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**European catfish (*Silurus glanis*) movements and diet ecology  
in a newly established population in the Tagus drainage**

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## Abstract

Biological invasions are one of the leading causes of biodiversity loss worldwide. The phenomena of species introduction has affected almost every ecosystem, but some are more resistant than others. Freshwater ecosystems are highly biodiverse containing about 50% of the world's known fish species and are vital to the development of society. These ecosystems are now under tremendous pressure, between the consequences of climate change, pollution and river regulation, and are more vulnerable to establishment of introduced species, and consequently more prone to impact by introduced species. Introduced species affect a multitude of aspects, from genes to entire ecosystems. In Portuguese freshwaters, there are currently 20 identified invasive fish species and more are arriving at an unprecedented rate of a new species every two years. One of the most recent arrivals has been the European catfish (*Silurus glanis*). This top predator native from Central and Eastern Europe was first introduced in Spain where it spread downstream along the Tagus River into Portugal, aided by human action. The fact that it is a recent arrival represents an opportunity to study the early stages of invasion and how this species might come to shape the community around it. This study focuses on two aspects of this species habits in order to offer ways to gauge its potential impact on the unique fauna found in Portuguese freshwaters: movements and dietary habits. Our findings suggest that this species is more active during summer months (used area peaked at 2.79 km<sup>2</sup>) and more lethargic during winter (used area decreased to a minimum of 0.45 km<sup>2</sup>). Activity was highly regulated by temperature and water flow, with other variables showing no significant influence on the extension of the area used. It exhibits high site-fidelity (84%) however some individuals (16%) are capable of relatively long distance movements (up to 11.5 km) that may aid in its dispersion. A trend towards aggregation during its breeding period was also observed. The analysis of one individual's depth use indicates that this species might make more use of shallow habitats than previously thought. It also pointed towards a stable level of activity throughout the day during spring, summer and autumn, but almost exclusively nocturnal activity during winter. The European catfish is able to predate over the full spectrum of available prey, exhibiting distinct diets in Lentic and Lotic environments. In the Lotic sections of the Tagus a great percentage of its diet is composed of native fishes and freshwater shrimp. Predation on European eel and sea lamprey constitutes an additional pressure source being placed over these already threatened species. Lentic catfish fed almost exclusively on crustaceans (freshwater shrimp and crayfish), with very few observable fish prey being mostly non-native. In general, smaller sized individuals also preyed on smaller prey. There was considerable ontogenic variation in the European catfish diet where in the Lentic system, piscivory was observed in large sized individuals, however, in the Lotic environment the smallest class fishes already included fish prey as part of their diet. Overall, the findings in this study can be important in understanding the spread of this species throughout Portugal and its potential impacts over native fauna. The results shown should prove invaluable when creating a plan for the mitigation of this species impacts or in any conservation plan directed towards species cohabiting with the European Catfish. As an example, the aggregations during the breeding period can represent valuable opportunities for removal of large numbers of individuals.

**Keywords:** Portugal; Invasive species; Top predator; Depth usage; Acoustic telemetry.

## Resumo

As invasões biológicas são uma das principais causas da perda de biodiversidade à escala mundial. O fenómeno da introdução de espécies afeta quase todos os ecossistemas. Os ecossistemas de água doce são altamente biodiversos, contendo cerca de 50% das espécies de peixe conhecidas e sendo vitais para o desenvolvimento da sociedade. No entanto, estão sob tremenda pressão, entre as consequências das alterações climáticas, da poluição, e regulação dos sistemas fluviais (e.g. barragens) que os deixaram vulneráveis, as espécies introduzidas têm tido maior facilidade em se estabelecer, tornando estes ambientes nuns dos mais fortemente afetados por espécies introduzidas. As espécies introduzidas afetam uma multitude de aspetos a diferentes escalas, de genes ao ecossistema. Ameaçam a biodiversidade única encontrada nestes ambientes aquáticos através da hibridação, a introdução de novos agentes patogénicos, competição e predação. Nos ambientes aquáticos portugueses, estão identificadas 20 espécies de peixe invasoras com novas espécies a serem detetadas a um ritmo sem precedentes de uma nova espécie a cada dois anos. O Peixe-gato-europeu, foi uma das introduções mais recentes. Este predador de topo, nativo da Europa Central e de Leste, foi primeiro introduzido em Espanha tendo dispersado ao longo do Tejo até chegar a Portugal, provavelmente ajudado por meios humanos. O facto de se tratar de uma chegada recente representa uma oportunidade de estudar os primeiros estágios de invasão e de como esta espécie pode vir a moldar a comunidade faunística aquática à sua volta. Este trabalho foca-se em dois aspetos dos hábitos desta espécie para tentar medir o seu potencial impacto na fauna que ocorre nas águas doces portuguesas: movimentos e dieta. Os resultados deste estudo sugerem que esta espécie se encontra mais ativa durante os meses de Verão (Área utilizada teve o seu máximo a 2,79km<sup>2</sup>) e mais letárgica durante o Inverno (Área utilizada desceu a um mínimo de 0,45km<sup>2</sup>). O nível de atividade revelado por esta espécie parece ser altamente condicionado pela temperatura e caudal, com outras variáveis testadas a não demonstrarem influência significativa sobre a extensão da área utilizada. Esta espécie exibiu grande fidelidade espacial (84%), mas, no entanto alguns indivíduos (16%) mostraram-se capazes de movimentos de longa distância (até 11.5km) que podem ajudar à sua dispersão. Uma tendência para a agregação durante o período de reprodução foi também observada. A análise ao nível da utilização de habitat ao longo da coluna de água, através da obtenção do registo de profundidades utilizadas por um dos indivíduos marcados, indica que esta espécie poderá fazer uso de habitats mais superficiais do que previamente pensado. Também apontou para um nível estável de atividade durante todo o dia na Primavera, Verão e Outono, mas atividade quase exclusivamente noturna no Inverno. Os hábitos alimentares observados com o estudo da dieta são preocupantes. Esta espécie tem a capacidade de predação sobre todo o espetro de presas disponíveis, exibindo dietas distintas nos habitats lênticos e lóticos. Nas secções lóticas do Tejo uma grande percentagem, da sua dieta é composta por peixes nativos e camarão-de-água-doce. A predação sobre Enguia-europeia e Lampreia-marinha representa uma fonte de pressão adicional sobre estas espécies ameaçadas. Os peixes-gato de habitats lênticos, por oposição, alimentaram-se quase exclusivamente de crustáceos (camarão-de-água-doce e lagostim-vermelho) com muito poucos peixes, que eram na sua maioria não-nativos. No geral, indivíduos mais pequenos também se alimentavam de presas mais pequenas. Observou-se grande variação ontogénica na dieta do Peixe-gato-europeu. Nos sistemas lênticos, a piscivoria só foi observada em indivíduos de grande porte, no entanto, nos sistemas lóticos, a classe mais pequena de peixes já incluía outras espécies de peixe como parte da sua dieta. Em suma, os resultados deste trabalho podem ser importantes para perceber a capacidade de dispersão desta espécie em Portugal e dos seus impactos na fauna nativa. As conclusões apresentadas deverão provar-se instrumentais aquando da criação de planos de mitigação dos impactos desta espécie, ou para qualquer plano de conservação direcionado para as espécies que coabitam com o Peixe-gato-europeu. Por exemplo, as agregações durante o período de reprodução podem representar uma oportunidade para a remoção de grandes quantidades de indivíduos.

**Palavras-chave:** Portugal; Espécie invasora; Predador de topo; Uso de profundidades; Telemetria

## Resumo Extenso

As invasões biológicas são uma das principais causas da perda de biodiversidade à escala mundial. O fenómeno da introdução de espécies afeta quase todos os ecossistemas, mas alguns são mais resistentes que outros. Os ecossistemas de água doce estão entre os mais diversos, contendo cerca de 50% das espécies de peixe conhecidas e sendo vitais para o desenvolvimento da sociedade. Estão, no entanto agora, sob tremenda pressão. Entre as consequências das alterações climáticas e da poluição, que os deixaram vulneráveis, as espécies introduzidas têm tido maior facilidade em se estabelecer, tornando estes ambientes nuns dos mais fortemente afetados por não-nativas. As espécies não-nativas são capazes de afetar negativamente as comunidades onde se inserem a diferentes níveis desde os genes até ecossistemas. Ameaçam a biodiversidade única encontrada nestes ambientes aquáticos através da hibridação, a introdução de novos patógenos, competição e predação. Nos ambientes aquáticos portugueses, estão identificadas 20 espécies de peixe não-nativos com novas espécies detectadas a um ritmo sem precedentes de uma nova espécie a cada dois anos. O Peixe-gato-europeu, é uma das chegadas mais icónicas. Este predador de topo, nativo da Europa Central e de Leste, foi primeiro introduzido em Espanha tendo descido o Tejo até chegar a Portugal. O facto de se tratar de uma chegada recente representa uma oportunidade de estudar os primeiros estágios de invasão e de como esta espécie pode vir a moldar as comunidades do rio. Este trabalho foca-se em dois aspetos da ecologia desta espécie para tentar medir o seu potencial impacto na fauna única encontrada nas águas doces portuguesas: movimentos e dieta. Na barragem de Belver, a barragem mais a jusante do Tejo, foi identificada uma população recém-estabelecida de Peixe-gato-europeu. Para delinear os padrões de movimento e atividade dos indivíduos pertencentes a esta população, montou-se uma rede de oito recetores acústicos a montante do paredão, abrangendo também um afluente da barragem (Ribeira de Eiras). Capturaram-se oito peixes pertencentes a esta população com o intuito de os marcar usando transmissores de biotelemetria acústica, um dos quais, equipado com sensores de pressão para medir profundidades. Após uma pequena cirurgia para a inserção do transmissor e um período de recuperação, estes peixes foram libertados de novo no local de captura, onde começaram a ser detetados pela rede de recetores. Ao todo, o período de seguimento passivo decorreu entre Junho de 2017 e Julho de 2018. Como complemento, efetuaram-se 30 sessões de seguimento manual com recurso a um recetor portátil, com o intuito de avaliar a eficácia da área de estudo escolhida e tentar uma melhor definição dos locais utilizados por estes peixes. Para calcular a área utilizada por estes peixes, optou-se por se utilizar estimadores de densidade espacial que estimam a probabilidade de nova ocorrência de um indivíduo para cada ponto geográfico com base em ocorrências prévias. Os objetos espaciais, produto desta análise, foram depois transpostos para um programa de informação geográfica (QGIS) para melhor entender a distribuição espacial das áreas utilizadas. Os valores de área foram depois associados a 13 variáveis independentes, 12 abióticas e 1 biótica, com o intuito de identificar os fatores que influenciam estas distribuições. Com esse objetivo, gerou-se um modelo linear misto através da seleção regressiva das variáveis selecionadas. Esta espécie exibiu grande fidelidade espacial, com um índice de residência de 84% dentro da área de estudo, no entanto alguns indivíduos (16%) mostraram-se capazes de movimentos de longa distância (até 11.5km a montante), principalmente durante o período de Inverno, que podem ajudar à sua dispersão. Os resultados deste estudo suportam estudos prévios sugerindo que esta espécie se encontra mais ativa durante os meses de Verão (Área semanal utilizada teve o seu máximo a 2,79km<sup>2</sup>) e mais letárgica durante o Inverno (Área semanal utilizada desceu a um mínimo de 0,45km<sup>2</sup>), sendo que a média de área semanal utilizada foi de 1,66km<sup>2</sup>. O modelo revelou que o nível de atividade foi altamente regulado pela temperatura e caudal. Uma tendência para a agregação durante o período de reprodução descrito para esta espécie foi também observada, este facto pode vir a ser determinante ao sucesso de ações de controlo direcionadas a esta espécie. A análise do uso de profundidades por um dos indivíduos marcados indica que esta espécie poderá fazer uso de habitats mais superficiais do que previamente

pensado. Também apontou para um nível estável de atividade durante todo o dia na Primavera, Verão e Outono, mas atividade quase exclusivamente noturna no Inverno, facto este também suportado por trabalhos prévios. Para avaliar os hábitos alimentares do Peixe-gato-europeu, obtiveram-se oportunisticamente, entre Abril de 2017 e Novembro de 2018, e com a ajuda de pescadores, 96 peixes. Esta metodologia acabou por criar constrangimentos na amostra recolhida pois a atividade piscatória concentra-se nos períodos de Primavera (N= 34) e Verão (N=30), gerando um enviesamento. Estes foram separados pela tipologia de habitat de onde eram oriundos, 35 originários de habitats lênticos e 61 de habitats lóticos, e os seus conteúdos estomacais analisados. Peixes onde nenhuma presa foi encontrada foram descartados da análise final. Para caracterizar a dieta desta espécie, tomaram-se duas abordagens distintas, a caracterização das diferenças entre os dois habitats selecionados e a caracterização das diferenças ontogénicas dentro de cada habitat. Os hábitos alimentares observados são preocupantes. As curvas de acumulação de presas desenhadas para cada habitat ainda não estabilizaram pelo que se estima que o número de *taxa* predados pode vir ainda a aumentar. Os resultados suportam o facto de esta espécie ter a capacidade de predação todo o espectro de presas disponíveis, exibindo dietas distintas em habitats lênticos e lóticos. A análise destes dados através de NMDS e gráficos de Costello modificados demonstram que a amplitude de nicho foi substancialmente maior nos habitats lóticos do que nos habitats lênticos. Nos habitats lóticos, esta espécie exibe um comportamento mais generalista, e, nos habitats lênticos esta parece tornar-se mais especializada na captura de crustáceos. Nas secções lóticas do Tejo uma grande percentagem, da sua dieta é composta por peixes nativos e camarão-de-água-doce. A inclusão de Enguia-europeia e Lampreia-marinha na dieta representa outra fonte de pressão sobre estas espécies ameaçadas. A predação sobre espécies pelágicas é interessante, visto que o Peixe-gato-europeu é considerado uma espécie bentónica. Este comportamento talvez possa ser explicado pela preferência por habitats mais superficiais encontrada durante o seguimento por biotelemetria. Em habitats lênticos, por oposição, os indivíduos alimentaram-se quase exclusivamente de crustáceos (camarão-de-água-doce e lagostim-vermelho) com muito pouca incidência de peixes, na sua maioria não-nativos. No geral, indivíduos mais pequenos também se alimentavam de presas mais pequenas. Observou-se grande variação ontogénica na dieta do Peixe-gato-europeu. Nos sistemas lênticos, a piscivoria só foi observada em indivíduos de grande porte, no entanto, nos sistemas lóticos, a classe dimensional mais pequena de siluros já consumia peixes. Apesar dos bons resultados obtidos neste estudo, um melhoramento dos esforços de amostragem, maior número de indivíduos recolhidos e marcados com melhor definição temporal e espacial, e a adoção de metodologias diferentes, avaliação da dieta por biomassa de presas ou identificação por DNA *metabarcoding*, pode vir a fortalecer os resultados e conclusões descritas. Este é o primeiro trabalho realizado em Portugal, que estuda os movimentos do siluro e avalia o seu potencial impacto nas comunidades de peixes do Tejo, tendo em conta o seu habitat. Esta informação poderá ser posteriormente utilizada para avaliar o risco de impacto e dispersão desta espécie e aplicada em programas de gestão para esta espécie e aquelas que com ela coabitam.

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## List of abbreviations, symbols and acronyms

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AIC – Akaike Information Criterion  
AmpFlow – Weekly Flow Amplitude  
AmpTemp – Weekly Temperature Amplitude  
 $A_N$  – Relative numerical prey abundance  
ANOSIM – Analysis of Similarity  
ANOVA – Analysis of variance  
APA – Portuguese Environmental Agency  
BIC – Bayesian Information Criterion  
df. resid – Residual degrees of freedom  
EN224 – Estrada Nacional 224  
EW – Eviscerated Weight  
 $F_0$  – Frequency of Occurrence  
FotoPer – Photoperiod  
GW – Gonad Weight  
 $h$  – Smoothing parameter  
 $H_0$  – Null hypothesis  
 $href$  – reference smoothing parameter  
 $i$  – Prey type  
ID – Identification  
IUCN – International Union for Conservation of Nature  
KDE50 – Kernel Density Estimation with 50% probability of occurrence  
KDE95 – Kernel Density Estimation with 95% probability of occurrence  
logLik – Logarithmical Likelihood  
LPhase – Lunar Phase  
LSCV – Least Square Cross Validation  
MaxFlow – Maximum Weekly Flow  
MaxTemp – Maximum Weekly Water Temperature  
MeanFlow – Mean Weekly Flow  
MeanTemp – Mean Weekly Water Temperature  
MinFlow – Minimum Weekly Flow  
MinTemp – Minimum Weekly Water Temperature  
 $n$  – Number of relocations  
 $N$  – Sample size  
 $N_{lent}$  – Lentic sample size  
 $N_{lentC1}$  – Lentic class 1 sample size  
 $N_{lentC2}$  – Lentic class 2 sample size  
 $N_{lot}$  – Lotic sample size  
NMDS – Non-metric multidimensional scaling  
 $P_i$  – Prey specific abundance  
 $Psi$  – amount of a given prey type  
 $Pst$  – total amount of prey items found in each stomach that contained a given prey  
Repro – Reproduction period

SL – Standard Length  
SNIRH – Sistema Nacional de Informação de Recursos Hídricos  
TL – Total Length  
TW – Total Weight  
UD – Utilization Distribution  
VarFlow – Between Week Flow Variation  
VarTemp – Between Week Temperature Variation  
VBA – Virtual Basic Applications  
W – Mann-Whitney statistic  
**X** – Independent variable  
**x** – Probability of occupation  
**X<sub>i</sub>** – *i*<sup>th</sup> relocation of any given point  
**Y** – Response variable  
**ε** – Random effect

## Chapter 1 – General Introduction

In the last century, we have witnessed a steep decline in biodiversity across the globe. Climate change, habitat loss, invasive species and other anthropogenic pressures have been the main drivers for this mass extinction (Sala et al., 2000). One of the largest causes of biodiversity loss, presently, are non-native species (Vitousek et al., 1996; Doherty et al., 2016; Clavero & García-Berthou, 2005). These species are often introduced for a myriad of reasons such as agricultural and animal production, ornamental purposes, fisheries enhancement and biocontrol among others (Lockwood et al., 2013). Other species arrive to new areas accidentally, as cargo contaminants (hitchhikers) or transported in ballast waters (Lockwood et al., 2013). Given the current growth in global trade, the number and the extent of these invasions will increase and consequently its impacts on the ecosystems and biodiversity (Jeschke & Strayer, 2005).

The number of non-native species is increasing at a global scale. For instance, it is estimated that over 50000 species were introduced in the U.S., while this number is around 14000 species in Europe, as described by the European Alien Species Information Network (EASIN). The possibility of one of these invasive species having a large impact on ecosystems increases as more species are introduced in a region (Ricciardi & Kipp, 2008). Species introductions have a plethora of impacts on biota, impacting from genes to whole ecosystems (Cucherousset & Olden, 2011). For instance, introduced fishes can hybridize with native species causing a loss of genetic integrity (Almodóvar et al., 2012), they can directly or indirectly compete for resources with native species, decreasing their fitness (Caiola & Sostoa, 2005) or bring with them other non-native pathogens that can wipeout entire populations (e.g. Gurevitch & Padilla, 2004). More directly, they can predate over native species (e.g. Leunda et al., 2008). These impacts may lead to the alteration of species abundance and the community structure, which may contribute to less diverse communities composed of mostly non-native species, as part of a process of Biotic Homogenization (Rahel et al., 2002)

In order to avoid this outcome, several entities, both public and private, are making efforts to slow or halt the spread of non-native species and minimize their impacts. Despite necessary, these efforts are quite economically straining, with the economic costs of non-native species in the United States adding up to 120 billion dollars per year and a total of 12.5 billion euros per year in the EU (Kettunen et al., 2009; Pimentel et al., 2005). Not only is the fight against non-natives species a costly endeavor, but the losses associated with the disappearance of important ecosystem services also amount to a great percentage of these costs (Kettunen et al., 2009; Pimentel et al., 2005).

Biological invasions are widespread and can occur in any ecosystem. Some of the most affected ecosystems are freshwater systems and freshwater fishes are one of the groups with high invasion rate (Welcomme, 1998; Jeschke & Strayer, 2005). Due to these invasive species becoming so common, we are witnessing a homogenization of freshwater communities (Villéger et al., 2011; Rahel, 2007). Freshwater systems are some of the most interesting areas for conservation worldwide. Despite only covering about 0.1% of the earth's surface, roughly half of the fish species are found in freshwater ecosystems globally (Nelson, 2015), these biomes can therefore be considered biodiversity hotspots and must be protected. Ironically, fishes are the most introduced vertebrate group in freshwaters (Welcomme, 1998; Jeschke & Strayer, 2005).

The main reasons behind the introduction of non-native fish species are varied and region dependent. They can be imported for aquaculture and later escape, which is more common in underdeveloped countries (Welcomme, 1998). In developed countries, introductions occur more commonly through the release of ornamental fishes or, more often, for the practices of sport and recreational fishing (Carpio et al., 2019; Cambray, 2003). Even within Europe the main reasons for animal introduction change, from

water transfers and channels in Germany and Austria (Rabtsch et al., 2012), to further west where most fish species were introduced for recreational fisheries like in Portugal and Spain (Elvira & Almodovar 2001; Ribeiro et al., 2009a).

The Iberian Peninsula is currently considered a hotspot for fish introductions with about 30% of the occurring species being non-native (Leprieur et al., 2008). Recently, in Portuguese freshwater ecosystems there have been 84 records of introduced animals of which about 30% were fish species (Anastácio et al., 2018). Indeed, in freshwater systems, we have witnessed to the establishment of 20 fish species at a rate of about one new species every two years in the last decade (Ribeiro et al., 2009b). This high rate of introduction and the typology of species introduced is a threat to the great diversity of species, many endemic, found in the Iberian Peninsula. Most introduced species are associated with angling practices either because they were used as bait or as the target for fishing (Ribeiro et al., 2015; Banha et al., 2017), with some being top predators and valued fishing trophies (Gago et al., 2016). These predators, such as northern pike or largemouth bass, can cause immediate impact over the freshwater ecosystems through competition with other predators, like otters or cormorant, by excluding them from suitable habitats, and predation over natives reducing their total abundance, being capable of local extinctions (Cucherousset & Olden, 2011; Smith & Darwall, 2006). However, their impacts on the ecosystems still lacks proper evaluation (Ribeiro & Leunda, 2012).

The biological invasion of Iberian freshwaters follows an east to west pattern (Elvira & Almodóvar, 2001). Initial populations are introduced in northeastern Iberian watersheds (Catalonia) from France (Elvira & Almodóvar 2001; Garcia-Berthou et al., 2005), and posteriorly are moved to other localities across the Iberian Peninsula, especially in the central plateau (Ribeiro et al., 2009b; Gago et al., 2016). The new established populations naturally disperse downstream westwards along the main international rivers (Douro, Tagus and Guadiana), and subsequently other secondary introductions occur in smaller drainages in the south and west parts of the Peninsula (Ribeiro et al., 2009b). Most of these secondary and tertiary introductions occur in reservoirs that are preferred fishing locations of recreational fishermen (Gago et al., 2016; Banha et al., 2017). In these ecosystems non-natives thrive and tend to dominate the fish communities dispersing downstream or upstream from these habitats (Clavero et al., 2013; Godinho et al., 1998). However, the processes of natural dispersion of non-native species, and how their movement patterns and spatial occupation occur during the initial stages of the invasion are still poorly known.

The European catfish (*Silurus glanis*, L., 1758) is a recently arrived predator to Portuguese freshwaters (Gkenas et al., 2015; Gago et al., 2016). This Silurid native to central and Eastern Europe as well as part of Asia, has been introduced in seven European countries namely the Netherlands, Italy, France, Belgium, England, Spain, later dispersing into Portugal, and was recently detected in Brazil (Elvira, 2001; Gkenas et al., 2015; Cunico et al., 2014). Most of these introductions aimed to improve sport fisheries given it attains a large size, reaching 2.8m in length and about 140 kg in weight making it the 2<sup>nd</sup> largest freshwater fish in Europe (Copp et al., 2009; Boulêtreau & Santoul, 2016). It was first detected in the Iberian Peninsula in 1974 in Mequinenza-Ribarroja reservoir in the Ebro drainage, and thereafter it was detected in the upper Tagus drainage reservoirs in Spain (Doadrio et al., 2001). Secondary occurrences in Catalonia were mostly in reservoirs (Benejam et al., 2007) and recently it has been reported for the Iznájar reservoir in the Guadalquivir drainage (Moreno-Valcárcel et al., 2012). The first *S. glanis* official record in Portugal was only in 2014, but its arrival was probably in 2006 (Gkenas et al., 2015; Gago et al., 2016). Currently, the European catfish occurs in most to the Portuguese Tagus mainstem being more prevalent in large reservoirs (Gago et al., 2016).

The European catfish seems to be an extremely tolerant fish capable of surviving under harsh habitat conditions that associated with high fecundity, longevity and generalized feeding behavior guarantees a

successful invasion in introduced regions. For instance, it survives in anoxic environments thanks to its high concentration of hemoglobin (30%-35%) in the blood (Lelek, 1987 *in* Copp et al., 2009; Mihálik, 1995 *in* Copp et al., 2009), being able to withstand very low levels of oxygen (Daněk et al., 2014). Moreover, this species occurs in waters with temperatures that range from below freezing to over than 30°C (Hilge, 1985; Copp et al., 2009), with its physiological optimum at 25-27°C (David, 2006). This fish is long-lived, with males attaining 22 years of age and females 16 years, exhibits high fecundities (25000-33000 of eggs/kg bodyweight) (Lever, 1977 *in* Copp et al., 2009) and builds nests in woody-vegetation areas. This species attains sexual maturity between the 3<sup>rd</sup> and 4<sup>th</sup> year of life (approximately 80 to 90 cm, total length), spawns between May and June (Alp et al., 2004). It is considered an opportunistic predator, mostly feeding on fish and crayfish (Copp et al., 2009), and has been classified as an Apex predator considering its predatory potential (Vejřík et al., 2017).

With its large size, very adaptable diet and economical interest making it an interesting study subject, the European catfish ecology is relatively well described within its native range. However, a look into information available reveals that much of the knowledge about this species predatory impact and movement patterns relate to long established populations (Capra et al., 2014; Carol et al., 2009). This information is crucial in predicting the potential impacts this Apex predator can have over the unique freshwater fauna found in Portugal, especially when native fish communities do not have piscivores.

This study aims to assess the European catfish impact during the early stages of invasion by understanding how it is preying on the native fish fauna and how the fish moves when it colonizes new environments. With this increase and integrated knowledge about this invasive fish, it is expect to understand the impact mechanisms of this species and improve management answers to current and future problems cause by European catfish.



## Chapter 2 – Movements of *Silurus glanis* in a reservoir of the river Tagus

### 2.1. Introduction

Fish movements have long been studied as a way to understand how these animals use the limited space around them to attend to their daily necessities of food and shelter as well as parts of their life cycle like reproduction or dispersion (Lucas & Baras, 2001). This allows for a better understanding of the structure of river communities and, in this way strengthens the creation of management plans for the conservation of these threatened habitats (Lucas & Batley, 1996). Tracking studies may also be applied to non-native introduced species as a way of understanding their dispersion capabilities to predict where and how fast they will spread across waterways (DeGrandchamp et al., 2008).

Studies about the movement of non-native fishes are uncommon (*but see* DeGrandchamp et al., 2008; Capra et al., 2014) therefore knowledge about how they colonize new environments and adapt to conditions that are often very different from their original range, is limited. The recent invasion of *Silurus glanis* (Gkenas et al., 2015) into the Portuguese Tagus River presents itself as an excellent opportunity to study how a recently established population uses the space available and disperses. Understanding the movement patterns can be a way of evaluating how widespread the impacts caused by this species can be, and how fast it can spread (DeGrandchamp et al., 2008).

The European catfish was thought to have high site fidelity, to be a slow swimmer, and did not display large scale/long distance movements, however little is known about its dispersion capabilities (*but see* Carol et al., 2007; Capra et al., 2014). Most studies have been conducted in its native range, almost exclusively in rivers (e.g. Daněk et al., 2016). These studies have found strong evidence of temporal variation in activity patterns with summer being the most active period and winter a period of near complete inactivity (Daněk et al., 2016; Slavík et al., 2007).

Information about the periods of highest activity during the day are contradictory with Daněk et al. (2016) in Czech Republic and Carol et al. (2007) in Spain finding evidence of mostly nocturnal behavior, and Slavík et al. (2007) describing that fishes maintained their activity level throughout the day also in Czech Republic. Findings regarding this species site fidelity seemed, again contradictory. In France, Capra et al. (2014) observed that the majority of fishes (54%) were not resident in their 35km long stretch of the river Rhône while, in Spain most of the fish monitored spent the totality of the tracking period inside the monitored area (Carol et al., 2007). Movements observed were also short in distance (Capra et al., 2014), likewise, in Spain the mean distance travelled between all fishes was of 18.6m/day (Carol et al., 2007). These works attempted to describe the influence of environmental variables such as temperature, flow and oxygen on movement patterns of *S. glanis* (Daněk et al., 2016; Slavík et al., 2007; Capra et al., 2014; Daněk et al., 2014) with Daněk et al. (2016) having a more thorough approach finding that temperature and flow were significant predictors in this species activity patterns. Nevertheless, these studies were based on manual tracking techniques, with tracking only occurring either very frequently in a short time span or at most in a weekly basis for longer periods. The methodology selected for the present study enables a long, continuous, tracking period that should present stronger results.

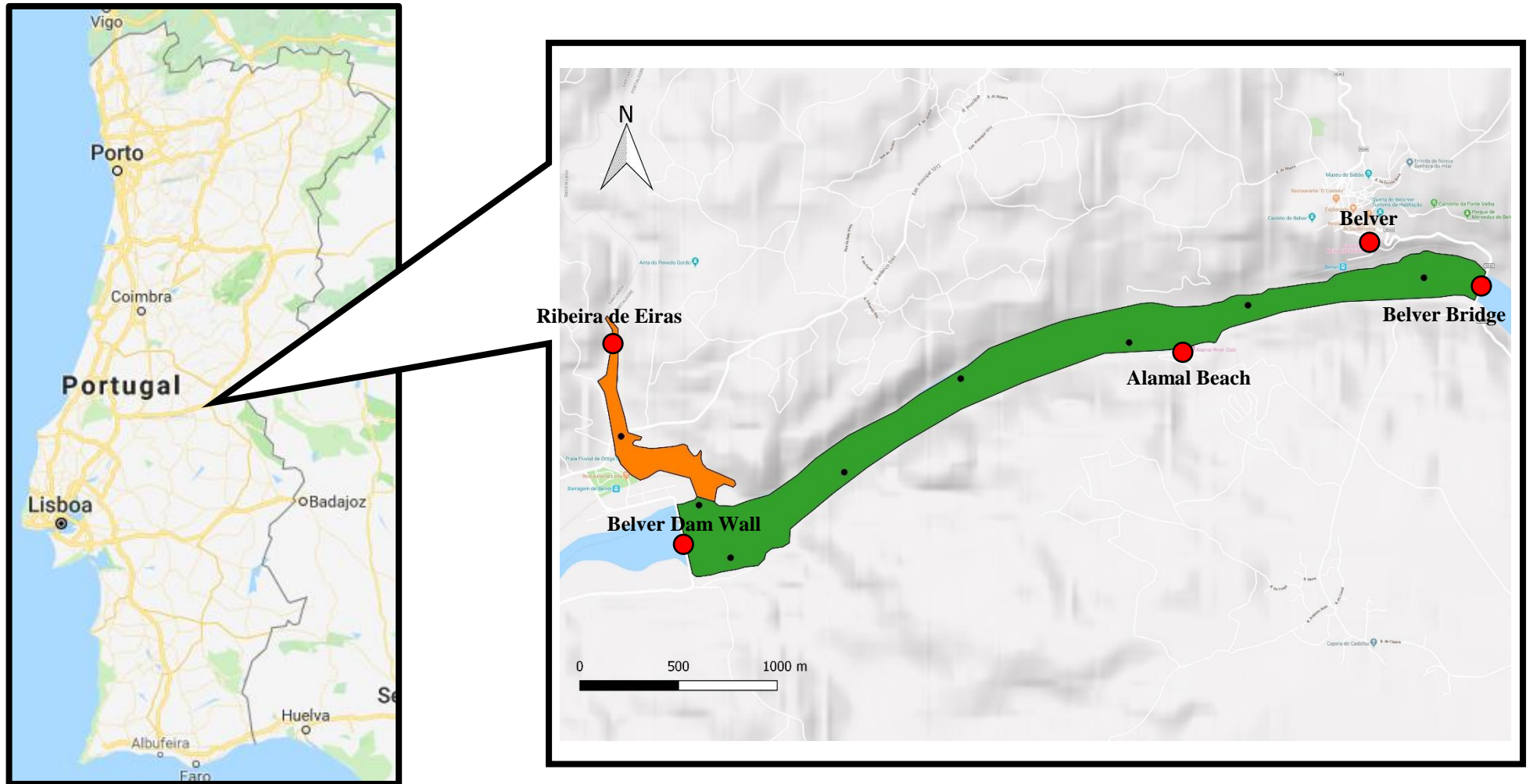
Passive acoustic biotelemetry is an increasingly powerful tool to track a large number of individuals in underwater habitats. It has become a popular method of understanding fine aspects of the ecology, behavior and life cycle of several fish species that aid in answering several management questions (Heupel et al., 2006). It has been used successfully in fish species in both marine and freshwater environments (Abecassis et al., 2009; Welch et al., 2009; Belo et al., 2016). This method requires the

assembly of a receiver array that will allow the continued tracking of any tagged fishes for potentially long periods of time (upwards of one year).

In this study, we aim to describe the European catfish movement patterns in a recently established population (Lower Tagus Drainage), in order to understand its site fidelity and its diel and seasonal activity. By evaluating the European catfish movements, we can try to predict its dispersal capacity, constituting a unique opportunity to understand how a non-native species spreads on the beginning of the invasion.

## **2.2. Study Area**

This study was conducted at the Belver Reservoir (Figure 2.1.) located at Belver, Gavião, Portalegre district (39°28'45.71"N, 7°59'51.83"W), the most downstream reservoir of the Tagus. The Tagus is the longest river in the Iberian Peninsula being 1007 km long crossing the international border between Spain and Portugal. It starts in the Albarracín Mountain, in Spain, and has its mouth in Lisbon, with its estuary starting in Vila Franca de Xira and being surrounded by the districts of Lisbon, Setúbal and Santarém. Across its length, there are several hydroelectric dams of various dimensions, with 14 dams in Spain and 2 in Portugal. The Belver reservoir was built in 1952 and houses a hydroelectric dam (Figure 2.2.) with an electrical production capacity of 80,7MW. The Portuguese Environmental Agency (APA) classifies this dam as an area of free use, meaning that there are no restraints to activities developed in the reservoir because it is destined for touristic or recreational uses. The study area corresponded to a 5.5km stretch of the Belver Reservoir, starting in a nearby stream called Ribeira de Eiras and prolonging itself to the Belver bridge, EN244 (Figure 2.1). The reservoir has a total capacity of 90000m<sup>3</sup> of water and has a maximum discharge rate of 18000m<sup>3</sup>/s. During the study period, the average water temperature was 17°C and varied between 11°C in the winter and 24°C in the summer. Average annual precipitation is 875mm and mean weekly flow, during the study period, was of 132.5 m<sup>3</sup>/s, with a maximum of 1922.54 m<sup>3</sup>/s and a minimum of 2.82 m<sup>3</sup>/s (Figure 2.3.). Baião & Boavida (2005) classified the reservoir from mesotrophic to meso-eutrophic.



**Figure 2.1. Bavel Reservoir location and representation of the Study Area.** Stream Section is represented by ■ and River Section is represented by ■; Receiver positions are indicated with ● and relevant landmarks are indicated with ●.

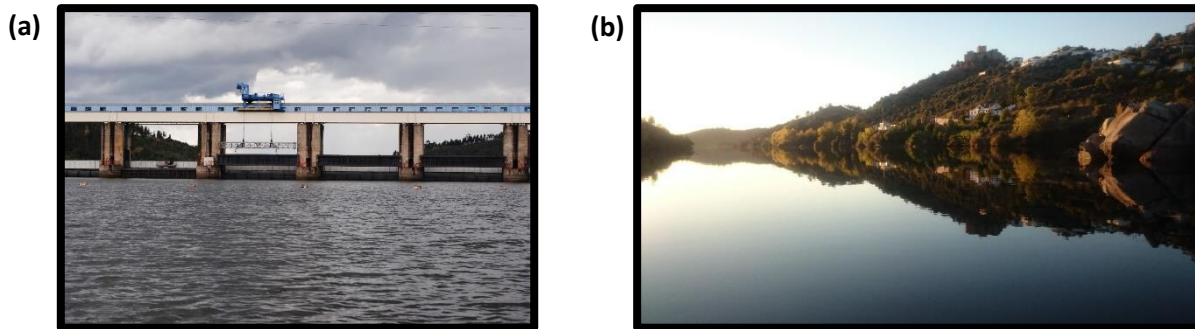


Figure 2.2. Depiction of the Hydroelectric Dam (a) and the Riverbank (b) along the study area.

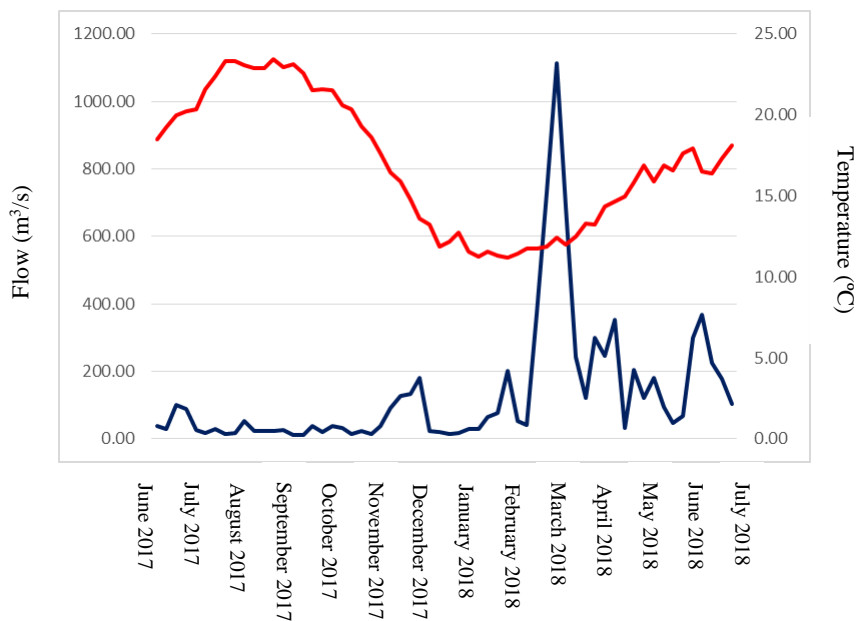


Figure 2.3. Flow and Temperature Variation along the study period. Depicted are flow (—) and water temperature (—).

### 2.3. Fish capture and tagging

Firstly, the fish were caught using gill nets placed in several points inside the study area. The eight fish used in this study were caught and tagged between September of 2016 and April of 2018 (Table 2.1.). Afterwards, the fishes were anesthetized in a solution of 2-phenoxyethanol (0.4ml per litter of water) and tags were inserted through a surgical incision in the peritoneal cavity. While anesthetized, individuals were measured to their total length and weighed (Table 2.1.), with exception of Sil#7, the measurements recorded would place all other individuals as adult fishes following Alp et al. (2004), where *S. glanis* is considered to have reached maturity at 78.82cm TL for males and 87.05cm TL for females. Sil#1, the first fish captured, tagged and released, was kept for approximately 1 month in a large tank to test the tagging procedure, ensuring that the tag did not influence the fish's behavior and wellbeing. Fishes tagged afterwards went through a shorter recovery period of about 1-2 hours, and when individual fish were responsive and swimming actively, they were then released back at the place of capture. All procedures were carried out in accordance with the Portuguese legislation regarding animal capture, manipulation and experimentation for scientific purposes. This includes certification requirements of FELASA level C courses to license those responsible for directing animal experiments and the Veterinary National Authority proper accreditation.

Six fishes were tagged with VEMCO V16 69kHz Hydro-acoustic Transmitters, with 16mm diameter, up to 98mm length and 17.3g (weight in water), 162 maximum power output and an expected battery life of 10 years, and two with the VEMCO V16P 69kHz Hydro-acoustic Transmitters, with 16mm diameter, up to 98mm length and 37g (weight in water), 162 maximum power output and an expected battery life of 10 years, equipped with pressure sensors to collect depth measurements (Table 2.1.). They were actively and passively tracked for a period of roughly one year from June 3<sup>rd</sup> 2017 to July 22<sup>nd</sup> 2018. Each transmitter released a pulse every interval of 60-90 seconds, being recorder by eight receivers deployed along the study area.

**Table 2.1. Data of the *Silurus glanis* tagged in the Belver reservoir.** Describing Fish ID, Date of Capture, Tagging and Release, Trasmmitter ID, Total Weight (Kg) and Total Length (cm). \*Transmitters equipped with pressure sensors. § Fish recaptured on 22-jul-17.

Fish ID	Capture	Tagging	Release	Transmitter ID	Weight (Kg)	Length (cm)
sil#1	26-sep-16	06-oct-16	25-oct-16	A69-9002-15298*	6,0	104,0
sil#2	08-feb-17	08-feb-17	08-feb-17	A69-9001-16502	6,0	94,0
sil#3	10-apr-17	11-apr-17	11-apr-17	A69-9001-16506	6,0	98,0
sil#4	10-apr-17	11-apr-17	11-apr-17	A69-9001-16504	6,3	98,0
sil#5 <sup>§</sup>	10-apr-17	11-apr-17	11-apr-17	A69-9001-16505	5,4	91,0
sil#6	10-apr-17	11-apr-17	11-apr-17	A69-9001-16503	6,9	97,5
sil#7	03-aug-17	03-aug-17	03-aug-17	A69-9002-15299*	2,9	71,0
sil#8	05-apr-18	05-apr-18	05-apr-18	A69-9001-16505	6,4	93,0

## 2.4 Tracking

An array of eight receivers (four VEMCO VR2W and four VEMCO VR2Tx) were installed along the study area to passively track the movements of the tagged individuals. These receivers were held by vertical steel cables attached to reservoir bedrock along the 5.5km stretch, at the top, each receiver had a rigid plastic buoy to ensure it would remain in a vertical position. This array was kept submerged (~10 m depth) for the entire study period to avoid tampering. The average distance between each receiver was approximately 600m with them distancing a maximum of 900m and a minimum of 310m (Supplementary Figure 1.1; Supplementary Table 1.1). A range test was not conducted as detection range would variate given receiver position and orography of the riverbank. The receivers placed in the stream and closer to the wall, had less distance between them to ensure that observations were registered as it was assumed that the noise produced by the hydroelectric dam's functioning and the meandering observed between the stream and the main stem would greatly affect detection efficiency. The VEMCO VR2Tx Receivers also have the capacity of collecting temperature data, which was later used to create a temperature profile of the study area during the tracking time frame.

The fish were actively tracked, about every three weeks, for a total of 30 tracking sessions, by boat using a VEMCO VR100 hydro-acoustic receiver. The entire stretch of the study area was scanned and if one or more fishes were found to be missing, the area would be extended another 6km upstream from the initial study area (Figure 2.3.). This tracking period usually occurred in the morning between 9AM and 12PM.

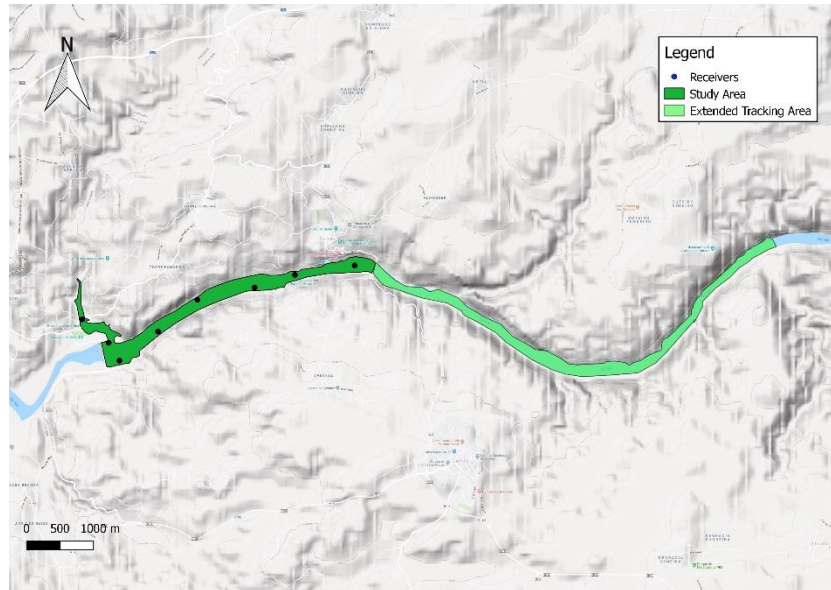


Figure 2.3. Study Area (■), receiver positions (●) and active tracking extension area (■).

## 2.5. Data analysis

Spatial data was separated for each fish using excel VBA macros, while also removing false detections. If subsequent detections of a fish were observed within 30 seconds of the first detection those were considered duplicate detections and were not taken into account for this study.

Site Fidelity, defined as the tendency to remain in or return to a previously occupied location (Switzer, 1993), was used as a measurement of the effectiveness of the installed receiver array and study area dimensions. To evaluate this aspect, a Weekly Residence Index was calculated as the percentage of total days of a week spent within the study area (a day would be considered as spent inside the study area if there was at least one observation in a receiver). Two main subareas of the study site were defined, Stream section and Main River section (Figure 2.1.) and residence time within each of these subareas was also calculated. The Residence Index for both areas was calculated as the percentage of days spent within each subarea relative to the number of days spent within the study area.

Using the package *adehabitatHR* for R, kernel estimation was used in the interpretation of the Utilization Distribution (UD) by the individuals tagged. UD is a function that attempts to evaluate the probability of a given individual to have relocated to a different point in space knowing that he has occupied the coordinates (x, y). The kernel estimation allows the visualization of these probabilities through a spatial object. Each point's probability of occupation ( $\mathbf{x}$ ) is calculated through the function (Equation 2.1):

$$\hat{f}(\mathbf{x}) = \frac{1}{nh^2} \sum_{i=1}^n K \left\{ \frac{1}{h}(\mathbf{x} - \mathbf{X}_i) \right\}$$

Equation 2.1. Function of probability of occupation of any given spatial point for a Kernel Utilization Distribution.

Where  $h$  is a smoothing parameter,  $n$  is the number of relocations and  $\mathbf{X}_i$  is the  $i^{th}$  relocation of the sample point. The smoothing parameter  $h$  controls the width of the kernel functions placed over each point, it can be calculated automatically using a reference bandwidth ( $href$ ) for each observation however this approach is not ideal for animals with several centers of activity as it will result in an



oversmoothing of the data. Another method of smoothing parameter calculus is the Least Squared Cross Validation (LSCV) that minimizes the difference in volume between the true UD and the estimated UD. Literature, however, suggests that a successive trial approach, wherein several distributions are generated with either different  $h$  values and the most fitting is chosen or where the  $href$  of several distributions are considered and an average is calculated, is best suited for most occasions (Silverman, 1986; Wand and Jones, 1994). This was the methodology chosen for this study and it resulted in  $h=150$  which took into account the average  $href$  values attained for each kernel produced. Using this methodology, kernels were produced for each fish's UD per month and per week (when possible).

Afterwards, polygons containing all points with probabilities of occurrence higher than 5% were obtained as a metric of **Area Usage (KDE95)**, being determined for both the monthly and weekly data. Another set of polygons containing all points where probability of occurrence was above 50% was also obtained as a measure of **Core Area Usage (KDE50)** for both the monthly and weekly data. The monthly polygons were then transposed to a QGIS project, where they were cut using a mask layer of the study area (Figure 2.1.), a final area value was then calculated for both KDE95 and KDE50 using the QGIS calculus tools. For the weekly areas, the direct output from *adehabitat*'s area calculation was used, without it being cut with the mask layer, and the deviation error was assumed.

The building of a linear mixed model was the methodology selected to assess which environmental aspects, and to which degree, influence the space use of the studied population. A linear mixed model is ideal for the determination of the value of a continuous response variable (**Y**), in this case **KDE95** or **Area Usage**, as a function of **X** independent variables taking into account random effects ( $\epsilon$ ), such as in this case, the individual behavior of each fish (Equation 2.2.).

$$Y \sim X_1 + X_2 + \dots + \epsilon$$

**Equation 2.2. Linear mixed model baseline function.**

A set of thirteen explanatory variables (Table 2.2.) was selected based on previous publications, with implications in the dial activity patterns of this species (Slavík et al; 2007; Capra et al., 2014; Danek et al., 2016).

Of these thirteen variables only Reproductive Period was non-environmental, with the months of June to August being considered as reproductive period and the remaining months as non-reproductive period, following Alp et al. (2004).

**Table 2.2. Independent variables description.** Identifying each variable's name, abbreviation and the originating source. SNIRH refers to the Serviço Nacional de Informação de Recursos Hídricos.

Variable Name	Abbreviation	Source
Mean Weekly Water Temperature	MeanTemp	VR2Tx Measurements
Minimum Weekly Water Temperature	MinTemp	VR2Tx Measurements
Maximum Weekly Water Temperature	MaxTemp	VR2Tx Measurements
Weekly Temperature Amplitude	AmpTemp	MaxTemp – MinTemp
Between Week Temperature Variation	VarTemp	MeanTemp <sub>n</sub> – MeanTemp <sub>n-1</sub>
Photoperiod	FotoPer	Observatório Astronómico de Lisboa
Lunar Phase	LPhase	Observatório Astronómico de Lisboa
Reproductive Period	Repro	Alp et al., 2004
Mean Weekly Flow	MeanFlow	SNIRH
Minimum Weekly Flow	MinFlow	SNIRH
Maximum Weekly Flow	MaxFlow	SNIRH
Weekly Flow Amplitude	AmpFlow	MaxFlow – MinFlow
Between Week Flow Variation	VarFlow	MeanFlow <sub>n</sub> – MeanFlow <sub>n-1</sub>

Oxygen concentration (mg/L) in the water was the final variable selected however it was not included in the final analysis as the data had several periods missing.

After the variable selection, a correlation test between all variables was done in order to eliminate strongly correlated variables, a correlation value of  $\pm 0.7$  was the cutoff value used. The method used was Pearson's correlation coefficient, as it is widely used to test for correlation in these variable types (Ng et al., 2007; Childs et al., 2008), and its result indicated strong correlations between several variables so all but MeanTemp, FotoPer, LPhase, MeanFlow and Repro were discarded. Individual behavior (ID) was the random effect selected for this model, which consisted on the individual component in the variation of area used.

Using R-package “lme4” a null model was created without the inclusion of any variables and taking into account only the random effects to establish a comparative ground. Model fitting was done through a backwards stepwise selection, starting with the model containing all the variables and eliminating variables one by one until only the most significant was left. Model comparison and selection took into account the Akaike Information Criterion (AIC), an estimator of statistical models' quality that penalizes models for the number of variables included in function of number of observations, and likelihood (logLik) that indicates which model explains most variability. ANOVAs were used to compare the AIC values of each successive model as an aid to model fitting ( $p$ -value < 0.05). Furthermore, Bayesian Information Criterion (BIC), an estimator of statistical model quality that further penalizes the number of variables included, was also used as an aid to model selection

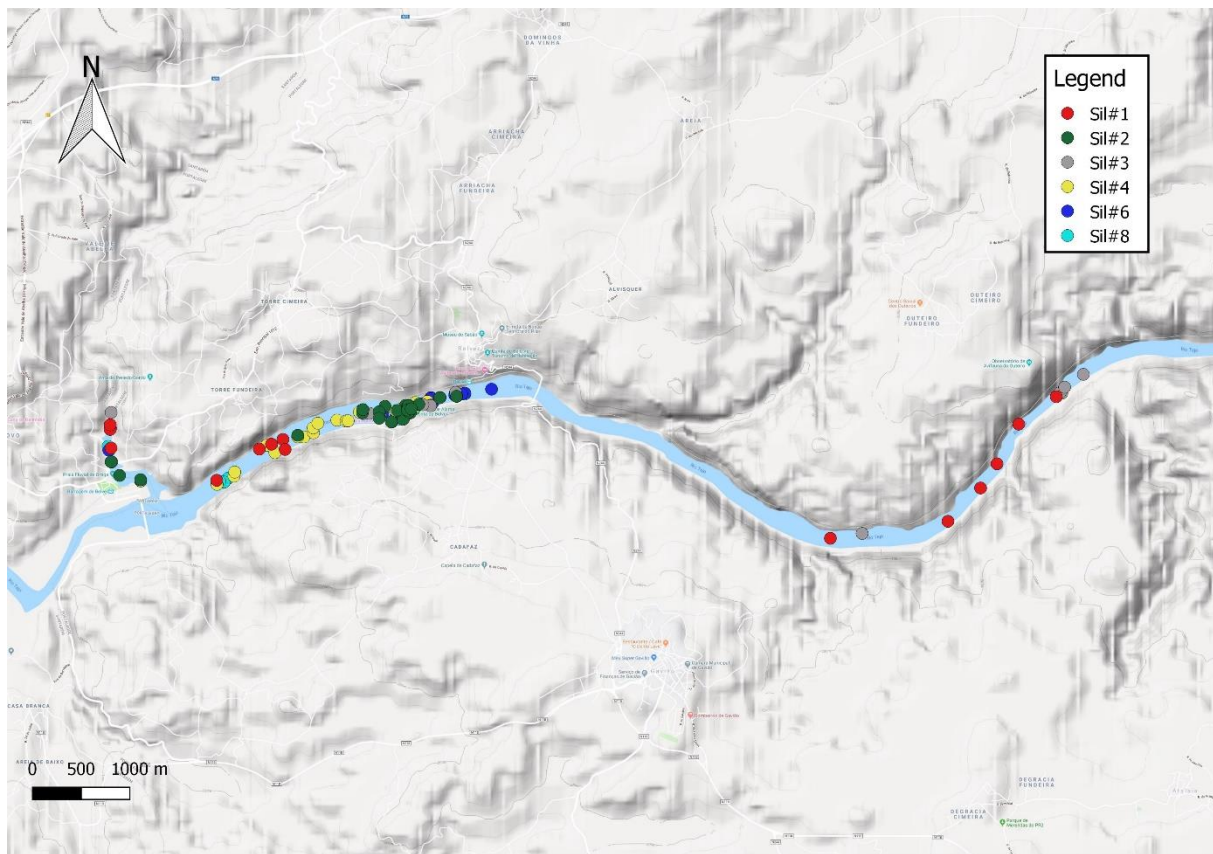


## 2.6. Results

The individuals tagged were all within the same size and weight range, the measurements shown in Table 2.1. indicate that all would have already reached maturity. Sil#7 is the exception, being too small to be considered mature. Sex was not discernable and so was not registered.

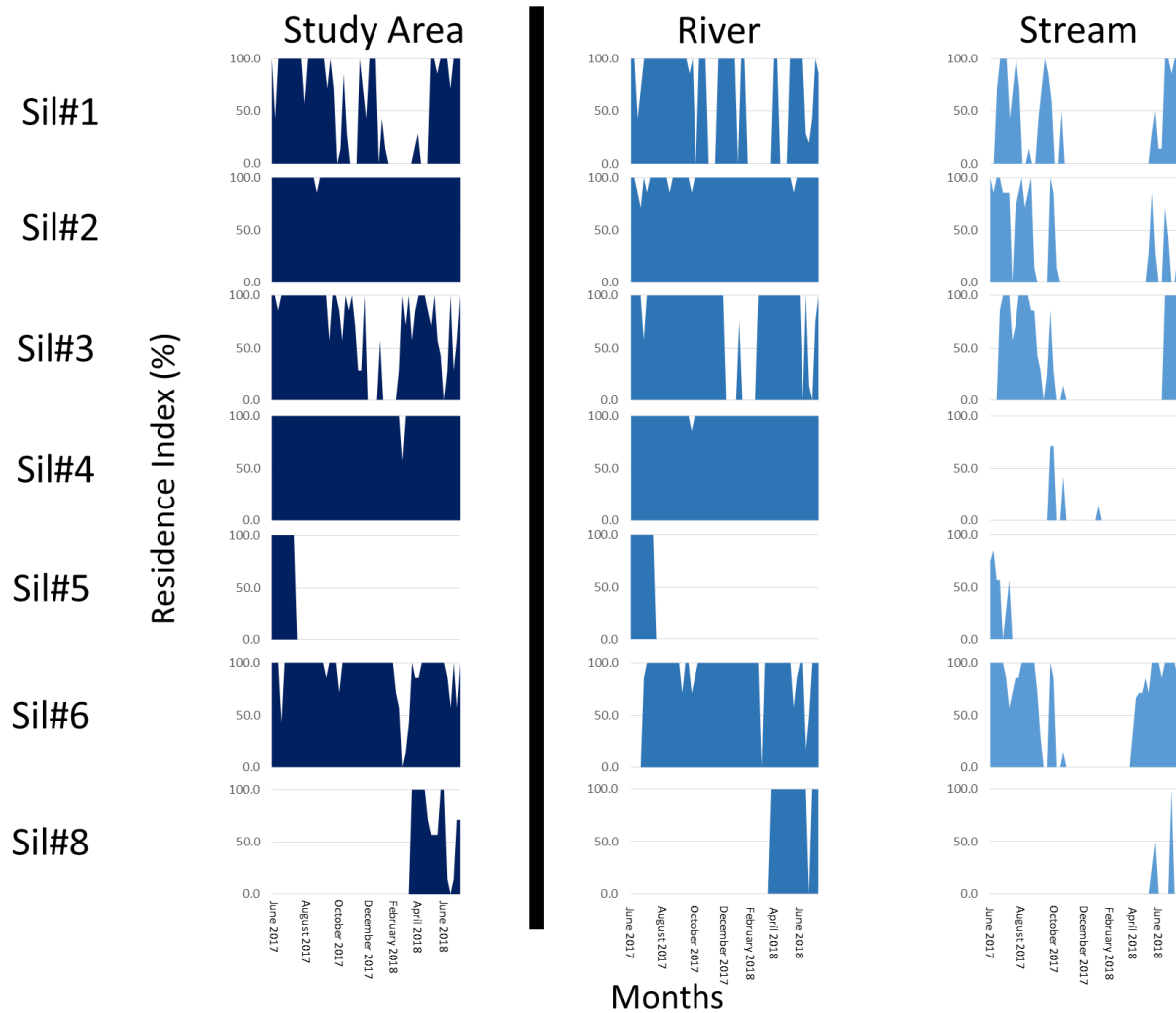
Three weeks after tagging, Sil#7's mark began only registering on the Belver#3 and Belver#4 receiver and with very low observation periods, this fish was treated as having died soon after his release and its data was not considered for the analysis. Sil#5 was captured 3 months after release by an angler, his tag was recovered and re-used in the tagging of Sil#8 in April 2018.

Manual tracking (Figure 2.5.), though used mostly to determine the effectiveness of the study area and mark viability, allowed for the detection of one individual (Sil#3) 4,5km upstream from the study area border, another individual Sil#1 was also found near this point during some of the sessions. It is also of note that some fishes were detected in every receiver in the span of one day indicating a total distance covered of approximately 7km. Most individuals were almost always found close to the reservoir banks in places with boulders, reeds or root systems belonging to nearby trees.



**Figure 2.5. Manual Tracking observations.** Each point represents the coordinate where each individual was observed during the Manual Tracking sessions. Sil#1 ●; Sil#2 ●; Sil#3 ●; Sil#4 ●; Sil#6 ●; Sil#8 ●

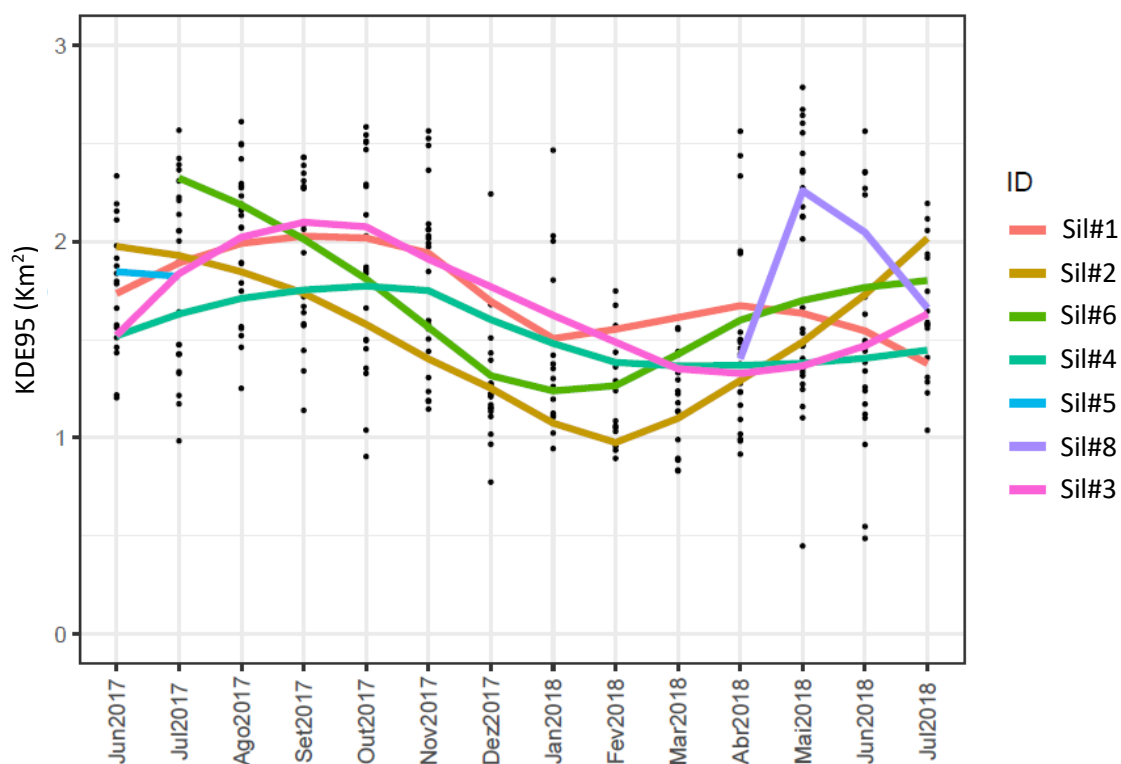
On average fishes were present inside the study area for 84% of the time (Figure 2.6.). Sil#1, Sil#3 and Sil#6 left the study area during the winter period. Usage of the River section of the study area was constant across the year while the Stream section was only used during the summer months (May to August) and a small period in October (Figure 2.6.).



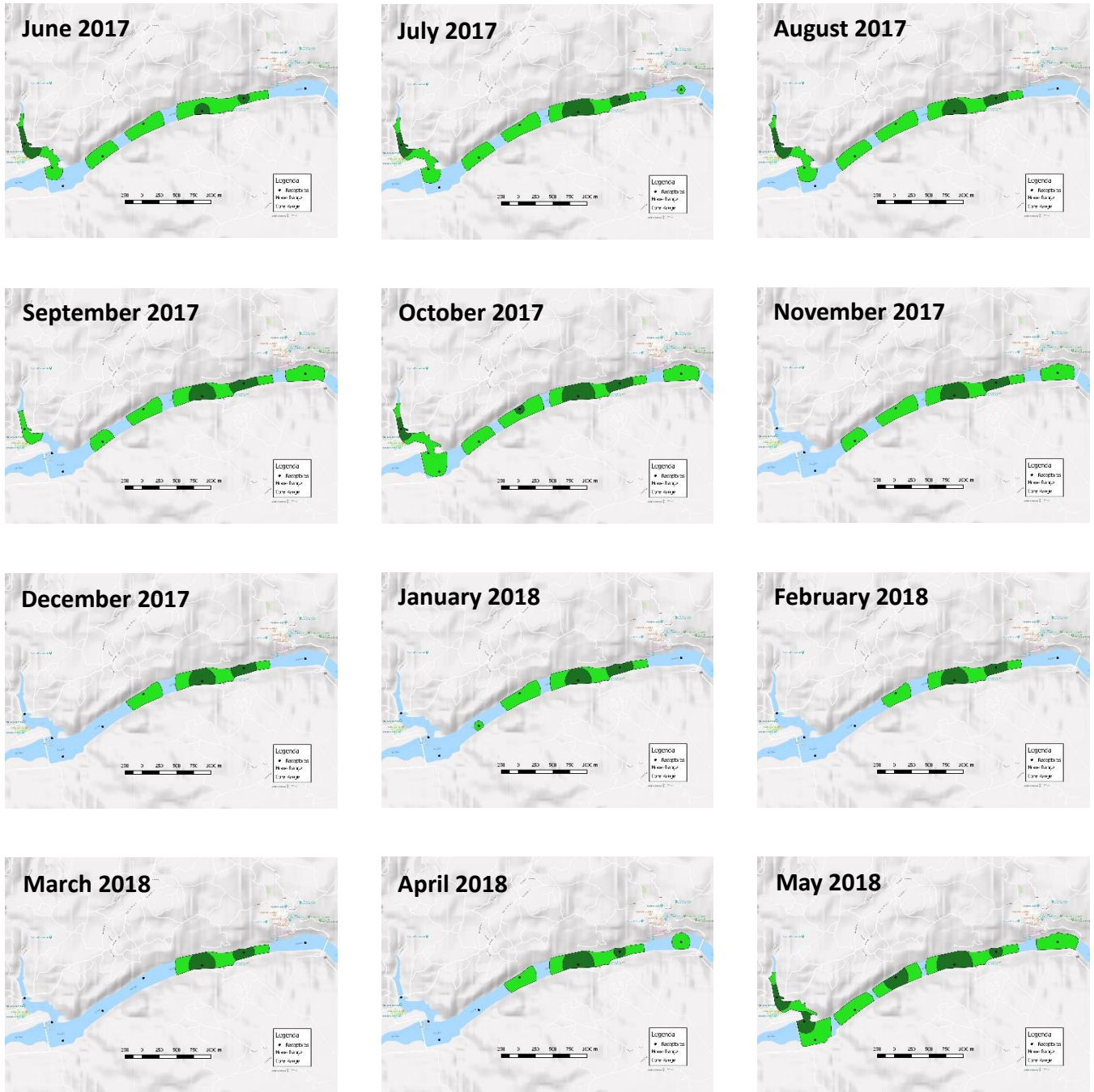
**Figure 2.6. Weekly Residence Index during the tracking period, inside the Study Area, the River and the Stream.**

Overall, the mean KDE95 size of all individuals throughout the year was 1.66km<sup>2</sup>, varying between 2.79km<sup>2</sup> (Maximum) and 0.45km<sup>2</sup> (Minimum). However, there was considerable seasonal variation along the year, in all individuals, with minimum area usage observed during the winter period (December, January and February) while maximums were reached during summer months (May, June, July and August) (Figure 2.7.).

The European catfish spatial occupation varied along the year, using different sections within the study area (Figure 2.8.) (Supplementary Figure 1.2). From spring to summer individuals tended to make full use of the study area including both the river and stream sections. During fall, the stream was abandoned in favor of the upper stretch of the reservoir. During winter, there was an abrupt decrease in the area used with most fishes remaining only in the stretch directly in front of the Alamal beach, while others like Sil#1 or Sil#3 exiting the area for up to one month.



**Figure 2.7. Graphical representation of monthly KDE95 size variation of all tagged fishes.** Each line represents the average KDE95 for each month while the black dots represent the KDE95 sizes calculated for each week within the month for each individual.



**Figure 2.8. Representation of monthly KDE95 and KDE50 size variation and geographical distribution of Sil#2 used here as an example of typical yearly behavior. ■ Represents KDE50; ■ Represents KDE95; ● Represents receiver positions. The remaining maps of this and other individuals may be found in Supplementary Image 1.2..**

The best fitted model was Model 4 with an AIC of 352.2 (Table 2.3.) that indicated that **KDE95** (y) is a function of Mean Temperature and Mean Flow and ID (Equation 2.3.), with a logLikelihood value of -171.10 being the fourth best in explaining variation. According to the model selected the equation to calculate **KDE95** is:

$$KUD95 = 0.05MeanTemp - 0.0004 MeanFlow + 0.85$$

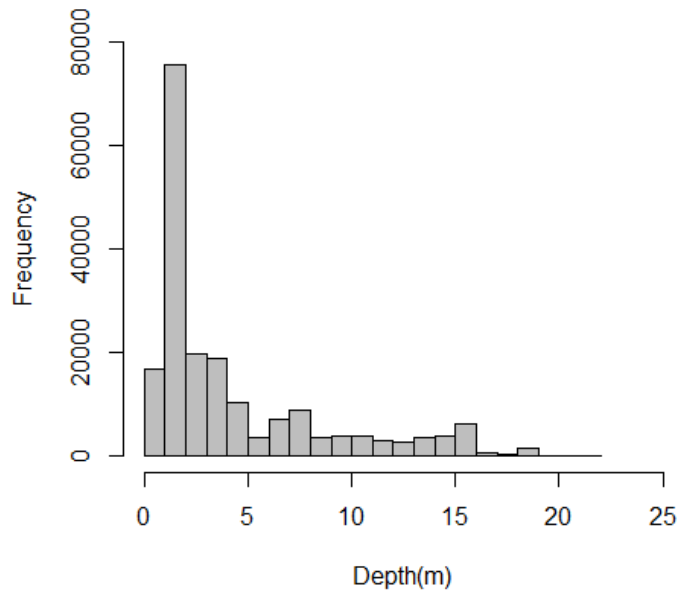
**Equation 2.3. Selected linear mixed model function.**

Mean Temperature positively influences KDE95, with higher temperatures increasing the size of the area used, while flow exerts a negative influence over KDE95, with higher flow decreasing the size of the area used.

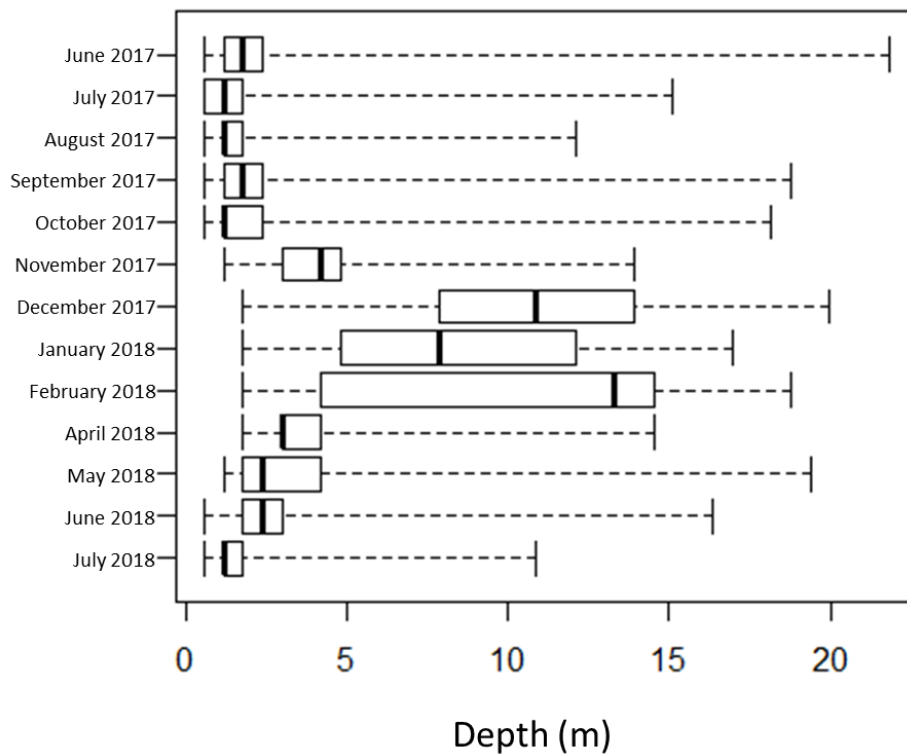
**Table 2.3. Model fitting process through backwards stepwise selection of variables.** With Model name, Model Formula, Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), logLikelihood (logLik), deviance and residual degrees of freedom (df. resid). \* Model selected as the one with the best fit.

Model Name	Model Formula	AIC	BIC	logLik	deviance	df.resid
0	y ~ 1+(1 ID)	419.1	430.1	-206.56	413.20	281
1	y ~ MeanTemp + Repro+FotoPer+MeanFlow+LunarPhase(1 ID)	359.6	396.1	-169.81	339.62	274
2	y ~ MeanTemp + Repro+FotoPer+MeanFlow+(1 ID)	355.7	381.7	-170.90	341.70	277
3	y~MeanTemp+FotoPer+MeanFlow+(1 ID)	354.0	375.9	-171.00	342.00	278
<b>4*</b>	<b>y~MeanTemp+MeanFlow+(1 ID)</b>	<b>352.2</b>	<b>370.4</b>	<b>-171.10</b>	<b>342.20</b>	<b>279</b>
5	y~MeanTemp+(1 ID)	355.5	370.1	-173.75	347.50	280

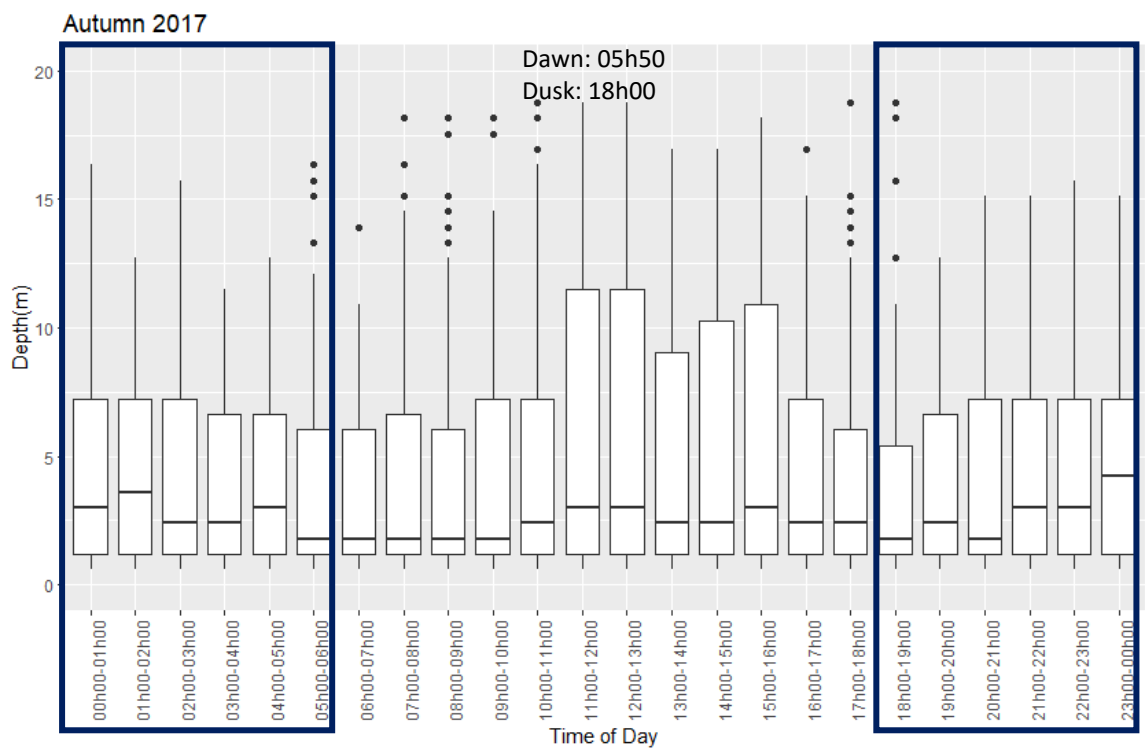
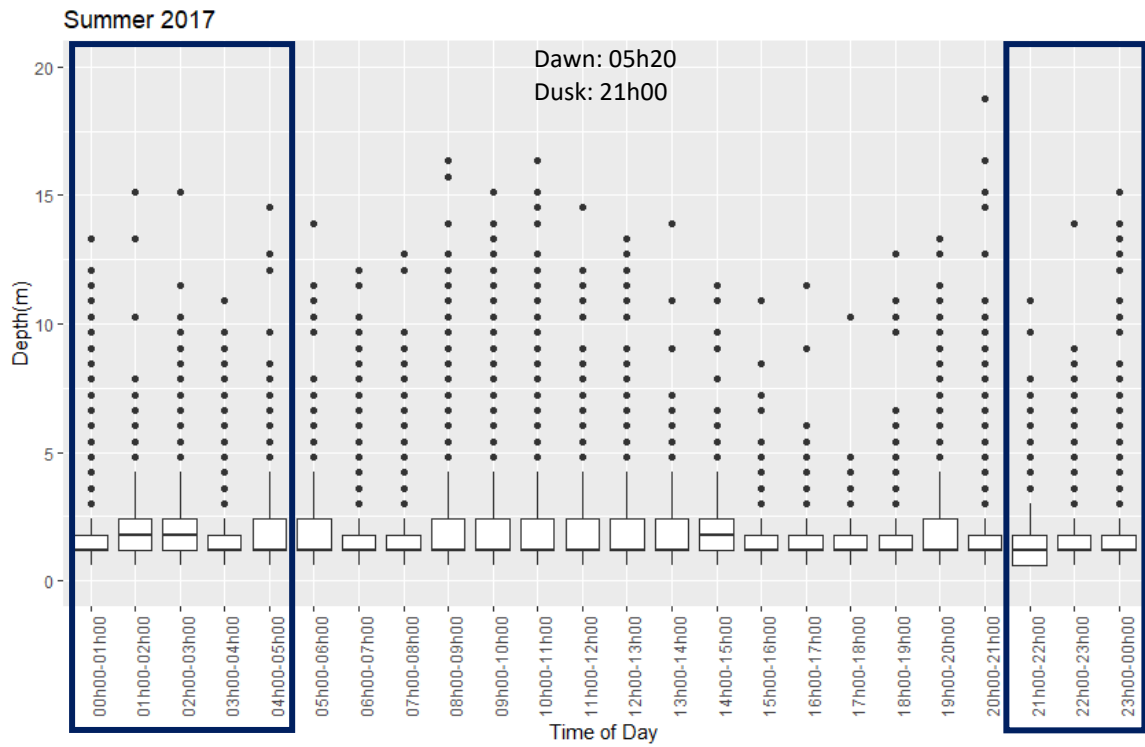
The tagged individual (Sil#1) used mostly shallow habitats, between 1m and 2m deep, but reached a maximum depth of 22m (Figure 2.9). Shallower habitat use was predominant during summer ( $\approx 2m$ ) and early fall ( $\approx 3m$ ), while during late fall depths increased ( $\approx 5m$ ), the Fish remained in very deep waters ( $\approx 11m$ ) during winter before coming closer to the surface in spring ( $\approx 3m$ )(Figure 2.10.). During spring, summer and autumn, depth use was similar throughout the day (Figure 2.11.). In winter the individual made an incursion into deeper habitats at dawn and remained there for the day, coming back to shallower habitats during the night.



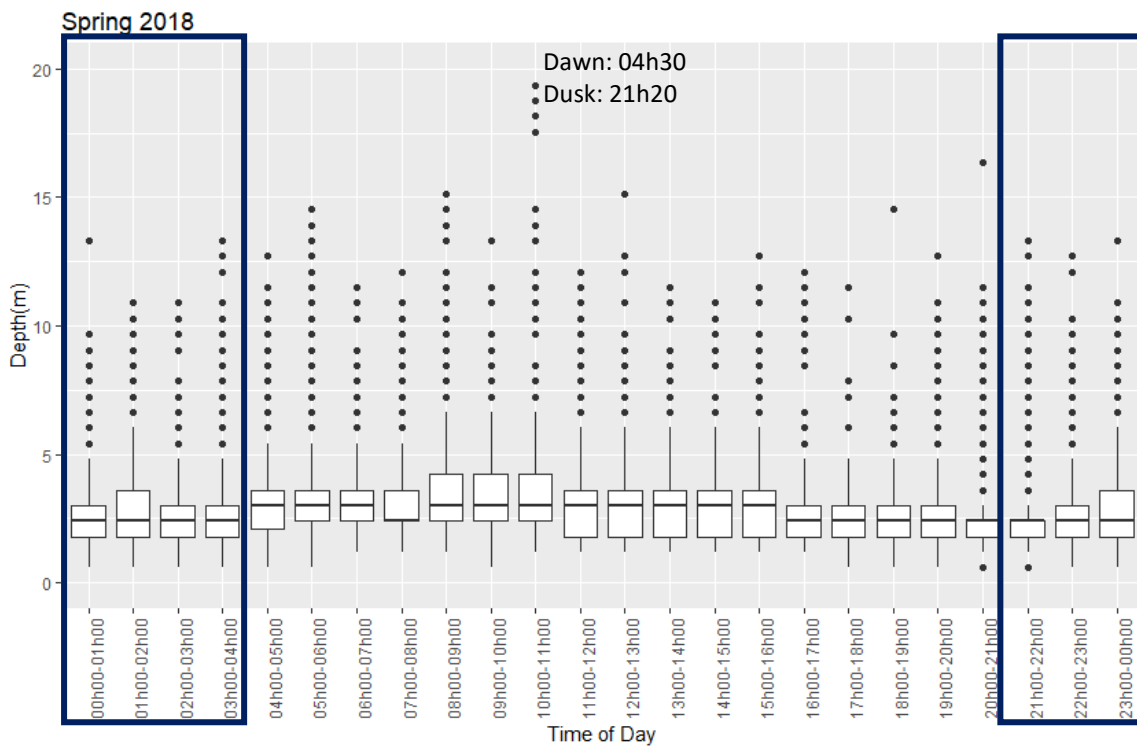
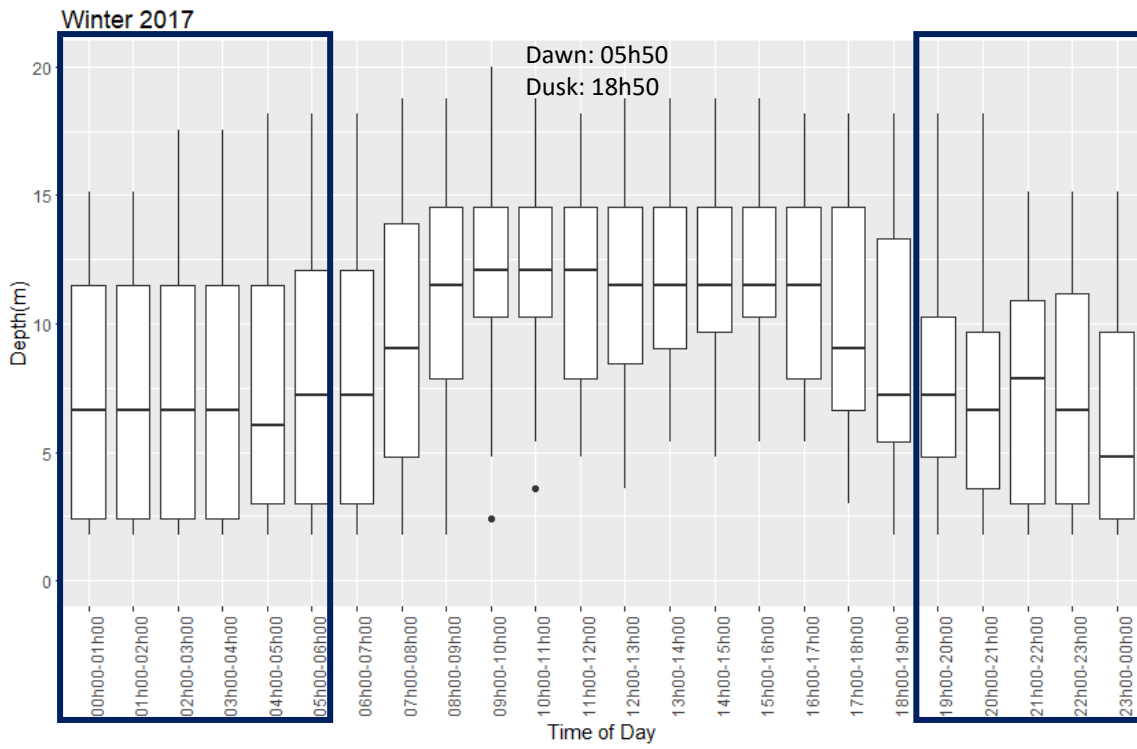
**Figure 2.9. Histogram showing depth (m) use by Sil#1 during the entire study period.**



**Figure 2.10. Boxplot of monthly depth use by Sil#1 throughout the study period.** There is no data for the month of March 2018 as the tagged individual was outside the study area for the entire timeperiod.

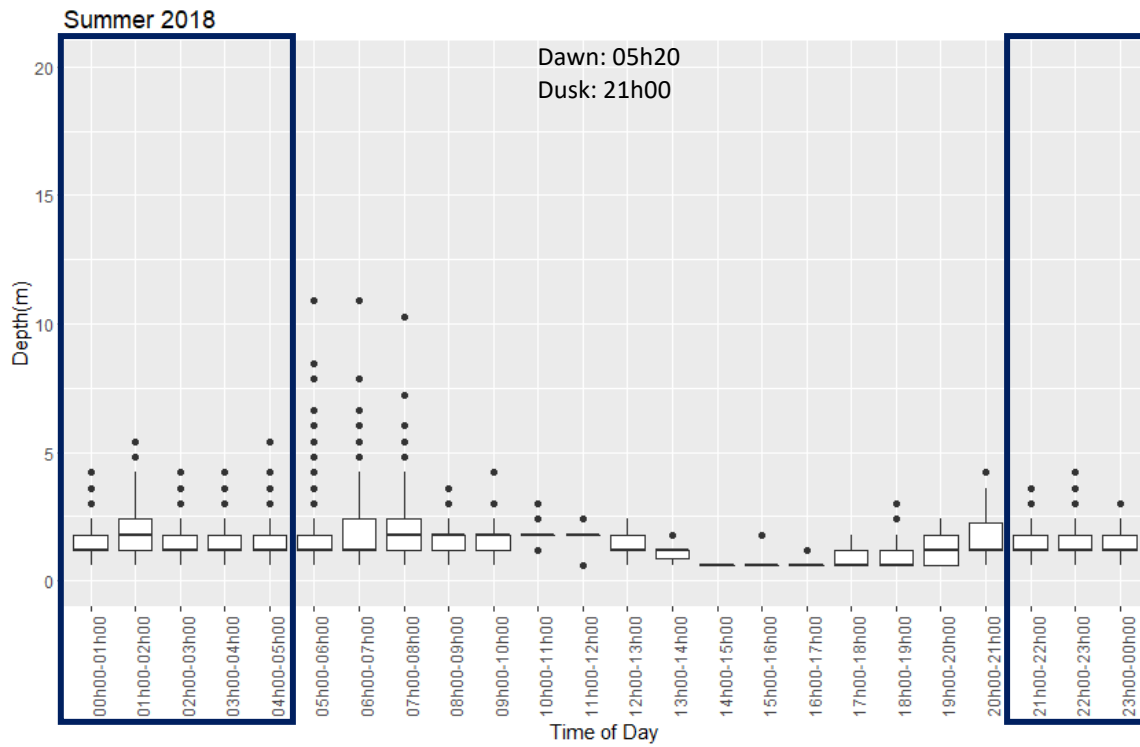


(Image continues in the next page)



(Image continues in the next page)





**Figure 2.11.** Series of Boxplots depicting circadian changes in depth use across the entirety of the study period for Sil#1. Time of sunrise (Dawn) and sunset (Dusk) from the Observatório Astronómico de Lisboa are present in the upper section for reference. . Night time measurements are represented inside the blue rectangles.

## 2.7. Discussion

The present work is one of few studies about European catfish movements that was done outside its native range (*but see* Carol et al., 2007; Capra et al., 2014) and the first one done in Portuguese freshwater ecosystems. It is also the first to have a more exhausting exploration of depth usage by this species. It evaluated this species space use habits in a reservoir of the Tagus river. This species exhibits high site-fidelity, however, its capacity for relatively long distance movements potentiates the danger of its dispersion. Aggregations of individuals of this species might occur during its reproductive period, an important finding in delineating plans for population control.

With this being the preliminary findings of a larger study, there were some shortcomings. Firstly, the small sample size, eight fishes, creates some constraints to the strength of the findings. The unfortunate death of two of the individuals tagged, one with the only other pressure sensor (depth) tag that was never recovered, meant the loss of very important data that might have been used to better support the conclusions drawn. The spatial resolution provided by the passive receiver array is not ideal, but this method allowed for a long continuous tracking period unlike those in previous works (Slavík et al., 2007; Carol et al., 2007; Capra et al., 2014; Danek et al., 2016). Despite this, the convergence of behaviours found in this small sample serves to at least establish trends and principles for a more exhaustive approach in the near future.

The relationship between mean water temperature, mean flow and area usage, is well supported by findings of other authors (Capra et al., 2018; Slavík et al., 2007; Danek et al., 2016). Periods of high temperature are associated with larger area usage and therefore longer movements across the study area. The winter period leads fish to a more lethargic state with sharp reductions, of upwards of 60%, in area usage. On the other hand, periods of high flow reduce this species movements, probably because energy expenditure is larger in such conditions.

The array assembled and area selected proved to be well suited for the task given that the tagged individuals were detected for 84% of the total study duration, consistent with Carol et al. (2007) observations of high site-fidelity. In this measure two behavioral trends were found, most fishes remained inside the reservoir area throughout the entire study period, three individuals, however, exhibited a more exploratory tendency with constant abandonments of the study area to upstream enclaves, perhaps with an enlarged array it would be possible to find even larger home range sizes for these individuals. It is also of note that most of these exits happened during the coldest periods of winter, meaning that a more suitable wintering habitat may be found further upstream.

The detection of Sil#1 and Sil#3 more than 10km upstream from the dam wall represents the largest movement described between two localizations so far, compared to previous works such as Capra et al., (2014). These long distance movements were centered in the months of winter, from December to February, meaning that a more suitable habitat to weather out these harsher months may be found at this location. Nevertheless, they paint a worrying picture for they show this species has an even greater capacity of movement, contrary to what was previously described.

Manual tracking also allowed for a more defined description of where these fishes would spend some of their time. These locations were normally characterized by large agglomerates of boulders, areas filled with reeds or large root systems, that could be classified as resting areas or feeding locations. To confirm this, however, it will be required the usage of markers equipped with accelerometers that will indicate which of these behaviours the fish undertakes at these locales.

Depth use has never been described in previous works, however, results must be looked at with some caution because they represent the behaviour of only one fish, if the trends shown are to be confirmed

at a later date, and supported with a bathymetric scan of the reservoir's bottom, it is surprising that such a great portion of these fishes daily activity is spent in shallow habitats given its fame as a bottom dweller. There are many potential explanations for this finding, such as the shallow habitats being home to more prey or prey that are easier to catch, water temperature being higher at the surface allowing for optimum energy expenditure or the presence of burrows being predominant at this level thus leading to their occupation by these individuals. The circadian variations found during winter, with shallower habitats being used during night time, are interesting as they support Danek et al., (2016) findings that this fish might be noctivagal solely during winter.

The convergence of six out of the eight fishes tagged to the stream section of the study area between the months of May to August, corresponding to the breeding period described by Alp et al. (2004), might be indicative of an aggregation event for reproduction. *S. glanis* is normally associated with higher order streams and main rivers (Copp et al., 2009; Gago et al., 2016), however low order streams are used mostly for breeding (Wolter and Bischoff, 2001). This must be confirmed by future works, however if it is verified, it might prove invaluable for the efforts to contain this species advances. One technique that may be used is that of a "Judas fish" (Bajer et al., 2011), fishes equipped with a transmitter that would lead researchers to aggregation sites, where large concentrations of this species could be removed at once.

In the future, a larger sample size must be obtained to increase robustness in these findings. Furthermore, a more in depth analysis of the habitats found throughout the study area might prove valuable to the explanation of several of these patterns. An enlargement of the study area may be a good solution to get a better sense of where some of the individuals go when they exit the current one. Additionally, this approach should be applied to flowing rivers as well in order to measure dispersion downstream as indicated by Gago et al. (2016).

The main cause of this species spread across any region is still, however, the work of recreational anglers that desire a bigger trophy. Much effort must be placed in the sensibilization of this sector for the dangers and impacts introductions have on native fauna.

## Chapter 3 – Diet ecology of *Silurus glanis* in lentic and lotic habitats of the Tagus River

### 3.1. Introduction

As a major driver of biodiversity loss, invasive species impact multiple levels of the environments they are introduced in, by hybridizing with native species, proliferating foreign pathogens, enacting competitive displacement of native species, and the most visible impact being, perhaps, predation (Didham et al., 2005). The introduction of top predators in freshwater systems has the potential to reduce or locally extirpate populations of native species (Ribeiro & Leunda, 2012). For Portugal, comprehensive studies focusing on the diet of invading top predators, or piscivores, are limited to two species the largemouth bass (*Micropterus salmoides*, Lacépède, 1802) (Godinho et al., 1994) and the pikeperch (*Sander lucioperca*, L., 1758) (Ribeiro, 2017). The rate of fish introduction in Portugal is high, with the most recent introductions being related to sport angling with a couple of predatory species (Ribeiro et al., 2009b; Gkenas et al., 2015; Banha et al., 2017). It is therefore important to evaluate the impacts of predatory fishes on aquatic organisms, especially sensitive species.

The most recent top predator introduced in Portuguese watersheds, *Silurus glanis*, has been described as a large sized opportunistic omnivorous predator (Stolyarov, 1985 in Copp et al., 2009) with its acute predatory senses that make him an apex predator (Vejřík et al., 2017). It feeds mostly on benthic organisms and is able to adapt its diet to the available spectrum of preys found within a system (Bekbergenov & Sagitov 1984 in Copp et al., 2009; Mihálik 1995 in Copp et al., 2009). Typically the predominant prey type found in *S. glanis* diet is the most abundant prey in the system, provided its size and habitat use are suitable for *S. glanis* (Omarov & Popova 1985 in Copp et al., 2009). Its capacity to adapt to different prey types is so astounding that records of this species preying on birds found in river banks are commonplace in regions of France (Cucherousset et al., 2012).

Works about the diet habits of European catfish were mostly done within its native range, where the fish community structure and environmental conditions are very different than those found in Portuguese rivers (see review Copp et al., 2009). For its introduced range there are few works, most of which are preliminary or based on low sample sites (e.g. Haubrock et al., 2019; Guillerault et al., 2017). Nevertheless, these works indicate that, in invasive contexts, *S. glanis* seems to continue to be highly adaptable to the prey supply available and incorporates native species into its diet (Syväranta et al., 2010). In Spain, a study by Carol et al. (2009), focusing on reservoir populations, observed that the basis of the diet consisted on crustaceans, mostly crayfish (*Procambarus clarkii*, Girard, 1852), with fish, mostly non-native species, representing the largest portion of biomass consumed. More recently, Guillerault et al. (2017) showed that this species predaes in migratory fish, so its impact could be larger than expected. This was previously supported by Syväranta et al. (2010) that found European eel (*Anguilla anguilla*, L., 1758) to be of the diet of *S. glanis* in France.

The previously described *S. glanis* habits are worrying in the Portuguese context, because this species invaded the Tagus river drainage (Gago et al., 2016). Some threatened native fish such as the European eel, the long-snouted barbel (*Luciobarbus comizo*, Steindachner, 1864) and sea lamprey (*Petromyzon marinus*, L., 1758) occupy benthic areas, the same spatial niche as *S. glanis*, making them potential prey for this species and adding to the pressure these species' populations are already under. In addition, Portuguese watersheds are not home to any native piscivorous fish (Cabral et al., 2005), the introduction of predators of this kind may have its impacts enlarged by a form of fish naivety (Ribeiro & Leunda 2012). Furthermore, the water temperature in Portugal is generally higher than in its native range, so a

higher metabolism is expected and possibly related to higher activity levels, which may lead to a larger impact on aquatic communities in the rivers where it occurs.

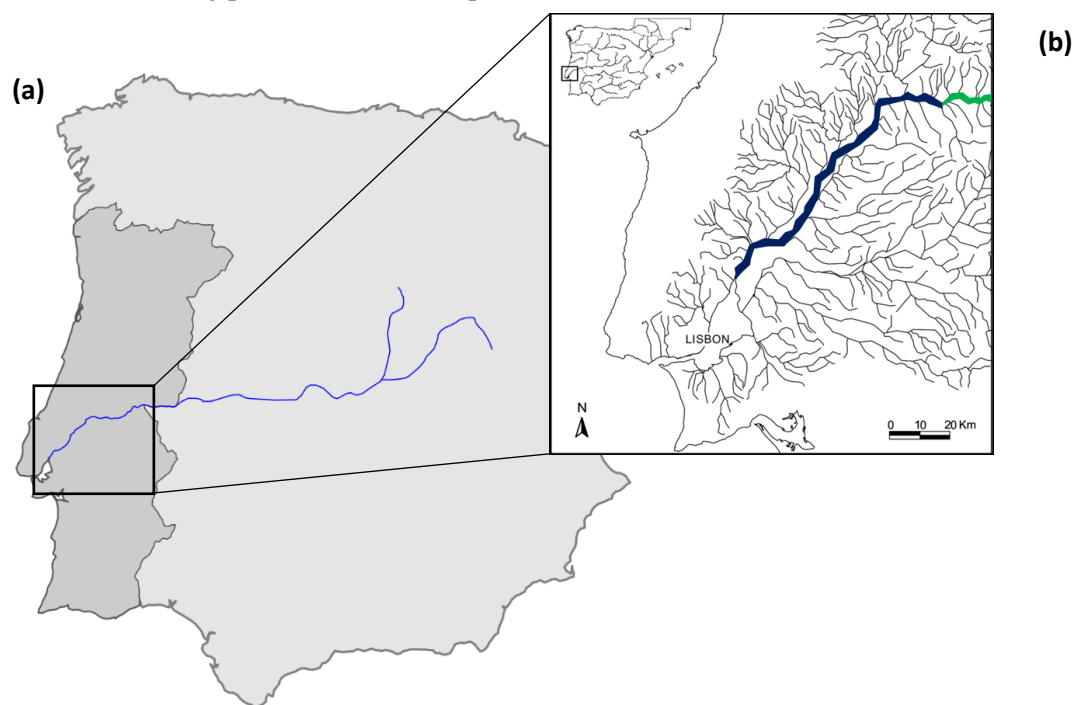
## 3.2. Methodology

### 3.2.1. Study Area

The Tagus is the longest river in the Iberian Peninsula. A river with great historical importance, it was once one of the main routes for the trade of goods between both Iberian countries. Despite retaining some of its economic importance traversing the river is now nearly impossible due to the many dams and weirs found along its course with the main objective of energy production (Sabater, et al., 2009). Due to its great extension, the river crosses many different ecosystems of extreme ecological importance like the Spanish meseta, the mediterranean forest valleys and the marshlands close to its mouth. A place of refuge during the last ice ages, it is home to many endemic species, many of which threatened, like the long-snouted barbel or the Lisbon arched-mouth nase (*Iberochondrostoma olisiponense*) to name a couple (Veríssimo et al., 2018). It is also an important habitat for diadromous species like the European eel, Sea lamprey, Allis shad (*Alosa alosa*, L. 1758) and Twait shad (*Alosa fallax*, Lacépède, 1800). Sadly, in recent years, the ever increasing episodes of severe pollution, drought and arrival of invasive species have accentuated the loss of this rich biodiversity (Veríssimo et al., 2018). It is fundamental that these impacts be measured to mitigate their effects, hence an understanding of the dietary habits of *S. glanis*, an apex predator that recently arrived to this region with a unique fauna.

### 3.2.2. Fish Sampling

The fish used in this study were obtained opportunistically between April 2017 and November 2018 by fishermen located in two distinct habitats: Flowing section of the Tagus river (Santarém-Azambuja), hereafter Lotic Environment and reservoirs of the Tagus main stem (Belver, Fratel and Cedilho) – Lentic Environment (Figure 3.1.). Fish were generally captured with stationary nets of various sizes and lengths. The Tagus is a river system with many obstacle through its course like weirs and dams. Belver, Fratel and Cedilho are hydroelectric dams with relatively small reservoirs that allow for constant flow of water, maximized during periods of electrical production.



**Figure 3.1. Location of the Tagus (a) and Portuguese Tagus with both sections highlighted. Lotic section (—) and Lentic section (—)**

### 3.2.3. Stomach content analysis

In the laboratory, fish were measured for total length (TL – nearest millimeter), whenever possible, and standard length (SL - nearest millimeter), were weighed (TW – total weight, nearest gram) and a gut incision was made to extract the digestive tract, liver and gonads, which were posteriorly weighed separately (GW - Gonad weight, nearest gram). The fishes were then weighed for eviscerated weight (EW, nearest gram). Stomach content analysis followed Knoppel (1970). Contents were placed in a petri dish and observed under a stereomicroscope (Leica MZ 125) and posteriorly identified to the lowest possible taxa using identification keys (Escala & Miranda, 2002) as well as expert opinions.

### 3.2.4. Data analysis

Fishes were separated into two groups by place of capture, Lentic Environments and Lotic Environments, and then sub-divided into three size classes for each group using TL measurements, Class 1: 0-50cm, Class 2: 50-100cm, Class 3: >100cm. Relative numerical prey abundance ( $A_N$ , %) was calculated as described in Hyslop (1980). Frequency of occurrence ( $F_O$ , %) is the result of, given the total number of stomachs containing prey, what percentage contained each of the preys listed. A Mann-Whitney U test was used to determine how significant the differences between each system and within each size class were, specifically pertaining to the four most preyed items (*Atyaephyra desmaresti*, *Procambarus clarkii*, Fish and Other Prey)

A Modified Costello (Amundsen et al., 1996) graphical method was used to illustrate the feeding behavior for each system. This method plots  $F_O$  (%) and prey specific abundance ( $P_i$ , %) (Equation 3.1.) to classify a species prey selection behavior.

$$P_i = \left( \frac{Psi}{Pst} \right) \times 100$$

Equation 3.1. Prey specific abundance.

$P_i$  (%) is calculated in function of  $Psi$ , the amount of prey items for a prey type ( $i$ ) found in stomachs, and  $Pst$  the total amount of prey items of each type of prey found in each stomach that contained prey  $i$ . The graphical representation allows for the characterization of feeding behavior by analyzing the distribution of points along the diagonals and axes of the diagram.

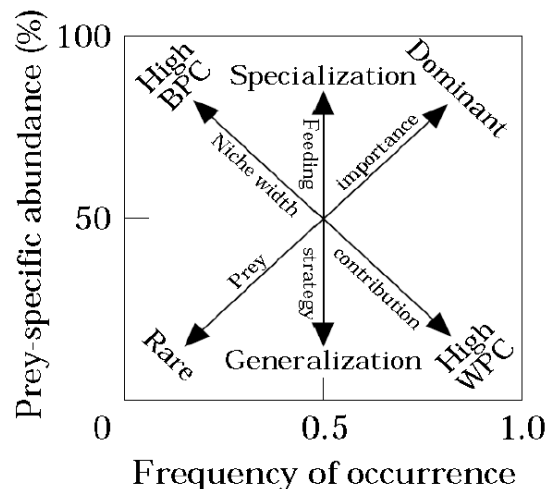


Figure 3.2. Diagram explanation of the modified Costello method as per Amundsen et al. (1996).

Feeding strategy is characterized by the vertical axis with the bottom representing Generalization and top representing Specialization. The importance of each prey is characterized in the diagonal from rare preys in the lower left and dominant preys in the upper right. Individuals with a narrow predatory niche will be associated with preys found in the upper left region and individuals with a broad niche will be associated with prey items in the lower right region (Amundsen et al., 1996) (Figure 3.2.). This analysis was produced using Excel 2013.

An NMDS (Non-metric multidimensional scaling analysis) was done utilizing R package Vegan: Community Ecology Package (Oksanen et al., 2010) to visualize the prey spectrum variation between and within each group of fish (Lentic and Lotic and size class within each environment). This approach was based in the Bray-Curtis similarity index (Clarke et al., 2014). The prime components were understood as tendencies for prey niche dimensions. A larger amount of iterations had to be used in order to reduce stress values to  $<0.2$ , as the standard approach resulted in lack of component convergence, this is possibly due to low sample size. A follow up ANOSIM (Analysis of Similarity) was attempted but trust in the results was not satisfactory and therefore it is not included following Gkenas et al. (2019).

A prey accumulation curve, a measure of number of different prey items in function of sample size, was obtained, for each environment (Lotic and Lentic). This was done using the Vegan R package, using a randomizing methodology with one thousand permutations adapted from (Rojas et al., 2013).

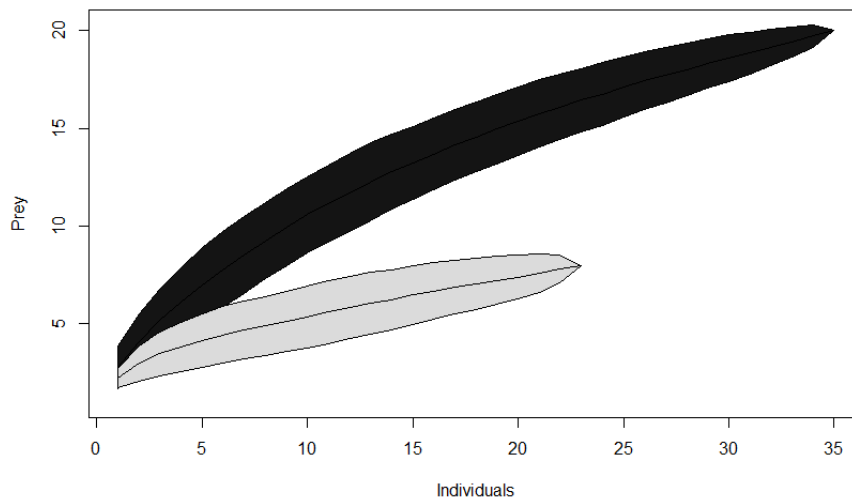
### 3.3. Results

In total, of the 96 fishes captured, 35 were collected in Lentic and the remaining 61 in Lotic habitats of the river Tagus. Fish where no prey was found, were discarded for the remaining analysis, leaving us with a final sample size of 28 for Lentic habitats and 38 for the Lotic habitats (Table 3.1.). Overall, the mean length in each size class did not vary between environments (Table 3.1.).

**Table 3.1. Sample size, size metrics and sex ratio for each of the size classes identified.** In the Lotic environment none of the fishes measuring >101cm had identifiable prey in their stomachs and were disregarded for the remaining analysis. \*Sex is represented by Male (M), Female (F) and Immature (I).

Size Class (cm)	Lotic Environment				Lentic environment			
	N	Average TL (cm)	Average EW (g)	Sex (M:F:I)	N	Average TL (cm)	Average EW (g)	Sex (M:F:I)
0-50	16	36.8 ± 4.6	306.2 ± 119.7	3:0:13	8	35.5 ± 1.5	301.6 ± 43.5	2:2:4
51-100	22	59.3 ± 7.3	1364.6 ± 740.6	10:8:4	15	71.4 ± 13.3	2392.1 ± 1353.2	4:7:4
>101	-	-	-	-	5	118.4 ± 9.5	10572 ± 2858.3	3:2:0

Prey cummulation curves showed that the number of preys continues to increase, as new stomachs are analyzed and have yet to stabilize (Figure 3.3.). The more accentuated curvature observed for the Lotic system when compared to the Lentic system is indicative of how broader the dietary niche seems to be in this environment. In fact, there is a new prey item registered for every two individuals in the Lotic system, and one new prey item registered every three individuals in the Lentic system.



**Figure 3.3. Prey cummulation curves for *S. glanis* captured in both habitats.** ■ Lotic habitats and ■ Lentic habitats. Calculated using a randomized method with 1000 permutations with variation being represented by polygons extending from the solid line.



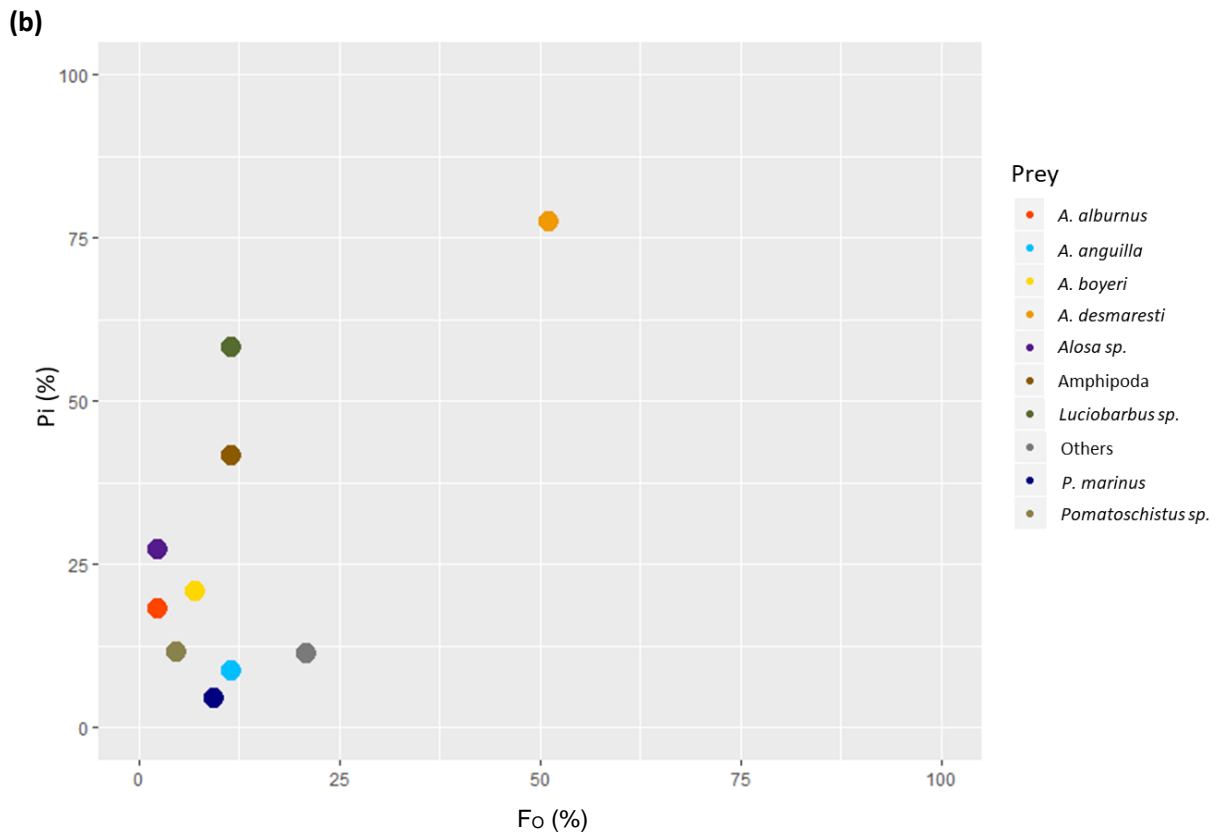
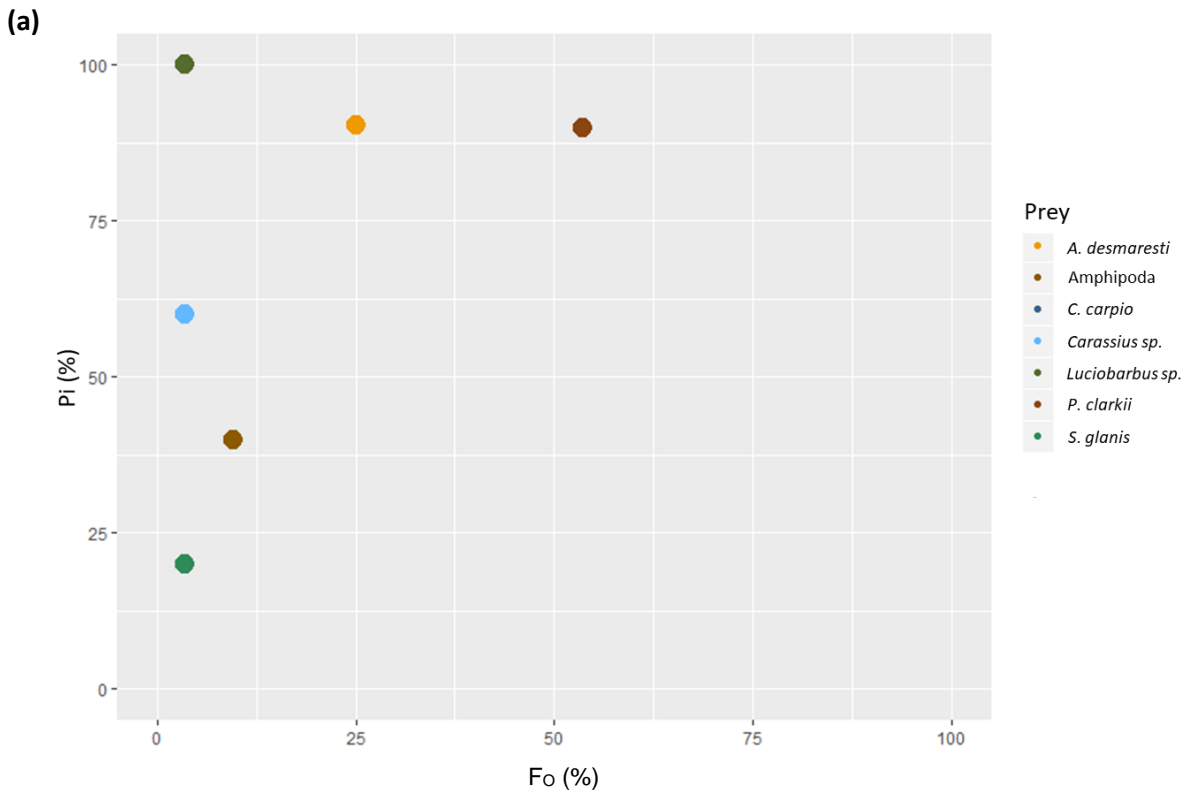
### 3.3.1. Habitat Comparison

In the 38 Lotic fish a total of 355 prey items were found, while in the 28 Lentic fishes, 111 prey items were found. The diet of the fishes captured in the Lotic section of the River differed greatly from that of the fishes from the Lentic sectors (Table 3.2.). Despite both relying heavily on crustaceans, the Lentic diet is almost solely based on this prey type, it also has a much larger crayfish component when compared to the Lotic system (Mann-Whitney U:  $W=499$ ;  $N_{lot}=38$ ;  $N_{lent}=28$ ;  $P<0,001$ ). In Lotic environments fish represented 18.3% of the diet, being comprised mostly of benthic fish (76.7% of fish prey found) but also by a portion of pelagic fishes (33.3% of fish prey found) exceeding the number of fish prey found in the Lentic system (Mann-Whitney U:  $W=533$ ;  $N_{lot}=38$ ;  $N_{lent}=28$ ;  $P<0,01$ ). In the Lentic systems, fish prey represented less than 10% of the total number of prey found and came from only two of the individuals sampled. In the Lentic environment, aside from the freshwater shrimp (*Atyaephyra desmaresti*, Millet, 1831) and amphipods, the remainder of the diet is constituted mostly by non-native species and one native fish (*Luciobarbus* sp.).

In the lentic environment, there seems to be some degree of specialization towards the consumption of crustaceans, while fish prey are rare in the diet (Figure 3.4.). As for the Lotic environment a broad dietary niche seems to be forming due to the number of different fish species found without any of them becoming dominant in the diet, however the same degree of specialization is observable towards the most abundant prey, *A. desmaresti* (Figure 3.4.).

**Table 3.2. Relative Numerical Prey Abundance ( $A_N$ , %) and frequency of occurrence ( $F_O$ , %) in the stomachs of *Silurus glanis* in the two main habitats of the Tagus River.** Statistical differences among habitats were obtained by Mann-Whitney tests \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; n.s. – not significant. Sample size (N) is represented bellow each environment. (n) represents number of prey items found in all stomachs for each habitat.

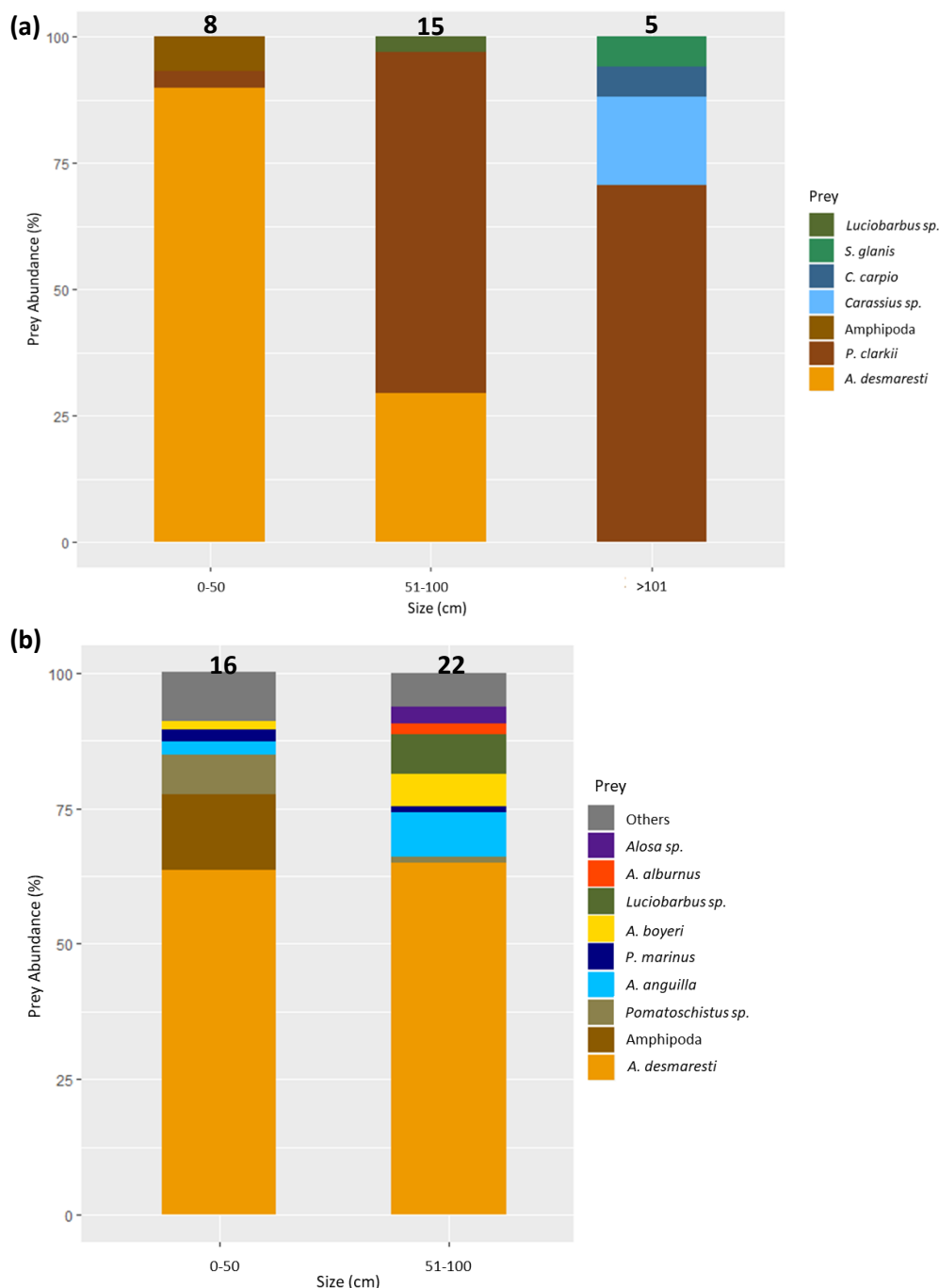
Food Item	Lotic Habitat N=38, n=355		Lentic Habitat N=28, n=111	
	$A_N$ (%)	$F_O$ (%)	$A_N$ (%)	$F_O$ (%)
Crustaceans	74.5	55.8	92.6	71.4
<i>Atyaephyra desmaresti</i> **	63.4	51.2	45.7	25.0
<i>Amphipoda</i>	10.5	11.6	2.5	9.5
<i>Procambarus clarkii</i> ***	0.6	2.3	44.4	53.6
Fish**	18.3	39.5	7.3	7.1
<i>Pomatoschistus</i> sp.	5.5	4.7	-	-
<i>Anguilla anguilla</i>	3.9	11.6	-	-
<i>Atherina boyerii</i>	2.8	7.0	-	-
<i>Luciobarbus</i> sp.	2.5	11.6	1.2	3.6
<i>Petromyzon marinus</i>	1.9	9.3	-	-
<i>Alosa</i> sp.	0.8	2.3	-	-
<i>Alburnus alburnus</i>	0.6	2.3	-	-
<i>Chelon ramada</i>	0.3	2.3	-	-
<i>Carassius</i> sp.	-	-	3.7	3.6
<i>Cyprinus carpio</i>	-	-	1.2	3.6
<i>Silurus glanis</i>	-	-	1.2	3.6
Other prey**	7.2	20.9	-	-



**Figure 3.4. Modified Costello graphs for the Lentic (a) and Lotic (b) diets. With Prey proportion per stomach (Pi) in function of Frequency of Observation (Fo).**

### 3.3.2. Ontogenic comparison

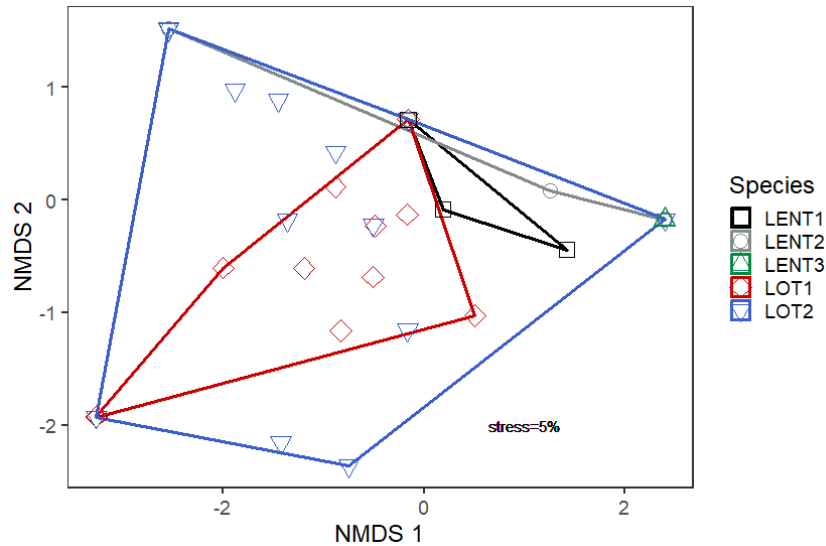
In the Lentic environments, small sized fish (Class 1) had a diet that comprised almost exclusively of crustaceans, nearly 90%, while in the Lotic environment it was about 65%, being also complemented with small fishes (Figure 3.5.). However, while in the lotic environment the main crustacean prey was the *A. desmaresti*, in the lentic environment it was crayfish, being dominant in Class 2 and Class 3. Furthermore, in the Lotic environment we can observe a remarkable prey diversity, with small european catfish eating mostly benthic fish (such as eel) while medium sized fishes have a larger proportion of pelagic fish prey (such as shad, *Alosa sp.*) (Figure 3.5.). Mann-Whitney U test revealed no differences between Class 1 and Class 2 for the Lotic system, however, it showed significant differences between Class 1 and Class 2 in the Lentic system in the amount of *A. desmaresti* consumed (Mann-Whitney U:  $W=52$ ;  $N_{\text{lentic1}}=8$ ;  $N_{\text{lentic2}}=15$ ;  $P<0,05$ ) and of crayfish (Mann-Whitney U:  $W=11$ ;  $N_{\text{lentic1}}=8$ ;  $N_{\text{lentic2}}=15$ ;  $P<0,05$ ).



**Figure 3.5. Ontogenic variation in the diet of *S. glanis*.** Representing Prey Abundance (%) and size class of both environmental populations. (a) Lentic and (b) Lotic of *S. glanis* in the Tagus main stem. Number above each column represents sample size.

### 3.3.3. Non-metric multidimensional scaling analysis (NMDS)

The NMDS (Figure 3.6.) showed that fishes in the Lotic system exhibited greater prey diversity than those in the Lentic system. As size increases in the Lotic system, there is an expansion in prey diversity, with medium sized individuals' (LOT2) prey spectrum overlapping that of small sized individuals (LOT1). For the Lentic system, the different size classes exhibit diverging predatory niches.



**Figure 3.6. Non-metric multidimensional analysis of the niche for *S. glanis* in both the lentic (LENT) and lotic (LOT) habitats.** The numbers 1,2,3 in each habitat group refer to individual size with 1 representing individuals sized 0-50cm, 2 representing individuals sized 51-100cm and 3 representing individuals sized 101cm+.

### 3.4. Discussion

This is the first dietary study of European catfish in Portuguese freshwaters, assessing its feeding behaviour in two different habitats in the Lower Tagus region. It clearly shows that this species appropriates the most common food resource available in each habitat. In the lentic environment, non-native fish eats predominantly crustaceans, such as the freshwater shrimp and red swamp crayfish, and in lotic systems the European catfish ate mostly freshwater shrimp, but closely followed by fish. Piscivory tends to increase in larger individuals in both habitats, and its diet is broader in lotic environments. This first data suggests that non-native predators can have different predatory impacts depending on environment, and in this case, the European catfish can have an important impact on the fish communities in the lotic habitats of the Lower Tagus river.

Firstly, this study relied on fishermen's fishing efforts (predominately towards fishing season – February and July), and consequently the number of fish captured varied along the year, with the bulk of the data coinciding with spring and early summer. However, the fish during the winter presented a low activity (Chapter 2) and, most likely, any collected European catfish will present low number of preys and prey diversity. Additionally, the size of the collected fish is highly dependent from fishing nets mesh size. Nevertheless, the observed tendency for piscivory seems to be consistent in both studied habitats and compared with other studies (e.g. Carol et al., 2009; Syväranta et al., 2010).

The Lotic habitats have, generally, communities that are more diverse, this fact increases the available prey diversity for *S. glanis* and allows for a wider dietary niche. Veríssimo et al. (2018) documented a total of 14 fish species in the Lower Tagus. The European catfish in the Tarn river, a tributary of the Garrone in France, seems to also feed on most of the available species there found (Syväranta et al., 2010). Most of the diet in the Lotic habitat consists on native species, with *A. desmarestii*, a small freshwater shrimp, being the most abundant prey (over 60%). The presence of prey like *A. anguilla*, *Alosa sp.* and *P. marinus* is worrying for two main reasons, firstly, the populations of these three species have seen a sharp decline over the past few decades, therefore, an added pressure on these populations can further aggravate this trend (ICES, 2015; ICES, 2018). In addition, these species are central to the economy of anglers and fisheries, as well as the restaurants that depend on them, across the Tagus River, and their decline will most definitely affect the future prospects of these businesses. In fact, Guilleraut et al. (2017) mentioned that the European catfish could represent an important predatory pressure on migratory fish, namely shad and lamprey.

On Lentic habitats, the diet consists mostly of invasive species, with the staple prey being *P. clarkii*, this observation is consistent with what was found in some Spanish reservoirs (Carol et al., 2009), however other reservoir populations exhibit fairly different niches. Prey diversity was lower than in lotic environments but such was to be expected given that, reservoirs are areas of high stress and disturbance mostly populated by non-native species. Although the Lentic populations of *S. glanis* feed on invasive species, such as crayfish or carp, its predation pressure might be relatively low given the low number of preys found in each stomach. Moreover, the determination of prey importance through biomass in addition to numerical abundance may clarify the predation pressure of catfish and prey importance to the species success.

It was observed that the typology of prey found within *S. glanis* stomachs seems to be directly related with the size of the mouth given that larger prey were found on larger individuals (Personal observation). In Lotic habitats, individuals of the Class 1 were already preying on other, small, fish species like goby (*Pomatoschistus sp.*) and juveniles of both eel and lamprey. Class 2 individuals fed on benthonic fishes, such as *Luciobarbus sp.* and eel, with 10% of the diet surprisingly consisting on pelagic species such as

*A. boyerii* and shad (*Alosa sp.*). It is also worth noting that in these habitats, almost the entirety (81.1%) of this fish's diet is comprised of native species and of these, roughly 20% are native fish species.

As for the Lentic habitats, the mouth size/prey size relation was easier to observe with Class 1 individuals feeding strictly on crustaceans with a vast majority of freshwater shrimp, Class 2 fishes replacing the shrimp with crayfish and, finally, Class 3 individuals including large fish such as carp in their diet. The predation on pelagic prey, states the adaptive capacity of this species predatory behavior and is in line with previous works (Cucherousset et al., 2012). In fact, the different dietary habits observed in both environments attests to this broad adaptive capacity. Indeed Copp et al. (2009) observed that the European catfish presented an enormous variation across the species range.

The prey cummulation curves (Figure 3.2.) have not yet stabilized indicating that a larger sampling effort is needed to evaluate the full predatory niche this species is using in its current distribution. However when compared to the work of Guillerault et al. (2017), we can see that for the same number of individuals sampled (N=11) they had found 6 different prey types in the stomach contents, while in the Tagus 14 prey were identified. The same work also describes DNA metabarcoding as a more efficient method of diet composition analysis, perhaps if the same had been applied in this case the number of identified prey would be higher. Although, the number of species predated by *S. glanis* in the Portuguese Tagus river seems larger than that found in the Garonne river, this comparison must be carefully examined in future works.

The larger prey diversity observed in the present study could be a result of native fish prey naivety, considering that this non-native fish only established about a decade. As stated previously, in the Portuguese freshwater fish communities lack of native piscivorous, consequently native fishes did not developed strategies to evade fish predation. This "inadaptation" towards this threat may explain why smaller *S. glanis* are able to prey on fish in the Lotic system. Most non-native fishes found on reservoirs share the same native distribution with *S. glanis*, therefore, fishes like carp and goldfish may have some innate mechanism of defense that has not yet been silenced. Studying the diet of this recent apex predator constitutes an unique oportunity to demonstrate the early and considerable impact, due to predation, on a unique fish community of the Lower Tagus river that could encompass more than 20 native fish, some endemic to this region.

## Chapter 4 – Final Considerations

The Iberian Peninsula is part of the Mediterranean biodiversity hotspot due mainly to the fact that it was an area of refuge during the glacier periods which led to occurrence of many endemisms, in its fauna there is also a large representation of species from many families. In Portugal alone, there are a total of 45 native fish species with 28 being endemic to the peninsula and 10 to the Portuguese watersheds (Almeida et al., 2019). According to the Portuguese red book of vertebrates (Cabral et al., 2005), fishes are the most threatened group in the Portuguese territory mainly due to commercial fishing, dams and the introduction of non-native species (Cabral et al., 2005)

The impacts of non-native species on the native fish fauna are potentially large considering the high percentage of threatened species of freshwater fishes (Hermoso & Clavero, 2011), associated with an increasing rate of arrival of new taxa (Ribeiro et al., 2009b; Anastácio et al., 2018). Consequently, it is now, more important than ever, to understand their movement and dispersal patterns along these weakened watersheds and evaluate the impact on these species exerted by their diet.

Towards this end, this study focused on the dietary habits and movements, of the recently arrived European Catfish, an apex predator (Vejřík et al., 2017), constituting the first data on a newly arrived predator in Portuguese watersheds. The findings here reported, show some worrying trends and allow for more informed decisions when strategies are delineated for the mitigation of this species dispersion and impact on Portuguese freshwaters. The distance covered by some movements showed this species potentially high dispersion capacity and exhibited how it may represent a danger to threatened fish fauna of the Portuguese freshwater habitats through predation.

Firstly, Portugal provides an ideal environment for this species. Warm water temperatures throughout most of the year allow this species to be close to its physiological optimum enabling more efficient, and therefore for longer periods, dispersive movements and bettering their predatory capacity (David, 2006). The lack of native piscivorous fishes, and the degree of fish naivety that comes with it, may also have its weight in the success of this species as an invader (Ribeiro & Leunda, 2011).

The presence of migratory species, like eel, lamprey and shad, in the catfish diet is demonstrative of this species capacity to adapt and predate even on cryptic prey like lamprey larvae (Guillerault et al., 2017). These species have witnessed a decline in abundance in the past decades their future is uncertain and *S. glanis* is now exerting added pressure on these vulnerable populations. In general, the broad dietary niche found in the Tagus flowing river population, is worrying as it shows this species lack of prey selectivity or preference over any particular prey typology. If allowed to spread further into tributaries of the Tagus, its range will include the spawning habitats of many smaller, also threatened species present in the basin, like the endemic and critically endangered Lisbon-arched-mouth-nase (*Iberochondrostoma olisiponense*, Gante 2007) (Veríssimo et al., 2018).

Our findings also confirm previously described movement trends allowing for a better understanding of how its range varies along the year. The relatively long-range movements detected, up to 11.5km, indicate that these fishes can make large exploratory dislocations that might potentiate species dispersion. These movements occurred predominantly during winter, when fishes were shown to be more lethargic (Figure 2.7. & Figure 2.8.) with this being supported by previous works (Slavík et al., 2007). This lower level of activity is shown to be due to the lower temperatures and increased flow, therefore these upstream habitats must be more suitable for wintering. These wide range movements, might be associated to search for food resources, given the observed narrow diet in the Lentic environment may also be an important factor (Figure 3.4.), given that the main prey, the red swamp

crayfish (*P. clarkii*) is generally inactive during winter (Beja, 1996). Consequently, the species impact might not be spatially restricted to the sites where this fish occur, but might have impacts at a wider scale.

The predominantly shallow habitats inhabited by one of the individuals would be supported by the fact that fishes in the Lentic habitat of the same size class as those tagged feed exclusively on crustaceans like *P. clarkii* and *A. desmaresti* which would be prevalent in these shallow habitats close to aquatic vegetation like root systems and reed (Anastácio & Marques, (1997); Van den Brink & Van Den Velde, (1985)), exactly the places where fishes were located during manual tracking. If the same idea is applied to the Lotic system, it would explain the appearance of pelagic prey in the stomach contents of this species thought of as mostly benthic. This prey, mostly small sized, would take refuge in shallower, more marginal habitats where the catfish might hunt demonstrating a large adapting capacity to appropriate new food resources as observed elsewhere (Cucherousset et al., 2012).

#### **4.1. Management options for catfish**

There is, however, hope in reducing this species spread in the Portuguese waterways. Recreational anglers are attributed to have facilitated the spread of this species across the Iberian Peninsula (Gago et al., 2016), yet they could have a role to play in the removal of this species. By reaching out to this community about the dangers and impacts of this species, we may convince them to abandon the practice of moving individuals from river to river, basin to basin. Additionally, professional fishermen see this species as potential threat to their high valued fish (mostly migratory), and might contribute to species remove and discard. Recently Vejřík et al. (2019) proposed hook lines as a very efficient technique to remove large amounts of individuals, the author modelled that 20 individual fishermen, using this method, could reduce populations of this fish to 10% of the starting level in one fishing season. This method is actually going to be implemented soon in Extremadura. Combining this technique with the Judas fish (Bajer et al., 2011), where a tagged fish could identify the catfish agregations, like the ones shown in this work and previous studies (Boulêtreau et al., 2011), could greatly increase capture efficiency by using a seine net, and therefore drastically reduce this species population at a local level.

Aside from local fishermen, efforts must be made by official entities to limit the spread of this species. Removal campaigns could serve as a tool to limit population growth. However, through the use of environmental DNA combined with monitoring sampling of reservoirs and rivers, a system that could alert to the presence of *S. glanis* could be put in place to allow for swift action in preventing this species spread in new systems.

#### **4.2. Future directions**

As it has been discussed along the previous chapters, this study suffered from some sampling restraints.

In the future, it is paramount that, for diet studies, sampling be more thorough, with greater representation in numbers, season, size and sex. The use of cutting edge research tools like DNA metabarcoding could prove invaluable in a finer approach to the identification of prey in the stomachs. An approach centered on prey biomass, instead of number, could also change the view represented in this work, perhaps showing that fishes, and not crustaceans, are the top prey.

As for studies focused on a bioacoustics telemetry approach, a larger sample size and wider receiver array can aid in truly understanding the size of this species vital areas. The use of tags equipped with activity sensors such as 3-axis accelerometer transmitters (Almeida et al., 2013) can improve the detail of the information that is being collected. Following this study, the utilization of the multiple detections



as a way to fill the gaps between the used areas can also aid in refining the spatial detail of the collected information.

In sum, *S. glanis* presence in Portuguese freshwater systems represents a danger to their biodiversity. It is a top predator that has found in these environments a suitable habitat and is capable of efficiently and quickly spreading. The findings here described provide valuable information for decision processes and acting plans to be implemented in this ecosystem, and the unique species therein found. The measures here depicted, will hopefully aid in the management of this species in the Portuguese context. It is up to us to protect this ecosystem's unique biodiversity so that future generations may have the opportunity to learn from it.

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## Annex 1

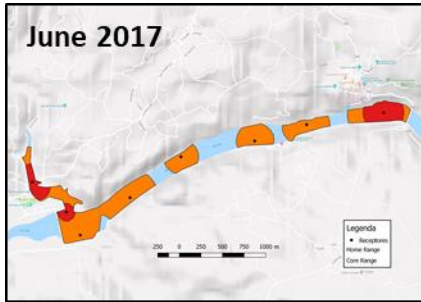


Supplementary Figure 1.1. Receiver identifications and spatial distribution within the Belver Reservoir.

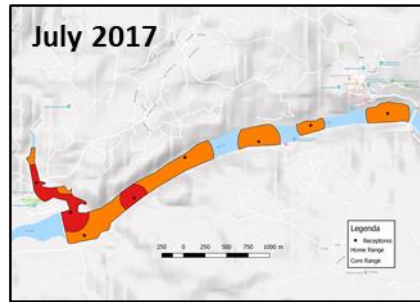
Receiver ID	Latitude	Longitude	Typology
Belver#0	39°29'3.26"N	8° 0'5.71"W	VR2Tx
Belver#1	39°28'52.00"N	7°59'49.20"W	VR2Tx
Belver#2	39°28'43.40"N	7°59'42.50"W	VR2W
Belver#3	39°28'57.40"N	7°59'18.40"W	VR2W
Belver#4	39°29'12.70"N	7°58'53.70"W	VR2W
Belver#5	39°29'18.60"N	7°58'18.00"W	VR2Tx
Belver#6	39°29'24.70"N	7°57'52.80"W	VR2W
Belver#7	39°29'29.20"N	7°57'15.50"W	VR2Tx

Supplementary Table 1.1. Receiver identification, placement coordinates and typology.

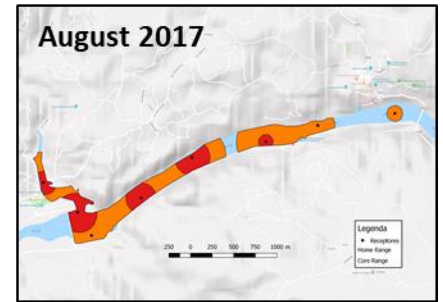
# Sil #1



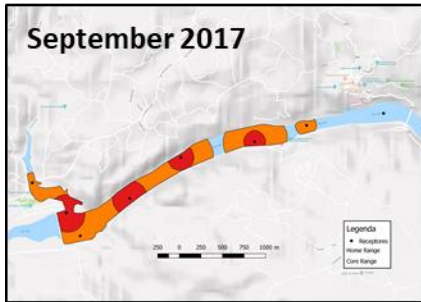
KDE95 = 0,771; KDE50 = 0,170



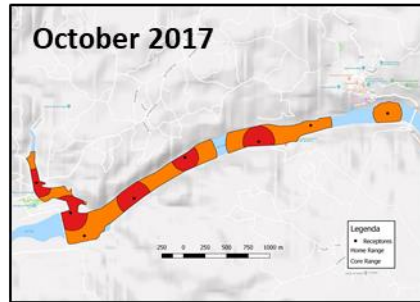
KDE95 = 0,800; KDE50 = 0,206



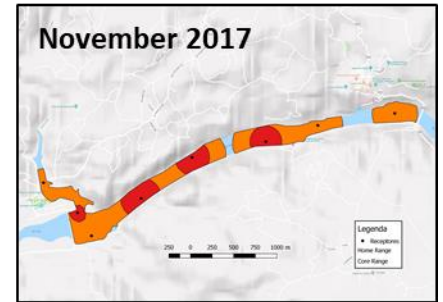
KDE95 = 0,803; KDE50 = 0,276



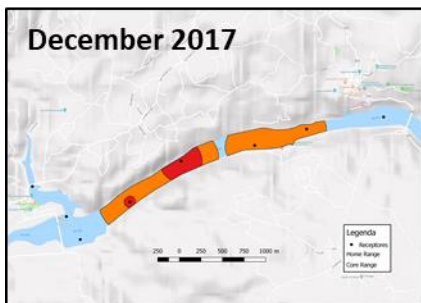
KDE95 = 0,734; KDE50 = 0,246



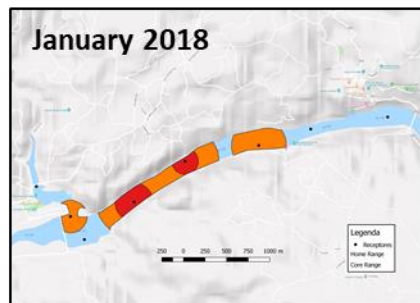
KDE95 = 0,856; KDE50 = 0,309



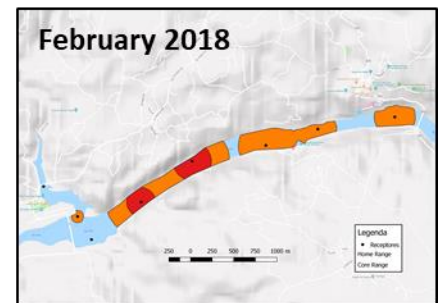
KDE95 = 0,894; KDE50 = 0,250



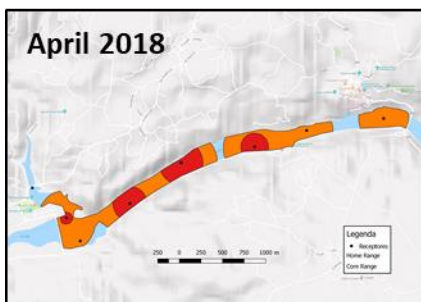
KDE95 = 0,514; KDE50 = 0,101



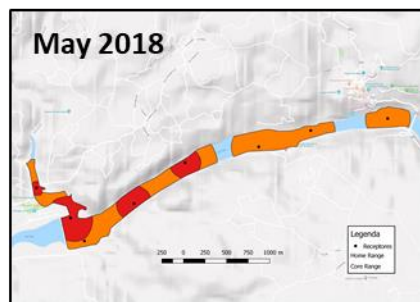
KDE95 = 0,503; KDE50 = 0,128



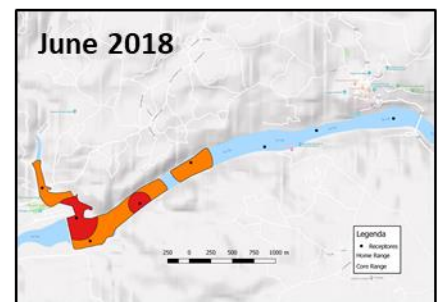
KDE95 = 0,620; KDE50 = 0,140



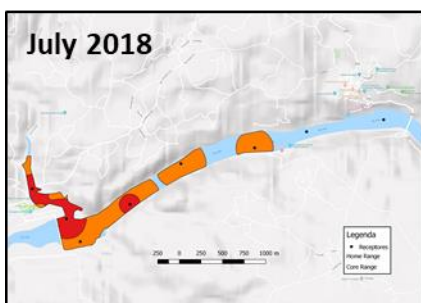
KDE95 = 0,836; KDE50 = 0,230



KDE95 = 0,884; KDE50 = 0,284



KDE95 = 0,503; KDE50 = 0,155

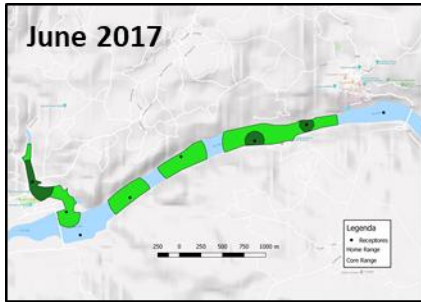


KDE95 = 0,611; KDE50 = 0,182

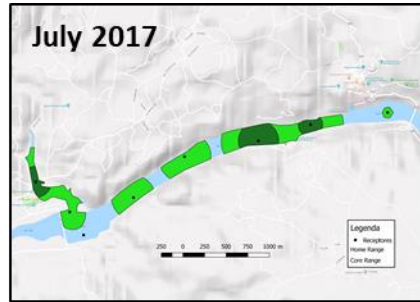
*(Image continues in the next page)*



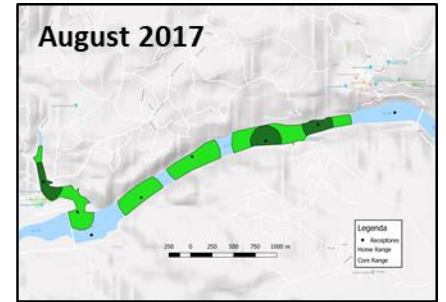
## Sil #2



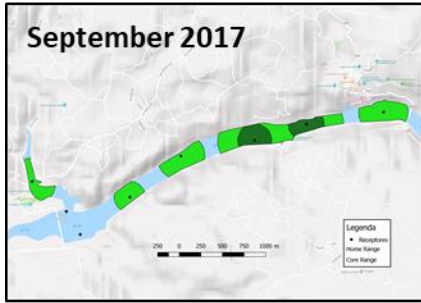
KDE95 = 0,605; KDE50 = 0,107



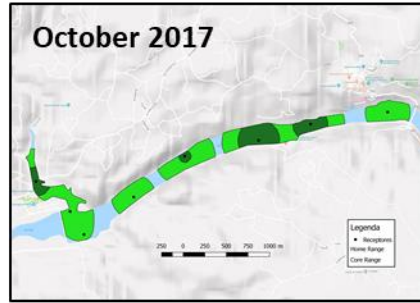
KDE95 = 0,642; KDE50 = 0,171



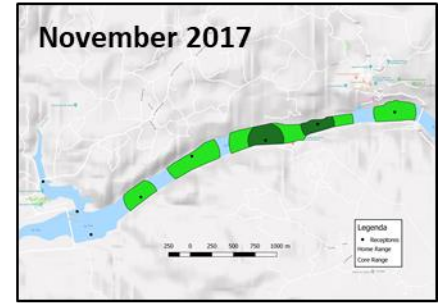
KDE95 = 0,644; KDE50 = 0,165



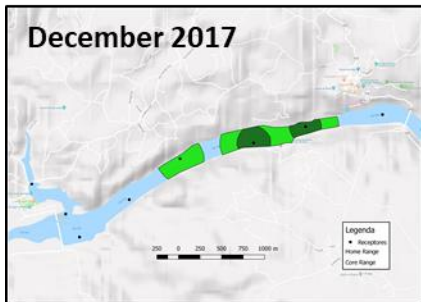
KDE95 = 0,607; KDE50 = 0,132



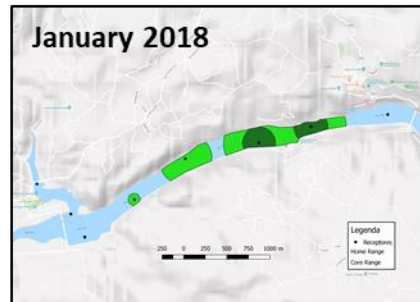
KDE95 = 0,818; KDE50 = 0,204



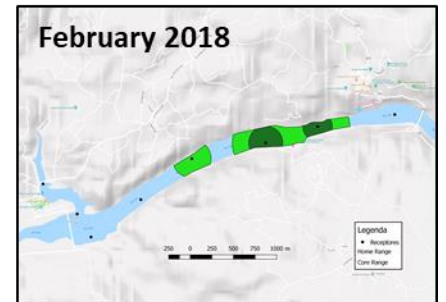
KDE95 = 555; KDE50 = 0,135



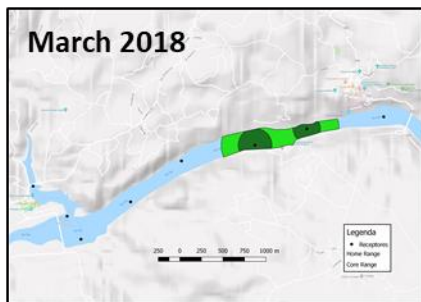
KDE95 = 0,354; KDE50 = 0,126



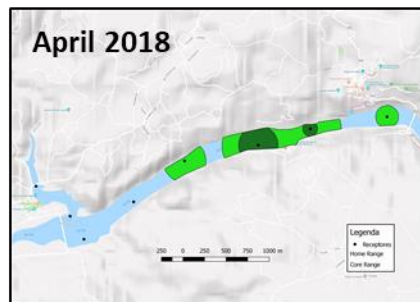
KDE95 = 0,390; KDE50 = 0,125



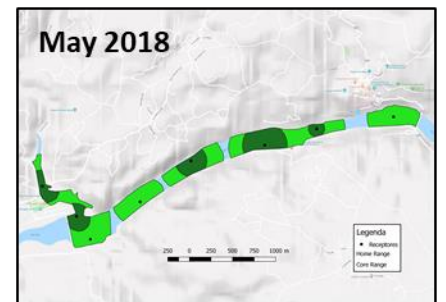
KDE95 = 0,331; KDE50 = 0,123



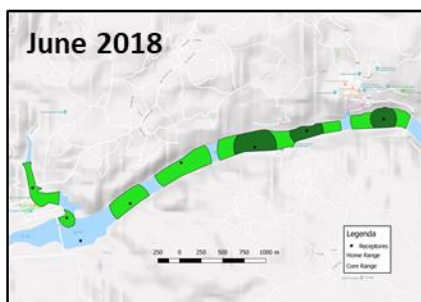
KDE95 = 0,254; KDE50 = 0,119



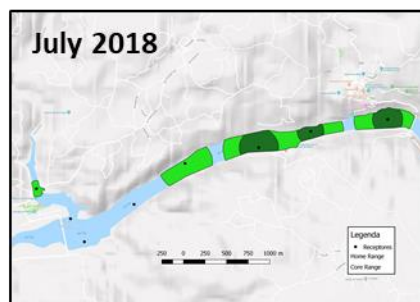
KDE95 = 0,399; KDE50 = 0,115



KDE95 = 0,896; KDE50 = 0,292



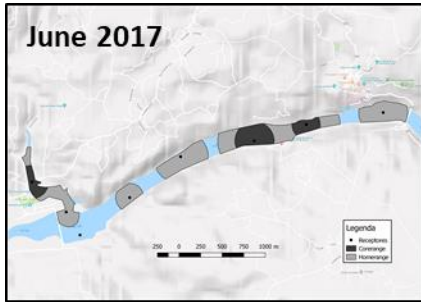
KDE95 = 0,718; KDE50 = 0,218



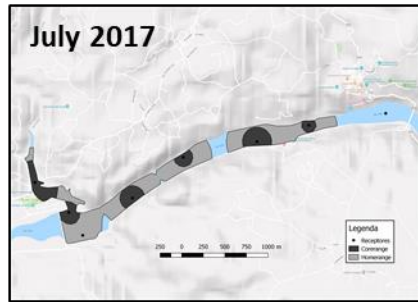
KDE95 = 0,514; KDE50 = 0,206

(Image continues in the next page)

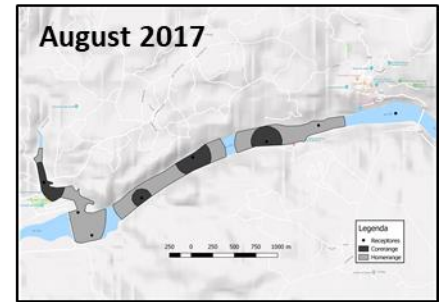
### Sil #3



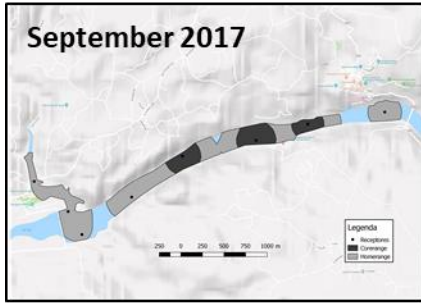
KDE95 = 0,695; KDE50 = 0,178



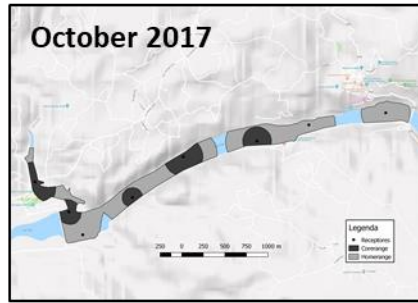
KDE95 = 0,773; KDE50 = 0,255



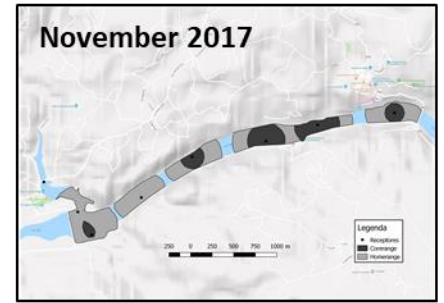
KDE95 = 0,760; KDE50 = 0,227



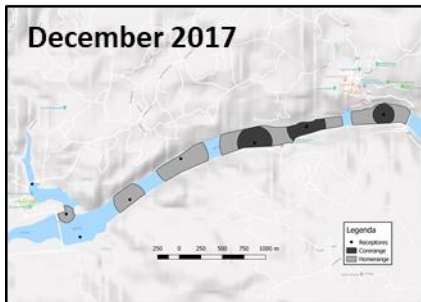
KDE95 = 0,846; KDE50 = 0,219



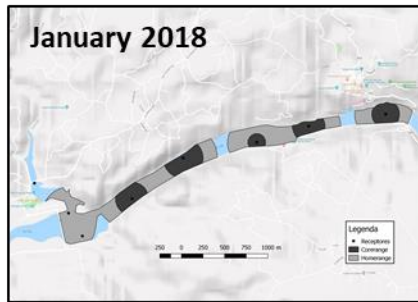
KDE95 = 0,899; KDE50 = 0,276



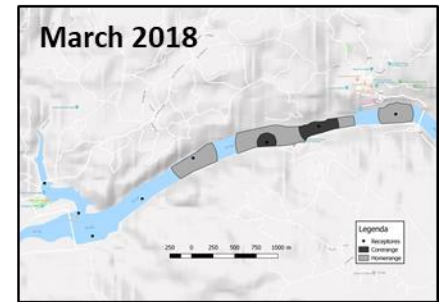
KDE95 = 0,841; KDE50 = 0,262



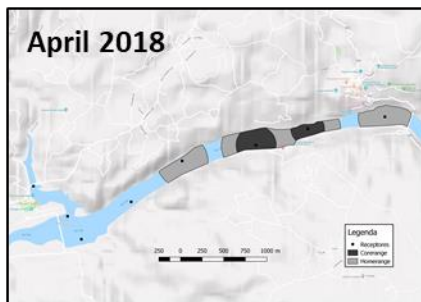
KDE95 = 0,598; KDE50 = 0,186



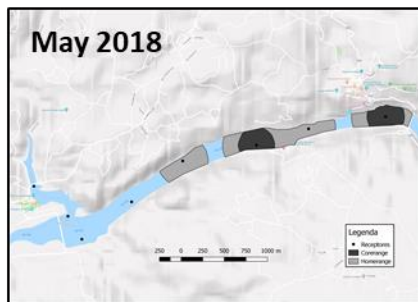
KDE95 = 0,837; KDE50 = 0,291



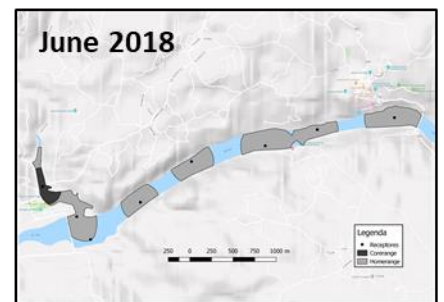
KDE95 = 0,435; KDE50 = 0,092



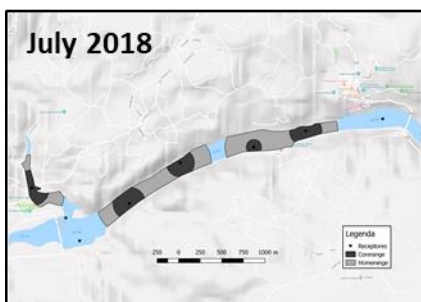
KDE95 = 0,483; KDE50 = 0,148



KDE95 = 0,485; KDE50 = 0,173



KDE95 = 0,700; KDE50 = 0,049

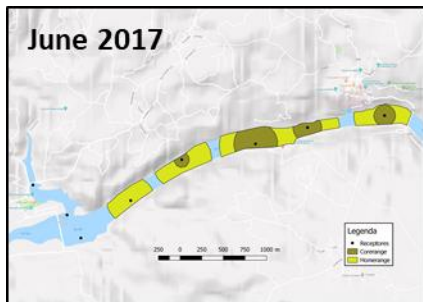


KDE95 = 0,601; KDE50 = 0,234

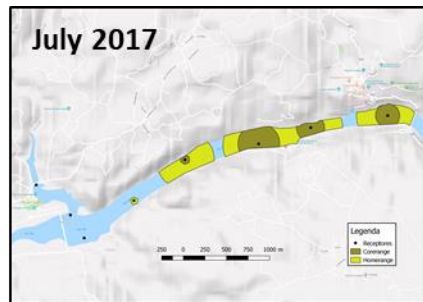
(Image continues in the next page)



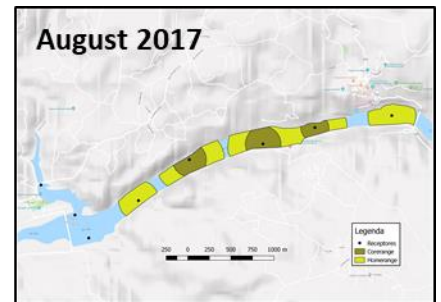
# Sil #4



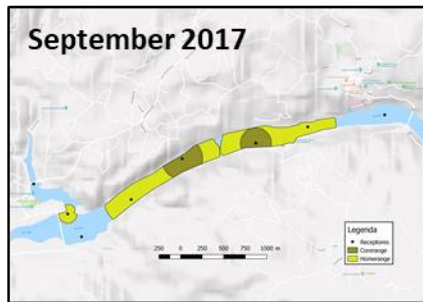
KDE95 = 0,615; KDE50 = 0,216



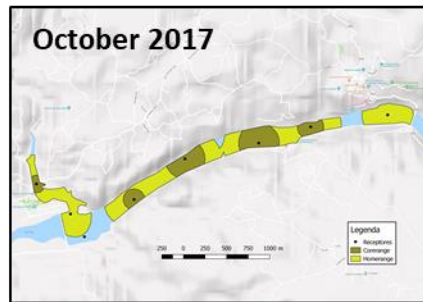
KDE95 = 0,515; KDE50 = 0,207



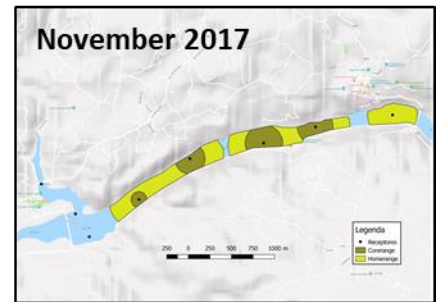
KDE95 = 0,602; KDE50 = 0,193



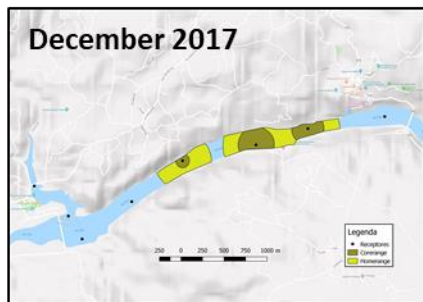
KDE95 = 0,563; KDE50 = 0,153



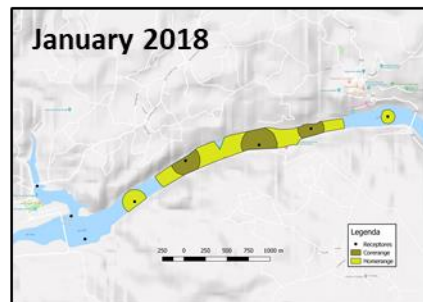
KDE95 = 0,848; KDE50 = 0,276



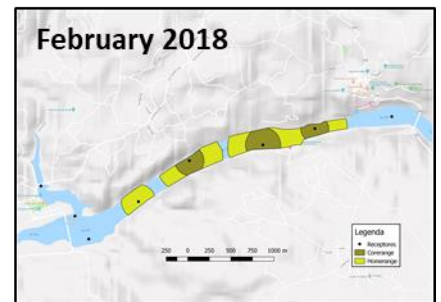
KDE95 = 652; KDE50 = 0,224



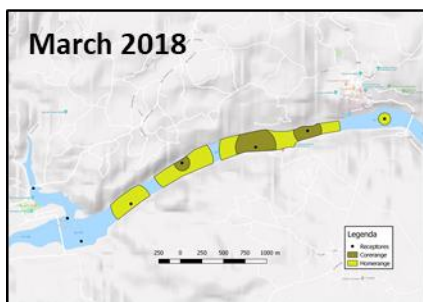
KDE95 = 0,374; KDE50 = 0,151



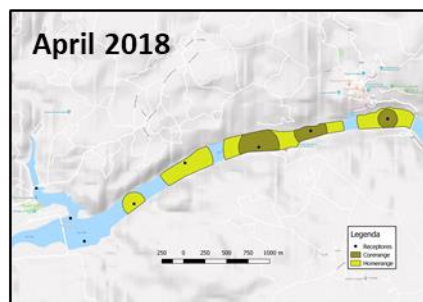
KDE95 = 0,485; KDE50 = 0,179



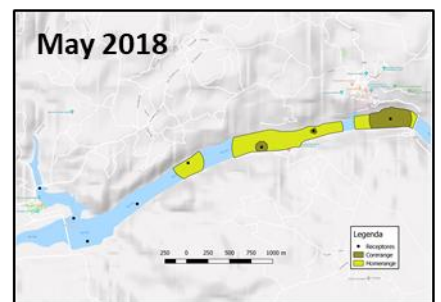
KDE95 = 0,475; KDE50 = 0,180



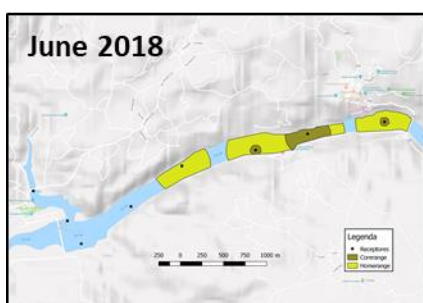
KDE95 = 0,497; KDE50 = 0,161



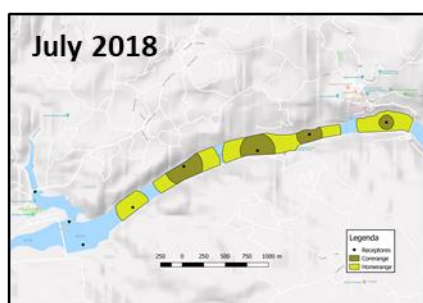
KDE95 = 0,546; KDE50 = 0,188



KDE95 = 0,436; KDE50 = 0,115



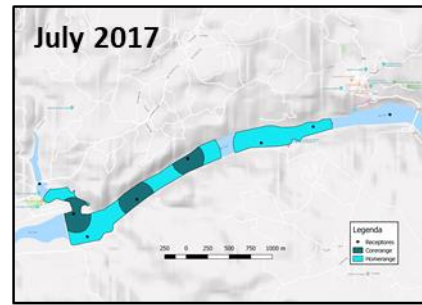
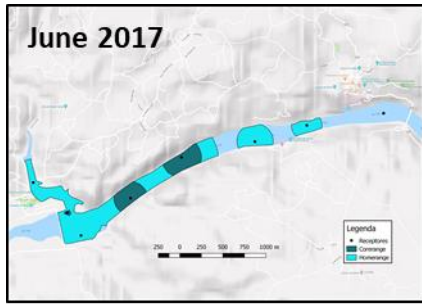
KDE95 = 0,492; KDE50 = 0,092



KDE95 = 0,605; KDE50 = 0,219

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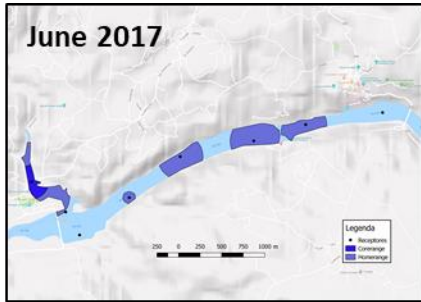
# Sil #5



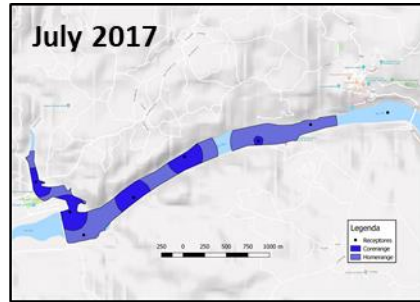
(Image continues in the next page)



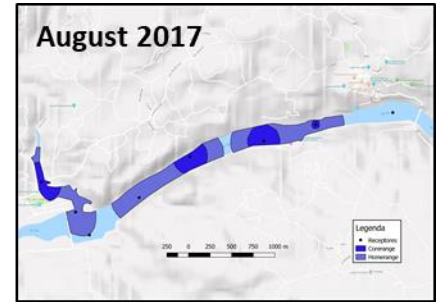
## Sil #6



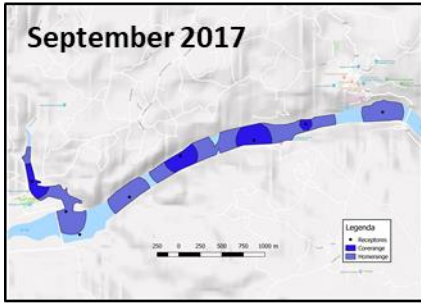
KDE95 = 0,411; KDE50 = 0,041



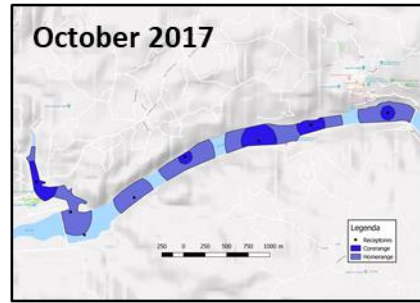
KDE95 = 0,789; KDE50 = 0,281



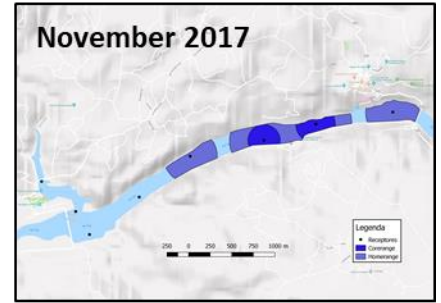
KDE95 = 0,715; KDE50 = 0,202



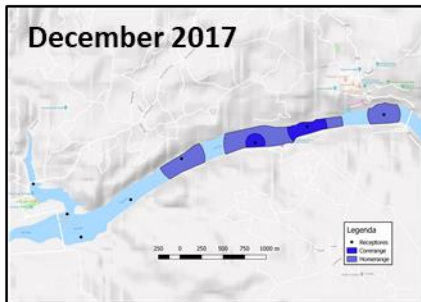
KDE95 = 0,795; KDE50 = 0,256



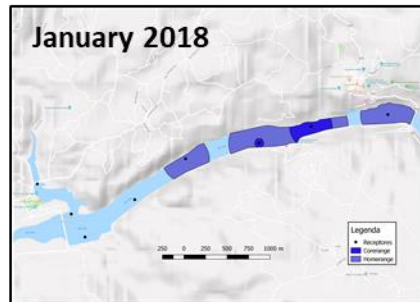
KDE95 = 0,790; KDE50 = 0,218



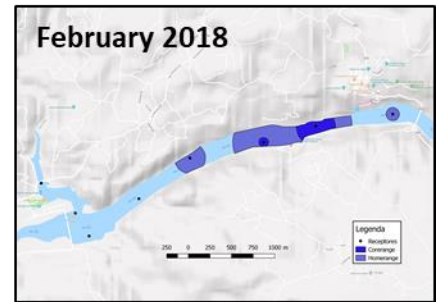
KDE95 = 495; KDE50 = 0,133



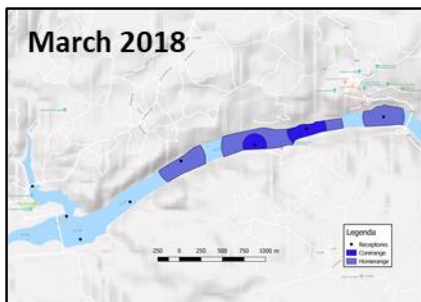
KDE95 = 0,427; KDE50 = 0,092



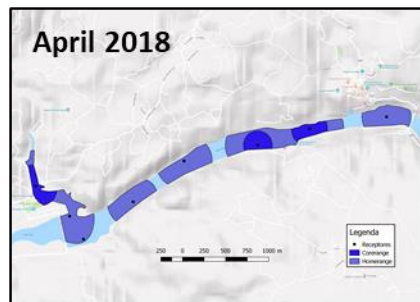
KDE95 = 0,469; KDE50 = 0,076



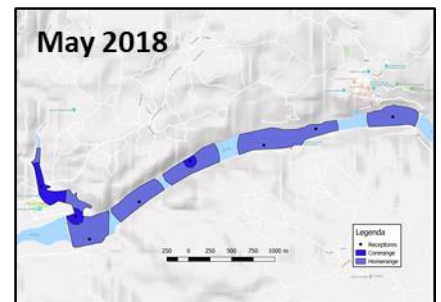
KDE95 = 0,329; KDE50 = 0,070



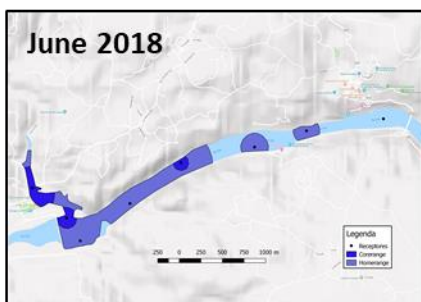
KDE95 = 0,459; KDE50 = 0,102



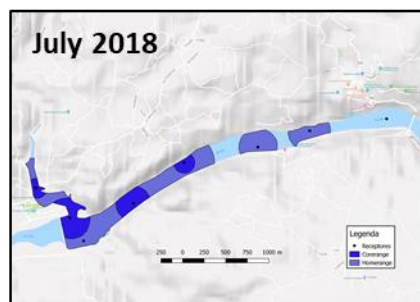
KDE95 = 0,814; KDE50 = 0,172



KDE95 = 0,854; KDE50 = 0,104



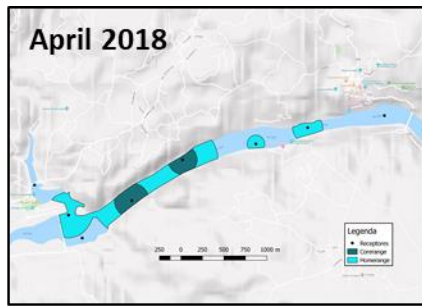
KDE95 = 0,640; KDE50 = 0,115



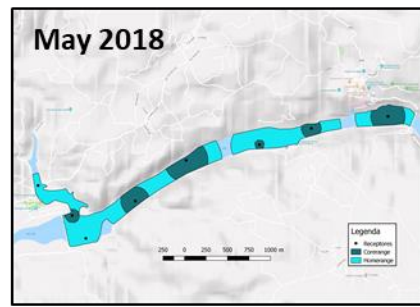
KDE95 = 0,709; KDE50 = 0,232

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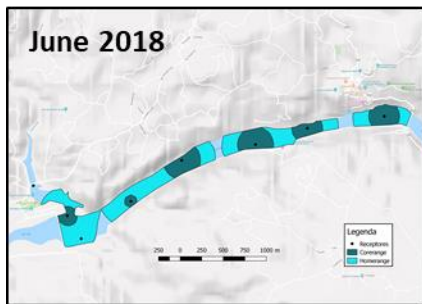
## Sil #8



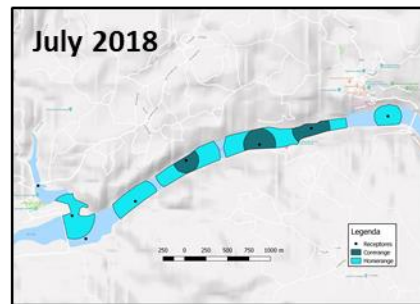
KDE95 = 0,495; KDE50 = 127



KDE95 = 0,898; KDE50 = 0,287



KDE95 = 0,857; KDE50 = 0,327



KDE95 = 0,696; KDE50 = 0,177

**Supplementary Figure 1.2. Maps of monthly KDE95 and KDE50 size variation and geographical distribution for all tagged fish throughout the entire study period.** Lighter colors represent KDE95 and darker colors represent KDE50; Below each map is represented the KDE95 and KDE50 area values in km<sup>2</sup>.