Research Article

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Feeding Properties of the Translocated Marine Fish Sand Smelt *Atherina boyeri* Risso, 1810 (Atherinidae) in a Freshwater Reservoir

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Abstract: The feeding ecology of the translocated marine fish sand smelt *Atherina boyeri* was studied in Hirfanlı Reservoir, a freshwater dam lake located in Central Anatolia. The data revealed that the sand smelt fed mainly on larger zooplankton, such as adult copepods and cladocerans. Fish eggs and juvenile fish were also found in the stomach content. There was a considerable dietary overlap among size groups larger than 30 mm total length. Larger individuals have also cannibalistic behaviour as they consumed juveniles of sand smelt. The proportion of empty stomachs was maximum in winter and minimum in summer. The trophic niche breadth was the highest in summer. The results suggested that the sand smelt may have a remarkable impact on zooplankton communities in the Hirfanlı Reservoir.

Keywords: Sand smelt, diet composition, stomach content, invasive fish, Hirfanlı Reservoir, Turkey

Introduction

Large scale introductions of non-indigenous fish species into new geographic regions is considered a new phenomenon since the 1980s (Welcomme 1988). Currently, the introduction of non-native species into new environments and their rapid invasion over natural areas is among the most serious threats to indigenous environments and biodiversity worldwide (Primack 2010). Turkey has the richest freshwater ichthyofauna in the Mediterranean Region with approximately 380 species (Froese & Pauly 2017) and it is considered as a hotspot of freshwater fish diversity and endemism (Fricke et al. 2007). Invasive species are considered to be one of the most important threats to the unique freshwater ichthyofauna of Turkey (Tarkan et al. 2015).

The sand smelt, *Atherina boyeri* Risso, 1810 (Atherinidae), is a marine fish naturally distributed in the Mediterranean, Black, Azov and Caspian Sea basins and eastern costs of the Atlantic Ocean. This species can enter estuaries and lagoons due to its euryhaline character. Furthermore, *A. boyeri* has been translocated into freshwater environments for fisheries purposes and established permanent freshwater populations in some countries (Kottelat & Freyhof 2007). Its traits, such as the broad salinity tolerance, early sexual maturity, prolonged reproductive period, high reproductive capacity and short life-span (Gençoğlu & Ekmekçı 2016), are usually attributed to successful invaders (Grabowska & Przybylski 2015).

The sand smelt naturally inhabits all the sea coastal waters of Turkey and over the last 40 years it has been translocated into many freshwater lakes and reservoirs isolated from the sea. It was firstly recorded from the İznik Lake. Although, its introduction time and purpose are still unclear, Tarkan et al. (2015) suggested that the sand smelt was initially introduced for fishery purposes, and secondarily spread by deliberate introduction. Thereafter, it has rapidly spread through the main freshwater

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basins in Anatolia and has established landlocked populations in many reservoirs. Currently, it is one of the most widespread introduced fish species in freshwater lakes and reservoirs in Turkey (Ekmekçi et al. 2013, Tarkan et al. 2015) and it is considered an invasive and problematic species (Tarkan et al. 2015). Although the sand smelt is a commercially valuable fish species (Atalay et al. 2017), it has adverse effects on ecosystems and inland fisheries in Turkey. Despite the existence of many records on expanding distribution area of the sand smelt, its life-history traits and ecological impacts in the recipient freshwater environments have not been adequately studied in Turkey. Knowledge on feeding habits of the sand smelt and its role in the food chain of lakes and reservoirs is also limited.

Therefore, the aim of this study was to reveal the diet composition, as well as the seasonal and ontogenetic dietary variations in sand smelt living in the Hirfanlı Reservoir. The results obtained can provide insights into understanding the feeding strategy of the translocated species as a potential threat on native biota.

Materials and Methods

Study site and fish sampling

The Hirfanlı Reservoir is located on the Kızılırmak River (N 39°16'22.2''; E 33°31'07.16''; Fig. 1). The average surface area of the reservoir is 218.81 km^2, while the average depth is 20.15 m. The dam has been constructed mainly for hydroelectric power generation and flood control and the reservoir is an important site for inland fisheries in Turkey. The

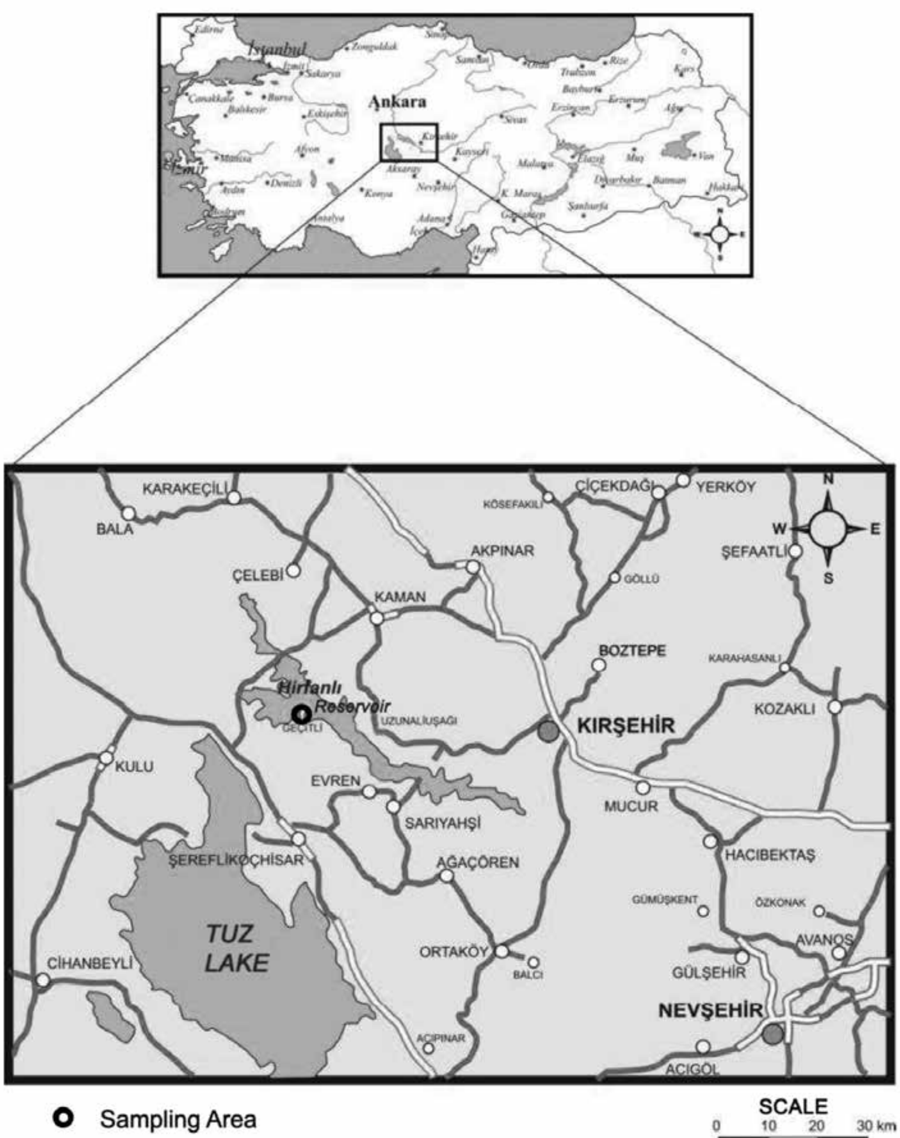

Fig. 1. The Hirfanlı Reservoir study site (modified from Ekmekçi et al. 2010)

fish fauna of the Hirfanlı Reservoir has changed significantly over the years due to anthropogenic activities. Even though the origin of introduction is unknown, *A. boyeri* has become dominant species in the reservoir along with the non-native topmouth gudgeon (*Pseudorasbora parva*) and the native killifish (*Aphanius marassantensis*) (Ekmekçi et al. 2010).

Within the time period of the study, water temperature was the lowest in January 2009 (5.08°C) and the highest in August 2009 (26.84°C). The dissolved oxygen (DO) ranged from 3.55 (November 2008) to 12.98 mg/l (April 2009), while pH values varied from 6.62 (January 2009) to 9.0 (August 2009). The electrical conductivity (EC) of water ranged from 1588 µS/cm (August 2009) to 1859 µS/cm (June 2008).

A total of 472 sand smelt specimens were caught monthly between April 2008 and March 2009. Only in January 2009 the sampling was not possible because of unfavourable weather and field conditions. A beach seine net with mesh size of 5 mm was used for the sampling. The specimens were preserved in 4% formaldehyde solution immediately after capture for further analysis.

Laboratory procedures, diet analysis and statistical methods

The total length (TL) of all specimens was measured to the nearest 0.01 mm. The digestive tracts were removed and preserved in 70% alcohol until examination. The stomachs were weighed to the nearest 0.001 g. Their content was removed and the empty stomachs were reweighed to the nearest 0.001 g. The volumes of the stomach contents were measured to the nearest 0.5 ml, using a graduated measuring cylinder (Hyslop 1980) and preserved in 70% alcohol. The prey composition was identified to the lowest possible taxonomic level with a Sedgewick Rafter counting chamber and counted under a binocular microscope.

The number of stomachs containing at least one individual prey was recorded. The vacuity index (VI%, the percentage of empty stomachs) and the percentage of stomach fullness (F%) were calculated. The index of relative importance (IRI) of each food item was assessed as given by Hyslop (1980): $IRI_i = (\%N_i + \%V_i) \times \%FO_i$. Here, $\%N_i$ is the percentage of the total number of prey *i* in relation to the total number of food items; $\%V_i$ is the percentage of volume of prey *i* in relation to the total volume of all food items and $\%FO_i$ is the percentage of all stomachs containing prey *i*.

The Shannon-Wiener index (*H*) was used to measure the diet diversity (Krebs 1989):

$H = -\Sigma p_i \times \ln p_i$, where p_i was the proportion of a specific prey category for 'i' categories of prey.

The total number of species in the stomach contents was regarded as species richness (S), and the evenness (E) was calculated using the following formula:

$E = H/\log(S)$.

The evenness is measured on a scale ranging from near 0 to 1. Low evenness values indicate high single-species dominance and high values indicate equal abundance of all species (Routledge 1980, Alatalo 1981, Stirling & Wılsey 2001).

The diet specialisation degree or diet breadth (B_i) was calculated as:

$B_i = 1 / \Sigma p_i^2$, where p_i is the proportion of prey species (i). According to the values of the diet breadth received, the following categories were considered: specialised feeders ($1 < B_i < 2$), less specialised feeders ($2.1 < B_i < 3$), and generalist feeders ($B_i \geq 3$) (Ludwig & Reynolds 1998, Rosas-Alayola et al. 2002).

The sand smelt specimens were grouped into nine size classes and accordingly competition among size groups was assessed by the Schoener index (Similarity index):

$PSI_{xy} = 1\text{-}0.5(\Sigma \mid P_{xi}\text{-}P_{yi} \mid)$, where P_{xi} and P_{yi} are the proportions of $IRI_i\%$ in the diet of the groups being compared based on the size classes. The index ranged from 0.0 to 1.0 and when it exceeded 0.60, it was considered as significant (Schoener 1970).

The mean TL of the specimens with empty stomachs were compared with the TL of the specimens, which had at least one prey item in their stomachs, using Student's *t*-test. The monthly variation of stomach fullness was also tested by ANOVA. The Kruskal-Wallis test was used for comparison of dietary breadth for each season and each size-class (Sokal & Rohlf 1995).

Results

The total length of sand smelt specimens, including immature specimens, ranged from 24.35 to 107.77 mm. The total length of female and male specimens varied from 39.45 to 107.77 mm and from 39.73 to 86.36 mm, respectively, and the sex ratio was 0.57:1 (M:F). Of the total 472 stomachs of sand smelt examined, 124 were empty (26%).

Feeding intensity

The average VI% values for all fish samples were 26.3%, while for the female, male and immature specimens were 18.8%, 32.5%, and 41.4%, respectively. The females had higher feeding intensity than males and immature individuals, with lower VI% values throughout the year. There was significant difference between the total lengths of specimens, which had empty stomachs and stomachs containing at least one individual prey (Student's *t*-test, t=-6.62, P<0.05). The monthly proportion of stomach fullness varied significantly over the year (ANOVA, F=15.67, P<0.05), with a maximum value (100%) in May and June 2008 and a minimum value (17.95%) in March 2009 (Fig. 2).

Diet composition

The stomach contents was grouped under 15 food categories (Table 1). Some of the food items (*Chironomus* sp., ostracods, *Gammarus* sp., insects, rotifers, and fish eggs) were represented with low importance (IRI%<0.01). The most common prey categories were cladocerans and copepods (IRI%>30), more specifically *Bosmina* sp. (IRI%=64.5) and the cyclopoid copepods (IRI%=35.2). The juveniles of sand smelt as prey organisms were also notable (Table 1).

Stomach contents in relation to fish size

The IRI% values of prey items in terms of size classes of sand smelt is given in Table 2. The Kruskal-Wallis test showed that %IRI of the dominant prey items (*Bosmina* sp. and Cyclopoid copepods), the juvenile sand smelt, and *Gammarus* sp. was significantly different among the size classes (H=27.93; d.f.=8; *P*<0.001). The highest species richness was for the size class of 70.0-79.9 mm, while the lowest was for the size classes of 20.0-29.9 and 100.0-109.9 mm. The smallest individuals had high evenness values, with high single species dominance. The diet specialisation degree (B_i) of size classes indicated that all size classes of sand smelt in the Hirfanlı Reservoir were specialised feeders ($B_i \leq 2$).

The results of ontogenetic competition (Schoener index; PSI_{xy}) are given in Table 3, where the values higher than 0.60 are indicated in bold. According to the Schoener index values, there was a dietary overlap between most of the size classes.

Seasonal variation in diet composition

The seasonal differences in the diet of the sand smelt were statistically insignificant (Kruskal-Wallis test, H=1.172; d.f.: 3; *P*<0.001). The niche breadth and evenness were the highest in summer 2008, and the lowest in autumn 2008. The species richness was the highest in spring 2008, and the lowest in winter 2009. The diet breadth values were lower than 2.1 over the year, which means that the diet of the sand smelt was specialised in all seasons (Table 4).

Discussion

The relationship between the percentage of empty

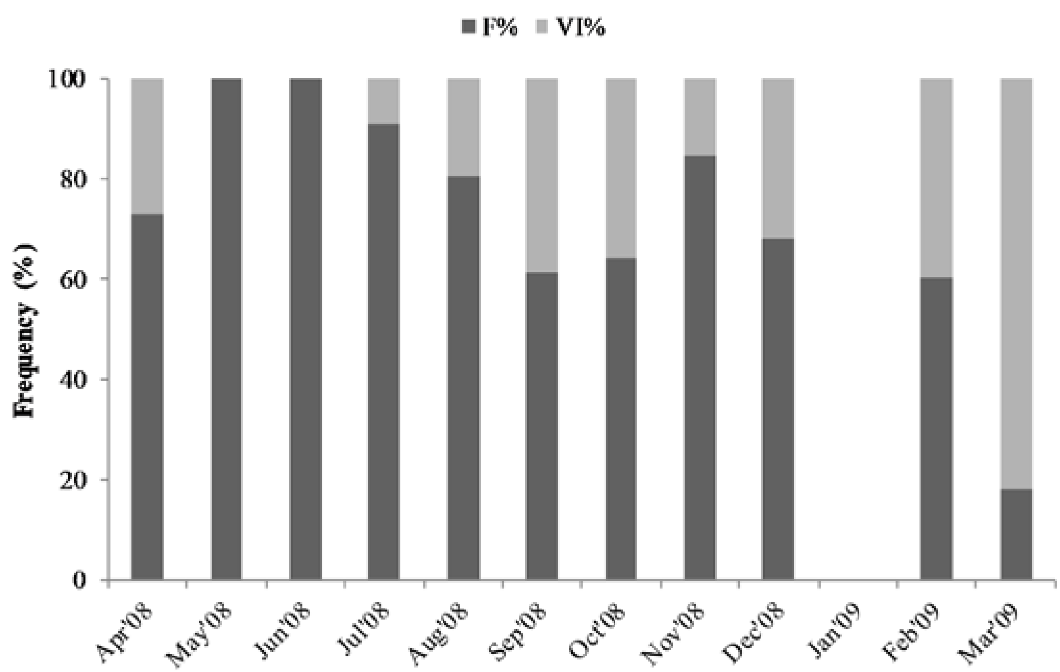

Fig. 2. Monthly variation in frequency of stomach fullness of sand smelt specimens in the Hirfanlı Reservoir. F% – percentage of stomach fullness; VI% – vacuity index (percentage of empty stomachs)

Table 1. Overall diet composition of sand smelt in the Hirfanlı Reservoir. V% – percentage of volume of prey; FO% – percentage of all stomachs containing prey; N% – percentage of the total number of prey; IRI% – index of relative importance

Prey Items	V%	FO%	N%	IRI%
Daphnia sp.	<0.01	1.09	16.56	0.27
Bosmina sp.	<0.01	61.29	70.86	64.50
Eurycercus sp.	<0.01	0.18	3.31	0.01
Chydorus sp.	<0.01	0.25	6.62	0.02
Cyclopoid Copepods	<0.01	36.85	64.24	35.15
Megacyclops larvae	<0.01	0.09	1.99	<0.01
Ostracoda	0.06	<0.01	0.66	<0.01
Keratella sp.	<0.01	0.01	1.33	<0.01
Other Rotifera	<0.01	0.09	3.97	<0.01
Copepoda egg	<0.01	0.09	0.66	<0.01
Chironomus sp.	0.61	<0.01	0.66	<0.01
Gammarus sp.	59.59	<0.01	3.97	<0.01
Insecta	6.62	<0.01	2.65	<0.01
Fish egg	<0.01	<0.01	0.66	<0.01
Atherina boyeri (juvenile)	33.10	0.04	13.91	0.03

Table 2. The IRI% values of prey items in terms of size classes (mm) of sand smelt in the Hirfanlı Reservoir. H – Shannon-Wiener index; S – species richness; E – species evenness; B_i – diet breadth

Prey Items		Size Classes (mm)							
	20.0-29.9	30.0-39.9	40.0-49.9	50.0-59.9	60.0-69.9	70.0-79.9	80.0-89.9	90.0-99.9	100.0-109.9
	IRI%								
Daphnia sp.	0.13	0.03	0.03	0.19	0.09	0.29	0.32	2.42	0
Bosmina sp.	98.31	98.50	98.50	87.78	51.92	39.79	26.66	13.55	99.88
Eurycercus sp.	0	0	0	0	0	0.07	0.24	0	0
Chydorus sp.	0	0	0	0.01	0	0.19	0.94	0	0
Cyclopoid Copepods	1.56	1.43	1.43	11.93	47.95	59.61	72.11	83.90	0
Megacyclops larvae	0	0	0	0.07	0	0	0.03	0	0
Ostracoda	0	0	0	0	0	0	0	0	0
Keratella sp.	0	0	0	0.01	0	0	0	0	0
Other Rotifera	0	0	0	0	0	0.01	0.03	0.02	0
Copepoda egg	0	0.02	0.02	0	0	0	0	0	0
Chironomus sp.	0	0	0	0	0	0	0	0	0
Gammarus sp.	0	0	0	0	0	0	0.07	0.06	0.12
Insecta	0	0	0	0	0	0	0	0	0
Fish egg	0	0	0	0	<0.01	0	0	0	0
Atherina boyeri (juvenile)	0	0	0	0.02	0.03	0.03	0.30	0.06	0
H	0.69	0.09	0.08	0.39	0.70	0.71	0.69	0.52	0.009
S	2	3	5	7	7	11	9	6	2
E	2.30	0.19	0.12	0.46	0.84	0.68	0.73	0.67	0.032
B_i	2.00	1.03	1.03	1.27	2.00	1.95	1.69	1.38	1.00

Table 3. The PSI_{xy} values in terms of size classes of sand smelt in the Hirfanlı Reservoir

Size class (mm)	30.0-39.9	40.0-49.9	50.0-59.9	60.0-69.9	70.0-79.9	80.0-89.9	90.0-99.9	100.0-109.9
20.0-29.9	0.02	0.01	0.12	0.48	0.50	0.50	0.52	0.00
30.0-39.9		**1.00**	**0.89**	0.54	0.41	0.28	0.15	**0.98**
40.0-49.9			**0.89**	0.53	0.41	0.28	0.15	**0.99**
50.0-59.9				**0.64**	0.52	0.38	0.26	**0.88**
60.0-69.9					**0.88**	**0.74**	**0.62**	0.52
70.0-79.9						**0.87**	**0.73**	0.40
80.0-89.9							**0.86**	0.26
90.0-99.9								0.14

Table 4. The IRI% values of food items of sand smelt in terms of seasons in the Hirfanlı Reservoir. N - number of individuals; for other symbols see Table 2

	IRI%			
	Spring 2008	**Summer 2008**	**Autumn 2008**	**Winter 2009**
Prey Items	N=46	N=45	N=41	N=19
Daphnia sp.	0.28	2.05	<0.01	0
Bosmina sp.	5.15	40.82	99.56	98.59
Eurycercus sp.	0.03	0	0	0.04
Chydorus sp.	0.13	0	<0.00	0
Cyclopoid Copepods	94.38	57.06	0.35	1.33
Megacyclops larvae	0.02	0	0	0
Ostracoda	0	0	<0.01	0
Keratella sp.	<0.01	0	0	0
Other Rotifera	0	0	0.02	<0.01
Copepoda eggs	0	0	<0.01	0
Chironomus sp.	<0.01	0	0	0
Gammarus sp.	<0.01	0	<0.01	<0.01
Insecta	<0.01	0	<0.01	0
Fish eggs	0	0	<0.01	0
A. boyeri (juvenile)	0	0.07	0.04	0.01
H	0.24	0.77	0.03	0.08
S	4	3	1	0
E	0.39	1.62	0.00	0.00
$\mathbf{B_i}$	1.12	2.03	1.01	1.03

stomachs and feeding intensity often tend to be negative (Bowman & Bowman 1980). The feeding intensity of the sand smelt in the Hirfanlı Reservoir was mostly over 50% throughout the sampling period, with an exception in March 2009 (Fig. 2). Feeding activity of fish is often highly correlated with the water temperature (Nikolsky 1963). During the study period, water temperature decreased month by month from December to March in the Hirfanlı Reservoir. The declined average VI% values in December, February and March were consistent with the decline in water temperature.

Feeding habits of sand smelt have been studied in various environments, such as freshwater lakes, estuaries and lagoons, using both stomach content analyses (Gon & Ben-Tuvia 1983, Mantilacci et al. 1990, Danilova 1991, Rosecchi & Crivelli 1992, Trabelsi et al. 1994, Bartulovic et al. 2004, Chrisafi et al. 2007, Doulka et al. 2012, Apaydin Yağci et al. 2013) and stable isotope analyses (Vizzini & Mazzola 2002-2005). These studies emphasised that the sand smelt has a diverse diet and opportunistic feeding behaviour, consuming the most abundant and large-sized organisms in marine and estuarine ecosystems. However, the sand smelt feeds on zooplankton, mostly cladocerans and copepods in freshwater ecosystems (Rosecchi & Crivelli 1992, Chrisafi et al. 2007, Doulka et al. 2012, Apaydin Yağci et al. 2013). According to the optimum foraging theory, the probability of prey capture is mainly dependent on density, size, motion and visibility of the prey (Lazzaro 1987). In the Hirfanlı Reservoir, the sand smelt fed mainly on *Bosmina sp.* and cyclopoid copepods (Table 1). There was a seasonal variation between these two prey items (Table 4), which is consistent with the seasonal variation in the zooplankton community as reported by previous studies on zooplankton fauna in the Hirfanlı Reservoir (Yiğit & Altindağ 2005, Baykal et al. 2006).

The diet of the sand smelt was specialised in all seasons (Table 4). These data support that the feeding behaviour of the sand smelt in the Hirfanlı Reservoir was also opportunistic. Although rotiferan species were abundant in the Hirfanlı Reservoir (Yiğit & Altindağ 2005), the sand smelt consumed the most efficient and large-sized food organisms and avoided the small-sized rotiferans (Table 1). This feeding strategy of the sand smelt was also reported in many other studies (Mantilacci et al. 1990, Rosecchi & Crivelli 1992, Bartulovic et al. 2004, Chrisafi et al. 2007, Doulka et al. 2012).

In previous studies, juvenile fish were recorded in the stomach contents of the sand smelt inhabiting freshwater (Rosecchi & Crivelli 1992, Chrisafi et al. 2007), marine and brackish water habitats (Gon & Ben-Tuvia 1983, Bartulovic et al. 2004). However, species identification for juvenile fish preys was provided only in two studies: Doulka et al. (2012) identified juveniles of an endemic species *Economidichthys trichonis* and *A. boyeri,* while Apaydin Yağci et al. (2013) found juveniles of the endemic *Aphanius anatoliae*, *Knipowitschia caucasica* and *A. boyeri* in the stomachs. In the Hirfanlı Reservoir, the sand smelt specimens larger than 50.0 mm TL fed occasionally on its own juveniles during summer, autumn and winter. The spawning period of the sand smelt in the reservoir is from May to August; additionally, both fecundity and recruitment are high in the population (Gençoğlu & Ekmekçi 2016). Therefore, the juveniles of the sand smelt are abundant during summer and autumn. Due to the opportunistic feeding behaviour of the sand smelt, it could be expected that the species occasionally consumes its own juveniles especially when juveniles are the most available potential food organism around. Still it is hard to say whether it is chasing its juveniles or consuming them occasionally, however, this cannibalistic behaviour may become a negligible factor in regulating the population size. According to the Schoener Index values (Table 3), there was an ontogenetic competition almost in all size classes with the exception of the smallest one. This could also be implied to competition avoidance of juveniles by segregating their dietary niche from the larger size groups.

Conclusions

The impact of invasive organisms on native species and on ecosystem functioning can only be determined by observational and experimental studies. In order to determine the possible detrimental effects of the sand smelt in freshwater ecosystems, data about its life-history traits including feeding habits are needed. The present study provides knowledge on feeding pattern of the sand smelt in a typical freshwater reservoir in Anatolia. This species is considered as invasive for the inland waters of Turkey and has established successful and abundant populations in freshwater ecosystems. In the Hirfanlı Reservoir, its typical invasive features, such as the short life span, prolonged reproductive period and broad environmental tolerance, were accompanied by the opportunistic feeding, with a preference for zooplankton. Therefore, it seemed to affect the zooplankton community and could be a strong competitor among planktivorous fish species in the habitat due to its selective predation on cladoceran groups. The sand smelt presented a dietary overlap among size classes and adaptation to temporal diet availability followed. Finally, although the present study provided some data on the ecological role of the sand smelt in the Hirfanlı Reservoir, more comprehensive observations on the ecological relationships of this species are required in order to determine its invasive potential and detrimental impacts.

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