Herbivory and seasonal changes in diet of a highly endemic cyprinodontid fish (*Aphanius farsicus*)

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Abstract Trophic ecology is essential to understand ecosystem functioning and structure and assist biological conservation. Here we investigate, for the first time, the feeding ecology of *Aphanius farsicus*, a cyprinodontid fish endemic of a single landlocked river basin in central Iran. We sampled monthly a population of this fish species during a year and examined differences in food across seasons, sexes and sizes. Similarly to other cyprinodonts, *A. farsicus* showed sexual dimorphism and more abundance of females. Size structure and individual condition varied across seasons, with larger fish in spring and better condition in summer and less in winter. We found no empty guts, suggesting that these fish feed all year round due to the warm climate of its native distribution. Farsi toothcarp diet was based on detritus, algae (particularly diatoms, green algae, and cyanobacteria), and small invertebrates. Seasonal variation in diet was more important than variation due to fish size and the Farsi toothcarp consumed more green algae in spring and early summer and more diatoms and insects the rest of the year. Herbivory was considerable, similarly to a few other cyprinodonts, and increased with fish size, particularly because of higher consumption of green algae. As with species composition in diet, season was more important than size in the variation of number, biovolume, mean size, and diversity of prey captured, with higher number, richness and size of prey captured in summer. The ontogenetic diet shift was less marked in this cyprinodont than in many other *Aphanius* species, probably due to its reduced size and the resource availability of its habitat, but was also shown by size-dependent feeding selectivity for a few invertebrates.

Keywords Herbivory · Temperature · Cyprinodontidae · Farsi toothcarp · Semi-arid regions · Maharlu Basin · Iran

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Introduction

Trophic ecology is essential to understand ecosystem functioning and structure and assist biological conservation (Stephens et al. 2007). Herbivory is a dominant ecological interaction in both terrestrial and aquatic ecosystems. Herbivorous fish profoundly affect the

distribution, abundance and diversity of plant communities, mediate competition between fast- and slow-growing species, and are consequently a critical component of nutrient cycling and energy transfer through food webs (Arthur et al. 2006; Hoey and Bellwood 2011). Previous studies, however, have shown that some fish species previously considered herbivores might actually be facultative and that even carnivores often present plant material in their diets (e.g., Stephens et al. 2007). Changes in the importance of herbivory in diet may be mediated not only by changes in prey availability but also by different energetic demands related to body size, morphological constraints and changes in habitat use during ontogeny (Stephens et al. 2007; Ramos-Jiliberto et al. 2011). Obtaining accurate dietary information is thus important for the management and conservation of aquatic ecosystems.

Aphanius is the only genus of the family Cyprinodontidae (sensu Parenti 1981) native to Eurasia (Teimori et al. 2012). Species of this genus inhabit freshwater habitats or coastal marine environments generally characterized by warm water and mesosaline to hypersaline conditions (Kottelat and Freyhof 2007; Alcaraz et al. 2008a,b; Coad 2014). They are present from the Mediterranean coasts of the Iberian Peninsula to the Red Sea and the Persian Gulf basins (Kottelat and Freyhof 2007; Coad 2014). Fourteen *Aphanius* species are currently recognized in Iran, a diversity hotspot for this genus, occurring in restricted areas that reflect the geologic units of the country (Coad 2000; Hrbek et al. 2006; Coad 2014; Teimori et al. 2012). However, the taxonomy of this genus is uncertain and several new *Aphanius* species in Iran have been recently described or redescribed (e.g., Hrbek et al. 2006; Teimori et al. 2011, 2012) and *Aphanius* diversity is expected to be higher than currently recognized (Hrbek et al. 2006). Most of these *Aphanius* species are threatened because of drought, habitat degradation and loss, water pollution, and the introduction of invasive species (Coad 2014); but little is known on the ecology of these endemic, threatened species, despite being essential for their conservation (Abell 2002; Olson et al. 2002).

The Farsi toothcarp (*Aphanius farsicus* Teimori et al. 2011), originally described as *A. persicus* (Jenkins, 1910), is a small cyprinodont, endemic only to the Maharlu Basin (Fars Province, central Iran), where it inhabits small freshwater springs and pools of varying salinity (Teimori et al. 2011). Because of human-mediated droughts (e.g., caused by water infrastructure

development and climate change), water pollution, habitat alteration and the introduction of exotic species, there is an urgent need for its conservation (Coad 2014; Teimori et al. 2011). However, similar to other Iranian cyprinodonts, there are few studies on Farsi toothcarp, mostly focusing on its taxonomy and distribution (e.g., Hrbek et al. 2006; Teimori et al. 2011), reproductive biology (Esmaeili and Shiva 2006), and parasitology (Amin et al. 2013). As far as we know, no study has been published on the food habits of *Aphanius farsicus*, despite being important for understanding its population dynamics, ecology and interaction with native and exotic species, and hence its conservation (Brown and Ewins 1996; Ramos-Jiliberto et al. 2011; Santos et al. 2012). The objectives of the present paper are to provide the first data on the diet of this endemic species, to analyze its ontogenetic and seasonal variation and to compare them with other *Aphanius* species. We also provide data on the seasonal variation in size structure and condition of this species. Based on the previous knowledge of this species and other cyprinodontid fishes, we hypothesized that *Aphanius farsicus* would display an omnivorous diet with seasonal diet and limited ontogenetic variation, due to its small size.

Material and methods

Study area

The study was conducted in Small Barm-e-Shoor (29° 27′ 56.01″ N, 52° 42′ 4.30″ E), a mesohaline spring located at an altitude of 1,465 m, about 500 m to the south of Large Barm-e-Shoor Spring (29° 28′ 7.05″ N, 52° 41′ 43.50″ E); in the northwest corner of the Maharlu Basin (Fig. 1). The inundated area, and consequently the water level, of the Barm-e-Shoor spring system varies widely with rainfall, from covering a maximum extent of about 1.5 km^2 during heavy rainy seasons to a number of scattered ditches and pools up to 2 m deep in the dry season. During the study, the Small Barm-e-Shoor Spring had clear and slow-flowing water with a muddy bottom, with a maximum inundated area of about 3300 m^2. The permanently inundated zone of the spring is vegetated with common reed (*Phragmites australis* (Cav.) Trin. ex Steud.), rush (*Juncus* sp.) and bulrush (*Typha* sp.); and dense floating mats of green algae, which are more abundant in summer when large blooms occur. The temporary salt marsh is dominated

by glasswort (*Salicornia* sp.), salt cedar (*Tamarix* sp.) and sedges (Cyperaceae) (see also BirdLife International, http://www.birdlife.org). The main physical and chemical features of the spring are summarized in Table 1. *Aphanius farsicus* was the only fish species found in the Small Barm-e-Shoor Spring, although one fish species of uncertain origin, the abu mullet, *Liza abu* (Heckel, 1843), and one introduced, the highly invasive mosquitofish (*Gambusia holbrooki* Girard, 1859) had been recorded cohabiting with the Farsi toothcarp in nearby springs, such as the Large Barm-e-Shoor Spring (H.R. Esmaeili, pers. obs.). The presence of the Caspian turtle (*Mauremys caspica ventrimaculata* Wischuf and Fritz, 1996) was also recorded.

The Maharlu Basin, with 4,270 km^2 of surface area, is a landlocked basin located in the central region of Iran, extending 160 km from northwest to southeast. The Maharlu Lake (29° 57′ N, 52° 14′ E) is an endorheic hypersaline lake located 23 km southeast of Shiraz in Fars province at an altitude of 1,454 m (Fig. 1), the lowest point in the plain. The climate is semi-arid with a mean annual precipitation of about 326 mm and a mean annual air temperature of 17.3 °C (Rahnemaei et al. 2005). Salinity ranges from 120 to 280 g/L and its surface area from 175 to 250 km^2 (during the rainy season) (Abatzopoulos et al. 2006), being the largest water body in the basin. It is also a fishless lake, inhabited mostly by the crustacean *Artemia* sp. and the unicellular algae *Dunaliella* sp. (Najafi et al. 2011), and supporting a diverse assemblage of resident and migratory waterfowl. The Maharlu Lake is only fed by small seasonal streams, other minor ephemeral streams, and by small springs around its shore (Abatzopoulos et al. 2006; Coad 2014).

Fish sampling and laboratory procedures

Small Barm-e-Shoor Spring was sampled monthly from August 2006 to July 2007. Fish were captured during daylight hours with a fine-mesh (stretched mesh size of 3 mm) dip net (*N*=561) at different points along the spring shore to avoid sampling bias for larger individuals, and different habitat use patterns by group types. Because *A. farsicus* is an endemic and threatened species (Coad 2014; Teimori et al. 2011; Amin et al. 2013), dip-netting was preferred over passive techniques to minimize fish duration time in a net, and only 30 individuals (15 males and 15 females) from each monthly collection were randomly selected and preserved in situ in 5 % formaldehyde for posterior stomach content analysis. The rest of the individuals were counted, sexed (from external sexual dimorphism, see Fig. 1) and released at the capture place. In the laboratory, all individuals were measured with a Vernier caliper (total length (TL) to the nearest 0.1 mm), weighed (total weight to the nearest 0.001 g), sexed (generally from external sexual dimorphism or by microscopic observation of the gonads), and eviscerated. The entire gut of each individual was slit open, placed in a Petri dish and examined macroscopically for the presence of coarse sediment or large prey. Subsequently, gut content was transferred to a 5 mL vial, filled with distilled water, mixed, and three pipette subsamples were mounted on a glass microscope slide and covered with a cover slip. Diet was determined by analyzing 20 random photos taken per subsample (60 photos per gut content) under a dissecting microscope. All food items present in the photos were classified, usually to genus level, counted and their area were measured, volume was subsequently estimated to the nearest 0.000005 mm^3 from the formula for the most similar geometric form with Image Tool 3.0 (UTHS CSA, San Antonio, TX), an image processing and analysis program.

Data analysis

The seasonal variation of sex ratio and their deviation from 1:1 were tested with *G*-tests of independence (Sokal and Rohlf 1995). The statistical analyses of diet data follow our previous work (e.g., García-Berthou 1999; Alcaraz and García-Berthou 2007a). Percent number (% number), percent biovolume (% biovolume), and frequency of occurrence were used to estimate the dietary importance of the different food categories. 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 biovolume is the equivalent measure for biovolume 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 % biovolume vs. frequency of occurrence (%). The most important prey items are closer to the top right corner. The other diagonal corresponds to feeding strategy: prey items with low occurrence but important by number or biovolume correspond to some sort of specialization and are closer to
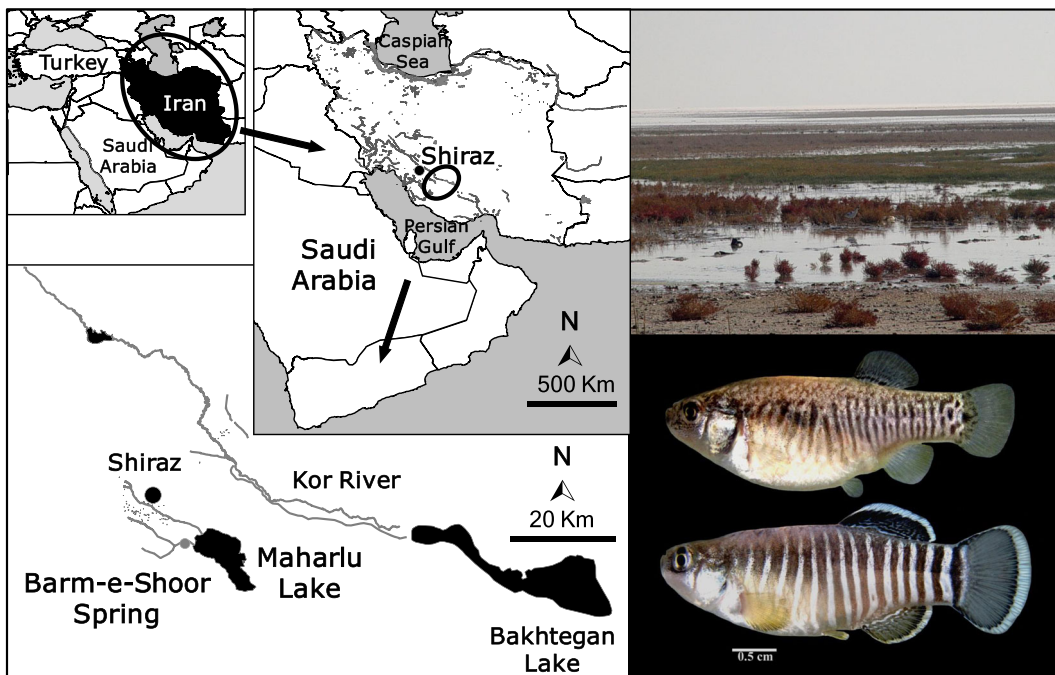
Fig. 1 Location of the study area (Barm-e-Shoor Spring) in the Maharlu Lake Basin. The photos show the Barm-e-Shoor Spring and a female (*on top*) and male (*bottom*) of the Farsi toothcarp (*Aphanius farsicus*)

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. Correspondence analysis (CA) was used to describe the main sources of diet variation, separately for number and biovolume data. CA is an ordination technique that reduces a species × sample matrix to a few dimensions explaining most of the variation. CA is better than the traditional procedure of a priori pooling food categories, which is based usually on taxonomic rather than on ecological criteria (Graham and Vrijenhoek 1988). Food items dominating solutions as outliers were excluded from the CA (Greenacre 2013; Tabachnick and Fidell 2013). Correlation analysis and analysis of variance (ANOVA) of the resulting scores were used to interpret the dimensions in terms of the measured features of the samples.

Analysis of covariance (ANCOVA) was used to compare variables, such as fish condition (length-weight relationship), gut diversity or prey biovolume,

among sex groups and months, using fish length as the covariate. Dietary descriptors were analyzed with multivariate analysis of covariance (MANCOVA); which is suitable when several dependent variables are measured for each sampling unit (Sokal and Rohlf 1995). We started with the most complex model, introducing all possible interactions [including interactions of covariates × factors, following García-Berthou and Moreno-Amich (1993)]. We then simplified the model by removing non significant interactions (*P*>0.10) to increase the statistical power of the remaining sources of variation. When the covariate was not significant (*P*>0.10) it was also deleted from the model (so an ANOVA was used). 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. Partial η^2 has an advantage over η^2 in that it does not depend on the number of sources variation in the ANOVA design used (Tabachnick and Fidell 2013). Most quantitative variables were log-transformed for the analyses because homoscedasticity and linearity were clearly improved. All statistical analyses were performed with SPSS 20 for Windows, except for CAs (CANOCO 4.5 was used).

Results

Farsi toothcarp population structure

Of 561 fish captured in Barm-e-Shoor Spring, 340 were females (60.6 %) and 221 males (39.4 %), thus overall sex ratio differed significantly from 1:1 ($G=25.4$, $df=1$, $P<0.0001$), being skewed towards females (1.54:1). Sex proportion did not differ significantly among months ($G=0.79$, $df=11$, $P=1.0$) (Table 1). Overall, mean total length was 23.4 mm ($SD=4.08$, range= 14.1–41.9 mm, $n=357$), and females were slightly longer (mean $TL=24.03$ mm, $SD=4.69$) than males (mean $TL=22.81$ mm, $SD=3.25$) (ANOVA, $F_{1, 333}=11.30$, $P=0.001$, partial $\eta^2=0.03$), in agreement with clear sexual dimorphism. There was also monthly variation in size structure ($F_{11, 333}=23.15$, $P<0.0001$, partial $\eta^2=0.43$), and sex × month interaction ($F_{11, 333}=2.29$, $P=0.010$, partial $\eta^2=0.07$) (Fig. 2). Toothcarp population was dominated by smaller individuals from July to January, and by larger individuals from May to June (Fig. 2), probably due to the species reproductive cycle. After accounting for fish length (ANCOVA of total weight with TL as covariate, $F_{11, 332}=6273.8$, $P<0.0001$, partial $\eta^2=0.95$), fish condition varied significantly among months ($F_{11, 332}=1.98$, $P=0.030$, partial $\eta^2=0.06$) being lower from November to March (Fig. 2). The same pattern was observed also in both male and female toothcarps but males were significantly heavier than females, for a given length ($F_{1, 332}=10.03$, $P=0.002$, partial $\eta^2=0.03$) (Fig. 2). There was also a marginal month × length interaction ($F_{11, 332}=1.70$, $P=0.073$, partial $\eta^2=0.05$) in fish condition.

Overall diet description

All the 357 toothcarp stomachs examined contained at least one food category, so there were no empty guts. Farsi toothcarp food spectrum was narrow and its diet was mainly based on detritus and plant material, including diatoms and green algae (Table 2, Fig. 3). Overall, similar results were obtained when percent number and percent biovolume were compared to frequency of occurrence (Fig. 3). Detritus was the most prevalent food category, being present in almost all stomachs (98.6 %), but diatoms were the dominant food category by number (53 %) and also by biovolume (15 %), after digested material (Table 2). Cyanobacteria and green algae (without accounting for filamentous green algae) were also common food categories (frequency of occurrence >71 %) and both accounted for ca. 28 % of the diet by number (Table 2). Cyanobacteria, diatoms and green algae were more important by number because of their smaller biovolume (i.e., size). Filamentous green algae were numerically important but less frequently consumed than cyanobacteria or green algae (Table 2, Fig. 3). Other food items such as insects, copepods, desmids or fish scales, although were present in more than 10 % examined stomachs, were unimportant categories both by number or biovolume (Table 2, Fig. 3). The relationship between number, biovolume, and occurrence did not point to any feeding specialization among individuals (top left corner is empty in Fig. 3).

The relative importance of prey eaten was highly dependent on toothcarp size (Fig. 4). Overall, green algae (both filamentous and not filamentous) were dominant in larger fish, but its importance was reduced in smaller fish, being replaced by diatoms and cyanobacteria (Fig. 4). Overall diet diversity depended significantly but slightly ($r^2=0.024$) on toothcarp length (ANCOVA; $F_{1, 347}=8.65$, $P=0.003$, partial $\eta^2=0.02$) but there were no significant differences between sex groups ($F_{1, 347}=0.072$, $P=0.79$) ($D=0.430$, $SD=0.211$; $D=0.434$, $SD=0.209$, for males and females respectively). In contrast, the number of different food items in gut contents was not related to toothcarp TL ($F_{1, 354}=2.07$, $P=0.15$) or sex ($F_{1, 354}=0.147$, $P=0.70$); and there were no significant differences either in number of prey consumed, separately for each prey type, or between sex groups ($P>0.30$ in all cases).

Diet variation

The first two dimensions of the correspondence analysis of gut contents explained 39.6 % of the total variance (eigenvalues=0.118 and 0.103 respectively) (Fig. 5). The first CA dimension scores (CA1) differed significantly among sampling months (ANOVA, $F_{11, 340}=24.96$, $P<0.0001$, partial $\eta^2=0.55$); of the 189 fish with negative CA1 scores, 138 were captured from mid-summer to late-autumn (August to December) (Fig. 5). Although the diet of these fish was mostly based on diatoms and cyanobacteria, other food items were also common (food categories distinguished on the left of the CA1); for instance, rotifers, fish scales, copepods, invertebrate eggs and insects (Figs. 5 and 6). Overall, CA1 mostly differentiated (positive scores) green algae and protozoa (Fig. 5). Green algae were more abundant in

Table 1 Features of Barm-e-Shoor Spring during the sampling period, from August 2006 to July 2007

Month	Sexual proportion		pH	Water conductivity (mS/cm)	Salinity (mg/L)	Dissolved oxygen (mg/L)	Water Temp. (°C)	NO3− (mg/L)	NO2− (mg/L)	NH4+ (mg/L)	PO4−2 (mg/L)	Ca+2 (mg/L)	Meteorological data			
	♂	♀											Min. Tª (°C)	Mean Tª (°C)	Max. Tª (°C)	Rainfall (mm)
August	38.2	61.8	7.8	18.92	11.57		23.6	0.3	0.025	0.37	0.18	27.2	18.94	29.26	36.99	9.90
September	37.8	62.2	6.5	13.57	8.76		20.0	0.1	0.021	0.17	0.81	20.6	14.50	24.89	33.16	0.00
October	39.6	60.4	7.2	22.69	14.96		21.0	0.3	0.014	0.58	1.35	32.5	10.30	20.37	29.00	0.00
November	37.2	62.8	7.5	11.39	6.78	4.12	22.9	3.3	0.102	0.45	0.47	20.5	5.24	12.62	20.44	3.00
December	40.5	59.5	7.4	11.15	7.52	3.87	17.5	3.5	0.052	1.56	0.76	22.3	2.77	4.41	10.15	102.40
January	40.0	60.0	6.5	11.12	7.78	3.26	16.0	2.8	0.011	0.48	0.45	22.3	2.82	4.23	11.49	29.80
February	37.8	62.2	7.4	11.25	7.60	5.82	17.5	4.7	0.012	0.31	0.34	25.3	2.30	8.81	15.48	37.90
March	42.2	57.8	7.3	11.00	6.99	4.32	19.9	2.8	0.010	0.43	1.28	30.2	4.39	11.69	18.47	74.10
April	40.5	59.5	7.1	11.27	6.96	3.70	21.3	3.4	0.012	0.7	0.20	19.8	9.39	17.01	23.98	43.60
May	42.6	57.4	7.1	11.39	7.07	2.71	21.1	1.2	0.011	0.21	0.16	21.6	14.40	24.30	31.68	1.70
June	39.6	60.4	7.2	11.37	6.70	3.23	23.4	0.8	0.039	0.47	0.32	23.2	19.26	29.57	37.56	0.00
July	36.7	63.3	7.2	11.21	6.70	3.66	22.7	4.2	0.017	0.85	0.26	29.1	21.40	31.05	38.82	0.00

All values refer to the sampling date, except for the meteorological data, which are monthly averages obtained from Shiraz airport meteorological station (OISS 408480), situated at 1491 m.a.s.l. (See Fig. 1). ♂ and ♀ are the percentages of male and female toothcarp, respectively. Water salinity concentration was converted from conductivity by equations in APHA (2001)

the toothcarp diet from mid-winter (January) to early-summer (July), whereas protozoa were more frequent from March to June (Figs. 5 and 6). Furthermore, filamentous green algae were more consumed from May to July, when diatoms and green algae were less common in diet (Fig. 6). CA1 scores were also significantly correlated with fish length (Pearson's $r=0.36$, $n=354$, $P<0.0001$), given the seasonal variation in mean fish length (Fig. 2).

The second CA dimension scores (CA2) were significantly correlated with fish length (Pearson's $r=-0.17$, $n=354$, $P=0.001$) and also varied significantly among sampling months (ANOVA, $F_{11, 340}=4.73$, $P<0.0001$, partial $\eta^2=0.13$). Fish with larger CA2 scores were mostly individuals captured in October (Fig. 5), showing a diet mainly based on diatoms but also other food categories such as insects, invertebrate eggs, copepods and scales were abundantly found in their gut contents (Figs. 5 and 6). Fish with lower CA2 scores were individuals mainly captured in February. The diet of these fish was characterized by lower numbers of both insects and copepods and by a large consumption of desmids. Similar results were obtained for biovolume data and are thus not shown here.

Additional insight into the relative contribution of fish length (ontogeny) and month to variation in toothcarp diet was obtained by analyzing some simple descriptors (Table 3). Although fish sex was incorporated into the model, no significant differences were found between sexes in the multivariate test ($P=0.595$) or in all univariate tests ($P>0.10$ in all cases), thus fish sex was removed from the analysis design to increase statistical power and facilitate interpretation. The parallelism assumption, i.e., homogeneity of slopes (García-Berthou and Moreno-Amich 1993), was satisfied according to the multivariate test (fish length × month, $P=0.203$) and for all diet descriptors ($P>0.27$ in all cases); therefore, the standard ANCOVA design was appropriate (Table 3). Although MANCOVA tests were significant, diversity ($D=0.43\pm SD=0.21$) did not depend significantly on fish length or sampling months (Table 3). Total prey number and total biovolume increased significantly with fish length ($r=0.27$ and $r=0.29$, respectively) and also varied among sampling months (Table 3, Fig. 7) and followed a similar pattern, with maximums in summer and early autumn, minimums in March and secondary peaks in February and April (Fig. 7). Mean volume of prey was not significantly
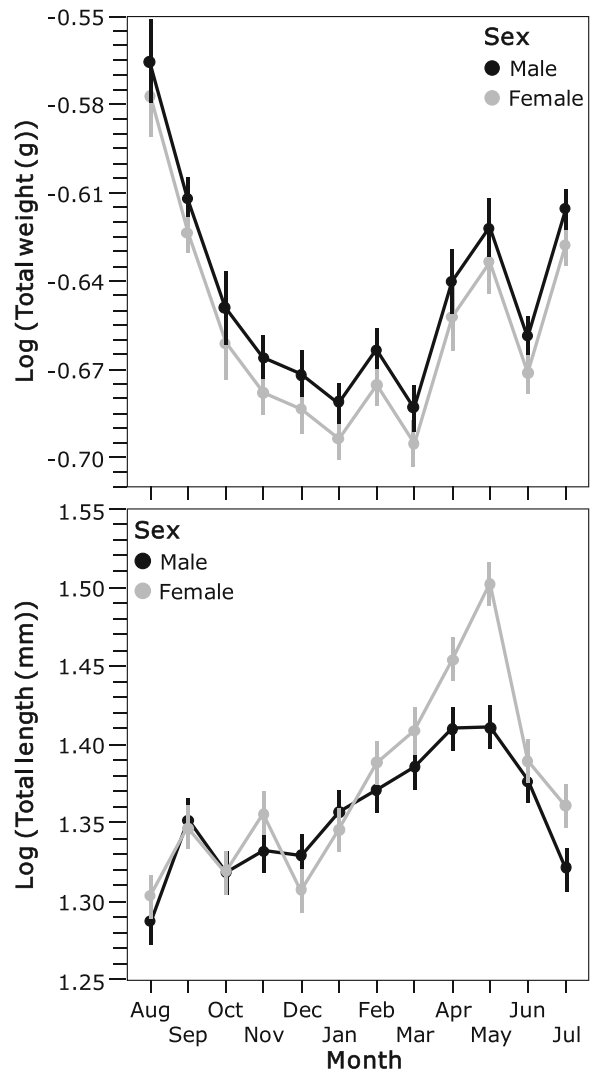

Fig. 2 ANOVA adjusted means of Farsi toothcarp total length by sex and month (*bottom*); and ANCOVAs size-adjusted means of total weight by sex and month (*top*). ANCOVA adjusted means are the population means after adjusting for fish length. *Bars* are standard errors

related to fish length but varied seasonally, whereas number of food items also differed significantly among months and was marginally related to fish length ($r=0.10$, Table 3, Fig. 7). In summary, the Farsi toothcarp captured larger prey and its diet had more richness of food items from August to November, whereas both mean volume of prey and number of food items decreased in December and January (Fig. 7). Overall, sampling month was a more important factor than fish length to the variation of dietary descriptors (see partial η^2 values in Table 3).

Table 2 Diet of the Farsi toothcarp in Barm-e-Shoor Spring: %
number, % biovolume, and frequency of occurrence of the main
food components

Food component	% Number	% Biovolume	Frequency of occurrence
Detritus	–	7.82	98.60
Cyanobacteria	11.45	4.09	71.99
Filamentous green algae	14.73	8.29	32.21
Other green algae	16.32	0.42	73.67
Desmids	0.72	0.03	13.73
Diatoms	52.96	15.14	93.28
Digested material	–	60.75	100.00
Protozoa	0.60	0.07	1.96
Rotifers	0.20	0.04	3.92
Copepods	0.74	1.07	13.45
Cladocerans	0.04	0.01	1.12
Artemia cysts	0.02	0.00	0.56
Insect adults	1.26	0.51	25.77
Insect larvae	0.17	0.00	0.56
Invertebrate eggs	0.41	0.04	5.88
Fish scales	0.38	1.72	12.04

Number of guts analyzed =357

The analysis of the relationship between mean prey
biovolume and toothcarp length, separately for each
prey type, showed that only three prey categories were
significantly related to fish length (Fig. 8). However,
only two of these prey showed a positive correlation
with fish length, insects (ANCOVA, $F_{1, 81}=17.64$,
$P<0.0001$) and rotifers ($F_{1, 11}=9.74$, $P=0.010$), while
invertebrate eggs were negatively related to fish length
($F_{1, 19}=8.48$, $P=0.009$) (Fig. 8). Therefore, the ontoge-
netic diet shift of toothcarp involves not only a change in
species composition but also in mean volume (i.e., size)
of certain prey items.

Discussion

Population structure

Like many other cyprinodontids (e.g., Al-Daham et al.
1977; Meffe and Snelson 1989), the Farsi toothcarp
exhibited a marked sexual dimorphism, with females
being larger and more abundant than males (1.54:1).
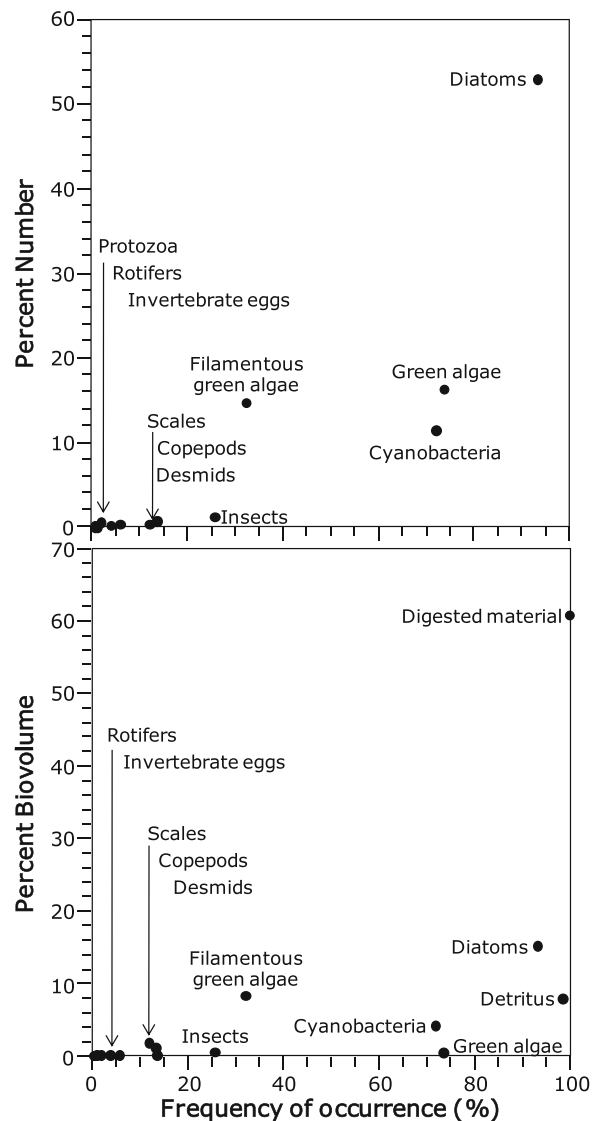Esmaeili and Shiva (2006) attributed this biased sex

Fig. 3 Relationship among % number, % biovolume, and fre-
quency of occurrence of the main food items in the Farsi toothcarp
diet. Plots based on Costello's (1990) method (see Methods). Food
items highlighted are the most important components in diet

ratio to differences in survival rate between sexes (i.e.,
female greater longevity or selective predation on male
toothcarp) since there were no significant differences
from 1:1 in fish smaller than 25 mm. However, alterna-
tive hypotheses to explain the sex ratio variation have
been proposed in other cyprinodontids, such as distinct
maturation patterns (Meffe and Snelson 1989). The
observed life history for this toothcarp species agrees
with the previous results in Barm-e-Shoor Spring
(Esmaeili and Shiva 2006), as well as with other
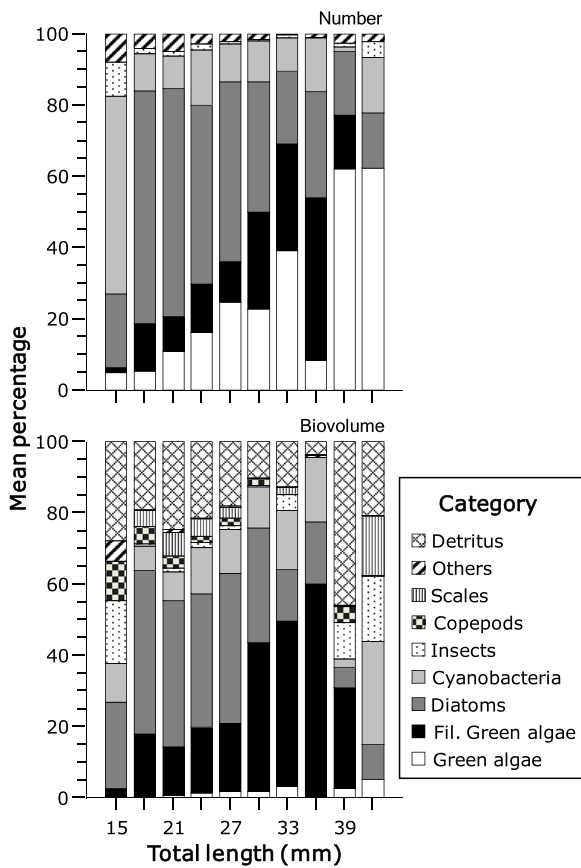*Aphanius* species in the Persian Gulf and the

Fig. 4 Ontogenetic variation in the Farsi toothcarp diet. Data are the percentage of gut content biovolume (*bottom*) and number (*top*), averaged for 3 mm length classes
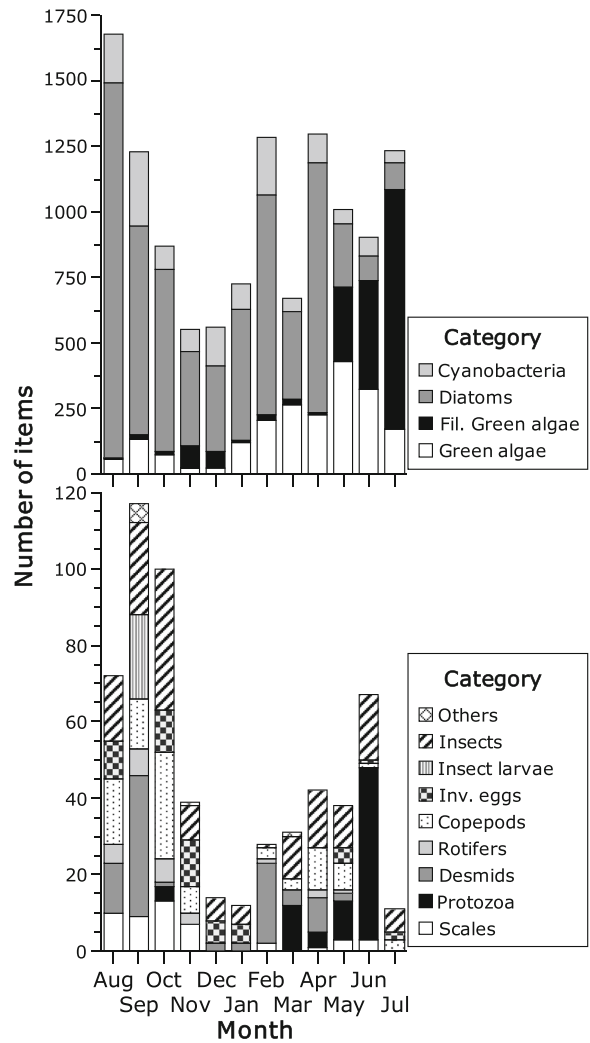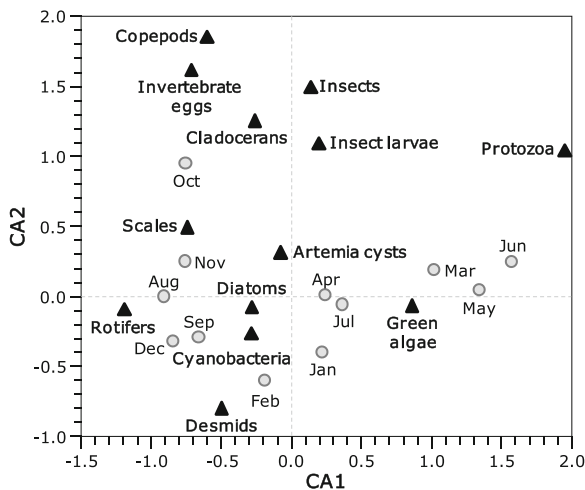

Fig. 5 Correspondence analysis of Farsi toothcarp gut contents by number: food item scores (▲) for the first and second axes and mean fish scores by month (◯). Filamentous green algae were omitted of the analysis because they dominated the solution as outlier

Fig. 6 Seasonal variation in the Farsi toothcarp diet. The most important (*top*) and less frequent (*bottom*) food items are presented separately

Mediterranean basin (e.g., Al-Daham et al. 1977; Fouda 1995; Kamal et al. 2009). *Aphanius farsicus* biology is characterized by fast growth and reduced longevity, usually do not live more than 2 years (maximum recorded age is 3 years), it is an asynchronous batch spawner reproducing from late spring to November (Esmaeili and Shiva 2006; Monsefi et al. 2009). However, after the first spawning event most of the largest individuals died and were replaced by newborns, consequently toothcarp population was dominated by smaller fish from July to January. Furthermore, fish condition was reduced during this period, probably both because of the reproductive effort (Esmaeili and Shiva 2006) and a decrease in water temperature (Table 1). Thereafter, fish

Table 3 ANCOVAs of the Farsi toothcarp diet-descriptors with month (factor) and total length (covariate)

Variable	Among sampling months				Total length (mm)			
	F	df	P	Partial η^2	F	df	P	Partial η^2
Multivariate test	4.72	55, 1535.7	<0.0001	0.135	15.50	5, 331	<0.0001	0.178
Total number of prey	11.29	11, 335	<0.0001	0.270	32.82	1, 335	<0.0001	0.089
Total biovolume	6.60	11, 335	<0.0001	0.178	57.53	1, 335	<0.0001	0.147
Mean prey volume	6.59	11, 335	<0.0001	0.178	0.07	1, 335	0.784	0.001
Number of food items	4.74	11, 335	<0.0001	0.135	3.37	1, 335	0.067	0.010
Diversity	1.41	11, 335	0.168	0.044	1.27	1, 335	0.261	0.004

All variables were $\log_{10}$ transformed. Multivariate statistics correspond to Wilks's lambda
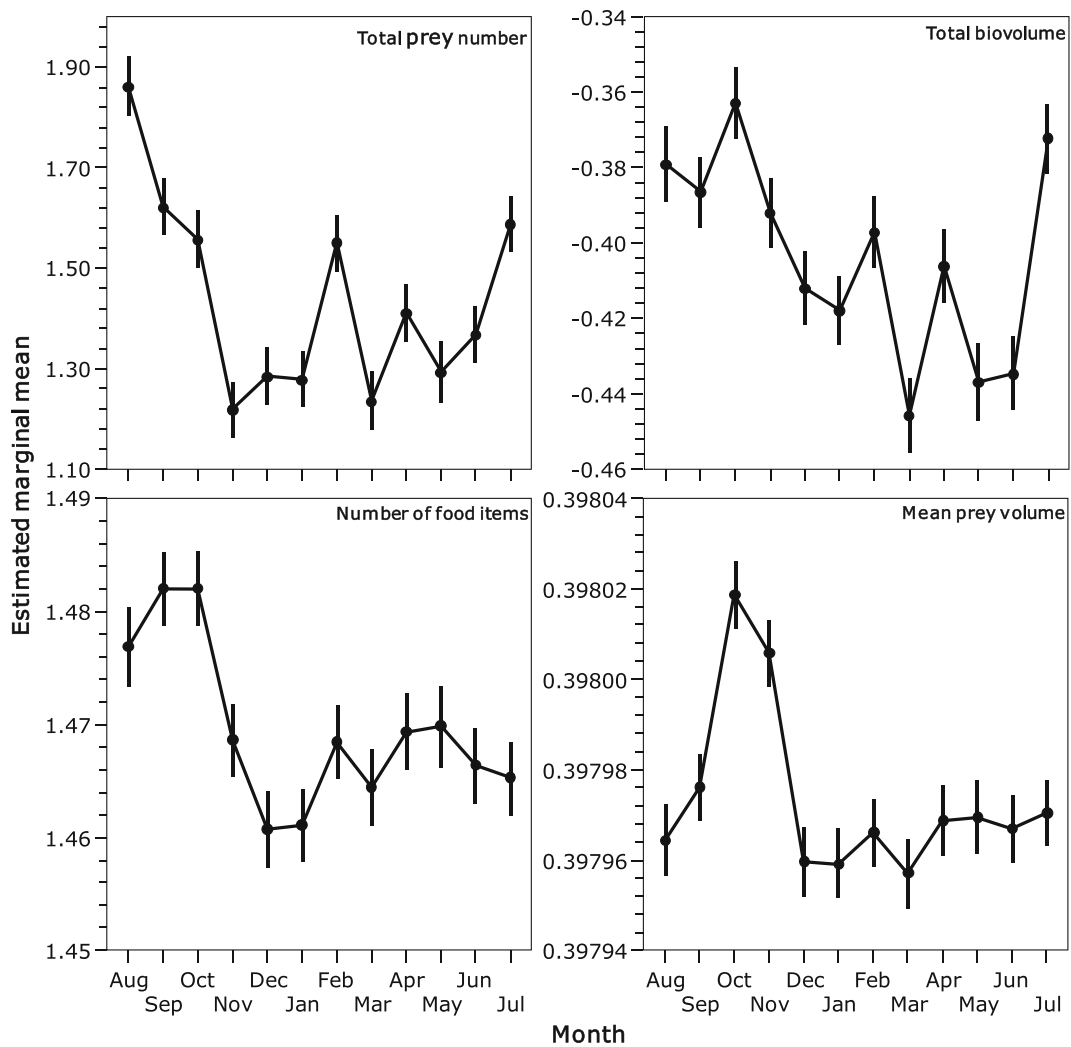

Fig. 7 Seasonal variation of the diet descriptors in the Farsi toothcarp. The size-adjusted means (ANCOVAs of Table 3) and standard errors are shown. The adjusted means are the population means after adjusting for fish length
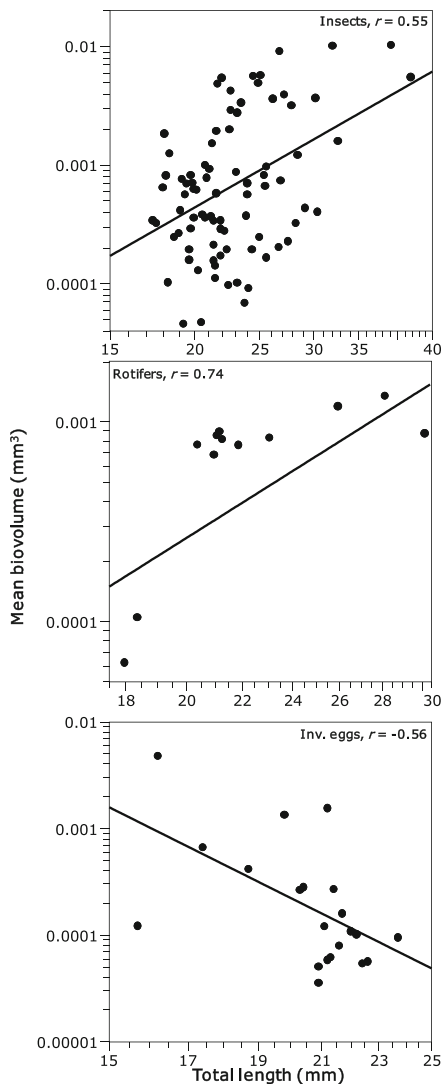
Fig. 8 Ontogenetic variation in mean prey volume of Farsi toothcarp. Only the three prey items significantly related to toothcarp length are shown

grew faster from January to May (i.e., higher population mean length) and condition increased with increasing water temperature and large individuals were dominant in abundance from May to June, at the start of the next spawning event.

Farsi toothcarp diet

Aphanius farsicus diet consisted of cyanobacteria, algae, detritus and invertebrates, but food items of plant origin were more common than those of invertebrate animal origin. The most common prey in the Farsi toothcarp diet, by number and occurrence, were algae,

particularly diatoms and green algae, followed by cyanobacteria. The feeding on invertebrate prey was restricted to only a few different prey types, mainly insects (adults) and copepods. There was an ontogenetic diet shift, as well as seasonal variation in diet, likely related to variation in resource availability.

Although there are no previous studies on the food of *A. farsicus*, its diet combining animal prey (mainly insects and crustaceans) with algae and detritus is similar to other *Aphanius* species, such as *A. dispar* (Rüppell, 1829), *A. mento* (Heckel, 1843), and *A. sophiae* (Heckel, 1847) (Al-Daham et al. 1977; Haas 1982). Although these studies were more limited in time and in number of guts analyzed, they found that all three species were mainly herbivorous, feeding mainly on both unicellular (e.g., diatoms and desmids) and microscopic filamentous algae but also consuming some animal prey, such as rotifers, copepods and insects (Al-Daham et al. 1977; Haas 1982). Nevertheless, when fish were acclimated to aquarium experimental conditions, only *A. mento* preferred plant food, and both *A. dispar* and *A. sophiae* preferred mosquito larvae to plant food (Al-Daham et al. 1977; Haas 1982). Conversely, the Farsi toothcarp diet was different from the diet based on invertebrates previously reported for other *Aphanius* species, such as *A. iberus* (Valenciennes, 1846), *A. fasciatus* (Valenciennes, 1821), and *A. vladykovi* (Coad, 1988) (e.g., Keivany and Soofiani 2004; Alcaraz and García-Berthou 2007a; Leonardos 2008), as well as for other cyprinodontiforms (e.g., Kneib 1986; Abilhoa et al. 2010; Kalogianni et al. 2010). According to both histology and morphology of its digestive tract, *A. farsicus* was classified as omnivorous but with similar characteristics to carnivorous fish (Monsefi et al. 2010). Thus, diet differences with other species may be explained not only by variation in selectivity but also in resource availability (Al-Daham et al. 1977; Alcaraz and García-Berthou 2007a; Bakhtiyari et al. 2011); further studies (e.g., seasonal variation of prey resources) are needed to clarify the mechanisms involved. *Gambusia holbrooki* is also a good example of feeding flexibility; although it is often considered a zooplanktivorous fish (e.g., García-Berthou 1999), it can feed on a wide variety of prey types, including insects of both aquatic and terrestrial origin, crustaceans, mollusks, algae, plant material and diatoms (reviewed by Pyke 2005), thus potentially competing with the Farsi toothcarp. The mosquitofish has been

introduced into more than fifty countries (García-Berthou et al. 2005); it was first introduced to Iran (Ghazian Marsh) in 1922–1930, but transferred to over 3000 water bodies throughout the country in 1966, as part of a malaria control programme, being the most widespread fish species in Iran (Tabibzadeh et al. 1970; Coad 2014). Its strong ecological impacts are well known and several studies have demonstrated its effects and its ability to outcompete small native fish, such as endemic cyprinodontids (Pyke 2005; Alcaraz and García-Berthou 2007b; Alcaraz et al. 2008a). Consequently, both conservation and management measures must be taken to prevent mosquitofish introduction into Small Barm-e-Shoor Spring.

Seasonal and ontogenetic diet shifts

Both male and female toothcarps showed similar feeding habits, since there were no significant differences between sexes in dietary descriptors, such as the richness of prey items and the mean volume of prey. Nevertheless, the relative importance of prey consumed was highly dependent on toothcarp size and sampling date, the latter being the most important source of variation (partial η^2) for the dietary descriptors and gut content composition. Overall, the Farsi toothcarp captured more prey, fed on a greater richness of prey items and had significantly more food biovolume in summer and autumn. Temperature is recognized as one of the most important factors in cyprinodontid fish biology, for instance affecting metabolism (i.e., behaviour and feeding activity), reproduction, and biotic interactions (Meffe and Snelson 1989; Leonardos 2008; Benejam et al. 2009). Therefore, similarly to other *Aphanius* species, this increase in feeding activity during warmer months (Table 1) is probably not only because of greater food and prey availability (Alcaraz and García-Berthou 2007a; Alcaraz et al. 2008b; Leonardos 2008) but also because of an increase in energy requirements for metabolism maintenance, reproduction and growth mediated by water temperature (Frenkel and Goren 1997, 2000; Esmaeili and Shiva 2006). In autumn there is a progressive reduction in feeding activity following the temperature decrease, but contrary to other studies (e.g., Leonardos 2008) colder temperatures were not enough to completely inactivate fish feeding, since no empty guts were found. Interestingly, similar feeding patterns both in composition and seasonal variation

have been described in several species of the cyprinodontiform genus *Cyprinodon*, such as the Devil's Hole pupfish (*Cyprinodon diabolis* Wales, 1930), Amargosa pupfish (*C. nevadensis*, Eigenmann & Eigenmann, 1889), Maya pupfish (*C. maya*, Humphries & Miller, 1981), Thicklip pupfish (*C. labiosus*, Humphries & Miller, 1981), Blackfin pupfish (*C. beltrani*, Álvarez, 1949) and Boxer pupfish (*C. simus*, Humphries & Miller, 1981) (Minckley and Deacon 1975; Naiman 1979; Stevenson 1992). *Cyprinodon* is a genus of the family Cyprinodontidae endemic to southwestern United States and northern Mexico (Naiman 1979), sharing a common origin with *Aphanius* from a common ancestral cyprinodontid lineage of the Tethys sea about 80 MA (Parker and Kornfield 1995). Both cyprinodontid genus currently presents fragmented populations in isolated habitats (e.g., springs, coastal lagoons) subjected to increasing stress because of water diversion to other uses, habitat destruction and alteration, species introduction, climate change, and other human impacts (Naiman 1979; Stevenson 1992; Alcaraz et al. 2008a, b).

Aphanius farsicus is a generalist predator with many different prey items from several microhabitats. Farsi toothcarp diet composition also showed large seasonal variation probably related to both food availability and energy requirements, but less pronounced ontogenetic shifts, as suggested by our results. Overall diet diversity, richness of prey items in gut contents and mean biovolume of prey captured were not related to fish length. Therefore, toothcarp ontogenetic diet shift was associated with a change in mean size of certain prey items and a higher consumption of a certain food type (namely green and filamentous algae); and it was not linked to a variation in prey categories consumed. Larger toothcarp consumed more algae, larger insects and rotifers and smaller egg masses. Although a more pronounced ontogenetic shift may be masked by the level of taxonomic precision used or by pooling prey items in a few food categories such as rotifers or insects (García-Berthou 1999), the feeding pattern reported here seemed clearly due to a relative constant feeding habitat and niche breadth, with only addition of some larger prey during growth. Further studies on the behaviour and ecology of this species and its habitats are needed to understand its adaptation to global change and help its conservation.

References

Abatzopoulos TJ, Agh N, Van Stappen G, Rouhani SM, Sorgeloos P (2006) *Artemia* sites in Iran. J Marine Biol Assoc UK 86: 299–307. doi:10.1017/S0025315406013154

Abell R (2002) Conservation biology for the biodiversity crisis: a freshwater follow-up. Conserv Biol 16:1435–1437. doi:10.1046/j.1523-1739.2002.01532.x

Abilhoa V, Vitule JRS, Bornatowski H (2010) Feeding ecology of *Rivulus luelingi* (Aplocheiloidei: Rivulidae) in a coastal Atlantic rainforest stream, southern Brazil. Neotropical Ichthyol 8:813–818. doi:10.1590/S1679-62252010005000012

Alcaraz C, García-Berthou E (2007a) Food of an endangered cyprinodont (*Aphanius iberus*): ontogenetic diet shift and prey electivity. Environ Biol Fish 78:193–207. doi:10.1007/s10641-006-0018-0

Alcaraz C, García-Berthou E (2007b) Life history variation of invasive mosquitofish (*Gambusia holbrooki*) along a salinity gradient. Biol Conserv 139:83–92. doi:10.1016/j.biocon.2007.06.006

Alcaraz C, Bisazza A, García-Berthou E (2008a) Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. Oecologia 155:205–213. doi:10.1007/s00442-007-0899-4

Alcaraz C, Pou-Rovira Q, García-Berthou E (2008b) Use of a flooded salt marsh habitat by an endangered cyprinodontid fish (*Aphanius iberus*). Hydrobiologia 600:177–185. doi:10.1007/s10750-007-9230-y

Al-Daham NK, Huq MF, Sharma KP (1977) Notes on the ecology of fishes of genus *Aphanius* and *Gambusia affinis* in Southern Iraq. Freshw Biol 7:245–251. doi:10.1111/j.1365-2427.1977.tb01672.x

Amin OM, Gholami Z, Akhlaghi M, Heckmann RA (2013) The description and host-parasite relationships of a new quadrigyrid species (Acanthocephala) from the Persian tooth-carp, *Aphanius farsicus* (Actinopreygii: Cyprinodontidae) in Iran. J Parasitol 99:257–263. doi:10.1645/GE-3247.1

APHA, AWWA, WPCF (2001) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington D. C

Arthur R, Done TJ, Marsh H, Harriott V (2006) Local processes strongly influence post-bleaching benthic recovery in the Lakshadweep atolls. Coral Reefs 25:427–440. doi:10.1007/s00338-006-0127-4

Bakhtiyari M, Kamal S, Abdoli A, Esmaeili HR, Ebrahimi M (2011) Comparison of the feeding behaviour and strategy of

the Killifish, *Aphanius sophiae* Heckel, 1847, at two different localities in Iran. Zool Middle East 52:47–56

Benejam L, Alcaraz C, Sasal P, Simon-Levert G, García-Berthou E (2009) Life history and parasites of the invasive mosquitofish (*Gambusia holbrooki*) along a latitudinal gradient. Biol Invasions 11:2265–2277. doi:10.3897/zookeys.215.1731

Brown KM, Ewins PJ (1996) Technique-dependent biases in determination of diet composition: an example with ring-billed gulls. Condor 98:34–41. doi:10.2307/1369505

Coad BW (2000) Distribution of *Aphanius* species in Iran. J Am Killifish Assoc 33:183–191

Coad BW (2014) Freshwater fishes of Iran. Available at http://www.briancoad.com. Retrieved September 2014

Costello MJ (1990) Predator feeding strategy and prey importance: a new graphical analysis. J Fish Biol 36:261–263. doi:10.1111/j.1095-8649.1990.tb05601.x

Esmaeili HR, Shiva AH (2006) Reproductive biology of the Persian Tooth-carp, *Aphanius persicus* (Jenkins, 1910) (Cyprinodontidae), in southern Iran. Zool Middle East 37:39–46

Fouda MM (1995) Life history strategies of four small-size fishes in the Suez Canal, Egypt. J Fish Biol 46:687–702. doi:10.1111/j.1095-8649.1995.tb01104.x

Frenkel V, Goren M (1997) Some environmental factors affecting the reproduction of *Aphanius dispar* (Rüppell, 1828). Hydrobiologia 347:197–207. doi:10.1023/A:1003000225293

Frenkel V, Goren M (2000) Factors affecting growth of killifish, *Aphanius dispar*, a potential biological control of mosquitoes. Aquaculture 184:255–265. doi:10.1016/S0044-8486(99)00326-9

García-Berthou E (1999) Food of introduced mosquitofish: ontogenetic diet shift and prey selection. J Fish Biol 55:135–147. doi:10.1006/jfbi.1999.0983

García-Berthou E, Moreno-Amich R (1993) Multivariate analysis of covariance in morphometric studies of the reproductive cycle. Can J Fish Aquat Sci 50:1394–1399. doi:10.1139/f93-159

García-Berthou E, Alcaraz C, Pou-Rovira Q, Zamora L, Coenders G, Feo C (2005) Introduction pathways and establishment rates of invasive aquatic species in Europe. Can J Fish Aquat Sci 62:453–463. doi:10.1139/F05-017

Graham JH, Vrijenhoek RC (1988) Detrended correspondence analysis of dietary data. T Am Fish Soc 117:29–36. doi:10.1577/1548-8659(1988)117%3c0029:DCAODD%3e2.3.CO;2

Greenacre M (2013) The contributions of rare objects in correspondence analysis. Ecology 94:241–249. doi:10.1890/11-1730.1

Haas R (1982) Notes on the ecology of *Aphanius dispar* (Pisces, Cyprinodontidae) in the Sultanate of Oman. Freshw Biol 12:89–95. doi:10.1111/j.1365-2427.1982.tb00605.x

Hoey AS, Bellwood DR (2011) Suppression of herbivory by macroalgal density: a critical feedback on coral reefs? Ecol Lett 14:267–273. doi:10.1111/j.1461-0248.2010.01581.x

Hrbek T, Keivany Y, Coad BW (2006) New species of *Aphanius* (Teleostei, Cyprinodontidae) from Isfahan Province of Iran and a reanalysis of other Iranian species. Copeia 244–255. doi:10.1643/0045-8511(2006)6%5b244:NSOATC%5d2.0.CO;2

Kalogianni E, Giakoumi S, Andriopoulou A, Chatzinikolaou Y (2010) Feeding ecology of the critically endangered *Valencia letourneuxi* (Valenciidae). Aquat Ecol 44:289–299. doi:10.1007/s10452-009-9253-8

Kamal S, Bakhtiyari M, Abdoli A, Eagderi S, Karami M (2009) Life-history variations of killifish (*Aphanius sophiae*)

populations in two environmentally different habitats in central Iran. J Appl Ichthyol 25:474–478. doi:10.1016/S0165-7836(98)00082-4

Keivany Y, Soofiani N (2004) Contribution to the biology of Zagros tooth-carp, *Aphanius vladykovi* (Cyprinodontidae) in central Iran. Environ Biol Fish 71:165–169. doi:10.1007/s10641-004-0106-y

Kneib RT (1986) The role of *Fundulus heteroclitus* in salt marsh trophic dynamics. Am Zool 26:259–269

Kottelat M, Freyhof J (2007) Handbook of European freshwater fishes. Publications Kottelat, Cornol

Leonardos I (2008) The feeding ecology of *Aphanius fasciatus* (Valenciennes, 1821) in the lagoonal system of Messolongi (western Greece). Sci Mar 72:393–401

Meffe GK, Snelson FF Jr (1989) Ecology and evolution of livebearing fishes (Poeciliidae). Prentice-Hall, New Jersey

Minckley CO, Deacon JE (1975) Foods of the devil's hole pupfish, *Cyprinodon diabolis* (Cyprinodontidae). Southwest Nat 20:105–111

Monsefi M, Shiva AH, Esmaeili HR (2009) Gonad histology of the Persian tooth-carp *Aphanius persicus* (Jenkins, 1910) (Cyprinodontidae) in Southern Iran. Turk J Zool 33:27–33. doi:10.3906/zoo-0712-9

Monsefi M, Gholami Z, Esmaeili HR (2010) Histological and morphological studies of digestive tube and liver of the Persian tooth-carp, *Aphanius persicus* (Actinopterygii: Cyprinodontidae). IUFS J Biol 69:57–64

Naiman RJ (1979) Preliminary food studies of *Cyprinodon macularius* and *Cyprinodon nevadensis* (Cyprinodontidae). Southwest Nat 24:538–541

Najafi G, Ghobadian B, Yusuf TF (2011) Algae as a sustainable energy source for biofuel production in Iran: a case study. Renew Sust Energ Rev 15:3870–3876. doi:10.1016/j.rser.2011.07.010

Olson DM, Dinerstein E, Powell GVN, Wikramanayake ED (2002) Conservation biology for the biodiversity crisis. Conserv Biol 16:1–3. doi:10.1046/j.1523-1739.2002.01612.x

Parenti LR (1981) A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). Bull Am Mus Nat Hist 168:335–557

Parker A, Kornfield I (1995) Molecular perspective on evolution and zoogeography of cyprinodontid killifishes (Teleostei; Atherinomorpha). Copeia 1995:8–21

Pyke GH (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki*. Rev Fish Biol Fish 15:339–365. doi:10.1007/s11160-006-6394-x

Rahnemaei M, Zare M, Nematollahi AR, Sedghi H (2005) Application of spectral analysis of daily water level and spring discharge hydrographs data for comparing physical characteristics of karstic aquifers. J Hydrol 311:106–116. doi:10.1016/j.jhydrol.2005.01.011

Ramos-Jiliberto R, Valdovinos FS, Arias J, Alcaraz C, García-Berthou E (2011) A network-based approach to the analysis of ontogenetic diet shifts: an example with an endangered, small-sized fish. Ecol Complex 8:123–129. doi:10.1016/j.ecocom.2010.11.005

Santos AFGN, Alcaraz C, Santos LN, Hayashi C, García-Berthou E (2012) Experimental assessment of the effects of a Neotropical nocturnal piscivore on juvenile native and invasive fishes. Neotropical Ichthyol 10:167–176. doi:10.1590/S1679-62252012000100016

Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research. Freeman, New York

Stephens DW, Brown JS, Ydenberg RC (2007) Foraging: behavior and ecology. University of Chicago Press

Stevenson MM (1992) Food habits within the Laguna Chichancanab *Cyprinodon* (Pisces: Cyprinodontidae) species flock. Southwest Nat 37:337–343

Tabachnick BG, Fidell LS (2013) Using multivariate statistics. Pearson Education Inc, Boston

Tabibzadeh I, Behbehani G, Nakhai R (1970) Use of *Gambusia* fish in the malaria eradication programme of Iran. Bull World Health Organ 43:623–626

Teimori A, Esmaeili HR, Reichenbacher B (2011) *Aphanius farsicus*, a replacement name for *A. persicus* (Jenkins, 1910) (Teleostei, Cyprinodontidae). Zootaxa 3096:53–58

Teimori A, Esmaeili HR, Gholami Z, Zarei N, Reichenbacher B (2012) *Aphanius arakensis*, a new species of tooth-carp (Cyprinodontidae) from the endorheic Namak Lake Basin in Iran. ZooKeys 215:55–76. doi:10.3897/zookeys.215.1731