Food of an endangered cyprinodont (*Aphanius iberus*): ontogenetic diet shift and prey electivity

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Synopsis

We studied the ontogenetic diet shift and prey electivity of an endangered cyprinodontid fish endemic to the Iberian Peninsula, the Spanish toothcarp (*Aphanius iberus*). The toothcarp's diet was omnivorous, dominated by harpacticoid copepods (*Mesochra lilljeborgi* and *Tisbe longicornis*), copepod nauplii and detritus. Diet composition varied greatly among habitats, depending on prey availability. In a rarely inundated habitat (glasswort), there was more consumption of the isopod *Protracheoniscus occidentalis* and the harpacticoid copepod *Mesochra lilljeborgi*, while in algal mats another harpacticoid (*Tisbe longicornis*), chironomid dipterans and invertebrate eggs were more important in diet. Although a benthic feeding habitat has previously been suggested, in our study the diet was based rather on water column organisms for both glasswort and algal mat habitats. There was also an ontogenetic diet shift, with an increase of mean prey length with fish length, clearly linked to a microhabitat change. Smaller fish showed positive electivity and greater reliance on planktonic prey (e.g. copepod nauplii, the harpacticoid copepods *Mesochra lilljeborgi* and *Tisbe longicornis*, the rotifer *Brachionus plicatilis*, and ostracods), while larger fish elected and preyed on more benthic organisms (e.g. *Canuella perplexa*, *Mesochra rapiens*, and ephydrid dipterans).

Introduction

The Spanish toothcarp (*Aphanius iberus*) is a cyprinodontid fish endemic to the Mediterranean coast of Spain and is considered in danger of extinction by the National Catalogue of Endangered Species and the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) (Doadrio 2001). It originally inhabited a wide range of lowland waters but now-a-days its distribution is reduced to brackish and hypersaline waters in salt marshes and coastal lagoons. This is due to the impact of invasive species such as the poeciliid fish *Gambusia holbrooki*, habitat destruction by wetland desiccation, and water

pollution (García-Berthou & Moreno-Amich 1992; Doadrio 2001; Rincón et al. 2002). The Atlantic populations of the Spanish toothcarp have recently been distinguished as a new species (*A. baeticus*) because of differences in morphometrics and genetics (Doadrio et al. 2002).

Although the Spanish toothcarp (*Aphanius iberus*) is one of the few protected fish species in Spain, 12 of the known 28 Mediterranean populations have disappeared and one is extinct in the wild with only a few individuals still preserved in captivity (Doadrio 2001). It is a small fish (total length usually <5 cm), with external sexual dimorphism, short longevity (aged up to 2+) and strongly euryhaline and eurythermal (Fernández-

Delgado et al. 1988; García-Berthou & Moreno-Amich 1992; Vargas & de Sostoa 1997). It reproduces from April to October, laying up to 900 eggs in successive spawns, and reaches sexual maturity in a few months (at a total length of less than 20 mm). The only published studies on *A. iberus* feeding ecology are Vargas & de Sostoa (1999) and experimental work by Rincón et al. (2002) and Caiola and Sostoa (2005). Vargas & de Sostoa (1999) showed that the Spanish toothcarp in the Ebro river delta is omnivorous, with a diet composed of both animal prey (mainly benthic crustaceans such as harpacticoid copepods and amphipods) and plant debris and detritus. This population also showed a seasonal change in diet related to the hydrological cycle of the lagoon (Vargas & de Sostoa 1999). Rincón et al. (2002) and Caiola and Sostoa (2005) studied the interactions of toothcarp and mosquitofish and found that small toothcarp captured less prey in the presence of adult conspecifics and adult mosquitofish. However, the role of resource availability in toothcarp feeding is largely unknown and there is no study on its prey electivity. In general, resource availability and electivity is often not considered in fish feeding studies, although without its measurement little can be said about niche overlap and niche breadth (Hurlbert 1978). The study of electivity is important in order to understand the response of the invertebrate community and the patterns of intraspecific and interspecific competition of predators (Gerking 1994). Knowledge of the specific prey types consumed at different sites also allows a more mechanistic understanding of habitat selection (Nemerson & Able 2004).

In a previous study (Alcaraz et al., submitted manuscript), we described the effect of habitat variation on the distribution and composition of the invertebrate community and the population structure of the Spanish toothcarp. The objectives of the present paper are (1) to analyse the effect of habitat variation on the Spanish toothcarp diet and, in particular, to assess the role of a rarely inundated habitat (glasswort); and (2) to study the toothcarp's prey electivity and its relationship with ontogeny and habitat variation. This is the second diet study of a natural population of this endangered toothcarp and the first measuring resource availability and electivity. Such data are essential to enhance its conservation programs. By simultaneously measuring diet and resource availability in the water column and the benthos, we also aim to assess its main feeding micro-habitat.

Methods

Study area

The study was undertaken in Fra Ramon, a coastal lagoon located behind the dune line of the Baix Empordà salt marshes (NE Spain) (Figure 1). It is situated at 42° 1′ 49″ N, 3° 11′ 29″ E and 1.75 m below the sea level. The salinity ranges from 28 ppt (i.e. brackish) during rainfall precipitation to 49 ppt in summer (i.e. hypersaline) and the temperature ranges from 5 to 30 °C. It is eutrophic according to the concentrations of total nitrogen and chlorophyll but hypereutrophic according to total phosphorous (A. Badosa, unpublished data). There is no freshwater inflow to the lagoon and, as is usual in Mediterranean wetlands, no regular tidal influence, so water entries occur mainly through heavy rainfall and rough sea periods. The water area of the lagoon ranges from ca. 2200 m^2 during the dry season (summer) to 13,000 m^2 in heavy rainfall periods (when the lagoon was sampled) and water level oscillates from −5 cm to 1 m above mean sea level (A. Badosa, unpublished data) (Figure 1).

The permanently inundated zone of the lagoon presents dense submerged meadows of ditch-grass (*Ruppia cirrhosa*) and floating mats of green algae (*Enteromorpha* sp. and *Chaetomorpha* sp.). Algal mats are more abundant in summer (when high blooms occur) and rare in winter. The salt marsh is dominated by the *Puccinellio-Arthrocnemetum fruticosi* plant association. However, in the zone closest to the lagoon that is occasionally inundated there is the *Suaedo-Salicornietum patulae* plant association, dominated by glasswort (*Salicornia patula*) and other succulent plants (e.g. *Suaeda maritima* and *Suaeda splendens*). This habitat is only occasionally flooded, usually for about 2 months per year (Figure 1). Only *Aphanius iberus* presents stable fish populations in the lagoon, although other species of marine origin, such as eel (*Anguilla*
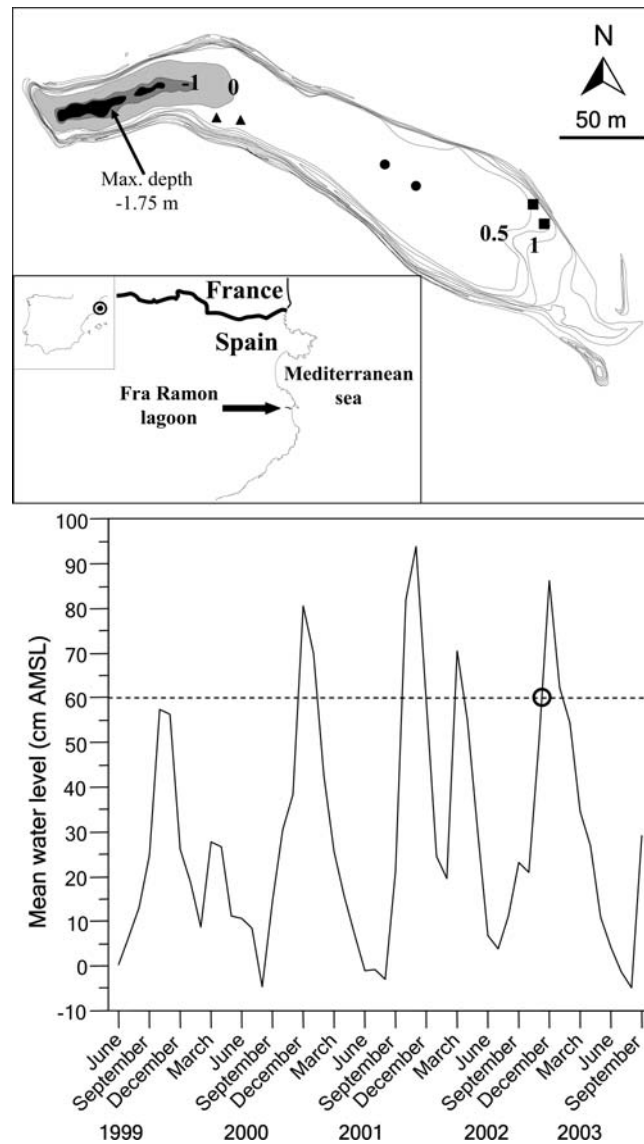
Figure 1. Top, location of the study area and map of the Fra Ramon lagoon with the main sampling points per habitat (■ = Glasswort, ● = Open water, and ▲ = Algal mats); depths are meters above mean sea level. Bottom, mean water level (in cm above mean sea level) in the lagoon from 1999 to 2003; dashed line shows the water level above which glasswort is flooded and available for fish and the circle marks the sampling date.

anguilla) and mullets (Mugilidae), are occasionally observed (Alcaraz et al., pers. obs.). The Spanish toothcarp population was monitored once every 2 months for 1 year to estimate its abundance, population structure, and habitat use. Our study was undertaken during one of the rare periods of flooding to examine whether the toothcarp used infrequently inundated habitats and assess the differences among habitats.

Field and laboratory methods

The lagoon was sampled on the mornings of 21–22 November 2002, during a period of flooding caused by heavy rainfall. We sampled three different habitats: 'glasswort', corresponding to the *Suaedo-Salicornietum patulae* plant association that is rarely inundated; 'algal mats', dominated by *Enteromorpha* sp. and *Chaetomorpha* sp. and

mostly present close to the littoral; and 'open water', unvegetated habitat that dominates most of the lagoon. At the sampling sites, these three habitats were 14, 37 and 33 cm deep, respectively. In each of the habitats we sampled organisms in the water column, the benthos (in both cases making two replicates per habitat), and fish (Figure 1). Zooplankton and macroinvertebrates in the water column were sampled with a 1 m long transect of 50 μm mesh dip net (volume sampled $= 0.038$ m^3). All the samples were immediately preserved in 10% formalin. All the material collected, including algal mats when present, was included in the samples and invertebrates were sorted later in the laboratory under a dissecting microscope. Benthic organisms were sampled with a 15×15 cm benthic grab and frozen in situ with a portable freezer. In the laboratory, the benthos samples were unfrozen and sifted with a 0.1 mm sieve. All the organisms were then fixed in 10% formalin.

Organisms were usually identified to the genus or species level, except in the cases of Nematoda and Ostracoda. All organisms were counted and a minimum of 20–30 individuals (if available) of each taxon were measured under a dissecting microscope with an ocular micrometer. The measurements were converted to dry mass according to published length-dry mass relationships (mainly Dumont et al. 1975; Smock 1980).

The fish sampling technique consisted of quickly throwing a 1 m high cylinder of 90 cm in diameter and then removing all the fish with a dip net. The mean depth of each sampling site was measured and the fish captured were expressed as densities (fish l^{-1}). Although this sampling technique may underestimate total density, it allows abundance comparison among habitats. Fish were sampled with 38 throws of the cylinder, systematically distributed throughout the lagoon, and habitat was then characterised. The number of sampled points thus resulted in 9 in glasswort, 9 in open water and 20 in algal mats, due to the dense cover of the latter habitat in the lagoon. Fish were frozen in situ with a portable freezer to avoid digestion of the stomach contents. In the laboratory, all individuals were measured (standard and total lengths to the nearest 0.5 mm), eviscerated, sexed (generally from external sexual dimorphism), and weighed (total and eviscerated weight to the nearest 0.1 mg). The entire gut was preserved in 10% formalin until analysis. The entire gut contents of the toothcarp were examined under a dissecting microscope; in the largest samples a sub-sample of 31 (glasswort) and 26 (algal mats) fish were examined. All the prey present in the gut were sorted, usually to the species or genus level, counted, and a minimum of 20–30 individuals (if available) of each prey taxon were measured with an ocular micrometer. As for the water and benthos samples, the measurements were converted to dry mass according to published length-dry mass relationships. The volume of uncountable categories (detritus, plant debris, plant leaves, invertebrate eggs and digested material) in the gut contents was estimated to the nearest 0.00025 mm^3 with a Neubauer counting chamber (haemacytometer) as in García-Berthou (1999) and the volume later transformed to biomass using a conversion of 0.27 mg of dry weight/mm^3 (Dumont et al. 1975; Lindegaard 1992).

Data analysis

Differences in density and mean length among habitats were analysed with analysis of variance (ANOVA), followed by Games–Howell post-hoc tests (hereafter, GH tests). GH tests are among the most powerful and most robust to unequal variances of post-hoc multiple comparison methods (Day & Quinn 1989). The frequencies of sex categories among habitats were compared with a G-test of independence.

The analysis of diet data follows our previous work (García-Berthou 1999; García-Berthou & Moreno-Amich 2000; García-Berthou 2001). Percent number (% number), percent biomass (% biomass), and frequency of occurrence were used to estimate the dietary importance of each food category. Percent number is the number of individuals of a prey type divided by the total number of individuals and expressed as a percentage, after pooling the gut contents of all fish. Percent biomass is the equivalent measure for biomass data. Frequency of occurrence is the percentage of guts in which a food category was present. To describe prey importance and feeding strategy, Costello's (1990) graphical method was used, i.e. a plot of % number or % biomass vs. frequency of occurrence. The most important prey are closer to the top right corner. The other diagonal corresponds to feeding strategy: prey with low occurrence but important

by number or biomass correspond to some sort of specialisation and are closer to the top left corner. Diet diversity (for each fish) was measured with the complement of Simpson's index (D), calculated as

$$D = 1 - \sum_i \frac{n_i(n_i - 1)}{N(N - 1)}$$

where n_i is the number of individuals of prey type i, and N is the total number of prey (Hurlbert 1971).

Analysis of covariance (ANCOVA) was also used to compare variables (e.g. fish weight or prey length) among sex groups and habitats, using fish length as the covariate. We started with the most complex model, introducing all possible interactions (including interactions of covariates×factors, following García-Berthou & Moreno-Amich (1993)). We then simplified the general linear model by removing nonsignificant interactions ($p \gg 0.10$) to increase the statistical power of the remaining sources of variation, which would otherwise be seriously compromised. When the covariate was not significant ($p > 0.10$) it was also deleted from the model (so an ANOVA was used). Quantitative variables were log-transformed for the analyses because homoscedasticity and linearity were clearly improved. Open water was omitted for most analyses because only three fish were captured in that habitat.

All factors were considered fixed effect factors. We used partial η^2 (partial eta squared) as a measure of effect size (i.e. importance of factors). Similarly to r^2, partial η^2 is the proportion of variation explained for a certain effect (effect SS/effect SS + error SS). Partial η^2 has an advantage over η^2 (effect SS/total SS) in that it does not depend on the number of sources variation in the ANOVA design used, because it does not use the total sum-of-squares (SS) as the denominator (Tabachnick & Fidell 2001).

Following Lechowicz (1982), comparison between diet composition and resource availability was made using Vanderploeg & Scavia's (1979) relativised electivity index (E^*):

$$E_i^* = \frac{[W_i - (1/n)]}{[W_i + (1/n)]}, \text{ where } W_i = \frac{r_i/p_i}{\sum r_i/p_i},$$

r_i is the relative (proportional) abundance of prey i in the diet, p_i is the relative abundance of prey i in the environment and n is the number of prey types included in the analysis. This index ranges from $+1$ (positive selection) to -1 (negative selection) and values near zero indicate neutral selectivity. Although other electivity indices such as Ivlev's or Strauss's are more widely used, these have several undesirable properties and Vanderploeg and Scavia's is the single best electivity index (Lechowicz 1982). The relative abundances of prey in the environment (p_i) were computed as the average of the proportions in the zooplankton and benthos samples; however, similar results were obtained when the electivity was analysed separately for zooplankton and benthos data. The E^* index was arcsine transformed (arcsine $\sqrt{[(E_i^* + 1)/2]}$) for statistical analysis, because homoscedasticity and normality were clearly improved. To test whether electivity significantly deviated from 0, a one-sample Student's t-test was used. Electivity was further analysed with ANCOVA (see above), using fish length as the covariate. All statistical analyses were performed with SPSS 12 for Windows.

Results

Habitat use and resource availability

As in previous studies, the toothcarp population size structure was bimodal (Figure 2) and composed of two groups: immature young-of-the-year ($0+$) born in summer and mature fish ($0+$, born mostly in spring, and $1+$). Toothcarp density differed significantly in the three habitats ($F_{2,35} = 14.87$, $P < 0.001$). Only three fish were captured in open water, and density was also higher in glasswort than in algal mats (GH test, $P < 0.05$). However, the higher density in glasswort was only significant for mature fish ($F_{1,27} = 22.82$, $P < 0.001$) and not for immature fish ($F_{1,27} = 0.93$, $P = 0.34$), showing size-specific differences in habitat use (Figure 2).

Mean standard length (SL) of the toothcarp was 18.4 mm (SE = 3.9, range 7 – 30). Fish were larger in glasswort (mean = 19.1 mm, range 10 – 30) than in algal mats (15.6 mm, range 7 – 25) ($F_{1,13} = 9.02$, $P = 0.10$) (Figure 2). This difference was due to a higher proportion of immature fish in algal mats ($G = 54.6$, df = 4, $P < 0.001$), since 52% of the
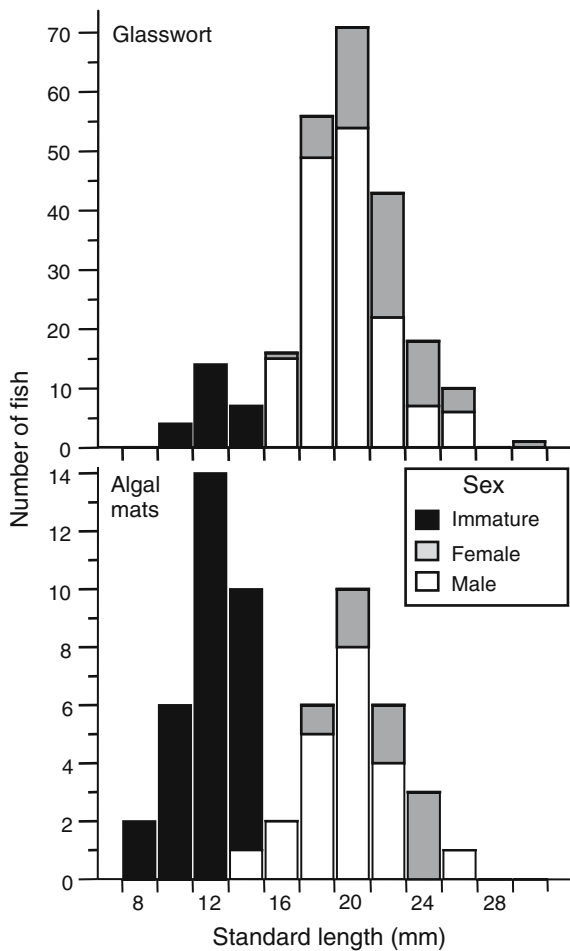
Figure 2. Length-frequency distribution of the toothcarp in Fra Ramon lagoon by habitat (top, glasswort; bottom, algal mats) and sex category (immature, female and male). The open water habitat is omitted because only three fish were captured.

individuals in algal mats were immature compared with only 10% in glasswort (Figure 2). Taking mature fish alone, although females (mean = 21.2 mm, range 16.5 – 30) were larger than males (19.4 mm, range 14.5 – 26) ($F_{1,17} = 9.71$, $P = 0.006$) due to sexual dimorphism, habitat produced no significant effect on standard length ($F_{1,17} = 0.50$, $P = 0.49$) or on sex group composition ($G = 0.82$, df = 2, $P = 0.66$) (Figure 2). Sex ratio significantly differed from 1 : 1 ($G = 45.7$, df = 1, $P < 0.001$), males (71.5%) being more abundant than females (28.5%).

The spatial heterogeneity of benthos and plankton is analysed in detail elsewhere (Alcaraz

et al., submitted manuscript) and briefly summarised herein. The density of organisms (ANOVA, $F_{2,3} = 117.6$, $P = 0.001$) and total dry biomass ($F_{2,3} = 10.41$, $P = 0.045$) of water column invertebrates showed significant differences among habitats. In both cases, the algal mat habitat presented higher densities of organisms. While density of invertebrates in glasswort was lower than in open water, dry biomass was higher because open water contained only smaller organisms (mainly copepod nauplii and rotifers). In glasswort and algal mat habitats, the water column was dominated in number by small organisms, mainly copepod nauplii, harpacticoids and, in algal mats, rotifers. By biomass the results differed and while algal mats were dominated by amphipods (*Gammarus aequicauda*) and gastropods (*Hydrobia* sp.),

Table 1. Diet of the Spanish toothcarp in Fra Ramon lagoon: % number, % biomass, and frequency of occurrence of the main food components.

Food category	% number	% biomass	Frequency of occurrence
Detritus	–	24.69	67.74
Plant debris	–	0.07	14.52
Plant leaves	–	0.10	3.23
Brachionus plicatilis	0.21	0.02	12.90
Testudinella clypeata	0.01	0.00	1.61
Nereis diversicolor	0.20	1.46	24.19
Unid. Acari	0.01	0.15	1.61
Unid. Ostracoda	0.37	0.05	32.26
Mesochra lilljeborgi	61.24	5.11	93.55
Mesochra rapines	0.04	0.01	4.84
Tisbe longicornis	15.20	4.63	82.26
Canuella perplexa	0.27	0.47	8.06
Copepoda nauplii	5.84	0.05	91.94
Gammarus aequicauda	0.02	3.59	3.23
Protracheoniscus occidentalis	0.18	39.13	11.29
Unid. Crustacea	0.01	0.05	1.61
Chironomidae (l.)	0.48	14.34	41.94
Stratiomyidae (l.)	0.05	0.35	3.23
Stratiomyidae (ex.)	0.01	0.00	1.61
Rhagionidae (l.)	0.02	2.11	3.23
Ephydridae (l.)	0.03	0.18	4.84
Ephydridae (ex.)	0.01	0.00	1.61
Unid. Hymenoptera (a.)	0.02	1.14	3.23
Invertebrate eggs	15.68	2.32	46.77
Terrestrial snails	0.12	0.00	16.13

Number of guts analysed = 62; total number of prey in the gut contents = 10,839; total biomass = 42.454 mg. l. = larvae, ex. = exuviae, a. = adult, unid. = unidentified.

glasswort was dominated by gastropods (*Hydrobia* sp.) and dipterans (mostly Stratiomyidae).

Total dry biomass of the zoobenthos also differed significantly among habitats ($F_{2,3} = 10.51$, $P = 0.044$), being lowest in algal mats, but not the total density of organisms ($F_{2,3} = 1.32$, $P = 0.39$). The three habitats presented a high abundance of harpacticoids (mainly *Mesochra lilljeborgi* in glasswort and *Canuella perplexa* in open water and algal mats) but open water and algal mats were clearly dominated by ostracods. By biomass, the most important taxa were chironomid larvae (in all three habitats) and ostracods in open water and algal mats.

Ontogenetic and habitat variation in diet

The toothcarp diet was mostly based on the harpacticoids copepods *Mesochra lilljeborgi* and *Tisbe longicornis*, copepod nauplii and detritus (Table 1 and Figure 3). The relationship between number, biomass, and occurrence (Figure 3) pointed to some feeding variation among individuals. First, some prey were important by biomass but with low occurrence, particularly the semiaquatic isopod (*Protracheoniscus occidentalis*) but also the amphipod *Gammarus aequicauda*, rhagionid dipterans and hymenopterans. These were the largest prey, dominating in only a few large fish (see
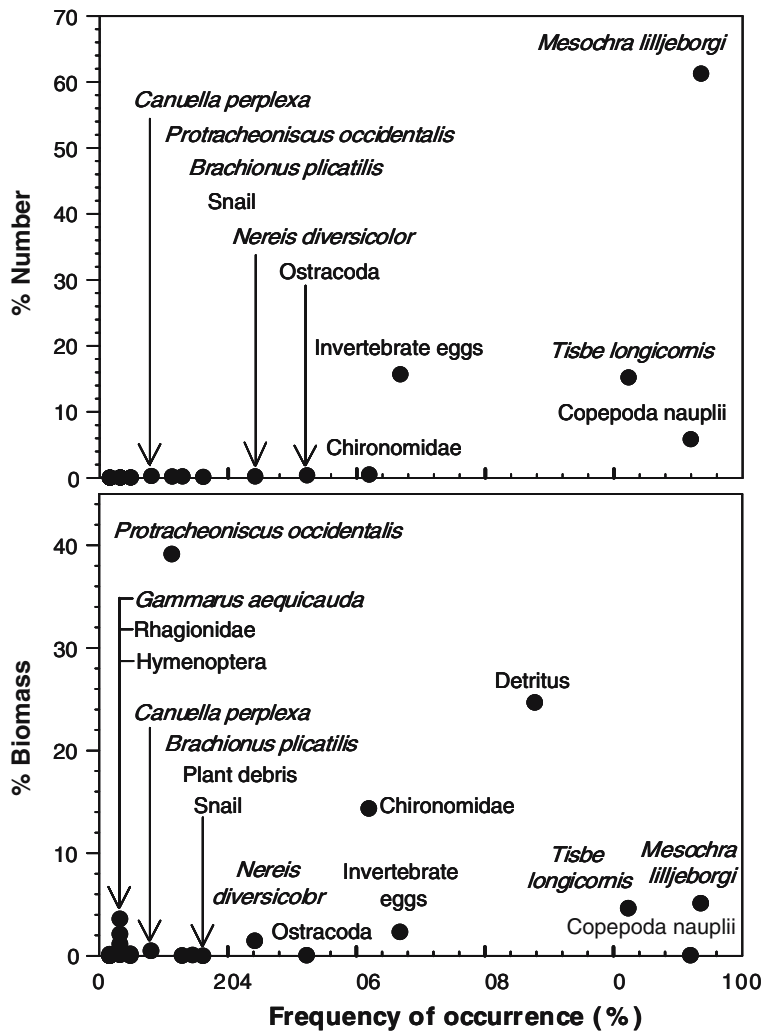

Figure 3. Relationship among % number, % biomass, and frequency of occurrence of food categories in the Spanish toothcarp diet. Plots based on Costello's (1990) method (see Methods). Food categories described are the items most important in diet.

below). Chironomid larvae were more important by biomass and invertebrate eggs by number and both were eaten more in algal mats than in glasswort (Figure 4).

The relative importance of prey eaten was highly dependent on habitat and toothcarp size (Figure 4). The harpacticoid copepod *Mesochra lilljeborgi* was important for small (juvenile or immature) fish in glasswort, but consumed less in algal mats, where it was replaced by chironomid larvae and invertebrate eggs. The isopod *Protracheoniscus occidentalis* was very dominant in larger toothcarp in glasswort, but replaced by
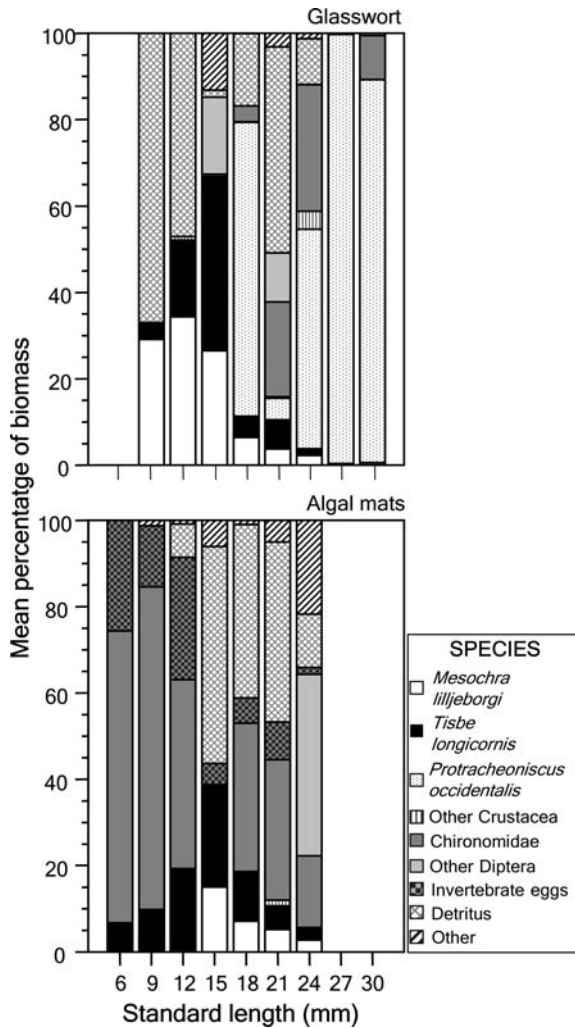
chironomids and other dipterans (mainly rhagionids) in algal mats (Figure 4). Detritus was important for toothcarp present in both glasswort and algal mats; its importance significantly decreased with size in glasswort but increased in algal mats.

Despite the ontogenetic variation in diet, the overall diversity of the toothcarp's prey did not significantly depend on length (ANCOVA, $F_{1,55} = 0.47$, $P = 0.50$) but it was significantly higher in algal mats ($D = 0.40 \pm SD = 0.18$) (ANOVA, $F_{1,56} = 4.96$, $P = 0.030$) than in glasswort ($D = 0.31 \pm SD = 0.16$). Females ($D = 0.40 \pm SD = 0.16$) presented a marginally higher diversity than males ($D = 0.32 \pm SD = 0.17$) ($F_{1,42} = 3.92$, $P = 0.054$). In contrast, the number of different prey items in gut contents did not show any significant relationship with standard length (ANCOVA, $F_{1,55} = 1.94$, $P = 0.17$) or depend on habitat (ANOVA, $F_{1,56} = 0.98$, $P = 0.33$).

The mean length of prey captured increased significantly with the standard length of the toothcarp (ANCOVA, $F_{1,55} = 10.9$, $P = 0.002$), but only in glasswort and not in algal mat habitat (habitat×SL interaction, $F_{1,55} = 4.53$, $P = 0.038$) (Figure 5). Total biomass of gut contents also depended significantly on fish size (ANCOVA, $F_{1,55} = 87.38$, $P < 0.001$), habitat ($F_{1,55} = 11.13$, $P = 0.002$), and their interaction ($F_{1,55} = 9.01$, $P = 0.004$). Food biomass in the gut was higher in algal mats than in

Figure 4. Ontogenetic and habitat variation in the diet of the toothcarp. Data are the percentage of gut content biomass, averaged for 3 mm-length classes of toothcarp.
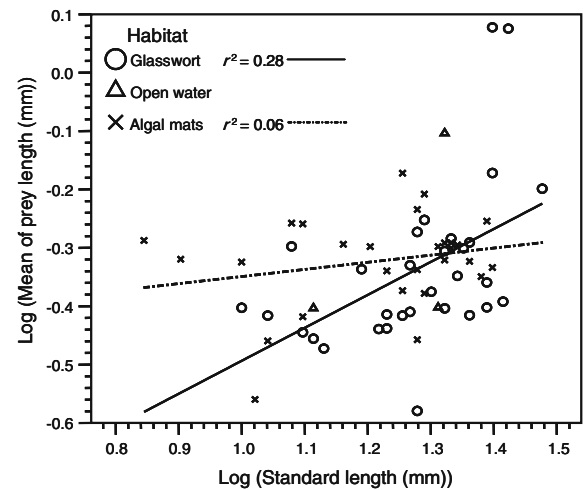

Figure 5. Relationship of mean prey length in the gut contents with standard length of toothcarp by habitat. The linear regression functions are also shown.
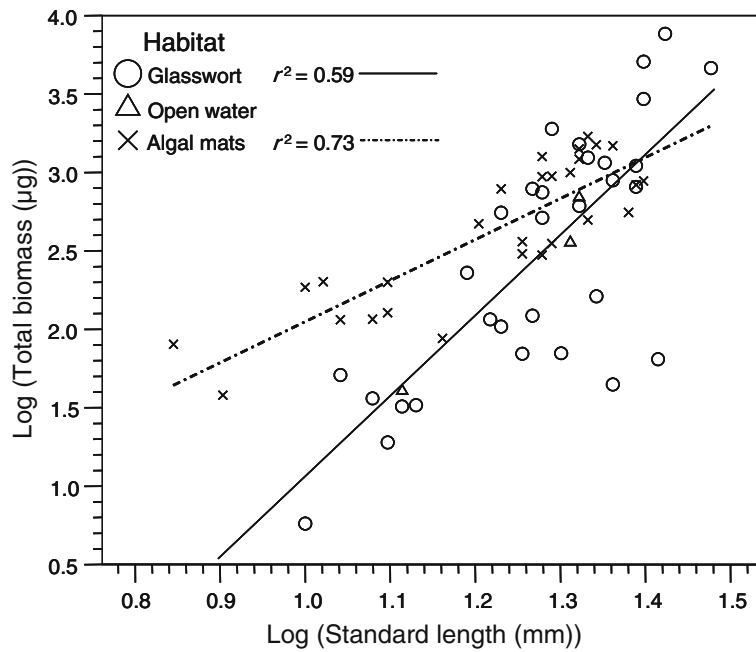
Figure 6. Relationship of total food biomass in the gut contents with standard length of toothcarp by habitat. The linear regression functions are also shown.
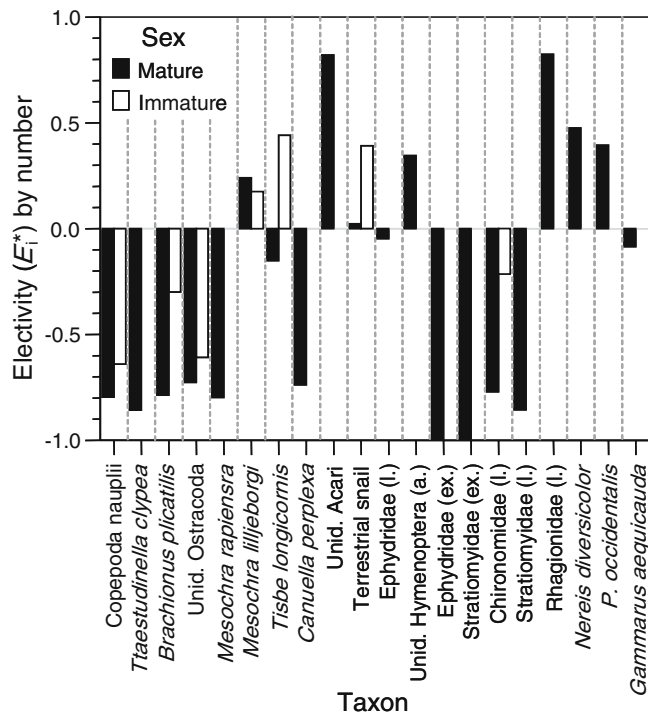

Figure 7. Electivity (Vanderploeg and Scavia's selectivity coefficient) in number by habitat for immature and mature Spanish toothcarp. The different prey items are ordered by species mean length in the lagoon. l. = larvae, ex. = exuviae, a. = adult, unid. = unidentified.

glasswort, after accounting for fish size, but only for small, immature fish (Figure 6).

Habitat (partial $\eta^2 = 0.088$) was more important than fish size (partial $\eta^2 = 0.013$) in explaining variation of prey diversity and composition in the toothcarp's diet; whereas for mean prey length and total food biomass, standard length (partial $\eta^2 = 0.17$ and 0.61, respectively) was more important than habitat (partial $\eta^2 = 0.084$ and 0.17, respectively).

Toothcarp's electivity

Mature toothcarp showed significant positive electivity (*t*-tests, $P < 0.05$) for the harpacticoid copepod *Mesochra lilljeborgi*, rhagionid dipterans and the polychaete *Nereis diversicolor*, and significant negative electivity for copepods nauplii, the rotifer *Brachionus plicatilis*, ostracods, the harpacticoids *Mesochra rapiens* and *Canuella perplexa*, chironomids, and stratiomyid dipterans (Figure 7). For the remaining categories (e.g. *Tisbe longicornis*, snail, ephydrid dipterans, hymenopterans, *Protracheoniscus occidentalis*, and *Gammarus aequicauda*) electivity did not significantly deviate from zero ($P > 0.10$) (Figure 7). Immature toothcarp selected positively for *Tisbe longicornis*, while copepod nauplii and ostracods were negatively selected (Figure 7). Electivity could not be statistically tested for some prey that only
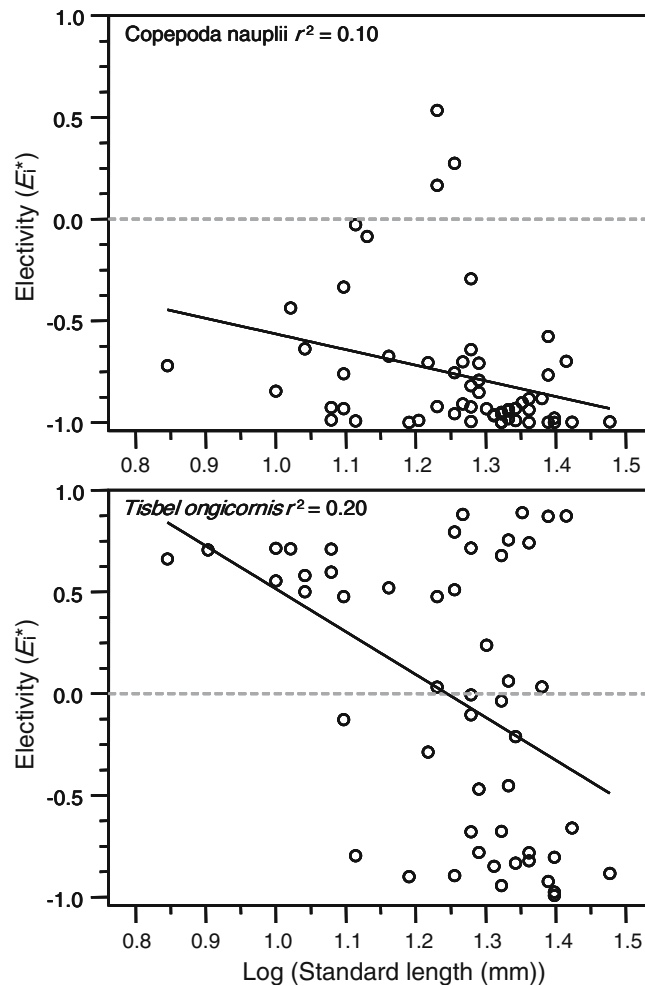

Figure 8. Relationship of electivity with standard length of toothcarp for copepod nauplii (top) and the harpacticoid copepod *Tisbe longicornis* (above).

appeared in a single fish, but it was clearly positive for water mites (Acari) and terrestrial snails due to lower relative abundance in the water, while it was negative for the rotifer *Testudinella clypeata* and

exuviae of ephydrid and stratiomyid dipterans, which were widely available (Figure 7).

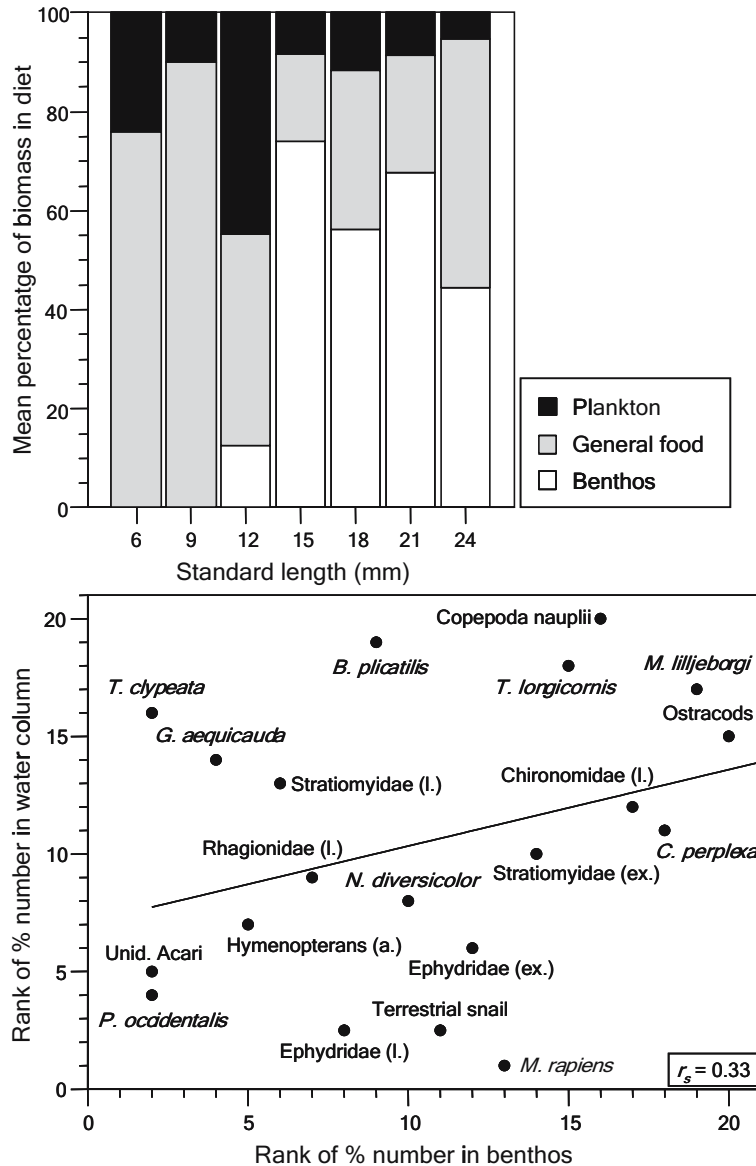Furthermore, electivity of prey depended significantly on standard length for *Tisbe longicornis*

Figure 9. Discrimination of planktonic and benthic prey (bottom) and relative importance of both with ontogeny in the toothcarp's diet (top). The figure at the bottom shows the relationship of the abundance (% number) of invertebrates in the water column and the benthos (rank transformed, in ascending order, because of strong asymmetry and nonlinearity). Species on top were the most abundant in the water column, while species on the right were the most abundant in the benthos. A larger distance from the linear regression function indicates taxa disproportionately more abundant in the water column (positive residuals) or the benthos (negative residuals). The figure at the top shows the relative importance of plankton and benthos in the toothcarp's diet in the algal mat habitat. Data are biomass proportion of planktonic (prey with standardised residual ≥1 in the bottom figure), benthic (residual ≤−1), and general food (not discriminating, present in both the water column and the benthos) in gut contents, averaged for 3 mm-length classes of toothcarp.

($F_{1,46} = 18.4$, $P < 0.001$), chironomids ($F_{1,21} = 29.9$, $P < 0.001$), copepod nauplii ($F_{1,51} = 10.6$, $P = 0.002$), ostracods ($F_{1,15} = 4.49$, $P = 0.051$) and snails ($F_{1,7} = 5.32$, $P = 0.054$) (see e.g. Figure 8). The remaining categories were not significantly related to standard length ($P \gg 0.10$). In general, electivity for many small prey was positive in small fish and negative in large fish.

Electivity also showed differences among habitats. Prey such as copepod nauplii ($F_{1,51} = 4.78$, $P = 0.033$), *Tisbe longicornis* ($F_{1,46} = 4.65$, $P = 0.036$), terrestrial snails ($F_{1,7} = 6.47$, $P = 0.038$), and *Nereis diversicolor* ($F_{1,11} = 31.2$, $P < 0.001$) showed higher electivity in glasswort than in algal mats, while *Mesochra lilljeborgi* ($F_{1,52} = 25.6$, $P < 0.001$) and chironomids ($F_{1,21} = 9.84$, $P = 0.005$) were more selected in algal mats. Furthermore, prey such as *Testudinella clypeata* and water mites only appeared in toothcarp from algal mats, while *Mesochra rapiens*, larvae and exuviae of ephydrids and stratiomyids, *Protracheoniscus occidentalis*, and *Gammarus aequicauda* were only present in fish from glasswort.

Microhabitat use and toothcarp feeding

Although some taxa were very abundant in both the water column and the benthos (e.g. *Mesochra lilljeborgi*, *Tisbe longicornis* or copepod nauplii), abundances in both microhabitats were not significantly related (Spearman's $r_s = 0.33$, $N = 20$, $P = 0.16$, by % number and $r_s = 0.26$, $N = 20$, $P = 0.26$ by % biomass) because some taxa were abundant in the water column but not in the benthos (e.g. *Testudinella clypeata*, *Gammarus aequicauda* or stratiomyids dipterans) and vice versa (e.g. *Mesochra rapiens*, snails or ephydrid dipterans) (Figure 9). This relationship (see caption to Figure 9) allows us to distinguish objectively between planktonic prey (e.g. rotifers such as *Brachionus plicatilis* and *Testudinella clypeata*, *Tisbe longicornis*) and benthic prey (e.g. *Mesochra rapines*, ephydrid dipterans) and thus examine whether the toothcarp feeds more from the water column or from the benthos.

In glasswort, the importance of prey in diet was positively correlated with percent abundance in the water column ($r_s = 0.54$, $N = 18$, $P = 0.021$) but not with abundance in the benthos ($r_s = 0.33$, $N = 18$, $P = 0.18$), because some typically planktonic taxa

(e.g. copepod nauplii) are very important in the toothcarp diet. In algal mats, percent number of prey categories was correlated with percent number in both the water column ($r_s = 0.64$, $N = 13$, $P = 0.020$) and the benthos ($r_s = 0.68$, $N = 13$, $P = 0.011$). By percent biomass, importance in diet was correlated only with percent biomass in the water column for both glasswort ($r_s = 0.52$, $N = 18$, $P = 0.028$) and algal mats ($r_s = 0.49$, $N = 13$, $P = 0.090$).

The ontogenetic diet shift previously described (Figure 4) was clearly related to variation in microhabitat use. Small fish showed a preference for feeding on plankton or general food prey (organisms present in both the water column and the benthos), whereas larger fish fed on benthic prey, such as ephydrid and stratiomyid dipterans or the harpacticoid copepods *Canuella perplexa* and *Mesochra rapines* (Figure 9). This ontogenetic shift in microhabitat and diet was clearer in algal mats than in glasswort (Figure 4), as expected, given the shallowness of the latter habitat.

Discussion

Toothcarp's diet

The most common prey, by number and occurrence, in the toothcarp's diet were microcrustaceans, particularly harpacticoid copepods (*Mesochra lilljeborgi* and *Tisbe longicornis*). There was an ontogenetic diet shift, as well as among-habitat variation in diet, as a result of different prey availability (e.g. the presence of the isopod *Protracheoniscus occidentalis* on glasswort, or the higher abundance of chironomids on algal mats habitat). The only field study on the diet of *Aphanius iberus* is the study carried out in the Ebro delta (Vargas 1993; Vargas & de Sostoa 1999), which did not measure prey availability. The main difference between our study (hereafter Fra Ramon) and the Ebro delta population was in the occurrence and relative importance of different prey. Harpacticoid copepods were the most important prey by number in both populations, with a similar percentage in number but a higher frequency of occurrence in Fra Ramon (> 90%) than in the Ebro (36% overall, and less important in autumn and winter), while amphipods were more abundant in the Ebro (15%

in number and 36% in occurrence on average, more in autumn and winter). Ostracods and chironomids presented similar occurrence in both populations but were more important by number in the Ebro population, while polychaete were more important in Fra Ramon. These differences were probably due to differences in resource availability. Percentage of guts with detritus or plant material was very similar in both populations (69% in the Ebro and 68% in Fra Ramon). By contrast, the number of empty guts in the Ebro population was very high (50%) and the mean number of prey per gut very low (7.8), whereas in Fra Ramon no gut was absolutely empty and the mean number of prey per gut was 174.8. These differences may be due to much larger prey sizes or methodological problems in the Ebro study.

The Spanish toothcarp's diet, combining prey of both animal (mainly crustaceans) and plant or detritic origin, is similar to that of other *Aphanius* species (Al-Daham et al. 1977; Haas 1982; Al-Akel et al. 1987) and other cyprinodontiform fish such as *Fundulus luciae* (Kneib 1978). However, *A. dispar*, *A. mento*, and *A. sophiae* presented a more herbivorous diet (Al-Daham et al. 1977; Haas 1982; Al-Akel et al. 1987), although under experimental conditions *A. mento* ate plants but *A. dispar* and *A. sophiae* preferred animal food (Al-Daham et al. 1977; Haas 1982). By contrast, another Spanish endemic and threatened cyprinodontiform (*Valencia hispanica*) showed a diet based only on invertebrate prey, mainly amphipods (Caiola et al. 2001). Two introduced species competing with the Spanish endemic cyprinodontiforms present a similar diet, with *Fundulus heteroclitus* also preferring harpacticoid copepods (Kneib 1986) and *Gambusia holbrooki* being zooplanktivorous and consuming cladocerans, ostracods and copepods (Crivelli & Boy 1987; García-Berthou 1999).

Habitat variation in diet

The toothcarp diet presented significant differences among habitats. Habitat was more important than fish size (power analysis, partial η^2) in explaining the variation of diversity and prey composition on toothcarp guts. There was higher diet diversity in algal mats and immature fish presented more biomass in gut contents here than in glasswort. In glasswort, larger toothcarp captured larger prey and there was a closer relation-

ship between mean prey length and fish length. Furthermore, glasswort toothcarp fed on larger prey than those in algal mats. Similarly, *F. heteroclitus* showed positive electivity for larger prey in the presence of less dense vegetation (Vince et al. 1976).

Immature fish were relatively more abundant in algal mats habitats, where they had more gut biomass, and they were also in better condition (Alcaraz et al., submitted manuscript). Rincón et al. (2002) showed in a mesocosm experiment that young stages of *A. iberus* captured significantly less prey in the presence of adult conspecifics. Our results point to interactive segregation in habitat use among size classes of toothcarp.

Food electivity and ontogenetic diet shift

Toothcarp presented significant positive electivity for a reduced number of prey. Electivity depended on prey availability and fish size. Overall, smaller fish positively selected small prey such as harpacticoid copepods (*Mesochra lilljeborgi* and *Tisbe longicornis*), while larger fish showed a greater preference for diverse larger prey (e.g. rhagionids dipterans, *Nereis diversicolor* and *Protracheoniscus occidentalis*). The positive electivity for terrestrial or semiaquatic prey, such as the terrestrial snail, the isopod *Protracheoniscus occidentalis* and hymenopterans, suggests the importance of these large, visible prey as a food resource for the toothcarp.

In Fra Ramon, the toothcarp diet was diverse but displayed an ontogenetic shift from harpacticoid copepods to larger prey (isopods and dipterans larvae). Standard length was a more important factor than habitat (power analysis) for explaining differences among mean prey length and total gut biomass. Mean prey length increased with fish length in glasswort, where harpacticoid copepods and detritus were very important for small fish, but were replaced by chironomids and mainly isopods as the fish grew in length. In algal mats, smaller fish consumed fewer harpacticoids, preferring chironomids and invertebrate eggs, and the importance of detritus and other dipterans increased with fish length. A similar ontogenetic diet shift was shown by Vargas (1993): in autumn, the smallest fish showed a preference for chironomids, increasing the abundance in their diet of other prey such as

harpacticoids copepods as they grew in size (Vargas 1993).

In Fra Ramon the ontogenetic diet shift in glasswort toothcarp involved other prey items, very different from those of algal mats or the Ebro population, demonstrating the importance of habitat variation and prey availability in dietary studies. In glasswort, harpacticoids copepods were more abundant than they were in algal mats (Alcaraz et al., submitted manuscript) being consumed more by small fish, and progressively replaced by larger prey such as dipterans or the isopod *Protracheoniscus occidentalis*. This pattern agrees with the Ebro study, where in spring harpacticoid copepods were more abundant than chironomids and more important in diet (Vargas 1993).

However, the overall diversity and number of prey categories in gut contents did not change significantly with fish length. This pattern was surely due to the fact that with growth, the Spanish toothcarp did not completely change its trophic niche and feeding habitat, but only added some larger prey to its diet (new species or larger sizes of the same species), these being more important in biomass.

Although several authors suggest a benthic feeding habitat (Vargas & de Sostoa 1999; Doadrio 2001; Rincón et al. 2002), the toothcarp diet was more closely correlated with organisms in the water column in both the glasswort and algal mats habitats. This pattern might be due to the fact that *A. iberus* is the only fish with a stable population in the Fra Ramon lagoon. In the presence of planktivorous aggressive species, such as *G. holbrooki*, the toothcarp restricts its microhabitat to a lower position in the water column (Rincón et al. 2002), producing niche segregation and compression in synoptic populations (Vargas 1993). There are no field studies showing the generality of these niche dynamics between the toothcarp and invasive species.

In Fra Ramon, where *A. iberus* is the only fish species with a stable population, we have shown that the ontogenetic diet shift is linked to a microhabitat change. Smaller fish showed positive electivity and the more importance of planktonic prey (e.g. copepods nauplii, the harpacticoid copepods *Mesochra lilljeborgi* and *Tisbe longicornis*, the rotifer *Brachionus plicatilis*, and ostrac-

ods), while larger fish presented more benthic prey (e.g. *Canuella perplexa*, *Mesochra rapiens*, and ephydrids dipterans) and showed more electivity for benthic prey such as rhagionids dipterans or the polychaete *Nereis diversicolor*. Among habitats, this microhabitat change in diet was more apparent in algal mats than glasswort. This was as expected, due to the shallowness of the latter habitat. Our findings demonstrate the usefulness of measuring resource availability and electivity in dietary studies.

References

Al-Akel, A.S., M.J.K. Shamsi & H.F. Al-Kahem. 1987. Selective feeding behaviour of the Arabian freshwater fish, Aphanius dispar. Pakistan J. Zool. 19: 211–215.

Al-Daham, N.K., M.F. Huq & K.P. Sharma. 1977. Notes on the ecology of fishes of genus Aphanius and Gambusia affinis in Southern Iraq. Freshwater Biol. 7: 245–251.

Caiola, N. & A. de Sostoa. 2005. Possible reasons for the decline of two native toothcarps in the Iberian Peninsula: evidence of competition with the introduced Eastern mosquitofish. J. Appl. Ichthyol. 21: 358–363.

Caiola, N., M.J. Vargas & A. de Sostoa. 2001. Feeding ecology of the endangered Valencia toothcarp, Valencia hispanica (Actinopterygii: Valenciidae). Hydrobiologia 448: 97–105.

Costello, M.J., J. Edwards & G.W. Potts. 1990. The diet of the two-spot goby, Gobiusculus flavescens (Pisces). J. Mar. Biol. Assoc. UK 70: 329–342.

Crivelli, A.J. & V. Boy. 1987. The diet of the mosquitofish Gambusia affinis (Baird & Girard) (Poeciliidae) in Mediterranean France. La Terre et la Vie, Revue d'Ecologie Appliquée 42: 421–435.

Day, R.W. & G.P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr. 59: 433–463.

Doadrio, I. (ed.). 2001. Atlas y libro rojo de los peces continentales de España. Ministerio de Medio Ambiente, Madrid.

Doadrio, I., J.A. Carmona & C. Fernández-Delgado. 2002. Morphometric study of the Iberian *Aphanius* (Actinopterygii, Cyprinodontiformes), with description of a new species. Folia Zoologica 51: 67–79.

Dumont, H.J., I. Van de Velde & S. Dumont. 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia 19: 75–97.

Fernández-Delgado, C., J.A. Hernando-Casal, M. Herrera & M. Bellido. 1988. Age, growth and reproduction of Aphanius iberus (Cu. & Val., 1846) in the lower reaches of the Guadalquivir river (south–west Spain). Freshwater Biol. 20: 227–234.

García-Berthou, E. 1999. Food of introduced mosquitofish: ontogenetic diet shift and prey selection. J. Fish Biol. 55: 135–147.

García-Berthou, E. 2001. Size- and depth-dependent variation in habitat and diet of the common carp (*Cyprinus carpio*). Aquat. Sci. 63: 466–476.

García-Berthou, E. & R. Moreno-Amich. 1992. Age and growth of an Iberian cyprinodont, *Aphanius iberus* (Cuv. & Val.), in its most northerly population. J. Fish Biol. 40: 929–937.

García-Berthou, E. & R. Moreno-Amich. 1993. Multivariate analysis of covariance in morphometric studies of the reproductive cycle. Can. J. Fish. Aquat. Sci. 50: 1394–1399.

García-Berthou, E. & R. Moreno-Amich. 2000. Food of introduced pumpkinseed sunfish: ontogenetic diet shift and seasonal variation. J. Fish Biol. 57: 29–40.

Gerking, S.D. 1994. Feeding Ecology of Fish. Academic Press, Inc, San Diego, CA.

Haas, R. 1982. Notes on the ecology of *Aphanius dispar* (Pisces, Cyprinodontidae) in the Sultanate of Oman. Freshwater Biol. 12: 89–95.

Hurlbert, S.H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology 52: 577–586.

Hurlbert, S.H. 1978. The measurement of niche overlap and some relatives. Ecology 59: 67–77.

Kneib, R.T. 1978. Habitat, diet, reproduction and growth of the spotfin killfish, *Fundulus luciae*, from a North Carolina Salt Marsh. Copeia 1: 164–168.

Kneib, R.T. 1986. The role of *Fundulus heteroclitus* in Salt Marsh Trophic Dynamics. Am. Zool. 26: 259–269.

Lechowicz, M.J. 1982. The sampling characteristics of electivity indices. Oecologia 52: 22–30.

Lindegaard, C. 1992. Zoobenthos ecology of Thingvallavatn: vertical distribution, abundance, population dynamics and production. Oikos 64: 257–304.

Nemerson, D.M. & W.A. Able. 2004. Spatial patterns in diet and distribution of juveniles of four fish species in Delaware Bay marsh creeks: factors influencing fish abundance. Mar. Ecol. Prog. Ser. 276: 249–262.

Rincón, P.A., A.M. Correas, F. Morcillo, P. Risueño & J. Lobón-Cerviá. 2002. Interaction between the introduced eastern mosquitofish and two autochthonous Spanish toothcarps. J. Fish Biol. 61: 1560–1585.

Smock, L.A. 1980. Relationships between body size and biomass of aquatic insects. Freshwater Biol. 10: 375–383.

Tabachnick, B.G. & L.S. Fidell. 2001. Computer-assisted research design and analysis. Allyn and Bacon, Boston 748 pp.

Vanderploeg, H.A. & D. Scavia. 1979. Calculation and use of selectivity coefficients of feeding: zooplankton grazing. Ecol. Model. 7: 135–149.

Vargas, M.J. 1993. Interacción entre Aphanius iberus y Gambusia holbrooki en el Delta del Ebro: sus ciclos biológicos y ecologías tróficas. Ph.D. Dissertation, University of Barcelona. 312 pp.

Vargas, M.J. & A. de Sostoa. 1997. Life-history pattern of the Iberian toothcarp *Aphanius iberus* (Pisces, Cyprinodontidae) from a Mediterranean estuary, the Ebro delta (Spain). Netherlands J. Zool. 47: 143–160.

Vargas, M.J. & A. de Sostoa. 1999. Ecología trófica del fartet, Lebias ibera, en el Delta del Ebro. pp. 133–161. *In*: M. Planelles-Gomis, (eds.), Peces ciprinodóntidos ibéricos. Fartet y Samaruc, Generalitat Valenciana, València.

Vince, S., I. Valiela & N. Backus. 1976. Predation by the salt marsh killifish Fundulus heteroclitus (L.) in relation to prey size and habitat structure: consequences for prey distribution and abundance. J. Exp. Mar. Biol. Ecol. 23: 255–266.