Received: 16 April 2016 | Revised: 6 September 2016 | Accepted: 9 October 2016
DOI 10.1002/aqc.2735

WILEY

RESEARCH ARTICLE

Defining critical habitat conditions for the conservation of three endemic and endangered cyprinids in a Mediterranean intermittent river before the onset of drought

Leonidas Vardakas[1,2] | Eleni Kalogianni[1] | Christina Papadaki[1] | Theocharis Vavalidis[1] | Angeliki Mentzafou[1] | Drosos Koutsoubas[2] | Nikolaos Skoulikidis Th.[1]

[1] Hellenic Centre for Marine Research, Institute of Marine Biological Resources & Inland Waters, Attica, Greece

[2] University of the Aegean, Department of Marine Sciences, Mytilene, Greece

Correspondence
Leonidas Vardakas, Hellenic Centre for Marine Research, Institute of Marine Biological Resources & Inland Waters, 46.7km Athens-Souniou Av. P.O. 19013 Anavissos, Attica, Greece.
Email: louisvard@gmail.com

Abstract

1. Identifying key factors in species' habitat requirements can be of use in defining critical habitats for their conservation, as well as in assisting the prioritization of habitat restoration actions. So far, most studies on habitat use by freshwater fishes have been focused on widespread and economically important species (e.g. salmonids).
2. This study aimed to identify the early summer habitat use (i.e. before the start of the drought period) of three endemic and endangered Greek cyprinids – the Evrotas chub *Squalius keadicus*, the Spartian minnowroach *Tropidophoxinellus spartiaticus* and the Evrotas minnow *Pelasgus laconicus*, with regard to depth, water velocity, substrate and macrophyte cover. In the case of the chub, habitat use by juvenile and adult fish was assessed separately. Data were collected for each fish group from four habitat types (riffles, runs, glides, pools) by using a modified point-abundance sampling with an electrofishing device. In total, 120 sampling points were sampled, in two near-reference perennial reaches of the Evrotas River (southern Greece) in early summer 2014, when there was continuous flow and full connectivity between habitats.
3. All three target species had their highest densities in deeper habitats with low water velocities and depositional substrates such as pools and runs. A high overlap in habitat use was evident for the three species. Habitat use curves based on microhabitat data were created for all species. Minnowroaches, minnows and large chubs actively selected deep habitats. Minnowroaches and minnows favoured slow-flowing, vegetated habitats with fine substrate located close to the river bank, while chubs had no clear affinity for particular velocities or substrate types. However, size class comparisons in chub indicated differences in both water depth and velocity.
4. Overall, the results of this study provide the first detailed report of the habitat use of these endangered fish species. These patterns of habitat use highlight the importance of deep habitats that must be preserved as refugia while the drought events progress.

KEYWORDS

cyprinids, habitat use curves; intermittent stream, microhabitat, threatened species

1 | INTRODUCTION

Intermittent rivers comprise a large proportion of the world's inland waters with research on these aquatic systems driven at present by both observed and projected shifts in flow regimes from perennial to intermittent. These shifts are associated with climate change, as well as land and water use changes, superimposed on natural intermittency (for a review, see Leigh et al., 2015). Hydrological variations are natural and frequent events in intermittent rivers, with aquatic organisms adapted to a fluctuating environment (Gasith & Resh, 1999; Hermoso

& Clavero, 2011; Maceda-Veiga, 2012). The worst effects of drought, however, are expected in intermittent rivers where drying is caused by a combination of natural causes and overexploitation of water resources (Skoulikidis et al., 2011), and thus aquatic species do not have sufficient time to develop drought resistant structures or physiological adjustments (Stanley, Fisher, & Grimm, 1997). Most studies agree that prolonged and consecutive droughts, expected under altered future climates when combined with human pressures, will lower the capacity of most fauna to persist in intermittent rivers and may result in declines or local extinctions of the most sensitive species and their potential replacement by more resistant species (Leigh et al., 2015; Magalhães, Beja, Schlosser, & Collares-Pereira, 2007; Matthews & Marsh-Matthews, 2003). This is especially crucial in the case of range-restricted endemic species where the consequences may be irreversible, as river intermittency has been linked both to population bottlenecks and population extinctions (Huey, Schmidt, Balcombe, Marshall, & Hughes, 2011; Meffe & Vrijenhoek, 1988; Sousa, Penha, Pala, Chikhi, & Coelho, 2010).

Fish populations inhabiting Mediterranean intermittent streams and rivers are very susceptible to such processes, owing to the high fluctuations in discharge, both natural and anthropogenic, of these aquatic systems (Gasith & Resh, 1999; Hermoso & Clavero, 2011; Maceda-Veiga, 2012). From a conservation viewpoint, a critical step towards maintaining populations of threatened native fish species in Mediterranean intermittent rivers is to assess their habitat ranges and explore their habitat preferences. Such studies can be instrumental in setting priorities for specific restoration measures for critical habitats of Mediterranean stream fishes, as well as in developing management tools for environmental flow assessments (Martelo, Grossman, Porto, & Magalhães, 2014; Martínez-Capel, García, Werenitzky, Baeza, & Rodilla, 2009; Santos & Ferreira, 2008). Furthermore, they can contribute to the development of indices for assessing ecological status, according to the provisions of the European Water Framework Directive (Leigh et al., 2015; Morais, Pinto, Guiherme, Rosado, & Antunes, 2004). Such knowledge is also important when considering the increase of drought events in the European Mediterranean area during recent decades, as well as the expected increase in their intensity and frequency in the near future (Estrela, Peñarrocha, & Millán, 2000; Livada & Asimakopoulos, 2005; Parry, Canziani, Palutikof, van der Linden, & Hanson, 2007; Skoulikidis et al., 2017).

At present, there is limited information on fish habitat use in rivers and streams with high fluctuation in hydrology, such as Mediterranean streams, derived mainly from studies conducted in the western Mediterranean, mostly in the Iberian Peninsula (Grossman & de Sostoa, 1994; Rincón, Barrachina, & Bernat, 1992; Santos & Ferreira, 2008; Santos, Godinho, & Ferreira, 2004). In contrast, fewer studies are available in the eastern Mediterranean area and the Balkan Peninsula in particular (see Muñoz-Mas et al., 2016 and Papadaki et al., 2016 focusing on the estimation of ecological flows based on trout habitat use), despite its high degree of fish endemism and river intermittency, that render its fish populations very vulnerable to human pressures (Barbieri et al., 2015; Economou et al., 2007). Intermittent rivers and streams dominate surface runoff in the southern Balkans, as in other areas of Mediterranean Europe, with semi-arid climatic conditions (Estrela, Marcuello, & Iglesias, 1996; Skoulikidis et al., 2017; Tockner et al., 2009). As a result, most southern Balkan rivers are intermittent, owing to natural seasonal variability as well as overexploitation of water resources (Skoulikidis et al., 2011; Tzoraki, Nikolaidis, Amaxidis, & Skoulikidis, 2007).

Most studies on fish–habitat relationships in Europe and North America have focused on salmonids, owing to their economic importance and ubiquity. This study focuses on three endemic cyprinids of special conservation concern, listed in both the IUCN Red List (IUCN, 2014) and the Greek Red List (Economidis, 2009), of the Evrotas River in Southern Peloponnese (Greece). These species are the Evrotas chub *Squalius keadicus* (Stephanidis 1971), (EN) endangered, the Spartian minnowroach *Tropidophoxinellus spartiaticus* (Schmidt-Ries 1943), (V) vulnerable, and the Evrotas minnow *Pelasgus laconicus* (Kottelat & Barbieri, 2004), (CR) critically endangered.

Owing to the complex geological and climatic history of the Evrotas area, combined with geographical isolation, these three species are range-restricted; the Evrotas chub is confined exclusively to the Evrotas River and the Vassilopotamos River, a small river once connected to Evrotas River near its outflow to the sea. This is a relic cyprinid species, remarkably interesting for its evolutionary history, since it holds one of the two basal branches of leuciscins in the phylogenetic tree, dating back to the late Miocene or possibly even earlier (Tsigenopoulos & Karakousis, 1996; Zardoya & Doadrio, 1999). Similarly to the chub, the Spartian minnowroach and the Evrotas minnow are also confined to a small number of southern Peloponnese rivers (Economou et al., 2007). Elucidating the habitat preferences of these threatened species is a prerequisite for any informed effort for their conservation and could contribute also to the conservation of other related and threatened cyprinid species of non-perennial rivers and streams of the wider Mediterranean Basin, one of the world's biodiversity hotspots (Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000).

The aim of the present study was to examine the early summer habitat use, i.e. before the period of drought that evidently alters the habitat use of lotic species, as only stagnant pools are present, of three cyprinid species in terms of water velocity, depth, substrate and macrophyte cover in two near-reference, perennial reaches. Interspecies and ontogenetic differences in habitat use were explored also in order to obtain species-specific habitat use curves based on microhabitat data, for assessing habitat selection by these three species.

2 | MATERIALS AND METHODS

2.1 | Study area

The hydrology, topography and ecosystem attributes of the Evrotas River have been described in detail in Skoulikidis et al. (2011). Briefly, the Evrotas (length 91 km) drains a medium-sized (2418 km^2), mid-altitude (150–600 m) Mediterranean basin, with numerous intermittent streams, discharging into the main course. The mountainous area of the basin is formed by Mesozoic-Palaeogene limestones and impermeable rocks, such as flysch and schists, while the lower parts of the valley are filled with Pliocene and Quaternary sediments. The climate is typically Mediterranean, with mild and cool winters and prolonged hot and dry summers, a mean annual temperature of 16°C and a mean annual precipitation of 803 mm (2000–2008, Nikolaidis, Skoulikidis, Kalogerakis, & Tsakiris, 2009), but with rainfall varying markedly between years. Most rainfall occurs during the months of October to March. Besides surface runoff resulting from rainfall and snowmelt, the river system is also fed by numerous karstic and alluvial springs. Highest flows normally occur between February and April, with lower but annually variable flows occurring between June and September. The dry period varies markedly in intensity from year to year, as in other Mediterranean rivers (Gasith & Resh, 1999), but normally in late summer–early autumn there is partial desiccation of the river, with approximately 20% of the Evrotas main channel, as well as most of its tributaries, drying out.

The current study was conducted in June 2014 when there was continuous flow and full connectivity between habitats (riffles, runs, glides, pools) that permitted free movement of the fish between habitats. The two study reaches (USK and VIV, Figure 1) are located at the upper and middle part of the Evrotas main channel (elevation 357 m asl and 280 m asl, respectively), with an approximate length of 300 m each. The USK reach is part of the uppermost 4 km perennial section of the Evrotas main channel, with low agricultural activities, sparse human settlement and almost pristine riparian forest. The VIV reach is part of a 12 km perennial section of the river, 20 km downstream from USK (Figure 1). Both reaches, have never been dried or reduced in pools for over a decade (pers. observation). However, between these two perennial sections, there is an intermittent segment of the river which dries almost every year during the summer period. Both river sections are also fed by several karstic springs and constitute two near-reference reaches of the river with minimal human disturbance (Skoulikidis et al., 2011). They were selected on the basis of accessibility, microhabitat variability and representativity of different habitat types (riffles, runs, glides and pools), as well as their reference status, since most of the other sections of the Evrotas River are hydromorphologically affected (Skoulikidis et al., 2011). These habitats, inferred by visual observation of surface flow character and verified by hydraulic measurements and quantitative substrate types (Aadland, 1993; Gosselin, Petts, & Maddock, 2010), included riffles (shallow/fast flowing with coarse substrate), runs (deep/moderately fast), glides (shallow/slower moving) and pools (deep/low flow or no flow with fine substrate).

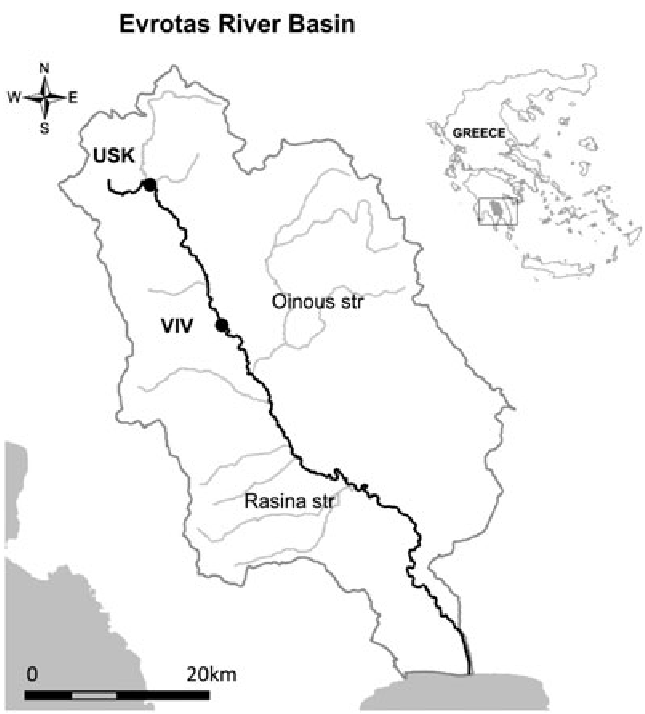

FIGURE 1 Map of the Evrotas River basin, with the Evrotas and its main tributaries; the location of the two reaches surveyed for fish microhabitat use is marked.

2.2 | Fish and microhabitat data

2.2.1 | Fish sampling

Fish data were collected using a modified point-abundance sampling by electrofishing (PASE, Copp, 1989; Santos & Ferreira, 2008) conducted during daylight hours, with an EFKO electrofishing DC unit (Honda 7 kVA generator, 150 m cable, 1.5 m anode pole, 6 A DC output, voltage range 300–600 V). Four operators participated in each survey, with one operator handling the anode, one operating the 'dead man' switch and acting as data recorder and two operators carrying out fish capture. Starting at the downstream edge of each reach, sampling proceeded upstream in a zigzag manner to sample all types of habitats; at each reach, 60 point samples were randomly collected at equidistant locations (*c*. 5 m) along cross-sectional transects, spaced approximately 10 m apart, along the reach (Santos & Ferreira, 2008). To minimize fish fright bias during sampling and avoid displacement of individuals from their original position, the team moved discreetly towards each sampling point; then, from a distance of 3–4 m, the anode was thrown in the air and upon landing in the water it was activated for approximately 20 s, depending on habitat size, and then retrieved using the power cord. At the same time, the team moved towards the sampling point, collecting the stunned fish. Captured fish were identified to species level, counted, their size class recorded at 5 cm intervals, and then they were returned alive to the river. There was a minimum interval of 5 min between consecutive point samplings to allow fish to relocate, if affected by the electricity. In the few cases that fish were attracted from a nearby habitat with different characteristics, these results were discarded.

Since size-related variation in microhabitat use is common in stream fishes (Grossman & de Sostoa, 1994; Martelo et al., 2014; Santos & Ferreira, 2008), separate analyses were conducted for small chub (<10 cm total length) and large chub (> 10 cm total length), roughly corresponding to juveniles and adults respectively. For the much smaller minnowroaches (max. Size 12 cm TL) and minnows (max size 7 cm TL), no separate size analysis was conducted. Frequencies of occurrence (FO, %) and densities (individuals per m^2) were also calculated for each fish group (small chub, large chub, minnowroach and minnow) at each microhabitat point. Furthermore, for each habitat type (riffles, runs, glides and pools) percentage fish abundance per habitat type was also calculated, i.e. the percentage of each fish group that occupied a given habitat type and percentage habitat use, i.e. the percentage of each habitat type in which the presence of a given fish group was recorded.

2.2.2 | Microhabitat measurements

After fish sampling at each discrete sampling point, the four microhabitat variables most commonly measured for stream fish were recorded: water velocity, water depth, substrate type and instream macrophyte cover (Carter, Copp, & Szomlai, 2004; Simonson, 1993). Water velocity (V, m s^{-1}) and depth (D, cm) were measured using an OTT C20 flow meter. Substrate coarseness was defined at each sampling point by using a modified Wentworth scale (Cummins, 1962); coarse substrate was defined as substrate >63 mm (including cobbles and boulders), while fine substrate was defined as substrate <63 mm (pebbles, gravel, sand, etc.). Visual estimates of macrophyte cover were expressed as the percentage cover of the sampling point with aquatic vegetation (moss and submerged helophytes). The presence of thick root mats and leaf cover were also recorded at each sampling point; however, this was omitted from any analysis since these features were sparse in both reaches. In order to estimate fish densities at each point, the fished area of each sampling point (m^2) was calculated by multiplying the length of the river stretch in which the anode remained energized by the diameter of the field shock; this has been empirically found at previous trials in this river to be approximately 2 m (including the 30 cm anode diameter), by energizing the specific electrofishing unit and recording the distance to which fish were stunned. Distance from nearest bank was also measured. In addition, at each sampling point measurements were made of dissolved oxygen (mg L^{-1}), water temperature (°C), conductivity (μS cm^{-1}), and pH, using a portable multiparameter Aquaprobe AP-200 with a GPS Aquameter (Aquaread).

Microhabitat availability measurements were made using depth and velocity variables by quantifying five points along seven equidistant transects, perpendicular to the flow at each sampling reach.

2.3 | Analysis of microhabitat use

Spatial overlap in microhabitat use by the four fish groups (small chub, large chub, minnowroach and minnow) using pair-wise comparisons, was assessed by Schoener's Index (C) (Schoener, 1970):

$$C = 1 - \frac{1}{2}\sum_{n=1}^{i} |Pxi - Pyi|$$

where *Px* and *Py* are the proportions of fish groups *x* and *y* using habitat *i*, i.e. riffle, run, glide or pool. *C* ranges from 0 (no habitat overlap) to 1 (full habitat overlap). Overlap is generally considered significant when *C* exceeds or equals 0.60 (Santos & Ferreira, 2008).

Spearman's rho was applied to test for correlations between fish densities and habitat parameters. To further elucidate the explained variance of fish densities by environmental habitat variables (velocity, depth, macrophyte cover and coarse substrate), a Redundancy Analysis (RDA) was conducted, following a Detrended Correspondence Analysis (DCA) that indicated that the RDA ordination method best responded to the fish data. Results of DCA showed a linear response, since the lengths of the gradient for the first axis were estimated at 2.93 < 3 (TerBraak & Smilauer, 1998). Before the RDA analysis, a Monte Carlo test was performed, with 499 permutations, in order to test the independence of fish densities from the habitat variables. Fish densities and habitat data were log (x + 1) transformed before all multivariate analyses, except for those variables (substrate coarseness and macrophyte cover) that were recorded as percentages, which were arcsine transformed. In the ordination diagram, vector position indicates the direction in which the variable increases, pointing concurrently to possible correlations of species and environmental variables. An almost right angle between two vectors indicates low correlation between them, while an acute angle indicates positive correlation and obtuse negative. The length of the vector expresses their contribution to the ordination diagram, with higher values representing greater strength of the variable in the analysis. Canoco 4.5 software was used for all the above analyses, and CanocoDraw for Windows for the projection of the ordination diagram (TerBraak & Smilauer, 1998).

2.4 | Microhabitat use curves

Point measurements of depth and velocity at each point with fish presence were used to estimate habitat use curves. Habitat use curves were generated following a standard approach (Bovee, 1986) and with a data-driven procedure, i.e. with minimum intervention of expert knowledge, based only on the collected data. In order to downweight the influence of the extreme values, number of fish per sampling point (N) was log (N + 1) transformed, as in similar studies (Brosse & Lek, 2000; Fukuda, Mouton, & De Baets, 2011). Once the histograms for the continuous variables were developed (depth and velocity), a smooth curve was adjusted to encompass them. The entire procedure was carried out in R (R Development Core Team, 2012); more specifically, the selected function was the *smoothspline*. This function adjusts smooth curves to the input data with 3rd-order polynomials, each one of them allowing for a turn within the adjusted curve. In addition, in order to be coherent with the ecological gradient theory (Austin, 2007), the number of splines was properly adjusted for every developed curve.

3 | RESULTS

3.1 | Habitat composition and fish abundances

Both reaches had similar depth ranges (0.1–1.1 m) and mostly coarse substrate (>63 mm), with sparse macrophyte cover. Mean wetted width varied from 6 m to 9.5 m and water velocity from 0.31 m s^{-1} to 0.55 m s^{-1} respectively. Maximum recorded water velocities were 0.98 m s^{-1} in USK and 1.3 m s^{-1} in VIV. Table 1 summarizes the variation in habitat and physicochemical parameters among the four habitat types sampled, indicating differences in depth, velocity, substrate coarseness and macrophyte cover among riffles, runs, glides and pools. There were no differences in the physicochemical parameters measured among the four habitat types (Table 1).

During the current survey, 20 of the 120 microhabitat sampling points were fishless (16.7%). At the remaining 100 points, 475 fish of the three target species were caught: 169 small chub, 187 large chub,

TABLE 1 Variation (means ± S.D.) in habitat and physicochemical parameters among the four habitat types in the Evrotas study area. Number of sampled microhabitats of each type in parenthesis

	Habitats			
	Riffles (N = 25)	Runs (N = 36)	Glides (N = 36)	Pools (N = 23)
Fished area (m^2)	6.09 ± 2.5	8.2 ± 2.5	7.07 ± 2.4	5.7 ± 2.5
Depth (m)	0.3 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.7 ± 0.2
Maximum depth (m)	0.4	1.10	0.55	1.10
Water velocity (m s^{-1})	0.7 ± 0.3	0.5 ± 0.2	0.4 ± 0.2	0.1 ± 0.2
Maximum velocity (m s^{-1})	1.30	1.10	0.73	0.78*
Coarse substrate (> 63 mm, %)	100.0 ± 0.0	73.3 ± 29.8	40.3 ± 28.7	40.1 ± 30.0
Macrophyte cover (%)	0.0 ± 0.0	6.1 ± 12.6	8.4 ± 17.5	9.3 ± 22.6
D.O (mg L^{-1})	9.3 ± 0.1	9.3 ± 0.1	9.3 ± 0.1	9.3 ± 0.1
Temperature(°C)	19.7 ± 0.7	19.7 ± 0.8	19.6 ± 0.8	19.9 ± 0.7
Conductivity (μS cm^{-1})	431.1 ± 22.8	427.5 ± 33.7	423.0 ± 42.6	420.8 ± 32.5
pH	7.9 ± 0.0	7.9 ± 0.0	7.9 ± 0.0	7.9 ± 0.0

*A few pool habitats presented higher flows and could be classified as pool-runs

76 minnowroaches and 43 minnows. Six European eels *Anguilla anguilla* (L.,1758) and two rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) were also caught in VIV, but were excluded from any analysis. Chubs were dominated by those 5–10 cm long (43.8%) and 10–15 cm individuals (47.4%), with very few individuals below 5 cm or exceeding 15 cm (max TL caught 17 cm); minnowroaches were mostly 6–10 cm individuals (82.9%, max TL caught 10 cm), while minnows rarely exceeded 6 cm (max caught 7 cm).

3.2 | Interspecies and ontogenetic variation in microhabitat use

Microhabitat use overlap between the four fish groups was high, with all pair-wise comparisons of fish groups' microhabitat use presenting significant values (i.e. Schoener's index >0.60), as all values ranged from 0.8 to 0.9.

Chubs were present in 77% of the microhabitats sampled (small chub in 61%, large chub in 53%), followed by the minnowroach in 33% of the microhabitats, while minnows were much less common (in 18% only of the microhabitats sampled). Most small chubs (40%) were recorded in runs (Table 2), 24% in pools, 23% in glides and only 13% in riffles, although riffle use by this group was 56%, i.e. small chub were present at 56% of all riffles sampled (Table 2). Runs were also the habitat type that small chub most frequently occupied (small chub were present in 69% of the total runs, Table 2). In the case of the large chub, most individuals were again recorded in runs and pools, 44% and 32% respectively (with maximum use of 78% in pools, Table 2), while only 16% and 8% of large chub were found in the shallower glides and riffles. This association with deep habitats, i.e. pools and runs, was more pronounced in the minnowroach, with most individuals found in pools (46%, with also maximum habitat use of 65% of pools) followed by runs (28%) and glides (22%), and with very low presence (4%) in the faster-flowing riffles (Table 2). The minnow, which was much scarcer, was also found mostly in the deeper habitats, i.e. runs and pools. When fish densities were compared among habitat types, all four fish groups exhibited highest densities in pools, followed by runs, glides and riffles.

TABLE 2 Percentage abundance of each fish group at each habitat type and percentage use of each habitat type by each fish group, for small chub, large chub, the minnowroach and the minnow

	% Abundance			
	Small chub	Large chub	Minnowroach	Minnow
Riffle	13	8	4	2
Run	40	44	28	40
Glide	23	16	22	21
Pool	24	32	46	37
	% use			
	Small chub	Large chub	Minnowroach	Minnow
Riffle	56	36	4	4
Run	69	69	36	22
Glide	61	31	28	17
Pool	52	78	65	30

The minnowroach and, to a lesser degree, the minnow appeared to favour vegetated microhabitats with finer substrate, as indicated by frequency of occurrence data and density data (Figure 2). Thus, 35.9% of the microhabitats occupied by the minnowroach were vegetated, in contrast to only 9.9% of those where the species was absent. Also, the densities of the minnowroach and the minnow were higher in microhabitats closer to the river bank (Figure 2). Conversely, both small and large chub did not appear to favour any habitat in terms of substrate and vegetation (Figure 2); for example, a similar percentage of the microhabitats occupied by the small chub and those not occupied by this group were vegetated (20.6% and 15.8% respectively), and the same was evident for the large chub too. Distance to the nearest bank did not appear relevant to small chub, which were present at high densities at various locations across the river, while for the large chub no clear tendency could be discerned (Figure 2).

According to Spearman's rho, minnowroach and minnow densities were negatively associated with water velocity (r = −0.27, P = 0.00 for minnowroach; r = −0.28, P = 0.00 for minnow) and positively associated with macrophyte cover (r = 0.30, P = 0.00 for

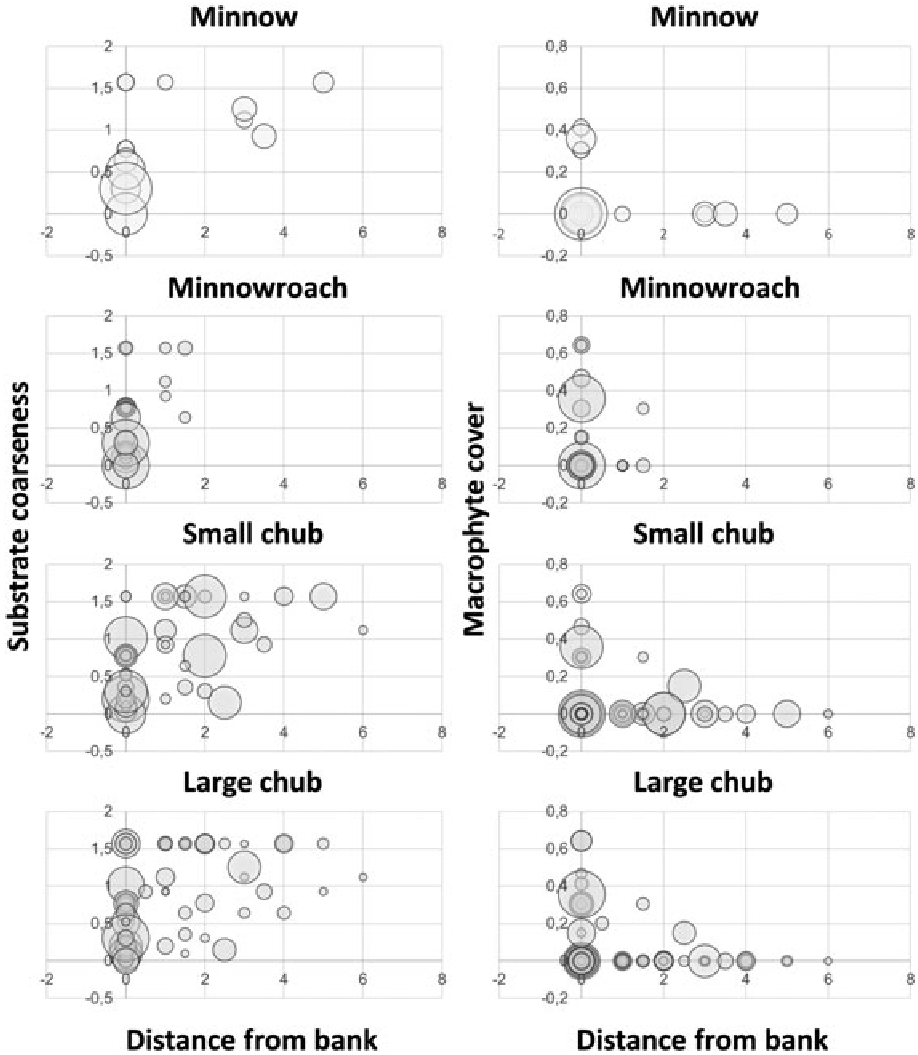

FIGURE 2 Microhabitat fish densities (bubbles) of small chub, large chub, the minnowroach and the minnow relative to: (a) substrate coarseness (>63 mm, arcsin values) and distance from nearest bank (m), (b) microhabitat fish densities relative to macrophyte cover and distance from bank.

minnowroaches; $r = 0.21$, $P = 0.02$ for minnow), with minnowroach densities also positively associated with depth ($r = 0.30$, $P = 0.00$) and negatively correlated with coarse substrate ($r = -0.30$, $P = 0.00$). Small chub densities were only associated negatively with water velocity ($r = -0.22$, $P = 0.01$), while large chub densities were associated positively with depth ($r = 0.27$, $P = 0.00$), and negatively with velocity, albeit marginally insignificantly ($r = -0.17$, $P = 0.07$). Both small and large chub exhibited no association with substrate coarseness and macrophyte cover.

The Monte Carlo test showed the significant effect of water velocity on fish density variation ($P = 0.004$). The ordination space defined by the first two axes (Figure 3) accounted for a large proportion of variability in species–habitat relationships (95.6%). Distribution of microhabitats in the ordination space reflected an association of pool microhabitats with increased fish densities (Figure 3). In the first canonical axis of the RDA, velocity, which was negatively correlated with fish densities, had the higher value, thus reflecting the tendency of all species' densities to increase in slower-flowing habitats. Macrophyte cover had the higher value for

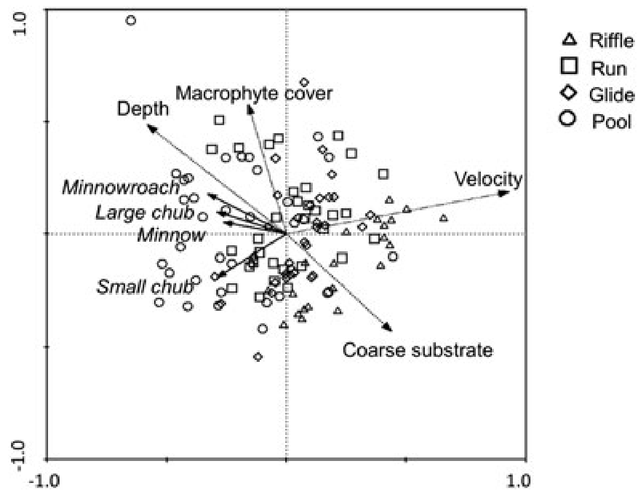

FIGURE 3 Redundancy Analysis (RDA) ordination diagram depicting the effects of habitat variables on fish group densities in the Evrotas catchment. Solid arrows are species and dashed arrows habitat variables. Superimposed are the microhabitat points of the various habitat types (riffles, runs, glides, pools).

the second canonical axis and was positively correlated with the minnowroach, the minnow and the large chub, reflecting the association of these groups with vegetated habitats. Furthermore, the RDA ordination diagram indicates a positive correlation of the minnowroach, the minnow and the large chub also with depth and a negative correlation with coarse substrate. Velocity was the only variable that was associated, negatively, with the small chub (Figure 3).

3.3 | Habitat use curves

According to the Kolmogorov–Smirnov test, only the minnow appeared to exhibit random habitat use for both microhabitat variables (depth, $P = 0.21$; velocity, $P = 0.42$). This, however, could be due to the small sample size ($n = 43$). The significance of the test for depth indicated that the minnowroach ($P = 0.01$), the small chub ($P = 0.01$) and the large chub ($P = 0.04$) were selective in their depth use. In contrast, the significance of the test for velocity for the minnowroach ($P = 0.67$), the small chub ($P = 0.78$) and the large chub ($P = 0.88$) indicated that habitat use resembled habitat availability.

Habitat use curves, based on 100 microhabitat point data, show that all four fish groups most frequently used depths between 0.3 and 0.65 m (Figure 4a). The depth use curves of minnowroach and minnow rose from approximately 0.05 m to an optimum of 0.35 m, while the small and the large chub had an optimum at 0.45 m. Depths >0.8 m were used only by the minnowroach and, to a lesser degree, by the minnow and the large chub. A comparison of depth habitat availability and depth habitat use indicates that the minnowroach, the large chub and the minnow actively selected deeper habitats (Figure 4a).

The minnowroach and the minnow used flow velocities below 0.7 m s^{-1} and 0.8 m s^{-1}, with an optimum at 0.26 m s^{-1} and 0.36 m s^{-1} respectively (Figure 4b). Velocity use curves for both size classes of chub showed an optimum at a higher velocity (0.5 m s^{-1}), with the large chub being frequent also at very low velocities (0.2 m s^{-1}), as well as exhibiting tolerance for the higher available velocities (0.8–1 m s^{-1}). The minnowroach velocity use curve showed a shift to lower velocities than those used by the other groups, as well as those available, with this species presenting high frequencies in the virtually still-water habitats, with minimal velocities of 0–0.1 m s^{-1} (Figure 4b).

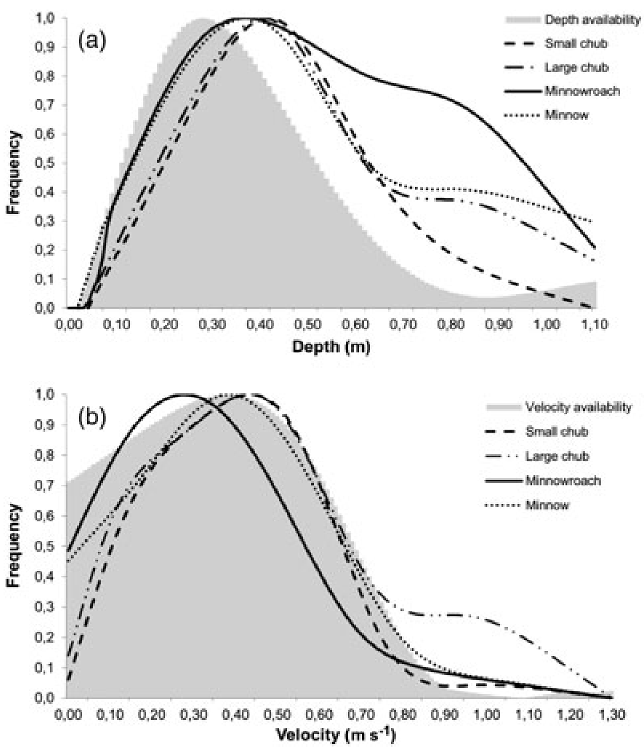

FIGURE 4 Habitat use curves of depth (a) and velocity (b) for small chub, large chub, the minnowroach and the minnow in the Evrotas River.

4 | DISCUSSION

The present study aimed at identifying the early summer habitat use patterns of the endemic fish species of the Evrotas River, as a first step towards creating a knowledge base for prioritizing habitats for fish conservation and also to gain some understanding of which habitat features are potentially limiting in areas to be restored. The results indicated that all three target species occurred at their highest densities in deep habitats, with low water velocities and depositional substrates, such as pools and, to a lesser degree, but equally important, in runs. Pools and runs are deep habitats that may provide cover from sight-feeding predators (Cowx & Harvey, 2003; Vlach, Dusek, Svatora, & Moravec, 2005). Furthermore, particulate organic matter flowing in the stream, such as vegetation or detritus, is often deposited in pools, influencing the composition of the benthic fauna and increasing microhabitat diversity, as well as acting as cover, primarily for juvenile fish (Carter et al., 2004). This concentration of fish in the relatively stable deep habitats, may be a response to the extreme seasonal fluctuation in flow rates in the Evrotas River, thus minimizing the energy expenditure associated with the stress of a constantly varying environment (Webb, Gerstner, & Minto, 1996).

The results of this study indicate a high overlap in early summer habitat use by the three Evrotas species, after the end of the reproductive period. A similar high overlap in summer habitat use, as opposed to other periods of the year with higher flow, has been found also for other cyprinid species and has been attributed to a gradual decrease of deep-sheltered habitats (Copp, 1992; Santos & Ferreira, 2008). This would imply that fish start to be confined to a reduced space, which in turn would increase food competition (Santos & Ferreira, 2008). Studies of seasonal habitat use in the Evrotas are therefore required to examine habitat overlap in periods of higher as well as lower discharge and possible shifts in habitat use during reproduction. The influence of feeding strategies should also be examined to test the hypothesis of increasing trophic competition (Santos & Ferreira, 2008).

Despite the high overlap in habitat use, there were, nevertheless, differences among the species. More specifically, minnowroaches actively selected deep, vegetated habitats with fine substrate, usually close to the shore, and avoided faster-flowing habitats, suggesting that this species exhibits limnophilic behaviour. Its related species *Tropidophoxinellus hellenicus* (Stephanidis 1939) has also been reported inhabiting deep stream areas and lakes (Economidis, 1991; Kottelat & Freyhof, 2007). Similarly, the Evrotas minnow mostly occupied slower flowing and vegetated habitats; this prevalence in lentic habitats has been reported also for other minnows in Greece (Kottelat & Barbieri, 2004; Perdikaris, Nathanailides, Gouva, Karipoglou, & Paschos, 2005) and elsewhere in European aquatic systems (Erős, Botta-Dukát, & Grossman, 2003; Prenda, Armitage, & Grayston, 1997) indicating limnophilic behaviour. In a similar study, Santos and Ferreira (2008) characterized the endangered Iberian nase also as limnophilic, as this species was significantly overrepresented in deeper areas with depositional substrates, compared with its co-occuring Iberian chub. The Evrotas chub, in contrast, had no clear affinity for particular velocities, since it occupied both slow and faster-flowing microhabitats and used a wider range of substrate types than the other species. This indicates that the Evrotas chub is more of a habitat generalist, as suggested elsewhere for Iberian chub species (Santos & Ferreira, 2008). However, data on seasonal habitat use are required, especially during the spawning period (April to May), as a shift to higher velocity areas has been observed in other Mediterranean chub species in spring (Pires, Cowx, & Coelho, 2000; Santos & Ferreira, 2008). Flexible microhabitat use strategies, i.e. microhabitat use according to availability, are common among fishes inhabiting Mediterranean streams and it has been suggested they may reflect adaptations to highly variable hydrological regimes (Grossman & de Sostoa, 1994; Martelo et al., 2014). According to an unpublished study that assessed the spatiotemporal variations of Evrotas endemic species during the drying process (Vardakas et al., unpublished), the minnowroach and the minnow were more resilient to drought, showing signs of coping with stagnant habitat conditions, attributed to their limnophilic behaviour. In contrast, the Evrotas chub was significantly affected by drought, with high mortality rates, even in the early phase of the drought. It is thus assumed that the Evrotas chub is less tolerant to drought conditions, and under future climate scenarios this species will reach the edge of extinction, if no conservation actions are implemented.

Overall, depth and velocity appeared to be involved in microhabitat selection by minnowroach, minnow and large chub. In comparison, Santos and Ferreira (2008) identified velocity and substrate as the most important variables in microhabitat use for the Iberian chub, and cover and depth for the Iberian nase in four river catchments of south-western Portugal, while substrate and depth were the most important variables for cyprinid microhabitat use in the river Matarraña in Spain (Grossman & de Sostoa, 1994) and the Torgal stream in Portugal (Martelo et al., 2014) at periods of persistent flow. Substrate and bottom vegetation were also relevant to the microhabitat use of minnowroaches and minnows in the Evrotas River, indicating that a number of variables probably interact dynamically in the microhabitat use of the three Evrotas species. No differences were observed among the physicochemical variables of habitats mainly due to the fact that the study was conducted when there was still adequate flow and habitats were connected.

There were also size class differences in microhabitat use, with large Evrotas chubs favouring deeper habitats, a pattern of habitat use documented in the literature (Erős et al., 2003), while small chub occupied shallower habitats than the adult fish, in accordance with previous studies on Iberian and other European cyprinids (Grossman & de Sostoa, 1994; Lamouroux, Capra, Pouilly, & Souchon, 1999; Santos et al., 2004). As suggested elsewhere (Santos & Ferreira, 2008), an increased predation risk for larger fish in shallower areas from birds may account for the presence of large chub in deeper water. Similarly, a reduction of predation pressure, e.g. from otters that seem to concentrate in deeper areas, may account for the presence of juvenile chub in shallower water (Copp, 1992). Size-related variation in microhabitat use is common in stream fishes, with depth being the most frequent variable eliciting size-related differences in microhabitat use by cyprinids, as evident in an extended study conducted in Río Matarraña, a relatively undisturbed river during the early 1980s in eastern Spain (Grossman & de Sostoa, 1994). Larger chub also occupied faster-flowing habitats than smaller individuals, as reported elsewhere for Iberian chubs and attributed to higher food availability in the coarser substrate of faster-flowing stream sections (Grossman & de Sostoa, 1994; Magalhães, 1993; Santos & Ferreira, 2008). Different size-class patterns of habitat use may indeed be linked to trophic adaptations, since it has been shown that diet may affect microhabitat use by different age groups (García-Berthou, 1999; Nunn, Harvey, & Cowx, 2007). Thus, the study of food availability in the various habitats should be incorporated in any future studies, in order to better understand habitat use by the Evrotas cyprinids; this would also elucidate their intra-species, as well as interspecies interactions, both trophically and spatially, enhancing the knowledge on their coexistence in the adverse conditions of an intermittent Mediterranean river.

This study highlights the importance of deep habitats, such as pools and runs, which appear to be important for the smaller minnowroaches and minnows as well as for the larger chubs. Several studies have stressed the critical importance of the quantity and quality of permanent pools that constitute available refugia during drought events and can secure fish survival (Hermoso, Ward, & Kennard, 2013; Larned, Datry, Arscott, & Tockner, 2010; Magalhães, Beja, Canas, & Collares-Pereira, 2002; Magoulick & Kobza, 2003; Pires, Cowx, & Coelho, 1999). Even where other habitat modifications appear to be successful during restoration efforts, protecting and restoring refuge habitats in streams subjected to large variations in discharge should be a conservation priority (Bond & Lake, 2003). Deep but faster-flowing habitats, such as runs, are also of critical and possibly equal importance, especially for larger fish, and should also be preserved within the frame of future conservation actions in the Evrotas River, where they are used by the large chub, as evident by the present study. A main issue pertinent to fish habitat restoration is its scale in space, as often localized habitat restoration actions are of limited effectiveness when larger scale disturbances occur, as in the case of rivers with large dams (Bond & Lake, 2003). Other large-scale disturbances occurring throughout the whole catchment (e.g. sediment inputs) may also be responsible for the failure of localized restoration actions addressing habitat limitation, as was the case for the bull trout (*Salvelinus confluentus* (Suckley, 1859) and the westslope cutthroat trout (*Oncorhynchus clarki lewisi* Suckley, 1856) in the Grave Creek, located in the Upper Kootenai River basin, USA (Bohn & Kershner, 2002). However, in smaller catchments free of dams, as in the Evrotas and other intermittent streams, localized habitat restoration actions, if properly designed, can have a very positive outcome. The retention of natural longitudinal connectivity, in the absence of dams, allows species to recolonize rapidly drought-affected areas after flow resumption during spring. Localized habitat enhancement and restoration actions could include, for example, increasing pool habitats by using large wood to improve hydromorphology (Kail & Hering, 2005) or artificial in-stream structures such as bank rip-rap and artificial dead wood (Pander & Geist, 2010) but with the prerequisite of ensuring high water quality in these modified habitats, as well as the ability of species to disperse to and from habitats (Santos & Ferreira, 2008).

Knowledge of fish habitat use can contribute to the formulation and implementation of effective habitat rehabilitation and restoration measures in intermittent streams. However, studies of habitat use by endangered and endemic fish species in Mediterranean intermittent streams are extremely limited, despite the fact that the Mediterranean region is considered a hot spot for freshwater fish biodiversity (Myers et al., 2000) and intermittent streams dominate surface runoff in Mediterranean Europe (Estrela et al., 1996; Skoulikidis et al., 2017; Tockner et al., 2009). Fish populations in Mediterranean intermittent streams are thought to be adapted to high seasonal fluctuations in flows (Hermoso & Clavero, 2011; Maceda-Veiga, 2012); however, the hydrological variability in these ecosystems is projected to increase in the near future owing to human activities, increasing the extent of the dry sections as well as the duration of the dry spells and, consequently, reducing habitat availability for fish populations. Predictive studies focused on fish assemblages inhabiting Mediterranean streams are scarce, but it is predicted that future communities will depend on position along the upstream–downstream gradient (Hermoso & Clavero, 2011); assemblages located at medium elevations are likely to undergo increases in species richness and species reassembly as the result of the expansion of non-native species and the local extinctions of native species (Buisson, Blanc, & Grenouillet, 2008; Buisson, Grenouillet, Casajus, & Lek, 2010). Forecasts also indicate that both cold-water and warm-water fish species will probably face habitat losses and will move to higher altitude habitats if those are available (Buisson & Grenouillet, 2009; Buisson et al., 2010; Lassalle & Rochard, 2009). Thus, any extreme increase in river intermittency due to unnatural factors can compromise the conservation of the endemic and endangered fish populations inhabiting the vulnerable Mediterranean stream ecosystems.

ACKNOWLEDGEMENTS

The authors wish to thank M.Th. Stoumboudi, D. Kommatas, P. Kouraklis and N. Pavlineri for their participation in the field work and C. Perdikaris for his valuable comments on the manuscript. Thanks also to Brian Zimmerman for improving the language of the English text. This study forms a part of L. Vardakas' PhD thesis at the University of the Aegean, Department of Marine Sciences, Greece. In order to conduct the field work, a permit was secured by the Ministry for Environment and Energy of Greece.

REFERENCES

Aadland, L. (1993). Stream habitat types: Their fish assemblages and relationship to flow. *North American Journal of Fisheries Management*, *13*, 790–806.

Austin, M. (2007). Species distribution models and ecological theory: A critical assessment and some possible new approaches. *Ecolological Modelling*, *200*, 1–19.

Barbieri, R., Zogaris, S., Kalogianni, E., Stoumboudi, M., Chatzinikolaou, Y., Giakoumi, S., … Economou, A.N. (2015). *Freshwater fishes and lampreys of Greece: An annotated checklist. Monographs on Marine Sciences No. 8*. Athens: Hellenic Centre for Marine Research.

Bohn, B. A., & Kershner, J. L. (2002). Establishing aquatic restoration priorities using a watershed approach. *Journal of Environmental Management*, *64*, 355–363.

Bond, N. R., & Lake, P. S. (2003). Characterizing fish-habitat associations in streams as the first step in ecological restoration. *Austral Ecology*, *28*, 611–621.

Bovee, K. D. (1986). Development and evaluation of habitat suitability criteria for use in the Instream Flow Incremental Methodology. Instream Flow Information Paper No. 21, FWS/OBS-86/7, Biological Report 86 (7). Washington, DC: DC USDI Fish and Wildlife Service.

Brosse, S., & Lek, S. (2000). Modelling roach (*Rutilus rutilus*) microhabitat using linear and nonlinear techniques. *Freshwater Biology*, *44*, 441–452.

Buisson, L., & Grenouillet, G. (2009). Contrasted impacts of climate change on stream fish assemblages along an environmental gradient. *Diversity and Distributions*, *15*, 613–626.

Buisson, L., Blanc, L., & Grenouillet, G. (2008). Modelling stream fish species distribution in a river network: The relative effects of temperature versus physical factors. *Ecology of Freshwater Fish*, *17*, 244–257.

Buisson, L., Grenouillet, G., Casajus, N., & Lek, S. (2010). Predicting the potential impacts of climate change on stream fish assemblages. *American Fisheries Society Symposium*, *73*, 327–346.

Carter, M. G., Copp, G. H., & Szomlai, V. (2004). Seasonal abundance and microhabitat use of bullhead *Cottus gobio* and accompanying fish species in the River Avon (Hampshire), and implications for conservation. *Aquatic Conservation: Marine Freshwater Ecosystems*, *14*, 395–412.

Copp, G. H. (1989). Electrofishing for fish larvae and 0+ juveniles: Equipment modifications for increased efficiency with short fishes. *Aquaculture and Fisheries Management*, *20*, 177–186.

Copp, G. H. (1992). Comparative microhabitat use of cyprinid larvae and juveniles in a lotic floodplain channel. *Environmental Biology of Fishes*, *33*, 181–193.

Cowx, I. G., & Harvey, J. P. (2003). *Monitoring the bullhead,* Cottus gobio. *Conserving Natura 2000 Rivers Monitoring Series No. 4*. Peterborough: English Nature.

Cummins, K. W. (1962). An evaluation of some techniques for the collection and analysis of benthic samples with emphasis on lotic waters. *The American Midland Naturalist*, *67*, 477–504.

Development Core Team, R. (2012). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Economidis, P. S. (1991). *Checklist of the freshwater fish of Greece (recent status of threat and protection)*. Athens: Bulletin of the Hellenic Society for the Protection of Nature, Special Publication.

Economidis, P. S. (2009). Freshwater fishes of Greece. In A. Legakis, & P. Maragou (Eds.), *Red book of endangered species in Greece*). Athens: Hellenic Zoological Society (in Greek).

Economou, A. N., Giakoumi, S., Vardakas, L., Barbieri, R., Stoumboudi, M., & Zogaris, S. (2007). The freshwater ichthyofauna of Greece: An update based on a hydrographic basin survey. *Mediterranean Marine Science*, *8*, 91–168.

Erős, T. Z., Botta-Dukát, Z., & Grossman, G. D. (2003). Assemblage structure and habitat use of fishes in a Central European submontane stream: A patch-based approach. *Ecology of Freshwater Fish*, *12*, 141–150.

Estrela, M. J., Peñarrocha, D., & Millán, M. (2000). Multi-annual drought episodes in the mediterranean (Valencia region) from 1950 to 1996. A spatio-temporal analysis. *International Journal of Climatology*, *20*, 1599–1618.

Estrela, T., Marcuello, C., & Iglesias, A. (1996). Water resources problems in southern Europe: An overview report. European Topic Centre on Inland Waters, European Environment Agency.

Fukuda, S., Mouton, A. M., & De Baets, B. (2011). Abundance versus presence/absence data for modelling fish habitat preference with a genetic Takagi-Sugeno fuzzy system. *Environmental Monitoring and Assessment*, *184*, 6159–6171.

García-Berthou, E. (1999). Spatial heterogeneity in roach (*Rutilus rutilus*) diet among contrasting basins within a lake. *Archiv für Hydrobiologie*, *146*, 239–256.

Gasith, A., & Resh, H. (1999). Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology and Systematics*, *30*, 51–81.

Gosselin, M. P., Petts, G. E., & Maddock, I. P. (2010). Mesohabitat use by bullhead (*Cottus gobio*). *Hydrobiologia*, *652*, 299–310.

Grossman, G. D., & de Sostoa, A. (1994). Microhabitat use by fishes in the lower Rio Matarraña, Spain: 1984–1987. *Ecology of Freshwater Fish*, *3*, 123–136.

Hermoso, V., & Clavero, M. (2011). Threatening processes and conservation management of endemic freshwater fish in the Mediterranean basin: A review. *Marine and Freshwater Research*, *62*, 244–254.

Hermoso, V., Ward, D. P., & Kennard, M. J. (2013). Prioritizing refugia for freshwater biodiversity conservation in highly seasonal ecosystems. *Diversity and Distributions*, *19*, 1031–1042.

Huey, J. A., Schmidt, D. J., Balcombe, S. R., Marshall, J. C., & Hughes, J. M. (2011). High gene flow and metapopulation dynamics detected for three species in a dryland river system. *Freshwater Biology*, *56*, 2378–2390.

IUCN (International Union for Conservation of Nature). (2014). The IUCN Red List of Threatened Species. Version 2014.3. www.iucnredlist.org. Accessed on 23 March 2015.

Kail, J., & Hering, D. (2005). Using large wood to restore streams in Central Europe: Potential use and likely effects. *Landscape Ecology*, *20*, 755–772.

Kottelat, M., & Barbieri, R. (2004). *Pseudophoxinus laconicus*, a new species of minnow from Peloponnese, Greece, with comments on the West Balkan *Pseudophoxinus* species (Teleostei: Cyrpinidae). *Ichthyological Exploration of Freshwaters*, *15*, 147–160.

Kottelat, M., & Freyhof, J. (2007). *Handbook of European freshwater fish*. Berlin: Kottelat, Cornol and Freyhof.

Lamouroux, N., Capra, H., Pouilly, M., & Souchon, Y. (1999). Fish habitat preferences at the local scale in large streams of southern France. *Freshwater Biology*, *4*, 673–687.

Larned, S. T., Datry, T., Arscott, D. B., & Tockner, K. (2010). Emerging concepts in temporary-river ecology. *Freshwater Biology*, *55*, 717–738.

Lassalle, G., & Rochard, E. (2009). Impact of twenty-first century climate change on diadromous fish spread over Europe, North Africa and the Middle East. *Global Change Biology*, *15*, 1072–1089.

Leigh, C., Boulton, A. J., Courtwright, J. L., Fritz, K., May, C. L., Walker, R. H., & Datry, T. (2015). Ecological research and management of intermittent rivers: An historical review and future directions. *Freshwater Biology*, *61*, 1181–1199.

Livada, I., & Asimakopoulos, D. N. (2005). Individual seasonality index of rainfall regimes in Greece. *Climate Research*, *28*, 155–161.

Maceda-Veiga, A. (2012). Towards the conservation of freshwater fish: Iberian rivers as an example of threats and management practices. *Reviews in Fish Biology and Fisheries*, *23*, 1–22.

Magalhães, M. F., Beja, P., Schlosser, I. J., & Collares-Pereira, M. J. (2007). Effects of multi-year droughts on fish assemblages of seasonally drying Mediterranean streams. *Freshwater Biology*, *52*, 1494–1510.

Magalhães, M. F. (1993). Effects of season and body size on the distribution and diet of the Iberian chub *Leuciscus pyrenaicus* in a lowland catchment. *Journal of Fish Biology*, *42*, 875–888.

Magalhães, M. F., Beja, P., Canas, C., & Collares-Pereira, M. J. (2002). Functional heterogeneity of dry-season fish refugia across a Mediterranean catchment: The role of habitat and predation. *Freshwater Biology*, *47*, 1919–1934.

Magoulick, D. D., & Kobza, R. M. (2003). The role of refugia for fishes during drought: A review and synthesis. *Freshwater Biology*, *48*, 1186–1198.

Martelo, J., Grossman, G. D., Porto, M., & Magalhães, M. F. (2014). Habitat patchiness affects distribution and microhabitat use of endangered Mira chub *Squalius torgalensis* (Actinopterygii, Cypriniformes). *Hydrobiologia*, *732*, 93–109.

Martínez-Capel, F., García, D. J., Werenitzky, D., Baeza, D., & Rodilla, M. A. (2009). Microhabitat use by three endemic Iberian cyprinids in Mediterranean rivers (Tagus River Basin, Spain). *Fisheries Management and Ecology*, *16*, 52–60.

Matthews, W. J., & Marsh-Matthews, E. (2003). Effects of drought on fish across axes of space, time and ecological complexity. *Freshwater Biology*, *48*, 1232–1253.

Meffe, G. K., & Vrijenhoek, R. C. (1988). Conservation genetics in the management of desert fishes. *Conservation Biology*, *2*, 157–169.

Morais, M., Pinto, P., Guiherme, P., Rosado, J., & Antunes, I. (2004). Assessment of temporary streams: The robustness of metric and multimetric indices under different hydrological conditions. *Hydrobiologia*, *5156*, 229–249.

Muñoz-Mas, R., Papadaki, C., Martínez-Capel, F., Zogaris, S., Ntoanidis, L., & Dimitriou, E. (2016). Generalized additive and fuzzy models in environmental flow assessment: A comparison employing the West Balkan trout (*Salmo farioides*; Karaman, 1938). *Ecological Engineering*, *91*, 365–377.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*, 853–858.

Nikolaidis, N., Skoulikidis, N., Kalogerakis, N., & Tsakiris, K. (2009). Environmental Friendly Technologies for Rural Development, Final Report 2005–09, LIFE-ENVIRONMENT LIFE05ENV/Gr/ 000245 EE.

Nunn, A. D., Harvey, J. P., & Cowx, I. G. (2007). The food and feeding relationships of larval and 0+ year juvenile fishes in lowland rivers and connected waterbodies. I. Ontogenetic shifts and interspecific diet similarity. *Journal of Fish Biology*, *70*, 726–742.

Pander, J., & Geist, J. (2010). Seasonal and spatial bank habitat use by fish in highly altered rivers – a comparison of four different restoration measures. *Ecology of Freshwater Fish*, *19*, 127–138.

Papadaki, C., Soulis, K., Muñoz-Mas, R., Ntoanidis, L., Zogaris, S., Dercas, N., & Dimitriou, E. (2016). Potential impacts of climate change on flow regime and fish habitat in montane rivers of the southwestern Balkans. *Science of the Total Environment*, *540*, 418–428.

Parry, M. L., Canziani, O. F., Palutikof, J. P., van der Linden, P. J., & Hanson, C. E. (2007). *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press.

Perdikaris, C., Nathanailides, C., Gouva, E., Karipoglou, C., & Paschos, I. (2005). Collapse of Epirus minnow, *Phoxinellus epiroticus* (Steindachner, 1895) fisheries in lake Pamvotis, Greece. *Ichthyological Exploration of Freshwaters*, *16*, 371–374.

Pires, A. M., Cowx, I. G., & Coelho, M. M. (1999). Seasonal changes in fish community structure of intermittent streams in the middle reaches of the Guadiana basin, Portugal. *Journal of Fish Biology*, *54*, 235–249.

Pires, A. M., Cowx, I. G., & Coelho, M. M. (2000). Life history strategy of *Leuciscus pyrenaicus* (Cyprinidae) in intermittent streams of the Guadiana basin (Portugal). *Cybium*, *24*, 287–297.

Prenda, J., Armitage, P. D., & Grayston, A. (1997). Habitat use by fish assemblages of two chalk streams. *Journal of Fish Biology*, *51*, 64–79.

Rincón, P. A., Barrachina, P., & Bernat, Y. (1992). Microhabitat use by 0+ juvenile cyprinids during summer in a Mediterranean river. *Archiv für Hydrobiologie*, *125*, 323–337.

Santos, J. M., & Ferreira, M. T. (2008). Microhabitat use by endangered Iberian cyprinids nase *Iberochondrostoma almacai* and chub *Squalius aradensis*. *Aquatic Sciences*, *70*, 272–281.

Santos, J. M., Godinho, F. N., & Ferreira, M. T. (2004). Microhabitat use by Iberian nase *Chondrostoma polylepis* and Iberian chub *Squalius carolitertii* in three small streams, north-west Portugal. *Ecology of Freshwater Fish*, *13*, 223–230.

Schoener, T. W. (1970). Non-synchronous spatial overlap of lizards in patchy habitats. *Ecology*, *51*, 408–418.

Simonson, T. D. (1993). Correspondence and relative precision of stream habitat features estimated at two spatial scales. *Journal of Freshwater Ecology*, *8*, 363–373.

Skoulikidis, N., Vardakas, L., Karaouzas, I., Economou, A., Dimitriou, E., & Zogaris, S. (2011). Assessing water stress in Mediterranean lotic systems: Insights from an artificially intermittent river in Greece. *Aquatic Sciences*, *Special Issue: Recent Perspectives on Temporary River Ecology*, *73*, 581–597.

Skoulikidis, N. T., Sabater, S., Datry, T., Morais, M. M., Buffagni, A., Dorfilnger, G., … Tockner, K. (2017). Non-perennial Mediterranean rivers in Europe: Status, pressures, and challenges for research and management. *Science of The Total Environment*, *557*, 1–18.

Sousa, V., Penha, F., Pala, I., Chikhi, L., & Coelho, M. M. (2010). Conservation genetics of a critically endangered Iberian minnow: Evidence of population decline and extirpations. *Animal Conservation*, *13*, 162–171.

Stanley, E. H., Fisher, S. G., & Grimm, N. B. (1997). Ecosystem expansion and contraction in streams. *BioScience*, *47*, 427–435.

TerBraak, C. J. F., & Smilauer, P. (1998). *Canoco reference manual and user's guide to Canoco for Windows: Software for Canonical community ordination version 4.0*. Ithaca, New York: Microcomputer Power.

Tockner, K., Uehlinger, U., Robinson, C. T., Tonolla, D., Siber, R., & Peter, F. D. (2009). Introduction to European rivers. In K. Tockner, U. Uehlinger, & C. T. Robsinson (Eds.), *Rivers of Europe* (pp. 1–23). London: Academic Press.

Tsigenopoulos, C. S., & Karakousis, Y. (1996). Phylogenetic relationships of *Leuciscus keadicus*, an endemic cyprinid species from Greece, with other species of the genus. *Leuciscus. Folia Zoologica*, *45*, 87–93.

Tzoraki, O., Nikolaidis, N. P., Amaxidis, Y., & Skoulikidis, N. T. (2007). In-stream biogeochemical processes of a temporary river. *Environmental Science and Technology*, *41*, 1225–1231.

Vlach, P., Dusek, J., Svatora, M., & Moravec, P. (2005). Fish assemblage structure, habitat and microhabitat preference of five fish species in a small stream. *Folia Zoologica*, *54*, 421–431.

Webb, P. W., Gerstner, C. L., & Minto, S. T. (1996). Station holding by the mottled sculpin, *Cottus bairdi* (Teleostei: Cottidae), and other fishes. *Copeia*, *2*, 488–493.

Zardoya, R., & Doadrio, I. (1999). Molecular evidence on the evolutionary and biogeographical patterns of European cyprinids. *Journal of Molecular Evolution*, *49*, 227–237.

How to cite this article: Vardakas L, Kalogianni E, Papadaki C, Vavalidis TH, Mentzafou A, Koutsoubas D, Skoulikidis TN. Defining critical habitat conditions for the conservation of three endemic and endangered cyprinids in a Mediterranean intermittent river before the onset of drought. *Aquatic Conserv: Mar Freshw Ecosyst*. 2017. doi: 10.1002/aqc.2735