MULTIVARIATE APPROACHES IN SPECIES DISTRIBUTION MODELLING: APPLICATION TO NATIVE FISH SPECIES IN MEDITERRANEAN RIVERS

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ABSTRACT

Worldwide, human activities such as water withdrawals, storing, hydropoeaking and climate change are negatively impacting freshwater biota. To evaluate the threats posed by these phenomena the development of scientifically sophisticated tools has now become a fundamental area of research within the scientific community. The physical habitat simulation approach has proved to be adequate to evaluate the effect of management alternatives, restoration actions and climate change. The physical habitat methods, which can be implemented at different spatial scales, assess the quantity and quality (i.e. suitability) of the physical habitat for different flows integrating hydrological, hydraulic and biological data (i.e. habitat suitability models). The development of the habitat suitability models can manifold be approached, although machine learning techniques steadily gained adepts over the classical methods and thus they became common practice.

Scientific literature reported a number of studies employing machine learning techniques. However, there are still knowledge gaps and room to test new and elder approaches for exploratory freshwater ecology and environmental flow (e-flow) assessment. This dissertation focused in the comprehensive analysis of the capabilities of some non–tested types of Artificial Neural Networks, specifically: the Probabilistic Neural Networks (PNN) and the Multi–Layer Perceptron (MLP) Ensembles. The analysis of the capabilities of these techniques was performed using the native brown trout (Salmo trutta; Linnaeus, 1758), the bermejuela (Achondrostoma arcasii; Robalo, Almada, Levy & Doadrio, 2006) and the redfin barbel (Barbus haasi; Mertens, 1925) as target species. The analyses focused in the predictive capabilities, the interpretability of the models and the effect of the excess of zeros in the training datasets, which for presence–absence models is directly related to the concept of data prevalence (i.e. proportion of presence instances in the training dataset). Finally, the effect of the spatial scale (i.e. micro–scale or microhabitat scale and meso–scale) in the habitat suitability models and consequently in the e–flow assessment was studied in the last chapter.

PNN presented a good trade–off between accuracy (classification) and generalization. Nonetheless, the performance in the evaluation site was higher than the one achieved during model training. The classificatory capability of the PNN was not significantly affected by prevalence. However, they presented some limitations regarding the output range (i.e. they presented trimmed probabilistic output range), which may limit their application in e–flow assessment if the training datasets present large overlapping between categories.

The MLP Ensemble paradigm presented better performance than the PNN for classification and it also performed well in the regression of fish densities. The result with active selection of the MLPs eventually included in the Ensemble outperformed the results after including the whole set of MLPs, highlighting the benefits of the over–produce–and–choose approach, which consists of first initiating a large number of MLPs and then
selecting the best performing subset. Two methods to do so were compared; the Forward selection of MLPs demonstrated to be able to marginally outperform the more complex approach based on bit strings and a Genetic Algorithm (GAs). However, there are several suitable methods to encode and optimise MLP Ensembles with GAs. The last analysis followed the Wang and Alhamdoosh approach, which iteratively increases the ensemble size by the stepwise addition of MLPs while in every step the GA searches for the best combination of them. The optimal MLP Ensembles developed with this approach provided accurate small–sized Ensembles. Consequently, although we did not perform a comparison with previous approaches, we considered it a worthwhile methodology. Furthermore, the MLP Ensembles also proved able to deal with unbalanced datasets, either with excess of zeroes or with low data prevalence. Consequently, due to its high predictive performance and its ability to deal with unbalanced datasets, the MLP Ensemble paradigm was considered a promising tool in ecology.

With regard to the modelled habitat suitability and fish ecology, the large brown trout preferences did not differ significantly to previous knowledge about the species. Therefore the highest suitability was assigned to relatively deep microhabitats with slow flow and medium–to–coarse substrate.

The microhabitat preferences of the bermejuela had never been studied before thus the study provided valuable insight on the species habitat suitability. The bermejuela can be classified as a shelter–orientated limnophilic species, because cover was the most important variable. The other relevant variables indicated the maximum suitability for slow and deep microhabitats, related with the natural deposition of silt in the riverbed.

The redfin barbel habitat preferences were explored at the meso–scale using fish density as the dependent variables. The redfin barbel preferred middle–to–upper river segments, but not the higher and steeper reaches. The importance of the variable depth confirmed that redfin barbel prefer pool–type habitats. Finally, the European eel (a critically endangered species) and the cyprinid species present in the study sites had similar habitat requirements thus the MLP Ensembles encountered great correlation between their densities.

The habitat suitability (i.e. probability of presence) for the redfin barbel was modelled at two different spatial scales (the meso–scale and the micro–scale). The micro–scale MLP Ensemble showed high suitability of relatively deep areas with coarse substrate and corroborating the cover–orientated and rheophilic nature of the redfin barbel. The meso–scale model highlighted the advantages of using cross–scale variables, since elevation (a macro–scale variable) was selected in the optimal model suggesting that the redfin babel eludes the uppermost river segments. The redfin barbel selects deep areas and, at this scale, the MLP Ensemble partially contradicted the micro–scale counterpart because velocity had a clearer positive effect on habitat suitability, which we related with the current flow during the sampling year. Finally, the redfin barbel showed a preference for fine
substrate, which may be associated different substrate patches appearing in each mesohabitat type.

The last addressed issue was the influence of the scale modelling approach in e–flow assessment because some authors suggested that the meso–scale renders more accurate results. Spanish legislation stated that the minimum e–flow assessment based on physical habitat simulation must be performed at the microhabitat scale. In our study case the micro–scale model predicted a slightly higher minimum e–flow, thus providing a more conservative result for the river ecosystem. Therefore, from the legal viewpoint, this scale can be considered a choice at least equally adequate than the meso–scale one.

The present dissertation rendered valuable methodological input yet relevant ecological knowledge that may prioritize sound monitoring and may eventually guide ecologically–friendly management actions.
RESUM

A nivell mundial, les activitats humanes, l'extracció d'aigua, l'emmagatzematament, els cabals punta i el canvi climàtic estan afectant negativament la biota d'aigua dolça. El desenvolupament de sofisticades eines científiques per a l'avaluació de les amenaces representades per aquests fenòmens ha esdevingut una àrea fonamental en la investigació científica. La simulació de l'hàbitat físic ha demostrat esser una eina adequada per a l'avaluació dels efectes derivats de les distintes alternatives, restauracions o del canvi climàtic. Els mètodes basats en l'hàbitat físic, que poden esser desenvolupats a distintes escales, avaluen la quantitat i la qualitat (i.e. idoneïtat) de l'hàbitat físic per a diferents cabals integrant hidrologia, hidràulica i informació biològica (i.e. models d'idoneïtat de l'hàbitat). El desenvolupament dels models d'idoneïtat de l'hàbitat pot ser enfocat de diverses formes no obstant però aquells basats en l'ús de tècniques d'aprenentatge computeritzat han anat guanyant adeptes així, a hores d'ara són pràctica habitual.

La literatura científica engloba nombrosos estudis emprant tècniques d'aprenentatge computeritzat. No obstant existeixen mancances en el coneixement i espai per a provar noves tècniques amb l'objectiu últim d'explorar l'ecologia de les espècies d'aigua dolça o bé per a l'avaluació de cabals ecològics. Aquesta tesis se centra en l'anàlisi comprensiu de les capacitats d'alguns tipus de Xarxa Neuronal Artificial que encara no han estat testats: les Xarxes Neuronal Probabilístiques (PNN) i els Conjunts de Perceptrons Multicapa (MLP Ensembles). Les anàlisis sobre les capacitats d'aquestes tècniques es varen desenvolupar emprant la truita comuna (Salmo trutta; Linnaeus, 1758), la madrilla roja (Achondrostoma arcasi; Robalo, Almada, Levy & Doadrio, 2006) i el barb cua-roig (Barbus haasi; Mertens, 1925) com a espècies objecte d'estudi. Les anàlisis se centren en la capacitat predictiva, interpretabilitat dels models i en l'efecte de l'excés de zeros a la base de dades d'entrenament, l'anomenada prevalença de les dades (i.e. la proporció de casos de presència sobre el conjunt total). Finalment, l'efecte de la escala (micro–escala o microhàbitat i meso–escala) en els models d'idoneïtat de l'hàbitat i conseqüentment en l'avaluació de cabals ambientals es va estudiar a l’últim capítol.

PNN va presentar un bon balanç entre precisió i generalització, atès que l'acompliment al lloc d'avaluació fou major que l'aconseguit durant l'entrenament del model. La capacitat classificatòria no es va veure compromesa per la prevalença. No obstant, aquest tipus de xarxes va presentar certes limitacions pel que fa a la predició de probabilitats (i.e. presentaren un rang d'eixida retallat), la qual cosa podria limitar la seua aplicació en avaluacions de cabals ambientals sempre que la base de dades d'entrenament presente un gran solapament entre categories.

El paradigma de les MLP Ensembles va presentar millor acompliment que l'aconseguit amb les PNN en problemes de classificació a més a més de funcionar bé durant la regressió de densitats de peixos. La selecció activa dels MLPs finalment inclosos al
conjunt (Ensemble) presentà millor acompliment que incloure el conjunt de tots els candidats entrenats. Aquest fet destaca els beneficis del mètode de sobreproducció–i–tria, que consisteix en iniciar primerament un nombre gran de MLPs, seleccionant a posteriori aquell subconjunt que presenta el millor acompliment combinat. Es testaren dos mètodes per fer aquesta selecció; la selecció basada en el pas endavant (Forward) va demostrar un acompliment marginalment superior al mètode en què s’empraren cadenes de bits i un Algorisme Genètic (GA). No obstant, existeixen diversos mètodes per a codificar i optimitzar MLP Ensembles amb GAs. Així l’última anàlisi va utilitzar el mètode de Wang and Alhamdoosh. Aquest mètode incrementa iterativament la mida de l’Ensemble mitjançant l’adició pas per pas de MLPs, mentre que a cada iteració el GA busca la millor combinació. Els MLP Ensembles optimitzats mitjançant aquest mètode foren en tots els casos precisos i de petita mida per tant, malgrat no haver-hi comparativa amb els mètodes anteriors, es va considerar que aquest mètode és digne de menció. Els MLP Ensembles demostraren esser capaços de tractar amb bases de dades desequilibrats, tant amb excés de zeros com amb una baixa prevalença. Conseqüentment, atesa la seua alta capacitat predictiva i l’habilitat per a tractar amb bases de dades desequilibrades, el paradigma dels MLP Ensembles ha d’ésser considerat una eina molt prometedora en ecologia.

Pel que fa a la idoneïtat de l’hàbitat modelitzada i la ecologia dels peixos, la truita comuna no va diferir significativament del coneixement previ que es tenia de l’espècie. D’aquesta manera la màxima idoneïtat va ser assignada a microhàbitats relativament profunds amb fluxe lent i substrat de mida mitjana i grossa.

Les preferències de microhàbitat de la madrilla roja no havien estat estudiades amb anterioritat el que proporciona informació valuosa sobre la idoneïtat de l’hàbitat per a aquesta espècie. La madrilla roja pot ser classificada com a espècie limnofílica orientada cap al refugi ja que aquest va esdevenir la variable més important del model. La resta de variables indicaren que la màxima idoneïtat s’assolia a microhàbitat lents i profundes, relacionats amb la deposició natural de llims a la llera del riu.

Les preferències d’hàbitat del barb cua–roig foren explorades a meso–escala emprant la seua densitat com a variable dependent. El barb cua–roig va preferir segments de riu al tram mig i alt dels rius però evitant els trams més alts i escarpats. La importància de les variables va confirmar la preferència del barb cua–roig pels hàbitats de tipus tolla. Finalment, l’anguila i la resta de ciprínids present als llacs d’estudi demostraren tindre similars requeriments d’hàbitat ja que el MLP Ensemble va trobar una gran correlació entre les seues densitats.

La idoneïtat de l’hàbitat (i.e. probabilitat de presència) del barb cua–roig va ser modelada a dues escales diferents (micro–escala i meso–escala). El MLP Ensemble a miro–escala va mostrar una alta idoneïtat en àrees relativament profundes amb substrat gruix i va corroborar la seua condició d’espècie reofílica així com la seua preferència pel refugi.
El model a meso–escala va destacar els avantatges d'encreuar escales de treball donat que l’elevació (una variable a macro–escala) va ser seleccionada al MLP òptim, suggerint que el barb cua–roig evita els trams més alts mostrejats. El barb cua–roig seleccionà àrees profundes i, a aquesta escala, el MLP Ensemble va contradir parcialment la seua contrapart a micro–escala. En aquest cas la velocitat mostrà una efecte clarament positiu en la idoneïtat de l’hàbitat, la qual cosa es va relacionar amb el cabal de l’any de mostreig. Finalment, el barb cua–roig va mostrar preferència pels substrats fins, el que es pot associar als diferents tipus de substrat (clapes o taques) que acostumen a apareixes a cada mesohàbitat.

L’últim element analitzat fou la influència de la escala de modelització en l’avaluació de cabals ambientals ja que alguns autors han suggerit que la meso–escala esdevé sempre en millors resultats. La legislació espanyola determina que l’avaluació de cabals ambientals basada en l’hàbitat físic ha de fer–se a escala de microhàbitat. En el nostre cas d’estudi el model a micro–escala va determinar un cabal de manteniment major. Per tant, des d’un punt de vista legal, aquesta escala ha d’esser considerada una elecció igualment adequada que la meso–escala.

Aquesta tesi compila informació valuosa des del punt de vista metodològic i proporciona nou coneixement ecològic de relleu, el qual hauria ajudar en la prioritzarció de seguiments adequats, in en última instància, d’accions de manieig ecològicament amigables.
RESUMEN

A nivel mundial, las actividades humanas, la extracción de agua, el almacenamiento, los picos de caudal y el cambio climático están afectando negativamente la biota dulceacuícola. El desarrollo de sofisticadas herramientas científicas para evaluar las amenazas representadas por estos fenómenos ha devenido un área fundamental en la investigación científica. La simulación del hábitat físico ha demostrado ser adecuada para la evaluación de los efectos derivados de distintas alternativas, restauraciones o del cambio climático. Los métodos basados en el hábitat físico, que pueden ser desarrollados a distintas escalas, evalúan la cantidad y calidad (i.e. idoneidad) del hábitat físico para diferentes caudales integrando hidrología, hidráulica e información biológica (i.e. modelos de idoneidad del hábitat). El desarrollo de los modelos de idoneidad del hábitat puede ser enfocado de muchos modos pero aquellos basados en el uso de técnicas de aprendizaje computarizado han ido ganando adeptos, de este modo son actualmente práctica habitual.

La literatura científica engloba numerosos estudios empleando técnicas de aprendizaje computarizado. No obstante existen aún lagunas en el conocimiento y espacio para testar viejos y nuevos métodos con el objetivo último de explorar la ecología de las especies dulceacuícolas o para la evaluación de caudales ecológicos. Esta tesis se centra en el análisis comprensivo de las capacidades de algunos tipos de Red Neuronal Artificial aún no testados: las Redes Neuronales Probabilísticas (PNN) y los Conjuntos de Perceptrones Multicapa (MLP Ensembles). Los análisis sobre las capacidades de estas técnicas se desarrollaron utilizando la trucha común (Salmo trutta; Linnaeus, 1758), la bermejuela (Achondrostoma arcasi; Robalo, Almada, Levy & Doadrio, 2006) y el barbo colirrojo (Barbus haasi; Mertens, 1925) como especies nativas objetivo. Los análisis se centraron en la capacidad de predicción, la interpretabilidad de los modelos y el efecto del exceso de ceros en las bases de datos de entrenamiento, la así llamada prevalencia de los datos (i.e. la proporción de casos de presencia sobre el conjunto total). Finalmente, el efecto de la escala (micro–escala o escala de microhábitat y meso–escala) en los modelos de idoneidad del hábitat y consecuentemente en la evaluación de caudales ambientales se estudió en el último capítulo.

PNN presentó un buen balance entre precisión y generalización. Dado que el desempeño en el sitio de evaluación fue mayor que el conseguido durante el entrenamiento del modelo. La capacidad clasificatoria no se vio significativamente afectada por la prevalencia. No obstante, este tipo de redes presentó ciertas limitaciones en lo que respecta predicción de probabilidades (i.e. presentaron un rango de salida recortado), lo cual podría limitar su aplicación en evaluación de caudales ambientales siempre y cuando la base de datos de entrenamiento presente un gran solapamiento entre categorías.

El paradigma de las MLP Ensembles presentó mejor desempeño que el conseguido con las PNN en problemas de clasificación además de funcionar bien en la regresión de
densidades de peces. La selección activa de los MLPs finalmente incluidos en el conjunto (Ensemble) presentó mejor desempeño que incluir el conjunto todos los candidatos entrenados. Eso destaca los beneficios del enfoque de sobreproducción–y–elección, que consiste en iniciar primero un conjunto grande de MLPs, seleccionando a posteriori aquel subconjunto que presenta el mejor desempeño combinado. Se testaron dos métodos para llevar a cabo esta selección; la selección basada en el paso delante (Forward) demostró un desempeño marginalmente superior al método empleando cadenas de bits y un Algoritmo Genético (GA). No obstante, existen diversos métodos para codificar y optimizar MLP Ensembles con GAs. Así el último análisis utilizó el método de Wang and Alhamdoosh. Este método incrementa iterativamente el tamaño del Ensemble con la adición paso a paso de MLP, mientras que en cada iteración el GA busca la mejor combinación de ellos. Los MLP Ensembles optimizados por este método fueron en todo caso precisos y de pequeña dimensión por tanto, a pesar de no existir comparación con los métodos anteriores, se consideró que es un enfoque digno de mención. Los MLP Ensembles demostraron ser capaces de lidiar con bases de datos desbalanceadas, tanto con exceso de ceros como con una baja prevalencia. Consecuentemente, debido a su alta capacidad predictiva y su habilidad para lidiar con bases de datos desbalanceadas el paradigma de los MLP Ensembles debe ser considerado una herramienta prometedora en la ecología.

Por lo que respecta a la idoneidad del hábitat modelada y la ecología de los peces, la trucha común no difirió significativamente del conocimiento previo que se tenía de la especie. De este modo la máxima idoneidad se asignó a microhábitats relativamente profundos con flujo de caudal lento y sustrato medio o grueso.

Las preferencias de microhábitat de la bermejuela no habían sido estudiadas con anterioridad lo que proporciona información valiosa sobre la idoneidad del hábitat para esta especie. La bermejuela puede ser clasificada como una especie limnofílica orientada hacia el refugio ya que este devino el elemento más importante del modelo. El resto de variables indicaron la máxima idoneidad en microhábitats lentos y profundos, relacionados con la deposición natural de limos en el lecho del río.

La preferencias de hábitat del barbo colirrojo fueron exploradas a meso–escala utilizando la densidad de estos como variable dependiente. El barbo colirrojo prefirió segmentos de río en el tramo medio y alto pero no los más altos y empinados. La importancia de las variables confirmó la preferencia del barbo colirrojo por los hábitats de tipo poza. Finalmente, la anguila y el resto de especies de ciprínidos presente en los sitios de estudio demostraron similares requerimientos de hábitat porque el MLP Ensemble encontró una gran correlación entre sus densidades.

La idoneidad del hábitat (i.e. probabilidad de presencia) del barbo colirrojo fue modelada a dos escalas espaciales diferentes (la meso–escala y la micro–escala). El MLP Ensemble a micro–escala mostró alta idoneidad en áreas relativamente profundas con sustrato grueso y corroboró la naturaleza reofílica del barbo colirrojo así como su preferencia por el
Refugio. El modelo a meso–escala destacó las ventajas de cruzar escalas de trabajo ya que la elevación (una variable de macro–escala) fue seleccionada en el modelo óptimo sugiriendo que el barbo colirrojo elude los tramos más altos muestreados. El barbo colirrojo seleccionó áreas profundas y, a esta escala, el MLP Ensemble parcialmente contradijo su contraparte a micro–escala. En este caso la velocidad tuvo un efecto claramente positivo en la idoneidad del hábitat, lo que nosotros relacionamos con el caudal circulante en el año de muestreo. Finalmente, el barbo colirrojo mostró preferencia por sustratos finos, lo que se puede asociar a los diferentes tipos de sustrato ( parches) que aparecen en cada tipo de mesohabitat.

El último elemento analizado fue la influencia de la escala de modelización en la evaluación de caudales ambientales ya que algunos autores han sugerido que la meso–escala deviene en mejores resultados. La legislación española determina que la evaluación de caudales ambientales basada en la simulación del hábitat físico debe llevarse a cabo a la escala de microhábitat. En nuestro caso de estudio el modelo a micro–escala predijo un caudal ambiental mayor. Por tanto, desde un punto de vista legal, esta escala debe ser considerada una elección, al menos, igualmente adecuada que la meso–escala.

Esta tesis compila valiosa información desde un punto de vista metodológico y proporciona conocimiento ecológico relevante que debería priorizar seguimientos adecuados y, en última instancia, acciones de manejo ecológicamente amigables.
# TABLE OF CONTENTS

I. GENERAL INTRODUCTION .................................................................................................................. 2
   I.1 GENERAL PROSPECT .................................................................................................................... 2
   I.2 STATE OF THE ART ....................................................................................................................... 5
   I.3 OBJECTIVES AND OVERVIEW ...................................................................................................... 11

II. APPLICATION OF PROBABILISTIC NEURAL NETWORKS TO MICROHABITAT SUITABILITY
    MODELLING FOR ADULT BROWN TROUT (*Salmo trutta* L.) IN IBERIAN RIVERS .................. 16
    ABSTRACT .................................................................................................................................................... 16
    II.1 INTRODUCTION .................................................................................................................................. 17
    II.2 METHODS ........................................................................................................................................... 19
    II.3 RESULTS .............................................................................................................................................. 29
    II.4 DISCUSSION ........................................................................................................................................ 36

III. MULTI-LAYER PERCEPTRON ENSEMBLES (MLP ENSEMBLES) IN MODELLING MICROHABITAT
    SUITABILITY FOR FRESHWATER FISH ................................................................................................. 46
    ABSTRACT .................................................................................................................................................... 46
    III.1 INTRODUCTION .............................................................................................................................. 47
    III.2 METHODS ....................................................................................................................................... 48
    III.3 RESULTS .......................................................................................................................................... 52
    III.4 DISCUSSION .................................................................................................................................... 54
    III.5 GRAPHICAL SENSITIVITY ANALYSIS – EXAMPLE ............................................................................. 56

IV. CAN MULTILAYER PERCEPTRON ENSEMBLES MODEL THE ECOLOGICAL NICHE OF
    FRESHWATER FISH SPECIES? .............................................................................................................. 59
    ABSTRACT .................................................................................................................................................... 59
    IV.1 INTRODUCTION .............................................................................................................................. 60
    IV.2 METHODS ....................................................................................................................................... 61
    IV.3 RESULTS .......................................................................................................................................... 69
    IV.4 DISCUSSION .................................................................................................................................... 73

V. ON SPECIES DISTRIBUTION MODELLING, SPATIAL SCALES AND ENVIRONMENTAL FLOW
    ASSESSMENT WITH MULTI–LAYER PERCEPTRON ENSEMBLES: A CASE STUDY ON THE REDFIN
    BARBEL (*Barbus haasi*; Mertens, 1925) ............................................................................................. 79
    ABSTRACT .................................................................................................................................................... 79
    V.1 INTRODUCTION .............................................................................................................................. 80
    V.2 METHODS ....................................................................................................................................... 82
    V.3 RESULTS .......................................................................................................................................... 90
    V.4 DISCUSSION .................................................................................................................................... 97

VI. GENERAL DISCUSSION ......................................................................................................................... 104
    VI.1 PROBABILISTIC NEURAL NETWORKS – PNN ................................................................................. 104
    VI.2 MULTI-LAYER PERCEPTRON ENSEMBLES – MLP ENSEMBLES .................................................. 106
Chapter I

GENERAL INTRODUCTION
I. GENERAL INTRODUCTION

1.1 GENERAL PROSPECT

Worldwide, human activities such as water withdrawals (Benejam et al., 2010), storing for irrigation purposes (Costa et al., 2012) or hydropoaking (Yao et al., 2015), directly altered river flow regimes impacting freshwater biota (Döll et al., 2009). Moreover, indirectly, human activities significantly modified precipitation patterns by altering land use (Döll et al., 2009) and climate (Kalogeropoulos & Chalkias, 2013) thus flow regimes in unregulated streams are not exempt of anthropogenic impacts (Li et al., 2015). The most evident impact correspond to the alteration of the natural flow regime (sensu Poff et al., 1997), which plays a vital role in the sustainment of the fluvial ecosystems (Poff et al., 1997; Richter et al., 1997; Tharme, 2003). River regulation typically affects all the basic components of the natural flow regime (magnitude, frequency, duration, seasonality and rate of change), which are associated with a range of biological and physical thresholds that determine river dynamics and the integrity and presence of the different communities of flora and fauna (Magdaleno & Fernández, 2011). Consequently, setting aside water quality, a major issue in river conservation consist of the retrieval of the natural flow regime (González Del Tánago et al., 2012). In order to improve the analysis, Bunn and Arthington (2002) summarized the major issues in four groups; first, flow is a major determinant of physical habitat in streams (e.g. Yao et al., 2015), which in turn is a major determinant of biotic composition (Snelder & Lamouroux, 2010). Second, aquatic species have evolved life history strategies primarily in direct response to the natural flow regimes thus disruptions in the flow patterns affect directly their populations, their distribution and their abundances (e.g. Belmar et al., 2013; Mims and Olden, 2013). Third, flow maintains the natural patterns of longitudinal and lateral connectivity, it is consequently essential for the viability of populations of many riverine species so reductions on flooding events often reduce breeding and recruitment success (e.g. Garófano–Gómez et al., 2012) and finally, the invasion and success of exotic and introduced species in rivers is facilitated by altering the intra– and inter–annual flow variability because most of them are usually lentic species (Clavero et al., 2004).

Spain is especially sensitive to such negative effects because there are currently more than 1200 large dams located in its river systems, with a total capacity of 56 000 hm$^3$, which corresponds to the fifth highest number of large dams worldwide, exceeded only by China, the United States, India and Japan (Magdaleno & Fernández, 2011). Moreover water scarcity and shortage caused by climate change is likely to significantly affect the patterns of running flows, especially in the semiarid areas of Mediterranean–climatic regions (Chirivella Osma et al., 2014), causing the need of significant water abstractions (either directly or via groundwater withdrawal) in areas that combine high human population densities and agricultural development (Benejam et al., 2010). Broadly all of...
these negative effects are currently affecting the composition and abundance of species—the most studied groups of taxa are macroinvertebrates, fish and riparian vegetation—altering the age structure of their communities (Magdaleno & Fernández, 2011). Thereby, in Spanish Mediterranean rivers, flow regulation has proved to alter fish (Benejam et al., 2010; Navarro-Llácer et al., 2010; Olaya-Marín et al., 2012) and macroinvertebrates communities (Navarro-Llácer et al., 2010; Filipe et al., 2013) by impoverishing the richness and abundance of their populations, with different time scale, and by altering the composition and distributions of riparian vegetation (Magdaleno & Fernández, 2011; Garófano-Gómez et al., 2013). Dams were the most important factor determining the conservation status of fish communities, with a positive association with the number of introduced species (most of them invasive species) (Clavero et al., 2004). Although these introduced species may have contributed to the decline of native fishes, the ecological status proved to be primarily related with the construction of dams and water diversions because it fragments river continuum and isolates habitats and native fish communities (Aparicio et al., 2000). Thus these communities are increasingly being cornered to the upper part of the stream networks without connection between these groups (Aparicio et al., 2000; Alcaraz et al., 2014).

The methodologies addressed to quantify and to evaluate the quality of the flow regime (environmental flow assessment or e-flow assessment), allowing the implementation of mitigation actions or the inference of ecologically–friendly management protocols were brilliantly summarized by Tharme (2003) at the beginning of the millennium. They were classified into four different categories: hydrological methods (e.g. Mathews and Richter, 2007), hydraulic methods (e.g. Lamouroux and Souchon, 2002), physical habitat methods (e.g. Bovee et al., 1998; Parasiewicz, 2001) and holistic methods (e.g. McClain et al., 2014). The hydrological methods rely on the statistical analysis of hydrological data whereas the hydraulic methods analyse changes in simple hydraulic variables, such as wetted perimeter or maximum depth, as proxies of limiting factors for freshwater biota. Physical habitat methods assess the quantity and suitability of the available physical habitat for target species or assemblages under different flows, on the basis of integrated hydrological, hydraulic and biological data (Maddock, 1999). This approach encompasses a hydrodynamic model, to simulate spatial and temporal variations in hydraulic parameters and a habitat suitability model, usually developed at the microhabitat scale (i.e. in small areas of few m² with uniform hydraulic conditions) for the target species, thus overstepping the simplicity of the hydraulic methods at the expense of increasing the cost rates (Lamouroux & Souchon, 2002). Finally the holistic approach goes beyond any of them integrating the physical habitat approach and considering several facets of the riverine ecosystems as well as social and economic modules (King et al., 2003; Poff et al., 2010).

This concern about the ecological status of the different water bodies, especially rivers, overstepped the academic world and thus, nowadays, legislative frameworks in many countries reflect modern societal needs for improved ecological conditions, even requiring
the implementation of environmental flow regimes (Katopodis, 2012). In European countries, the development of the Water Framework Directive (WFD) (European Parliament and Council, 2000) meant a transformation of the guidelines for the monitoring and assessment of water bodies across all EU Member States (Martinez-Haro et al., 2015) since it is a legislative framework that stated a set of environmental objectives for water bodies with the time frame of the year 2015 (Conallin et al., 2010) that triggered programs of restoration measures to prevent further deterioration and ameliorate the ecological status of water bodies (González Del Tánago et al., 2012). Consequently, its ratification resulted in various legislative measures to prevent further degradation and to assure biological conservation such as the Spanish norm for hydrological planning (MAGRAMA, 2008) among others. However, although the WFD implicitly assumes the link between the physical and biological components of freshwaters ecosystems (Conallin et al., 2010), the requirements and the methods to assess the ecological status strongly depend on the considered jurisdiction (Tharme, 2003; Conallin et al., 2010). For instance, within the Greek context, another Mediterranean country with similar specificities, the strategies to assess environmental flows in altered rivers have been based on simplifications of the hydrological methods (Ministry of Environment, Energy and Climate Change, 2011). Conversely, the Spanish norm for hydrological planning (MAGRAMA, 2008) specifically states that environmental flows must be based on dedicated hydrological and habitat simulation methods (i.e. physical habitat studies).

The aforementioned hydrological methods generally apply statistical analyses on natural (unaltered) historical daily mean flows (Mathews & Richter, 2007). Worldwide, the most broadly applied method has been the Tennant method (Tennant, 1976) whereas the Palau method was formerly the most popular in Spain (Palau & Alcazar, 1996). Up–to–date hydrological methods provide satisfactory results (e.g. Mathews and Richter, 2007). However, they are not exempt of criticisms because in some cases the approach has been excessively simplified thus they have been reduced to mere rules–of–thump (Tharme, 2003). Furthermore, the confounding influence of channel morphology prevents streamflow statistics being an adequate surrogate for the assessment of hydraulic alteration (Turner & Stewardson, 2014). Thus, although some methods improved the hydrological analysis by including many aspects of the hydrological alteration (Mathews & Richter, 2007), it can be easily concluded that those based on the physical habitat simulation may render better results than the hydrological methods alone (Tharme, 2003). Accordingly, physical habitat methods have demonstrated adequate to evaluate the effect of different management alternatives (Yao et al., 2015), restoration actions (Mouton et al., 2007a) and the potential effects of climate change (Belgiorno et al., 2013). Furthermore, it has been considered by some environmental flow practitioners as the most scientifically and legally defensible methodology available for Environmental Flow Assessment (EFA) (Tharme, 2003).
I.2 STATE OF THE ART

The physical habitat simulation approach typically encompasses three elements (Person et al., 2014). Namely:

1. the hydraulic model component
2. the habitat suitability model component
3. the hydrologic and/or water temperature and quality models component

Regarding the hydrodynamic model, nowadays, there are plenty of alternatives specifically addressed for the physical habitat simulation. They vary in the number of dimensions used to perform the simulation. For instance, PHABSIM (Milhous, 1979) or RHYHABSIM (Jowett, 1999) and SEFA (Payne & Jowett, 2012) develop 1D models whereas River–2D (Steffler & Blackburn, 2002) can be used to develop 2D models. In addition, other alternatives link the hydraulics, simulated with a commercial and non–specific software, with the habitat suitability models; i.e. CASiMiR (Jorde, 1997; Schneider, 2001). Accordingly, hydraulic models vary in complexity, in increasing order, from simple 1D models based on hydraulic geometry (Jowett, 1998) to those based on 2D and 3D hydraulic equations (Olsen & Stokseth, 1995; Leclerc et al., 2003; Pasternack et al., 2004). As the computational power increased the feasibility of more complex hydraulic models for instream habitat analysis increased (Jowett & Duncan, 2012) especially taking into account that topographic data can now be acquired at spatial resolutions equal to or smaller than the size of fish themselves, and can therefore be used to characterize their physical habitat at an ecologically relevant scale (e.g., suitability of depth or velocity to support a specific activity such as spawning) (Wheaton et al., 2010). Practitioners must decide then about the form of hydraulic model that best suits their purpose and budget (Jowett & Duncan, 2012). Nevertheless, although theoretically feasible, 3D simulations are still restricted to the field of scientific research (Mouton et al., 2007).

In accordance with the previous comments and the statutory provisions described in the corresponding legislations, it can be easily inferred that the development of reliable habitat suitability models play a key role in a proper habitat assessment (Larocque et al., 2011). In this regards, Waters (1976) suggested the application of continuous curves representing a suitability index. The so–called Habitat Suitability Criteria (HSC) became by far the most common approach in studies involving the physical habitat simulation (Conallin et al., 2010; Muñoz-Mas et al., 2012). The HSC range from 0 (unsuitable) to 1 (completely suitable) and the corresponding value is interpolated for the whole set of microhabitat variables — the most important at the microhabitat scale are the aforementioned depth, velocity, substrate and cover (Gibson, 1993; Conallin et al., 2010; Boavida et al., 2014) — then the set of suitability values is typically aggregated to infer global indices describing the quantity and quality of the available habitat. The Weighted Usable Area (WUA) is the
most renowned general indicator of habitat quality and quantity and it corresponds to the sum of the areas (i.e. cells or pixels) weighed by the inferred suitability within the entire domain of the hydrodynamic model (Bovee et al., 1998). The WUA is usually calculated for each of the simulated flows thus becoming the WUA–flow curve (Boavida et al., 2014). For instance, the Spanish norm for hydrological planning (MAGRAMA, 2008) states that the minimum environmental flow should be selected within the range of 50–80% of the maximum WUA. In addition, upon the WUA–flow curve further calculations can be made for the EFA; for instance the comparison of alternative flow regimes and/or scenario analysis via habitat time series (Milhous et al., 1990).

However, the variables used in the development of HSC are treated independently for the estimation of the HSC even though interactions among them should be expected (Orth & Maughan, 1982). Consequently, there are examples of multivariate approaches (e.g. logistic regression) that demonstrated a greater ability in the determination of the presence or absence of some species (Guay et al., 2000). Multivariate analysis methods take into account the interaction of predictors and determine species response to cumulative effect of a set of environmental predictors (Ahmadi-Nedushan et al., 2006). The applications in physical habitat studies range from the relatively simple multiple linear regressions (Yu & Lee, 2002) to the pretty complex genetically optimized multilayer perceptron ensembles (Muñoz-Mas et al., 2014a). Multiple linear regression (MLR) approaches consider the joint effect of multiple explanatory variables and their interactions (Conallin et al., 2010) thus logistic regression, a variant of the ordinary MLR where the dependent variable is categorical (i.e. presence or absence), proved proficient to develop habitat suitability models (Guay et al., 2000; Garland et al., 2002). However species responses to environmental predictors are likely to be unimodal, often skewed, rather than straight–lines (Austin, 2007). Furthermore, the requirements of MLR are pretty restrictive (e.g. normality and homoscedasticity) so modern regression techniques such as Generalized Lineal Models (GLM) have proved preferable (Ahmadi-Nedushan et al., 2006). GLMs are a more flexible family of regression models, which relax the assumption of MLR also allowing other distributions for the response variable (e.g. binomial or Poisson). However, they do not solve the drawback related to linearity. Thereby when the species–habitat relationships are nonlinear, which is the case for most commonly used data (Ahmadi-Nedushan et al., 2006), researches advocated for semi– or non–parametric approaches such as Generalized Additive Models (GAMs) (e.g. Jowett and Davey, 2007), fuzzy logic based models (e.g. Muñoz–Mas et al., 2012) or artificial neural networks (e.g. Brosse and Lek, 2002). Among ANNs’ umbrella the most commonly applied technique has been the feed forward Multi–Layer Perceptron (MLPs).

GAMs (Hastie & Tibshirani, 1990) are semi–parametric models, indicated to deal with non–linearity, since they do not presuppose any specific type of distribution of the input variables because smooth functions, with different degree and number of curvatures, are used to model their effects (Jowett & Davey, 2007). The fuzzy logic approach, firstly
introduced by Zadeh (1965), takes into account the inherent uncertainty of ecological variables by discretizing the inputs in fuzzy sets (e.g. Low velocity, Medium velocity, High velocity etc.) due to the fuzzy nature of these sets, a given value may belong to more than one fuzzy sets with different proportion. Furthermore, the fuzzy logic approach enables expressing non-linear relations in a transparent manner because the relationship between the different combinations of fuzzy sets are articulated in IF–THEN sequences, known as fuzzy rules (Muñoz-Mas et al., 2012). For instance, IF velocity is High and depth is Medium and substrate is Low THEN the habitat suitability is Low. Artificial neural networks (ANNs), even the MLP, is a modelling paradigm inspired in the structure of the human brain and thus, its processing capability is fostered by the large number of highly interconnected elements called neurons, working in unity to solve specific problems (Olden et al., 2008). MLPs are able to identify non-linear relationships between input and output data, even if the data are imprecise or noisy and automatically assumes interactions between the environmental predictors (Conallin et al., 2010). Although these are the most relevant modelling approaches, currently GAMs and fuzzy models are likely to gain practitioners since these techniques are actually implemented in commercial software packages; GAMs have been implemented in SEFA (Payne & Jowett, 2012) whereas CASiMiR allows the use of fuzzy models (Jorde, 1997; Schneider, 2001). Conversely, the ANN users need to be experienced in computer programming and models’ building so its popularity has been traditionally restricted to the field of scientific research (Conallin et al., 2010). Nevertheless, they represent by far the most popular technique among those encompassed in the group of computational intelligence and machine learning in the bio–environmental sciences (Fukuda & De Baets, 2012). Nevertheless, scientific community and EFA practitioners are constantly innovating and searching for novel and accurate techniques. Therefore, the previous enumeration is not exhaustive since there are successful studies that used other techniques of lesser popularity. For instance; Fukuda et al. (2014), used classification and regression trees (Breiman et al., 1984), Martelo et al. (2014) used hurdle models (Mullahy, 1986) or Tirelli et al. (2012) used support vector machines (Vapnik, 1995) all of them to relate fish presence and abundance with the habitat characteristics achieving pretty competitive results such as random forests (Breiman, 2001).

Despite those successful studies, it has been demonstrated that every single modelling technique do not necessarily perform consistently, resulting in divergent predictions (Fukuda et al., 2013; Bouska et al., 2015). Such phenomenon is especially remarkable in those techniques (e.g. MLPs or genetic fuzzy models) whose initial conditions drive the optimization process (Raudys, 2000; Fukuda et al., 2010) thus concluding that even the same modelling approach could be able to render different models. In some cases it has demonstrated difficult to advocate for a specific model (Fukuda et al., 2013; Bouska et al., 2015). Therefore the use of model ensembles has been emphasised to overcome these limitations (Araújo & New, 2007). The very basic principle of ensemble modelling is to
learnt several models, developed with a unique methodology or with several different techniques and then, combining each single forecast into a single prediction (Wang & Alhamdoosh, 2013). As the computation capability and the programming skills of researches raised the ensemble approach gained popularity so nowadays almost every single technique have their ensembles counterpart such as GAMs ensembles (Bock et al., 2010), MLP Ensembles (Hansen & Salamon, 1990) or fuzzy models ensembles (Scherer, 2012), as well as combinations of different models (Thuiller et al., 2009). Nevertheless random forests (Breiman, 2001) is the only approach that can be considered widespread in EFA–related studies (e.g. Vezza et al., 2015).

Regarding the hydrologic and water chemistry and temperature component, process–based (i.e. physically–based) models have traditionally been preferred (van Vliet et al., 2012), and there is a myriad of capable models available for hydrologic simulation (Zhuo et al., 2015), some with specific modules dedicated to water temperature forecasting (e.g. Luo et al., 2013). However, machine learning–based approaches (data–based) such as neuro–fuzzy rule base models (Lohani et al., 2012) or Artificial Neural Networks (ANNs) (Rabi et al., 2015) have recently gained appreciable visibility (Zhuo et al., 2015).

Once the three components are set they can be combined to inspect temporal trends by developing the Habitat Time Series (HTS), which correspond to the interpolation of corresponding quality index (usually WUA) from the pertinent habitat–flow curve (typically the WUA–flow curve), whereas the habitat conditions under different flow regimes are usually analysed by summarizing the HTS in the so–called Habitat Duration Curves (HDC) (Milhous et al., 1990). The HDCs depict the probability of exceedance of a given quantity of habitat. Therefore, such curves are very useful to evaluate the frequency and duration of suitable habitat, allowing conservationists or managers to discern the long term impact of different scenarios and possible bottlenecks for aquatic organisms (Parasiewicz, 2008).

The levels of the spatial scales used to sample the habitat utilization and consequently to model the habitat suitability for the target species can be grouped in three main classes: macro–scale (e.g. Olaya–Marín et al., 2012), meso–scale (e.g. Vezza et al., 2015) and micro–scale (e.g. Muñoz–Mas et al., 2012). Typically, the flow–related changes in physical habitat have been modelled at the microhabitat scale (few m²) using data of one or more hydraulic variables (e.g. depth and velocity) collected at multiple locations within a river reach (Bovee, 1986; Tharme, 2003). The hydraulic models are thus developed to fit these levels of granularity by rendering cells, patches or pixels in accordance with required precision (Jowett & Duncan, 2012). The micro–scale approach is the most widely accepted technique used to determine how flow alterations affect the habitat characteristics (Vezza et al., 2014). It demonstrated a proficient ability to predict fish location (Guay et al., 2000; Muñoz-Mas et al., 2014b) and consequently it is the approach described in the Spanish norm for hydrological planning (MAGRAMA, 2008). However, the use of the microhabitat scale has been criticized as being time–consuming (Parasiewicz, 2001) and for
emphasizing cross-sectional variation over the longitudinal one (Vezza et al., 2012). Consequently, some studies highlighted the benefits of the mesohabitat scale among the possible spatial scales that can be used to analyse fish habitat requirements (Costa et al., 2012; Vezza et al., 2015). Using the mesohabitat scale it is possible to describe the environmental conditions around an aquatic organism not only limiting the analysis at the point where it is observed (Vezza et al., 2015). Mesohabitats, generally corresponding in size and location to Hydro-Morphological Units such as pool, riffle or rapid, can be used to describe fish ecology with a broader range of habitat variables and even considering biotic variables such as the density of the accompanying species (Muñoz-Mas et al., 2015; Vezza et al., 2015). Furthermore, in some study cases, the mesohabitat approach demonstrated a greater ability to properly relate the habitat-suitability predictions and fish presence (e.g. Parasiewicz and Walker, 2007). However, more research is needed to incontestably assert that the mesohabitat approach outperforms the micro-scale one and what will be the ultimate flow recommendations based on the different approaches.

Finally, the macro-scale approach allows to develop reliable conservation planning tools that encompass at least entire river basins sampled at different segments (Olaya-Marín et al., 2012) which can be used to evaluate the trade-offs of different management and conservation strategies under broad scenarios (Bouska et al., 2015). Nevertheless, environmental variables rarely act at a single spatial scale (Boulangeat et al., 2012), and there are studies that demonstrated how fish species’ richness, composition and distribution are hierarchically conditioned by a suite of environmental predictors, from the large-scale to the reach-scale (Morán-López et al., 2012).

Riparian vegetation (e.g. Magdaleno and Fernández, 2011), macroinvertebrates (Belmar et al., 2013) and fish species (e.g. Schmutz et al., 2007) can be used as indicators of ecological status but fish species present some advantages that make them especially suited for that purpose. Freshwater fish are not only the most diverse group of vertebrates; they also feature the greatest proportion of threatened species (Clavero et al., 2004). Fish can occupy high trophic levels (Sánchez-Hernández & Amundsen, 2015), they are relatively easy to sample and to identify, and generally are known to indicate in-stream habitat constraints (Lorenz et al., 2013). Furthermore, fish are mobile species compared to other aquatic organism groups, e.g. benthic invertebrates, and often undergo ontogenetic shifts in their habitat preferences (Ayllón et al., 2010). Thus, to complete their life cycle, all required habitats must be present (Lorenz et al., 2013). Consequently the state of fish populations and fish habitats has served as indicators of aquatic ecosystem health (Katopodis, 2012), especially in areas such as the Iberian Peninsula, which is considered one of the freshwater fish biodiversity hotspots within Europe (Reyjol et al., 2007) with several species in imminent risk of extinction (Leunda, 2010).

Finally, modellers have to deal with another remarkable issue in order to render reliable models; the data prevalence (i.e. the proportion of presence in the entire data set).
Although there were previous studies pointing out the importance of such parameter in the discrimination capability of habitat models (Fielding & Bell, 1997), it was after the seminal Manel’s et al. (2001) study when the concern about the limitations imposed by such parameter were profusely studied by the scientific community (e.g. Fukuda, 2013; Jiménez–Valverde et al., 2009; Mouton et al., 2009). Low values of such parameter can be caused by several different reasons (Jiménez-Valverde et al., 2009) such species rarity (Fukuda, 2013) but also sampling biases related with the excessively fine granularity (Guisan et al., 2007). Fukuda (2013) brilliantly described the information provided by presence and absence data. Thereby, presence data indicate clear evidence that a target species was observed at a given location whereas absence data contain essentially uncertainty. For instance, there could be the case where the target species was absent due to the presence of enemy species or simply because the habitat was not yet occupied by the species.

Consequently the capability of a given model to balance the error committed for the presence and absence data can lead to different conclusions. Its impact is no exempt of controversy. For instance, Jiménez–Valverde et al. (2009) argued that unbalanced habitat suitability data are not such a problem from a statistical viewpoint, highlighting that the effects of unbalanced prevalence should not be confused with those of low quality data affected by false absence data as described by Mouton et al. (2009). However Jiménez–Valverde et al. used GLMs, which perhaps are able to deal with low prevalence but, as described above, present some other limitations. Each technique has their merits and demerits and some of them can be not irrespective to data prevalence. For instance the GAMS (Leathwick et al., 2006) or fuzzy logic (Fukuda, 2013) have proven to be affected whereas some other proved pretty robust in front of such parameter (Muñoz-Mas et al., 2014b). Therefore, several strategies have been developed from the simply case weighting or down–sampling the absence dataset (getting 0.5 prevalence datasets) (Hirzel et al., 2001; Maggini et al., 2006) to the application of parallel distributed genetics–based approaches that permute the absence cases across several computers to optimize fuzzy models (Nojima et al., 2012). Nevertheless, modellers must take into account data prevalence and its effects on the novel techniques prior to advocate for any of them.
I.3 OBJECTIVES AND OVERVIEW

Despite the number of studies described above there are still knowledge gaps and room for new approaches. The present dissertation focused in the comprehensive analysis of the capabilities of some novel modelling techniques (i.e. non-tested before) for environmental flow assessment and exploratory ecology, specifically: the Probabilistic Neural Networks (PNN) and the Multi-Layer Perceptron (MLP) Ensembles. The analysis of the capabilities of these techniques was performed using Iberian native fish species as the target species. Furthermore, the studies specially focused in the capability these techniques to deal with low prevalence or the excess of zeros in the training datasets thus it was common across chapters. The remaining specificities are listed below.

Main objective:

1. Testing the capabilities of PNN and MLP Ensembles to model the habitat suitability for native fish species and its usefulness in environmental flow assessment.

Specific objectives:

2. To evaluate the effect of data prevalence or a high percentage of zeros in the performance of these techniques (i.e. PNN and MLP Ensembles).

3. To inspect the uncertainty associated with the predictions carried out with these techniques.

4. To evaluate different approaches to determine the optimal MLP Ensemble.

5. To model the habitat suitability for freshwater fish species: brown trout (*Salmo trutta*; Linnaeus, 1758), Bermejuela (*Achondrostoma arcassi*; Steindachner, 1866) and redfin barbel (*Barbus haasi* Mertens, 1925).

6. To evaluate the effect of the modelling spatial scale (micro-scale and meso-scale) on environmental flow assessments in Mediterranean rivers.

I.3.1 Chapter II

There are several kinds of ANN such as aforementioned MLPs (McCulloch & Pitts, 1943; Rumelhart et al., 1986) but also the PNN (Specht, 1990) or the Elman (Elman, 1990) and Jordan ANN (Jordan, 1997). Each type is especially addressed for a different task. For instance the MLPs are especially addressed for regression (Olden et al., 2008), although they can also be used for classification (Tirelli & Pessani, 2011). PNN are theoretically able to cope with low prevalence (Specht, 1990) making them excellent candidates to
swell the group of available habitat suitability modelling techniques. PNN basically compare the assessed conditions, typically termed as input patterns (e.g. the pixels of a hydraulic models), with the measured conditions included in the training database and determines the probability of membership of the input pattern to each of the categories present in the training database (typically restricted to two categories, presence or absence). To deal with differences on the intensity of the output the weight of each category in the database is inversely proportional to the number of cases in the corresponding category. Then the classification to a given category depends on the values of the variables to be assessed but not on the number of cases from a given category.

PNN had been applied successfully in pattern classification in some areas related to fish (e.g. classification of sonar signals) (Moore et al., 2003) and in the assessment of the suitability for bacteria growth in given environmental conditions (Hajmeer & Basheer, 2002) but to our knowledge this chapter (and the corresponding paper) corresponded to the very first application in fish habitat modelling and environmental flows. The study aimed at testing the suitability of PNN as a tool for brown trout habitat suitability modelling at the micro–scale and for environmental flow assessment. To achieve this general aim, i) presence–absence PNN were generated and trained, ii) the effect of prevalence on models performance and habitat assessment was thoroughly analysed, iii) the modelled brown trout habitat suitability was analysed in a multivariate way, iv) the PNN were evaluated in an independent river under similar ecological conditions to those where the training database was collected; and finally, v) the applicability of the PNN models to assess minimum legal e–flows at the evaluation site was discussed by calculating the WUA – flow curve.

1.3.2 Chapter III

Multi–Layer Perceptron (MLP) Ensembles (Hansen & Salamon, 1990) are the ensemble counterpart of ANNs. MLP Ensembles can be used for classification (Watts & Worner, 2008) and regression tasks (Boucher et al., 2010). Its development typically follows the overproduce–and–choose approach (Partridge & Yates, 1996). First, a large pool of MLPs is trained, usually by bagging the complete dataset (resampling with replacement), and the combination of MLPs that produces the best mean prediction (arithmetic mean) on the out–of–bag dataset is sought. In some cases the surplus MLPs are effectively removed (e.g. Wang and Alhamdoosh, 2013) whereas in other cases they are simply weighted by a sufficiently small value to almost eliminate any influence on the predictions (e.g. Opitz and Shavlik, 1996). In this chapter i) two approaches to select the MLPs included in the final ensemble, one based on the Forward selection of the candidates and the second one used a Genetic Algorithms to select the ultimate set of MLPs included in the Ensemble. The target species was the presence–absence (classification) of the Bermejuela
(Achondrostoma arcasii; Robalo, Almada, Levy & Doadrio, 2006) at the micro–scale and ii) a graphical sensitivity analysis was applied to the model that presented the best performance to inspect the habitat preferences of the Bermejuela. Finally results in terms of habitat suitability were discussed.

1.3.3 Chapter IV

Scientists, stakeholder and managers require sound ecological knowledge in order to take substantiated decisions to adequately deal with predicted climate–induced changes in the flow regime (Santiago et al., 2015; Muñoz-Mas et al., 2016b). Ecological models can be used to explore fish ecology thus they have demonstrated to be useful elements to understand the realized niche of species and to species conservation in relation to global change, which includes climate change (Austin, 2007). Ecological systems often exhibit non–linear complex data structures and thus they usually cannot be analysed with simple statistical approaches (Crisci et al., 2012). In this context, the MLP Ensembles could be especially suited to develop ecological models.

Fish ecology can be addressed at different spatial scales. Some authors highlighted the benefits of the mesohabitat scale among the other spatial scales to analyse fish habitat requirements (Costa et al., 2012; Vezza et al., 2015) because using this scale is possible to describe the environmental conditions around an aquatic organism not only limiting the analysis to the point where it is observed (Vezza et al., 2015). Furthermore, mesohabitats – generally corresponding in size and location to Hydro–Morphological Units (HMU) such as, pool, riffle or rapid – can be used to describe fish ecology with a broader range of variables even including biotic predictors (Vezza et al., 2015).

The importance of dealing with uncertainty has been stressed as a key challenge in ecological modelling (Larocque et al., 2011) (Larocque et al., 2011). Consequently, this chapter inspected the uncertainty associated with the MLP aggregation with regard to the ranges and distribution of the selected input variables.

The principal objectives of this chapter were, i) to test the proficiency of the MLP Ensembles to model the ecological niche of freshwater fish species, and ii) to test whether biotic variables affect the distribution of redfin barbel. To achieve these aims, and using MLP Ensembles, two different models of redfin barbel were developed. The first considered only physical habitat variables collected at the meso–scale, the second model also included biotic predictors (fish and macroinvertebrates). Finally, the uncertainty associated to the process of aggregation of each individual forecast within the MLP Ensemble was inspected in both models.
I.3.4 Chapter V

In environmental flow assessment the instream habitat has been typically evaluated at the micro–scale with habitat suitability models developed at this scale (Tharme, 2003; Conallin et al., 2010). However, as we described above, some authors suggested that the meso–scale could be a better scale to describe the habitat preferences of the inhabiting fish and hence rendering more accurate e–flows (Parasiewicz & Walker, 2007; Vezza et al., 2012). However, more research is needed to dispel any doubt about the advisability of the meso–scale over the micro–scale one and about significant differences in the e–flow eventually assessed.

In this chapter i) MLP Ensembles were used to develop presence–absence models for the redfin barbel at two different scales (micro–scale and meso–scale and ii) a graphical sensitivity analysis was performed to compare our models between them and with previous literature. The two MLP Ensembles were linked with a hydraulic model to infer e–flows iii) evaluating the practical applicability of these models and iv) we briefly discussed the merits and demerits of our models and the differences in the e–flow assessment.
APPLICATION OF PROBABILISTIC NEURAL NETWORKS TO MICROHABITAT SUITABILITY MODELLING FOR ADULT BROWN TROUT (*Salmo trutta* L.) IN IBERIAN RIVERS

II. APPLICATION OF PROBABILISTIC NEURAL NETWORKS TO MICROHABITAT SUITABILITY MODELLING FOR ADULT BROWN TROUT (*Salmo trutta* L.) IN IBERIAN RIVERS

**ABSTRACT**

Probabilistic Neural Networks (PNN) have been tested for the first time in microhabitat suitability modelling for adult brown trout (*Salmo trutta* L.). The impact of data prevalence on PNN was studied. The PNN were evaluated in an independent river and the applicability of PNN to assess the environmental flow was analysed. Prevalence did not affect significantly the results. However PNN presented some limitations regarding the output range. Our results agreed previous studies because trout preferred deep microhabitats with medium–to–coarse substrate whereas velocity showed a wider suitable range. The 0.5 prevalence PNN showed similar classificatory capability than the 0.06 prevalence counterpart and the outputs covered the whole feasible range (from 0 to 1), but the 0.06 prevalence PNN showed higher generalisation because it performed better in the evaluation and it allowed a better modulation of the environmental flow. PNN has demonstrated to be a tool to be into consideration.

**Keywords:** Probabilistic Neural Networks; brown trout; microhabitat suitability; prevalence; spatially explicit evaluation; Mediterranean rivers
II.1 INTRODUCTION

The environmental impact of hydrological alteration is of major concern for researchers (Marsili-Libelli et al., 2013). Therefore, scientists and managers have developed a vast body of methodologies to assess the consequences of changes in running river flows (Acreman and Dunbar, 2004, Ahmadi–Nedushan et al., 2006). This concern overstepped the merely academic environment yielding the Water Framework Directive (WFD) (European Parliament & Council, 2000), a legislative framework that stated a set of environmental objectives for water bodies with the time frame of the year 2015. The WFD implicitly assumes an underlying link between ecological status and abiotic quality elements; thus, a key aspect is the identification and assessment of the links between the physical and biological components of streams (Conallin et al., 2010). Accordingly, freshwater fish are considered good indicators of water quality and biotic integrity in freshwater ecosystems (Pont et al., 2006).

The Instream Flow Incremental Methodology (IFIM) (Bovee et al., 1998) was the first methodological framework for the environmental impact assessment and negotiation in water allocation schemes (Paredes–Arquiola et al., 2013). Moreover it has been stressed as the most scientifically and legally defensible methodology available (Tharme, 2003). The physical habitat simulation is a part of the IFIM methodology that permits to understand the impact of flow alterations on stream habitat (Maddock, 1999). Consequently it has been considered in the transposition of the WFD to the Spanish norm for hydrological planning (MAGRAMA, 2008). Among the fish species considered in the physical habitat simulation, in the Iberian context brown trout (Salmo trutta L. 1758) has been specifically used as an indicator of ecological status (Ayllón et al., 2012). Therefore, insight into the habitat suitability of brown trout is crucial for the implementation of the WFD and for environmental flow (e–flow) assessments, especially in areas vulnerable to global change such as the Mediterranean streams (García–Ruiz et al., 2011).

The continuous univariate Habitat Suitability Curves (HSCs) are a simple and common modelling approach in studies involving physical habitat simulation (Payne and Allen, 2009); hence several researchers have developed habitat suitability models in the form of HSCs (Ayllón et al., 2010, Bovee, 1978, Hayes and Jowett, 1994, Raleigh et al., 1986, Vismara et al., 2001). The relationship between Weighted Usable Area (WUA) and river flow (Bovee et al., 1998) derived from these models has been used extensively in e–flow assessments (Payne, 2003). However, several authors have suggested that considering each hydraulic variable independently may be questionable because ignoring significant interactions between variables may induce a bias (Orth and Maughan, 1982). As a consequence, the multivariate approaches have gained popularity (De Pauw et al., 2006). Several data–driven multivariate techniques have been applied in brown trout habitat suitability modelling. Specifically at the microhabitat scale, these studies ranged from simple bivariate polynomial functions (Lambert and Hanson, 1989, Vismara et al., 2001) to
more complex fuzzy rule base models (Jorde et al., 2001). Thereby, logistic regression has been used by some researchers (Ayllón et al., 2010, Hayes and Jowett, 1994), as well as Generalized Additive Models (GAMs) (Jowett and Davey, 2007) to develop habitat suitability models for brown trout. Among the machine learning techniques, Artificial Neural Networks (ANN) and specifically the Multilayer Perceptron, have also been applied to model habitat suitability for brown trout (Reyjol et al., 2001). In the eastern Iberian Peninsula, the fuzzy logic approach has been applied to develop models for brown trout with the mesoscale as the central resolution (Mouton et al., 2011) whereas at the microscale Muñoz–Mas et al. (2012) developed fuzzy rule base models for middle–size brown trout (body length from 10 to 20 cm).

Overall, each approach for habitat modelling has advantages and disadvantages and due to their different model structures they are distinct in their data needs, transferability, user–friendliness and presentable outputs (Conallin et al., 2010). Therefore, the habitat simulation methodologies are in a permanent evolution driven by their imperfections and inherent constrains (Lamouroux et al., 1998) (Lamouroux et al., 1998). Probabilistic Neural Networks (PNN) (Specht, 1990) are a promising type of ANN. These were applied successfully in pattern classification in some areas related to fish (e.g. classification of sonar signals) (Moore et al., 2003). But to our knowledge, this technique has never been applied before in habitat suitability modelling at the microscale.

An important aspect in data–driven habitat suitability modelling is the prevalence (i.e., proportion of presence in the entire data set) because it can have a strong effect on model performance (Fukuda, 2013, Manel et al., 2001). The decreasing trends in brown trout populations (Almodóvar et al., 2012) or the temporary absence of the species (Lütolf et al., 2006) in addition to the sampling protocols, can lead to low prevalence databases. The capability to deal with low prevalence has been the main focus in several studies. For instance, Mouton et al. (2009) developed a prevalence–adjusted method addressed to fuzzy rule base models, and Freeman et al. (2003) tested the ability of Random Forests (Breiman, 2001) to deal with low prevalence datasets. In this context, PNN are theoretically able to cope with low prevalence databases (Specht, 1990), thus suggesting its suitability to construct fish habitat models with unbalanced databases. Another remarkable issue in ecological modelling is the over–fitting. Some techniques are prone to that phenomenon in a different degree. Therefore, some authors highlighted the importance of making a successful evaluation (sensu Guisan and Zimmermann, 2000) using independent data to improve the reliability of the models (Bennett et al., 2013).

Our study aimed at testing the suitability of PNN as a tool for brown trout habitat suitability modelling at the microscale. To achieve this general aim, (i) presence–absence PNN were generated and trained; (ii) the effect of prevalence on models performance and habitat assessment was analysed; (iii) the modelled brown trout habitat suitability was analysed in a multivariate way by checking how the PNN assess a synthetic database covering all the
possible combinations of velocity, depth and substrate within the survey range; (iv) the PNN were evaluated in an independent river under similar ecological conditions to those where the training database was collected; and finally, (v) the applicability of the PNN models to assess minimum legal e–flows at the evaluation site was discussed by calculating the WUA – flow curve.

II.2 METHODS

II.2.1 Microhabitat data collection

The target species of this study at the microscale was the adult (body length > 20 cm) brown trout. The data samplings were carried out at low–flow conditions during late spring, summer and early autumn in the period 2007–2009 in the Guadiela and Cuervo Rivers (within the Tagus River Basin; TB) and in the Jucar and Senia Rivers (within the Jucar River Basin District; JRBD) (Fig. 1).

Fig. 1. In the Iberian Peninsula (left), location of the sites where microhabitat data of brown trout were collected in rivers within the Tagus River Basin and the Jucar River Basin District. Red circle shows the location where the models were evaluated in the Cabriel River.

The microhabitat study was undertaken in complete and connected HydroMorphological Units (hereafter, HMUs) and classified as pool, glide, riffle and rapid. A sort of modification
of the equal effort approach was applied (Bovee et al., 1998) with the selection of equal areas of slow and fast water HMUs, grouping pools with glides (slow) and riffles with rapids (fast). Each HMU was studied by underwater observation (snorkelling) during daylight with minimum disturbance to the fish according to common procedures (Heggenes et al., 1990, Martínez–Capel et al., 2009). This technique allows the observation of the fish behaviour, thus only adult brown trout that were ‘feeding’ or ‘holding a feeding position’ were considered because it is assumed that they are occupying such positions because the most energetically profitable (Rincon and Lobon–Cervia, 1993). Microhabitat conditions, termed as training patterns, were measured along the HMU in cross–sections, classifying fish abundance into two groups as ‘absence’ (no fish observed) and ‘presence’ (at least one fish observed). The resulting sampled area per training pattern (measurement) ranged from 1.23 m$^2$ to 7.96 m$^2$. The high number of absence patterns versus presence patterns led to a low prevalence (average prevalence being 0.06) that ranged from 0.02 to 0.11 depending on the river (Table 1).

<table>
<thead>
<tr>
<th>River</th>
<th>Year</th>
<th>N Presence</th>
<th>N Absence</th>
<th>Prevalence</th>
<th>Area surveyed (m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jucar (JRBD)</td>
<td>2007</td>
<td>7</td>
<td>339</td>
<td>0.02</td>
<td>910</td>
</tr>
<tr>
<td>Guadiela (TRB)</td>
<td>2009</td>
<td>51</td>
<td>411</td>
<td>0.11</td>
<td>3189</td>
</tr>
<tr>
<td>Senia (JRBD)</td>
<td>2007</td>
<td>11</td>
<td>346</td>
<td>0.03</td>
<td>922</td>
</tr>
<tr>
<td>Cuervo (TRB)</td>
<td>2009</td>
<td>29</td>
<td>361</td>
<td>0.07</td>
<td>3065</td>
</tr>
</tbody>
</table>

Depth was measured with a wading rod to the nearest cm and the mean flow velocity of the water column (hereafter velocity) was measured with an electromagnetic current meter (Valeport®). The percentage of each substrate class was visually estimated around the sampling point or fish location. The substrate classification was simplified from the American Geophysical Union size scale: bedrock, boulders (>256), cobbles (64–256 mm), gravel (8–64 mm), fine gravel (2–8 mm), sand (62 mm–2 mm), silt (< 62 mm) and vegetated soil (i.e. substrate covered by macrophytes), similarly to a previous work in Iberian rivers (Martínez–Capel et al., 2009). Substrate composition was converted into a single value through the Substrate index (hereafter substrate), by summing weighted percentages of each substrate type as follows: Substrate index=0.08 ·% bedrock + 0.07 ·% boulder + 0.06 ·% cobbles + 0.05 ·% gravel + 0.04 ·% fine gravel + 0.03 ·% sand (Mouton et al., 2011) (Table 2). Finally, the three input variables (velocity, depth and substrate) were normalized $N(0,1)$. 

Table 1. Sample sizes of the four campaigns for microhabitat data collection of the adult brown trout, in rivers within the Tagus River Basin (TRB) and Jucar River Basin District (JRBD).
Table 2. Characteristics of the selected rivers within the Tagus River Basin (TRB) and Jucar River Basin District (JRBD) where the microhabitat data were collected. Substrate (Dominant) corresponds to the larger aggregated percentage.

<table>
<thead>
<tr>
<th></th>
<th>Cuervo (TRB)</th>
<th>Guadiela (TRB)</th>
<th>Jucar (JRBD)</th>
<th>Senia (JRBD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2009</td>
<td>2009</td>
<td>2007</td>
<td>2007</td>
</tr>
<tr>
<td>Mean Width (m)</td>
<td>8.8</td>
<td>9.6</td>
<td>8.4</td>
<td>6.6</td>
</tr>
<tr>
<td>Survey Flow (m$^3$/s)</td>
<td>0.27</td>
<td>0.61</td>
<td>1.05</td>
<td>1.17</td>
</tr>
<tr>
<td>Strahler Order</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mean Flow Velocity (m/s)</td>
<td>0.21</td>
<td>0.18</td>
<td>0.11</td>
<td>0.31</td>
</tr>
<tr>
<td>Max. Flow Velocity (m/s)</td>
<td>1.03</td>
<td>1.15</td>
<td>1.15</td>
<td>1.75</td>
</tr>
<tr>
<td>Mean Depth (m)</td>
<td>0.48</td>
<td>0.59</td>
<td>0.5</td>
<td>0.59</td>
</tr>
<tr>
<td>Max. Depth (m)</td>
<td>1.24</td>
<td>1.78</td>
<td>1.01</td>
<td>1.4</td>
</tr>
<tr>
<td>Substrate (Dominant)</td>
<td>Gravel</td>
<td>Cobble</td>
<td>Vegetation</td>
<td>Boulder</td>
</tr>
</tbody>
</table>

II.2.2 Development of the Probabilistic Neural Network

II.2.2.1 PNN theory

PNN are radial–basis neural networks based on a Bayes–Parzen classifier (Specht, 1990). PNN basically compare how close the input pattern is to the patterns of each category in the training database and assign the category that presents the highest number of patterns in the vicinity. The number of patterns on each category can vary; in order to deal with these differences the weight of each pattern is inversely proportional to the number of training patterns in the corresponding category. Thus, the classification within a given category depends on the values of the input but not on the number of training patterns included in that category.

From a theoretical point of view but considering the present classification problem, where two categories were considered (i.e. adult brown trout ‘presence’ or ‘absence’), the Bayes’ theorem considers an input $x=[x_{\text{velocity}}, x_{\text{depth}}, x_{\text{substrate}}]$ which will be classified in the category ‘presence’ if the following inequality is fulfilled: $h_{\text{Presence}} \cdot i_{\text{Presence}} \cdot f_{\text{Presence}}(x) > h_{\text{Absence}} \cdot i_{\text{Absence}} \cdot f_{\text{Absence}}(x)$ where $h_{\text{cat}}$ is the a priori probability of occurrence, $i_{\text{cat}}$ is the cost associated with misclassification and $f_{\text{cat}}(x)$ is the Probability Density Function (PDF) of the corresponding category. The aggregation of these three parameters defines the membership function. The Bayes’ theorem tend to increase the probability of the class (‘presence’ or ‘absence’) with the higher density of training patterns in the vicinity of the unknown input ($f_{\text{cat}}(x)$), or if the cost of misclassification ($i_{\text{cat}}$) or prior probability ($h_{\text{cat}}$) of the corresponding category are higher (Hajmeer and Basheer, 2002). The cost of misclassification ($i_{\text{cat}}$) and the prior probability ($h_{\text{cat}}$) allow the development of over–predictive models where false–positives are preferred, for instance in cancer diagnosis (Berrar et al., 2003) and should be adjusted for every specific problem. In our study, the a priori probability of occurrence ($h_{\text{cat}}$) was considered 0.5 and no misclassification costs
(i_{cat}) were applied, thus both factors were neglected. In this case, the training patterns must provide the information to estimate the underlying multivariate PDF \( f_{cat}(x) \) of each category (Specht, 1990), following the equation 1:

\[
f_{cat}(x) = \frac{1}{(2\pi)^{p/2} \prod_{j=1}^{p} \sigma_j \sqrt{n}} \sum_{i=1}^{n} \exp \left[ -\sum_{j=1}^{p} \left( \frac{x_j - x_j^i}{2\sigma_j} \right)^2 \right]
\]

(Equation 1)

where \( x \) is the input pattern to be classified (\( x_{\text{velocity}}, x_{\text{depth}}, x_{\text{substrate}} \)) and \( x_j^i \) is the \( i^{th} \) training pattern for the corresponding category (\( x_{\text{velocity}}^i, x_{\text{depth}}^i, x_{\text{substrate}}^i \)). The \( \sigma_j \) are the smoothing parameters (\( \sigma_{\text{velocity}}, \sigma_{\text{depth}}, \sigma_{\text{substrate}} \)) that represent the standard deviation around the mean of the 3 input variables (\( p=3 \)) and they control the degree of influence of every pattern in the corresponding axe; velocity, depth or substrate. Finally, the \( n \) parameter corresponds to the total number of training patterns in that category; it varies depending on the considered PDF, and it was set to 1457 in the ‘absence’ PDF and to 98 in the ‘presence’ PDF.

PNN architecture differs from other ANN such as the Multilayer Perceptron (MLP) (Fig. 2). Unlike MLP, the activation functions or the architecture are not optimised and the main reason to include PNN within the ANN discipline is that the input is compared or connected to every training pattern. The PNN have a unique type of activation function, the so called radial basis function, and the network architecture depends on the training database, thus presenting equal amount of nodes as training patterns (Bishop, 1995).

In the case of presence–absence classification, the PNN calculated two PDF \( f_{cat}(x) \) in parallel, one for each output category. The first layer (the input layer) is a distributing layer where \( x \) is the input pattern (i.e. a combination of velocity, depth and substrate), and it is connected to every node in the second layer (the hidden layer). The hidden layer has a number of neurons equal to the number of training patterns (i.e. the 1555 collected patterns; \( n=98 \) presences, \( m=1457 \) absences). In the hidden layer, the ‘distance’ between the input pattern and each training pattern in the corresponding node \( (X_{P1}, X_{P2}, ..., X_{P98}, \text{ and } X_{A1}, X_{A2}, ..., X_{A1457}) \) is calculated. The third layer executes the summation of the signals (‘distances’) produced in the previous layer, but each category has an independent summation of signals, as demonstrated in Fig. 2. Once the \( \sigma_j \) parameters are selected, the network is already prepared to assess any pattern. That is the main reason why PNN are considered a one–pass learning method because they are automatically trained by the patterns in the training database (Specht, 1989). Finally, the output of both nodes is standardized between 0 and 1, by dividing the results with the sum of both outputs, in order to agree with other habitat suitability models.
Fig. 2. General architecture of a Probabilistic Neural Network (PNN) in a presence–absence classification problem. *x* corresponds to the assessed pattern. *XP* and *XA* correspond to the presence training patterns (*n*=98) and the absence training patterns (*m*=1457) respectively.

The $\sigma_j$ parameters have a decisive impact on the PNN performance (Fig. 3). Therefore, its optimisation is recommended to obtain an optimal PNN (Hajmeer and Basheer, 2002). If the smoothing parameter is too small, the PDF would be highly over–fitted to the training patterns, thus reducing the capability of the network to generalize (i.e. to assess properly an unseen pattern). If the $\sigma_j$ parameters are too large, the output value would be almost constant and proportional to the number of training patterns in the considered class ('presence' or 'absence'); in this case, the values of the inputs would not play any role in the assessment of a given pattern (Zhong et al., 2005). The present study considered a single smoothing parameter, thus resulting in $\sigma_{\text{velocity}}=\sigma_{\text{depth}}=\sigma_{\text{substrate}}$. 
II.2.2.2 PNN optimisation

The PNN was implemented in the R environment (R Core Team, 2015) considering the complete database (hereafter PNNC). The \( \sigma \) parameter was optimised by leave-one-out cross-validation with the subplex algorithm proposed by Rowan (1990) and implemented in the R environment (R Core Team, 2015) by King (2008). Waters (1976) introduced the use of univariate Habitat Suitability Curves (HSC) assessing the degree of suitability of the usual microhabitat variables, such as depth or velocity, ranging from 0 and 1. Accordingly, several studies comprising difference techniques ranged the habitat suitability from 0 to 1 (Ayllón et al., 2010, Jowett and Davey, 2007, Muñoz–Mas et al., 2012). However, large values of \( \sigma \) typically do not provide the extreme feasible outputs (Fig. 3). The use of single performance criterion may lead to counterproductive results such as favouring models that do not reproduce important features of a system (Bennett et al., 2013). Therefore, two main goals were included in the objective function, the maximization of the classification strength and the maximization of the output range. The classification strength was quantified by means of a performance measure arising from the confusion matrix components (true positive \([TP]\), false positive \([FP]\), false negative \([FN]\) and true negative \([TN]\)). The selected performance criterion was the True Skill Statistic (TSS) (Equation 2) because it has been demonstrated suitable in models dealing with unbalanced prevalence databases and it favours a good balance between Sensitivity (Sn) and Specificity (Sp) (Allouche et al., 2006, Fukuda et al., 2013, Mouton et al., 2010). Sn is the true positive rate \([=TP/(TP+FN)]\) and Sp is the true negative rate \([=TN/(FP+TN)]\). The output range was considered by subtracting the minimum output value, which was expected to be 0, to the maximum output value which was expected to be 1. The objective function was the aggregation of both indices.
\[ TSS = \text{Sensitivity} + \text{Specificity} - 1 \] (Equation 2)

\section*{II.2.3 Effect of the data prevalence on performance of the PNN}

We analysed the effect of prevalence on PNN performance by altering the prevalence of the training dataset but keeping the optimised $\sigma$ parameter constant. Bagging was an alternative but it has been reported as extremely time-consuming (Zhong et al., 2005). Therefore, the selected datasets had to be statistically similar to the original database, presenting similar distributions for depth, velocity and substrate. The sub-sampling methodology presented in Muñoz-Mas et al. (2012) was used to generate each of the five alternative datasets with prevalence of 0.1, 0.2, 0.3, 0.4, and 0.5. The statistical analysis, a robust generalization of Welch test (Welch, 1951) and a robust generalization of Kruskal-Wallis test (Rust and Filgner, 1984) showed no significant differences with the complete database (prevalence=0.06). Therefore, these new five datasets were considered suitable for further analyses. Considering the optimal $\sigma$ calculated for the PNN$_C$, the leave-one-out cross-validation was carried out for each alternative dataset, assessing the corresponding training patterns. The TSS and the output distribution (minimum, maximum, quartiles, median and mean) were calculated and compared with those obtained from PNN$_C$. Finally, the results were univariately plotted (hereafter univariate habitat suitability plots) and these plots were used to check differences in the predicted suitability derived from changes in the prevalence. Subsequently, the PNN based on the 0.5 prevalence dataset (hereafter PNN$_{0.5}$) which corresponds to the ideal situation (i.e. the training dataset presents equal number of training patterns per category) was used for further analyses.

\section*{II.2.4 Model transparency and ecological relevance}

Formerly, a great effort has been made to improve the transparency of Neural Networks (Olden and Jackson, 2002, Gevrey et al., 2006). Following this premise, our PNN models (PNN$_C$ and PNN$_{0.5}$) were used to assess the habitat suitability over a synthetic database that covered all possible combinations of velocity, depth and substrate within the surveyed range. With velocity ranging from 0 to 1.75 m/s, depth from 0 to 1.75 m and the substrate ranging from 0 to 8. The entire process represented a modification of the assessment presented in Hajmeer and Basheer (2002). The assessed three-dimensional database was simultaneously plotted (hereafter multivariate habitat suitability plot) with the training database assessed by means of the leave-one-out procedure; both datasets were separated in slices representing the classes of substrate (from 0 to 8), thus 9 plots were made. The consideration of all possible combinations of velocity and depth disagree with the usual distribution of these variables (Coarer, 2007) that tend to fit the distribution found
in the training database. However, these plots allowed the inspection of the modelled suitability for every combination of velocity, depth and substrate within the feasible range, as well as the capability of PNN to extrapolate. The multivariate habitat suitability plot and the univariate habitat suitability plot derived from the PNN\textsubscript{C} were discussed in comparison with previous studies.

Brown trout has been successfully introduced due to its ecological flexibility and its reputation as fine food and good sport (Klemetsen et al., 2003), therefore it presents a worldwide distribution. Accordingly, it has been the target species of several studies covering a huge range of ecological conditions and spatial scales. Given the large amount of information about its habitat selection at the microscale, it was necessary to prioritize by selecting some benchmarking studies that were mainly located in the Mediterranean context and had applied multivariate techniques (Ayllón et al., 2010, Lambert and Hanson, 1989, Vismara et al., 2001). However, the multivariate approach is not as widespread as the univariate one. Therefore, we finally considered other studies from Europe and other continents that used multivariate approaches, as well as others of great international relevance for the target fish species (Bovee, 1978, Heggenes, 1996, Jorde et al., 2001, Jowett and Davey, 2007, Rincon and Lobon–Cervia, 1993).

II.2.5 Model evaluation and transferability
To assess the transferability of the generated PNN (PNN\textsubscript{C} and PNN\textsubscript{05}), a spatially explicit evaluation was carried out in a river reach of the Cabriel River (main tributary of the Jucar River), where a hydraulic model was available (Muñoz–Mas et al., 2012). The selected reach met different requirements for the study of habitat suitability; underwater visibility (to observe fish underwater with minimum disturbance), abundance of the target fish species within the region where it is native, habitat heterogeneity and representativeness (all the habitat types present in the river appeared in the selected reach in similar proportion), neither morphological alteration nor relevant human impacts in the aquatic environment, and high water quality (not limiting the fish community in any aspect, and without any health risk for the diver) (Martínez–Capel et al., 2009).

II.2.5.1 Hydraulic modelling
A 2D hydraulic simulation was done in an approximately 300 m long reach of the Cabriel River. The topographic data of the river channel and banks were collected using a Leica© Total Station. The average area per topographic measurement was approximately 2 m\textsuperscript{2}, surveying the wetted area more intensively, thus ranging from 0.51 m\textsuperscript{2} in the wetter area to 3.72 m\textsuperscript{2} in banks. Substrate composition was visually estimated as described in the Microhabitat data collection. The hydrometry was performed in 11 cross–sections, with
depth and velocity measured along these sections and the resulting information was used to gauge the flow rate. Measurements were performed at three different flow rates (0.54, 1.04 and 2.75 m$^3$/s) and these were used for model calibration. The hydraulic modelling was carried out with River–2D© (University of Alberta, 2002) and the bed roughness was used to calibrate the model based on the depth and mean column velocity at each transect in accordance with previous studies (Jowett and Duncan, 2012). The model was considered acceptable when errors in water surface elevation were smaller than 5 cm at any cross-section, and when the patterns of the generated velocities and those measured at each cross-section were similar (Fig. 4). The topographic data obtained in the biological evaluation survey (section 2.5.2) were used to check differences and changes in the river bed. An average difference between channel elevation in the model and control measures of 0.04±0.13 m was obtained. Therefore, the topography was considered similar and the hydraulic model acceptable for further analyses. Thirty four different flows were simulated, ranging from 0.05 to 6.5 m$^3$/s; in all the cases the water level was below the bankfull stage of the river channel.

![Fig. 4. Water Surface Elevation (WSE) along the thalweg (left) and observed versus predicted WSE (right).](image)

**II.2.5.2 Biological evaluation**

A new field campaign was carried out in the Cabriel River in the early summer 2012, at a flow rate of 0.89 m$^3$/s. Unlike the previous campaigns, the diver did not snorkel entire HMUs, but the whole area included in the hydraulic model. The survey was performed according to the previous microhabitat surveys (Heggenes et al., 1990, Martínez–Capel et al., 2009). Rather, instead of recording velocity, depth and substrate only the locations
(coordinates X, Y, Z) of observed adult brown trout were recorded using a FOIF© Total Station.

II.2.6 PNN evaluation and applicability in e–flow assessment

The flow occurring during the biological evaluation survey was simulated with the hydraulic model. The two PNN ($PNN_C$ and $PNN_{05}$) were used to assess the habitat suitability in the entire simulated reach. The frequency analyses of the habitat suitability assessed in the trout locations and over the entire reach were compared in order to check the generalization capability of the PNN. The main purpose of the transferability tests is the quantification of model accuracy at destination (Fukuda, 2010, Randin et al., 2006, Thomas and Bovee, 1993). Among the transferability tests, the most popular in studies involving the microhabitat suitability modelling for fish species was the test of Thomas and Bovee (1993). However, those authors recognized that the $X^2$ test was affected by sample sizes. Therefore, we calculated the Sn, Sp and TSS to quantify the transferability success and to allow the comparison with the training results based on the same performance criteria.

The Spanish norm for hydrological planning (MAGRAMA, 2008) specifies that the minimum legal e–flow should be proposed based on the analysis of WUA versus flow curves. The WUA corresponds to the aggregation of the simulation cells (e.g. pixels) weighted by the corresponding suitability (i.e. the output of the habitat suitability model based on the velocity, depth and substrate of each of the simulation cells) (Equation 3).

$$WUA = \sum_{i=1}^{n} f(Velocity, Depth, Substrate)_i \times Area_i \text{ (Equation 3)}$$

where, $f(Velocity, Depth, Substrate)$ is the prediction of the PNN employing the velocity depth and substrate of the pixel $i$, and the $Area_i$ corresponded to $1\ m^2$.

To check the applicability of PNN for habitat assessment at different flows and for the identification of that minimum legal e–flow, the simulated flows were assessed with the $PNN_C$ and the $PNN_{05}$ and the WUA–flow curves were constructed. The patterns and potential implications were then discussed.
II.3 RESULTS

II.3.1 Effect of the data prevalence on performance of the PNN

The PNN\(_C\) showed an acceptable value of the True Skill Statistic (TSS=0.35) in addition to an acceptable output range (0–0.86) and the Sn was higher than the Sp (Table 3).

The univariate habitat suitability plots of mean flow velocity showed a suitable habitat between 0 and 1 m/s, and a maximum around 0.35 m/s (Fig. 5). The highest mean velocity that was classified as 'presence' was 1.031 m/s. Depth showed two trends in the univariate habitat suitability plots. The majority of the data showed an increase of suitability as depth increased including the hull of the training patterns whereas a secondary branch showed a decrease as depth increased (Fig. 5). This effect was mainly produced by differences in the underlying substrate and is clarified in the multivariate habitat suitability plot (Fig. 7). The minimum depth classified as 'presence' was 0.16 m. The trend of the univariate habitat suitability plot for substrate was parabolic with an optimal around substrate indices corresponding to medium–to–coarse substrates, ranging from 4 to 7 (on average gravels to boulders) with a maximum at 6 (on average cobbles).

![Fig. 5. Univariate habitat suitability plots based on the PNN\(_C\). Green dots represent 'presence' data and grey dots represent 'absence' data. The patterns were classified as 'presence' when they were higher than the threshold (0.5 suitability), and as 'absence' below that threshold. The presence data were mostly correctly classified (Sn=0.77), thus they appear over the 0.5 threshold.](image)

Considering the different training datasets and their corresponding PNN, prevalence did not appear to affect the model results in a significant manner because the univariate habitat suitability plots (Fig. 6) were similar to the one based on the PNN\(_C\) (Fig. 5). However, slight differences were observed in the multivariate plot (see Fig. 6 for the PNN\(_C\) and Fig. 8 for the PNN\(_{0.05}\)) and numerically (Table 3). Regarding the numerical analysis, the mean and median output values decreased with decreasing prevalence whereas the performance (TSS) slightly increased (Table 3). Although there were some inconsistencies in these trends, they were considered mainly due to the difficulty to extract exactly the same distribution by means of the sub–sampling procedure from a limited amount of data,
rather than to an unclear pattern. Therefore, the results indicated that the prevalence slightly affected the intensity of the output signals (i.e. the maximum modelled suitability increased as prevalence increased) but did not produce a higher classificatory strength because the TSS decreased as prevalence increased.

Table 3. Performance criteria (Sensitivity – Sn; Specificity – Sp; True Skill Statistic – TSS) and output statistics (minimum – Min; first quartile – 1st Q; Median; Mean; third quartile – 3rd Q; maximum – Max) corresponding to the PNN_C and the five alternative datasets with different prevalence and a constant smoothing parameter (σ=0.31).

<table>
<thead>
<tr>
<th>PNN</th>
<th>Prevalence</th>
<th>Sn</th>
<th>Sp</th>
<th>TSS</th>
<th>Min</th>
<th>1st Q</th>
<th>Median</th>
<th>Mean</th>
<th>3rd Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNN_C</td>
<td>0.06</td>
<td>0.77</td>
<td>0.58</td>
<td>0.35</td>
<td>0.00</td>
<td>0.27</td>
<td>0.46</td>
<td>0.44</td>
<td>0.58</td>
<td>0.86</td>
</tr>
<tr>
<td>PNN_01</td>
<td>0.1</td>
<td>0.79</td>
<td>0.57</td>
<td>0.35</td>
<td>0.00</td>
<td>0.27</td>
<td>0.48</td>
<td>0.45</td>
<td>0.60</td>
<td>0.89</td>
</tr>
<tr>
<td>PNN_02</td>
<td>0.2</td>
<td>0.77</td>
<td>0.54</td>
<td>0.31</td>
<td>0.00</td>
<td>0.31</td>
<td>0.51</td>
<td>0.46</td>
<td>0.61</td>
<td>0.85</td>
</tr>
<tr>
<td>PNN_03</td>
<td>0.3</td>
<td>0.78</td>
<td>0.54</td>
<td>0.31</td>
<td>0.00</td>
<td>0.33</td>
<td>0.53</td>
<td>0.48</td>
<td>0.63</td>
<td>0.87</td>
</tr>
<tr>
<td>PNN_04</td>
<td>0.4</td>
<td>0.71</td>
<td>0.57</td>
<td>0.29</td>
<td>0.00</td>
<td>0.34</td>
<td>0.52</td>
<td>0.49</td>
<td>0.61</td>
<td>1.00</td>
</tr>
<tr>
<td>PNN_05</td>
<td>0.5</td>
<td>0.76</td>
<td>0.55</td>
<td>0.31</td>
<td>0.00</td>
<td>0.39</td>
<td>0.54</td>
<td>0.51</td>
<td>0.64</td>
<td>0.94</td>
</tr>
</tbody>
</table>

II.3.2 Model transparency and ecological relevance

In the multivariate habitat suitability plots based on the PNN_C, the combination of high depth and high flow velocity values resulted in the greatest habitat suitability. However, the locations with high depth and simultaneously high flow velocity are rare or absent in the considered Mediterranean river systems of small size –order 2 or 3–; such locations did not appear in any training dataset, neither during the maximum simulated flow. Therefore, this specific aspect of the results was considered an anomaly due to the extrapolation of the model (Fig. 7, top–right corners) and was not analysed further. The PNN_C over–predicted the ‘presence’ in most cases as indicated by the Sn (Table 3; Fig. 7). The finest substrates appeared almost unsuitable (Fig. 7, substrate = 0, and 1) corresponding to the secondary branch in the univariate habitat suitability plots (Fig. 5). The habitat suitability increased broadly at medium–sized substrates, but in a small fringe corresponding to the shallower areas which remained unsuitable (Fig. 7, substrate from 2 to 5); that fringe enlarges for higher values of substrate and the suitable habitat was restricted to areas with depth larger than 0.5 m and velocity lower than 1 m/s (Fig. 7, substrate from 6 to 8).
Fig. 6. Univariate habitat suitability plots based on the alternative PNN developed varying the prevalence from 0.1 to 0.5. Green dots represent 'presence' data and grey dots represent 'absence' data. The patterns were classified as 'presence' when they were higher than the 0.5 suitability threshold, and as 'absence' if lower than that threshold.
Fig. 7. Multivariate habitat suitability plot based on the PNN_C (prevalence=0.06). Asterisks correspond to the training data of ‘presence’ classified as ‘presence’ in dark green and ‘presence’ classified as ‘absence’ in light blue. Circles correspond to ‘absence’ classified as ‘absence’ in black and ‘absence’ classified as ‘presence’ in light green.

The multivariate analysis of habitat suitability for the PNN_{05} (Fig. 8) showed similar patterns to the PNN_C. The plots showed the anomaly in the assessment of large depth combined with high velocity, where the model indicated the maximum suitability (Fig. 8 top–right corners). Obviating these areas, the PNN_{05} also showed large unsuitable areas over the finer substrates, except a fringe centred on the velocity of 0.5 m/s (Fig. 8, substrate = 0 and 1). On average the habitat suitability increased as substrate increased, achieving the maximum suitability at medium–sized substrates (Fig. 8, substrate = 3, 4 and
However, for these substrates PNN\textsubscript{0.5} did not show a region with lower habitat suitability in deeper areas. At larger values of substrate, the aforementioned fringe increased and the suitable habitat was also restricted to areas deeper than 0.5 m with velocity lower than 1 m/s (Fig. 8 substrate = 6, 7 and 8). In general the multivariate analysis of PNN\textsubscript{0.5} showed a higher degree of over prediction (with larger areas of maximum suitability in Fig. 8 than in Fig. 7) but it did not result in significant differences of the performance criteria (Table 3).

**Fig. 8.** Multivariate habitat suitability plot based on the PNN\textsubscript{0.5} (prevalence=0.5). Asterisks correspond to the training data of 'presence' classified as 'presence' in dark green and 'presence' classified as 'absence' in light blue. Circles correspond to 'absence' classified as 'absence' in black and 'absence' classified as 'presence' in light green.
II.3.3 Model evaluation and transferability

During the biological evaluation survey, we observed thirty one adult brown trout in the reach within the hydraulic model ($Q=0.89\ m^3/s$). The current flow was simulated and indicated a maximum depth of 1.4 m and a maximum velocity of 0.53 m/s, whereas the dominant and subdominant substratum were boulders (substrate=6; 39 %) and very fine substrate (substrate=0; 22 %). The PNN$_C$ and the PNN$_{05}$ had a similar performance, showing the higher suitability in the deeper areas (dark–green and green) and the unsuitable habitats (orange and red) in shallow areas as a consequence of the presence of low depth and fine substrate (Fig. 9 Left). Both PNN provided a good trade–off between areas assessed as ‘presence’ and as ‘absence’, thus showing crossed distributions, although the PNN$_C$ gave a maximum suitability of 0.8 and the PNN$_{05}$ of 1 (Fig. 9 Right). These observations confirm the aforementioned results about the effect of prevalence on model performance. On an equal footing, low prevalence (e.g. PNN$_C$) slightly reduces the intensity of the signal providing lower output values. Nevertheless, PNN$_C$ presented better transferability (i.e. higher TSS) because it outperformed PNN$_{05}$ due to the larger Sn. Both PNN were underpredictive because Sp was higher than Sn (Table 4).

Table 4. Transferability evaluation (Sensitivity – Sn; Specificity – Sp; True Skill Statistic – TSS corresponding to the PNN$_C$ and PNN$_{05}$ at the evaluation site with a constant smoothing parameter ($\sigma=0.31$).

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Sn</th>
<th>Sp</th>
<th>TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNN$_C$</td>
<td>0.06</td>
<td>0.65</td>
<td>0.75</td>
</tr>
<tr>
<td>PNN$_{05}$</td>
<td>0.5</td>
<td>0.55</td>
<td>0.77</td>
</tr>
</tbody>
</table>
Fig. 9. Left side, comparison of the habitat assessment on the evaluation site (Q=0.89 m$^3$/s) using the PNN$_C$ (A) and the PNN$_{05}$ (B). Red colour means unsuitable locations and dark green means locations with the maximum suitability. Black squares represent adult brown trout locations during the survey. On the right side, frequency histograms of the assessment of both PNN. General is represented by black bars (assessment of the entire simulated reach) and trout locations (assessment at fish locations) by grey bars. It is notable that only the assessment based on PNN$_{05}$ provided the maximum suitability (range 0.8–1).

The Weighted Usable Area (WUA)–Flow curves differed depending on the considered PNN (Fig. 10). The PNN$_C$ presented a WUA–Flow curve with an asymptotic shape (curve A). It presented an increasing trend until 3.5 m$^3$/s. Then the curve slightly decreased onwards that point but rising again for the higher simulated flows (Fig. 10). However, the PNN$_{05}$ presented a monotonic increasing trend (curve B), without any relevant change in the trend of the curve.
II.4 DISCUSSION

II.4.1 Optimization results

Mediterranean streams are characterised by strong intra and inter–annual flow variations thus becoming one of the main drivers on the observed oscillations of trout populations (Ayllón et al., 2010). Moreover, the sampling protocols can also introduce false negatives on the training database because a higher resolution increases the probability of sampling sites of ‘absence’ with similar characteristics as the observed at ‘presence’ locations. From an ecological viewpoint, some of these ‘absence’ records should be assessed as suitable in a different degree (Mouton et al., 2008, Mouton et al., 2009). Therefore, the selected modelling techniques might deal with this issue. In this study the Probabilistic Neural Networks (PNN) demonstrated proficient to cope with this kind of databases and provided acceptable results, with an adequate True Skill Statistic (TSS) value (Table 3) that is similar to those in studies using performance criteria based on the confusion matrix and databases with similar prevalence that were collected following the same protocols (Muñoz–Mas et al., 2012). Additionally, the PNN over–predicted the ‘presence’ providing larger values of Sensitivity (Sn) in contrast with the Specificity (Sp) (Table 3), which has been demonstrated preferable as stated above (Mouton et al., 2008, Mouton et al., 2009).

Fig. 10. Weighted Usable Area (WUA)–Flow curves for adult brown trout calculated with the 34 simulated flows ranging from 0.05 to 6.5 m³/s. The curve A based on PNN_C presented an asymptotic shape, whereas the curve B based on PNN_05 showed a monotonic increasing trend.
II.4.2 Analysis of prevalence effect on PNN performance

The analysis of performance criteria versus prevalence (Table 3) in combination with the model evaluation (Fig. 9) showed that the predictive accuracy was practically unaffected (confidence interval of 5.6 % of the mean TSS) but the maximum output value presented a decreasing trend. Some inconsistencies in these trends were observed with small fluctuations (Table 3). However, we considered they were mainly due to the difficulty in extracting exactly the same distribution (by subsampling) from a limited amount of data. The procedure certainly kept the original distribution and the shapes of the univariate habitat suitability plots (Fig. 6) were fairly similar than those based on the PNN_C (Fig. 5).

The interpolation of the theoretical aggregation of velocity, depth and substrate that should be selected is straightforward. However, the sub-sampling method selects a real measurement that can differ from the theoretical value. This issue can be caused by the inherent discretization in the measurement of any continuous variable (Giri and Banerjee, 2012) (i.e. velocity at the nearest cm/s, depth at the nearest cm and the substrate in the percentage of 8 classes) or because the database does not contain enough training patterns. Nevertheless, we considered these differences negligible and the analysis robust enough to conclude that PNN is a suitable technique to deal with unbalanced databases; but the output values must be taken into account if values along the whole feasible range are desired (Table 3). The use of an artificial database was considered because it could facilitate the subsampling procedure. However, uncertainty about the effect of prevalence in a real database remains a primary reason not to use artificial databases in this way.

The results agreed with previous analyses that tested several databases and demonstrated that PNN are not constrained by the undesirable effects of unbalanced databases (Zhong et al., 2005), but the range of outputs was not considered in the aforementioned study. The range of outputs has been of major concern in the present study in order to fit the output range in common microhabitat suitability models (Jowett and Davey, 2007, Muñoz–Mas et al., 2012, Payne and Allen, 2009). Other techniques such as the fuzzy logic approach had to contend with trimmed output ranges and practical solutions have been proposed. For example, CASiMiR© (Jorde, 1997, Schneider, 2001) allow the rescaling of the outputs between 0 and 1. Nevertheless, we considered the outputs close enough to the maximum and discarded that option. The reduction on the σ parameter would solve this issue, thus providing values along the whole range. But it would produce overfitting because PNN are sensitive to this phenomenon (Grim and Hora, 2010, Zhong et al., 2005). The modeller should select a larger σ in order to improve the model generalization, which could lead to a smaller output range (Fig. 3). A possible solution is the consideration of the classificatory capability of PNN isolatedly. Although we cannot ensure that a dichotomous output would not provide us with reliable results, the PNN will clearly be disadvantaged in comparison with other modelling techniques. Brown trout has been categorized as ecologically flexible (Klemetsen et al., 2003) and accordingly we observed a large overlapping between ‘presence’ and ‘absence’ datasets.
Therefore we considered that databases with better defined discriminant surfaces (i.e. lesser overlapping between categories) would not tend to produce trimmed outputs regardless of the prevalence in the training database. However these issues should be thoroughly explored in future research.

II.4.3 Model transparency and ecological relevance

The multivariate and the univariate habitat suitability plots based on the PNN$_C$ (Fig. 5 and Fig. 7) in combination with the model evaluation (Fig. 9) showed on average, a remarkable agreement with some of the most important studies. However, differences within the literature were also observed, mainly related to several factors such as differences in fish size or river types (Jowett and Davey, 2007) or in the selected sampling protocol (Heggenes et al., 1990) and their limitations. Direct observation is accurate in specific conditions and tend to underestimate the amount of individuals in shallow waters (Heggenes et al., 1990). Electrofishing is characterized by coarser resolution (Heggenes, 1996) and presents a bias related to the displacement caused by galvanotaxis (Gatz Jr et al., 1987). Neither the direct observation nor the electrofishing allow the easy observation of fish behaviour, thus databases could become noisy when including different activities (Heggenes et al., 1990). The observed differences could be due to the selected modelling approach. A recent study proposed that each modelling technique could be focused on different aspects of the training database, even predicting different habitat suitability (Fukuda et al., 2013).

Habitat selection patterns of brown trout are well established in broad terms (Ayllón et al., 2010). In near–natural rivers it has been reported to prefer relatively deep pools, occupying near–bottom locations with slow flow and medium–to–coarse substrate (Armstrong et al., 2003, Ayllón et al., 2010, Heggenes, 1996, Moyle, 2002). Accordingly, our results showed an increase of suitability as depth increases (Fig. 5) but it presented an important interaction with substrate (Fig. 7). Specifically, the substrate index of 0 and 1 has been assessed as unsuitable regardless the considered depth, suggesting that, in some cases, substrate cannot be compensated with the other variables. Conversely, depth did not show restrictions of habitat suitability from the substrate index of 5 onward (Fig. 7). Velocity has been reported as an important constraint on habitat suitability (Ayllón et al., 2010, Heggenes, 1996). The maximum sampled mean velocity was 1.75 m/s (Senia River), although most of the training data were below 1 m/s. The maximum velocity classified as ‘presence’ was 1.031 m/s (Fig. 5), therefore our results indicated a wider suitable range in comparison with previous studies (Armstrong et al., 2003) and similar in magnitude with studies conducted on larger rivers (Jowett and Davey, 2007). Mediterranean climate is characterized by the marked seasonality in climate events, intermittent periods of torrential rains and droughts, and high inter and intra–annual flow variation (Baeza et al., 2005, Gasith and Resh, 1999). Summer is associated with low flow
Probabilistic neural networks

Chapter II

39

and consequently with an increment on water temperature (Lorig et al., 2013). This study was carried out mostly in summer during low flow; therefore the Mediterranean climate could influence trout behaviour via the water temperature. We did not consider specifically water temperature as an input, although in the Iberian context it is expected to cover warmer waters than previous studies (Nicola et al., 2009). For instance, the maximum temperature in the Cabriel River corresponded to 22.32 °C during 2010 (unpublished). Brown trout selects more slow flowing water in winter in comparison with summer due to differences in water temperature (Klemetsen et al., 2003) and the consequent effect on the rate of biochemical reactions (Kingsolver, 2009). Moreover, local adaptations of brown trout populations to environmental conditions even in populations subjected to stocking activities have been reported (Keller et al., 2011). Therefore, in accordance with the expected differences in water temperature, we considered plausible that adult brown trout endure higher velocity in Mediterranean rivers, thus supporting the idea that our results are ecologically significant.

Regarding previous studies with multivariate approaches for habitat suitability modelling, the heterogeneity on the applied techniques and consequently on the outputs suggested that any comparison should be considered in broad terms. Vismara et al. (2001) collected data by electrofishing from an Italian alpine river thus the degree of Mediterraneity is expected to be buffered because lower temperatures were reported, as approximately 10–11 °C. The bivariate polynomial functions from Italy, based on velocity and depth, showed a monotonic increment of the suitability as depth increases from 0 to 1 m, whereas velocity had an inverse influence on it. Thereby the maximum suitability was set to be at the velocity of 0 m/s. This pattern generally matched our findings because they presented also a positive correlation between depth and suitability (Fig. 5) whereas velocity had a negative impact on suitability, especially over coarse substrate (Fig. 7, substrate = 6, 7 and 8). The substrate size has a positive effect on the endurance of higher velocity (Greenberg et al., 1996) in contrast to the aforementioned pattern (Fig. 7, substrate = 6, 7 and 8). However we imputed this phenomenon to the unrealistic extrapolation because the suitable range was wider than the observed in previous studies (Armstrong et al., 2003) and this decrement appears in the area with no training patterns. Nevertheless, Vismara et al. (2001) did not consider substrate, thus the comparison cannot be carried out reliably.

Lambert and Hanson (1989) also developed bivariate polynomial functions from data collected by snorkelling in small streams of the King River Basin in the Sierra Nevada of California, with noticeable Mediterranean influence, but no temperatures were reported. The results strongly differed, since the optimal velocity and depth corresponded to 0.0 m/s and around 0.5 m respectively, and both gradually tailed off as they approached their maxima, corresponding to 0.75 m/s and 1.5 m. It has been reported that the optimal depth for adults increases in accordance with the proportion of pools and with the maximum depth of that pools (Ayllón et al., 2009, Ayllón et al., 2010). The samplings by Lambert and Hanson (1989) were conducted in river stretches at an elevation of 1500 to 1800 m above
datum, with running flows ranging from 0.7 to 0.03 m$^3$/s. Therefore we suggest that the observed shift of habitat suitability could be partially produced by differences in habitat availability because the study sites are likely to be shallower than in our study. However, Lambert and Hanson (1989) developed the model based only on ‘presence’ data whereas Vismara et al. (2001) apparently developed the model by applying the forage ratio (Voos, 1981) thus considering ‘presence’ and ‘absence’ data. The forage ratio tends to displace the optima to higher depth and velocity (Bovee and Zuboy, 1988). Vismara et al. (2001) surveyed a smaller range (maximum depth was 0.9 m and maximum velocity 1 m/s) than our study, thus we cannot discard the effect of the selected approach as the main reason for these differences. In addition, the polynomial functions were considered rigid in the adjustment of a smooth surface to the collected data (Lambert and Hanson, 1989, Vismara et al., 2001). In contrast, the PNN was versatile in the encompassment of the suitable microhabitats (Fig. 7). However, their capability to adjust the suitable space is not exempt of criticism (Grim and Hora, 2010). The development of reliable PNN must face the bias and variance dilemma (Geman et al., 1992), thus the selection of a larger $\sigma$ shall yield smoother transitions between categories (Fig. 3) whereas a very small $\sigma$ would overfit the discriminant surface to the training patterns. The selected $\sigma$ provided a continuous suitable niche (Fig. 7) but it was not completely insusceptible to that phenomenon, consequently some irregularities can be observed in the discriminant surface (Fig. 7).

The fuzzy logic approach has been used to develop and evaluate expert–knowledge habitat suitability models in an alpine river, the Brenno River (Jorde et al., 2001). This river showed the maximum suitability within the velocity range of 0.3–0.9 m/s and the depth range of 0.15 – 0.5 m over medium–to–coarse substrate. Deeper areas showed high suitability in accordance with our results. However, the maximum depth did not present the highest suitability. The morphology of braided gravel–bed rivers and the consideration of cover did not allow the proper comparison. Nevertheless, habitat selection in salmonids is based on their competitive abilities and the profitability of territories in terms of both potential net energy intake rate and predation risk (Grand and Dill, 1997, Railsback and Harvey, 2002). Pools usually provide sufficient depth and cover to obscure fish from avian predators (Bunt et al., 1999). However, turbidity plays a fundamental role as a constraint on predator–prey interactions (Abrahams and Kattenfeld, 1997). Consequently, salmonids become more unwary when turbidity increases (Gregory and Griffith, 1996). According to the literature, the Brenno River is expected to present higher turbidity than our rivers (Brunke, 2002); therefore, we hypothesize that turbidity could partially explain the differences on microhabitat suitability.

Jowett and Davey (2007) applied GAMs in modelling habitat suitability for large brown trout with data collected by snorkelling in a large New Zealand river (average flow > 226 m$^3$/s). The partial plots showed a pointed curve for velocity with an optimal around 0.5 m/s, whereas the depth showed a wider curve with the optimum in a range between 2 and 4 m. Although body length (> 40 cm) and the river size strongly differed with our study, their
results lend credibility to ours. These outcomes remarked the observed ontogenetic shift towards the selection of deeper habitats and as the availability of deep microhabitats increases (Ayllón et al., 2010). In this regard, we considered our target rivers of intermediate size in comparison with previous studies (Ayllón et al., 2010, Heggenes, 1996, Jowett and Davey, 2007, Lambert and Hanson, 1989, Rincon and Lobon–Cervia, 1993) especially concerning the available depth. Thus, the observed pattern in the multivariate habitat suitability plot (Fig. 7) (i.e. positive correlation between suitability and depth) was considered reliable. Regarding the Iberian context Ayllón et al. (2010) surveyed by electrofishing some northern Iberian rivers and developed habitat suitability models with logistic regression. When considering only the rivers that better fit our range of sampling conditions (river types 4, 5 and 7) these models demonstrated a negative correlation with velocity and a positive correlation with depth, although both variables were summarized in the Froude number and the model also included cover and mesohabitat type. Therefore, proper comparison was considered unreliable.

Regarding previous studies with univariate approaches, Ayllón et al. (2010) also developed univariate Habitat Suitability Curves (HSC). The curves for depth were typically stable (horizontal) at the right of the optimum depth from 0.8 m onwards. Some studies on brown trout indicated such stable suitability for deep habitats (Bovee, 1978, Vismara et al., 2001). However, other studies indicated a decrease (Hayes and Jowett, 1994, Heggenes, 1996, Lambert and Hanson, 1989) with optima approximately ranging from 0.5 to 1 m. This phenomenon could be a result of the modelling approach because in some cases only ‘presence’ data were used (Category II HSC, after Bovee et al., 1998) or it could be a result of the absence of incoming drift (Hauer et al., 2012) in deep waters, instead of the direct negative effect of depth on the habitat suitability. Unfortunately, our results do not provide information about its discernment.

From a univariate perspective, the mean velocity showed a wider suitable range in comparison with the optima in most of the studies in the Iberian Peninsula (0.0–0.4 m/s) (Ayllón et al., 2010, Rincon and Lobon–Cervia, 1993). Nevertheless, our results were comparable with other authors (Hayes and Jowett, 1994) who did not attribute the highest suitability to very slow microhabitats, in contrast with other studies (Heggenes, 1996, Vismara et al., 2001) where habitat availability was more limited than in the present study. Moreover, it has been stressed that habitat simulations based on HSC presenting optimum suitability at low current velocity are prone to predict biased maximum WUA values at very low flows (Railsback, 1999), thus highlighting the value of the model presented here.

The substrate presented fewer opportunities for comparison because in some cases it has been neglected (Jowett and Davey, 2007, Lambert and Hanson, 1989) or because in some other cases few substrate types appeared (Rincon and Lobon–Cervia, 1993, Vismara et al., 2001). The model showed a maximum suitability for medium–to–coarse substrate. In general, suitability tended to increase from fine–gravel to bedrock (Fig. 7).
These results partially agreed with previous studies in the Iberian peninsula (Rincon and Lobon–Cervia, 1993) and abroad (Bovee, 1978, Heggenes, 1996). However, those studies also showed a decrease over the bedrock whereas our model did not (Fig. 7). This could be produced by the deficient substrate distribution (Rincon and Lobon–Cervia, 1993, Vismara et al., 2001) or due to the use of the substrate index (Mouton et al., 2011). The substrate index restricted the number of ‘presence’ patterns on pure bedrock to one (substrate=8) whereas the alternative of using the dominant substrate could result in different suitability over bedrock. The effect of different substrate aggregation methods should be thoroughly analysed in further research.

Although the range of microhabitat availability was generally larger than previous European studies, the spatial distribution of the training patterns (Fig. 7) suggests that the whole distribution range of adult brown trout was not completely covered. The model assessed some extrapolated conditions unreliably (Fig. 7 top–right corners) then, the modeller should be cautious when applying PNN outside the range of observations. In this regard PNN presented a deficiency in comparison to fuzzy logic, which allows the modification of models in areas outside of the surveyed range (Mouton et al., 2009). To overcome this problem, further sampling campaigns should comprise extremer conditions (velocity > 1.75 m/s, depth > 1.78 m) to accurately define the suitable habitat. Fortunately these extreme conditions are rare or non–existent in the considered Mediterranean river systems (e.g. maximum simulated velocity equalled 1.3 m/s and maximum simulated depth 1.73 m), allowing the application of these new models in the brown trout habitat assessment.

II.4.4 Model evaluation and transferability

Some authors have pointed out the difficulty to decide which models are the best, even when good model performance is achieved (Vaughan and Ormerod, 2005). Often, independent data to evaluate models is lacking, and the best model is then selected based on comparison of different performance criteria (e.g. TSS, Sn and Sp in our study). However, our results showed that evaluation based on independent data (Guisan and Thuiller, 2005) may provide valuable additional information on model performance and its generalisation capability. Specifically, the PNN05 performed better when comparing different performance criteria (i.e. similar TSS and larger maximum output) but the PNNC showed larger generalisation capability when applied to independent data. Certainly, the optimal $\sigma$ for PNNC might differ from the optimal for PNN05. Thereby a reoptimised $\sigma$ could improve the PNN05 performance or its generalisation capability. Nevertheless, once in the ideal situation (prevalence=0.5) the range of modelling techniques shall become very large and the use of PNN may become unnecessary. Aside from this, we considered that modifications of the training database would reduce the model reliability by reducing the
considered variability, especially when few cases are sub–sampled. Consequently, these factors suggested that the use of the complete database should be the first option.

The transferability of habitat suitability models has been of major concern for researchers (Fukuda, 2010, Randin et al., 2006, Thomas and Bovee, 1993). Failures on model transferability have been reported due to site–specificity and seasonal or size–related changes on habitat preferences (Fukuda, 2010). The $\text{PNN}_C$ showed a good transferability with a $\text{Sn}$ of 0.65 and high $\text{Sp}$. This success highlighted the capability of the PNN to properly model the microhabitat suitability. However, it showed underpredictive (i.e. $\text{Sp} < \text{Sn}$) which has been reported to be less defensible from an ecological viewpoint (Mouton et al., 2008, Mouton et al., 2009). The modification of the classification threshold (0.5) (Fukuda et al., 2013), the inclusion of misclassification costs or the alteration of the prior probability should improve the transferability. However, it was not considered within the scope of this piece of research, because the main purpose of the study was not the development of an optimal model at the evaluation site but testing the PNN capabilities.

Once a single $\sigma$ ($\sigma=0.31$) was selected, the Weighted Usable Area (WUA)–Flow curve presented two different patterns depending on the prevalence of the training dataset (Fig. 10). The curve calculated with the $\text{PNN}_C$ (curve A) presented a close–to–asymptotic shape, in comparison the curve based on the $\text{PNN}_{0.5}$ (curve B) presented a monotonic increment. The Spanish norm for hydrological planning (MAGRAMA, 2008) established that the minimum legal e–flow released to stakeholders or water managers should be selected within the range of 50–80 % of the maximum WUA or considering a relevant change in the slope of the WUA–flow curve. A monotonously increasing curve akin to curve B, could only produce a single e–flow if the break of slope was detected and not a range of minimum e–flow. Thereby, the $\text{PNN}_C$ (curve A) may be more appropriate for the public agreement about the minimum legal flow, allowing for its better modulation within the legal range (from 50 % to 80 % of the maximum WUA).

**II.4.5 Implementation on further studies**

Habitat suitability for the adult brown trout has been the main focus in many scientific and research projects (see aforementioned studies). The previous knowledge gained from these has allowed us to discern broadly the reliability of the developed model. In the present study we used an optimisation algorithm, but certainly with a single $\sigma$ it was not obligatory. Therefore, we propose the following optimisation approach to deal with an unstudied species, thereby improving the applicability of PNN in habitat suitability modelling. The PNN optimisation should start from an arbitrary but large $\sigma$ and the modeller should reduce the $\sigma$ in a step–by–step procedure. The results of the leave–one–out cross–validation procedure for each step should be plotted and the modeller should select an intermediate $\sigma$ when a good trade–off between the bias and variance is
achieved. Depending on the considered problem and the selected $\sigma$, this procedure starts from a general scope on the habitat suitability for the target species and ends with an over-fitted model lacking ecological relevance because it is likely to yield suitable microhabitats inserted within an unsuitable frame. The goal is to select an optimal model in an intermediate stage thus providing a unique continuous microhabitat. In that sense, the PNN could be considered an intermediate step between purely data-driven models (e.g. Multilayer Perceptron) and the expert knowledge-based models such as the fuzzy rule base systems or the Category I HSC (after Bovee et al., 1998).

A recent study focused on modelling the habitat suitability for the spawning of the European grayling (*Thymallus thymallus* L.), using a broad range of modelling techniques (Fukuda et al., 2013), showed that Random Forest outperform any other modelling technique. Although previous studies demonstrated PNN as less competitive than other approaches (Zhong et al., 2005), results were strongly dependent on the considered databases. Therefore, once the capability of PNN to model habitat suitability is confirmed, subsequent research should focus on the comparison of PNN performance with other popular modelling techniques. Moreover, researchers are frequently introducing novel techniques for e-flow assessment (Lamouroux et al., 1998). Recently, the use of random forests (Breiman, 2001) has been coupled to a habitat simulation system (MesoHABSIM) (Parasiewicz, 2001) providing valuable results (Vezza et al., 2012). Similarly, a new study applied Takagi–Sugeno fuzzy logic models to improving the IFIM approach, allowing the analysis of a wider range of scenarios (Marsili–Libelli et al., 2013). Likewise we expect that in the near future PNN could be taken into consideration in microhabitat suitability modelling and e-flow assessments.
MULTI-LAYER PERCEPTRON ENSEMBLES (MLP ENSEMBLES) IN MODELLING MICROHABITAT SUITABILITY FOR FRESHWATER FISH

III. MULTI-LAYER PERCEPTRON ENSEMBLES (MLP ENSEMBLES) IN MODELLING MICROHABITAT SUITABILITY FOR FRESHWATER FISH

ABSTRACT

Some Iberian native fish species should be the targets of conservation actions, given the decline of their populations and their vulnerable/threatened status of conservation. In this context, Species Distribution Models (SDMs) play a significant role in understanding habitat requirements guiding further conservation actions. Multi–Layer Perceptrons (MLPs) have been proficiently used in the development of SDMs at different spatial scales. However, many real–world problems are too complex for a single MLP. Therefore the use on MLP Ensembles, which perform co–ordinate predictions, achieved superior accuracies. The active selection of the MLP involved in the Ensemble could outperform the inclusion of every trained MLP. Then, we tested two approaches in the selection of the proper MLPs in the development of a SDM at the microhabitat scale for an endemic freshwater fish species, the Bermejuela (*Achondrostoma arcasii*; Robalo, Almada, Levy & Doadrio, 2006). The first method was based on the Forward selection and the second one in Genetic Algorithms. The results proved that the Forward methodology can outperform the more complex Genetic Algorithm–based approach. The sensitivity analysis showed that the Bermejuela is a shelter–orientated limnophilic species.

Keywords: *Achondrostoma arcasii*, MLP Ensemble, Genetic algorithms, Microhabitat.
III.1 INTRODUCTION

The native fish species of the Iberian Peninsula present a high degree of endemicity; therefore, they should be the target of biodiversity conservation actions (Doadrio, 2002a). Species Distribution Models (SDMs) are being used in nearly all branches of life and environmental sciences (Guisan & Thuiller, 2005), playing a significant role in understanding habitat requirements of fish species and providing a framework from which spatial and temporal prediction in their distribution patterns can be done (Olden et al., 2002). Therefore they might improve the effectiveness of any restoration and management action (Mouton et al., 2007a). The most popular Artificial Neural Networks architecture has been the Multi-Layer Perceptron (MLP) appearing most often in the ecological literature because it proved to be a universal approximation of any continuous function (Olden et al., 2008). There are several examples about the use of a single MLPs in the development of SDMs at the microhabitat scale (hereafter microhabitat suitability models) (Brosse & Lek, 2000; Laffaille et al., 2003) as well as in other modelling tasks addressed to fish conservation (Olaya-Marín et al., 2012). However several studies have demonstrated that SDM do not perform equally even providing discrepant forecasts depending on several factors, such as the initial conditions or the selected modelling technique (Buisson et al., 2010). To deal with this drawback, and considering that many real-world problems are too large and too complex for a single monolithic system to solve alone (Yao & Xu, 2006), the use of MLP Ensembles was proposed (Hansen & Salamon, 1990). The main idea behind ensemble learning is to minimize those discrepancies by combining several models that can be generated using either different subsets of training examples or the whole training dataset and different models settings. Finally, the individual predictions are combined into a single forecast (Wang & Alhamdoosh, 2013). In regression problems, the aggregated prediction is usually calculated by averaging the prediction of each of the considered MLPs, whereas in classification problems the majority vote or the winner-takes-all approaches are common (Hansen & Salamon, 1990). Originally in ensemble modelling, the ensemble forecast included every developed MLP (Hansen & Salamon, 1990), but it was promptly demonstrated that the active selection of the proper networks improve the final predictions (Opitz & Shavlik, 1996; Zhou et al., 2002b). There are several methods to produce and select the proper MLPs (Zhou et al., 2002b; Yao & Xu, 2006; Wang & Alhamdoosh, 2013) but some hints have suggested that in some cases more straightforward methodologies can outperform more complex algorithms achieving at least similar performance (Akhand & Murase, 2010; Lofstrom et al., 2010).

In the present study we modelled the microhabitat suitability with MLP Ensembles for the Bermejuela (*Achondrostoma arcassi*; Steindachner, 1866), an Iberian endemic fish classified as vulnerable by the IUCN (International Union for Conservation of Nature). Two approaches were used in the active selections of the MLPs included in the ultimate ensemble. The first one was based on the Forward selection of candidates and the second one was performed using a Genetic Algorithm. A graphical sensitivity analysis was applied.
to the outperforming MLP Ensemble, and results in terms of habitat suitability were discussed.

III.2 METHODS

III.2.1 Microhabitat data collection

The microhabitat data collection took place by snorkelling in 2009 during spring and summer in the Guadiela and Escabas Rivers, both within the Tagus River basin (Fig. 11).

![Fig. 11. Location of the study sites where the surveys of microhabitat selected by brown trout were performed, in rivers of the Tagus River Basin.](image)

The microhabitat study was done in complete Hydro–Morphological Units (HMU), such as pools or riffles with similar proportion of fast and slow HMU areas. In general this approach reduces the bias on the dataset, surveying the different habitat conditions in similar proportions (Muñoz-Mas et al., 2012). The physical environmental parameters used by the Bermejuela were surveyed during daylight with minimum disturbance to the fish accordingly to standard procedures (Heggenes et al., 1990). The Bermejuela proved to be a schooling fish and thus it appeared in groups with individuals of different sizes. Nevertheless, presence–absence was considered the proper approach in modelling fish habitat requirements at the micro–scale to better fit the requirements of ulterior habitat evaluations (e.g. environmental flow assessment). Fish were observed in 87 locations, whereas the habitat in the surrounding area was measured in 798 locations organized in
cross-sections uniformly distributed along the HMUs, finally providing a Prevalence of 0.10.

The four usual variables on microhabitat studies were measured; mean water column velocity, water depth, substrate composition and the presence (or absence) of different cover types (Gibson, 1993). Velocity was measured with an electromagnetic current meter (Valeport®) and depth was measured with a wading rod at the nearest cm.

The percentages of the different substrates classes present were visually estimated around the sampling point or fish location (Bovee & Zuboy, 1988). The substrate classification was simplified from the American Geophysical Union size scale in: bedrock, boulders (>256 mm), cobbles (256–64mm), gravel (64–8 mm), fine gravel (8–2 mm), sand (2 mm – 62 µm), silt (< 62 µm) and vegetated soil (i.e. substrate covered by macrophytes); accordingly, a Substrate index (hereafter abbreviated as S) (Mouton et al., 2011) was calculated.

The cover types were vegetation, shade, boulders, caves, roots or woody debris. The maximum surveyed mean velocity was 1.01 m/s and the maximum depth was 2.05 m, the most abundant substrate type was cobble (Substrate index = 6) and the largest number of cover types present at a single location was 3.

III.2.2 Microhabitat suitability modelling

III.2.2.1 MLP candidates training

The selected modelling technique was the Multilayer Perceptron Ensemble (MLP Ensemble) (Hansen & Salamon, 1990). In accordance with Waters (1976), who firstly introduced the use of habitat suitability curves, the microhabitat suitability was desired to be standardised in a value ranging from zero to one, with zero (absence) being unsuitable and one (presence) fully suitable. Therefore, the hyperbolic tangent and linear transformation functions were used to build each of the MLP candidates. The MLP training was carried out in R (R Core Team, 2015) with the package monmlp (Cannon, 2012), which allows a versatile generation of different MLP architectures and optimizes the MLP weights using the non-linear minimization (nlm) routine (R Core Team, 2015). The number of neurons in the hidden layer was set to two because it represents an acceptable trade-off between the bias and variance (Geman et al., 1992).

In MLP Ensemble modelling each member of the committee (or ensemble) should complements each other, improving the predictive capability (Akhand & Murase, 2010), but differences between the members of the Ensemble are also advisable. There are a variety of ways to construct an MLP Ensemble with certain diversity between candidates; using different training sets, architectures or learning methods, although Bagging has been
demonstrated to be sufficiently effective for most of the situations (Opitz & Maclin, 1999; Brown et al., 2005; Akhand & Murase, 2010). Therefore each MLP was trained with the 60% randomly selected presence cases (52 cases), and considering that prevalence may have an strong effect on model performance with equal number of Absence cases (Manel et al., 2001), which were likewise randomly selected. Thus each training dataset had 104 cases and 0.5 prevalence. The remaining cases were gathered in the validation dataset. It has been reported that the diversity among classifiers generally compensates for the increase in error rate of any individual classifier, both in the training and in the validation datasets (Opitz & Maclin, 1999). Therefore, no care was taken about the over–fitting and the training algorithm was left unconstrained. However, for the active selection of the considered MLPS in the Ensemble, it is advisable to analyse the error in training and validation for each of the ensemble members. Therefore we analysed the error that each member of the MLP Ensemble would commit on each training and validation datasets of the selected candidates.

The objective function was the one that minimised the Mean Absolute Error (MAE). Additionally, the objective function included a penalty if the outcomes did not cover the whole considered range (i.e. from 0 to 1). The amount of trained MLPS was heuristically determined, because testing all the possible combinations of 0.5 Prevalence datasets was considered unaffordable. The number of trained MLPS was the one that produced stable MAE considering every trained MLP. Therefore, 2000 MLPS were trained and pooled. Finally, the classification strength of the developed MLP Ensembles was also considered, then the Sensitivity (i.e. ratio of presences classified as presences), the Specificity (i.e. ratio of absences classified as absences) and the global performance criterior True Skill Statistic (TSS) (Allouche et al., 2006) were also calculated.

III.2.2.2 Forward selection of candidates

Considering previous studies that applied this approach (Akhand & Murase, 2010; Lofstrom et al., 2010), the forward selection of the best combination of MLPS was carried out as follows: starting from each of the pooled MLP, the remaining were added one by one, and their predictions were averaged. The one that produced the largest improvement on the model performance (largest reduction of the MAE) was selected and the remaining MLPS were tried next following the same procedure. The process continued until no improvement of the objective function was obtained.

III.2.2.3 Genetic Algorithms for candidates selection

Genetic Algorithms (GA) comprise search and optimization algorithms that work based on the process of natural selection (Akhand & Murase, 2010) and they have proved proficient
for Network selection in the optimization of MLP Ensembles (Zhou et al., 2002b; Akhand & Murase, 2010). The optimization was carried out in R (R Core Team, 2015) by means of the *rgenoud* package (Mebane Jr & Sekhon, 2011). This function combines evolutionary algorithm methods with a derivative based (quasi–Newton) method to solve difficult optimization problems. *Rgenoud* presents 9 operators driving the optimization which correspond to: cloning, uniform mutation, boundary mutation, non–uniform mutation, polytope crossover, simple crossover, whole non–uniform mutation, heuristic crossover and local–minimum crossover (see Mebane Jr and Sekhon, 2011 for further details). These operators were set to, 0.5, 0.5, 0, 0, 0, 0.5, 0.1, 0.2 and 0 respectively. The 2000 available MLPs were encoded in chromosomes of 2000 length bits. The population size was formed by 40000 individuals and 100 generations were considered.

### III.2.3 Graphical sensitivity analysis

A graphical sensitivity analysis (Plate et al., 2000) was applied to investigate the effect of the input variables on the output. This methodology presents an easy interpretation and has been successfully applied in the analysis of several problems (Cannon & McKendry, 2002). The graphical method uses modified scatter plots which permits the assessment of the effects of inputs on the output (see Cannon and McKendry, 2002 for a thoroughly explanation). For each input variable (i), the variation of the model output due to variation of i (Δi) is calculated considering an arbitrary baseline (the mean value) and plotted as segments, with slope equal to the partial derivative of the model output with respect to Xi. The visualization of the partial derivatives as segments allows the identification of trends and types of non–linear relationships between each input variable and the output. The plots contain information about:

1. The effect of input variables on the output. Variables with no effect on the model appear as horizontal lines.

2. The variable importance, described by the overall Δi vertical range. The greater the overall Δi vertical range, the greater the influence of the variable on the model.

3. The interaction with other variables, described by the spread of Δi along the y–axis. Variables with no interaction appear as single lines.

4. Trends and non–linearity shown by trends and non–linearity of the derivatives. If the small segments describe curves, the function computed by the network is not linear.

A dedicated example can be consulted at the end of the present chapter.
III.3 RESULTS

III.3.1 Training results
The Forward selection of MLPs showed a low value of the objective function 0.15 whereas the Genetic Algorithm (GA) provided a slightly higher value of 0.17 (Table 5). The whole MLPs set (the one without MLP selection) provided a larger value 74.00 whereas the best single net provided a value of 0.31. The Forward procedure selected 6 MLPs whereas the GA only 3. However, regarding the classificatory strength they did not differed substantially (Table 5) because sensitivity (Sn), specificity (Sp) and TSS were similar. Therefore, the MLP Ensemble developed by means of the Forward procedure was selected for the sensitivity analysis. The inspection of the errors distribution in training and validation showed differences (Fig. 12). However, considering that most of the data belonged to the validation dataset, it was finally considered acceptable; thus the difference was not extreme, even presenting a slight overlap, and the classificatory strength was high (Fig. 12).

Table 5. Results summary, Number of selected networks, Value of the Objective function (weighted aggregation of MAE), Sensitivity (Sn), Specificity (Sp) and TSS obtained using two approaches, the Forward method and the Genetic Algorithm

<table>
<thead>
<tr>
<th></th>
<th>Forward selection</th>
<th>Genetic Algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of selected Nets</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Fitness</td>
<td>0.15</td>
<td>0.17</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.92</td>
<td>0.91</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.74</td>
<td>0.75</td>
</tr>
<tr>
<td>True Skill Statistic</td>
<td>0.66</td>
<td>0.65</td>
</tr>
</tbody>
</table>
Fig. 12. Distribution of the training and validation Mean Absolute Errors (MAEs) committed by each of the selected datasets on every training and validation dataset. The optimal ensemble rendered by the forward approach encompassed 6 MLPs involving 36 training and 36 validation datasets. As expected, the selection of datasets with 0.5 Prevalence (104 cases) produced a larger error in validation, although it resulted in a relatively small overlap.

### III.3.2 Sensitivity analysis

The sensitivity analysis (Fig. 13) showed that all the variables presented interactions because they presented a vertical spread at each of the considered values. Velocity showed a negative effect on the presence of Bermejuela, thus no positive values were observed beyond 0.2 m/s. Depth showed a positive effect on its presence, but shallow waters also presented positive effects due to some combinations with the remaining variables. Substrate showed a negative effect as it gets coarser, thus the larger positive effects corresponded to substrate index around 0. Cover presented the largest positive effect if present, but the increment on the suitability was mitigated as the amount of elements of cover increased. The ranking of variables showed that the most important variable was cover, followed by depth and velocity and lastly by substrate.
Fig. 13. Sensitivity analysis. Dashed line represents the base line corresponding to the mean value. The four considered variables showed interactions thus presented local and general spread. Red values represent the variable importance. The most important variable was cover.

### III.4 DISCUSSION

The developed MLP Ensemble could be considered good and the results consistent for further applications. The microhabitat suitability model achieved higher values of the performance criteria than previous studies on freshwater fish at the micro–scale, specifically considering its classificatory strength (Mouton et al., 2008; Muñoz-Mas et al., 2012).

The results agreed previous studies (Zhou et al., 2002b), regarding that the active selection of the MLP Ensemble outperformed the inclusion of the whole set. However, the extreme difference observed (0.15 versus 74) could be certainly attenuated by constraining the overfitting to the training dataset. The results partially agree with previous studies where more straightforward methodologies outperform more complex algorithms, achieving at least similar performance (Akhand & Murase, 2010; Lofstrom et al., 2010). Theoretically the use of GAs is a proficient approach that allows testing alternatives that
could not be tested in the Forward procedure, and it has been recognized that relatively bad models can provide satisfactory results since errors are compensated (Opitz & Maclin, 1999). However, the optimization problem was relatively simple whereas the searching space was too large. Therefore the Forward successfully searched for the best core of complementary MLPs eventually providing a lower MAE. The GA started from a broader searching space which hindered the achievement of better results. The main factors controlling the results derived from the rgenoud are the population size and the amount of generations (Mebane Jr & Sekhon, 2011), then an alternative will rely on the increment of that parameters, but the GA lapsed longer than the Forward procedure dissuading us from using this procedure. Recently the use of GA has been satisfactorily applied in the selection of MLPs (Wang & Alhamdoosh, 2013) following a different approach. In that study, the amount of MLPs in the ensemble was increased iteratively thus (Wang & Alhamdoosh, 2013) thus first the best pair of MLPs was found by means of GA, then the best trio, and so on. That approach could be faster than the Forward approach and the combinations would be not conditioned by previous steps. However, we considered that regarding the present problem the results shall not differ substantially. Nevertheless it should be thoroughly studied in future research. At this point we recommend the Forward methodology. However, other alternatives such as the negatively correlated MLPs Ensembles (Yao & Xu, 2006) or the aforementioned approach should be tested in order to determine without peradventure the prominence of a given methodology.

Regarding the sensitivity analysis, the Bermejuela can be classified a shelter–orientated limnophilic species, because cover was the most important variable. The other relevant variables indicated the maximum suitability for slow and deep microhabitats, related with the natural local deposition of silt in the riverbed. The observed interactions corroborated the critics to the univariate approach, who stated that the consideration of each habitat variable independently would produce larger errors (Orth & Maughan, 1982). That occurs because fish, and Bermejuela as well, do not select the habitats based on single variables, but considering them as a whole. No examples of microhabitat suitability appear in the literature, but their status and distribution (Doadrio, 2002a) and some technical reports at the local level (Martínez-Capel et al., 2009, 2011). Therefore, the selected model is considered valuable in its application on restoration efforts, which should be encouraged because the Bermejuela has disappeared from some of the main streams of the Iberian Peninsula (Elvira, 1995).
III.5 GRAPHICAL SENSITIVITY ANALYSIS – EXAMPLE

The following example originally appeared in Cannon and McKendry (2002) and has been adapted with illustrative purposes. The *in silico* problem states that a given phenomenon is affected by six different variables named from V₁ to V₆ (Equation 4). V₁ and V₂ interact and have a non-linear effect on the response (Y). V₃ and V₄ do not interact. The effect of V₃ is parabolic whereas the one for V₄ is linear. Finally V₅ and V₆ interact and have a linear effect on the response variable.

\[
Y = 5 \cdot \sin(V_1 \cdot V_2) + 20 \cdot (V_3 - 0.5)^2 - 10 \cdot V_4 + 20 \cdot V_5 \cdot V_6 \quad \text{(Equation 4)}
\]

The V₁ and V₂ interaction is characterized by the butterfly shape of the effects (similar to the one observed in Fig. 13) whereas the sinusoidal effect is observed through the pattern described by the plotted segments (Fig. 14).

![Figure 14](image1.png)

*Fig. 14. Example of the effects plots for inputs to the function in Equation 4*

V₃ and V₄ are not involved in any interaction consequently they have a purely additive effect appearing as single lines, although some error derived from the modelling approach is observable (Fig. 15). The parabolic shape of the V₃ effects fit its theoretical effect on the response whereas V₄ presents a linear response.
Finally $V_5$ and $V_6$ present interactions consequently they present the butterfly–like shape of the effects (Fig. 16). However responses are linear therefore, in contrast to $V_1$ and $V_2$, the effects present a linear disposal.
IV. CAN MULTILAYER PERCEPTRON ENSEMBLES MODEL THE ECOLOGICAL NICHE OF FRESHWATER FISH SPECIES?

ABSTRACT

The potential of Multilayer Perceptron (MLP) Ensembles to explore the ecology of freshwater fish species was tested by applying the technique to redfin barbel (*Barbus haasi* Mertens, 1925), an endemic and montane species that inhabits the North-East quadrant of the Iberian Peninsula. Two different MLP Ensembles were developed. The physical habitat model considered only abiotic variables, whereas the biotic model also included the density of the accompanying fish species and several invertebrate predictors. The results showed that MLP Ensembles may outperform single MLPs. Moreover, active selection of MLP candidates to create an optimal subset of MLPs can further improve model performance. The physical habitat model confirmed the redfin barbel preference for middle–to–upper river segments whereas the importance of depth confirms that redfin barbel prefers pool–type habitats. Although the biotic model showed higher uncertainty, it suggested that redfin barbel, European eel and the considered cyprinid species have similar habitat requirements. Due to its high predictive performance and its ability to deal with model uncertainty, the MLP Ensemble is a promising tool for ecological modelling or habitat suitability prediction in environmental flow assessment.

**Keywords:** Artificial neural networks, *Barbus haasi*, data mining, species distribution modelling, uncertainty analysis.
IV.1 INTRODUCTION

Ecological models for the quantitative prediction of species distributions are key to understanding the realised niche of species and its implication for species conservation in relation to global change (Austin, 2007). Therefore, ecological models have increasingly received attention due to their wide management applications in the context of biogeography, conservation biology and climate change studies (Mouton et al., 2010). Many studies on ecological modelling have focused on explanation rather than prediction (Elith and Leathwick, 2009); however, differences in the life–history or in the gene flow of fish assemblages could result in different realised niches (Mouton et al., 2010). Abiotic factors, together with dispersal and biotic interactions, are often considered the three elements that shape the ecological niche by determining species distribution and abundance (Barve et al., 2011). However, ecological models have usually focused on abiotic factors only (Boulangeat et al., 2012), and very few studies in freshwater fish ecology have explicitly included biotic variables (Elith and Leathwick, 2009) to explore biotic interactions and consumer–resource dynamics (Soberón, 2007). The consideration of these three elements (i.e. abiotic, biotic and dispersal factors) do not allow for simple statistical analysis because the data collected often exhibit non–linear and complex data structures (Crisci et al., 2012). Consequently, there is a need for new and innovative approaches to understand the complex structure of living systems (Larocque et al., 2011).

Several sophisticated modelling techniques have been applied in the ecological modelling of fish species, ranging from linear to multivariate and machine learning techniques such as Artificial Neural Networks (ANN) (Brosse and Lek, 2000, Muñoz–Mas et al., 2014, Palialexis et al., 2011). The most popular ANN architecture has been the Multilayer Perceptron (MLP) paradigm because it is considered to be able to approximate any continuous function (Olden et al., 2008). Formerly, MLP was referred to as a ‘black box’ because it provided little explanatory insight into the relative influence of variables in the prediction process (Olden and Jackson, 2002). To date, an enormous effort has been made to develop methods that clarify variable importance and interactions (Gevrey et al., 2006, Lek et al., 1996, Olden and Jackson, 2002), and consequently, MLPs should no longer be treated as ‘black box’ models (Özesmi et al., 2006).

There are several examples of single MLP applications in freshwater fish ecology (Park and Chon, 2007). For instance, MLPs have been successfully applied to model fish ecology through a broad range of ecosystems (Brosse and Lek, 2000, Gevrey et al., 2006, Kemp et al., 2007, Laffaille et al., 2003) and in some cases outperforming other statistical approaches (Baran et al., 1996, Lek et al., 1996). Despite those successful studies, it has been demonstrated that single models (e.g. a single MLP) do not necessarily perform consistently, resulting in divergent predictions (Buisson et al., 2010, Fukuda et al., 2011, Fukuda et al., 2013). The use of model ensembles has been emphasised to overcome this phenomenon (Araújo and New, 2007). The Multilayer Perceptron Ensemble (MLP
Ensemble, Hansen and Salamon, 1990) has proven to be proficient in several areas of ecology (Palialexis et al., 2011, Watts and Worner, 2008), but has rarely been applied in freshwater ecosystems (Muñoz–Mas et al., 2014).

Fish communities in Mediterranean rivers are an interesting targets to develop these novel statistical approaches (Hopkins II and Burr, 2009), particularly communities dominated by cyprinids, as they are characterised by a high number of endemic species for which there is insufficient knowledge about their ecology (Ferreira et al., 2007). Furthermore, endemic species tend to facilitate a more robust analysis of species–environment relationships. In this paper, we focused on the redfin barbel (*Barbus haasi* Mertens, 1925), a rheophilic small barbel (maximum body–length 30 cm) that is endemic to the Iberian Peninsula (Bianco, 1998) and categorised as vulnerable (Freyhof and Brooks, 2011). Their populations have decreased markedly, with pollution and the presence of exotic species being the main factors involved in the decline (Perea et al., 2011). Although redfin barbel has been the subject of numerous studies addressing its life–history, home–range, habitat preferences and the effects of pollutants (Aparicio and De Sostoa, 1999, Aparicio, 2002, Figuerola et al., 2012, Grossman and De Sostoa, 1994), a knowledge gap remains on the impact of biotic variables such as the density of accompanying fish species or invertebrate predictors in its ecological niche.

Therefore, the objective of this study was: (1) to test the proficiency of the MLP Ensembles to model the ecological niche of freshwater fish species, and (2) to test whether biotic variables affect the distribution of redfin barbel. To achieve these aims using MLP Ensembles, two different models of redfin barbel were developed. The first considered only physical habitat variables, the second included biotic and physical habitat variables.

### IV.2 METHODS

#### IV.2.1 Data collection

The study was conducted at the meso–scale in every summer, between 2003 and 2006. The study sites were located in the headwaters of the Ebron and Vallanca Rivers (Turia River tributaries), the Palancia River and the Villahermosa River (Mijares River Tributary) (Fig. 17) which approximately correspond to the southern limits of redfin barbel distribution (Perea et al., 2011). All the study sites were in unregulated streams and therefore a wide flow range was sampled (*i.e.* from 0.02 m³/s to 1.84 m³/s). For complete climatic description of the study area, see Alcaraz–Hernández et al. (2011) and Mouton et al. (2011).
Fig. 17. Location of the target river basins in the Iberian Peninsula (left) and study sites in the Mijares, Palancia and Turia River basins.

IV.2.1.1 Physical habitat survey

The physical habitat was assessed in every 300 m reach using an adaptation of the Basinwide Visual Estimation Technique (Dolloff et al., 1993). The approach stratifies the study site by HydroMorphological Units (hereafter called HMUs) classified as: pools, glides, riffles, and rapids (see Alcaraz–Hernández et al., 2011 for further details). Once an HMU was categorised, its physical attributes were recorded. They were, length, average width, obtained from three cross–sections corresponding to ¼, ½, and ¾ of the total length, mean depth (hereafter as depth), calculated from nine points corresponding to the measurements taken at each of the aforementioned cross–sections and the maximum depth, measured at the corresponding point. Percentage of shading over the channel, percentage of embeddedness, pieces of woody debris and percentage of the substrate types following a simplified classification from the American Geophysical Union (Martínez–Capel et al., 2009, Muñoz–Mas et al., 2012) were visually estimated and summarised in the substrate index (Mouton et al., 2011). In addition, the cover index (García de Jalón and Schmidt, 1995) was determined. This index characterises the available refuge due to caves, shading, substrate, submerged vegetation and water depth by assigning six scores from 0 (no refuge) to 5 (maximum score), and the weighted aggregation of these scores produces an index range from 0 to 10 (Table 6).

The river flow was gauged in at least one cross–section using an electromagnetic current meter (Valeport®), and flow velocity was calculated by dividing the flow by the average
cross–section area. Elevation and slope were extracted from cartography in a geographic information system, whereas habitat variability was estimated with the Shannon–Weaver diversity index, taking into account the number of habitat types (i.e. number of pools, glides, riffles or rapids) from the visual stratification of each study site (Table 6).

IV.2.1.2 Biological survey
The biological survey was undertaken by electrofishing, and all captured fish species were recorded. In each study site, one slow (i.e. pool or glide) and one fast (i.e. riffle or rapid) HMU were selected and surveyed (3–passes removal) after netting off the HMU. Due to a severe drought, some study sites were dry, resulting in 93 HMUs being sampled. Redfin barbel males are mature at approximately 45 mm, while females are mature at 100 mm (Aparicio, 2002), and therefore it was regarded as conservative to consider all specimens larger than 45 mm, resulting in a prevalence of 0.42. No size restrictions were imposed on the remaining fish species, and thus all the individuals were considered in the data analysis. Since the fish community varied across streams, the cyprinid species were grouped in a single variable (Table 7), and following previous studies fish densities were log(x+1) transformed (Brosse and Lek, 2000, Fukuda et al., 2011).
<table>
<thead>
<tr>
<th>Variable code</th>
<th>Min.</th>
<th>1st Qu.</th>
<th>Median</th>
<th>Mean</th>
<th>3rd Qu.</th>
<th>Max.</th>
<th>Units</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>River.Reach</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>8.108</td>
<td>12</td>
<td>16</td>
<td>–</td>
<td>Study site code</td>
</tr>
<tr>
<td>Year</td>
<td>2003</td>
<td>2004</td>
<td>2005</td>
<td>2005</td>
<td>2006</td>
<td>2006</td>
<td>–</td>
<td>Date</td>
</tr>
<tr>
<td>Meso.type</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2.95</td>
<td>4</td>
<td>4</td>
<td>–</td>
<td>Mesohabitat type</td>
</tr>
<tr>
<td>Meso.diversity</td>
<td>0.2</td>
<td>0.62</td>
<td>0.7</td>
<td>0.68</td>
<td>0.76</td>
<td>0.99</td>
<td>–</td>
<td>Reach mesohabitat diversity</td>
</tr>
<tr>
<td>Length</td>
<td>8.6</td>
<td>19.1</td>
<td>24.36</td>
<td>26.92</td>
<td>31.5</td>
<td>54.7</td>
<td>m</td>
<td>Length</td>
</tr>
<tr>
<td>Width</td>
<td>1.26</td>
<td>3.43</td>
<td>4.79</td>
<td>4.66</td>
<td>5.83</td>
<td>8.8</td>
<td>m</td>
<td>Width</td>
</tr>
<tr>
<td>Depth</td>
<td>0.04</td>
<td>0.22</td>
<td>0.32</td>
<td>0.35</td>
<td>0.46</td>
<td>0.79</td>
<td>m</td>
<td>Mean depth</td>
</tr>
<tr>
<td>M.Depth</td>
<td>0.15</td>
<td>0.43</td>
<td>0.63</td>
<td>0.64</td>
<td>0.83</td>
<td>1.23</td>
<td>m</td>
<td>Maximum depth</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.01</td>
<td>0.09</td>
<td>0.24</td>
<td>0.3</td>
<td>0.42</td>
<td>1.06</td>
<td>m/s</td>
<td>Mean flow velocity</td>
</tr>
<tr>
<td>Substrate</td>
<td>2.65</td>
<td>4.9</td>
<td>5.2</td>
<td>5.22</td>
<td>5.7</td>
<td>8</td>
<td>–</td>
<td>Substrate index</td>
</tr>
<tr>
<td>Embeddedness</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>29.35</td>
<td>50</td>
<td>100</td>
<td>%</td>
<td>% mud covering substrate</td>
</tr>
<tr>
<td>Cover</td>
<td>1</td>
<td>2.75</td>
<td>3.5</td>
<td>3.67</td>
<td>4.25</td>
<td>7.5</td>
<td>–</td>
<td>Cover index</td>
</tr>
<tr>
<td>Shadow</td>
<td>0</td>
<td>20</td>
<td>60</td>
<td>54.95</td>
<td>85</td>
<td>100</td>
<td>%</td>
<td>% shading</td>
</tr>
<tr>
<td>Wood.debris</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.01</td>
<td>0</td>
<td>0.16</td>
<td>pieces/m²</td>
<td>Woody debris</td>
</tr>
<tr>
<td>Elevation</td>
<td>605</td>
<td>655</td>
<td>743</td>
<td>745.8</td>
<td>792</td>
<td>968</td>
<td>m</td>
<td>Reach elevation above datum</td>
</tr>
<tr>
<td>Slope</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>m/m</td>
<td>Reach slope</td>
</tr>
<tr>
<td>D.redfin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.61</td>
<td>2.66</td>
<td>31.22</td>
<td>ind./100 m²</td>
<td>Density of <em>Barbus haasi</em></td>
</tr>
<tr>
<td>D.b.trout</td>
<td>0</td>
<td>1.32</td>
<td>4.48</td>
<td>11.19</td>
<td>16.12</td>
<td>86.47</td>
<td>ind./100 m²</td>
<td>Density of <em>Salmo trutta</em></td>
</tr>
<tr>
<td>D.eel</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.9</td>
<td>0</td>
<td>20.74</td>
<td>ind./100 m²</td>
<td>Density of <em>Anguilla anguilla</em></td>
</tr>
<tr>
<td>D.r.trout</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.64</td>
<td>2.18</td>
<td>42.58</td>
<td>ind./100 m²</td>
<td>Density of <em>Oncorhynchus mykiss</em></td>
</tr>
<tr>
<td>D.cyprinids</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12.31</td>
<td>11.09</td>
<td>198.3</td>
<td>ind./100 m²</td>
<td>Cyprinids density</td>
</tr>
<tr>
<td>Inv.density</td>
<td>0</td>
<td>1930</td>
<td>4680</td>
<td>7910</td>
<td>9590</td>
<td>56010</td>
<td>ind./ m²</td>
<td>Invertebrates density</td>
</tr>
<tr>
<td>Inv.richness</td>
<td>0</td>
<td>16</td>
<td>19</td>
<td>18.55</td>
<td>22</td>
<td>34</td>
<td>–</td>
<td>Invertebrates richness</td>
</tr>
<tr>
<td>Inv.diversity</td>
<td>0</td>
<td>0.34</td>
<td>0.41</td>
<td>0.39</td>
<td>0.46</td>
<td>0.56</td>
<td>–</td>
<td>Invertebrates diversity</td>
</tr>
<tr>
<td>Inv.biomass</td>
<td>0</td>
<td>0</td>
<td>0.07</td>
<td>0.39</td>
<td>0.27</td>
<td>6.02</td>
<td>g/m²</td>
<td>Invertebrates biomass</td>
</tr>
</tbody>
</table>
Benthic invertebrates were collected with a Hess sampler (0.5 m$^2$) following the International Standard ISO 8265:1988, official version of the European Standard EN 29265 (January 1994). Samples were later identified to the lowest possible taxonomic level (predominantly at family level), sorted and counted to obtain the density of invertebrates. Specimens were dried in an oven at 65 ºC for 24 h and the dry residue was weighed to obtain invertebrate biomass. Finally, two additional predictors were derived: invertebrate richness (i.e. the sum of present taxa in each sample) and invertebrate diversity by applying the Shannon–Weaver diversity index based on the number of individuals per taxa at each sampled HMU (Table 6).

Table 7. Fish community in the four rivers. The cyprinid fish community varied across rivers and was summarized in a single variable.

<table>
<thead>
<tr>
<th>Ebron</th>
<th>Vallanca</th>
<th>Palancia</th>
<th>Villahermosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmo trutta</td>
<td>Salmo trutta</td>
<td>Salmo trutta</td>
<td>Salmo trutta</td>
</tr>
<tr>
<td>Oncorhynchus mykiss</td>
<td>Oncorhynchus mykiss</td>
<td>Oncorhynchus mykiss</td>
<td>Oncorhynchus mykiss</td>
</tr>
<tr>
<td>Babus haasi</td>
<td>Babus haasi</td>
<td>Babus haasi</td>
<td>Babus haasi</td>
</tr>
<tr>
<td>Luciobarbus guiraonis</td>
<td>Luciobarbus guiraonis</td>
<td>Luciobarbus guiraonis</td>
<td>Luciobarbus guiraonis</td>
</tr>
<tr>
<td>Anguilla anguilla</td>
<td>Achondrostoma arcasii</td>
<td>Anguilla anguilla</td>
<td>Squalius valentinus</td>
</tr>
</tbody>
</table>

IV.2.2 Models’ development

The physical habitat and biotic models were developed by means of MLP Ensembles (Hansen and Salamon, 1990).

The development of the optimal MLP Ensembles followed the overproduce–and–choose approach. This approach consists of the generation of an initial large pool of MLP candidate classifiers (overproduce) whereas the second phase is devoted to select the best performing subset of MLPs (choose). The choose phase was performed by means of the step–forward algorithm. Thus, starting from every MLP candidate classifier the best complementary MLP candidate is iteratively searched until no improvement in the Mean Squared Error (MSE) was achieved (Fig. 18 A).

To render parsimonious models, the optimal input variables’ subsets for both models were also selected by means of the step–forward algorithm. First, the best pair of input variables was determined by developing a MLP Ensemble for every uncorrelated pair following the aforementioned procedure and then this pair became the base for the following step forward variable selection. The algorithm continued until no more variables were available and the selected model was the one with the lowest number of variables and error. Finally,
in order to rule out overfitting, we visually estimated differences between the distributions of the MSE based on the training and validation datasets of the selected MLPs (Fig. 18 B).

**Fig. 18.** Flowchart of the steps followed in the development of the physical habitat and biotic models.

**IV.2.2.1 Multilayer Perceptron Ensemble development**

Building a MLP Ensemble involves training several individual models (MLPs) and combining them to produce aggregated predictions (Hansen and Salamon, 1990). The construction of the individual models (hereafter MLP candidates) was carried out in R (R Core Team, 2015) with the package `monmlp` which optimises the model weights using the non–linear minimisation (`nlm`) routine (Cannon, 2012). The activation functions were the hyperbolic tangent and the linear transformation, while the number of nodes was restricted to improve generalisation (Özesmi et al., 2006) following equation 5.

\[
N_{\text{nodes}} = \max\left(\{1,\left(\frac{\text{number of variables} + 1}{2}\right)\}\right) \quad (\text{Equation 5})
\]
The aggregated forecast was determined by averaging the individual predictions of each selected model. Since model training depends on initial conditions, every MLP candidate was optimised five times with 500 iterations each.

Heterogeneity, or diversity, between MLP candidates is crucial because MLP Ensembles achieve better generalisation when models are complementary (Opitz, 1999). Several approaches allow for the construction of an MLP Ensemble with heterogeneous candidates, such as using different training datasets, architectures or learning methods (Brown et al., 2005). To increase heterogeneity among models, the database was divided in 63 different training and validation datasets corresponding to all possible combinations of 66% of the cases for training, and 33% for validation (i.e. following the \(k\)-fold approach). Consequently, 63 different MLP candidates were trained for every tested combination of input variables.

Originally the MLP Ensembles included all the developed models (Hansen and Salamon, 1990), but it was promptly demonstrated that active selection of the MLP candidates improved the final predictions (Opitz and Shavlik, 1996, Zhou et al., 2002). There are several methods to apply the overproduce–and–choose approach (Soares et al., 2013, Wang and Alhamdoosh, 2013, Yao and Xu, 2006, Zhou et al., 2002), but to our knowledge those sophisticated methods have not been coupled to a variable selection procedure. Consequently, we applied a step–forward selection of the MLP candidates which has been proved to perform similarly to more complex algorithms (Muñoz–Mas et al., 2014). Our step–forward selection was run starting from each of the 63 MLP candidates, searching for the best combination and stopping when no improvement was achieved. This was in contrast to the usual step–forward routine where the procedure would start from the best single model.

Since the optimal MLP Ensemble may not include all the MLP candidates, the observed performance could be affected by overfitting because the selected models may be trained only with some parts of the training database. Therefore the role of test data was twofold; first we applied an \textit{a priori regularisation} method with the early stop regularisation (\textit{sensu} Ludwig et al., 2014) by calculating the MSE on the validation dataset every 100 iterations of the \textit{nlm} routine and then we visually estimated for each selected MLP differences between the distributions of the MSE based on the training and validation datasets of all the selected MLPs. In the case of dissimilar distributions, the number of nodes and the number of iterations ran between calculations of the MSE on the validation dataset were readjusted.

To allow for the comparison with previous studies that either included all trained networks (Palialexis et al., 2011) or based the model selection on a ranking of the individual performances, including the top MLPs (Watts and Worner, 2008), the MSE of the best MLP candidate and of the MLP Ensemble Complete (\textit{i.e.} the one without any models'
selection) and then for the best five, ten and fifteen models were calculated and compared with our optimal MLP Ensembles.

### IV.2.2.2 Variable selection

To identify the most important variables shaping the ecological niche, for both models an input variable subset was selected based on the step–forward procedure because it has proven computationally efficient and tends to result in relatively small input variables’ subsets (May et al., 2011). In contrast to some other approaches (e.g. Generalised Additive Mixed Models, Lin and Zhang, 1999) the MLP Ensemble approach does not specifically allow for the consideration of spatial or temporal autocorrelation among training data. To rule out any influence of study site and sampling year, they were included as input variables (Table 6). Their absence on the ultimate models would indicate their irrelevance, thus corroborating the properness of the data packing. In addition, to render parsimonious models, instead of the usual step–forward procedure that discontinues when no improvement is achieved, the procedure was sustained until no more variables were available. The performance of the best model (MLP Ensemble) and the number of variables considered at every iteration were rescaled between 0 and 1 (1 being optimal), with the optimal MLP Ensemble being the one that maximised the sum of both criteria. The step–forward procedure may fail to consider variable interactions and may depend on the variable that was selected first. To overcome this limitation, one model was developed for each pairwise combination of variables. The best pair of variables was selected as the starting set of variables in the step–forward procedure. Additionally, during the entire process, neither correlated ($r^2 > 0.5$) nor collinear (variable inflation factor; $vif > 5$) combinations of variables were considered. Since the input database was a combination of ordinal and continuous variables, the function `hetcor` in the package `polycor` (Fox, 2010) was used to calculate the variables’ correlation.

### IV.2.3 Partial dependence plots and uncertainty analysis

Model reliability and transparency is of major concern for ecological modelling (Austin, 2007, Guisan and Thuiller, 2005, Özesmi et al., 2006) and is fundamental when models are used with exploratory purposes. Therefore, to graphically characterise the relationship between the input variables and the predicted densities obtained by the optimal MLP Ensembles, partial dependence plots (Friedman, 2001) implemented in the package `randomForests` (Liaw and Wiener, 2002) were developed.

The importance of dealing with uncertainty has been stressed as a key challenge in ecological modelling (Larocque et al., 2011). Consequently, partial dependence plots were developed also for every model in the optimal MLP Ensemble, and the function
densregion.normal in the package denstrip (Jackson, 2008) was used to visually inspect the uncertainty associated to the MLP aggregation in comparison with the input variable distribution.

### IV.3 RESULTS

#### IV.3.1 Training results

The optimal physical habitat model included five variables (three nodes): elevation, embeddedness, depth, slope and cover (Fig. 19) with a maximum correlation of 0.33 and a variable inflation factor of 1.41. The optimal biotic model also included five variables (three nodes): density of eel, cyprinids’ density, width, invertebrates’ density and cover (Fig. 19) with a maximum correlation of 0.38 and variable inflation factor of 1.51. In addition, the spatiotemporal correlation was considered negligible since study site and sampling year were not selected as inputs in the ultimate models (i.e. the physical habitat and the biotic models).

![Fig. 19. Sequence of the variable selection during the step–forward procedure to develop the MLP Ensemble (from left to right). The plots show the Mean Squared Error, MSE, in function of the number of variables. The circle indicates the selection of the optimal model.](image)

Although the two models showed similar performance (i.e. similar values of the Mean Squared Error, MSE) and the relatively large amount of zeros in the training dataset slightly biased their outputs, the biotic model slightly outperformed the physical habitat model with MSE of 0.12 and 0.13 respectively (Fig. 20 & Table 8).

The physical habitat model selected 15 MLPs. Consequently, 15 training datasets and 15 validation datasets were involved in its development. Cross–evaluation (i.e. the evaluation
of every training and validation dataset with every selected MLP candidate) showed that MSEs were distributed equally for the training and the validation datasets (Fig. 21), and therefore we considered the physical habitat model not overfitted. The biotic model selected eight MLPs, with eight training and validation datasets involved in the development of the selected MLPs. Likewise, the distribution of the training and validation MSE clearly overlapped, and therefore the biotic model was also considered not overfitted.

The best MLP candidate, the MLP Ensemble Complete (i.e. considering all sixty three MLPs) and the top five, top ten and top fifteen MLPs yielded higher MSEs than the optimal MLP Ensembles. The highest difference appeared between the MLP Ensemble Complete and the optimal physical habitat model (Table 8).

![Graphs of predicted vs observed values for physical habitat and biotic model](image)

**Fig. 20.** Relation between the observed and predicted values of the optimal MLPs. The optimal physical habitat and biotic models show transformed output.
Fig. 21. Frequency analysis of the Mean Squared Error (MSE) of the selected MLP candidates based on the corresponding training and validation datasets.

Table 8. Mean Squared Error (MSE) of the best MLP candidate, the optimal MLP Ensemble, the MLP Ensemble Complete (considering all MLPs; 63) and of the top five, top ten and top fifteen MLP. The amount of considered networks appears in brackets.

<table>
<thead>
<tr>
<th>Model</th>
<th>Physical habitat model</th>
<th>Biotic model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best MLP candidate</td>
<td>0.31 (1)</td>
<td>0.27 (1)</td>
</tr>
<tr>
<td>Optimal MLP Ensemble</td>
<td>0.14 (15)</td>
<td>0.13 (8)</td>
</tr>
<tr>
<td>MLP Ensemble Complete</td>
<td>0.95 (63)</td>
<td>0.31 (63)</td>
</tr>
<tr>
<td>Top MLP – 1 to 5</td>
<td>0.23 (5)</td>
<td>0.19 (5)</td>
</tr>
<tr>
<td>Top MLP – 1 to 10</td>
<td>0.21 (10)</td>
<td>0.18 (10)</td>
</tr>
<tr>
<td>Top MLP – 1 to 15</td>
<td>0.18 (15)</td>
<td>0.18 (15)</td>
</tr>
</tbody>
</table>

IV.3.2 Partial dependence plots – physical habitat model

The optimal physical habitat model showed a unimodal response between redfin barbel density and elevation, with a maximum density at 738 m above sea level. Embeddedness showed an almost flat trend but an exponential increase from 75 % onwards. Depth showed a steep positive linear trend, thus suggesting the major impact among the selected variables, whereas slope and cover were negatively and almost linearly related to redfin barbel density. As expected, uncertainty was higher at the extremes of the variables’ distributions and therefore trends at these extreme values could be unreliable (Fig. 22).
Fig. 22. Partial dependence plots of the physical habitat model (black line). Light lines (Blue lines on the e–version) correspond to the partial dependence plot of every selected MLP candidate. Faded background corresponds to uncertainty analysis based on mean and standard deviation of predictions, and the darker the colour the smaller the uncertainty.

IV.3.3 Partial dependence plots – Biotic model

The optimal biotic model showed a positive linear relation between eel and redfin barbel densities. Cyprinids’ density presented a unimodal response with the peak around 4 individuals/100 m$^2$. Width showed an almost positive linear influence on redfin barbel density whereas invertebrates’ density presented a unimodal response inflecting at 21718 individuals/ m$^2$. Likewise the physical habitat model cover presented a linear trend but with smaller uncertainty and slope. Uncertainty was higher than in the physical habitat model although it presented a similar pattern with the extreme values being more uncertain than the central part of the input variables’ distributions (Fig. 23).
Fig. 23. Partial dependence plots of the biotic model (black line). Light lines (Blue lines on the e–version) correspond to the partial dependence plot of every selected MLP candidate. Faded background corresponds to uncertainty analysis based on mean and standard deviation of predictions, and the darker the colour the smaller the uncertainty.

IV.4 DISCUSSION

IV.4.1 MLP Ensemble development

Our results indicated that the MLP Ensemble paradigm can be considered proficient to model the ecological niche of freshwater fish species, in line with previous studies that modelled fish density with neural networks (Baran et al., 1996, Brosse and Lek, 2000, Laffaille et al., 2003). The presented optimal models also outperformed any single MLP, which agrees with previous research (Palialexis et al., 2011). We also demonstrated that active selection of MLP candidates to create an optimal subset can further improve MLP Ensembles’ performance. This is consistent with Zhou’s et al. (2002) statement that “many could be better than all”. Moreover, our candidates’ selection approach resulted in a better performance in contrast with the selection approach based on the individual performance (i.e. top five, ten and fifteen). We recommend this procedure in contrast to previous studies that selected the best subset based on the individual performance of the MLPs (Watts and Worner, 2008). However, our step–forward process is determined by the first selected model, and despite the fact that the procedure started from every single neural network,
the possibility to get stuck at a local minimum exists. Therefore, untested combinations of models could outperform those obtained by the step–forward algorithm.

Genetic algorithms may overcome the aforementioned constraints (Soares et al., 2013, Wang and Alhamdoosh, 2013). There are successful applications of genetic algorithms in variable selection procedures (May et al., 2011, Olden et al., 2008) and also within the selection of optimal MLP Ensembles (Soares et al., 2013, Wang and Alhamdoosh, 2013). Although the use of genetic algorithms for both variable selection and MLP candidates’ selection could exponentially increase the computation effort, this approach is certainly promising and should be the subject of future research.

The effect of the relatively large amount of zeros in the training dataset was a remarkable issue and slightly biasing models’ outputs. There are specific techniques in count data modelling to deal with excess of zeros with either parametric (Mullahy, 1986, Lambert, 1992) and non–parametric (Liu and Chan, 2010) responses. Certainly the comparison of the capability of MLP Ensembles and these techniques in ecological modelling would be of interest, although these techniques by definition do not easily account for variable interactions and thus do not easily assure better performance. Nevertheless, our results were considered satisfactory since they provided an acceptable balance between model complexity, performance and computational effort, and they were devoted to explore rather than to predict fish density in further analysis.

**IV.4.2 Ecological relevance of the physical habitat model**

The optimal physical habitat model included five variables: elevation, slope, depth, embeddedness and cover. Elevation is broadly accepted as a proximal predictor of temperature (Elith and Leathwick, 2009), and consequently we considered that it may reflect the effect of climate on redfin barbel distribution. Similarly, the negative trend of redfin barbell density versus slope agrees with its preference for middle–to–upper stream reaches of mountainous rivers (Perea et al., 2011). The positive impact of depth corresponds with previous studies that considered the redfin barbel a pool dweller (Aparicio and De Sostoa, 1999). Our results may also suggest the importance of backwaters or stagnated areas as resting habitats. This could also explain the positive relationship between redfin barbell density and embeddedness, since pool substrates are generally more embedded. Despite the negative relationship between cover and redfin barbel density in our study, some authors classified the redfin barbel as a shelter–orientated fish (Grossman and De Sostoa, 1994). Aparicio (2002) reported the active use of cover in an ephemeral river but related its use to the absence of deep pools in this specific river, rather than to a redfin barbel preference for cover. Our study suggests that in more complex river systems with well–developed pool–riffle patterns, redfin barbel may tend to avoid excessive cover complexity.
IV.4.3 Ecological relevance of the biotic model

In contrast to previous studies (Vezza et al., 2015, Watts and Worner, 2008) where the combination of physical habitat and biotic variables outperformed the model developed only with physical habitat variables, our biotic model did not significantly perform better than the physical habitat model. Moreover, uncertainty was higher in the biotic model, which underlines the previously reported complexity related to the assessment of biotic interactions (Leathwick and Austin, 2001). Although larger datasets may reduce this uncertainty, our study nevertheless suggests interesting and plausible relationships. In addition, the observed associations between biotic variables and redfin density agreed with the ecological gradient theory because responses were quasi-linear or unimodal (Austin, 2007).

The biotic model demonstrated a positive association between redfin barbel and European eel, which confirms the work of Laffaille et al. (2003). They modelled eel habitat suitability in a small coastal catchment with a single MLP. Eels were more abundant in deep and low flow shaded areas without aquatic vegetation (Laffaille et al., 2003). Such a pattern broadly concurs with the requirements of redfin barbel. Also the suggested relationship between redfin barbel and cyprinids corresponds with previous studies on the Iberian Peninsula that reported the presence of multi-species shoals as well as an overlap in microhabitat use (Martínez–Capel et al., 2009). Fish schooling benefits include the enhancement of hydrodynamics and the protection against predators (Landa, 1998). Moreover, similar positive interactions with cyprinid species have been reported for Iberian chub (Squalius pyrenaicus, Günther, 1868) and eastern Iberian barbel (Luciobarbus guiraonis, Steindachner, 1866) (Vezza et al., 2015). This indicates that restoration actions focused on redfin barbel could also result in habitat enhancement for other cyprinid species.

Although previous work positively correlated invertebrate density to fish density (Mas–Martí et al., 2010), our results show a maximal redfin barbel density at 21718 individuals/m². This could be related to food availability. However, the preferred prey invertebrates of redfin barbel (*i.e.* Chironomidae, Ephemeroptera and Trichoptera following Miranda et al., 2005) were strongly correlated with the invertebrate density applied in our model. Therefore, we attributed the avoidance of the higher invertebrate densities to a habitat correlation. Previous studies suggested that in Mediterranean rivers, with very unstable climatic conditions, riffles tend to host higher invertebrate density than pools (Bonada et al., 2006). Therefore the decrement of the partial dependence plot could be showing the necessity for larger depth rather than a preference for intermediate invertebrate densities. Nevertheless, the discrepancy between our results and the literature can also be a consequence of the applied model complexity (*e.g.* linear versus non-linear models), and the impact of model complexity should be thoroughly analysed in further studies modelling the relationship between invertebrate and fish density. The biotic
model also selected two physical habitat variables, cover index and width, and the relationship with cover index being similar to that shown in the physical habitat model. The positive association between redfin barbel density and width may be attributed to the negative correlation between width and elevation, in line with the aforementioned redfin barbel preference for middle–to–upper stream reaches, but with a slightly different response because the study encompassed four different rivers.

Although the biotic model appears to suggest interactions between redfin barbel and other species, significant positive or negative correlations between species does not imply a causative effect (Wisz et al., 2013). A simple correlation does not mandatorily correspond to any species interaction, neither mutualism nor facilitation, and therefore further research should clarify the true impact of species interactions. Furthermore, changes in the habitat available may result in a substantial increment in the competition between species (Wisz et al., 2013). The cyprinids density partial dependence plot showed a decrement at the tail of the curve. Therefore, in spite of being uncertain, it could suggest that, under different habitat conditions than those in our study, the positive interaction with cyprinid fish species may become habitat competition, thus emphasising the necessity of close monitoring in the near future to avoid ecological loss.

**IV.4.4 Model uncertainty**

Relatively few studies address uncertainty in ecological modelling and its effects on model predictions and decision making (Elith and Leathwick, 2009). In accordance with previous studies (Peters et al., 2009), the largest uncertainty tended to appear in the regions of the input variables that were poorly represented in the training database. In contrast to the high uncertainty demonstrated by the different MLP candidate predictions, the optimal MLP Ensembles produced sound and smoother partial dependence plots that allowed general trends to be derived from a wide range of model outputs. This has been stimulated by three approaches applied in our study. First, the bias and variance dilemma (Geman et al., 1992) was addressed by limiting model complexity (i.e. limiting the number of nodes and variables), leading to less complex models than in previous studies (Dedecker et al., 2004, Lek et al., 1996). Second, the early stop regularisation considered the errors committed on the training and validation datasets (Ludwig et al., 2014). Third and most importantly, we assessed overfitting by checking whether species responses to environmental variables were consistent with the ecological gradient theory (Austin, 2007). Inconsistent model results would have suggested a more restrictive modelling approach by limiting model complexity or adjusting the early stop parameters. Uncertainty could also arise when samples from different periods are combined, since fish density is a density–dependent phenomenon (Mas–Martí et al., 2010); however, the sampling year was not selected as an important variable in the optimal MLP Ensembles. The results from our study suggested that temporal packing can be considered admissible when focusing on a
short time span and consequently it can be concluded that MLP Ensembles can be used to model the ecological niche of freshwater fish species.
ON SPECIES DISTRIBUTION MODELLING, SPATIAL SCALES AND ENVIRONMENTAL FLOW ASSESSMENT WITH MULTI-LAYER PERCEPTRON ENSEMBLES: A CASE STUDY ON THE REDFIN BARBEL (Barbus haasi; MERTENS, 1925)
V. ON SPECIES DISTRIBUTION MODELLING, SPATIAL SCALES AND ENVIRONMENTAL FLOW ASSESSMENT WITH MULTI–LAYER PERCEPTRON ENSEMBLES: A CASE STUDY ON THE REDFIN BARBEL (*Barbus haasi*; MERTENS, 1925)

ABSTRACT

Inconsistent performance of Species Distribution Models (SDMs), which may depend on several factors such as the initial conditions or the applied modelling technique, is one of the greatest challenges in ecological modelling. To overcome this problem, ensemble modelling combines the forecasts of several individual models. A commonly applied ensemble modelling technique is the Multi–Layer Perceptron (MLP) Ensemble, which was envisaged in the 1990s. However, despite its potential for ecological modelling, it has received little attention in the development of SDMs for freshwater fish. Although this approach originally included all the developed MLPs, Genetic Algorithms (GA) now allow selection of the optimal subset of MLPs and thus substantial improvement of model performance. In this study, MLP Ensembles were used to develop SDMs for the redfin barbel (*Barbus haasi*; Mertens, 1925) at two different spatial scales: the micro–scale and the meso–scale. Finally, the potential of the MLP Ensembles for environmental flow (e–flow) assessment was tested by linking model results to a hydraulic model. MLP Ensembles with a candidate selection based on GA outperformed the optimal single MLP or the ensemble of the whole set of MLPs. The micro–scale model complemented previous studies, showing high suitability of relatively deep areas with coarse substrate and corroborating the need for cover and the rheophilic nature of the redfin barbel. The meso–scale model highlighted the advantages of using cross–scale variables, since elevation (a macro–scale variable) was selected in the optimal model. Although the meso–scale model also demonstrated that redfin barbel selects deep areas, it partially contradicted the micro–scale model because velocity had a clearer positive effect on habitat suitability and redfin barbel showed a preference for fine substrate in the meso–scale model. Although the meso–scale model suggested an overall higher habitat suitability of the test site, this did not result in a notable higher minimum environmental flow. Our results demonstrate that MLP Ensembles are a promising tool in the development of SDMs for freshwater fish species and proficient in e–flow assessment.

Keywords: Artificial neural network, genetic algorithm, Iberian Peninsula, Mediterranean river, meso–scale, micro–scale.
V.1 INTRODUCTION

Species distribution models (SDMs) play a significant role in understanding habitat requirements of fish species, providing a framework from which spatial and temporal distribution patterns can be predicted (Olden et al., 2008). Thereby SDMs are useful tools to select cost–efficient restoration or management actions (Mouton et al., 2010). To date, a wide range of SDMs is available, encompassing several modelling techniques. Examples include Generalized Additive Models (Fukuda et al., 2013), Fuzzy Rule Base Systems (Mouton et al., 2011), or Artificial Neural Networks (ANNs) – most commonly Multi–Layer Perceptrons (MLPs) – (Olaya–Marín et al., 2012). All these techniques are typically applied to generate a single monolithic SDM, which often has proven to be sufficient for ecological modelling (Olden et al., 2008). The MLP paradigm, for instance, has been widely used due to its high predictive performance and its versatility to cope with different kinds of datasets (Olden et al., 2008, Olaya–Marín et al., 2012, Fukuda et al., 2013). Therefore, there are successful examples of single MLPs modelling fish habitat requirements at different scales, from the micro–scale (Brosse and Lek, 2000, Gevrey et al., 2006, Laffaille et al., 2003) to the macro–scale (Olaya–Marín et al., 2012) while at the meso–scale some studies have demonstrated that a single MLP can outperform other statistical approaches (Baran et al., 1996, Lek et al., 1996). Yet, many real–world problems, like demonstrated for marine ecosystems (Meier et al., 2014), are too large and too complex for a single monolithic model (Yao and Xu, 2006). Moreover, SDMs may not perform consistently and even provide discrepant predictions, depending on several factors such as the initial model conditions or the applied modelling technique (Thuiller et al., 2009, Fukuda et al., 2013).

To deal with these inconsistencies, ensemble modelling is now an emerging field of research in ecological modelling (Araújo and New, 2007). Ensemble modelling is based on the minimization of the error through the integration of several models by combining their different predictions into a single forecast. Despite their promising potential for species distribution modelling, only few applications of ensemble modelling have been reported, including an example combining several different techniques to develop SDMs for fish species (Thuiller et al., 2009). To date, Random Forests is the only ensemble modelling technique that could be considered widespread (Mouton et al., 2011, Fukuda et al., 2013, Mostafavi et al., 2014). Although the ANN ensemble modelling counterpart, the MLP Ensemble, was conceived more than twenty years ago (Hansen and Salamon, 1990) and may have the same potential as Random Forests, it has been rarely applied in fish SDMs so far (e.g. Muñoz–Mas et al., 2014a, 2015, 2016). Originally MLP Ensembles included all trained MLPs (Hansen and Salamon, 1990) but it was soon demonstrated that active selection of the considered MLPs improved the final predictions (Wang and Alhamdoosh, 2013), and that Genetic Algorithms (GA) were appropriate for this selection (Soares et al., 2013, Wang and Alhamdoosh, 2013).
Therefore, the present study developed SDMs for the redfin barbel (*Barbus haasi* Mertens, 1925) with GA–optimised MLP Ensembles. The redfin barbel is a small rheophilic barbel mainly inhabiting middle–to–upper stream reaches of mountainous rivers. Although redfin barbel occurs in the North–East quadrant of the Iberian Peninsula, its distribution area has been halved mainly due to pollution and the presence of invasive species (Aparicio, 2002, Perea et al., 2011). Thus, it is a particularly suitable target species for development and testing for these models since it is considered threatened in the Mediterranean region (Freyhof and Brooks, 2011), but a sustainable extant population is still present in our study area. From the ecological viewpoint, the redfin barbel is considered a cover–orientated fish (Grossman and De Sostoa, 1994) preferring deep and slow–flowing pools with abundant cover (Aparicio and De Sostoa, 1999).

In environmental flows (e–flow) assessment the instream habitat has been typically evaluated at the micro–scale (few m\(^2\) of the instream area) using data and SDMs concordant with the scale (Conallin et al., 2010). The micro–scale SDMs have demonstrated proficient ability to predict fish location (e.g. Muñoz–Mas et al., 2014) and accordingly, this scale is specified in the Spanish norm for hydrological planning as the legal standard in e–flow assessment (MAGRAMA, 2008). However, the use of the micro–scale has been criticized for being time–consuming (Parasiewicz, 2001) and for emphasizing cross–sectional variation over the longitudinal one (Vezza et al., 2012). Consequently, some studies highlighted the benefits of the meso–scale – which typically correspond with Hydro–Morphological Units (HMUs) such as pools, riffles or rapids – among the possible spatial scales that can be used to analyse fish habitat requirements (Costa et al., 2012; Vezza et al., 2015). Using the meso–scale it is possible to describe the environmental conditions around an aquatic organism, even using biotic predictors, and not only limiting the analysis to the point where fish were observed (Vezza et al., 2015). Therefore, meso–scale models demonstrated great ability to properly relate the habitat–suitability predictions and fish presence (Parasiewicz & Walker, 2007). However, more research is needed to dispel any doubt about the advisability of the meso–scale over the micro–scale one and about significant differences in e–flow assessment.

In this paper, we hypothesized i) that the spatial scale affects the SDMs performance and structure and ii) that these differences may lead to differences in the assessed e–flows. We developed models at two different scales; the micro–scale and the meso–scale. Once models were developed, a graphical sensitivity analysis was performed to compare our results with previous literature. To evaluate the practical applicability of these models, the two SDMs were linked with a hydraulic model to infer e–flows. Finally, the merits and demerits of our models and differences in the e–flow assessment are briefly discussed.
V.2 METHODS

V.2.1 Micro–scale data collection

The presence of adult redfin barbel (body length > 5 cm) (Aparicio, 2002) 'feeding' or 'holding a feeding position' (i.e. active specimens) was observed by snorkelling in the Mijares River (Jucar River Basin District, east of the Iberian Peninsula) during the early summer of 2012 (Fig. 24). The survey included the area covered by the hydraulic model (Fig. 24) and we surveyed complete HMUs classified as: pools, glides, riffles, and rapids, by selecting a similar area of slow (pools and glides) and fast (riffles and rapids) HMUs (Alcaraz–Hernández et al., 2011). Four abiotic variables were measured in cross–sections randomly distributed over each HMU: mean water velocity (velocity), water depth (depth), the substrate composition (substrate) and the presence of several types of cover (cover) because these variables have been reported to be the most relevant for fish distribution at the micro–scale (Gibson, 1993). Velocity and depth were measured with an electromagnetic flow velocity meter (Valeport®, UK) and a wading rod, respectively. Both substrate and cover were visually estimated. The substrate was classified in bedrock, boulders, cobbles, gravel, fine gravel, sand, silt and macrophytes (Muñoz–Mas et al., 2012), and the percentages of the different substrate types were summarized in a single substrate index (Mouton et al., 2011). The considered types of cover were large boulders, undercut banks, woody debris, roots, shade (intense) and vegetation, and the number of different cover types present at each location was summed to calculate the cover index (e.g. no cover = 0, boulders + undercut banks = 2, etc.) (Table 9). The initial dataset included 92 presences, and 341 instances where redfin barbel was absent, resulting in a data prevalence (proportion of presence data in the entire dataset) of 0.21.

Table 9. Code, summary, description and units of the variables included in the micro–scale MLP Ensemble.

<table>
<thead>
<tr>
<th>Code</th>
<th>Min.</th>
<th>1st Qu.</th>
<th>Median</th>
<th>Mean</th>
<th>3rd Qu.</th>
<th>Max.</th>
<th>Variable &amp; units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velocity</td>
<td>0.00</td>
<td>0.29</td>
<td>0.60</td>
<td>0.59</td>
<td>0.86</td>
<td>2.13</td>
<td>Mean water velocity [m/s]</td>
</tr>
<tr>
<td>Depth</td>
<td>0.09</td>
<td>0.38</td>
<td>0.54</td>
<td>0.60</td>
<td>0.75</td>
<td>2.75</td>
<td>Water depth [m]</td>
</tr>
<tr>
<td>Substrate</td>
<td>1.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.12</td>
<td>5.56</td>
<td>8.00</td>
<td>Substrate index [-]</td>
</tr>
<tr>
<td>Cover</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.36</td>
<td>1.00</td>
<td>3.00</td>
<td>Cover index [-]</td>
</tr>
</tbody>
</table>
Fig. 24. Location of the target river basins in the Iberian Peninsula (upper–right corner) and study sites in the Mijares, Palancia and Turia River basins.

V.2.2 Meso–scale data collection

Electrofishing surveys for adult redfin barbel were conducted every summer between 2003 and 2006 in the headwaters of four rivers in the Jucar River Basin District: the Ebrón River and the Vallanca River (both tributaries of the Turia River), the Villahermosa River (a Mijares River tributary) and the Palancia River (Fig. 24). Note that the micro–scale study site was not included in the four meso–scale study sites, although it encompassed the segment of the hydraulic model. Four study sites per river were surveyed following an adaptation of the Basinwide Visual Estimation Technique (BVET, Dolloff et al., 1993; for additional details, see Alcaraz–Hernández et al. (2011) and Mouton et al. (2011)). Two HMUs per reach, one slow HMU (pool or glide) and one fast HMU (riffle or rapid), were sampled and, in addition to the HMU type, 13 abiotic habitat variables were assessed in each HMU: length, mean width, mean depth, maximum depth, the percentage of shading, the percentage of embeddedness, the density of woody debris, the substrate index (following the aforementioned classification), the cover index, mean flow velocity, the elevation and slope of the reach and the habitat variability. Length and mean width were measured with tape. Width was measured three times at cross–sections located at $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ of the total HMU length whereas depth was measured with a wading rod in three uniformly distributed locations per cross–section. For each HMU, depth measurements were then averaged to obtain mean depth and the maximum depth was measured in the corresponding location. The percentage of shading, the percentage of embeddedness (i.e. the percentage of the HMU area covered by silt), the number of woody debris particles and
the substrate classification were visually estimated. The number of woody debris particles was divided by the HMU area to obtain the density of woody debris. The cover index was calculated as a weighted aggregation of scores assigned to the presence of undercut banks, shade, large substrate, submerged vegetation and the mean depth (García de Jalón and Schmidt, 1995). The river flow at the time of the survey was gauged with an electromagnetic flow velocity meter (Valeport®, UK) and the mean flow velocity was calculated by dividing the gauged flow by the mean cross-section area. Finally, elevation and slope were derived from digital elevation models (National Centre for Geographic Information, CNIG) and the habitat variability was estimated by the Shannon–Weaver diversity index, considering the number of different HMUs in a 300–m–length stretch surrounding each study site (Table 10). The initial dataset included 39 presences, and 54 HMUs where redfin barbel was absent, resulting in a prevalence of 0.42.

<table>
<thead>
<tr>
<th>Code</th>
<th>Min.</th>
<th>1st Qu.</th>
<th>Median</th>
<th>Mean</th>
<th>3rd Qu.</th>
<th>Max.</th>
<th>Variable &amp; units</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMU type</td>
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<td>2.0</td>
<td>3.0</td>
<td>3.0</td>
<td>4.0</td>
<td>4.0</td>
<td>HMU type [#]</td>
</tr>
<tr>
<td>Depth</td>
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<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.8</td>
<td>Mean depth [m]</td>
</tr>
<tr>
<td>M.Depth</td>
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<td>0.4</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8</td>
<td>1.2</td>
<td>Maximum depth [m]</td>
</tr>
<tr>
<td>Length</td>
<td>8.6</td>
<td>19.1</td>
<td>24.4</td>
<td>26.9</td>
<td>31.5</td>
<td>54.7</td>
<td>Length [m]</td>
</tr>
<tr>
<td>Width</td>
<td>1.3</td>
<td>3.4</td>
<td>4.8</td>
<td>4.7</td>
<td>5.8</td>
<td>8.8</td>
<td>Mean width [m]</td>
</tr>
<tr>
<td>Substrate</td>
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<td>4.9</td>
<td>5.2</td>
<td>5.2</td>
<td>5.7</td>
<td>8.0</td>
<td>Substrate index [-]</td>
</tr>
<tr>
<td>W.Debris</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>Woody debris [pieces/m²]</td>
</tr>
<tr>
<td>Elevation</td>
<td>605.0</td>
<td>655.0</td>
<td>743.0</td>
<td>745.8</td>
<td>792.0</td>
<td>968.0</td>
<td>Elevation a.s.l. [m]</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>Slope [m/m]</td>
</tr>
<tr>
<td>Embeddedness</td>
<td>0.0</td>
<td>0.0</td>
<td>15.0</td>
<td>29.4</td>
<td>50.0</td>
<td>100.0</td>
<td>Percentage of embeddedness [%]</td>
</tr>
<tr>
<td>Shade</td>
<td>0.0</td>
<td>20.0</td>
<td>60.0</td>
<td>55.0</td>
<td>85.0</td>
<td>100.0</td>
<td>Percentage of shade [%]</td>
</tr>
<tr>
<td>Cover</td>
<td>1.0</td>
<td>2.8</td>
<td>3.5</td>
<td>3.7</td>
<td>4.3</td>
<td>7.5</td>
<td>Cover index [-]</td>
</tr>
<tr>
<td>Diversity</td>
<td>0.2</td>
<td>0.6</td>
<td>0.7</td>
<td>0.7</td>
<td>0.8</td>
<td>1.0</td>
<td>HMU diversity [-]</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>1.1</td>
<td>Mean flow velocity [m/s]</td>
</tr>
</tbody>
</table>

V.2.3 Variable selection

Due to the limited number of variables included in the micro–scale sampling and the assumed relevance of these variables for fish distribution (Gibson, 1993), all four variables were included in the micro–scale model. Nevertheless, none of these variables appeared significantly correlated (spearman $r^2 < 0.5$) or collinear (variable inflation factor; vif < 5).
Since the meso–scale surveys resulted in a high number of explanatory variables, for the final meso–scale model, a parsimonious suite of variables were selected following the step–forward procedure (May et al., 2011); this procedure consists of adding iteratively one variable at a time while the performance is being improved, and stops adding as soon as performance decreases. This approach has proved computationally efficient and tends to result in relatively small input variable sets (May et al., 2011). During the step–forward procedure, neither correlated \((\text{spearman } \rho^2)\) nor collinear \((\text{vif})\) combinations of input variables were allowed.

**V.2.4 Multi–Layer Perceptron (MLP) Ensemble development**

**V.2.4.1 MLP Candidates training**

The overproduce–and–choose approach (Partridge and Yates, 1996) generates the optimal MLP Ensemble by first initiating a large number of MLP Candidate classifiers and then selecting the best performing subset of classifiers (Soares et al., 2013). Diversity among the selected MLP Candidates is a key factor of a MLP Ensemble (Wang and Alhamdoosh, 2013) because the diversity among classifiers generally compensates for the increase in error rate of any individual classifier (Opitz, 1999). In diverse ensembles, each candidate complements the others, and thus improves the aggregated forecast (Akhand et al., 2009). There are several methods to construct a diverse MLP Ensemble (Wang and Alhamdoosh, 2013). However, bagging has proven better than several of the more sophisticated methods (Akhand et al., 2009) and can be easily implemented. Bagging splits the initial dataset in training and a test dataset. First, \(k\) training \((\text{bag})\) datasets of size \(m\) are generated by sampling, with replacement, the initial dataset of size \(n\), with \(m < n\). For each training dataset, the test dataset (or the \(\text{out–of–bag}\) dataset) then consists of the non–sampled instances from the initial dataset. MLP Candidates are developed based on these \(k\) training datasets and the aggregated forecast is finally obtained by averaging the predictions of the individual MLP Candidates. Since the prevalence of the training dataset may affect the result of SDMs (Mouton et al., 2009, Fukuda, 2013), \(m\) was chosen in accordance to the prevalence of the initial dataset.

The micro–scale dataset contained a number of absences that exceeded by far the number of presences and therefore, 66 % of the presences \((\text{i.e. } 61\text{ instances})\), and the same number of absences were randomly selected \((m=122)\). The micro–scale dataset did not allow training all possible combinations with a prevalence of 0.5. Therefore an arbitrary but large number of MLP Candidates \((k=2000)\) were trained in order to ensure that every ‘presence’ instance was linked to every ‘absence’ instance several times.

To reduce the number of input variables, the optimisation of the MLP Ensemble at the meso–scale was inserted in the step–forward variable selection procedure (May et al.,
2011). The meso-scale dataset had a more balanced prevalence (0.42), therefore instead of the bagging approach the \( k \)-fold approach was performed. This approach can be seen as a systematic \textit{bagging} without replacement. Thus, the training datasets consisted of all possible combinations of 66% of the observed cases with varying prevalence \((m=61)\). Thus, sixty three MLP Candidates \((k=63)\) were trained, with each combination of input variables in the step-forward procedure.

MLP Candidates were trained with the R (R Core Team, 2015) package \textit{monmlp} (Cannon, 2012) which optimizes the MLP weights using the non-linear minimization \((nlm)\) routine (R Core Team, 2015). The number of neurons corresponded to the integer of half the number of variables included in the MLP Ensemble. Hyperbolic tangent and logistic transformation functions were used in the hidden and the output layers, respectively. In line with the Habitat Suitability Index (Bovee et al., 1998), the model output will hereafter be referred to as suitability index. To assess the degree of overfitting of the model results, we compared the performance, quantified by the True Skill Statistic (TSS) \([-1, 1]\) (see Mouton et al., 2010 for additional details about performance criteria), of each selected MLP Candidate on both the \textit{bag} and the \textit{out-of-bag} datasets.

\textbf{V.2.4.2 Selection of candidates – Genetic algorithms}

GAs are search and optimization algorithms based on the process of natural selection (Olden et al., 2008). From the wide range of GA approaches to select the optimal subset of MLP Candidates (Wang and Alhamdoosh, 2013, Soares et al., 2013, Muñoz–Mas et al., 2014), we followed Wang and Alhamdoosh (2013) since satisfactory results were obtained with small-sized ensembles. This approach iteratively increases the ensemble size by the stepwise addition of MLPs while in every step the GA searches for the best combination of MLPs. That is to say, the GA first finds the best ensemble of two MLPs, subsequently it finds the best ensemble of three MLPs and so on.

We applied the \textit{rgenoud} package (Mebane Jr and Sekhon, 2011), including nine operators driving the optimization which correspond to cloning, uniform mutation, boundary mutation, non-uniform mutation, polytope crossover, simple crossover, whole non-uniform mutation, heuristic crossover and local–minimum crossover (Mebane Jr and Sekhon, 2011). The phenomenon whereby GAs get stuck on local optima is known as premature convergence (Fogel, 1994). To avoid this, the population diversity and the selection pressure should be balanced (Pandey et al., 2014). Therefore, the cloning operator was restricted \((0.25)\) whereas the operators that increase diversity \(i.e.\) uniform mutation, simple crossover and heuristic crossover) were set relatively high \((0.6, 0.6 and 0.4)\). In summary, the whole set of operators were set to \(0.25, 0.6, 0.05, 0.05, 0.05, 0.6, 0.05, 0.4 and 0\) respectively. On the other hand, the population size as well as the number of generations varied in accordance with the ensemble size \((\text{Ens}_{\text{size}})\). The population size followed
\[
\log_{1.5}(\text{Ens}_{\text{size}}) \cdot 4000 \text{ and } \log_{1.5}(\text{Ens}_{\text{size}}) \cdot 2000 \text{ in the micro–scale and the meso–scale models respectively whereas the number of generations was set to } (\text{Ens}_{\text{size}}) \cdot 10 \text{ in both models. The models were optimised based on a multi–objective function. Specifically, the GA maximized the TSS while stimulating overprediction (sensitivity > specificity) (Mouton et al., 2010) and forcing model outputs to span the whole output range (from 0 to 1) following equation 6,}
\]

\[
\text{Objective} = TSS + \min\{0, \text{Sensitivity} - \text{Specificity}\} - \min\{\text{MLP Ensemble}(\text{Database})\} - [1 - \max\{\text{MLP Ensemble}(\text{Database})\}](\text{Equation 6})
\]

where MLP Ensemble corresponds to the aggregated forecast based on the different predictions performed by each MLP component (i.e. \(\sim f(x)\)) and Database to the training dataset (i.e. \(\sim x\)).

To assess the quality of the MLP Ensembles obtained with the Wang and Alhamdoosh approach (Wang and Alhamdoosh, 2013), the performance of three different ensembles was compared: the ensemble containing only the best single MLP Candidate (Best MLP Candidate), the ensemble aggregating all MLPs (Complete MLP Ensemble) and the GA optimised MLP Ensemble (Optimal MLP Ensemble).

V.2.5 Graphical sensitivity analysis

The applied sensitivity analysis uses modified scatter plots to assess the effects of the model inputs on the output. For each variable \(V_i\), the variation of the model output due to variation of \(V_i\) (\(\Delta_i\)) is calculated and plotted as segments, with the slope of these segments equalling the partial derivative of the model output related to \(V_i\). The visualization of the partial derivatives as segments allows the identification of trends and non–linear relationships between each input variable and the output but also provides other advantages:

1. The general trend provides information about the overall impact of \(V_i\) on the response variable;
2. The variable importance is quantified by the overall vertical range of all the segments;
3. The interaction with other variables is described by the spread along the y–axis and thus variables with no interaction appear as single lines.
To avoid that the sensitivity analysis would focus only on the conditions sampled during the data collection, two artificial datasets with 1000 instances were generated with the function `runif` (R Core Team, 2015) and both the training and the artificial datasets, were used to perform the sensitivity analysis.

### V.2.6 Experimental application of the SDMs

Although 2D hydraulic models can be considered the general standard in micro–scale e–flow assessment (e.g. Muñoz–Mas et al., 2016) it has been demonstrated that the adequate implementation of 1D model can perform similarly, even over complex river morphologies such as braided river channels (Jowett & Duncan, 2012). The meso–scale model was based on one single value of each input variables per surveyed HMU thus the use of 2D models would have required the oversimplification of hydraulics and thus worthless modelling effort. Therefore, to balance the modelling effort and the requirements of each scale (the micro–scale and the meso–scale) and following previous studies (Costa et al., 2011), the hydraulics were simulated with RHYHABSIM (Clausen et al., 2004) in a Mijares River segment that overlapped only with the area surveyed for the micro–scale model (Elevation = 659 m a.s.l) (Fig. 25). RHYHABSIM is a one–dimensional hydraulic model based on cross–sections and the water surface elevation. The habitat simulation encompassed a river segment of 383.94 m length where 20 cross–sections were placed covering all the significant elements in the river channel (mean distance = 20.2 m). The cross–sections were marked so that they could be located for subsequent measurements. Detailed topography (mean distance between measurements = 0.58 m) was surveyed over the study site and both water surface elevation and water velocity along the cross–section were surveyed twice at two different flow rates (0.372 and 1.525 m³/s) (Fig. 26). Substrate composition and the presence of cover were assessed and an additional survey was carried out at a flow rate of 4.21 m³/s to ascertain the stability of the limits of the HMU. We simulated 50 evenly distributed flows and for each flow the habitat suitability was simulated using the optimal MLP Ensembles. In addition the Weighted Usable Area (WUA) (Bovee et al., 1998) was calculated and to assess the practical applicability of our models in e–flow assessment, a minimum e–flow was derived from the WUA–flow curves based on Spanish legislation. Specifically, the Spanish norm for hydrologic planning (MAGRAMA, 2008) establishes that the minimum e–flow should correspond with 50 % to 80 % of the maximum WUA. If no maximum could be observed in the WUA–Flow curve, the inflection point should determine the minimum e–flow.
Fig. 25. General view of the model, located in the Mijares River (Elevation = 659 m a.s.l.), used to simulate hydraulics at the working scales (micro-scale and meso-scale). The figure depicts the plain, lateral and isometric views of the topographic data (coloured dots), the wetted perimeter and the water surface elevation for one of the calibration flows (0.372 m$^3$/s). The data are depicted in meters and local coordinates.
V.3 RESULTS

V.3.1 Training results

Following the step–forward algorithm, the meso–scale model with the highest performance (Optimal MLP Ensemble) contained four variables: elevation, velocity, maximum depth and substrate (in order of selection) (Table 11). For both the micro–scale and the meso–scale model, the complexity of the MLPs involved in the Ensemble was low since only two nodes were considered for each MLP.

The GA–optimised MLP Ensemble (Optimal MLP Ensemble) outperformed the Best MLP Candidate and the ensemble aggregating the prediction of all candidates (Complete MLP Ensemble) (Table 11). For the micro–scale model, the predictions of the absent and present instances strongly overlapped, which revealed lower discriminant (classificatory) capability (Fig. 27 – Left). Nevertheless, the TSS was high (0.62) and the number and values predicted for the present instances were higher than the absent instances (i.e. sensitivity > specificity). The meso–scale model showed a stronger distinction between the predictions of absent and present instances and consequently it presented higher, almost perfect (TSS=0.92), discriminant capability (Fig. 27 – Right).
Fig. 27. Observations versus predictions for the micro–scale and the meso–scale models. Solid line depicts the regression line whereas the dashed line corresponds to the perfect discrimination.

The micro–scale model encompassed four MLPs whereas the meso–scale model involved seven MLPs. Consequently, four bag and four out–of–bag datasets were involved in the micro–scale MLP Ensemble, and seven by seven in the meso–scale counterpart. Cross–evaluation (i.e. the evaluation of every bag and out–of–bag dataset with every selected MLP Candidate) rendered similar distributions (i.e. they presented evident overlapping) of the TSS, thus it revealed low overfitting to the data. As a consequence both models were considered suitable for further analysis (Fig. 28).

Fig. 28. Distribution of the TSS (True Skill Statistic) rendered by each MLP over each bag (red) and out–of–bag (green) datasets involved in the Optimal MLP Ensembles (4 × 4 for the micro–scale model and 7 × 7 for the meso–scale). The distributions for both models,
with 16 and 79 data respectively, presented significant overlapping revealing low overfitting to the data.

**Table 11.** Summary of the performance TSS (True Skill Statistic) of developed MLP Ensembles at the micro–scale and the meso–scale (Optimal MLP Ensemble) and the counterparts without MLP selection (Complete MLP Ensemble) and considering no MLP aggregation (Best MLP Candidate).

<table>
<thead>
<tr>
<th>Scale</th>
<th># MLPs</th>
<th>TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro–scale</td>
<td>Best MLP Candidate</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Optimal MLP Ensemble</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Complete MLP Ensemble</td>
<td>2000</td>
</tr>
<tr>
<td>Meso–scale</td>
<td>Best MLP Candidate</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Optimal MLP Ensemble</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Complete MLP Ensemble</td>
<td>63</td>
</tr>
</tbody>
</table>

**V.3.2 Sensitivity analysis – Micro–scale model**

All the input variables presented interactions, as demonstrated by the spread over the ordinate axis (Fig. 29). Although differences in variable importance appeared small, depth was the most important variable. Depth demonstrated a quadratic relationship with a parabolic trend inflecting around 0.85 m, while velocity appeared negatively linearly related to redfin barbel presence. Cover had an asymptotic relationship with a remarkable increment from absence of cover (0) to presence of cover (1). Substrate was the least important variable and showed a positive trend. The sensitivity analysis of the artificial dataset showed a similar response than the one based on the original dataset.
Fig. 29. Micro–scale sensitivity analysis of the original dataset and the artificial dataset; black segments correspond to the original dataset and yellow ones to the artificial dataset. The variable importance is indicated in the upper right corner.

V.3.3 Sensitivity analysis – Meso–scale model

In the meso–scale model, also all included variables showed interactions (Fig. 30). Elevation showed a clear linear negative effect on fish presence and was the most important variable. Velocity showed an asymptotic trend with positive effects beyond 0.25 m/s. Substrate presented a small negative trend, in contrast to the micro–scale model. Maximum depth was the least important variable and only showed a slightly positive trend. Sensitivity analysis of the artificial dataset also matched the one based on the training dataset.
Fig. 30. Meso–scale sensitivity analysis of the original dataset and the artificial dataset, black segments correspond to the original dataset and yellow ones to the artificial dataset. The variable importance is indicated in the upper right corner. M. Depth = Maximum depth.

V.3.4 Habitat assessment

The WUA–Flow curves for of the micro–scale and the meso–scale models neither presented an asymptote nor a clear optimum (Fig. 31). The smooth.spline function in R (R Core Team, 2015) was used to remove curve irregularities and to calculate the inflection points of both curves. The inflection point of the micro–scale WUA–Flow curve appeared at 1.1 m$^3$/s whereas the meso–scale WUA–Flow curve inflection point occurred at 0.7 m$^3$/s.
Habitat conditions at the minimum simulated flow (0.2 m$^3$/s) and at the flows corresponding to the inflection points (1.1 and 0.7 m$^3$/s for the micro–scale and the meso–scale respectively) where then evaluated and visualised for spatially explicit inspection (Fig. 32). The habitat assessment at the micro–scale yielded suitable areas all along the hydraulic model for the minimum simulated flow and the inflection flow, although habitat suitability was significantly higher at the latter flow. Conversely, the meso–scale model assessed most of the low flow with low to middle suitability but a very little narrow rapid whereas practically all of the HMUs at the inflection flow were assessed with high or very high suitability.
Fig. 32. Assessment of the minimum simulated flow (0.2 m$^3$/s) and of the minimum environmental flow corresponding to 1.1 m$^3$/s for the micro–scale SDM and to 0.7 m$^3$/s for the meso–scale SDM. Black lines indicate the cross–sections.
V.4 DISCUSSION

V.4.1 General prospect

The approach employed by Wang and Alhamdoosh (2013) proved proficient to develop optimal MLP Ensembles since it provided small sized ensembles, and the Optimal MLP Ensembles outperformed the corresponding Best MLP Candidate and the Complete MLP Ensemble. The sensitivity analysis indicated that the effects of the artificial dataset matched the effects derived from the training datasets, emphasizing the reliability of the two SDMs. The outputs of both models also covered the whole feasible range (from 0 to 1), which facilitates the interpretation by inexperienced readers, but especially by stakeholders and managers. Furthermore, the output span allows its treatment as probabilistic–like outputs and its comparison with previous physical habitat modelling studies (Bovee et al., 1998) as being analogous to the outputs rendered by the more traditional univariate Habitat Suitability Curves (HSCs) (Muñoz–Mas et al., 2012). Altogether should encourage the use of MLP Ensembles in e–flow assessment studies (e.g. Muñoz–Mas et al., 2016).

V.4.2 Micro–scale model

The micro–scale model achieved a TSS similar to previous studies that used ensemble techniques at this scale (i.e. Random Forests) (Fukuda et al., 2013) and it showed a good trade–off between specificity and sensitivity, regardless of the prevalence of the original training dataset. However, it achieved the lowest TSS between the two models. Nevertheless, the results were considered satisfactory because it achieved high values of TSS in comparison with previous studies (Fukuda et al., 2013, Muñoz–Mas et al., 2014). MLP Ensembles are sensitive to prevalence like other techniques (Fukuda, 2013), but training the MLP Candidates with 0.5 prevalence datasets contributed to our objectives, which included maximising TSS, obtaining a sensitivity higher than the specificity and overlapping bag and out–of–bag TSS distributions. Therefore, we strongly recommend this approach in the development of micro–scale suitability models with MLP Ensembles.

The habitat suitability for the redfin barbel was optimal from 0.5 m to 1 m depth and where cover and medium–to–coarse substrate were present; on the other hand, flow velocity presented a general negative influence on fish presence, although positive effects were found all along the surveyed range even at the maximum surveyed velocity (2.13 m/s). The differences in variable importance were small, although we consider the ranking coherent with the prior knowledge about the species (Grossman and De Sostoa, 1994, Aparicio, 2002). The redfin barbel certainly should be categorized within the group of rheophilic barbels (Aparicio, 2002) because our results modelled high velocity as suitable. Interestingly, this result contrasts with previous HSCs for this species that suggest a more
limnophilic nature (Sostoa et al., 2005), although our results on depth agree with those of
the aforementioned study. These differences could have been influenced by
circumstances at the time when data were collected (Copp, 2008) or the kind of available
microhabitats (Ayllón et al., 2009). Unfortunately the reasons for such differences cannot
be revealed based on the available information. Literature disagrees on substrate
suitability, either suggesting a preference for algae and organic matter (Grossman and De
Sostoa, 1994) or the opposite, for coarse substrates (Sostoa et al., 2005). This
discrepancy probably originates from the fact that those previous studies have joined
some substrate and cover types rather than providing a clear preference for any of them.
Yet, substrate appeared to be of lesser importance within our micro–scale model. Finally,
the results agreed with previous studies demonstrating that the redfin barbel is a cover–
orientated species (Grossman and De Sostoa, 1994), based on the influence of cover on
fish presence. Consequently, the micro–scale model combines novel insights and
information from previous studies, and thus it improves the knowledge about redfin
barbel’s habitat preferences at the micro–scale.

V.4.3 Meso–scale model

The meso–scale model proved the competence of the GA–optimised MLP Ensembles
because it yielded a similar performance to previous studies that modelled the presence–
absence of freshwater fish species with Random Forests (Mouton et al., 2011, Vezza et
al., 2015). Some studies indicated that models based on multiple spatial scales usually
outperform single–scale analyses (Olden et al., 2006) mainly because environmental
variables rarely act at a single spatial scale (Boulangeat et al., 2012). The optimal meso–
scale model included not only three purely meso–scale variables (velocity, substrate and
maximum depth) but elevation as one meso–to–macro scale variable. The selected
variables significantly interacted, thus modifying the predicted effects positively or
negatively. However, despite the higher performance, the step–forward algorithm for
selecting variables may have been conditioned by the first selected variable (elevation),
which could lead the algorithm to get stuck in a local minimum (May et al., 2011). There
are examples of the use of GAs in variable selection procedures (May et al., 2011, Olden
et al., 2008) and in MLP candidates’ selection (Soares et al., 2013, Wang and
Alhamdoosh, 2013). Therefore, further research should be performed in order to inspect
the capabilities of GAs to simultaneously undertake the selection of the variables and the
MLP Candidates.

Elevation had a linear and negative effect on redfin barbel presence. This variable is
broadly accepted as a proximal predictor of water temperature (Elith and Leathwick, 2009).
In the Iberian Peninsula, cyprinids increase their dominance in fish assemblages in the
lower river segments (Santos et al., 2004) thus we considered such a pattern reliable.
However elevation may also partially explain the effect of slope and the fact that the upper
segments had very low flow and thus shallow HMUs. In contrast with the micro–scale model, velocity had purely a positive effect on redfin barbel’s presence, which agrees with its rheophilic classification (Aparicio, 2002). Nevertheless, the meso–scale model involved data from several years in contrast to the micro–scale study, which was performed in one single campaign, and thus we cannot discard that such discrepancy is not reflecting differences on the sampled running flows. In a previous study that involved the redfin barbel’s meso–scale dataset (Muñoz-Mas et al., 2015) the influence of the study site and sampling year, which can be univocally related to the running flow at the time of the sampling, was ruled out. However, flow significantly varied among years thus some of the uppermost river stretches became completely dried up during two sampling campaigns. The redfin barbel have demonstrated a strong site–fidelity, which is only contravened when the habitat suitability significantly degrades (e.g. by noticeable reductions on the available water depth) (Aparicio & De Sostoa, 1999). In such situation the redfin barbel undertakes the largest displacements in search of suitable habitats, typically moving towards extant lowland pools (Aparicio & De Sostoa, 1999). Consequently, the patterns observed for flow velocity could be depicting such type of migrations toward suitable habitats, which in our study area would be associated with higher flows and, given the slope or the study sites, also with higher flow velocity. Maximum depth surprisingly was the least important variable, in contrast to depth being the most important variable in the micro–scale model. However, the deepest surveyed HMUs were predicted to be most suitable for redfin barbell, which matches the aforementioned studies that considered the redfin barbel a pool dweller (Aparicio and De Sostoa, 1999). The meso–scale analysis for substrate indicated an inverse pattern compared to the micro–scale model. Such discrepancy in the response across scales has been reported previously (Gosselin et al., 2010). However it is remarkable that the meso–scale results are not necessarily different from the micro–scale model because the substrate index is calculated as the average value of the different types of substrate present. Therefore, the micro–scale model depicts the substrate observed at fish locations and due to the small sampled area around the fish, it is unlikely to encompass a heterogeneous group of substrates. Conversely, the meso–scale model depicts the mean value for the patches appearing at the sampled HMUs and they may encompass multiple types of substrate, as this patchy distribution is common at the meso–habitat scale (e.g. Inoue and Nunokawa, 2002). Substrate heterogeneity has previously been considered in the study of the redfin barbel (Aparicio & De Sostoa, 1999) and it certainly could clarify these apparent discrepancies, although given the accuracy of the developed model it was considered unnecessary. Nevertheless, the values of maximum depth in the meso–scale data corresponded to the median depth in the micro–scale model, and, in contrast to the micro–scale study, the meso–scale survey assessed several rivers. Therefore, comparison between them should be taken cautiously in broad terms; the meso–scale model might be considered a regional model focusing on broader scale aspects and the microscale model was more specific for the Mijares River.
V.4.4 Habitat assessment and implications of developed SDMs

Previous comparisons of micro- and meso-scale models also yielded differences in the assessed suitability (Parasiewicz and Walker, 2007). The sampling methods have been identified as potential sources of bias in the development of SDMs, since no method can ensure that all fish are detected (Mcmanamay et al., 2014). At the micro-scale, snorkelling has been proved preferable over electrofishing (Brosse et al., 2001); while every HMU was netted off before carrying out any survey at the meso-scale. Moreover, the use of presence-absence data rather than abundance data can be a cost-effective and accurate approach to monitor aquatic species (Joseph et al., 2006). Consequently, we considered the effect of the sampling method negligible and assumed the observed differences mainly occurred due to ecological and mathematical aspects. The micro-scale model could be assumed to represent ‘feeding’ or ‘holding a feeding position’ behaviour because it is assumed that such positions are the most energetically profitable (Rincón and Lobón-Cerviá, 1993) and hiding and/or disturbed fish observations were ruled out. However, the redfin barbel was observed several times in multi-species shoals mainly composed by cyprinids (e.g. Squalius valentinus; Doadrio y Carmona, 2006), with which the redfin barbel has shown evident affinity (Muñoz-Mas et al., 2015), and these observations were included in the ultimate dataset. These shoals were wandering nearby elements of cover (e.g. logs and woody debris) with some individuals foraging on the debris and substrate. There are no specific studies on the redfin barbel’s diet (e.g. Squalius valentinus; Doadrio y Carmona, 2006), although it has been suggested its preference for drifting invertebrates such as Chironomidae, Ephemeroptera and Trichoptera (Miranda et al., 2005). Other akin Iberian species (Gante et al., 2015) (i.e. Barbus and Lucioababus spp.) typically ingest a great variety of items without any clear preference (omnivory, eurifagy), even presenting significant proportions of the diet composed by vegetation (Magalhães, 1993; Collares-Pereira et al., 1996). In accordance with these generalist feeding behaviour we considered our choice adequate, although based on previous studies about the diel dynamics of habitat use of the European barbel (Barbus barbus; Linnaeus, 1758) (Baras & Nindaba, 1999) our dataset could be including a mixture of activities. Then, despite of a great uncertainty, these data could be depicting the so-called activity centre or the daily activity area, which can be roughly estimated as the HMU encompassing the residence and the feeding area (Baras, 1997).

Conversely, the meso-scale model is based on fish catches in HMUs where the fish develop any of the diel activities such as ‘feeding’ but, in this case, it surely encompassed also ‘hiding’ or ‘resting’ individuals as long as electrofishing does not allow the differentiation of the activity undertaken by fish captures. Therefore, in the meso-scale model, the training data considered all the fish in the HMU without any distinction of activity and assuming that any potential migration occurred in spring, before sampling (Aparicio and De Sostoa, 1999). Significant changes in habitat use have been demonstrated for the European barbel depending on the time of the day and the season
(Baras and Nindaba, 1999). Therefore, these two SDMs could represent different habitat needs.

Despite the potential ecological differences between models and the ranges of the sampled input variables, the patterns of the two WUA curves were similar. However, the micro–scale approach assessed the hydraulics in a very detailed way (every cell can be assessed differently) whereas the meso–scale approach presented a coarser resolution, and thus as soon as it considered an HMU suitable it added the entire HMU area to the WUA. Consequently, there is a difference in magnitude between both WUA flow rating curves, which would principally be caused by the discrepant resolution used in the habitat assessment (i.e. the mean size of the assessed cells were larger in the meso–scale model). The use of a density–based suitability index could provide more gradual information on species habitat selection in the meso–scale model (Fukuda et al., 2011) and may thus lead to more similar WUA–flow curves, although it should be corroborated by dedicated studies.

Compared to the traditional micro–scale evaluation the meso–scale approach permitted the survey of longer river segments, involving a wider range of habitat variables that could consider diverse fish behaviour at larger spatial scales (Vezza et al., 2012). Indeed, by sacrificing some detail it is possible to reveal larger spatial and temporal ecological patterns (Jewitt et al., 2001). Consequently, in this study a hydraulic model developed on a longer river segment may enable a more thorough and varied meso–scale assessment. However, this issue was already partially dealt with by simulating water depth and flow velocity for unmeasured discharge conditions (following RHYHABSIM) in contrast to some other approaches, which are based in a finite number of observations (MesoHABSIM; Vezza et al., 2012). Taking into account that no habitat time series analysis has been performed (Milhous et al., 1990), the differences in the magnitude of the WUA–Flow curves did not result in notable differences in the minimum legal e–flow (1.1 m$^3$/s and 0.7 m$^3$/s ). Nevertheless, the micro–scale models, which is the scale specified in the Spanish norm for hydrological planning for e–flow assessment (MAGRAMA, 2008), remained on the conservative side because it has determined a slightly higher e–flow.

Previous research already demonstrated that a lower e–flow is derived from a WUA–Flow curve that presented larger values of the WUA (Muñoz–Mas et al., 2012), which suggests that a revision of these legal specifications may be appropriate. The capability to simulate large numbers of flows has risen along the decade and thus the WUA–Flow curves nowadays present smooth transitions from flow to flow. In this case the inflection point is determined by a very little difference and could vary by reducing the number of simulated flows. Further, the Mijares River is subject to severe droughts, with one of the calibration flows being 0.372 m$^3$/s. The species naturally occurs in this river segment and its adaptation to droughts has been confirmed (Aparicio and De Sostoa, 1998, 1999). Therefore, it can be concluded that the minimum legal e–flow derived from both SDMs
would not eventually pose any threat to the species. Altogether, our results demonstrated that MLP Ensembles are a promising tool in the development of SDMs for freshwater fish species and proficient in e–flow assessment.
VI. GENERAL DISCUSSION

The present dissertation focused in the comprehensive analysis of the capabilities of some non-tested modelling techniques for environmental flow assessment and exploratory ecology. The tested techniques have been the Probabilistic Neural Networks (PNN) (Specht, 1990) and the Multi–Layer Perceptron (MLP) Ensembles (Hansen & Salamon, 1990). The analyses of the capabilities of these techniques were performed using exclusively native Iberian fish species. Specifically, the fish species were, brown trout (*Salmo trutta*; Linnaeus, 1758), bermejuela (*Achondrostoma arcassi*; Robalo, Almada, Levy & Doadrio, 2006) and redfin barbel (*Barbus haasi*; Mertens, 1925) as targets of the tested modelling routines. The analyses principally focused in the predictive capability, without loss of generalization, thus great interpretability (i.e. the capability to express the behaviour of the real system through the model in a comprehensible way) (Casillas et al., 2005) was also chased. The evaluation of the impact of low data prevalence (i.e. the ratio of presence data within the entire dataset) and excess of zeros in the training datasets was common across the analyses. Finally, the effect of the spatial scale in the habitat suitability models and the consequent differences in the assessed e–flow has been studied in the last chapter.

VI.1 PROBABILISTIC NEURAL NETWORKS – PNN

The optimal PNN, modelling the presence-absence of brown trout, was considered proficient because it achieved an acceptable value of the True Skill Statistic (TSS) and the sensitivity was higher than the specificity, as recommended by some other authors (Mouton et al., 2008). Furthermore, the PNN rendered a slightly higher value of the TSS in the evaluation site, which highlighted the validity of the model, and the accuracy was only marginally lower than other studies employing similar datasets of salmonids (i.e. *Salmo farioides*; Karaman, 1938) (Muñoz-Mas et al., 2016b). Accordingly, it would be expected further studies employing PNN to model the microhabitat suitability or to calculate e–flows.

However, in a follow up paper that involved the same dataset (i.e. the one for brown trout) to study the potential impact of climate change (Muñoz-Mas et al., 2016a), we achieved similar or superior accuracy with four out of five of the employed machine learning techniques (i.e. generalized additive models, MLP Ensembles, random forests, support vector machines and fuzzy rule base systems). Thus the TSS obtained with the PNN was solely comparable to the value achieved by the fuzzy rule base systems (i.e. Takagi and Sugeno, 1985) whereas the remaining techniques achieved higher values. Interestingly, the highest value was achieved by Support Vector Machines (SVMs) (Vapnik, 1995) an approach that may resemble PNN, especially when the SVMs are developed employing radial basis functions.
The basic idea with SVMs is constructing a discriminant (classificatory) hyperplane with the maximum discriminant margin (Huang & Wang, 2006). The radial basis functions are the most common employed functions to do so (Howley & Madden, 2005; Hoang et al., 2010); therefore, any derived graphical sensitive analyses may resemble the one for the PNN (i.e. the contour plot depicted in Fig. 7 and Fig. 8, Chapter II). Furthermore the training of the support vector machines was significantly faster than the lapse for the PNN, which may tip the balance towards the use of SVMs. However, researchers soon developed some methods to improve PNN (Specht, 1992). Between them, those based on Berthold and Diamond’s (1998), which have some similarities with SVMs training, are among the most promising (Li & Ma, 2008; Sunay et al., 2009; Qader & Adda, 2014). The term “support vectors” refers to the training patterns used to define the lower and the upper margins of the discriminant hyperplane, which are selected during models’ training. In contrast, the approaches based on Berthold and Diamond’s rely in the selection of some prototypes (i.e. a subset of training patterns) exhibiting noticeable similarity with the former technique. As a consequence of the pattern subsampling, the training process is significantly accelerated and the effect of outliers is to be reduced (Li & Ma, 2008; Sunay et al., 2009; Qader & Adda, 2014). Although scientific literature nowadays covers several comparisons between SVMs and PNN where SVMs outperformed PNN they employed the standard implementation of PNN (Muniz et al., 2010; Modaresi & Araghinejad, 2014); thus the aforementioned enhanced algorithms should be the subject of further research before to indubitably advocate for one or another.

Despite the limitations regarding the output range described above, PNN performance kept mostly constant for different prevalence of the training datasets. SVMs that employ Platt’s (2000) approach to calculate probabilities demonstrated unable to adequately deal with such dataset characteristics because the probabilistic output of SVMs was sufficiently affected by class overlapping and low prevalence to ban its predictions from ulterior analyses (Muñoz-Mas et al., 2016a). The interaction between dataset nature and the optimal modelling algorithm has been evidenced in a number of studies (e.g. Eugster et al., 2014) and thus it would be expected the trimmed output range observed for PNN to be reduced for databases with lesser overlapping between categories. Therefore, studies with coarser resolutions (i.e. meso–scale and broader) and better balances between classed should not suffer from such drawbacks (Platts et al., 2008).

Regarding the target species, the modelled habitat suitability for the large brown trout obtained with the PNN was similar to previous studies where the preference for relatively deep microhabitats (i.e. pools) with slow flow and medium-to-coarse substrate was reported (Armstrong et al., 2003; Ayllón et al., 2010; Heggenes, 1996 and Moyle, 2002). Therefore, from an ecological viewpoint PNN, and the kin SVM (Muñoz-Mas et al., 2016a), rendered trustworthy results, especially because both of them predicted a decrement of the suitability for the higher flow velocity (Muñoz-Mas et al., 2016a). As long as several authors have suggested a pronounced decrease in habitat suitability beyond a water
velocity of ca. 0.5 m/s (Jowett & Davey, 2007; Ayllón et al., 2010) and considering that such decrease is certainly reasonable due to increasing energy costs to stand higher velocities (Rincón & Lobón-Cerviá, 1993), the habitat suitability modelled with the PNN and the SVM would be plausible. In contrast, the remaining techniques (i.e. generalized additive models, MLP Ensembles and random forests) suggested high habitat suitability beyond 0.5 m/s. The use of several modelling techniques (ensemble modelling) typically renders predictions ecologically more reliable than the ones obtained using a single technique (Muñoz-Mas et al., 2016a). Therefore, it can be inferred that the inclusion of the predictions obtained with the PNN is likely to improve the ultimate predictions of a given ensemble. PNN have proven to be a useful tool able to render ecologically sound models and thus further applications either in ecological modelling or e–flow assessment, would be desirable.

VI.2 MULTI-LAYER PERCEPTRON ENSEMBLES – MLP ENSEMBLES

Ensemble modelling typically becomes in an improvement in models’ accuracy either employing ensembles based on multiple techniques (e.g. BIOMOD; Thuiller et al., 2009) or based in a single technique (e.g. random forests; Breiman, 2001). Therefore, the better results obtained with MLP Ensembles were expected. The main theoretical evidence behind ensemble methods is the bias–variance–covariance decomposition, which offers theoretical justification for the improved performance of an ensemble over its constituent base predictors (Ren et al., 2016). Although it is actually subject to scientific debate (Didaci et al., 2013), the main school of thought assumes that the key issue in ensemble methods is diversity, which includes data diversity, parameter diversity or structural diversity among others (Ren et al., 2016); and therefore, the approach followed should be considered embedded within sound theoretic background.

Although MLP ensembles have been profusely used in many scientific research areas such as running flow forecasting (Abrahart et al., 2012) or lung cancer diagnosis (Zhou et al., 2002a) they have received little attention in ecology. Consequently, although the number of published papers involving Artificial Neural Networks showed a steady increment during the last decade (Fukuda & De Baets, 2012), they have been mainly restricted to training single MLPs. As described in the introduction, habitat suitability and species distribution modelling with ensemble approaches have principally employed tree–based techniques such as random forests (e.g. Vezza et al., 2015), although boosted regression trees are not far behind (e.g. Elith et al., 2008). Indeed, these techniques involve noteworthy capabilities such as their ability to handle strongly nonlinear relationships with high order interactions and different variable types (e.g. numerical or nominal) (Olden et al., 2008; Grubinger et al., 2014), which may tip the balance towards them. However tree–based approaches typically separate the feature space by axis–
parallel hyperplanes, which may be sub-optimal (Truong, 2009) and ecologically unreliable because they render irregular stair-like decision surfaces (Menze et al., 2011). Furthermore, with some of these techniques we observed erratic discriminant surfaces (Muñoz-Mas et al., 2016a) that disagree with the precepts of the ecological gradient theory (Austin, 2007). Consequently, from our personal viewpoint, these techniques should not automatically relegate other alternatives such as the MLP Ensembles (Muñoz-Mas et al., 2016). MLP Ensembles presented satisfactory accuracy and smooth partial dependence plots (Fig. 22 and Fig. 23, Chapter IV), which fit better the aforementioned ecological gradient theory, and they demonstrated great versatility dealing with classificatory and regression problems. In addition, they showed noticeable aptitude to deal with low data prevalence and excess of zeros. Therefore, despite the inconclusive results about the best overproduce–and–choose approach (i.e. Forward or GA-based), we consider MLP Ensemble an appealing technique.

In the past, the training of MLPs proved to be slower compared to other approaches (Olden et al., 2008). However, despite Moore’s law is showing signs of slowing (Yeric, 2015), computers are becoming faster and faster (Schmidhuber, 2015). Calculus parallelization (employing multiple CPUs) is becoming widespread (e.g. Bryan, 2013) and, although at slower pace than desired (Valle & Berdanier, 2012), programming skills of ecologists are increasing significantly. Nowadays there are plenty of software packages of free distribution allowing the implementation of MLP Ensembles such as the one employed here (monmlp; Cannon, 2012). Consequently, we expected the present dissertation and the accompanying publications to foster the use of MLP ensembles in ecological studies.

Nevertheless, neural network topologies are recently increasing its complexity with networks involving hundreds of millions of weights, and billions of connections between units (i.e. deep neural networks) (Lecun et al., 2015). Training such large networks lasted weeks few years ago but progress in hardware, software and algorithm parallelization have reduced training times to a few hours (Lecun et al., 2015). These new topologies demonstrated unprecedented capacity for pattern recognition (Schmidhuber, 2015) whereas dropout has become a successful yet simple approach to sustain good generalization (Srivastava et al., 2014). Thus, this set of improvements is likely to help ecologists to incrementally extract knowledge from observational datasets. Big data scientist have already developed ensembles of deep neural networks (Wenhao Huang et al., 2015; Ren et al., 2016). Consequently, testing the capabilities of these novel approaches shall be the subject of further research and we hope the publications associated with the dissertation may additionally turn the focus towards these more complex approaches.
VI.3 BERMEJUELA

Translocated species have been traditionally viewed acquiescently, although there is evidence that they can produce negative impacts likewise non–natives (Oscoz et al., 2006; Alcaraz et al., 2014). Consequently, in accordance with Gozlan et al. (2010), it is crucial to improve our abilities to forecast the risks resulting from translocations. The Cabriel River harbours the most important populations, in terms of presence and fish density of the Jucar nase (Parachondrostoma arrigonis; Robalo, Almada, Levy & Doadrio, 2006) (Alcaraz et al., 2014), the Iberian fish species more susceptible of extinction (Doadrio, 2002b). The Jucar nase nowadays cohabits with the apparently translocated bermejuela (sampled during the development of Olaya-Marín et al. 2012), which showed great similarity with the habitat selection of the Jucar nase by selecting microhabitats with slow flow velocity, fine substrate but shallow to medium depth (Fig. 13). Accordingly, in a follow-up study (Muñoz-Mas et al., 2016 – under review), it has been concluded that this species may poses the largest threat for the endangered Jucar nase. Fortunately, very few specimens of bermejuela were found in the Cabriel River (Olaya-Marín et al., 2012), in areas that they share with the brown trout, suggesting that either the propagule pressure (sensu Gozlan et al. 2010) or the recruitment success were low. Therefore, bermejuela is actually not considered an invasive fish species. However, its presence could be masked due to its hybridization with the Jucar nase since there is previous evidence of hybridization between bermejuela and the Iberian nase (i.e. Pseudochondrostoma polylepis × Achondrostoma arcassi) (Collares-Pereira & Coelho, 1983). Altogether, the habitat suitability modelling and the associated physical habitat simulation approach demonstrated to be a valuable and versatile tool dealing with different exigencies either in risk or e-flow assessment.

VI.4 REDFIN BARBEL

The Eastern Mediterranean region of the Iberian peninsula is likely to be characterized by a continued decrease in water yield (Chirivella Osma et al., 2014; Salmoral et al., 2015), which may create a bottleneck for species survival. This general concern has been confirmed in dedicated studies employing the last set of climate change scenarios (IPCC, 2014), the so-called Representative Concentration Pathways (RCPs) scenarios (Muñoz-Mas et al., 2016a). The rear edge of species distribution ranges have proven to be of enormous importance for the survival and evolution of biota (Hampe & Petit, 2005). Therefore the coupled studies on the redfin barbel rendered valuable information about potential impacts on the species at the southern edge of its historical distribution area (Aparicio, 2002; Perea et al., 2011). The habitat preferences confirmed the redfin barbel selection of pool-type habitats, although velocity had also a positive effect on its presence (Fig. 29 and Fig. 30, Chapter IV). In accordance with these general habitat preferences the
reduction in the magnitude of the running flow is likely to have synergistic impacts eventually displacing the species towards benign locations. Redfin barbel has several adaptations to Mediterranean flow regimes being able to inhabit intermittent rivers (Aparicio, 2002). Therefore, females have multiple spawning (releasing two to five egg batches) whereas the timing and length of the breeding season are related to high flow periods (Aparicio & De Sostoa, 1998). However, the frequency and recurrence of peak temperatures and droughts are likely to increase by the end of the century (Santiago et al., 2015) and then, the adaptive traits of the species could be insufficient for the species survival. Furthermore, the observed correlation between babel density and cyprinid density suggest that any negative impact to the species would be extendable to most of the inhabiting ichthyofauna. These conclusions highlight the interest of the herein presented results on the habitat preferences and ecology of the redfin barbel, especially regarding interspecific relationships.

VI.5 MICRO–SCALE AND MESO–SCALE IN E–FLOW ASSESSMENT

The comparison between the micro–scale and meso–scale in e–flow assessment proved that none of the working spatial–scales is applicable in every case study. Some studies achieved better correlation between habitat suitability and fish densities employing meso–scale approaches (e.g. Parasiewicz and Walker, 2007) whereas our results suggested that better performance not necessarily implies higher and more ecologically friendly e–flows. A large suite of elements and conditionals converge in e–flow assessment affecting each of its components such as data availability (Auerbach et al., 2015) or site specific constraints (Vezza et al., 2015). Despite some modelling techniques may render more accurate yet reliable models, each one has its merits and demerits thus increasing uncertainty on the ultimate predictions (Lin et al., 2015). Model evaluation (sensu Guisan and Zimmermann, 2000) is obviously a fundamental task that may tip the balance towards the most accurate approach (e.g. Parasiewicz and Walker, 2007). Then, the evaluation of model’s generalization and transferability is a fundamental task that comes recurrently out in scientific literature (Vaughan & Ormerod, 2005; Wenger & Olden, 2012; Huang & Frimpong, 2016).

However, the target species can be occasionally extirpated and transferability evaluation must rely on traditional cross-validation to disfavour over–fitted models (Wenger & Olden, 2012). In such a situation the approaches tested in this document may represent a step forward towards improved results. Furthermore, our results highlighted the importance of the legal requirements thus they should not be underrated because they can tip the choice towards option that would be most likely ruled out. Therefore we subscribe that none of the existing methods can be neglected and its choice may depend on a balance between legal requirements, ecological goals, economic costs and scientific uncertainties (Poff et al.,
Therefore, low intensity hydraulic habitat assessment methods may be applicable to generalise the habitat assessment over large river segments (e.g. Lamouroux and Souchon, 2002) whereas MesoHABSIM (Parasiewicz, 2007) and physical habitat methods (Bovee et al., 1998) should be employed in increasing order to gain resolution in e–flow studies. Finally, all of them should feed broader scale methodologies such as the ELOHA (Ecological Limits of Hydrologic Alteration) framework to perform holistic evaluation of the ultimate effect of changing running flows on freshwater ecosystems (Poff et al., 2010). The present dissertation rendered valuable methodological input yet relevant ecological knowledge that may prioritize sound monitoring protocols eventually guiding ecologically–friendly management actions.
Chapter VII

CONCLUSIONS & FURTHER RESEARCH
VII. CONCLUSIONS & FURTHER RESEARCH

VII.1 CONCLUSIONS

The present dissertation generated conclusions that were split in two different sections. The first one addressed to describe the advantages of the tested techniques and the second one referred to the insights of fish ecology and habitat selection and the consequences in the e–flows eventually calculated.

VII.1.1 Conclusions on modelling techniques

In general, Probabilistic Neural Networks (PNN) have proven to be a useful tool in modelling habitat suitability, especially considering the use of raw datasets. Results on this approach presented two major issues, i) prevalence did not significantly affect its performance (it held constant regardless of the prevalence) and ii) PNN may present limitations regarding the output range. Nevertheless, the performance stability prevails over output trimming thus, it is expected that PNN will play a relevant role in microhabitat suitability modelling and e–flow assessment, although its popularity will certainly depend on the availability of user–friendly software packages.

Broadly, the Multi–Layer Perceptron (MLP) Ensemble presented better performance than the PNN. In regard to the MLP Ensemble for the Bermejuela, it was considered to be proficient because it achieved high values of the performance criteria. Furthermore, the active selection of the MLPs included in the Ensemble outperformed the results including the whole set, demonstrating the virtues of the over–produce–and–choose approach. The Forward selection of MLPs demonstrated to be able to marginally outperform the more complex approach based on Genetic Algorithms.

The MLP Ensembles for the redfin barbel density also corroborated the value of the over–produce–and–choose approach and the uncertainty related with models aggregation released valuable information about the uncertainty inherent to models assembling. Therefore, in both models, the largest uncertainty tended to appear in the regions of the input variables distribution that were poorly represented in the training database. In accordance to their high predictive capability and its ability to deal with model uncertainty, the MLP Ensemble paradigm was considered a promising tool in exploratory ecology.

The MLP Ensembles developed following the Wang and Alhamdoosh approach provided accurate small–sized Ensembles thus, despite the lack of comparison with the approaches employed for the bermejuela and redfin barbel density, it was deemed a worthwhile methodology. The mesoscale model stood out because it presented almost a perfect accuracy (TSS = 0.93) with only four variables.
The legal norm in Spain stated that studies on e–flow assessment based on the physical habitat simulation approach must be performed at the microscale, which eventually predicted a slightly higher e–flow. Therefore, from the legal viewpoint, this scale can be considered potentially a choice at least equally adequate or better than the meso–scale one in the context of the Spanish water planning.

**VII.1.2 Conclusions on fish ecology**

The results on habitat suitability for the large brown trout did not differ significantly from the previous knowledge about the species. Therefore, our results agreed with previous studies where large brown trout has been reported to prefer relatively deep pools with slow flow and medium–to–coarse substrate.

The microhabitat preferences of the Bermejuela had never been studied before thus the study provided valuable insight on the species. It was concluded that the Bermejuela can be classified as a shelter–orientated limnophilic species, because cover was the most important variable. The other relevant variables indicated the maximum suitability for slow and deep microhabitats, related with the local deposition of silt in the riverbed.

The two MLP Ensembles for the redfin barbel density provided great insight on the species ecology. The redfin barbel preferred middle–to–upper river segments, but not the higher and steeper reaches. The importance of depth confirmed that redfin barbel prefer pool–type habitats. The redfin barbel, the European eel and the cyprinid species present in the study sites had similar habitat requirements.

The comparison of the habitat preferences at the micro–scale and the meso–scale confirmed previous studies and complemented existing knowledge on the habitat preferences of redfin barbel. The micro–scale MLP Ensemble showed high suitability of relatively deep areas with coarse substrate and corroborating the cover–orientated and rheophilic nature of the redfin barbel. The meso–scale model highlighted the advantages of using cross–scale variables, since elevation (a macro–scale variable) was selected in the optimal model suggesting that the redfin babel inhabits preferably midland river segments. The redfin barbel selects deep areas; at this scale the MLP Ensemble partially contradicted the micro–scale counterpart because velocity had a clearer positive effect on habitat suitability, which was associated with the current flow. Finally, the redfin barbel showed a preference for fine substrate.
VII.2 FURTHER RESEARCH

Researchers have proposed several methods to improve PNN (Specht, 1992). Those based on Berthold and Diamond’s (1998), which rely on data clustering or subsetting, are among the most promising (Li & Ma, 2008; Sunay et al., 2009; Qader & Adda, 2014). However, in any of them the smoothing parameter is constant across the input space. The Bayesian rule in the pattern layer estimates the conditional probability of each class given an input pattern without considering any probable local densities or heterogeneity in the training data (Ahmadlou & Adeli, 2010). Recently, an Enhanced PNN (EPNN) was presented using local decision circles to overcome the aforementioned shortcoming and improve its robustness to noise in the data (Ahmadlou & Adeli, 2010).

For the foregoing one research topic will be testing the capabilities of several of these approaches, principally the most promising one based on data sub-setting and clustering and the one based on local decision circles. The capabilities of these improved PNN will be compared with benchmark approaches to develop and train Support Vector Machines (SVMs) (Vapnik, 1995) and the dedicated R code will be released to facilitate modellers and ecologists to make use of these novel approaches. This study is actually under development.

Models ensembles, including MLP Ensembles, achieved noticeable accuracy in the follow up paper addressed to study the potential impact of climate change in the suitable habitat for brown trout (Muñoz-Mas et al., 2016a). In that paper three out of five of the assembled techniques are uncommon in the development of model ensembles (i.e. MLP Ensembles, support vector machines and fuzzy rule base systems). In that case, ensemble modelling rendered predictions ecologically more reliable than the ones obtained using a single technique (Muñoz-Mas et al., 2016a). However, SVMs were ruled out from the analysis based on probabilistic outputs (employing the Weighted Usable Area) because they rendered trimmed outputs that did not allow comparison. Therefore, another research topic will rely in the study of the benefits or disadvantages of including additional techniques in standard models ensembles. These tested modelling techniques could be then included in standard models assembling platforms such as BIOMOD (Thuiller et al., 2009).

The use of deep neural networks for pattern recognition is gaining momentum thus their paramount capabilities has caused most major technology companies (e.g., Google, Facebook or Microsoft) to initiate research and development projects related with deep nets (Lecun et al., 2015). However, its popularization is not exempt of some criticism since there is empirical demonstration that shallow feed-forward nets can learn the complex functions previously learned by deep nets and achieve accuracies previously only achievable with deep models (Ba & Caruana, 2014). In this regard another research topic will be testing the usefulness of deep architectures in ecological studies.
Finally, the relationship between the redfin barbel and the accompanying cyprinids presented in chapter IV corroborated other studies; for instance, the one of Vezza et al. (2015) performed in the Jucar River basin. These results suggest that ecological models and e–flow assessment could be done employing guilds instead of single species to provide global indicators about the effects of different scenarios on the ichthyofauna present. This approach based on guilds has demonstrated useful in other studies (e.g. Ferreira et al., 2007), although the strength of species association and hence the validity of derived models requires dedicated studies (e.g. Vadas and Orth, 2001). Therefore, the last research topic will focus in testing the strength and validity of ecological models based on guilds. The last proposal is under development at the time of finishing the present dissertation.
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Chapter VIII

135

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