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Distribution patterns of fish assemblages in an Eastern Mediterranean intermittent river

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ABSTRACT

Key-words: *distribution patterns, fish assemblages, Mediterranean, intermittent, drought*

The distribution patterns of fish assemblages within streams can provide insights for river type classifications and may warrant specific conservation actions. However, there is limited knowledge of how fish assemblages assort along a longitudinal axis in Mediterranean intermittent streams. Patterns in spatial and temporal distribution of fish communities were analysed in a Mediterranean intermittent river (Evrotas River) located in Southern Greece, hosting three endemic range restricted species of high conservation concern, during the period 2007–2009, with 80% of the river's total length desiccating in the 2007 and 2008 droughts. The general trend was an increase in fish density and species richness along an upstream-downstream gradient. Fish assemblages from upstream to downstream were characterized by a decrease of the most rheophilic species (*Squalius keadicus*) and an increase of the most stagnophilic species (*Tropidophoxinellus spartiaticus*). Three river segments, characterized by a high degree of homogeneity were delineated. Habitat and environmental preferences for the studied fish species were identified, with elevation and low flowing habitats being the most important environmental factors affecting fish distribution patterns. The current study provides evidence that even in an intermittent river an assemblage pattern following a longitudinal gradient can be identified, mainly due to the lack of instream barriers that allows recolonization after flow resumption.

RÉSUMÉ

Modèles de distribution des assemblages de poissons dans un cours d'eau intermittent de la Méditerranée orientale

Mots-clés : *modèles de distribution, assemblages de poissons,*

Les modèles de distribution des assemblages de poissons dans les cours d'eau peuvent fournir des indications pour les classifications des types de rivière et peuvent justifier des mesures spécifiques de conservation. Cependant, il y a une connaissance limitée de la façon dont les assemblages de poissons sont répartis le long de l'axe longitudinal dans les ruisseaux intermittents méditerranéens. Les schémas de la distribution spatiale et temporelle des communautés de poissons

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Méditerranée, intermittent, sécheresse

ont été analysés dans une rivière intermittente méditerranéenne (rivière Evrotas) située dans le sud de la Grèce, habitat de trois espèces endémiques peu répandues de préoccupation pour la conservation, au cours de la période 2007-2009, avec 80 % du total de linéaire de la rivière à sec lors des sécheresses de 2007 et 2008. La tendance générale était une augmentation de la densité de poissons et de la richesse en espèces le long d'un gradient amont-aval. Les assemblages de poissons d'amont en aval ont été caractérisés par une diminution des espèces les plus rhéophiles (*Squalius keadicus*) et une augmentation des espèces les plus lentophiles (*Tropidophoxinellus spartiaticus*). Trois segments de rivière, caractérisés par un haut degré d'homogénéité ont été délimités. L'habitat et les préférences environnementales pour ces espèces de poissons étudiées ont été identifiés, avec l'altitude et les habitats à faible courant étant les facteurs environnementaux les plus importants qui affectent les modes de distribution du poisson. L'étude actuelle fournit la preuve que même dans une rivière intermittente un modèle d'assemblage suivant un gradient longitudinal peut être identifié, principalement en raison de l'absence de barrières dans le cours d'eau qui permet la recolonisation après la reprise de l'écoulement.

INTRODUCTION

Understanding how biological communities are structured along a river's longitudinal axis in lotic ecosystems remains a challenge for stream ecologists (Lorenz *et al*., 1997). Vannote *et al*. (1980) described a conceptual synthesis based on expected species patterns and physical environmental parameters along the longitudinal continuum (The River Continuum Concept – RCC). Even though the RCC remains valuable as a concept, several more recent contributions emphasized that other external influences may also affect fish distribution dynamics, such as the effects of river form (geomorphology), climate variability, presence of tributaries and floodplains, riparian vegetation, channel discontinuities and human disturbances (Osborne and Wiley, 1992; Ward and Stanford, 1995; Benda *et al*., 2004; Thorp *et al*., 2006). The current consensus is that stream communities are structured by a combination of local and regional processes, local habitat conditions and species ecological requirements, combined with inter- and intra-species interactions (Townsend *et al.*, 2003; Thorp *et al.*, 2006; Hoeinghaus *et al.*, 2007).

Apart from elucidating the underlying mechanisms of fish distribution along a river continuum, identification of fish assemblage distribution patterns within streams also provides valuable insights into biotically-based river type classifications. Many early river studies, dated from the beginning of the 20th century, developed a zonal distribution of aquatic animals, with Huet (1959) refining this 'fish zonation' concept in European streams. This typological concept connotes a predictable longitudinal succession of stable communities that have distinct breakpoints, as stream environments change (Miranda and Raborn, 2000). These fish assemblage types, or 'ichthyofaunal river types' (also referred to as river zones) have long been used as baselines for interpreting and predicting in-stream conditions and are also relevant to modern water management and conservation policy. The EU Water Framework Directive 2000/60/EC (WFD) which utilizes fishes as ecological status indicators, dictates that all EU member states classify their surface water bodies on the basis of a typology that is relevant to natural species assemblage structure and at the same time promotes the development of type-specific reference conditions (Schmutz *et al*., 2000; Aarts and Nienhuis, 2003). In addition, ecological classifications of aquatic species assemblages are also of pivotal importance in biodiversity conservation, since ecological attributes of individual community units, types or zones may warrant specific conservation measures (Angermeier and Schlosser, 1995), with fish communities being key units of conservation interest (Angermeier and Winston, 1999; Maitland, 2004).

Traditionally, fish assemblages in temperate streams and rivers have indicated a predictable longitudinal change in species composition, associated with specific lotic conditions

(Schlosser, 1982; Moyle and Vonderacek, 1985; Lobb and Orth, 1991). Despite the worldwide application of this ichthyofaunal typology, few river zonation studies have been conducted in Mediterranean streams and rivers (Carmona *et al.*, 1999; Vila-Gilspert *et al.*, 2002; Clavero *et al.*, 2005; Franchi *et al.*, 2014), which are characterized by a lack of sentinel fish species, such as trout, grayling and bream, typically widespread in temperate rivers. Mediterranean streams are often characterized by a depauperate native fish fauna (despite the high freshwater fish biodiversity of the Mediterranean region), by assemblage structures dominated by range-restricted endemic species -often with wide niche-breadths-, by high temporal and spatial fish assemblage variability, due to frequent hydrological instability and by non-longitudinal species' distribution patterns often complicated by extensive anthropogenic modifications impacting rivers under high water stress (Ferreira *et al*., 2007). Furthermore, anthropogenic pressures in the Mediterranean region have altered systems to the point that some species have been extirpated from entire river basins and, at the same time, human-induced species' translocations from nearby basins blur even more the natural composition of Mediterranean rivers (for Greece, see Barbieri *et al.*, 2015). However, even though more sophisticated guild-based approaches (Welcomme *et al*., 2006) and visualization tools using modelling methods are now being developed (Stojkovic *et al*., 2014), longitudinal distribution pattern analyses, such as the fish-based zonation frameworks, are still being widely utilized, even beyond Europe, where zonation pattern descriptions have proven consistently useful in describing river ecosystem structure (*e.g.* McGarvey and Hughes, 2008).

This study aimed to describe ichthyofaunal assemblage compositions and distributions along a longitudinal river gradient and attempted to interpret fish assemblage types in a hydrologically disturbed Mediterranean intermittent river. Confounding effects of severe drought-event disturbance and the influence of anthropogenic pressures on the assemblages are also discussed.

MATERIALS AND METHODS

> *STUDY AREA*

This study, was conducted at the Evrotas River, a biogeographically isolated basin in the southernmost river valley of the Balkans, in Southern Greece (Figure 1). Biogeographically the river belongs to the Ionian Ecoregion having however the most distinctive fish species composition in this ecoregion (Zogaris *et al.* 2009; Barbieri *et al.*, 2015). The river harbours three range restricted species of special conservation concern listed in the IUCN Red List (2014), *i.e.* the Evrotas Chub *Squalius keadicus* (Stephanidis, 1971) classified as "Endangered" by IUCN and endemic to the Evrotas; the Spartian Minnowroach *Tropidophoxinellus spartiaticus* (Schmidt-Ries, 1943) classified as "Vulnerable" and endemic to the southern Peloponnese peninsula; and the Laconian Minnow *Pelasgus laconicus* (Kottelat and Barbieri, 2004) classified as "Critically Endangered" by IUCN and endemic to the Evrotas and upper Alphios river basins. Apart from the endemic freshwater fish species, the river hosts two more native fishes of conservation interest, the European Eel (*Anguilla anguilla* L.) assessed as "Critically Endangered" and the River Blenny (*Salaria fluviatilis* Asso, 1801), a peri-Mediterranean endemic that has shown marked decline in several Mediterranean countries (Zogaris *et al.* 2012) although not listed as threatened due to its wide geographical distribution. The river also hosts two non-indigenous species, the Eastern Mosquitofish *Gambusia holbrooki* (Girard, 1859), which has been successfully established in the lower section of the river and the Rainbow Trout *Oncorhynchus mykiss* (Walbaum, 1792), which has probably not established viable populations, since the few individuals (TL $>$ 10 cm) captured during this study were quite possibly from unofficial stocking actions and/or escapees from an aquaculture unit.

The topographical, hydrological and some ecosystem attributes of the Evrotas River have been described in Skoulikidis *et al.* (2011). Briefly, it is a medium-sized (2.418 km^2), mid-altitude (150–600 m) Mediterranean basin. The river flows unobstructed without dams impeding its flow for about 90 km to its outlet in the Laconic Gulf, after a small deltaic formation

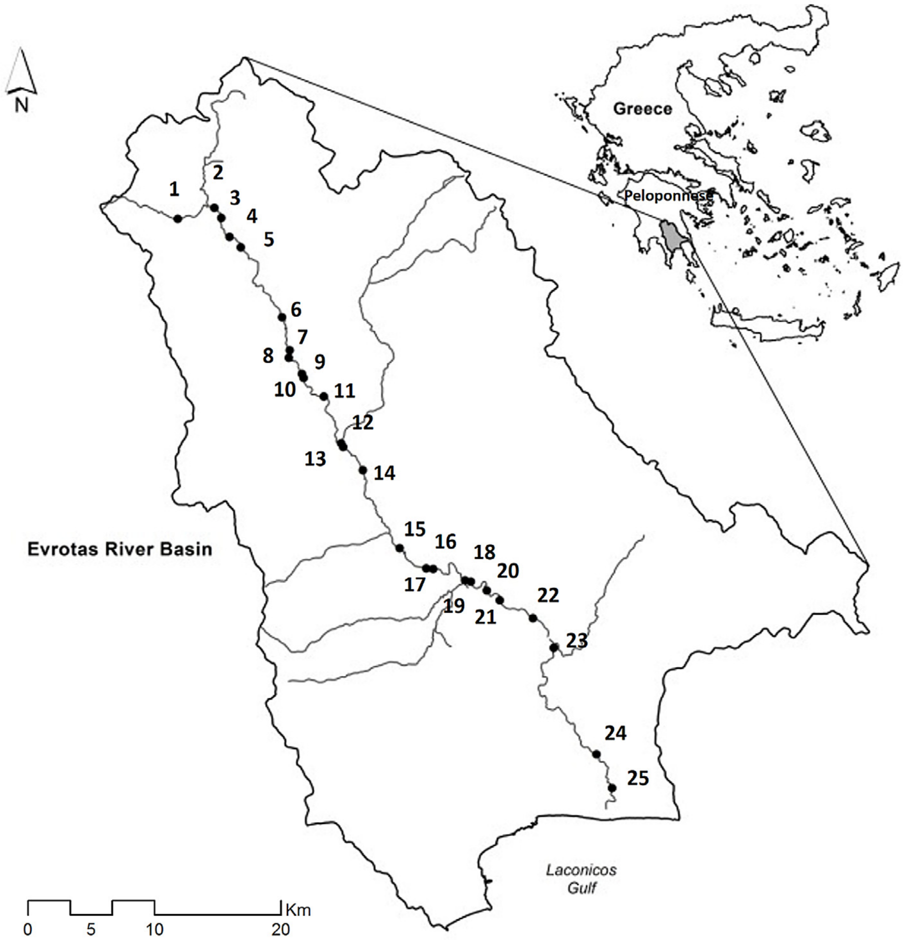

Figure 1
Location of the 25 sampling sites in the Evrotas river basin.

(53 km^2). The Evrotas River has undergone, however, substantial hydromorphological alteration especially during the past 60 years, *i.e.* channelization, wetland and riparian zone reduction, as well as severe water abstraction (Skoulikidis *et al.*, 2011). Past ichthyological research in the Evrotas basin (Stephanidis 1971, 1974) indicate that all native fish species were then widely distributed in its main channel and its tributaries. However, in the 1980s there was already evidence of a reduction of the species' local ranges and of the absence of certain species in some of the river's smaller tributaries (Economidis, 1992). Severe and prolonged droughts that occurred between the summer periods of 1987 and 1992, combined with extensive water abstraction for irrigation, caused the almost complete desiccation of the river and its tributaries during that period. Fish populations were extirpated from most of the basin, with remnant populations only in a few spring-fed sections of the main channel. Currently, the river's variable flow regime is partly human-induced due to the over-abstraction of both surface and ground waters throughout the river valley rendering it an "artificially intermittent Mediterranean river", a term introduced in Skoulikidis *et al.* (2011).

> SAMPLING

Fish samplings were conducted at 25 sites along the longitudinal axis of the main channel of Evrotas River during the low flow (summer) periods (July–October) of 2007, 2008 and 2009 (Figure 1). Droughts are regular natural disturbances that impact the Evrotas River, when small

Table I
Descriptive information of the sampling campaigns and geographical location of the study sites (• = sampled, ns = not sampled, dry = the site was dry during the investigation).

Sites	Sampling Period			Coordinates	
	2007	2008	2009	*N*	*E*
1	•	ns	•	345 950	4 125 403
2	ns	•	•	348 847	4 126 458
3	dry	•	•	349 445	4 125 586
4	dry	dry	•	350 167	4 123 943
5	dry	dry	•	351 347	4 123 062
6	dry	dry	•	354 788	4 117 073
7	•	ns	•	355 292	4 113 550
8	ns	•	•	355 407	4 114 179
9	•	ns	ns	356342	4112006
10	•	•	•	356 620	4 111 837
11	•	•	ns	358 302	4 110 157
12	ns	•	•	359 977	4 105 897
13	dry	•	•	362 902	4 104 290
14	dry	dry	•	361 799	4 103 897
15	dry	dry	•	364 842	4 113 006
16	•	ns	ns	367 644	4 095 077
17	ns	•	•	367 819	4 094 956
18	ns	•	ns	370 779	4 094 991
19	ns	•	ns	371 454	4 080 995
20	•	ns	ns	372 157	4 093 194
21	ns	ns	•	373 490	4 092 655
22	dry	•	ns	376 368	4 090 912
23	dry	dry	•	378 128	4 088 467
24	dry	dry	•	381 725	4 079 209
25	•	•	•	383 022	4 076 283

sections of the river dry out; 2007 and 2008, however, were exceptionally dry years, when almost 80% of the river dried out (Skoulikidis *et al.*, 2011). Most sites within the established sampling network dried up quickly and were unavailable for sampling, thus, during 2007 and 2008, fish sampling was conducted in the remaining perennial segments (see Figure 1 and Table I). Sampling sites were selected based mainly on generic macro-habitat representativeness (all habitats present, *i.e.* riffles, runs, glides and pools), though accessibility to riverbanks was an additional factor considered in their selection. The sampling network was quite dense, with one site almost every 4 km, as evenly as possible distributed and with a representative cover of all available habitats. Sampling consisted of a single pass of a 100 m river stretch without using a stop-net; however, during every pass, sampling was conducted at a river stretch demarcated by physical barriers (*e.g.* shallow riffles) to minimize fish escape in either direction. In all sampling sessions, the same field crew participated, to ensure that effort was consistent among sites and years. Throughout the surveys, a Hans-Grassl GmbH battery-powered backpack electrofisher (Model IG200-2, DC pulsed, 1.5 KW output power, 35–100 Hz, max. 850 V, range used 450–600 V) was routinely used. On some occasions, usually in sites where depth exceeded 1.5 m, a generator-powered unit was employed (EFKO Elektrofischereigeräte GmbH, Model FEG 6000, DC (unpulsed), 7.0 KW output power, 600 V, range used 300–600 V). Fish were identified to species level, counted, measured to size-class intervals and then released alive, back to the river. Species nomenclature and common English names follow Barbieri *et al.* (2015).

For each study site, various environmental parameters were recorded/calculated (Table II). More specifically, physicochemical parameters, *i.e.* conductivity (μS·cm^{-1}, Cond), dissolved oxygen (mg·L^{-1}, DO), pH and water temperature (°C, *T*) were recorded at random points at each site. In addition, habitat parameters, *i.e.* mean wetted width (m, WW), mean depth (cm, *D*), shadedness (%, Sh) and substrate coarseness *i.e.* $>$ 63 mm (%, CSU) were recorded at transects every 10 m, as well as instream generic habitats categorized as low flow, *i.e.*

Table II
Average, median and range values of environmental parameters at the three Evrotas river segments.

River segments	Upland (UP)				Middle (MD)				Lowland (LW)			
	Average	Median	Min	Max	Average	Median	Min	Max	Average	Median	Min	Max
Distance from source (km)	7.95	7.95	3.54	12.50	38.03	35.67	21.33	58.30	84.20	83.00	66.00	100.50
Elevation (m)	342.13	343.00	314.00	354.00	209.85	231.00	129.00	277.00	59.50	67.00	5.00	111.00
Conductivity ($\mu S \cdot cm^{-1}$)	0.48	0.49	0.36	0.57	0.59	0.58	0.50	0.70	0.59	0.59	0.47	0.76
DO ($mg \cdot L^{-1}$)	9.00	9.00	7.00	10.30	8.36	8.45	4.99	9.80	8.05	8.08	6.76	10.00
pH	7.87	8.00	7.00	8.27	7.75	7.86	6.12	8.65	7.64	7.71	6.70	8.41
To water (°C)	20.72	20.72	15.80	25.60	20.50	20.70	16.40	25.40	25.39	25.19	21.50	31.70
Wetted width (m)	6.13	6.13	2.00	8.50	10.33	8.75	4.00	25.00	9.25	9.25	3.00	18.00
Mean Depth (m)	0.36	0.40	0.15	0.50	0.44	0.40	0.25	0.75	0.53	0.50	0.25	0.80
Shadedness %	36.25	10.00	0.00	90.00	28.84	17.50	0.00	90.00	19.80	15.00	0.00	80.00
site width < 10	100.00	100.00	100.00	100.00	69.40	98.00	0.00	100.00	54.50	60.00	0.00	100.00
site width > 10	0.00	0.00	0.00	0.00	30.60	2.00	0.00	100.00	45.50	40.00	0.00	100.00
site depth < 0.5	90.63	90.63	75.00	100.00	71.25	80.00	0.00	100.00	66.00	66.00	10.00	100.00
site depth > 0.5	9.38	9.38	0.00	25.00	28.75	20.00	0.00	100.00	34.00	34.00	0.00	90.00
coarse substrate (CSU, %)	35.71	32.86	5.00	90.00	26.50	25.00	2.00	70.00	23.50	15.00	0.00	70.00
fine substrate (%)	64.29	67.14	10.00	95.00	73.50	75.00	30.00	98.00	76.50	85.00	30.00	100.00
slow habitats (%)	68.29	74.00	40.00	85.00	57.47	61.24	0.00	100.00	57.30	55.00	5.00	100.00
fast habitats (%)	31.71	26.00	15.00	60.00	42.53	38.76	0.00	100.00	42.70	45.00	0.00	95.00

pools (P) and glides (G) and fast flow, *i.e.* runs (R) and rifles (RF). Finally, spatial parameters, *i.e.* distance from source (m, Dist) and elevation (m, El) were calculated from topographical maps (1:50 000).

> DATA ANALYSIS

Fish abundance data at each site (number of fish per single run) were converted to area densities (*i.e.* inds·m^{-2}). The surface area sampled at each site was estimated from its geometrical characteristics (fished length and cross-sectional width). Introduced species were excluded from any further analysis, since their contribution in the catches was very low ($<$ 3% of total catch).

Assessment of the importance of the different physicochemical, habitat and spatial variables was conducted by using multivariate statistics, such as Principal Components Analysis (PCA) using a matrix, which included temperature, dissolved oxygen, pH, conductivity, elevation, distance from source, wetted width and depth, shadedness, substrate coarseness and fast flow habitats. Parameters expressed as percentage values were arcsin transformed. Data were normalized prior to the analysis.

To elucidate the spatial pattern of fish distribution, to identify areas with similar fish assemblages and to compare these assemblages among areas, a non-parametric multidimensional scaling (MDS) was performed using fish densities (inds·m^{-2}) which were square-root transformed to calculate Bray-Curtis dissimilarity. Subsequently, an analysis of similarities (ANOSIM) was performed to identify differences between river sections and a similarity breakdown using similarity percentages (SIMPER) tests was performed to identify similarity within and dissimilarity between river segments, as well as those species that account for similarities within a segment and for dissimilarities between segments. Finally, fish species' densities were superimposed on the PCA to visualize variations in the different river segments. PCA, ANOSIM, SIMPER tests and MDS plots were performed with PRIMER v. 5.0 for Windows (Clarke and Warwick, 1994).

To test the heterogeneity of the fish community composition in the sampling sites, Detrended Correspondence Analysis (DCA) was performed. Length of gradients for the first axis was estimated at 2.185 ($<$3) indicating linear response of the data. Thus, Redundancy Analysis (RDA) was used to analyze species-environmental/habitat variance relation. Prior to RDA, forward selection was carried out with Monte Carlo Permutation Tests in order to estimate the contribution of each variable to the explained variance of the response (species) variables. The inflation factor was tested to be lower than 20 in the data, in order to avoid variables that were strongly correlated to each other (Ter Braak and Smilauer, 1998). Fish densities and physicochemical/habitat/spatial data were log (X + 1) transformed, while for explanatory variables expressed as percentage, the arcsin transformation was used. Both DCA and RDA analyses were carried out with CANOCO 4.5 (Ter Braak and Smilauer, 1998).

RESULTS

> ENVIRONMENTAL FACTORS

The PCA biplot of the study sites and the different variables indicated the first two axes accounting for 45.6% of the variance (Figure 2). The first axis (PC 1, 30.9%) arranged sites along spatial gradients (elevation and distance from source), while the second axis (PC 2, 14.7%) explained variables associated to substrate coarseness flow conditions and water temperature. Since PCA distinguished the various river segments based mainly on altitude and distance from source, they were given conventional names related to their geographic distribution in the basin, namely Upland (UP), Middle (MD) and Lowland (LW, Figure 2). Although the vast majority of the sites were largely classified as acceptable within the UP, MD

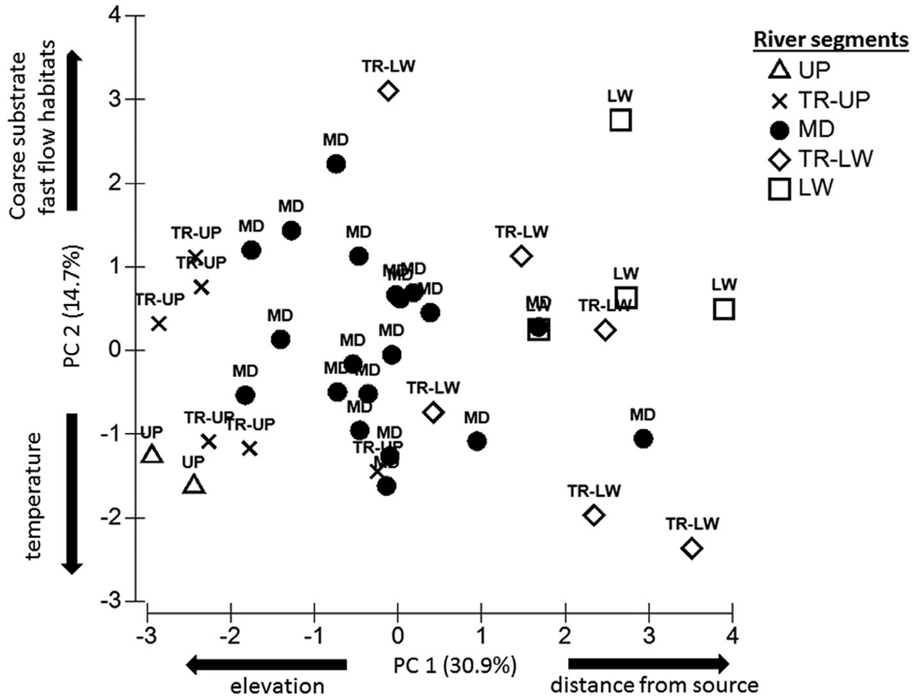

Figure 2
PCA biplot of the Evrotas River sampling sites indicating vectors of the variables considered with high loadings (>0.4), and ordination of study sites.

and LW river segments, some sites falling in between these areas were characterized as transitional, *i.e.* Upper Transitional (TR-UP) and Lower Transitional (TR-LW), and were reassigned through expert judgement (Figure 2). For the analyses, the TR-UP was reassigned to the UP segment and the TR-LW to the LW segment.

The upland river segment (UP) comprises a rather restricted section of the river that runs a narrow channel (mean wetted width of 6.13 m, Table II), through a hilly valley characterized by the presence of karstic springs along the main channel. Water is well oxygenated (mean DO 9 mg·L^{-1}, Table II) and generally cool with an average summer temperature of 20.7 °C, while mean riparian canopy cover is over 36.25% (UP, shadedness, Table II).

The middle section (MD) constitutes the largest and morphologically most heterogeneous segment of the Evrotas River. It includes wide braided channels with alluvial gravel and sand beds, remnants of floodplains (mean wetted width of 10.3 m, MD in Table II). Water temperature values (mean of 20.5 °C) are similar to the UP segment, as also oxygen values (mean DO of 8.36 mg·L^{-1} Table II). Riparian canopy cover does not exceed 30% (MD, shadedness, Table II).

The LW segment is relatively short and extends only within the main channel of the Evrotas Delta. Water is warmer than in the other two segments (mean temperature value of 25.4 °C, Table II) with sparser riparian canopy cover (LW, shadedness, Table II). Generally, from upland to lowland, sites become on average wider and deeper, with sparser canopy cover and finer substrate.

> *ICHTHYOFAUNAL ASSEMBLAGES*

A total of 14269 individuals belonging to five native species were collected during this survey (2007–2009). Species richness per site ranged from two to five species (mean 3.5, SD 0.7). The general trend was an increase of fish density and species richness from upstream to downstream (Figure 3, with the three segments delineated). More specifically the density

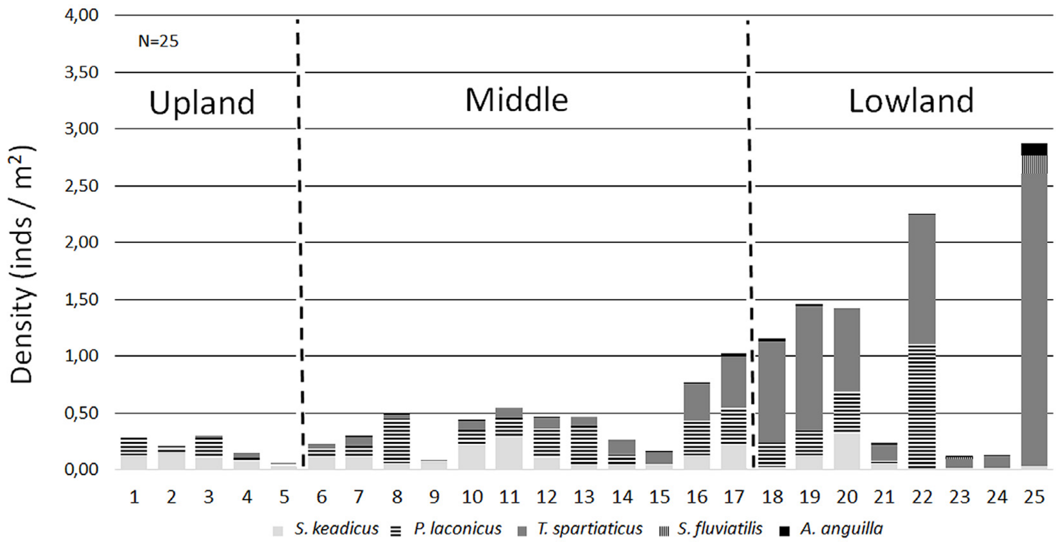

Figure 3
Longitudinal gradient of fish densities (inds·m^{-2}) and species richness during the entire study period (average values).

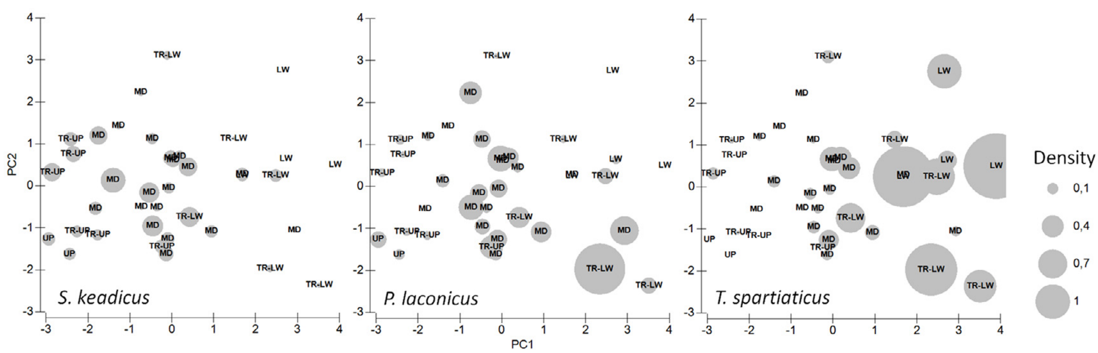

Figure 4
PCA plots with (superimposed) fish densities for each of the three native species in the UP, TR-UP, MD, TR-LW, and LW river segments of the Evrotas River.

of chub *S. keadicus* was higher at the upper (UP) and middle sections (MD) of the basin, decreased at the lower section (LW) and the species was almost absent near the river mouth (Figures 3 and 4a). The minnowroach *T. spartiaticus*, in contrast, was absent at the upper part (UP) of the river, present in the middle section (MD), with the species finally being dominant near the river mouth (Figures 3 and 4c). The minnow *P. laconicus* displayed an irregular pattern with just a slight increase in its density values from upstream to downstream (Figures 3 and 4b). Blennies were caught mainly in the lower reaches of the river (Figure 3), while the European Eel had a sporadic distribution being more abundant near the river mouth with individuals however caught also near the river springs (in TR-UP sites) confirming that no barriers affected the species' distributional range (Figure 3). Frequency of occurrence data revealed a similar longitudinal distribution pattern with that of density data variation, characterized by a decreasing percentage contribution of chub and an increasing percentage contribution of the minnowroach from the headwaters to the river mouth (Figure 5). MDS ordination plot provided evidence for three tentatively delineated river segments, characterized by distinct fish communities related to a longitudinal gradient (Figure 6). The consistent distinction among fish assemblages was confirmed by means of ANOSIM analyses (UP *vs.* MD, $R = 0.344$, $p < 0.05$; UP *vs.* LW, $R = 0.737$, $p < 0.01$; MD *vs.* LW, $R = 0.536$, $p < 0.01$). According to SIMPER analysis (Table III) within the UP and MD, species percentage contribution

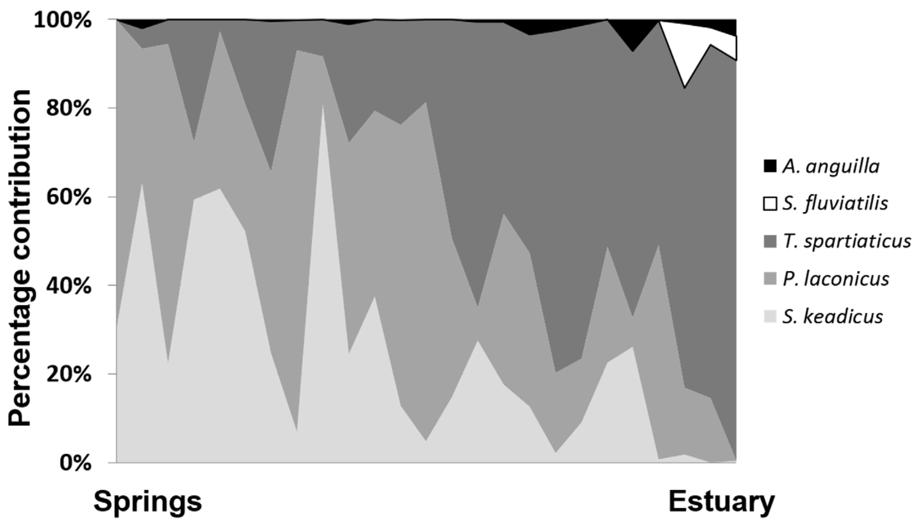

Figure 5
Species percentage contribution along a longitudinal gradient in the Evrotas River.

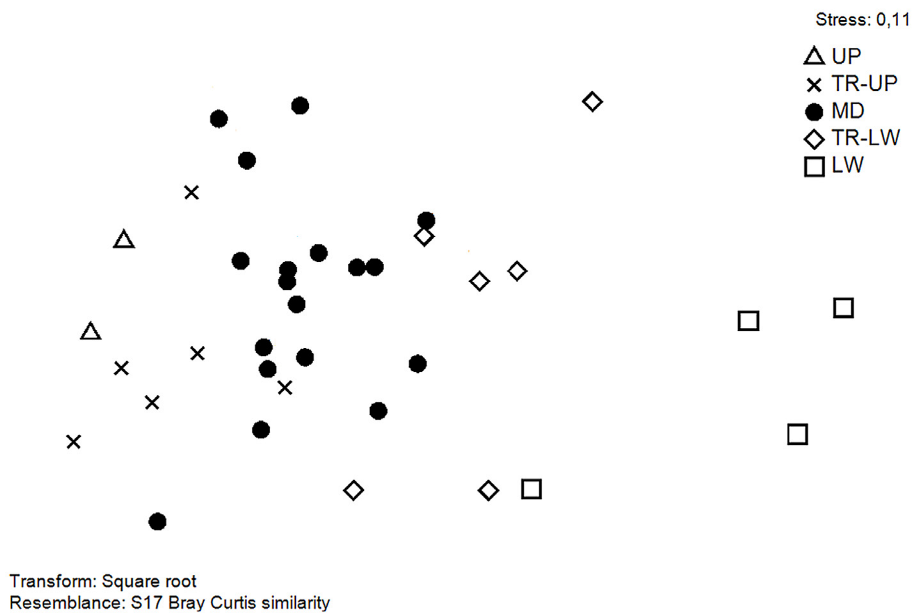

Figure 6
MDS ordination of sampling sites using square-root transformed fish density data (inds·m^{-2}).

responsible for uniformity was shared between the chub and the minnow, while in the LW, the minnowroach was the dominant species (69.6%), being responsible for the homogeneity within this segment. The highest average dissimilarity (88.7%) was observed between the UP and LW, indicating that in these areas species composition is the most distinct.

> RELATIONSHIP BETWEEN FISH SPECIES AND ENVIRONMENTAL VARIABLES

Inflation factor values revealed that fine substrate material and run flow type were correlated with coarse substrate material and pool flow type respectively and therefore the former were excluded from the RDA. According to the Monte Carlo Permutation test, elevation, pools and pH had statistically important correlation ($p < 0.05$) with fish densities data (Table IV). Together, these three variables explained 0.65 of the species composition variance, while all environmental variables explained 0.75.

Table III
Simper analysis results with species contribution to similarity within and dissimilarity between the Evrotas river segments.

UP (*Ave. similarity: 86.60*)		MD (*Ave. similarity: 68.80*)		textbfLW (*Ave. similarity: 61.33*)	
Species	%	Species	%	Species	%
S. keadicus	54.50	*P. laconicus*	36.00	*T. spartiaticus*	69.60
P. laconicus	45.50	*S. keadicus*	33.40	*S. fluviatilis*	17.02
		T. spartiaticus	28.90	*A. anguilla*	13.38
UP & MD (*Ave. dissimilarity = 36.84*)		**UP & LW (*Ave.dissimilarity = 88.75*)**		**MD & LW (*Average dissimilarity = 65.31*)**	
Species	%	Species	%	Species	%
T. spartiaticus	51.50	*T. spartiaticus*	50.80	*T. spartiaticus*	42.06
P. laconicus	25.20	*S. keadicus*	13.40	*P. laconicus*	16.98
S. keadicus	17.30	*S. fluviatilis*	12.80	*S. keadicus*	15.9
		P. laconicus	12.80	*S. fluviatilis*	14.89
		A. anguilla	10.20	*A. anguilla*	10.16

Table IV
Species composition variance explained by environmental variables used in RDA analysis and Monte Carlo test results.

Variable	Variance explained	*p*-Value
Elevation	0.48	0.002
Pool	0.58	0.004
pH	0.65	0.004
D.O.	0.66	0.216
Riffle	0.68	0.132
Glide	0.70	0.152
Distance from source	0.71	0.236
Mean Depth	0.72	0.328
Conductivity	0.73	0.448
Water Temperature	0.74	0.438
Wetted weight	0.74	0.658
Shadedness	0.75	0.762
Course substrate	0.75	0.812

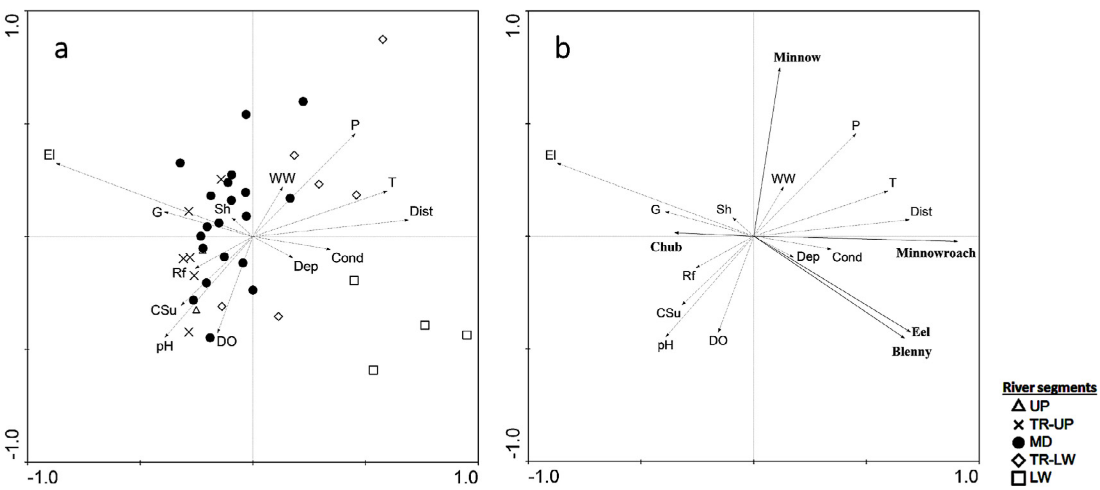

Figure 7
RDA biplot, (a) sites and environmental and habitat variables; (b) environmental and habitat variables and fish species. El: Elevation, Dist: Distance from source, Cond: Conductivity, DO: Dissolved Oxygen, pH: pH, T: Water Temperature, WW: Wetted width, Dep: Mean Depth, Sh: Shadedness, CSu: Coarse substrate, Rf: Riffle, G: Glide, P: Pool.

All environmental variables recorded/calculated – apart from those excluded by high inflation factor values – were used in RDA in order to visualize their contribution to the final result. The RDA produced a biplot (Figures 7a, 7b) in which the first two axes account for 97.3% of the variance (79.8% on the first axis). For the first axis, elevation and distance from source had the higher values in the correlation matrix, while the second was mostly associated with slower flowing deeper habitats (Pools) and pH. Generally, upstream sites were plotted on the left side of the diagram due to higher elevation, which is also associated to shallower habitats (*i.e.* Glides, Riffles), coarser substrate and higher percentage of shadedness. All lowland sites, in contrast, were plotted on the right side of the diagram due to higher water temperature, distance from source, slower flowing habitats (Pools), higher conductivity values, as well as having larger wetted width and higher mean depth. According to the RDA species-environmental variables diagram, the minnowroach seems to have a positive association with distance from source, temperature and conductivity, and to a lesser degree with pool habitats; the chub a positive association with elevation and shallower habitats, while the minnow a positive association with pool habitats (Figure 7b). Eels and blennies were positively associated with conductivity and increased in numbers in lowland sites.

DISCUSSION

> FISH COMMUNITY LONGITUDINAL GRADIENT

Overall, the findings of the current study agree with the general pattern for fish communities in lotic ecosystems studied mostly in Central and Western Europe, *i.e.*, that species richness and abundance gradually increase from upstream to downstream (Bayley and Li, 1994; Cowx and Welcomme, 1998); species at Evrotas River, a Mediterranean intermittent river, do also assort along environmental gradients and there is a longitudinal distribution pattern, with species richness and density increasing downstream. In this, the current study conforms also with some studies conducted in other Mediterranean streams, mostly in Iberia (see Vila-Gispert *et al.*, 2002; Clavero *et al.,* 2005). Clavero *et al.* (2005) examining fish distribution in 14 coastal stream stretches of Southern Spain indicated that the main sources of variation in fish community composition and habitat characteristics in the different stretches were related to a clear upstream-downstream gradient, along which total species richness increased. Mediterranean streams, however, in contrast to streams in Central and Western Europe that are more homogenous and gradually changing, are highly fluctuating environments with high habitat heterogeneity in ecological factors that may influence fish distributions and species compositions (Vila-Gispert *et al*., 2002). In addition, most large- and medium-sized watercourses in the Mediterreanean countries have regulated flows and are fragmented by dams (for Iberia see Prenda *et al.*, 2002; for Greece see Skoulikidis *et al.*, 2009). Furthermore, intermittent flow during the summer periods in Mediterranean streams creates even more fragmented habitats, often isolated by physical and ecological gradients (Balon, 1981) that obviously pose a challenge to the traditional river zonation concept. The study of Carmona *et al*. (1999) on the distribution patterns of indigenous freshwater fishes in the Tagus River basin (Spain) is an example of a Mediterranean river that does not conform to the typical river zonation pattern, *i.e.* on the one hand, the headwater stretches of the Tagus river are less rich in fish species than the lower course stretches, but it is characterized by a lack of graduality. Similarly, Franchi *et al.* (2014) studying the fish community of the upper Tiber River (Italy) showed that the construction of the Montedoglio Dam, had interrupted the typical longitudinal zonation of the species in the river. In the case of the Evrotas River, with the retention of its natural longitudinal connectivity (absence of dams) this could be of critical importance, as it allows species to recolonize drought-affected areas after flow resumption during the winter and spring period, in contrast to most other Mediterranean streams and rivers. Since natural refuges (spring-fed sections) exist within the main channel of the Evrotas River, the absence of anthropogenic barriers allows fish to disperse and produce more or less natural fish community dynamics.

Undoubtedly, an important methodological issue and major requirement in order to identify distinct ichthyological assemblages and to interpret changes along a longitudinal gradient is to utilize a sufficient number of samples from undisturbed or relatively undisturbed conditions and this is particularly difficult to find in medium-small Mediterranean streams. During our study, water flow was exceptionally low during the years 2007 and 2008, with some segments in the main river course being already dry or showing signs of desiccation. Although droughts are frequent natural disturbances in the Evrotas River, the surveys of 2007 were performed in the most severe drought year recorded since 1992 (Economou *et al.*, 1999), when almost 80% of the river's main channel dried out (Skoulikidis *et al.*, 2011). The harsh hydrological conditions resulted in a temporary alteration of fish community structure. Obviously, species density and their relative contribution to the assemblages have been influenced by anthropogenic degradation, mainly due to severe water abstraction. Despite these complexities in fish community dynamics, however, a general pattern of succession assemblage types is derived, with three river segments characterized by high degree of homogeneity being delineated.

The RDA analysis revealed that environmental variables influence substantially fish assemblage structuring in the Evrotas River. Variables such as water depth, substrate size, canopy cover, available habitats, water temperature, dissolved oxygen and conductivity can be considered fundamental in the maintenance of fish assemblage structure. The lotic longitudinal gradient of fish species composition, richness and density has indeed been linked to gradual variation in water depth, current velocity, substratum and habitat complexity (Gorman and Karr, 1978; Pires *et al.*, 1999; Filipe *et al*., 2002; Magalhes *et al.*, 2002). Carmona *et al.* (1999) showed, for example, that indigenous fishes were distributed through the Tagus river basin (Spain) forming geographical communities (chorotypes), some of which can be associated with environmental factors like river morphology, water quality or geographical location.

> *FISH-HABITAT RELATIONSHIPS AND IMPLICATIONS FOR CONSERVATION*

According to our data, *S. keadicus* was mostly associated to shallower habitats, an aquatic habitat type which is being severely impacted by water abstraction in the Evrotas River. In contrast, *T. spartiaticus* and to a lesser degree *P. laconicus* are more stagnophilic species (Kottelat and Barbieri, 2004) that show preference for slow flowing, vegetated habitats (Vardakas *et al.*, unpublished data). Both species are assumed to be strongly phytophilic and depend on vegetation for reproduction, foraging and protection from natural enemies. However, recurrent droughts may affect helophytes that these species may need in order to reproduce. In addition, the blenny that was once widely distributed in the middle and lower section of the Evrotas River (Economou *et al.*, 1999), is now restricted to the lower section of the river. However, individuals from this species were caught in the TR-LW sub-segment. Larvae of the species are planktonic until about 15 mm, drifting to and remaining in pools (Kottelat and Freyhof, 2007). We thus speculate that human interventions, particularly hydromorphological alterations and widespread desiccation, that have caused the destruction of the specialized habitats needed for the survival of larvae, caused the disappearance of this species from the middle part of the basin (see also Vinyoles and de Sostoa, 2007).

Using fish assemblages to describe stream ecosystem properties is variously applied in river classifications worldwide; it is a classical descriptive approach with important policy-relevant applications (Angermeier and Winston, 1999; Aarts and Nienhuis, 2003). The ensuing ichthyofaunal assemblage units or fish assemblage types are usually mapped and help define a river's biotic typology. Based on the current study, the fish assemblage pattern at Evrotas River is contingent with the concept of fish community variation along a longitudinal axis. Evrotas provides a rare opportunity as a case study river, since there is an absence of barriers to dispersal along its entire main channel. It is evident that future work should focus on efforts to better interpret the designated fish assemblage units in order to validate the ichthyofaunal assemblage types described during this study. Furthermore, the Evrotas River includes one critically endangered, one endangered and one vulnerable fish species; in addition, European eel occurs at higher altitudes than many other Greek systems, owing to the widespread fragmentation of rivers elsewhere by damming. Therefore, in the context of future environmental changes, such as climate change and the expected severity of its effects, the present study outlines baselines that should be carefully monitored in the coming years.

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