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- Normalized Abundance Spectra of fish community reflect
- 2 hydro-peaking on a Mediterranean large River

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#### 15 Abstract

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The European Water Framework Directive requires the integration of body size characters as an important part of fish-based bioassessment tools for freshwaters ecological status determination. The study of the entire fish community size-structure provides valuable information about food web capacity, food web stability and ecological efficiency of aquatic ecosystems. One of the most used representations of community size structure is the Normalized Abundance Spectra (NAS) that provides an approximation of the total fish abundance and food web capacity (through the parameter y-intercept) and an estimation of food web efficiency (through the slope of the linear regression). In this study we explored NAS of the lower Ebro River fish community by integrating data from monthly electrofishing samplings during a whole year (November 2014- October 2015). We found that the percentage of total alien and alien-prey individuals were directly related with yintercept and inversely related with slope of NAS. This is because the bulk of the community consists of introduced species of small body length. Furthermore, we detected significant relationships between NAS-related parameters and the hydrological variables describing diel flow oscillations and daily flow variability. Based on this, we suggest that high flow variability conditions and, above all, high hydro-peaking conditions, caused a diminution of the total abundance of fish and a decrease of the proportion of small sized fish (i.e. lower y-intercept and flatter slopes of NAS, respectively). Finally, a significant interaction between hydro-peaking and the percentage of alien-predators suggests that high hydro-peaking conditions benefit predation by facilitating predator-prey encounters. This is reflected by strong linear relationship between NAS parameters and percentage of piscivorous at high hydropeaking conditions that disappears at low hydro-peaking fluctuations. We concluded that the high proportion of alien fishes and the presence of a hydropower generation plant that operates by hydro-peaking are important factors determining fish size structure in the lower Ebro River.

## Key words

Size Structure, fish community, Ebro River, hydro-peaking, alien species, size spectra

## Highlights

- 1- Small sized species of alien fish dominate the lower Ebro River fish community.
- 2- Normalized Abundance Spectra (NAS) of fish were evaluated monthly for one year.
- 3- NAS reflected hydro-peaking impacts on the fish community.
- 45 4- High hydro-peaking conditions may increase predatory efficiency of piscivorous fish.

#### 1. Introduction

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Since the implementation of the European Water Framework Directive (WFD) (European Community, 2000) a huge effort has been done to find suitable bioassessment tools for the assessment of freshwater ecosystems health. Indeed, the WFD requires the use of size characteristics as a normative condition for fish-based bioassessment tools (Murphy et al., 2013; Reyjol et al., 2014), and it is certainly true that size structures of aquatic communities are shaped by biotic interactions and environmental factors, and may therefore reflect changes occurred on the surrounding conditions, whether of anthropogenic or natural origin (Blanchard et al., 2017; Emmrich et al., 2014; Murry and Farrell, 2014; Sprules and Barth, 2016). There are many approaches to visualize the size distribution of organisms in a sample that have become increasingly popular since the formulation of the biomass size spectrum theory in the mid-1960s. This theory states that in aquatic ecosystems, the sum of biomass is approximately constant across equal logarithmic intervals of body size from the smallest to the biggest organism (Sprules and Barth, 2016). From this idea emerged the Normalized Abundance Spectra (NAS) that can be adjusted to a linear model and provide information about food-web capacity (through the y-intercept of the spectrum) and ecological efficiency of communities (through the slope of the spectrum) (Daan et al., 2005; Mehner et al., in press; Rice and Gislason, 1996). Size structure approaches have been extensively used to study the ecosystems in marine environments (Andersen and Beyer, 2006; Blanchard et al., 2017, 2005; Kimmel et al., 2006; Platt and Denman, 1977; Sheldon et al., 1972), and in lakes and lagoons (Arranz et al., 2015; Brucet et al., 2010, 2005, Emmrich et al., 2014, 2011). But there are very few studies on rivers (Benejam et al., 2018, 2015; Broadway et al., 2015; Murry and Farrell, 2014). In this study we analysed Normalized Abundance Spectra (NAS) of the fish community of the lower Ebro River, a Mediterranean large river in the Iberian Peninsula, to test their ability to detect environmental and biotic disturbances.

The Ebro River is affected by several human impacts: water abstraction, dam regulation with subsequent altered hydrological regime (Batalla et al., 2004) and low suspended sediments (Rovira et al., 2007), industrial pollution (Benejam et al., 2010; Huertas et al., 2016), thermal pollution (Prats et al., 2012, 2010), proliferation of macrophytes (Ibáñez et al., 2012) and introduction of many alien fish species (Caiola et al., 2014; Elvira, 1995a, 1995b), among others. It is well known that non-native fish introductions can result in local decline and even extinction of native species through different mechanisms such as genetic introgression, introduction of parasites and diseases, competition for habitat, habitat alteration and predation (García-Berthou, 2007). Specifically in the lower Ebro River, where this study is focused, some of the new introduced species are voracious piscivorous (e.g. *Silurus glanis*) that can cause critical changes in the original fish populations because many

indigenous species have evolved in communities with none native piscivorous fishes (Carol et al., 2009; Ribeiro and Leunda, 2012). Another important source of alteration from the natural ecological status in the lower Ebro River is the presence of a hydroelectric generation power plant that operates by hydro-peaking. This is the procedure by which hydroelectricity is produced to cover the daily fluctuating demands of the energy market and it results on a daily peaking flow regulation that affects the biological dynamics of downstream communities. Many authors reported adverse impacts of hydro-peaking on fish caused by, inter alia, stranding, habitat abandonment, downstream displacement, inhibition of spawning, migration obstruction, habitat modification, increase of physiological stress and depleted food production (Almeida et al., 2017; Boavida et al., 2015; Schmutz et al., 2015; Young et al., 2011). Among all the mentioned environmental disturbances, we looked for potential causes of alteration on NAS through an extensive sampling of fish stocks by electrofishing.

The main objective of the present paper is to identify the natural and anthropogenic factors affecting size structure of fish community in a large Mediterranean river. In this way, we want to evaluate the potential of Normalized Abundance Spectrum parameters as size-related bioindicators to complement the fish-based indices currently in use.

## 2. Material and methods

#### 2.1. Study area

The Ebro River is located in the NE of the Iberian Peninsula (Fig. 1a). With a total drainage area of 85.569 km<sup>2</sup> and a mean annual flow of 294 m<sup>3</sup>·s<sup>-1</sup>, provides valuable ecosystem services to people such as water for agriculture uses (irrigation area of 906.000 ha), urban supply (basin population of 3.176.091) and hydro-power generation (458 stations) (Confederación Hidrográfica del Ebro, 2009).

In terms of hydrology the study area can be divided in two regions: the river section upstream from Xerta weir that comprises the stations E05 to E02 where the mean annual flow is of approximately 320 m<sup>3</sup>·s<sup>-1</sup>, and the reach after Xerta weir that comprises sampling station E01. This weir is the last human obstacle before the river mouth and its function is to divert water for agricultural uses and human consumption. The mean annual flow in station E01 is about 280m<sup>3</sup>·s<sup>-1</sup>. Hydro-peaking oscillations generated in a hydroelectrical power plant located in Flix dam are perceptible in all the study area (Figs. 1 and 2).

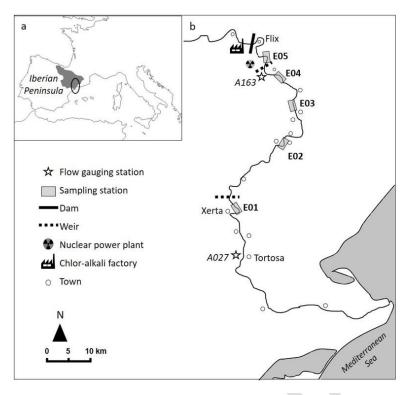


Figure 1. (a) Location of the Ebro River and (b) the five sampling stations.

## 2.2. Sampling

Data of fish species abundance and body size were collected by electro-fishing in 5 stations on the lower Ebro River (Fig. 1). The stations were randomly selected to cover all the hydromorphological variability of the study area (for more details see Caiola et al., 2014). Each station consisted in a 2 km length stretch to fulfil the requirements of EN 14011 European standard (CEN. European Committee for Standardization, 2003) that establishes a minimum sampling stretch length of ten times the river width (in the study area the mean width is *ca*. 180 m). Ten equidistant points located in the littoral zone were sampled within each station, randomly alternating left and right banks, with a total length sampled at each sampling station of about 200m. A boat based electro-fishing gear (Model: EL63IIGI, HANS GRASSL GmbH, Schönau am Königssee, Germany) was used at 600 V and 10 A pulsed D.C. The fish were collected with a dip net of 2.5 m long, 47 cm of diameter and 7 mm of mesh size. The specimens were identified to species level, counted, measured and weighted. Native specimens were returned to the river and alien fishes were sacrificed with an overdose of anesthetic (MS-222). We normalized abundances dividing by fished area and time of fishing, obtaining catches per unit effort (CPUE: individuals·ha<sup>-1</sup>·min<sup>-1</sup>) and biomass per unit effort (BPUE: kg·ha<sup>-1</sup>·min<sup>-1</sup>).

In addition to fish community metrics, we measured several environmental factors and habitat variables: water temperature (°C), conductivity (mS·cm<sup>-1</sup>), dissolved oxygen (mg·l<sup>-1</sup>) and total dissolved solids (mg·l<sup>-1</sup>) were measured with a multi-parameter probe (YSI model 556 MPS); water depth (m) was measured using a portable depth-meter (Hondex model PS-7) and water flow velocity (m·s<sup>-1</sup>) was measured with a current-meter (Global Water model FP101). The presence, absence and area covered by macrophytes was recorded by visual approximation following the recommendations for macrophytes assessment of the STAR project (Dawson, 2002). Daily and diel flow data series available at the Ebro Water Authority (CHE) web site (http://www.chebro.es/) were used to calculate hydrological indices (see section 2.5. Hydrological variables calculation).

Monthly sampling campaigns were conducted between November 2014 and October 2015. Due to safety questions, we avoided sampling on December 2014, February and March 2015, when the water flow at the study area was higher than 500 m<sup>3</sup>·s<sup>-1</sup> and it was impossible to guarantee the safety of the crew.

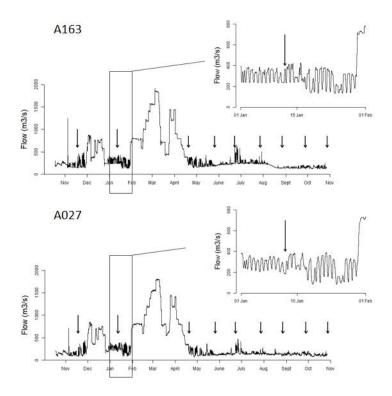


Figure 2. Hydrographs on gauging station A163 (upper figure) and on gauging station A027 (lower figure) during the study period November 2014 – October 2015. Arrows indicate sampling dates. Note the hydropeaking oscillations.

# 2.3. Fish size spectrum calculation

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For each station and sampling period we modeled the Normalized Abundance Spectrum (NAS) with body lengths of the entire fish community. NAS is obtained through a binning method in which body size measurements are grouped into logarithmic size intervals and the abundance of individuals is represented for each size class in a log<sub>2</sub>-log<sub>2</sub> plot. We standardized abundance by dividing it by the linear width of the size interval as described on Sprules and Barth (2016). The linear size spectra was calculated from NAS as the linear regression between the log<sub>2</sub> midpoint of size classes (abscissas) and the log<sub>2</sub> of normalized abundance per size class (ordinates) (Fig. 3). We considered six size classes following a  $\log_2$  scale (1<sup>st</sup> class,  $\leq 2^6 = 64$  mm; 2<sup>nd</sup> class,  $> 2^6 = 64$  mm to  $2^{7}$ =128 mm;  $3^{rd}$  class, > $2^{7}$ =128 mm to  $2^{8}$ =256 mm;  $4^{th}$  class, > $2^{8}$ =256 mm to  $2^{9}$ =512 mm;  $5^{th}$  class,  $>2^9=512$  mm to  $2^{10}=1024$  mm;  $6^{th}$  class,  $>2^{10}=1024$  mm to maximum length). Electro-fishing has been shown as a suitable method to estimate size structure of fish in rivers (Benejam et al., 2015). However, although it has been demonstrated to be less selective than other sampling methods, it can imply an underestimation of small fish in some species due to the fact that the electric field has greater effects on large fish (Barbour et al., 1999; Borgstrøm and Skaala, 1993; Millar et al., 2016). For this reason, the smallest size classes were grouped in the first class (<64mm) (Benejam et al., 2015). We estimated the intercept and the slope for each linear size spectrum of the entire fish community. The y-intercept of the linear size spectrum can be interpreted as an approximation of food web capacity (Gaedke and Straile, 1994; Murry and Farrell, 2014) whereas the slope equals to a measure of energy transfer or ecological efficiency (Emmrich et al., 2011, Mehner et al. in press). We only considered for the analysis the size distributions that fit a linear spectrum, so we discarded 3 regressions with p values >0.1 (Arranz et al., 2015) from a total of 45 (5 stations per 9 sampling campaigns).

# 2.4. Hydrological variables calculation

There are two official gauging points for water flow measurement in the study area: the gauging stations A163 in Ascó and A027 in Tortosa (Fig. 1). They belong to the Automatic System for Hydrological Information (SAIH) of the Ebro River (Confederación Hidrográfica del Ebro, 2002) and provide a flow measurement every 15 minutes. We used the A027 series as an approximation of the flow on sampling station E01 and the A163 series for the sampling stations E02, E03, E04 and E05.

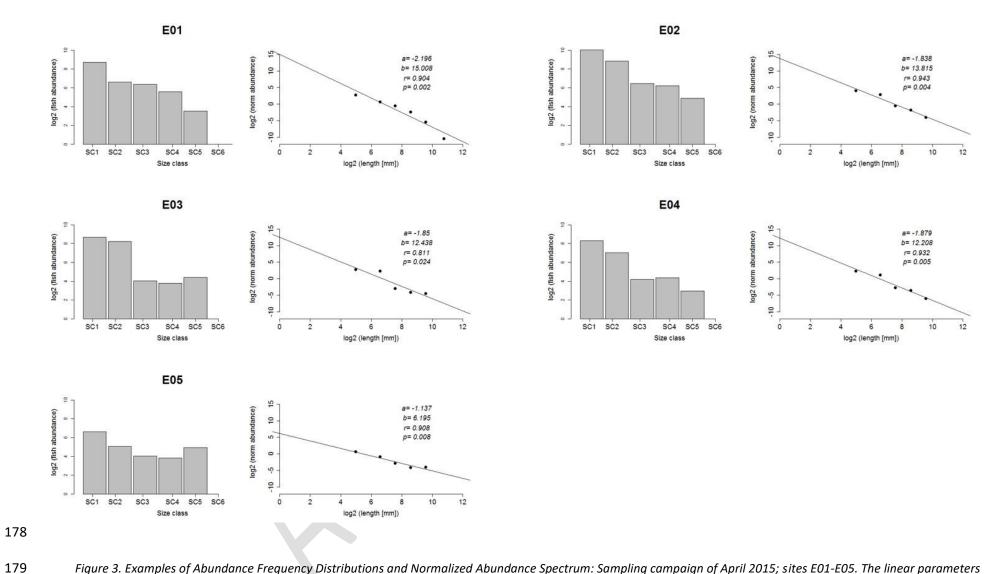


Figure 3. Examples of Abundance Frequency Distributions and Normalized Abundance Spectrum: Sampling campaign of April 2015; sites E01-E05. The linear parameters a (slope) and b (y-intercept) are shown, as well as their r and p values.

Index	Calculation
Daily indices	
M <sub>A</sub> 3	Coefficient of variation in daily flows
M <sub>A</sub> 44	Variability in daily flows divided by median monthly flow, where variability is calculated as $90^{th}$ - $10^{th}$ percentile
M <sub>L</sub> 13	Coefficient of variation in minimum flows
M <sub>L</sub> 14	Lowest monthly flow divided by median monthly flow
M <sub>H</sub> 17	25 <sup>th</sup> percentile from the flow duration curve divided by median daily flow
Мн 20	Monthly maximum flow
F <sub>L</sub> 1	Number of occurrences during which the magnitude of flow remains below the 25 <sup>th</sup> percentile
F <sub>H</sub> 5	Number of occurrences during which the magnitude of flow remains above the median daily flow
D <sub>H</sub> 12	Mean 7-day maximum divided by median flow
R <sub>A</sub> 8	Number of negative and positive changes in water conditions from one day to the next
$M_A5$	Skewness in daily flows: Mean daily flows divided by median daily flows
Subdaily indic	es
dmin	Daily minimum
dmax	Daily maximum
dD	Daily delta or range
dSD	Daily Standard deviation
dramp	Maximum hourly ramp rate
dpath	Daily path length (the geometric distance of the daily hydrograph of flow versus time
Drev	Number of changes between rising and falling periods)
Drf	Rise and fall counts difference
dAstD	Annually standardized delta (monthly mean of daily delta divided by annual mean)
dCV	Coefficient of variation (daily standard deviation divided by the mean monthly daily flow)
dstMHramp	Standardized maximum hourly ramping rate (maximum daily ramp rate divided by the mean
	monthly daily flow
dflash	Richards-Baker flashiness index (daily path length of oscillations divided by the daily mean
	over each 24-h period)

Table 1 – Hydrological indices adapted from Olden & Poof (2003) and Bevelhimer et al (2014) for a period of one month before date of sampling. Daily indices were calculated from a daily data series and sub-daily indices from an hourly data series.

A collection of hydrological indices adapted from literature was calculated (Table 1). The set of daily indices were described in Olden and Poff (2003) as key variables to characterize the hydrological regime for our type of river (with snow and rain influence) while avoiding redundancy. We included adapted descriptors of magnitude of flow events ( $M_A3$ ,  $M_A44$ ,  $M_L13$ ,  $M_L14$ ,  $M_H17$  and  $M_H20$ ), frequency of flow events ( $F_L1$  and  $F_H5$ ), duration of flow events ( $D_H12$ ) and the rate of change in flow conditions ( $R_A8$ ). We also included the skewness in daily flows ( $M_A5$ ), described by Kinsolving and Bain (1993) as important in the response of fish to anthropogenic flow alterations. Sub-daily indices were proposed by Bevelhimer et al. (2014) as good descriptors of diel flow variations such as the produced by power generation plants. Among them, there are two descriptors of magnitude (dmin and dmax) and one descriptor of the frequency of oscillations (drev). The rest are different ways of expressing the amplitude of flow fluctuations (dD, dSD, dramp, dpath, drf, dAstD, dCV,

dstMHramp, dflash). We adapted all the indices to assess flow variations in a short temporal scale and calculated them for the period of one month before each sampling.

#### 2.6. Biotic factors

To assess how the presence of alien species affects the size structure of the community, three biotic factors were considered: percentage of alien individuals, percentage of alien-piscivorous individuals (over total catches) and percentage of alien-prey individuals (over total catches). It should be noted that the only native piscivorous fish that we found was the sea bass (*Dicentrarchus labrax*), a marine species that occasionally migrate between freshwater and the sea (in both directions), for feeding purposes. We only fished 12 individuals of this species, always in the transect E01 (the station furthest downstream), so we could say that the piscivorous population is almost entirely constituted by alien fish (Table 2).

To calculate the number of piscivorous, juvenile individuals considered too young to feed on fish, were subtracted for each predator species. That is: only individuals greater than 120 mm were considered piscivorous for *Perca fluviatilis* (Kottelat and Freyhof, 2007) and *Sander lucioperca* (Aparicio et al., 2016), while this threshold was 50 mm for *Silurus glanis* (Copp et al., 2009) and *Micropterus salmoides* (García-Berthou, 2002). To determine the percentage of alien-prey individuals, all the non-piscivorous alien fishes were considered susceptible to predation, so we calculated it as the subtraction of percentage of alien minus percentage of alien-piscivorous.

#### 2.7. Statistical Analyses

To check for relationships between the response variables y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency) and the potential explanatory variables (macrophyte coverage, water temperature, conductivity, dissolved oxygen, total dissolved solids, water depth, water velocity, percentage of alien fish, percentage of alien-piscivorous, percentage of alien-prey and the hydrological indices on Table 1), we performed simple lineal regressions with autocorrelation structures for time and space when necessary. The large number of explanatory variables that we wanted to include in the analysis prevented us from applying multiple regressions and thus, we performed simple lineal regressions in this first step. As our sampling design consists of several observations along time within each river stretch, it results on a data set with a possible lack of independence, both spatial and temporal. To deal with this limitation we used Mixed Effects Models with river reach as random factor and tested the inclusion of temporal auto-correlation structures to improve the models as described in Zuur et al. (2009). Firstly, we tested 5 types of auto correlation structures: AR-1, linear correlation, Gaussian correlation, exponential correlation and

Species	Common name	Reproduction	Spawning time	Migration	Habitat	Feeding	Status	First detected	Total	CPUE	BPUE
Anguilla anguilla	Eel	Pelagophilic <sup>3</sup>	late winter or spring <sup>7</sup>	Diadromous <sup>1,2</sup>	Eurytopic <sup>1</sup>	Omnivorous <sup>1</sup>	N	-	1879	321.2	75.45·10 <sup>3</sup>
Dicentrarchus labrax	Seabass	Pelagophilic <sup>5</sup>	January-March <sup>7</sup>	Amphidromous <sup>5</sup>	Limnophilic <sup>7</sup>	Piscivorous <sup>5</sup>	N	-	12	1.7	$0.88 \cdot 10^{3}$
Gobio lozanoi	Iberian gudgeon	Polyphilic⁵	May-July <sup>7</sup>	Resident <sup>1</sup>	Rheophilic <sup>2</sup>	Invertivore <sup>2</sup>	N	-	332	54.6	$0.11 \cdot 10^3$
Undetermined Mullet	Mullet	Pelagophilic <sup>5</sup>	-	Amphidromous <sup>5</sup>	Limnophilic <sup>7</sup>	Detritivorous <sup>5</sup>	N	-	398	56.1	8.14·10 <sup>3</sup>
Luciobarbus graellsii	Ebro barbel	Litophilic <sup>5</sup>	May-July <sup>7</sup>	Potamodromous <sup>5</sup>	Limnophilic <sup>5</sup>	Omnivorous <sup>5</sup>	N	-	85	10.9	$2.72 \cdot 10^3$
Salaria fluviatilis	Freshwater blenny	Litophilic <sup>2,3</sup>	May-August <sup>7</sup>		Eurytopic <sup>5</sup>	Invertivore <sup>2,3</sup>	N	-	746	200.9	1.49·10 <sup>3</sup>
Squalius laietanus	Catalan chub	Litophilic <sup>2</sup>	April-July <sup>7</sup>	Potamodromous <sup>5</sup>	Rheophilic <sup>2</sup>	Omnivorous <sup>2</sup>	N	-	1371	203.1	$2.95 \cdot 10^3$
Alburnus alburnus	Bleak	Litophilic <sup>4</sup>	April-June <sup>7</sup>	Potamodromous <sup>5</sup>	Limnophilic <sup>5</sup>	Omnivorous <sup>2</sup>	Α	1992	18567	2661.9	113.95.10
Carassius auratus	Goldfish	Phytophilic <sup>2,3</sup>	May-July <sup>7</sup>	Potamodromous <sup>6</sup>	Limnophilic <sup>5</sup>	Omnivorous <sup>2,3</sup>	Α	s.XVII	174	24.5	10.49·10 <sup>3</sup>
Cyprinus carpio	Common carp	Phytophilic <sup>2,3</sup>	May-July <sup>7</sup>	Resident <sup>1</sup>	Limnophilic <sup>1</sup>	Omnivorous <sup>1,2,3</sup>	Α	s. XVI	343	48.4	188.34.10
Gambusia holbrooki	Eastern mosquitofish	Ovoviviparous	<sup>3</sup> May- September <sup>7</sup>	Resident <sup>1</sup>	Limnophilic <sup>1,2</sup>	Invertivorous <sup>2,3</sup>	Α	1921	1448	273.8	0.33·10 <sup>3</sup>
Lepomis gibbosus	Sunfish	Polyphilic <sup>3</sup>	May-June <sup>7</sup>	Resident <sup>1</sup>	Limnophilic <sup>1,2</sup>	Invertivorous <sup>1,2,3</sup>	Α	80's	113	15.0	$0.44 \cdot 10^3$
Micropterus salmoides	Largemouth black bass	Polyphilic <sup>3</sup>	late spring or early summer <sup>7</sup>	Resident <sup>1</sup>	Limnophilic <sup>1,2</sup>	Piscivorous <sup>1,2,3</sup>	Α	1955	1	0.1	0.04·10 <sup>2</sup>
Pseudorasbora parva	Razbora	Polyphilic <sup>5</sup>	April-June <sup>7</sup>		Limnophilic <sup>7</sup>	Omnivorous <sup>2</sup>	Α	1999	1118	157.7	0.82·10 <sup>3</sup>
Perca fluviatilis	European perch	Phytophilic <sup>5</sup>	April-May <sup>7</sup>	Resident <sup>5</sup>	Limnophilic <sup>5</sup>	Piscivorous <sup>2</sup>	Α	1970	35	4.9	$0.16 \cdot 10^3$
Rutilus rutilus	Roach	Polyphilic <sup>7</sup>	April-June <sup>7</sup>		Limnophilic <sup>5</sup>	Omnivorous <sup>2</sup>	Α	80's	528	74.5	$4.48 \cdot 10^3$
Scardinius erythrophthalmus	Rudd	Phytophilic <sup>2</sup>	April-June <sup>7</sup>		Limnophilic <sup>2</sup>	Omnivorous <sup>2</sup>	Α	1910-13	79	11.1	0.60·10 <sup>3</sup>
Silurus glanis	Wels catfish	Phytophilic <sup>2,7</sup>	April-June <sup>7</sup>		Limnophilic <sup>7</sup>	Piscivorous <sup>2</sup>	Α	1974	213	30.0	101.37.10
Sander lucioperca	Zander	Litophilic <sup>5</sup>	March-April <sup>7</sup>	Potamodromous <sup>5</sup>	Limnophilic <sup>5</sup>	Piscivorous <sup>5</sup>	Α	1990	203	28.6	$2.37 \cdot 10^3$

Table 2 – Latin names, common names, reproductive, migratory, habitat and feeding behaviours, status refereed to the Ebro River (N=native; A=alien), total catches,

mean CPUEs and mean BPUEs. Blank means no classified (1-Alexandre et al, 2013; 2- Garcia-Berthou et al, 2015; 3- Magalhaes et al, 2008; 4- Pinder AC, 2001; 5- Odreix et

<sup>230</sup> al, 2014; 6- Riede K, 2004; 7- Aparicio et al, 2016)

spherical correlation. Once we knew the best temporal auto-correlation expression we proceeded to choose the best model comparing: a) a linear regression model without any autocorrelation; b) a linear regression model with the best autocorrelation structure for time; c) a mixed effects model with river reach as random factor and d) a mixed effects model with river reach as random factor and the best autocorrelation structure for time. The models were adjusted using the R package nlme (Pinheiro et al., 2017; R Core Team, 2016) and compared with the Akaike Information Criterion (AIC) (Akaike, 1974). The most parsimonious model (with the lowest AIC) was selected each time.

In a second step, from the initial set of hydrological variables, we considered only those that were significantly related with size spectra parameters in the previous analysis to investigate possible multiplicative effects between hydrology and biotic factors. To avoid redundant tests, we first calculated the correlation matrix for all factors and discarded correlated hydrological indices with r > 0.6. Then, interactions were tested for all the possible combinations between the three biotic factors (percentage of alien, percentage of alien-prey and percentage of alien-piscivorous) and the three uncorrelated hydrological indices (dAstD,  $F_L1$  and  $M_A5$ ), with models of the type:

Response variable ~ hydrological factor + biotic factor + hydrological factor : biotic factor

Were "response variable" refers to y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency). To avoid multicollinearity, the predictors were centered before the calculation of the interaction term by subtracting their means. We applied the same model selection procedure than before.

## 3. Results

## 3.1. Fish community composition

The study area was clearly dominated by alien fishes, with an average of 79.7% of alien individuals and 82.2% of alien biomass abundances. The bleak (*Alburnus alburnus*) was the most abundant species in number of individuals, followed by the European eel (*Anguilla anguilla*). Common carp (*Cyprinus carpio*) and again, the bleak, were the most abundant in terms of biomass. In regards to feeding functional group, most of the fish were omnivorous (86.4%), followed by the invertivorous (10.2%), the piscivorous (1.8%) and the detritivorous (1.5%) (Table 2).

Concerning average fish size, native individuals were on average  $42.93 \pm 3.77$  mm bigger than the community mean, while alien fish were  $15.47 \pm 2.57$  mm smaller. As expected, alien-piscivorous had a large mean body length,  $344.97 \pm 25.07$  mm above average, and alien-preys had a mean size  $26.16 \pm 2.2$  mm below average.

## 3.2. Effects of environmental and biotic variables on size structure

As shown on Table 3, y-intercept of NAS (food web capacity) was negatively related with hydrological indices describing the amplitude of sub-daily flow oscillations (dD, dSD, dpath, and dAstD) and their frequency (drev), hydrological variables estimating high (F<sub>H</sub>5) and low (F<sub>L</sub>1) flow events frequency and the skewness in daily flows (M<sub>A</sub>5). Thus, the greater the flow variability, the lower the y-intercept of NAS (food web capacity), indicating a fewer total abundance of fish in the entire community when hydrological oscillations were high. Furthermore, y-intercept of NAS was also negatively related with the percentage of alien piscivorous fish. This has a direct interpretation, since we can expect that a higher number of piscivorous would imply higher prey consumption, and so, a diminution of total abundance (i.e. y-intercept or food web capacity). In the other hand, y-intercept was positively related with the percentage of total alien individuals and percentage of alien-prey, indicating that these groups of organisms contribute to increase the total number of fish, and so, the amount of energy available in the ecosystem.

Response variable	Predictor	AIC <sub>0</sub>	AIC <sub>f</sub>	Model	Estimate	SE	p_value	R <sup>2</sup>
y-intercept of NAS	dD	219.53	201.72	M2	-0.034	0.011	<0.01	0.52
(food web capacity,	) dSD	217.37	199.96	M2	-0.091	0.030	<0.01	0.53
	dpath	220.93	200.82	M2	-0.019	0.005	<0.001	0.54
	drev	217.37	202.24	M2	-0.074	0.024	<0.01	0.56
	dAstD	207.17	189.76	M2	-13.461	4.360	< 0.01	0.52
	F <sub>L</sub> 1	219.25	202.18	M2	-0.100	0.022	<0.001	0.40
	F <sub>H</sub> 5	219.57	204.37	M2	-0.087	0.018	< 0.001	0.31
	M <sub>A</sub> 5	204.75	184.90	M2	-15.056	3.326	< 0.001	0.63
	% alien	205.95	183.58	M3	0.118	0.022	<0.001	0.31
	% alien prey	205.98	183.52	M3	0.113	0.021	<0.001	0.22
	% alien piscivorous	212.06	195.00	M3	-0.724	0.333	< 0.05	0.05
slope of NAS (food	dD	55.14	43.57	M2	0.004	0.001	<0.01	0.49
web efficiency)	dpath	56.63	44.45	M4	0.002	0.001	< 0.05	0.01
	drev	53.71	41.96	M4	0.006	0.003	<0.05	0.52
	dAstD	43.02	31.45	M2	1.672	0.570	< 0.01	0.49
	F <sub>L</sub> 1	54.57	40.91	M4	0.011	0.005	<0.05	0.00
	$M_A5$	43.04	29.96	M2	1.289	0.439	< 0.01	0.53
	% alien	45.87	28.13	M4	-0.014	0.003	<0.001	0.22
	% alien prey	45.95	28.39	M4	-0.013	0.003	<0.001	0.22

Table 3 – Results of the models adjusted between single predictors and the response variables y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency). Only significant models are shown. The column "Model" indicates: M2) Mixed Effects Model with river reach as random factor and an autocorrelation structure for time; M3) Generalized Least Squares Model with a corLin autocorrelation structure for time and M4) Generalized Least Squares Model with an AR-1 autocorrelation structure for time. AIC, Akaike information criteria; SE, Standard error. R² corresponds to a linear regression of fitted versus real values.

The slope of NAS (food web efficiency) was significantly and positively related with some hydrological indices describing the amplitude (dD, dpath and dAstD), and frequency (drev) of subdaily flow oscillations and daily flow variability ( $F_L1$  and  $M_A5$ ) (Table3), suggesting a diminution of the proportion of small sized fishes when flow variability was high. The slope (food web efficiency) was strongly and negatively related with the percentage of total alien individuals and percentage of alien-prey that means that a higher percentage of these groups lead to higher relative abundances of small fishes, and so, steeper slopes of NAS.

## 3.3. Interactions between hydrological and biotic factors

In the second part of the analysis, we investigated possible multiplicative effects between hydrology and biotic factors. To avoid redundant tests and given the high degree of autocorrelation among variables shown in Table 4, we selected dAstD as a descriptor of sub-daily flow, and  $F_L1$  and  $M_A5$  as indicators of daily regime flow and discarded dD, dSD, dpath, drev and  $F_H5$  for being highly correlated with the first.

	dD	dSD	dpath	drev	dAstD	F <sub>L</sub> 1	Fн5	M <sub>A</sub> 5	% alien	% alien prey	% alien piscivorous
dD	-	0	0	0	0	0.709	0.718	0.002	0.54	0.667	0.284
dSD	0.948	-	0	0	0	0.994	0.551	0.009	0.88	0.996	0.26
dpath	0.942	0.869	-	0	0	0.11	0.137	0	0.926	0.953	0.363
Drev	0.788	0.747	0.860	-	0	0.082	0.051	0	0.337	0.368	0.311
dAstD	0.987	0.922	0.915	0.773	-	0.715	0.683	0.001	0.281	0.37	0.377
F <sub>L</sub> 1	0.061	-0.001	0.257	0.278	0.060	-	0	0	0.262	0.246	0.122
F <sub>H</sub> 5	0.059	-0.097	0.239	0.310	0.067	0.711	-	0.005	0.069	0.077	0.076
$M_A5$	0.485	0.407	0.625	0.628	0.495	0.596	0.434	-	0.404	0.444	0.721
% alien	-0.100	-0.025	-0.015	-0.156	-0.175	-0.182	-0.290	-0.136	-	0	0
% alien prey	-0.070	-0.001	0.010	-0.146	-0.146	-0.188	-0.283	-0.124	0.996	-	0
% alien piscivorous	-0.174	-0.182	-0.148	0.164	-0.143	0.249	0.284	0.058	-0.616	-0.652	-

Table 4– Correlation matrix of the independent variables that showed significant relationship with size related variables in simple models. The shaded matrix contains Spearman's r values; the open matrix contains p values. In bold significantly correlated coefficients (r>0.6 and p<0.05).

As shown in Table 5, a highly significant multiplicative effect was found between dAstD and the percentage of alien-piscivorous fish when modelling their effects on the response variables derived from NAS (y-intercept or food web capacity and slope or food web efficiency). These results mean that the level of one factor (in this case dAstD that describes hydropeaking) modifies the way in which the other factor (here, percentage of alien-piscivorous) affects the response variable. To visualize these results, we represented in Figure 4 the y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency) as a function of the percentage of alien-piscivorous grouping data in high dAstD (values above their mean) and low dAstD (values bellow their mean). The variables dAstD and percentage of alien-piscivorous interact in such a way that in situations of high dAstD that indicate high hydropeaking oscillations, the relationship between the percentage of piscivorous and the y-intercept of NAS (trophic chain capacity) was strong (p<0.01 and  $R^2$ =0.44), whereas under low dAstD conditions (i.e. low diel oscillations of flow), the linearity between these two parameters was lost. Similarly, the slope of NAS (trophic chain efficiency) presented a significant linearity with the percentage of piscivorous in high dAstD conditions (p<0.01 and R<sup>2</sup>=0.43) but no linearity was found in periods of low dAstD. The interactions between the other hydrological variables (F<sub>L</sub>1 and M<sub>A</sub>5) and the rest of biotic factors (percentage of alien species and percentage of alien-prey) were no significant.

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Response variable	Significant predictors	Mode	el AIC₀	AICf	Estimate	SE	p value	R <sup>2</sup>
y-intercept of NAS	% of piscivorous	M1	195.75	176.32	-1.491	0.385	<0.001	0.74
(food web capacity)	dAstD				-22.376	4.387	<0.001	
	% of pisc.*:dAstD*				-17.052	4.619	<0.001	
slope of NAS (food	% of piscivorous	M1	41.68	24.16	0.146	0.047	<0.001	0.71
web efficiency)	dAstD				2.489	0.532	<0.001	
	% of pisc.*:dAstD*				2.184	0.560	<0.001	

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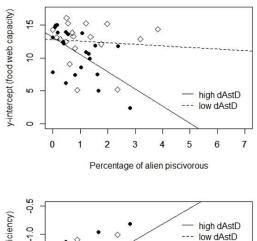
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Table 5 – Results of the models showing hydrological index dAstD, predatory pressure (% of piscivorous fishes) and their interaction affecting y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency). AIC, Akaike information criteria; SE, Standard error; R<sup>2</sup> corresponds to a linear regression of fitted versus real values; M1 means Mixed Effects Model with river reach as random factor. (\*) Indicates that predictors were centred to avoid autocorrelation.

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— high dAstD — low dastD — low

Figure 4. The response of y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency) to the percentage of piscivorous is dAstD depending. Note the different grades of regression in high and low dAstD conditions.

#### 4. Discussion

Our results confirm the high abundance of introduced fish species in the Ebro River previously reported by Almeida et al. (2017), Aparicio et al. (2016), Caiola et al. (2014), Elvira (1995a, 1995b) and López et al. (2012) among others. When we looked at how these species were represented in the size structure, we found that, although there were very large introduced species such as the Wels catfish (*Silurus glanis*) and de common carp, the most abundant alien species were characterized for being small sized fish with very high reproduction rates such as the bleak and the mosquitofish (*Gambusia holbrooki*). More concretely, 78% of small fish (lengths below the mean length of the entire community) were alien individuals while only a 22% were native fish. This high proportion of small introduced fish is related with higher values of food web capacity (i.e. higher y-intercept of NAS, calculated from the entire fish community) and lower food web efficiency (i.e. steeper slopes of NAS, calculated from the entire fish community). Thus, according to the models, an increase of the percentage of total alien individuals was related with an increase of food web capacity (i.e. total abundance), as well as with a decrease of food web efficiency and a greater

proportion of small fish. The same happened with the percentage of alien-prey since the correlation between total alien and alien-prey was very high (r=0.996). These results indicate that total and prey alien fish contribute to increase the amount of energy available in the ecosystem. Presumably, if the community were exclusively composed of autochthonous species, there would be fewer proportion of small sized individuals (there would be no *Gambusia holbrooki* nor *Pseudorasbora parva*) and the NAS would present flatter slopes and lower y-intercept values, although this is something that we cannot guarantee since all the trophic dynamics would be altered and the community would be totally different from the current one.

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Among the hydrological variables that we included in the analysis, only those describing variability of flow conditions were significantly related with fish community size structure. FH5 and F<sub>L</sub>1 are the occurrences of high and low extreme flow events while M<sub>A</sub>5 gives an idea of the asymmetry in the distribution of flow measurements. The others are directly linked to diel flow variability, and they can be considered hydro-peaking descriptors (Bevelhimer et al., 2014) that take higher values when the amplitude of flow sub-daily oscillations increase (dD, dSD, dpath, dAstD) or the frequency of fluctuations is higher (drev). A hydroelectric power plant that operates through hydro-peaking to cover daily rises of energy demand is located 4 km upstream from the station E05. Although the hydro-peaking was not constant during the whole year, it persisted for long periods with average ranges of flow fluctuation between 165 m<sup>3</sup>·s<sup>-1</sup> (in February) and 79 m<sup>3</sup>·s<sup>-1</sup> (in July). The negative relationship between hydrological indices and food web capacity can be read as a decrease of total fish abundance and the portion of small fish in conditions of high hydropeaking. Other authors previously observed a decrease of small sized fishes and juveniles in the river banks immediately downstream hydroelectric power stations that operate by hydro-peaking (Bain et al., 1988; Bond and Jones, 2015; Enders et al., 2017). The causes reported to explain this decline are several: Jones (2013) found a reduced population of benthic invertebrates in the margin area influenced by dewatering, and Bond and Jones (2015) hypothesized that this could lead to a lack of bentivorous fishes and juveniles that would move to deeper waters looking for more feeding resources. Enders et al (2017) pointed to the loss of suitable habitat, increased mortality or altered behaviour of small-bodied fishes as the causes of their observations, but they also pointed out that desiccation and scouring of spawning locations during the incubation period could suppose a big loss of individuals for the next generation. Our results further suggest that the decrease of total fish abundance and of small fish at high hydropeaking conditions may have consequences on the whole food web capacity.

Our results also showed that another biotic factor was significantly related with food web capacity and efficiency: the percentage of alien-piscivorous fish. As stated for the first time by Elton (1927) in the beginnings of trophic ecology, the predatory-prey interaction is size-depending in a manner that big fishes feed on the smaller ones. Thus, a big presence of piscivorous will reduce by consumption, the number of small sized fishes and this fact will be reflected on the shape of bodysize distributions (Allen et al., 2006). Similar trends have been observed by other researchers in marine environments, (Bianchi, 2000; Rice and Gislason, 1996) as well as in lakes (Emmrich et al., 2011) and rivers (Murry and Farrell, 2014). But going deeper into the linkage of predation and size structure, our results suggest that hydro-peaking has a multiplicative effect on this relationship, so that under conditions of high flow oscillations, the predation by alien-psicivorous may become more efficient, triggering to a higher modification of size spectrum parameters (Fig. 4). Under low hydropeaking conditions, the linearity between the amount of predators and size spectrum parameters was lost, suggesting a low efficiency of predation. This could be explained, as Boavida et al. (2013) and Bond and Jones (2015) suggested, by the action of flow fluctuation caused by hydro-peaking that would force small fishes to avoid the riverbed area that is continuously wetted and dried, moving to deeper waters where the encounters with larger piscivorous would be more frequent. Also Bain et al (1988) proposed the increase of predation risk in shallow areas during high flows as one of the most important causes of the reduction of small fish in high hydro-peaking conditions.

To summarize, we found that some descriptors of flow variability as well as the biotic factors alien fish, alien prey and alien piscivorous, significantly affected the fish community size structure, while environmental variables such as temperature, conductivity, dissolved oxygen, dissolved solids, water depth, water velocity and the presence of macrophytes, did not show any significant relationship with size structure. Nevertheless, other authors demonstrated that environmental variables such as water temperature can deeply affect the size structure of fish communities, especially when dominated by cyprinids, as is the case (Mills & Mann, 1985; Wolter, 2007). The fact that our data did not reflect this relationship or others, may be due to a narrow range of variability of these environmental factors in our study area and period. Therefore, it would be very interesting to expand the analysis, both spatially and temporally, to detect other factors that can affect the fish community size structure but that couldn't be identified in the present work.

## 5. Conclusions

This study suggest that hydro-peaking may negatively affect the populations of small fishes in the lower Ebro River. Furthermore, it shows that Normalized Abundance Spectra of the fish

community in the lower Ebro River is highly determined by the presence of alien species, and thus they have the potential to be developed as indicators of these two alterations (alien invasions and hydro-peaking). In addition, although more investigation is required to better understand it, we observed a possible interaction between hydro-peaking and the presence of piscivorous fish that would make fish community size structure more susceptible to change by the action of predation in high hydropeaking conditions. These results should be taken into account by water authorities and hydropower managers when designing possible mitigation measures for hydro-peaking impacts on fish, since it has been demonstrated that not only affects certain individuals, but can also alter the trophic dynamics of the community.

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