

## Effects of forest management on physical habitats and fish assemblages in Iberian eucalypt streams



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### ABSTRACT

In Portugal, more than 150,000 ha of eucalypt plantations are managed under best management practices (BMP), in compliance with international certification systems. Some of these forestry areas extend to streams, and the riparian areas are thus protected from productive land uses. However, many other eucalypt plantations present riparian zones that are not managed under BMP and are often occupied by agricultural uses. To test the effects of forest management on physical habitats and fish assemblages in Iberian eucalypt streams, we selected three different stream reach types: (1) reaches in catchments dominated by native vegetation associations, and presenting semi-natural riparian zones (reference-REF); (2) reaches surrounded by certified eucalypt plantations (i.e., managed under BMP), and consequently, presenting protected riparian buffer zones (PRZ); and (3) reaches surrounded by eucalypt plantations (dominant) and some oak forests, and presenting disturbed riparian zones with agricultural land use (DRZ). All sites represented small, low-gradient, warmwater streams in Central Portugal (Tagus basin), and their upstream land cover was dominated by natural/semi-natural uses. PRZ sites were abiotically similar to REF sites, although PRZ sites generally presented lower potential cover for fish and tended to support a riparian vegetation that was a little more fragmented. PRZ sites supported fish assemblages that were similar to those in reference reaches, although at the functional level we found a higher abundance of native invertivores in REF sites. DRZ reaches, on the other hand, differed substantially from the other two groups. DRZ sites presented disturbed riparian vegetation (removal or substitution of native trees by exotic and/or non-woody species), eroded and modified channels, and a degradation of stream habitats (lack of riffle–pool complexes, higher silt contents, and lower potential cover for fish). The fish assemblages were also quite different, with a higher abundance of alien specimens (mainly *Gobio lozanoi*) and generalist spawners, and a lower proportion of native invertivores and lithophils. Riffles and morphological alteration explained the greatest proportion of variance in species composition. Our study showed that protecting stream riparian zones by implementing BMP may mitigate the effects of eucalypt forestry on Iberian fish assemblages. However, given that there are a few differences in physical condition and biological integrity between streams associated with certified plantations and reference streams we suggest additional riparian management measures in the certified areas, such as restoration of the native vegetation, removal of alien plants, and improvement of the stream habitat.

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### 1. Introduction

The Australian native tree *Eucalyptus globulus* (eucalypt) was introduced to Portugal in the second half of the 19th century (Barreiro and Tomé, 2012). Nowadays, this species is common and widespread, occupying more than 800,000 ha in 2010 – i.e. approximately 26% of the total forested area in the country (ICNF, 2013). More than 150,000 ha of these eucalypt plantations are managed under best management practices (BMP), in compli-

ance with international certification systems (CELPA, 2013). When the forestry management areas extend to river systems, one of the most common practices in certified eucalypt plantations is the protection and restoration of riparian buffer zones – i.e. stream-side areas that include terrestrial and aquatic ecosystems and are not managed for agriculture or forestry. BMP associated with the riparian areas include reduced-impact logging practices (such as setting many roads back from riparian areas) and the protection of native riparian vegetation. As a rule for small-sized streams in our study area, the protected riparian buffers possess a total width of about 60 m (30 m per margin strip). Important functions of the riparian zones include thermal buffering, provision of invertebrates as food

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for fishes, nutrient and sediment trapping, enhancement of bank stability, and provision of woody debris and rootwads as habitat for fish (Gregory et al., 1991; Naiman et al., 1993; Pusey and Arthington, 2003). Particularly in Mediterranean-type environments, riparian corridors represent ecosystems with high habitat patchiness and biodiversity (Corbacho et al., 2003; Aguiar and Ferreira, 2005; Stella et al., 2013), and their protection is thus critical to ensuring the integrity of the aquatic systems. However, in many other Portuguese eucalypt-dominated landscapes, especially in non-certified plantations, the riparian zones are frequently occupied by several agricultural uses, such as cropping and livestock grazing.

Research has highlighted the importance of forestry BMP in maintaining the physical and water quality characteristics of stream ecosystems (see reviews: Clinnick, 1985; Hutchens et al., 2004; Northcote and Hartman, 2004; Edwards and Williard, 2010). The bioassessment studies on BMP effectiveness also suggest these measures provide an overall benefit in terms of protecting stream biota, although most studies are focused on macroinvertebrate assemblages (e.g. Graynoth, 1979; Adams et al., 1995; Kreutzweiser et al., 2005; McCord et al., 2007). In commercial harvest areas in Central Amazonia that were certified as practicing sustainable forestry, reduced-impact logging also did not have significant impacts on fish communities and appeared to be a viable alternative to clear-cut practices (Dias et al., 2010). The protection of native riparian vegetation is an important-albeit not the only component of BMP guidelines. In forestry areas dominated by exotic tree species, native riparian vegetation can play a particularly important role in mediating the impacts of plantations on rivers (Graça et al., 2002). This mitigating role was evident for the invertebrate communities in Iberian streams flowing through eucalypt plantations (Abelho and Graça, 1996; Ferreira et al., 2015). In a project carried out in New Zealand landscapes dominated by exotic pine forests, riparian buffer strips also had an overall benefit on native fish (Rowe et al., 2002) and invertebrate assemblages (Quinn et al., 2004).

From these studies, we conclude that the knowledge of BMP effectiveness in relation to aquatic systems comes mostly from non-Mediterranean areas (particularly in North America and Oceania), with a lack of scientific information concerning overall fish-forestry interactions in Iberian eucalypt plantations. We are unaware of any studies that evaluate the role BMP play in catchments subjected to logging in Mediterranean areas, when it comes to maintaining the biological quality of river systems (*sensu* Water Framework Directive) and the biotic integrity of fish communities. Fishes are good environmental quality indicators because they are sensitive to a range of biological, physical and chemical disturbances (Simon and Lyons, 1995; Karr and Chu, 1999; Pont et al., 2006; Ferreira et al., 2007b), providing a useful broad guideline of BMP, including an assessment of their success.

Agricultural practices are the most widespread cause of stream degradation, increasing nonpoint inputs of nutrients and sediments, and altering habitats (Wang et al., 1997; Henley et al., 2000; Allan, 2004; Héros and Clavero, 2011). Agricultural land use is generally considered a key variable for measuring the human impacts on stream ecosystems and a good predictor of both physical habitat quality and in-stream biotic integrity. Biological communities are structured by factors that operate across multiple spatial scales, from microhabitats to ecoregions (Ricklefs, 1987; Lawton, 1999), and thus the effects of agricultural land use are pervasive at the catchment and local levels (Wilson and Xenopoulos, 2008; Feld, 2013). Previous studies on agricultural-dominated catchments have indicated that agricultural uses within the riparian areas can affect the structure of fish assemblages and the stream environment (Lammert and Allan, 1999; Stewart et al., 2001; Heitke et al., 2006). However, only a few studies that address

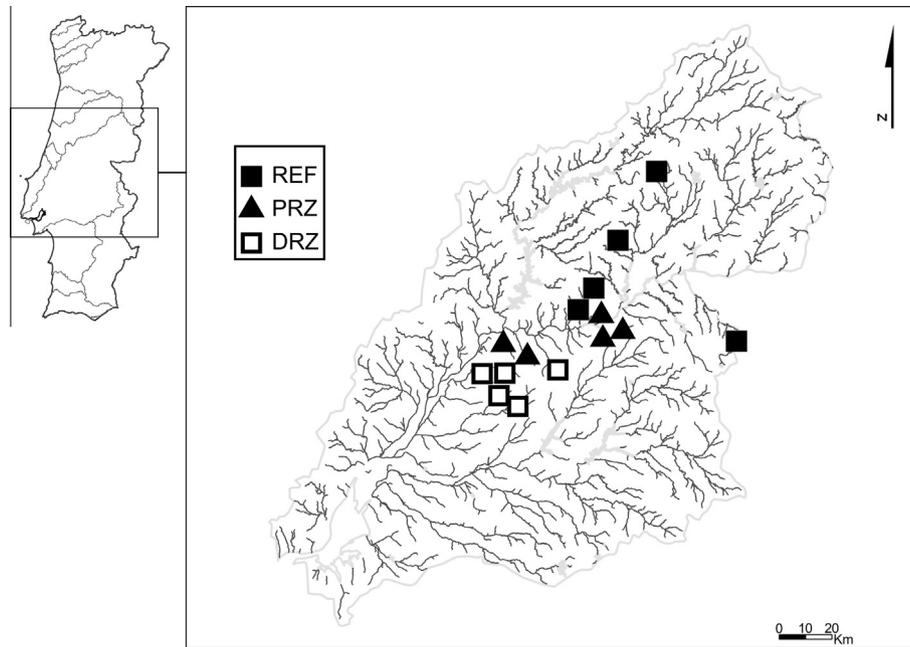
largely forested watersheds have focused on the importance of land use at smaller spatial scales (e.g. Jones et al., 1999), and little is known about the effects that riparian agricultural land use in forestry landscapes has on streams, as in Iberian eucalypt plantations where BMP do not extend to the lotic systems.

Against this background we designed a study to investigate to which extent protected riparian buffer zones can mitigate the impacts of eucalypt plantations on Mediterranean fish assemblages, while simultaneously attempting to clarify the ecological consequences of not protecting those areas from high-impact uses (mainly agricultural land uses). We selected the middle part of a Portuguese river basin – the Tagus basin – where the patchy nature of land uses allowed us to compare reference sites dominated by native vegetation to eucalypt plantations presenting both protected and degraded riparian zones. We hypothesised that protecting riparian buffers by implementing BMP would mitigate the impacts of eucalypt silviculture. We therefore expected harvested sites with good quality riparian areas to present stream habitats and fish assemblages similar to those in reference sites. We also tested our expectation that harvested sites with agricultural riparian land use would present altered and impaired habitats, and that these changes would be associated with changes in species composition and functional attributes of fish assemblages.

## 2. Material and methods

### 2.1. Study area and site selection

We selected fifteen sites that drain small catchments in the central part of the Portuguese River Tagus basin (Fig. 1). The study area is characterized by a Mediterranean climate, with high floods occurring from autumn to late winter, and a gradual decline in flow during late spring and summer (Gasith and Resh, 1999). The study reaches were similar in catchment size and channel dimensions for each reach type and represented small, low-gradient warmwater streams (Table 1). Only two sites occurred within the same catchment, but they were spaced out 4.5 km from each other, thus reducing problems of spatial autocorrelation (Santos et al., 2011; Oliveira et al., 2012). Typically in these Mediterranean streams, fish assemblages are poor in native species, but with a high proportion of endemic forms, mostly cyprinids, and may include a few widespread alien species (Oliveira, 2006). Although study sites presented some differences in land use at the catchment scale, land cover was dominated by natural/semi-natural uses (e.g., forests, Mediterranean woodlands) (Table 1), and no major impacts (e.g. hydrological disturbance, pollutant sources) were detected. In most of the study area the population density was low – generally less than 50 inhabitants/km<sup>2</sup>. Riparian forests were typically dominated by the common alder (*Alnus glutinosa*), the narrow-leaved ash (*Fraxinus angustifolia*), and willows (*Salix* spp.). Three different reach types were compared, each with five replicates, hereafter reference (REF), protected riparian zone (PRZ), and disturbed riparian zone (DRZ) sites (Fig. 1): (1) REF sites had catchments dominated by native forests and Mediterranean shrublands, with most of the riparian vegetation and immediate riparian zones in a semi-natural state; (2) PRZ sites were surrounded by certified eucalypt plantations (i.e., managed under BMP), and consequently, presented riparian zones protected from productive activities. These areas were thus not managed for agriculture or forestry and supported a riparian vegetation that was composed of several strata and was slightly cleared or fragmented by human intervention; (3) DRZ reaches were surrounded by eucalypt plantations (dominant) and some oak forests, and presented riparian zones with agricultural land use, including cropping and livestock grazing; in all but one of the DRZ sites, the riparian vegetation exhibited a



**Fig. 1.** Map of the Tagus basin showing the location of the sampling sites; REF = reference sites; PRZ = protected riparian zones; DRZ = disturbed riparian zones.

**Table 1**

Median and range of environmental variables in the three reach types. To test for significant differences in natural variability among reach types we employed a multivariate PERMANOVA. This non-parametric method was based on Euclidean distance resemblance matrix. Non-redundant variables were used for this analysis (mean annual temperature, catchment area, channel slope, and stream depth). The multivariate PERMANOVA did not detect a significant effect of reach type on natural variability (pseudo- $F_{2,12} = 1.615$ ,  $P = 0.196$ ); REF = reference sites; PRZ = protected riparian zones; DRZ = disturbed riparian zones.

Variable	Forest and riparian category		
	REF	PRZ	DRZ
Mean annual temperature (°C)	15.0 (13.3–15.4)	15.4 (15.3–15.4)	15.5 (15.4–15.5)
Catchment area (km <sup>2</sup> )	54 (27–106)	42 (29–62)	55 (36–99)
Catchment with natural/semi-natural land uses (%)	90 (75–98)	88 (78–93)	85 (81–88)
Channel slope (%)	1.0 (0.7–1.3)	1.2 (0.8–1.8)	0.7 (0.3–1.0)
Mean width (m)	5.2 (3.3–6.2)	3.8 (2.8–5.7)	4.0 (2.5–4.5)
Mean depth (cm)	45 (32–70)	34 (25–64)	30 (22–45)

highly discontinuous row of trees and was generally dominated by non-woody invasive species (mainly *Arundo donax* and *Rubus* spp.).

## 2.2. Fish assemblages

### 2.2.1. Fish sampling

In 2013 all sites were electrofished (DC, 300–700 V, or pulsed DC, 400–1000 V) during late spring–early summer base flow. During this period stream flows were lower (but still had full connectivity between habitats), thus ensuring a higher fishing efficiency. Simultaneously, we avoided situations in which the extreme-flow events that typically occur during the rainy season could causing bias in fish sampling or in the measurement of local habitat variables (Santos et al., 2011; Oliveira et al., 2012). Electrofishing distances followed CEN standards for assessing fish species composition and abundance for wadeable sites (CEN, 2003). Thus, the electrofishing distance for each site was 20 times the mean wetted width of its channel to encompass complete sets of the characteristic stream form (e.g. riffles, pools, runs), with a minimum sampled length of 100 m to ensure a representative sample of the fish assemblage (Santos et al., 2011; Oliveira et al., 2012). The entire widths of streams were electrofished by wading slowly upstream during daylight, using natural barriers – mostly shallow habitats – to delimit survey zones. Although capture efficiency

estimates, which were assumed to be constant across sites, were not available for the study area, previous works indicated that this sampling effort was sufficient to ensure accurate characterization of fish species composition and abundance (Ferreira et al., 2007a; Oliveira et al., 2009, 2012; Santos et al., 2011). Fish were identified and measured in the field; native specimens were returned alive to the water and aliens were killed in Tricane (MS-222), in accordance with Portuguese legislation. Given the time required for these procedures (fish processing), we assumed that electrofishing did not influence water quality measurements that followed fish sampling. Juveniles of *Lampetra* species cannot be reliably identified to species and were therefore grouped into *Lampetra* spp.

### 2.2.2. Fish metrics

In order to test whether the functional attributes of fish assemblages responded to our three study reach types, we considered several metrics related to the density or percentage of fish guilds grouped into ecological functions (for details see Oliveira et al., 2012). The fish species were generally assigned to these guilds on the basis of the European EFI+ project (<http://efi-plus.boku.ac.at/>; finished in 2009) (Logez et al., 2013) with a few modifications supported by more recent published data (Oliveira et al., 2012; Silva et al., 2014), completed by expert judgment when necessary. Metrics presenting Spearman correlation coefficients higher than

**Table 2**  
Results of univariate PERMANOVA models for the effects of reach type on both compositional and functional fish metrics (densities (individuals per 100 m<sup>2</sup>) and percentages are expressed as median and range); REF = reference sites; PRZ = protected riparian zones; DRZ = disturbed riparian zones. Significant test statistics at  $P < 0.05$  are marked in bold, and different superscript letters indicate significant differences ( $P < 0.05$ ) among reach types.

Variable	Pseudo- $F_{2,12}$	P	Forest and riparian category		
			REF	PRZ	DRZ
<i>Compositional metrics<sup>a</sup></i>					
Alien specimens (percentage)	18.896	<b>0.005</b>	0 <sup>a</sup> (0–6)	0 <sup>a</sup> (0–20)	55 <sup>b</sup> (14–59)
Alien specimens (density)	8.312	<b>0.013</b>	0 <sup>a</sup> (0–1.2)	0 <sup>a</sup> (0–5.9)	30.4 <sup>b</sup> (2.4–44.3)
Native species (number)	0.309	0.880	4 (1–5)	4 (1–6)	3 (3–4)
Native specimens (density)	3.001	0.104	29.4 (18.5–43.4)	15.3 (6.5–24.1)	24.4 (9.1–54.9)
Red-list specimens (percentage)	5.863	<b>0.023</b>	85 <sup>a</sup> (64–100)	71 <sup>a</sup> (40–100)	37 <sup>b</sup> (25–51)
<i>Functional metrics<sup>a</sup></i>					
Invertivores_natives (percentage)	6.112	<b>0.018</b>	85 <sup>a</sup> (64–100)	51 <sup>ab</sup> (25–100)	13 <sup>b</sup> (0–77)
Invertivores_natives (density)	4.066	<b>0.009</b>	24.9 <sup>a</sup> (12.6–43.4)	6.8 <sup>b</sup> (3.5–15.4)	3.1 <sup>b</sup> (0.2–13.6)
Omnivores (percentage)	0.933	0.447	15 (0–31)	29 (0–67)	8 (0–19)
Omnivores (density)	0.005	0.999	4.3 (0–9.1)	8.7 (0–14.6)	4.5 (0–12.2)
Lithophilics_cyprinids (percentage)	49.748	<b>0.003</b>	100 <sup>a</sup> (92–100)	97 <sup>a</sup> (80–100)	23 <sup>b</sup> (5–43)
Generalist spawners (percentage)	5.447	<b>0.015</b>	0 <sup>a</sup> (0–8)	0 <sup>a</sup> (0–7)	8 <sup>b</sup> (2–43)

<sup>a</sup> Density metrics were  $\log(x + 1)$  transformed, and proportional metrics were  $\arcsin(\sqrt{x})$  transformed.

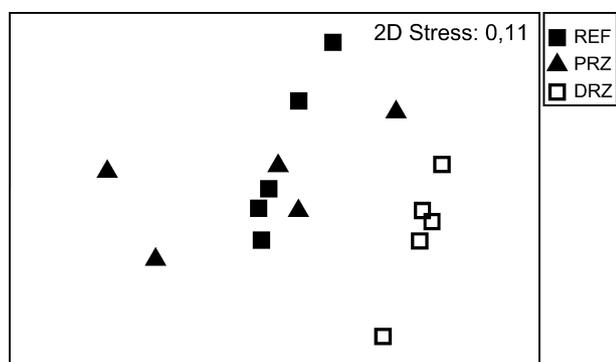
[0.85] were considered redundant. Final metrics for analysis included two trophic guilds (invertivores\_natives and omnivores), based on food items in the diet of adult individuals, and two reproductive guilds (lithophilics and generalist spawners), based on spawning substrate (Table 2; see Appendix A for species functional guilds). Fish metrics based on species composition have proven useful for assessing human impacts in Mediterranean streams (Ferreira et al., 2007a,b), and thus we also considered non-redundant compositional metrics (Table 2).

### 2.3. Environmental variables

After fish processing, the following environmental variables were measured or estimated from the whole electrofished reaches: (1) pH and conductivity, that were measured with a multiparameter meter (Extech Instruments, EC500), and dissolved oxygen, that was measured with an oximeter (Extech Instruments, DO600); (2) mean wetted width, mean depth, and substrate composition, which was visually assessed and expressed in 7 classes (CEN, 2003): 1 = silt, <0.02 cm; 2 = sand, 0.02–0.2 cm; 3 = gravel, 0.2–1.6 cm; 4 = pebble, 1.6–6.4 cm; 5 = cobble, 6.4–26.0 cm; 6 = boulder, >26.0 cm; 7 = bedrock; these three measurements were taken at 30 m intervals, for a total 3–5 cross-sectional transects depending on distance fished; (3) major habitat types; we defined four habitat types (Ohio EPA, 2006) – glide, pool, riffle, and run – that were measured and expressed as percent of reach length; (4) tree canopy shading, overhanging vegetation (<1 m above water surface), woody cover (woody debris and submerged roots), macrophyte cover, undercut banks, and potential cover for fish (any submerged structure that could provide protection for fish); all these variables were visually assessed and scored on a grade scale for cover with the following classes: 1 = <5%; 2 = 5–25%; 3 = 25–50%; 4 = 50–75%; 5 = >75%; (5) morphological alteration (MORPH), bank erosion (EROS), longitudinal continuity and coverage of riparian corridor (RIPCONT), and composition and structure of riparian vegetation (RIPCOMP) (all evaluated from professional judgment and scored to the degree they deviated from minimally disturbed conditions (from 1 for no deviation, to 5 for highly degraded): MORPH ranged from negligible (1) to complete channelization and bank hardening (5), EROS ranged from none (1) to severely eroded streambanks (5), RIPCONT ranged from natural condition (1) to complete riparian vegetation removal (5), and RIPCOMP ranged from natural condition (1) to high coverage of exotic and/or non-woody species (5).

### 2.4. Statistical analyses

We used univariate and multivariate analyses to determine the differences in fish assemblages and environmental variables among reach types. All statistical analyses were performed using the software package PRIMER v.6 with the PERMANOVA+ add on (Clarke and Gorley, 2006; Anderson et al., 2008). First, we used univariate permutational analysis of variance (PERMANOVA) to compare fish metrics and environmental descriptors among reach types (REF, PRZ and DRZ). This method is particularly appropriate for data that do not conform to the assumptions of parametric tests (Anderson et al., 2008). Environmental descriptor analyses were based on Euclidean distance resemblance matrices, and fish metric analyses were performed on Bray Curtis distance resemblance matrices of transformed data ( $\log(x + 1)$  for densities and  $\arcsin(\sqrt{x})$  for proportions). The transformation procedure made it possible to reduce the effect of high-abundance species while still giving the most common species greater weight. To test for significant differences in fish assemblage structure among reach types we employed a multivariate PERMANOVA, and to visualize the relationships among the sampled sites we used a non-metric multidimensional scaling (MDS) ordination technique. These two non-parametric methods were based on the same Bray Curtis matrix of  $\log(x + 1)$  transformed density data, complementing each other in the comparison of fish assemblage structure between fish types.  $P$  values for all PERMANOVA tests (pseudo- $F$  values) were based on 999 permutations, according to Manly's (2006) recommendations for tests at an  $\alpha$ -level of 0.05; where significant differences were found, pair-wise *a posteriori* comparisons among reach types were performed under 999 permutations. Since multivariate PERMANOVA is sensitive to differences in dispersion among groups, we used the PERMDISP routine to test for homogeneity of multivariate dispersion. A similarity percentage analysis (SIMPER) was subsequently employed on transformed density data to determine which species best typified each of the three reach types and to reveal the most discriminating species among those groups. Finally, to explore and visualize which group of environmental descriptors best explained the variation in fish assemblage composition, we used the distance-based linear modelling (DISTLM) routine and a distance-based redundancy analysis (dbRDA). The resemblance matrix used in DISTLM was based on the Bray Curtis similarity of  $\log(x + 1)$  transformed density data, using all specified selection procedure combined with the AIC (Akaike Information Criterion) selection criteria. Before the DISTLM analysis, we used



**Fig. 2.** Non-metric multi-dimensional scaling (MDS) plot of sampling sites based on Bray Curtis matrix of  $\log(x + 1)$  transformed density data; REF = reference sites; PRZ = protected riparian zones; DRZ = disturbed riparian zones. The MDS plot revealed that fish composition in DRZ sites (degraded reaches) was clearly different from that in REF and PRZ sites (non-degraded reaches). REF and PRZ sites supported fish assemblages with high biotic integrity, whereas DRZ sites displayed altered fish assemblages, including an increase of aliens and several changes at the functional level.

a draftsman plot and a Spearman correlation matrix to access the existence of highly correlated environmental variables and any need for data transformation; variables with Spearman correlation coefficients higher than  $|0.7|$  were considered redundant, and one of a group of collinear variables was included for analysis. A total of 10 environmental variables were considered for modelling: rifle, run, MORPH, RIPCONT, tree canopy shading, overhanging vegetation, undercut banks, pH, conductivity and oxygen. The total number of studied reaches was thus higher than the number of environmental predictors, which is an assumption that is required for the DISTLM modelling approach (Anderson et al., 2008). Variables were normalised automatically as part of the DISTLM routine (Anderson et al., 2008).

### 3. Results

We collected 12 species of fish from 6 families (Appendix A), representing a total of 2132 individuals. About 65% of all individuals were endemic cyprinids. The catch for the sampling sites ranged from 6.5 to 43.4 and had a median of 29.2 individuals per 100 m<sup>2</sup>. Alien fish metrics (density and percentage) were significantly higher in DRZ sites than in REF and PRZ sites (Table 2). Although no significant differences were observed for the total density and number of native species among reach types, DRZ reaches showed a significantly lower percentage of individuals belonging to threatened species – i.e. taxa classified as at least vulnerable on the Portuguese Red List of Threatened Vertebrate Species (Cabral et al., 2005). Of the trophic guilds we compared, the

percentage of native invertivores varied among reach types and was significantly lower in DRZ sites than in REF sites, with PRZ reaches presenting intermediate values and not differing significantly from the other two groups. When native invertivores are represented by density, the values in REF reaches are significantly higher not only than those in DRZ sites, but also than those in PRZ sites. No significant differences among reach types were observed for the omnivorous metrics. The two reproductive metrics we compared differed significantly among reach types, with DRZ reaches presenting a lower percentage of lithophils and a higher proportion of generalist spawners than REF and PRZ sites (Table 2).

The multivariate PERMANOVA detected a significant effect of reach type on fish assemblage structure (pseudo- $F_{2,12} = 4.417$ ,  $P = 0.001$ ); no evidence of heterogeneity in multivariate dispersions among the three groups was found by the PERMDISP test ( $F_{2,12} = 2.135$ ,  $P = 0.292$ ). Pairwise comparisons revealed that DRZ sites showed highly significant differences in fish species composition in comparison with REF ( $P = 0.007$ ) and PRZ ( $P = 0.014$ ) reaches, but did not show significant differences between REF and PRZ sites ( $P = 0.320$ ). These results were confirmed by the MDS ordination plot (Fig. 2), which showed a clear separation (high degree of dissimilarity) between DRZ reaches (which formed a distinct group) and all the other REF and PRZ sites. The stress value for the MDS was 0.11, which offers a fairly small prospect of misleading interpretations – i.e. it represented an acceptable goodness-of-fit of the non-parametric regression to the similarity data (Clarke and Warwick, 2001). The most discriminating species between REF and PRZ sites was *Squalius pyrenaicus*, which occurred with higher abundance in “natural” stream reaches (Table 3). The alien *Gobio lozanoi* and *Lampetra* spp. – the two taxa most typical of DRZ sites – were the species that best discriminated between this group and both REF and PRZ stream reaches. *Squalius alburnoides*, which significantly contributed to the average similarity of REF and PRZ groups, was also a relevant discriminating species between these groups and the DRZ group (where it was absent).

The univariate PERMANOVAS also showed significant effects of reach type on habitat variables (Table 4). Pools and riffles were much more representative in REF and PRZ sites than in DRZ stream reaches, which exhibited high habitat homogeneity, with a lack of riffle–pool complexes. The variables MORPH, EROS, RIPCOMP and silt were all significantly higher in DRZ reaches than in the REF and PRZ sites; in comparison, these two groups had significantly coarser substrates. No significant differences were observed for the variables RIPCONT and canopy shading, although fragmentation of the riparian vegetation tended to be higher in DRZ sites (Table 4). The DRZ stream reaches thus displayed higher impacts on streambanks, a degradation of the riparian vegetation, and higher accumulations of fines in the streambed. Of the instream cover variables we compared, woody cover and potential cover for fish showed a significant difference among reach types. These two descriptors were significantly higher in REF sites than in DRZ

**Table 3**

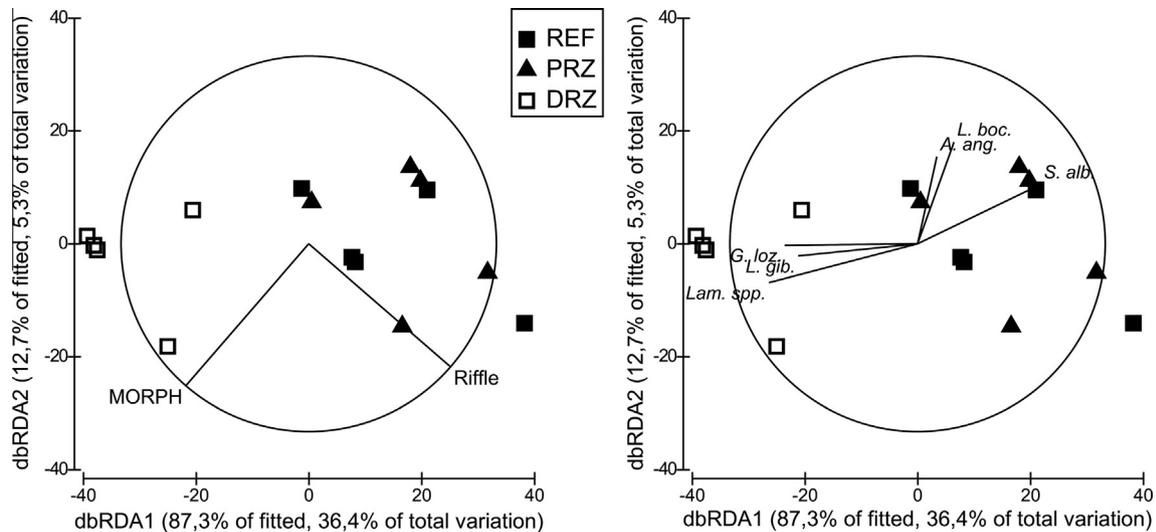
Species contributions to similarity within groups and to differences (dissimilarity) among groups based on SIMPER analyses for  $\log(x + 1)$  transformed density data ( $n = 100 \text{ m}^{-2}$ ); REF = reference sites; PRZ = protected riparian zones; DRZ = disturbed riparian zones. Only species with contributions greater than 10% are shown.

Species	Mean density and contribution			Mean dissimilarity and contribution		
	REF	PRZ	DRZ	REF vs. PRZ (47%)	REF vs. DRZ (71%)	PRZ vs. DRZ (75%)
<i>Gobio lozanoi</i> <sup>a</sup>	0	1.3 (2.9)	21.6 (33.0)	3.8 (6.6)	14.7 (20.8)	11.8 (15.7)
<i>Lampetra</i> spp.	0	0	15.5 (28.5)		13.2 (18.7)	13.0 (17.3)
<i>Lepomis gibbosus</i> <sup>a</sup>	0.2 (0)	0	1.9 (10.8)	1.7 (3.1)	5.8 (8.3)	6.4 (8.5)
<i>Luciobarbus bocagei</i>	2.6 (11.5)	1.9 (14.0)	0.9 (0)	7.6 (13.4)	6.1 (8.6)	5.5 (7.3)
<i>Pseudochondrostoma polylepis</i>	1.9 (17.1)	3.1 (15.9)	3.6 (7.1)	7.8 (13.7)	6.0 (8.6)	6.2 (8.3)
<i>Squalius alburnoides</i>	9.0 (21.8)	4.5 (43.1)	0	9.1 (16.0)	9.1 (12.9)	8.9 (11.8)
<i>Squalius pyrenaicus</i>	16.5 (48.9)	3.2 (15.5)	4.0 (19.2)	12.7 (22.3)	5.9 (8.4)	6.2 (8.2)

<sup>a</sup> Alien species.

**Table 4**  
Results of univariate PERMANOVA models for the effects of reach type on environmental variables (expressed as median and range); REF = reference sites; PRZ = protected riparian zones; DRZ = disturbed riparian zones. Significant test statistics at  $P < 0.05$  are marked in bold, and different superscript letters indicate significant differences ( $P < 0.05$ ) among reach types.

Variable	Pseudo- $F_{2,12}$	P	Forest and riparian category		
			REF	PRZ	DRZ
<i>Channel morphology and riparian integrity</i>					
Riffle (%)	6.158	<b>0.015</b>	20 <sup>a</sup> (5–44)	15 <sup>a</sup> (8–35)	0 <sup>b</sup> (0–20)
Pool (%)	9.849	<b>0.007</b>	60 <sup>a</sup> (20–95)	33 <sup>a</sup> (29–87)	0 <sup>b</sup> (0–20)
Run (%)	0.867	0.404	20 (0–60)	36 (0–60)	80 (0–100)
MORPH (1–5)	33.250	<b>0.001</b>	2 <sup>a</sup> (1–2)	1 <sup>a</sup> (1–2)	4 <sup>b</sup> (3–4)
EROS (1–5)	6.348	<b>0.012</b>	2 <sup>a</sup> (1–3)	1 <sup>a</sup> (1–3)	3 <sup>b</sup> (3–5)
RIPCONT (1–5)	2.279	0.158	2 (1–2)	3 (1–4)	4 (1–5)
RIPCOMP (1–5)	6.452	<b>0.019</b>	2 <sup>a</sup> (1–3)	2 <sup>ab</sup> (1–4)	4 <sup>b</sup> (3–5)
<i>Instream cover and substrate composition</i>					
Tree canopy shading (1–5)	1.313	0.412	4 (3–4)	4 (2–5)	2 (2–5)
Overhanging vegetation (1–5)	0.125	0.956	3 (1–4)	3 (1–4)	3 (1–4)
Woody cover (1–5)	6.348	<b>0.017</b>	4 <sup>a</sup> (3–4)	3 <sup>ab</sup> (2–5)	2 <sup>b</sup> (1–3)
Macrophyte cover (1–5)	1.059	0.536	2 (1–2)	2 (1–3)	2 (1–3)
Undercut banks (1–5)	1.357	0.329	2 (1–2)	1 (1–3)	3 (1–4)
Potential cover for fish (1–5)	11.692	<b>0.010</b>	5 <sup>a</sup> (4–5)	4 <sup>ab</sup> (3–5)	3 <sup>b</sup> (2–3)
Substrate dominant class (1–7)	8.432	<b>0.008</b>	5 <sup>a</sup> (5–7)	5 <sup>a</sup> (3–7)	3 <sup>b</sup> (2–4)
Silt (%)	5.933	<b>0.011</b>	8 <sup>a</sup> (2–10)	5 <sup>a</sup> (2–10)	15 <sup>b</sup> (8–35)
<i>Water quality</i>					
pH	1.806	0.207	7.6 (7.0–8.1)	7.0 (6.9–7.4)	6.8 (6.4–8.2)
Conductivity ( $\mu\text{S cm}^{-1}$ )	1.470	0.252	77 (61–164)	134 (63–174)	141 (113–162)
Oxygen (%)	7.058	<b>0.010</b>	92 <sup>ab</sup> (84–97)	88 <sup>a</sup> (75–89)	95 <sup>b</sup> (92–100)



**Fig. 3.** Distance-based redundancy analysis (dbRDA) plot for the fitted model of fish data (based on Bray Curtis matrix of  $\log(x + 1)$  transformed density data) with explanatory variables (left) and fish species (right). Riffle and MORPH together accounted for 42% of the total variance. The dbRDA plot showed a clear separation between DRZ sites (degraded reaches which formed a distinct group) and all the other REF and PRZ sites (non-degraded reaches). *Squalius alburnoides* was positively correlated with Axis 1, indicating an association with REF and PRZ sites, and two alien species (*G. lozanoi* and *Lepomis gibbosus*) together with *Lampetra* spp. were negatively correlated with Axis 1, indicating an association with stream reaches impaired by habitat degradation. Only species with correlations  $>0.35$  with either axis are plotted; MORPH = morphological alteration; REF = reference sites; PRZ = protected riparian zones; DRZ = disturbed riparian zones. Species abbreviations are as follows: *A. ang* = *Anguilla anguilla*; *G. loz* = *Gobio lozanoi*; *Lam. spp.* = *Lampetra* spp.; *L. gib* = *Lepomis gibbosus*; *L. boc* = *Luciobarbus bocagei*; *S. alb* = *Squalius alburnoides*.

sites, with PRZ reaches presenting intermediate values without differing significantly from the other two groups. Finally, we found significant differences for the dissolved oxygen levels, which interestingly tended to be higher in the DRZ sites than in the other two groups, although the measured values in all stream reaches indicated unpolluted (or almost unpolluted) waters as measured by the Portuguese water quality classification system (SNIRH: <http://snirh.pt/>).

Riffle and MORPH both significantly explained the structure of fish assemblages (marginal tests,  $P < 0.05$ ), together accounting

for 42% of the total variance (sequential tests,  $P < 0.05$ ). A good agreement was evident between the constrained (dbRDA) and the unconstrained ordination (MDS) methods (Figs. 2 and 3), indicating a good fit for the DISTLM model. In fact, the dbRDA diagram also showed a clear separation between DRZ sites with significant morphological alterations and structurally homogeneous habitats on the one hand, and REF and PRZ sites with higher habitat heterogeneity and well-preserved channels on the other. *S. alburnoides* was positively correlated with Axis 1, indicating an association with non-degraded streams, and two alien species (*G. lozanoi* and

*Lepomis gibbosus*) together with *Lampetra* spp. were negatively correlated with Axis 1, indicating an association with stream reaches impaired by habitat degradation (Fig. 3).

#### 4. Discussion

Differences in the condition of the riparian zone in eucalypt plantations are likely to contribute significantly to differences in fish composition and functional attributes of Iberian fish assemblages. Our predictions – that good quality riparian areas might mitigate the impacts of eucalyptus silviculture and that highly degraded riparian zones would have detrimental effects on fish assemblages – were supported by our findings. Considering that the studied reach types presented overlapping ranges in their natural variables (i.e., catchment area, slope, width, etc.), and that the land use at the catchment level in the two non-reference groups was dominated by non-agricultural areas, we conclude that even in forested catchments the naturalness of the riparian areas is of paramount importance to maintaining streams with high biological integrity (*sensu* Karr, 1991).

The influence of riparian buffers on aquatic systems is disproportionate to its total land area, when considering their size relative to watershed areas (Nerbonne and Vondracek, 2001; Stewart et al., 2001; Feld, 2013). In fact, due to their immediate proximity to the surface water, the physical conditions at the riparian buffer scale play a key role in determining the abiotic and biotic features of streams (Feld, 2013). Best management practices within eucalyptus plantations, including the significant reduction of forestry and silvicultural practices in the riparian areas and the conservation of native vegetation buffers, appeared to greatly reduce changes in stream geomorphology and consequently in aquatic habitats. In fact, we did not find significant differences for most of the predictor variables between REF and PRZ sites, although PRZ sites generally presented lower potential cover for fish and tended to support a riparian vegetation that was a little more fragmented. This relationship is not surprising, since a structurally complex and more continuous riparian vegetation may increase stream habitat availability by contributing more cover in the form of branches, trunks, large logs or root wads (Pusey and Arthington, 2003; Baker et al., 2004; Arizpe et al., 2008). In spite of these differences in stream condition, PRZ sites supported fish assemblages that were similar to those in reference reaches, although we have detected a few differences between these two groups at the functional level (as discussed below). It appears that stream reaches associated with certified eucalypt plantations are protected from forestry practices, thus presenting fish assemblages with high biotic integrity.

These differences we found in riparian vegetation continuity and structure between REF and PRZ sites are consistent with results from Boothroyd et al. (2004), who found that native forests were more effective at protecting high value riparian vegetation compared to streams in exotic forestry areas with riparian buffers. According to Cunjak et al. (2004), even wide buffer strips and careful harvesting practices may not preclude damage to aquatic ecosystems during forestry operations. However, our findings may also partly reflect the past land use in our study area. The fact is that most of the areas managed in accordance with BMP endured higher human impacts in the past (including agriculture and non-sustainable forestry), and although the protection of riparian zones has occurred over the last two decades, it is possible that riparian vegetation has not had time to fully recover to its natural state. For streams crossing Savannah-type evergreen oak woodlands – a Mediterranean agroforestry system that is managed for multiple uses – the positive effects of forest certification were only measurable after five years of certification (Dias et al., 2015). The differences we observed between REF and PRZ sites may thus reflect

some impacts of eucalyptus forestry, but also the disturbance of the riparian zones in certified sites before the implementation of sustainable forestry practices.

Conversely, we observed extensive degradation of the physical environment of streams where riparian areas were occupied by productive crop and grazing lands (DRZ group). The DRZ sites were frequently devoid of riparian vegetation, and their streams presented eroded and modified channels with lower potential cover for fish, high habitat homogeneity, and substrates revealing non-negligible deposition of fine sediments. However, we found an increase of dissolved oxygen levels in DRZ sites. We do not have additional data that could clarify this difference, but we believe that this result has minimal implications on our findings, as measured values for all studied streams indicate waters with adequate oxygen levels for warmwater fish communities (Alabaster and Lloyd, 1982). In DRZ stream reaches the variation in the species composition of fish assemblages was higher, aliens were common and abundant (*G. lozanoi* was the most characteristic species of this group), the proportion of native invertivores and lithophils was lower, and generalist spawners tended to increase. Despite the 'natural' stochasticity in our study area, these results are consistent with those found by other researchers in agricultural landscapes where declines in the morphological and physicochemical stream environment were observed, affecting the quality of fish habitat and the integrity of fish communities (Pusey et al., 1993; Lammert and Allan, 1999; Stewart et al., 2001; Heitke et al., 2006). In particular, livestock can have a strong effect on riparian condition where it has unrestrained access to streams, eroding the streambanks and altering the riparian plant community through grazing (Kauffman and Krueger, 1984; Belsky et al., 1999). Even in watersheds with high forest cover, riparian deforestation and its detrimental effects on stream habitats, may impact the biological integrity of fish assemblages (Jones et al., 1999). We thus conclude that the non-protection of the riparian areas in DRZ stream reaches clearly promoted the structural degradation of stream morphology and compromised the functionality of the riparian vegetation, with obvious impacts at the habitat scale.

This conclusion may, however, be complicated by other factors at different scales (Feld, 2013). In these DRZ sites, riparian and habitat condition may be mostly explained by local land use, but may also reflect differences in non-measured variables such as soils, or subtle differences of land use at larger scales. Nevertheless, the degradation of the riparian vegetation and the atypical values of habitat descriptors at DRZ sites (e.g. absence of riffle-pool complexes, over-silted streambeds, little potential cover for fish, highly eroded banks) support the conclusion that agricultural practices at the riparian level in our forest landscapes have strongly affected stream habitats and consequently fish assemblages. Unlike watershed-scale impacts, riparian disturbance may be directly detrimental to aquatic habitats due to decreased bank stability, loss of cover, or increased bed sedimentation (Steedman et al., 2004).

The high abundance of alien specimens in DRZ sites was unquestionably the most important factor for the impairment of its fish assemblages. This is largely explained by the invasion of *G. lozanoi* – a species endemic to north-eastern Spain and south-western France – which has been translocated to several Iberian basins, where it is now a highly successful regional invader (Ribeiro et al., 2009). *Gobio lozanoi*, which is considered tolerant to stream degradation, comprised a large proportion of fish assemblages in DRZ sites, and it is thus not surprising that the percentage of threatened species was significantly lower in this group. Although captured in smaller numbers, the highly tolerant alien species *L. gibbosus* was present in all DRZ sites, also contributing to a loss of the biological integrity of these communities. The ability of alien fishes to thrive in degraded streams, and consequently

to impact natural fish assemblages, has also been detected in other studies (Brown, 2000; Kennard et al., 2005; Ferreira et al., 2007a), and represents one of the main causes of a decline in biotic integrity in lotic systems.

We did not find significant differences in native fish richness and density among reach types. However, at the functional level our study was able to detect significant effects of reach type on fish metrics. First, we found higher abundance in both percentage and density of native invertivores in the streams with the highest quality riparian areas (i.e. the REF group). These results are in line with those of Oliveira et al. (2012) from central Portugal, who also reported higher abundance of this guild in minimally disturbed streams that were densely shaded by deciduous vegetation. These systems receive large amounts of terrestrial invertebrates provided by the riparian vegetation (Mason and McDonald, 1982; Pusey and Arthington, 2003; Studinski and Hartman, 2015), and fish assemblages may thus include high densities of invertivore species (Vannote et al., 1980). Terrestrial invertebrates can comprise more than 50% of energy intake by stream fishes (Allan et al., 2003), and may significantly contribute to the diet of invertivore cyprinids endemic to Iberia (Magalhães, 1993; Coelho et al., 1997). Although no significant differences were observed for native invertivores between PRZ and DRZ sites, this guild (mostly represented by *Squalius* spp.) tended to be more abundant in the least-degraded group. In this context, we believe that this difference is mainly due to the contrasting condition of the riparian vegetation between the two reach types, which is likely to influence the volume of the food supply to fishes. The higher abundance of *Squalius* spp. in REF sites could also have reflected the higher availability of diverse habitats in these stream reaches. The fact is that these species are less tolerant to general habitat degradation (Ferreira et al., 2007a; Oliveira et al., 2012), and a reduction in their density could thus be associated with a decrease in the physical quality of reach types (at its simplest, REF > PRZ >> DRZ).

Second, we also found significant differences in the reproductive guilds between DRZ sites and REF and PRZ sites. We believe that this observation may partly reflect differences in stream substrate composition. The degradation or removal of riparian vegetation is quite often followed by an increase in the sediment loads within the stream channel (Rabeni and Smale, 1995). These changes in substrates influence the abundance of reproduction guilds, and a lower proportion of lithophilic individuals is to be expected in streams with soft bottoms (Rabeni and Smale, 1995; Belliard et al., 1999; Bramblett et al., 2005). The higher relative abundance of both *Lampetra* spp. – a group that seems to present considerable hatching success in degraded spawning habitats (Silva et al., 2014) – and generalist spawners in the DRZ group may thus be partly explained by the increased sedimentation of fine sands and silt we observed in these “agricultural sites”.

The nature and extent of forestry impacts on aquatic ecosystems and ecological processes depend on a number of factors, including landscape characteristics, river type, silvicultural practices, or planted trees (Hartman, 2004). In exotic forestry areas, Quinn et al. (2004) concluded that New Zealand pine plantations with BMP supported macroinvertebrate assemblages that were very similar to native forests in small and steep headwater streams; but in streams in central Portugal, Abelho and Graça (1996) reported that although the presence of a buffer strip of riparian trees attenuated the impact of eucalyptus monoculture on invertebrate communities, the latter were different from those in native forests. In fact, the allochthonous organic matter produced in eucalyptus plantations differs considerably from that occurring in native forests, thus directly affecting the structure, abundance and biomass of aquatic macroinvertebrates (Graça et al., 2002; Larrañaga et al., 2009). We believe that these types of effects of eucalyptus plantations on stream biota ought to be

more evident for these benthic communities than for fish, largely due to the strong dependence of many aquatic macroinvertebrates on the leaf litter produced in forests. In contrast, most of our native fish species are not so directly related to the type of forest litter and generally present high plasticity in response to variations in the communities at lower trophic levels (Granado-Lorencio, 1992; Encina et al., 2006). Nevertheless, the leaching of phenols and other soluble compounds from eucalyptus leaves can also have a significant impact on both aquatic invertebrates (Bunn, 1988; Canhoto and Laranjeira, 2007) and fish (Gehrke et al., 1993; Morrongiello et al., 2011), for example by inhibiting fish reproduction in high leachate conditions. It might be interesting to study whether the impact of the leaves influenced the differences we found between PRZ and REF sites at the functional level. However, these conclusions should be tested in the more general context of the links between the carbon fluxes in the Iberian stream ecosystems and the functional attributes of fish assemblages (Pouilly et al., 2006; Oliveira et al., 2012).

In summary, we infer from our data that the potential impacts of eucalyptus silviculture on Iberian fish assemblages may be mitigated at sites where BMP are employed. However, given that there are a few differences in riparian condition and biological integrity between the reference sites and eucalyptus plantations managed under BMP, we suggest that these stream reaches require more proactive management measures. These include techniques such as restoration of the native vegetation, removal of alien plants, or even improvement of the stream habitat. Stream rehabilitation techniques may prove effective at restoring natural processes that create and maintain habitats, improving instream conditions and increasing the integrity of fish communities (Roni et al., 2008), although there is some doubt as to whether aquatic communities can recover by simply restoring their habitat (Palmer et al., 2010; Nilsson et al., 2015).

Our study was an initial attempt at identifying the effects of different riparian management on stream fishes in Iberian catchments dominated by eucalyptus plantations. However, the results of our study should be viewed with caution, as they represent an ecological snapshot. Temporal variation in Mediterranean streams and consequently non-negligible stochasticity in compositional and functional characteristics of fish assemblages, which could not be detected by our single sampling at each site, may play a role in understanding faunal responses to riparian condition. Also, while studies with small size groups in the published literature are common (e.g. Jones et al., 1999; Lorion and Kennedy, 2009; Ferreira et al., 2015), this may have limited our ability to identify additional differences, although we attempted to overcome this by selecting homogeneous stream reaches across a relatively large spatial extent. The detection of impacts on fish assemblages may be also complicated by the overall low species richness at the local scale. In Iberia most stream reaches typically have a fish assemblage of five/six or fewer native species, which are well adapted to survive in Mediterranean systems with strong environmental variability (Encina et al., 2006; Ferreira et al., 2007a). We should recognize that natural (i.e., non-anthropogenic) variability, determined by the location of each site in the study area, may also have had a role in driving the differences in fish assemblages between the reach types. However, the results of the PERMANOVA, exclusively based on variables describing natural environmental variability (catchment size, slope, temperature, etc.) (Table 1), showed no significant differences between the three reach types, confirming the overlapping ranges of these variables for each group. Although there was some correspondence between the geographical location of the sites (Fig. 1) and the patterns that emerged from the ordination analyses (Figs. 2 and 3), we believe that the differences found in our study were thus strongly connected with forest management. Ultimately, we agree with Rowe et al. (2002)

that the duration of the favourable effects of BMP needs to be tested in order to determine whether such ecological mitigation is sustainable over longer terms. Despite these caveats, the consistency of faunal differences between reach types strongly suggests a causal role for forest management and riparian condition. Future studies should consider larger groups of sites and appropriate temporal scales, so as to test for the consistence and persistence of BMP in protecting stream ecosystems in Iberian eucalyptus plantations.

## 5. Conclusions

We compared two distinct riparian conditions in Iberian streams surrounded by eucalypt-dominated landscapes, with a group of reference streams dominated by native vegetation and with high quality riparian zones. Eucalypt streams with protected riparian zones presented physical habitats and fish assemblages similar to those in reference sites. However, riparian vegetation and instream cover appeared to be in slightly better condition in the reference group, probably due to past land use practices and effects of forestry in eucalypt streams. These differences may partly explain the higher abundance of native invertivores we observed in the reference group. On the other hand, we found significantly degraded streams and consequently an impairment of fish assemblages in eucalypt streams with agricultural riparian zones. The strongest faunal change in these streams was the invasion of *G. lozanoi*, a tolerant alien species. This emphasizes the crucial importance to river conservation of maintaining good quality riparian areas, even in forested catchments.

Best management practices (BMP) in Iberian eucalypt plantations, including the significant reduction of productive activities in the riparian zone and the protection of the native riparian vegetation, seem to mitigate the potential effects of eucalypt forestry in stream habitats and fish assemblages. Research considering larger temporal scales would be useful, since it would provide a more detailed understanding of the effectiveness of BMP in protecting stream ecosystems in long terms.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foreco.2015.12.011>.

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