Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Biologia da Conservação

Dissertação

Distribuição e preferências de habitat do esgana-gata (*Gasterosteus aculeatus* L.) em Portugal: implicações para a sua gestão e conservação

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Orientador(es) | Carlos M. Alexandre
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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

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Resumo

Distribuição e preferências de habitat do esgana-gata (*Gasterosteus aculeatus* L.) em Portugal: implicações para a sua gestão e conservação.

O esgana-gata (*G. aculeatus* L.) é um peixe de água doce, classificado como *Em Perigo* (EN) pelo Livro Vermelho dos Vertebrados de Portugal. Contudo, devido à falta de conhecimentos relativamente à sua distribuição e preferências de habitat, poucas foram as medidas propostas com o objetivo de proteger as suas populações. Este estudo permitiu modelar a potencial distribuição da espécie, através de um método de previsão por *ensemble.* Verificou-se que o esgana-gata tende a ocorrer em habitats aquáticos onde o substrato arenoso é dominante e os níveis de escoamento e de precipitação durante o mês mais seco são mais elevados, evitando áreas de declive acentuado e com elevados níveis de precipitação média anual. Baseado nos resultados obtidos, foi elaborado um mapa de probabilidades de ocorrência da espécie, através do qual foram categorizados diferentes troços de rios, de acordo com os seus diferentes níveis de prioridade de conservação para a espécie.

Palavras-chave: *Gasterosteidae*; modelos de previsão; *ensemble forecasting methods*; distribuição e conservação; Península Ibérica.

Abstract

Distribution and habitat preferences of stickleback (*Gasterosteus aculeatus* L.) in Portugal: implications for their management and conservation.

The threespine stickleback (*G. aculeatus* L.) is a small freshwater fish that has been listed as *Endangered* (EN) in Portugal on the national Red List of Threatened Vertebrates. However, due to the lack of knowledge about its distribution and habitat preferences, few measures have been proposed aiming at the conservation of populations of this species. This study allowed to model the potential distribution of the species, using an ensemble forecasting method. It was found that stickleback tends to occur in aquatic habitats where the sandy substrate is dominant and the levels of flow and precipitation during the driest month are higher, avoiding areas of steep slope and with high levels of average annual precipitation. Based on our results, a map of the species probability of occurrence was generated, and based on this, some river sections were categorized, according to distinct priority levels for the species conservation.

Keywords: *Gasterosteidae*; Prediction models; Ensemble forecasting methods; species distribution and conservation; Iberian Peninsula.

Índice

1. Enquadramento geral

1.1. O esgana-gata

O esgana-gata (*Gasterosteus aculeatus* Linnaeus, 1758), ou também denominado comummente como espinhela, é um pequeno peixe teleósteo, pertencente à classe Actinopterygii, à ordem Gasterosteiformes e à família *Gasterosteidae* (Cabral *et al.,* 2005). Esta espécie foi descrita como um complexo de populações dulciaquícolas, fenotipicamente diferentes, que derivaram de ancestrais marinhos, através de radiação adaptativa, após as glaciações do período Quaternário (Foster *et al*., 2003; Alexandre & Almeida, 2009). O esgana-gata é uma espécie com uma ampla distribuição geográfica, estando presente em grande parte do hemisfério norte (Berna, 2001; Alexandre & Almeida, 2009; Clavero *et al.*, 2009), e apresentando o seu limite sul de distribuição na região mediterrânica (Crivelli & Britton, 1987). A presença desta espécie está descrita em grande parte do continente europeu, distribuída não só pelos lagos e pelos rios que desaguam no Mediterrâneo e na costa europeia do Atlântico, mas também pelos diversos habitats das Ilhas Britânicas, Ilhas Faroé e Islândia (Bell & Foster, 1994; Alexandre & Almeida*,* 2009). O esgana-gata surgiu na Península Ibérica após as glaciações do Quaternário, durante as quais, muitas espécies aquáticas se refugiaram no sul da Europa (Araguas *et al.,* 2011). Estas flutuações climáticas provocaram grandes alterações ao nível da estrutura e dinâmica fluvial, levando ao isolamento de inúmeras populações, o que permitiu a acumulação de diferenças genéticas e o desenvolvimento de linhagens divergentes (Araguas *et al.,* 2011; Sanz *et al.,* 2015). Atualmente, embora o esgana-gata apresente um ciclo de vida anádromo, estuarino ou totalmente marinho noutros países, em Portugal, ainda só foram capturados indivíduos holobióticos (Alexandre & Almeida, 2009; Araguas *et. al,* 2011), isto é, indivíduos que realizam a totalidade do seu ciclo de vida em habitats de água doce.

Pelo facto da espécie *G. aculeatus* apresentar uma grande plasticidade fenotípica, a sua identificação e caracterização pode ser difícil. Contudo, na generalidade das suas populações, os indivíduos são de pequenas dimensões e apresentam um corpo fusiforme e hidrodinâmico, que pode atingir no máximo 8 cm em indivíduos dulciaquícolas residentes (Fig. 1), e cerca de 11 cm na forma anádroma (Bell & Foster, 1994). As suas maxilas são de pequenas dimensões e apresentam dentes bastante aguçados, sendo a pré-maxila superior protráctil, (i. e., prolonga-se para a frente), e a maxila inferior ligeiramente proeminente (Berna, 2001). Um dos aspetos morfológicos mais característicos desta espécie, são os 3 espinhos isolados no dorso, na zona anterior à barbatana dorsal, o que lhe dá o nome, na terminologia anglo-saxónica, de "*threespine stickleback*" (Alexandre & Almdeida, 2009). Por sua vez, a barbatana dorsal apresenta entre 10 a 14 raios simples, e a anal entre 7 a 11 raios, sendo um deles espinhoso. A espécie apresenta ainda um raio espinhoso na região pélvica (Bell & Foster, 1994; Alexandre & Almeida, 2009; Page & Burr, 2011). Relativamente às barbatanas peitorais, estas possuem geralmente entre 8 a 11 raios (Kristjánsson *et al.,* 2005), e correspondem ao principal órgão propulsor para a locomoção, sendo ainda utilizadas pelos machos para ventilar os ovos depositados pelas fêmeas durante a reprodução (Bell & Foster, 1994; Alexandre & Almeida, 2009). Por fim, e também com influência na locomoção da espécie, esta apresenta uma barbatana caudal constituída por cerca de 12 raios ramificados (Pereira, 1994) (Fig. 2).

Fig. 1 Fotografia de um esgana-gata holobiótico capturado num afluente da bacia do rio Vouga, com cerca de 5 cm de comprimento total.

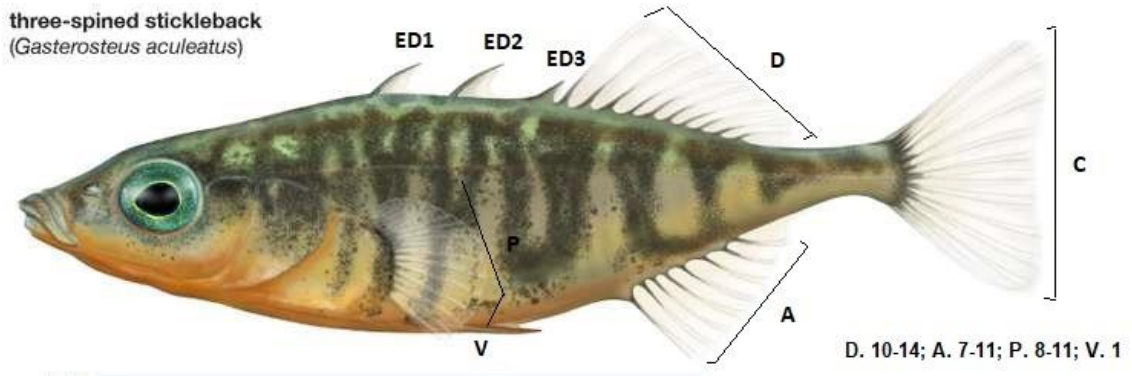

Fig. 2 Imagem demonstrativa da posição das diferentes barbatanas, e respetivos espinhos, do esgana-gata, correspondendo: D à barbatana dorsal e ED1, ED2 e ED3 aos espinhos isolados da região dorsal; P à barbatana peitoral; A à barbatana anal, na qual um dos seus raios é espinhoso; C à barbatana caudal; e V à barbatana pélvica, que é constituída apenas por um raio espinhoso. No canto inferior direito podemos observar a "fórmula das barbatanas" para esta espécie (imagem adaptada de Bell & Vincent, 2017).

Ao contrário da maioria dos peixes teleósteos, em detrimento de escamas, o esgana-gata apresenta placas ósseas, isto é, formações ósseas rígidas provenientes de escamas modificadas (Bell & Foster, 1994; Foster *et al.,* 2003). Tanto os espinhos como as placas ósseas podem variar numericamente consoante as características do habitat que a espécie ocupa, e têm funções de proteção. Enquanto as placas ósseas formam uma "armadura" rígida (Martins & Carneiro, 2018), os espinhos podem ser eretos e bloqueados como medida defensiva na presença de predadores (Foster *et al.,* 2003; Alexandre & Almeida, 2009). A cor do corpo destes indivíduos é também influenciada pelos diferentes tipos de habitat em que as populações residem, apresentando as formas holobióticas geralmente um tom castanho esverdeado, enquanto que as formas anádromas podem apresentar uma gama de cores desde o verde prateado até ao preto azulado. No geral, todas as formas apresentam uma linha lateral ao longo do corpo formada por inúmeros poros microscópicos (Page & Burr, 2011).

O esgana-gata é uma espécie que depende da visão para realizar a maioria dos seus comportamentos (alimentação, escolha de parceiros sexuais, entre outros), pelo que é maioritariamente encontrado durante o dia e perto da coluna de água (entre 10 a 15 metros de profundidade) (Fernández *et al.,* 2006). Contudo, em condições de elevada turbidez do meio, ou outros fatores que diminuam a sua visibilidade, esta espécie pode também recorrer ao olfato, especialmente para detetar as suas presas (Johannesen *et al.,* 2012). A técnica de predação desta espécie consiste em busca, perseguição, ataque e captura da presa (Mattern, 2006). Relativamente à sua alimentação, esta espécie é considerada eurifágica, isto é, a sua dieta varia consoante a disponibilidade alimentar, sendo maioritariamente constituída por invertebrados bentónicos (e.g., pequenos crustáceos), insetos aquáticos e suas larvas, insetos terrestres afogados, larvas de peixes e até pequenos peixes (Alexandre & Almeida, 2009; Page & Burr, 2011). Alguns autores afirmam ainda que esta espécie pode demonstrar comportamentos canibais, alimentando-se dos seus próprios ovos (Page & Burr, 2011), representando um comportamento adaptativo da espécie com o objetivo de repor a energia que foi despendia pelos machos durante os cuidados parentais e defesa do território (Whoriskey & FitzGerald, 1985).

Embora seja um predador voraz, o esgana-gata é considerado também um recurso alimentar essencial para outros predadores, como a perca-sol (*Lepomis gibbosus* Linnaeus, 1758) e o achigã (*Micropterus salmoides* Lacepède, 1802), e até para algumas aves piscívoras (Foster *et al.,* 2003; Alexandre & Almeida, 2009). Contudo, para além de morfologicamente apresentar algumas características que evitam a sua predação, como os espinhos e as placas ósseas, a espécie pode adotar alguns comportamentos anti-predatórios, como as fugas em zigue-zague e a formação de cardumes, que dificultam a sua captura por parte dos predadores (Fernandez *et al.,* 2006; Thünken *et al.,* 2014).

Um dos comportamentos que mais caracteriza o *G. aculeatus*, e que o diferencia de outras espécies de peixes teleósteos, é o seu sistema de acasalamento bastante flexível, podendo as suas populações ser poliândricas, isto é, uma fêmea pode acasalar com vários machos, ou poliginândricas, em que, tanto os machos como as fêmeas podem ter múltiplos parceiros durante a época de reprodução. A flexibilidade do seu sistema de acasalamento pode representar uma estratégia para aumentar o tamanho do efetivo populacional e favorecer a diversidade genética (Araguas *et al.,* 2011). A reprodução desta espécie corresponde a um dos seus comportamentos mais estudados e característicos (Fig. 3). A época de reprodução ocorre, geralmente, entre abril e junho, quando a temperatura da água está entre os 14ºC e os 16ºC (Doadrio, 2001; Alexandre & Almeida, 2009), período no qual os machos desenvolvem uma coloração avermelhada na zona ventral do corpo (característica epigâmica) para atrair as fêmeas e intimidar os machos rivais (Alexandre & Almeida, 2009; Page & Burr, 2011). Nesta época, é bastante comum alguns machos apresentarem ainda uma coloração azul esverdeada nos olhos, especificamente na zona da íris (Berna, 2001). Quando se inicia a época de reprodução, os machos demarcam o seu território e tornam-se bastante agressivos com os outros machos, o que não acontece fora desta época (Alexandre & Almeida, 2009). Neste período, fazem uma depressão no solo do seu território, onde constroem o seu ninho de formato tubular, com os materiais vegetais disponíveis no meio aquático, como algas filamentosas e pequenos detritos lenhosos. O ninho é fixado ao substrato através de uma proteína renal, denominada espigina, secretada pelos machos durante este processo (Alexandre & Almeida, 2009; NatureServe, 2015). O macho irá cortejar uma fêmea através de uma série de movimentos em ziguezague, e esta, caso esteja recetiva, irá segui-lo até ao seu ninho, no qual irá entrar e depositar entre 50 a 100 oócitos (Alexandre & Almeida, 2009; Page & Burr, 2011; NatureServe, 2015). Após a desova, as fêmeas são expulsas do ninho pelo macho, de modo a que este possa entrar e fertilizar os oócitos (Alexandre & Almeida, 2009). Nesta espécie, os machos são responsáveis pela totalidade dos cuidados parentais após a desova, que consistem em guardar e reparar o ninho e ventilar os ovos, para que estes sejam repetidamente banhados por uma nova água, de modo a facilitar a sua oxigenação, através de movimentos das suas barbatanas peitorais (Alexandre & Almeida, 2009; NatureServe, 2015). Cerca de 7-8 dias após a desova, os juvenis eclodem, mas continuam a ser protegidos pelo macho até que atinjam uma dimensão em que são considerados uma ameaça, sendo então afugentados, ou abandonados no ninho (Alexandre & Almeida, 2009; NatureServe, 2015).

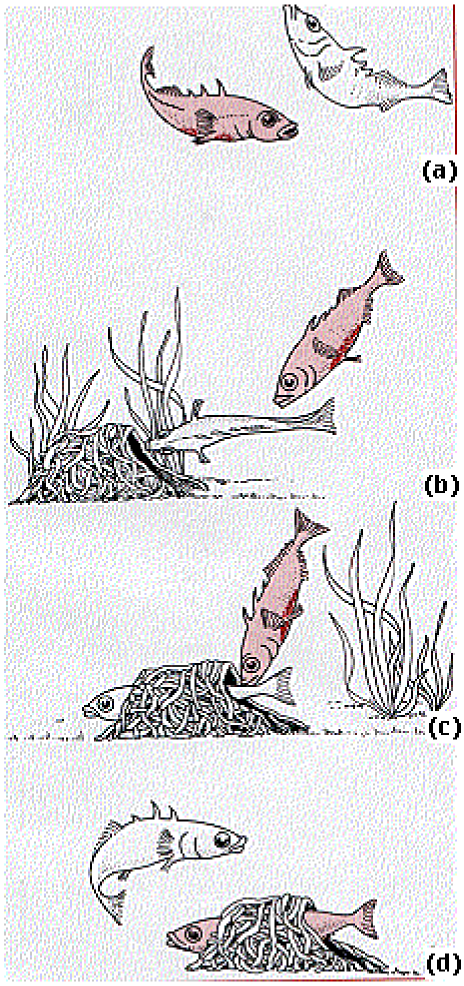

Fig. 3 Ilustração descritiva do processo de acasalamento do esgana-gata. a) inicialmente, ocorre a corte da fêmea, por parte do macho com movimentos em ziguezague; b) de seguida a fêmea segue o macho até ao seu ninho; c) a fêmea entra no ninho e deposita os oócitos, e em seguida, d) o macho irá expulsar a fêmea e fertilizar os oócitos (adaptado de Kimball, 2021).

A ampla distribuição geográfica, o alto grau de plasticidade fenotípica e a ampla diversidade morfológica, comportamental e genética, fez com que o esgana-gata se tornasse uma espécie-modelo com grande importância para várias áreas de estudo, como a fisiologia animal, biologia do desenvolvimento, genética, evolução, entre outras (Bell & Foster, 1994; Poizat *et al.,* 2002; Alexandre & Almeida, 2009; Sanz *et al.,* 2015; Fang *et al.,* 2018). Porém, apesar da sua importância científica, e relevância para o equilíbrio dos ecossistemas aquáticos enquanto espécie nativa, o seu baixo valor económico e a falta de conhecimentos relativos à sua distribuição, ecologia e preferências de habitat, fez com que poucas medidas específicas tenham sido implementadas para a conservação das suas populações que, nos últimos anos, têm estado em declínio (Clavero *et al.,* 2009), sobretudo aquelas que se encontram próximas dos limites da sua distribuição, como é o caso da Península Ibérica (Araguas *et al.,* 2011). As populações de esgana-gata, ao longo da sua distribuição, enfrentam a maioria das ameaças conhecidas por alterarem a estrutura dos ecossistemas aquáticos, como a sedimentação, a redução da qualidade e/ou quantidade da água, a eutrofização e a introdução de espécies exóticas (Foster *et al.,* 2003; Alexandre & Almeida, 2009). Tendo em conta todas estas ameaças e o facto de ter desaparecido de grande parte da sua área de distribuição, esta espécie foi classificada como *Em Perigo* (EN) em Portugal, e em Espanha, com base nos critérios da União Internacional para a Conservação da Natureza (IUCN) (Cabral *et al.,* 2005; Hermida *et al.,* 2005; Clavero et al., 2009; Vila *et al.,* 2017). Alexandre & Almeida (2009) e Clavero *et al.,* (2009) foram dos poucos autores a realizar trabalhos direcionados ao estudo da biologia do esgana-gata e ao seu uso do habitat em rios intermitentes na região mediterrânica, afirmando ser necessário, obter dados comparáveis nesta região, de modo a que se estabeleçam e iniciem programas de monitorização e conservação desta espécie a longo prazo. Em Portugal, por constituir o limite sul da sua distribuição, e visto que a espécie se encontra em perigo de extinção regional, é crucial a coleta de mais informações acerca das suas populações, a nível regional e local, e que sejam propostas e implementadas medidas de conservação de modo a evitar a sua extinção em Portugal (Alexandre & Almeida, 2009).

1.2. Objetivos

O presente estudo tem como objetivo geral avaliar e atualizar a informação sobre o estado das populações de esgana-gata, e sobre a sua distribuição em Portugal Continental, bem como os fatores ambientais de macro-escala que condicionam a sua ocorrência, como forma de identificar áreas prioritárias para a conservação desta espécie no limite sul da sua distribuição natural.

Mais especificamente, neste estudo pretende-se:

- Identificar os locais de ocorrência da espécie na área de estudo e caracterizar a sua distribuição;

- Identificar fatores ambientais responsáveis pela seleção do habitat a um nível geográfico regional, com abrangência nacional;

- Desenvolver um modelo de probabilidades de ocorrência para o esgana-gata;

- Definir locais prioritários para a sua conservação, com base nas suas preferências de habitat, e nas ocorrências confirmadas e potenciais.

1.3 Metodologia de Amostragem

Os dados de ocorrência de *G. aculeatus* em Portugal Continental foram obtidos a partir de bases de dados existentes, resultantes de campanhas de amostragem da mesma equipa de investigação (MARE - Centro de Ciências do Mar e Ambiente) em projetos anteriores, e de bases de dados oficiais das autoridades nacionais (p.e., ICNF - Instituto Nacional de Conservação da Natureza e Florestas). Só foram utilizados dados de 2010 em diante, pois foi considerado que dados anteriores precisariam de uma nova validação *in loco*, podendo estar desatualizados. As lacunas das bases de dados, resultantes da subamostragem de algumas áreas do país, foram compensadas com novos dados obtidos em campanhas de amostragem específicas, desenvolvidas entre abril e outubro de 2019, principalmente no âmbito do projeto "Colmatação de lacunas de informação sobre a distribuição e abundância de peixes dulciaquícolas e migradores (diádromos) em Portugal Continental no âmbito da elaboração do Livro Vermelho", que está atualmente a decorrer.

O procedimento utilizado nas campanhas de amostragem realizadas em 2019 foi, em parte, adaptado do *Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Diretiva Quadro da Água: Protocolo de amostragem e análise para a fauna piscícola* (INAG, 2008). A captura de indivíduos de esgana-gata foi efetuada através de pesca elétrica (Fig. 4), utilizando um gerador dorsal (Hans Grassl ELT 60 II-HI 500 V-DC 10 A) ou um gerador fixo operado da margem ou a partir de uma embarcação (Hans Grassl EL 62 600 V-DC 10 A), dependendo das características dos locais de amostragem, auxiliado por um ânodo. A pesca elétrica é, atualmente, uma das formas mais eficazes para capturar espécimes piscícolas em ambientes dulciaquícolas de características lóticas, como é o cado da espécie-alvo deste estudo. O ânodo (rede camaroeira) e o cátodo (fita de cobre) que são ligados ao gerador, quando acionados por um interruptor, criam um campo elétrico que provoca a natação involuntária dos peixes na direção do ânodo, seguida de paralisia, que facilita a sua captura (Dodds & Whiles, 2020).

Fig. 4 Fotografia do método de captura através da pesca elétrica, onde podemos observar o (1) ânodo, o (2) cátodo e o (3) gerador dorsal.

O comprimento do troço do rio amostrado foi definido como sendo 20 vezes a largura média do leito inundado, para rios com uma largura inferior a 30 metros, e 10 vezes a largura média do rio, para rios com mais de 30 metros de largura. O comprimento do troço amostrado, sempre que possível, foi no mínimo 100 metros, e o método de captura utilizado dependeu da profundidade e largura do rio, sendo os rios menores amostrados a vadear, e os maiores e de profundidade mais elevada de barco. A prospeção foi realizada em sistema aberto, isto é, sem recorrer à utilização de redes de barreira a fechar o troço pescado. Os locais foram amostrados apenas uma vez durante o decorrer do estudo, tendo sido efetuada apenas uma passagem e de sentido único, em direção a montante. Os indivíduos capturados foram identificados, contabilizados, medidos e pesados, e devolvidos novamente ao rio. Em todos os locais amostrados foram anotadas as coordenadas utilizando um GPS Garmin 78st (*Global Positioning System*), produzindo dados muito precisos (erros menores que 3-5 m) (Graham *et al.*, 2008).

Em todas as estações prospetadas, realizou-se ainda uma caracterização do habitat, com a medição de variáveis como a profundidade (m), velocidade de corrente (m s^{-1}) (com o auxílio de um fluxómetro HYDRO-BIOS, precisão 0.01 m/s), pH, oxigénio dissolvido (mg/l), entre outros. Contudo, para os objetivos deste trabalho, apenas foi utilizada a informação relativa à ocorrência da espécie e a sua localização, tendo os restantes parâmetros sido registados para eventuais estudos futuros.

2. Manuscrito 1

Distribution and habitat preferences of stickleback (*Gasterosteus aculeatus* L.) in Portugal: implications for their management and conservation

Abstract

The threespine stickleback (*G. aculeatus* L.) is a small freshwater fish that has been listed as *Endangered* (EN) in Portugal on the national Red List of Threatened Vertebrates. However, due to the lack of knowledge about its distribution, ecology and habitat preferences, few measures have been proposed aiming at the conservation of this species. From existing databases and electric fishing sampling campaigns developed in 2019, we obtained occurrence data of threespine stickleback for a total of 646 sites, from which 51 were presences and 595 absences. The occurrence data, together with 15 environmental predictors of macrohabitat variables, were used to model the potential distribution of the species through an ensemble forecasting method. Through the results of our final ensemble, it was found that threespine stickleback may occur predominantly at lower stretches of river systems, where sandy substrate is dominant, and flow is higher. Sticklebacks are also more likely to occur in sites with high levels of rainfall in the driest month. The species also tends to avoid steep slope areas, with high levels of annual precipitation. Based on our results, a map of the species probability of occurrence was generated, and based on this, some river sections were categorized, according to distinct priority levels for the species conservation.

Keywords:

Gasterosteidae; Prediction models; Ensemble forecasting methods; species distribution and conservation; Iberian Peninsula.

1. Introduction

The threespine stickleback (*Gasterosteus aculeatus* L.), is a small teleost fish (Bell & Foster, 1994; Cabral *et al*., 2005), that is widely distributed around the world, being present in almost the entire northern hemisphere (Hagen, 1967; Berna, 2001) and presenting its southern limit of distribution in the Mediterranean region (Crivelli & Britton, 1987; Rind *et al*., 2020). This species is characterized by its complex of phenotypically different populations, resulting from its different adaptations to biotic and abiotic factors imposed by the different types of habitats (Bell & Foster, 1994; Foster *et al*., 2003). Currently, in most of its distribution area, *G. aculeatus* comprises two main forms: a freshwater holobiotic form and an anadromous form, which resides in marine or estuarine environments, migrating to freshwater to reproduce (Hagen, 1967; Bell & Foster, 1994; Sanz *et al*., 2015). In the Iberian Peninsula, all the individuals observed presented holobiotic characteristics and the scientific community believes that populations from this region are derived, post-glacially, from oceanic ancestors that took refuge in this region during Quaternary glaciations (Sanz *et al*., 2015). The isolation of threespine stickleback populations, in this region, led to the accumulation of genetic differences (Araguas *et al*., 2011; Sanz *et al*., 2015), which gave rise to phenotypically distinct populations, but not enough to promote their speciation (Bell & Foster, 1994).

Rivers vary in geomorphology and physical properties along their courses, and these attributes have been shown to influence the distribution of fish species (Tomanova *et al*., 2007). Considering habitat preferences, holobiotic populations of threespine sticklebacks are mostly found in clear water streams (Alexandre & Almeida, 2009; Page & Burr, 2011), since some of its ecological behaviours are based on visuals clues (e.g. feeding or choosing sexual partners) (Candolin & Voigt, 1999; Doadrio, 2001). So, the threespine stickleback will tend to avoid rivers that cross intense forests (Clavero *et al*., 2009) due to the shading of the aquatic environment. These fish also show a preference for waters with high oxygen levels and with substrates of sand, pebbles or gravel (Fernández *et al*., 2006), which can be used as a refuge in the existence of predators (Alexandre & Almeida, 2009; Page & Burr, 2011). During the breeding season, areas with a predominance of silty or sludge substrate and places with stagnant water, are avoided because eggs and juveniles would be exposed to low oxygen concentration (Alexandre & Almeida, 2009; Clavero *et al*., 2009). It's uncommon to find individuals of this species in large rivers and in places with a high current speed, as riffles, especially during the breeding season (Page & Burr, 2011), because it hinders the construction of the nest and represents a danger for juveniles (Bell & Foster, 1994; Alexandre & Almeida, 2009). In the breeding season, shallow habitats are preferentially chosen for nest construction, probably because at low depths the water temperature is more convenient for the development of eggs and juveniles (Alexandre & Almeida, 2009).

In a study of Candolin & Voigt (1999), it was demonstrated that threespine stickleback prefers open places without aquatic vegetation in the absence of predators, because females were more visible and easily found. However, in the presence of predators, as is common in most Iberian habitats, the threespine stickleback shows a preference for places with some aquatic vegetation (Clavero *et al*., 2009), such as reeds or cane fields (Fernández *et al.,* 2006). The vegetation is used to build the nest (Alexandre & Almeida, 2009; Clavero *et al*., 2009), and as a refuge, to reduce the risk of predation of adult individuals and their eggs. With the increased risk of predation, the male's courtship activity decreases in places with no vegetation, resulting in a decrease in the interest shown by the females (Candolin & Voigt, 1999). The introduction of non-native species, like pumpkinseed sunfish (*Lepomis gibbosus,* Linnaeus, 1758) and northern pike (*Esox Lucius* Linnaeus, 1758), which are common species in Mediterranean rivers, can lead to the low occurrence of *G. aculeatus* in these habitats, due to competition for refuge and food, and they can also be direct predators (Foster *et al*., 2003). Invasive species may also transform the habitat, which occurs with red crayfish (*Procambarus clarkii* Girard, 1852), which reduces the amount of submerged macrophytes used by the threespine stickleback as a refuge (Clavero *et al*., 2009).

As described in previous paragraphs, most of the existing studies on habitat preferences of stickleback are focused at a meso and microhabitat scales, and usually directed to locally based populations (e.g., Crivelli & Britton, 1987; Alexandre & Almeida, 2009; Clavero *et al.,* 2009). There is a lack of scientific knowledge regarding the macro-scale environmental factors influencing the occurrence and distribution of this species at national or regional levels, that can be used to define priority sites, at a large national scale, for the development of concerted and effective actions for the species conservation. Our study was carried out in mainland Portugal, which represents the southern limit of the species Atlantic distributions, where most regions are characterized by a Mediterranean climate with strong inter-annual variations in water flow, with peak flows in winter and extremely dry periods in the summer (Gasith & Resh, 1999). This climate is usually not favourable to threespine stickleback survival, because it brings this species close to its tolerance limits, especially during summer, due to extreme drought periods (Alexandre & Almeida, 2009; Clavero *et al*., 2009; Araguas *et al*., 2011). In the Iberian Peninsula, beside seasonal variability, river network is quite complex and comprises many independent river basins, leading to the isolation of populations. In addition to these factors, human activities, such as water abstraction, agricultural runoff, domestic and industrial effluents, caused dramatic declines and, in some cases, local extinction of threespine stickleback populations (Clavero *et al*., 2004; Araguas *et al*, 2011). Based on the International Union for Conservation of Nature (IUCN) criteria, after threespine stickleback disappeared from most of its regional distribution and taking into account all its threats, the species was listed as *Endangered* (EN), both in Portugal and Spain (Cabral *et al*., 2005; Hermida *et al*., 2005; Clavero *et al*., 2009; Vila *et al*., 2017; Rind *et al*., 2020).

In the Iberian Peninsula, the threespine stickleback is extremely valuable, as a native species, for the aquatic ecosystem balance (Foster *et al*., 2003), and also from the scientific standpoint, due to its wide geographical distribution, evolutionary history, and high genetic, morphological and behavioural diversity (Bell & Foster, 1994; Poizat *et al.,* 2002; Araguas *et al*., 2011; Sanz *et al*., 2015; Fang *et al*., 2018). According to Araguas *et al*. (2011), some of these populations can be considered as Evolutionarily Significant Units (ESUs) in this region, because some of their individuals are genetically distinct from other populations in Europe. Despite that, until now, large-scale studies have never been conducted in the Iberian Peninsula with the aim of improving the knowledge of the populations, distribution, and environmental factors that influence the occurrence this species.

Factors that determine the spatial and temporal distribution of species have always been questioned by biologists and ecologists (Domisch *et al*., 2015). To predict possible distribution of species, the scientific community have been using Species Distribution Models (SDM's), which correlate species occurrence data with environmental variables through statistical models, with the aim of protecting wild species, environmental planning and creating fauna management programs (Guisan & Thuiller, 2005; Diniz-Filho *et al*., 2009; Marmion *et al*., 2009; Domisch *et al*., 2015; Nezer *et al*., 2016; Zhang & Vincent, 2018). Nevertheless, these models have been rarely applied to conserve freshwater fish species (Buisson *et al*., 2008).

Considering existing knowledge gaps about *G. aculeatus*, this study aims to correlate its occurrence with macro-scale environmental factors, and predict the potential distribution of this species, using SDM's, to identify which environmental factors influence its regional distribution. Ultimately, this information will contribute to the conservation of the threespine stickleback populations in mainland Portugal through the definition of the most important areas for the protection and management of the target species. More specifically, we aim to: i) identify the occurrence sites of this species in the study area and characterize its distribution; ii) identify the macro-scale environmental factors that determine their distribution in the study area; iii) develop a model of occurrence probability for the threespine stickleback; and, based on previous results, iv) define priority sites for the species conservation.

2. Materials and Methods

2.1 Study area

This study was conducted in mainland Portugal, which comprises an area of approximately 89 015 km², and is located at the western end of the Iberian Peninsula, bordered by Spain on the north and east, and the Atlantic Ocean on the west and south. While in the central and northern regions of the country the landscape is mountainous with plateaus interspersed with areas of intensive agriculture, in the south the plains predominate with a warmer and drier climate than in the north, where the climate is colder and rainier. Most of Portugal regions face a typical Mediterranean climate characterized by cool, wet winters and hot, dry summers. Seasonality and variability in rainfall are the main attributes of the Mediterranean-type climate (Gasith & Resh, 1999; ICA, 2011). The annual mean temperature in mainland Portugal is 15.6°C (± 4.7°C), reaching their lowest values in the northern region of the country, and the highest values on the southern coast. The annual mean precipitation, in turn, is 834 mm (± 211 mm), reaching their highest values in the mountainous areas of the northern region, and the lowest in the Alentejo region, in the south of Portugal (Portal do Clima, 2015; IPMA, 2020). Most land use in mainland Portugal is associated with agriculture and natural or semi-natural forests (CIA, 2010).

2.2 Species occurrence data

The *G. aculeatus* occurrence data in mainland Portugal were obtained from existing databases (that is, previous projects of the research team responsible for this study, combined with official data from national authorities) and sampling campaigns specifically developed to fill gaps in areas with lower sampling effort (mainly within the scope of the revision of the National Red List of Threatened Freshwater and Migratory Fish Species, currently underway). Among the data collected from other authors, past sampling campaigns and other existing databases (such as, for example, ICNF - National Institute for Nature Conservation and Forests), the occurrence data with geographic coordinates errors and duplicate records were removed. Only data from 2010 onwards were used, as we considered previous data would need *in situ* confirmation. The sampling campaigns, developed to fill the existing spatial gaps in the occurrence data, were carried out during 2019, between April and October (avoiding periods of high flow that could reduce sampling efficiency) in all Portuguese hydrographic basins. Sticklebacks were sampled using electrofishing (Hans Grassl ELT 60 II-HI 500 V-DC), with two different methods, depending on the river depth and width: smaller rivers were sampled by wading and larger ones by boat, in accordance with the standard sampling protocol defined by national authorities in the scope of Water Framework Directive (WFD) (INAG, 2008). The length of the sampled river section was defined as 20 times the average width of the flooded riverbed, for rivers up to 30 meters wide, and 10 times the average width of the river, for rivers with more than 30 meters wide. Caught sticklebacks were identified, counted, measured and released back into the river.

After combining presence/absence data from existing databases and specific sampling campaigns, some procedures were adopted to clean the data. As the prevalence of the dataset was low (n= 837; presences= 51; absences= 786), and bias could be induced in the analyses (Phillips *et al*., 2009), some of the absences located too close were removed. For that, the absence points were resampled in a grid of 5 km x 5 km cells (Arcgis 10.7.1) and only the centroid from each cell was selected. The final dataset (n = 646; presences=51; absences= 595) was used for modelling the distribution of *G. aculeatus* (Fig. 5).

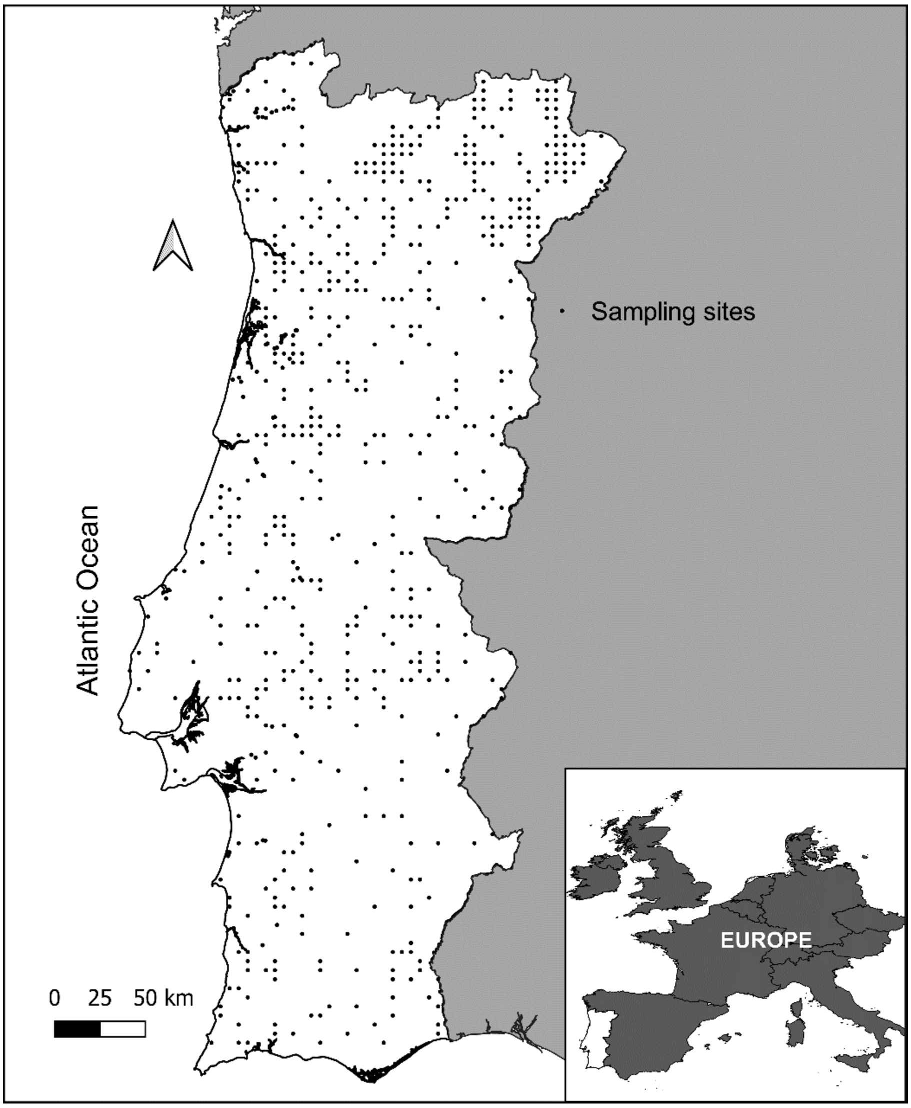

Fig. 5 Location of occurrence sites (from existing databases and specific sampling campaigns), throughout mainland Portugal, used to model threespine stickleback distribution.

2.3 Environmental predictors

The environmental variables used to predict the distribution of *G. aculeatus* were chosen for their ecological relevance in the macroscale distribution of many freshwater species, as for their availability for the study area. Initially, seventeen variables were selected and categorized according to their typology: geomorphology, climate, environmental stressors and hydrology, as described in Table 1 (please check Appendix 1 for maps of these variables).

Table 1 Variables initially selected to model the threespine stickleback's distribution.

Variables	Code	Range
Geomorphology		
Altitude (m)	*altitude*	0 - 1 959
Slope (°)	*slope*	0 - 48 689
Distance to coast (m)	*dist_coast*	0 - 357 420
Silt (%)	*silt*	3.6 - 20.3
Sand (%)	*sand*	19.1 - 85.9
Climate		
Annual mean temperature (°C)	*tempmean*	6.6 - 17.8
Maximum temperature of warmest month (°C)	*tempmax*	20.4 - 33.8
Annual precipitation (mm)	*precip*	477 - 1 880
Precipitation of driest month (mm)	*precipdriest*	1 - 35
Hydrology		
Flow accumulation (no. of cells)	*f_accum*	0 - 9 764 683
Flow weight with rainfall (no. of cells)	*f_weight_rain*	0 - 267 597 088
WTI	*wti*	0 - 20.07
SPI	*spi*	-3.5 × 10^9 to 4.5 × 10^8
Envirnmental stressors		
Artificial surfaces (no. of cells)	*use_art*	0 - 6 083
Agricultural areas (no. of cells)	*use_agr*	0 - 218 888
Forest and semi-natural areas (no. of cells)	*use_forest*	0 - 161 346
Population (n/km²)	*populat*	0 - 15 304

The environmental variables of geomorphological typology, used in this analysis were: altitude, river slope, distance to coast and the type of substrate (percentage of silt and percentage of sand in the soil). Altitude ("altitude") was obtained from WorldClim version 2.1 (Fick & Hijmans, 2017), with a resolution of

1 km², derived from the SRTM (Shuttle Radar Topography Mission) elevation data (SRTM, 2010). Elevation is a topographic variable that is correlated with variables such as temperature and rainfall, and consequently influencing the water flow and the substrate characteristics (Lassalle *et al*., 2008). The variable slope (“slope”) was included in our study because it’s also correlated with river velocity and the type of substrate (Rosgen, 1994). Distance to coast (“dist_coast”) was included in our analysis as it reflects the changes in physical and chemical characteristics of the rivers from the headwaters to downstream extent (Vannote *et al*., 1980). Both slope and distance to coast were calculated using the SRTM Digital Elevation Model in ArcGIS (ESRI, 2009). The type of substrate was recognized as an important factor limiting the distribution of *G. aculeatus* because it is correlated with its refuge, essentially in the presence of predators (Fernández *et al*., 2006). So, in order to evaluate the importance of this parameter at a macrospatial scale, we used two types of substrate in our analysis, being these the percentage of silt (“silt”, grain size between 0.002 and 0.063 mm) and the percentage of sand (“sand”, grain size between 0.063 and 2 mm). The percentage of these types of substrate was obtained from the Harmonized World Soil Database (FAO/IIASA/ISRIC/ISSCAS/ JRC, 2009; 1 km² /resolution).

In addition to the geomorphological variables, we also added, to the species distribution model, hydrology predictors. In rivers and streams, the habitat structure is defined largely by physical processes, especially by the movement of water, whereby variation of the river flow is responsible for shaping and organize the physical habitat and associated biotic communities (Poff *et al*. 1997; Zeiringer *et al*., 2018). In addition, the flow variation is directly correlated with the species distribution in the different river habitats (Poff *et al*., 1997). In Portugal rivers, the variation in flow is also one of the most important predictors in distribution of freshwater species, because most rivers have a temporary regime (Ferreira, 2013). In this study, flow in target river basins was estimated using two flow accumulation functions in ArcGIS and two flow indexes in Saga (SAGA, 2010). The flow accumulation (“f_accum”) function corresponds simply to the total area that drains to each location, while flow (“f_weight_rain”) was calculated using the flow accumulation weighted by the mean annual precipitation. The SAGA Wetness Index (“WTI”, Böhner *et al*., 2002) and the Stream Power Index (“SPI”; Moore *et al*., 1988) are measures of the wetness and the erosive power of flowing river water, respectively.

To understand how and what are the climatic variables that influence the distribution of sticklebacks, four climate predictors were also tested: annual mean temperature (“tempmean”), maximum temperature of the warmest month (“tempmax”), annual precipitation (“precip”) and precipitation of the driest month (“precipdriest”). Temperature and precipitation are variables that generally influence the distribution of freshwater species because they are directly correlated with the water temperature and the amount of water received in the hydrographic basins (Erickson & Stefan, 2000; Lassalle *et al*., 2008). In Mediterranean climatic regions, especially during the summer, variables such as temperature of the warmest month and precipitation of driest month are very important because they represent extreme periodic climatic events and can limit the distribution of species more sensitive to ecological changes. All climatic predictors were obtained from WorlClim version 2.1 database (Fick & Hijmans, 2017), at a 1 km² cell resolution.

One of the biggest threats to the freshwater ecosystem is pollution from human activities such as industrial effluents, agricultural activities, urban waste management issues, and increase in urbanized areas (Araguas *et al*., 2011; Amoatey & Baawain, 2019). Since there are no databases with sufficient information on all polluting sources in mainland Portugal and assuming that water quality is associated with uses of drained soil (Kroll *et al*., 2009), we tried to derive water quality from Corine Land Cover 2000 land use data (CLC; EEA, 2010; 100 m²/ resolution). Land uses were divided into three types: (1) artificial surfaces (“use_art”), (2) agricultural uses (“use_agr”) and (3) forest and semi-natural areas (“use_forest”). Other environmental stressor included in our study was demographic information (“populat”, total population). This information was obtained from LandScan (LandScan, 2010; 1 km²/ resolution) and used as a proxy of organic pollution level from domestic effluents.

Initially, Pearson’s correlation was used to explore the correlation between the different environmental variables and the occurrence of the target species (Table 2), as well as within all environmental variables (Appendix 2). Pearson *r* correlation (Freedman et al., 2007) is the most widely used correlation statistic to measure the degree of the relationship between linearly related variables. Significance was also accessed for each correlation.

All environmental variables were resampled to the same grid used for occurrence data (5x5 km). Collinearity was accessed using VIF (variance inflation factor), which according James *et al*. (2017) and Naimi & Araujo (2016) is the best method to assess collinearity between variable, as it measures how strongly each predictor can be explained by the rest of predictors. “Annual average temperature” and the “forest and semi-natural areas” variables were removed from the analyses, because VIF values exceeded the defined threshold (10), and could lead to the increase of model error and uncertainty (Graham, 2003; Guisan & Thuiller, 2005; Braunisch *et al*., 2013; Naimi & Araujo, 2016). Therefore, only 15 environmental variables were used in the analyses, but as the removed variables were highly correlated with some of the remaining predictors, no environmental information was lost.

2.4 Statistical modelling

In order to reduce the uncertainty associated with the Species Distribution Models (SDM), we implemented an ensemble forecasting method, that combine the weighted projections of all statistical models used (Araújo & New, 2007; Marmion *et al*., 2009; Boavida-Portugal *et al*., 2018; Roy *et al*., 2020) and is reported to outperform the predictions of individual statistical models (Crossman & Bass, 2008). Nineteen different statistical models were available and, for each model, optimal parameterization and fit evaluation were conducted using the True Skill Statistic (TSS) (Allouche *et al*., 2006) threshold and models performing poorly (with TSS values < 0.6) were excluded from the final ensemble (according to Landis & Koch, 1977 classification scheme).

The final ensemble, included 10 of 19 statistical techniques and was built using weighted median consensus (Marmion *et al.,* 2009): Generalized linear model (Glm), Random forest (Rf), Flexible and discriminant analysis (Fda), Multiple Discriminant Analysis (Mda), Model occurrence probability using presence-only data (Maxlike), Lasso and Elastic-Net Regularized Generalized Linear Models (Glmnet), Multivariate Adaptive Regression Splines (Mars), Boost regression trees (Brt), Recursive Partitioning and Regression Trees (Rpart) and Maximum entropy (Maxent).

All models were calibrated using 70% of random occurrence data and the performance of each model were evaluated against the remaining 30% of the data (Boavida-Portugal *et al.*, 2018; Roy *et al.*, 2020). Beyond creating independent or at least partially independent sets for model calibration and validation, partition also allows to take data uncertainty into account (Hawkins *et al*. 2008). The procedure was repeated 50 times and final ensemble was built using 100% of the data, as data partitions have been shown to add significant uncertainty to forecasts (Araújo *et al.,* 2009). Data processing was performed using SDM package (Naimi & Araujo, 2016) in R program (R Development Core Team, 2010) version 3.2.2.

2.5 Definition of conservation priorities

The results of our ensemble forecasting model, the map with the probabilities of occurrence of the target species, and the confirmed occurrences of *G. aculeatus* in mainland Portugal, were used to define the base criteria for creating 4 levels of river stretch conservation priorities, being these:

- Level 0 (no conservation interest): river stretches belonging to watersheds where *G. aculeatus* presence was not confirmed and in watersheds with presence of the species but in river stretches presenting an occurrence probability of less than 20%;
- Level 1 (moderate conservation priority): river stretches with absence of the species but belonging to watersheds where the presence of species is confirmed and where the occurrence probability ranges between 20% and 40%;
- Level 2 (high conservation priority): river stretches with absence of the species but belonging to watersheds where the presence of specie is confirmed and where the probability of occurrence is above to 40%;
- Level 3 (maximum conservation priority): river stretches with confirmed presence of *G. aculeatus*, with geographical limit, downstream and upstream of river, defined by a probability of occurring less than 10%.

Some of the confirmed presences of threespine stickleback occurred at isolated sites, mostly located in upstream river sections of southern river basins, surrounded by species absences, and thus, low or null probability areas. As this isolated populations are of the uttermost importance for species conservation, and otherwise defined protection sites would be of residual extension and poorly effective, we opted for a very conservative approach to the protection of the species, by limiting maximum priority areas only at 10% probability. Moreover, when confirmed occurrences of the species were in null probability areas (black areas in probability map), usually in upstream very small tributaries, we defined the whole stretch as of maximum conservation priority.

In some cases, the same water line had different probabilities of occurrence, which would lead to different levels of conservation priority being assigned to it. However, the definition of several priority levels in relatively small watercourses is neither practical nor effective conservation units, so the level of priority assigned corresponded to the level that had the greatest extension in that waterline.

Although these do not belong to any specific level of conservation priority, river stretches that still maintain connectivity between level 3 areas and the sea have also been highlighted as sections where particular attention should be given to the maintenance of longitudinal connectivity, due to the probability, not yet confirmed for Portugal, that this species can develop an anadromous form (Cabral *et al.,* 2005). Medium-to-large reservoirs, resulting from the existence of dams and similar impassable structures, were superimposed to the probability and conservation priority maps and defined as sites of null probability of occurrence with no conservation interest, regardless of the probability of occurrence obtained by the final ensemble. These are habitats where waterflow was interrupted, with lentic characteristics and dominated by introduced species, some of them piscivorous, and so, considered by expert judgement as places with not conducive conditions to the occurrence and survival sticklebacks.

3. Results

3.1 Distribution of *G. aculeatus*

Data both from databases and sampling campaigns comprised our final dataset with 646 points. However, the presence of threespine stickleback was detected only at 7.9% (presences=51) of the total sampled sites (Fig. 6). The confirmed presences of threespine stickleback were mainly observed close to the Portuguese coast, specifically in Minho, Âncora, Lima, Cávado, Vouga, Mondego, Lis, Tagus, Sado and Mira basins. Based on our data, a wider distribution of stickleback was observed in the Vouga basin, where about 37% of its overall presences was found.

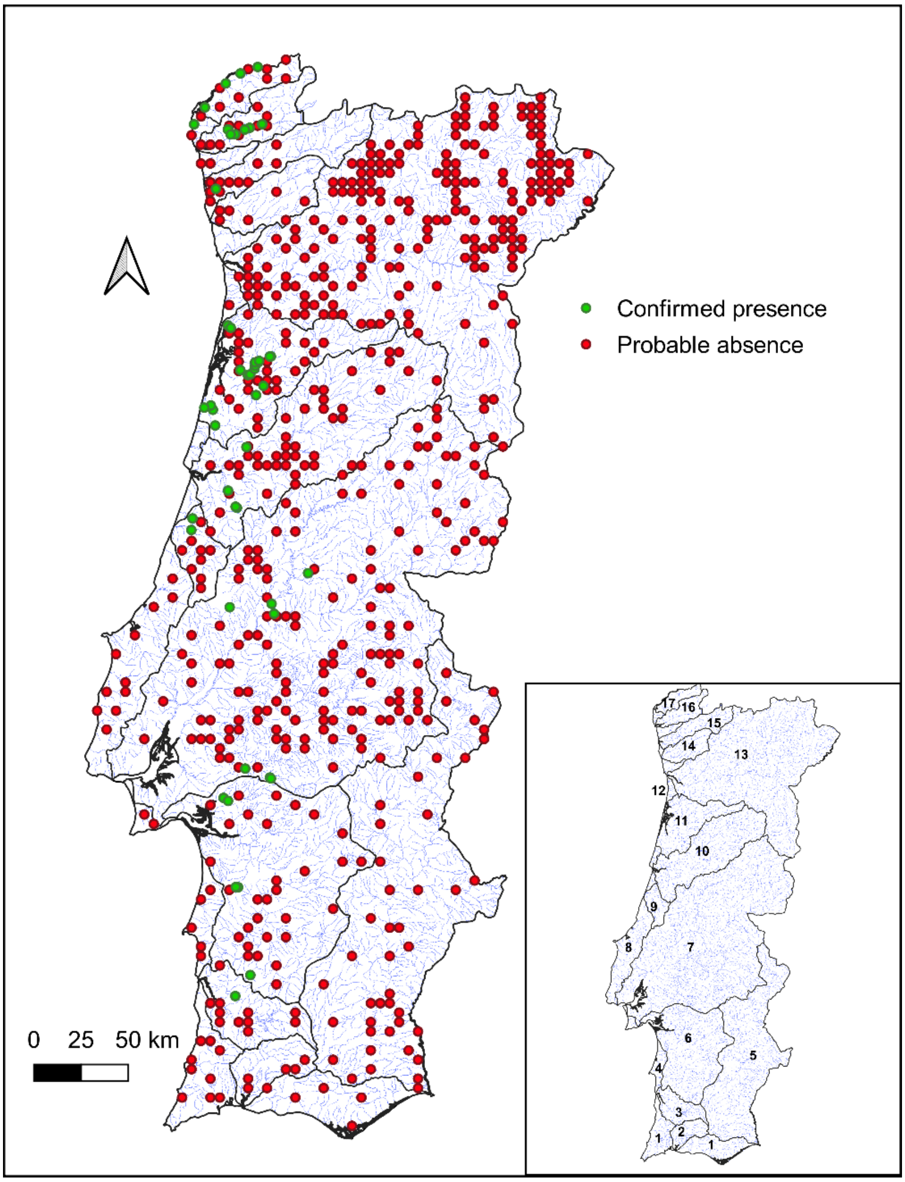

Fig. 6 Distribution of the sampling sites (N =646) according the presences and absences observed for the target species. Green circles indicate sites where the presence of *G. aculeatus* was confirmed, and red circles probable absence. The map in the lower right corner of the figure shows the location of the main Portuguese river basins: (1) small independent streams of Algarve, (2) river Arade, (3) river Mira, (4) small independent streams of Alentejo, (5) river Guadiana, (6) river Sado, (7) river Tagus, (8) small independent systems of Oeste, (9) river Lis, (10) river Mondego, (11) river Vouga, (12) small independent streams between Douro and Vouga river basins, (13) river Douro, (14) river Ave, (15) river Cávado, (16) river Lima and (17) river Minho.

3.2 Relationship between *G. aculeatus* occurrence and environmental predictors

Through Pearson's correlation coefficient, we understand how the variables are correlated with the presence of the species. Knowing that when coefficient of determination (R^2) = 0, there is no linear relationship between the variables, and the relationship becomes stronger as the absolute value of r increases and ultimately approaches a straight line as the coefficient approaches –1 or +1 (Schober *et al.,* 2018), we can conclude that the variables with the stronger relationship with the presence of the species are the "slope" (*r*= - 0.27) and the "f_accum" (*r*= - 0.26), suggesting that about 27% of presences can be explained by the relationship with the "slope" and 26% explained by the relationship with de "f_accum" (Tab. 2)

According to our model results, distribution of *G. aculeatus* is mostly defined by river geomorphology ("slope" and "sand") and hydrology ("f_accum" and "f_weigth_rain") and by climate ("precip" and "precipdrie") (Fig.7). The selected set of environmental variables and the selection of models with TSS values greater than 0.6, allowed the development of a final ensemble with a very good performance, with a mean TSS of 0.68 (±0.05). According to TSS values of all models, "brt", "maxent" and "rf" were the models with the best performance, with TSS values of 0.74, 0.72 and 0.75 respectively. The variables that had higher influence on the distribution and occurrence of *G. aculeatus* were the geomorphological variables, with the "slope" contribution being around 49.5% and the "sand" with 9.5% of contribution (total of 59% of contribution). The contribution of the remaining most important variables were: "precipdrie" with 23.9% of contribution; "precip" with 15.5%; "f_weight_rain" with 8.4% and "f_accum" with 6.6% of contribution.

Figure 8 shows the response curves of the target species occurrence with the variation of the six more important predictors. According to the response curves, we can conclude that threespine stickleback presence is mostly associated to river segments combining slopes lower than 150°, from which the probability of occurrence begins to decrease. The occurrence of this species is also associated to habitats with predominant substrates of sand, low values of average annual precipitation, but high values of precipitation in the driest month, occurring mainly in areas where these variable values are around 20 and 30 mm. The model and resultant curves also demonstrate a preference of the species for sites with higher flow, but not necessarily for rivers with larger drainage areas, which reflects a dependence of the species from the local climate, especially in what concerns to water availability.

Table 2 Values for Pearson correlation between all environmental variables and the presence of the species. NS no significant correlation; *Correlations significant at the 95 %; **correlations significant at 99 %; correlations significant at 99.9%.

Variables	**Code**	***r***	**p-value**
Altitude (m)	altitude	-0.11	**
Slope (°)	slope	-0.27	***
Distance to coast (m)	dist_coast	-0.16	***
Silt (%)	silt	0.097	*
Sand (%)	sand	0.032	NS
Annual mean temperature (°C)	tempmean	0.087	*
Max Temperature of warmest month (°C)	tempmax	-0.16	***
Annual precipitation (mm)	precip	0.042	NS
Precipitation of driest month (mm)	precipdriest	0.081	*
Flow accumulation (no. of cells)	f_accum	-0.26	***
Flow weight with rainfall (no. of cells)	f_weight_rain	0.13	***
WTI	wti	0.18	***
SPI	spi	0.0053	NS
Artificial surfaces (no. of cells)	use_art	0.11	**
Agricultural areas (no. of cells)	use_agr	0.043	NS
Forest and semi-natural areas (no. of cells)	use_forest	0.12	**
Population (n/km²)	populat	0.053	NS

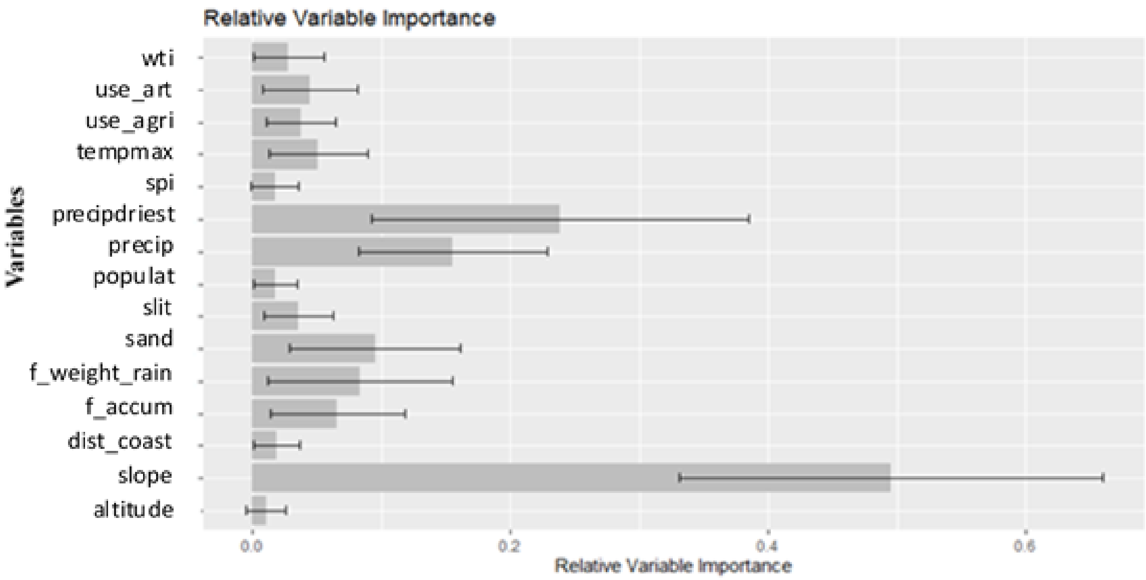

Fig. 7 Relative importance of each considered environmental variable to explain the distribution and occurrence of *G. aculeatus*.

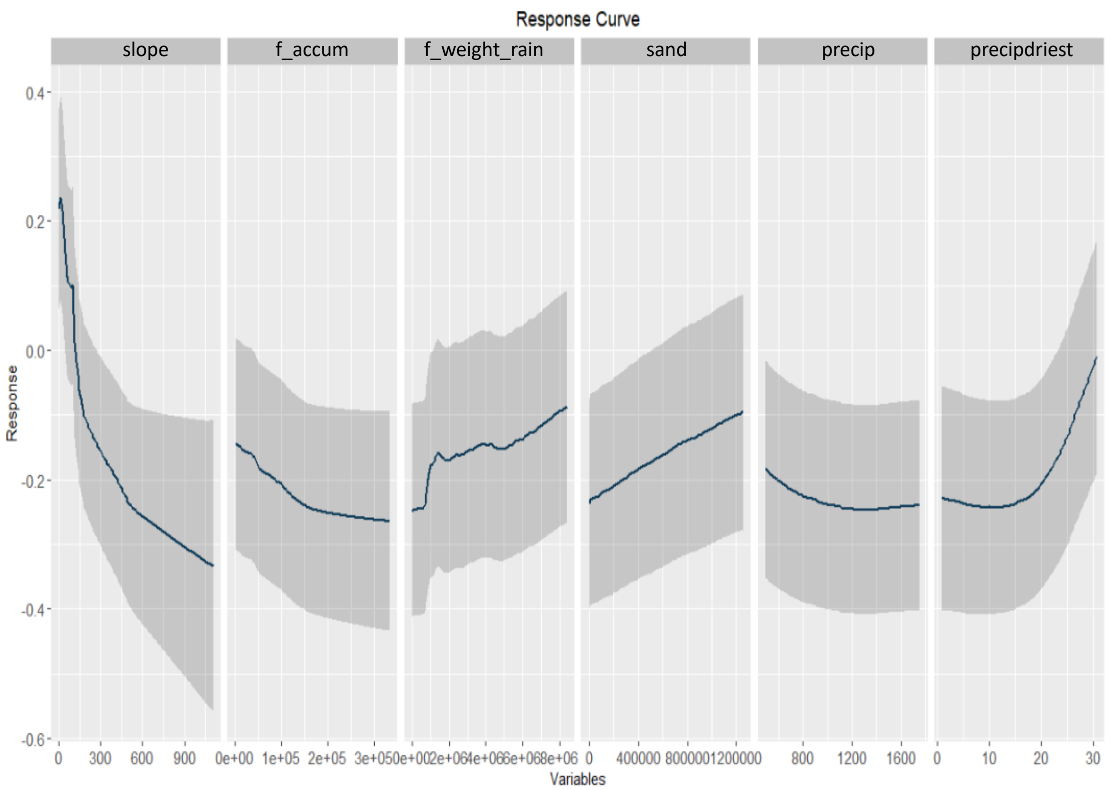

Fig. 8 Response curves for the variables that had higher influence on the species distribution and occurrence. It demonstrates how the probability of occurrence of *G. aculeatus* varies within the range of each variable.

3.3 Spatial predictions of *G. aculeatus* probability of occurrence

The probability of occurrence of *G. aculeatus* predicted for the mainland Portugal, according to our model, is displayed in Figure 9. The probability of occurrence of sticklebacks in the study area ranges from 0% to (≈)56%. Sites with confirmed occurrences of *G. aculeatus* had an average (± standard deviation) occurrence probability of 25.2% (± 20.1%). The highest values of occurrence probability were found in the northern region of mainland Portugal, and near the coast, in the hydrographic basins of the Minho, Lima and Vouga. The probability of occurrence was also moderately high (30%-40% probability of occurrence) in some watercourses located in basins where the species was not detected, for example in the Neiva and Ave river basins. The lowest probabilities of occurrence were in the east zone of the country, close to the border with Spain, and in the south, specifically in the Algarve region, where the probability of occurrence values rarely exceeded 0%.

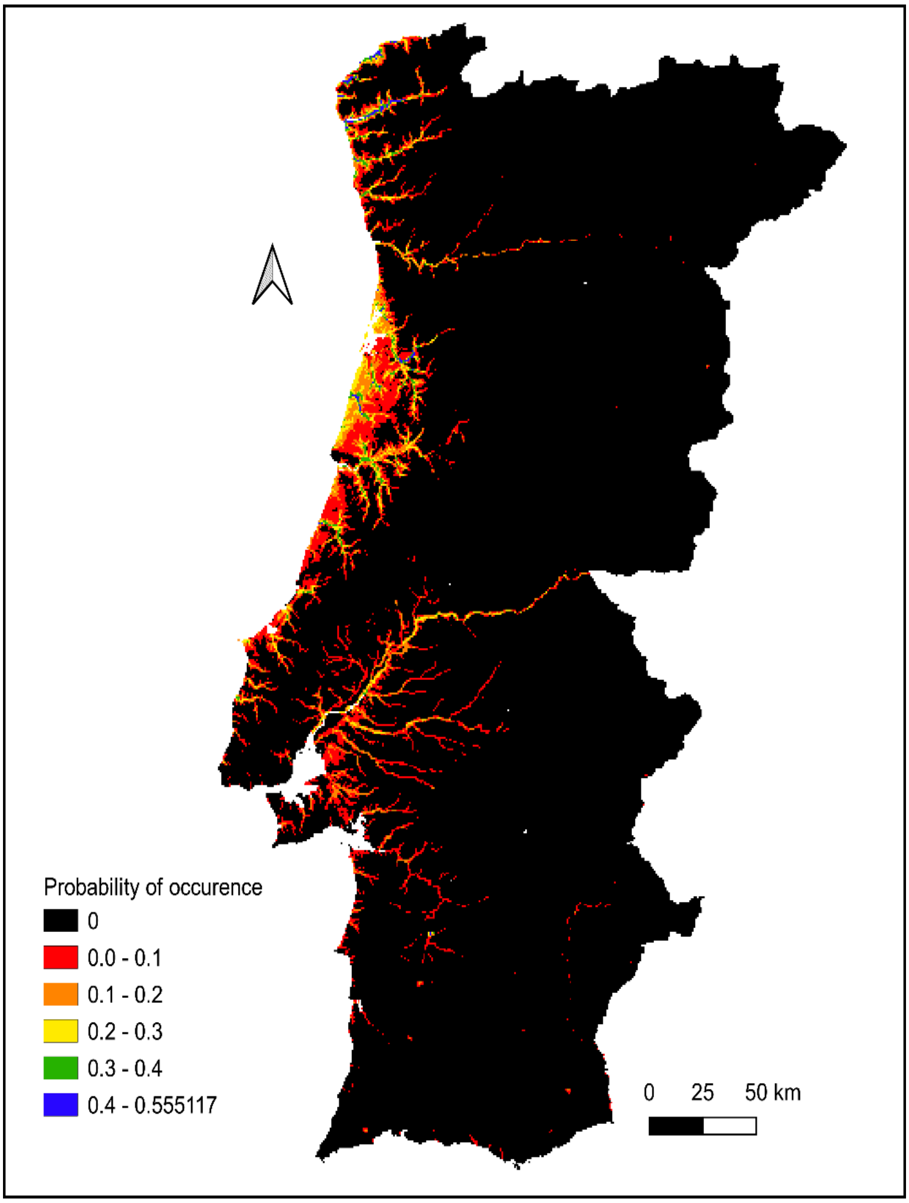

Fig. 9 Distribution of *G. aculeatus* probability of occurrence in mainland Portugal.

3.4 Map of conservation priorities

All rivers and streams of mainland Portugal and the levels of conservation priority for those where the species occur (or is projected to occur) are represented in Fig. 10. In total, priority conservation levels were assigned to about 1 021 km of river sections, which represents about 3.6% of the total length of Portuguese hydrographic systems. The conservation priority level that covered a greater number of kilometres of river sections was the maximum level, covering about 540 km (Table 3), followed by the moderate priority level that covered about

313 km and, finally, the high priority level, covering just 48 km. Due to the possible existence of the anadromous form of threespine stickleback and knowing that these fish need to migrate between marine/brackish and freshwater systems to complete its life cycle, and that even the smallest structure can represent an obstacle for this species, about 120 km of important river sections for the maintenance of longitudinal connectivity with the sea have been identified. At least one of the priority conservations levels has been assigned to almost all major river basins, except for the Douro, Guadiana and Ave basins, and also except some of the smaller independent streams that exist along the country littoral region, namely the Algarve, North and West streams. The Figure 10 also shows the distribution of reservoirs, resulting from the presence of dams and weirs, in mainland Portugal, in which the target species was considered, by expert judgement, to have null probability of occurrence.

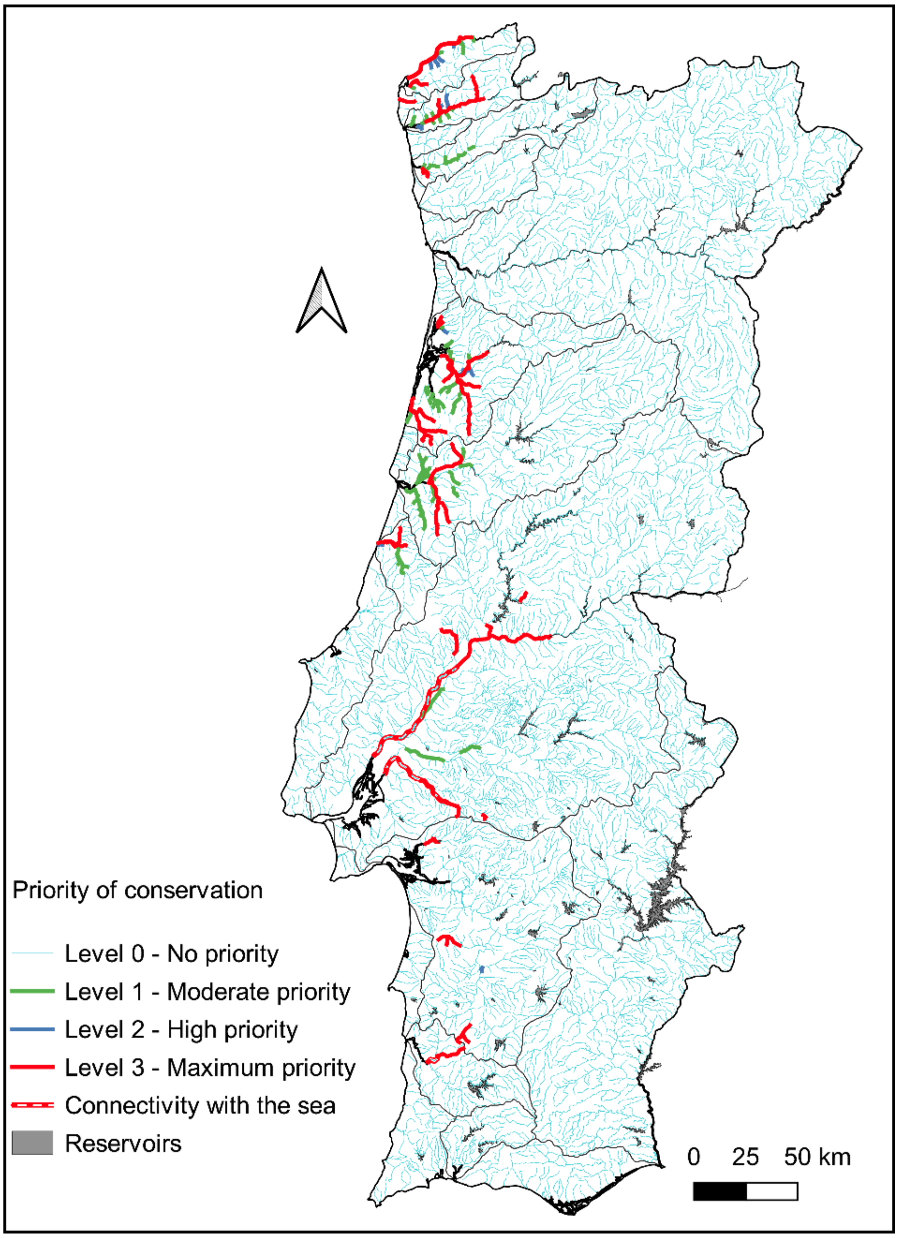

Fig. 10 Map of conservation priority for *G. aculeatus* in mainland Portugal following the criteria defined in Section 2 (Section 2.5). For the identification of the main river basins see Figure 6.

Table 3 Table with rivers categorized with the maximum level of conservation priority, number of kilometers covered, presence/absence of the species and the need for measures directed to the maintenance of connectivity with the sea.

Catchment	River	River stretch (km)	Presence / Absence	Maintenance of connectivity with sea
Mira	Pilriteiro stream	15	Presence	Yes
Sado	Gema stream	11	Presence	No
	Barranco Vale Rodrigo stream	6	Absence	No
	Grândola stream	17	Presence	No
	Fonte dos Narizes stream	4	Absence	No
	Marateca stream	8	Presence	Yes
Tejo	Almansor river	5	Presence	Yes
	Vale das Pegas stream	5	Absence	Yes
	Tejo river	57	Presence	Yes
	Almonda river	18	Presence	No
	Zêzere river	7	Presence	Yes
	Pisão stream	5	Presence	No
Lis	Lis river	9	Absence	Yes
	Carreira stream	6	Presence	Yes
	Leça stream	15	Presence	Yes
Mondego	Arunca river	28	Presence	Yes
	Malavenda stream	1	Absence	Yes
	Anços river	11	Absence	Yes
	Fornos river	17	Absence	Yes
	Ança stream	10	Presence	Yes
Vouga	Vale da Corujeira stream	27	Presence	Yes
	Arneiro stream	4	Absence	Yes
	Varziela stream	13	Presence	Yes
	Palhal stream	15	Presence	Yes
	Vouga river	36	Presence	Yes
	Águeda river	14	Presence	Yes
	Cértima river	30	Presence	Yes
	Negra stream	13	Presence	Yes
	Seixo stream	3	Presence	Yes
	Cáster stream	5	Presence	Yes
Cávado	Caveiro stream	1	Absence	Yes
	Milhases stream	2	Presence	Yes
	Outeiro stream	2	Absence	Yes
Lima	Lima river	32	Presence	Yes
	Vez river	13	Presence	Yes
	Estorãos river	8	Presence	Yes
Âncora	Âncora river	8	Presence	Yes
Minho	Minho river	48	Presence	Yes
	Coura River	11	Presence	Yes

4. Discussion

4.1 Influence of environmental variables on the distribution of *G. aculeatus*

The large survey conducted for this study demonstrated that in mainland Portugal, *G. aculeatus* is mostly distributed along the coast, from the Minho river, in the north of the country, to the Capelinha (or Pilriteiro) stream (Mira river basin) in the south. The map of probability of occurrence was consistent with the data collected during the field work. The areas with the highest probability of occurrence, were found close to the coast (less than about 40 km of the sea), from the central area of Portugal, in Mondego river basin, to the far north of the country, specifically on the Minho river. With the increasing distance to the coast, the probability of occurrence of the species decreases, being 0% in all the east zone of mainland Portugal, except for Tagus river, which presents a moderate probability of occurrence throughout its extension. The areas with the lowest probability of occurrence were in most cases, as we expected, those where no sticklebacks were captured, such as the Guadiana basin and Algarve streams. Stagnant water ecosystems, such as reservoirs, are avoided by threespine sticklebacks, because they have low and highly fluctuating levels of oxygen, especially near to the bottom, which are not conducive to the development of their eggs and juveniles (Clavero *et al*., 2009). In the case of the Douro basin, where no individual of *G. aculeatus* was ever captured, and because it's a river basin with a high number of large reservoirs (reservoirs = 7) (SNIRH, 2020), associated with the construction of dams and similar structures, we considered it as a watershed not favourable to the occurrence of the species, particularly in its main stem. Our study showed that the distribution of the threespine stickleback in mainland Portugal is explained mainly by six environmental variables: "slope", "percentage of sand", "precipitation of driest month", "annual precipitation", "flow accumulation" and "flow".

"Slope" is the strongest predictor to explain the distribution of *G. aculeatus* in the study area. The peak probability of occurrence of this species occurs at very low slope values and begins to decrease in areas with slope values greater than 150 °. As we can see in Figure 9 and Appendix I - 2, in the mountainous areas of mainland Portugal where the slope values are quite high, as in the interior areas of the northern and central regions of the country, and in the mountains of the Algarve, in the south, the probability of occurrence of threespine stickleback is 0%. On the contrary, along the coast, where slope values are low, the probability of occurrence reaches the highest values. "Slope" is a variable with an indirect effect on species distribution. This variable usually has a great influence on fish species distribution because it is correlated with many other variables, such as annual mean temperature, water flow, granulometric composition and, mainly, with altitude (Apaydin *et al*., 2010; Camana *et al*., 2016). Although “annual mean temperature” was removed from our analyses due to its collinearity issues, this are highly correlated with the slope (Appendix II - 1). With the increase in altitude, there is an increase in the slope and, consequently, a decrease in the "annual mean temperature" and in the water temperature of rivers (Erikson & Stefan, 2000). Water temperature is a determining factor in the distribution of fish species because influences spawning periods, growth rates and mortality of fish specimens (Cluis, 1972). Experimental work has indicated that most sticklebacks fails to reach sexual maturity when exposed to temperatures below 10° C (Baggerman, 1957; Borg & VanVeen, 1982; Crivelli & Britton, 1987). So, knowing that the threespine stickleback needs an ideal temperature between 14° C and 16° C, to reach sexual maturity and for the development of its eggs and juveniles (Mori, 1994; Alexandre & Almeida, 2009), it is expected that this species will tend to avoid rivers stretches with low water temperatures associated with steep slopes.

The slope of the river channel has a vital role in streamflow and in the water accumulated by the basins (Dubeux & Sollenberger, 2020), determining the hydrologic and geomorphologic characteristics of the river, and consequently influencing its specific richness of freshwater fish (Oberdoff *et al*., 1995; Mims & Olden, 2013; Camana *et al*., 2016; Cohen *et al*., 2018; Shumie, 2018). In our final ensemble model, two variables related with the river flow, that were used as indicators of the amount of water received in a basin (e.g., Ferreira *et al*., 2013), had an important influence in the distribution and occurrence of *G. aculeatus*, being these the flow ("f_wheight_rain") and flow accumulation ("f_accum"). The way in which these two variables influences the distribution of the target species cannot be interpreted and evaluated independently. The "f_accum" variable, which is influenced by altitude, allows the identification of river stretches or sites with the largest drainage basins. However, as we can conclude from the analysis of the graph in Figure 8, what most influences the occurrence of sticklebacks in mainland Portugal is the amount of water that the basin receives from precipitation (“f_weight_rain”) and not the dimension of the drainage basin only by itself. The increase in the probability of occurrence of this species with the increase of flow (“f_weight_rain) and with the increase of the “precipitation in the driest month”, which is also one of the variables with higher influence in the distribution of *G. aculeatus*, is an indicator that the species avoids rivers with temporary regimes, as usually can be observed in areas with typically Mediterranean characteristics (Bonada & Resh, 2013). These temporary rivers, which can mainly be found in the south of Portugal, even if they have large drainage basins, are not favourable to the occurrence and survival of *G. aculeatus*, due to the severe reduction in the amount of water and the consequent degradation of the aquatic habitat in the driest months (Alexandre & Almeida, 2009; Clavero *et al.,* 2009; Rind *et al.,* 2020). The few isolated populations of the target species that were found and persist in this type of temporary habitats (e.g., Sado and Mira basins) have probably found adaptations to survive in these adverse conditions (Crivelli & Britton, 1987; Rind *et al*., 2020), which remain to be properly studied (Foster *et al.,* 2003). Although this species tends to avoid temporary rivers, it also tends to avoid rivers with large drainage basins that can accumulate too much water, from high levels of "annual precipitation", and even too high levels of "f_weight_rain". In rivers with these characteristics, mainly in steep channel slopes, the effects of flash floods associated with this large amount of water, are more intense, and the current velocity is higher, potentially causing higher mortality and displacement of individuals, in absence of refuge. Downstream displacement occurs especially with individuals at earlier development stages (Harvey, 1987), or with species with low swimming performance (Chun *et al*. 2011), as it is the case of stickleback. Moreover, in habitats with these characteristics, rivers tend to have higher current speed, requiring greater energy expenditure by aquatic organisms. However, the stickleback prefers rivers with low current speed (Taylor & McPhail, 1986), to save energy for nest building and parental care (Page & Burr, 2011).

As mentioned above, the slope, influencing the water flow, will consequently influence the type of sediment and substrate of each section of the river. From our results, we observed that in mainland Portugal the percentage of sand is also one of the factors that most influences the distribution of threespine stickleback, corroborating other studies (e.g., Alexandre & Almeida, 2009) where individuals of this species were captured in habitats constituted mostly by sand and gravel. In this study, the probability of occurrence of *G. aculeatus* increases with increasing percentage of sand, probably because this species uses this smaller, and easier to dig, substrate as a refuge in the presence of predators and to build its nest (Mori, 1994; Alexandre & Almeida, 2009; Page & Burr, 2011). By comparing our map of probabilities of occurrence (Fig. 9) with our map of distribution of the percentage of sand (Appendix I - 5), we can see that the highest probability of occurrence occurs in places along the coast in the north of Portugal and in Tagus river, where the percentage of sand presents medium-to-high values.

4.2 Prioritizing conservation of *G. aculeatus*

The scientific community claims that the threespine stickleback, among other fish species, took refuge in the fresh waters of the Mediterranean during the Pleistocene glaciations, and that its isolation during this period allowed the species to reach large levels of genetic variability, making their populations quite important for evolutionary studies, as they are genetically different from all others present in Europe (Araguas *et al*., 2011; Rind *et al*., 2020). However, despite their ecological and conservationist value as a native species and importance as model species for scientific studies, populations of threespine sticklebacks in the Iberian Peninsula are declining due to various types of threats, such as the introduction of non-native fish species, pollution and human activities (e.g. such as water abstraction or stream flow regulation) (Foster *et al*., 2003; Clavero *et al*., 2004; Cabral *et al.,* 2005; Araguas *et al*., 2011). Its critical situation is also exacerbated by the Mediterranean climate, with alternate dry and wet periods with unpredictable flash floods (Gasith & Resh, 1999).

Although these threats are known by the scientific community, the lack of knowledge regarding distribution and ecological requirements of the species has been an obstacle to the definition of effective conservation measures for *G. aculeatus*. However, with the information obtained in this study, it is now possible to properly address this issue and define hot spots for the conservation of threespine sticklebacks in Portugal. First, with our maps of presence sites and projected distribution for *G. aculeatus*, we can conclude that most of the confirmed presences and the places with the highest probability of occurrence are found in places not covered by the National Network of Protected Areas of Portugal (ICNF, 2018), so the majority of the areas presented here as potentially important and of priority for the conservation of this species, are not covered by any type of legal protection (ICNF, 2018).

To protect this species, we propose the definition and creation of Special Areas of Conservation (SAC) that cover the areas defined in this study as having a high probability of occurrence. To show which aquatic ecosystems are in most need of conservation measures, we categorize the different rivers with four different conservation priority levels, depending on the confirmed occurrences and the probability of occurrence obtained in the final ensemble. The maximum priority conservation level was assigned to all sections of the river where the species was captured, even if the probability of occurrence given by the final model to these locations was 0%. The populations residing in these areas with low or null probability, as observed in the Sado and Mira basins and in some tributaries of the Tagus river (streams with Mediterranean characteristics), are isolated populations that probably have developed adaptations to survive the harsh environment during the summer. The conservation of these populations is of the uttermost importance, because they can represent alternative strategies and genetic profiles distinct from all other populations (Araguas *et al*., 2011; Rind *et al*., 2020), so they should be effectively protected in order to conserve the evolutionary heritage of the stickleback adaptive radiation (Foster *et al.,* 2003; Clavero *et al.,* 2009). Also, despite the attempt to cover all the geographic area of mainland Portugal it is possible that we did not capture all the environmental space where the species occur, so the final model might be underestimating the probability of occurrence in these areas (Bystriakova *et al*., 2012). The limit of the maximum level of conservation priority ends when the value of the probability of occurrence in these stretches becomes less than 10%, that is, the area of maximum conservation priority in a river, where the presence of the species has been confirmed, only ends when the habitat has few or none environmental conditions to support the species, which clearly represents a conservative approach to the problem, but that we consider essential for the effective protection of this species and their habitats, specially of the southern isolated populations.

Associated with the highest conservation priority level, a special level of conservation protection has been created for rivers that, although might have low values of probability of occurrence, are important for the maintenance of the connectivity with the sea. This connection with the marine environmental is important for the anadromous form of this species, which potentially can occur on the study area, and needs to move freely between freshwater, estuarine and marine environments to complete its life cycle (Taylor & McPhail, 1986; Raeymaekers *et al*., 2005; Arai *et al.,* 2020). Independently of the presence or absence of the anadromous sticklebacks in Portuguese river basins, implementing conservation measures in downstream sections of these watersheds directed to the maintenance of longitudinal connectivity between freshwater and marine habitats, will improve the conservation of aquatic ecosystem in general. In particular, this type of measure will also help the migration and completion of the life cycle of other endangered diadromous species that share similar occurrence areas with *G. aculeatus,* such as the anadromous river lamprey (*Lamprey fluviatilis* L.) (Ferreira *et al*., 2009), in the Tagus river, or allis shad (*Alosa alosa* L.) (Mota & Antunes, 2011), the sea trout (*Salmo trutta* L.) and the Atlantic salmon (*Salmo salar* L.) in the northern and central river basins of Portugal (Almeida *et al.,* 2018).

The high and moderate priority conservation levels were created to categorize the rivers that have no presence confirmed but that have environmental characteristics conducive to the existence and survival of the species. Supposedly, these river stretches present environmental conditions capable of supporting the existence of threespine stickleback populations, therefore, in basins where the species is present, if there is a need to restock some rivers with sticklebacks, these would be the areas signalled in this study as having high and moderate conservation priority levels. Also, as environmental conditions are expected to vary due to climate change, these areas might become important refuges in future scenarios.

The anadromous form of threespine stickleback is thought to occur in Portugal, although its presence was never confirmed, which is probably related with the lack of studies directed to this objective, based on an adequate selection of sampling areas (e.g., downstream brackish sections of main rivers and respective tributaries, coastal lagoons) and methods (e.g., minnow traps, hand nets) (Karve *et al.,* 2008; Arai *et al*., 2020). Therefore, we suggest the development of specific sampling campaigns, carried out with other fishing techniques and including a genetic component directed to the confirmation of the occurrence of anadromous form of *G. aculeatus* in mainland Portugal. Although the results obtained in this study can be used *per se* in the definition of conservation measures for this species, it is necessary to consider the possibility of carrying out further studies in the future, to corroborate these results. The fishing data are subject to several sources of uncertainty, since not capturing individuals in one location does not mean that the species does not exist in that location, so our study may have underestimated the presence data, distorting our results. In addition, sometimes there may be undeclared catches, uncertainty in species identification, and incorrect use of capture techniques due to a lack of knowledge of species ecology, among others. As much as these sources of uncertainty have been reduced, the difference in the amount of data between presences and absences of the species was very high, so the area where the probability of occurrence is very low may have been overestimated. In addition to the uncertainties associated with occurrence data, sometimes the lack of more recent information for some environmental variables can also cause a slight bias in results, such as the lack of more recent data for land use. Future efforts should also focus on addressing these topics to improve the projections of species distribution models, with the aim of corroborating and even improving the conclusions obtained in this study.

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3. Considerações finais

Neste estudo, a utilização de modelos de distribuição de espécies (SDM's), forneceu informações importantes sobre a distribuição e preferências de habitat do esgana-gata, uma espécie nativa em Portugal Continental, classificada como *Em Perigo* (EN) no Livro Vermelho dos Vertebrados de Portugal Continental (Cabral *et al.,* 2005), que podem servir de base para a criação e implementação de medidas de conservação para alguns rios em território nacional. Neste estudo, o modelo de previsão por *ensemble* utilizado, que combina ponderadamente as projeções de todos os modelos estatísticos (Araújo & New, 2007; Marmion *et al*., 2009; Boavida-Portugal *et al*., 2018; Roy *et al*., 2020), permitiu identificar as principais variáveis ambientais que influenciam a distribuição do esgana-gata em Portugal Continental, sendo estas: o declive, a percentagem de areia, a precipitação máxima média do mês mais seco, a precipitação média anual, o escoamento e o fluxo acumulado de drenagem. Em geral, o esgana-gata encontra-se preferencialmente em locais com declives suaves, onde predominem substratos de areia, e os níveis de precipitação no mês mais seco e de escoamento sejam elevados. O facto de os altos níveis de precipitação no mês mais seco, e elevados níveis de escoamento, estarem relacionados com o aumento da probabilidade de ocorrência da espécie, demonstra que o esgana-gata tende a evitar rios de regime temporário, ou seja, rios que possam secar, parcial ou totalmente, durante os meses mais secos. Acredita-se que a preferência por rios em que predomine um substrato arenoso esteja relacionada com a maior facilidade da espécie em "escavar" este substrato, para se refugiar de predadores. Além disso, este tipo de substrato permite uma maior oxigenação dos ovos (Alexandre & Almeida, 2009; Page & Burr, 2011). Ao contrário destas variáveis, o declive está inversamente correlacionado com a probabilidade de ocorrência da espécie, e tem uma influência indireta na sua distribuição, pois está correlacionado com outras variáveis, como a altitude, a temperatura média anual e a velocidade de corrente dos rios (Apaydin *et al*., 2010; Camana *et al*., 2016; Dubeux & Sollenberger, 2020). A espécie tende ainda a evitar rios com elevado escoamento acumulado resultante da área de drenagem, em locais onde a precipitação média anual seja muito elevada, possivelmente devido ao aumento da probabilidade de ocorrerem inundações nestes locais, e ao possível aumento das velocidades de corrente em rios afetados por estas variáveis (Taylor & McPhail, 1986; Page & Burr, 2011). Posto isto, podemos concluir que a espécie demonstra preferência por rios com níveis de caudal e fluxo de água intermédios, tendendo a evitar rios com grandes bacias de drenagem que possam levar a um aumento extremo do caudal, e também rios de regime temporário, que sequem parcial, ou totalmente, durante os meses mais secos. Além disso, observou-se a inexistência da espécie em rios com elevado número de barreiras intransponíveis (p.e., barragens e açudes), e respetivas albufeiras, observáveis por exemplo ao longo da bacia do rio Douro. As barreiras intransponíveis, para além de influenciarem o caudal do rio, têm impactos adversos na desova dos peixes nativos e na sua alimentação, e fazem com que este tipo de habitats seja dominado maioritariamente por espécies não-indígenas (Minckley, 1995; Arthington *et al.,* 2016; Coleman *et al.,* 2018), como o perca-sol (*Lepomis gibbosus*) e o achigã (*Micropterus salmoides*).

Posto isto, podemos concluir que é próximo da zona costeira, na região centro e norte do país, que se encontram as características ambientais mais favoráveis à existência do esgana-gata, pelo que a maioria dos locais de máxima prioridade para a conservação da espécie foram definidos nessas regiões. A bacia do rio Vouga foi a bacia mais abrangida pelo nível de prioridade máxima, tendo sido categorizados cerca de 160 km de rios, e ribeiras, com este nível. Com exceção da bacia do Tejo, à qual também foi atribuído o nível de prioridade máxima de conservação a uma parte significativa da sua extensão (cerca de 97 km), a maioria dos níveis de prioridade máxima de conservação encontram-se distribuídos pelas bacias da região norte e centro do país (Fig. 10). Pelo contrário, nas bacias a sul do Tejo, foram poucos os cursos de água classificados como sendo de prioridade máxima para a conservação da espécie, abrangendo uma extensão de apenas 61 km. Contudo, embora as características ambientas favoráveis à existência da espécie se encontrem maioritariamente na região norte e centro do país, as populações encontradas na região sul, nas bacias do Mira e do Sado, que ocorrem, principalmente, em rios de regime temporário, são de extrema importância para a conservação da espécie. As poucas populações registadas nesta região, encontram-se em habitats isolados e fragmentados, tendo estas desenvolvido ao longo dos anos, estratégias de vida que lhes permitem residir nas condições adversas encontradas nesses locais (Crivelli & Britton, 1987; Arthington *et al.,* 2016). As adaptações adquiridas pela espécie para residir neste tipo de habitat, considerado inóspito à sua sobrevivência, podem fazer com que estas populações apresentem uma genética distinta de todas as outras populações da espécie (Araguas *et al.,* 2011; Arthington *et al.,* 2016; Kääriäinen *et al*., 2017). Contudo, o isolamento destas populações, juntamente com a sua pequena dimensão, faz com estas estejam mais vulneráveis a eventos estocásticos, como por exemplo longos períodos de seca extrema (Coleman *et al*., 2018), e ainda que estejam mais suscetíveis à perda de diversidade genética, devido ao "efeito de gargalo" (Fischer e Lindenmayer, 2007), o que também contribui para o aumento do seu risco de extinção, reduzindo o seu potencial de adaptação a novas ameaças (Coleman *et al*., 2018). Estas populações isoladas de esgana-gata, que ocorrem em locais com características totalmente desfavoráveis à sua existência, necessitam de medidas urgentes de conservação, para que a diversidade e o funcionamento dos ecossistemas aquáticos, nestes locais, se mantenham intactos e para que não se percam fontes genéticas importantes. Na impossibilidade de criar e implementar medidas de conservação para todos os troços definidos como sendo de prioridade máxima, e na eventualidade de ser necessário selecionar as populações que mais necessitam de medidas urgentes, é importante ter em conta a singularidade genética destas populações isoladas e a extrema dificuldade em repovoar estes habitats hostis com indivíduos da mesma espécie mas provindos de outras populações que, eventualmente, não estejam tão bem adaptados a estas condições adversas.

No futuro, é necessário que sejam realizados estudos que visem clarificar a possível existência da forma anádroma de *G. aculeatus* em Portugal Continental. Visto que, no decorrer deste estudo não foram capturados quaisquer indivíduos de ciclo de vida anádromo, num novo estudo deverão ser adotados métodos de amostragem distintos, como a realização de campanhas de amostragem em locais mais próximos da costa e com métodos de captura diferentes (p.e., armadilhas com isco) (Karve *et al.,* 2007). De modo a minimizar os custos para a conservação da espécie em geral, antes de serem implementadas medidas específicas para a conservação da forma holobiótica da espécie, seria benéfico confirmar a eventual existência da forma anádroma, para que, no caso desta forma migradora existir, serem definidos locais prioritários para a conservação paralela de ambas as formas. Ainda assim, para que a conservação da espécie não esteja dependente de possíveis estudos futuros, foi desenvolvido um mapa de locais prioritários de conservação, onde são indicadas as linhas de água que garantem a manutenção da conectividade com o mar, de modo a que possam ser protegidos os rios ou ribeiras importantes no trajeto migratório de algumas espécies migradoras, incluindo a possível forma anádroma de *G. aculeatus.*

A concentração de populações humanas, e das suas atividades, em torno dos sistemas de água doce e a crescente demanda por água, tem levado a que estes ecossistemas aquáticos (rios, ribeiras, lagos e albufeiras), sejam alvo de inúmeras perturbações e ameaças, como a poluição, represas e desvios de água, perda e fragmentação de habitat, entre outras, provocando declínios populacionais críticos em inúmeras espécies, e até, em casos extremos, eventos de extinção local ou global (Leidy & Moyle, 1998; Arthington *et al.,* 2016; Darwall e Freyhof, 2016). Visto que estas espécies são de extrema importância para a diversidade e funcionamento dos ecossistemas aquáticos e para a saúde, o bem-estar e as economias das sociedades em todos os domínios geográficos (Hughes, 2015), é necessário que mais estudos como este sejam realizados com a finalidade de obter conhecimentos mais aprofundados sobre a ecologia, preferências de habitat, biologia e evolução das espécies e respetivas populações, tendo em vista a criação e implementação de medidas viáveis para a sua gestão e conservação. Para além da proteção das espécies através da criação de reservas naturais ou por meio de legislação de proteção da própria espécie e do respetivo habitat, devem também ser direcionados esforços para o restauro dos habitats mais ameaçados (Arthington *et al.,* 2016).

Pese embora as campanhas de amostragem terem sido realizadas em 2019, o tratamento de dados e a redação do trabalho foi maioritariamente executada em 2020, ano no qual fomos assolados pela pandemia de Covid-19. As medidas de quarentena associadas à pandemia, provocaram o adiamento de algumas reuniões de carácter científico e fez com que estas decorressem por videochamadas, o que, por falta de prática sedimentada, se tornou menos prático na discussão de temas e no esclarecimento de dúvidas, do que as habituais reuniões presenciais. As restrições impostas no combate à pandemia, tornaram quase impossível o contacto pessoal, o que dificultou, também, a aprendizagem de alguns conceitos fundamentais, relativos principalmente à utilização de modelos estatísticos, pelo que, se não fosse o empenho dos vários membros da equipa de investigação MARE, este trabalho seria mais difícil de ser realizado.

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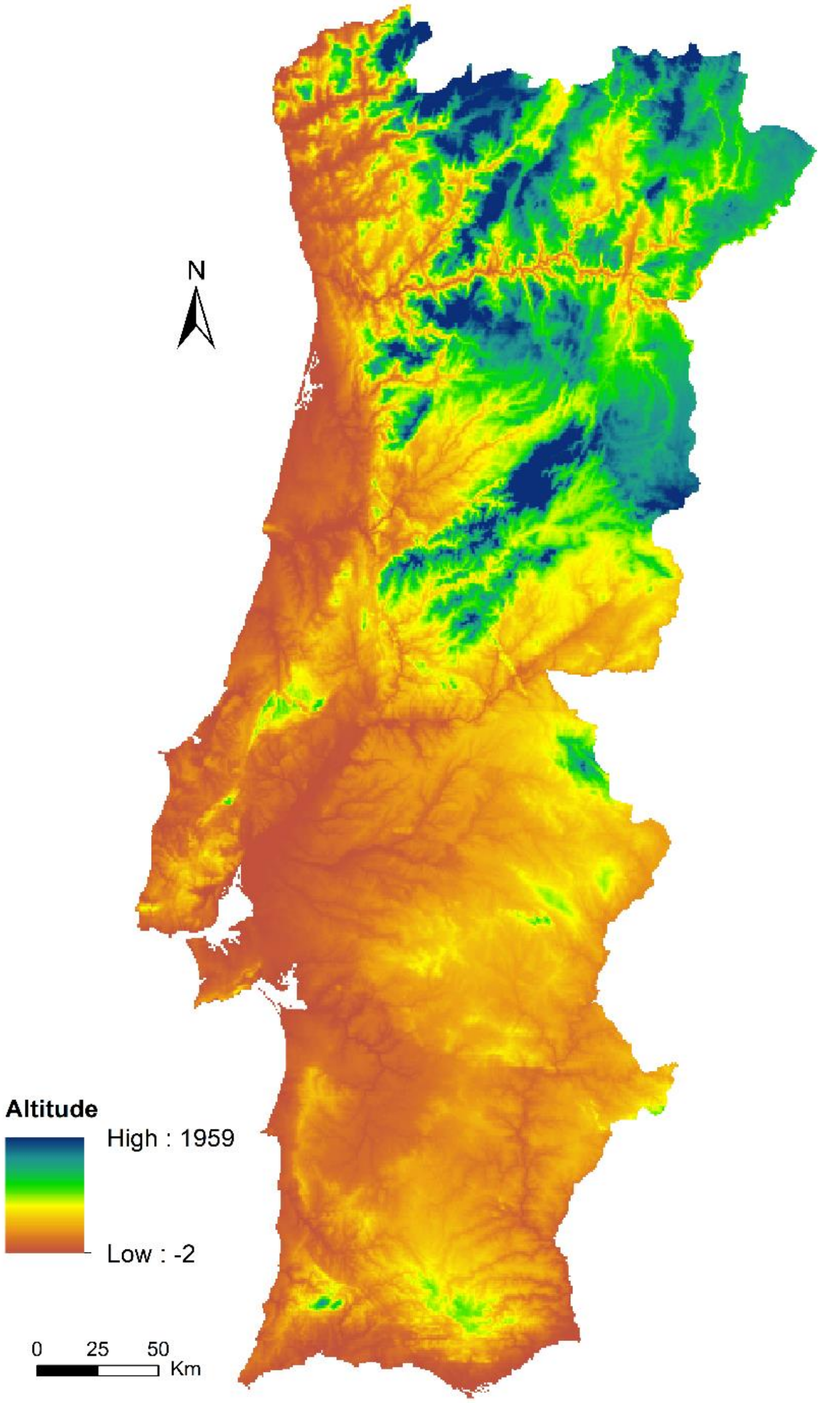
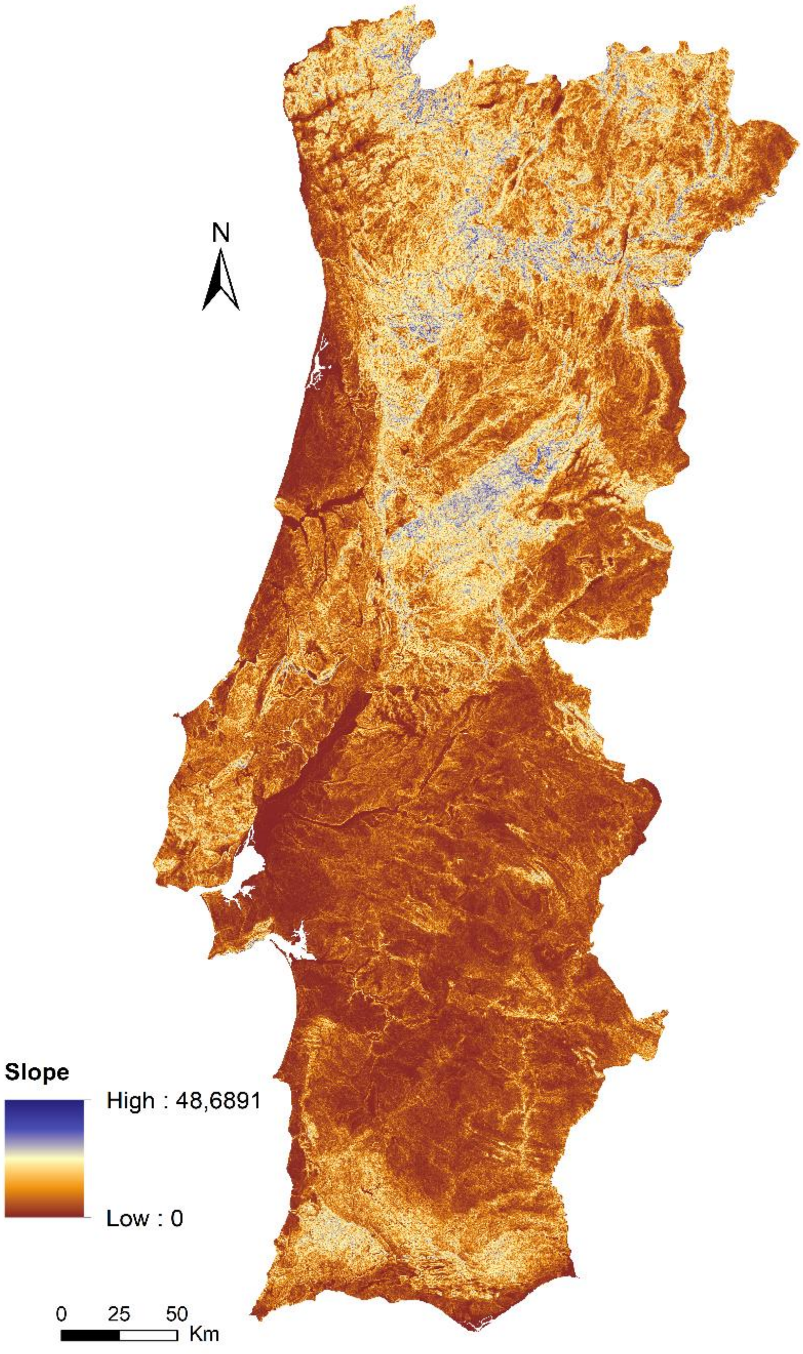
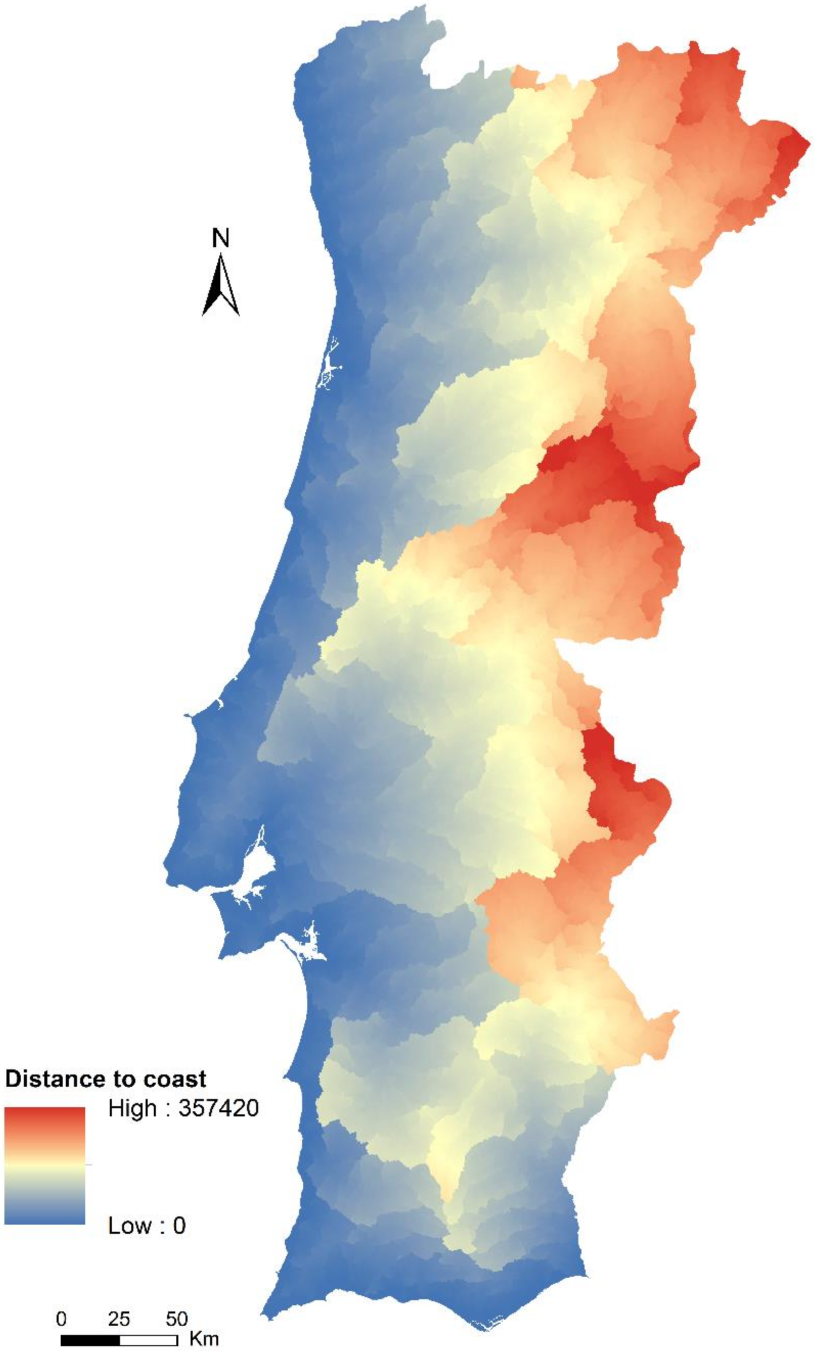
Appendix I - Maps for the study area of all the environmental predictors used for modelling the habitat of *G. aculeatus*



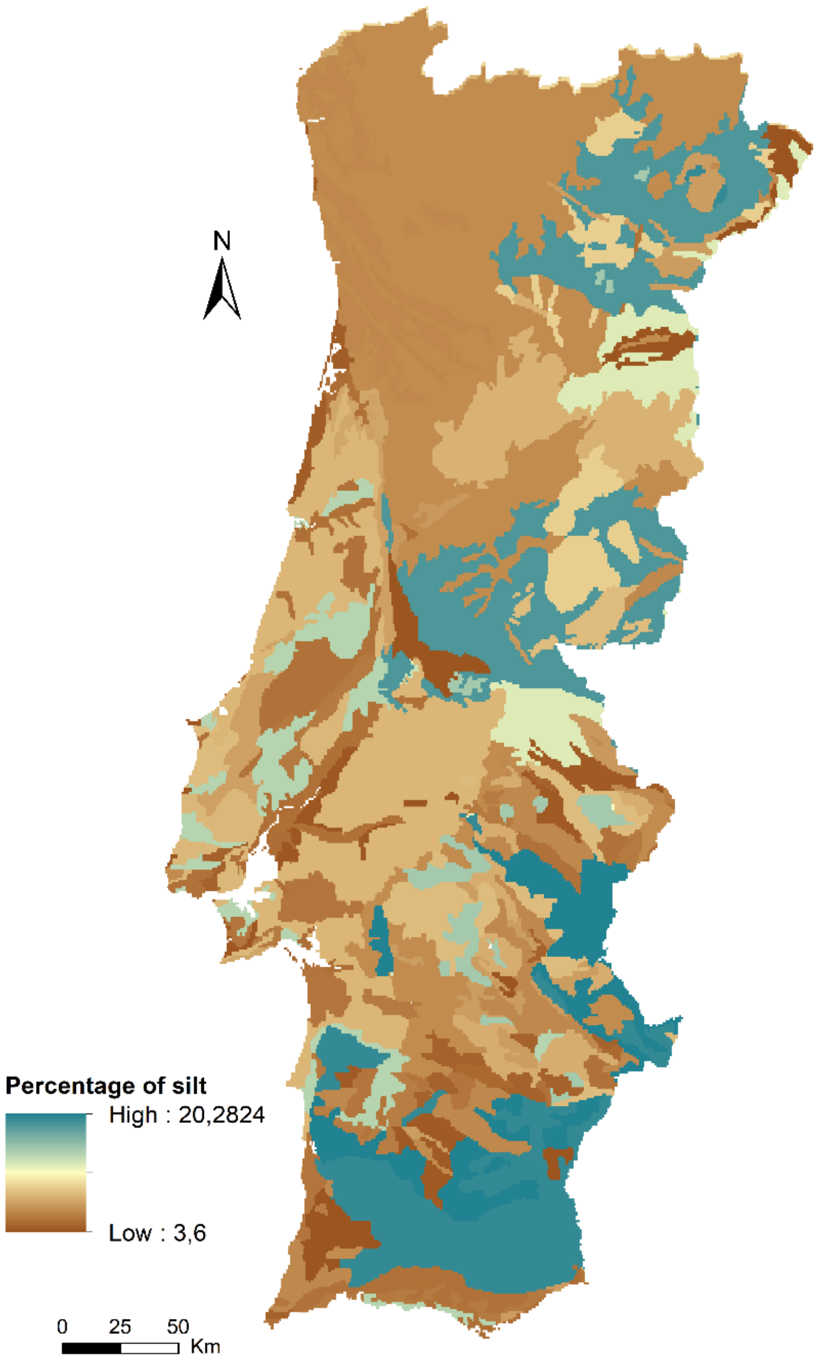
1 - Altitude (m)



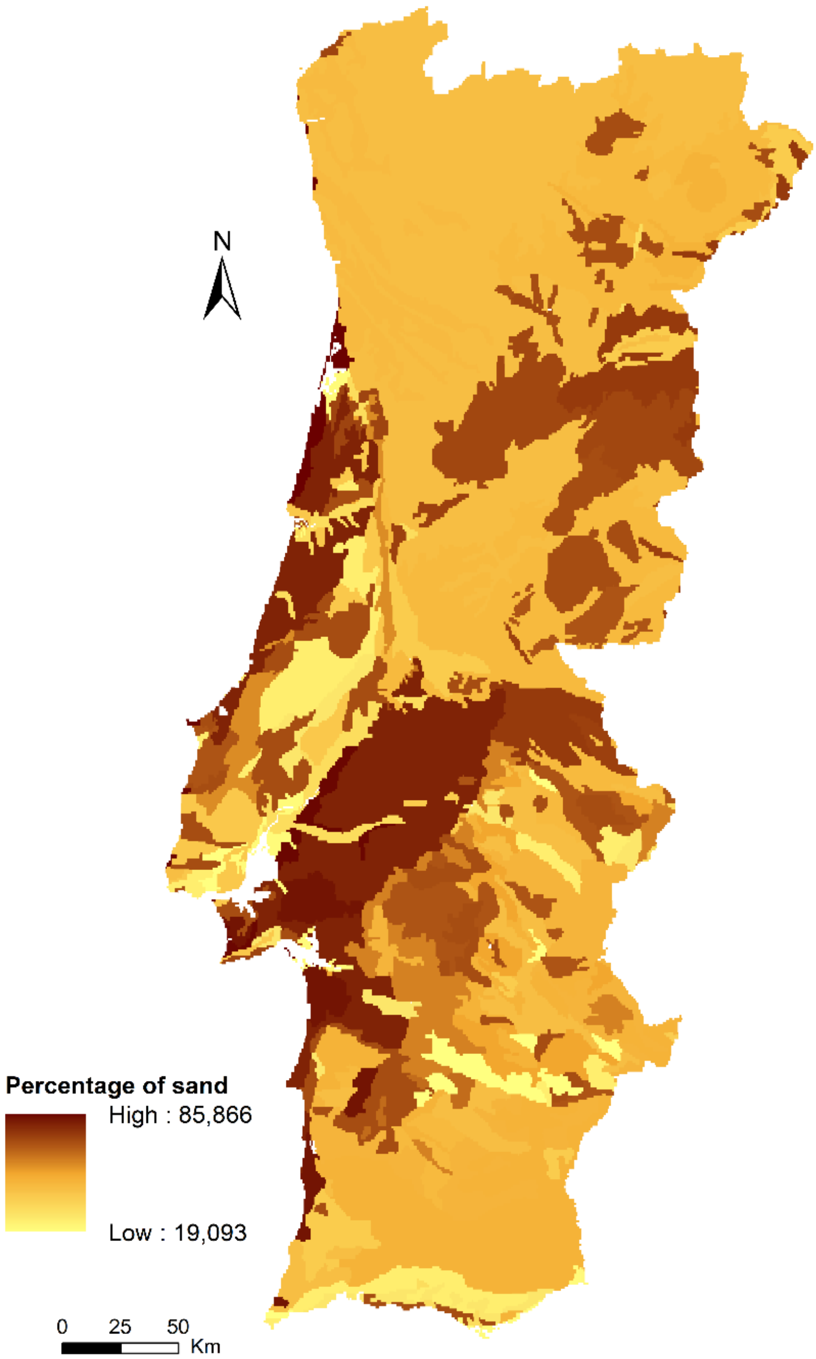
2 - Slope (°)



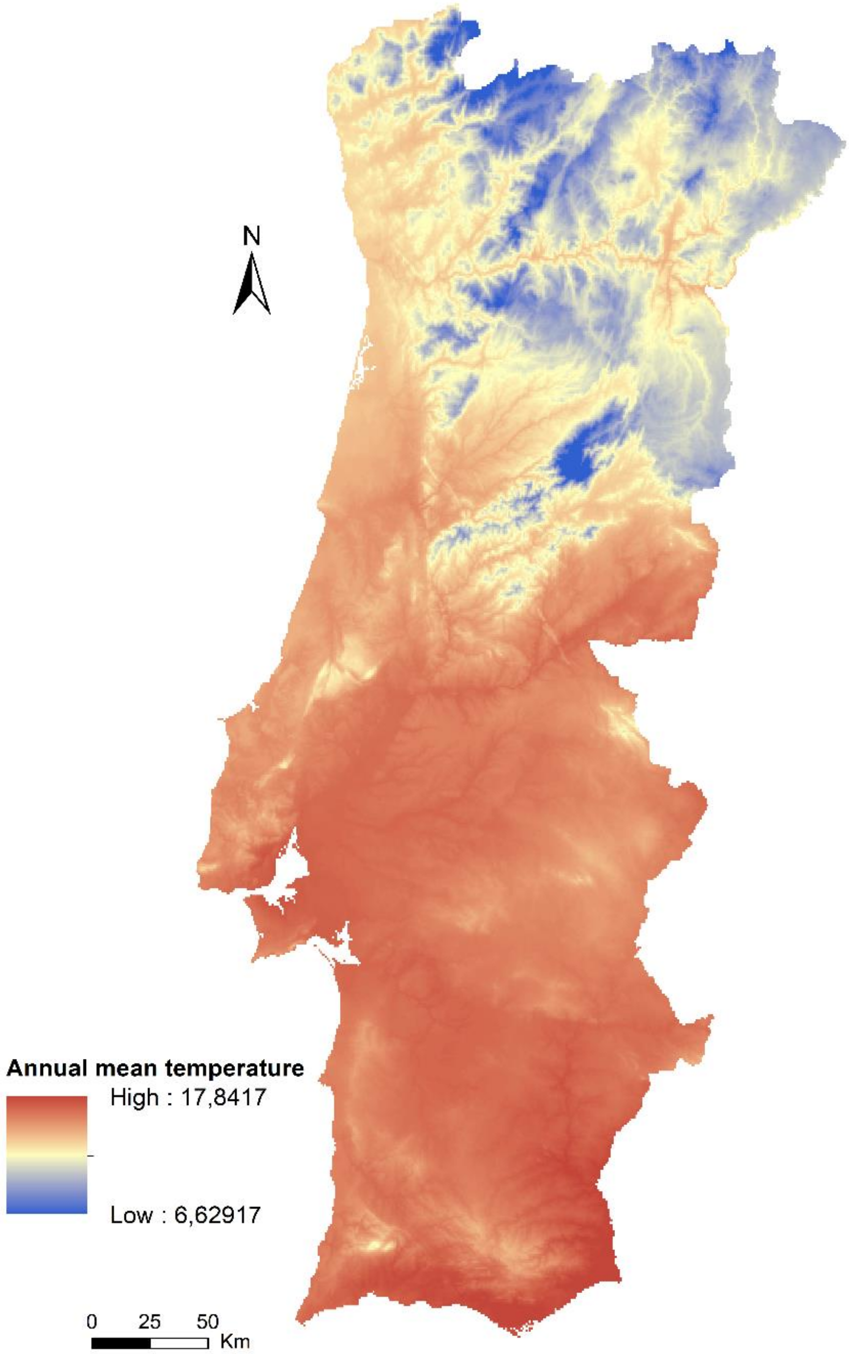
3 - Distance to coast (m)



4 – Percentage of silt (%)

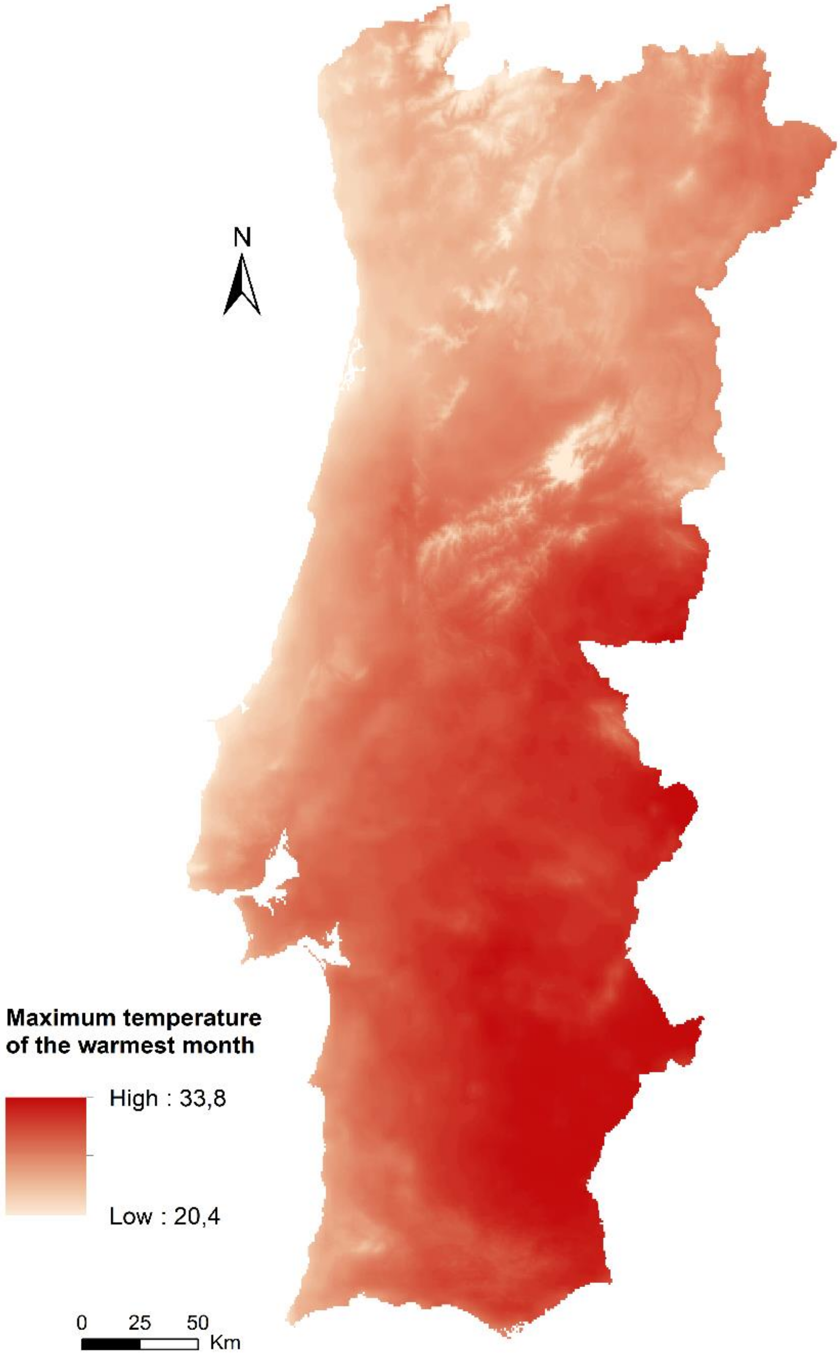


5 – Percentage of sand (%)

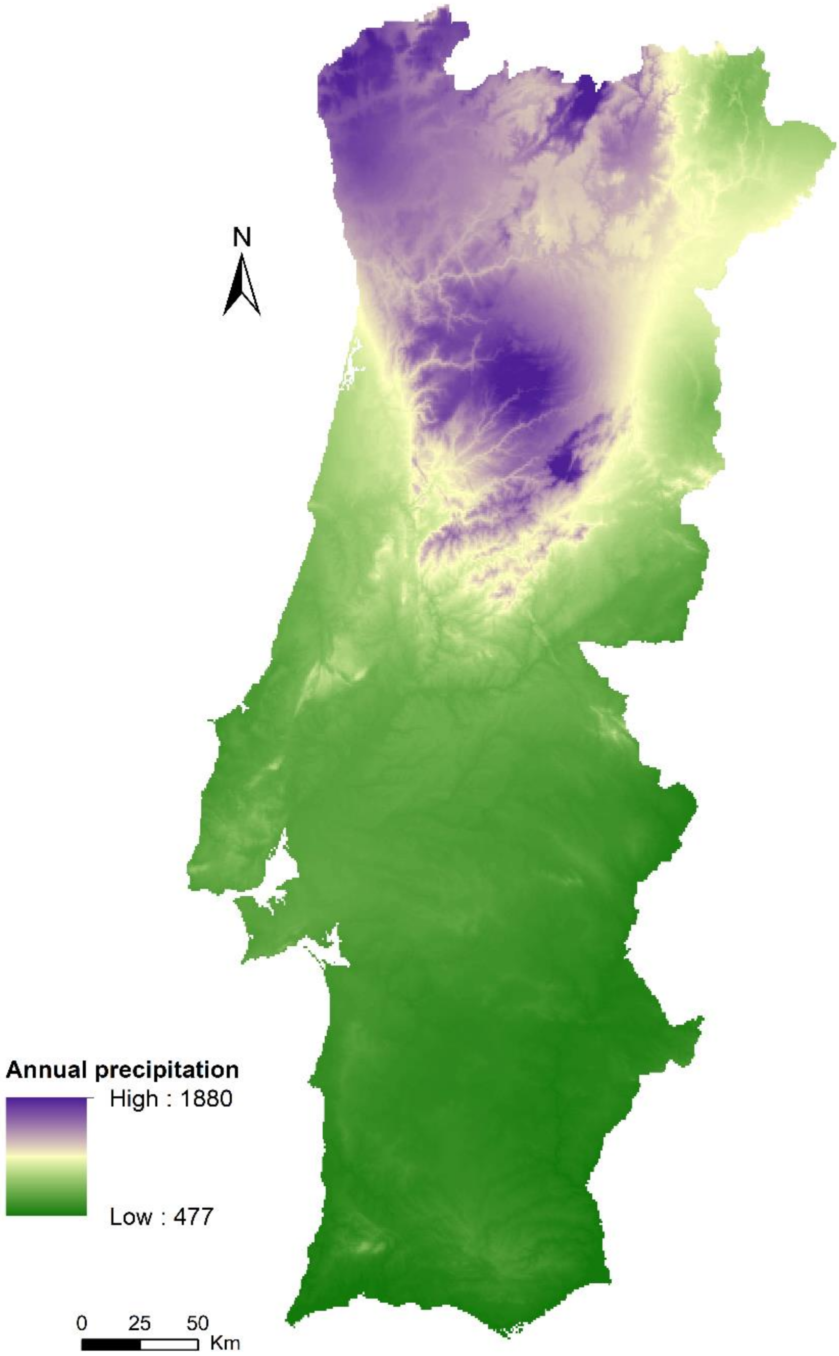


6 - Annual mean temperature (°C)

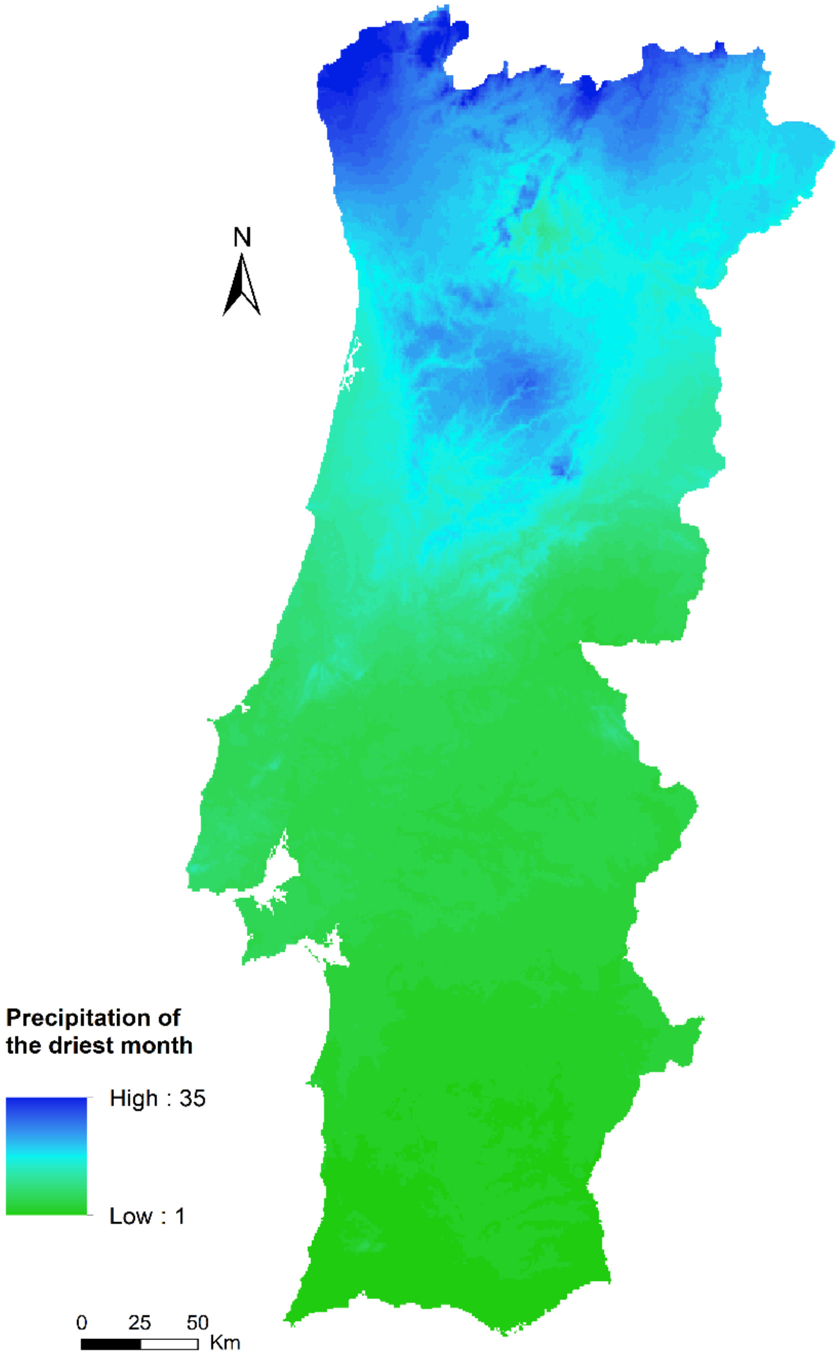
(The variable "average annual temperature" was not used in modelling the distribution of the species due to its collinearity problems).



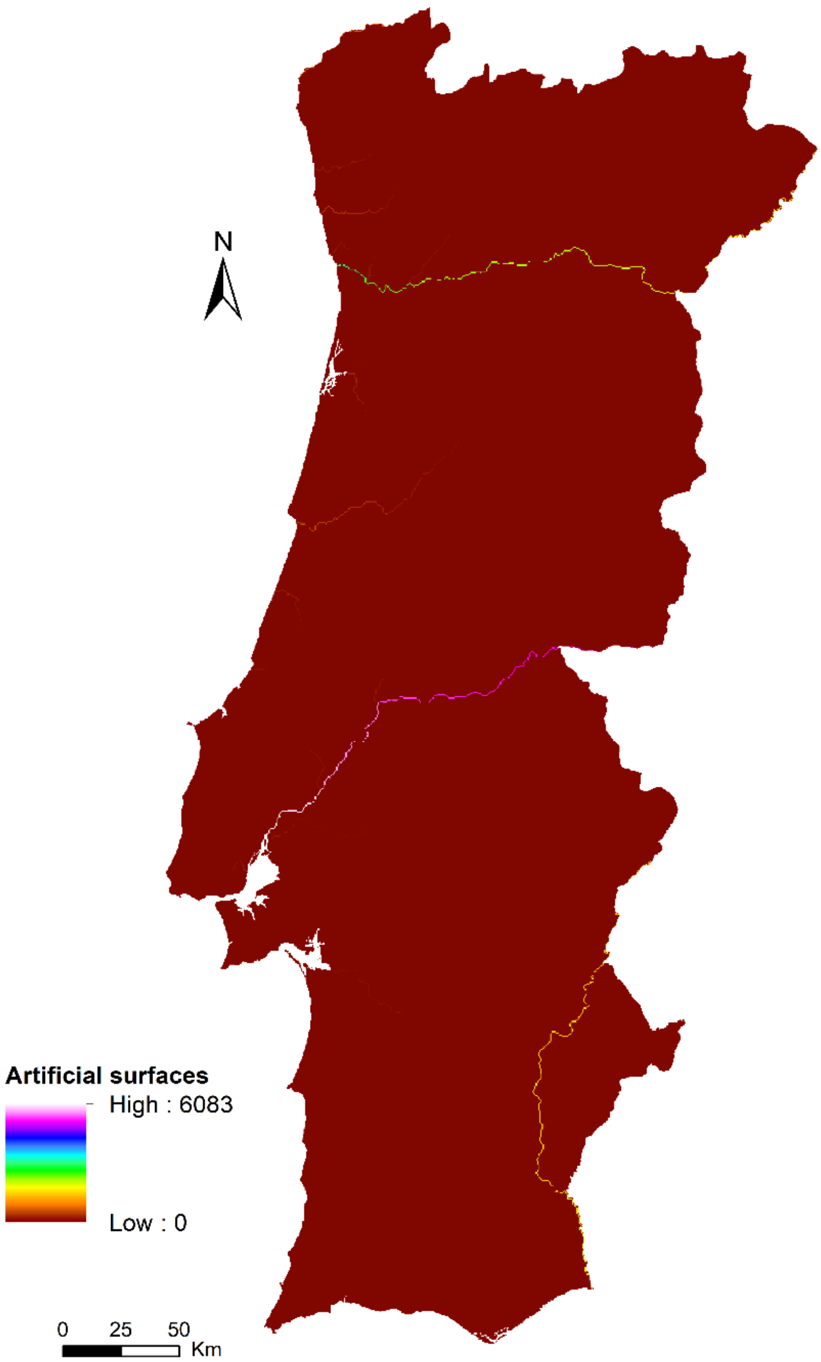
7 - Maximum temperature of the warmest month (°C)



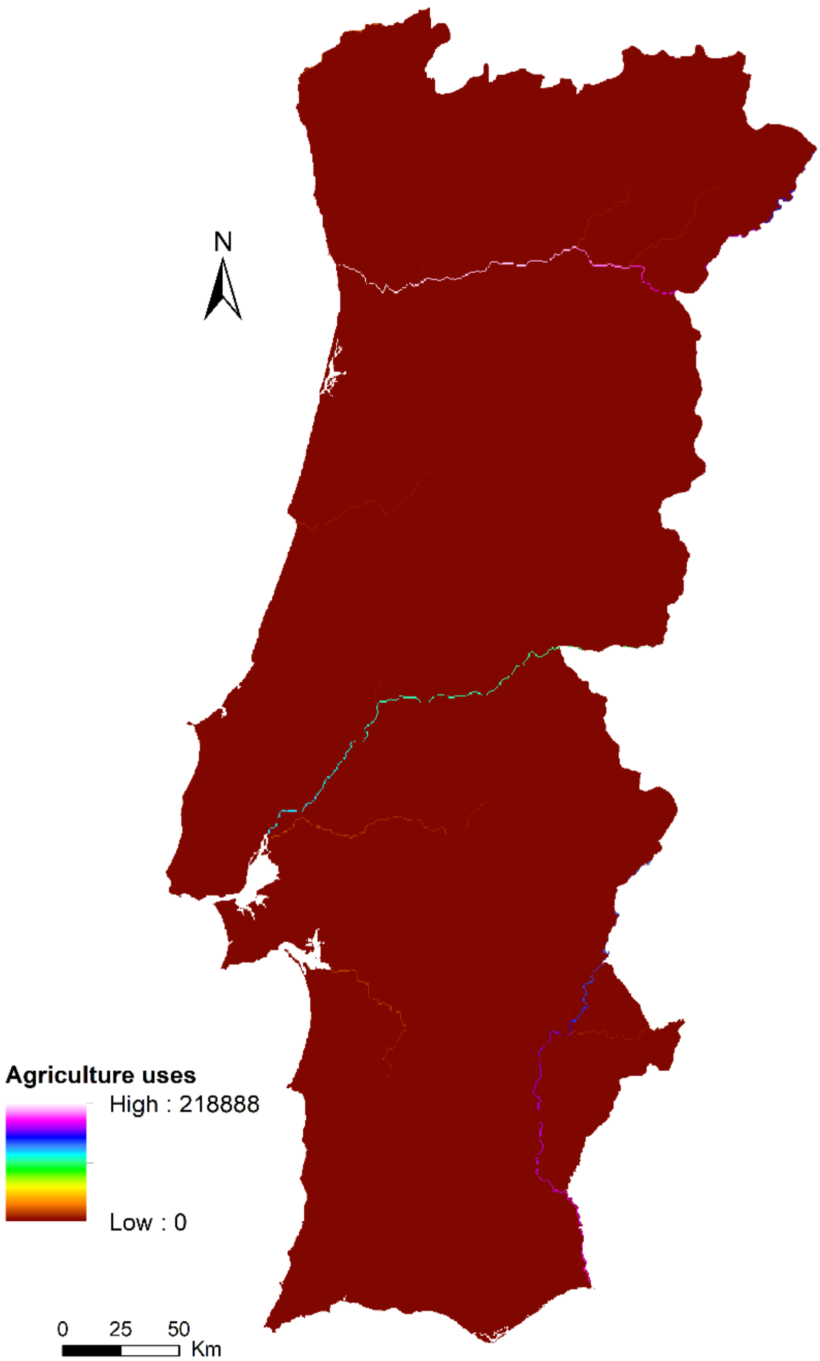
8 - Annual precipitation (mm)



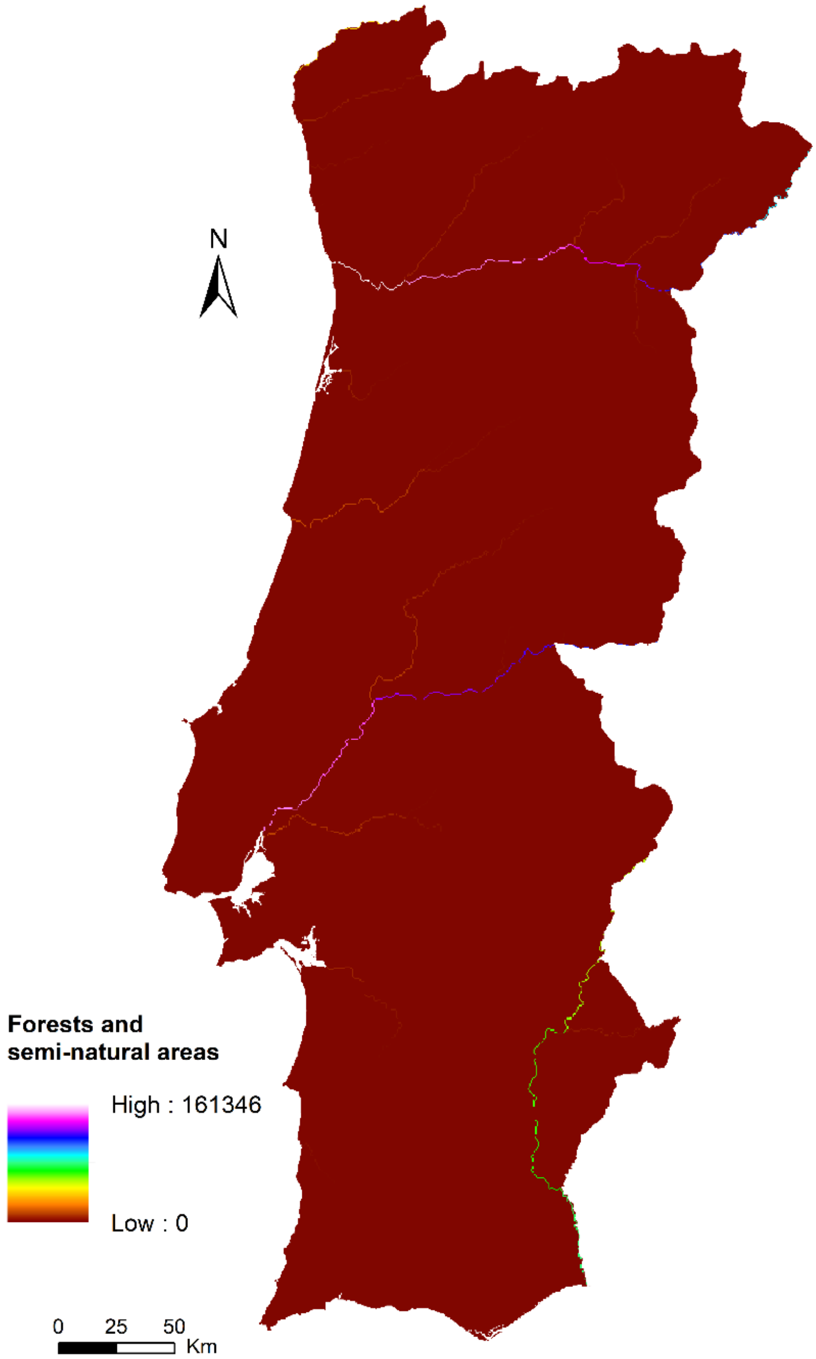
9 - Precipitation of the driest month (mm)



10 - Land uses, included in the group of artificial surfaces

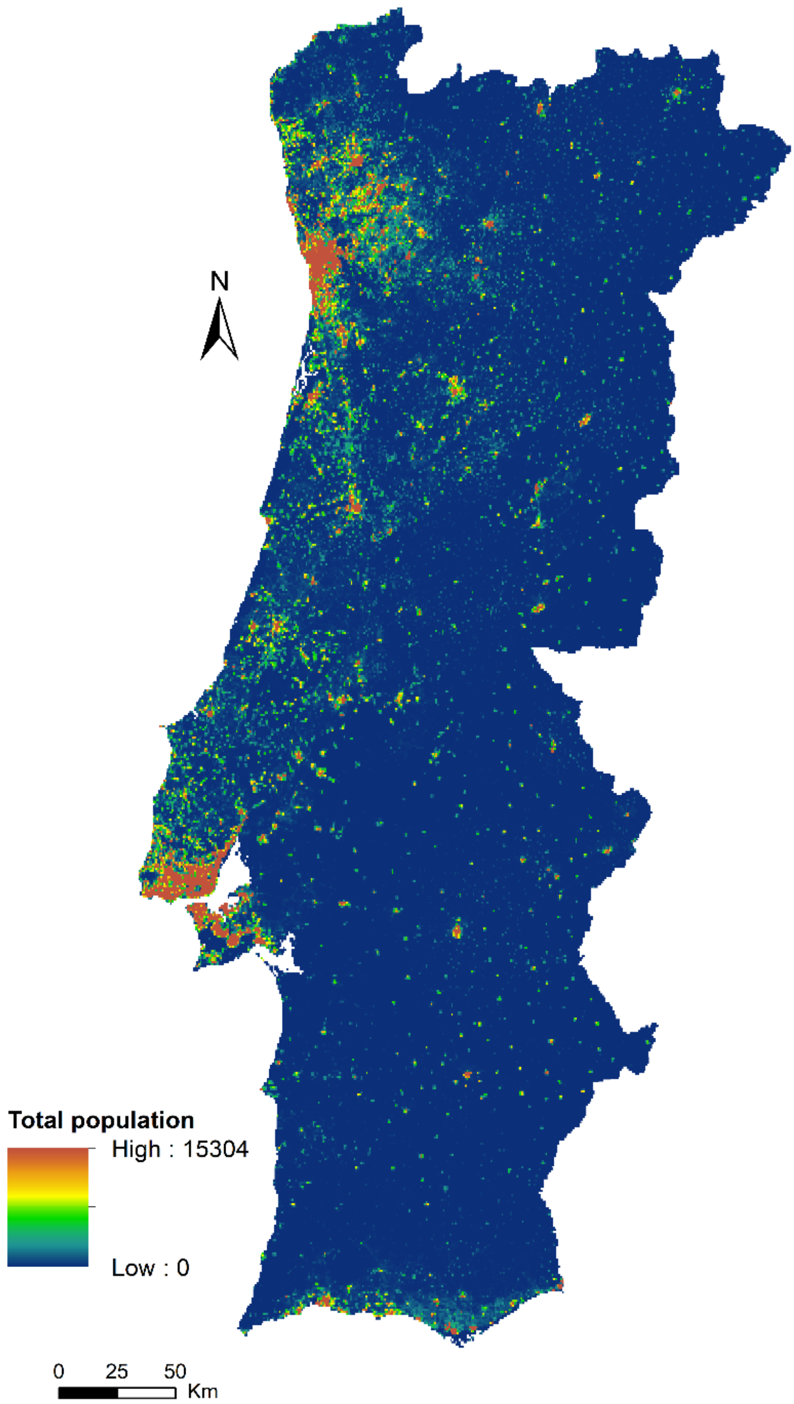


11 - Land uses, included in the group of agricultural areas



12 - Land uses, included in the group of forests and semi-natural areas

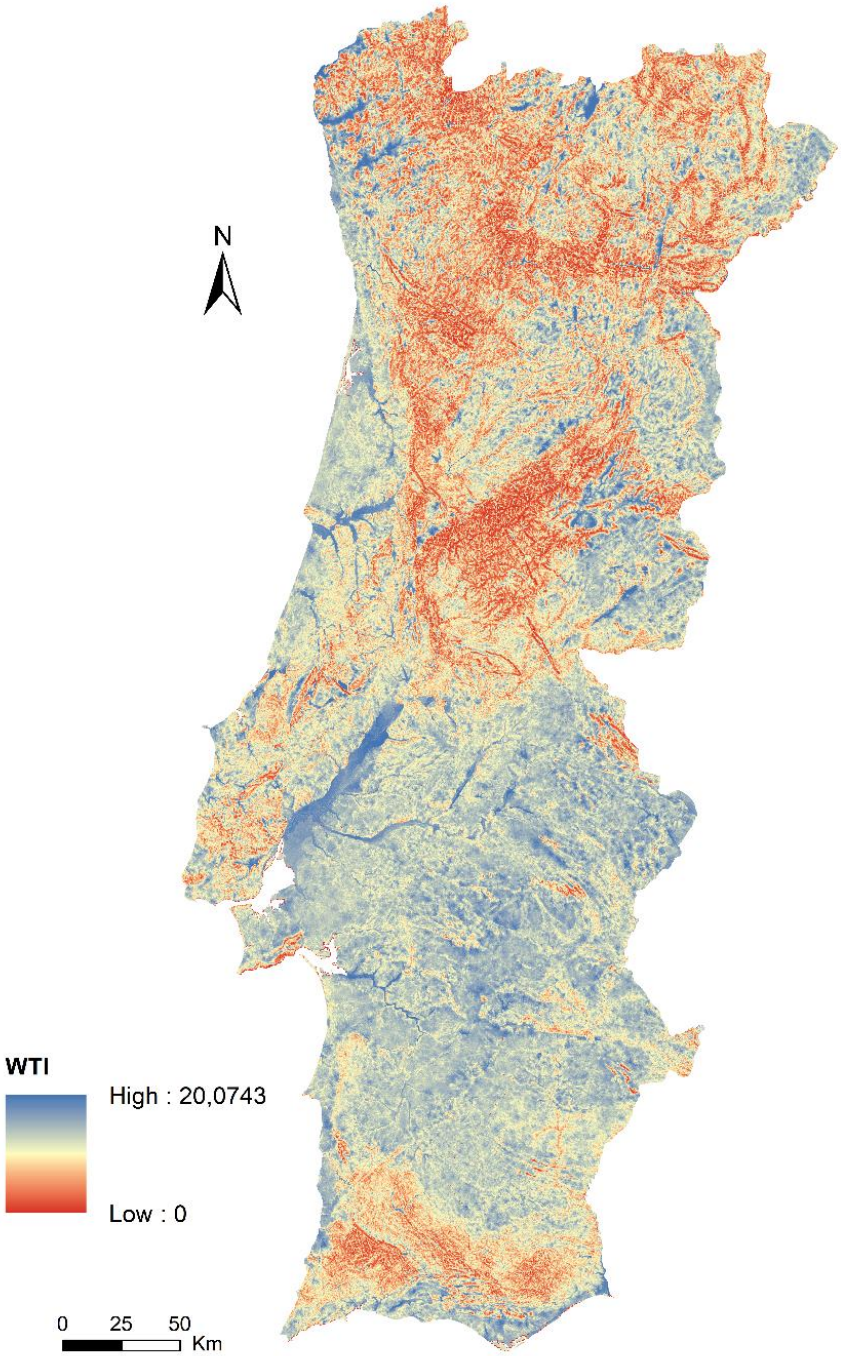
(The variable "forests and semi-natural areas" was not used in modelling the distribution of the species due to its collinearity problems)



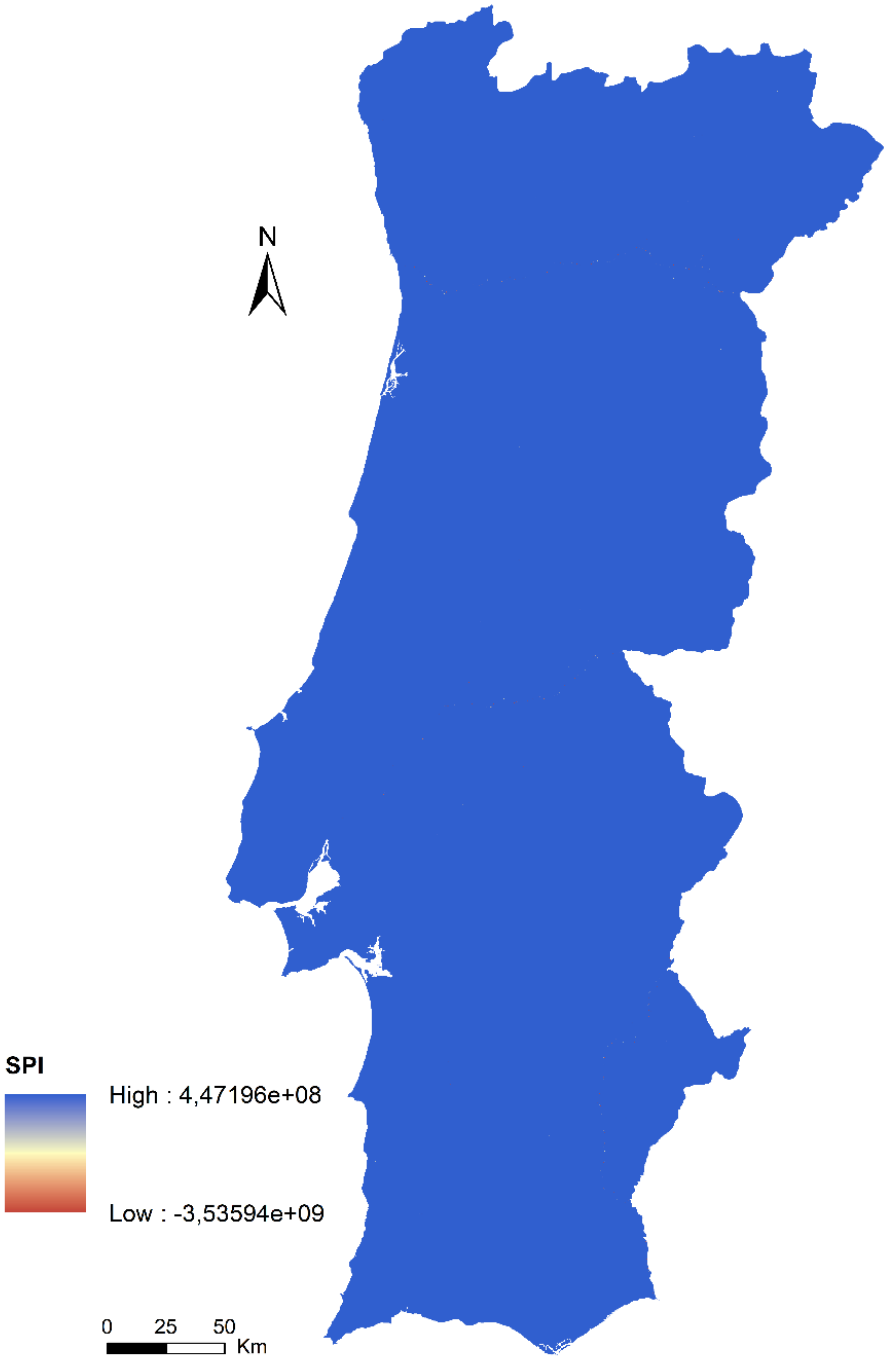
13 - Total population

14 - Flow accumulation

15 – Flow



16 – SAGA Wetness Index (WTI)



17 – SAGA Power Index (SPI)

Appendix II - Pearson's correlation between the 17 environmental variables

1 - Table with values for Pearson correlation between the 17 environmental variables initially identified to include in the model. *Correlations significant at the 95 %; **correlations significant at 99 %; correlations significant at 99.9%.

	altitude	slope	dist_coast	silt	sand	tempmean	tempmax	precip	precipdriest	f_accum	f_weight_rain	wti	spi	use_art	use_agr	use_forest	populat
altitude	1	0.089 *	0.13 **	0.051	0.084 *	0.088 *	0.31 ***	-0.22 ***	-0.17 ***	0.016	0.30 ***	-0.17 ***	0.011	0.063	0.09 *	0.083 *	-0.14 ***
slope	0.089 *	1	0.49 ***	-0.21 ***	-0.063	-0.83 ***	-0.18 ***	0.37 ***	0.39 ***	-0.12 **	0.70 ***	-0.42 ***	-0.018	-0.11 **	-0.076	-0.11 **	-0.15 ***
dist_coast	0.13 **	0.49 ***	1	-0.27 ***	0.058	-0.54 ***	-0.32 ***	0.44 ***	0.39 ***	0.011	0.26 ***	-0.79 ***	-0.088 *	0.012	0.075	0.051	-0.10 **
silt	0.051	-0.21 ***	-0.27 ***	1	-0.0094	0.29 ***	0.21 ***	-0.26 ***	-0.29 ***	-0.078 *	-0.054	0.21 **	-0.005	0.0021	-0.0096	-0.016	-0.069
sand	0.084 *	-0.063	0.058	-0.0094	1	0.061	0.052	-0.033	-0.03	0.011	-0.012	-0.043	-0.013	0.32 ***	0.50 ***	0.43 ***	-0.012
tempmean	0.088 *	-0.83 ***	-0.54 ***	0.29 ***	0.061	1	0.64 ***	-0.74 ***	-0.79 ***	0.032	-0.43 ***	0.40 ***	-0.0062	0.057	0.024	0.037	-0.029
tempmax	0.31 ***	-0.18 ***	-0.32 ***	0.21 ***	0.052	0.64 ***	1	-0.74 ***	-0.72 ***	-0.065	0.29 ***	0.21 ***	0.024	-0.019	-0.03	-0.04	-0.24 ***
precip	-0.22 ***	0.37 ***	0.44 ***	-0.26 ***	-0.033	-0.74 ***	-0.74 ***	1	0.92 ***	0.042	0.011	-0.28 ***	-0.012	-0.0071	0.024	0.031	0.18 ***
precipdriest	-0.17 ***	0.39 ***	0.39 ***	-0.29 ***	-0.03	-0.79 ***	-0.72 ***	0.92 ***	1	0.051	0.11 **	-0.22 ***	-0.0087	-0.00091	0.015	0.038	0.16 ***
f_accum	0.016	-0.12 **	0.011	-0.078 *	0.011	0.032	-0.065	0.042	0.051	1	-0.076	0.064	0.012	0.26 ***	0.25 ***	0.28 ***	0.046
f_weight_rain	0.30 ***	0.70 ***	0.26 ***	-0.054	-0.012	-0.43 ***	0.29 ***	0.011	0.11 **	-0.076	1	-0.19 ***	-0.0051	-0.052	-0.0089	-0.04	-0.23 ***
wti	-0.17 ***	-0.42 ***	-0.79 ***	0.21 **	-0.043	0.40 ***	0.21 ***	-0.28 ***	-0.22 ***	0.064	-0.19 ***	1	0.085	0.022	-0.018	0.00054	0.12 **
spi	0.011	-0.018	-0.088 *	-0.005	-0.013	-0.0062	0.024	-0.012	-0.0087	0.012	-0.0051	0.085	1	0.038	-0.033	0.038	0.017
use_art	0.063	-0.11 **	0.012	0.0021	0.32 ***	0.057	-0.019	-0.0071	-0.00091	0.26 ***	-0.052	0.022	0.038	1	0.78 ***	0.89 ***	0.018
use_agr	0.09 *	-0.076	0.075	-0.0096	0.50 ***	0.024	-0.03	0.024	0.015	0.25 ***	-0.0089	-0.018	-0.033	0.78 ***	1	0.94 ***	-0.0013
use_forest	0.083 *	-0.11 **	0.051	-0.016	0.43 ***	0.037	-0.04	0.031	0.038	0.28 ***	-0.04	0.00054	-0.016	0.89 ***	0.94 ***	1	0.012
populat	-0.14 ***	-0.15 ***	-0.10 **	-0.069	-0.012	-0.029	-0.24 ***	0.18 ***	0.16 ***	0.046	-0.23 ***	0.12 **	0.017	0.018	-0.0013	0.012	1