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Can a restocking event with European (glass) eels cause early changes in local biological communities and its ecological status?

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ABSTRACT

In an attempt to assist the recovery of the panmictic population of the European eel, declining since the late 1980s, the restocking of areas with low or no natural recruitment has been one of the measures adopted to reverse this trend. However, the main focus in several monitoring programmes for these actions, has been in the best interest of its viability and cost/benefit relationships and, for that, the condition of the released stocks has been the main concern. Yet, so far, no studies have assessed the potential ecological impacts that restocking might have on other biological communities. This pioneer pilot study aimed to evaluate the early ecological impact of a restocking event on other biological communities, considering inter-specific competition (other fish species) and feeding impact (macroinvertebrates).

The reference condition of the biological communities of an inland tributary of the Mondego river, the River Ceira, was determined in three sites inaccessible to the natural recruitment of eels, followed by a post-stocking assessment. The results showed no significant changes in the fish assemblages in restocked areas, contrary to the macroinvertebrate community. However, the ecological status for the macroinvertebrate community showed no deleterious effects, with the results suggesting exactly the opposite. This may be related to the low density of the restocked eels and factors influencing the local trophic web. This study confirms the suitability of the habitat for restocking with glass eels, during its early stages, without disrupting the local ecological status, using densities close to those of natural recruitment.

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1. Introduction

The 1980s marked the onset of a drastic decline in the recruitment of the panmictic population of the European eel, *Anguilla anguilla* (Linnaeus, 1758), which raised major concerns as to the future of this species. Since then, and over the following decades, recruitment has decreased to alarming values, with some estimates reaching values of 1% in 2000 (reviewed in Harrison et al., 2014). In an attempt to assist the recovery of the stock and reduce human-induced mortality to a minimum, the Council Regulation (EC) No. 1100/2007 imposed on member states the development of management plans ruling the establishment measures to allow the escapement of 40% of the silver eel biomass relative to the best estimate of escapement that would have existed if no anthropogenic influences had impacted the stock. Most causes for the population decline are believed to occur in the continental phase of the species, with habitat loss acknowledged as one of the main factors. Apart from the direct mortality caused by glass eel fishing, there are numerous obstacles along the freshwater systems that have reduced the available habitat for settlement and growth (Moriarty and Dekker, 1997), which can generate density dependent lethal and sub lethal effects, below obstacles. These include short-term effects such as mortality caused by scarcity of food or shelter, parasite dissemination, and long-term effects as spawning biomass reduction due to sex ratio changes, favouring males, as a consequence of increased densities (Costa et al., 1993; Davey and Jellyman, 2005; Bevacqua et al., 2011).

One of the actions undertaken to increase recruitment, either to benefit local fisheries or, more recently, to support the recovery of the population, is to restock continental systems where natural recruitment is meagre or inexistent (e.g. Moriarty and McCarthy, 1982; Andersson et al., 1991; Wickström et al., 1996; Pedersen, 1998; Simon and Dörner, 2014; Ovidio et al., 2015).

In stocking and recruitment studies, when available, only data about the released specimens, such as growth rates data, is collected (e.g. Rosell, 1997; Simon et al., 2013; Simon and Dörner, 2014; Ovidio et al., 2015; Nzau Matondo et al., 2019). In fact, even this latter assessment is a relatively new scientific exercise. Although restocking is a nearly a one-hundred-year practice, it has only been in recent decades that monitoring the condition of the released individuals has been carried out within the framework of scientific programmes (e.g. Andersson et al., 1991; Pedersen, 1998). Thus, important questions addressing ecological issues, such as the impact caused on other species, either through predation (over lower trophic groups) or competition (other fish species) remain unanswered or fall short. Although eels show a high dietary plasticity on a spatial and temporal scale (Belpaire et al., 1992; Yalçın-Özdilek and Solak, 2007), in freshwater, insect larvae compose the main diet in early stages (Belpaire et al., 1992; Tesch, 2003; Yalçın-Özdilek and Solak, 2007) and, thus, having the potential to be highly impacted. Inter-specific competition for food, on the other hand, depends on the level of dietary overlap. Hence, species that, at a given life-cycle stage, feed on the same prey-items as other competitors may also be potentially affected, if resources are limited.

Densities used for stocking have varied considerably but are usually established based on natural recruitment and yield *per* recruit estimates (Moriarty and Dekker, 1997; Knights and White, 1998). However, without a clear ecological assessment it is not possible to conclude if these densities have the potential to impact other biological communities, what is the threshold density for attaining a disruptive condition, or if, depending on local ecological conditions, lower densities yield better growth rates, lower mortalities and, ultimately, a higher production. Since the longitudinal fragmentation of most European watercourses has made several inland reaches inaccessible for natural recruitment, using these areas and testing its suitability for restocking would increase the potential reproductive success of the population. However, its viability, and sustainability, requires additional measures, at the risk of resulting in a fruitless endeavour. In short, a reasonable proportion of stocked eels needs to contribute to the spawning biomass (where fisheries have a significant impact) and be able to escape as silver eels (hydropower-induced mortality during the downstream migration) (Winter et al., 2006).

This pilot study aims to evaluate the ecological impact of a restocking event in the first year, in order to assess the suitability of its future application as a rehabilitation measure for eel populations in areas affected by the loss of longitudinal connectivity. To achieve this goal, during the first year, immediately before and after a restocking event with glass eels, a selected river stretch was monitored to detect changes in the fish assemblage (for inter-specific competition) and macroinvertebrate community (for predation impact). The underlying hypothesis is the existence of differences in biological communities between areas stocked with glass eels (treatment) and areas without the occurrence of eels (control).

2. Materials and methods

2.1. Study area

The study was conducted in River Ceira (Fig. 1), one of the main and most fragmented tributaries of the Mondego River, with more than 20 low-head weirs in the first 40 km, from the confluence with Mondego. The study site is located 45 km upstream of that confluence. The river stretch selected for the stocking experiment (experimental area) was located just upstream of the hydroelectric dam of Monte Redondo, near the small village of Cortecega (Site 1) and extended up to the village of Cabreira, at Site 4 (Downstream limit: 40° 8.410′N 8° 6.551′W; Upstream limit: 40° 8.508′N 8° 3.936′W, Fig. 1). The limits of the experimental river stretch were set based on the presence of major obstacles, considered potentially difficult to overpass by eels. In fact, the Monte Redondo dam, with a height of 7 m, is the largest unsurpassable downstream obstacle between the study site and its confluence with the River Mondego, contributing to the absence of eel specimens in this river

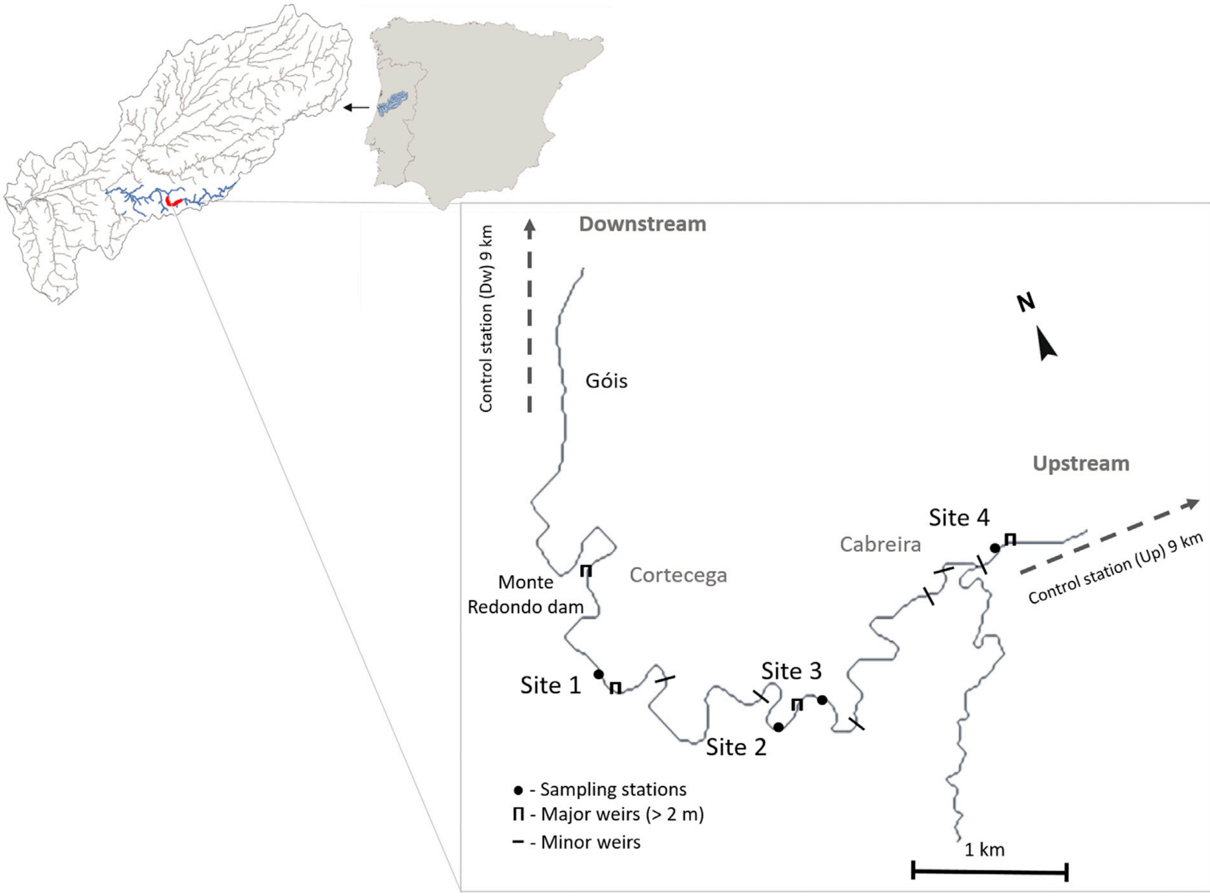

Fig. 1. Map of the study site, with indication of sampling stations and weirs, including the upstream (CUp) and downstream (CDw) control stations. The selected river stretch starts downstream at the Monte Redondo dam and ends upstream at Cabreira.

stretch. Several local recreational fishermen stated that yellow eels used to occur in this river stretch, but they have disappeared several decades ago. The current absence of eels was also confirmed through exploratory electrofishing campaigns.

The complete study area has approximately 27.5 km in length (all sampling stations, including controls) and the experimental area approximately 9.5 km (area of glass eel release and observed dispersion limits), flowing in a relatively deep and narrow valley. The experimental area has a variable width (from 3 m to 25 m, the latter located upstream of the weirs – average 7 m) and depth (from 20 cm to 2 m – the latter typically upstream of the weirs), representing a waterbody with an area of 0.07 km^2, with a regular flow rate and a current velocity ranging from 0.3 m s^{-1} to 1 m s^{-1} at the release sites (measured during sampling campaigns), which vary depending on the depth and width along the stretch. However, as a consequence of these features, flash floods are common during periods of intense rainfall. The riparian gallery is dense, hampering the access to the river, and, although not always continuous, provides shading on the margins. The riverbed is relatively homogeneous throughout the reach, with a substrate ranging from cobble to exposed bedrock although large boulders composed most of the riverbed, providing an extensive instream cover area (GRADISTAT grain size classification, as in Blott and Pye, 2001). There were eight weirs in the selected reach, most breached and with low heights and slopes, allowing the migration of the eels stocked and potamodromous fish that still occur in this area. However, within the limits of the experimental area, there are two of these weirs, with a vertical slope and a height over 2 m, that were considered potentially difficult to overpass by eels and other species during migratory movements, namely upstream of Site 1 and between Sites 2 and 3 (Fig. 1).

2.2. Experimental design

Three sites were selected for restocking glass eels, namely Sites 1, 2 and 3, according to accessibility and location (upstream and downstream of the major weirs), as represented in Fig. 1. The selection of three sites allows a replication of the assessment of the restocking and provides a more robust analysis. An additional sampling location (Site 4) was set in the upstream reach limit to identify expected eel dispersal movements, as no unsurpassable obstacles exist between Site 3 and Site 4. Two additional control stations were selected 9 km upstream (40° 9.007′N 8° 1.588′W) and downstream (40° 10.220′N 8°

6.984′W) of the experimental reach, with ecological and hydromorphological conditions similar to the remaining sites to guarantee similarity between biological communities and habitats. Given the distance and obstacles between the release sites and the two control stations (CDw: downstream; and CUp: upstream), the recapture of stocked individuals was not expected in these reference areas. The use of control stations allowed the concomitant monitoring of the biological communities in the absence of glass eels and to discard or identify potential confounders, such as temporal variations or episodic phenomena that might otherwise bias the results. The duplication and location of these control stations increase the probability of ensuring the integrity of the study under potential stochastic events, natural or anthropogenic, such as extreme weather conditions during the study period.

The experiment was conducted in a one-year period, comprising two homologous periods (April 2014 and April 2015) and comprised three major stages:

Stage 1) Reference condition, prior to the glass eel restocking – To accomplish this task, a sampling campaign was carried out in all sites in late April 2014, prior to the restocking of glass eels, to sample macroinvertebrates and fish species. Additionally, it confirmed the absence of glass eels or elvers in the study area.
Stage 2) Restocking with Glass eels – Glass eels were released immediately after the first sampling campaign, late April 2014.
Stage 3) Ecological assessment – In order to assess possible differences in the composition of macroinvertebrate and fish communities as a consequence of restocking, a sampling campaign was carried out in the following homologous period, late April 2015, thus, eliminating the confounding effect of any seasonal variability associated with the life-cycle of both biological communities.

2.3. Glass eel restocking

Restocking was carried out with 18 kg of glass eels, captured in the River Minho (Fig. 1), as this is the only watercourse, in Portugal, where glass eel fishing is allowed. Additionally, the proximity with River Mondego should reduce possible translocation effects on the glass eels restocked.

The glass eels were transported to the release location in a controlled environment with proper life support systems, assuring constant aeration and a constant temperature of 12 °C. Once in the release sites the glass eels were acclimatized to the water conditions before release. The number of specimens released at each location (Site 1, Site 2 and Site 3) was estimated considering the average individual weight sampled ($N = 60$) of 0.29 g ± 0.07 (SD), totalizing a number close to 60,000 specimens. Hence, the number of individuals released in Site 1 was $N = 18{,}157$, in Site 2 was $N = 21{,}750$, and in Site 3 was $N = 19{,}385$. Considering the entire river stretch, the estimated density of stocked glass eels was approximately 0.9 ind.m^{-2}, *i.e.* 0.0003 kg m^{-2}. This relatively low density, together with the selection of release sites located upstream and downstream of major weirs, was set as a cautious measure to allow a clear assessment. That is, higher densities in unstudied conditions such as these inland river stretches, might start-off with increased mortality or density dependent factors, originating missing steps on the subsequent assessment.

2.4. Sampling methodology

Sediment samples were collected at each site for determination of total organic matter (TOM), which was obtained by loss on ignition (480 °C). The sediment samples were collected from beneath the boulder cover. However, in the downstream control station (CDw), a marginal zone of the sampling area was composed of fine sand and silt. The TOM results for this site was based on two samples (boulder and silt areas) and weighted according to the whole sampled area for macroinvertebrates and electrofishing.

Macroinvertebrates were collected by kick sampling, following an adaptation of the standard sampling protocol defined by INAG (2008a), developed to comply with the EU Water Framework Directive (Directive nº 2000/60/CE). Sampling was carried out using a triangular net 30 × 30 × 30 cm, with a 500 μm mesh size, always operated by the same researcher and covering all microhabitats present in the study area. Three 60 s replicates were collected for each sampling station at each sampling event. Time was used to calculate CPUE, in this case, individuals of each taxon *per* unit of time. The heterogeneous characteristics of the riverbed did not allow a sampling area estimate for CPUE, achieved when a continuous sample is taken (e.g. in sand bottoms). Samples were fixed in 96° ethanol and transported to the laboratory, where they were rinsed using a sieve of 500 ìm mesh size. Each sample was sorted to separate all individuals, using a binocular stereomicroscope, which were then counted and identified to the lowest possible taxon.

Fish species, including eels, were sampled with a backpack electrofishing apparatus (Hans Grassl ELT60 HIII 300/500V), in the daytime, following an adaptation of the standardized sampling protocol defined by INAG (2008b), developed to comply with the WFD. According to this protocol, the minimum length of the sampling reach should be 20 times its mean width, but never less than 100 m. At each sampling occasion, a zigzag progression upstream was made wading, covering the area defined in the protocol (on average 110 m at 1.6 m min^{-1}). Fishing time and area were recorded to calculate CPUE. The specimens captured were anaesthetized by immersion in 2-phenoxyethanol at a concentration of 0.4 ml/L (v/v) for eels and 0.3 ml/L (v/v) for all the other species and total length and total weight were measured to the nearest 1 mm and 0.01 g, respectively. After full recovery, all individuals were released at the original site of collection.

2.5. Data analysis

The experimental design required the testing and fulfilment of a main assumption: similarity between all sites (for the composition of fish and macroinvertebrate communities) in the absence of glass eels, *i.e.* there should be no significant differences between sampling stations whenever glass eels were absent. The sites that had to meet this condition were, not only, all sampling stations in April 2014, but also the control stations of April 2015. This guarantees that both biotic communities remain similar in the absence of the stocked eels. To confirm this assumption, single fixed factor Permutational Analysis of Variance − PERMANOVA (Anderson, 2001) was used to test the differences between all sampling periods (for fish species and macroinvertebrates) and all sampling stations.

The approach to the data, concerning the assessment of ecological impact caused by the restocking with glass eels, encompassed two main analyses, using PERMANOVA routines. The occurrence of changes in the macroinvertebrate community, caused by glass eel restocking, was determined using a mixed design with two factors, testing the differences in the macroinvertebrate communities between sites:

- Factor *Eel* (fixed) − Four levels: (1) *Restock* (indicating sites in April 2015 where glass eels had been released and recaptured), (2) *Absence* (the same sites as *Restock*, but prior to the glass eel release, in April 2014), (3) *Control14* (the control stations in April 2014, prior to the glass eel release) and (4) *Control15* (the control stations, in April 2015, one year after the glass eel release);
- Factor *Station* (random, nested in *Eel*) − Three levels, nested in *Restock* and *Absence*, respectively: (1) *Site1-15*, *Site2-15* and *Site3-15*, (2) *Site1-14*, *Site2-14* and *Site3-14*, − and Two levels nested in *Control14* and *Control15*: (1) *CUp-14* and *CDw-14*, (2) *CUp-15* and *CDw-15*;
- Each level of *Station* with three replicates.

To identify changes in the fish community due to the presence of glass eels, a single fixed factor design (no replicates *per* sampling event), with four levels, was carried out, testing differences in the composition of the fish community: *Restock* (Site 1, Site 2 and Site 3 in April 2015); *Absence* (Site 1, Site 2 and Site 3 in April 2014); *Controls in 2014* (CUp-14 and CDw-14); and *Controls in 2015* (CUp-15 and CDw-15). The absence of replicates for the fish community is justified by the available area at each site together with sampling method used (electrofishing), as the whole area of each site was covered with a single wading passage (from downstream to upstream). Any attempt to replicate would create dependent samples from same area, whereas for invertebrates the area covered with a kick sampling drag, not only leaves room for replication, as it is required for representativeness.

For the biological communities that showed differences between *Absence* and *Restocking* levels, an additional single-factor permutational analysis of variance was used to test the dissimilarities between restocking sites in 2015 (Site 1, Site 2 and Site 3), to assess the heterogeneity (or homogeneity) of the restocking impact at different locations.

For the PERMANOVA analyses the abundance data was log $(X + 1)$ transformed and Bray-Curtis similarity coefficient was used as a resemblance measure. The Monte Carlo permutation tests (P(MC)) were used whenever there were not enough possible permutations (number of possible permutations <100) to get a reasonable test in PERMANOVA.

To assess the ecological status and identify quality changes in the macroinvertebrate community, different tools, such as *taxa* richness, total number of individuals and the Shannon-Wiener diversity index (Shannon and Weaver, 1963), were calculated for both April 2014 and 2015. Additionally, an assessment of ecological status for the macroinvertebrate community was also carried out using the biotic index IBMWP (Iberian BMWP) and the $IPtI_N$ (Índice Português de Invertebrados Norte − the Portuguese index for invertebrates developed for northern freshwater tributaries) (Alba-Tercedor et al., 2002; INAG, 2009). The Iberian BMWP scores each sample based on all the *taxa* collected and, as a proxy bioindicator, reflects the quality of the benthic community, through the occurrence of the different *taxa*. The $IPtI_N$ provides an ecological status score based on five different weighted metrics that include the IBMWP (INAG, 2009) and was adopted within the scope of the WFD for rivers in the northern Portuguese region, as a result of intercalibration protocols (WFD, 2009). Additionally, and based on IBMWP scores, a taxa tolerance was assigned (categorical, between tolerant and sensitive), where pollution-intolerant taxa have higher scores, whereas pollution-tolerant taxa have lower scores (Armitage et al., 1983; Jáimez-Cuéllar et al., 2002).

The PERMANOVA routines and Shannon-Wiener index computation were run in Primer v6 + PERMANOVA statistical package (Clarke and Gorley, 2006; Anderson et al., 2008).

3. Results

3.1. Pre-stocking condition and study viability

Pre-stocking sampling confirmed the absence of glass eels or elvers in the selected river stretch, at both the release and control sites. The composition of the fish community is presented in Table 1. Seven fish species, mainly Cyprinidae, composed

Table 1
Fish species captured in the selected river stretch of River Ceira (glass eel release sites and controls): average number of individuals *per* m^2 ($Ind.m^{-2}$) and median total length (TL) with range (square brackets), for each fish species, April 2014 and April 2015. The estimates of *Anguilla anguilla* for April14 (*), represent the theoretical densities upon release of the glass eels (stocked specimens).

	April14		April15	
	$Ind.m^{-2}$	TL (mm)	$Ind.m^{-2}$	TL (mm)
Anguilla anguilla (Linnaeus, 1758) *	0.90	66 [54–80]	0.09	110 [101–141]
Achondrostoma oligolepis (Robalo, Doadrio, Almada & Kottelat, 2005)	0.03	62 [38–96]	0.03	63 [40–90]
Cobitis paludica (de Buen, 1930)	0.03	70 [31–93]	0.05	66 [43–101]
Luciobarbus bocagei (Linnaeus, 1758)	0.07	87 [30–227]	0.07	97 [32–261]
Pseudochondrostoma polylepis (Steindachner, 1864)	0	–	0.01	66 [43–110]
Salmo trutta Linnaeus, 1758	0	–	0.01	50 [41–55]
Squalius alburnoides (Steindachner, 1866)	0.07	65 [23–105]	0.09	65 [33–150]
Squalius carolitertii (Doadrio 1988)	0.01	55 [25–122]	0.01	62 [36–75]
Cyprinidae ni	<0.01	30 [21–38]	0.01	32 [23–37]

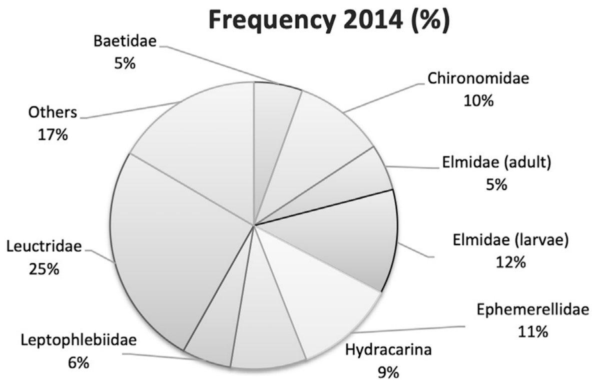

Fig. 2. Numeric relative frequency (%) of macroinvertebrate taxa (higher than 5%) in the selected stretch of River Ceira for all sampling stations, before glass eel restocking.

the local fish assemblage, in which the most abundant species were the Iberian barbel, *Luciobarbus bocagei*, along with the bordalo, *Squalius alburnoides*. The least abundant species were the Iberian nase, *Pseudochondrostoma polylepis*, and the brown trout, *Salmo trutta*, both caught only in April 2015. The macroinvertebrate community sampled accounted for 79 different taxa, most of which were insect larvae. The dominant *taxa* were consistent throughout the study and is depicted in Fig. 2.

For both macroinvertebrate and fish communities, the Permutational Analysis of Variance showed no significant differences between release sites and control stations in 2014, prior to the release of glass eels (macroinvertebrates: $p = 0.293$; fish: $p = 0.621$), enabling the study design and comparisons after restocking.

3.2. Ecological impact of restocking

The results of the PERMANOVA, performed to test the differences in the composition of the benthic community between homologous periods, are presented in Table 2. The following pairwise tests, applied to the significant factor 'Eel', showed a change in the macroinvertebrate community only for the sites where the stocked eels were present (Table 3). These changes were generally represented by a decrease in several *taxa*, most of which tolerant to environmental stress (Table 4). Between these restocking sites (Sites 1, 2 and 3, all in 2015) no differences were found ($p = 0.51$), which means that there was no variation in the impact caused by the presence of eels. The control stations showed no inter-annual fluctuations in the macroinvertebrate community, as no difference was found between April 2014 and its homologous period, April 2015. Although with a benthic community statistically similar to all other sites, the downstream control station had a marginal riverbed cover consisting of silt and fine sand. Furthermore, the relative percentage of silt and fine sand in this sampling station ranged from 20% in April 2014 (in the left margin) to 60% in April 2015. This change was followed by an increase in TOM content in the sediment (Table 5) between homologous periods, and a decrease in all remaining sites, located upstream of CDw.

When compared with 2014, the macroinvertebrate *taxa* richness and total number of individuals in April 2015 tend to be higher at the sites where glass eels were released, except for Site 2, where there was a smaller difference (Fig. 3). The H′ diversity index was also slightly higher in the release sites, although the downstream control station displayed a decrease in 2015. The IBMWP and $IPtI_N$ indices expressed an agreement classifying all sampling sites as 'Very Good' (Class I) and 'Excellent', respectively, despite the spatial variations. In the release sites, the balance between tolerant and sensitive *taxa*

Table 2
PERMANOVA results for the 2-factor, mixed-model, assessing the differences in the composition of the benthic community, between 2014 (pre-stocking with glass eels) and 2015 (in the homologous period after restocking). Significant fixed factor in bold.

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Eel	3	9484.1	3161.4	3.5914	**0.0017**	4987
Station (Eel)	6	5281.5	880.25	1.51	0.0040	9823
Residuals	20	11659	582.94			
Total	29	26424				

A two-factor mixed design: Eel, fixed factor with four level: Restock (sites in April 2015 were glass eels were release and recaptured), Absence (the same sites as Restock, but prior to the glass eel release, April 2014), Control14 (the control stations in April 2014, prior to the glass eel release), and Control15 (the control stations, in April 2015, one year after the glass eel release); and the random factor Station, nested in Eel, with three levels for Restock and Absence: Site 1, Site 2 and Site 3, and two levels for Control14: CUp14 and CDw14, and Control15: CUp15 and CDw15.

Table 3
Pairwise tests for the PERMANOVA significant factor 'Eel' of a 2-factor, mixed-model, assessing the differences in the composition of the macroinvertebrate community, between 2014 (pre-stocking with glass eels) and 2015 (in the homologous period after restocking). Significant values in bold.

Groups	t	P(perm)	perms	P(MC)[a]
Absence, Control14	1.1956	0.1047	10	0.2463
Absence, Restock	2.5346	0.1006	10	**0.0017**
Absence, Control15	1.5571	0.1	10	0.0613
Control14, Restock	2.6245	0.0988	10	**0.0033**
Control14, Control15	0.98946	0.6691	3	0.4742
Restock, Control15	2.4371	0.1024	10	**0.0071**

[a] The Monte Carlo permutations, P(MC), used due to the low number of permutations (perms).

Table 4
Numeric relative frequency (%) of macroinvertebrate taxa (higher than 5% in, at least, one year) in the homologous periods of 2014 and 2015, for the stations in River Ceira were glass eels were released, and indication of trend. Additional indication of taxa tolerance to stressful environmental conditions, as (T) tolerant and (S) sensitive (from Jáimez-Cuéllar et al., 2002).

	2014	Trend	2015	Tolerance
Baetidae	2%	↑	10%	**T**
Chironomidae	10%	↑	31%	**T**
Corixidae	5%	↓	<1%	**T**
Elmidae (adult)	6%	↓	2%	**T**
Elmidae (larvae)	12%	↓	5%	**T**
Ephemerellidae	10%	↓	6%	**S**
Heptageniidae	1%	↑	5%	**S**
Hydracarina	11%	↓	2%	**T**
Leptophlebiidae	5%	↑	9%	**S**
Leuctridae	27%	↓	16%	**S**
Others	13%	–	19%	–

Table 5
Percentage of total organic matter (TOM) in the sediment of the sampling stations from both years and indication of the trend at each site.

	TOM % April 2014	Trend	TOM % April 2015
CDw	***1.79***	↑	***3.25***
Site 1	3.90	↓	1.86
Site 2	2.22	↓	1.83
Site 3	3.74	↓	2.14
CUp	2.96	↓	2.78

over the one-year period, favoured sensitive species, as the abundance of 4 out of 6 tolerant *taxa* decreased, and 2 out of 2 sensitive taxa increased (Table 4). For these indices, Site 3 was the sampling station with the highest increase, and the area with the highest glass eel dispersion. This eel movement was acknowledged due to the occurrence of elvers in the most upstream station, Site 4, in 2015, which implied a 3.6 km upstream dispersion, considering the closest release site, Site 3.

The PERMANOVA performed for the fish community rendered no significant differences between homologous periods (Table 6).

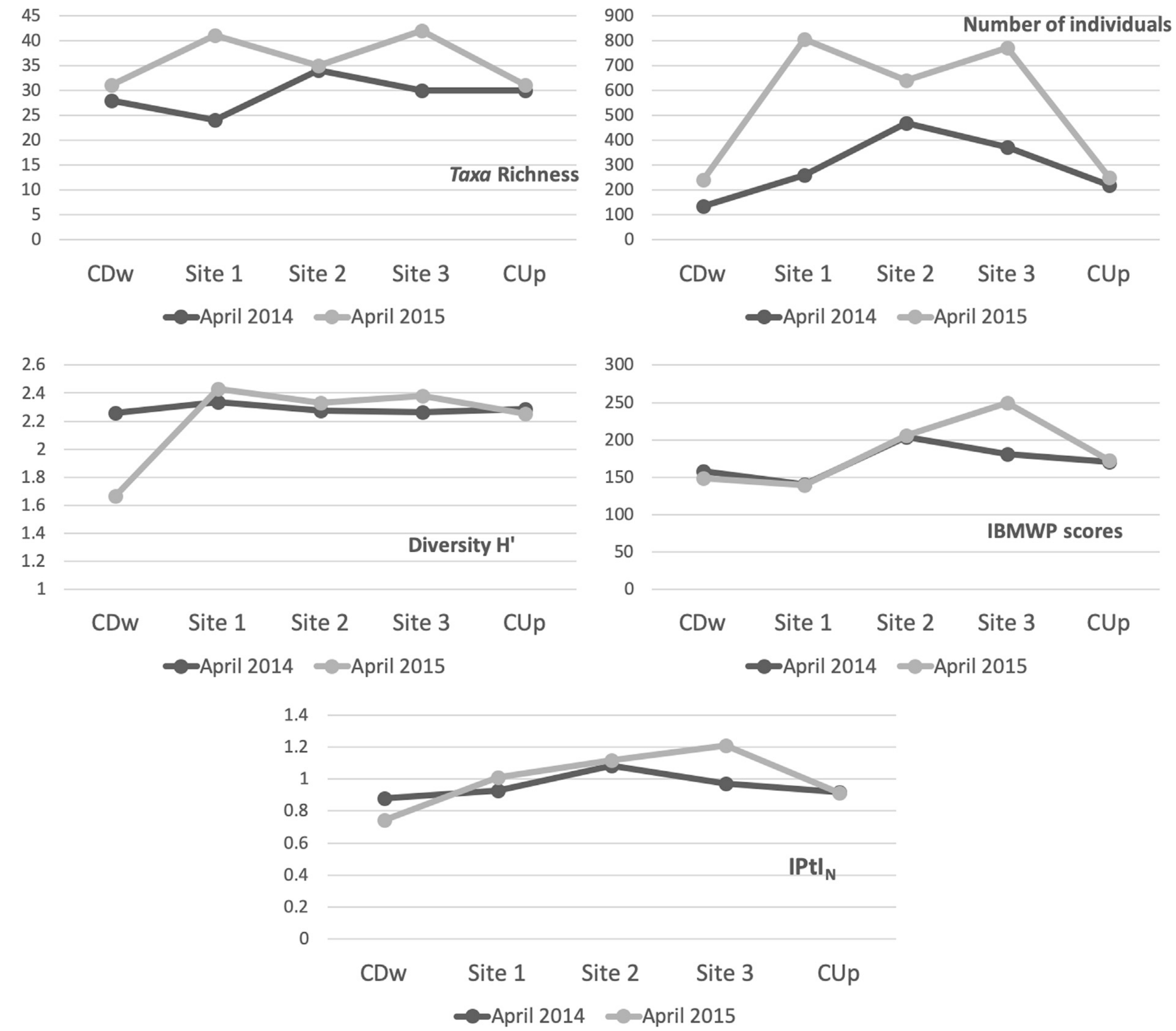

Fig. 3. Values for *taxa* richness; Total number of individuals; Shannon Wiener diversity index (H′); and Scores for the IBMWP and IPtI$_N$ indices, for both periods (2014 and 2015), where glass eels were released (Site 1, Site 2 and Site 3) and in control stations (CDw and CUp).

4. Discussion

The average density estimated from the recapture of elvers was ten times lower than the estimated theoretical density at the time of release of glass eels. Different factors may have contributed to explain this result: (i) dispersion movements guided by site preference (locations not sampled); (ii) early mortality; and (iii) the efficiency of recapture. Dispersion phenomena are very likely, as no obstacles preclude these migratory movements. In fact, the upstream migration of eels shows a negative correlation with size, even after restocking, with elvers displaying a high migratory activity (Nzau Matondo and Ovidio, 2018; Nzau Matondo et al., 2019). Determination of mortality rates under these experimental conditions is a problematical issue, however, this river stretch seems to provide favourable conditions for elvers. The cover provided by boulders in the riverbed offers protection against predation (concealment in crevices) and a richer feeding ground (Nzau Matondo et al., 2019). Moreover, in 2015 this river stretch was affected by a flash-flood caused by the collapse of an upstream dam. Notwithstanding, the ability of elvers to migrate upstream along with their absence in the downstream control station, demonstrates the ability of this species to thrive in these environments. The low densities estimated from recapture may also be explained by the small size of elvers, as the efficiency of electrofishing increases with body size (Naismith and Knights, 1990; Lambert et al., 1994). This problem has been frequently reported in other studies (e.g. Simon and Dörner, 2014; Ovidio et al., 2015).

The restocking of River Ceira had an impact on the macroinvertebrate community in the first year after restocking, but it did not significantly change the fish assemblages. Although fish species present in the restocking area prey on benthic fauna to some degree, which can vary opportunistically or seasonally (Magalhães, 1992; Crivelli, 1996; Lagarrigue et al., 2002;

Table 6
Results of the PERMANOVA for the single factor model assessing the differences in the composition of the fish community, between 2014 (pre-stocking with glass eels) and 2015 (in the homologous period, after restocking).

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Pre-stock/Restock	3	2614.8	871.6	1.2814	0.2315	9030
Residuals	8	5441.7	680.22			
Total	11	8056.5				

Valladolid and Przybylski, 2003; Gomes-Ferreira et al., 2005; Santos et al., 2013; Sánchez-Hernández, 2014), the first year after the stocking of glass eels did not appear to have an impact on the structure and abundance of local fish species. This indication of an absence of inter-specific competition may be related to a high carrying capacity of the habitat but also to prey biometry. Nonetheless, the well-known dietary plasticity of the European eel (Belpaire et al., 1992; Yalçın-Özdilek and Solak, 2007), may allow a change in feeding preferences, depending on potential competitors, which, in turn, favours a good adaptation to different habitat types observed throughout the eel distribution range.

The induced change in the macroinvertebrate community did not cause, however, a disruption and a decrease in ecological status, rather suggesting the opposite. One year after the glass eel restocking, the release sites showed an increase in the macrobenthic indices, which was not observed in the control stations. The exception, Site 2, showed a smaller or negligible increase, much similar to that of the control stations. Also, the control station CDw displayed a large decrease in diversity and ecological quality in April 2015, when compared to the previous homologous period. Here, the duplication of the control sites proved to be an added value in this particular study, as the downstream control station experienced a change in the TOM content of sediments and an increase in the abundance of chironomids in 2015. The most likely cause for this change was the flash-flood in early 2015, which resulted in an organic matter enrichment, setting favourable conditions for these Diptera to thrive (Armitage et al., 1997). This sediment variation and TOM enrichment can account for the temporal decrease observed in diversity (e.g., Battle et al., 2007). The apparent overall improvement of the ecological status at the release sites (IBMWP and $IPtI_N$), was mainly influenced by Site 3, as no other site presented such a relevant change. The main difference between this release site and the remaining sites was the higher degree of dispersion of elvers that occurred, as this was the stocking site with the largest upstream area free of obstacles (confirmed by the recaptures in Site 4). Nonetheless, there is an overall agreement between the estimated metrics obtained for the release sites. At sites where the results suggested an improvement of the ecological status, there was also an increase in *taxa* richness and diversity (De Pauw et al., 2006) and, generally, among the most represented *taxa*, the balance between tolerant and sensitive groups favoured sensitive species.

The changes observed in the macroinvertebrate communities suggest, to some degree, a relation with prey availability. Many macroinvertebrate *taxa* can avoid fish predation by building tube-like enclosures, and using it as fastening structures (Hershey, 1987), and by burrowing behaviours (van de Bund and Groenendijk, 1994; Gosselin and Hare, 2003). Interestingly, the *taxa* that increased their numeric frequency belonged to families that include species with burrowing behaviour (Leptophlebiidae, Chironomidae and Corixidae), contrasting with a decrease in those that generally do not have species exhibiting this type of behaviour (Leuctridae, Ephemerellidae, Elmidae, Hydracarina). This unlikely coincidence is possibly related to the easier predation of eels upon bottom-dwellers and other exposed invertebrates, rather than burrowers, which may require a higher energy cost.

A plausible explanation for the improvement rather than the degradation of benthic ecological status may stem in the fact that this river stretch was, formerly, an area with natural recruitment of eels. The gradual loss of longitudinal connectivity in recent decades, has possibly led to significant changes in the fish community due to the removal of the European eel from the ecosystem. Such a change may have resulted in significant ecological disruptions (Heithaus et al., 2008). Additionally, natural recruitment in these reaches, located further upstream in the river basin, is typically very low, so the possible re-equilibrium of the ecosystem may also be a result of the low density of restocked eels, which was close to natural recruitment.

The selected stretch of the River Ceira, despite the absence of eels due to the loss of longitudinal connectivity, demonstrates habitat suitability to receive low densities of eels in their early stages, without disrupting the local ecological status. Likewise, although the fish community was not disturbed by glass eel restocking during the first year, eels may grow to create a competition effect as prey biometry and dietary needs increase with size. Hence, the ecological status should be monitored to characterize its evolution as the restocked eels age. This is a necessary step to be able to support future restocking actions.

Declaration of competing interest

The authors declare no conflicts of interest.

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