Research Article

Prey utilisation and trophic overlap between the non native mosquitofish and a native fish in two Mediterranean rivers

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Abstract

Non native freshwater fish species have been long implicated in the decline of native Mediterranean ichthyofauna, through hybridization, disease transmission, competition for food and habitat, predation and/or ecosystem alteration; our knowledge, however, on the underlying mechanisms of these ecological impacts remains very limited. To explore the potential for trophic competition between the widespread Eastern mosquitofish *Gambusia holbrooki* and its co-occurring native toothcarp *Valencia letourneuxi* we compared resource use, feeding strategies, trophic selectivities and diet niche overlap. For this purpose, we studied two populations of the two species from a freshwater and a brackish habitat respectively, characterized by different food resource availabilities. In both habitats, the mosquitofish consumed a greater diversity of invertebrates and preyed on terrestrial invertebrates more frequently than the native toothcarp. Furthermore, in the less diverse and less rich brackish habitat, the non native relied heavily on plant material to balance a decrease in animal prey consumption and modified its individual feeding strategy, whereas these adaptive changes were not apparent in the native species. Their diet overlapped, indicating trophic competition, but this overlap was affected by resource availability variation; in the freshwater habitat, there was limited overlap in their diet, whereas in the brackish habitat, their diets and prey selectivities converged and there was high overlap in resource use, which is indicative of intense interspecific trophic competition. Overall, it appears that the underlying mechanism of the putative negative impacts of the mosquitofish on the declining Corfu toothcarp is mainly trophic competition, regulated by resource variability, though there is also evidence of larvae predation by the mosquitofish.

Keywords: *Gambusia holbrooki, Valencia letourneuxi,* Corfu toothcarp, Resource competition, Diet overlap.

Introduction

Invasive fish species are regarded as a major cause of native fish decline and a main threat to biodiversity, often in combination with other factors, such as habitat loss or degradation (Clavero & García-Berthou, 2005; García-Berthou, 2007). However, evidence of such adverse ecological impacts remains descriptive and controversial and their true nature largely unknown; therefore, non native freshwater fish impacts on the native ichthyofauna are the subject of a continuing scientific debate (Gozlan, 2008; Leprieur *et al.*, 2009; Leunda, 2010). This problem is even more acute in freshwater Mediterranean systems where non native freshwater fishes represent more than a quarter of all fish species in drainage basins, and where high endemicity makes native fish extinctions much more likely because of their small natural ranges (Tricarico, 2012; Ribeiro & Leunda, 2012). Up to now, very few studies have addressed the impacts of non native species in Mediterranean systems, and even less have explored such impacts in aquatic systems of the Hellenic Peninsula.

To our knowledge, there are only two relevant studies available, namely, Rosecchi *et al.* (1993) and Apostolidis *et al.* (2008), both, however, are largely descriptive and do not assess impact mechanisms.

Furthermore, most of the non-native freshwater fish impact studies in the Mediterranean region have been conducted on salmonids or piscivorous species (such as pikeperch and largemouth bass), with much fewer focusing on widely spread non natives, such as the common carp *Cyprinus carpio* Linnaeus, 1758 and the Eastern mosquitofish *Gambusia holbrooki* Girard, 1859 (for a review, see Ribeiro & Leunda, 2012). *Gambusia holbrooki*, in particular, is the second most widespread non native fish species in the Mediterranean region and the most widespread in the Hellenic Peninsula, with confirmed occurrence in 49.5% of its river basins (Economou *et al.*, 2007). This species has been implicated, together with habitat loss, in the decline of many native Mediterranean fish species and, particularly, through competition effects, to the decline of two threatened Iberian endemics, the Valencia toothcarp *Valencia hispanica* (Valenciennes, 1846) and the Ibe-

Medit. Mar. Sci., 15/2, 2014, 287-301

287

rian toothcarp *Aphanius iberus* (Valenciennes, 1846) (see Rincón *et al.*, 2002; Caiola & de Sostoa, 2005). These authors have identified experimentally that food resource competition has been the principal impact mechanism. Studies, however, which could demonstrate similar trophic impacts in other Mediterranean regions are still lacking.

Competition for food resources between non native and native species in Mediterranean freshwater systems can indeed be an important impact mechanism given the hydrological instability of many of these aquatic systems, especially during the dry season, coupled with the dietary flexibility of non native species (Blanco *et al.*, 2004; Ribeiro *et al.*, 2007). However, most studies of the putative detrimental impacts of non native fish on Mediterranean native species have focused on hybridization and disease transmission as the underlying mechanisms (Ribeiro & Leunda, 2012). Furthermore, the few diet competition studies in systems of this region have been descriptive, providing only potential competition evidence with native species. In addition, few studies calculated diet overlap indexes (Lorenzoni *et al.*, 2002; Teixera & Cortes, 2006) and, to our knowledge, only a recent study also assessed dietary selectivity and resource availability (Ruiz-Navarro *et al.*, 2013; for an overview see Ribeiro & Leunda, 2012). This gap in our knowledge is crucial, since ecological interactions between non native and native freshwater species can be context-specific and may vary across different aquatic ecosystem types, ecological conditions and/or fish community characteristics (Leunda, 2010; Ribeiro & Leunda, 2012). Thus, it can hamper effective conservation management at a local scale, since context-specific trophic interactions are important mechanisms affecting the distribution of aquatic communities (Declerck *et al.*, 2002; Oscoz *et al.*, 2005).

This study investigates resource use, feeding strategies, diet selectivity and niche overlap of the introduced *G. holbrooki* and the endangered Corfu toothcarp *Valencia letourneuxi* (Sauvage, 1880). This critically endangered species, endemic to Western Greece and Southern Albania, with a highly fragmented geographical distribution, low population densities and high habitat specificity, has undergone a rapid population decline in recent years, attributed partly to competition with and aggression by *G. holbrooki* (Kottelat & Freyhof, 2007; Kalogianni *et al.*, 2010a). In the current study, we explored (a) the potential for trophic competition and/or predation between the two species and (b) the role of resource availability in the pattern of these interactions by comparing resource use, feeding strategies, diet selectivity and niche overlap in two different aquatic systems of Western Greece (Louros and Acheron rivers). By studying the form and plasticity of the trophic relationships between the two species, our aim was to contribute to both the elucidation of the competitive interactions between non indigenous competitors and native species, and the formulation of effective conservation and management plans for the native species.

Materials and Methods

Study sites

The study area comprised of two localities in Western Greece that host *V. letourneuxi* and the non native poeciliid *G. holbrooki*, namely the freshwater Skala stream (Louros River drainage) and the brackish Acheron canal (Acheron River drainage), both sampled in late June 2009 (Fig.1a). In many aspects, these localities are typical *V. letourneuxi* habitats, i.e. they are both low altitude spring-fed aquatic systems, with slow flowing, clear waters and rich submerged and surface vegetation that provide food, spawning substrate and shelter from predators to this native fish (Barbieri *et al.*, 2000; Kalogianni *et al.*, 2010a).

(a)

(b)
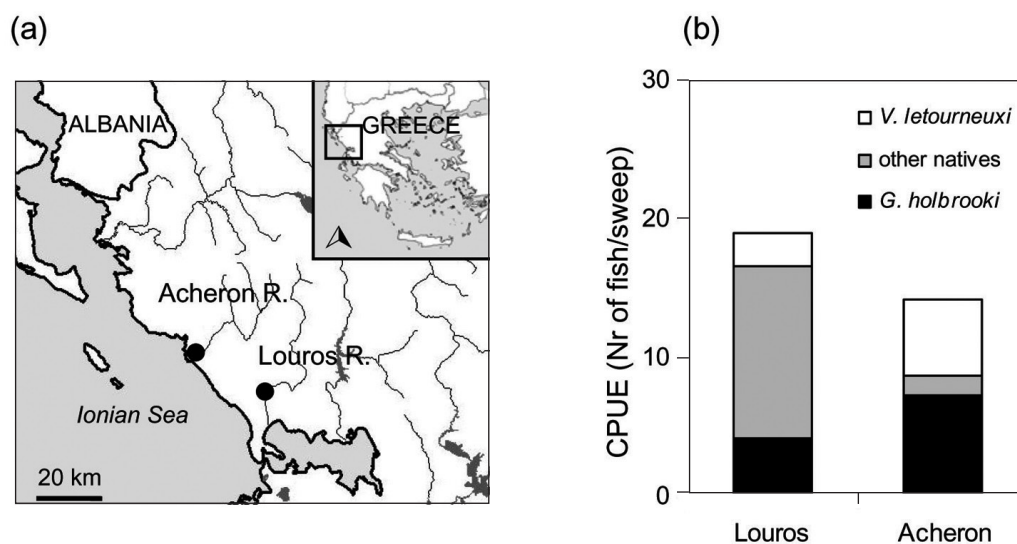
Fig. 1: (a) Location of sampling sites at Acheron and Louros' drainages in Western Greece; (b) local fish assemblage composition and abundance variation.

288

Medit. Mar. Sci., 15/2, 2014, 287-301

Site selection

These two sites were selected for this study, because in Acheron and to a lesser degree in Louros it was possible to collect an adequate number of native fish specimens, compared to all the other aquatic systems of Western Greece where the two species live in sympatry (in all other locations, *V. letourneuxi* is both locally restricted and occurs at low densities, see Kalogianni *et al.*, 2010a). Furthermore, preliminary macroinvertebrate sampling revealed marked differences in their macroinvertebrate fauna richness and diversity. Therefore, their selection enabled the study of dietary patterns and inter-species interactions under two different sets of abiotic and biotic conditions.

Site description

The Louros site (39° 10' 31" N, 20° 46' 00" E), located in the Skala stream, is a fairly deep (1.2 m) and wide (9 m) canal fed by karstic springs, with some riparian and rich aquatic vegetation. In June 2009, *V. letourneuxi* was found there in association with *G. holbrooki* and three native species, the minnow *Pelasgus thesproticus* (Stephanidis, 1939) the goby *Economidichthys pygmaeus* (Holly, 1929) and the stickleback *Gasterosteus gymnurus* Cuvier, 1829, the latter at very low densities. The observed water temperature was 17.3°C and salinity 0.1‰.

The Acheron site (39° 14' 55" N, 20° 28' 50" E) is a 1.2 m deep, 13 m wide drainage canal at the northern edge of the Acheron river delta. This canal is fed by alluvial springs, has relatively sparse riparian vegetation but rich aquatic vegetation. In June 2009, temperature was 25.3°C and salinity 6.5‰. *Valencia letourneuxi* was found in association there with two native species, the minnow *P. thesproticus* and the eel *Anguilla anguilla* (Linnaeus, 1758), as well as the non native *G. holbrooki*.

Macroinvertebrate and fish sampling

Three macroinvertebrate samples (from the floating vegetation, the vegetated bank face and the stream bed respectively, with 1 min sampling duration for each microhabitat) were collected with a pond net (surface 625 cm², mesh size 900 µm) from the two study localities by the same individual. The samples were preserved in 4% formaldehyde and transferred to the laboratory for further identification. At the laboratory, macroinvertebrates were sorted, identified down to family level, according to Tachet *et al.* (2000), counted and weighed. Environmental site characterization was also conducted in the field; temperature, conductivity, dissolved oxygen concentration, pH and salinity were measured *in situ* with a Consort C535 multi-parameter analyzer.

Fish sampling was also conducted by the same person, during daylight hours, using a large net with a D-shaped frame (2 mm mesh size). Fish samples were collected from a 30-60 m long and 2 m wide stretch of the littoral zone of the study sites, with similar depths and aquatic vegetation cover. After sampling, fish were identified to species level, counted and measured. Total catches consisted of 200 *G. holbrooki* (SL range 8.2-35 mm) and 131 *V. letourneuxi* (SL range 6.8-45.5 mm). Fish abundances were estimated considering the effort (number of net sweeps) in order to determine catch per unit effort (CPUE), i.e. number of fish per net sweep. Prior to preservation in 10% formalin for subsequent laboratory analysis, the fish were anesthetized with quinaldine solution.

Data analysis

Macroinvertebrate fauna composition and diversity

For an estimate of food availability, macroinvertebrate abundance and biomass were calculated, pooling the data of the three microhabitats sampled. Estimates of prey richness (*S*, taxa count) were also obtained by counting the numbers of different categories. Prey diversity was assessed using the Shannon-Weaver index $H' = -\Sigma\ p_i\ \mathrm{log}p_i$, where p_i is the fraction of items in the macroinvertebrate sample that are of category *i* (Shannon & Weaver, 1963) and differences in *H'* were tested with Student's *t*-test, as modified by Magurran (1988). To assess the evenness of the distribution of the various taxa in each of the two habitats, equitability *J'* was calculated (Pielou, 1966). To compare the dominance pattern, the distributional structure and the diversity profiles of the macroivertebrate fauna in the two localities, *k*-dominance abundance curves were plotted. In these plots, species are ranked in decreasing order of dominance along the x-axis and the percentage cumulative abundance (*k*-dominance) is then plotted against the species rank *k* (Platt *et al.*, 1984).

General diet patterns and feeding intensity

At the laboratory, only fish larger than 16 mm SL were included in the dietary analysis (a size range roughly corresponding to the adult stage of both species, see Barbieri *et al.*, 2000; Pyke, 2005; Kalogianni *et al.*, 2010b), in order to minimize ontogenetic variation in their diet. Specimens were weighed before and after being eviscerated (TW and NW, nearest 0.01 mg). The gut contents of 107 *G. holbrooki* (max SL 35.0 mm) and 59 *V. letourneuxi* (max SL 45.5 mm) were analyzed under a dissecting stereoscope. Animal prey items were identified to the lowest possible taxonomic level (mostly family), according to Tachet *et al.* (2000) and counted.

The relative importance of each dietary category was expressed as frequency of occurrence (F_o, %) and abundance (A_i, %), where F_o is the percentage of non-empty guts containing a specific prey and A_i is the percentage of a given prey of the total prey items found in each gut sample (Hyslop, 1980). Gut contents were grouped in

Medit. Mar. Sci., 15/2, 2014, 287-301

289

32 aquatic categories (plus fish) and four terrestrial prey categories. Plant material occurrence was also recorded. Binomial Test on population proportions, based on 10000 Monte Carlo permutations, was applied to test the significance of differences in aquatic prey, terrestrial prey and plant material occurrence. Feeding intensity was evaluated from the vacuity index (percentage of empty guts) and the mean number of animal prey items. Differences were tested with the Binomial Test and Mann-Whitney U-test, respectively.

Prey electivity

Prey electivity with respect to the macroinvertebrate community was quantified using Savage's index (Savage, 1931; Oscoz *et al.*, 2006), $Wi = Ai/Di$, where Ai is the relative abundance of prey i in the alimentary tract content and Di is the relative availability of this resource at the study site. The values of Wi vary between 0 and ∞, where 1 means no selection of prey i, and values lower and higher than 1 show avoidance (negative preference) and selection (positive preference), respectively. This index was selected because it enables the verification of its statistical significance (Manly *et al.*, 1993) after applying an adjustment. Due to the dichotomous variable nature of tests per prey category (Y/N), the Binomial Test was applied (Daniel, 1995) to test the statistical significance. Because of the large number of comparisons, instead of Bonferroni adjustment the Holm's (1979) adjustment method [a/number of categories - k, let k be the ascending order value (n-1) of the p values of the n tests, see also Peres-Neto (1999)] was used.

Diet diversity and feeding strategy

Diet diversity, at the population and the individual level, was evaluated with the Shannon-Weaver population diversity index (H') and the individual diversity index (H' ind*)* respectively (Shannon & Weaver, 1963) and differences were tested with Student's *t*-test, as described by Magurran (1988); at the population level, these indices were absolute values, whereas at the individual level, they were average values, i.e., means of H' values of all individual fish in each sample; standard error for H' ind was also calculated. Finally, evenness was evaluated with equitability J' (Pielou, 1966). To detect differences in feeding strategy, plots were constructed, following Costello's graphical method (Costello, 1990), modified by Amundsen *et al.* (1996). These plots are based on a two-dimensional representation, where each point represents frequency of occurrence ($\%F_o$) and prey specific abundance ($\%P_i$). Information about prey importance and feeding strategy of the predator can be obtained by examining the distribution of points along the diagonals and the axes of the diagram; for further details consult Amundsen *et al.* (1996). Furthermore, a Tokeshi plot (Tokeshi, 1991) was constructed to examine intra- and inter-species differences in individual *versus* population diet, by plotting individual diet diversity (H'_{ind}) *versus* population diet diversity (H').

Diet overlap

To explore the potential for trophic competition between the two species, dietary overlap was measured using the Morisita-Horn index (C_{mh}, Morisita, 1959; Horn, 1966). This index ranges from zero (no overlap) to one (complete overlap); with values above 0.6 denoting a biologically significant overlap.

Intraspecific and interspecific differences in diet were further examined with multivariate analysis techniques, using Primer 6β software. Dietary data were square root transformed and a similarity matrix was produced, using the Bray-Curtis similarity coefficient. NMDS ordination analysis (Clarke & Warwick, 1994) was performed to provide a visual non scale ordination between samples. Multivariate dispersal (MVDISP) was used to consider the level of dispersion on the ordination plots (Clarke & Warwick, 1994). One-way analyses of similarities (ANOSIM) were performed to test for significant intra- and interspecific dietary differences.

Results

Fish abundance

Both *V. letourneuxi* and *G. holbrooki* were less abundant in Louros with CPUE values of 2.4 and 4.3 respectively (Fig. 2b), compared to Acheron (CPUEs values of 5.6 and 7.3 respectively). The fish assemblage in Louros was dominated by other native species (the dominant species was *E. pygmaeus*), while in Acheron by *G. holbrooki*. At both sites, the non-native *G. holbrooki* was relatively more abundant than *V. letourneuxi* (*Gambusia* / *Valencia* ratio in Louros 1.8:1; in Acheron 1.3:1).

Macroinvertebrate sample composition and food availability

The abundance and biomass of potential macroinvertebrate prey was lower (almost half) in Acheron than in Louros (Fig. 2a, b). Prey richness (S) and diversity (H') indices showed that faunal diversity too was significantly lower in Acheron (H', Acheron vs. Louros, $t = -12.92$, $p < 0.01$), as well as equitability (J', Fig. 2c). Furthermore, at the Acheron site, macroinvertebrate fauna was dominated almost exclusively by gastropods and crustaceans, whereas at the Louros site several other taxa, such as Diptera, Heteroptera and Trichoptera were well represented (Fig. 2a, b). Abundance *k*-dominance curve plots of macroinvertebrate categories at the family level confirmed that the Louros habitat had a more diverse and evenly distributed assemblage of macroinvertebrates (Fig. 2c).

Examination of the structure of the two macroinvertebrate communities at the family level (Table 1) showed

290

Medit. Mar. Sci., 15/2, 2014, 287-301
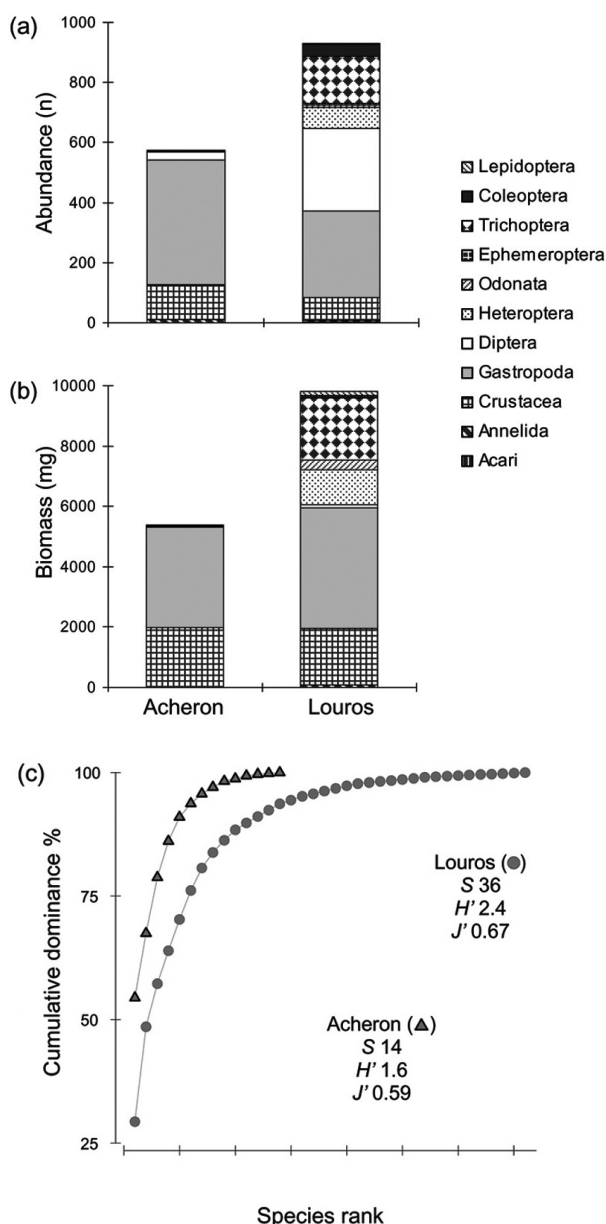
Fig. 2: (a) Abundance and (b) biomass of invertebrate taxa in the samples for the two habitats; (c) Abundance *k*-dominance curves plots of macroinvertebrate categories at the two study sites. The lowest curve (Louros) represents a more diverse assemblage.

that in Acheron, it was dominated by Hydrobiidae gastropods (relative abundance exceeding 50%) with another 13 categories present in the sample. At the Louros site, in contrast, Chironomidae larvae was the dominant available prey in terms of relative abundance (almost 30%), with some representation, however, of another 35 prey categories. Furthermore, at the Louros site there were invertebrates requiring high-quality waters, such as Ephemeroptera and Trichoptera larvae, as well as specific categories, such as Calopterygidae (Odonata), Limnephilidae (Trichoptera), Hydraenidae and Gyrinidae (Coleoptera), sensitive to human-induced disturbance, which were absent at the Acheron site (Table 1).

Dietary variation

Feeding intensity

From a total of 107 *G. holbrooki* and 59 *V. letourneuxi* examined, the gut of 10 *G. holbrooki* (Acheron site) and 4 *V. letourneuxi* (2 from the Acheron site and 2 from the Louros site) were found empty, and therefore were not further analysed (Table 2). The Vacuity index (%VI) of *G. holbrooki* increased significantly from Louros to Acheron (Binomial test, $p = 0.000$), indicating lower feeding intensity at the Acheron site, whereas for *V. letourneuxi* there was no statistically significant spatial variation in the %VI.

Mean number of animal prey items consumed (as an additional measure of feeding intensity) decreased significantly in both species from Louros to Acheron (Table 2, Mann-Whitney U-test, $p = 0.000$). Overall, these shifts, based on animal prey consumption, indicate that both species reduced their feeding intensity in Acheron, with this effect being more pronounced in *G. holbrooki*.

Overall diet patterns

Aquatic prey were the main animal prey of both species in the two habitats, both with respect to relative abundance and occurrence (Table 2); this decreased significantly in *G. holbrooki* from Louros to Acheron ($p = 0.000$), whereas in *V. letourneuxi* there was no significant shift. Terrestrial prey were also consumed, and terrestrial prey occurrence decreased significantly from Louros to Acheron in both species (for *G. holbrooki*, $p = 0.000$; for *V. letourneuxi* $p = 0.002$). Finally, plant material occurrence increased from Louros to Acheron in both species (however, this was significant in *G. holbrooki only*, $p = 0.001$). Overall, from Louros to Acheron, there was a strong spatial shift in *G. holbrooki* diet to high plant material consumption, whereas *V. letourneuxi* continued to rely on aquatic and to a lesser degree terrestrial prey.

Interspecies comparison showed that in Louros, their aquatic prey consumption did not differ significantly, whereas in Acheron, *G. holbrooki* consumed aquatic prey less frequently than *V. letourneuxi* ($p = 0.000$). Frequency of consumption at both sites of terrestrial prey (such as drifting insects) as well as plant material was significantly higher for *G. holbrooki* compared to *V. letourneuxi* (for terrestrial prey in Louros $p = 0.000$, in Acheron $p = 0.003$; for plant material in Louros $p = 0.029$, in Acheron $p = 0.000$). Overall, *G. holbrooki*, apart from aquatic prey, also relied more on terrestrial prey and plant material at both habitats, compared to *V. letourneuxi*. Finally, fish had a minor contribution to the diet of both species, indicating an opportunistic fish predation by both *G. holbrooki* and *V. letourneuxi*.

Though a broad number of animal prey categories were identified in all populations, the main forage base of both species consisted of a much smaller subgroup of main prey items that also presented spatial variation

Table 1. Relative abundance (%Ai) and biomass (%Bi) of the various macroinvertebrate categories in Louros and Acheron rivers (L: larvae, A: adult). Categories sensitive to human induced disturbance (Artemiadou & Lazaridou, 2005) are only present in Louros (see asterisks).

Macroinvertebrate categories	Louros		Acheron	
	%Ai	%Bi	%Ai	%Bi
Acari				
Hydrachnidae	0.43	0.02		
Annelida				
Glossiphonidae	0.21	0.46		
Erpobdellidae	0.21	0.27		
Oligochaeta			1.91	0.16
Crustacea				
Gammaridae	5.91	4.26	11.30	1.82
Atyidae	2.15	14.97	1.22	0.17
Palaemonidae			7.30	34.75
Asselidae			0.35	0.02
Gastropoda				
Planorbidae	1.29	2.51	2.78	5.11
Neritidae	2.47	6.97	13.04	21.82
Hydrobiidae	3.11	5.48	**54.43**	28.27
Bithyniidae	19.23	20.79	0.52	0.38
Lymnaeidae	0.54	0.47	1.39	6.07
Valvatidae	4.51	4.46		
Diptera				
Chironomidae (L)	**29.32**	1.09	4.87	0.67
Ephydriidae (L)	0.11	0.01		
Tipulidae (L)	0.11	0.02		
Heteroptera				
Veliidae	0.11	0.02		
Naucoridae	0.21	0.95	0.17	0.37
Corixidae	6.34	10.77		
Gerridae	0.11	0.01		
Aphididae	0.54	0.01		
Odonata				
Coenagrionidae			0.52	0.30
Platycnemidae	0.75	2.02		
Calopterygiidae*	0.11	1.18		
Ephemeroptera				
Baetidae	0.75	0.17		
Trichoptera				
Hydroptilidae	8.70	0.63		
Limnephilidae*	6.66	19.58		
Leptoceridae	1.29	0.70	0.17	0.07
Hydropsychidae	0.11	0.10		
Coleoptera				
Hydraenidae (A)*	0.11	0.00		
Hydrophilidae (L)	0.11	0.01		
Hydrophilidae (A)	0.21	0.03		
Dytiscidae (L)	0.54	0.09		
Dytiscidae (A)	0.21	0.11		
Haliplidae (A)	1.29	0.19		
Elmidae (L)	0.21	0.02		
Elmidae (A)	1.40	0.36		
Gyrinidae (L)*	0.11	0.02		
Lepidoptera	0.54	1.25		

(Table 2). More specifically, in Louros, *G. holbrooki* consumed mostly small amphipods, Ostracoda and Chironomidae larvae, while in Acheron its staple food was Gammaridae, and to a much lesser degree Formicidae ants, a surface prey (Table 2). In addition, four prey categories were also common in its Louros diet (Chironomidae pupae, Veliidae and two terrestrial preys, Diptera adults and Araneae) but almost absent in Acheron. *G. holbrooki* prey categories were more evenly distributed in Acheron than in Louros (*J'* value in Acheron 0.78, in Louros 0.54). The two *G. holbrooki* populations shared 10 prey categories, but their main forage base did not include any category abundant in the diet of both populations (Table 2), indicating a strong spatial shift in *G. holbrooki*'s diet.

Valencia letourneuxi in Louros consumed mostly Ostracoda and Chironomidae larvae too, while in Acheron, Gammaridae and to a lesser degree, Chironomidae larvae (Table 2), indicating a similar but much less pronounced spatial shift in the animal diet of *V. letourneuxi* from Louros to Acheron. Again, prey categories were more evenly distributed in Acheron than in Louros (*J'* value in Acheron 0.64, in Louros 0.50). In terms of diet similarity, the Acheron population shared only seven categories with Louros, with Chironomidae being the only common abundant prey in the diet of the species in both habitats.

Qualitative interspecies diet comparison indicates that the animal diet of both species in Louros was less similar that in Acheron (Table 2). More specifically, in Louros, the main animal prey of the two species differed (small amphipods in *G. holbrooki* as opposed to Chironomidae larvae in *V. letourneuxi*), but they shared three other relatively abundant prey items in their diet (Ostracoda, Chironomidae larvae and pupae). In Acheron, in contrast, their main prey and by far the most abundant in the diet of both species was the same (Gammaridae crustaceans), with all other prey having low average contributions to their diet. The above indicate a convergence of the diet of the two species in Acheron.

Prey electivity

As regards prey selection (Table 3), *G. holbrooki* consistently refused Bithyniidae and Hydrobiidae gastropods although abundant in Louros and Acheron respectively. In contrast, two rare categories (Ephydridae larvae and Veliidae) were positively selected in both habitats. Interestingly, Gammaridae amphipods that were refused in Louros, were positively selected in Acheron, where they constituted the third most abundant prey available, after Hydrobiidae and Neritidae gastropods (see also Table 1).

Similarly, *V. letourneuxi* also avoided Bithyniidae and Hydrobiidae gastropods, and positively selected Chironomidae larvae, Ephydridae larvae and Veliidae. Also in this species, differential selectivity of Gammaridae was observed, which were refused in Louros, but

292

Medit. Mar. Sci., 15/2, 2014, 287-301

Table 2. Dietary composition of *G. holbrooki* and V. letourneuxi from Louros and Acheron, displayed as frequency of occurrence (Fo, %) and numeric abundance (Ai, %). The dominant categories in the diet of the two species are printed in bold. L: larvae; P: pupa; A: adult.

| | | *G. holbrooki* | | | | *V. letourneuxi* | | | |
| | | Louros (*n* = 43) | | Acheron (*n* = 54) | | Louros (*n* = 19) | | Acheron (*n* = 36) | |
Prey categories		%Fo	(%Ai)	%Fo	(%Ai)	%Fo	(%Ai)	%Fo	(%Ai)
	Aquatic								
Acari	Hydrachnidae	34.9	(1.9)			5.3	(1.0)		
Crustacea	Amphipoda	**69.8**	**(36.9)**	1.9	(1.5)	5.3	(0.5)	5.6	(1.8)
	Atyidae			1.9	(1.5)				
	Gammaridae	2.3	(0.1)	**29.6**	**(39.7)**			**77.8**	**(52.8)**
	Ostracoda	**60.5**	**(26.3)**			**31.6**	**(12.4)**	2.8	(0.6)
	Palaemonidae			1.9	(1.5)			8.3	(3.7)
	Asselidae							8.3	(4.9)
	Sphaeromatidae							13.9	(4.3)
Gastropoda	Planorbidae					5.3	(0.5)		
	Neritidae	9.3	(0.4)	3.7	(4.4)	10.5	(1.5)	19.4	(7.4)
	Hydrobiidae							11.1	(4.3)
	Lymnaeidae			1.9	(1.5)			5.6	(1.2)
Diptera	Chironomidae L	**81.4**	**(20.8)**	1.9	(1.5)	**100.0**	**(64.2)**	**27.8**	**(10.4)**
	Chironomidae P	48.8	(4.0)	5.6	(4.4)	52.6	(7.0)	2.8	(0.6)
	Ceratopogonidae L					5.3	(0.5)		
	Stratiomyidae L	2.3	(0.1)			5.3	(1.0)		
	Ephydridae L	9.3	(0.7)	5.6	(4.4)	5.3	(3.0)	8.3	(2.5)
	Dixidae L	4.7	(0.2)			10.5	(2.0)		
Heteroptera	Veliidae	37.2	(3.2)	3.7	(8.8)	5.3	(0.5)	2.8	(0.6)
	Naucoridae A			5.6	(4.4)				
	Notonectidae A	2.3	(0.1)						
Odonata	Coenagrionidae							2.8	(0.6)
	Libellulidae							2.8	(0.6)
Ephemeroptera	Baetidae	2.3	(0.1)	3.7	(2.9)				
Trichoptera	Hydroptilidae	2.3	(0.1)			5.3	(0.5)		
	Hydropsychidae L	2.3	(0.1)						
Coleoptera	Hydraenidae L	2.3	(0.1)						
	Hydraenidae A	7.0	(0.3)						
	Hydrophilidae L	9.3	(0.4)						
	Hydrophilidae A			1.9	(1.5)				
	Dytiscidae L	4.7	(0.2)			21.1	(3.0)		
Lepidoptera	Lepidoptera L					5.3	(1.0)		
Fish	Fish L	2.3	(0.1)	1.9	(5.9)			2.8	(2.5)
	Total aquatic	*97.7*	*(96.3)*	*48.1*	*(83.8)*	*100.0*	*(98.5)*	*94.4*	*(98.8)*
	Terrestrial								
Diptera	Diptera A	32.6	(1.9)	5.6	(4.4)	10.5	(1.0)		
Heteroptera	Aphididae					5.3	(0.5)		
Hymenoptera	Formicidae A	7.0	(0.3)	**9.3**	**(11.8)**				
Araneae	Araneae	27.9	(1.4)					5.6	(1.2)
	Total terrestrial	*46.5*	*(3.7)*	*14.8*	*(16.2)*	*15.8*	*(1.5)*	*5.6*	*(1.2)*
	Algae and plants	46.5		70.4		31.6		38.9	
	Total preys	898		68		201		163	
% VI (*n* total fish examined)		0 (43)		15.6 (64)		9.5 (21)		5.3 (38)	
Mean *n* items consumed		20.88		1.26		10.58		4.53	
Diversity *H'*		1.71		2.16		1.42		1.83	
Evenness *J'*		0.54		0.78		0.50		0.64	

Table 3. Prey selection by *G. holbrooki* and *V. letourneuxi* from Louros and Acheron rivers. Positive selectivity (preference, p); negative selectivity (avoidance, a). There was no selection apparent for the remaining macroinvertebrate categories (not shown in the Table).

| | Louros | | | | | | Acheron | | | | | | |
| | | *G. holbrooki* | | | *V. letourneuxi* | | | | *G. holbrooki* | | | *V. letourneuxi* | |
Prey group	Di	Ai	Wi		Ai	Wi		Di	Ai	Wi		Ai	Wi	
Acari														
Hydrachnidae	0.43	1.89	**4.41****	p	1.00	2.32^{ns}		-	-	-		-	-	
Crustacea														
Gammaridae	5.91	0.11	**0.02****	a	0.00	**0.00****	a	11.30	39.71	**3.51****	p	52.76	**4.67****	p
Asselidae	-	-	-		-	-		0.35	0.00	0.00^{ns}		4.91	**14.11****	p
Gastropoda														
Bithyniidae	19.23	0.00	**0.00****	a	0.00	**0.00****	a	0.52	0.00	0.00^{ns}		0.00	0.00^{ns}	
Hydrobiidae	3.11	0.00	**0.00** *	a	0.00	0.00^{ns}		54.43	0.00	**0.00****	a	4.29	**0.08****	a
Valvatidae	4.51	0.00	**0.00****	a	0.00	**0.00** *	a	-	-	-		-	-	
Diptera														
Chironomidae L	29.32	20.82	**0.71****	a	64.18	**2.19****	p	4.87	1.47	0.30^{ns}		10.43	**2.14****	p
Ephydriidae L	0.11	0.67	**6.22****	p	2.99	**27.79****	p	0.00	4.41	∞	p	2.45	∞	p
Heteroptera														
Veliidae	0.11	3.23	**30.07****	p	0.50	4.63^{ns}		0.00	8.82	∞	p	0.61	∞	p
Naucoridae	0.21	0.00	0.00^{ns}		0.00	0.00^{ns}		0.17	4.41	**25.37****	p	0.00	0.00^{ns}	
Corixidae	6.34	0.00	**0.00****	a	0.00	**0.00****	a	-	-	-		-	-	
Trichoptera														
Hydroptilidae	8.70	0.11	**0.01****	a	0.50	**0.06****	a	-	-	-		-	-	
Limnephilidae	6.66	0.00	**0.00****	a	0.00	**0.00****	a	-	-	-		-	-	
Coleoptera														
Dytiscidae L	0.54	0.22	0.41^{ns}		2.99	**5.56****	p	-	-	-		-	-	
Hydraenidae A	0.11	0.33	**3.11** *	p	0.00	0.00^{ns}		-	-	-		-	-	
Hydrophilidae L	0.11	0.45	**4.15****	p	0.00	0.00^{ns}		-	-	-		-	-	

Di: prey availability; *Ai* prey use; *Wi* Savage index, ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$

positively selected in Acheron (Table 3). Overall, both species exhibited spatial plasticity in their diet selectivity, evident especially from the differential selectivity for Gammaridae in the two habitats.

Comparing the positive selectivities of the two species, in Louros only Ephydridae larvae were positively selected by both species, whereas in Acheron three categories (Ephydridae larvae, Veliidae and Gammaridae), were positively selected by both species, indicating a convergence of their dietary preferences in the brackish Acheron (Table 3).

Diet diversity

The group trophic diversity (*H'*) of both species increased significantly from Louros to Acheron (for *G. holbrooki,* $t = 2.44$, $p = 0.017$, for *V. letourneuxi*, $t = -2.63$, $p = 0.009$, see also Table 2). In contrast, individual trophic diversity (*H'*$_{ind}$) tended to decrease (in *G. holbrooki* from 1.100 ± 0.063 in Louros to 0.291 ± 0.080 in Acheron, in *V. letourneuxi* from 0.709 ± 0.101 in Louros to 0.560 ± 0.085 in Acheron; only statistically significant in *G. holbrooki*, $t = 8.09$, $p = 0.000$). These opposite trends in population and individual diet diversity are also evi-

dent in the Tokeshi plot, where values of *H'*$_{ind}$ are plotted against *H'* values (Fig. 3a). In terms of population niche width (*H'*), the Acheron points for both species indeed suggest a more diverse group diet. In terms of individual niche width (*H'*$_{ind}$), the Acheron points for both species are located lower than the Louros ones, indicating a less diverse *individual* diet, mostly in *G. holbrooki* (Fig. 3a).

Interspecies comparison shows that group trophic diversity (*H'*) of *G. holbrooki* was higher than that of *V. letourneuxi* in both habitats (statistically significant difference only in Louros, $t = 2.80$, $p = 0.005$). A similar trend was also evident in the Tokeshi plot (Fig. 3a), as both *G. holbrooki* points were located more to the right of the plot than *V. letourneuxi* points, indicating a wider population trophic niche for *G. holbrooki* and a more diversified diet.

Feeding strategy

According to the Costello graphs, at the population level, *G. holbrooki* exhibited a generalist feeding strategy, as no prey point was located at the upper right of the diagram (Fig. 3b). At the individual level, however, there was a shift in its feeding strategy, i.e. in Louros,

294

Medit. Mar. Sci., 15/2, 2014, 287-301

most prey points were located in the lower half of the diagram, denoting that the population consisted *solely* of generalist individuals. In Acheron, however, a fraction of the population adopted a specialist strategy, with little or no overlap in resource use (Fig. 3b). These varied individual feeding strategies of *G. holbrooki* in Acheron resulted in its wider population niche width, as well as in the narrower individual niche widths in this habitat, described above.

In contrast, at the population level, *Valencia letourneuxi* exhibited, in both sites, some group specialization directed towards Chironomidae larvae (in Louros) and Gammaridae (in Acheron, Fig. 3b). This group specialization was less pronounced in Acheron than in Louros, resulting in the higher population niche width of the Acheron population. At the individual level, all but one of the remaining prey points were located in the lower half of the diagram, indicating, in contrast to *G. holbrooki,* wide *individual* niche widths at both sites.

Overall, as prey availability decreased (from Louros to Acheron), both species retained their population strategies; however, *G. holbrooki* modified its *individual* strategy from generalization to some degree of specialization.

Diet overlap

NMDS ordination plots of abundance dietary data revealed similar spatial trends in the diet of both species, namely a high degree of spatial dietary plasticity (Fig. 4). More specifically, in *G. holbrooki* the Acheron samples tended to be distinguished from the Louros samples, as they were arranged at their periphery (Fig. 4a). The Louros samples were also more tightly grouped together and characterized by lower dispersion (MVDISP index = 0.867) than the Acheron samples (1.281, higher between-individual variation). ANOSIM showed a statistically significant dissimilarity between the two samples ($R = 0.616$, $p = 0.001$) while the Morisita-Horn overlap index value revealed no biologically significant diet overlap between the two *G. holbrooki* populations ($C_H = 0.068$).

In *V. letourneuxi* too, the two groups tended to be distinguished (Fig. 4b). In terms of dispersion, the diet of *V. letourneuxi* was also more homogenous in Louros (MVDISP in Louros: 0.933) compared to the Acheron population (1.020, higher ingroup variation). ANOSIM revealed a statistically significant dissimilarity between the diets of the two populations (ANOSIM Louros *vs* Acheron, $R = 0.637$, $p = 0.001$) and, again, the Morisita-Horn overlap index values revealed no biologically significant diet overlap between the two populations (Louros *vs* Acheron $C_H = 0.189$). Overall, NMDS ordination plots confirmed the high spatial feeding plasticity exhibited by both species at the population level, with both shifting to a different and more heterogeneous diet at the Acheron site.

Interspecies comparison, however, revealed dif-
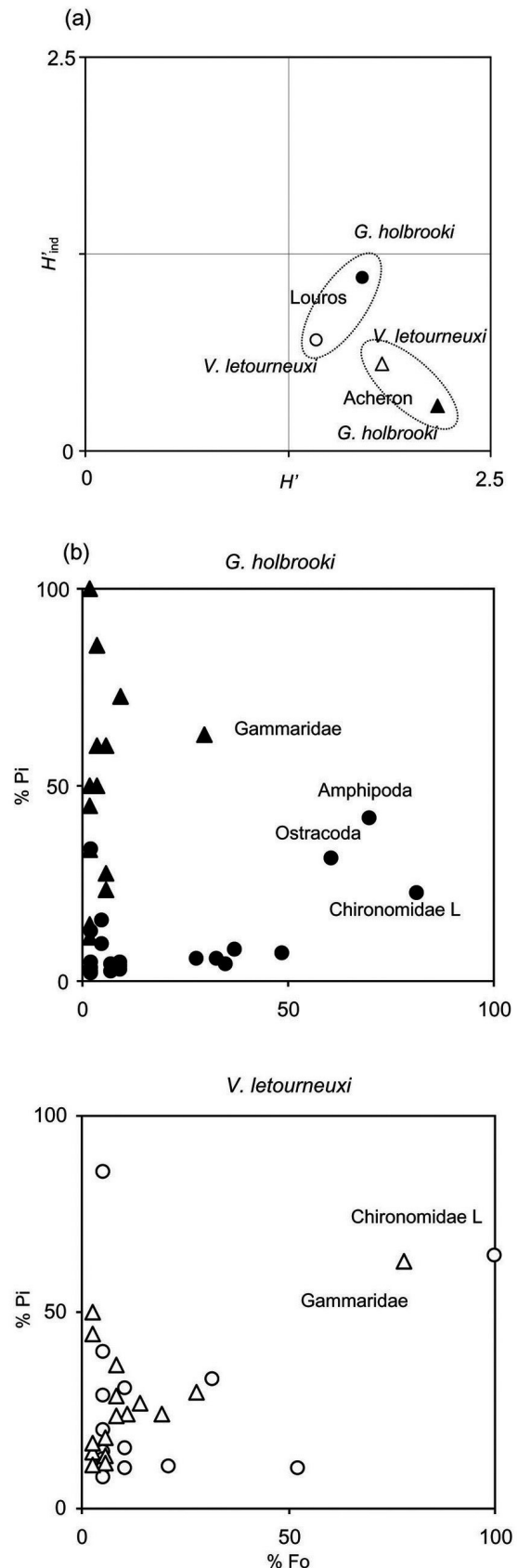
Fig. 3: (a) Tokeshi plot of the feeding patterns of *G. holbrooki* (black) and *V. letourneuxi* (white) in Louros (circles) and Acheron (triangles) drainages. (b) Costello feeding strategy plots of *G. holbrooki* and *V. letourneuxi*. Frequency of occurrence (%Fo), Prey specific abundance (%Pi).

Medit. Mar. Sci., 15/2, 2014, 287-301

295
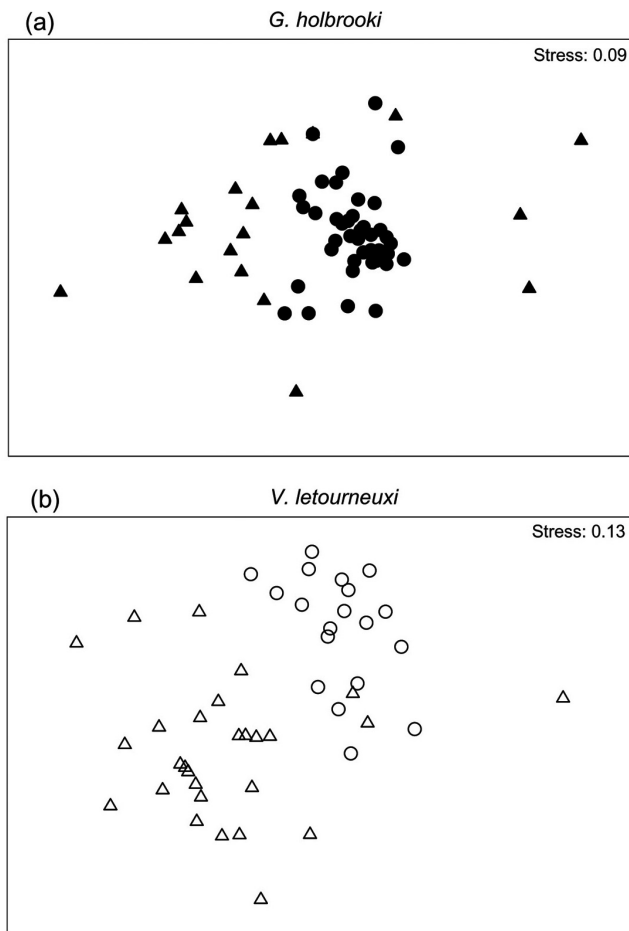
Fig. 4: MDS ordination plots of between-habitat variation (Louros *vs* Acheron) in the diet of *G. holbrooki* (a) and *V. letourneuxi* (b). Circles: Louros; Triangles: Acheron.

ferences in their trophic interactions between the two habitats (Fig. 5). In Louros (Fig. 5a), there was limited overlap between the two samples, with *G. holbrooki* having a higher dispersion value (MVDISP index = 1.039, higher between-individual variation) than *V. letourneuxi* (MVDISP index = 0.928). ANOSIM revealed a dissimilarity between the samples ($R = 0.285$, $p = 0.001$) and the Morisita-Horn index confirmed that there was marginally no biologically significant diet overlap between the two species in Louros ($C_H = 0.500$).

In Acheron NMDS ordination plots in contrast (Fig. 5b), there was a clear overlap between the two samples, with *G. holbrooki* again having a higher dispersion value (MVDISP index = 1.186) than *V. letourneuxi* (MVDISP index = 0.856). ANOSIM revealed a dissimilarity between the samples, but with a very low R value ($R = 0.115$, $p = 0.001$) and the Morisita-Horn index showed very high dietary overlap between the two species in Acheron ($C_H = 0.877$).

Overall, the diet of the two species overlapped highly in Acheron, while in Louros there was marginally no diet overlap.

Discussion

This study focused on the trophic patterns and dietary interactions of the Eastern mosquitofish and its co-occurring native *V. letourneuxi* in order to explore putative trophic derivative impacts, such as competition for resources. This highly successful non native fish species exhibited a more diverse, opportunistic and adaptive feeding and, furthermore, readily switched to locally available resources as well as to higher plant material consumption, under conditions of lower animal food availability. The current study also revealed a diet overlap between the two species suggesting that resource competition could potentially result in a negative ecological impact of *G. holbrooki* on *V. letourneuxi*. However, the intensity of this competition appears to be mediated by resource availability, since diet overlap varied considerably between the two different sets of macroinvertebrate fauna attributes at the two study sites.

Conducting this study only in the summer period is potentially a shortcoming, since there are often marked seasonal fluctuations in resource availability in Mediterranean aquatic systems that can profoundly affect food resource use. However, the wet season is actually characterized by lower macroinvertebrate and algae biomass
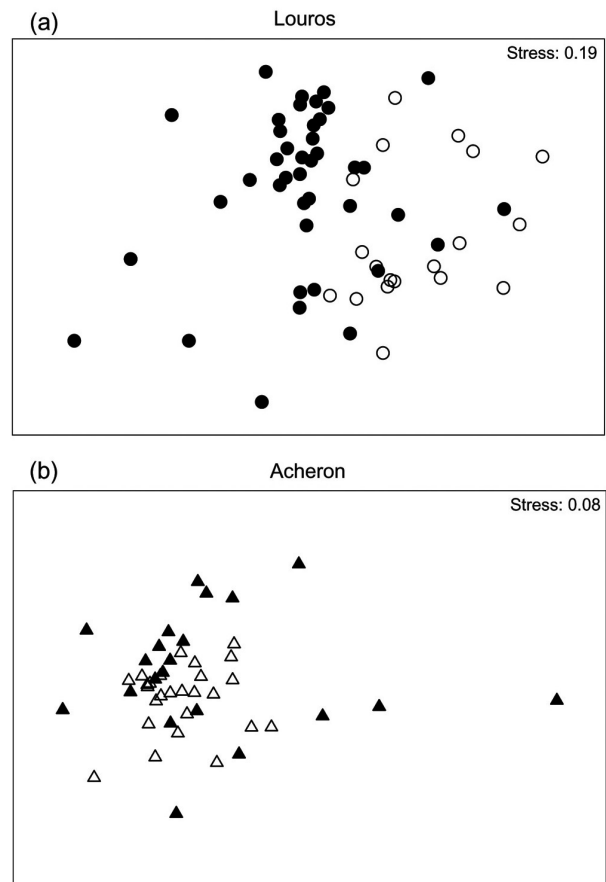
Fig. 5: MDS ordination plots of between-species diet variation (*G. holbrooki vs V. letourneuxi.*) in Louros (a) and Acheron (b). Black marks *G. holbrooki*; White marks: *V. letourneuxi*.

availabilities (for the seasonality of macroinvertebrates in a typical *V. letourneuxi* habitat, see Kalogianni *et al.*, 2010b); consequently, the intensity of the competitive interactions inferred here for the summer period could be even more enhanced in winter. Furthermore, the profound differences of the two study sites as regards macroinvertebrate fauna availability, enabled a comparative study of the ecological interactions of the two species under two very distinct abiotic and biotic conditions. Therefore, limiting our study in these two habitats does not in any way affect the results presented here. Consequently, our approach could be a useful insight into the impact of the widespread non native mosquitofish on native ichthyofauna and its underlying mechanism(s).

Comparison of feeding patterns

In the freshwater Louros habitat, both species consumed mostly Chironomidae larvae, which were the dominant available prey, while in Acheron, they both switched to the readily available Gammaridae crustaceans. Thus, both *G. holbrooki* and *V. letourneuxi* displayed a flexible animal feeding, denoting their ability to exploit locally available resources. The two habitats varied indeed significantly in terms of food availability and composition, with Louros characterized by a richer and more diverse macroinvertebrate fauna, in sharp contrast to the brackish Acheron with its typically lower macroinvertebrate abundance and diversity (Williams *et al.*, 1990; Merritt & Wallace, 2003). Furthermore, there was evidence of anthropogenic disturbance in Acheron, based on the absence there of either invertebrates requiring high-quality waters, such as Ephemeroptera and Trichoptera larvae (Tachet *et al.*, 2000; Ribeiro *et al.*, 2007) or of macroinvertebrate categories sensitive to human-induced disturbance, according to the Greek river macroinvertebrate index (Hellenic Evaluation Score, HES, Artemiadou & Lazaridou, 2005), all present in Louros. Anthropogenic pressures, such as urban settlements and land reclamation schemes have heavily modified the deltaic area of Acheron. This, together with the brackishness of the habitat (that would explain, for example, the absence of Trichoptera), possibly account for its less rich and less diverse macroinvertebrate fauna.

Relative food scarcity in Acheron resulted in lower feeding intensity rates in both species, coupled however, especially in the case of *G. holbrook*i, with higher consumption of algae and plants (a readily available food source, albeit with low absorption rate and energy content, Ribeiro *et al.*, 2007). Diet composition, however, usually reflects not only prey availability, but also a trade-off between other factors, such as prey susceptibility, prey handling capability, energy intake and risk of predation, that could further explain the observed prey selectivities (Oscoz *et al.*, 2008). Thus, limited prey handling capability (gape size limitations and/or prolonged

handling times for their energy intake) might explain the negative selection in both habitats and by both species of the readily available gastropods, such as Hydrobiidae and Bithyniidae, which are large hard-bodied prey that must be actively extracted from the substratum (Maldonado *et al.*, 2009). Conversely, high prey susceptibility (low mobility) and high energy intake could explain the major contribution of Chironomidae larvae to their diet in Louros where it was the commonest animal prey available, as well as its positive selection by *V. letourneuxi* in both habitats. Finally, a result of the interplay of prey availability and energy intake could be the differential selectivity of Gammaridae that were refused by both species in Louros, probably due to their intense sclerotization (and thus lower energetic value) and the relative availability of other preferential preys, but positively selected in the much poorer Acheron habitat, where their availability was high and other preferred preys were lacking, as evidenced by our macroinvertebrate sampling data (see also Oscoz *et al.*, 2008).

Moreover, the increase in the trophic niche breadth of both species from Louros to Acheron was particularly striking and evidenced by their higher diet diversity and evenness indexes. This increase appears to be in accordance with the optimal foraging theory (OFT), which predicts an increase in population niche width as resource availability decreases (MacArthur & Pianka, 1966; Schoener, 1971). Overall, at a spatial level, both species exhibited high dietary plasticity, modified their dietary preferences and increased their group diet diversity under conditions of macroinvertebrate scarcity.

However, although both species displayed the above adaptive traits in response to food availability differences, interspecies comparison, showed that *G. holbrooki*, at both study sites, had a more generalist diet at the population level, with wider diet breadths compared to *V. letourneuxi*, thus supporting the notion that successful invasive species generally have more diverse diets than native species (Arthington & Marshal, 1999; Ribeiro *et al.*, 2007). This is in agreement with the results of Ruiz-Navarro *et al.* (2013), which also showed that both the mosquitofish and its co-occurring Iberian toothcarp *A. iberus* presented, under three salinity conditions in an Iberian wetland, high dietary spatial plasticity. Overall, however, the mosquitofish fed more broadly than *A. iberus*. Having a generalist diet could indeed confer good adaptability to any habitat, and this biological feature has been considered advantageous for different stages of the invasion process involving non native species (Ribeiro *et al.*, 2007).

In addition, plant material consumption was much more frequent in *G. holbrooki* compared to *V. letourneuxi*; high levels of algae consumption by the mosquitofish have been previously reported elsewhere (e.g. for the *G. holbrooki* population of Lake Hévíz, Hungary, Specziár, 2004), while low levels of plant consumption were also

Medit. Mar. Sci., 15/2, 2014, 287-301

297

reported for the only *V. letourneuxi* population previously studied (Kalogianni *et al.*, 2010b). The higher plant material consumption of the mosquitofish, especially in the Acheron habitat, however, denotes a shift of *G. holbrooki* to a suboptimal but easily accessible and readily available food resource, possibly as an efficient buffer strategy when animal resources are scarce (Magalhães, 1993; Ribeiro *et al.*, 2007). Coupled with its more diverse diet, this strategy may favour *G. holbrooki* even more than *V. letourneuxi* as regards the exploitation of available resources.

Furthermore, *G. holbrooki* consumed, much more frequently, terrestrial preys such as drifting insects, which are easier to detect and have lower evasive ability during their emergence (McLaughlin *et al.*, 1994) and can thus constitute an important and valuable prey. This difference in terrestrial prey consumption cannot be attributed to differences in their morphology, since both species have a flattened head and a terminal, upward mouth that would in both cases enhance surface feeding (Barbieri *et al.*, 2000; Pyke, 2005). It could, however, indicate a displacement of *V. letourneuxi* from the surface in the habitats where the two species co-occur, as a form of competition induced niche shift, similar to that induced to the Andalusian toothcarp *Aphanius baeticus* Doadrio, Carmona & Fernández Delgado, 2002 by its co-occurring species, and to other native fishes by the mosquitofish (Clavero *et al.*, 2007; Arthington, 1991; Keller & Brown, 2008). Indeed, preliminary data comparing the diet of a sympatric *versus* an allopatric *V. letourneuxi* population in two similar freshwater habitats seem to corroborate the above assumption (Kalogianni *et al.*, unpublished data).

Finally, in our study, feeding strategy plots revealed that in Louros, where both species have lower population densities and resources are more abundant, both followed a generalist *individual* strategy, consisting of generalist individuals. In Acheron, however, where both species have higher population densities and resources are scarcer, only *G. holbrooki* shifted to a mixture of generalist and specialist individual strategy, with a fraction of the population becoming specialists. This individual feeding specialization (high between-individual diet variation) led to a significant decrease in the individual niche breadth of the mosquitofish. Previous studies have indeed demonstrated a positive relationship between population density (a proxy for intraspecific competition) and individual specialization (Svanbäck *et al.*, 2008; Araújo *et al.*, 2008; Svanbäck & Persson, 2009; Araújo *et al.*, 2011), and others showed that lower resource abundance also coincided with the inclusion of novel resources via increased inter-individual variation (Svanbäck *et al.*, 2011). We therefore tentatively postulate that in Acheron, under conditions of resource scarcity and stronger intraspecific competition, due to higher population densities, *G. holbrooki* modified its individual feeding strategy shifting to individual specialization, in order to alleviate intraspecific competition.

Furthermore, this shift led to the observed increase in its population niche, albeit through a different mechanism, i.e. individual specialization, rather than an increase in individual niche breadth predicted by OFT (see Araújo *et al.*, 2008). Finally, individual feeding specialization may also reflect clumped prey distributions (Ward *et al.*, 2006), i.e. short-term increases in the local abundance of certain prey items. In the Acheron, with its relatively impoverished macroinvertebrate fauna, this shift of the mosquitofish to individual specialization could result in a competitive advantage over the native *V. letourneuxi*, through more efficient exploitation of even temporally available resources.

Overall, *G. holbroooki* consistently exhibited a much more diverse diet, a wider feeding spectrum and a more generalist feeding strategy at the population level compared to the native species. In addition, under conditions of relative animal food scarcity, it greatly modified its diet by increasing plant material consumption and adapting its individual feeding strategy, to alleviate intraspecific competition and better explore even temporal fluctuations in prey availability. We suggest that all the above would confer an advantage to the mosquitofish over the native species, ultimately affecting the stability and abundance of its populations, in the aquatic systems where the two species co-occur and thus compete for the same (often limited) resources.

Underlying mechanisms of interspecies interactions

The mechanisms proposed by various authors for the negative impact of the mosquitofish on *V. letourneuxi* populations were competition with and aggression from *G. holbrooki*, as well as predation on its young developmental stages (Stephanides, 1964; Bianco & Miller, 1989; Economidis, 1995). In the current study, there was some evidence of opportunistic mosquitofish piscivory, which is in agreement with the experimental observation of mosquitofish predation on the small *A. iberus*, an Iberian cyprinodontiform (Rincón *et al.*, 2002). However, despite the circumstantial evidence for fish predation by *G. holbrooki* presented here, this apparently is not a major form of interaction with the native Corfu toothcarp.

The overlap, however, in the diet of the two species is indicative of competition for trophic resources that can have a negative impact on the native species. Theoretically, overlap of trophic position and feeding habits of non-native fishes with native species can result in trophic competition, due to reduced amounts of available food resources. Classical ecological theory predicts that, in that case, trophic competition will lead to resource partitioning or niche divergence. Several modifications of the classical competition theory have been proposed, such as that of Wiens (1993) that related niche overlap to competition strength and resource level variation. According to Wiens, when resources become limiting, there is indeed niche divergence and consequently low overlap in the

298

Medit. Mar. Sci., 15/2, 2014, 287-301

diet of co-occurring species, but when resources become extremely limited, species are forced to converge in order to exploit the remaining resources, and overlap becomes high. The increase in diet overlap between our two target species from Louros to Acheron fits well to Wien's theory. Namely, in Louros, differences in overall diet and prey electivities, coupled with low diet overlap indicate some degree at least of resource partitioning, an indirect result of trophic competition. In Acheron, the higher similarity in their overall diet, the convergence of their prey electivities and the substantial diet overlap indicate strong trophic competition. Previous dietary studies have indeed postulated resource partitioning or conversely niche overlap between the mosquitofish and other native species by comparing overall diet patterns (mostly in Australian aquatic systems, see Pen *et al.*, 1993; Arthington & Marshall, 1999), but these were not corroborated by data on resource availability. The current study, by exploring trophic overlap in a comparative context and simultaneously assessing differential prey availability, is to our knowledge the first to present concrete evidence of a resource mediated trophic competition between a widespread non native fish species and an endemic species. In contrast, a recent study on the diet of the mosquitofish and its co-occuring *A. Iberus* in three sections of an Iberian wetland, correlated their trophic overlap not to prey availability, but to mosquitofish relative abundance as well as salinity, which is in turn related to mosquitofish aggression (Ruiz-Navarro *et al.*, 2013). In this wetland, however, there were no marked differences in prey availability among the three sections studied, in contrast to the two Greek rivers studied here. Furthermore, the variations of the invasive/native species ratio and of salinity in the three wetland sections were much more pronounced, compared to those in the two Greek habitats. Nevertheless, Ruiz-Navarro *et al.* (2013) reached the same conclusion with the authors of the current study, namely that the native species could be subjected to a decrease in its population viability, as a direct consequence of trophic competition with the invasive species.

Theoretically, the intensity of trophic competition between the mosquitofish and the Corfu toothcarp could be reduced through mechanisms such as segregation of microhabitats or differential diet activity patterns (Oscoz *et al.*, 2008). However, the feeding zones of the two species tend to overlap in the water column, and both appear to be diurnal feeders (for the mosquitofish see Pyke, 2005; Ling & Willis, 2005; for *V. letourneuxi*, unpublished data).

Aggressive behavioural interactions (interference competition) could be a second mechanism of negative interactions between the Eastern mosquitofish and *V. letourneuxi*, such as the one shown between *G. holbrooki* and two Iberian endemics in experimental settings (Rincón *et al.* 2002; Caiola & de Sostoa, 2005). Indeed, aggressive interactions between our two target species have been ob-

served in the field, as both use similar vertical positions in the water column. Furthermore, this type of competition between the two species could be condition-specific, affected by abiotic factors too, such as temperature. A recent study has indeed shown that *G. holbrooki* food capture rates and aggression towards the endemic Iberian toothcarp *A. iberus* increased at higher temperatures (Carmona-Catot *et al.*, 2013). We can therefore infer that, especially in Acheron, with its higher water temperatures and relatively limited resources, interference competition could act synergistically with exploitative competition, leading to negative changes in the breeding success, survival and ultimately population demographics of the native species. Furthermore, globally increasing temperatures could exacerbate these negative interactions between the highly invasive, warm water mosquitofish and native Mediterranean freshwater species.

Conservation implications

From a conservation point of view, as stated by Ribeiro & Leunda (2012), it is very important not only to identify the impacts of non native freshwater fish species without uncertainties, but also to evaluate whether these vary across locations and aquatic systems. This information is particularly important for the design of appropriate conservation management policies, especially in the highly endemic Mediterranean systems where such information is lacking. This lack of knowledge is even more acute in the case of the Corfu toothcarp *V. letourneuxi*, which, together with the Valencia toothcarp *V. hispanica*, - native to Spain and the only other member of the genus- are two rare, critically endangered Mediterranean species, with very restricted distributions and low population densities. Therefore, the study of their interactions with invasives, such as the widespread mosquitofish, are critical for the formulation of appropriate and urgently needed conservation measures.

In that respect, the current study presents evidence, firstly, that the performance (condition, growth, reproduction) of the native *V. letourneuxi* populations may be adversely affected by the presence of the mosquitofish, through trophic competition (and possibly interference competition) and, secondly, that these potential detrimental impacts could be more severe in habitats with higher fish densities, resource limitations, and/or environmental stress. Therefore, preventing mosquitofish invasion in the three remaining habitats in Greece, which support high density allopatric populations of *V. letourneuxi*, should become a major priority (Kalogianni *et al.*, 2010a; Giakoumi *et al.*, 2010). Moreover, biotic and abiotic factors (such as animal resource availability and high water temperatures) should be additional criteria employed in the identification of those *V. letourneuxi* populations targeted for immediate conservation intervention, in order to reverse the decline of the species due to the adverse effects of the invasive mosquitofish.

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300

Medit. Mar. Sci., 15/2, 2014, 287-301

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Medit. Mar. Sci., 15/2, 2014, 287-301

301