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DOCTORAL THESIS:

ECOHYDRAULIC STUDY OF IBERIAN FISH MIGRATION

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ABSTRACT

Many fish species need to move between different habitats in order to complete their life cycles. Migration respond to both internal and external factors, with environmental variables, such as river flow and thermal regimes, acting as main stimuli for the onset and maintenance of migratory behavior in freshwater fish. River fragmentation and anthropogenic alterations on these variables (e.g. river regulation) may have strong impacts on fish populations, which could be aggravated by future projections of climate change and increasing water demand. Thus, to assess these impacts and define mitigation measures, it is vital to understand fish movement patterns and the environmental variables affecting them.

Native Iberian fish fauna presents the greatest European percentage of endemism and it is characterized by a low number of families, with most species belonging to Cyprinidae family. The most representative species from this family are barbels and nases, as for example the Iberian barbel and the Northern straight-mouth nase. However, there is still scarce information regarding the ecological requirements of these endemic species during their upstream migration. Other important family in the Iberian Peninsula, in terms of recreational fishing and ecological traits, is the Salmonidae, being the brown trout one of the most representative salmonids worldwide. Despite this species has been deeply studied, most available research has been focused on anadromous populations and studies in the south of its natural distribution are still scarce.

The present thesis aims to cover some of these research gaps regarding upstream migration patterns of Iberian barbel, Northern straight-mouth nase and brown trout (both potamodromous and anadromous ecotypes), as well as to determine the environmental conditions which trigger these movements. In addition, this information is used to evaluate the effect of human impacts and to assess the effect of mitigation measures on these species.

To achieve this, long-term movement data of these three species in four different locations of the Iberian Peninsula (Porma River (León), Tormes River (Salamanca), Marín River (Navarra) and Bidasoa River (Navarra)) were gathered, analyzed and discussed. In all the studied cases, data were collected in fishways, since they are mandatory points of passage during upstream migration. Survival analysis techniques were used to study median migration dates and patterns. Random forest regression was used to evaluate the influence of environmental variables on the number of captures, to develop models able to predict when upstream movements were more likely to occur, to evaluate different scenarios of river discharge and water temperature, and to assess the possible effects of management and restoration actions.

The long-term monitoring has demonstrated to be an essential tool, not only for identify population changes, migration patterns and cues, but also to evaluate the overall effect of changes in the parameters involved in the migration and to assess the effects of mitigation measures.

Continuous monitoring studies are a must to define adaptive strategies and reliable management plans to ensure the conservation of Iberian freshwater fish.

RESUMEN

Muchas especies piscícolas realizan desplazamientos entre diferentes hábitats para completar sus ciclos vitales. Estos movimientos responden tanto a factores internos como externos, siendo las variables ambientales como, por ejemplo, los regímenes de temperatura y caudal de los ríos, los principales estímulos para el inicio y mantenimiento del comportamiento migratorio en peces de agua dulce. La fragmentación de los ríos y las alteraciones antrópicas de esos regímenes (por ejemplo, la regulación de los ríos), pueden tener graves consecuencias en las poblaciones piscícolas. Estos impactos pueden verse agravados por los escenarios futuros de cambio climático y aumento de la demanda de los recursos hídricos. Por lo tanto, es vital entender los patrones de movimiento de los peces y las variables ambientales que afectan dichos movimientos.

La fauna autóctona de la Península Ibérica presenta el mayor porcentaje de endemismos de Europa. Así mismo, está caracterizada por un número pequeño de familias, perteneciendo la mayoría a la familia Cyprinidae. Las especies más representativas de esta familia son los barbos y las bogas, como por ejemplo el barbo común y la boga del Duero. Sin embargo, existe poca información sobre sus requerimientos ecológicos durante sus migraciones ascendentes. Otra familia importante de la Península Ibérica, tanto en términos ecológicos como por su importancia en la pesca deportiva, es la familia Salmonidae, siendo la trucha común uno de los salmónidos más representativos a nivel mundial. A pesar de que esta especie ha sido ampliamente estudiada, la mayor parte de estos estudios se han centrado en poblaciones anádromas, y los estudios en la parte sur de su distribución natural son todavía escasos.

Los objetivos de la presente tesis tratan de rellenar estas lagunas de información sobre los patrones de migración ascendente del barbo común, la boga del Duero y la trucha común (tanto el ecotipo potamódromo como el anádromo), así como de determinar las condiciones ambientales que influyen en dichos movimientos. Además, esta información será utilizada para evaluar los efectos de algunos impactos antrópicos y para valorar los efectos de ciertas medidas de mitigación de impactos.

Para lograr estos objetivos, se ha llevado a cabo un seguimiento a largo plazo de estas tres especies en cuatro localizaciones diferentes de la Península Ibérica (río Porma (León), río Tormes (Salamanca), río Marín (Navarra) y río Bidasoa (Navarra)). En todos los casos de estudio se utilizaron pasos para peces como sitios de monitorización, ya que éstos son puntos obligatorios de paso durante las migraciones ascendentes. Se utilizaron técnicas estadísticas de análisis de supervivencia para el estudio de las fechas y patrones migratorios, y técnicas de regresión de *Random Forest* tanto para evaluar la influencia de las variables ambientales en las capturas, como para desarrollar modelos capaces de predecir cuándo se van a producir esos movimientos, así como para evaluar diferentes escenarios de caudales y temperaturas, y para valorar los posibles

efectos de medidas de gestión y restauración.

El seguimiento de la migración a largo plazo ha demostrado ser una herramienta esencial, no solo para identificar cambios poblacionales, patrones de migración y efectos de las variables ambientales, si no para evaluar el efecto de los cambios producidos en los parámetros que influyen en las migraciones, así como para valorar los efectos de las medidas de mitigación de impactos. Los estudios de seguimiento continuo son imprescindibles para poder definir estrategias de manejo adaptativo y planes de gestión fiables, que logren así asegurar la conservación de los peces ibéricos.

LIST OF ORIGINAL ARTICLES

This thesis has been developed under the category “thesis by compendium of publications”, that, as stated by university regulations (BOCYL 15/06/2016 and subsequent modifications), it must include at least three scientific articles prepared by the doctoral student and accepted or published in any kind of important media, according to the criteria of the ANECA (the National Agency for Quality Assessment and Accreditation of Spain) for the area of knowledge in which the thesis is presented. In addition, these articles must have been published within the period in which the student has been enrolled in the doctoral program, that in this case corresponds to the period 2015-2020.

The work of this thesis is based on the following five original articles:

(1) García-Vega, A., Sanz-Ronda, F.J. & Fuentes-Pérez, J.F. 2017. Seasonal and daily upstream movements of brown trout *Salmo trutta* in an Iberian regulated river. *Knowledge & Management of Aquatic Ecosystems*, 418: 9.
DOI: https://doi.org/10.1051/kmae/2016041.
Journal metrics (Journal Citation Reports, JCR) in 2017: Impact factor = 1.525. Rank: 24/51 (Q2) in Fisheries; 54/106 (Q3) in Marine & Freshwater biology.

(2) García-Vega, A., Sanz-Ronda, F.J., Fernandes Celestino, L., Makrakis, S. & Leunda, P.M. 2018. Potamodromous brown trout movements in the North of the Iberian Peninsula: Modelling past, present and future based on continuous fishway monitoring. *Science of the Total Environment*, 640–641: 1521–1536.
DOI: https://doi.org/10.1016/j.scitotenv.2018.05.339
Journal metrics (JCR) in 2018: Impact factor = 5.589. Rank: 27/251 (Q1) in Environmental Sciences.

(3) García-Vega, A., Fuentes-Pérez, J.F., Bravo-Córdoba, F.J., Ruiz-Legazpi, J., Valbuena-Castro, J. & Sanz-Ronda, F.J. 2020. Reproductive movements of potamodromous cyprinids in the Iberian Peninsula: when environmental variability meets semipermeable barriers. [In review]

(4) García-Vega, A., Fuentes-Pérez, J.F., Leunda, P.M., Ardaiz, J. & Sanz-Ronda, F.J. 2020. Upstream migration of anadromous and potamodromous brown trout: patterns and triggers in a 25-year overview. [In review]

(5) García-Vega, A., Leunda, P.M., Ardaiz, J. & Sanz-Ronda, F.J. 2020. Effect of restoration measures in Atlantic rivers: A 25-year overview of sea and riverine brown trout populations in the River Bidasoa. Fisheries Management and Ecology, 27: 580-590.
DOI: https://doi.org/10.1111/fme.12458
Journal metrics (JCR) in 2019 (last update): Impact factor = 1.733. Rank: 22/53 (Q2) in Fisheries.

OTHER RELATED JOURNAL PUBLICATIONS

List of journal publications that this thesis is not directly based on but where the author of the thesis has contributed during the dissertation period (2015-2020) as member of the research group “Group of Applied Ecohydraulics” (GEA-Ecohidráulica, www.gea-ecohidraulica.org) of the University of Valladolid:

- Fuentes-Pérez, J.F., Sanz-Ronda, F.J., Martínez de Azagra-Paredes, A. & García-Vega, A. 2016. Non-uniform hydraulic behavior of pool-weir fishways: A tool to optimize its design and performance. *Ecological Engineering*, 86: 5–12.
  DOI: https://doi.org/10.1016/j.ecoleng.2015.10.021
- Fuentes-Pérez, J.F., García-Vega, A., Sanz-Ronda, F.J. & Martínez de Azagra-Paredes, A. 2017. Villemonte’s approach: validation of a general method for modeling uniform and non-uniform performance in stepped fishways. *Knowledge and Management of Aquatic Ecosystems*, 418: 23.
  DOI: https://doi.org/10.1051/kmae/2017013
- Fuentes-Pérez, J.F., Silva, A.T., Tuhtan, J.A., García-Vega, A., Carbonell-Baeza, R., Musall, M., Kruusmaa, M., 2018. 3D modelling of non-uniform and turbulent flow in vertical slot fishways. *Environmental Modeling and Software*, 99: 156-169.
  DOI: https://doi.org/10.1016/j.envsoft.2017.09.011
- Sanz-Ronda, F.J., Bravo-Córdoba, F.J., Sánchez-Pérez, A., García-Vega, A., Valbuena-Castro, J., Fernandes-Celestino, L., Torralva, M. & Oliva-Paterna, F.J. 2019. Passage Performance of Technical Pool-Type Fishways for Potamodromous Cyprinids: Novel Experiences in Semiarid Environments. *Water*, 11: 2362.
  DOI: https://doi.org/10.3390/w11112362
- Valbuena-Castro, J., Fuentes-Pérez, J.F., García-Vega, A., Bravo-Córdoba, F.J., Ruiz-Legazpi, J., Martínez de Azagra-Paredes, A. & Sanz-Ronda, F.J. 2020. Coarse fishway assessment to prioritize retrofitting efforts: a case study in the Duero River basin. *Ecological Engineering*, 155: 105946.
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A complete list of publications including previous articles to the dissertation period, conference communications, book chapters, etc. can be consulted in the ORCID (https://orcid.org/0000-0002-3765-9007) and ResearchGate profiles of the author (www.researchgate.net/profile/Ana_Garcia-Vega).

THESIS OUTLINE

The thesis consists of five original articles. All of them are focused on the study of upstream migration patterns of three of the most important and representative Iberian fish species: brown trout (*Salmo trutta*), Iberian barbel (*Luciobarbus bogaei*) and Northern straight-mouth nase (*Pseudochondrostoma duriense*), which is the main objective of this thesis.

Potamodromous brown trout in upper basin river sections	Iberian barbel and Northern straight-mouth nase	Potamodromous & anadromous brown trout in lower basin river sections
Chapter I Chapter II	Chapter III	Chapter IV Chapter V

Figure 1. Relation between studied species and chapters.

In order to do achieve this main objective, several steps were followed, that correspond to the specific sub-objectives (see Objectives chapter). First, the periods with major amount of fish movements were identified. With this, differences by species (or ecotypes) and/or rivers could be identified. Secondly, the most significant environmental factors affecting these movements (e.g. photoperiod, water temperature, river discharge, etc.) were studied, as variations on these factors could alter migration ~~dates~~ and patterns. After that, possible influence of human activities in flow and thermal river regimes was also considered. For this, the effect of river regulation for irrigation and water abstraction for hydropower was studied. In addition, possible alterations on the migration patterns as consequence of future projections of climate change and increasing water demand was also considered. Finally, mitigation measures of human impacts were assessed. Specifically effects of different management (fishing closures, size limits and quotas, fish stoking) and connectivity measures (fishways and weir removals) were analyzed.

	Peak movements	Environmental variables	River regulation	Climate change	Restoration measures
Chapter I	●	●	●		
Chapter II	●	●	●	●	
Chapter III	●	●	●		
Chapter IV	●	●			
Chapter V					●

Figure 2. Relation between chapters and specific objectives.

Fish migration

Migration is the movement of individuals or populations between two well-defined habitats, occurring with a regular periodicity, from daily to seasonal and also once-a-lifetime, but certainly within the lifespan of an individual (Brönmark et al., 2014; Dingle and Drake, 2007; Northcote, 1984). This behavior is found in all major animal groups, including birds, mammals, crustaceans, reptiles, amphibians, insects, and fish (Hoare, 2009).

Many fish species need to move between different habitats to complete their life cycles (Figure 3). In order to distinguish the type of movement, migration has been classified by function, i.e. fish migrate in order to mate and look for spawning grounds (reproductive migration), to search for food and therefore improve their growth (feeding migration) and to refuge in periods of unfavorable circumstances or to search for adequate habitat and climatic conditions (refuge migration) (Brönmark et al., 2014; Lucas et al., 2001; Northcote, 1984).

Figure 3. Reproductive migrations of Ebro barbel (*Luciobarbus graellsii*) in the Ebro River (Población de Valdivielso, Burgos, Spain) [left] and brown trout (*Salmo trutta*) in the Arlanzón River (Burgos, Spain) [right]. Source: Sebastián Camarero Pascual.

When the differentiation comes from habitat usage, things become more complex, as migration can be classified in oceanodromy, with movements occurring exclusively in the sea (e.g. Atlantic Bluefin tuna (*Thunnus thynnus*)), potamodromy, with movements occurring exclusively in freshwater (e.g. Iberian barbel (*Luciobarbus bocagei*)) and diadromy, with movements occurring between both environments. Diadromy have three modalities: anadromy, where most part of feeding and growth are at sea and adults migrate into freshwater to reproduce (e.g. Atlantic salmon (*Salmo salar*)), catadromy, where most part of feeding and growth are at freshwater and adults migrate into sea to reproduce (e.g. European eel (*Anguilla anguilla*)) and amphidromy, where there are movements between sea and freshwater (and vice versa) but without reproductive purposes (e.g. grey mullet (*Mugil cephalus*)) (Lucas et al., 2001). Furthermore, there are species that can display diverse life history tactics, such as brown trout (*Salmo trutta*), that presents migratory dimorphism, with anadromous (sea trout) and potamodromous (riverine trout) individuals, or even partial migrations i.e. where populations are composed of a mixture of resident and migratory individuals (Chapman et al., 2012).

Stimuli for migration respond to internal and external factors. On the one hand, both genetics (genetic signal, adaptations to move between salt and freshwater environments, homing behavior, etc.) and internal physiological state of the fish (maturation state, hormones, metabolic balance, etc.) affect and control migration, although they are strongly influenced by the environment (Lucas et al., 2001). On the other hand, environmental factors (such as water temperature, hydrology, water chemistry, lighting (e.g. photoperiod, day–night alternation, etc.) and meteorological factors) act as stimuli for the onset and maintenance of migration (Lucas et al., 2001; Smith, 1985). The relative importance of each parameter is different for each species or population and, in general, it is the combination of several variables which triggers migration (Lucas et al., 2001; Rodriguez-Ruiz and Granado-Lorencio, 1992). Occasionally, when a relevant environmental cue is missing, this is replaced by alternative stimuli, which will trigger movement and, thus, avoids important delays in the migration (DWA, 2005; Teichert et al., 2020).

Photoperiod, water temperature and river discharge are usually considered as the most important environmental variables affecting fish migration timing (Jensen and Aass, 1995; Jonsson and Jonsson, 2011; Ovidio et al., 1998; Zimmer et al., 2010), although other variables such as water quality, lunar cycle, atmospheric pressure, etc. can motivate their movements (Bravo-Córdoba et al., 2018; Lucas et al., 2001). Fish use photoperiod, i.e. day length or time between sunrise and sunset, as an indicator of the season and it intervenes in hormonal regulation during sexual maturation (Jonsson, 1991; Norberg et al., 2004). As ectotherms, fish are strongly influenced by water temperature, which influences swimming (Ruiz-Legazpi et al., 2018; Videler, 1993; Wardle, 1980), growth and physiological functions (such as endocrine function, lipid accumulation, metabolism, etc.) (Boltaña et al., 2017; Dell et al., 2011), internal process for gonadal development (Jonsson and Jonsson, 2011; Lahnsteiner and Leitner, 2013), as well as incubation period and emergence time (Elliott and Hurley, 1998; Murray and McPhail, 1988). Increase in river discharge generally actuates as a stimulant signal (Clapp et al., 1990) and as a facilitator for overcoming obstacles (Ovidio and Philippart, 2002). However, there are different flow requirements in different life stages, with high flows during upstream migrations and flow reduction in fish initial development (Nicola et al., 2009). Therefore, alterations on these factors can lead in a shift on the phenology and a consequent mismatch between available and necessary resources (Otero et al., 2014), endangering the persistence of many migratory fish species (Shuter et al., 2012).

Threats to migratory fish

Aquatic environments are the most vulnerable ecosystems as the majority of the human populations are concentrated close to streams, lakes, estuaries, and coastal areas (Moyle & Leidmigratiy, 1992). Freshwater environments are subject to multiple stressors derived from the use that human society makes of rivers (e.g. irrigation, power generation, flood control or industrial and domestic supply), which can alter ecological patterns and processes (Branco et al., 2016; Segurado et al., 2016). Dudgeon et al. (2006) identified five major threat categories that affect freshwater biodiversity: overexploitation, water pollution, flow modification, habitat degradation and species invasion. These threats still continue to be persistent, have escalated or even evolved in new hazards (Reid et al., 2019). Among the stressors derived from these threats, river fragmentation and alterations on natural river flow and thermal regimes are the most important affecting freshwater fish (Feng et al., 2018; Jones and Petreman, 2015; Nilsson et al., 2005), due to the need of moving between different habitats to complete their life cycles (Brönmark et al., 2014; Lucas et al., 2001) and to the differential requirements by life stages (Armstrong et al., 2003).

Spain is in the top 10 of countries around the world with the largest number of dams (ICOLD, 2019). Over 1,200 large dams (dams with more than 15 m height or 10 m with an storage capacity greater than 1 hm^3) are documented in the national inventory of dams and reservoirs (MITECO, 2000). However, despite current inventories by the different river basin authorities determine the existence of about 26,000 barriers, it has been estimated that the actual number approaches 50,000 obstacles (Rincón Sanz and Gortázar Rubial, 2016). Dams obstruct the migration of fish to spawning or feeding grounds (Figure 4) and alter and fragment habitat, thus affecting species distributions (Jager et al., 2001; Liermann et al., 2012). Moreover, even smaller barriers such as weirs, bridge foundations, gauging stations or culverts (among other obstacles), also hinder or block fish movements and have effects on fish populations (Benejam et al., 2014; Branco et al., 2017). In addition, the blockage is not only in the upstream direction. In general, the way of going downstream is through the top of the weir or by the spillways, in which the survival depends on the height of the obstacle and the pool depth below it (Larinier and Travade, 2002), or in the case of a hydropower plan, throughout the turbines, in which, fish is not only exposed to strikes by the blades while crossing the turbines, but also to a barotrauma (Brown et al., 2012; Carlson, 2012). As consequence, the hindrance to migrate can provoke alterations in the reproductive behavior (spawning delays or spawn in non-adequate locations) (Gosset et al., 2006; Marschall et al., 2011; Van Leeuwen et al., 2016) or in their fitness (energy expenditure, injuries, low food access, etc.) (Araújo et al., 2013; Morán-López and Uceda-Tolosa, 2020). This can lead into an alteration of the community structure by means of size population reduction, isolation of populations, diversity reduction and genetic erosion (Benejam et al., 2014; Branco et al., 2017), what in turn can reduce their resistance against diseases and invasive alien species (Marvier et al., 2004), and lead to a drastic reduction of fish populations or even to their disappearance (Dias et al., 2017; Elvira, 1996).

Figure 4. Iberian barbel (*Luciobarbus bocagei*) trying to overcome (without success) the weir of Villahoz (Arlanza River, Spain). Source: GEA-Ecohidráulica.

Iberian river's hydrology is featured by seasonality and inter-annual variability, with high flows during autumn-winter, floods occur during a few months in late autumn, winter, and early spring, and strong summer droughts (Gasith and Resh, 1999). Irrigation represents the main reason for flow regulation in Spain (more than 80% of total water use) (González del Tánago et al., 2012). This supposes a significant alteration in the natural Mediterranean pattern, with lower discharges in winter when water is stored in reservoirs and higher discharges in summer when water is released for irrigation (González del Tánago et al., 2016) (Figure 5a). Hydropower generation is in the second place (González del Tánago et al., 2012), and the alteration of hydrographs depends on the type of hydropower scheme (run of river, storage or pumped-storage) (Kaunda et al., 2012). This alteration comes from the discontinuous release of turbined water to attend peaks of energy demand, that causes artificial flow fluctuations downstream, together with the water saving to attend the demand (Figure 5b). In addition, in both irrigation and hydropower, there is an additional effect in the thermal regimen (Casado et al., 2013; Dickson et al., 2012). The release of water from a reservoir has attached artificial changes in water temperature depending on if the water is released by the spillways or by bottom outlets (Michie et al., 2020).

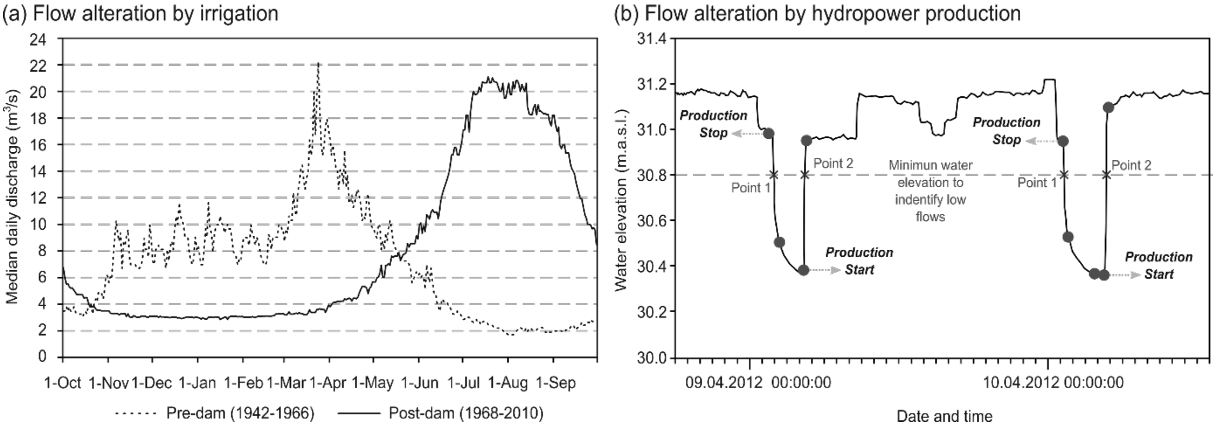

Figure 5. (a) Annual pattern of median daily discharge of Porma River (León, Spain) before and after Juan Benet dam operation for irrigation (source: González del Tánago et al., 2016). (b) Illustration of two low-flow events (between stop and start of production) during hydropeaking in the Lundesokna River (central Norway) (source: Casas-Mulet et al., 2015).

Furthermore, future scenarios of climate change show potential alterations, not only in water temperature, but also in the magnitude, intensity and frequency of rainfall and consequently in river discharge (Solomon et al., 2007). This, together with the expected water scarcity as a result of the increasing water demand and pollution for industrial, domestic and agricultural supply and their waste water (Pittock and Lankford, 2010; Seckler et al., 1999), as well as river fragmentation (Nilsson et al., 2005), may negative affect freshwater populations (Almodóvar et al., 2012; Branco et al., 2016; Sánchez-Hernández and Nunn, 2016; Segurado et al., 2016; Van Vliet et al., 2013; Vörösmarty et al., 2000).

As previously mentioned, freshwater fish use flow and thermal regimes (among other variables) as main ecological timers for initiating and maintaining behavioral reactions such migration, feeding and spawning (Lucas et al., 2001). Therefore, alterations on these regimens can lead into a loss of the migration signal and a consequent migration delay, difficult obstacle ascent and reduction of habitat connectivity (Ovidio and Philippart, 2002), shift on the phenology and consequent mismatch between available and necessary resources (Otero et al., 2014; Shuter et al., 2012), population and diversity reductions caused by mismatch among offspring and ecological requirements (Nicola and Almodóvar, 2002) and changes on fish assemblages (Shea and Peterson, 2007). In addition, the alterations in the morphological, hydrological and climatic processes are expected to provoke a reduction of suitable physical and thermal habitat availability (Almodóvar et al., 2012; Boavida et al., 2015), with the acting associated co-stressors (e.g. oxygen depletion) (Branco et al., 2016; Segurado et al., 2016).

Protection of migratory fish

The Iberian freshwater fauna faces important conservation threats, with most native species suffering a progressive and generalized decline (Aparicio et al., 2000; Hermoso and Clavero, 2011). The effective protection of migratory fish requires long-term conservation efforts and is part of national legal requirements (Spanish Fluvial Fishing Act, 1942; Spanish Water Act, 2001) and also in regional acts (e.g. Act of Natural Heritage of Castile and Leon, 2015). According to these regulations, fish movement have to be guarantee and those structures that hinder or limit it, should be modified (or removed when possible) for allowing this purpose. Additionally, two European directives have arisen to protect freshwater ecosystems. On the one hand, the Habitats Directive (1992) aims to guaranteeing the conservation of natural habitats and wild fauna and flora. In particular, this directive encourages the management of those linear and continuous structures (such as rivers) that are essential for the migration, dispersal and genetic exchange of wild species. On the other hand, the Water Framework Directive (2000) commits European Union member states to achieve a good ecological status of the aquatic ecosystems by 2027. This directive defines fish, hydrological regimen and river continuity (among others) as biological and hydromorphological indicators of the river quality, and it was transposed to Spanish Water Act in 2001.

Therefore, strong efforts to reverse global trends in freshwater degradation and fulfill legal requirements have been carried out during last decades (Geist, 2015) and new multidisciplinary sciences have arisen to understand the interaction between biotic and abiotic components of a riverine ecosystem (Rice et al., 2010). Ecohydraulics is one of these emerging fields on research. It combines the study of the hydraulic properties and processes associated with moving water typical of hydraulic engineering and their influence on aquatic ecology and biology (Maddock et al., 2013). This discipline can be understood as the study of a trilogy (Katopodis, 2016): (1) movements, abilities and passage of aquatic organisms; (2) environmental, ecological or instream flow regimes for aquatic flora and fauna; and (3) restoration of aquatic habitats and ecosystems.

Regarding actions to allow fish passage and improve river longitudinal connectivity, the installation of fishways (Figure 6) is one of the most widely adopted solution to solve the impact of transversal river structures (Bunt et al., 2016, 2012; Sanz-Ronda et al., 2013a), when the removal of the obstacle is not possible. Fishways (also known as fish passages, fish passes or fish ladders) are structures that facilitate or allow the passage of fish from one side to the other in transversal obstacles to the river (Martínez de Azagra, 1999). There are many type of fishways such as lifts, locks, stepped fishways, block ramps, baffle fishways, etc. (Clay, 1995; FAO/DVWK, 2002), although the most common type worldwide are the stepped fishways (Fuentes-Pérez et al., 2017).

Figure 6. Stepped fishway (submerged notch and orifice type) in Guma dam (Duero River, Spain). Source: GEA-Ecohidráulica.

Environmental flow definition is essential to guarantee the survival of freshwater organisms and reduce the effects of flow regimen alterations. An environmental flow can be defined as the quantity, timing, duration, frequency and quality of water flows required to sustain freshwater, estuarine and near-shore ecosystems and the human livelihoods and well-being that depend on them (Acreman and Ferguson, 2010) (Figure 7). There are many methods (more than 250) for its quantification and application (Tharme, 2003) and all of them has their own advantages and disadvantages (Acreman, 2016). In general, they can be grouped in 4 major groups: hydrological methods (e.g. Tennant, Q50), hydraulic methods (e.g. Wetted-Perimeter), hydrobiological methods (e.g. IFIM, PHABSIM) and holistic methods (e.g. Building Block Methodology) (Tharme, 2003).

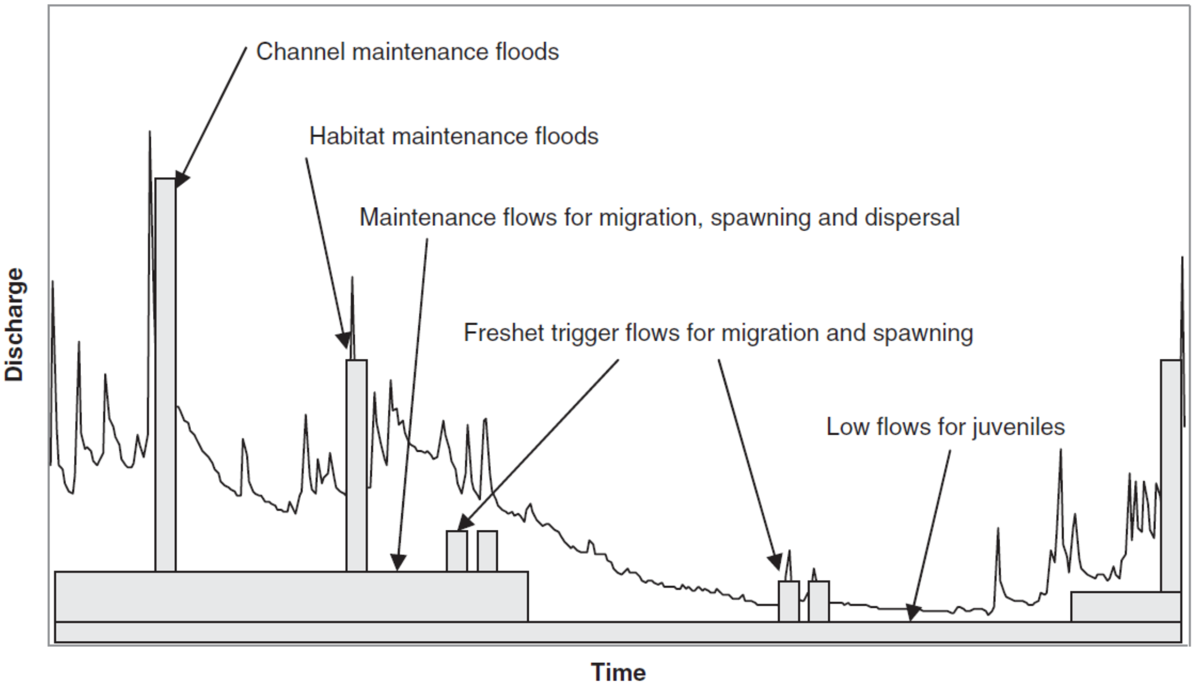

Figure 7. Environmental flow conceptual approach. The continuous line represents the natural flow hydrograph for a year. The blocks represent the flow regime required to maintain a healthy ecosystem. Source: Acreman and Ferguson, 2010.

Restoration projects in aquatic habitats are highly diverse (Geist and Hawkins, 2016). Projects for migratory fish conservation can include not only the restoration of river connectivity and flow regimen, but also measures to provide refuge during hydropeaking events (Costa et al., 2019), thermal regimen control by management of water releases in reservoirs (Sherman, 2000) or spawning habitat improvement (Lapesa et al., 2016), as well as management actions, such as fishing regulations (Cowx and Gerdeaux, 2004) or fish stocking (George et al., 2009).

However, any conservation effort should be followed by a monitoring study, to assess the population status and to know if the applied measures are really effective (Bernhardt and Palmer, 2011). In addition, there are some factors inherent to the general knowledge of fish ecology that can hinder the conservation of migratory fish, such as (1) the limited information on basic taxonomy, life history requirements, or seasonal aspects of riverine fishes, (2) the limited understanding of fish–flow relationships, or for example, (3) the difficulty to predict responses of river fish and river ecosystems to both environmental change and restoration/management actions (Cooke et al., 2012).

Iberian fish fauna

As a result of long-term geographical isolation, native Iberian fish fauna present a high degree of endemism (the greatest percentage of Europe) (Clavero et al., 2004): from the 61 native fish species, 41 are endemic (Doadrio et al., 2011), and according to the International Union for Conservation of Nature (IUCN) Red List, 6 of them are classified as "critically endangered", 11 as "endangered" and 21 as "vulnerable" (CeUICN, 2019). Near the 65% of native species (80% when considering only endemic species) clearly migrate during their life cycle (Sanz-Ronda et al., 2013a), with moments of lesser or greater extent, i.e. from a few kilometers, such as barbels (genus *Barbus* and *Luciobarbus*), nases (genus *Pseudochondrostoma* and *Parachondrostoma*), chubs (genus *Squalius*), riverine brown trout or Pyrenean gudgeon (*Gobio lozanoi)*, to hundreds of kilometers, such as the European eel, the sea brown trout, the Atlantic salmon, shads (genus *Alosa*), the European river lamprey (*Lampetra fluviatilis*) or the sea lamprey (*Petromyzon marinus*) (Sanz-Ronda et al., 2017).

In addition, there are 33 non-native fish species inhabiting Iberian rivers (Elvira and Almodóvar, 2019) from which, 19 are included in the Spanish Inventory of Invasive Alien Species (2013). This high number of invasive alien supposes an additional important threat to native ichthyofauna, as invasive species have shown important ecological impacts, including hybridization and genetic introgression, parasite and disease transmission, resource competition for habitat and food, predation and ecosystem alterations (Ribeiro and Leunda, 2012).

From a taxonomic point of view, native Iberian fish fauna is characterized by a low number (16) of families, with most species belonging to Cyprinidae family (Doadrio et al., 2011). The most abundant species are barbels (genus *Barbus* and *Luciobarbus*) and nases (genus *Pseudochondrostoma*, and *Parachondrostoma*), that are characteristic and dominate medium-sized rivers in Iberian Peninsula (Oliveira et al., 2012), being Iberian barbel (*Luciobarbus bogaei*) and Northern straight-mouth nase (*Pseudochondrostoma duriense*) the most representative endemic cyprinids of the largest Iberian river, the Duero River (Martínez Jiménez, 2006) (Figure 8).

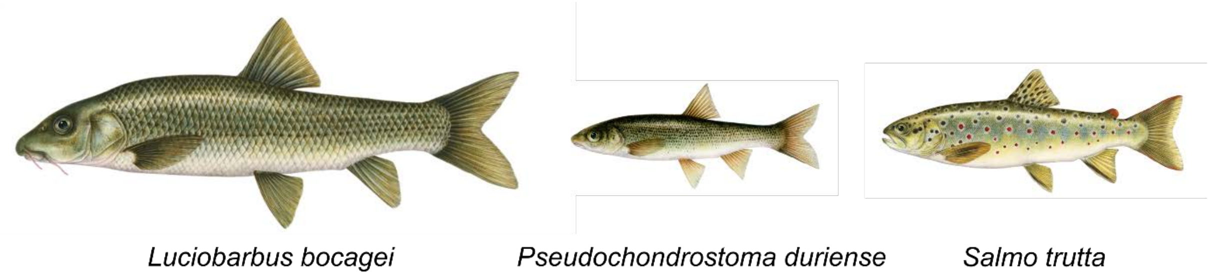

Figure 8. From left to right: Iberian barbel, Northern straight-mouth nase, and brown trout. Source: SIBIC (2017); Artist: Claudia Baeta.

Iberian barbel is listed as "least concern" (IUCN, 2020) whereas Northern straight-mouth nase is more threatened, being categorized as "vulnerable" (IUCN, 2020) and it is mentioned on Annex II of the European Union Habitats Directive (1992). These potamodromous cyprinids perform upstream migrations with reproductive purposes. Their reproductive season is usually in spring, between April and June (Doadrio, 2002), and they ascend to headwaters looking for reaches of shallow waters with high level of oxygen and bottoms of sand and pebbles where they place their eggs (Almaça, 1996; SIBIC, 2017). However, there is still scarce information regarding the migration patterns and ecological requirements of these species during their upstream migration.

On the other hand, the Salmonidae family is one of the most iconic one worldwide, as it gathers fish species of great importance in terms of recreational fishing and their evolutionary and life-history tactics (Jonsson and Jonsson, 2011; Lobón-Cerviá, 2018). One of this specie is the brown trout (*Salmo trutta*) (Figure 8). It is a world-wide distributed species. Its natural distribution spreads over Europe, North Africa and West Asia, but it has been also introduced in South Africa, Russia, North and South America among others (Klemetsen et al., 2003; MacCrimmon and Marshall, 1968). It is categorized as "least concern" in a global scope (IUCN, 2020); however, in a Spanish level, it is defined as "vulnerable" in the "Red Book of vertebrates in Spain" (Doadrio, 2002; González et al., 2017). In the Iberian Peninsula brown trout exhibits different life history tactics, i.e., there are both anadromous (sea trout) and potamodromous (riverine trout) populations. Sea trout can be found in catchments of the Cantabrian and Galicia region, reaching the River Limia (Portugal) in the South (SIBIC, 2017), whereas riverine trout is mainly found in headwaters of rivers located in the Cantabrian, Catalonia and Galicia regions, as well as Duero, Tagus, Guadalquivir, Segura, Júcar and Ebro catchments (SIBIC, 2017).

Brown trout reproductive season is usually in autumn-winter, from November to January (Almodóvar, 2002), and occasionally until April (Gortázar et al., 2007; Larios-López et al., 2015). This species performs reproductive upstream migration, from the sea (in the case of sea trout) or from middle/upper reaches (in the case of riverine trout) to upper part of the river basin (Ovidio et al., 1998; Saraniemi et al., 2008), where they look for adequate spawning sites, with cold and highly oxygenated waters and gravel beds where their eggs are laid (SIBIC, 2017). This species can also exhibit feeding migrations, with a variable distance range in the case of the riverine trout (from nursery stream to a larger river when they grow older), and to the sea in the case of the sea trout (Hendry, 2004; Jonsson and Jonsson, 2011; Northcote, 1992; Olsson et al., 2006). In addition, thermal refuge searching movements in the upstream direction have been also reported during summer for riverine trout (Clapp et al., 1990; Kaeding, 1996; Ovidio, 1999) and in winter (overwinter migration) for the sea trout (Jensen et al., 2015).

Brown trout has been deeply studied due to its economic and historical importance (Northcote and Lobón-Cerviá, 2008). Actually, changes in photoperiod, water temperature and river discharge have been described as possible triggers of brown trout movements (Jensen and Aass, 1995; Jonsson and Jonsson, 2011; Ovidio et al., 1998; Svendsen et al., 2004; Thorpe, 1989). However, most of available research on brown trout migration has been focused on anadromous populations, mainly during reproductive season or limited time period, and studies in the south of its natural distribution range are still scarce. Thus, possible discrepancies in requirements by life-history need to be defined (Ferguson et al., 2019), over all considering that migration patterns can be affected by latitude, with a local variation dependence on environmental conditions (Aarestrup et al., 2018). Moreover, studies from the southern ranges are essential in the context of climate change, as many species are expected not only to shift habitat upriver (Hari et al., 2006), but also to shift coldwards (i.e. usually northwards on distribution ranges) (Jonsson and Jonsson, 2009), and intermediate and northern ranges are likely to become more similar to the currently in southern areas.

The present thesis tries to cover some of the research gaps regarding migration patterns of Iberian barbel, Northern straight-mouth nase and riverine and sea brown trout, as well as their environmental requirements during these movements. In addition, this information will be used to evaluate the effect of human impacts and to assess the effect of mitigation measures. The outcomes of this thesis will be useful to predict responses of river fish and river ecosystems to future environmental changes, allowing the definition of adaptive strategies and reliable management plans to ensure the conservation of Iberian freshwater fish.

OBJECTIVES

The main objective of this thesis is to study the upstream migratory patterns of three of the most important Iberian species: Iberian barbel (*Luciobarbus bocagei*), Northern straight-mouth nase (*Pseudochondrostoma duriense*) and brown trout (*Salmo trutta*).

In order to do this, several specific objectives are defined to know and model the main dates of fish migration and possible affecting factors:

1) to determine the periods when major amounts of fish upstream movements are concentrated;
2) to determine the most significant environmental factors affecting these movements;
3) to evaluate the influence of river regulation (e.g. irrigation, hydropower generation) on fish movements;
4) to analyze possible alterations on the migration patterns as consequence of future projections of climate change;
5) to assess the effects of mitigation measures (such as weir removal, fishways, fishing closures, etc) on fish migration.

Fish species

Iberian barbel, Northern straight-mouth nase and brown trout were selected as key species to study Iberian fish migration.

On the one hand, both Iberian barbel (hereinafter referred to as barbel) and Northern straight-mouth nase (hereinafter referred to as nase) are potamodromous cyprinids that occupy a wide range of freshwater habitats, from floodplains to headwaters and play an important role in trophic interactions within their ecosystems (Collares-Pereira et al., 1996; Kottelat and Freyhof, 2007). Their broad distribution and similarities with several cyprinids regarding their physical habitat (benthic for barbel and mid-column for nase), reproduction strategy (lithophilic), and migratory behavior (potamodromous) as well as similar biomechanical features (Doadrio, 2002; Kottelat and Freyhof, 2007) make them representative cyprinid species from the Mediterranean area (Branco et al., 2016; Sanz-Ronda et al., 2019; Silva et al., 2020). However, there is still scarce information regarding the migration patterns and ecological requirements of these species.

On the other hand, brown trout (hereinafter referred to as trout) is one of the two native species of salmonids currently inhabiting the Iberian Peninsula (the other is the Atlantic salmon). It is an important angling species in Spain, and consequently has high socio-economic status (Almodóvar and Nicola, 2004). It has a great importance in terms of its trophic interactions within streams, and its evolutionary and life-history tactics, with both anadromous (sea trout) and potamodromous (riverine trout) ecotypes in the Iberian Peninsula (Lobón-Cerviá, 2018). In addition, studies in its southern distribution range are useful to predict the global evolution of this species under climate change future scenarios. Despite this specie has been deeply studied (Northcote and Lobón-Cerviá, 2008), most of available research has been focused on anadromous populations, with almost no attention to life-history comparative studies (Ferguson et al., 2019), and studies in the south of its natural distribution range are still scarce (Benitez et al., 2015; Thurow, 2016).

Study cases

Four locations were used to study the migration of barbel, nase and trout. All study sites were located in the Iberian Peninsula (Figure 9), specifically two of them in the Duero River basin (Porma River and Tormes River) and the other two in the Bidasoa River basin (Bidasoa River and Marín River) (Figure 9).

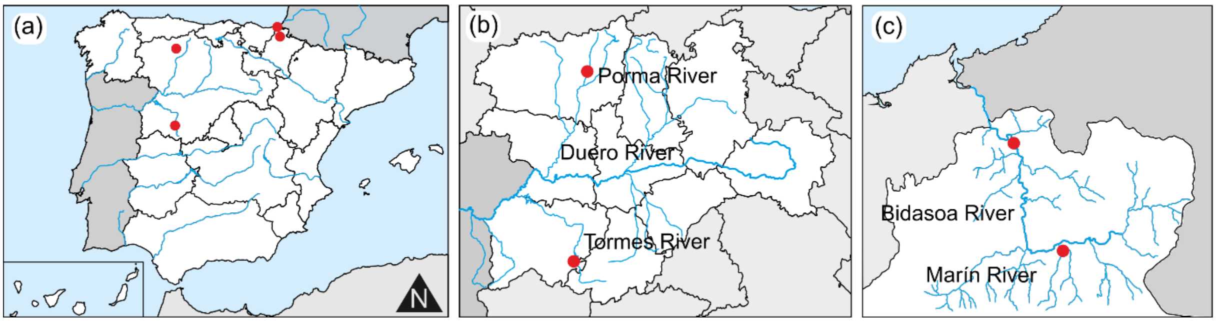

Figure 9. Location of the study sites (red dots). a) Iberian Peninsula, b) Duero River basin (Castile and Leon) and c) Bidasoa River Basin (Navarre).

The Porma River (Figure 9b) it is a tributary of the Esla River, in the northwestern part of the Duero catchment. The study site was located in Vegas del Condado village (León, ETRS89 42°41’N, 5°21’W), downstream of the Porma reservoir (Juan Benet dam). The study reach has an annual average discharge of 14.21 m³/s, with an altitude of 860 m a.s.l. The riverine trout is the most abundant species of this river reach, although presence of bermejuela (*Achondrostoma arcasii*) and Pyrenean gudgeon (*Gobio lozanoi*) occurs in low density.

The Tormes River (Figure 9b) is a tributary of the Duero River. The study reach started (from downstream) at the Cespedosa water supply weir (Salamanca, ETRS89 40°31’00’’N, 5°35’10’’W), located at the tail of the Santa Teresa Reservoir (496 hm³), and it ended at San Fernando hydropower plant dam (ETRS89 40°30’42’’N, 5°33’41’’W). This river reach comprises a 1.8 km length canyon river stretch, with an altitude around 900 m a.s.l. and a mean annual discharge of 23.74 m³/s. Barbel and nase are the most abundant species and comprises the largest proportion of biomass of the river reach. Besides them, the species composition includes riverine trout, bermejuela, Iberian chub (*Squalius carolitertii*) and calandino (*Squalius alburnoides*).

The Marín River (Figure 9c) is a small-size river, tributary of the Bidasoa River. The study site was located in Oronoz-Mugairi village (Navarre, ED50 43°8′N, 1°36.5′W), with an altitude of 160 m a.s.l. and a mean annual discharge of 2.08 m³/s. The main species in this river reach is the riverine trout, although sea trout, Atlantic salmon and European eel also occur in very low densities. The distance from the sea (51.4 km) and the presence of several transversal obstacles in the Bidasoa River, downstream of Mugairi fishway restricts the presence of these diadromous fish in this tributary (Gosset et al., 2006).

Finally, the Bidasoa River (Figure 9c) has a total length of 69 km and a catchment area of 710 km². The study site was located between the villages of Bera and Lesaka (Navarre, ETRS89 43°16’N, 1°41’W; Navarre, Spain), 21.7 km upstream from the sea, at an altitude of 40 m a.s.l. with a mean annual discharge of 24.2 m³/s. In the study reach, the brown trout population comprised anadromous (sea) and potamodromous (riverine) components. The fish assemblage also included other diadromous species, such as Atlantic salmon, European eel, sea lamprey, and Allis shad (*Alosa alosa*). Other potamodromous species included were the Ebro nase (*Parachondrostoma miegii*), Pyrenean gudgeon, Pyrenean minnow (*Phoxinus bigerri*), and stone loach (*Barbatula barbatula*) (Government of Navarre, 2016a; SIBIC, 2017). It is important to note that, during last decades, the regional government has been carrying out different management (fishing closures, size limits and quotas, fish stoking) and connectivity measures (fishways and weir removals) in the study reach, some of them as part of the European project LIFE IREKIBAI (LIFE 14 NAT/ES/00186, www.irekibai.eu), in order to recover the river connectivity and improve fish fauna.

Sampling procedures

Data collection in all study sites was carried out in fishways. Fishways, besides allow fish migration when a transversal obstacle is present in a river, are ideal places to monitor fish migration, because they are mandatory points of fish passage. All fishways were stepped type, in exception of Bera location. There, the fish pass system consisted of a combination of a stepped fishway and a fish lift since it works as a permanent monitoring station (a detailed description of each fishway can be found the respective chapters).

Different dates and fish sampling methodologies were used depending on the study case. The fishway of the Porma River (Figure 10) was continuously monitored from October 2011 to January 2013. A fish counter (VAKI Riverwatcher) was installed in the most upstream slot of the fishway. It registered the day and time of the passage, the fish silhouette, the fish size, the direction of the movement (up/down) and the water temperature.

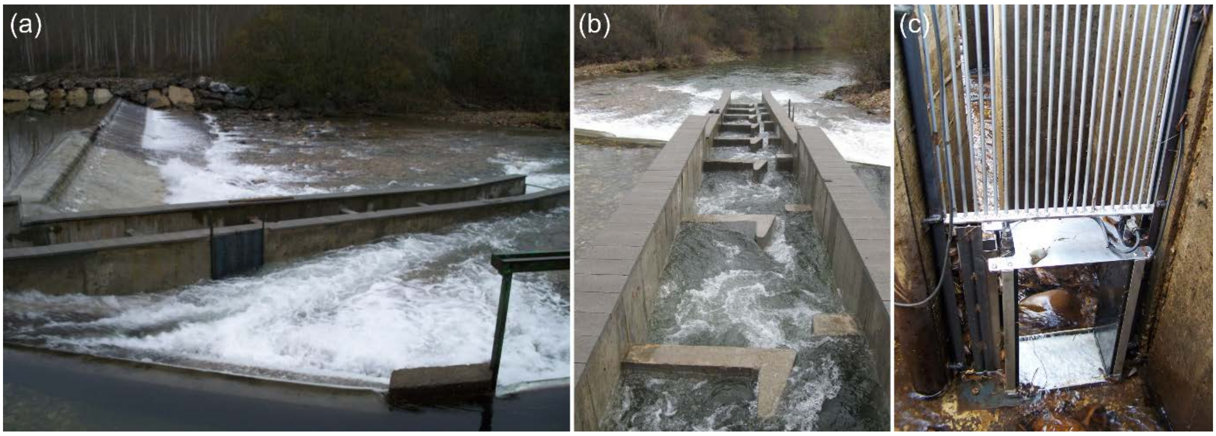

Figure 10. Porma River: (a) dam and fishway; (b) inside of the fishway; (c) fish counter (VAKI Riverwatcher) installed in the most upstream slot of the fishway. Source: GEA-Ecohidráulica.

The fishway of the Tormes River (Figure 11) was monitored between mid-April and end-July from 2012 to 2016 (season when cyprinid reproductive movements were expected) by means of a trap installed in a pool of the upstream part of the fishway (the 5th pool of 44 pools in total). The trap consisted in the installation of funnels in the downstream notch and orifice and a mesh in the upper cross-wall for avoiding fish to escape. The frequency of trap checking was variable depending on the year, from 2-4 times a week (2012-2013) to once a day (2014-2016). Captured fish were identified, counted and measured (fork length and weight). Data were gathered by the staff of the hydropower plant, and by the Group of Applied Ecohydraulics of the University of Valladolid.

Figure 11. Tormes River: (a) old fishway (2012–2013); (b) retrofitted fishway and small attraction weir (2014–206); (c) plant view of the dam and old fishway; (d) capture pool with funnel only in the notch; (d) funnel in both notch and orifice. Source: GEA-Ecohidráulica.

The fishway located at the Marín River (Figure 12) was full-year monitored from September 2008 to March 2017. For sampling, the fishway was closed by means of portable gates in the most downstream cross-wall of the fishway and in the turning pool. The gate of the fishway was equipped with a fish net that prevented fish from exiting the fishway in the upstream direction. The fishway was revised two-three times a week during the whole year, increasing the monitoring frequency to once a day when high migration rates occurred. The captured fish were identified, measured (fork length) and sex identified. Data were gathered by the Fishing Service of the Government of Navarre.

Figure 12. Marín River: (a) fishway, measure station (waiting tank and measure table) and the weir at the background of the picture; (b) detail of a portable gate (empty fishway). Source: GEA-Ecohidráulica.

Finally, the fishway of the Bidasoa River was full-year monitored from September 1995 to December 2019. The monitoring station comprises a stepped fishway of five pools and a fish lift. The cage of the lift works as capture trap (it has a funnel in the entrance) and is located into the upper pool. It is lifted to transport the fish to the measuring room, where fish are identified, measured (fork length and weight) and sex identified. The frequency of monitoring was two-three times per week during the whole year, increasing to once a day when high migration rates were observed. Data were gathered by the staff of the Fishing Service of the Government of Navarre.

Figure 13. Bidasoa River: (a) weir; (b) monitoring station (stepped fishway and fish lift); (c) stepped fishway; (d) cage of the fish lift inside the upper pool of the stepped fishway; (e) detail of funnel to enter into the cage of the fish lift. Source: GEA-Ecohidráulica.

In all cases, the only possible way for upstream migration was through the fishways as they were the only possible route to pass the weirs. Downstream migrants could not descend through the fishways due to the configuration for fish trapping (in exception of the Porma River fishway where the fish counter could detect them), and thus, they cannot be counted, and only upstream migration was studied.

All experiments and procedures were performed following European Union ethical guidelines (Directive 2010/63/UE) and Spanish Act RD 53/2013, with the approval of the competent authorities (Regional Governments on Natural Resources and Water Management Authorities).

Data analysis

The statistical analyses performed throughout the whole document can be classified in three main analysis types: 1) frequency analysis, 2) survival analysis and 3) random forest. All computations and statistical analysis were performed in R (R Core Team, 2019).

(1) Frequency analysis is an important area of statistics that allows to quantitatively describe the characteristics of a set of data. It deals with the number of occurrences (frequency) and analyzes measures of central tendency, dispersion, percentiles, etc., which allows to quantitatively describe the characteristics of a set of data (Hampton and Havel, 2006). In this thesis, frequency analysis of number of captures were carried out to identify major upstream migration periods, considering possible differences by year and by groups (i.e. species, ecotype, sex, etc.).

(2) Survival analysis is a collection of statistical procedures for data analysis for which, the outcome variable of interest is time until an event occurs (Kleinbaum and Klein, 2010). These techniques were designed primarily for the study of deaths in medicine and epidemiology (Miller, 1998). However, they have proved great potential in biological and ecological studies where variables are time dependent (e.g. Castro-Santos and Haro, 2003; Chambers and Leggett, 1989). In this thesis, survival analysis techniques were used to describe migration patterns, by applying the concept of survival time (time until an event occurs) to migration time (time until a fish is captured) (Figure 14).

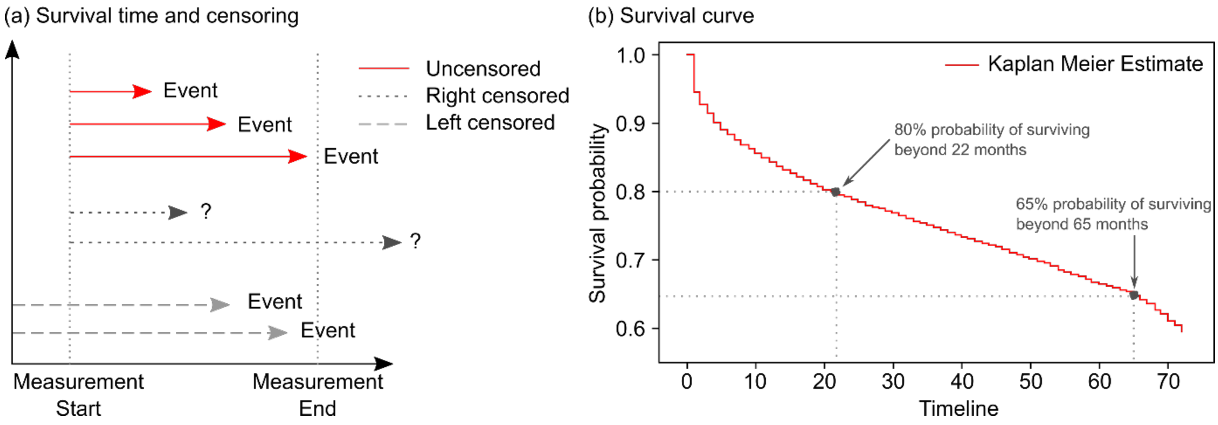

Figure 14. Survival analysis definition: (a) survival times and types of censoring (modified from WebFOCUS, 2017); (b) survival curve (representation of the survival function with Kaplan Meier estimator) (modified from Pandey, 2019).

Because in all study cases fish were not previously tagged, some assumptions were made:

(i) Once a fish was captured, it continued its migration. That is to say, as repeat observations of the same individual could not be distinguished, it was assumed that all fish were only captured once.

(ii) The captured fish were the only ones that participated in the experiments and the exact survival time (capture date) of all participating individuals (captured fish) was known, i.e. there were not censored data.

(3) Random forest is a statistical ensemble learning method for classification, regression and other tasks that is based on the combination of a multitude of decision trees (Figure 15), which is used to determine the mode (classification) or mean prediction (regression) of the individual trees (Breiman, 2001).

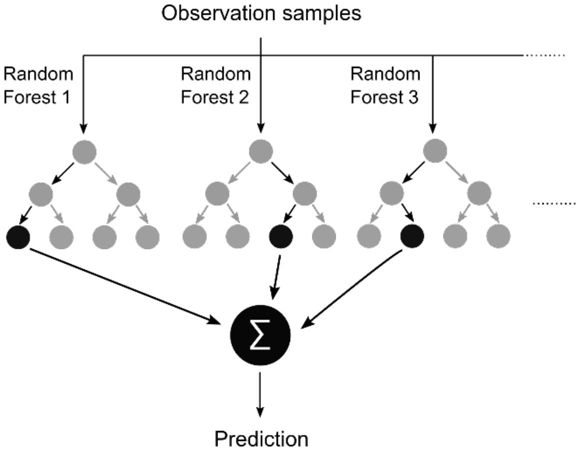

Figure 15. Random Forest conceptual approach (modified from AVB, 2019).

This method has been widely applied in ecology (Breiman, 2001; Cutler et al., 2007) and more recently in freshwater fish studies to predict fish abundance and species response to environmental (Vezza et al., 2015; Ward et al., 2014) and to assess and predict the effects of restoration and management actions (Cochran-Biederman et al., 2015; Flanagan and Richardson, 2010; Teichert et al., 2016). Random forest can capture the over-dispersion or zero-inflation inherent in count data (Garcia-Marti et al., 2019), allows freedom from normality and homoscedasticity assumptions and does not require previous data transformation or a separate test set for cross-validation as it is performed internally during the run (Breiman, 2001). In this thesis, random forest regression was used for four main purposes: (i) to evaluate the influence of environmental variables on the number of captures; (ii) to develop models able to predict when upstream movements were more likely to occur; (iii) to evaluate different scenarios of river discharge and water temperature; and (iv) to assess the possible effects of management and restoration actions.

CHAPTER I:

Seasonal and daily upstream movements of brown trout *Salmo trutta* in an Iberian regulated river

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Abstract

Migrating fish are vulnerable to anthropogenic disturbances and, to assess the impact of human activities in freshwater ecosystems, it is vital to understand their movement patterns. The aim of this study is to describe the upstream movements of potamodromous brown trout *Salmo trutta* (seasonal and daily) and the potential environmental triggers in a regulated river in the northwest of the Iberian Peninsula (Porma River, León, Spain). Data collected in a fishway from October 2011 to January 2013 with a fish counter showed two important migratory periods, one associated with reproduction (October-December) and another one during summer (July-August). Both were significantly correlated with changes in solar radiation, flow and water temperature. Although in all seasons movements were identified throughout the day, they were more frequent early in the morning and in the afternoon during the spawning migration and in the morning during summer. River regulation of the Porma Reservoir significantly influenced movements by providing non-natural high flows during summer, which increased the chance of migration, but also colder water that could delay the thermoregulatory movements. In contrast, it provided lower flows in the spawning season. This highlights how susceptible brown trout movements are to human influence on flow and thermal regimes.

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1. Introduction

Many fish species move between functional areas in order to complete their life cycle. To ensure adequate timing in their arrival to the new habitats, fish require reliable timers (Jonsson and Jonsson, 2002). Environmental factors (such as light, water temperature, hydrology, water chemistry and meteorological factors) act as stimuli for the onset and maintenance of migratory behaviour (Lucas et al., 2001). The relative importance of each parameter is different for each species or population and, in general, it is the combination of several variables which triggers migration (Lucas et al., 2001). Occasionally, when a relevant environmental cue is missing, this is replaced by an alternative stimuli which will trigger movement and, thus, avoids important delays in the migration (DWA, 2005).

Brown trout *Salmo trutta* (Linnaeus 1758) shows a great plasticity in its migratory behaviour (Lucas et al., 2001). This species has successfully adapted to a wide geographical range and exhibits different life history tactics (anadromous and potamodromous). It has also shown a wide range of variation in its reproductive traits. For instance, many authors have observed significant latitudinal variations in the mean spawning date and in the duration of the spawning period (Gortázar et al., 2007; Klemetsen et al., 2003; Larios-López et al., 2015). Furthermore, during its life cycle, brown trout migrates both long distances for spawning, and shorter ones for feeding or wintering (Jonsson and Jonsson, 2011; Klemetsen et al., 2003). Daily movements patterns also differ during the different seasons (Bunnell et al., 1998; Klemetsen et al., 2003; Ovidio et al., 2002) and among the different populations (Clapp et al., 1990; Ovidio et al., 2002; Zimmer et al., 2010).

In general, the most important upstream movements of the southern populations of brown trout are linked to their search for adequate spawning sites (García de Jalón, 1992). Their spawning period in the Iberian Peninsula expands from November to January (Doadrio, 2002), and occasionally until April (Gortázar et al., 2007; Larios-López et al., 2015). However, the dates and environmental triggers of the spawning movements in the Iberian Peninsula for potamodromous populations have not been sufficiently studied. Only fishway evaluations in Iberian rivers have registered upstream movements in movements in autumn and winter (Ordeix et al., 2011; Santos et al., 2005, 2002), later than in northern latitudes (from August to October) (Jensen and Aass, 1995; Jonsson and Jonsson, 2002; Rustadbakken et al., 2004) or in mid-latitudes (from October to November) (Benitez et al., 2015; Ovidio, 1999).

Due to their migratory behavior and dependence on environmental cues, brown trout is vulnerable to diverse anthropogenic disturbances such as river fragmentation and flow and thermal alterations (Jonsson and Jonsson, 2009). Dams, weirs and other river structures can not only hinder or limit the movements of freshwater organisms but also vary the natural water regime (Nilsson et al., 2005). Flow regulation modifies inter and intra-annual seasonality and variability, providing lower mean flows in winter and higher flows in drought season (e.g. irrigation dams), daily rapid changes in flow (e.g. hydropeaking in hydropower generation) or damping flood peaks (e.g. dams for flood control) (Almodóvar and Nicola, 1999; González del Tánago et al., 2016). These non-natural flow variations might affect the density, biomass and species composition (Almodóvar and Nicola, 1999; Benejam et al., 2014), as well as affect the daily fish behaviour and the time of spawning and migration periods (Jonsson and Jonsson, 2011). Therefore, it is vital to improve our understanding of the migratory patterns of the different populations and of the potential impact of environmental disturbances, especially in those less studied and more threatened populations, such as the Iberian populations (Clavero et al., 2004).

The aim of this study is to improve the understanding of the upstream migration of potamodromous brown trout in an Iberian regulated river. Both seasonal and daily movements were considered in order to highlight the possible effects of river regulation on the environmental cues which trigger fish movements. Specifically, the main goals of the study were: to (1) identify the time periods when most upstream movements occur; (2) determine the times of day with the greatest activity; (3) uncover the most significant environmental cues that influence these movements; and (4) evaluate the possible effects of river regulation on fish migration.

2. Materials and methods

2.1. Study area

The study area (Figure I.1) is located in the middle section of the Porma River (a tributary of the Esla River, in the north-western part of the Duero Basin), in Vegas del Condado village (León, Spain, northwest of the Iberian Peninsula) (42°41’ N, 5°21’ W), with an average altitude of 860 meters above mean sea level.

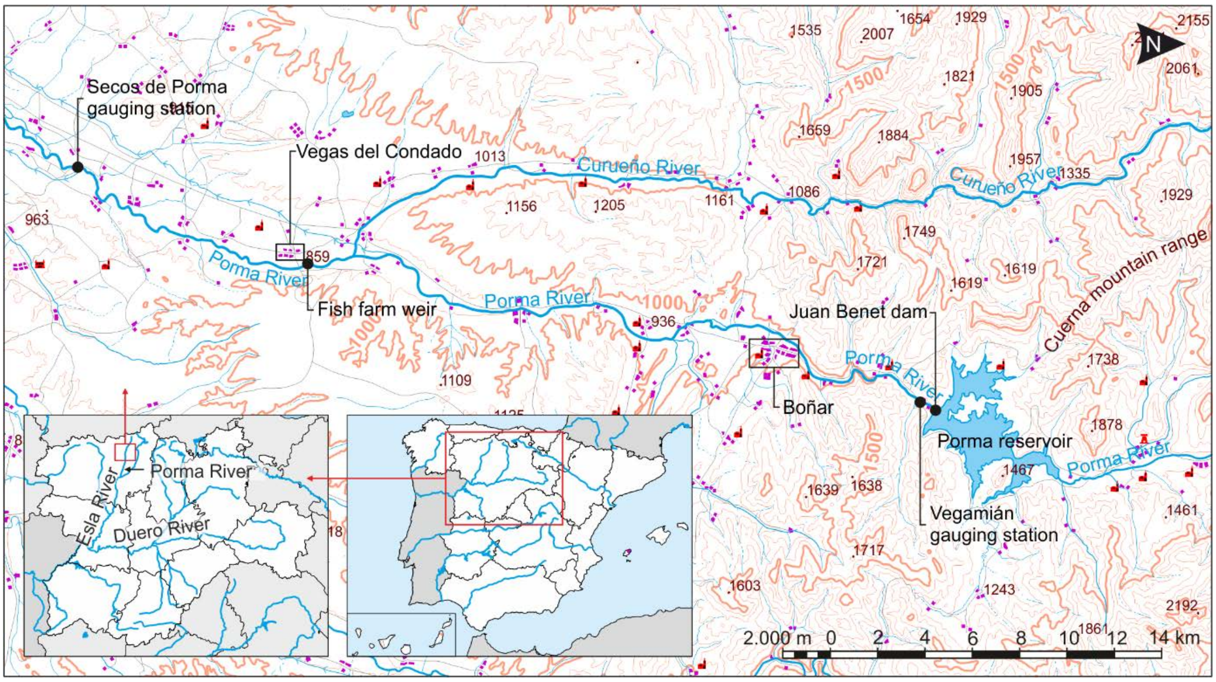

Figure I.1. Location of the study area: Vegas del Condado village (León, Spain) in the Porma River, downstream of the Porma reservoir.

The reach under study is in the trout zone (Huet, 1954), specifically in the metarhitron zone (Illies and Botoseanu, 1963), and it belongs to the C4 category (gravel bed stream, moderate sinuosity and a slope of 0.001 to 0.020 m/m) (Rosgen and Silvey, 1996).

The study reach has an annual average discharge of 14.21 m^3/s and an annual average water temperature of 10.2ºC. It is located downstream of the Porma reservoir (Juan Benet dam) (Figure I.1). The main functions of the reservoir are to supply water for irrigation and domestic needs, flood control and power generation. The reservoir has a water storage capacity of 317.4 hm^3, representing 106% of the annual natural runoff. The presence of this reservoir causes a non-natural thermal and flow regime (González del Tánago et al., 2016). In contrast to the summer drought in Mediterranean non-regulated streams, the release of cold water from the bottom outlet for irrigation generates higher flows in summer and lower flows during the winter months when water is stored (Figure I.2a). However, the effect of flood control in autumn and winter is diminished due to the long distance of the study reach from the reservoir (34 km) and the flow contribution of the Porma Basin (Figure I.2b). Likewise, the hydropower production of the reservoir does not produce hydropeaking as it only takes advantage of the water released for supply.

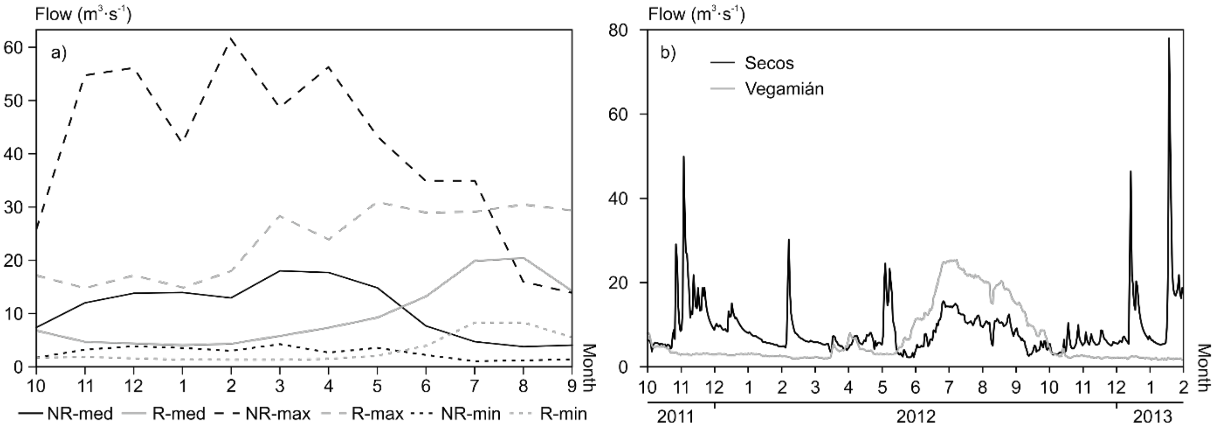

Figure I.2. (a) Comparison between non-regulated [NR, (1940/41-2005/06) (CHD, 2015)] and regulated [R, after dam start-up in 1968 (1969/70-2006/07) (CHD, 2008)] situations of the Porma River for medium (med), maximum (max) and minimum (min) monthly flow data (m^3/s) in Vegamián gauging station (Figure I.1). NR data has been modelled by CHD via the numerical model SIMPA (Estrela and Quintas, 1996). (b) Mean daily flow data for the study period in Secos del Porma gauging station compared with Vegamián gauging station.

Data collection was carried out in a vertical slot fishway located in a derivation weir of a brown trout fish farm (Figure I.1 and Figure I.3). This weir is a gravity dam of 1.80 m high and 35 m wide. The fishway is the only way for the upstream migration. It has 9 slots of 0.2 m of width and its pools have an average width and length of 1.6 m and 2.4 m respectively. The average topographic difference between slots is 0.2 m. The fishway design flow was 0.350 m^3/s, with a volumetric power dissipation between 180 and 190 W/m^3, depending on river discharge. The hydraulic variables inside this structure, for the different discharge ranges of the river, are within the preferences of brown trout. Likewise, the fishway has shown a high passage success and it is not selective in terms of fish size (Bravo-Córdoba, 2011).

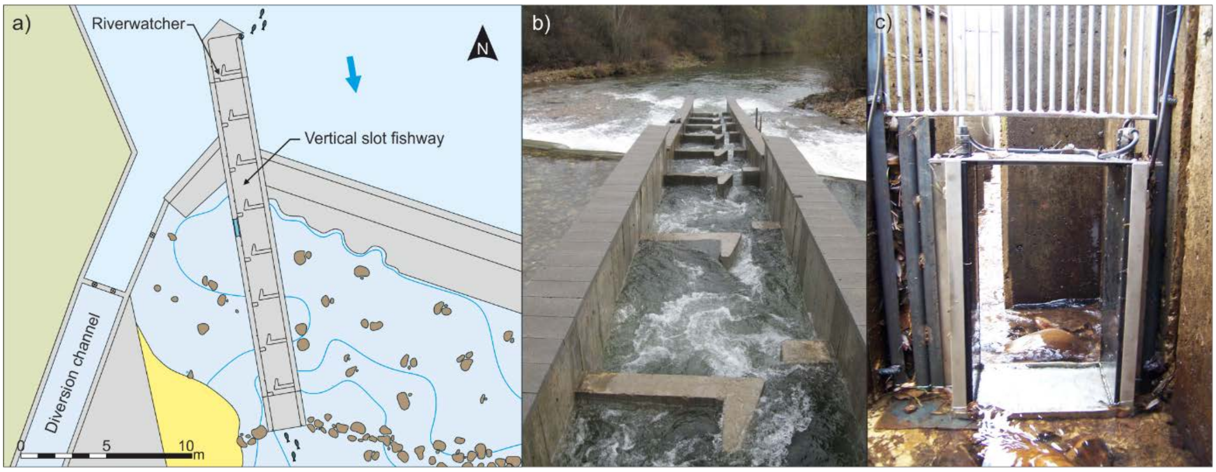

Figure I.3. Fishway plan (a) and pictures of the fishway (b) and the Riverwatcher fish counter (c) in the weir of Vegas del Condado in the Porma River.

2.2. Sampling procedure

To determine brown trout upstream movement patterns, data from October 2011 to January 2013 from a fish counter (VAKI Riverwatcher) located in the most upstream slot of the fishway were used (Figure I.3). The fish counter registered the day and time of the passage, the fish silhouette, the fish height, the direction of movement (upstream/downstream), the speed of the fish and the water temperature.

The fish height (mm) was translated to fish length (mm) by means of a biometric relation for brown trout. This relation was calculated using data from an electrofishing experiment (Erreka; 2200 W, 5 A) performed in November 2010 in the study area [($n = 48$; fork length range = 145−320 mm; $R^2 = 0.9243$): fork length = 4.9614 · height + 4.4805].

The fish counter has a minimum detection height of 40 mm (which corresponded to a fish length of 203 mm) and accuracies up to 95−99% for salmonid migrations (Gudjonsson and Gudmundson, 1994; Shardlow and Hyatt, 2004; VAKI, 2016). Due to the absence of a camera in the fish counter, several electrofishing (November 2010, 2013 and 2015) were carried out, 1 km up and downstream of the fishway, to determine the specific composition of the river reach. The 80% of captures were brown trout and the other 20% corresponded to bermejuela *Achondrostoma arcasii* (Steindachner, 1866) and gudgeon *Gobio lozanoi* (Linnaeus, 1758), both species with fork length up to 10 and 15 cm respectively (Doadrio, 2002). Hence all the registers in the fish counter (after filtering false positives) were considered brown trout.

2.3. Environmental data

During the study period the following environmental variables were recorded: river flow (m^3/s) (SAIH 2112 Secos del Porma gauging station, data every 10 minutes), ambient temperature (ºC), rainfall (mm) and solar radiation (MJ/m^2) (SIAR LE02 Mansilla Mayor weather station, data every

30 minutes), atmospheric pressure (hPa) (2661 Vírgen del Camino weather station, data every 6 hours), water temperature (ºC) (Riverwatcher fish counter, data every 3 hours; Vegas del Condado fish farm, data 3 times at day), and moon phase (numeric value referred to the length of the lunar month).

2.4. Data processing and statistical analysis

Fish counter records were processed using Winari Software (version 4.33). Every record was visually checked to minimize counting errors, such as false counts [bubbles, tree branches or otters *Lutra lutra* (Linnaeus 1758)], records with more than one fish simultaneously or multiple counts of the same fish. If a record had more than one silhouette, it was managed as different records and the height of each fish was estimated by means of the height of the silhouettes. On the other hand, multiple counts of the same fish were defined as those registers that, recorded a similar height (± 0.5 cm), in a short time range (± 2 min), were recorded several times and alternatively in both directions (i.e. upstream-downstream-upstream-etc.).

To identify major upstream migration periods, frequency analysis of the number of upstream movements were performed. In order to check whether size selectivity occurred throughout the year, records were classified according to length in 3 classes: small (200–270 mm), medium (270–350 mm) and large (>350 mm), which corresponded with the age ranges from 3+ to 5+, from 6+ to 8+ and more than 8+, respectively for brown trout in rivers of León (Gallego, 2009). Kruskal-Wallis test was carried out among the monthly fish number for each length class.

Once major upstream migration periods were identified, in order to test if the number of migrants could be related to the environmental factors, Spearman rank correlations were carried out between the daily fish number and the daily mean value of the considered environmental variables for each migration period. Furthermore, since the study covers almost two years, differences between years were determined using the Mann-Whitney U-test.

In addition, in order to identify the hours with more upstream movements, an hourly frequency analysis (considering solar hour) was performed by season (winter records: December, January and February; spring records: March, April and May; summer records: June, July and August; and autumn records: September, October and November) and for the major migration periods. The data were further classified as recorded during dawn (sunrise ± 1 hour), morning (from dawn to 12 h), afternoon (from 12 h to dusk), dusk (sunset ± 1 hour) and night, to determine whether records were more prevalent during a particular time of day by means of Chi-squared test. Spearman rank correlations were used to determine whether a relation between the number of hourly records and environmental variables existed (except atmospheric pressure due to the low sampling rate).

3. Results

3.1. Characteristics of the recorded fish

A total number of 670 upstream fish were recorded during the study period. Fork length ranged from 203 to 689 mm (mean length 298 ± 107 mm) (Figure I.4). According to their classification by length, 385 (58%) small fish (200–270 mm), 115 (17%) medium fish (270–350 mm) and 170 (25%) large fish (> 350 mm) were recorded.

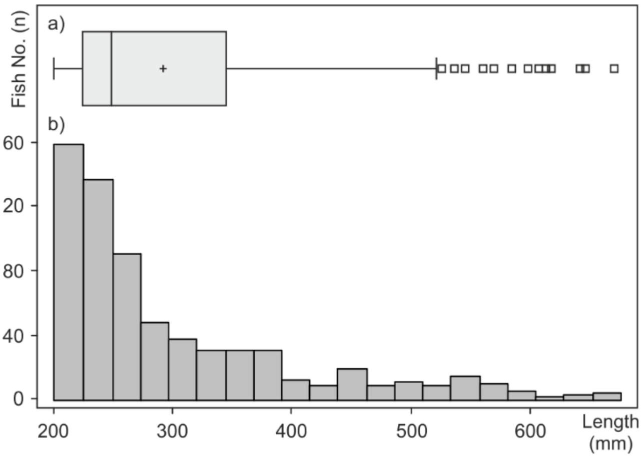

Figure I.4. (a) Box-plot and (b) histogram of the size distribution of the recorded fish in the Porma River.

3.2. Seasonal upstream movement patterns

Two periods of upstream movements could be distinguished (Figure I.5), one during autumn and winter (spawning migration) (83% of the total records, 48% for 2011 and 35% for 2012) and another one during the summer months (summer migration) (15%). In contrast, few spring movements were recorded (2% of the total records).

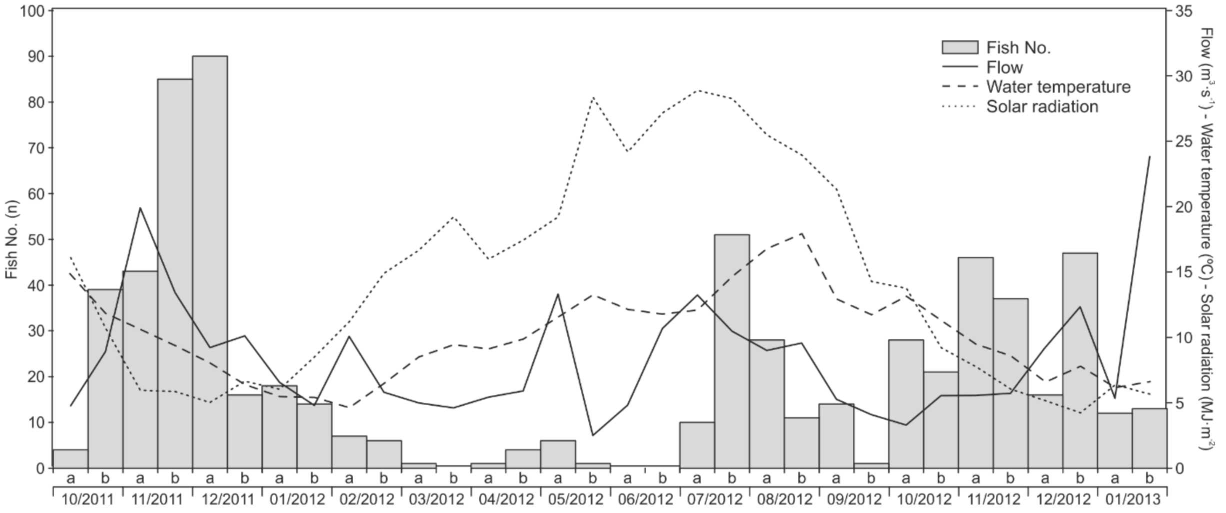

Figure I.5. Representation of the fortnightly records (*a* corresponds to the first fortnight of the month and *b* to the second one) for the study period compared to the average fortnightly flow, water temperature and solar radiation.

There was no statistically significant difference between 2011 and 2012 migrations (*U* = -

223; *p*-value = 0.5142) and no significant differences were detected between the sizes of fish and upstream migration periods (*K* = 0.2925; *p*-value = 0.8639). Only statistically significant differences in mean daily river flow (*U* = -1630.5; *p*-value = 0.0024) and atmospheric pressure (*U* = -3096.0; *p*-value < 0.0001) were found between 2011 and 2012 for the overlapping period (October – January).

The movements associated with spawning migration were recorded between October and December (*n* = 277 for 2011; *n* = 195 for 2012). November was the month with most upstream movements during both registered spawning migration periods (n = 128 for 2011; n = 83 for 2012) while the maximum number of movements were recorded in the first half of December 2011 (n = 90) and in the second half of December 2012 (*n* = 47) (Figure I.5). In both years, spawning movements were negatively correlated with solar radiation (2011: *ρ* = -0.3394, *p*-value = 0.0015; 2012: *ρ* = -0.3524, *p*-value = 0.0004) and with water temperature in 2011 (*ρ* = -0.2762, *p*-value = 0.0096) [ambient temperature was strongly correlated with water temperature (ρ = 0.6942, *p*-value < 0.0001) so it was not considered as an independent cue]. Fish movements during the spawning migration period occurred between 4.6−16.1 °C, with no movements recorded below 4.5ºC [minimum water temperature reached was 2.0ºC (04/02/2012)]. Also, a positive association between the number of migrants and flow was found in both years (2011: *ρ* = 0.2953, *p*-value = 0.0056; 2012: *ρ* = 0.2099, *p*-value = 0.0349), with fish movements recorded in the flow range of 2.52−59.06 m^3/s.

The summer upstream movements (*n* = 114) occurred from July to the first half of September, with a peak in the second half of July (*n* = 51), and were positively correlated with water temperature (*ρ* = 0.2972, *p*-value = 0.0046) and flow (*ρ* = 0.3222, *p*-value = 0.0021). Summer movements occurred with flows between 4.54 and 15.68 m^3/s and water temperatures between 10.5−19.9ºC. No movements were detected above 19.9ºC [maximum water temperature reached in the study period was 20.8ºC (22/08/2012)].

3.3. Daily upstream movement patterns

In autumn 2011, upstream movements were spread throughout the day, while in 2012 they were significantly higher during the afternoon (*p*-value = 0.0160) (Figure I.6 and Figure I.7a). In winter, the movements were also spread throughout the day in both years (Figure I.6 and Figure I.7b), with a peak after dawn (23% in 2011 and 21% in 2012). In spring, migration occurred throughout the day, with a peak (31%) at midday (Figure I.6 and Figure I.7c). During the summer, and more specifically during the summer migration, most movements occurred during the morning (*p*-value = 0.0136 and *p*-value = 0.0045 respectively), with a migration peak (16%) after sunset (Figure I.6 and Figure I.7d). During the spawning migration, upstream movements were spread throughout the day in 2011, while in 2012 they were significantly higher during the afternoon (*p*-value = 0.0103) (Figure I.6 and Figure I.7e).

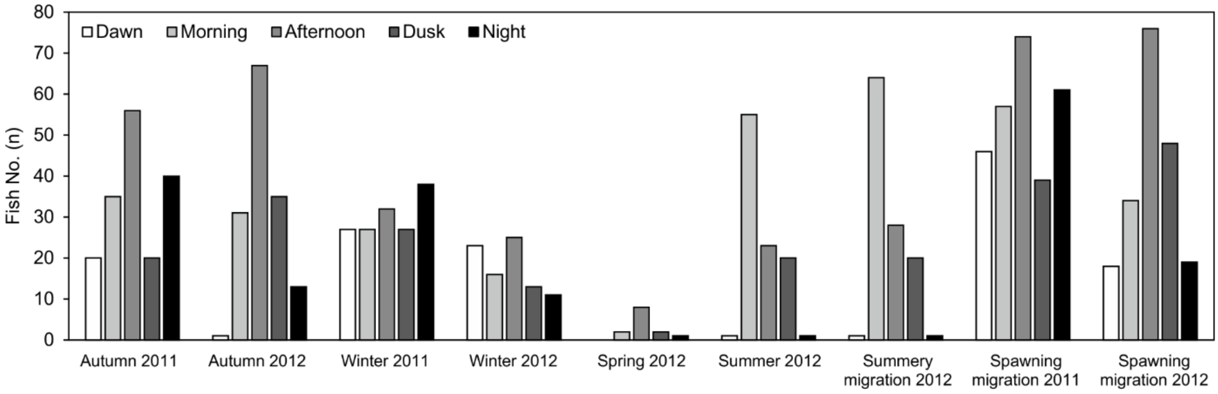

Figure I.6. Daily distribution of the number of upstream migrants for each season.

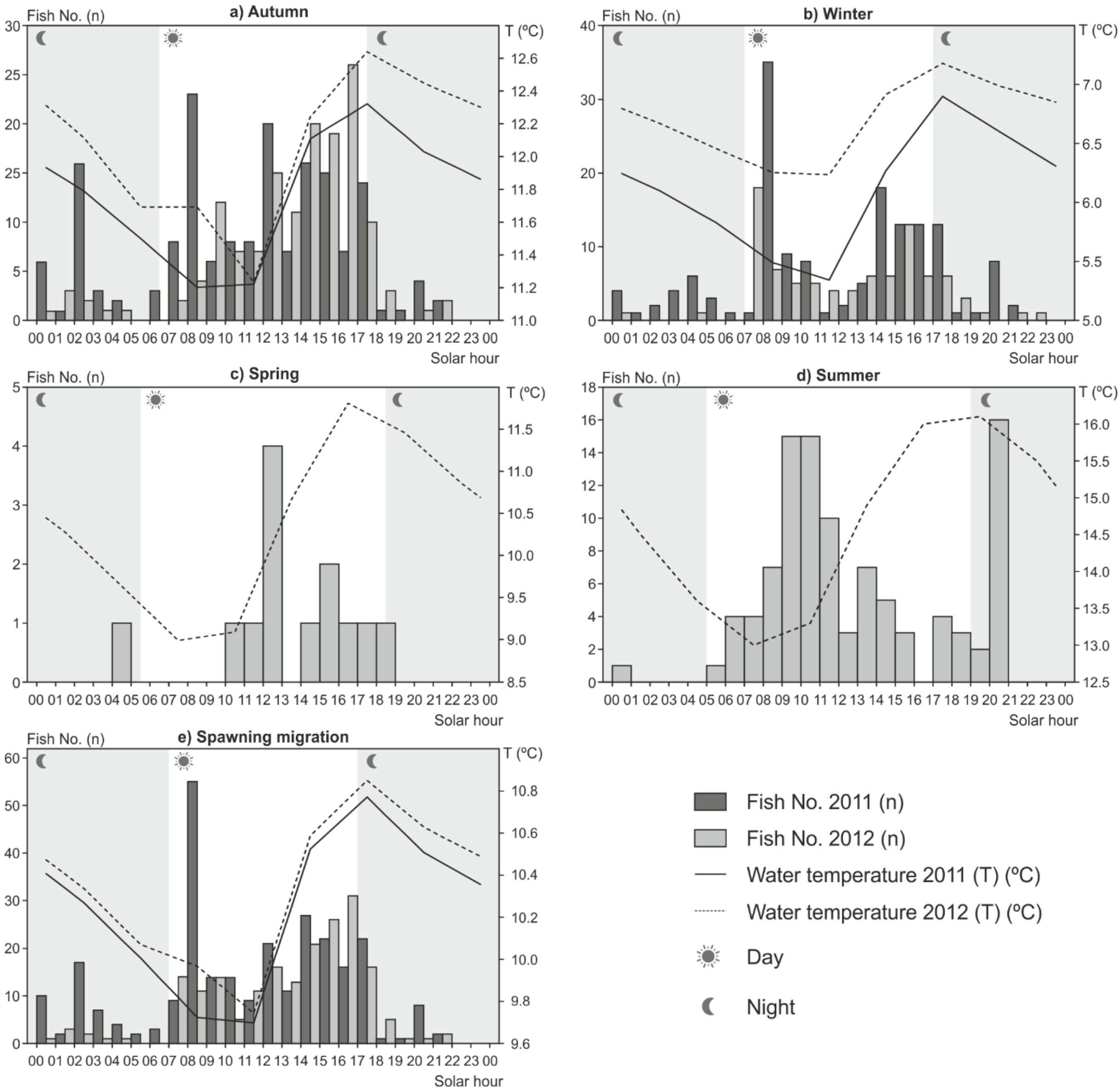

Figure I.7. Number of records by solar hour and season: a) autumn (September-November); b) winter (December-February); c) spring (March-May); d) summer (June-August); e) spawning migration (October-December). The average time of sunrise and sunset and the average hourly water temperature of each season/period are indicated.

A positive correlation between hourly number of migrants and water temperature was found in winter 2011 and spring 2012, while this correlation was negative in autumn (for both 2011 and 2012) and during the spawning migration of 2011 (Table I.1). During all seasons (except autumn 2012) movements were positively correlated with solar radiation. Likewise, a positive correlation with the flow was found in all seasons except for the winter of 2012 and during the spawning migration of 2012. Rainfall was only positively correlated with fish movements in autumn of both years and in winter 2011.

Table I.1. Correlations between hourly number of migrants and environmental variables (n is the number of fish and ρ is the Spearman correlation coefficient).

Season	Date range	n	Water temperature		Flow		Solar radiation		Rainfall	
			ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value
Autumn (2011)	From: 04/10/2011 To: 30/11/2011	171	-0.1396	0.0000	0.1425	0.0000	0.1478	0.0000	0.3356	0.0258
Autumn (2012)	From: 01/09/2012 To: 30/11/2012	147	-0.0742	0.0005	0.0488	0.0226	0.0246	0.2497	0.0606	0.0046
Winter (2011)	From: 01/12/2011 To: 28/02/2012	151	0.1637	0.0000	0.0984	0.0000	0.0657	0.0022	0.0460	0.0318
Winter (2012)	From: 01/12/2012 To: 30/01/2013	88	0.0215	0.4117	0.0367	0.1603	0.1127	0.0000	0.0194	0.4591
Spring (2012)	From: 01/03/2012 To: 31/05/2012	13	0.0450	0.0345	0.0524	0.0139	0.0495	0.0201	-0.0189	0.3750
Summer (2012)	From: 01/06/2012 To: 31/08/2012	100	0.0594	0.0052	0.0487	0.0222	0.1769	0.0000	0.0100	0.6398
Summer migration (2012)	From: 01/07/2012 To: 15/09/2012	114	-0.0125	0.5903	0.0427	0.0668	0.2130	0.0000	0.0222	0.3404
Spawning migration (2011)	From: 04/10/2011 To: 31/12/2011	277	-0.0516	0.0169	0.1054	0.0000	0.1428	0.0000	0.0301	0.1640
Spawning migration (2012)	From: 01/10/2012 To: 31/12/2012	195	-0.0026	0.9029	0.0044	0.8355	0.1110	0.0000	0.0175	0.4110

4. Discussion

Fish counters are an interesting non-contact method for monitoring free fish movements, as they enable data acquisition without handling (e.g. mark-recapture or radio telemetry) and without causing injury or stress to the fish (e.g. electrofishing or trapping) which could potentially have an effect on the acquired data. However, two major limitations were found in this study: (i) the minimum registered fish height (40 mm translated into a length of 203 mm in this case) which might have led to some fish not being recorded (fry or juvenile fish), and (ii) the difficulty in identifying species since the device was not equipped with a video camera system and fish identification relied on fish silhouettes. This could have resulted in some rare occasions on the counting of some individuals from other species, despite brown trout being the principal species with fork length higher than 20 cm.

The greatest number of fish movements were recorded during autumn and winter, which is in agreement with others studies carried out in the Iberian Peninsula (Ordeix et al., 2011; Santos et al., 2005, 2002). Solar radiation, water temperature and flow were the major environmental variables associated with these movements. The decrease in solar radiation is related to the change in photoperiod, which is a reproductive cue for salmonids (Jonsson and Jonsson, 2011;

Lucas, 2000). According to previous studies, the decrease in water temperature is an important trigger for spawning migrations (Benitez et al., 2015; Clapp et al., 1990; Jensen and Aass, 1995; Ovidio et al., 1998; Zimmer et al., 2010), since it affects the maturation, energetic and metabolic costs (Jonsson and Jonsson, 2011). Likewise, the increase in water flow is considered a stimulant factor (Clapp et al., 1990; Jonsson and Jonsson, 2002; Lucas, 2000; Ovidio, 1999), as it facilitates the overcoming of obstacles (Ovidio and Philippart, 2002) and contributes to predator avoidance (Svendsen et al., 2004). However, extremely high flows during upstream migrations may limit the migratory activity because it is energetically demanding to swim against strong currents (Jonsson and Jonsson, 2002). In contrast to this assumption, in our study the day with the highest number of records (n = 23) occurred during a high flood (03/11/2011 with flows between 33.17 to 59.06 m^3/s). The Porma reservoir provides lower flows than in a non-regulated situation during the spawning migration season (Figure I.2a) due to the water storage for summer irrigation and the flood control that decreases the magnitude, duration and timing of floods (González del Tánago et al., 2016). However, in the study area this effect is slightly diminished compared to the area immediately downstream of the reservoir due to the flow contributions of the Porma Basin between the reservoir dam and the fish farm weir (Figure I.2b).

The increase in water temperature and flow rate were the key factors in the summer migration. Salmonids have low tolerance to high water temperatures (Jonsson and Jonsson, 2009) and move upstream to search for more convenient water temperatures (Clapp et al., 1990; Ovidio, 1999; Zimmer et al., 2010). In the Iberian Peninsula, important upstream movements have been observed in May and June (Santos et al., 2002; Sanz-Ronda et al., 2016b). The cold water released from the bottom outlet of the Porma Reservoir for irrigation during the summer could have delayed these upstream movements until July. Also, the non-natural high flow rates increased the chance of migration of fish fauna. Irrigation is one of the most significant human alterations in Mediterranean streams (González del Tánago et al., 2012) causing higher discharges in summer (instead of drought) when water is released for irrigation, and lower discharges in winter when water is stored.

Daily activity patterns of brown trout differ among populations as a consequence of the different environmental conditions (variation in light conditions, food abundance, presence of predators or temperature regimes) (Clapp et al., 1990). For example, studies of daily activity patterns in potamodromous brown trout natural populations reported activity predominantly during dusk in autumn and winter, during the dusk and night in summer (Ovidio et al., 2002), and during the night during the spawning migration (Ovidio et al., 1998; Rustadbakken et al., 2004). However, studies of fishway monitoring in Iberian potamodromous populations showed more diurnal records during autumn and winter, and more nocturnal records during the rest of the year (Santos et al., 2005, 2002). In our study, upstream movements in the fishway occurred throughout the day, although they were more frequent during day-time with different hourly peaks in each season. During summer, movements were more frequent in the morning when water temperature was lower and flow higher (due to the water release for irrigation), while in the spawning season movements were more frequent in the early morning, as a change in light conditions after sunrise, and during the afternoon, when water temperature was higher. Only in autumn results showed a correlation with rainfall. Rainfall induces changes in ambient (noise, habitat availability, water level, current, transparency, conductivity, oxygen content and temperature), that could induce movements (Lucas et al., 2001). However, the reservoir may affect these natural water flow variations, reducing floods and providing lower water levels and habitat availability (González del Tánago et al., 2012).

5. General conclusions

Brown trout movements were observed throughout the year and were mainly correlated with water temperature and flow rate. This dependence underlines the susceptibility of brown trout migrations to human influence on flow and thermal regimes. River regulation for summer irrigation might have a positive effect on migration due to the high flow rates and a possible delay in these thermoregulatory movements caused by the colder water release. However, during the spawning season regulation may have the opposite effect due to the decrease in the flow as a result of water storage, and the flood control, which in turn decreases the magnitude, duration and timing of flow peaks. These effects may influence the onset and maintenance of migration.

In conclusion, the study of regulated as well as non-regulated streams is vital for a correct management of rivers and in order to identify the potential effects of human activity on the natural behaviour of fish fauna.

6. Acknowledgements

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CHAPTER II:

Potamodromous brown trout movements in the North of the Iberian Peninsula: modelling past, present and future based on continuous fishway monitoring

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Abstract

Brown trout uses river flow and thermal regimens as main stimuli for initiating and maintaining behavioral reactions such as migration and spawning. Therefore, anthropogenic alterations on these factors may have strong impacts on its populations. The aim of this work is to understand these consequences by assessing potamodromous brown trout movements in the past and present, and to model future responses. For this, brown trout movements in a fishway in the Marin River (Bidasoa basin, Northern Iberian Peninsula) have been monitored from 2008 to 2017. Random forest regression has been used to assess the influence of environmental variables on brown trout movements and to model the response under hypothetical climatic and hydrological scenarios. Results show that brown trout uses the fishway during the whole year, with more upstream movements during the spawning season. The model is able to predict accurately the timing and number of migrants. Its use under hypothetical climate change and flow regulation scenarios shows a delay in the migration time. Therefore, modelling using large time series can be a powerful tool to define management and conservation strategies and prepare compensation measures for future scenarios.

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1. Introduction

Freshwater environments are subject to multiple stressors derived from the use that the human society makes of rivers (e.g. irrigation, power generation, flow control or industrial and domestic supply), which can alter ecological patterns and processes (Branco et al., 2016; Segurado et al., 2016). Among these stressors, river fragmentation and alterations on natural river flow and thermal regimens are the most important affecting freshwater ecosystems (Feng et al., 2018; Jones and Petreman, 2015; Nilsson et al., 2005). Near future scenarios suggest an aggravation of this situation, with an increase of the magnitude and number of stressors acting upon river ecosystems and an increase of the possible interactions (Segurado et al., 2016). In one hand, climatic change shows potential alterations in water temperature and in the magnitude, intensity and frequency of rainfall and consequently in river flow (Solomon et al., 2007). In the other hand, human population growth can cause water scarcity due to the increase in water demand and water pollution as result of industrial, domestic and agricultural waste water (Almodóvar et al., 2012; Branco et al., 2016; Segurado et al., 2016; Vörösmarty et al., 2000). Understanding the isolated and combined impact of all these alterations is necessary for the prediction of responses to changing environments and for the establishment of impact mitigation and compensation measures (Segurado et al., 2016).

Fish are one of the most sensible aquatic organisms to these stressors (Clavero et al., 2004). Freshwater fish use flow and thermal regimens as ecological timers for initiating and maintaining behavioral reactions such migration, feeding and spawning (Lucas et al., 2001). More specifically, water temperature has effects on the developmental rate, growth, sexual maturation and food consumption across life-stages whereas water flow influences shelter, feeding, migration and spawning opportunities (Jonsson and Jonsson, 2011). Alterations on these factors can lead in a shift on the phenology and a consequent mismatch between available and necessary resources (Otero et al., 2014), endangering the persistence of many migratory fish species (Shuter et al., 2012), such as the brown trout (*Salmo trutta*, Linnaeus 1758).

Brown trout performs multiple movements along its life cycle, from small-scale migrations, such as alevin post-emergence dispersion from spawning ground over the nearby streambed, to longer ones in order to find feeding, refuge or spawning habitats (Aarestrup et al., 2018; Lucas et al., 2001). This species can display diverse life history tactics, from anadromy (sea trout) to potamodromy (riverine trout), or even partial migrations, with both short (resident trout) and long-distance movements (migratory trout) (Jonsson and Jonsson, 1993). This ecological plasticity gives them the ability to survive and adapt, up to some extent, to potential perturbations over time (e.g. population imbalances, river fragmentation or climatic alterations) (Aarestrup et al., 2018).

Brown trout is a world-wide distributed species. Its natural distribution spreads over Europe, North Africa and West Asia, but also it has been introduced in South Africa, Russia, North and South America among others (Klemetsen et al., 2003). Its migration patterns and cues are affected by latitude, with a local variation dependence on environmental conditions (Aarestrup et al., 2018). In general, most important upstream movements of southern populations occur in autumn and winter (Doadrio, 2002; García-Vega et al., 2017). These movements are related to the search of adequate spawning sites (García de Jalón, 1992) and are usually triggered by changes in photoperiod and water temperature (due to its influence on biological functions (Jonsson and Jonsson, 2011; Thorpe, 1989)) and are favoured by high flows (e.g. overcoming obstacles, predator avoidance (Ovidio and Philippart, 2002; Svendsen et al., 2004)). In addition, thermoregulatory movements outside of the reproductive season have been reported (García-Vega et al., 2017; Ovidio, 1999).

Brown trout has been deeply studied due to its economic and historical importance (Northcote and Lobón-Cerviá, 2008). However, most of available research on brown trout migration has been focused on anadromous populations, mainly during reproductive season or limited time period, and studies in the south of its natural distribution range are still scarce (Benitez et al., 2015; Thurow, 2016). Here, we present a long-term and full-year study (from 2008 to 2017) of potamodromous brown trout (hereafter referred to as trout) migration in a small-size river in the Northern Iberian Peninsula. The main aim of this work is to assess and model trout movements in the past, present and future, using a holistic approach, encompassing biological, climatic and hydrological variables. The specific objectives to achieve this are to: (1) identify periods with most upstream movements and the possible variations among years; (2) evaluate the relation of these movements with biological and environmental variables; (3) develop a model able to predict when upstream movements are more likely to occur; and (4) evaluate the consequences of climate change and flow regulation scenarios in the migration timing.

Unravelling the movement patterns of potamodromous species is therefore important to enhance conservation efforts, overall taking into account the future projections on water demand and climate change. Models based on long time series and environmental and climatic variables can have direct application in the management and conservation strategies. Particularly in climate change and flow regulation scenarios these models can be an essential tool to stablish mitigation measures (e.g. environmental flows, fishways), adequate scheduling of river restoration activities and fisheries stock management.

2. Materials and Methods

2.1 Study area

The study was carried out in the Marin River, a small-size river, tributary of the Bidasoa River (Navarre, Spain). The study reach is located in Oronoz-Mugairi village (ED50 43°8'N, 1°36.5'W) (Figure II.1), with an altitude of 160 m above mean sea level. It is placed in the *trout zone* (Huet, 1954), specifically in the *Epirhithron zone* (Illies and Botoseanu, 1963), and belongs to the A3 category (cobble bed river, low sinuosity and a slope of 0.04-0.099 m m^{-1}) (Rosgen and Silvey, 1996). The mean annual discharge is 2.08 m^3/s and the mean annual water temperature of

11.6°C. According to physical and chemical analysis (mean values of PO_4 = 0.03 mg L^{-1}, NH_4 = 0.03 mg L^{-1}, NO_3 = 5.29 mg L^{-1}, O_2 = 9,77 mg L^{-1}, pH = 8.08; Government of Navarre, 2015), the water quality is *very good* (based on Spanish law RD 817/2015). The main species in this river reach is the potamodromous brown trout, although Atlantic salmon *Salmo salar*, Linnaeus 1758 and European eel *Anguilla anguilla* (Linnaeus 1758) also occur in very low densities. The distance from the sea (51.4 km) and the presence of at least 10 transversal obstacles in the Bidasoa River, downstream of Mugairi fishway (weirs and dams, not all of them were equipped with a fishway, as far as the end of the study date) restricts the presence of anadromous trout in this tributary (Gosset et al., 2006).

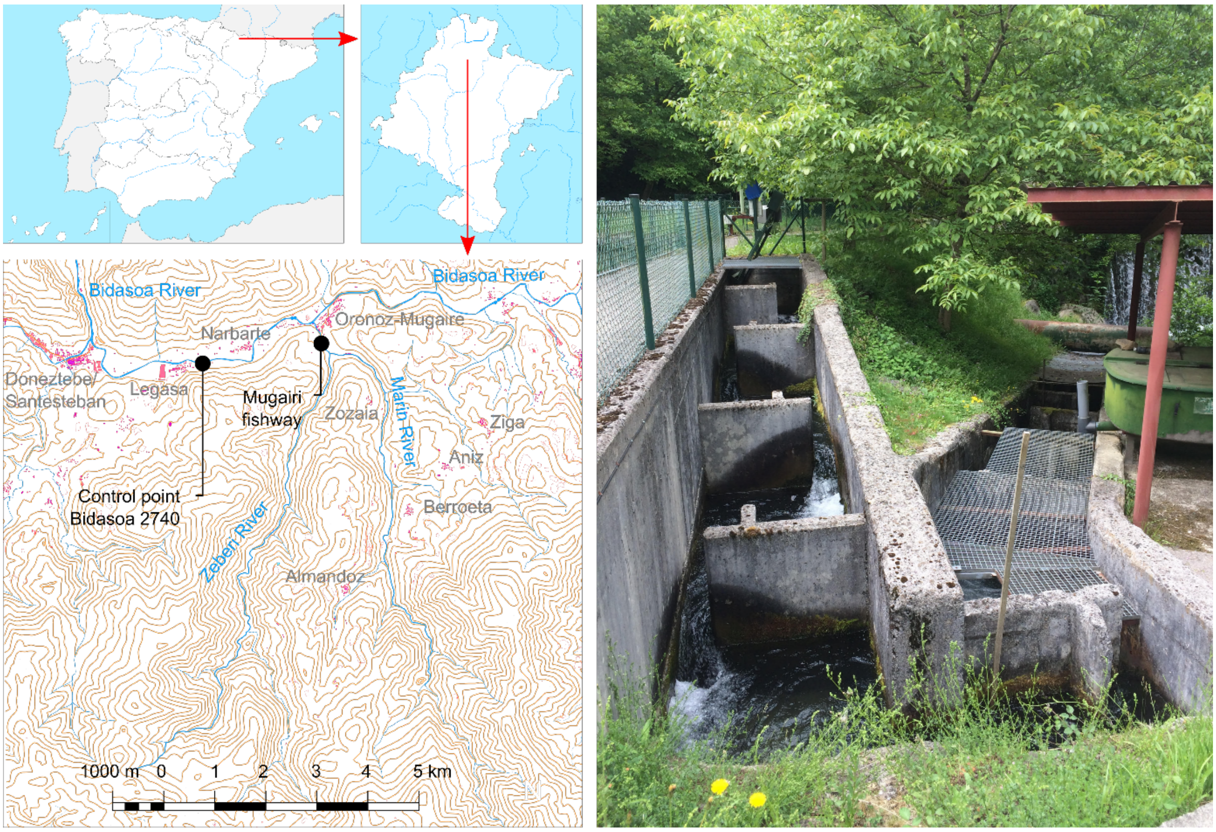

Figure II.1. Location of the Mugairi fishway in the Marin River (Oronoz-Mugairi, Navarre), in the north of the Iberian Peninsula, and picture of the fishway.

To obtain information about trout movements, a continuous monitoring of a fishway was carried out. Fishways, in addition to facilitate the free movement of fish through cross-sectional obstacles in rivers, can be used as structures to study upstream fish movements, as they act as corridors of mandatory passage (Travade and Larinier, 2002). The Mugairi fishway was built in 1994 in a derivation weir of a salmonid fish farm that belongs to the Government of Navarre (Figure II.1). It is a gravity weir of 4.5 m high and 24 m wide. The fishway is a pool and weir type with 14 pools. Pools have an average width and length of 1.3 m and 2.1 m respectively, with a slope of 11%. Each cross-wall has a submerged notch of 0.2 m of mean width and 0.65 m of mean sill height and a squared bottom orifice of 0.17 m mean side length. The fishway design flow was 0.15 m^3/s, with a mean water depth of 0.9 m, water drop of 0.25 m and volumetric power dissipation of 140 W/m^3 during design discharge (Fuentes-Pérez et al., 2017). The geometric and hydraulic parameters inside the fishway are in the range of fishway design recommendations (FAO/DVWK, 2002; Larinier, 2002).

2.2. Sampling procedure

The fishway was monitored by the fish farm staff from 01/09/2008 to 17/03/2017 two-three times a week during the whole year, increasing the monitoring frequency to once a day when high migration rates occurred. Study years were considered starting on 1st September (when spawning movements were more likely to start) and ending on 31st August, and were named with the number of the both integral year (e.g. the study year 2008-09 goes from 01/09/2008 to 31/08/2009).

For sampling, the fishway was closed by means of portable gates in the most downstream cross-wall of the fishway and in the turning pool. The gate of the fishway is equipped with a fish net that prevents fish from exiting the fishway in the upstream direction. Fish were captured with a fish net within the fishway and then transferred to a 1000 L tank with continuous water supply provided by a water pump. Visual identification of the species was carried out. The captured trout were measured (fork length, FL, in mm) and sex identified based on secondary external sexual characters. Mature brown trout usually exhibit sexual dimorphism which allows sex differentiation (male and female), whereas immature individuals are monomorphic, which hinders the sex differentiation (sex unidentified) (Reyes-Gavilán et al., 1997). Finally, trout were released upstream of the weir to continue their migration.

2.3. Environmental variables

Photoperiod, water temperature and river flow were selected as environmental variables that could trigger or be related to the trout migration (García-Vega et al., 2017; Jonsson and Jonsson, 2011). Photoperiod (P, in h) corresponded with the daylength (time between sunrise and sunset) and was calculated with the Brock model (Brock, 1981). Water temperature (T, in °C) was monitored (HOBO Data Logger U14-002, Onset ©) by the Government of Navarre throughout the day at 6 h intervals (water temperature previous to the equipment installation (06/02/2009) and missing values due to technical problems (from 09/10/2013 to 30/01/2014) were completed with a linear regression ($R^2 = 0.8905$) with air temperature (Bertiz weather station, daily frequency) as dependent variable (Webb et al., 2003)). Due to the absence of gauging stations in the Marin River, flow data (Q, in m^3/s) were obtained by river basin comparison (Martínez de Azagra and Navarro, 1996) from its main tributary, the Zeberi River (AN943 Oronoz-Mugariri gauging station, daily frequency, Government of Navarre, 2016a) (Figure II.1).

2.4. Data analysis and modelling

2.4.1. Captured fish characteristics

Frequency analysis of the number of captures by size and sex categories (male, female and unidentified sex) were performed. Binomial test was performed to find differences between number of captures by sex and test for equality of proportions was performed to find differences among years. In addition, Mann-Whitney Wilcoxon (MW) test was used to find differences in FL by sex and Kruskall-Wallis (KW) test was performed to find differences in FL among years. These non-parametric tests were applied as variables were not normally distributed. When KW test was significant, post hoc Dunn's Multiple Comparison Test with Bonferroni correction was performed. In order to identify life stages, distinction between adult or juvenile trout was made. Trout sexual maturation in Iberian rivers is achieved between 2+ and 3+ ages (Doadrio, 2002), which coincides approximately with 20 cm FL in the Bidasoa basin (Laplanche et al., in prep.). This FL is also the minimum catch size described in the Navarre fishing law (Government of Navarre, 2018a). Therefore, 20 cm FL was established as cut-off for adult size.

2.4.2. Captures throughout time

Frequency analysis of the number of captures by month and year were performed. In addition, linear regression between number of captures in the fishway (during the period autumn-winter, i.e. from September to February) and the population density estimates from the Bidasoa River (during the previous August) next to the confluence with the Marin River was carried out to find a relation between the captures in the fishway and the population in the river. Trout population estimates where gathered by the Fishing Service of the Government of Navarre from annual electrofishing surveys (captures of adult and juvenile trout during August) in a control point (ref. Bidasoa 2740) next to Legasa village (Figure II.1).

To check differences in number of captures among months and years, KW and Dunn tests were performed. Moreover, to detect whether pattern of movements varies among years and sexes (no distinction by life stage was considered because the low number of captured juveniles), survival analysis techniques were used, by applying the concept of survival time (time until an event occurs) to migration time (time until a fish is captured in the fishway). This approach was selected because is a powerful statistical analysis to assess differences among categorical independent variables (sex or years) when the dependent variable is time or some function time-dependent (Lee and Wang, 2003). For this Kaplan-Meier (KM) survival curves (Kaplan and Meier, 1958) were determined to show different patterns by sex and to determine the median migration date (the week when the 50% of the captures in the fishway has occurred), for the whole year as well as for the two periods autumn-winter and spring-summer. As the revision periodicity of the fishways varied over time, captures were grouped (summed) by week for the analysis, considering the starting point the first week of September. Log Rank test was used for KM curve comparison (Mantel, 1966). For survival analysis we used the *survival* package (Therneau and Grambsch, 2000) of the *R* software (release 3.2.3, *R* Core Team, 2016). In addition, linear regression between mean weekly FL and time (in weeks) was carried out in order to identify different movement pattern by size throughout the year. Mean FL by week was used as dependent variable in order to get a representative value because the number of captures were different each week.

2.4.3. Environmental variables

Comparisons of the environmental variables among years were performed using KW and Dunn tests. The relation among the environmental variables (mean weekly P, T and Q and their variations with respect to the previous week (actual minus previous) ΔT and ΔQ) was analyzed by means of Spearman correlations.

2.4.4. Modelling past and present scenarios

The influence of the environmental variables on the number of captures was modelled by means of Random Forest (RF) regression. RF has been widely applied in ecology (Breiman, 2001; Cutler et al., 2007) and more recently in freshwater fish studies (Markovic et al., 2012; Vezza et al., 2015), showing good performance in fish abundance prediction and response to environmental alterations (Ward et al., 2014). RF is a statistical ensemble method based on the combination of a multitude of decision trees which are used to determine the mean prediction of the individual trees (Breiman, 2001). For the RF regression, number of weekly captures was considered as dependent variable while the environmental variables (T, ΔT, Q, ΔQ and P) were the independent ones. Due to the low number of captures during the period spring-summer (from March to August), RF regression was only applied for the data of the period autumn-winter (from September to February). Besides quantifying the migration (weekly number of captures), in order to check if the model was able to predict the migration timing (weeks with more number of captures), the predicted values were transformed into proportions for comparison with the observed ones.

We used the *randomForest* (Liaw and Wiener, 2002) *R* package, in which the number of trees to grow was set at 500 while the number of variables randomly sampled as candidates at each split was set at the square root of the number of input variables (recommended default settings). In RF, there is no need for a separate test set for cross-validation as it is performed internally during the run (Breiman, 2001), so RF model was built without data splitting to fully extract the ecological information from the observed data. However, validation of the model was evaluated by the coefficient of determination (R^2) for both, the number of captures (migration quantification) and the proportion of captures by week (migration timing). Because in RF extreme observations are estimated using averages of response values that are closer to those observations, large values of the regression function may be underestimated and small values of the regression function may be overestimated (Zhang and Lu, 2012). This issue was resolved by applying a linear bias correction. The importance of the environmental variables was measured using the increase in mean squared error (MSE) of predictions, which represents how much the model fit decreases when a variable drops of the model (the higher number, the more important). Partial dependence plots for environmental variables were obtained from RF in order to characterize the marginal effect of a variable in the model.

2.4.5. Modelling hypothetical future scenarios

In order to evaluate the influence of climate change and a possible increase in water demand, three hypothetical future scenarios were evaluated with the developed RF model. The scenario 1 represents a climate change scenario with a water temperature increase similar to the increase of air temperature in the A1B scenario of the Intergovernmental Panel on Climate Change (IPCC) for the Southern Europe and Mediterranean (SEM) region: +3.3°C in autumn (September-November) and +2.6°C in winter (December-February) (Solomon et al., 2007), and rest of variables equal to the observed ones. A1B scenario was chosen because, despite the IPCC did not state that any of the climate change scenarios of the report were more likely to occur than others, they used it as baseline scenario for the future regional projections. The scenario 2 represents a river water regulation, with a poor environmental flow, in order to recreate an intensive river regulation, based on the Tennant method: constant flow of 10% of the mean annual flow, that provides the minimum protection of the physical habitat for aquatic fauna (Tennant, 1976), i.e. $Q = 0.208$ m^3/s, but without considering seasonality, i.e. $\Delta Q = 0$, and rest of variables equal to the observed ones. Finally, the scenario 3 is a combination of the scenarios 1 and 2. Due to significant differences on median migration dates and environmental variables among years, the nine studied years were used as base for nine future projections instead only one projection of an averaged scenario.

3. Results

3.1. Captured fish characteristics

In the whole study period, 11228 trout were captured in the fishway, where 39% were male, 60% were female and 1% were sex unidentified (Table II.A.1). Individuals were mainly presented in adult stage (96%). Global sex rate was 1.5 female for each male (test for equality of proportions: $\chi^2 = 57.525$; p-value < 0.0001), with the number of females significantly greater than the number of males in all years, in exception of 2015-16, where both proportions were similar (p-value $= 0.1163$) (Table II.A.1)

FL was in the range 40 - 650 mm, with an average of 297 ± 66 mm and a median of 290 mm. Results of KW test showed that there were significant differences in FL among years ($\chi^2 = 291.28$; p-value < 0.0001), with significantly shorter FL on 2014-15 and longer on 2011-12 and 2013-14 (Table II.A.1 and Table II.A.2). Males were significantly larger than females (except for the years 2009-10 and 2013-14 when there was no difference), and the unidentified sex trout were smaller than the identified ones (Table II.A.1 and Figure II.2).

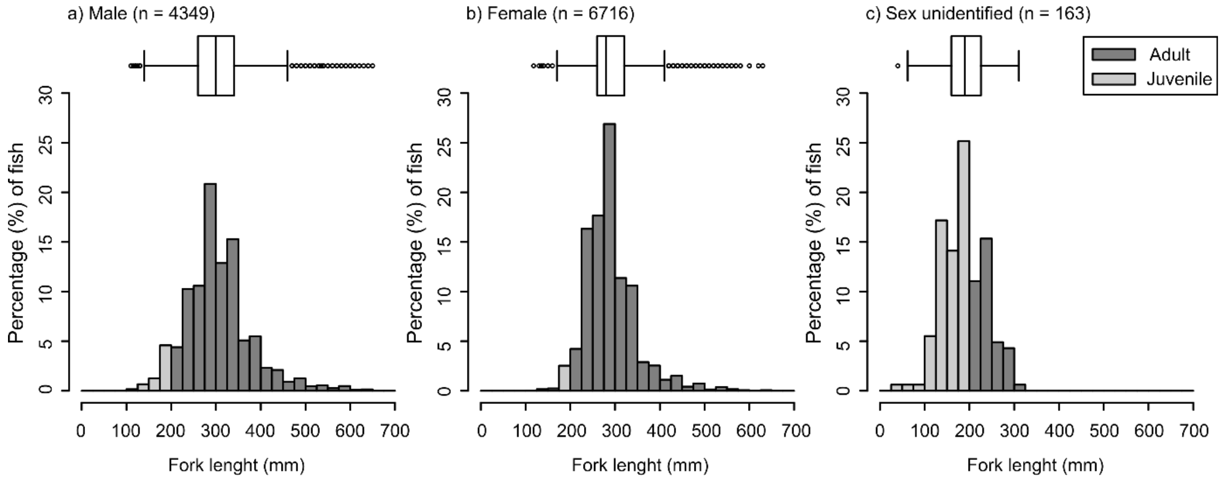

Figure II.2. Boxplot and histogram of the size distribution of the all captured trout by sex and life stage.

3.2. Captures throughout time

Number of captures in the fishway varied among months (Figure II.3 and Table II.A.3). In general, November and December were the months with the highest number of captures (88% of captures). Results of KW test showed that there were significant differences in number of captures among months (χ^2 = 69.193; *p*-value < 0.0001), with November, December and January significantly different to the most part of the remaining months (Table II.A.4). On the other hand, there were not significant differences on number of captures among years (χ^2 = 8; *p*-value = 0.4335). Linear regression showed a positive relation between the number of captures in the Bidasoa River in summer with the captures in the fishway the following autumn-winter (Figure II.4).

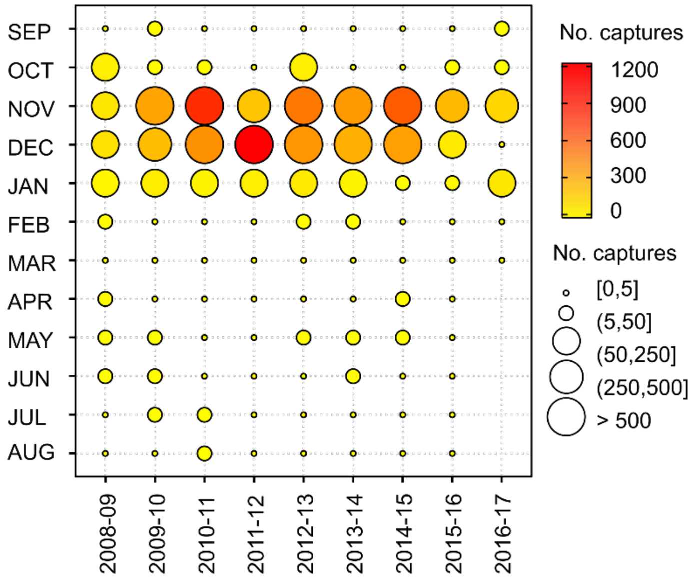

Figure II.3. Heatmap of the number of captures along time. Detailed information about number of captures by month and year, as well as mean values, can be found in Appendix A.

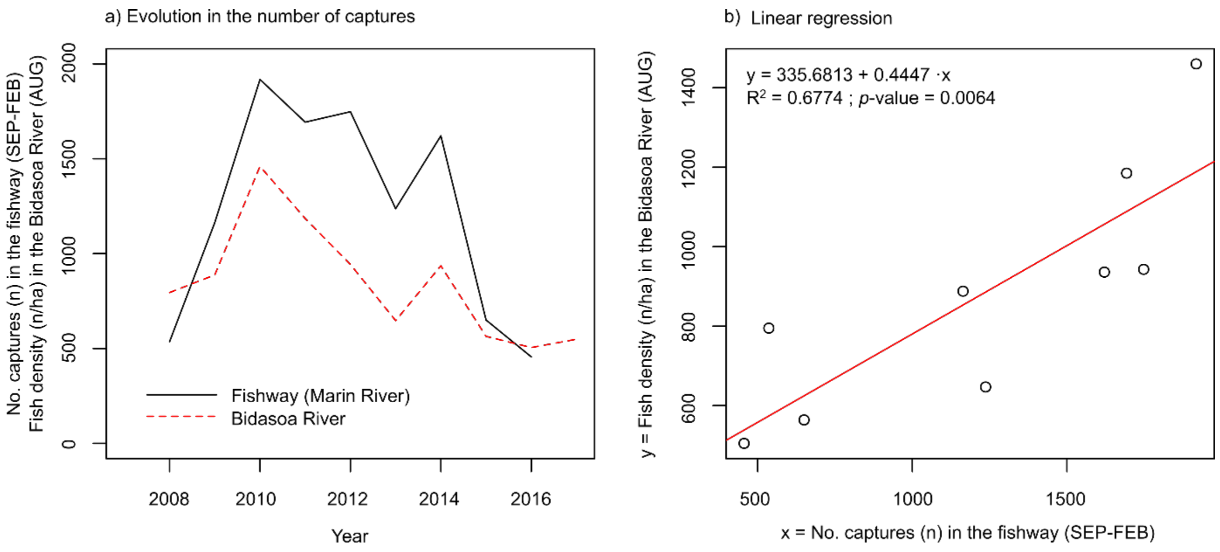

Figure II.4. Comparison between the number of captures in the fishway (Marin River) from September to February and the electrofishing captured trout in the Bidasoa River in August. a) Evolution in the number of captures. b) Linear regression between the number of fish captured in the fishway (Marin River) in September-February (x) and the fish density captured in the Bidasoa River in August (y).

Migration patterns were quite similar in all years (Figure II.5, black solid lines), with more number of captures in November and December. Nevertheless, Log Rank test showed that the global migration curves were significantly different by year (χ^2 = 389, *p*-value < 0.0001) and in 2011-12 the median migration date was considerably longer than other years, whereas in 2009-10, 2010-11 and 2016-17 was shorter (Table II.1).

Table II.1. Comparisons of median migration date (week when the 50% of the captures in the fishway has occurred, starting from September) by year. Results of Log Rank test for Kaplan-Meier curve (Figure II.5) comparison are shown (p stands for p-value).

	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	Total
All captures whole period (SEP-AUG) median migration date	14 (n = 595)	12 (n = 1192)	12 (n = 1953)	16 (n = 1693)	13 (n = 1769)	13 (n = 1263)	14 (n = 1653)	13 (n = 650)	11.5 (n = 460)	14 (n = 11229)
All captures spring-summer (MAR-AUG) median migration date	35 (n = 59)	41 (n = 28)	47 (n = 35)	- (n = 0)	37 (n = 21)	40 (n = 26)	35.5 (n = 32)	- (n = 0)	28 (n = 4)	38 (n = 205)
All captures autumn-winter (SEP-FEB) median migration date	13 (n = 536)	12 (n = 1164)	12 (n = 1918)	16 (n = 1693)	13 (n = 1748)	12 (n = 1237)	14 (n = 1621)	13 (n = 650)	11 (n = 456)	14 (n = 11024)
Male autumn-winter (SEP-FEB) median migration date	12 (n = 151)	11 (n = 491)	12 (n = 741)	16 (n = 624)	13 (n = 659)	12 (n = 495)	14 (n = 654)	13 (n = 304)	11 (n = 203)	13 (n = 4322)
Female autumn-winter (SEP-FEB) median migration date	14 (n = 385)	14 (n = 673)	12 (n = 1176)	16 (n = 1043)	13 (n = 1089)	13 (n = 742)	14 (n = 964)	13 (n = 345)	12 (n = 253)	14 (n = 6671)
Log Rank test (KM curve comparison) male vs female autumn-winter (SEP-FEB)	χ^2 = 2.6 p = 0.107	χ^2 = 12.6 p = 0.0004	χ^2 = 13.0 p = 0.0003	χ^2 = 24.0 p < 0.0001	χ^2 = 12.8 p = 0.0003	χ^2 = 7.7 p = 0.0055	χ^2 = 12.5 p = 0.0004	χ^2 = 0.1 p = 0.8060	χ^2 = 0 p = 0.8570	χ^2 = 66.3 p < 0.0001

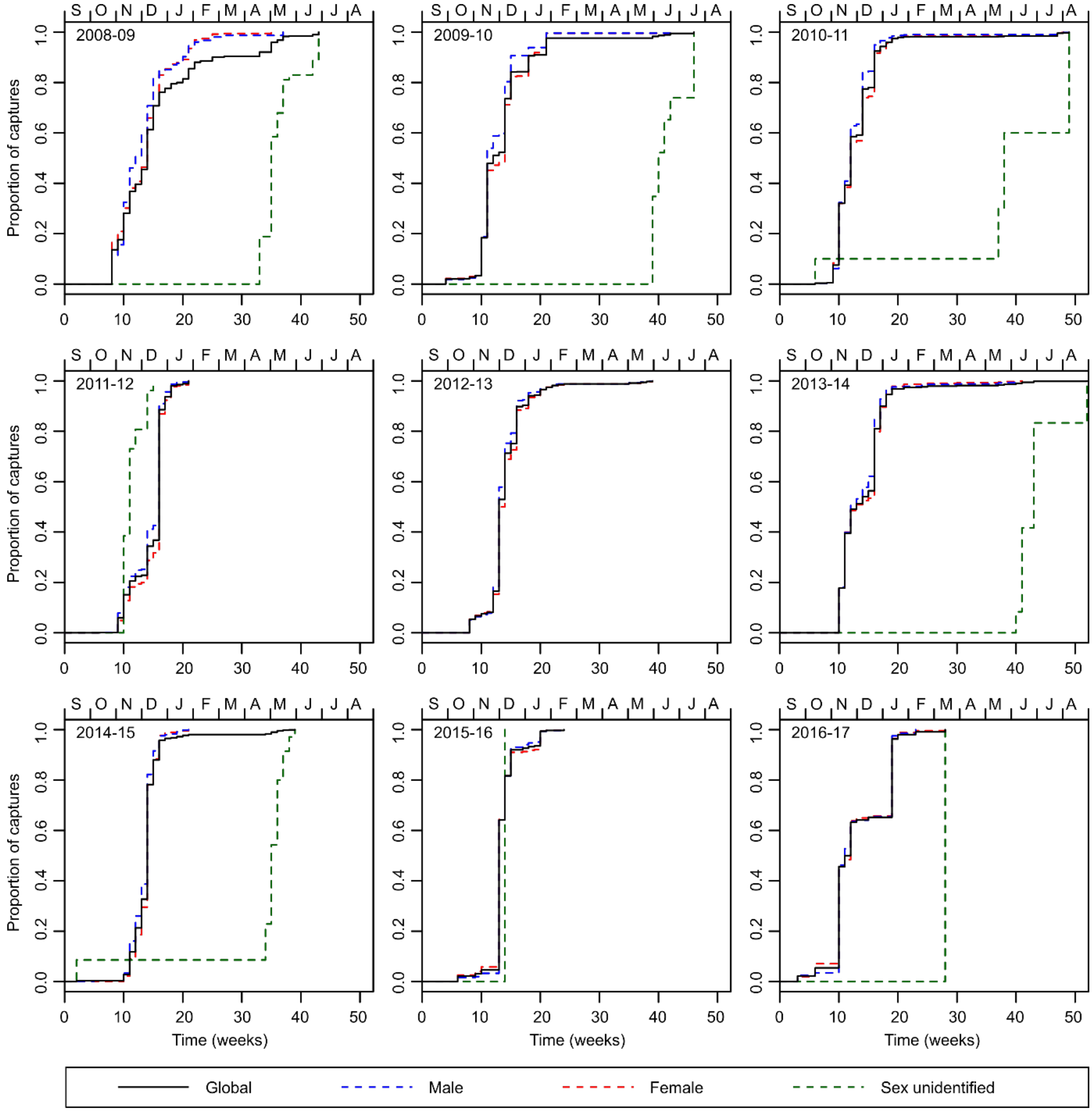

Figure II.5. Kaplan-Meier survival curves by sex category (representation of 1 – S(t) where S(t) is the survival function). Number of fish for each category, year and month are in Table II.A.1 and Table II.A.3.

In the years 2008-09, 2015-16 and 2016-17, years that corresponded with the lowest number of captures, KM curves of females and males were found no significantly different (Figure II.5, red and blue dashed lines respectively, and Table II.1). However, in the remaining years, although the patterns of both curves were quite similar, they were significantly different, with males captured slightly earlier than females and being the curves for the latter slightly more staggered. In the other hand, sex unidentified trout (Figure II.5, green dashed lines, and Table II.1) were captured in all years significantly later than identified ones, except for 2011-12 that they were captured significantly before and in 2015-16 that there were no differences (there was only one sex unidentified trout). Moreover, mean FL significantly decreased with the time, i.e., trout with longer FL (in general and for both sexes) moved, in general, earlier than the shorter ones (Figure II.6).

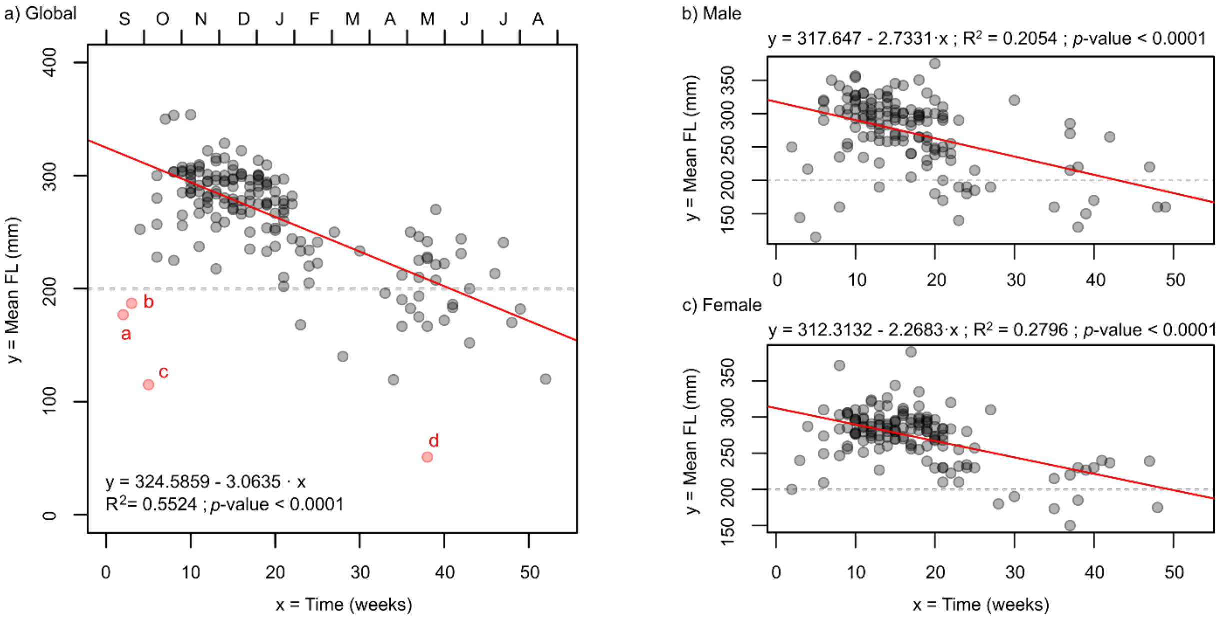

Figure II.6. Scatterplot of the mean FL (y) against time in weeks (x) considering, for a) all captures (where, four outliers (caused by few fish caught) were excluded in the analysis: point a (week 2 of 2014-15, n = 5), b (week 3 of 2016-17, n = 10), c (week 5 of 2014-15, n = 1) and d (week 38 of 2014-15, n = 2)), b) male and c) female captured trout. Red lines represent the regression lines whereas the grey dashed lines are the limit of the size between adults and juveniles.

3.3. Environmental variables

There were not significant differences in the environmental variables among years except for the flow (Table II.A.5), as 2010-11 was the year with the lowest mean annual flow (1.208 m^3/s) with very low values during autumn-winter as well as in spring-summer, and in the other hand, 2012-13 was the year with the highest spring flows (Figure II.7). Table II. 2 shows the range of the environmental variables during the study period as well as the range of each variable within trout movements occurred. For the period autumn-winter, all variables were significantly correlated among them (Table II. 2), in exception of their variation, with only Q and ΔQ significantly correlated.

Table II. 2. Description of the environmental variables. a) Range of variation of the environmental variables (variable range) and range of each variable within trout movements occurred (movement range). b) Spearman correlation test (correlation coefficients (ρ) and p-values). Values above the diagonal correspond to the period March-August and below the diagonal to the period September-February.

a) Range of environmental variables and trout movements						
	Water temperature (°C)		River flow (m³/s)		Photoperiod (h)	
	SEP-FEB	MAR-AUG	SEP-FEB	MAR-AUG	SEP-FEB	MAR-AUG
Variable range	6.7-15.1	7.9-16.5	0.19-24.62	0.23-16.37	8.99-13.24	11.16-15.38
Movement range	7.4-14.8	9.2-14.5	0.20-19.09	0.23-7.73	8.99-12.86	11.26-15.38

Table II. 2. (cont.)

b) Spearman correlations between variables

	Water temperature (T)	Variation in water temperature (ΔT)	River flow (Q)	Variation in river flow (ΔQ)	Photoperiod (P)
Water temperature (T)	1	ρ = 0.0818 *p*-value = 0.2345	ρ = -0.6738 *p*-value < 0.0001	ρ = 0.1020 *p*-value = 0.1377	ρ = 0.5692 *p*-value < 0.0001
Variation in water temperature (ΔT)	ρ = 0.0829 *p*-value = 0.2073	1	ρ = -0.0037 *p*-value = 0.9567	ρ = -0.3473 *p*-value < 0.0001	ρ = -0.0012 *p*-value = 0.9858
River flow (Q)	ρ = -0.6195 *p*-value < 0.0001	ρ = 0.0085 *p*-value = 0.8975	1	ρ = 0.0622 *p*-value = 0.3667	ρ = -0.3941 *p*-value < 0.0001
Variation in river flow (ΔQ)	ρ = 0.0816 *p*-value = 0.2148	ρ = -0.0844 *p*-value = 0.1990	ρ = 0.2438 *p*-value = 0.0002	1	ρ = 0.0472 *p*-value =0.4928
Photoperiod (P)	ρ = 0.6787 *p*-value < 0.0001	ρ = -0.0158 *p*-value = 0.8108	ρ = -0.4282 *p*-value < 0.0001	ρ = 0.0230 *p*-value = 0.7265	1

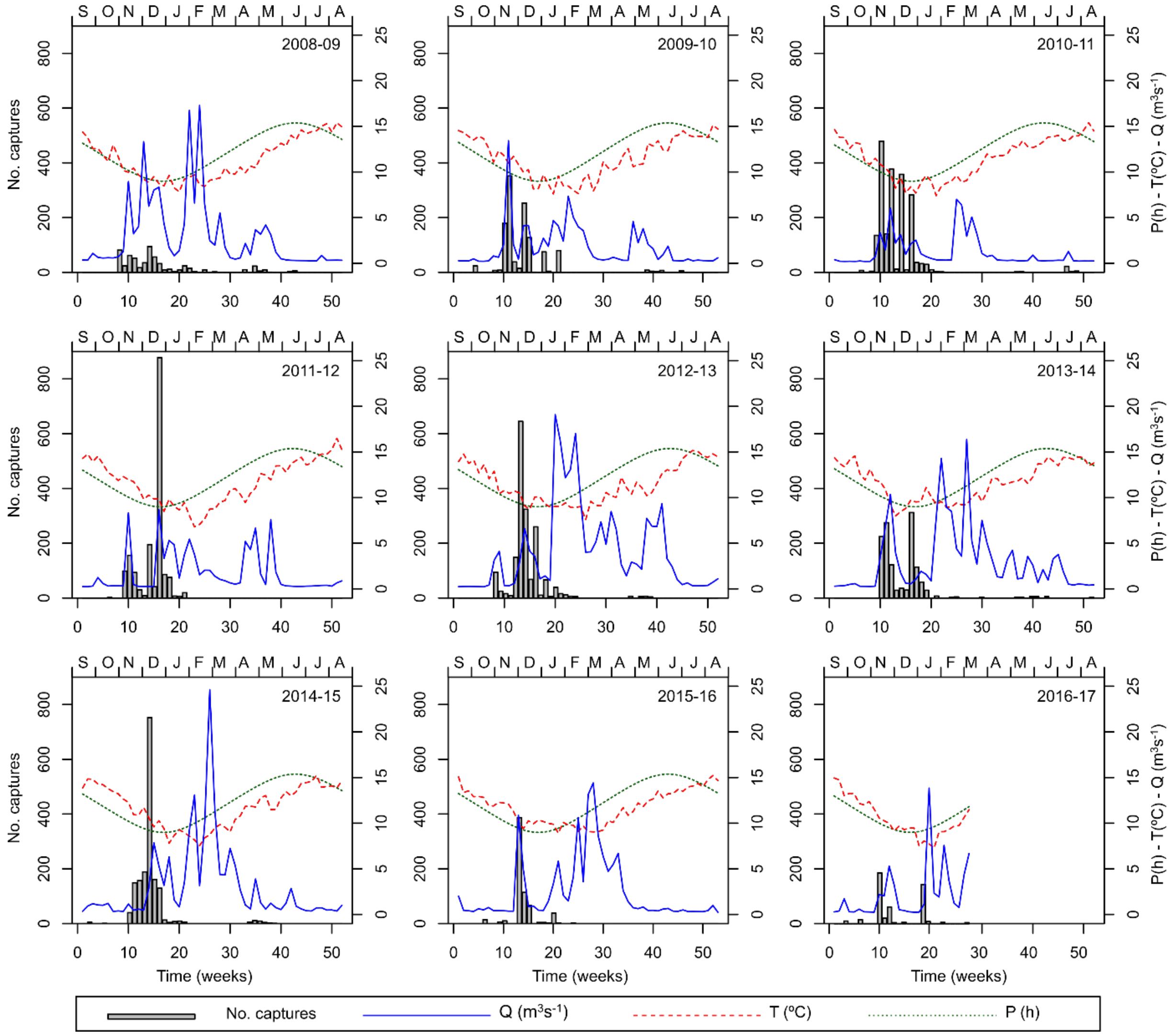

Figure II.7. Mean weekly river flow (Q, in m³/s), water temperature (T, in ºC), photoperiod (P, in h) and number of captures by year for the study period (detailed information of number of captures by month and year can be found in Table II.A.3).

3.4. Modelling past and present scenarios

RF regression model showed a good performance in the prediction of number of captures (R^2 = 0.84, MSE = 2041.77), improving the model to a great extent after the linear bias correction (R^2 = 0.91, MSE = 1250.05; Figure II.8). In addition, RF model was able to predict accurately the migration timing (Figure II.9).

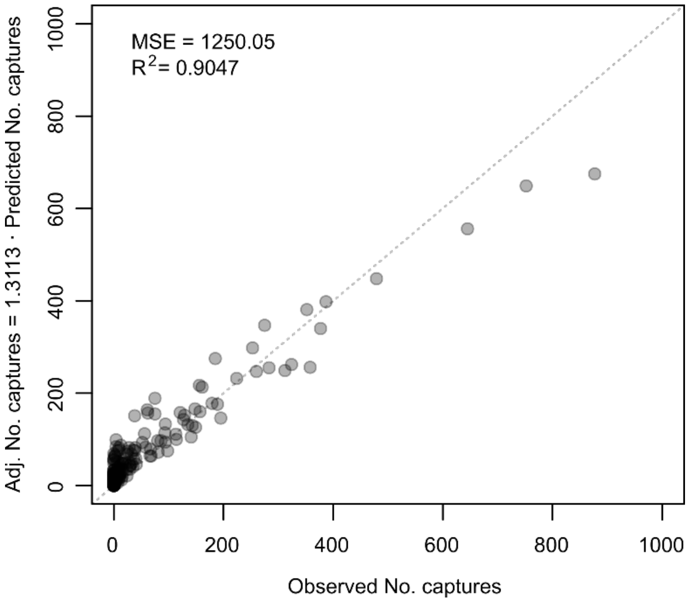

Figure II.8. Observed against predicted counts for RF regression after linear bias adjustment. RF regression was only applied for the data of the period autumn-winter (from September to February) with n = 11023 captured trout.

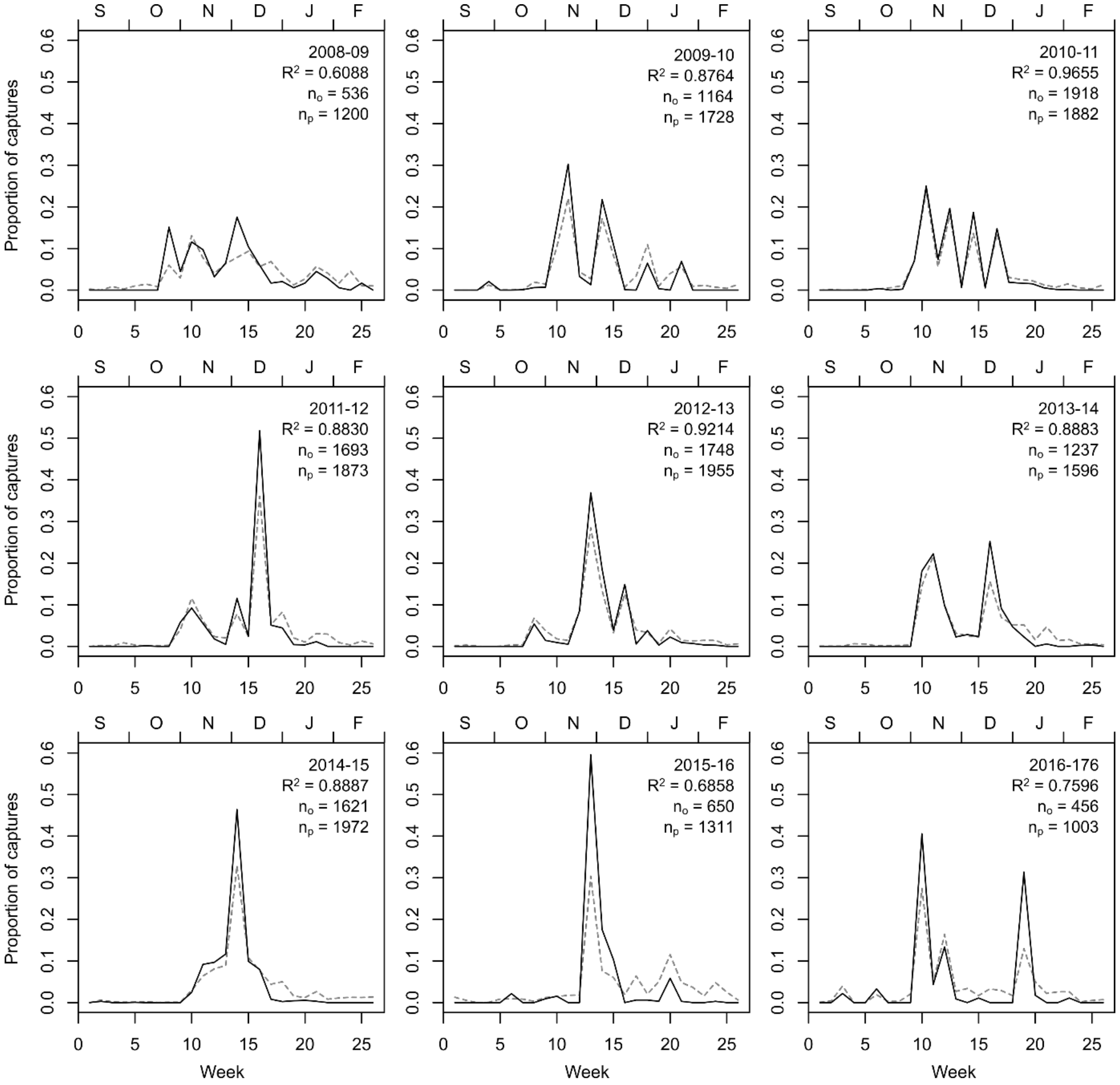

Figure II.9. Evaluation of the migration timing: observed (black solid line) and predicted (gray dashed line) proportion of captures for the period September-February (R^2 = coefficient of determination of the adjustment between the proportion of observed and predicted captures; n_o = observed number of captures; n_p = predicted number of captures).

The most important variable for predicting the number of captures was the water temperature followed by the flow variation respect to the previous week and the river flow, then the photoperiod and in last place the water temperature variation with respect to the previous week (Figure II.7 and Figure II.10a). According to the partial dependence plots, the range 10°C-12°C of water temperature was associated with more captures (Figure II.10b), without a clear pattern in its variation with respect the previous week (Figure II.10c). In addition, low values of photoperiod (< 10 h) showed a high influence on the number of captures (Figure II.10d). Finally, high values of mean weekly flows (Figure II.10e) and positive differences with respect to the previous week (i.e. flow increase) (Figure II.10f) were related to more number of captures, especially in the range from 2 to 4 m^3/s.

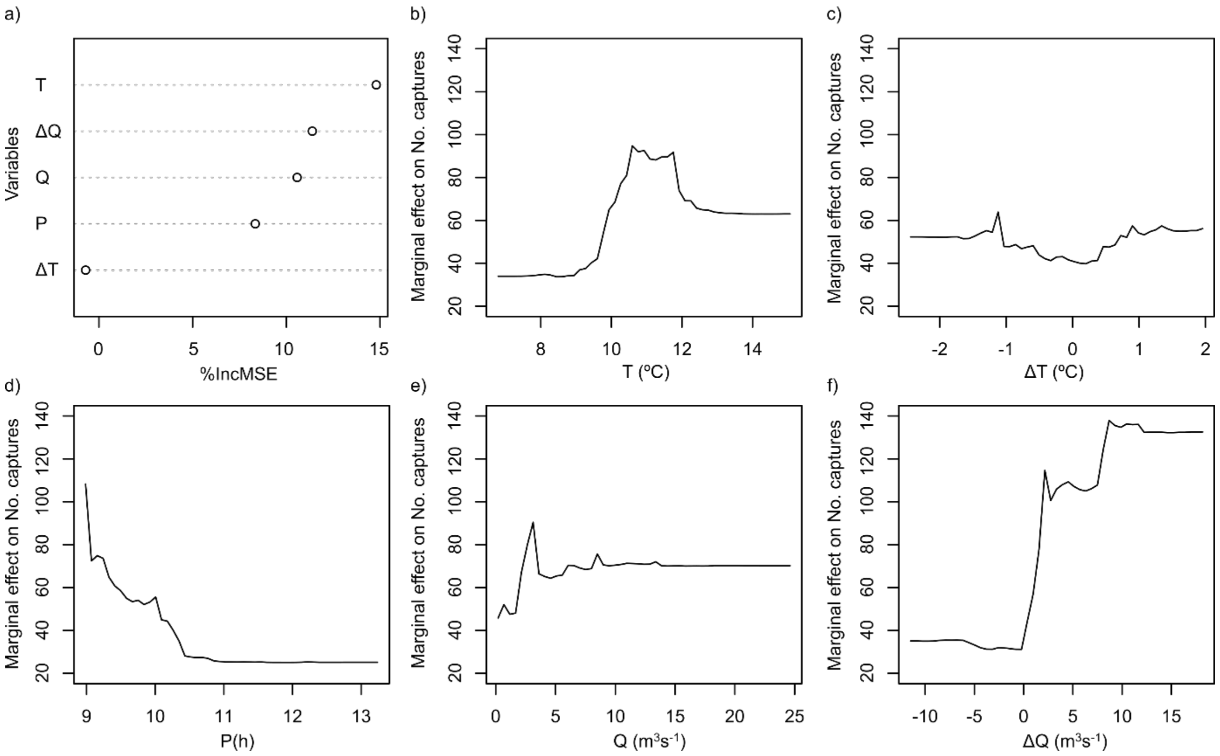

Figure II.10. a) Variable importance of the RF model in terms of increase in mean squared error (IncMSE); b) to f) partial dependence plots.

3.5. Modelling hypothetical future scenarios

Simulated patterns of captures were different for the three hypothetical considered scenarios (Figure II.11). For scenario 1, the captures along time were delayed with respect to the observed in past scenarios (mean delay in median migration date of 4 ± 2 weeks) and more number of captures were expected, whereas scenario 2 and scenario 3 showed a continuous pattern of migration with lower number of captures and with also a delayed median migration date (3 ± 1 weeks and 4 ± 1 weeks respectively) respect to the observed in past scenarios (Figure II.11).

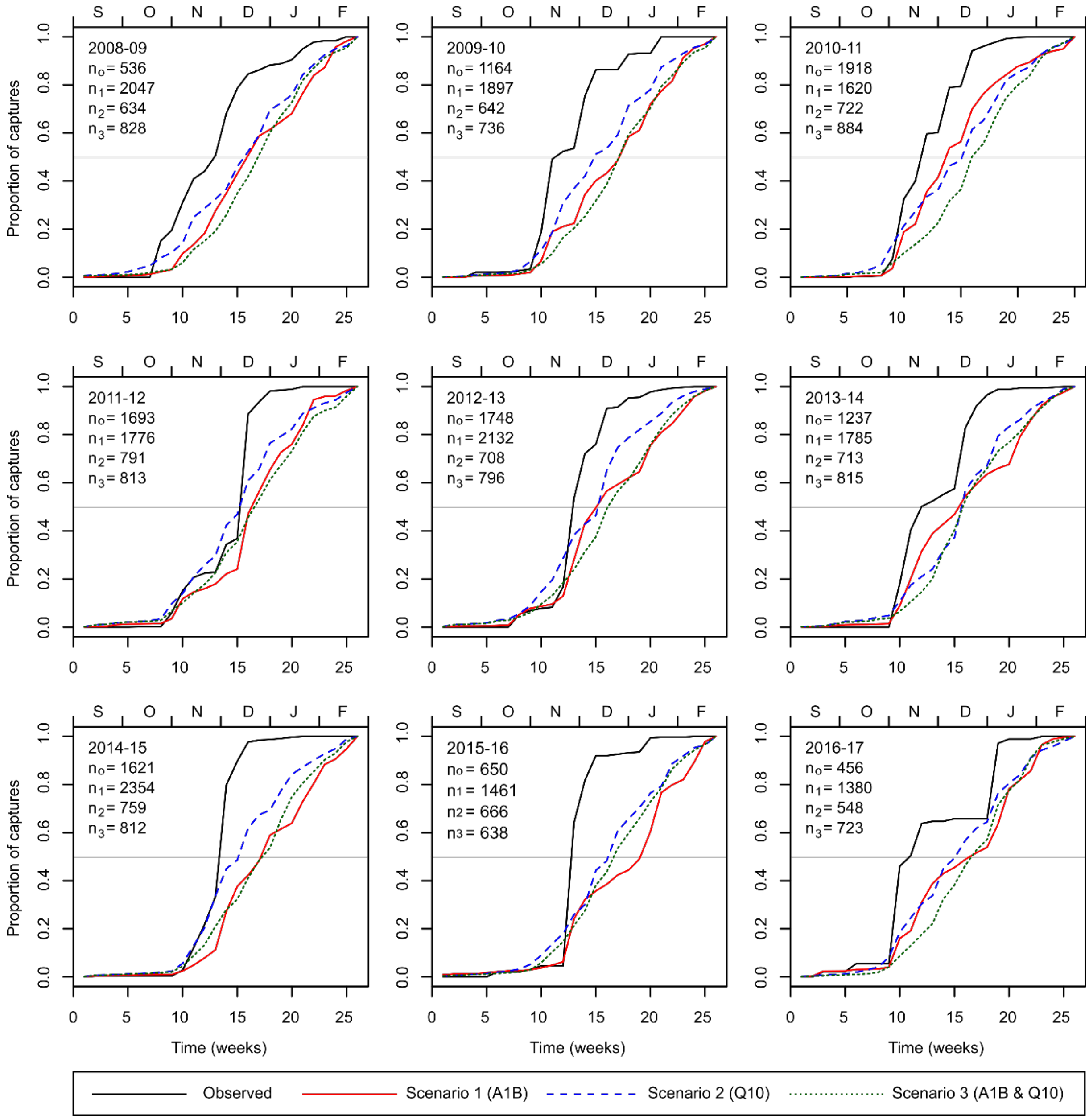

Figure II.11. Cumulative proportions of captures against time for past and future modelled scenarios for the period September-February: (o) observed past scenario; (1) scenario of water temperature increase (same increase as air temperature of IPCC A1B scenario); (2) scenario of water regulation (constant flow of 10% of the mean annual flow); and (3) combination of the scenarios 1 and 2.

4. Discussion

This study analyzes and models, through a long-term and full-year monitoring and with a holistic approach, the upstream migration of potamodromous brown trout over nine years in a river system of the Iberian Peninsula and shows its application to estimate future migration responses to possible climatic change and flow regulation scenarios.

To achieve this, fish captures in a fishway were used. Upstream migration occurred through the fishway. However, due to the configuration of the capture system, downstream migration occurred by the volitional drop of fish over the weir, and therefore, it was not possible to monitor. The number of captures in the fishway was also likely to correspond to high part of the migratory population of the river reach, as captures in the fishway depend on its attraction and passage efficiency, and also on the distance from the original point that fish start the migration (Bunt et al., 2012). Regarding the use by species, apart from a single salmon (female, 810 mm FL, captured on November 2010), potamodromous brown trout was the only species found using the fishway. In this sense, due to the high number of captured trout and their FL range, Mugairi fishway could be considered non-selective by size. Moreover, the found relation between the population in the main stream Bidasoa River (sampled in summer) and the number of trout in the Marin River (captured in autumn-winter) could mean that adult trout live in the Bidasoa River because offers the habitat conditions (water depth and flow velocity among others) within the trout preference range for life and development, and then they migrate upstream to this smaller tributary for searching adequate conditions to spawn during the reproductive season (cf. Armstrong et al. (2003) for a review about trout habitat requirements depending on life stages).

4.1. Past and present scenarios

Captures were concentrated in November and December, extending in some years from October to January. These movements were mainly performed by sex identified adults and with large FL. This time interval agrees with the spawning migration period in the Iberian Peninsula (Doadrio, 2002; García-Vega et al., 2017), although in more southern populations spawning can extend until April (Gortázar et al., 2007; Larios-López et al., 2015).

Migrant female proportion was greater than male proportion, which differs with the observed in the mainstream Bidasoa River and some potamodromous populations of the center of the Iberian Peninsula (Duero and Tajo Basins) where both proportions were found to be similar (Almodóvar et al., 2006; Nicola and Almodóvar, 2002). Nevertheless, it is consistent with the values for anadromous Atlantic populations of the north of the Iberian Peninsula (2-3 females per male), where females are more likely to become anadromous, whereas males tend to stay in the river (Caballero et al., 2018, 2012). So, similary, it is possible that, in short Atlantic rivers as Bidasoa, females move from tributaries to the main stream for improving their growth while males stay in the tributaries becoming more resident.

In addition, our results showed that males migrated slightly earlier than females and larger individuals earlier than shorter ones. This agrees with the fact that, in salmonids, males usually enter the spawning grounds before females, where large males display agonistic behavior to establish and maintain dominance (Esteve, 2017; Jonsson and Jonsson, 2011).

Outside of the spawning period, less movements were observed. This movements were mainly performed by sex unidentified trout with shorter FL, which were associated with juvenile stages, and seem to be related to their ecological requirements as thermoregulatory behavior, feeding or exploration (Lucas et al., 2001). The existence of movements outside the spawning migration period demonstrates the importance of guaranteeing a correct performance of fishways throughout the whole year (García-Vega et al., 2017).

Regarding the developed RF model for predicting the timing and quantification of the migration, it showed a very good performance in the prediction of the migration timing. However, although it also showed a good performance in the prediction of number of captures ($R^2 = 0.91$), some deviations were detected. This can be explained by the existence of other variables not considered in the model, for example, the relation with the population in the Bidasoa River, i.e., captures depend not only on the environmental conditions but also in the available stock in the river. This available stock is the reflect of environmental conditions of previous years, that, in turn, conditions the spawning, larval development, juvenile survival and recruitment of the trout population (Caudill et al., 2013; Flitcroft et al., 2016; Moraes and Deverat, 2016; Young, 1999).

The decrease in photoperiod and water temperature, as well as the increase in river flow were identified as predictable variables in the model. Fish use the photoperiod as indicator of the season because it intervenes in the hormonal regulation during fish maturation (Jonsson, 1991). However, its importance in the model was lower than other variables. This can be explained because the day length is the same on each specific day every year. Thus, it had an important effect to identify the season when spawning migration is more likely to occur, but inter-annual variation in time of migration were induced by other environmental variables. These variables may shift from one river to other or even within the same river (Benitez and Ovidio, 2018). The decrease in water temperature was identified as the most important cue in the Marin River. This variable influences internal physiological processes for gonadal development (Lahnsteiner and Leitner, 2013). However, these changes in water temperature must be inside of a specific range. Lower temperature than this favorable range can retard maturation in fishes (due to its influence on the endocrine function, lipid accumulation and, in general, in the metabolism), whereas higher temperatures can have deleterious effects (gametogenesis inhibition and gamete viability) (Jonsson and Jonsson, 2011). In combination with the decrease in photoperiod and water temperature, river flow showed influence on trout upstream movements. Usually, the increase in water flow is considered a stimulant factor (Clapp et al., 1990; García-Vega et al., 2017) and a facilitator for overcoming obstacles (Ovidio and Philippart, 2002). Our results showed that discrepancies on the onset of migration between years were mainly due to the timing of the flow rate increase.

4.2. Future scenarios

The effects of alterations on river flow and thermal regimens, as climate change or river regulation, particularly affect to the Salmonidae family, which comprises oxygen demanding species that require cold and clean water (Jonsson and Jonsson, 2011), and specially to the brown trout, as it has lower tolerance to high temperatures than other salmonid species (Elliott and Elliott, 2010) and needs relatively high flows during its upstream migrations (Cragg-Hine, 1985) and moderate flows in post-spawning periods (Nicola and Almodóvar, 2002). The effects of climate change are especially relevant in its southern distribution area, where higher temperatures and longer and more often droughts are expected (Hermoso and Clavero, 2011).

RF model showed that water warming in SEM region as consequence of climate change (scenario 1) may suppose mainly a delay on the median spawning migration date. In addition, as the model maintained invariant the rest of parameters, a higher number of migrants was expected. However, this increment does not mean a higher reproductive success and it is expected that climate change will suppose a decline of brown trout population, overall in its southern distribution range (Almodóvar et al., 2012), due to its influence on physiological, behavioral and ecological traits. Physiologically, water warming reduce the gamete quality, and consequently the fertility. Lahnsteiner and Leitner (2013) analyzed the quality of gametes of brown trout under different water temperatures, obtaining fertility rates higher than 65% for males and 80% for females in the when 7.4 ± 4.6 °C, with negative effects on the reproductive potential when temperature surpassed 12 °C. In terms of behavior, water warming may produce a mislead among the cues that affect the timing of the spawning migration, leading a delay on the reproduction. In addition, with warmer climate, a shorter incubation period and earlier emergence time are expected (Jonsson and Jonsson, 2009). Moreover, a mismatch among offspring hatch and their ecological requirements, as food availability (Dingle and Drake, 2007; McLaughlin et al., 2013) or the flow reduction necessary to fish initial development (Nicola et al., 2009) could occur. Finally, the different co-stressors of the water warming, could affect to the ecology of the brown trout, with loss of suitable thermal habitat as the most important impact affecting its populations (Almodóvar et al., 2012).

When a scenario of constant low flow was modelled (scenario 2 of water regulation), in addition of a delay in the median migrate date, it results on a progressive migration, with lower expected number of migrants. This was evident when a mixed scenario of constant low flow and water warming was modelled (scenario 3). Results of the past models showed that the migration occurred in pulses triggered by the local increase in flow rate. So, the anthropogenic alteration in flow regimen may produce the loss of this signal for upstream migration, which enhances the need of an optimal design and delivery of environmental flows. In addition, low flows can affect to the habitat availability, connectivity of habitats, obstacle ascent and increase the vulnerability to predation (Jonsson and Jonsson, 2009). On the other hand, flow reduction in post-spawning periods is essential to promote the survival of early life stages and recruitment (Nicola and Almodóvar, 2002).

5. Conclusions

This study showed that climate change and flow regulation may endanger potamodromous brown trout populations by directly affecting the reproductive migration timing. For past and present migrations, our study showed that a wide size range of potamodromous brown trout performs upstream movements throughout the year, more concentrated during spawning season, but also outside of the reproductive window, with a combination of variables as stimulus for the migration.

The knowledge about migration patterns and the monitoring of fish populations is essential to assess the effect of human impacts (e.g. alterations of river flow and thermal regimens, overfishing, etc.) and in the management and decision making (optimal delivery of environmental flows, correct fishway design and operation, adequate scheduling of river restoration activities or fishing periods and quotas). In addition, modelling large time series can give us information about reach cues of the overall effect of changes in the parameters involved in the migration and, thus, prepare compensation measures and actions for possible impacts.

6. Acknowledgments

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7. Appendix A. Auxiliary tables

Table II.A.1. Characteristics of the captured trout by year and results of comparison tests (n = number of captured trout; FL = fork legth; SD = standar deviation, H0 = null hypothesis of the test).

Variable	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17*	Total
Total n	595	1192	1953	1693	1769	1263	1653	650	460	11228
n male	154 (25.9%)	493 (41.4%)	748 (38.3%)	624 (36.9%)	667 (37.7%)	502 (39.7%)	654 (39.6%)	304 (46.8%)	203 (44.1%)	4349 (38.7%)
n female	388 (65.2%)	676 (56.7%)	1195 (61.2%)	1043 (61.6%)	1102 (62.3%)	749 (59.3%)	964 (58.3%)	345 (53.1%)	254 (55.2%)	6716 (59.8%)
n sex unidentified	53 (8.9%)	23 (1.9%)	10 (0.5%)	26 (1.5%)	0	12 (1%)	35 (2.1%)	1 (0.1%)	3 (0.7%)	163 (1.5%)
Sex rate (female:male)	2.5:1	1.4:1	1.6:1	1.7:1	1.7:1	1.5:1	1.5:1	1.1:1	1.3:1	1.5:1
p-value binomial test (H_0: n male = n female)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1163	0.0192	<0.0001
n adult	585 (94.5%)	1116 (95.6%)	1909 (97.8%)	1629 (96.2%)	1712 (96.8%)	1223 (96.4 %)	1593 (96.7%)	634 (96.1%)	427 (94.9%)	10828 (96.4%)
n juvenile	34 (5.5%)	52 (4.4%)	44 (2.2%)	64 (3.8%)	57 (3.2%)	45 (3.6%)	55 (3.3%)	26 (3.9%)	23 (5.1%)	400 (3.6%)
FL range (mm) global	130-570	125-620	135-580	118-650	120-640	90-630	40-550	150-450	110-460	40-650
Mean FL ± SD (mm) global	287.7±56.4	291.7±63.0	292.4±53.3	318.9±82.0	302.1±75.7	303.3±67.7	280.7±54.5	290.9±51.2	285.8±57.4	296.8±66.1
Median FL (mm) global	290	290	290	300	290	300	280	290	290	290
FL range (mm) male	168-535	125-620	150-580	140-650	120-640	140-600	115-530	160-450	110-460	110-650
Mean FL ± SD (mm) male	303.6±63.6	296.6±75.3	305.1±57.8	341.9±97.4	310.0±82.6	307.6±72.5	289.5±56.6	302.6±52.0	296.4±65.0	307.5±74.1
Median FL (mm) male	310	300	300	330	300	300	290	300	300	300
FL range (mm) female	170-570	150-530	135-530	118-630	130-630	150-630	160-550	150-450	140-440	118-630
Mean FL ± SD (mm) female	291.6±45.8	290.7±51.2	285.248.2±	309.0±65.2	297.2±70.9	302.9±61.7	278.9±48.0	280.7±48.4	279.3±43.2	292.4±57.7
Median FL (mm) female	290	280	280	300	290	290	270	280	280	280
FL range (mm) sex unidentified	130-310	150-260	140-280	140-190	-	90-180	40-270	-	110-140	40-310
Mean FL ± SD (mm) sex unidentified	212.3±46.3	216.0±32.4	203.5±37.9	167.3±15.4	-	150.8±27.5	166.7±50.6	300.0±	126.7±15.3	189.8±47.6
Median FL (mm) sex unidentified	220	220	195	170	-	160	180	300	130	190
p-value MW test (H_0: median FL male = FL female)	0.0054	0.0994	<0.0001	<0.0001	0.0001	0.0762	<0.0001	<0.0001	<0.0001	<0.0001
p-value MW test (H_0: FL sex identified = FL sex unidentified)	<0.0001	<0.0001	<0.0001	<0.0001	-	<0.0001	<0.0001	0.8039	0.0032	<0.0001

* incomplet year of fishway monitoring which was ended at March.

Table II.A.2. Pairwise comparison for differences in FL among years. The upper number is Dunn's pairwise *z* test statistic and the lower number the *p*-value associated with the test.

	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16
2009-10	-0.2495 1.0000							
2010-11	-0.6191 1.0000	-0.4478 1.0000						
2011-12	-7.7696 < 0.0001	-9.4621 < 0.0001	-10.2788 < 0.0001					
2012-13	-2.0428 0.7393	-2.2492 0.4410	-2.0665 0.6980	8.0436 0.0000				
2013-14	-3.8764 0.0019	-4.4629 0.0001	-4.5355 0.0001	4.7750 < 0.0001	-2.6042 0.1658			
2014-15	3.8143 0.0025	5.1287 < 0.0001	6.3242 < 0.0001	15.9826 < 0.0001	8.1607 < 0.0001	10.0368 < 0.0001		
2015-16	-1.1290 1.0000	-1.0568 1.0000	-0.7744 1.0000	6.6367 0.0000	0.7142 1.0000	2.6660 0.1382	-5.3224 < 0.0001	
2016-17	0.2095 1.0000	0.4651 1.0000	0.8103 1.0000	7.2898 0.0000	2.0982 0.6459	3.7782 0.0028	-3.2126 0.0237	1.2647 1.0000

Table II.A.3. Number of captures by month and year. Summaries for autumn-winter (from September to February) and spring-summer (from March to April) periods are shown.

	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	Sum	Mean	SD
SEP	0	24	0	0	0	0	5	0	10	39 (0.3%)	4	8
OCT	105	16	11	2	105	0	1	20	15	275 (2.4%)	31	43
NOV	167	585	1139	383	832	648	977	449	270	5450 (48.5%)	606	327
DEC	190	420	692	1201	662	512	613	137	5	4432 (39.5%)	492	362
JAN	62	119	74	107	138	70	25	42	151	788 (7.0%)	88	43
FEB	12	0	2	0	11	7	0	2	5	39 (0.3%)	4	5
MAR	2	0	0	0	0	3	0	0	4	9 (0.1%)	1	2
APR	33	0	0	0	0	0	16	0	-	49 (0.4%)	6	12
MAY	15	8	5	0	21	6	16	0	-	71 (0.6%)	9	8
JUN	9	14	0	0	0	15	0	0	-	38 (0.3%)	5	7
JUL	0	6	22	0	0	0	0	0	-	28 (0.2%)	4	8
AUG	0	0	8	0	0	2	0	0	-	10 (0.1%)	1	3
Sum	**595**	**1192**	**1953**	**1693**	**1769**	**1263**	**1653**	**650**	**460**	**11228**	**1248**	**563**
Sum SEP-FEB	**536 (90.1%)**	**1164 (97.7%)**	**1918 (98.2%)**	**1693 (100%)**	**1748 (98.8%)**	**1237 (97.9%)**	**1621 (98.1%)**	**650 (100%)**	**456 (99.1%)**	**11023 (98.2%)**	**1225**	**563**
Sum MAR-AUG	**59 (9.9%)**	**28 (2.3%)**	**35 (1.8%)**	**0 (0%)**	**21 (1.2%)**	**26 (2.1%)**	**32 (1.9%)**	**0 (0%)**	**4 (0.9%)**	**205 (1.8%)**	**23**	**19**

Table II.A.4. Pairwise comparison for differences in number of captures by month. The upper number is Dunn's pairwise *z* test statistic and the lower number the *p*-value associated with the test.

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AGO	SEP	OCT	NOV
FEB	2.8040										
	0.1666										
MAR	3.7576	0.9537									
	0.0057	1.0000									
APR	3.3538	0.6336	-0.2916								
	0.0263	1.0000	1.0000								
MAY	2.0950	-0.6252	-1.5504	-1.2233							
	1.0000	1.0000	1.0000	1.0000							
JUN	3.1492	0.4290	-0.4962	-0.1988	1.0245						
	0.0540	1.0000	1.0000	1.0000	1.0000						
JUL	3.5095	0.7893	-0.1359	0.1513	1.3746	0.3501					
	0.0148	1.0000	1.0000	1.0000	1.0000	1.0000					
AGO	3.7319	1.0117	0.0865	0.3674	1.5908	0.5663	0.2161				
	0.0063	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000				
SEP	3.4031	0.5991	-0.3546	-0.0524	1.2064	0.1522	-0.2081	-0.4305			
	0.0220	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000			
OCT	1.5528	-1.2512	-2.2049	-1.8474	-0.5886	-1.6428	-2.0031	-2.2255	-1.8503		
	1.0000	1.0000	0.9063	1.0000	1.0000	1.0000	1.0000	0.8595	1.0000		
NOV	-1.0433	-3.8473	-4.8010	-4.3660	-3.1072	-4.1614	-4.5217	-4.7441	-4.4464	-2.5961	
	1.0000	0.0039	0.0001	0.0004	0.0623	0.0010	0.0002	0.0001	0.0003	0.3112	
DEC	-0.6399	-3.4438	-4.3975	-3.9746	-2.7158	-3.7700	-4.1303	-4.3527	-4.0429	-2.1926	0.4035
	1.0000	0.0189	0.0004	0.0023	0.2182	0.0054	0.0012	0.0004	0.0017	0.9350	1.0000

Table II.A.5. Comparison tests for differences in the environmental variables among years: a) KW test (photoperiod was excluded as it is the same all years). b) Pairwise comparison for differences in mean weekly flow among years. Tthe upper number is Dunn's pairwise z test statistic and the lower number the p-value associated with the test. Values above the diagonal correspond to the spring-summer (MAR-AUG) period and below the diagonal to the autumn-winter (SEP-FEB) period.

a) KW test

Variable	**Whole year (SEP-AUG)**	**Autumn-winter (SEP-FEB)**	**Spring-summer (MAR-AUG)**
Water temperature	chi-squared = 1.6090 *p*-value = 0.9783	chi-squared = 5.5714 *p*-value = 0.6951	chi-squared = 1.1217 *p*-value = 0.9926
River flow	chi-squared = 37.1700 *p*-value < 0.0001	chi-squared = 17.6000 *p*-value = 0.0244	chi-squared = 41.7430 *p*-value < 0.0001

b) Dunn test for river flow variable

	2008-09	**2009-10**	**2010-11**	**2011-12**	**2012-13**	**2013-14**	**2014-15**	**2015-16**
2008-09	-	0.6876 1.0000	1.6733 1.0000	-0.1306 1.0000	-3.1810 0.0205	-2.8289 0.0654	-1.9885 0.6545	-1.0080 1.0000
2009-10	1.7446 1.0000	-	0.9952 1.0000	-0.8194 1.0000	-3.8985 0.0014	-3.5431 0.0055	-2.6948 0.0986	-1.7118 1.0000
2010-11	3.5280 0.0075	1.8006 1.0000	-	-1.8052 0.9947	-4.8842 0.0000	-4.5288 0.0001	-3.6806 0.0033	-2.7070 0.0951
2011-12	2.5819 0.1768	0.8373 1.0000	-0.9715 1.0000	-	-3.0504 0.0320	-2.6983 0.0976	-1.8579 0.8845	-0.8761 1.0000
2012-13	1.3290 1.0000	-0.4156 1.0000	-2.2121 0.4853	-1.2529 1.0000	-	-0.8761 1.0000	1.1924 1.0000	2.2029 0.3864
2013-14	1.5430 1.0000	-0.2016 1.0000	-2.0002 0.8186	-1.0389 1.0000	0.2140 1.0000	-	0.8404 1.0000	1.8475 0.9054
2014-15	0.9011 1.0000	-0.8435 1.0000	-2.6358 0.1511	-1.6808 1.0000	-0.4279 1.0000	-0.6419 1.0000	-	0.9993 1.0000
2015-16	1.9030 1.0000	0.1584 1.0000	-1.6437 1.0000	-0.6789 1.0000	0.5740 1.0000	0.3600 1.0000	1.0019 1.0000	-
2016-17	2.7218 0.1169	0.9772 1.0000	-0.8330 1.0000	0.1399 1.0000	1.3928 1.0000	1.1788 1.0000	1.8207 1.0000	0.8188 1.0000

CHAPTER III:

Reproductive movements of potamodromous cyprinids in the Iberian Peninsula: when environmental variability meets semipermeable barriers

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Abstract

This study aims to describe reproductive migration patterns of *Luciobarbus bocagei* and *Pseudochondrostoma duriense* in a canyon-stretch of the Tormes River (Spain), with high environmental variability and presence of semipermeable barriers. The main objectives were to identify peak migration dates and effects of environmental variables and to propose and test ensemble learning technics as a tool to model and manage fish migration. To achieve this, 5-year fish monitoring in a stepped fishway was carried out and Survival Analysis and Random Forest techniques were used for data analysis and modeling. Results showed a wider migration period than the one considered in literature and regional fishing laws. Movements were strongly related to environmental conditions, but also conditioned by the hydraulic scenario at the semipermeable barriers. Random Forest was able to include the effect of each barrier and predict accurately timing and number of migrants, classifying and ranking the importance of variables. In addition, models allowed the assessment of retrofitting actions and to test the effect of variable environmental flow scenarios on fish movements. This work shows that the study of fish movements must encompass the impacts affecting the river to quantify the importance of environmental factors and to propose cost-effective adaptive management strategies.

García-Vega, A., Fuentes-Pérez, J.F., Bravo-Córdoba, F.J., Ruiz-Legazpi, J., Valbuena-Castro, J. & Sanz-Ronda, F.J. 2020. Reproductive movements of potamodromous cyprinids in the Iberian Peninsula: when environmental variability meets semipermeable barriers. [In review]

1. Introduction

Freshwater ecosystems are among the most threatened in the world (Saunders et al., 2002) and the decline of freshwater fish is a generalized global problem (Jenkins, 2003). Freshwater environments are subject to multiple stressors derived from the use that human society makes of rivers (e.g. irrigation, power generation, flood control or industrial and domestic supply), which can alter ecological patterns and processes (Branco et al., 2016; Segurado et al., 2016). Dudgeon et al. (2006) identified five major threat categories that affect freshwater biodiversity: overexploitation, water pollution, flow modification, habitat degradation and species invasion. These threats still continue to be persistent, have escalated or even evolved in new threats (Reid et al., 2019). Among these stressors, river fragmentation and alterations on natural river flow and thermal regimes are the most important affecting freshwater fish (Feng et al., 2018; Jones and Petreman, 2015; Nilsson et al., 2005), due to the need of moving between different habitats to complete their life cycles (Brönmark et al., 2014; Lucas et al., 2001) and to the different requirements by life stages (Armstrong et al., 2003).

Native Iberian fish fauna presents the greatest European percentage of endemism (Clavero et al., 2004) and it is characterized by a low number of families, most belonging to Cyprinidae family (Doadrio, 2002). The most abundant species are barbels (genus *Barbus* and *Luciobarbus*) and nases (genus *Pseudochondrostoma*, and *Parachondrostoma*), that are characteristic and dominate medium-sized rivers in Iberian Peninsula (Oliveira et al., 2012), being Iberian barbel (*Luciobarbus bocagei* Steindachner, 1865*)* and Northern straight-mouth nase (*Pseudochondrostoma duriense* Coelho, 1985) the most representative cyprinids of the largest Iberian river, the Duero River (Martínez Jiménez, 2006). Both species are endemic of the Iberian Peninsula. Iberian barbel (hereinafter referred to as barbel) is listed as "least concern" by the International Union for Conservation of Nature (IUCN, 2020) whereas Northern straight-mouth nase (hereinafter referred to as nase) is more threatened, being categorized as "vulnerable" (IUCN, 2020) and it is mentioned on Annex II of the European Union Habitats Directive (92/43/EEC).

Both barbel and nase are rheophilic potamodromous cyprinids and occupy a wide range of freshwater habitats, from floodplains to headwaters and play an important role in trophic interactions within their ecosystems (Collares-Pereira et al., 1996; Kottelat and Freyhof, 2007). Their broad distribution and similarities with several cyprinids regarding their physical habitat, lithophilic reproduction strategy, and potamodromous migratory behavior as well as similar biomechanical features (Doadrio, 2002; Kottelat and Freyhof, 2007) make them representative species from the Mediterranean area (Branco et al., 2016; Sanz-Ronda et al., 2019; Silva et al., 2020). As many other species, they need to migrate in order to look for the spawning grounds and reproduce (Lucas et al., 2001). Reproductive season of Iberian cyprinids is usually in spring, between April and June according to specialized literature (Doadrio, 2002; Kottelat and Freyhof, 2007), and they ascend to headwaters looking for reaches of shallow waters with high oxygen concentration and bottoms of sand and pebbles where they place their eggs (Almaça, 1996; SIBIC, 2017). However, there is still scarce information regarding the driving factors during the upstream migration of these endemic species.

In general, fish use environmental variables such as light (e.g. photoperiod, moon cycle), water temperature, hydrology (e.g. river discharge, water depth), meteorology (e.g. rainfall, barometric pressure, etc.), and other chemical information (e.g. salinity, water quality, etc.) as ecological timers for initiating and maintaining behavioral reactions such migration, feeding and spawning (Lucas et al., 2001; Smith, 1985). Thus, alterations on flow and thermal regimes such as those derived from river regulation, water abstraction or pollution, can lead in a shift on the phenology and a consequent mismatch between available and necessary resources (García-Vega et al., 2018; Otero et al., 2014) endangering the persistence of freshwater fish (Shuter et al., 2012). Iberian river's hydrology is featured by seasonality and inter-annual variability. It usually presents high flows during autumn, winter and spring; floods during a few months in late autumn, winter and early spring; and strong summer droughts (Gasith and Resh, 1999). Future scenarios of climate change show potential alterations not only in water temperature but also in the magnitude, intensity and frequency of rainfall and consequently in river discharge (Solomon et al., 2007; Van Vliet et al., 2013), which may exacerbate the natural annual variability. This, together with the expected water scarcity as a result of the increasing water demand and pollution for industrial, domestic and agricultural supply and their waste water (Pittock and Lankford, 2010; Seckler et al., 1999), as well as river fragmentation (Nilsson et al., 2005), may negatively affect freshwater populations (Almodóvar et al., 2012; Branco et al., 2016; Sánchez-Hernández and Nunn, 2016; Segurado et al., 2016; Vörösmarty et al., 2000). Therefore, knowledge of migration patterns and environmental cues affecting them is vital not only to identify fish requirements and constraints, but also for the assessment of anthropogenic impacts and the effectiveness of mitigation measures.

Considering the complexity of interactions presented in some river reaches, modelling techniques based on monitoring time series, that encompass anthropogenic impacts and environmental variables, are essential to define management strategies. Particularly in climate change and flow regulation scenarios, ensemble modelling technics, such as Random Forest (Breiman, 2001), have the potential to establish and/or ~~asses~~ mitigation measures (e.g. environmental flows, fishways), to set adequate scheduling of river restoration activities or to establish smart management strategies of fisheries stock. These technics allow to consider the variability of complex scenarios where the passage is function on environmental conditions and anthropogenic impacts (García-Vega et al., 2020, 2018).

This study aims to describe reproductive migration patterns of Iberian barbel and Northern straight-mouth nase under complex variable scenarios. For this, 5-year monitoring (from 2012 to 2016) in a stepped fishway located in a hydropower complex in the Tormes River (Duero River basin, Spain) was carried out. The specific goals were to (1) detect dates with peak migration during their reproductive upstream movements, (2) identify environmental variables affecting these peak movements as well as the influence of semipermeable obstacles along the study reach, (3) model the migration patterns of these potamodromous cyprinids, and (4) evaluate retrofitting actions and propose adaptive management measures to maximize fish migration under semipermeable barriers.

2. Materials and methods

2.1. Study area

The study area is located in the Tormes River, a tributary of the Duero River (Salamanca, Spain). It starts (from downstream) at the Cespedosa water supply weir (ETRS89 40º31'00''N, 5º35'10''W), located at the tail of the Santa Teresa Reservoir (496 hm^3), and it ends at San Fernando hydropower plant (HPP) dam (ETRS89 40º30'42''N, 5º33'41''W) (Figure III.1). This river reach comprises a 1.8 km length canyon river stretch, with an altitude around 900 m a.s.l. and a mean annual discharge (under natural conditions) of 23.74 m^3/s. Barbel and nase are the most abundant species and comprise the largest proportion of biomass of the river reach. Besides them, the species composition includes brown trout (*Salmo trutta* Linnaeus, 1758), bermejuela (*Achondrostoma arcasii* Steindachner, 1866), Iberian chub (*Squalius carolitertii* Doadrio, 1987) and calandino (*Squalius alburnoides* Steindachner, 1866).

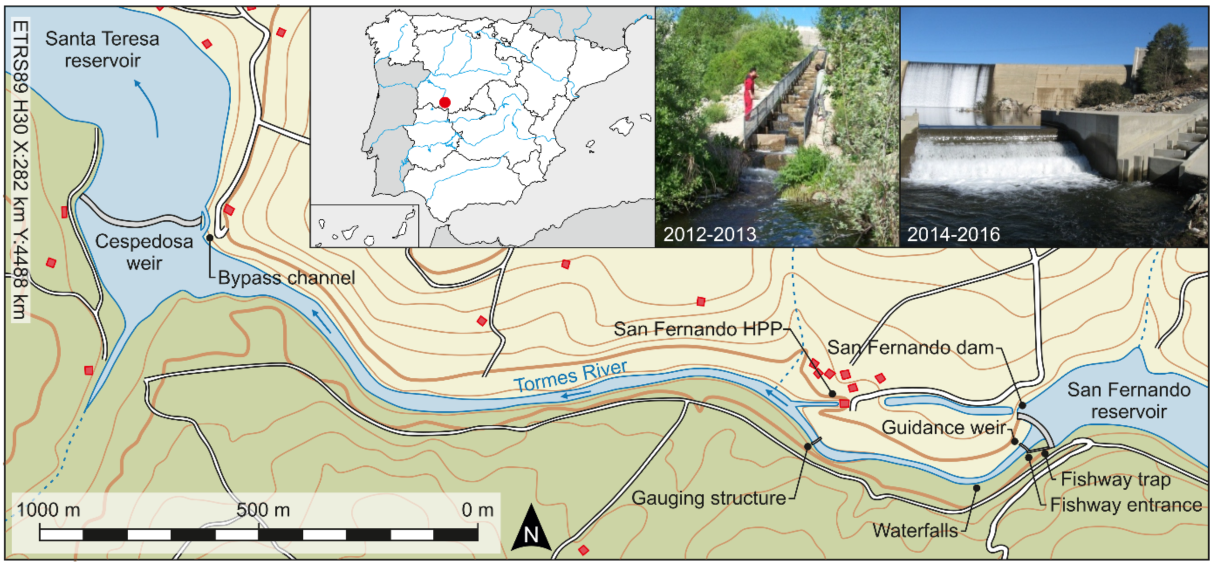


Figure III.1. Location of the study area: from Cespedosa weir to San Fernando dam (arrows indicate the flow direction) and main obstacles for fish migration in the river reach. Pictures of original (2012-2013) and retrofitted fishway with a fish-guiding weir (2014-2016).

The HPP is a run-of-river system, with a dam of 13.4 m height, a diversion channel of 525 m (400 m inlet, 125 m outlet, affecting 660 m length of Tormes River mainstem), a reservoir of 1 hm^3 and an operating capacity of 30 m^3/s and 5000 kW. Environmental flows are strictly fulfilled by the HPP, with a minimum of 2.5 m^3/s required in May and June (theoretical spawning season), and 1.05 m^3/s the rest of the year. Fish data (barbel and nase) were obtained by a trap situated in the stepped fishway associated to this HPP (Figure III.1). The original fishway consisted of 44 pools (pool length = 1.55 m; pool width = 1.75 m; pool water depth = 0.9-1m) connected each other by a free flow notch (sill height = 0.75 m) and a bottom orifice (0.15 m x 0.2 m), with a water drop between pools of 0.3 m (slope = 17.5%; volumetric dissipated power = 150-175 W/m^3) and a design discharge of 140 L/s. A posterior retrofitting of the fishway was carried out during late summer 2013. It consisted on the modification of its downstream part (the first upstream 25 pools and cross-walls were preserved as original), to reduce the power dissipation (125 W/m^3) by modifying pool volume (pool length = 1.6 m; pool width = 1.75 m), slope (water drop = 0.25 m; slope = 14.7 %) and connections between pools (submerged notch width = 0.17 m; notch sill = 0.6 m; orifice surface 0.15 m x 0.2 m). In addition, to improve the location of the entrance and guide the fish, a weir of 2 m height was built downstream of San Fernando dam (Figure III.1). This fish-guiding weir has a lateral notch close to the fishway to create an attractive velocity and turbulence field in the vicinity of the fishway entrance. However, when moderate-high discharge occurs, the excessive turbulence may hinder the fish entry. Further information about this fishway and river reach assessment can be found in Sanz-Ronda et al., 2015a, 2016; CHD, 2017; Pedescoll et al., 2019.

Before reaching the fishway, fish must overcome three main obstacles from Santa Teresa reservoir (Figure III.1):

(1) **Cespedosa water supply weir:** it is a weir of 240 m width and a max height of 2 m. This weir is a semipermeable obstacle, i.e. it is submerged (and passable for fish) when the water level in Santa Teresa reservoir is 882.6 m a.s.l. (i.e. weir top level). In March 2014 a small nature-like bypass channel (slope 10 % and 3 m width) was built for allowing fish migration in the right bank, but its performance is also conditioned by the reservoir water level and river discharge.

(2) **Gauging structure:** a small permeable weir of environmental discharge control operated by the HPP (1 m height with a central notch of 4 m width and 0.5 m depth).

(3) **Natural waterfalls**: natural barriers with variable water drops (between 1 and 3 m). Their permeability depends on the discharge through San Fernando dam.

2.2. Monitoring procedure and environmental variables

Fishway was monitored between mid-April and end-July from 2012 to 2016. Data of fish captures were gathered by HPP staff and authors, and annual reports (CEIBA, 2016; Sanz-Ronda et al., 2015b, 2014, 2013b, 2012) are available at the Fisheries Service of Junta de Castilla y León (www.jcyl.es) and at the Duero River Basin Water Authority (www.chduero.es). During monitoring periods, two sampling methodologies were applied:

(1) **During 2012 and 2013 (original fishway):** a trap in the 5th pool (from upstream, pool size: 3.9 m x 1.7 m) was installed. It consisted in the installation of a mesh in the upper cross-wall for avoiding fish to escape, and a funnel in the downstream notch to allow fish to enter but not to exit (downstream orifice was permanently closed). The trap was

checked 2-4 times a week in the morning (9 am; GMT+02:00). During trap checking, fishway gate was closed so discharge in the whole fishway was interrupted (pools were checked to look for fish before its complete empty). Capturing and handling lasted less than 2 hours. Captured fish were identified, counted and measured (fork length (FL, in cm; ±0.1 cm) and weight (W, in g; ±0.1 g)).

(2) **From 2014 to 2016 (retrofitted fishway):** the trap of the 5th pool was slightly modified with funnels in both downstream notch and orifice. In addition, trap checking periodicity was once a day (9 am) and a discharge bypass was made between 4th and 6th pool, so the fishway discharge was not interrupted during samplings. FL measures were only taken in 2014 (in 2015 and 2016 only the species were identified).

In all cases, before measuring, fish were sedated with a solution of 60-100 mg/L MS-222 (tricaine methanesulfonate) as minimum dosage recommended for cyprinids to measure biometric parameters (Neiffer and Stamper, 2009). As the checking of the trap was made in the first morning hours, captures were assigned to the previous day, as according to the annual monitoring reports fish likely climbed the fishway during the previous day (median transit time in the fishway < 9 h). Finally, fish were released upstream to continue their migration.

Photoperiod, water temperature, river discharge, and level of Santa Teresa Reservoir were considered as main variables influencing barbel and nase movements. Other variables such as rainfall (highly correlated to river discharge), moon cycle (different correlation in each year; usually more important for diadromous species due to the influence in tide height (Smith, 1985), with low influence reported for potamodromous Iberian cyprinids (Rodriguez-Ruiz and Granado-Lorencio, 1992)) or barometric pressure and chemical information (not available data) were discarded for the analysis. Photoperiod (in h) corresponded with the day length (time between sunrise and sunset) and it was calculated with the Brock model (Brock, 1981). Water temperature (in °C) was monitored in the fishway during samplings (at 9 am). Missing values were completed with a linear regression ($R^2 = 0.6711$; p-value < 0.0001; $y = 4.5216 + 0.8075 \cdot x$) with previous day air temperature (weather station ref. AV102 Losar del Barco, daily frequency, www.inforiego.org) as dependent variable (Webb et al., 2003). River discharge data were obtained from the gauging station ref. 2081 Puente Congosto (www.miteco.gob.es) located 5 km upstream the study reach. River section discharge was estimated considering the operating range of the HPP (4.5-30 m^3/s) and the required minimum environmental discharge (May and June: 2.5 m^3/s; rest of the year: 1.05 m^3/s). Finally, daily level data of Santa Teresa Reservoir were obtained from its gauging station ref. 2038 (www.miteco.gob.es).

2.3. Data processing and analysis

All statistical analyses were performed using *R* version 3.5.3 (*R* Core Team, 2019). As the periodicity of the fishway trap checking varied over time, captures were grouped (summed) every three days for all the analyses.

2.3.1. Descriptive statistics

Frequency analysis of the number of captures by species and years were performed. Chi-squared together with post-hoc pairwise chi-square tests were used in order to identify differences in number of captures among years. Kruskall-Wallis (KW) test was performed to find differences in fish size by year. When KW test was significant, post hoc Dunn's multiple comparison test with Bonferroni correction was performed. These non-parametric test were applied as variables were not normally distributed.

To detect whether pattern of movements varied among years, survival analysis techniques were used, by applying the concept of survival time (time (t) until an event occurs) to migration time (time until a fish is captured in the fishway). For this, Kaplan-Meier (KM) survival curves (Kaplan and Meier, 1958) were determined to show possible different patterns and to determine the median migration date (the 3-day period when the 50% of the captures has occurred). Analysis were performed from t = 1 (15th-17th April) to t = 35 (26th-28th July) (total = 3.5 months). Log Rank (LR) test was used for KM curve comparison (Mantel, 1966). For survival analysis the *survival R* package (Therneau and Grambsch, 2000) was used.

The environmental variables were compared among years by using KW and Dunn tests.

2.3.2. Random Forest modelling

To determine the influence of the environmental variables on the number of captures, assess the retrofitting actions in the study site, evaluate the effects of semipermeable obstacles and propose optimal managing strategies on the site, Random Forest (RF) regression was used. RF is a statistical ensemble method based on the combination of a multitude of decision trees which are used to determine the mean prediction of the individual trees (Breiman, 2001). RF has been widely applied in ecology (Breiman, 2001; Cutler et al., 2007) and more recently in freshwater fish studies showing good performance in fish abundance prediction and response to environmental alterations (García-Vega et al., 2018; Markovic et al., 2012; Vezza et al., 2015; Ward et al., 2014). The *randomForest* (Liaw and Wiener, 2002) *R* package was used, in which the number of trees to grow was set at 500 while the number of variables randomly sampled as candidates at each split was set at the square root of the number of input variables (recommended default settings). In RF, unlike linear regression, interactions between different predictor variables are automatically incorporated into the regression tree model (Smith et al., 2013). In addition, there is no need for a separate test set for cross-validation as it is performed internally during the run (Breiman, 2001), so RF model was built without data splitting to fully extract the ecological information from the observed data.

Due to the possible different requirements of both species (barbel and nase) as well as the methodological variability between the period 2012-2013 (original fishway) and 2014-2016 (retrofitted fishway, attraction weir, bypass in Cespedosa weir and sampling procedure) four RF regression models were developed in order to: (1) reduce possible bias, (2) compare retrofitting effects and (3) compare model results by species. For the regression, the number of captures was considered as the dependent variable. Three-day mean of photoperiod (P), river section discharge (Q), and water temperature (T) (continuous variables) of the moment of the capture (t) were selected as independent variables. In addition, variation of river discharge (ΔQ) with respect to the previous three-day period (t-1) (i.e. $\Delta Q = Q_t - Q_{t-1}$) was also included as a continuous independent variable. As descriptors of the passability of the semipermeable barriers, two new binary categorical variables (0,1) were created, one related to the passability of Cespedosa weir (Z_{prev}), that depends on the water level of Santa Teresa reservoir, and the other one related to the necessary discharge to overcome the waterfalls (Q_{prev}). For the former, $Z_{prev} = 1$ if the level of the reservoir was greater than a certain limit (Z_{limit}) (a priori unknown but will be optimized during the run) at least once during the considered period t-i, and $Z_{prev} = 0$ if otherwise. For the latter, $Q_{prev} = 1$ if Q was greater than a certain limit (QW_{limit}) at least once during the considered period t-i, and $Q_{prev} = 0$ if otherwise. In both conditional variables also water temperature limits (TZ_{limit} and TW_{limit} respectively) were considered due to its influence on swimming and jumping capacity of fish (Larinier et al., 2002; Ruiz-Legazpi et al., 2018). Lastly, to consider the possible effect of an excessive turbulence in the fishway as consequence of the notch at the fish-guiding weir (only for 2014-2016 period), an additional binary variable was defined ($Q_{entrance}$). For this variable, $Q_{entrance} = 1$ if Q was greater than a certain discharge limit (QE_{limit}) during the moment t, and $Q_{entrance} = 0$ if otherwise.

Z_{limit}, TZ_{limit}, QW_{limit}, TW_{limit} and QE_{limit} as well as the considered time period (t-i) for the definition of Z_{prev} and Q_{prev} were determined by an automatic optimization search in the four RF models, to maximize the coefficient of determination (R^2) and minimize the mean squared error (MSE).

Additionally, as in RF extreme observations are estimated using averages of response values that are closer to those observations, large values of the regression function may be underestimated and small values of the regression function may be overestimated (Zhang and Lu, 2012). This issue was resolved by applying a linear bias correction.

To get reliable and optimized models, a custom designed backward stepwise procedure was programmed in *R* to discard variables with low or none contribution in the model. Each model started with all *p* predictors. Then, the least important predictor (the one with lower contribution in R^2) was removed and a new RF model was estimated using $p - 1$ predictors, until all selected predictors contributed more than 0.02 units in R^2 respect to the starting full model. The final four models were evaluated by the R^2 for both, the number of captures (migration quantification) and the proportion of captures every 3-day period (migration timing). The importance of the variables was measured using the increase in mean squared error of predictions (%IncMSE), which represents how much the model fit decreases when a variable drops of the model and the increase in node purity (IncNodePurity), that is used to measure the quality of a split for every variable (node) of a tree (it is calculated by the difference between the sum of squared residuals before and after the split on that variable). For both metrics, the higher the number, the more important it is. Partial dependence plots for environmental variables were obtained from RF in order to characterize the marginal effect of a variable in the model (i.e. the impact that a unit change in one of the independent variables has on the outcome variable while all other variables remain constant).

Lastly, to assess the possible effects of the retrofitting actions during 2014-2016 and to show the potential of RF models as a managing and decision tool, two modelling scenarios were created. On the one hand, the first scenario consisted on the use of 2012-2013 environmental data to predict the potential number of captures if retrofitting actions were previously implemented. For this, the full model of 2014-2016 was applied to the 2012-2013 environmental data, to assess the differences in number of captures and timing with respect to 2012-2013 captures. On the other hand, due to the influence of environmental discharge in the passability of the semipermeable barrier prior to the fishway, a second scenario was proposed. It consisted on a variable managing strategy of the environmental discharge to improve fish migration in the river reach during reproductive season (April-July). For this, data of the period 2014-2016 was used, although with variations in the river discharge of the study section. This variation consisted in the periodical (every *i* three-day) augment of Q (and thus, Q_{prev}) up to QW_{limit} when the temperature was in an adequate range (>TW_{limit}) and such discharge was available in the river. With this modified environmental dataset, 2014-2016 full model was applied and the differences in the number of captures and timing were assessed.

3. Results

3.1. Fish characteristics

In the whole study period, 38908 fish were captured in the fishway during their reproductive upstream migration. Barbel was present in a higher proportion (n = 27890, 72%) than nase (n = 11018, 28%), although inter-annual differences in number were observed (all Chi-squared test p-values < 0.0001) (Figure III.2a). According to post-hoc pairwise chi-square tests, barbel only presented no differences in number of migrants in 2013 and 2015 (p-value = 0.18), whereas nase in 2014 and 2016 (p-value = 0.252).

Barbel fork length ranged from 17 to 55 cm (median = 33.5 cm; mean = 33.7 ± 3.4 cm) with significant differences among years (Figure III.2b, KW test p-value < 0.0001). In the case of nase, fork length ranged from 7 to 33 cm (median = 15 cm; mean = 14.9 ± 2 cm) with also significant differences among years for nase (Figure III.2b, KW test p-value < 0.0001) although without differences between 2012 and 2014 according to Dunn pairwise test (2012-2013 p-value = 0.0001; 2012-2014 p-value = 0.0684: 2013-2014 = 0.0001). Both fork length–weight resulted allometric growth models following the equations $W = 0.0199 \cdot FL^{2.8309}$ (R^2 = 0.8950) for barbel and $W = 0.0171 \cdot FL^{2.8642}$ (R^2 = 0.9605) for nase.

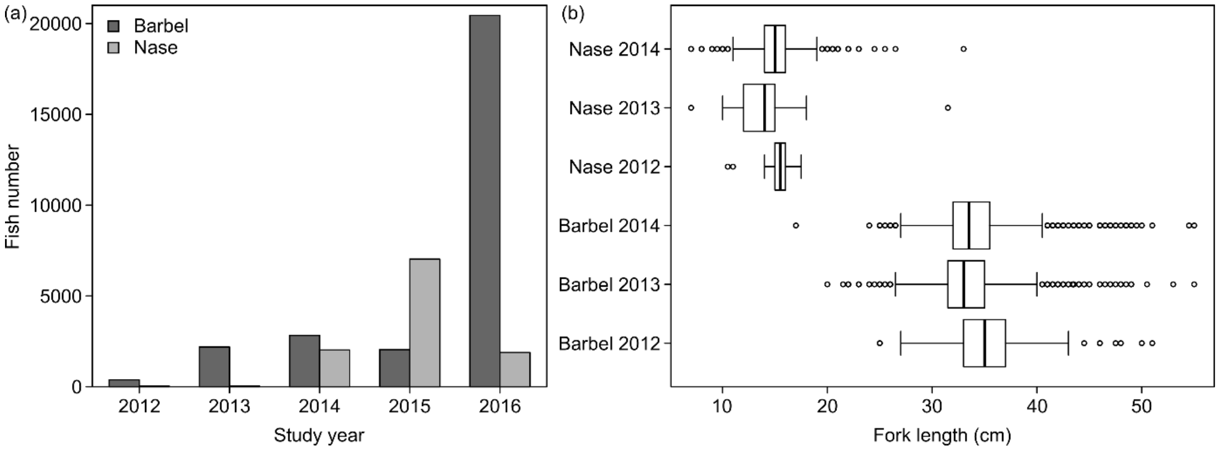


Figure III.2. (a) Number of captures by year and species. (b) Fork length boxplot by species and year (FL data available only in 2012-2014).

3.2. Migration dates and patterns

Most part of total captures in the fishway occurred from mid-May to mid-June (barbel 86.89%, nase 77.89%). However, the percentage of captures during this one-month period varied among years (barbel 2012 = 91.97%, 2013 = 87.22%, 2014 = 81.54%, 2015 = 46.64%, 2016 = 92.97%; nase 2012 = 88.00%, 2013 = 79.25%, 2014 = 60.14%, 2015 = 90.25%, 2016 = 52.25%), with migration extended to mid-July in some years (barbel 2013 and nase 2013, 2014 and 2016) (Figure III.3 and Figure III.4) or low number of captures during May (e.g. nase in 2016). Barbel and nase presented different global migration patterns (Figure III.4a; LR test *p*-value < 0.0001), with median migration dates of *t* = 18 (5 – 7 June) for barbel and *t* = 16 (30 May – 1 June) for nase Figure III.4a; LR test *p*-value < 0.0001). In addition, significant differences among years were found (Table III.1, Figure III.3 and Figure III.4b-f). In 2012, 2014 and 2016, barbel migration occurred earlier than nase, whereas in 2013 and 2015 nase migrated earlier than barbel. In addition, both histograms and KM curves showed that migrations in 2014-2016 occurred in several peaks along the migration period (Figure III.3 and Figure III.4).

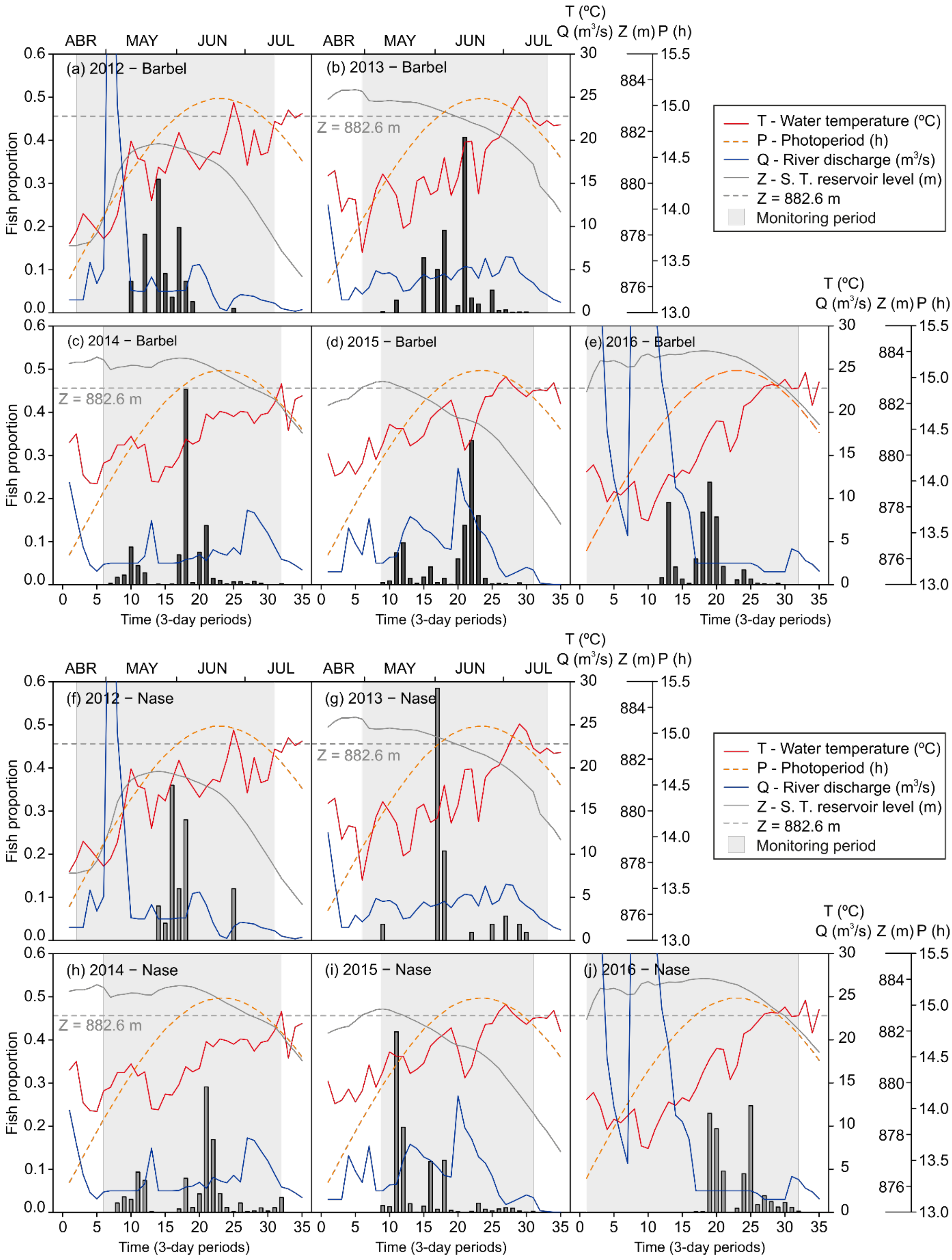


Figure III.3. Proportion of upstream migrants of barbel and nase. Mean (3-day period) of water temperature (T), photoperiod (P), river discharge in the study section (Q) and Santa Teresa reservoir level (Z) (horizontal dashed line = Cespedosa weir level = 882.6 m). Shadow area represents the monitoring period for each year.

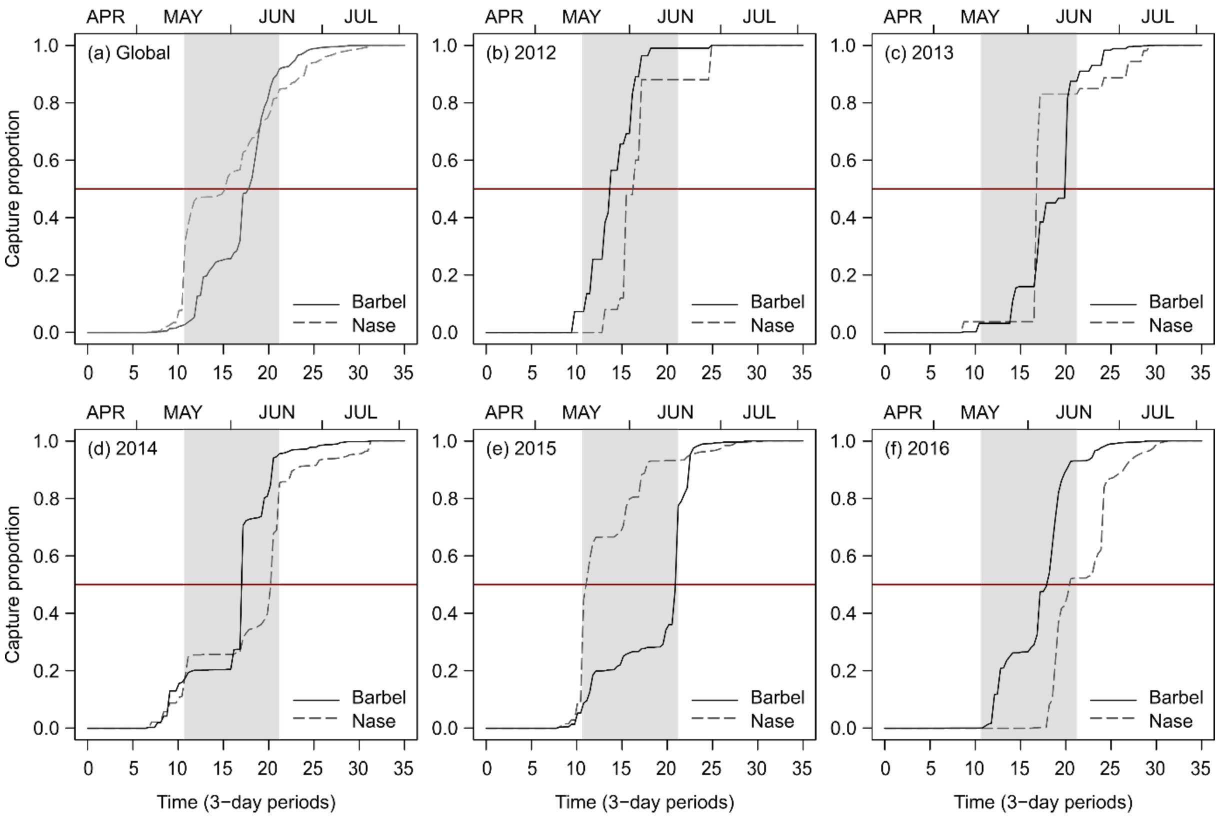


Figure III.4. Migration patterns (KM survival curves) by year and species (horizontal line = 50% of captures; shadow area: fishing closure period, from 15th May to 15th June).

Table III.1. Median migration dates (the 3-day period when the 50% of the total number of captures has occurred; captures were grouped every 3 days from $t = 1$ (15th-17th April) to $t = 35$ (26th-28th July)) and Long Rank (LR) tests for curve comparison among years and by species (n = fish number; 0.95LCL = lower 95% confidence limit; 0.95UCL = upper 95% confidence limit; p = p-value).

Species	Year	n	Median	Median (date)	0.95LCL	0.95UCL	Min	Max	LR test
Barbel	2012	384	14	24-26 May	14	14	10	25	All and pairwise: $p < 0.05$
	2013	2191	21	14-16 June	21	21	9	30	
	2014	2827	18	5-7 June	18	18	7	32	
	2015	2037	22	17-19 June	21	22	9	29	
	2016	20451	19	8-10 June	18	18	5	32	
Species	**Year**	**n**	**Median**	**Median (date)**	**0.95LCL**	**0.95UCL**	**Min**	**Max**	**LR test**
Nase	2012	25	17	2-4 June	16	18	14	25	All and pairwise: $p < 0.05$
	2013	53	17	2-4 June	17	18	9	30	
	2014	2027	21	14-16 June	21	21	8	32	
	2015	7026	12	18-20 May	12	12	9	31	
	2016	1887	21	14-16 June	21	23	17	32	
LR test			**Global**	**2012**	**2013**	**2014**	**2015**		**2016**
Barbel vs Nase			< 0.0001	0.0001	0.2	< 0.0001	< 0.0001		< 0.0001

3.3. Migration and environmental variables

There were significant differences in daily values among years (only considering period from mid-April to end-July) of water temperature (KW test: p-value < 0.0001; mean T = 17.3±4.5 ºC;

range = 6.4 – 26.8 ºC), river discharge (KW test: *p*-value < 0.0001; mean Q = 7±16.48 m^3/s; range = 0.032 – 164.606 m^3/s) and water level of Santa Teresa Reservoir (KW test: *p*-value < 0.0001; mean Z = 881.9±1.9 m; range = 876.3 – 884.0 m) (Figure III.3 and **Appendix A** in **Supplementary data**). Photoperiod (KW test: *p*-value = 1; mean P = 14.56±0.52 h; range = 13.24 – 15.07 h) is the same for all years.

Most part of fish captures in the fishway occurred between 16 and 21ºC of mean water temperature (73% and 94% of total captures of barbel and nase respectively), although with different thermal ranges varying among years and species (Figure III.3 and **Fig. A1** in **Appendix B** in **Supplementary data**). For example, in 2016 there was an important peak of barbel movements (31% of 2016 captures) between 12 and 14ºC (Figure III.3), whereas the 58.5% of 2013 nase captures occurred near 15ºC (Figure III.3). Regarding river discharge, most of the captures of barbel in the fishway occurred with river discharges from 2.5 to 3 m^3/s (near to the minimum legal environmental discharge) in 2012 (89%), 2014 (73%) and 2016 (67%) whereas they were above 4 m^3/s in 2013 (88%) and 2015 (90%). For the nase, most of the captures in 2012 (88%) and 2016 (96%) occurred with discharge similar to the minimum legal environmental discharge. In 2014 the range of peak captures (97%) in the fishway varied between 2.5 and 4 m^3/s, whereas they were between 3.5 and 8 m^3/s in 2013 (100%) and 2015 (91%).

Full RF models showed a good performance in the prediction of both number and timing of captures, with similar or even better results after variable reduction procedure (Table III.2 and Figure III.5).

Table III.2. RF models will all *p* predictors (full model) and after back stepwise procedure (optimized model) ($i = 5$, $Z_{limit} = 882.3$ m for barbel and $Z_{limit} = 882.4$ m for nase (no influence of a TZ_{limit} was detected during optimization), $QE_{limit} = 4$ m^3/s, $QW_{limit} = 10$ m^3/s and $TW_{limit} = 13$ºC).

		Barbel		Nase	
		Full model	**Optimized model**	**Full model**	**Optimized model**
2012-2013	**Predictors**	$Z_{prev} + Q_{prev} + \Delta Q + Q + T + P$	$Q + T$	$Z_{prev} + Q_{prev} + \Delta Q + Q + T + P$	$\Delta Q + T + P$
	Global *R*²	0.8164	0.8926	0.9714	0.9653
	MSE	3288.16	1923.86	0.57	0.69
	***R*² 2012**	0.3847	0.3473	0.8655	0.8449
	***R*² 2013**	0.7654	0.8436	0.9682	0.9847
2014-2016	**Predictors**	$Z_{prev} + Q_{prev} + \Delta Q + Q_{entrance} + Q + T + P$	$Z_{prev} + Q_{prev} + \Delta Q + Q + T + P$	$Z_{prev} + Q_{prev} + \Delta Q + Q_{entrance} + Q + T + P$	$\Delta Q + Q + T + P$
	Global *R*²	0.9228	0.928	0.9195	0.9203
	MSE	56125.72	52350.1	11598.6	11483.67
	***R*² 2014**	0.5331	0.5153	0.5267	0.5806
	***R*² 2015**	0.4634	0.2658	0.9515	0.9498
	***R*² 2016**	0.9241	0.9382	0.8795	0.8568

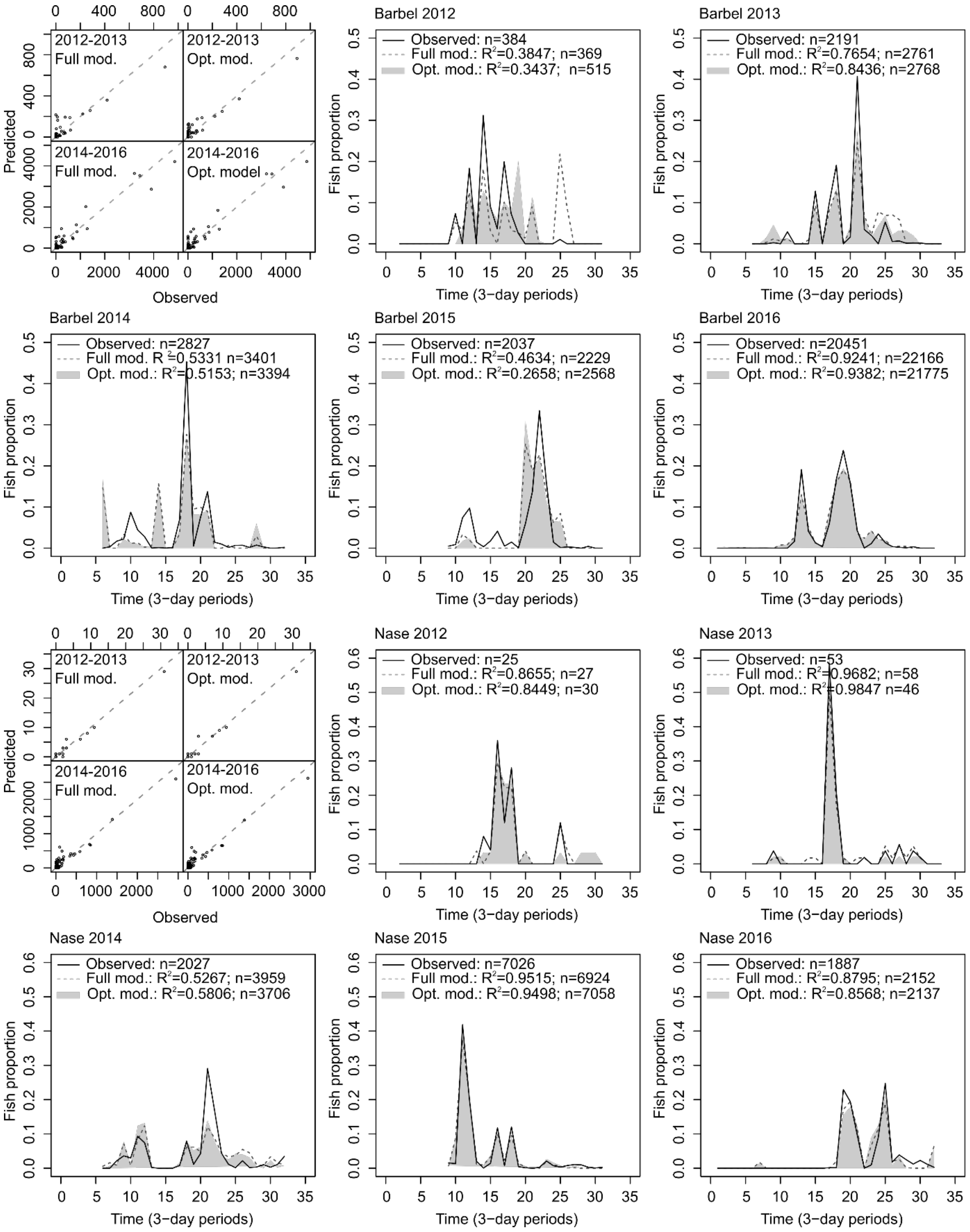


Figure III.5. Evaluation of model performance for both number of captures (n) and migration timing (proportion along time): comparison among the observed fish proportion in time t, the predicted with the full model and the predicted with the optimized (after variable reduction) model.

Variable importance was different for both species as well as different between periods 2012-2013 and 2014-2016 (Figure III.6). In the case of barbel, backward stepwise procedure showed that model of the period 2012-2013 considered as most explicative predictors those related to the 3-day periods of the capture (entrance in the fishway) (Figure III.6a), whereas in the period 2014-2016 the variables associated with previous obstacles were needed to explain its capture patterns (Figure III.6b). In the case of the nase, optimized models of the two periods considered only variables related to the capture, with a strong importance of the water temperature and photoperiod in both periods (Figure III.6c and Figure III.6d).

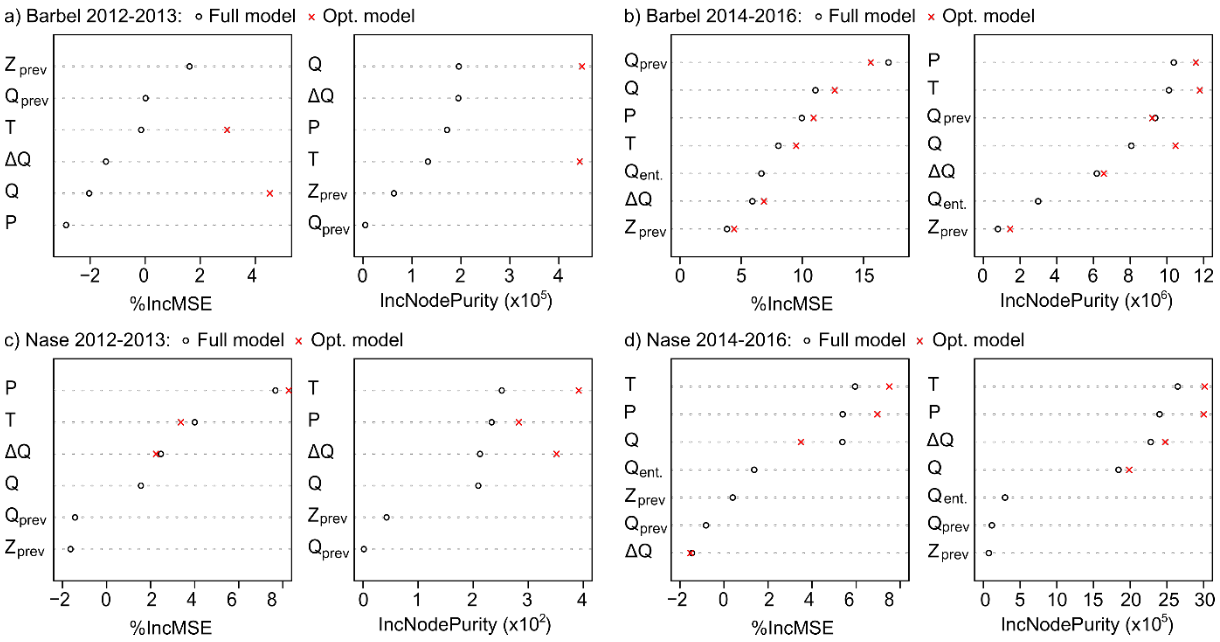
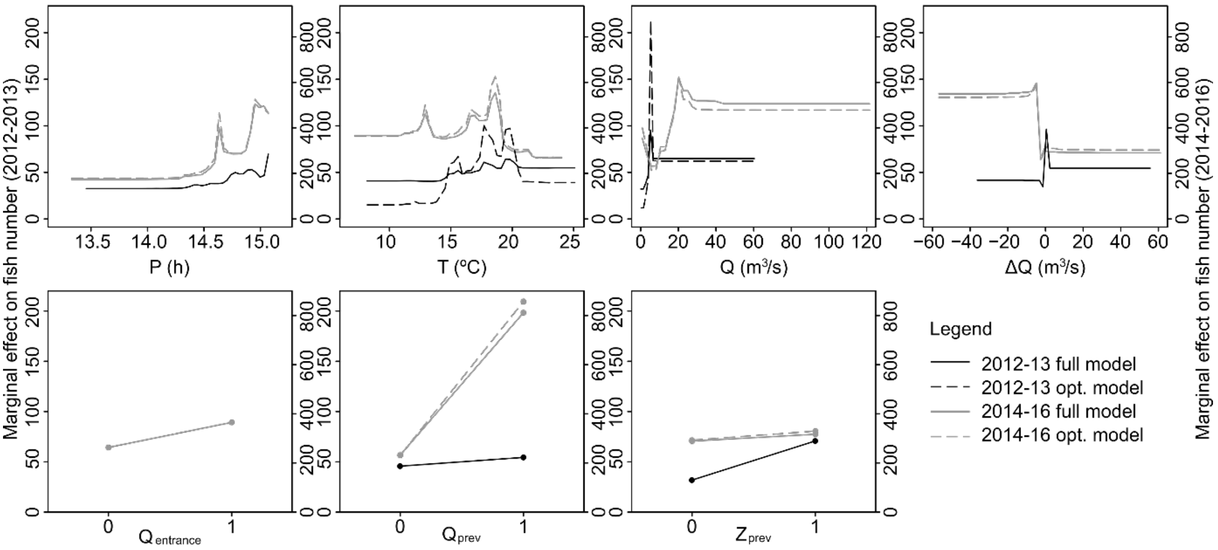


Figure III.6. Variable importance in terms of (1) increase in the mean squared error of predictions (%IncMSE), which represents how much the model fit decreases when a variable drops of the model and (2) increase in node purity (IncNodePurity), which measures the quality of a split (reduction in the sum of squared errors) (for both, the higher number, the more important). (T = water temperature; Q = river discharge; P = photoperiod; Z = reservoir level).

According to global partial dependence plots, barbel captures are expected with increasing photoperiod as well as increasing water temperature up to reach the maximum expected peak near 15 h, for both 2012-2013 and 2014-2016, and 18.5°C and 19.5°C respectively (Figure III.7a) (there are secondary peaks with lower values, such as 14.65 h and 13°C probably associated with 2016 captures, Figure III.3). In addition, more barbel captures will occur when increases in discharge also occur although in a moderated range for 2012-2013 model respect to 2014-2016 model, with more importance of the level of the reservoir for the former. Furthermore, more captures of barbel are expected when high discharges occur during previous days (Q_{prev}), and thus discharge reductions (ΔQ) when approximate to the fishway (although with lower effect in 2012-2013 model). A low discharge related to the entrance had also positive effects in the number of barbel captures.

a) Barbel - partial dependence plots



b) Nase - partial dependence plots

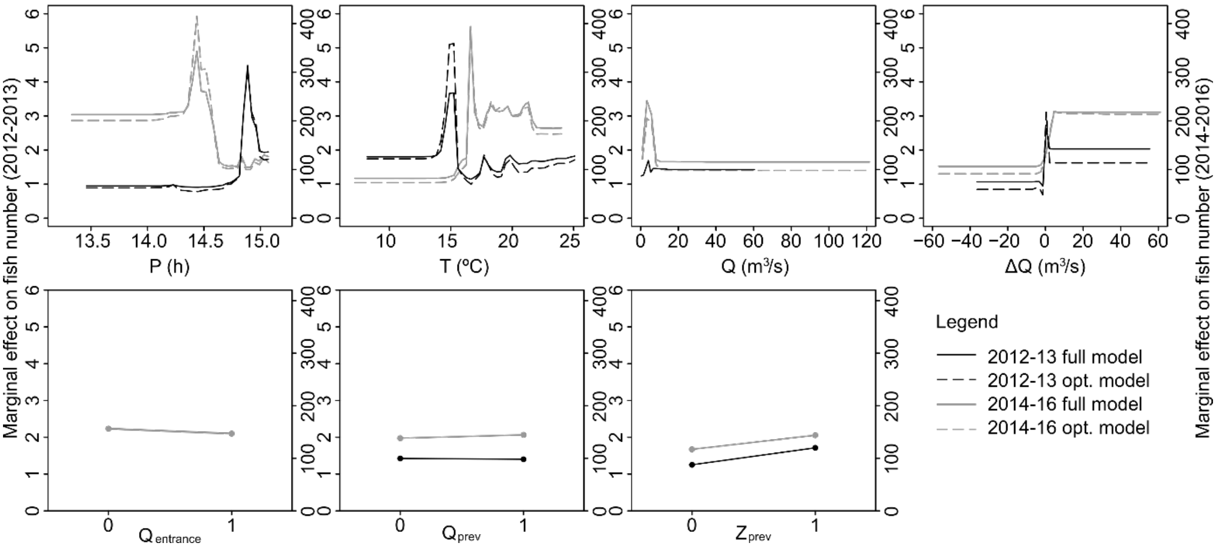


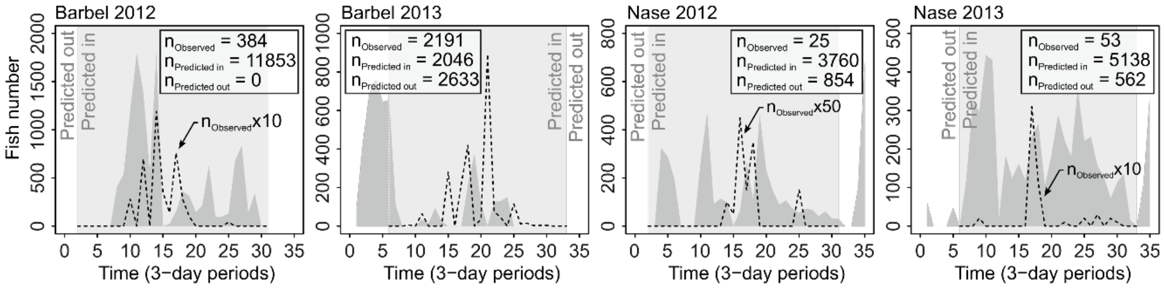
Figure III.7. Partial dependence plots to characterize the marginal effect of a variable in the model (i.e. the impact that a unit change in one of the independent variables has on the outcome variable while all other variables remain constant).

Nase models showed that more captures are expected with lower values of photoperiod (max in 14.9 h and 14.45 h for 2012-2013 and 2014-2016 models respectively) and water temperature (max in 15ºC and 16.5ºC for 2012-2013 and 2014-2016 models respectively) than barbel (Figure III.7b). Water level of the reservoir above Cespedosa weir (Z_{prev}) and moderate increases (Q and ΔQ) in river discharge will be also associated with more captures. However, discharge during previous days had lower effect in the models and no clear relation with the discharge for the entrance were found.

3.4. Scenario modelling

Simulated patterns for 2012-2013 scenario showed a potential greater number of captures for both barbel and nase when considering retrofitting actions (2014-2016 model) (Figure III.8a). In the case of the second modeling scenario, with a periodical increase on mean environmental river flow of 10 m³/s in a 3-day period (QW$_{limit}$) every 15 days (*i* = 5) (only when river discharge was higher than QW$_{limit}$ and temperature was equal or higher than TW$_{limit}$,Table II. 2), a greater number of captures was expected with these releases of environmental discharge (Figure III.8b).

a) Retrofittig scenario modelling



b) Periodical discharge release scenario modelling

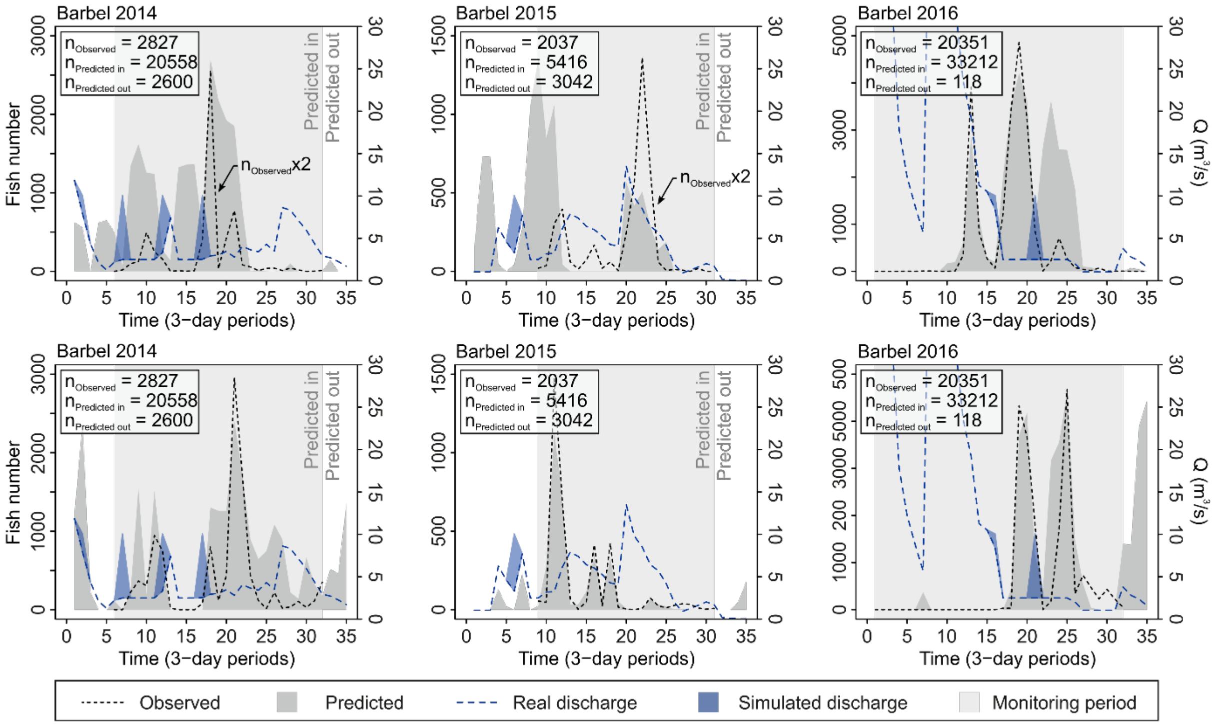


Figure III.8. Simulated scenarios with full models. (a) 2012-2013 environmental data but considering the fishway improvements, attraction weir and bypass in Cespedosa weir. (b) 2014-2016 release of 10 m³/s of environmental flow every 15 days (arrows indicate the simulated discharge releases). Number (n) of predicted inside (in) and outside (out) of the monitoring period (shadow area). It must be noted that to improve the illustration, different y-axis ranges have been used between graphs, and number of observed fish has been scaled in some of the graphs (i.e. n$_{observed}$ x factor).

4. Discussion

In this paper, reproductive migration patterns of Iberian barbel and Northern straight-mouth nase in a canyon section of the Tormes River have been described. The strong variability detected in the study reach, with presence of semipermeable barriers as well as flow abstraction for energy production made difficult to establish accurate ranges in such complex scenarios. However, the used ensemble modeling techniques have shown their potential to accurate model, assess effects of environmental variables and simulate both observed and hypothetical scenarios.

4.1. Migration patterns

Specialized literature reports reproductive migration between April and June for Northern straight-mouth nase whereas from February to June for Iberian barbel (Doadrio, 2002; Rodriguez-Ruiz and Granado-Lorencio, 1992).

In agreement with this, annual fishing closure was stablished between 15th May to 15th June (regulated by annual laws, www.medioambiente.jcyl.es) and a minimum environmental discharge was stablished for the HPP in May-June (2.5 m^3/s) respect to the rest of the year (1.05 m^3/s). However, this study reports pulsed movements with variable peak migration maxima among years, which also extended until July, outside literature limits as well as fishing closures. Thus, it would be necessary to re-define fishing closure season and re-study the timing of environmental flow delivery to adapt the legal requirements to the reality of the area. In addition, despite nase is considered as the cyprinid that firstly performs reproductive migration (Doadrio, 2002), these five-year study did not allow to establish a clear order of migration between barbel and nase. It seems, that the strong environmental variability as well as the flow depending semipermeable nature of the studied reach may actuate as a break to fish movement, delaying the movement of fish as well as diluting the natural migration order.

Nase migration was strongly affected by photoperiod and water temperature and with expected lower ranges than barbel, what agrees with a possible earlier migration. Other studies have shown that water temperature is the major factor that controls migration of Iberian cyprinids (Rodriguez-Ruiz and Granado-Lorencio, 1992; Santos et al., 2002) with ranges between 17-19ºC in the Guadalete River (SW Spain) (Rodriguez-Ruiz and Granado-Lorencio, 1992) or near 16ºC in the Vilariça River (NE Portugal) (Boavida et al., 2018). Benitez and Ovidio (Benitez and Ovidio, 2018) found that temperature requirements during cyprinid spawning migration may differ between rivers and also depending on the river position. Nevertheless, in absence of an adequate discharge in a semipermeable pathway, where passability is function of water levels and river discharge (Ovidio and Philippart, 2002), its migration can be delayed (Kelson et al., 2020; Newton et al., 2018), showing a strong variability between years in accordance with the timing of environmental variables. The combination of natural variability of the Mediterranean climate together with complex geomorphological and anthropized river systems strongly conditions the movement of fish. Thus, research studies in high variable scenarios should include multiple-year monitoring in order to get reliable results.

During 2012 (the year with the lowest number of captures), the level of the reservoir did not reach the top of the Cespedosa weir (without bypass channel during this year). Thus, fish had low possibilities to surpass this weir and all captured migrants were likely to correspond to individuals residing between this first weir and the fishway. In 2013 the number of captures increased, in accordance with a higher reservoir level but still conditioned by the inadequate location and attraction of the old fishway entrance. The increase was noticeable for the barbel, but not for the nase, as adequate temperature for the latter occurred when the level of the reservoir had already decreased. In 2014, captures of both species continue increasing, favored by all the retrofitting actions and by high water levels of the reservoir. In 2015, barbel captures went down to a similar number of 2013, which may be explained by the lower discharge (< 8 m^3/s) for this species during the time window with favorable reservoir water level (i.e. > 882.3 m) to access to the bypass or pass the weir at the beginning of the season, concentrating most of the captures in mid-July after discharge rates increases (10-15 m^3/s). In contrast, 2015 was the year with most captures of nase. These captures were concentrated during the adequate water level window of the reservoir level and with an adequate temperature range (18-20ºC) for this species. During 2016 was reported the highest number of captures for barbel. Despite that, temperatures were smaller than the optimal range for this species, considering the order of importance of environmental triggers for this species (Figure III.6), it seems that photoperiod and discharge during previous days induced their movements. In contrast, for nase, being the temperature the most important trigger for their migration, movements were observed only during their optimal temperature range, obtaining a similar number to those of 2014.

The variability related to the capture methodology, retrofitting actions and number of captures in each year made complex to use a single model to characterize fish response to environmental variables. Nonetheless, developed models showed a good performance to predict both number and migration timing. For the period 2012-2013, optimized models explained migration based only in predictors related to the 3-day periods of the capture (P, Q, T and ΔQ). This reinforces the assumption that the collected data during these years likely corresponds to individuals residing between Cespedosa weir and the fishway, or that fish that entered the study area before did not localize the old fishway entrance in the optimized time period ($i = 5$), reducing the effect of Q_{prev} and Z_{prev}.

In the case of the optimized model 2014-2016 for nase, predictors related to obstacles were also discarded during the backward stepwise procedure. However, these variables were indispensable to explain barbel captures. Models showed that more barbel captures are expected with discharges equal or higher than 10 m^3/s during previous days of the capture to allow the passage through the natural waterfalls.

4.2. Retrofitting effect

In the case under study, a general positive trend was observed along the years, however, high environmental variability made difficult to see the effect of a certain retrofitting action in a short monitoring period. For instance, the number of barbel in 2013 and 2015 were similar, but in 2015 the level of the reservoir was lower, what seems to have affected the passage through the identified semipermeable barriers not comprising the number of fish. When comparing years with similar favorable environmental conditions, such as mid-May to mid-June of 2013 (original fishway) and 2014 (retrofitted fishway) (Figure III.3), it can be seen that the number of captures is significantly greater once retrofitting actions have been carried out, concluding that these improvements were likely to be positive for fish.

Restoration measures and retrofitting actions can have an important influence on fish population size (Cowx and Gerdeaux, 2004). Simulated scenarios showed a considerable increase of expected captures during 2012-2013 considering the predictive model after the retrofitting actions. However, it is important to note that fish population size is not only dependent on the environmental conditions during migration, but also during recruitment, as non-adequate conditions during early life-stages (e.g. reduction in flow) can condition juvenile survival (Lobón-Cerviá and Rincón, 2004; Nicola and Almodóvar, 2002). This could have introduced a bias in the simulation results. Furthermore, as the number of potential migrants in the large Santa Teresa reservoir was unknown, it was assumed constant along the study period, what may have introduced another source of bias. However, during years with low reservoir levels when migration path is interrupted, such as those in 2012, fish could have spawned in suboptimal conditions in the Santa Teresa reservoir tail, just downstream Cespedosa weir.

4.3. Adaptive management

Iberian cyprinids have evolved and adapted to survive in the variable environmental conditions of Mediterranean basins (Clavero et al., 2004), thus they require such conditions to complete their life cycle. For these species, anthropogenic alterations in flow regimen may produce the loss of migratory signal, affect the habitat availability and reduce river connectivity (Jonsson and Jonsson, 2009; Lucas et al., 2001). In the study site, flow regulation is characterized by a decrease of river discharge and a removal of variability (i.e. constant discharge), which may have compromised the natural fragile equilibrium between fish and this river reach. This enhances the need of an optimal design and delivery of environmental flows. Environmental flow is usually defined as the quantity, timing, duration, frequency and quality of water flows required to sustain freshwater, estuarine and near-shore ecosystems and the human livelihoods and well-being that depend on them (Acreman and Ferguson, 2010). Therefore, it is important to consider the different flow scenarios along the year instead of setting only a constant discharge.

It is clear from our analysis that high flow peaks are essential not only to trigger fish movements but also to make permeable the obstacles in the case under study. Modelling techniques such as Random Forest (among others) allow to consider the variably of complex scenarios where the passage is function on environmental conditions (García-Vega et al., 2020, 2018) and have demonstrated here to be useful for the establishment of different variable environmental flows delivery. Results show that establishing periodical (once every 2 weeks) water releases (i.e. reach a 3-day mean discharge of 10 m^3/s), when possible (i.e. river discharge available) and considering water temperature (≥ 13ºC, due to its influence on swimming ability

(Ruiz-Legazpi et al., 2018) and effects on internal processes of maturation and migratory motivation (Lahnsteiner and Leitner, 2013; Lucas et al., 2001)), significantly enlarges the migratory window, increasing the total number of captured fish. In addition, and according to the frequency distribution of migrants (**Fig. A1**), it seems important that also discharges < 4 m^3/s are needed in order to optimize fishway attraction and entrance in the current configuration. These flow events have to be designed by considering and adequate duration and intensity to avoid negative effects in fish populations (Alonso-González et al., 2008). Nevertheless, it is worth mentioning that the cost of such strategies needs to be further analyzed in terms of energy production and fish recruitment increment, making compatible fish conservation and energy production.

Ensemble learning methods, such as RF models, allow to develop "smart management strategies" by adapting the ongoing management, in real time or under the uncertainty of unforeseen climate variations, and to propose new ones. In addition, new observations can be used to feedback the predictive model and continuously propose better strategies, including at one-point cost analysis measures of each action (e.g. relation between environmental flow and turbined flow) to elaborate optimized plans.

5. Acknowledges

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6. Supplementary data

Appendix A: Environmental variables description

Summary of environmental variables. They were compared among years by using Kruskall-Wallis (KW) test. When KW test was significant, post hoc Dunn's multiple comparison test with Bonferroni correction was performed. All statistical analyses were performed using R version 3.5.3 (R Core Team, 2019).

PHOTOPERIOD						
Min	1st Qu.	Median	Mean	SD	3rd Qu.	Max
13.24	14.27	14.74	14.56	0.5157	14.99	15.07

WATER TEMPERATURE **KW test:** χ^2 = 15.9921, df = 4, p < 0.0001

Post-hoc pairwise Dunn test: first number = Dunn's pairwise z test statistic; second number = raw p-value associated with the test. Alpha = 0.05; Reject Ho if p ☐ alpha/2

	2012	2013	2014	2015
2013	0.655297; p=1			
2014	0.425567; p=1	-0.229729; p=1		
2015	-2.474480; p=0.0667	-3.129778; p=0.0087*	-2.900048; p= 0.0187*	
2016	1.129768; p=1	0.474470; p=1	0.704200; p=1	3.604248; p=0.0016*

WATER TEMPERATURE

Year	Min	1st Qu.	Median	Mean	SD	3rd Qu.	Max
2012	7.1	14.4	18.1	17.2	4.9	20.6	26.8
2013	6.4	14.0	16.3	16.9	4.7	21.1	26.0
2014	9.4	14.5	17.0	17.2	3.3	19.9	23.7
2015	11.0	16.3	19.0	18.9	3.6	22.0	24.3
2016	6.6	12.0	15.9	16.4	5.4	21.9	25.0
Global	6.40	14.00	17.60	17.31	4.52	21.00	26.80

RIVER (ENVIRONMENTAL) FLOW **KW test:** χ^2 = 47.7939, df = 4, p < 0.0001

Post-hoc pairwise Dunn test: first number = Dunn's pairwise z test statistic; second number = raw p-value associated with the test. Alpha = 0.05; Reject Ho if p ☐ alpha/2

	2012	2013	2014	2015
2013	-5.197178; p=0.0000*			
2014	-4.258710; p=0.0001*	0.938468; p=1		
2015	-2.906695; p=0.0183*	2.290483; p=0.1100	1.352015; p=0.8819	
2016	-6.352006; p=0.0000*	-1.154827; p=1	-2.093295; p=0.1816	-3.445310; p=0.0029*

RIVER (ENVIRONMENTAL) FLOW

Year	Min	1st Qu.	Median	Mean	SD	3rd Qu.	Max
2012	0.080	1.420	2.310	4.929	11.317	2.812	76.473
2013	1.133	2.500	4.093	4.150	2.210	4.986	15.263
2014	1.474	2.500	2.838	3.971	2.629	4.020	16.019
2015	0.032	1.500	2.939	4.111	3.657	6.049	19.523
2016	1.447	2.500	3.185	17.863	32.654	15.982	164.606
Global	0.032	2.138	2.814	7.005	16.483	5.560	164.606

LEVEL OF SANTA TERESA RESERVOIR **KW test:** χ^2 = 301.7659, df = 4, p < 0.0001

Post-hoc pairwise Dunn test: first number = Dunn's pairwise z test statistic; second number = raw p-value associated with the test. Alpha = 0.05; Reject Ho if p ☐ alpha/2

	2012	2013	2014	2015
2013	-8.760081; p=0.0000*			
2014	-12.90561; p=0.0000*	-4.145537; p=0.0002*		
2015	-4.28815; p=0.0001*	4.471931; p=0.0000*	8.617469; p=0.0000*	
2016	-14.97383; p=0.0000*	-6.213757; p=0.0000*	-2.068219; p=0.1931	-10.68568; p=0.0000*

LEVEL OF SANTA TERESA RESERVOIR							
Year	Min	1st Qu.	Median	Mean	SD	3rd Qu.	Max
2012	876.30	877.82	880.16	879.61	1.69	881.21	881.54
2013	878.70	881.70	882.74	882.25	1.33	883.17	883.80
2014	880.60	882.66	883.41	883.06	0.81	883.60	883.90
2015	877.10	880.21	881.69	881.14	1.63	882.40	882.87
2016	880.96	883.02	883.63	883.32	0.80	883.91	884.05
Global	876.30	881.00	882.40	881.90	1.89	883.4	884.00

Appendix B: Movements and environmental variables

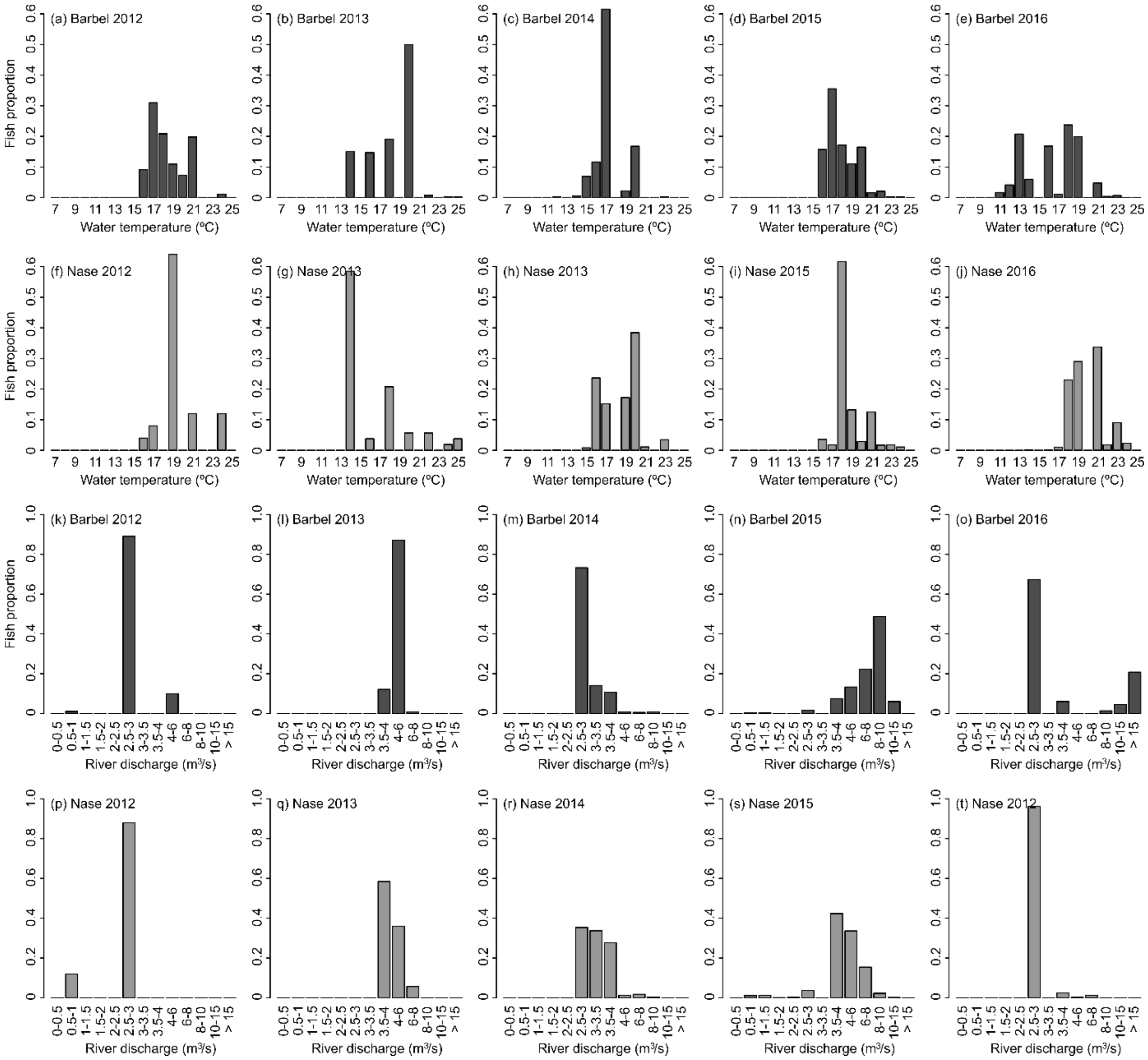

Figure III.B.1. Range of movements in relation to water temperature and river discharge.

CHAPTER IV:

Upstream migration of anadromous and potamodromous brown trout: patterns and triggers in a 25-year overview

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Abstract

River fragmentation and alterations on flow and thermal regimes are the main stressors affecting migrating fish, which could be aggravated by climate change and increasing water demand. To assess these impacts and define mitigation measures, it is vital to understand fish movement patterns and the environmental variables affecting. This study presents a long-term (1995-2019) analysis of upstream migration patterns of anadromous and potamodromous brown trout in the lower River Bidasoa (Spain). For this, captures in a monitoring station were analyzed using Survival Analysis and Random Forest techniques. Results showed that most upstream movements of potamodromous trout occurred in October-December, whereas in June-July for anadromous trout, although with differences regarding sex and size. Both, fish number and dates varied over time, and were related to the environmental conditions, with different influence on each ecotype. The information provided from comparative studies can be used as basis to develop adaptive management strategies to ensure freshwater species conservation. Moreover, studies in the southern distribution range can be crucial under climate warming scenarios, where species are expected to shift coldwards.

García-Vega, A., Fuentes-Pérez, J.F., Leunda, P.M., Ardaiz, J. & Sanz-Ronda, F.J. 2020. Upstream migration of anadromous and potamodromous brown trout: patterns and triggers in a 25-year overview. [In review]

1. Introduction

Current society needs a large volume of fresh water to keep its present lifestyle, whether for irrigation, power generation, flood control or industrial and domestic supply, what can alter hydrological and ecological patterns and processes in freshwater environments (Branco et al., 2016; Segurado et al., 2016). Near future scenarios of climate change suggest an aggravation of this situation, with potential alterations in water temperature and in the magnitude, intensity and frequency of rainfall and consequently in river discharge (Solomon et al., 2007) as well as an increasing human water demand and pollution (Pittock and Lankford, 2010; Seckler et al., 1999). The most important derived stressors affecting freshwater fish are river fragmentation and alterations on natural river flow and thermal regimes (Feng et al., 2018; Jones and Petreman, 2015; Nilsson et al., 2005), due to the need of moving between different habitats to complete their life cycles (Brönmark et al., 2014; Lucas et al., 2001) and to the differential requirements by life stages (Armstrong et al., 2003). Freshwater fish use flow and thermal regimens as main ecological timers for initiating and maintaining behavioural reactions such migration, feeding and spawning (Lucas et al., 2001). Alterations on these regimens can lead into a loss of the migration signal and a consequent migration delay (García-Vega et al., 2018), difficult obstacle ascent and reduction of habitat connectivity (Ovidio and Philippart, 2002), shift on the phenology and consequent mismatch between available and necessary resources (Otero et al., 2014; Shuter et al., 2012). In turn, this can derive in a population and diversity reductions caused by mismatch among offspring and ecological requirements (Nicola and Almodóvar, 2002) and changes on fish assemblages (Shea and Peterson, 2007), as well as a reduction of suitable physical and thermal habitat availability (Almodóvar et al., 2012; Boavida et al., 2015), endangering the persistence of many migratory fish species (Shuter et al., 2012), such as the brown trout (*Salmo trutta*).

Brown trout is a world-wide distributed species. Its natural distribution spreads over Europe, North Africa and West Asia, but also it has been introduced in South Africa, Russia, North and South America among others (Klemetsen et al., 2003; MacCrimmon and Marshall, 1968). This species can display diverse life history tactics, from anadromy (i.e. most part of feeding and growth are at sea and adults migrate into freshwater to reproduce) to potamodromy (i.e. movements occurring exclusively in freshwater), or even partial migrations (i.e. where populations are composed of a mixture of resident and migratory individuals) (Chapman et al., 2012; Lucas et al., 2001). Its migration patterns and cues are affected by latitude with a local variation dependence on environmental conditions (Aarestrup et al., 2018). In general, most important upstream movements of southern potamodromous populations occur in autumn-winter (Doadrio, 2002; García-Vega et al., 2017) and during summer-autumn in the case of anadromous ones (Caballero et al., 2018, 2012). These movements are related to the return to the spawning grounds (both ecotypes spawn in autumn and winter, earlier at higher altitudes and latitudes because of lower water temperatures and longer egg incubation period (Jonsson and Jonsson, 2011)) and are usually triggered by changes in photoperiod and water temperature (due to its influence on biological functions (Jonsson and Jonsson, 2011; Thorpe, 1989)) and favoured by high flows (e.g. overcoming obstacles (Ovidio and Philippart, 2002)).

Despite brown trout has been deeply studied due to its economic and historical importance (Northcote and Lobón-Cerviá, 2008), most of available research on brown trout migration has been focused on anadromous populations, with almost no attention to life-history comparative studies (Ferguson et al., 2019), and studies in the south of its natural distribution range are still scarce (Clavero et al., 2018; Doadrio et al., 2015). The knowledge of migration patterns and cues is fundamental to understand species requirements and to design adaptive management plans, considering all the range of possible ecotypes and different migration patterns, as different life histories can have different requirements (Ferguson et al., 2019). Moreover, studies from the southern ranges are essential in the context of climate change, as many species are expected not only to shift habitat up-river (Hari et al., 2006) but also to shift coldwards on their current distribution ranges (Jonsson and Jonsson, 2009).

Considering the above, a long-term study (from 1995 to 2019) of brown trout (both anadromous and potamodromous) upstream migration in the Northern Iberian Peninsula is presented here. For this, data from a salmonid monitoring station located in the lower River Bidasoa (Navarre, Spain) have been used. The main objective was to evaluate the upstream migration patterns of anadromous and potamodromous brown trout population in order to identify (1) periods with most upstream movements and possible differences by ecotype, sex and size, (2) the possible variation among years, considering also a possible trend in fish number and migration dates, and (3) evaluate the relation of these movements with environmental variables. This information can be used to stablish direct management and restoration measures to improve both anadromous and potamodromous trout populations, as well as to enhance conservation efforts, overall considering the future projections of climate change and increasing water demand.

2. Materials and methods

2.1. Study site

Data to study the upstream migration patterns of brown trout were collected from a salmonid monitoring station in the lower River Bidasoa. River Bidasoa has a total length of 69 km and a catchment area of 710 km^2. The monitoring station was built in 1991 at a weir of a foundry plant located between the villages of Bera and Lesaka (ETRS89 43°16’N, 1°41’W; Navarre, Spain), 21.7 km upstream from the sea, at an altitude of 40 m above mean sea level (Figure IV.1a). This weir is presently the second obstacle from the sea. There is a weir downstream of the monitoring station, that has a pool-type fishway (GAN-NIK, 2017) and during last decade other three weirs downstream were removed thanks to the great effort of the regional government to recover river connectivity and improve fish fauna (García-Vega et al., 2020). However, there are still numerous weirs upstream of this point, in which the regional government is also trying to restore the river connectivity (Rodeles et al., 2019).

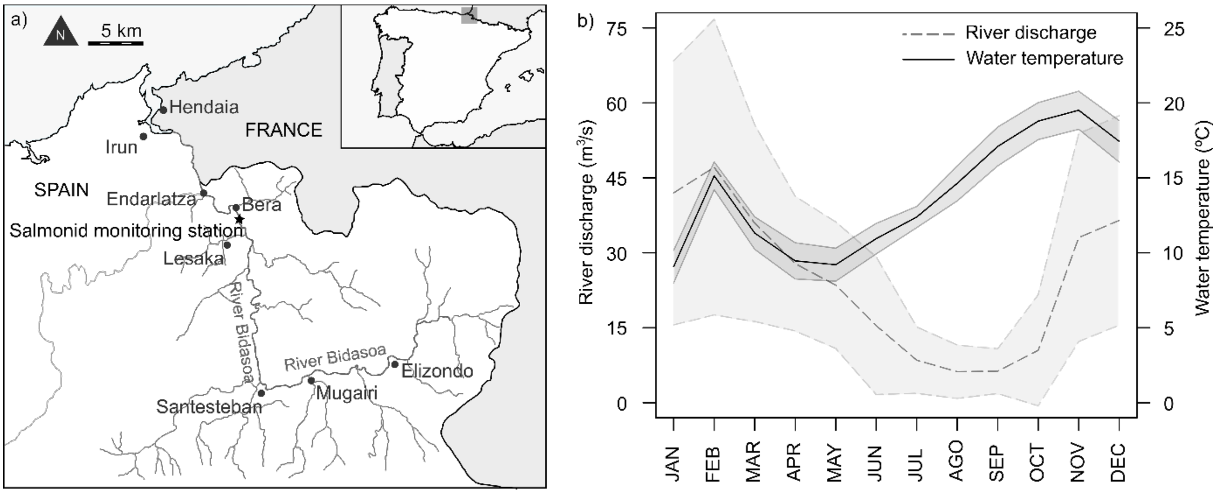

Figure IV.1. (a) Study area in the River Bidasoa Basin (Northern Iberian Peninsula). (b) Monthly flow and thermal regimen of the Bidasoa in the study reach and for the study period (1995-2019).

In the study reach, the brown trout population comprised anadromous (sea) and potamodromous (riverine) components. The fish assemblage also included other diadromous species, such as Atlantic salmon (*Salmo salar*), European eel (*Anguilla anguilla*), sea lamprey (*Petromyzon marinus*), and Allis shad (*Alosa alosa*). Other potamodromous species included were the Ebro nase (*Parachondrostoma miegii*), Pyrenean gudgeon (*Gobio lozanoi*), Pyrenean minnow (*Phoxinus bigerri*), and stone loach (*Barbatula barbatula*) (Government of Navarre, 2016a; SIBIC, 2017). The mean annual discharge in the study reach was 24.2 m^3/s and mean annual water temperature was 13.7 °C (MAPAMA, 2019) (Figure IV.1b). According to physical and chemical analyses (mean values: PO_4 = 0.04 mg/L, NH_4 = 0.03 mg/L, NO_3 = 3.32 mg/L, O_2 = 10.29 mg/L, pH = 7.87; (Government of Navarre, 2018b), water quality was "very good" (based on Spanish Act RD 817/2015).

2.2. Monitoring procedure

The monitoring station comprises a stepped fishway of five pools and a fish lift. The cage of the lift works as capture trap (it has a funnel in the entrance) which is located into the upper pool and is lifted to transport the fish to the measuring room. The only possible way for upstream migration is through the monitoring station as it is the only possible route to pass the weir. Downstream migrants cannot descend through the fishway (due to the configuration for fish trapping) and thus, they cannot be counted. Considering this, it can be deduced that captures in the monitoring station were likely to represent only a part of the population.

Data collected from 24 September 1995 to 24 December 2019 were used in the analyses. The frequency of monitoring was two-three times per week during the whole year, increasing to once a day when high migration rates were observed. The trap was checked and reset in the early daylight hours. Any trout captured were categorized by ecotype (riverine vs trout) based on external characteristics. A misclassification between ecotypes was possible when the anadromous ecotype has spent much time in the river. The possible misclassification error was evaluated in a previous scale analysis in the monitoring station, which showed a misclassification error of 1.6% for the riverine and 15.4% for the sea trout and possible doubts to 1.6% and 11.2% respectively (Tobes et al., 2012). The trout were also measured (fork length, FL, in cm; ±0.1 cm), weighed (W, in g; ±0.1 g; only since 2001) and the sex identified (only since 2001) based on external characteristics. Despite mature brown trout usually exhibit sexual dimorphism, which allows sex differentiation based on secondary external sexual characters (Reyes-Gavilán et al., 1997), the possibility of a sex misclassification could occur. After data collection, the trout were released upstream of the monitoring station to allow for migration.

2.3. Environmental variables

Photoperiod, water temperature and river discharge were selected as main environmental variables that could trigger or be related to trout migration (García-Vega et al., 2018, 2017; Jonsson and Jonsson, 2011). Photoperiod (in h) corresponded with the day length (time between sunrise and sunset) and was calculated with the Brock model (Brock, 1981). Water temperature (in °C) was monitored at the monitoring station (HOBO Water Temp Pro v2) from November 2007 to March 2018 throughout the day at 6 h intervals. From April 2018, a water quality station (SAICA-11) was installed at the monitoring station recording water temperature data every 10 min (www.agua.navarra.es). Water temperature previous to the equipment installation on November 2007 and other missing values were completed with a linear regression ($R^2 = 0.864$) with previous day air temperature (weather station 227 Bera, daily frequency; www.meteo.navarra.es) as predictor variable (Webb et al., 2003) (data from January to August 2007 were not available, and the weather station 158 Lesaka was used instead). River discharge data (in m^3/s, daily frequency) were obtained from the gauging station 1106 Endarlatza (www.chcantabrico.es).

2.4. Data processing and analysis

2.4.1. General fish characteristics

To fully define all the capture fish characteristics for the following analyses, firstly, each trout was categorized by ecotype (two categories: riverine and sea trout), sex (three categories: male, female and unidentified sex) and size (four categories: FL < first quartile; first quartile ≤ FL < median; median ≤ FL < third quartile; FL ≥ third quartile). In addition, missing values of weigh were completed with an allometric relationship $W=a{\cdot}FL^b$ by ecotype.

Secondly, and in order to have an overview of the collected data, a frequency analysis of the number of captures by ecotype and sex was carry out, and the test for equality of proportions (EP test) were used to find possible differences between groups. In addition, the Mann-Whitney Wilcoxon (MW) test was used to detect significant differences in fork length and weight by ecotype and sex. This non-parametric test was applied as variables were not normally distributed.

2.4.2. Intra-annual movements

In order to identify the periods with most upstream movements, fish were grouped (summed) by months, and the peak upstream migration period was considered as the months with the highest movements (>80%). This allow to identify two upstream migrations, one in summer (June-July) and other in autumn (October-December) (see Results section).

To describe migration patterns, survival analysis techniques were used, by applying the concept of survival time (time (*t*) until an event occurs) to migration time (time until a fish is captured in the monitoring station). For this, Kaplan-Meier (KM) survival curves (Kaplan and Meier, 1958) were determined to identify the median migration date (the week when the 50% of the captures has occurred) and to show possible different patterns by ecotype, sex and size. Log Rank (LR) test was used for KM curve comparison (Mantel, 1966). Analyses were performed from January to December (full year) considering the first week of January as $t = 1$ (data of 1995 were excluded because was not full-monitored). Because fish were not previously tagged, some assumptions were made: (1) Once a fish was captured, it continued its migration. That is to say, as repeat observations of the same individual could not be distinguished, it was assumed that all fish were only captured once. (2) The captured fish were the only ones that participated in the experiments and the exact survival time (capture date) of all participating individuals (captured fish) was known, i.e. there were not censored data.

In addition, in order to identify a possible order of arrival between males and females during peak migration, sex ratios and KM curve comparisons were also carried out, considering in this case only data of the periods of peak upstream migration, i.e. summer and autumn migrations, adding ± 1 month (i.e. May-August and September-January). Only data of these sub-periods were used in order to avoid a possible bias due to the influence of movements outside of these peaks with other possible biological meanings (e.g. thermoregulatory upstream movements (García-Vega et al., 2017; Ovidio, 1999)).

2.4.3. Inter-annual variations

To answer the second objective, i.e. identify possible variants among years, first, fish were grouped (summed) by month and year. This allowed to evaluate changes in number of captures and peak migration months throughout time. The trend over time in the total number of captured fish during peak migration period ± 1 month (i.e. May-August and September-January) was evaluated using linear regression. This allowed to not exclude data from the year 1995 in the analyses (monitoring started in September 1995) and fully extract the ecological information from the observed data. To detect possible significant differences in migration patterns between both ecotypes among years, annual KM curves by ecotype were also calculated, using LR test for curve comparison. In addition, the trend of median migration dates was evaluated using linear regression considering only data of the peak upstream migration periods ± 1 month (i.e. May-August and September-January).

2.4.4. Influence of environmental variables

In order to evaluate the relation between trout movements and the selected environmental variables, first weekly mean values of each considered variable were calculated, i.e. the mean weekly photoperiod (P), mean weekly water temperature (T), the variation in water temperature respect to the previous week ($\Delta T = T_t - T_{t-1}$), mean weekly river discharge (Q) and the variation in river discharge respect to the previous week ($\Delta Q = Q_t - Q_{t-1}$).

Secondly, in order to find possible significant differences of environmental variables among years, P, T and Q were compared by using Kruskall-Wallis (KW) test. Then, linear regression was applied to detect whether an increasing trend in mean annual water temperature or discharge occurred (photoperiod is the same all years). In addition, to assess the correlation among the environmental variables (P, T, ΔT, Q and ΔQ), Spearman correlations were calculated.

Finally, the relation between environmental variables and number of captures was studied. For this, the ranges of the environmental variables within movement occurred was evaluated by means of frequency analysis, and Spearman correlations between number of captures of the environmental variables were also calculated. Then, the influence of environmental variables on the number of captures was assessed by means of Random Forest (RF) regression. This method has been widely applied in ecology (Breiman, 2001; Cutler et al., 2007) and more recently in freshwater fish studies to predict fish abundance and species response to environmental alterations (García-Vega et al., 2018; Markovic et al., 2012; Vezza et al., 2015; Ward et al., 2014). RF is a statistical ensemble method based on the combination of a multitude of decision trees, which is used to determine the mean prediction of the individual trees (Breiman, 2001). It can capture the overdispersion or zero-inflation inherent in count data (Garcia-Marti et al., 2019), allows freedom from normality and homoscedasticity assumptions and does not require previous data transformation or a separate test set for cross-validation as it is performed internally during the run (Breiman, 2001).

Two models were created, one for each ecotype, considering the number of weekly captures as response variables. Only data of the peak migration periods ± 1 month were used in order to reduce seasonality effects (i.e. September-January and May-August for riverine and sea trout respectively). The predictor variables were: the mean weekly photoperiod (P), water temperature (T), the variation in water temperature respect to the previous week ($\Delta T = T_t - T_{t-1}$), river discharge (Q) and the variation in river discharge respect to the previous week ($\Delta Q = Q_t - Q_{t-1}$). Because in RF extreme observations are estimated using averages of response values that are closer to those observations, large values of the regression function may be underestimated and small values of the regression function may be overestimated (Zhang and Lu, 2012). This issue was resolved by applying a linear bias correction. The final models were evaluated using the coefficient of determination (R^2). The importance of the environmental variables was measured using the increase in mean squared error (MSE) of predictions (%IncMSE), which represents how much the model fit decreases when a variable drops of the model and the increase in node purity (IncNodePurity), which measures the quality of a split for every variable (node) of a tree and it is calculated by the difference between sum of squared residuals before and after the split on that variable. For both metrics, the higher number, the more important it is. Partial dependence plots for environmental variables were obtained from RF in order to characterize the marginal effect of a variable in the model (i.e. the impact that a unit change in one of the predictors has on the response variable while all other variables remain constant).

All data processing and statistical analyses were performed using *R* version 3.5.3 (R Core Team, 2019) and significance level was established at $\alpha = 0.05$ for all the analyses. The *survival* package (Therneau and Grambsch, 2000) was used for the survival analysis, the *Hmisc* package (Harrel, 2020) for the correlation analysis and the *randomForest* package (Liaw and Wiener, 2002) for the random forest regression, in which the number of trees to grow was set at 500, whereas the number of variables randomly sampled as candidates at each split was set at the square root of the number of input variables (recommended default settings).

3. Results

3.1. General fish characteristics

In total, 13646 trout were captured from 1995 to 2019 in the monitoring station during their upstream migration. Riverine trout was more abundant than sea trout (88.3% vs 11.7%; EP test p-value < 0.0001) but with smaller size (Table IV.1, MW test for FL and W p-values < 0.0001). Proportion of females was greater than males for both ecotypes (riverine trout: sex ratio F:M=1.41; sea trout sex ratio F:M=1.76; both EP test p-values < 0.0001). Males were significantly larger and heavier than females for both ecotypes (riverine male median FL = 30.5 cm and W = 300 g; female FL = 28.5 cm and W = 240 g; both MW test p-value < 0.0001; sea male median FL = 38.5 cm and W = 580 g; female FL = 36.0 cm and W = 480 g; MW test p-value FL = 0.0001 and W = 0.0060).

Table IV.1. General characteristics of the captured riverine and sea trout (n = fish number; FL = fork length; W = weight; SD = standard deviation; IqR ($q_1 - q_3$) = interquartile range (first quartile – third quartile); R^2 = determination coefficient).

Variable		Riverine trout	Sea trout
n	**Global**	12043	1603
	Male	4031	566
	Female	5671	994
	Unidentified	2341	43
FL (cm)	**Mean ± SD**	29.1 ± 5.3	39.3 ± 8.6
	Median	29	37
	IqR ($q_1 - q_3$)	26.1 – 32.0	33.1 – 42.8
	Range	8.5 – 69.6	17.4 – 71

Table IV.1. (cont.)

Variable		Riverine trout	Sea trout
W (g)	Mean ± SD	283.0 ± 174.5	734.9 ± 600.3
	Median	250	520
	IqR (q_1 – q_3)	186.6 – 340	380 – 840
	Range	7.6 – 3620	52.0 – 4600
$W = a \cdot FL^b$	a	0.0171	0.0086
	b	2.8527	3.0494
	R^2	0.8881	0.9415

3.2. Intra-annual movements

Brown trout upstream movements occurred throughout the year and varied among months. Riverine trout had a peak of migration from October to December (80.7% of captures, Figure IV.2a and Figure IV.2b) with a median date of migration on the second week of November, whereas sea trout migration peak was during June and July (81.3% of captures, Figure IV.2a and Figure IV.2c) with a median date of migration on the last week of June. In addition, sea trout presented a small increase of captures (9.2%) in the period October-December (median migration day on the third week of November) (the riverine ecotype did not present any sub-peak during summer). These substantially different migration patterns were also indicated by the estimated KM curves (Figure IV.3a and Figure IV.3d; LR test p-value < 0.0001).

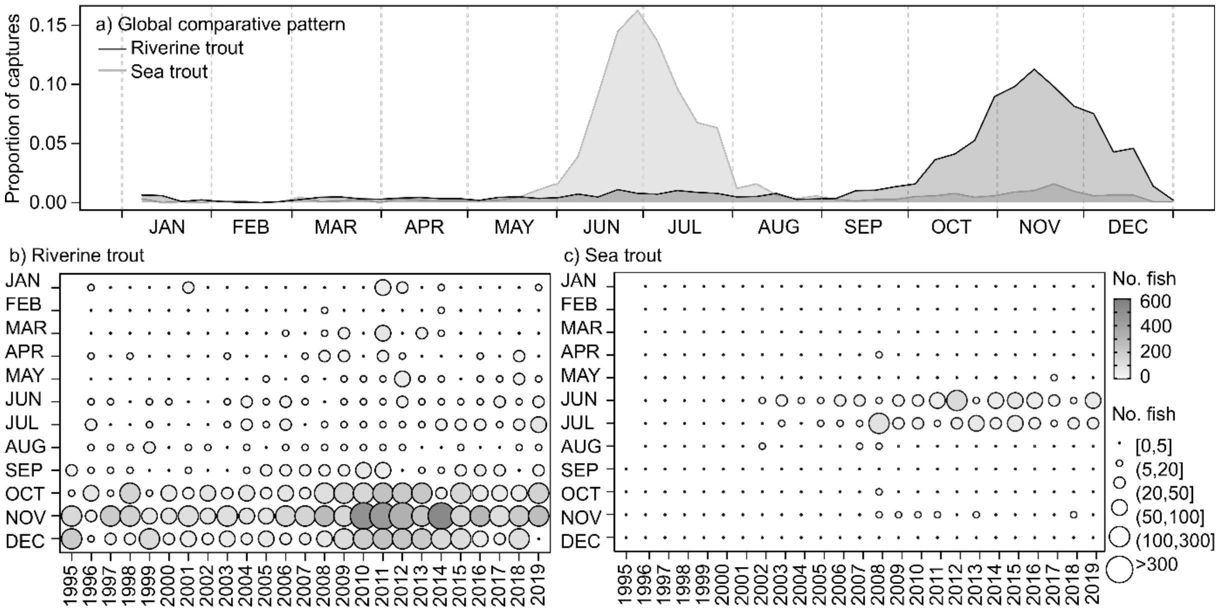

Figure IV.2. a) Global proportion of captures along the year (1995 not considered because incomplete). Number of riverine (b) and sea (c) trout observed in the monitoring station during their upstream migration by month and year.

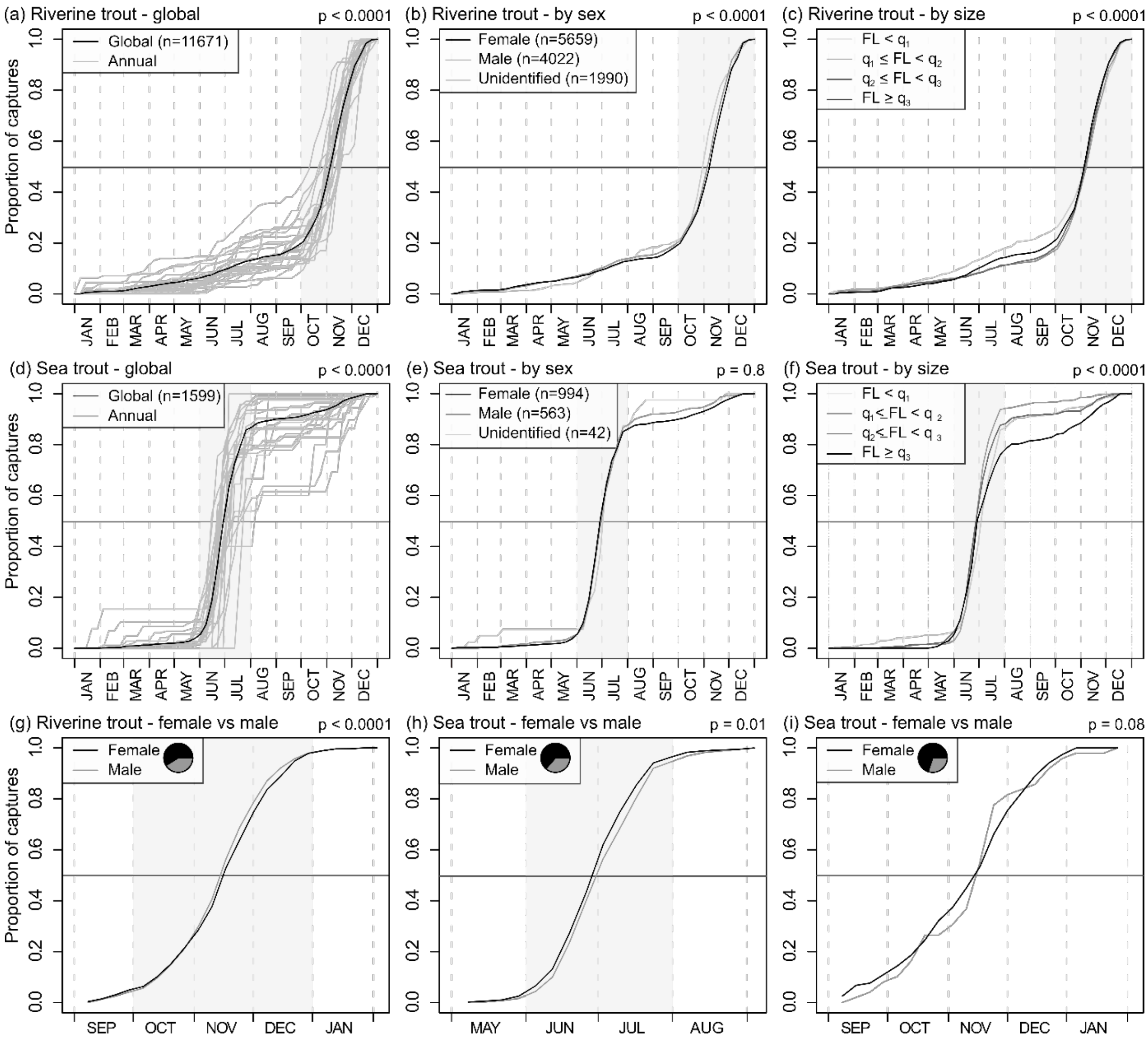

Figure IV.3. Kaplan Meier curves representing upstream migration patterns in the monitoring station regarding total number, by sex and by size (n = fish number; q_1 = first quartile; q_2 = second quartile (median); q_3 = third quartile; see Table IV.1) for riverine (a-c) and sea (d-f) trout respectively considering the full year (1995 was not considered because incomplete). In addition, female and male sub-period comparison for riverine (g) and sea trout (h and i) (pie chart of number of female vs male fish during peak migration). Log Rank test p-values ($\alpha = 0.05$) for comparison among curves. Shadow areas represent the peak migration period for each ecotype (i.e. the months with >80% of movements).

Proportion of females during peak upstream migration months was higher than males for both ecotypes (riverine trout: October-December sex ratio F:M=1.43; sea trout: June-July sex ratio F:M=1.75; all EP test p-values < 0.0001), as well as outside of peak migrations (riverine trout: January-September sex ratio F:M=1.33; sea trout: August-May sex ratio F:M=1.81; all EP test p-values < 0.0001). Despite its visual similarity, global KM curves for females and males were found significantly different for riverine trout (LR test p-value < 0.0001 considering both, the full year (Figure IV.3b) and only the autumn period (Figure IV.3g)), with males moving slightly earlier than females (close to one week shift in median migration date). Sea trout KM curves for females and males were statistically equivalent (LR test: full year p-value = 0.6 (Figure IV.3e); autumn p-value = 0.08 (Figure IV.3i)), meaning similar global migration patterns. However, when considering only the migration period summer, slightly significant differences were found (Figure IV.3h, p-value = 0.01), with similar median migration date but earlier (close to a week) patterns for female sea trout.

In addition, different migration patterns were observed regarding fish size, as KM curves were found not statistically equivalent (Figure IV.3c and Figure IV.3f, LR test p-values < 0.0001 for both ecotypes). In the case of riverine trout, despite most part of upstream movements occurred in October-December (median migration dates for all size groups between weeks 45-46), there were also movements outside this period. The individuals of the smallest size range performed more movements along the year (74.6% in October-December; 25.4% in January-September) when comparing with other size groups (that were more concentrated, with >80% in October-December). In addition, fish from the largest range (8.7% of individuals of this group) were detected migrating in June-July (Figure IV.3c). For the sea trout, while most part of movements of the three smaller size categories occurred in June-July, in the case of the largest size group, there was an additional small peak (17.3%) of migration during October-December.

3.3. Migration throughout time

Number of brown trout during peak migrations varied among years (Figure IV.2), with a significant increasing trend in number of both ecotypes long the study period (Figure IV.4a and Figure IV.4b). However, this trend was not significant for those sea trout captured during autumn migration (Figure IV.4c). In addition, despite the major proportion during all studied years was concentrated in October-December for riverine and June-July for sea trout (Figure IV.2b and Figure IV.2c), significant differences in migration patterns (i.e. KM curves) among years were found for each ecotype (both LR test p-values < 0.0001; Figure IV.3a and Figure IV.3d). Median migration dates of autumn movements of riverine trout ranged from last week of October to the second week of December, with a decreasing trend in median migration date, that is earlier migrations over time (trend slope = -0.08; p-value = 0.0415; Figure IV.4d). Regarding sea trout, median migration dates of summer movements ranged from the second week of June to the third week of July, whereas in autumn the range was from the last week of October to the second week of January, both migrations with a slightly decreasing trend over time (trend slope = -0.05 and -0.13 respectively) although without statistical significance (p-values = 0.2357 and 0.1508 respectively) (Figure IV.4e and Figure IV.4f).

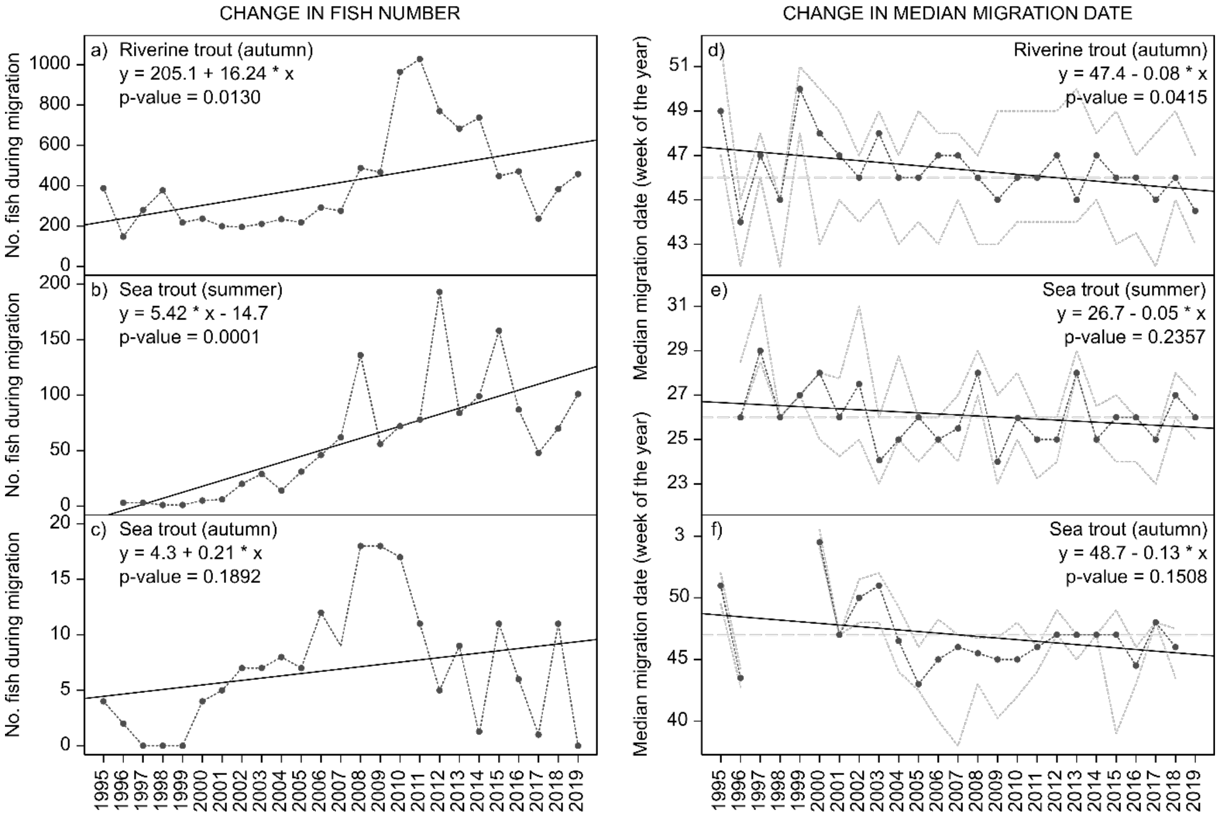

Figure IV.4. Changes in number and in median migration date (week of the year) along time for: (a, d) riverine trout during autumn migration ± 1 month (i.e. September-January); (b, e) sea trout during summer migration ± 1 month (May-August); and (c, f) sea trout during autumn ± 1 month (September-January). Grey dotted line represents the interquartile range of median migration date. Horizontal grey dashed line represents the global median migration date. Solid line represents the linear trend (α = 0.05).

3.4. Influence of environmental variables on trout migration

There were no significant differences among years in mean weekly photoperiod (KW test: p-value = 1; mean P = 12.18±2.21 h; range = 8.97 – 15.39 h) as it is the same for all years. Regarding mean weekly water temperature, there were no significant differences among years when considering the full year (KW test: p-value = 0.99; mean T = 13.75±4.03 ºC; range = 4.19 – 24.04 ºC) and a linear trend was not detected (p-value = 0.184) throughout the study period. However, significant differences among years were found when considering only riverine peak migration months (October-December p-value = 0.02) but not for sea trout peak months (June-July p-value = 0.37) (Figure IV.5). In addition, water temperature was found positively correlated with photoperiod (i.e. greater temperature as well as ΔT were expected with increasing photoperiod) (Table IV.2). In the case of mean weekly river discharge, there were significant differences among years for the full year (KW test: p-value < 0.0001; mean Q = 24.22±29.35 m^3/s; range = 0.71 – 273.55 m^3/s), as well as for peak migration months (both p-values < 0.0001) (Figure IV.5), with a significant increasing trend over the study years (p-value = 0.0001). In addition, discharge was found negatively correlated with both water temperature and photoperiod (Table IV.2).

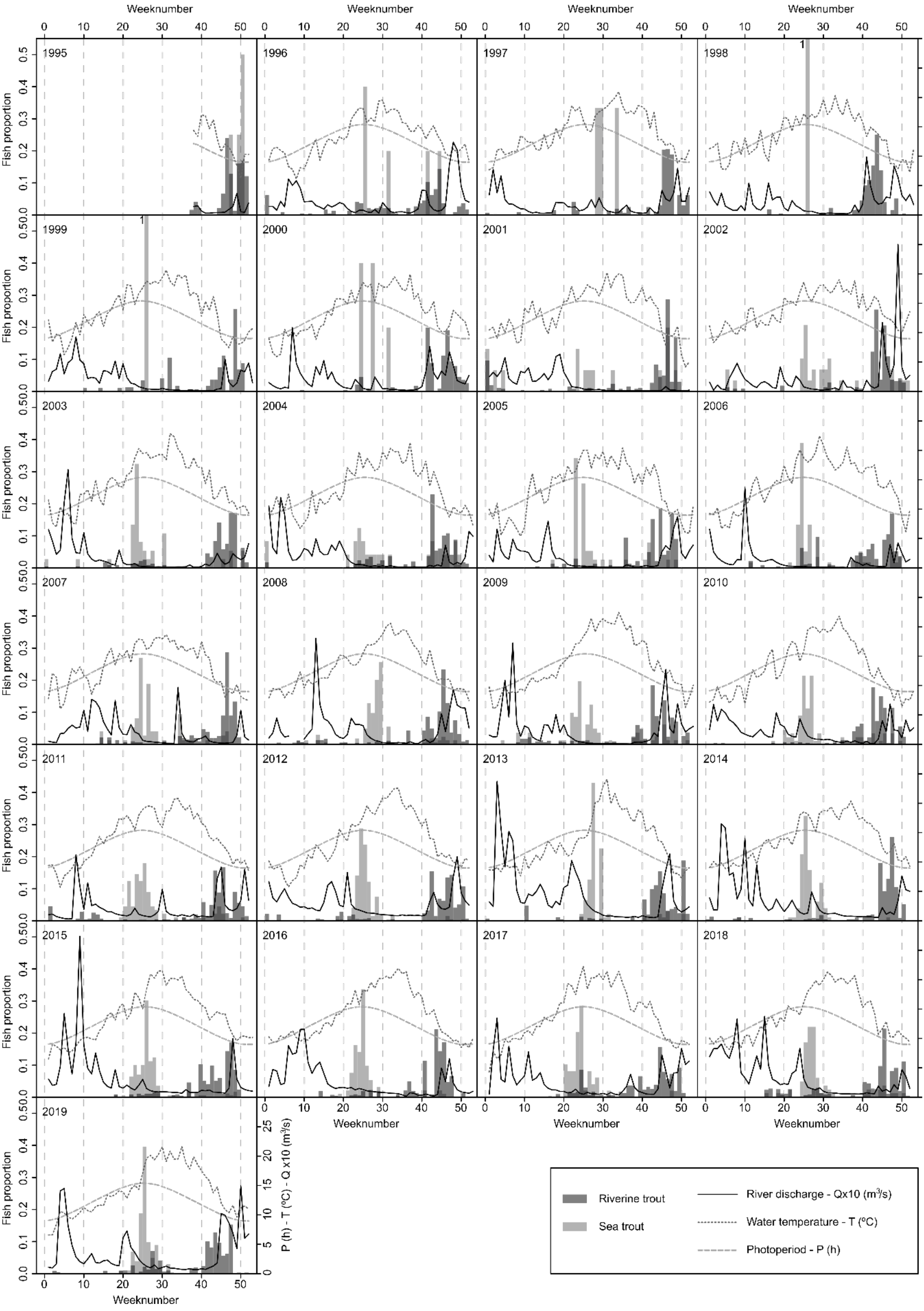

Figure IV.5. Proportion of upstream migrants of riverine and sea trout as well as mean weekly river discharge (Q), water temperature (T) and photoperiod (P) along 25 years of monitoring in the lower River Bidasoa. Weeknumber corresponds with the week of the year (from the first week of January).

Table IV.2. Spearman correlation among variables (n_{sea} = weekly number of sea trout; $n_{riverine}$ = weekly number of riverine trout, P = mean weekly photoperiod, Q = mean weekly river discharge, T = mean weekly water temperature, ΔQ = variation in river discharge respect to the previous week, ΔT = variation in water temperature respect to the previous week). Values above the diagonal correspond to the correlation coefficient. Values below the diagonal correspond to p-values of the correlation test (α = 0.05).

	n_{sea}	$n_{riverine}$	P	Q	T	ΔQ	ΔT
n_{sea}	-	0.40	0.31	-0.14	0.32	-0.03	0.09
$n_{riverine}$	<0.0001	-	-0.17	-0.03	0.08	0.03	-0.06
P	<0.0001	<0.0001	-	-0.34	0.74	-0.05	0.16
Q	<0.0001	0.2643	<0.0001	-	-0.62	0.11	-0.01
T	<0.0001	0.0050	<0.0001	<0.0001	-	0	0.20
ΔQ	0.2424	0.2505	0.0506	0.0002	0.9888	-	-0.24
ΔT	0.0014	0.0218	<0.0001	0.7187	<0.0001	<0.0001	-

Number of riverine trout was negatively correlated to photoperiod and variation in water temperature (Table IV.2, with most part of upstream movements (81%) occurred when $P < 10.5$ h and 64% with $9.5 < T < 14.5$ ºC (Figure IV.5). On the contrary, sea trout number was positively correlated to these variables (Table IV.2) and the 85% of movements occurred when $P > 14.5$ h and 75% with $16.5 < T < 20.5$ ºC (Figure IV.5). For both ecotypes, migration occurred in a range of medium-moderated river discharge. On the one hand, 71% of riverine trout movements occurred within discharges $5 < Q < 50$ m^3/s during discharge peak events (both ascending and descending phases) (Figure IV.5). On the other hand, 69% of sea trout movements in the monitoring station occurred within $5 < Q < 20$ m^3/s, during descending phase of peak discharges Figure IV.5), presenting a negative correlation to river discharge (Table IV.2).

RF regressions showed a good performance in the prediction of number of both riverine (Figure IV.6a) and sea trout (Figure IV.6b) during peak migration season. The most important variable for predicting number of riverine trout was the water temperature whereas for the sea trout it was the photoperiod (Figure IV.6c and Figure IV.6d), with the other way around for the second variable, and followed by the river discharge, and then, the changes of these variables (ΔT and ΔQ) respect to the previous week. According to partial dependence plots (Figure IV.6e to Figure IV.6i), a potential increase in riverine trout will occur when $P < 12$ h (with a peak near 10 h), T between 9.5 and 16.5 (with a peak near 13ºC), and moderate-high discharge (without a clear pattern respect to variation on Q and T respect to the previous week). In the case of sea trout, the model predicts an increase in number when $P > 14.7$ h (peak near the maximum P), $T > 15$ºC, during increasing temperatures (i.e. $\Delta T > 0$) and moderate discharge (with decreasing discharge respect to the previous week, i.e. $\Delta Q < 0$).

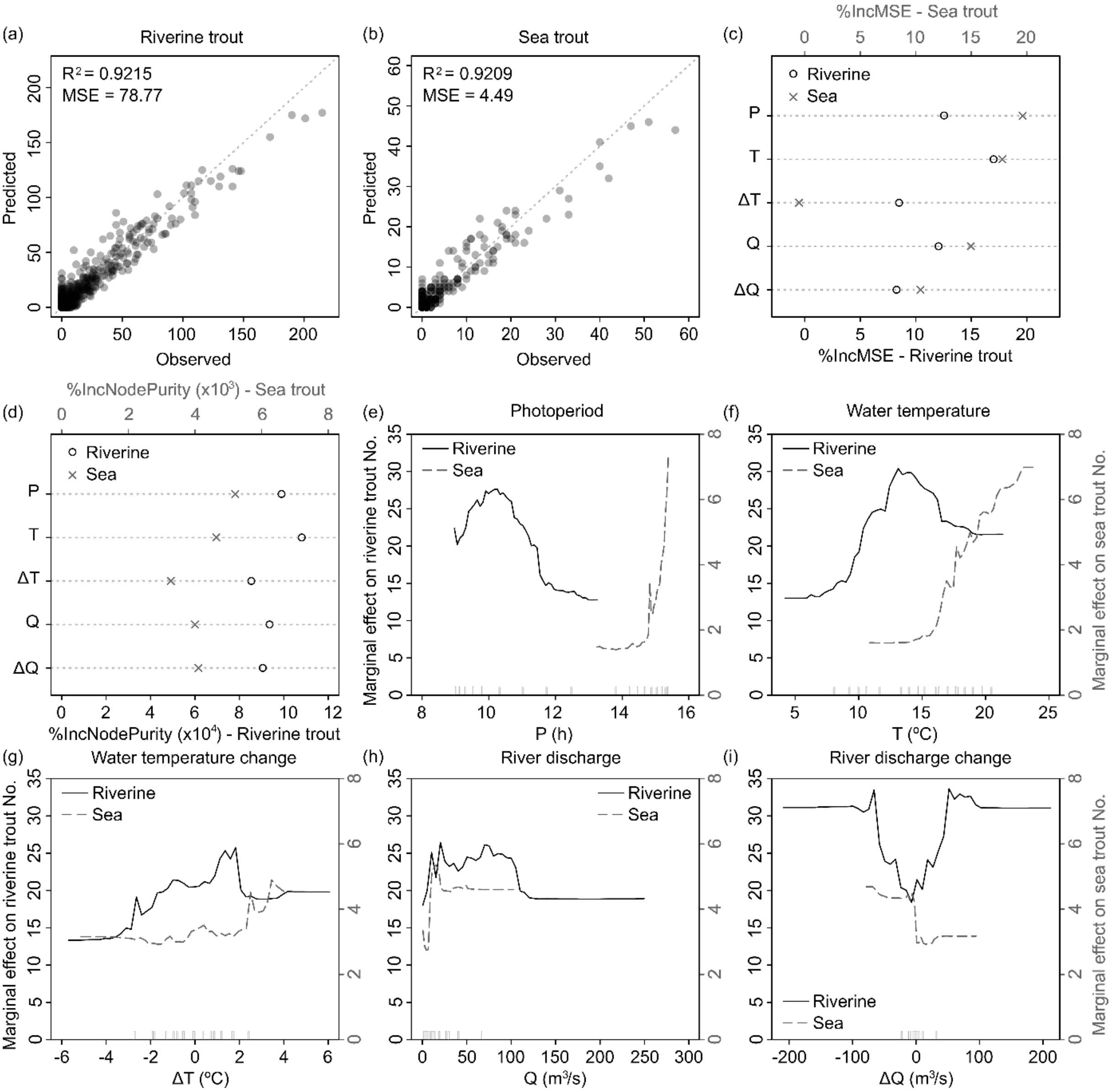

Figure IV.6. RF models outputs for evaluating the influence of environmental variables on riverine and sea trout upstream migration in the lower River Bidasoa. Evaluation of model performance (R^2= determination coefficient; MSE = mean squared error) for (a) riverine and (b) sea trout. Variable importance regarding c) increase in mean squared error (%IncMSE, which represents how much the model fit decreases when a variable drops of the model) and d) increase in node purity (IncNodePurity, which measures the quality of a split for every variable (node) of a tree and it is calculated by the difference between sum of squared residuals before and after the split on that variable) (for both metrics, the higher number, the more important the variable is). Partial dependence plots (f-i) for assessing the marginal effect of each variable in predicting number of riverine and sea trout migrants (i.e. the impact that a unit change in one of the predictors has on the response variable while all other variables remain constant).

4. Discussion

The contribution of this study is to bring a long-term (25 years) and full-year analysis of upstream migration patterns of both anadromous and potamodromous brown trout in the lower

River Bidasoa, in the southern natural distribution area of this species. Results showed a bimodal timing of upstream migration. While most upstream movements of riverine trout were concentrated from October to December (80.7%), movements of sea trout occurred much earlier, mainly focused on June and July (81.3%) with only a 9.2% from October to December. This separation in two migration runs in anadromous trout has been previously reported (Caballero et al., 2018; Hellawell et al., 1974). In general, the upstream migration of anadromous trout within a river may occur over several months (Aarestrup et al., 2018), and returning dates seems to be associated with the type of river where the natal spawning grounds are, being sooner in mainstem rivers and closer to the spawning seasons in tributaries and small rivers (Caballero et al., 2018). Atlantic anadromous trout populations has been reported to return to their natal river from May to September (Caballero et al., 2012), whereas return in Norwegian rivers has been observed from April through December, but the main upstream migration occurring from August to October (Jonsson and Jonsson, 2002). In the case of potamodromous populations of the Iberian Peninsula, upstream movements usually occur from October to January (Doadrio, 2002; García-Vega et al., 2017), showing a narrower window (movements more concentrated in November and December) in upper parts of the Bidasoa basin (García-Vega et al., 2018). At this regard, a two-week anticipation was detected between the median migration week of riverine trout in the monitoring station of the River Bidasoa and its tributary Marin River (García-Vega et al., 2018) 35 km upstream, near Mugairi village (Figure IV.1a). Despite the detected time life-history shift in the returning to the spawning grounds, both anadromous and potamodromous brown trout spawning occurs in late autumn or winter (González et al., 2017). Therefore, both ecotypes can co-exist in the spawning season and thus, spawn at the same time and place with interbreeding, i.e., making it possible for reproduction between the two ecotypes (Caballero et al., 2012; Ferguson et al., 2019; Goodwin et al., 2016).

Riverine trout males migrated slightly earlier than females, which agrees with other populations in upper parts of the River Bidasoa (García-Vega et al., 2018). In general, males of salmonids usually enter the spawning grounds before females to establish dominance (Esteve, 2017; Jonsson and Jonsson, 2011). However, in the case of sea trout, both sexes presented similar migration patterns in their comeback from the sea in the study reach This could be due to the wide period between return migration (summer) and spawning times (autumn), as well as to the distance to the final spawning grounds. In addition, the observed sex ratio was tipped in favour of females for both ecotypes. This agrees with other Atlantic anadromous populations of the north of the Iberian Peninsula (2-3 females per male, Caballero et al., 2012) and northern ones (Jonsson and Jonsson, 2011), as well as with potamodromous populations of upper tributaries of the River Bidasoa (1.5 females per male, García-Vega et al., 2018), with males usually more resident than females (Jonsson, 1989).

Besides the two main migration peaks detected in a year, there were also movements outside those intervals, with a greatly proportion of smaller fish (the largest trout (both riverine and sea) were found near the spawning season) but also occurring in all size ranges. These outside reproduction movements can be associated to the fulfilment of other ecological requirements, such as feeding, refuge (e.g. wintering, summer thermoregulation, etc.) or exploration (Lucas et al., 2001) and have been previously reported in several studies for both riverine (Benitez et al., 2015; García-Vega et al., 2017; Ovidio, 1999) and sea trout (Jensen et al., 2015; Jonsson and Gravem, 1985). This highlights the importance of guaranteeing longitudinal connectivity of rivers and environmental flows throughout the whole year.

The results showed a general decreasing trend in median migration dates (as well as similar pattern of interquartile dates), with signifincatly earlier migrations in the case of riverine trout. This may be explained by the connectivity measures (three weir removals in 2014-2016 and a fishway construction in 2008) carried out downstream of the monitoring station (see García-Vega et al. (2020) for the full analyses), which can be translated into lower delays during migrations. These actions, together with additional management measures (fishing closures (2008-2011) with a posterior establishment of size limits and quotas, and fish stocking (2003-2012)), seem also responsible of the increasing trend in number of returning sea trout and the number of riverine trout. However, the results of these measures, seemed to be also affected by the environmental conditions during the migration window as well as recruitment (if discharge reductions during early fry development stages do not occur, it is expected that the run size will be reduced (Lobón-Cerviá, 2007; Lobón-Cerviá and Rincón, 2004; Nicola et al., 2009)).

Environmental variables act as stimuli for the onset and maintenance of migration (Lucas et al., 2001; Smith, 1985). The relative importance of each parameter is different for each species or population and, in general, it is the combination of several variables which triggers migration (Lucas et al., 2001). Occasionally, when a relevant environmental cue is missing, this is replaced by alternative stimuli, which will trigger movement and, thus, avoids important delays in the migration (DWA, 2005). The random forest results showed that the most important variable for predicting number of riverine trout was the water temperature. This variable was in second place in the sea trout RF model, below the photoperiod. Other works have also shown that the influence of temperature on the river entrance of sea trout seems to be conflicting and inconclusive (Aarestrup et al., 2018). Water temperature has a strong influence on internal physiological processes for gonadal development (Lahnsteiner and Leitner, 2013) and had high influence on the timing of migration (García-Vega et al., 2018), more evident for riverine populations as movements were closer to breeding time. Photoperiod and water temperature showed a clear positive correlation, and in general, they act in combination to regulate movements of salmonids (Zydlewski et al., 2014). While most part movements of riverine trout occurred in autumn when decreasing photoperiod, as a signal of the proximity to the spawning season (it intervenes in hormonal regulation during maturation (Jonsson, 1991; Smith, 1985)), sea trout upstream movements occurred in summer, when the photoperiod is near maximum. This can be also a signal to entry from the sea to the river, with enough time to reach the spawning grounds in upper tributaries. However, even if favourable photoperiod are encountered, migration can be delayed if adequate water temperature do not occur (Teichert et al., 2020). Moreover, as photoperiod is the same among years (i.e. day length is the same on each specific day every year), inter-annual variations in time of migration were likely induced by changes in water temperature and the local increase in river discharge.

Increase in river discharge is considered a stimulant factor and a facilitator for overcoming obstacles (Clapp et al., 1990; Ovidio and Philippart, 2002). However, extremely high discharges may limit migration as it is energetically demanding to swim against strong currents (Jonsson and Jonsson, 2002). For riverine trout, discrepancies in migration dates among years could due to the timing of moderate discharge peak events. This effect has been also observed in upstream parts of the River Bidasoa (García-Vega et al., 2018). In the case of sea trout, the peaks in number occurred after moderate increase in discharge rate, during the descending phase. The increase in discharge has been demonstrated as an important stimulating factor for river entrance of salmonids, especially in smaller rivers (Aarestrup et al., 2018). Thus, sea trout probably entered from the Cantabrian Sea to the River Bidasoa during these increases in discharge, and then, when they passed through the monitoring station, the discharge peaks had already passed.

The differential discharge requirements of both ecotypes and the existence of movements throughout the year, reinforces the necessity of adequate scheduling of environmental flow deliveries as well as to provide full-year river connectivity in regulated rivers where these two ecotypes coexist. At this regard, the variations in hydrological conditions of a river can also compromised the efficiency of a fishway if this has not been considered during design (Fuentes-Pérez et al., 2018, 2016). Under certain hydrological conditions, fishways may be too much demanding and thus selective for small fish or fish with lower swimming abilities (Sanz-Ronda et al., 2019, 2016a), or even the attractiveness of the entrance can be hindered, so its localization rate can low down (Bravo-Córdoba et al., 2018). These can introduce some bias in the population size estimations. In order to solve this, studies of tagged individuals can constitute a highly valuable source of demographic data (Nater et al., 2020), and could be a good complement in long-term monitoring studies.

5. Summary and conclusion

This study presents a long-term (25 years) and full-year analysis of upstream migration patterns of anadromous and potamodromous brown trout in the lower River Bidasoa (Spain), in the southern natural distribution area of this species. Results showed different peak migration dates for both ecotypes, with different patterns regarding sex and size. Both, number of migrants and migration dates, varied over time, with a clear relation to the environmental conditions, that affected in different way to each ecotype, and also benefited from the conservation efforts carried out in the River Bidasoa during last decades.

Comparative studies of different migration patterns and cues within different life histories are essential not only to understand their ecology and environmental requirements, but also to evaluate the effect of human impacts as well as to assess the effect of mitigation measures and management decisions. In addition, the information provided from comparative studies as this, can be used as basis to develop adaptive management strategies that encompass the conservation of freshwater species together with the increasing demand of water resources as consequence of the current life style of human society and the effects of incoming global change. Moreover, studies in the southern range of species distribution can be very relevant under climate warming scenarios, where species are expected to shift not only up-river but also coldwards in their distribution ranges.

6. Acknowledgements

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CHAPTER V:

Effect of restoration measures in Atlantic rivers: a 25-year overview of sea and riverine brown trout populations in the River Bidasoa

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Abstract

Effective protection of migratory fish requires long-term conservation encompassing active restoration and management measures with follow-up studies. The main findings of long-term (1995–2019) monitoring of anadromous and potamodromous brown trout in the River Bidasoa are presented. The main aims were evaluating trout number and median migration date changes, and assessing the effects of management (closures, size limits and quotas, fish stoking) and connectivity measures (fishways and weir removals). Results showed an increasing trend in upstream migrants and decrease in migration dates (earlier migrations). Effects of overlapping measures were difficult to differentiate and affected by environmental conditions. Statistical analysis demonstrated that fishing closures (2008–2011) and fishway construction (2008) showed great importance, whereas the effect of fish stocking (2003–2012) was variable and unclear. The unexpectedly short-term effect of three weir removals (2014–2016) may have been due to unusually high discharges in March, affecting the recruitment during previous years.

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1. Introduction

Freshwater environments, which host the most diverse group of vertebrates, fish (Tedesco et al., 2017), are vulnerable to losses of native and endemic species (Hermoso and Clavero, 2011) due to the concentration of human populations close to inland waters (Moyle and Leidy, 1992). The effective protection of important freshwater fishes, such as the brown trout (*Salmo trutta* Linnaeus, 1758), requires long-term conservation efforts (Habitats Directive, 1992), which have necessitated the European legal requirement that the inland waters of Member States meet 'good' ecological status (Water Framework Directive, 2000). The recovery of threatened fish species requires conservation plans that encompass effective restoration and management measures (Maitland, 1995), such as river longitudinal connectivity improvement (Roscoe and Hinch, 2010), the restoration of aquatic habitats (Geist and Hawkins, 2016), environmental flow definition (Acreman et al., 2014), fishing regulation (Cowx and Gerdeaux, 2004) and a post-measure monitoring programme (Bernhardt and Palmer, 2011).

An iconic species in the Cantabrian rivers of the Iberian Peninsula (Northern Spain), brown trout is of great importance in the Bidasoa basin (Navarre region), which sustains both anadromous (sea) and potamodromous (riverine) ecotypes. This species' importance is in terms of recreational fishing, the evolutionary and life-history tactics of the species, and its trophic interactions within streams (Lobón-Cerviá, 2018). However, in the 20th century, brown trout populations suffered a drastic decrease throughout its native range, including the Bidasoa basin. The main possible causes of this are intense river fragmentation and water abstraction for energy production (Gosset et al., 2006), exacerbated by the loss of suitable thermal habitats due to global warming (Almodóvar et al., 2012).

To guarantee the survival of brown trout and other native fish species, the government of the Navarre region, over the last three decades, has been making a significant effort to protect and preserve native fish populations through restoration measures, fishery management decisions, and the long-term monitoring of fish population status. The aim of the present study was to analyse the long-term (25-year) data on brown trout (both riverine and sea trout) in the lower Bidasoa to: 1) evaluate changes in trout number and migration dates over time (1995–2019); and 2) assess the possible effects of management actions, which included the prohibition of trout fishing (2008–2011) through a change in fishing regulations, followed by fish stock enhancement (2003–2012) combined with improved river connectivity (construction of a fishway in 2008 and removal of three weirs, one in 2014 and two in 2016). The starting null hypotheses were as follows: i) the trout number of both ecotypes would not change over time as a consequence of any measures, and ii) migration dates would not be altered by enhanced river connectivity measures. This study represents a starting point for future management decisions as well as an example for other studies that intend to protect the populations of both riverine and sea brown trout.

2. Materials and methods

2.1. Study area and description of the measures

The River Bidasoa has a total length of 69 km and a catchment area of 710 km^2. The salmonid monitoring station is located between the villages of Bera and Lesaka (ETRS89 43°16'N, 1°41'W; Navarre, Spain), 21.7 km upstream from the sea, at an altitude of 40 m above mean sea level (Figure V.1). The mean annual discharge in the study reach was 24.2 m^3/s and mean annual water temperature was 13.7°C (MAPAMA, 2019). According to physical and chemical analyses (mean values: PO_4 = 0.04 mg/L, NH_4 = 0.03 mg/L, NO_3 = 3.32 mg/L, O_2 = 10.29 mg/L, pH = 7.87; Government of Navarre, 2018) the water quality was "very good" (based on Spanish Act RD 817/2015).

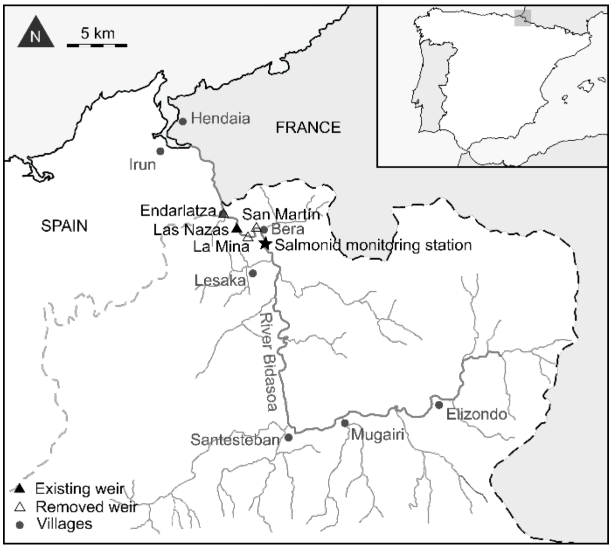


Figure V.1. Study area in the River Bidasoa basin (Northern Iberian Peninsula). Location of the salmonid monitoring station and the four weirs downstream.

A salmonid monitoring station was built in 1991 at a weir of a foundry and comprises a stepped fishway of five pools. It has a capture trap in the upper pool that is raisable to transport the fish to the measuring room. The only possible way for upstream migration is through the monitoring station as the fishway is the only possible route to pass the weir. Downstream migrants cannot descend through the fishway (due to the configuration for fish trapping) and thus, they cannot be counted. Considering this, it can be deduced that captures in the monitoring station were likely to correspond with only a part of the population.

In the study reach, the brown trout population comprised anadromous (sea) and potamodromous (riverine) components. The fish assemblage also included other diadromous species, such as Atlantic salmon *Salmo salar* Linnaeus, 1758, European eel *Anguilla anguilla* (Linnaeus, 1758), sea lamprey *Petromyzon marinus* Linnaeus, 1758, and allis shad *Alosa alosa* (Linnaeus, 1758). Other potamodromous species included were the Ebro nase *Parachondrostoma miegii* (Steindachner, 1866), Pyrenean gudgeon *Gobio lozanoi* Doadrio & Madeira, 2004, Pyrenean minnow *Phoxinus bigerri* Kottelat, 2007, and stone loach *Barbatula barbatula* (Linnaeus, 1758) (Government of Navarre, 2016a; SIBIC, 2017).

Four weirs, all licenced at the beginning of the 20th century for hydropower production, were located downstream of the salmonid monitoring station: “Endarlatza”, “Las Nazas”, “La Mina”, and “San Martín” (14.8, 16.8, 19.1, and 20.4 km from the sea respectively,Figure V.1). The “La Mina” weir (weir dimensions: height H = 2.5 m and width L = 130 m) had a pool-type fishway; however, this weir was removed in September 2014 as its exploitation licence expired. As part of the European project LIFE IREKIBAI (LIFE 14 NAT/ES/00186, www.irekibai.eu), among other activities, the demolitions of the “Endarlatza” and “San Martin” weirs occurred in October 2016 (both with expired licences). The “Endarlatza” weir (H = 2.8 m and L = 46.2 m) had two fishways: one non-functional and a more recent one (pool-type, functional) built in 1993 (IREKIBAI, 2016a). The “San Martín” weir (H = 2.9 m and L = 102.8 m) did not have any fishway (IREKIBAI, 2016b). Finally, the “Las Nazas” weir (H = 2.6 m and L = 81 m) was licenced in 1899 for hydropower production and is still in use. It had one non-functional fishway, so in January 2008, a new pool-type fishway was built (a submerged notch and orifice fishway, with nine pools, a slope of 8%, and an efficiency of 30% for riverine trout) (GAN-NIK, 2017). In addition, in August 2016, a control system of the environmental flow (e-flow) was installed in this weir as part of the Irekibai project. Thus, “Las Nazas” is presently the only obstacle downstream of the salmonid monitoring station. However, there are still numerous weirs upstream of this point, which the regional government is also working on to recover river connectivity (Rodeles et al., 2019).

From 2003 to 2012, the regional government conducted controlled fish stockings with native trout from the Bidasoa basin (GANASA, 2013) as another strategy for the recovery of wild fish populations. Adult mature riverine trout were captured in the monitoring station and transferred to a fish farm in Mugairi (Figure V.1). These trout were genetically analysed to ensure their Bidasoa origin before the breeding and creation of the F1 broodstock. Every year, in June, fry were released into the mainstem of the River Bidasoa, between the localities of Santesteban and Endarlatza (Figure V.1) (marked by adipose fin clipping), whereas the excess embryonated (eyed) ova were sowed in incubation boxes in twenty different small tributary streams during winter. Approximately 434 thousand fry and 1.25 million eyed ova were released, with a mean of 43,500 fry and 139,000 ova per year (GANASA, 2013).

As an additional protection measure for the recovery of the drastically reduced salmonid populations, there was an exceptional complete closure by a regional law (OF 48/2008) for brown trout fishing during the years 2008, 2009 and 2010 in the upper salmonid region of Navarre. This law extended to the whole Bidasoa basin. Following the fishing closure, a change in the fishing model was implemented: the annual number of fishing licences now depends on the population status and number, with catch levels and sizes regulated by annual regional laws.

2.2. Monitoring procedure, data processing and statistical analysis

Data collected from the period of 24 September 1995 to 24 December 2019 were used in the analyses. The frequency of monitoring was two to three times a week during the whole year, increasing to once a day when high migration rates were observed. The trap was checked and reset in the first morning hours. The captured trout were measured (fork length, FL, in cm: ± 0.1 cm), weighed (W, in g: ± 0.1 g) and the ecotype was identified (riverine vs sea trout) based on external characteristics. A misclassification between ecotypes is possible when the anadromous ecotype has spent much time in the river. A previous scale analysis in the monitoring station showed a misclassification error of 1.6% for the riverine and 15.4% for the sea trout and possible doubts to 1.6% and 11.2%, respectively (Tobes et al., 2012). After data collection, the trout were released upstream of the monitoring station to allow for migration.

All statistical analyses were performed using *R* version 3.5.3 (*R* Core Team, 2019). A frequency analysis of the number of captures by ecotype was carried out and the test for equality of proportions (EP test) was used to find possible differences in number between them. In order to evaluate changes in trout number over time, fish were grouped (summed) by year. The trend was evaluated by means of linear regression. In addition, the Mann-Whitney Wilcoxon (MW) test was used to detect significant differences in annual fish numbers between the period before any actions were taken (1996–2002; 1995 was not considered because the data were incomplete) and the period with conservation measures (2003–2019) for both ecotypes.

In order to evaluate changes in migration timing, i.e. dates, over years, the MW test was used to detect significant differences between the period before any river connectivity improvement measures were taken (until 2007) and the period after (2008–2019) for both ecotypes. First, captures were grouped (summed) by months. Then, to calculate migration dates and reduce the noise from the analyses, the months with the highest movements (>80%) were considered as the peak upstream migration period. Afterwards, within these months, captures were grouped (summed) by week for the analysis, to homogenise the variable periodicity of trap operation. Finally, a median migration date was calculated considering the week when 50% of the captures occurred. The trend of median migration dates was also evaluated using linear regression.

To assess the influence of management and restoration measures, the Random Forest (RF) regression was used. This method has been widely applied in ecology (Breiman, 2001; Cutler et al., 2007) and more recently in freshwater fish studies to predict fish abundances and species responses to environmental alterations (e.g. Vezza, Muñoz-Mas, Martinez-Capel, & Mouton, 2015; Ward, Holmes, Thorson, & Collen, 2014) and to assess and predict the effects of restoration and management actions (Cochran-Biederman et al., 2015; Flanagan and Richardson, 2010; Teichert et al., 2016). The RF is a statistical ensemble method based on the combination of a multitude of decision trees, which is used to determine the mean prediction of the individual trees (Breiman, 2001). It can capture the overdispersion or zero-inflation inherent in count data (Garcia-Marti et al., 2019), allows freedom from normality and homoscedasticity assumptions and does not require previous data transformation or a separate test set for cross-validation as it is performed internally during the run (Breiman, 2001) .

Two models were created, one for each trout ecotype. For this, the *randomForest* (Liaw and Wiener, 2002) *R* package was used, in which the number of trees to grow was set at 500, whereas the number of variables randomly sampled as candidates at each split was set at the square root of the number of input variables (recommended default settings). The final models were evaluated using the coefficient of determination (r^2). The importance of the variables was measured using the increase in node purity (IncNodePurity), which measures the quality of a split for every variable (node) of a tree, and calculated by the difference between the sum of squared residuals before and after the split on that variable (the higher the number, the more important it is). Partial dependence plots were obtained from RF in order to characterise the marginal effect of a variable in the model (i.e. the impact that a unit change in one of the independent variables has on the outcome variable while all other variables remain constant).

To exclude seasonality from the model, the annual number of upstream migrants during peak migration was selected as the dependent variable. The independent variables were management measures (categorical variables), connectivity improvement measures (binary variables), environmental variables (continuous variables), and effect of recruitment (continuous variable).

Management measures were the fishing closure and the fish stocking, both with three categories: Before, During and After (Table V.1). During the application of a management measure (considering other possible co-variables constant), first a progressive increase of the number of fish is expected until reaching a maximum (Figure V.2a), and, following its application, a progressive decrease will occur. Based on this behaviour, the period of influence of management measures was extended by two years as it is the approximate time for fish to reach 20 cm FL (Laplanche et al., 2019) and be detected in the capture station (93% and 99% of riverine and sea trout, respectively, presented FL ≥20 cm, with a median FL of 29 cm and 37 cm and interquartile minima–maxima of 26.1–32.0 cm and 33.1–42.8 cm for riverine and sea trout, respectively).

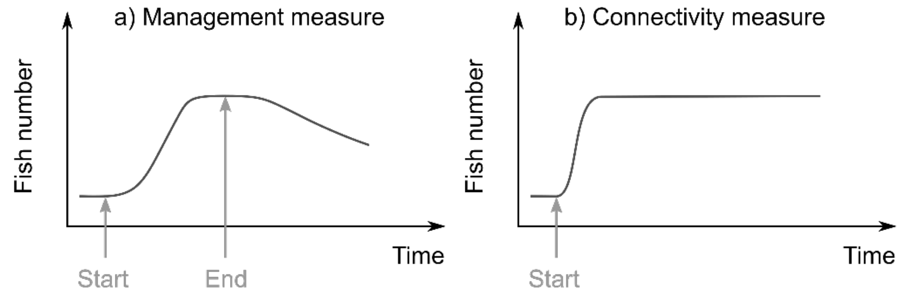


Figure V.2. Ideal expected behaviour of (a) management (fishing closure and fish stocking) and (b) river connectivity improvement measures (fishway and dam removals) when considering other possible affecting co-variables as constant.

Table V.1. Independent variables considered in the random forest model: description of the temporal effects of each management and restoration measure and environmental variable.

Variable	Application dates	Categories	Riverine trout	Sea trout†
Fishing closure	From: January 2008 To: December 2010‡	Before	1995–2007	1996–2007
		During	2008–2012	
		After	2013–2019	
Fish stocking	From: June 2003 To: June 2012‡	Before	1995–2002	1996–2002
		During	2003–2014	
		After	2015–2019	
Nazas fishway	January 2008	Before	1995–2007	1996–2007
		After	2008–2019	
Mina removal	September 2014§	Before	1995–2013	1996–2014
		After	2014–2019	2015–2019
Irekibai actions	October 2016§	Before	1995–2015	1996–2016
		After	2016–2019	2017–2019
River discharge during peak migration		Cont.	October–December	June–July
Water temperature during peak migration		Cont.	October–December	June–July
Discharge in March		Cont.	March of the second preceding year	

† As the study period was from September 1995, there were no data of sea trout peak migration in 1995.
‡ Extension of the effect by two years.
§ As peak migration of sea trout occurred from June–July, the effect was not evident until the following year.

River connectivity improvement measures were the following: the fishway construction in “Las Nazas” weir, the “La Mina” weir removal and Irekibai actions (removals of “Endarlatza “and “San Martín” weirs), all with two categories: Before and After (Table V.1). After the application of a connectivity improvement measure (considering other possible co-variables constant), it is expected that a rapid increase in the number of captures will occur until reaching a maximum, which will ideally remain constant over time (Figure V.2b).

The considered environmental variables were the mean discharge and water temperature during peak migration months. These variables influence the onset and maintenance of brown trout upstream migration (e.g. Jensen & Aass, 1995; Jonsson & Jonsson, 2011; Ovidio, Baras, Goffaux, Birtles, & Philippart, 1998).

Finally, to reflect the possible effect of the recruitment, i.e. the addition of new individuals to a population, as an important determinant of population size (Gaillard et al., 2008), river discharge in March was also used as a variable in the model. River discharge in March after trout emergence has been found to be a good predictor of annual recruitment (Lobón-Cerviá, 2007; Lobón-Cerviá and Rincón, 2004). Thus, the mean monthly discharge in March for the second preceding year was considered as a descriptor of the possible effect of recruitment on population size.

Water temperature (T, in °C) was recorded at the monitoring station (HOBO Water Temp Pro v2) from November 2007 to March 2018 at 6-hr intervals throughout the day. In April 2018, a water quality station (SAICA-11) was installed to the monitoring station to record water temperature data every 10 min (www.agua.navarra.es). Water temperature prior to the equipment installation in November 2007 and other missing values were determined with a linear regression ($r^2 = 0.864$) using previous day air temperature (weather station in Bera, daily frequency) as the dependent variable (Webb et al., 2003) (only data from January to August 2007 were not available and the Lesaka weather station was used instead). River discharge data (Q, in m^3/s, daily frequency) were obtained from the gauging station 1106 Endarlatza (www.chcantabrico.es).

3. Results

From 1995 to 2019, 13,646 trout were captured in the salmonid monitoring station during their upstream migration, of which 88.3% corresponded to riverine trout and 11.7% to sea trout (EP test $p < 0.0001$). The mean annual number (± standard deviation) of upstream migrants was 487 ± 274 for riverine trout and 67 ± 56 for sea trout. These numbers varied across years, with an increasing trend over time (Figure V.3a and Figure V.3b; riverine trout trend slope = 19.8 and $p = 0.0050$; sea trout trend slope = 5.6 and $p < 0.0001$) and significantly greater number for both ecotypes in the period with measures Figure V.3c and Figure V.3d; MW test: riverine $p = 0.0033$; sea: $p = 0.0002$).

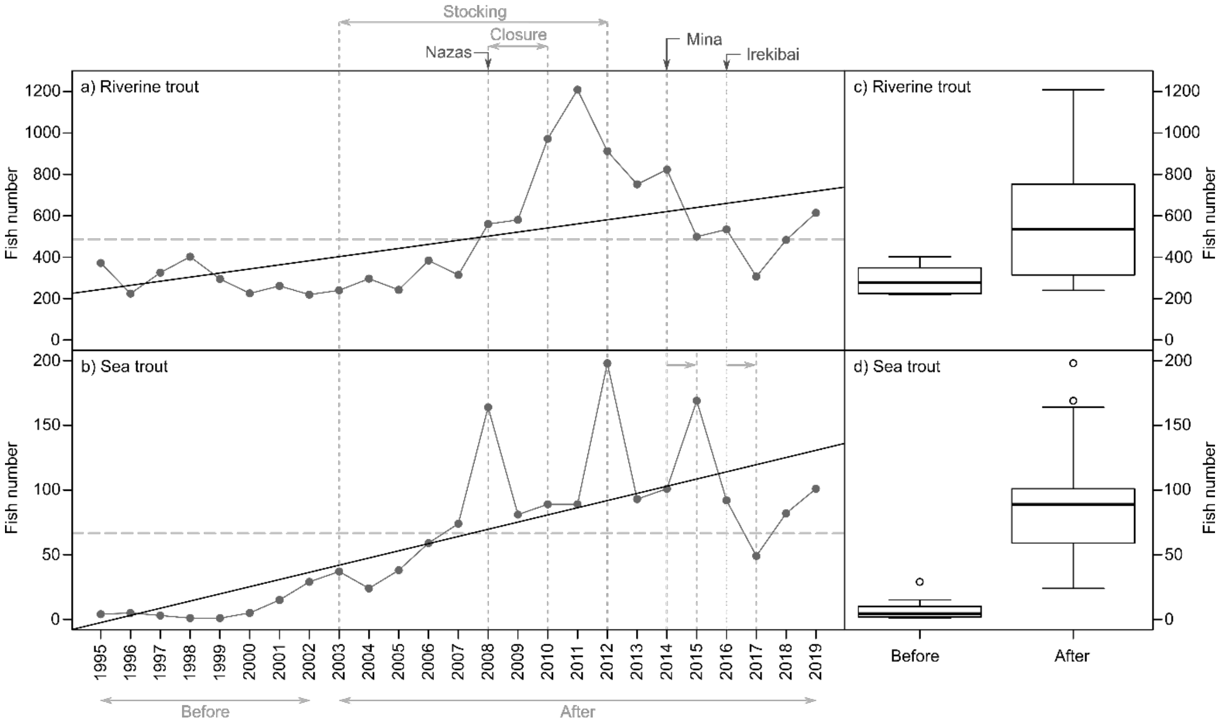


Figure V.3. Evolution over time (a, b) and boxplots (c, d) in annual number of upstream migrants of riverine and sea trout at the monitoring station in the lower River Bidasoa. The black solid line represents the linear trend (riverine trout: $y = 19.8 * x + 224.36$; sea trout: $y = 5.6 * x - 8.01$). The horizontal dashed line represents the global mean number. The vertical dotted lines represent the year of application of management measures (Stocking = Fish stocking; Closure = Fishing closure) and river connectivity improvement measures (Nazas = fishway construction in the "Las Nazas" weir; Mina = "La Mina" weir removal; Irekibai = Irekibai actions (removals of the "Endarlatza" and "San Martín" weirs)). As the peak migration of sea trout occurred from June to July, Mina (September 2014) and Irekibai (October 2016) actions were not meant to come into effect until the next year. ‘Before’ corresponds to the 1995–2002 period and ‘After’ to the 2003–2019 period.

Riverine trout showed a low number of annual migrants before any action and similar mean until 2007 (292 ± 64) (Figure V.3a). Between 2008 and 2011, the number increased considerably (830 ± 316), dropping later for the period 2012 to 2017 (638 ± 229), following which it started to recover again (549 ± 93). In the case of sea trout, the number of migrants increased gradually throughout the period (Figure V.3b), with a very low mean number before any action (8 ± 10), a slow increase in the period 2003–2007 (46 ± 20), and several high peaks during 2008–2015 (123 ± 46). In 2016–2017, the trend became negative (70 ± 30), recovering afterwards (92 ± 13).

The greater proportion of upstream migrations of riverine trout (80.7%) occurred from October to December each year, with the median migration date in the second week of November. The annual migration peak of sea trout occurred from June to July (81.3%) with a median date of migration in the last week of June. Significant differences were found in these median migration dates over time, ranging from the last week of October to the second week of December for riverine trout, with a decreasing trend, i.e. early migrations over time (trend slope = –0.08; *p* = 0.0415; Figure V.4a) and significant early migration dates after river connectivity improvement measures (MW test *p* = 0.0375; Figure V.4c). In the case of sea trout, the range was from the second week of June to the third week of July; Figure V.4b, also with a slightly decreasing trend over time (trend slope = –0.05, *p* = 0.23) but without significant differences before and after river connectivity improvement measures (MW test *p* = 0.5727; Figure V.4d).

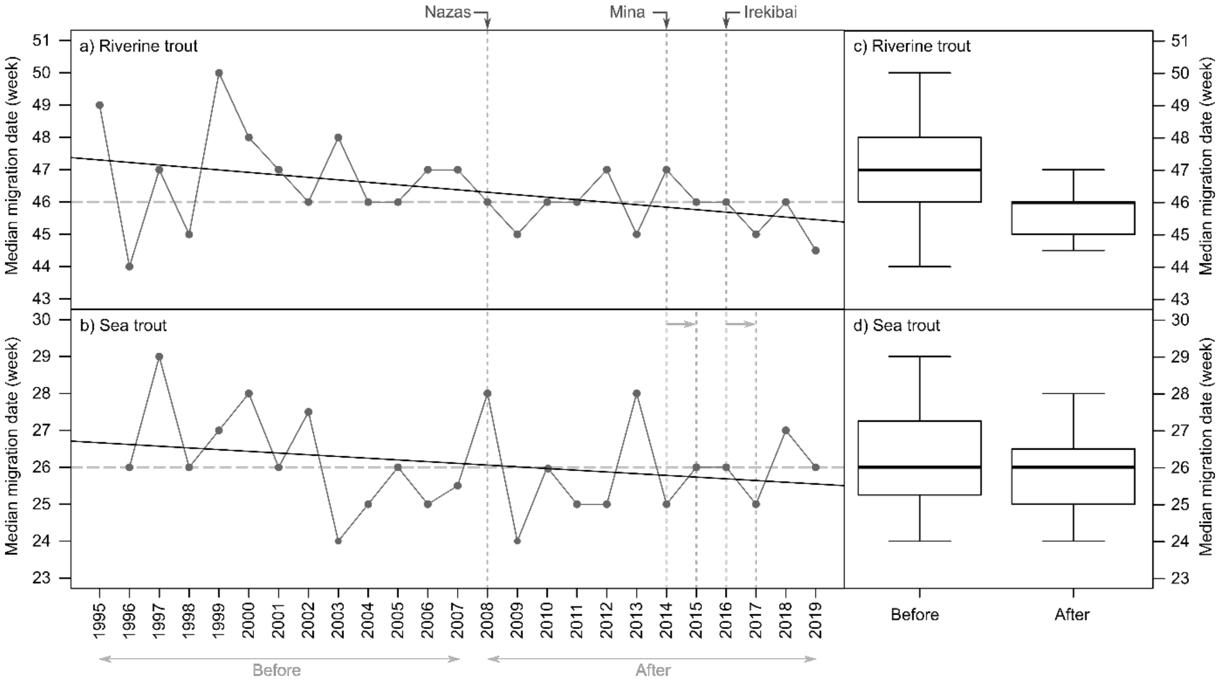


Figure V.4. Change over time (a, b) and boxplots (c, d) of median date (week of the year) of upstream migration for riverine sea trout at the monitoring station in the lower River Bidasoa. The black solid line represents the linear trend (riverine trout: y = 47.4 – 0.08 ∗ x; sea trout: y = 26.7 – 0.05 ∗ x). The horizontal dashed line represents the global median migration date. The vertical dotted lines represent the year of application of river connectivity improvement measures (Nazas = fishway construction in the “Las Nazas” weir; Mina = “La Mina” weir removal; Irekibai = Irekibai actions (removals of the “Endarlatza” and “San Martín” weirs)). As the peak migration of sea trout occurred from June to July, Mina (September 2014) and Irekibai (October 2016) actions were not meant to come into effect until the next year. ‘Before’ corresponds to the 1995–2007 period and ‘After’ to the 2008–2019 period.

Significant differences in environmental variables were discovered among years (all KW test $p < 0.0001$;Figure V.5). Figure V.6a shows the RF results to evaluate the influence of the management and restoration measures as well as environmental variables on the number of migrants. The RF model for riverine trout ($r^2 = 0.826$, Figure V.6a) showed that the fishing closure (‘During’ category) could have a great positive influence on the number of migrants followed by the “Las Nazas” fishway construction. River discharge in March was also very important, with low values related to more number of migrants two years later. A moderate effect of water temperature (but without a clear pattern) and river discharge (the greatest number was found between 20 and 40 m³/s), together with the fish stocking (more numbers coinciding with the years of its application, ‘During’ category), was observed. Both the “La Mina” removal and Irekibai actions showed much lower effects compared to the other variables.

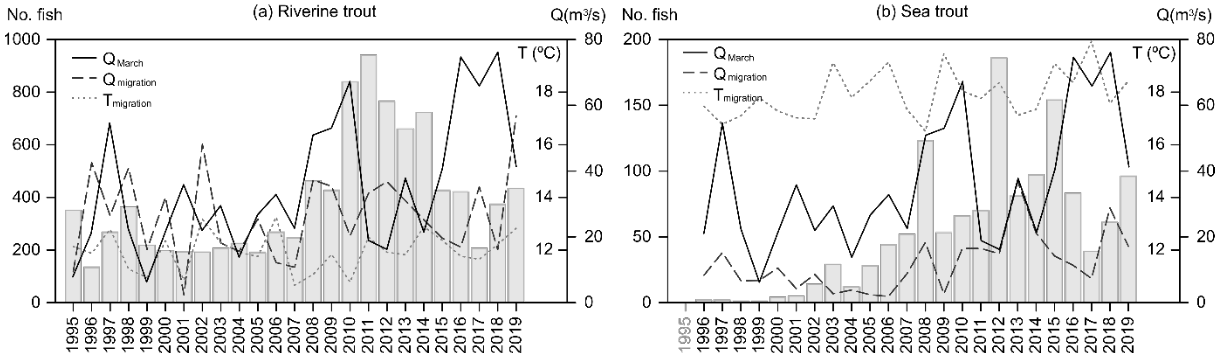
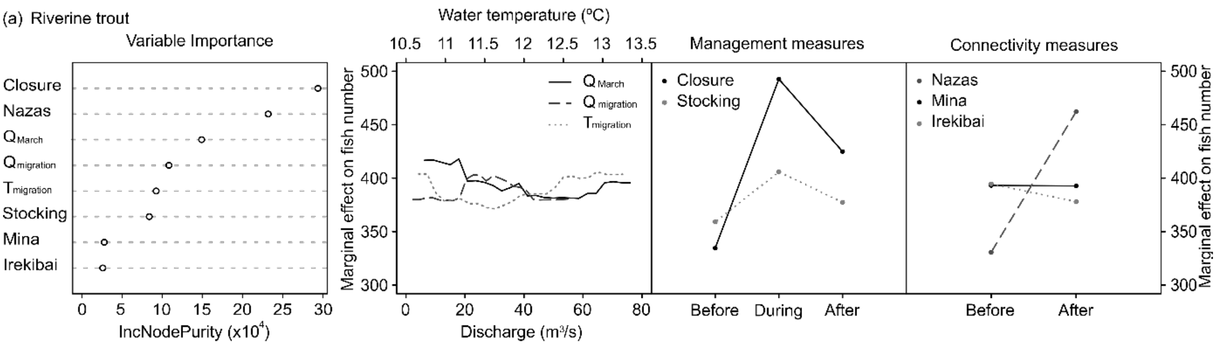


Figure V.5. Relationship between environmental variables (mean monthly discharge in March for the second preceding year as well as mean river discharge and water temperature) during peak migration months and fish migrants in the monitoring station.

(a) Riverine trout



(b) Sea trout

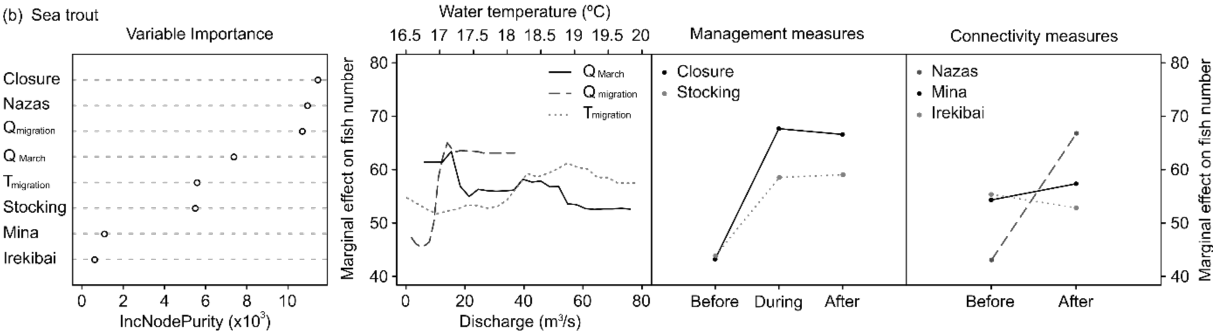


Figure V.6. Random forest regression models for (a) riverine and (b) sea trout. The variable importance in terms of increase in node purity (IncNodePurity), which measures the quality of a split (reduction in sum of squared errors) for every variable (the higher number, the more important). Partial dependence plots to assess the marginal effect of environmental variables (mean discharge and water temperature during peak migration and mean monthly discharge in March for the second preceding year), management measures (Stocking = Fish stocking; Closure = Fishing closure). and connectivity measures (Nazas = fishway construction in the “Las Nazas” weir; Mina = “La Mina” weir removal; Irekibai = Irekibai actions (removals of the “Endarlatza” and “San Martín” weirs)) in predicting number of riverine and sea trout migrants.

The RF model for sea trout (r^2 = 0.831, Figure V.6b) revealed the strong positive influence of the fishing closure with the “Las Nazas” fishway construction and the discharge during migration on the number of trout (more number with high values). For sea trout, the mean monthly discharge in March for the second preceding year exerted a moderate negative effect on sea trout run; water temperature at peak migration had a moderate positive effect whereas stocking had a lower marginal effect than closure but still at a moderate level as indexed by the IncNodePurity measure of variable importance. As in the case of riverine trout, the “La Mina” removal and Irekibai actions showed the lowest effects in the model when compared to the other variables.

4. Discussion

In general, the number of anadromous trout in a river decreases constantly with distance from the sea, forming a spatial continuum in migration strategies (Boel et al., 2014; Cucherousset et al., 2005; Klemetsen et al., 2003; Ruokonen et al., 2019). Despite the proximity of the salmonid monitoring station to the sea, nearly 22 km, the number of sea trout was much lower than the riverine trout. In recent decades, the number of returning sea trout has declined in many regions of Europe (Ferguson et al., 2019; Harris and Milner, 2006). This population decline was caused by multiple human-related factors and co-stressors during upstream and downstream migrations as well as in the destination habitats (Branco et al., 2012; Dudgeon et al., 2006; Segurado et al., 2016). The cumulative effects of habitat fragmentation and destruction together with discharge regime alterations (Nilsson et al., 2005) have resulted in both a reduction in survival and an increase in the hazards of migrations. Thus, the benefits of large migrations are reduced and the balance is potentially tipped in favour of in-river migrations (Ferguson et al., 2019).

Restoration measures and management decisions can have an important influence on the population size (Cowx and Gerdeaux, 2004). The number of returning sea trout as well as the number of riverine trout increased considerably after initiating different conservation actions downstream of the monitoring station. However, in many cases, the effects of the different measures cannot be quantified separately, and some can mask the effect of others (ideally requiring a specific before-after-control study design for each measure). Moreover, their effects are strongly conditioned by the environment.

Fishing closure (and the catch regulations later) was one of the variables that contributed significantly to the increase in number of trout for both ecotypes (RF regression). Temporal closures and other fisheries' regulations (e.g. decrease in fishing effort, catch control, and protection of spawning areas) together with stakeholder involvement can have positive benefits for fish populations (Almodóvar and Nicola, 2004; Cowx and Gerdeaux, 2004). The number of fishing licences gradually increased during the second half of the 20th century in Spain, which led to overexploitation and, therefore, the decrease of trout stocks in some Iberian rivers (Almodóvar and Nicola, 2004). This led the regional government to impose a complete fishing ban in the upper salmonid region of Navarre. This area included the Bidasoa basin in the years 2008, 2009, and 2010, followed by a tightening of fishing regulations to protect trout populations.

Coinciding with the salmonid fishing closure, in 2008, the construction of a new fishway in "Las Nazas" (GAN-NIK, 2017) was carried out, which demonstrated high relevance for the models for both ecotypes. This time overlap and the great importance of both measures in the models made it difficult to separately quantify their effects.

In the same manner, trout stocking between 2003 and 2012 also overlapped with the fishing closure and "Las Nazas" fishway, but it had a lower importance in both models as compared to closure. Trout stocking was carried out by the regional government as a measure to increase trout populations, which had been reducing during previous decades. The stocking programme lasted for ten years and was then stopped. According to the stocking technical report of the regional fishing service (GANASA, 2013), the increase of trout population density (i.e. the objective of the stocking) was not achieved. The contribution of the stocking to the population size was variable, without the expected increase with time, and most notable increases seemed to be related to the fishing closure years (GANASA, 2013). From the approximately 434,000 fry released in the mainstem of the River Bidasoa between the localities of Santesteban and Endarlatza (Figure V.1), only 765 marked trout (710 riverine and 55 sea trout) were counted in the monitoring station during the period 2003–2012 (GANASA, 2013). The lowest percentage of the stocking contribution to the captures in the monitoring station (0.6%) corresponded to 2004 whereas the greatest (15.8%) occurred in 2006, with a mean value of 11.7% for the period 2003–2012 (GANASA, 2013). Thus, the contribution of fry stocking to the migrants passing the monitoring station was very small, and its moderate as per the RF results could be possibly explained by the overlapping with the other measures.

The removals of the “La Mina” weir in 2014 and “Endarlatza” and “San Martin” weirs in 2016 (within Irekibai actions) showed the least influence in both models; however, these measures were assessed for only a short period (three years). These results were contrary to expectations. A possible explanation of this low effect is that a mismatch among ecological requirements and environmental variables could occur and thus affect the number of migrants. Low discharge has been shown to increase survival in early fry development (Nicola et al., 2009). The results showed very high discharge in the March months of previous years, which would be expected to reduce the relevant run size (Lobón-Cerviá, 2007; Lobón-Cerviá and Rincón, 2004).

In general, environmental factors act as timers for the onset and maintenance of fish migrations (Lucas et al., 2001); thus, discrepancies in migrations between years can be explained by the different timings of adequate thermal ranges and moderate discharge rate increases (García-Vega et al., 2018; Jonsson and Jonsson, 2011). On one hand, water temperature influences biological functions and internal physiological processes (Lahnsteiner and Leitner, 2013; Thorpe, 1989). Riverine trout movements were closer to breeding time, which can make water temperature an important predictor of migration timing (García-Vega et al., 2018). Sea trout was also observed to be influenced by water temperature; however, temperatures were much higher close to their thermal physiological limit (Jonsson and Jonsson, 2011). This could be related to the increase in photoperiod and solar radiation during summer months and the consequent increase in water temperature. Other works have also shown that the influence of temperature on the river entrance of sea trout seems to be conflicting and inconclusive (Aarestrup et al., 2018). On the other hand, river discharge is considered a stimulant factor (Clapp et al., 1990) and a facilitator for overcoming obstacles (Ovidio and Philippart, 2002). River discharge during peak migrations was very important in both RF models but with a greater importance for the sea trout. The increase in discharge has been demonstrated as an important stimulating factor for the river entrance of salmonids, especially in smaller rivers (Aarestrup et al., 2018). Considering this, it can be concluded that alterations in discharge and thermal regimes can produce delays in the migrations due to a loss of the migration signal, a difficult obstacle ascent, and/or a reduction of habitat connectivity (Aarestrup and Koed, 2003; García-Vega et al., 2018; Gauld et al., 2013; Oliveira et al., 2018), reducing the available time window for migration and thus the number of fish that can make the journey upstream.

The weak effects in the models of the connectivity measures in the models can be also explained because the weirs did not completely hinder migration. Both the “Endarlatza” and “La Mina” weirs had fishways and, despite the “San Martín” weir not having one, during high-discharge events, it allowed fish to pass through the top of the weir. Thus, in addition to connectivity improvements, these removals appear to have reduced the delay during migration. The results showed a decreasing trend in median migration dates, with significantly earlier migrations in the case of riverine trout after connectivity measures, which can translate into lower delays during migration. The reduction of barriers can contribute to a quicker ascent and a recolonisation of the upper parts of the basin (Clark et al., 2020) contributing to higher reproductive success as well as a higher recruitment in the following years. However, these effects cannot always be immediate as other factors can be at play. For example, an increase of population size was observed during the three last years of the study. Once the barriers in a river are removed, the migration to upper parts and small tributaries may be resumed. Moreover, new anadromous individuals can arise from potamodromous as the genetic potential for smolting can lie dormant or be maintained through time (Thrower et al., 2004). Further, although the initial generations can show poor smoltification and low marine survival, they are expected to improve over time (Ferguson et al., 2019). Thus, monitoring should continue in the future to determine the medium and long-term effects of these restoration measures and get stronger conclusions.

The present study has shown that long-term monitoring can identify population change, attribute them with reasonable confidence to measures, even when they overlap, and thus point to the potential benefits of future management options. However, environmental co-variates may be of equal importance and should be included in the analyses of monitoring results. The present work can be a starting point for future management decisions as well as an example for other studies aimed at protecting the populations of both riverine and sea brown trout.

5. Acknowledgements

This work has received funding from the project “Estudio de la migración de los peces en entornos mediterráneos” supported by ITAGRA.CT and FUNGE University of Valladolid. AGV's contribution was financed by a PhD grant from the University of Valladolid PIF-UVa 2017. The authors would like to thank the Fishing Service of the Government of Navarre for the field data collection, as well as J.F. Fuentes-Pérez for his valuable comments and active participation in the revision of this paper.

As a result of previous chapters, several general and global conclusions are derived. These conclusions are logically developed from the cumulative research of the presented compendium of articles.

About fish upstream migration periods

(1) *Median migration dates varied among years*. In all study cases, median migration dates of all studied species presented significant differences among years, shifting from some weeks or even fortnights.

(2) *Upstream movements of Iberian cyprinids (Iberian barbel and Northern straight-mouth nase) associated to reproductive migration occurred mainly from April to July, although without clear order of migration between barbel and nase*. Although peak movements (>80% of captures) were concentrated to mid-May to mid-June, pulsed movements with variable peak migration maxima among years were observed in the Tormes River, which also extended until July. In addition, the five-year study showed a changing order of migration between barbel and nase, that was conditioned by the river discharge and passability of the barriers.

(3) *Upstream movements of potamodromous brown trout associated to reproductive migration occurred mainly from September to January, although with variations depending on the study site and on the river position*. On the one hand, while peak movements of brown trout in upper basin rivers of the center of the Iberian Peninsula (i.e. Porma River) occurred between October and December, in upper basin rivers from the north (i.e. Marín River) peak movements occurred slightly later and were more concentrated, between November and December. On the other hand, peak movements in lower sections of the river (i.e. Bidasoa River) occurred earlier and in a wider temporal window when comparing to tributaries (i.e. Marín River) in the upper part of the basin (October-December vs November and December, with two-week anticipation in median migration dates).

(4) *Males of potamodromous brown trout migrated slightly earlier than females during reproductive migrations*. In both locations in the Bidasoa basin (i.e. Marín and Bidasoa Rivers), males were captured slightly earlier than females (close to a week of shift in median migration dates).

(5) *Peak upstream movements of brown trout varied depending on the ecotype*. Results in the lower Bidasoa river showed that brown trout presented a bimodal timing of upstream migration depending on the life history. While peak movements (> 80% of captures) of potamodromous trout were concentrated from October to December, movements of anadromous trout occurred much

earlier, mainly focused on June and July.

(6) *Large trout were more likely to migrate closer to the breeding season*. In both locations in the Bidasoa basin, the largest trout (both potamodromous and anadromous) were found near the spawning season. Movements outside of the spawning period were mainly performed by trout with shorter fork length.

(7) *Fish performed movements outside of spawning periods*. In all those studies with full-year monitoring, there were captures throughout the year. For example, important thermoregulatory upstream movements during summer time in the Porma River were observed.

(8) *Potamodromous trout upstream movements in fishways occurred throughout the daylight periods*. In the study of daily patterns in the Porma River, it was found that trout passed the fishway along the day, although movements were more frequent early in the morning and in the afternoon during the spawning migration and in the morning during summer.

(9) *Survival analysis techniques were useful to analyze migration patterns*. These techniques allowed to identify, describe and compare different migration patterns, both visually (e.g. Kaplan-Meier curves) and numerically (e.g. Log Rank Test).

Global conclusion: *Full-year river connectivity should be guarantee*. The existence of fish movements throughout the year, either for the existence of movements outside of spawning migration of a species or for a fish assemblage composed by different species with peak migrations in different seasons, reinforces the necessity of guarantee river connectivity along time. This can be achieved by removing obsolete obstacles, constructing fishways as well as by assessing the efficiency of constructed fishways in order to know that they really allow fish migration.

About environmental factors affecting fish movements

(1) *Environmental conditions affected the migration timing*. As median migration dates were not the same among years, differences could be attributed to the different timing of adequate ranges of environmental conditions that could affect fish migration.

(2) *Photoperiod, water temperature and river discharge were identified as essential variables to describe migration patterns*. These variables allowed to predict accurately number and timing of migrants of all species in all the study cases.

(3) *Ranges of environmental requirements varied between species and ecotypes*. Movements of the different studied species (trout, barbel and nase) and ecotypes (potamodromous vs anadromous) were observed within different range of the studied environmental variables, with slight variations depending on the year availabilities.

(4) *Environmental variables also influenced daily trout movements.* During summer, movements were more frequent when water temperature was lower and flow higher, while in the

spawning season movements were more frequent during changes in light conditions after sunrise (i.e. in the early morning), as well as during the afternoon, when water temperature was higher.

(5) *Environmental variables did not affect only in the timing, but also in the number of migrants*. Very high river discharge after trout emergence was found to produce a relevant reduction on run size, and thus, to condition the number of migrants two years later. Also non-adequate ranges of the environmental variables showed a decrease in the migration window, and thus, to reduce the opportunity of migrating.

(6) *Random forest regression resulted in a very useful tool to evaluate the relation between migration and environmental variables*. Besides to accurately estimate the number of migrants and timing of migration, this technique allowed to stablish an importance order of the variables as well as to assess the influence of each variable on the number of captures (e.g. partial dependence plots).

Global conclusion: *Migrating fish are susceptible to alterations on flow and thermal regimes*. The dependence on timing and quantity of river discharge and water temperature (among other factors) underlies the susceptibility of fish to alterations on freshwater ecosystems.

About the influence of river regulation on fish movements

(1) *River regulation for irrigation affected seasonal movement patterns*. Although effects in the discharge regimen during autumn-winter produced by the Porma reservoir to storage water (i.e. reduction in river discharge) were slightly damped by the existence of tributaries in the reach between the reservoir and the study site, a strong alteration occurred in summer. This was not only due to the water availability (in contrast of natural summer drought) but also to the cooler effect produced by the water releases from the bottom outlet of the Porma Reservoir dam, delaying thermoregulatory movements in brown trout until July.

(2) *River regulation for irrigation affected daily movement patterns*. Summer movements of brown trout in the Porma River occurred mainly during water releases for irrigation (occurring in the morning hours), when the water temperature was lower and the flow higher.

(3) *Flow abstraction affected the passage through semipermeable barriers*. Decrease of river discharge and removal of variability (i.e. constant discharge), conditioned the fish passage in the Tormes River, where the studied river reach presented obstacles where passability was function of water levels and river discharge.

(4) *Scheduled water releases during migration window benefits connectivity*. Results in the Tormes River showed that periodical water releases (when possible and considering water temperature), significantly enlarged the migratory window, increasing the potential number of migrating fish.

(5) *Variability in river discharge is needed during peak migrations in complex river scenarios*. In river reaches, such as the Tormes River, where fish had to deal with different structures in order to swim upstream, besides water releases to improve connectivity under semipermeable barriers, low flows were necessary to find fishway entrance. In addition, when constant flows were simulated in the Marín River, delays on migration dates (together with a reduction of the number of migrants) were expected.

(6) *Random forest regression resulted in a very useful tool to define environmental flows*. Besides to accurately estimate the number of migrants and timing of migration with observed values of environmental variables, this technique allowed to simulate different flow scenarios which in turn, allowed to identify limit values, and to maximize fish migration under semipermeable barriers.

Global conclusion: *Adequate quantity, quality and timing of environmental flows should be guarantee*. The existence of different discharge requirements along the year (such as high flows for habitat connectivity or low flows during early life-stages), makes necessary to provide variable environmental flows instead of setting only a constant discharge.

About possible alterations on migration patterns as consequence of future projections of climate change

(1) *Water warming may suppose a migration delay*. Simulated scenarios of water warming in the Marín River showed a potential delay on the median spawning migration dates.

(2) *Combination of water warming and intense river regulation may endanger migrating fish*. Besides delays in median migration dates due to thermal regimen alterations, a reduction of potential number of migrants were predicted in the Marín River if a combination of water warming and a heavy reduction in river flow occurred.

(3) *Models based on large time series can have direct application on fish population management*. Study and modelling of large time series, besides give us information about reach cues of the overall effect of changes in the parameters involved in the migration, can serve as tools to simulated scenarios of anthropogenic impacts (e.g. water warming (Marín River) and river discharge alterations (Marín and Tormes River)), and thus, to prepare compensation measures and actions for possible future impacts.

Global conclusion: *Knowledge of fish migration patterns and environmental variables affecting can be used as basis to develop adaptive management strategies*. Monitoring of fish migration are useful to simulate the effect of possible impacts expected under near future scenarios with increasing demand of water resources and the incoming global change. Moreover, studies in the southern range of species distribution can be very relevant under climate warming scenarios, where species are expected to shift not only up-river but also coldwards in their distribution ranges.

About the assessment of mitigation measures on fish migration

(1) *Restoration measures and management decisions had an important influence on the population size*. Both, the number of anadromous trout and potamodromous trout increased considerably after initiating different conservation actions in the Bidasoa River.

(2) *Connectivity measures, such as fishway construction (and retrofitting) and dam removal benefited migrating fish*. Besides showing an increase in number of migrants (both in the Marín and in the Tormes Rivers), earlier migrations were observed, that is to say, lower delays during migration.

(3) *Fishing closures contributed significantly to the increase of fish population size*. Results in the Bidasoa River showed that complete fishing closures and catch regulations later, significantly increased the number of trout for both ecotypes.

(4) *Fish stocking presented not clear effects*. The contribution of fry stocking in the Bidasoa River to the run size of trout migrants in the study reach was very small, and its possible contribution could be possibly explained by the overlapping with other management and restoration measures.

(5) *Effects of combined restoration and management measures can overlap*. Results in the Marín River showed that effects of the different measures could not be quantified separately, and some masked the effect of others. Therefore, for identify separately effects, a specific before–after-control study design for each measure would be required.

(6) *Environmental variables should be considering in the assessment of restoration and management measures*. Results in the Bidasoa River showed that the effect of high discharges during earlier stages conditioned the run size two years later, conditioning the short-term effects of restoration measures.

Global conclusion: *Long-term post-measure monitoring programs are needed*. Monitoring studies of fish migration are needed, not only to understand fish ecology and environmental requirements, but also to assess the population status and to know if the applied measures are really effective.

FUTURE STEPS

This thesis is not a conclusive work, but also a starting point for the study of the migration of other Iberian fish species, and to the assessment of the effects of anthropogenic impacts and mitigation measures on their movements. In addition, some of the conclusions defined here open new questions that will need further research and analysis.

Some of the results showed variations in median migration dates of a species depending on the river longitudinal position. Studies of multi-annual movement patterns at the scale of the river basin would contribute to the comprehension of the periodicity of the movements and would allow a more accurate understanding of the influence of environmental factors. Due to the long-time data series in different parts of the Bidasoa River (lower Bidasoa vs its tributary in the upper basin, the Marín River), a comparative article would provide interesting information regarding differential trout migration according to their position in a river basin. Other possible analysis became from the comparison of trout migration between rivers of the center of the Peninsula (Porma River) and in the north (Marín River). At this regard, a first approach (in Spanish) was presented in the congress "*IV Reunión del Grupo de Hidrología Forestal de la Sociedad Española de Ciencias Forestales*" (see García-Vega et al., 2019).

More study locations under different climatic and hydrological conditions, such as those rivers in Mediterranean basins and/or in the south of the Iberian Peninsula, would provide a more complete picture of the requirements of the studied species. Moreover, in this thesis only upstream migration was evaluated. However, as stated in the Introduction chapter, fish has bidirectional movements, with also downstream migrations. In addition, despite the three studied species are good representatives of Iberian fish species, monitoring of other Iberian fish species could offer variations in ~~dates~~ and patterns or environmental requirements. At this regard, thanks to the projects "FIThydro: Fishfriendly Innovative Technologies for Hydropower" (EU-H2020 Ref. 727830) (with a test case in the Iberian Peninsula) and "LIFE SEGURA RIVERLINK" (LIFE12 ENV/ES/001140), in which the Group of Applied Ecohydraulics have worked on, have provided data of fish migration in the Duero and Segura Rivers respectively, that are currently under analysis.

Furthermore, despite the importance of knowing the migratory behavior of native species for their conservation, information regarding the movement ~~patters~~ of invasive alien species would be key for their management. By knowing the migration patterns of invasive alien species, successful management strategies could be defined to reduce their arrival and spread rates, in order to get its controlling and eradication.

Some of the results of this thesis showed possible influence of fishways during fish migration (e.g. lower discharges near to the fishway would be needed in the Tormes River for improving the entrance of fish; lower delays and more number of captures occurred after new fishway construction and retrofitting (e.g. Bidasoa and Tormes Rivers.). This needs to be further investigate and, actually, it is one of the current research lines of the Group of Applied Ecohydraulics. Understand fish performance in fishways is vital in order to carry out proper fishway assessment and to evaluate their effects on fish migration.

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ANNEX 1

List of articles included in the compilation thesis

(As required by Section 4.1. of the Regulation concerning doctoral thesis defense at UVa)

TO THE CHAIRMAN OF THE PhD BOARD OF THE UNIVERSIDAD DE VALLADOLID

Thesis author: Mrs. Ana García Vega

Published articles:

Article 1 (Chapter I)

Full publication reference: García-Vega, A., Sanz-Ronda, F.J. & Fuentes-Pérez, J.F. 2017. Seasonal and daily upstream movements of brown trout *Salmo trutta* in an Iberian regulated river. *Knowledge & Management of Aquatic Ecosystems*, 418: 9. DOI: 10.1051/kmae/2016041.
Date of publication: 30 January 2017; Date of acceptance: 29 December 2016.
Indexation database: Journal Citation Reports (JCR) by Clarivate Analytics (www.jcr.clarivate.com)
Impact index (JCR) in 2017: Impact factor = 1.525. Rank: 24/51 (Q2) in Fisheries; 54/106 (Q3) in Marine & Freshwater biology.

Article 2 (Chapter II)

Full publication reference: García-Vega, A., Sanz-Ronda, F.J., Fernandes Celestino, L., Makrakis, S. & Leunda, P.M. 2018. Potamodromous brown trout movements in the North of the Iberian Peninsula: Modelling past, present and future based on continuous fishway monitoring. *Science of the Total Environment*, 640–641: 1521–1536. DOI: 10.1016/j.scitotenv.2018.05.339
Date of publication: 18 June 2018; Date of acceptance: 27 May 2018.
Indexation database: Journal Citation Reports (JCR) by Clarivate Analytics (www.jcr.clarivate.com)
Impact index (JCR) in 2018: Impact factor = 5.589. Rank: 27/251 (Q1) in Environmental Sciences.

Article 3 (Chapter V)

Full publication reference: García-Vega, A., Leunda, P.M., Ardaiz, J. & Sanz-Ronda, F.J. 2020. Effect of restoration measures in Atlantic rivers: A 25-year overview of sea and riverine brown trout populations in the River Bidasoa. *Fisheries Management and Ecology*, 27: 580-590. DOI: 10.1111/fme.12458
Date of publication: 09 October 2020; Date of acceptance: 10 September 2020
Indexation database: Journal Citation Reports (JCR) by Clarivate Analytics (www.jcr.clarivate.com)
Impact index (JCR) in 2019 (last update): Impact factor = 1.733. Rank: 22/53 (Q2) in Fisheries.

Unpublished articles:

Article 4 (Chapter III)

Provisional reference: García-Vega, A., Fuentes-Pérez, J.F., Bravo-Córdoba, F.J., Ruiz-Legazpi, J., Valbuena-Castro, J. & Sanz-Ronda, F.J. 2020. Reproductive movements of potamodromous cyprinids in the Iberian Peninsula: when environmental variability meets semipermeable barriers.

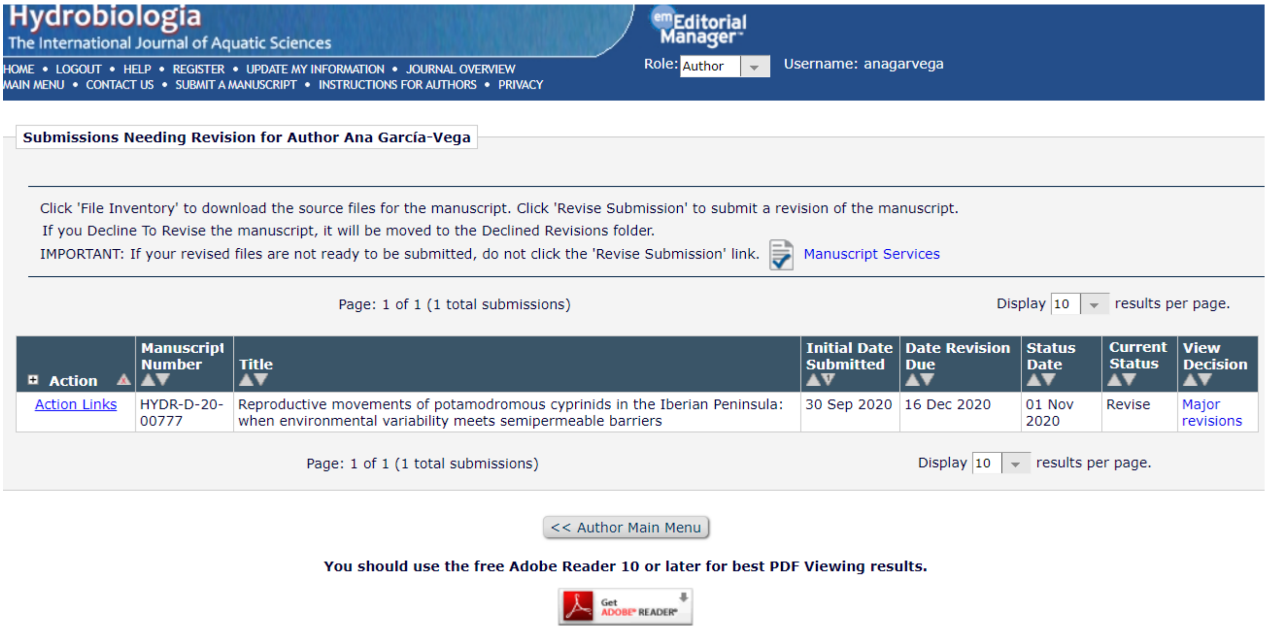
Submitted to: *Hydrobiologia.*

Status: Major revisions

Indexation database: Journal Citation Reports (JCR) by Clarivate Analytics (www.jcr.clarivate.com)

Impact index (JCR) in 2019 (last update): Impact factor = 2.385. Rank: 22/107 (Q1) in Marine & Freshwater biology.

Screenshot of the article management website:



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Submissions Needing Revision for Author Ana García-Vega

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Action	Manuscript Number	Title	Initial Date Submitted	Date Revision Due	Status Date	Current Status	View Decision
Action Links	HYDR-D-20-00777	Reproductive movements of potamodromous cyprinids in the Iberian Peninsula: when environmental variability meets semipermeable barriers	30 Sep 2020	16 Dec 2020	01 Nov 2020	Revise	Major revisions

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Article 5 (Chapter IV)

Provisional reference: García-Vega, A., Fuentes-Pérez, J.F., Leunda, P.M., Ardaiz, J. & Sanz-Ronda, F.J. 2020. Upstream migration of anadromous and potamodromous brown trout: patterns and triggers in a 25-year overview.

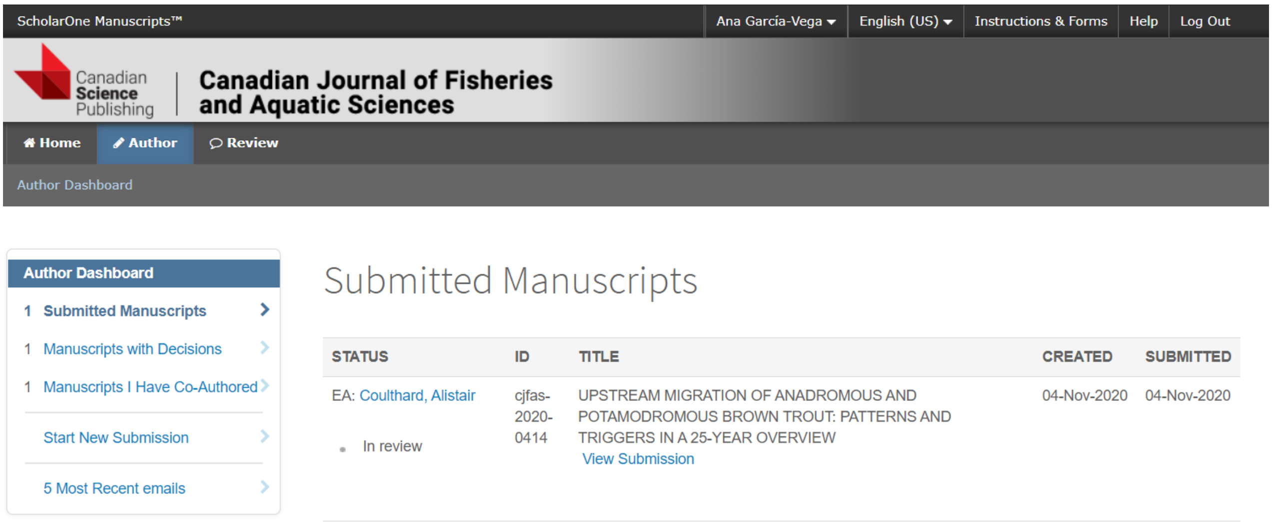
Submitted to: *Canadian Journal of Fisheries and Aquatic Sciences.*

Status: In review

Indexation database: Journal Citation Reports (JCR) by Clarivate Analytics (www.jcr.clarivate.com)

Impact index (JCR) in 2019 (last update): Impact factor = 2.849. Rank: 9/53 (Q1) in Fisheries; 16/107 (Q1) in Marine & Freshwater biology.

Screenshot of the article management website:



Submitted Manuscripts

STATUS	ID	TITLE	CREATED	SUBMITTED
EA: Coulthard, Alistair • In review	cjfas-2020-0414	UPSTREAM MIGRATION OF ANADROMOUS AND POTAMODROMOUS BROWN TROUT: PATTERNS AND TRIGGERS IN A 25-YEAR OVERVIEW View Submission	04-Nov-2020	04-Nov-2020

Palencia (Spain), 19 November 2020

Ana García Vega

NOTES: More than three articles may be included, providing the same information. All publications listed must be accepted or published within the period in which the student has been registered in the PhD program.

IMPORTANT: If articles are accepted but not yet published, a proof must be provided (e.g. a letter from the editor or a screenshot of the article management website).

ANNEX 2

CO-AUTHOR PERMISSION

(As required by Section 8.1. of the Regulation concerning doctoral thesis defense at UVa, sections e and f)

Mr. **Francisco Javier Sanz Ronda** with DNI number 15398294R as co-author of the publications:

- García-Vega, A., Sanz-Ronda, F.J. & Fuentes-Pérez, J.F. 2017. Seasonal and daily upstream movements of brown trout Salmo trutta in an Iberian regulated river. Knowledge & Management of Aquatic Ecosystems, 418: 9. DOI: https://doi.org/10.1051/kmae/2016041.
- García-Vega, A., Sanz-Ronda, F.J., Fernandes Celestino, L., Makrakis, S. & Leunda, P.M. 2018. Potamodromous brown trout movements in the North of the Iberian Peninsula: Modelling past, present and future based on continuous fishway monitoring. Science of the Total Environment, 640–641: 1521–1536. DOI: https://doi.org/10.1016/j.scitotenv.2018.05.339
- García-Vega, A., Leunda, P.M., Ardaiz, J. & Sanz-Ronda, F.J. 2020. Effect of restoration measures in Atlantic rivers: A 25-year overview of sea and riverine brown trout populations in the River Bidasoa. Fisheries Management and Ecology, 27: 580-590. DOI: https://doi.org/ 10.1111/ fme.12458
- García-Vega, A., Fuentes-Pérez, J.F., Bravo-Córdoba, F.J., Ruiz-Legazpi, J., Valbuena-Castro, J. & Sanz-Ronda, F.J. 2020. Reproductive movements of potamodromous cyprinids in the Iberian Peninsula: when environmental variability meets semipermeable barriers. [In review]
- García-Vega, A., Fuentes-Pérez, J.F., Leunda, P.M., Ardaiz, J. & Sanz-Ronda, F.J. 2020. Upstream migration of anadromous and potamodromous brown trout: patterns and triggers in a 25-year overview. [In review]

I give my consent for these to form part of the documentation for the deposit and defense of the Doctoral Thesis, presented at the University of Valladolid by Ms. Ana García Vega entitled "Ecohydraulic study of Iberian fish migration".

Likewise, I inform that the contribution of the doctoral student has been as follows: *She defined the research problem, analyzed the data and discussed the results.*

Finally, I inform that I have not used said publications and/or articles as part of the deposit and defense documentation of another doctoral thesis and that I refuse to use it in a future doctoral thesis.

Palencia (Spain), 23 November 2020

SANZ RONDA
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JAVIER - DNI
15398294R

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Fco. Javier Sanz Ronda

CO-AUTHOR PERMISSION

(As required by Section 8.1. of the Regulation concerning doctoral thesis defense at UVa, sections e and f)

Mr. Juan Francisco Fuentes Pérez with DNI number 78880235X as co-author of the publications:

- García-Vega, A., Sanz-Ronda, F.J. & Fuentes-Pérez, J.F. 2017. Seasonal and daily upstream movements of brown trout Salmo trutta in an Iberian regulated river. Knowledge & Management of Aquatic Ecosystems, 418: 9. DOI: https://doi.org/10.1051/kmae/2016041.
- García-Vega, A., Fuentes-Pérez, J.F., Bravo-Córdoba, F.J., Ruiz-Legazpi, J., Valbuena-Castro, J. & Sanz-Ronda, F.J. 2020. Reproductive movements of potamodromous cyprinids in the Iberian Peninsula: when environmental variability meets semipermeable barriers. [In review]
- García-Vega, A., Fuentes-Pérez, J.F., Leunda, P.M., Ardaiz, J. & Sanz-Ronda, F.J. 2020. Upstream migration of anadromous and potamodromous brown trout: patterns and triggers in a 25-year overview. [In review]

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- García-Vega, A., Leunda, P.M., Ardaiz, J. & Sanz-Ronda, F.J. 2020. Effect of restoration measures in Atlantic rivers: A 25-year overview of sea and riverine brown trout populations in the River Bidasoa. Fisheries Management and Ecology, 27: 580-590. DOI: https://doi.org/ 10.1111/ fme.12458
- García-Vega, A., Fuentes-Pérez, J.F., Leunda, P.M., Ardaiz, J. & Sanz-Ronda, F.J. 2020. Upstream migration of anadromous and potamodromous brown trout: patterns and triggers in a 25-year overview. [In review]

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Jorge Valbuena Castro