was a dispersion of wetlands along PC2.

The first two PC explained 66.1 % of the variation in landscape context, with PC1 (40.2%) mainly highlighting a gradient of decreasing Connectivity (-0.571), while PC2 (25.9%) reflected decreases in both Stream Order (-0.595) and Area (-0.561) (Figure 4.4b). There was a dispersion of wetlands along PC1, while Santana, Golegã and Caniceiras differentiated from the remaining wetlands along PC2.

The first two PC explained 82.3 % of the total variation in land use conditions. The PC1 (62.8 %) mainly described a gradient between LULC Change (0.539) and Extensive Agriculture (0.520) and Intensive Agriculture (-0.531), while PC2 (19.5 %) gradient mostly described by Urban/Industrial (0.848). Golegã, Pinhal Novo and Setil separated from Granho, Muge and Sto Estevão along PC1, while Caniceiras and Manique distinguished from Sto Estevão and Setil along PC2 (Figure 4.4c).

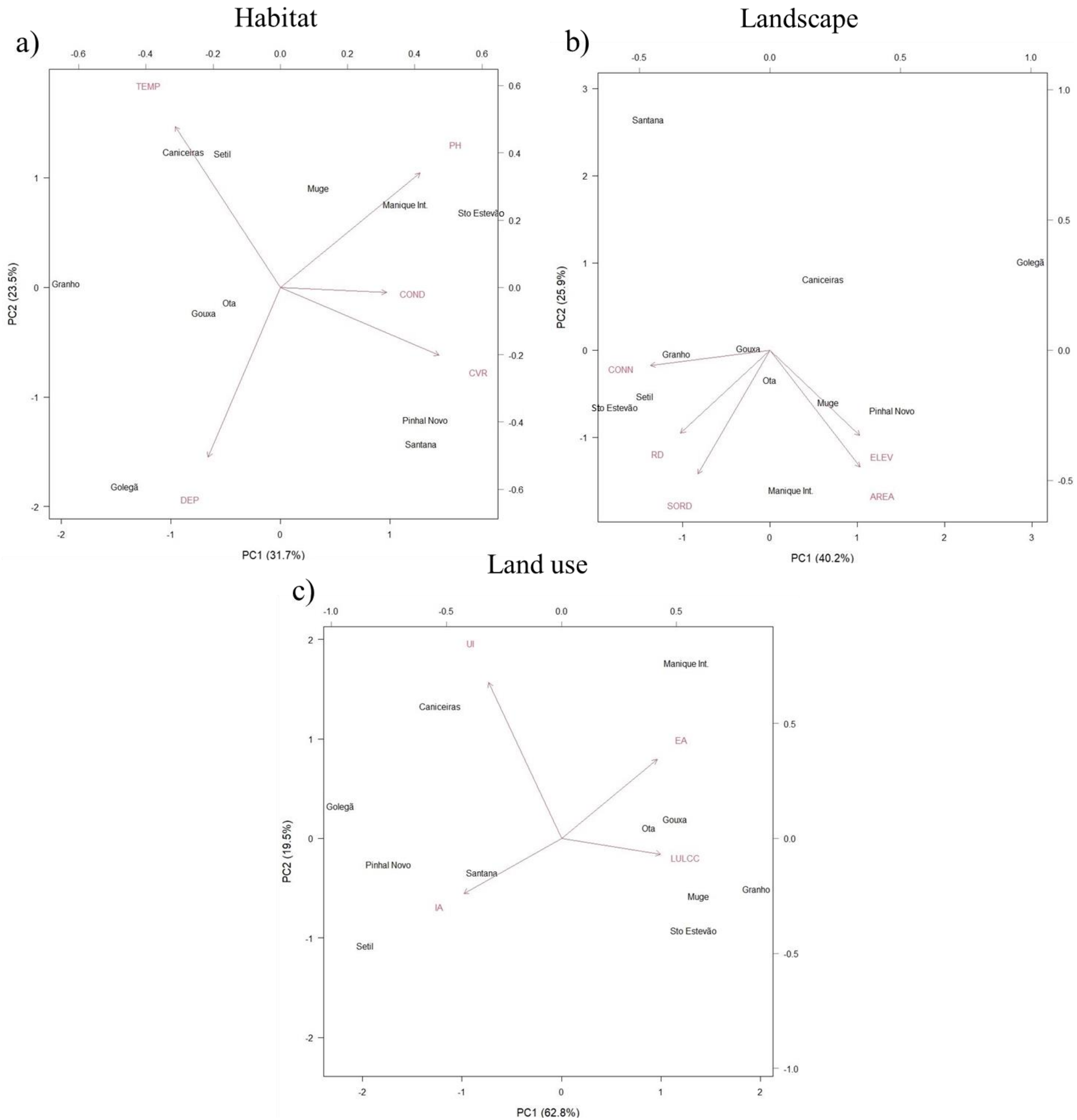

Figure 4.4 – Principal Components Analysis (PCA) of habitat (a), landscape (b) and land use (c) variables for the 11 selected wetlands in the Lower Tagus. Variable codes are in Table 4.1.

Table 4.4 – Results from PC1 and PC2 of the Principal Component Analysis (PCA) conducted on habitat, landscape and land uses. Main explanatory variables were retained for the Canonical Correspondence Analysis (CCA). Note: Variables included in the analysis in bold and variables excluded with *.

Variables		PC1	PC2
Habitat	TEMP*	-0.392	0.599
	COND*	0.395	-0.019
	PH*	0.520	0.424
	DEP	-0.269	**-0.630**
	CVR	**0.590**	-0.252
Landscape	AREA*	0.431	-0.561
	ELEV*	0.430	-0.410
	CONN	**-0.571**	-0.073
	SORD	-0.346	**-0.595**
	RD*	-0.428	-0.399
Land use	**UI**	-0.396	**0.848**
	IA*	-0.531	-0.299
	EA*	0.520	0.429
	LULCC	**0.539**	-0.085

4.5 Fish communities

Fish occurred in all wetlands except Pinhal Novo, where unforeseen excessive vegetation constrained sampling and precluded the use of seine nets. Therefore, this wetland was excluded from fish community analyses.

A total of 8 272 fish was captured, including 5 native and 8 non-native species. Native species were European eel (*Anguilla anguilla*), Thinlip grey mullet (*Chelon ramada*), Southern Iberian spined loach (*Cobitis paludica*), Lisbon arched-mouth nase (*Iberochondrostoma olisiponense*) and Iberian barbel (*Luciobarbus bocagei*). Non-native species were Bleak (*Alburnus alburnus*), Black bullhead (*Ameiurus melas*), Common carp (*Cyprinus carpio*), Goldfish (*Carassius* sp.), Eastern mosquitofish (*Gambusia holbrooki*), Pumpkinseed sunfish (*Lepomis gibbosus*), Largemouth bass (*Micropterus salmoides*) and Pikeperch (*Sander lucioperca*) (Table 4.5). European eel was the most widespread native species occurring in five wetlands. Eastern mosquitofish was found in all wetlands (Table 4.5).

Species richness *per* wetland ranged from three to eight species. The average richness of overall species was 5.3 ± 1.8 SD and richness of non-native was higher (4.1 ± 1.7 SD; range 2-7 species) than that of native species (1.2 ± 0.9 SD; range 0-3) (Table 4.6). The wetland with the highest overall species was Granho (8) and the poorest were Manique Intendente and Santana (3). Caniceiras presented the highest richness of native species (3), and no native species were found in Santana and Muge. The number of non-native species was particularly high in Granho (7), and there were never less than two species in wetlands (Table 4.6).

In terms of CPUE, the non-native Eastern mosquitofish dominated local communities, being particularly abundant in Manique Intendente, Muge and Santana (> 70 %) (Figure 4.5). Common carp was highly abundant in Ota (78.5 %), and Largemouth bass dominated the community in Golegã (56.9 %) and was also abundant in Gouxa (11.1 %). The Bleak was dominant in Granho (64.4 %), being also present in Setil (7.5 %) and Santo Estevão (2.1 %), although in lower abundances. Black bullhead showed higher abundance in Santo Estevão (40.2 %). Pikeperch was captured in Muge only (Figure 4.5). Native Thinlip grey mullet dominated fish community in Setil (~50 %), where Iberian barbel were also found. The European eel reached the highest abundances in Caniceiras (24.2 %), that also hosted the Lisbon arched-mouth nase (24.2 %) and the Southern Iberian spined loach (3 %). These species were also present in Granho and Golegã, but always in very low numbers (< 2%), respectively.

Shannon-Wiener Index diversity *per* wetland, ranged from 0.22 in Muge to 1.48 in Caniceiras. The highest diversity of native species was also found in Caniceiras (0.88), followed by Golegã (0.24) and Setil (0.11). Conversely, non-native diversity ranged from 0.22 in Muge and 0.26 in Manique Intendente to 1.09 in Granho (Table 4.6).

Table 4.5 - Fish species from the studied wetlands of the Lower Tagus, with indication of their origin (N, native; NN, non-native), conservation category in the IUCN Red List of Threatened Species (IUCN, 2022; www.iucnredlist.org) (CR, critically endangered; EN, endangered; VU, vulnerable; LC, least concern; *NA, Not Applicable), total number (N), frequency of occurrence (FO, Pinhal Novo excluded), and CPUE determined by multi-gear mean standardization (MGMS) (mean ± standard deviation and the range (minimum-maximum)).

Species	**Name**	**Origin**	**IUCN Red List**	**FO**	**N**	**CPUE**	
						Mean ± Std Dev	**Range (min – max)**
Anguilla anguilla	European eel	N	CR	0.50	35	0.01 ± 0.01	(0.00 - 0.02)
Chelon ramada	Thinlip grey mullet	N	LC	0.20	180	0.07 ± 0.17	(0.00 - 0.51)
Cobitis paludica	Southern Iberian spined loach	N	VU	0.20	2	0.00 ± 0.00	(0.00 - 0.00)
Iberochondrostoma olisiponense	Lisbon arched-mouth nase	N	CR	0.20	12	0.00 ± 0.01	(0.00 - 0.01)
Luciobarbus bocagei	Iberian barbel	N	LC	0.10	3	0.00 ± 0.00	(0.00 - 0.01)
Alburnus alburnus	Bleak	NN	*NA	0.30	191	0.09 ± 0.27	(0.00 - 0.85)
Ameiurus melas	Black bullhead	NN	*NA	0.60	163	0.09 ± 0.16	(0.00 - 0.45)
Cyprinus carpio	Common carp	NN	*NA	0.40	1196	0.47 ± 1.46	(0.00 - 4.62)
Carassius sp.	Goldfish	NN	*NA	0.80	140	0.05 ± 0.11	(0.00 - 0.35)
Gambusia holbrooki	Eastern mosquitofish	NN	*NA	1.00	4769	2.02 ± 4.75	(0.01 - 15.47)
Lepomis gibbosus	Pumpkinseed sunfish	NN	*NA	0.50	652	0.20 ± 0.34	(0.00 - 1.04)
Micropterus salmoides	Largemouth bass	NN	*NA	0.40	921	0.29 ± 0.86	(0.00 - 2.74)
Sander lucioperca	Pikeperch	NN	*NA	0.10	8	0.00 ± 0.01	(0.00 - 0.03)

Table 4.6 – Fish species richness (S) and diversity (H') from the studied wetlands of the Lower Tagus, with indication of mean ± standard deviation and the range (minimum-maximum). * NA - No fish captured.

Wetland	**Richness (S)**			**Diversity (H')**		
	Overall	Natives	Non-natives	Overall	Natives	Non-natives
Golegã	7	2	5	1.03	0.24	0.98
Gouxa	6	1	5	0.92	0.00	0.84
Manique Int.	3	1	2	0.34	0.00	0.26
Santana	3	0	3	0.60	0.00	0.60
Muge	4	0	4	0.22	0.00	0.22
Granho	8	1	7	1.16	0.00	1.09
Setil	6	2	4	1.02	0.11	0.51
Ota	4	1	3	0.60	0.00	0.59
Sto Estevão	7	1	6	1.29	0.00	0.98
Caniceiras	5	3	2	1.48	0.88	0.69
Pinhal Novo*	NA	NA	NA	NA	NA	NA
Mean ± Std Dev	5.3 ± 1.8	1.2 ± 0.9	4.1 ± 1.7	0.86 ± 0.41	0.12 ± 0.28	0.67 ± 0.30
Range (min – max)	(8 - 3)	(0 - 3)	(2 - 7)	(0.22 - 1.48)	(0.00 - 0.88)	(0.22 - 1.09)

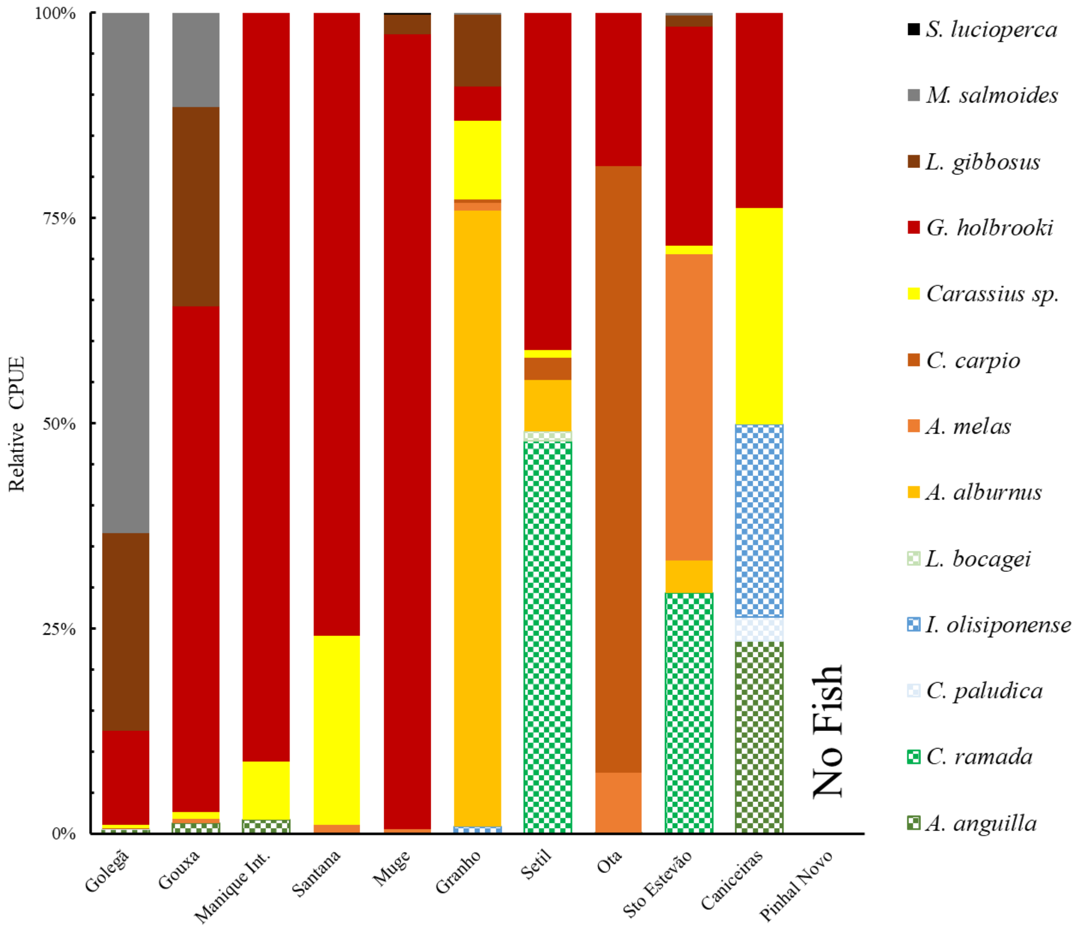

Figure 4.5 – Catch *per* Unit Effort (CPUE) determined using Multi Gear Mean Standardization (MGMS), for fish species captured in all the wetlands. Native species with pattern bar and non-native with full bar.

The DCA highlighted a considerable heterogeneity in local fish communities, dispersion of the fish communities and limited evidence of clustering. The first DC axis captured 25.2 % of total variation in fish data and the second DC axis 22.8 % (Figure 4.6). Site and species scoring reflected an association of Caniceiras with the Lisbon arched-mouth nase but also with the non-native Bleak, and of Ota with non-native Black bullhead and Common carp and of Golegã with non-native Largemouth bass and Pumpkinseed sunfish.

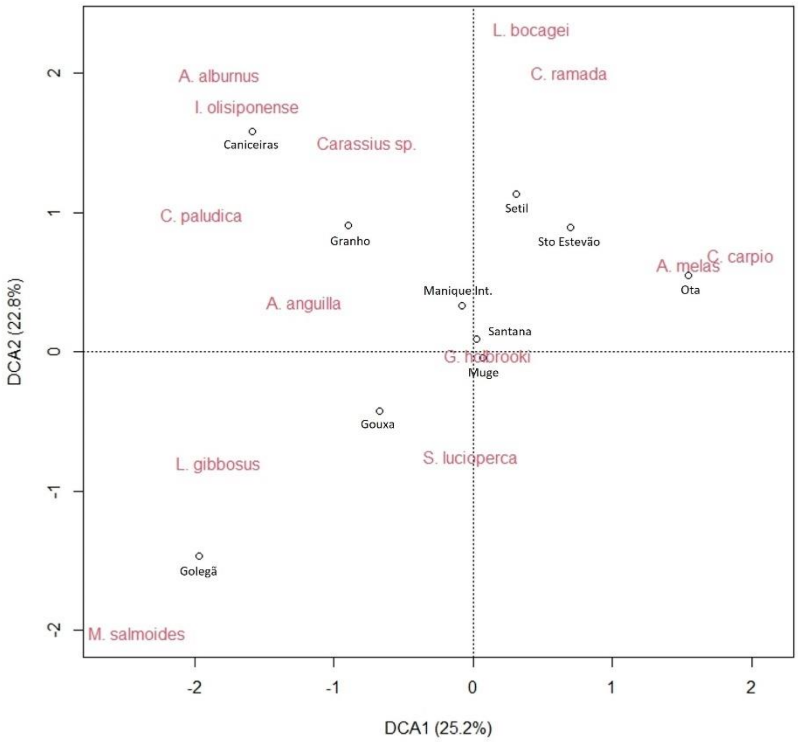

Figure 4.6 – Detrended Correspondence Analysis (DCA) ordination diagram of the fish communities in inland wetlands (excluding Pinhal Novo) in the Lower Tagus.

4.6 Fish-environment relationships

The variable selection approach to CCA revealed the occurrence of significant effects of habitat (DEP, CVR), landscape (CONN, SORD) and land use (LULCC) on variation in fish communities among wetlands (p-value = 0.008). Together the first two CC axes presented 42.7 % of the variance in fish data (Figure 4.7). Indeed, there were significant correlations between fish community structure and DEP and SORD (p = 0.004) and marginal associations with CONN (p = 0.100), but those with LULCC (p = 0.143) and particularly with CVR (p = 0.201) were weak and non-significant, see in detail in Tables S8, S9, S10, in Annex I.

Distribution of sites scores in the ordination space highlighted considerable differentiation in fish communities among wetlands (Figure 4.7). Fish communities in Setil and Sto Estevão which are directly connected to the main stem of Tagus River included Iberian barbel and Thinlip grey mullet, while the deeper and isolated Golegã and Gouxa tended to harbour higher abundance of Largemouth bass and Pumpkinseed sunfish and the shallow Caniceiras included European eel, Southern Iberian Spined loach and Lisbon arched-mouthed nase (Figure 4.7).

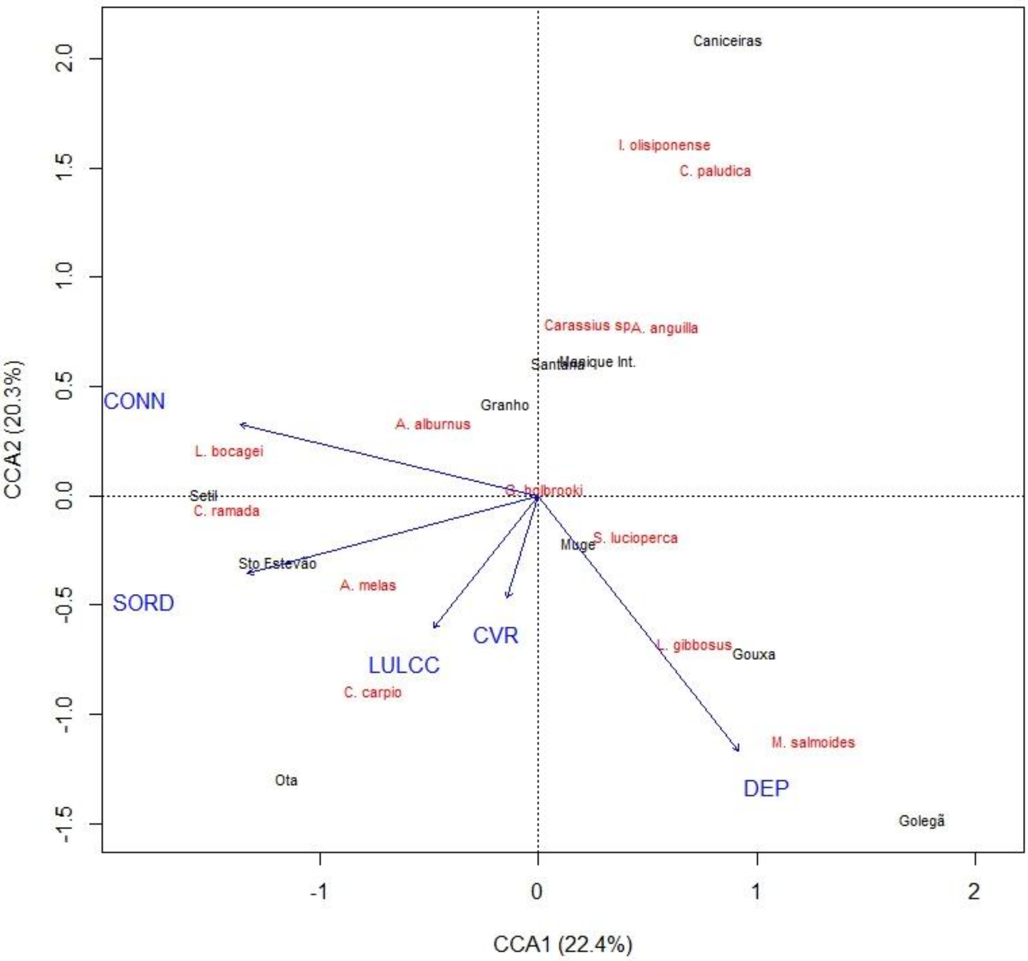

Figure 4.7 – Canonical Correspondence Analysis (CCA) ordination diagram depicting the effects of habitat, landscape and land use on fish communities in inland wetlands (excluding Pinhal Novo) in the Lower Tagus. Variables code and description presented in Table 4.1.

5. Discussion

This work presents the first approach to evaluate the current status of inland wetlands in the Lower Tagus and of is local fish communities, aiming to create a baseline of updated information for its conservation and restoration. Specifically, inland wetlands in the region were identified, and evaluation of land use and change and anthropogenic pressures on 11 of these was performed. Local fish communities were surveyed, and its general relationships with the habitat, landscape and land use and change contexts were quantified.

Results from this work indicate that at least 30 inland wetlands in the Lower Tagus may have the potential to support fish. Selected wetlands still considerably disturbed, though land use pressure has reduced in recent years, and there was a replacement of intensive agriculture by extensive agriculture and natural areas.

Although local fish communities are dominated by non-native fish, some inland wetlands still harbour threatened native species, including regional endemics. Variation in fish community structure appears to associate with habitat and landscape contexts, with migratory and potamodromous fish being found in wetlands connected to the main steam, and endangered species including regional endemics occurring in the shallowest wetland. There were no clear influences of land use change and wetland cover on fish community structure.

Based on this new evidence a first set of guidelines for conservation management of inland wetlands is presented. The need to raise awareness to inland wetlands protection and restoration is highlighted, and wetlands that should be prioritized to reverse the decline of native fish populations and the loss of freshwater biodiversity in the Lower Tagus have been identified.

5.1 Inland wetlands in the Lower Tagus

Using the Normalized Difference Water Index (NDWI) obtained from satellite image analysis, it was possible to identify 409 water bodies, widespread between Golegã and Lisbon, among which 30 maintain water during the dry period. This surpass previous inventories presented by Farinha & Trindade, (1994), and indicates that inland wetlands may be much more prevalent in the region than previously thought and should deserve further attention.

The use of remote sensing allowed the coverage of all the region and was not restricted by land propriety accessibility, either public or private, which are likely to promote larger and more accurate survey of current inland wetlands in the Lower Tagus than traditional methods. However, some improvements in methodology should be considered in future studies. The identification and monitoring of wetlands using remote sensing techniques has gained particular importance since the development of easily available and accurate datasets provided by satellites missions (Kaplan & Avdan, 2017; Pena-Regueiro *et al.*, 2020), which can be used for global, regional and local assessments. Nevertheless, there are still some constrains particularly in relation to human mediated identification of the inland wetlands, that should be recognized and addressed. Although, the NDWI analysis was complemented by using Google Earth

Historical images, the number of available Google Earth images is not the same across the Lower Tagus and does not include the same seasons. In particular, the use of automated or semi-automated methodologies can reduce or even eliminate these constraints and should thus be favoured in future approaches (Lefebvre *et al.*, 2019).

Another constrain in deriving NDWI was the presence of aquatic vegetation, which may introduce some biases in assessments of water bodies (Gao, 1996). The NDWI tends to hinder the reflection values from dense vegetation, thus underestimating inland water bodies (Perennou *et al.*, 2018). This may be particularly serious, since several sub basins in the Lower Tagus are invaded by the water hyacinth (*Eichhornia crassipes*) that completely cover the surface of water bodies (Pádua *et al.*, 2022).

Besides, in this work the NDWI was derived based on information for the dryer period in a single year (2019), which provides only a snapshot of the study area. Given the yearly variability in hydrological conditions prevailing in Lower Tagus, this problem should be overtaken by using multiple year datasets to identify and assess water bodies changes (e.g. Lefebvre *et al.*, 2015).

Irrespective of this, the identification of 30 inland waters with potential to support fish in the Lower Tagus is a new and relevant information that must be considered in conservation management planning at regional and national scales. Inland wetlands in the Lower Tagus have been long neglected, and the national classification of new areas did not change much since the classification of Paul do Boquilobo in the 80's (Araújo *et al.*, 2022). The only exception is the Gouxa inland wetland, also considered herein, that has been subject to a restoration project in 2005, to rehabilitate an abandoned quarry (see Mendes *et al.*, 2012), and classified as natural reserve in February of 2023, based on EU Habitats Directive classification of peatlands.

The outcomes of this work point to the occurrence of 28 inland wetlands in the Lower Tagus that were not previously identified and classified (excluding Paul do Boquilobo and Gouxa), 18 of which still require updated data on their importance for fish diversity conservation and restoration. Although some small wetlands have gained attention in the last years by local entities, municipalities, and public awareness, additional monitoring and management efforts are still required for their preservation. Municipalities and private landowners can use the new information provided here to support the integration of additional areas in the national conservation network (see Table 4.3).

5.2 Land uses and change in the Lower Tagus

Despite there were some positive evolution in extensive agriculture and natural uses in recent years, inland wetlands in the Lower Tagus were still under significant pressure in terms of land use and change. Intensive agriculture was still the major land use in the surroundings of the 11 inland wetlands assessed, likely reflecting changes in the type of agriculture practiced but also the abandonment of agricultural practices. In fact, similar transitions were observed in other rural areas of Portugal, and also across Europe, with countryside exodus leading to the abandonment of the rural areas since the mid-20th century (Ceaușu *et al.*, 2015; Meneses *et al.*, 2018; Daskalova & Kamp, 2023). This is also consistent with the study by Fernandes *et al.* (2020), indicating that the intensification of agriculture along Tagus River, that began in 1940s and lasted until 2000s, is starting to be replaced by other land uses, including not only extensive agriculture but also urban settlement.

Current land use patterns in inland wetlands in the Lower Tagus is concerning, because both intensive agriculture and urban/industrial uses are detrimental to biodiversity and aquatic environments and major drivers of inland wetland loss and degradation worldwide (Foley *et al.*, 2005). In fact, a similar pattern was already found in Pinhal Novo where there is an intensification of agriculture, a high vegetation growth, and no fish species. Moreover, dominant and impacting land uses altogether led to the classification of seven out of the eleven assessed inland wetlands as disturbed or heavily disturbed (Table 4.3). This may also, at least to some extent, contribute for the dominance of non-native species in fish communities (see Table 4.6), as commonly found in freshwater ecosystems (Clavero *et al.*, 2010, Radinger *et al.*, 2019).

Under this scenario, it is expectable that most of the 30 inland wetlands identified in the Lower Tagus (see Annex II) have high anthropogenic pressure in their surroundings and a low ecological condition. Nevertheless, further LULC assessments are required to better comprehend the status, trends, and anthropogenic pressures on inland wetlands at the regional scale (Mitsch & Gosselink, 2000; de Felipe *et al.*, 2023). Moreover, wetlands in the Mediterranean region not covered by protection measures, have been acknowledged to become more susceptible to be lost (Leberger *et al.*, 2020).

There have been substantial improvements in LULC classification, but datasets still present time lags and must be updated and be made available more regularly. The data series analysed covered 2007, 2010, 2015, 2018, with the most recent information being outdated four years in relation to the current survey. At date, improvements on data availability (*Sentinel-2*) and new approaches using automated geospatial analysis for global classification appear promising to improve LULC assessment (Karra *et al.*¸ 2021) and produce datasets substantially faster using near-real time information (Brown *et al.*, 2022), and further surveys in the Lower Tagus should consider the use of these techniques.

5.3 Fish communities in the Lower Tagus wetlands

Results from the fish surveys confirmed that inland wetlands in Lower Tagus are used by several species for spawning, feeding and as nursery grounds as previously suggested by Correia & Teixeira (2003), including highly threatened native species and Portuguese endemics. However, not surprisingly, native and endangered species were not that commonly found or abundant as in previous surveys conducted in other inland wetlands (see Collares-Pereira *et al.*, 1994; Correia & Teixeira 2003; Veríssimo *et al.*, 2018). Indeed, there was a dominance of non-native species indicating there is a high risk of replacement of the local native fish communities (e.g. Cucherousset *et al.*, 2007; Clavero *et al.*, 2021), which may represent an extra ecological pressure on inland wetlands of the Lower Tagus (Veríssimo *et al.*, 2018).

A total of five native and eight non-native species, was recorded in the eleven inland wetlands surveyed. Besides the Thinlip grey mullet and Iberian barbel classified as Least Concern (LC), native species included the European eel and the Lisbon arched-mouth nase considered Critical Endangered (CR) and the Southern Iberian spined loach Vulnerable (VU) (Table 4.5). However, in contrast to the European eel which has a wide distribution range, the Lisbon arched-mouth nase is endemic to the Lower Tagus, and includes populations only in three known sub-basins, in the Maior, Trancão and Muge streams and the Tagus main stem. These species may prefer lentic and shallow waters, with abundant aquatic and riparian vegetation, including seasonally flooded areas, and may thus use wetlands because they provide high food resources, spawning and nursery ground as pointed out in other works (Cucherousset *et al.*, 2008; Veríssimo *et al.*, 2018).

However, with the exception Setil and Caniceiras, the inland wetlands were dominated by non-native fishes which peaked at Santana and Muge. This represents a degradation of the local fish communities and is consistent with similar reductions and replacements of the native species in other Mediterranean freshwater habitats (see Ribeiro & Leunda, 2012; Anastácio *et al.*, 2019; Clavero *et al.*, 2021). Three species, Black bullhead, Eastern mosquitofish and Pumpkinseed sunfish, were widespread and found in abundant numbers in inland wetlands. This might be due to elevated physiological tolerance exhibited by these species (Cucherousset *et al.*, 2007; Ribeiro *et al.*, 2008), which are also reported to occur in high numbers in other wetlands in France and Spain (Cucherousset *et al.*, 2008; Clavero *et al.*, 2021).

Fish community composition showed little than expected relations to habitat, landscape and land use contexts, contrary to what is generally found (e.g., Magalhães *et al.*, 2002; Esselman & Allan, 2010). This is likely due, at least to some extent, to the very heterogeneous conditions naturally prevailing in the region, and even to the occurrence of stochastic events, such wetland clean up, that may have long-lasting and unmeasured effects on fish composition and numbers. For instance, Moyle *et al.* (2007) showed a high variability in the fish composition between years and additionally high spatial variation within the Cosumnes wetland.

Although, fish richness and Shannon-Wiener diversity showed no strong patterns across the wetlands, there was a slight tendency for inland wetlands connected directly to the watershed (open), harbouring richer and more diverse communities. Moreover, there was a tendency for migratory Thinlip grey mullet and potamodromous Iberian barbel being found in wetlands connected to the main steam, but Pumpkinseed sunfish and Largemouth bass peaking in deep Golegã and Gouxa. Perceived effects of connectivity are consistent with findings by Stojković Piperac *et al.* (2022), pointing to higher fish diversity in connected water bodies. The occurrence of Thinlip grey mullet in open wetlands only, namely in Setil and Sto Estevão, likely reflects its migration for feeding purposes, which can proceed several km upstream in the Tagus basin (Pereira *et al.*, 2021). Likewise, the Iberian barbel also present in wetland connected to the watershed (Setil) tend to migrate upstream during the Spring to spawn in gravel or sandy riverbed (Alexandre *et al.*, 2014). The associations with depth may reflect the preference by stable dependent species such as Largemouth bass (Ribeiro *et al.*, 2008), but also at least to some extent, the local practice of angling (personal observation), and the high value of this non-native species for anglers (Collares-Pereira *et al.*, 2021). Usually in Portugal, non-native species with higher value for angling tend to spread fast across and within the basins, being generally associated to lentic habitats, such as reservoirs, that function to as hotspot for freshwater fish invaders (Clavero *et al.*, 2013; Martelo *et al.*, 2021).

Non-native fish are a pressure to native fish but may also threaten other vertebrate groups such as amphibians, which may be preyed by Largemouth bass and Pikeperch (Godinho *et al.*, 1997; Ribeiro *et al.*, 2021) and be outcompeted by Eastern mosquitofish and Pumpkinseed sunfish (Rincón e*t al.*, 2002; Gkenas *et al.*, 2022). Also, the presence of Common carp has been reported to have a negative impact on threatened waterbirds and in other water dependent birds in SW Spain by destroying macrophyte beds (Macega-Veiga *et al.*, 2017).

The dominance of non-native species is particularly concerning, given the multi-gear fishing techniques used in the current study are expected to circumvent gear selectivity and provide the best possible picture of fish communities (Gibson-Reinemer *et al.*, 2017; Medhi *et al.*, 2021). Nevertheless, the current study included one unique sample occasion, which despite the maximized fishing effort, may have fail to fully capture the use of wetlands by freshwater fish. For example, in a survey performed in the summer of 2021 in Caniceiras, an additional fish species was captured, the national Endangered (EN) Three-spined stickleback (*Gasterosteus aculeatus*) as previously detected in 2016 (Verissimo *et al.*, 2018). Wetland use by different fish species may vary along the year and over the years as in other Mediterranean wetlands, as the Consumnes river, in Central California, stressing the need for continuous monitoring data (Moyle *et al.*, 2007). In particular, the adjustment of the sampling with the drying and flooding periods might be important to obtain more complete fish assessments (Cucherousset *et al.*, 2007). Although catchability constraints have been accounted by combining passive and active standard fishing methods, the use of gill nets in pools > 1m deep could also provide new and valuable information (Sánchez-González & Casals, 2022). Additionally, more recent and powerful techniques such as environmental DNA metabarcoding might also provide a broader look in the fish communities (Antognazza *et al.*, 2020; Kačergytė *et al.*, 2020). Combination of these techniques with citizen science data may complement the information for each particular wetland and be a powerful tool to engage local public and stakeholders in biodiversity conservation (Newman *et al.*, 2017; Martelo *et al.*, 2021).

5.4 Implications for conservation management

The protection and effective management of inland wetlands in Lower Tagus is essential for biodiversity conservation at national and international level (Darwall *et al.*, 2014). Wetlands functioning is highly dynamic and fish communities provide interest for scientific studies and strategic conservation planning. Consequently, long-term monitoring is important to address biodiversity variability, since has been often neglected, and captures crucial ecological response to anthropogenic and natural stressors (Moyle *et al.*, 2007). Moreover, the study of wetlands should be deepened given that water-land interactions are not fully understood and could reveal important ecosystem services (e.g. carbon capture) that reinforce the expansion of national wetlands network in Portugal.

This work provides additional novel information on freshwater fish communities, that play an important role for conservation and biodiversity since they figure as the most endangered freshwater group (Cabral *et al.*, 2005). Despite the high perturbation found in the inland wetlands in the Lower Tagus, and the prevalence of non-native species in local communities, the presence of European eel throughout the study wetlands, and the occurrence of Lisbon arched-mouth nase or three-spined stickleback in Caniceiras (see Veríssimo *et al*., 2018 for details) demonstrate the major conservation relevance of the Tagus small wetlands. Specifically, the occurrence of important native species, particularly of the critically endangered (CR) Lisbon arched-mouth nase should help achieve a protection classification for Caniceiras and Granho, and can be subject for the RAMSAR designation according to criteria 2 of Group B; *"A wetland should be considered internationally important if it supports vulnerable, endangered, or critically endangered species or threatened ecological communities."* (see RAMSAR criteria in *https://www.ramsar.org/sites/default/files/documents/library/ramsarsites_criteria_eng.pdf*).

Future strategic plans for inland wetlands should involve stakeholders, such as landowners in their active conservation action plans, farmers to change their agriculture practices and involve local municipalities to promote awareness of the importance of these habitats for the biodiversity conservation and their sustainable management. Restoration plans will be key to rehabilitate these wetlands, particular those classified as disturbed or heavily disturbed. For example, Gouxa wetland has been subject to a restoration project, through a rehabilitation of an old quarry and recently was classified as natural reserve of local interest. Also, the observed fish community degradation was mostly due to the high non-native fish abundances, however these enclosed habitats might benefit from control or eradication campaigns done at local level. These approaches have been performed with some degree of success and it resulted in the native fish community recuperation (Britton & Brazier, 2006; Bosch *et al.*, 2019; Martínez *et al.*, 2022). Native fish communities might also highly benefit from additional measurements such as habitat enhancement, that have proved to be more effective compared to traditional approaches such as fish stocking based on single species (e.g. Radinger *et al*., 2023).

Environmental outreach may also be beneficial to raise awareness of the general public about the importance of inland wetlands and to address the biodiversity impact caused by non-native species.

Future work in inland wetlands of the Lower Tagus River should involve dedicated surveys and regular monitoring along the year and between years, following previous and improved approaches (see Moyle et al., 2007), to obtain a more complete and in deep regional assessment of the current status and conservation management needs. The current expansion of extensive agriculture and natural areas provide an opportunity to implement better land use practices in the wetlands surroundings, which in association with restoration processes and proper management may create additional opportunities to halt non-native species increases and biodiversity loss. The Lower Tagus wetlands are highly degraded, but still hold unique fish endangered and/or endemic species being our responsibility to preserve these unique habitats for future generations in a world that is withstanding an increasingly global water crisis (Tickner *et al.*, 2020; de Filipe *et al.*, 2023).

7. References

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8. Annexes

Annex I – Supplementary Results Figures and Tables

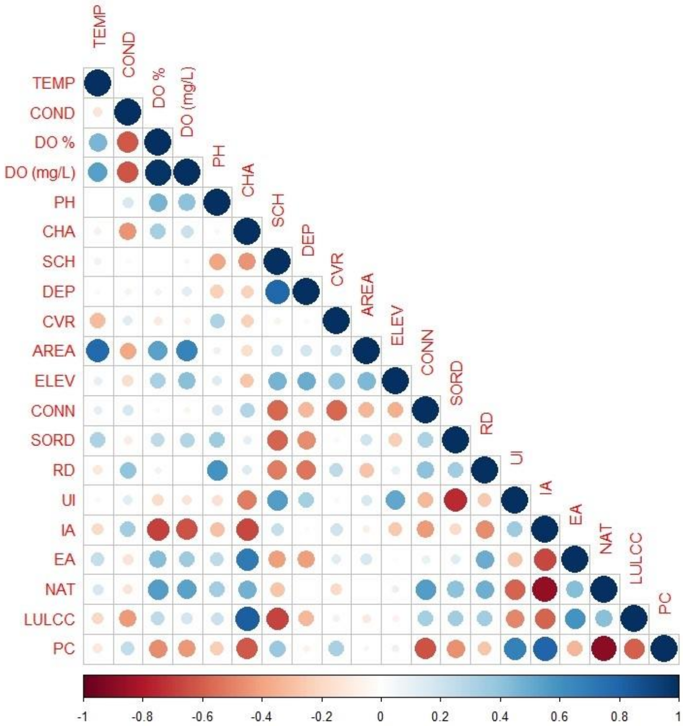

Figure S1 - Correlations between Habitat, Landscape and Land Use variables. Positive correlations are displayed in blue and negative in red. Colour intensity and circle size are proportional to correlation coefficients. See Table 4.1 for variables codes.

Table S1 - Gain/loss (%) of Land Use Land Cover (LULC) between LULC Period 1 (2007 – 2010 – 2015) and Period 2 (2018) for each studied wetland in the Lower Tagus: UI – Urban/Industrial; IA – Intensive Agriculture; EA – Extensive Agriculture; NA – Natural.

Wetland	**Gain/loss (%)**			
	UI	**IA**	**EA**	**NA**
Golegã	0.0	-0.1	0.2	0.0
Gouxa	0.0	-1.8	7.6	-5.8
Manique Intendente	1.0	-17.7	25.3	-8.6
Santana	0.0	-2.8	11.8	-9.0
Muge	0.0	-10.4	10.5	0.0
Granho	0.0	-16.4	7.0	9.5
Setil	0.0	1.2	0.0	-1.2
Ota	0.0	-6.4	11.5	-5.0
Sto Estevão	0.0	-15.2	-6.8	22.0
Caniceiras	-0.1	5.9	1.5	-7.3
Pinhal Novo	0.0	7.2	7.2	-14.4
Mean ± Std Dev	0.1 ± 0.3	-5.1 ± 8.8	6.9 ± 8.4	-1.8 ± 10.1
Range (min – max)	(-0.1 - 1.0)	(-17.7 - 7.2)	(-6.8 - 25.3)	(-14.4 - 22.0)

Table S2 - Results of the Principal Component Analysis on Habitat data obtained from the studied wetlands of the Lower Tagus.

PC	Eigenvalues	% Variation	Cumulative % Variation
1	1.58	31.7	31.7
2	1.18	23.5	55.2
3	0.90	18.0	73.2
4	0.81	16.1	89.3
5	0.54	10.7	100.0

Table S3 - Loadings of habitat variables on PC axes. Temperature (TEMP), Conductivity (COND), pH (PH), Depth (DEP) and Cover (CVR).

Variables	PC1	PC2	PC3	PC4	PC5
TEMP	-0.392	0.599	-0.099	-0.510	0.468
COND	0.395	-0.019	-0.910	-0.042	0.116
PH	0.520	0.424	0.166	-0.451	-0.564
DEP	-0.269	-0.630	-0.096	-0.695	-0.196
CVR	0.590	-0.252	0.353	-0.229	0.642

Table S4 - Results of the Principal Component Analysis on Landscape data obtained from the studied wetlands of the Lower Tagus.

PC	Eigenvalues	% Variation	Cumulative % Variation
1	2.01	40.2	40.2
2	1.29	25.9	66.1
3	1.02	20.3	86.4
4	0.51	10.1	96.5
5	0.17	3.5	100.0

Table S5 - Loadings of landscape variables for PC axes. Area (AREA), Elevation (ELEV), Connectivity (CONN), Stream order (SORD) and River distance (RD).

Variables	PC1	PC2	PC3	PC4	PC5
AREA	0.431	-0.561	0.350	0.297	0.538
ELEV	0.430	-0.410	-0.580	0.216	-0.514
CONN	-0.571	-0.073	-0.019	0.815	-0.057
SORD	-0.346	-0.595	0.458	-0.318	-0.465
RD	-0.428	-0.399	-0.576	-0.315	0.477

Table S6 - Results of the Principal Component Analysis on Land use data obtained from the studied wetlands of the Lower Tagus.

PC	Eigenvalues	% Variation	Cumulative % Variation
1	2.51	62.8	62.8
2	0.78	19.5	82.3
3	0.38	9.6	91.9
4	0.33	8.1	100.0

Table S7 - Loadings of land use variables for PC axes. Urban/Industrial (U/I), Intensive Agriculture (IA), Extensive Agriculture (EA), Natural (NA) and LULC Change (LULCC).

Variables	PC1	PC2	PC3	PC4
UI	-0.396	0.848	-0.290	0.199
IA	-0.531	-0.299	-0.545	-0.576
EA	0.520	0.429	0.038	-0.738
LULCC	0.539	-0.085	-0.786	0.290

Table S8 - Loadings from explanatory variables of Canonical Correspondence Analysis (CCA) axes. Depth (DEP), Cover (CVR), Connectivity (CONN), Stream order (SORD) and LULC Change (LULCC).

Variables	CCA1	CCA2	CCA3	CCA4	CCA5	*p-value*
DEP	0.536	-0.682	-0.222	-0.402	-0.193	0.004
CVR	-0.083	-0.274	-0.216	0.536	0.764	0.201
CONN	-0.798	0.190	0.142	-0.553	-0.041	0.004
SORD	-0.778	-0.207	0.435	0.351	-0.197	0.100
LULCC	-0.281	-0.354	0.540	0.060	0.708	0.143

Table S9 - Loadings from fish communities of Canonical Correspondence Analysis (CCA) axes.

Species	CCA1	CCA2	CCA3	CCA4	CCA5
Anguilla anguilla	0.583	0.772	-0.557	-0.236	-0.220
Chelon ramada	-1.412	0.208	-0.405	0.529	-1.896
Cobitis paludica	-1.420	-0.064	-0.289	0.389	-0.726
Iberochondrostoma olisiponense	0.814	1.487	-0.877	-0.247	-0.601
Luciobarbus bocagei	0.584	1.608	-0.361	-0.397	-0.517
Alburnus Alburnus	-0.479	0.338	1.434	-0.430	-0.169
Ameiurus melas	-0.775	-0.401	-0.058	-0.133	0.581
Cyprinus carpio	-0.754	-0.901	-0.663	-0.927	0.113
Carassius sp.	0.238	0.784	-0.065	-0.233	0.306
Gambusia holbrooki	0.028	0.030	-0.048	0.343	0.213
Lepomis gibbosus	0.718	-0.681	0.512	-0.018	-0.252
Micropterus salmoides	1.265	-1.124	-0.187	-0.073	-0.352
Sander lucioperca	0.450	-0.193	0.847	2.961	0.205

Table S10 - Loadings from wetlands of Canonical Correspondence Analysis (CCA) axes.

Wetlands	CCA1	CCA2	CCA3	CCA4	CCA5
Golegã	1.792	-1.339	-0.720	0.258	-0.245
Gouxa	0.663	-0.978	0.701	-0.789	-0.861
Manique Intendente	-0.239	0.214	-0.221	0.495	1.704
Santana	0.525	1.147	-0.603	-0.357	1.309
Muge	0.450	-0.193	0.847	2.961	0.205
Granho	0.006	0.545	2.322	-0.851	0.116
Setil	-1.412	0.208	-0.405	0.529	-1.896
Ota	-0.747	-1.220	-0.948	-1.242	0.509
Sto Estevão	-1.430	-0.411	-0.141	0.211	0.767
Caniceiras	0.699	1.820	-0.895	-0.306	-0.642

Annex II – Water bodies identified in the Lower Tagus using remote sensing detection

Table S1 - Water bodies identified (409) in the Lower Tagus using remote sensing, coordinates (Latitude, Longitude) and Exclusion criteria (E.C.1 to E.C.5), Visited wetlands and selected to be Sampled wetlands. Note: Exclusion Criteria (E.C.): E.C. 1 - subjected to complete drought; E.C. 2 - highly artificialized (e.g., dammed); E.C. 3 - completely isolated (e.g., by weirs or irrigation peats); E.C. 4 – inadequately sized (< 0.5 hectares (ha) or > 50.0 ha) and E.C. 5 - accessibility, sampling feasibility and safety.

#	Latitude	Longitude	Exclusion criteria	#	Latitude	Longitude	Exclusion criteria
1	39.394880	-8.532053	4	**206**	38.889435	-8.802345	3
2	39.503594	-8.451104	3	**207**	38.879567	-8.770094	2
3	39.510137	-8.453245	1	**208**	38.857196	-8.729629	1
4	39.510569	-8.443382	3	**209**	38.869502	-8.734957	2
5	39.433326	-8.525948	3	**210**	38.869502	-8.734957	4
6	39.422543	-8.508369	1	**211**	38.909395	-8.740811	2
7	39.397814	-8.481033	Sampled	**212**	38.909395	-8.740811	2
8	39.401596	-8.461697	Visited (5)	**213**	38.845719	-8.731841	3
9	39.397732	-8.468245	1	**214**	38.866444	-8.768979	Sampled
10	39.397545	-8.574984	2	**215**	38.755042	-8.722875	3
11	39.404002	-8.666493	3	**216**	38.800659	-8.746015	4
12	39.530614	-8.375925	4	**217**	38.822891	-8.742820	2
13	39.530362	-8.387128	2	**218**	38.822891	-8.742820	2
14	39.444651	-8.340033	2	**219**	38.822891	-8.742820	2
15	39.400028	-8.409587	2	**220**	38.822891	-8.742820	2
16	39.469123	-8.064680	3	**221**	38.823632	-8.732875	4
17	39.307262	-8.801565	3	**222**	38.822272	-8.757378	2
18	39.260735	-8.921193	3	**223**	38.844405	-8.782393	2
19	39.262963	-8.920637	3	**224**	38.845482	-8.792878	3
20	39.261983	-8.919440	3	**225**	38.901884	-8.824565	1
21	39.261983	-8.919440	3	**226**	38.898014	-8.808575	1
22	39.263533	-8.848114	3	**227**	38.896301	-8.803282	1
23	39.232829	-8.731604	3	**228**	38.859068	-8.796275	4
24	39.237060	-8.740415	1	**229**	38.859068	-8.796275	4
25	39.208324	-8.727256	3	**230**	38.861499	-8.812938	4
26	39.183232	-8.769063	3	**231**	38.861499	-8.812938	4
27	39.246849	-8.781007	3	**232**	38.861499	-8.812938	4
28	39.230828	-8.852609	3	**233**	38.861499	-8.812938	4
29	39.203998	-8.913100	3	**234**	38.878082	-8.838152	3
30	39.203998	-8.913100	3	**235**	38.878082	-8.838152	3
31	39.373910	-8.813918	3	**236**	38.868778	-8.879392	3
32	39.217467	-8.883387	Sampled	**237**	38.866225	-8.857485	3
33	39.217467	-8.883387	1	**238**	38.858807	-8.864072	3
34	39.358421	-8.806531	3	**239**	38.858807	-8.864072	3
35	39.387520	-8.859791	3	**240**	38.847348	-8.891635	3
36	39.336634	-8.833010	3	**241**	38.847348	-8.891635	3
37	39.326946	-8.838215	3	**242**	38.838516	-8.881192	3

38	39.296402	-8.795603	3	**243**	38.833682	-8.879141	3
39	39.319959	-8.922808	3	**244**	38.839914	-8.820793	4
40	39.323666	-8.908735	3	**245**	38.835215	-8.816677	4
41	39.340921	-8.939497	3	**246**	38.829920	-8.823148	4
42	39.241939	-8.916428	3	**247**	38.829920	-8.823148	4
43	39.241939	-8.916428	3	**248**	38.828352	-8.824941	4
44	39.239994	-8.918928	3	**249**	38.822622	-8.827863	4
45	39.262963	-8.920637	3	**250**	38.825253	-8.832217	4
46	39.193555	-8.894291	3	**251**	38.825253	-8.832217	4
47	39.193555	-8.894291	3	**252**	38.819058	-8.804001	4
48	39.193555	-8.894291	3	**253**	38.812201	-8.806641	4
49	39.193555	-8.894291	3	**254**	38.809275	-8.824888	4
50	39.379481	-8.444048	3	**255**	38.806394	-8.830611	4
51	39.358793	-8.637727	3	**256**	38.806394	-8.830611	4
52	39.389870	-8.602686	3	**257**	38.820759	-8.788337	3
53	39.337342	-8.620645	3	**258**	38.844977	-8.917782	Visited (5)
54	39.355997	-8.604447	3	**259**	38.815107	-8.920045	3
55	39.371058	-8.604185	3	**260**	38.793728	-8.878444	3
56	39.311764	-8.639147	3	**261**	38.807365	-8.777899	4
57	39.323441	-8.648073	3	**262**	38.797688	-8.787375	3
58	39.329367	-8.653406	3	**263**	38.774715	-8.802195	2
59	39.395658	-8.575221	2	**264**	38.770404	-8.771841	3
60	39.345098	-8.666055	3	**265**	38.770404	-8.771841	4
61	39.316076	-8.658794	2	**266**	38.934972	-8.823162	3
62	39.282074	-8.665057	3	**267**	38.934050	-8.825632	3
63	39.287493	-8.681093	2	**268**	38.934050	-8.825632	3
64	39.314397	-8.694355	3	**269**	38.934050	-8.825632	4
65	39.244110	-8.709644	3	**270**	38.764989	-8.772184	3
66	39.216747	-8.705352	1	**271**	38.739573	-8.802534	4
67	39.269659	-8.675529	1	**272**	38.747477	-8.818746	3
68	39.204265	-8.674722	3	**273**	38.753911	-8.841205	3
69	39.385805	-8.430166	4	**274**	38.757389	-8.837732	Visited (5)
70	39.246522	-8.626940	1	**275**	38.737892	-8.860333	3
71	39.288412	-8.549864	1	**276**	38.737892	-8.860333	3
72	39.304922	-8.572423	Visited (5)	**277**	38.732322	-8.875102	3
73	39.305927	-8.556086	3	**278**	38.752488	-8.861370	3
74	39.333455	-8.470205	3	**279**	38.896877	-9.027394	4
75	39.326098	-8.443541	3	**280**	38.928644	-8.818469	3
76	39.326098	-8.443541	3	**281**	38.729356	-8.842661	3
77	39.324485	-8.439338	3	**282**	38.919597	-8.811309	4
78	39.314775	-8.450264	3	**283**	38.909962	-8.819818	1
79	39.309456	-8.490909	4	**284**	38.933602	-8.416916	4
80	39.265709	-8.503546	4	**285**	38.940567	-8.522671	2
81	39.265709	-8.503546	2	**286**	38.906996	-8.507171	3
82	39.265709	-8.503546	2	**287**	38.876542	-8.536042	3
83	39.274006	-8.518034	4	**288**	38.880955	-8.537404	3
84	39.280135	-8.525476	Visited (5)	**289**	38.873954	-8.540001	3
85	39.288816	-8.533618	3	**290**	38.888972	-8.544098	2

86	39.258585	-8.585827	2	**291**	38.888972	-8.544098	3
87	39.246890	-8.586141	2	**292**	38.881247	-8.555343	3
88	39.235637	-8.585251	Sampled	**293**	38.916532	-8.562398	4
89	39.231488	-8.573077	2	**294**	38.916532	-8.562398	3
90	39.229805	-8.524868	1	**295**	38.941122	-8.426361	4
91	39.179456	-8.500408	3	**296**	38.912782	-8.582195	3
92	39.182560	-8.478389	4	**297**	38.912782	-8.582195	3
93	39.172912	-8.449859	3	**298**	38.915751	-8.589165	3
94	39.384059	-8.641441	3	**299**	38.915752	-8.589165	3
95	39.377821	-8.638822	1	**300**	38.927080	-8.613954	4
96	39.360990	-8.664796	2	**301**	38.944197	-8.586542	4
97	39.355086	-8.657168	3	**302**	38.948579	-8.528753	Visited (5)
98	39.318500	-8.387387	3	**303**	38.947644	-8.527844	Visited (5)
99	39.166927	-8.752932	2	**304**	38.923905	-8.628527	3
100	39.118121	-8.801953	3	**305**	38.948230	-8.428753	4
101	39.074178	-8.892006	4	**306**	38.931211	-8.637645	4
102	39.078395	-8.933732	1	**307**	38.950030	-8.712332	1
103	39.084706	-8.931581	1	**308**	38.908493	-8.722040	2
104	39.099330	-8.939801	4	**309**	38.888895	-8.625008	3
105	39.101042	-8.947929	Sampled	**310**	38.876671	-8.618140	3
106	39.144327	-8.947981	3	**311**	38.888261	-8.644395	Visited (5)
107	39.156315	-8.943715	Visited (5)	**312**	38.885387	-8.649358	3
108	39.129398	-8.964643	2	**313**	38.888261	-8.644395	2
109	39.075905	-8.954764	3	**314**	38.862786	-8.709190	3
110	39.162830	-8.754589	3	**315**	38.868692	-8.719473	3
111	39.075905	-8.954764	3	**316**	38.831588	-8.681359	3
112	39.079554	-8.946409	2	**317**	38.942777	-8.427885	Visited (5)
113	39.058473	-8.995562	3	**318**	38.823482	-8.666223	3
114	39.028884	-8.986249	4	**319**	38.820908	-8.650174	3
115	39.028237	-8.947430	3	**320**	38.811372	-8.641416	4
116	38.963877	-8.821358	1	**321**	38.811372	-8.641416	4
117	39.146943	-8.773629	2	**322**	38.808600	-8.643166	3
118	39.141594	-8.755085	Sampled	**323**	38.810608	-8.622479	4
119	39.113543	-8.767990	Sampled	**324**	38.810608	-8.622479	1
120	39.123632	-8.729127	3	**325**	38.809210	-8.617411	4
121	39.116086	-8.738982	4	**326**	38.806403	-8.610148	4
122	39.130617	-8.795454	2	**327**	38.812264	-8.597583	4
123	39.135227	-8.833292	3	**328**	38.812264	-8.597583	4
124	39.106365	-8.661870	Sampled	**329**	38.812264	-8.597583	4
125	39.104129	-8.673528	Visited (5)	**330**	38.805993	-8.601127	Visited (5)
126	39.094904	-8.633870	Visited (5)	**331**	38.807143	-8.579564	3
127	39.094573	-8.638887	Sampled	**332**	38.807187	-8.571347	3
128	39.124820	-8.560841	3	**333**	38.807187	-8.571347	3
129	39.134277	-8.509427	3	**334**	38.807187	-8.571347	3
130	39.119758	-8.473756	3	**335**	38.808384	-8.553994	3
131	39.118448	-8.468595	3	**336**	38.945328	-8.468197	3
132	39.111011	-8.492213	3	**337**	38.787770	-8.506448	3
133	39.118697	-8.507581	4	**338**	38.790587	-8.516483	3

134	39.118697	-8.507581	3	**339**	38.795714	-8.546752	3
135	39.119366	-8.518571	2	**340**	38.795714	-8.546752	3
136	39.094938	-8.608731	1	**341**	38.796859	-8.559165	3
137	39.094125	-8.597861	2	**342**	38.785579	-8.577476	3
138	39.064342	-8.590887	3	**343**	38.790288	-8.573936	4
139	39.064342	-8.590887	3	**344**	38.798105	-8.583101	Visited (5)
140	39.064342	-8.590887	3	**345**	38.800491	-8.600598	1
141	39.064342	-8.590887	3	**346**	38.910929	-8.459813	4
142	39.073094	-8.571567	4	**347**	38.798350	-8.610903	4
143	39.052267	-8.549892	4	**348**	38.801427	-8.625559	3
144	39.058926	-8.557682	3	**349**	38.801507	-8.625920	3
145	39.056139	-8.678450	3	**350**	38.786746	-8.621539	4
146	39.012886	-8.702480	3	**351**	38.758688	-8.600879	4
147	38.993949	-8.675977	2	**352**	38.760267	-8.577733	3
148	38.993949	-8.675977	3	**353**	38.780393	-8.643215	3
149	38.996502	-8.652978	3	**354**	38.766615	-8.661285	3
150	38.975718	-8.618726	2	**355**	38.803448	-8.663380	3
151	38.975718	-8.618726	Visited (5)	**356**	38.803448	-8.663380	3
152	38.983113	-8.584321	1	**357**	38.817468	-8.679141	3
153	38.969972	-8.567332	3	**358**	38.808980	-8.686641	3
154	38.969972	-8.567332	2	**359**	38.808980	-8.686641	3
155	38.965195	-8.545987	4	**360**	38.814881	-8.720513	2
156	38.966192	-8.542726	4	**361**	38.822994	-8.711656	3
157	38.966192	-8.542726	4	**362**	38.830468	-8.704971	3
158	38.964998	-8.543489	4	**363**	38.833157	-8.712483	3
159	38.986423	-8.497194	3	**364**	38.752115	-8.558141	3
160	38.991342	-8.496753	4	**365**	38.748772	-8.584578	3
161	38.999889	-8.467489	1	**366**	38.751645	-8.603614	3
162	39.006723	-8.443986	4	**367**	38.751645	-8.603614	4
163	39.012447	-8.417477	4	**368**	38.893520	-8.467416	3
164	38.993582	-8.445601	3	**369**	38.893520	-8.467416	4
165	38.983856	-8.462399	3	**370**	38.893087	-8.464998	4
166	38.984505	-8.461234	3	**371**	38.893815	-8.466619	4
167	39.166508	-8.684136	1	**372**	38.898834	-8.474438	4
168	38.951040	-8.510152	Visited (5)	**373**	38.904899	-8.470845	2
169	38.952801	-8.581825	1	**374**	38.908983	-8.297004	3
170	38.955896	-8.632308	4	**375**	38.929522	-8.394583	4
171	38.954013	-8.633956	4	**376**	38.936889	-8.412544	4
172	38.955928	-8.689850	3	**377**	38.928425	-8.413028	4
173	38.951437	-8.711083	4	**378**	38.922343	-8.406423	4
174	39.006786	-8.717525	3	**379**	38.919066	-8.400352	4
175	39.156799	-8.606021	3	**380**	38.903088	-8.389910	4
176	39.132247	-8.607676	4	**381**	38.918776	-8.294907	3
177	39.113535	-8.614627	3	**382**	38.917210	-8.315966	3
178	39.144158	-8.675598	2	**383**	38.913832	-8.323293	4
179	39.106016	-8.677751	2	**384**	38.892302	-8.330568	4
180	38.952384	-8.163477	2	**385**	38.896863	-8.323047	4
181	39.013220	-8.407450	3	**386**	38.913115	-8.343991	4

182	39.013220	-8.407450	3	**387**	38.926066	-8.347359	3
183	39.011945	-8.409611	3	**388**	38.933011	-8.359596	3
184	38.974938	-8.210056	2	**389**	38.525991	-9.048295	3
185	38.968784	-8.207445	2	**390**	38.537678	-9.048391	2
186	39.025690	-8.213583	3	**391**	38.537678	-9.048391	2
187	39.037771	-8.228375	3	**392**	38.537678	-9.048391	2
188	39.019090	-8.282743	3	**393**	38.664210	-9.164947	2
189	39.006904	-8.290115	3	**394**	38.606027	-9.026107	4
190	39.006904	-8.290115	2	**395**	38.686268	-8.834739	2
191	39.014724	-8.319652	4	**396**	38.644389	-8.824373	3
192	39.015357	-8.320028	3	**397**	38.683872	-8.743333	3
193	38.780385	-9.169187	2	**398**	38.700518	-8.728050	4
194	38.780385	-9.169187	2	**399**	38.632889	-8.749588	3
195	38.779620	-9.169125	2	**400**	38.723170	-8.900744	1
196	38.840506	-9.181201	4	**401**	38.674440	-8.870856	3
197	38.849556	-9.147281	Sampled	**402**	38.652897	-8.878746	Sampled
198	38.839087	-9.115088	1	**403**	38.655388	-8.940065	4
199	38.857702	-9.110754	Visited (5)	**404**	38.612622	-8.943519	3
200	38.945852	-8.891206	1	**405**	38.625305	-8.970287	3
201	38.905076	-8.803954	1	**406**	38.705066	-8.856663	3
202	38.940535	-8.769740	3	**407**	38.726540	-8.841484	4
203	38.940535	-8.769740	3	**408**	38.658034	-8.720381	3
204	38.938225	-8.758110	Visited (5)	**409**	38.626919	-8.707006	1
205	38.892408	-8.789438	Visited (5)				