

Feeding habits of the exotic black bullhead *Ameiurus melas* (Rafinesque) in the Iberian Peninsula: first evidence of direct predation on native fish species

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The feeding ecology of the exotic invasive black bullhead *Ameiurus melas* was conducted in the Iberian Peninsula for the first time. Dietary analysis based on the stomach contents of individuals caught in several Iberian basins was carried out as a first step to evaluate its potential threat for the native Iberian ichthyofauna. Aquatic macroinvertebrates (mainly Chironomidae) dominated the black bullhead's diet in all size-classes and sites, irrespective of natural riverine or artificial lentic habitats. Secondary prey items were responsible for the observed between-sites (microcrustaceans in artificial lentic habitat; oligochaeta and caddisfly larvae in natural riverine habitats) and ontogenetic diet differences (from microcrustaceans to larger prey). These diet variations were also detected in trophic diversity values and feeding strategy plots. Black bullheads consumed plant material, terrestrial prey and co-occurring fish species (native or exotic) and thus they could be considered as generalist or opportunistic, foraging on the most abundant and available prey. There was no positive relationship between black bullhead size (total length) and fish prey size, probably indicating piscivory on dead or dying vulnerable fishes as well as predation on smaller-sized active fishes. The results showed that the black bullhead could negatively affect native Iberian ichthyofauna throughout direct predation and competition. Aspects of potential conservation and management implications of fishes resulting from the undesirable presence of the black bullhead in Iberian water bodies are discussed.

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Key words: *Ameiurus melas*; diet; Ebro River basin; invasive; piscivory; Tagus River basin.

INTRODUCTION

Biological invasions after either intentional or unintentional introduction of exotic fish species is a worldwide environmental problem of growing concern. Despite biological invasions resulting from climatic or geotectonic events are

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commonplace in nature, humans have provided a variety of pathways (e.g. unauthorized and authorized stocking, water transfer systems, ship ballast water, fishing and angling and ornamental fish release) by which exotic fish species can circumvent historical biogeographic barriers (Rahel, 2007). Due to the unpredictability (the 'Frankenstein Effect'; Moyle *et al.*, 1987), and in most cases irreversibility, of biological invasions in aquatic environments, there is an increased concern over the potential effects of introduced species on native species, ecosystems and local or even national socio-economics (Copp *et al.*, 2005). Among the most dramatic effects of exotic fish species that may lead to the decline of native species are: (1) predation that results in displacement or density reduction of native species' populations (Elvira *et al.*, 1996; Moyle *et al.*, 2003); (2) competition for food and habitat often through aggressive behaviour (Rincón *et al.*, 2002; Caiola & de Sostoa, 2005); (3) hybridization among closely related species that threatens genetic integrity if they behave introgressively (Costedoat *et al.*, 2005; Chen *et al.*, 2007); (4) faunal homogenization when a few cosmopolitan species come to dominate communities at the expense of unique native species (Moyle & Mount, 2007; Rahel, 2007); (5) habitat alteration and degradation (Richardson *et al.*, 1995; Pinto *et al.*, 2005); and (6) pathogen and parasite transmission (Leberg & Vrijenhoek, 1994). Too often, the end result of these effects has been the extinction of native fish species (Clavero & García-Berthou, 2005).

Historically, biogeographic barriers to the movement of aquatic organisms at multiple spatial scales contributed to the development of unique regional faunas (Rahel, 2007). The biogeographic isolation of the Iberian Peninsula by surrounding oceans and the Pyrenees, together with catchment divides and discreteness, mountain ranges and climatic conditions allowed the development of a highly endemic freshwater fish fauna, composed mainly of small to medium sized cyprinids, cobitids and cyprinodontids in communities naturally lacking obligate piscivorous species (Crivelli, 1995; Elvira, 1995). Unfortunately, there is a long history of exotic fish introductions that has accelerated greatly over time as methods of transportation improved (Copp *et al.*, 2005). As a result, during the last century and more acutely in the last decades, many of the native Iberian species have been showing a decline both in their ranges and abundances, while as many as 25 exotic fish species successfully continue to spread (Almaça, 1995; Elvira & Almodóvar, 2001).

The black bullhead *Ameiurus melas* (Rafinesque) (Ictaluridae, Siluriformes) is a freshwater catfish indigenous to North America from the Great Lakes to northern Mexico (Page & Burr, 1991). This demersal species inhabits low-flowing habitats (limnophilic) with soft substrata in all riverine and lacustrine environments, including artificial ones such as ponds and reservoirs. Like other members of the Ictaluridae, this species is active mainly at night, feeding mainly on immature insects, leeches and crustaceans when young, while adults also feed on clams, snails, plant material and fishes (Page & Burr, 1991; Declerck *et al.*, 2002). Though the species has been reported to exceed 600 mm in total length (L_T) and 3.5 kg in mass, the individuals caught to date in Iberia do not reach these sizes (Doadrio, 2001). As Copp *et al.* (2005) stated, the black bullhead was introduced to some European countries in the late 19th and early 20th century (e.g. 1910–1913 in Spain; Elvira & Almodóvar, 2001),

but only began to appear in other countries in the late 20th and early 21st century (*e.g.* Portugal; Gante & Santos, 2002; Ribeiro *et al.*, 2006). In Iberia, where native obligate piscivorous fish species are naturally absent, the motivation for black bullhead official introduction was the 'improvement' of the fish communities by filling this 'vacant' niche in newly created reservoirs in Spain, which were also popular angling sites (Elvira & Almodóvar, 2001; Copp *et al.*, 2005). Despite these first black bullhead introductions in Spain being authorized and very localized (*e.g.* Lake Banyoles), the subsequent rapid and large distribution range expansion that continues in Iberia has been due to illegal and indiscriminate introductions for angling purpose (Elvira, 1984; Elvira *et al.*, 1998). Amongst the life history and ecological attributes that seem to characterize the success of the black bullhead as an invasive species are: high fecundity, parental care of the offspring, voracious and versatile feeding habits, habitat and water quality flexibility (withstanding water temperatures as high as 30° C, high turbidity, a wide range of pH and even hypoxia) and tolerance to pollution, as has been shown for many other invasive fish species in Mediterranean catchments (Alcaraz *et al.*, 2005; Vila-Gispert *et al.*, 2005; Ribeiro *et al.*, 2008). These characteristics constitute a real rather than a potential threat for the Iberian ichthyofauna (Gante & Santos, 2002).

In a situation where a highly endemic fauna of great ecological value such as the Iberian ichthyofauna is facing a growing impoverishment, and even a threat of extinction, due to exotic invaders, research is justified. Even if the real effect of most exotic fish introductions on Iberian fish species is still unknown (Elvira & Almodóvar, 2001), a growing research effort in recent years is contributing to the quantitative information available. In particular, feeding studies based on stomach content of some exotic fishes have already been carried out in Iberia (García-Berthou, 1999*a, b*; García-Berthou & Moreno-Amich, 2000*a*; García-Berthou, 2001), some of them showing clear negative interaction through predation on native fishes (Elvira *et al.*, 1996; Nicola *et al.*, 1996; Domínguez & Pena, 2000; García-Berthou & Moreno-Amich, 2000*b*; Domínguez *et al.*, 2002; García-Berthou, 2002; Rincón *et al.*, 2002). The goal of the present research was to evaluate for the first time the threat of the exotic black bullhead by direct predation on and competition with the native ichthyofauna through dietary analyses. Therefore, samples were collected from several localities within Iberia to describe for the first time the black bullhead's diet with emphasis on piscivory, and to test for dietary differences between sites and habitats and throughout ontogeny.

MATERIALS AND METHODS

STUDY SITES AND FISH SAMPLING

Specimens of *A. melas* were captured at various localities throughout the Arga, Ebro and Guadarrama Rivers in Spain and a small reservoir at Ribeira de Santo Estevão in Portugal (Fig. 1). The Arga River is a 145 km long tributary of the Ebro River that flows north-to-south and drains a catchment area of 2760 km² entirely within the province of Navarra. A total of 99 specimens were captured by means of electrofishing along the Arga River (77–167 mm total length, L_T), from which 11 specimens were



FIG. 1. Location map of the sampled water bodies in the Iberian Peninsula.

captured in November 2003, while the remaining 88 specimens were captured in October 2004 and October 2005 and deposited in the Museum of Zoology, University of Navarra (MZNA) collection (MZNA 136030 to 136033, 137460, 137461). In addition to *A. melas*, the fish assemblage in the sampling sites of the Arga River was determined to be composed of the native *Barbus graellsii* Steindachner, *Parachondrostoma miegii* (Steindachner), *Gobio lozanoi* Doadrio & Madeira, *Phoxinus phoxinus* (L.) and *Barbatula barbatula* (L.) and the exotic *Cyprinus carpio* L. In the mainstem Ebro River in the province of Navarre, 51 specimens (39–204 mm L_T) were caught by means of electrofishing in October 2005 and deposited in the MZNA collection (MZNA 137194 to 137202). Along with *A. melas*, the endemic *B. graellsii*, *P. miegii*, *G. lozanoi*, *P. phoxinus* and *Salaria fluviatilis* (Asso), and the exotics *C. carpio*, *Carassius auratus* (L.), *Alburnus alburnus* (L.), *Micropterus salmoides* (Lacépède) and *Silurus glanis* L. were also found to occur in this stretch of the Ebro River. The Guadarrama River is a right-sided tributary of the Tagus River that drains a catchment area of 1700 km² along its 130 km length in the provinces of Madrid and Toledo. Along the Guadarrama River, 74 specimens (31–265 mm L_T) were captured by means of electrofishing in July, September and October 2006. The fish assemblage was composed of the endemic *Barbus bocagei* Steindachner, *Achondrostoma arcasii* (Steindachner), *Pseudochondrostoma polylepis* (Steindachner), *Squalius alburnoides* (Steindachner), *Squalius pyrenaicus* (Günther) and *Cobitis paludica* (De Buen), and the exotics *G. lozanoi*, *C. carpio*, *C. auratus*, *Lepomis gibbosus* (L.) and *Gambusia holbrooki* Girard. An additional total of 32 specimens were lent for this study by Museu Bocage (Lisbon, Portugal) (MB/05-1808 and MB/05-1809). As described by Gante & Santos (2002), 31 specimens (107–123 mm L_T) were collected by angling with bait from a small reservoir at Ribeira de Santo Estevão (Tagus River basin) in August 2000, while another specimen (289 mm L_T) was collected with gillnets in February 2001 in the Guadiana River (Fig. 1), and constituted the very first records of *A. melas* in Portugal. Apart from *A. melas*, some other non-natives such

as *L. gibbosus* and *M. salmoides* also occurred in the small man-made reservoir at Santo Estevão (H. F. Gante, pers. comm.). All fishes captured were stored immediately on ice but not frozen and transported to the laboratory where the specimens were fixed in 4% formaldehyde solution for 48 h and afterwards preserved in 70% ethanol solution.

LABORATORY METHODS AND DIET ANALYSIS

Once in the laboratory, fishes were measured (L_T to the nearest mm), weighed (to the nearest 0.1 g), eviscerated and the entire gut preserved in 70% ethanol until analysis. The specimens analysed were assigned to three L_T classes: <100 mm L_T , 100–150 mm L_T and >150 mm L_T . The stomach contents of all *A. melas* ($n = 255$) were examined under a dissecting microscope ($\times 7$ to $\times 45$). Four prey item groups were identified: plant material (not quantified), terrestrial invertebrates, aquatic invertebrates and fishes. When possible, aquatic invertebrates were identified to family level, terrestrial invertebrates to order level and fishes to species level resulting in a total of 51 prey items. The vacuity index (% I_V) was calculated as the percentage of empty stomachs to the total number of stomachs examined (Hyslop, 1980).

Detrended correspondence analysis (DCA) was used to describe the main sources of diet variation (Legendre & Legendre, 1998). With dietary data, this statistical procedure allows grouping similar food items based on their common occurrence and identifies the axes explaining the highest proportion of variation. For the DCA, the original matrix of the absolute frequencies of each animal prey item in each non-empty stomach ($n = 178$) was reduced to 35 food items in order to reduce 'noise'. The reduction was done by pooling some food items in higher functional (microcrustaceans, terrestrial and larvae + pupae) or taxonomic resolution (Mollusca and Trichoptera) groups and not by elimination of rarely occurring food items. Sample scores of the extracted first two dimensions were tested for: (1) the site effect by means of the Kruskal–Wallis test and (2) the ontogenetic effect by means of Spearman's correlation (r_s) analysis against fish L_T . Horn's index was used as similarity measurement between each pair of sampling site groups and size classes ($n = 9$), and the resulting similarity matrix was clustered by the unweighted pair-group method using the arithmetic averages algorithm (UPGMA) (Krebs, 1999). These statistical analyses were performed with the PAST data analysis package (<http://folk.uio.no/ohammer/past>).

For each prey item, the frequency of occurrence (% F_i) of a given prey type is defined as the number of stomachs in which that prey occurs (N_i), expressed as a frequency of the total number of stomach in which prey are present (N). According to (Cailliet, 1977) this index represents population-wide food habits. In animal prey items, the relative abundance of a given prey (% A_i) is defined as the percentage of total stomach contents in all predators comprised by that given prey (Macdonald & Green, 1983). In mathematical terms, these can be described by the equations: % $F_i = 100 (N_i N^{-1})$ and % $A_i = 100(\sum S_i \sum S_i^{-1})$, where S_i is the stomach content (number) composed of prey i , and S_j the total stomach content of all stomachs in the entire sample. In addition, the use of the Shannon index of diversity $H' = -\sum (S_i S_i^{-1}) \ln(S_i S_i^{-1})$ provides a relatively objective indication of niche breadth (Marshall & Elliott, 1997). Therefore, the calculation of H' and the subsequent t -test between two given samples was performed with PAST data analysis package.

In order to determine the feeding strategy of *A. melas*, feeding strategy diagrams were constructed following Costello's (1990) method with the modifications suggested by Amundsen *et al.* (1996). These diagrams are based on a two-dimensional representation, where each point represents % F_i and the prey-specific abundance (% P_i), calculated according to the following formula: % $P_i = 100(\sum S_i \sum S_{ii}^{-1})$, where S_{ii} is the total stomach content only in those predators with prey i in their stomach. 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: (1) the prey importance is represented in the diagonal from the lower left (rare prey) to upper right (dominant prey), (2) the feeding strategy is represented in the vertical axis from the bottom (generalization) to top (specialization) and (3) the relationship between feeding

strategy and the between or within-phenotype contributions to the niche width is represented in the diagonal from the lower right (high within-phenotype component, WPC) to upper left (high between-phenotype component, BPC) (Amundsen *et al.*, 1996).

The prey fish's body size was calculated, when possible, from the bony remains of fishes found in black bullhead stomachs using relationships published elsewhere (Miranda & Escala, 2002). A Pearson's correlation analysis was performed to test for the relationship between the calculated prey fish L_T and predator L_T .

RESULTS

DIET COMPOSITION AND BETWEEN-SITE VARIATION

From the total number of fish examined ($n = 255$) the stomachs of 67 were found empty, and therefore were not further analysed. The remaining stomachs ($n = 188$) were then analysed: 10 stomachs had only algae and plant material, 78 stomachs had only animal prey and 100 stomachs had both trophic groups. A total of 4276 animal prey items were found and identified in the specimens analysed. More than a half of the stomachs analysed ($\%F_i = 58.5$) had algae and plant material, but generally not in large quantities and often along with pieces of substratum ($\%F_i = 23.4$).

The first two dimensions extracted by the DCA (eigenvalues = 0.808 and 0.556) performed on animal food item absolute abundances showed the differences between the site groups, as illustrated by the shape, amplitude and orientation of the 95% CI ellipses (Fig. 2). Moreover, sample scores of the site groups were significantly different in the first (Kruskal–Wallis test, $P < 0.001$) as in the second (Kruskal–Wallis test, $P < 0.001$) of the extracted dimensions. The DCA biplot also allowed the specific animal prey items responsible for these diet differences between site groups to be identified. Aquatic prey were the main item found in stomachs, both with respect to occurrence as well as relative abundance, although some differences were detected between localities (Table I and Fig. 2). In the localities where free-flowing-river habitat was dominant (Arga, Guadarrama and mainstem Ebro Rivers) Chironomidae larvae was the main prey item, whereas in the lentic artificial habitat (Santo Estevão Reservoir) microcrustaceans (Anomopoda, Copepoda and Ostracoda) were the most consumed food items. Fishes (Pisces) as prey items were present in nearly 10% of the specimens in both habitat types, but the abundance of this prey item was quite low (<2%). Both occurrence and abundance of fish prey items were lower in the reservoir than in the riverine habitats. Of the fish species consumed, there were clear differences between sites (Fig. 2). Exotic mosquitofish *G. holbrooki* and pumpkinseed *L. gibbosus* were the fish species consumed in the Guadarrama River (Table I). The exotic common carp *C. carpio* and the native *P. miegii* along with other undetermined fishes were found in black bullhead stomachs from the mainstem Ebro River (Table I). In the Arga River, *B. graellsii*, *G. lozanoi* and *P. miegii* along with other undetermined fishes were eaten (in decreasing abundance) by black bullheads (Table I). In the lentic Santo Estevão Reservoir, exotic *C. carpio* and Centrarchids were the only consumed fish prey items (Table I). Terrestrial prey occurrence, but not abundance, was higher in specimens from the Guadarrama River and Santo Estevão Reservoir (Tagus River basin) than in those from the Arga and mainstem Ebro Rivers

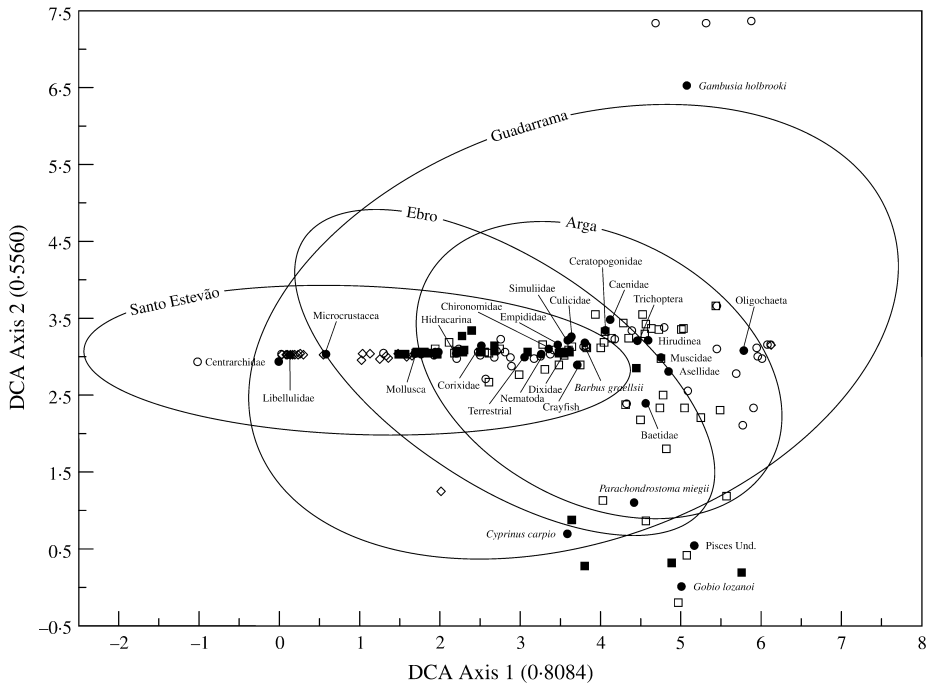


FIG. 2. Detrended correspondence analysis (DCA) of stomach contents (prey abundance, ●) of *Ameiurus melas* from the Iberian Peninsula (◇, Santo Estevão Reservoir; ■, Ebro River; □, Arga River; ○, Guadarrama River) (see Fig. 1). Prey item scores and specimen scores along with the 95% C.I. ellipses for the site groupings in the ordination space are defined by the first and second axes extracted by the DCA.

(Ebro River basin). The occurrence of plant material in the black bullhead stomachs was higher in the artificial lentic habitat than in the riverine habitats. Trophic diversity (H') was lower in the reservoir than in natural river habitats (Table I), although this difference was not statistically significant in the comparison between the Santo Estevão Reservoir and mainstem Ebro River (Santo Estevão Reservoir *v.* Ebro River *t*-test, $P > 0.05$; *v.* Guadarrama River *t*-test, $P < 0.001$; *v.* Arga River *t*-test, $P < 0.001$). The highest value of the % I_v was found in the specimens from the Arga River, while the lowest was found in those from the Guadarrama River (Table I).

ONTOGENETIC SHIFT

The moderate but highly significant positive correlation ($r_s = 0.40$, $P < 0.001$) between the fish L_T and the sample scores in the first dimension extracted by the DCA suggested an ontogenetic diet shift. A further similarity analysis performed by L_T classes and site groups allowed similarities or differences between these groups to be detected (Fig. 3). The first main division separated the specimens from the small reservoir at Ribeira de Santo Estevão at a similarity level of *c.* 31%. The next division at 44% similarity level

TABLE I. Diet composition of black bullhead *Ameiurus melas* specimens from the Arga, Guadarrama and Ebro Rivers and the Santo Estevão Reservoir. Data are presented for each total length class (L_T) and for the pooled data (Total) as the percentage of occurrence ($\%F_i$) and the relative abundance ($\%A_i$) (in parentheses). Trophic diversity (H'), vacancy index ($\%V$) and gut sample size (n) are also shown

Aquatic invertebrates	Arga River			Guadarrama River			Ebro River			Santo Estevão Reservoir		
	<100	100-150	>150	Total	>100	100-150	>150	Total	<100	>150	Total	
Oligochaeta	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	38.9 (63.4)	10.7 (1.9)	15.6 (11.2)	0 (0)	0 (0)	0 (0)	33.3 (0.5)
Erbodellidae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16.7 (3.0)	14.3 (0.7)	10.9 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)
Hirudinea cocoon	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3.6 (0.2)	1.6 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)
Nematoda	25.0 (1.9)	4.7 (0.5)	0 (0)	8.2 (0.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ancyliidae	0 (0)	4.7 (0.5)	16.7 (2.8)	4.9 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hydrobiidae	0 (0)	4.7 (1.3)	0 (0)	3.3 (0.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lymnaeidae	0 (0)	11.6 (2.1)	0 (0)	8.2 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Physidae	0 (0)	4.7 (0.8)	0 (0)	3.3 (0.5)	0 (0)	0 (0)	3.6 (0.1)	1.6 (0.1)	0 (0)	0 (0)	0 (0)	20.8 (1.2)
Planorbidae	0 (0)	2.3 (0.3)	0 (0)	1.6 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hidracarina	8.3 (9.7)	0 (0)	0 (0)	1.6 (2.4)	0 (0)	0 (0)	3.6 (0.1)	1.6 (0.1)	3.0 (0.3)	0 (0)	2.6 (0.2)	20.8 (0.3)
Anomopoda	0 (0)	2.3 (0.8)	0 (0)	1.6 (0.5)	16.7 (4.3)	0 (0)	0 (0)	4.7 (1.0)	12.1 (3.3)	0 (0)	10.3 (2.6)	87.5 (50.6)
Copepoda	25.0 (10.3)	0 (0)	0 (0)	4.9 (2.6)	50.0 (24.0)	0 (0)	0 (0)	14.1 (5.6)	21.2 (15.5)	0 (0)	17.9 (12.5)	83.3 (11.6)
Ostracoda	8.3 (0.6)	2.3 (0.3)	0 (0)	3.3 (0.3)	33.3 (2.0)	11.1 (2.1)	17.9 (1.8)	20.3 (1.9)	54.5 (20.1)	0 (0)	46.2 (16.3)	62.5 (32.6)
Asellidae	0 (0)	4.7 (0.5)	0 (0)	3.3 (0.3)	0 (0)	5.6 (0.8)	14.3 (0.6)	7.8 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)
Crayfish	16.7 (1.3)	4.7 (0.5)	16.7 (1.4)	8.2 (0.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16.7 (1.1)	2.6 (0.2)	0 (0)
Baetidae	8.3 (0.6)	30.2 (4.6)	33.3 (4.2)	26.2 (3.6)	22.2 (1.1)	16.7 (1.3)	3.6 (0.1)	12.5 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)
Caenidae	0 (0)	2.3 (0.3)	16.7 (1.4)	3.3 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ceratopogonidae	0 (0)	2.3 (0.3)	0 (0)	1.6 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chironomidae L	91.7 (56.1)	81.4 (54.6)	100 (40.3)	85.2 (53.3)	88.9 (63.7)	38.9 (18.7)	60.7 (78.1)	62.5 (65.3)	97.0 (54.3)	33.3 (86.2)	87.2 (60.4)	54.2 (2.2)
Chironomidae P	25.0 (1.9)	16.3 (3.4)	0 (0)	16.4 (2.6)	38.9 (2.6)	22.2 (2.1)	32.1 (2.7)	31.2 (2.6)	18.2 (1.6)	16.7 (1.1)	17.9 (1.5)	0 (0)
Culicidae L	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7.1 (8.5)	3.1 (5.2)	0 (0)	0 (0)	0 (0)	0 (0)
Culicidae P	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3.6 (1.9)	1.6 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)
Dixidae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	33.3 (2.3)	5.1 (0.4)	0 (0)
Empididae	0 (0)	2.3 (0.3)	16.7 (1.4)	3.3 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

TABLE I. Continued

Aquatic invertebrates	Arga River			Guadarrama River			Ebro River			Santo Estevão Reservoir		
	<100	100-150	>150	Total	>100	100-150	>150	Total	<100	>150	Total	100-150
Muscidae	0 (0)	2-3 (0-3)	0 (0)	1-6 (0-2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Simuliidae L	25-0 (5-8)	16-3 (2-3)	0 (0)	16-4 (2-9)	27-8 (1-4)	11-1 (4-3)	10-7 (0-9)	15-6 (1-5)	3-0 (0-3)	0 (0)	2-6 (0-2)	0 (0)
Simuliidae P	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3-6 (0-2)	1-6 (0-1)	0 (0)	0 (0)	0 (0)	0 (0)
Corixidae	8-3 (0-6)	2-3 (0-3)	0 (0)	3-3 (0-3)	0 (0)	0 (0)	3-6 (0-1)	1-6 (0-1)	21-2 (2-4)	0 (0)	17-9 (2-0)	0 (0)
Libellulidae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8-3 (0-1)
Ecnomyidae	8-3 (1-3)	20-9 (4-1)	0 (0)	16-4 (2-9)	0 (0)	0 (0)	0 (0)	0 (0)	6-1 (0-5)	0 (0)	5-1 (0-4)	0 (0)
Hydropsychidae	25-0 (5-2)	39-5 (14-4)	66-7 (44-4)	39-3 (15-6)	0 (0)	5-6 (0-4)	0 (0)	1-6 (0-1)	3-0 (0-3)	0 (0)	2-6 (0-2)	0 (0)
Hydroptilidae	0 (0)	4-7 (0-5)	16-7 (1-4)	4-9 (0-5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Psychomyiidae	0 (0)	9-3 (2-1)	16-7 (1-4)	8-2 (1-5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Trichoptera Und. L	0 (0)	2-3 (0-3)	0 (0)	1-6 (0-2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Trichoptera Und. P	0 (0)	0 (0)	16-7 (1-4)	1-6 (0-2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	100 (95-5)	95-3 (95-1)	100 (100)	96-7 (95-8)	100 (99-1)	55-6 (96-2)	78-6 (97-8)	78-1 (97-8)	100 (98-6)	50-0 (90-8)	92-3 (97-1)	100 (99-1)
Pisces												
<i>Parachannaostoma miegii</i>	8-3 (0-6)	2-3 (0-3)	0 (0)	3-3 (0-3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	33-3 (2-3)	5-1 (0-4)	0 (0)
<i>Barbus graellii</i>	8-3 (0-6)	7-0 (0-8)	0 (0)	6-6 (0-7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Gobio lozanoi</i>	0 (0)	4-7 (0-8)	0 (0)	3-3 (0-5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Cyprinus carpio</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3-0 (0-3)	33-3 (2-3)	7-7 (0-7)	4-2 (0-1)
<i>Gambusia holbrooki</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16-7 (1-3)	7-1 (0-2)	7-8 (0-3)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Lepomis gibbosus</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5-6 (0-4)	0 (0)	1-6 (0-1)	0 (0)	0 (0)	0 (0)	0 (0)
Centrarchidae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4-2 (0-1)
Pisces und.	8-3 (0-6)	0 (0)	0 (0)	1-6 (0-2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16-7 (1-1)	2-6 (0-2)	0 (0)
Total	25-0 (1-9)	11-6 (1-8)	0 (0)	13-1 (1-6)	0 (0)	22-2 (1-7)	7-1 (0-2)	9-4 (0-4)	3-0 (0-3)	50-0 (5-7)	10-3 (1-3)	8-3 (0-1)
Terrestrial prey												
Mollusca	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3-6 (0-1)	1-6 (0-1)	0 (0)	0 (0)	0 (0)	0 (0)
Arachnida	8-3 (0-6)	0 (0)	0 (0)	1-6 (0-2)	0 (0)	0 (0)	3-6 (0-1)	1-6 (0-1)	3-0 (0-3)	0 (0)	2-6 (0-2)	4-2 (0-1)
Collembola	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3-6 (0-1)	1-6 (0-1)	0 (0)	0 (0)	0 (0)	0 (0)

TABLE I. Continued

	Arga River			Guadarrama River			Ebro River			Santo Estevão Reservoir	
	<100	100-150	>150	Total	>100	100-150	>150	Total	<100	>150	Total
Aquatic invertebrates											
Coleoptera	16.7 (1.3)	2.3 (1.5)	0 (0)	4.9 (1.3)	0 (0)	11.1 (0.9)	0 (0)	3.1 (0.1)	3.0 (0.3)	0 (0)	2.6 (0.2)
Diptera	0 (0)	0 (0)	0 (0)	0 (0)	16.7 (0.9)	0 (0)	3.6 (0.1)	6.3 (0.3)	0 (0)	0 (0)	0 (0)
Hemiptera	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Formicidae	0 (0)	4.7 (0.5)	0 (0)	3.3 (0.3)	0 (0)	0 (0)	10.7 (0.3)	4.7 (0.2)	0 (0)	0 (0)	0 (0)
Trichoptera	0 (0)	2.3 (0.3)	0 (0)	1.6 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Terrestrial Und.	8.3 (0.6)	7.0 (0.8)	0 (0)	6.6 (0.7)	0 (0)	16.7 (1.3)	32.1 (1.2)	18.8 (0.9)	6.1 (0.5)	33.3 (3.4)	10.3 (1.1)
Total	33.3 (2.6)	14.0 (3.1)	0 (0)	16.4 (2.6)	16.7 (0.9)	27.8 (2.1)	53.6 (2.0)	35.9 (1.7)	12.1 (1.1)	33.3 (3.4)	15.4 (1.5)
Algae and plants	33.3	55.8	83.3	54.1	27.8	100	92.9	76.7	15.2	33.3	17.9
H'	0.73	0.81	0.57	0.84	0.48	0.55	0.43	0.62	0.59	0.29	0.58
%I _v	31.6	37.7	54.5	38.3	14.3	14.3	12.5	13.5	25.0	14.3	23.5
n	19	69	11	99	21	21	32	74	44	7	51

Und, undetermined; L, larvae; P, pupae.

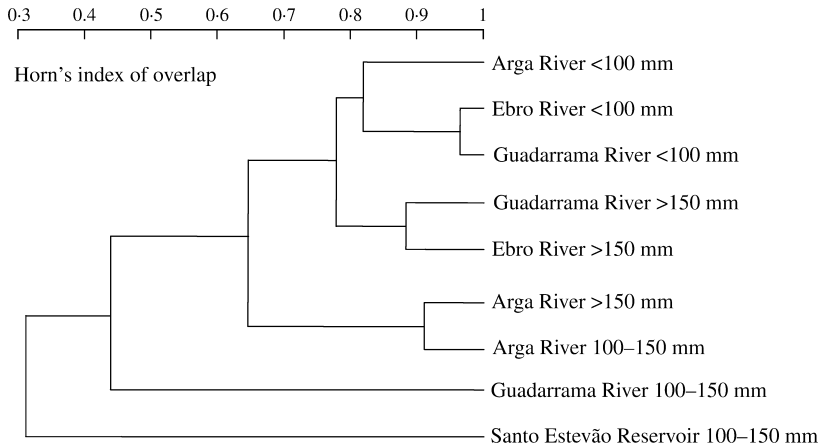


FIG. 3. Dendrogram resulting from the UPGMA cluster analysis of Horn's index of prey abundance pooled data by *Ameiurus melas* total length classes and site groups (see Fig. 1).

segregated the 100–150 mm specimens from the Guadarrama River, and the next split the cluster formed by the L_T classes 100–150 and >150 mm from the Argá River at a similarity level of *c.* 62%. The next division separated, at the 78% similarity level, the cluster of >150 mm specimens from the Guadarrama and Ebro Rivers, and the cluster of <100 mm specimens from the Argá, Ebro and Guadarrama Rivers. Even if there were ontogenetic differences, the diet remained mainly composed of aquatic prey. Specifically, Chironomidae were the most frequent and abundant prey items in almost all L_T classes in all sites. Thus, the observed ontogenetic diet shift was shown by other (secondary) prey items, such as microcrustaceans, upon which consumption decreased as fish grew larger (Table I). The prey items that replaced the microcrustaceans during ontogeny were different between sites. In the Argá River, mayflies (Baetidae and Caenidae) and caddisflies (Hydropsychidae, Ecnomyiidae, Psychomyiidae, Hydroptilidae and undetermined Trichoptera, in decreasing abundance) were consumed more as fish grew larger (Table I). In the Guadarrama River, consumption on Oligochaeta, total terrestrial prey and fishes increased with L_T (Table I), similar to that which occurred in the Ebro River (Table I). In all three rivers, the presence of plant material in black bullhead stomachs increased with L_T (Table I).

The ontogenetic shift was also detected in H' , with the lowest values in the >150 mm and the highest in the 100–150 mm L_T classes (Table I). Specifically, in the Argá River, trophic diversity of >150 mm L_T was significantly lower than the other size classes (>150 *v.* <100 mm, *t*-test, $P < 0.05$; >150 *v.* 100–150 mm *t*-test, $P < 0.001$), but the highest value in the 100–150 mm L_T class was not statistically significant compared with <100 mm (*t*-test, $P > 0.05$). In the Guadarrama River, the only significant comparison between trophic diversity values was the one between 100–150 *v.* >150 mm L_T classes (*t*-test, $P < 0.01$) (all others, *t*-test, $P > 0.05$). In the Ebro River, the >150 mm L_T class trophic diversity value was significantly lower than in the <100 mm (*t*-test, $P < 0.001$).

FEEDING STRATEGY

The feeding strategy plots (Fig. 4) show that black bullhead of all L_T classes in all studied sites had a generalist feeding strategy, *i.e.* with an important number of rare prey and a low number of dominant prey. In natural riverine habitats, Chironomidae, Hydropsychidae, Oligochaeta and some fish species were the dominant prey items, whereas in the artificial lentic habitat microcrustaceans (mainly Anomopoda and Ostracoda) were dominant prey items. Again, an ontogenetic shift was detected in the feeding strategy of black bullheads (Fig. 4). In the Arga River, dominance of Chironomidae larvae decreased as fish grew larger, whereas Hydropsychidae increased. In the Guadarrama River, dominance of microcrustaceans decreased as fish grew larger whilst Oligochaeta

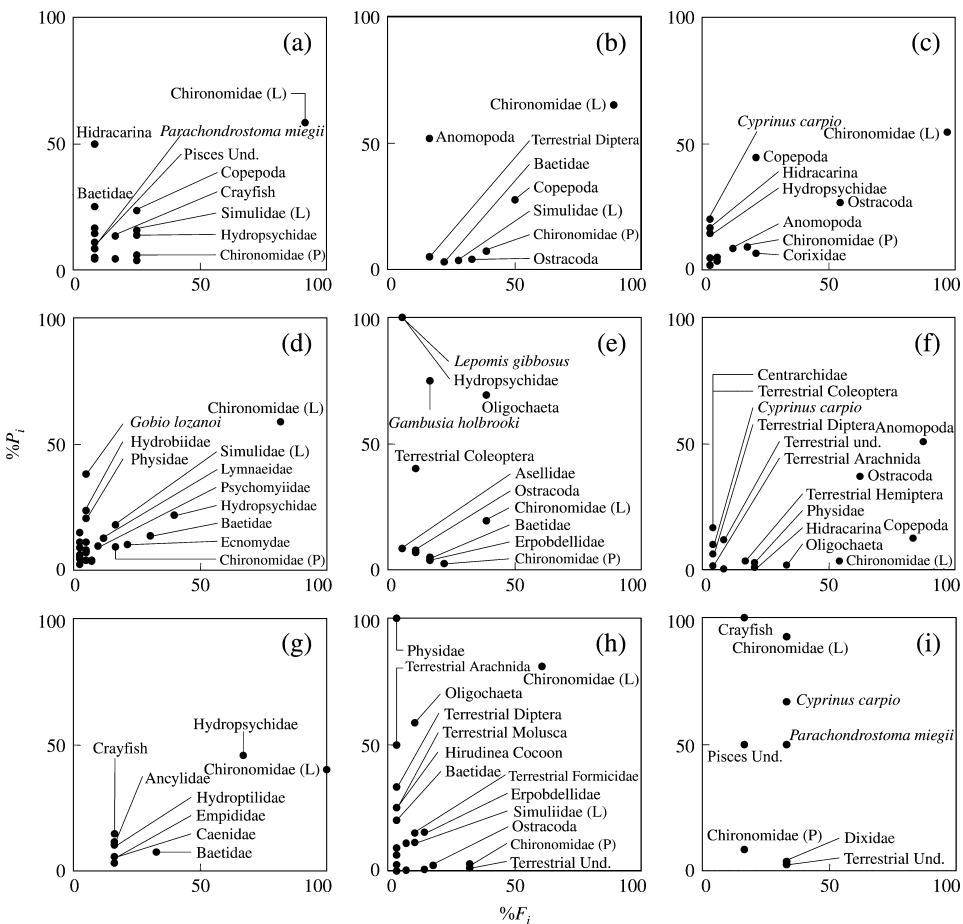


FIG. 4. Feeding strategy plots [percentage occurrence of prey ($\%F_i$) and prey-specific abundance ($\%P_i$)] for *Ameiurus melas*: (a) Arga River <100 mm total length (L_T), (b) Guadarrama River <100 mm L_T , (c) Ebro River <100 mm L_T , (d) Arga River 100–150 mm L_T , (e) Guadarrama River 100–150 mm L_T , (f) Santo Estevão Reservoir 100–150 mm L_T , (g) Arga River >150 mm L_T , (h) Guadarrama River >150 mm L_T and (i) Ebro River >150 mm L_T , (see Fig. 1). Each point represents a different prey item. Und, undetermined; L, larvae; P, pupae.

increased, and additionally, in the 100–150 mm L_T class the dominance of Hydropsychidae and exotic fish species increased. In the mainstem Ebro River, Chironomidae and microcrustaceans were the dominant prey items in the <100 mm L_T class, whereas Chironomidae, crayfish and, native and exotic fish species were dominant in the >150 mm black bullhead stomachs.

DISCUSSION

DIET COMPOSITION AND BETWEEN-SITE VARIATION

Traditionally, there has been some debate on the most appropriate fish collection method for dietary analysis. Problems such as regurgitation, post-capture digestion or atypical feeding behaviour have been pointed out as a result of inadequate sampling gear selection (Bowen, 1996). The only specimen captured from the Guadiana River was by means of gillnets, and may be due to this capture method its stomach was found to be empty. The rest of the specimens analysed in this study were captured by electrofishing and this capture technique appeared not to create enough stress to cause regurgitation. Other factors may also influence the amount and type of food found in fish stomachs such as the diel cycle, seasonal and interannual changes both on feeding ecology and availability, territoriality and differential digestion rates (Bowen, 1996). Since these factors were not controlled in the present study none can be attributed or excluded as responsible for the diet variability and for *c.* 26% of empty stomachs found. Indeed, the 95% CI ellipse of the Guadarrama River samples in the DCA is by far the broadest, probably accounting at least in part to the seasonal component (summer and autumn) of the samples collected in this river. In the same way, the 95% CI ellipse of the Arga River samples in the DCA is much narrower probably indicating that even seasonality was 'controlled': the interannual component introduced some variability in the aquatic invertebrate community available for the black bullheads.

Aquatic macroinvertebrates dominated the black bullhead's diet in all the sites studied, irrespective of natural riverine or artificial lentic habitat, something already underlined in other ictalurids (Kline & Wood, 1996; Declerck *et al.*, 2002; Michaletz, 2006). Since they also consumed plant material, terrestrial prey and co-occurring fish species, black bullhead should be considered as generalist or opportunistic, foraging on the most abundant and available prey. Algae and plant material were used in relatively small quantities (high % F_i but low % A_i), most likely due to its low absorption rate and energy content (Lien, 1981) and suggesting that animal prey items are energetically more profitable as a trophic resource (Penczak *et al.*, 1984). Consumption of large quantities of algae in other ictalurids has been suggested as that they are food-limited (Blanc & Margraf, 2002). Thus, the low consumption observed here would indicate that the black bullhead populations analysed in this study were not food-limited. Together with algae and plant material, most stomachs had detritus and substratum, which would indicate accidental ingestion during benthic foraging activity or even a detritivorous diet (Pouilly *et al.*, 2006). When piscivorous feeding upon live fishes is detected in gape-limited predators, a positive relationship between predator and prey size (*e.g.* L_T) is expected. The *a priori*

expected positive relationship was not found ($r = 0.077$, $n = 16$, $P > 0.05$) probably indicating a piscivory behaviour on dead or dying vulnerable fishes along with a predation on smaller live fishes.

The joint study of diet composition and the DCA on the absolute frequencies of prey items allowed both the common and the specific (secondary) prey items responsible for diet differences between sites to be identified. Although microcrustaceans, caddisfly larvae and Oligochaeta were also common in the black bullhead's diet in those sites with a predominant lotic habitat, Chironomidae were the most frequent and abundant prey items consumed. The predominance of this benthic prey pointed again towards the benthophagous behaviour of black bullheads, similarly to other species of the genus *Ameiurus* (Weisberg & Janicki, 1990). Differences in the use of secondary prey items could be related both to their availability in the different rivers and to the generalist or opportunistic trophic behaviour of black bullheads. In the Santo Estevão Reservoir, the greater consumption of microcrustaceans observed was not rare as it has already been reported in other benthic fish species in lentic habitats (Oscoz *et al.*, 2003; Encina *et al.*, 2004). Terrestrial prey consumption is closely dependent on the contact intensity between the riparian forest and the water body itself. Terrestrial prey consumption (%A_i) was low in all sites but even lower in the Santo Estevão Reservoir, almost certainly due to a poorly developed riparian forest and its reduced interaction with the reservoir (de Mérona *et al.*, 2005). Fish consumption was observed in nearly 10% of the specimens in both habitat types with quite low abundances (<2%), but were even lower in the artificial lentic habitat. The fish species consumed were those available for black bullheads in each site, regardless of their native or exotic origin. An additional difference between artificial lentic and natural riverine habitats was detected on the black bullhead's trophic diversity values, being lower in the reservoir population probably due to decreased prey species richness compared to free-flowing habitats (García de Jalón & Camargo, 1994).

ONTOGENETIC SHIFT AND FEEDING STRATEGY

Ontogenetic diet shift has been largely reported for many freshwater fishes (García-Berthou, 1999a, b, 2002; García-Berthou & Moreno-Amich, 2000b; Oscoz *et al.*, 2005a, 2006), including members of the Ictaluridae both in composition and towards larger prey items (Kline & Wood, 1996; Declerck *et al.*, 2002). In this study, the significant correlation between L_T and the sample scores in the first dimension extracted by the DCA, the similarity analysis, and the feeding strategy plots revealed an ontogenetic diet shift throughout black bullhead L_T classes within the site groups. Moreover, the feeding strategy plots clearly displayed the generalist (opportunistic) feeding behaviour, irrespective of habitat type (sites) and L_T class, furthermore showing the site-specific trajectories of these changes. Smaller fishes are confined to capture and ingest only small prey or soft-body prey, and as they grow morphological changes allow them to capture and consume effectively larger prey while reducing their ability to capture small ones (Walton *et al.*, 1992). Although ontogenetic differences were found, Chironomidae were the most frequently and abundantly consumed prey items in almost all L_T classes in all sites. As a result,

secondary prey items were responsible for the observed ontogenetic diet shift. Consumption upon microcrustaceans decreased in abundance as the fish grew larger, being replaced by larger prey such as mayflies, caddisflies, Oligochaeta, terrestrial prey or fishes, depending of the site. This diet variation could be related to site-specific prey availability, the accessibility to that prey and the risk of predation (Oscoz *et al.*, 2005a, 2006). Moreover, these ontogenetic diet differences could be related to ontogenetic shift in habitat use since smaller fish are more pelagic and adults more benthic (Cucherousset *et al.*, 2006). In addition, occurrence of plant material also increased with black bullhead L_T , as has been found in other ictalurids (Michaletz, 2006). On account of this ontogenetic shift in diet composition, trophic diversity also varied with L_T , with the lowest values in the largest black bullheads, while the highest were found in the intermediate L_T class. As the fish grow larger they could potentially capture and ingest a wider range of prey items, and therefore trophic diversity might increase. Beyond a specific L_T , however, the smallest prey items are not efficiently captured or handled or become not energetically profitable. This hypothesis could not be effectively proven due to reduced number of specimens of the largest L_T class in the populations studied except for the Guadarrama River.

CONSERVATION AND MANAGEMENT IMPLICATIONS

Some previous studies based on stomach content of other exotic fishes have already shown the trophic interaction with native Iberian fishes (Elvira *et al.*, 1996; Nicola *et al.*, 1996; Domínguez & Pena, 2000; Domínguez *et al.*, 2002; Oscoz *et al.*, 2005a). The present results suggest that the black bullhead could be negatively affecting native ichthyofauna in two ways. First, the results showed that black bullheads are preying on native fish species such as *B. graellsii*, *P. miegii* and *G. lozanoi*. Even if only fish bony remains (e.g. scales, opercula, cleithra and pharyngeal arches) were identified in black bullhead stomachs, egg predation could not be excluded. Probably, egg predation was not detected because of rapid digestion (García-Berthou & Moreno-Amich, 2000b). Secondly, this study showed that the diet composition of black bullhead is similar to the diet described for some co-occurring Iberian native species (Oscoz *et al.*, 2005b, 2006). Taking into account black bullhead's voracity and aggressive behaviour, the diet similarity might lead to an unfavourable competition for the same food resources, subsequently, displacing native fishes to suboptimal food resources. Some effects of exotic ichthyophagous fishes, such as on competition and predation, are habitat-dependent and might be favoured by habitat degradation. In this respect, aquatic habitat impoverishment, characterized by increased river regulation and creation of lentic areas, water pollution, and flow and thermal regime alteration, has been suggested to favour exotic limnophilous species in Iberian watersheds (Elvira *et al.*, 1998; Aparicio *et al.*, 2000; Clavero *et al.*, 2004). This type of habitat degradation occurs more frequently in downstream areas, probably resulting in the greatest susceptibility to invasion by exotic fish species such as black bullhead (Céréghino *et al.*, 2005). The success of black bullhead as an invasive species has been attributed to its life history and factors such as flexibility to habitat type, limnophilous

nature and tolerance to water quality, even pollution (Gante & Santos, 2002; Vila-Gispert *et al.*, 2005; Ribeiro *et al.*, 2008). Interestingly, river restoration programmes aiming to recover the lotic:lentic ratio and longitudinal connectivity, which may include removal of dams and weirs, should take into account that the barriers may be preventing the upstream expansion of exotic species (Rahel, 2007). The restoration of natural flow and thermal regimes and habitat conditions, however, may favour native species over exotic species (Moyle *et al.*, 2003; Rahel, 2007).

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