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# Feeding habits of sand smelt (*Atherina boyeri*, Risso 1810) in Trichonis Lake (Western Greece)

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## Summary

The feeding habits of sand smelt (*Atherina boyeri*, Risso, 1810) from Trichonis Lake (Western Greece) were investigated. Stomach contents were analyzed from 240 specimens with total lengths ranging from 35 to 112 mm. Samples were taken at monthly intervals (January–December 1997). Of the total number of stomachs examined, 53 were empty (22.1%). However, values varied greatly with season (maximum in January: 50%; minimum in August: 7.6%). Prey analyses of stomach contents identified 15 important items (%Rn > 0.05) belonging to six major groups: crustacean (copepods, cladocera), mollusca (bivalve: larvae), insects (larvae), cestode worms and finfish (fry and eggs). Dominant prey were larvae of the bivalve *Dreissena polymorpha* (%Rn = 33.8), the copepods *Eudiadomus drieschi* (%Rn = 26.4) and the cladocera *Diaphanosoma brachyurum* (%Rn = 24.2). The importance of cladocera and copepods decreased with increasing size of the sand smelt, while the importance of bivalve larvae, fish eggs and finfish fry increased with increasing sand smelt size. Seasonal changes in diet composition and prey abundance in sand smelt stomachs were recorded as coinciding with the seasonal composition and abundance of the zooplankton community in the surface layers of Trichonis Lake. Bivalve larvae were the dominant prey of *A. boyeri* during January to May, while copepods and cladocera dominated from June to December.

## Introduction

The sand smelt *Atherina boyeri*, Risso 1810, is a small, short-lived, euryaline fish usually living in hypersaline to fresh water and forming large local populations (Boscolo, 1970; Tortonese, 1975; Quignard and Pras, 1986). It is distributed in the Mediterranean, Black Sea, and Atlantic from the south of Spain to Morocco and Madeira, and in isolated populations on the coasts of England and The Netherlands (Quignard and Pras, 1986).

Aspects of the life history of *A. boyeri* have been recorded at various coastal and lagoonal locations in the Mediterranean (Kiener and Spillman, 1969; Boscolo, 1970; Kohler, 1976; Castel et al., 1977; Marfin, 1981; Ferrari and Rossi, 1983–84; Gon and Ben-Tuvia, 1983; Trabelsi et al., 1994; Fouda, 1995; Leonardos and Sinis, 2000) as well as in estuaries and inland waters (Moretti et al., 1959; Fernandez-Delgado et al., 1988; Mantilacci et al., 1990; Rosecchi and Grivelli, 1992; Economou et al., 1994; Stoumboudi et al., 1997; Leonardos, 2001; Bartulovic et al., 2004; Koutrakis et al., 2004).

Various studies have been carried out in terms of food and feeding habits of the species (Moretti et al., 1959; Kohler, 1976; Castel et al., 1977; Marfin, 1981; Ferrari and Rossi, 1983–84; Gon and Ben-Tuvia, 1983; Mantilacci et al., 1990; Danilova, 1991; Rosecchi and Grivelli, 1992; Bartulovic et al., 2004), but little is known about the diet of the sand smelt in fresh waters (Moretti et al., 1959; Mantilacci et al., 1990; Rosecchi and Grivelli, 1992).

Having arrived via the riverlines and channels of the Acheloos River, the sand smelt is found in lakes Trichonis and Ozeros in western Greece where it has acclimatized and expanded, occupying the ecological niche of pelagic planctophagous fish. In the Trichonis Lake this population is now extremely abundant and supports a valuable commercial purse-seine fishery. Fished during the night, *A. boyeri* composes the primary fish catch of the lake with respect to marketability (approximately 3 € $kg^{-1}$). The annual production is about 500 ton (Leonardos, 2001).

A mitochondrial DNA study of eight different sand smelt populations, including those of Lake Trichonis, was carried out by Klossa-Kilia et al. (2002). Analyses of the morphological and meristic characteristics variability (Chrisafi et al., 2001) have shown the relative isolation of the Trichonis Lake sand smelt population. The reproductive cycle (Stoumboudi et al., 1997), ecology and exploitation pattern (Leonardos, 2001), and the larval ecology and behavior (Economou et al., 1994) of this population have also been studied.

The aim of the present study was to assess the food and feeding habits of sand smelt in Lake Trichonis (Western Greece). In terms of ecosystem management, such studies are essential for evaluation of the ecological role of the species as well as the understanding of its position in the food web structure in the lake.

## Materials and methods

### The study area

Lake Trichonis is located in Western Greece (38°15′N, 21°30′E) and covers a total area of about 96.9 $km^2$, with a maximum depth of 58 m and an average depth of 30.5 m (Fig. 1). The lake is connected with the neighboring Lake Lysimachia, which in turn maintains an open connection to the sea via the Acheloos River. It is a warm monomictic lake, with water temperatures ranging from 10 to 28°C. The mean Secchi disk depth is about 9 m.

The physicochemical data and the phyto- and zooplankton compositions classify the lake as oligotrophic to mesotrophic

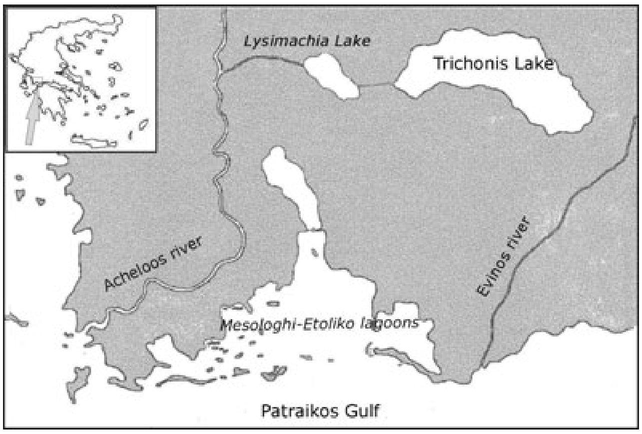

Fig. 1. Map of study area

(Koussouris, 1981; Overbech et al., 1982) while recent studies indicate a possible tendency to decrease the lake's trophic status (Kehayias et al., 2004; Doulka et al., 2005).

### Sampling

Samples of sand smelt were collected monthly by purse-seine (mesh size 22 mm) from January to December 1997 from various Lake Trichonis locations. Fish were preserved in 10% formalin solution for later laboratory investigation. Each fish was measured to the nearest 0.1 mm total length (TL). From the monthly sample a sub-sample of about 20 individuals was randomly selected. Stomachs were removed from a total of 240 specimens and the contents examined. Prey composition was determined under a binocular microscope to the lowest possible systematic level.

TL of fish examined ranged from 35 to 112 mm (mean = 78.4 ± 16.2 mm). In order to estimate variation in food habits as a function of size, the specimens were separated into five class sizes: $L_1$: TL < 54 mm (n = 22); $L_2$: 54.1 < TL < 72 mm (n = 58); $L_3$: 72.1 < TL < 84 mm (n = 63); $L_4$: 84.1 < TL < 95 mm (n = 61); and $L_5$: TL > 95.1 mm (n = 38). Length class size boundaries correspond to TL at the $1^+$ to $4^+$ age groups of species in Trichonis Lake (Leonardos, 2001).

To estimate stomach fullness, the empirical scale present in Table 1 was used. The percentage of each stomach fullness category was estimated. *Frequency of Occurrence* ($F_{pi}$): $F_{pi} = (N_{1i}/N_p)$, where $N_{1i}$ is the number of the stomachs in which the food item $i$ was found and $N_p$ is the number of non-empty stomachs.

Table 1
Empirical scale of sand smelt stomach fullness divided into four categories as defined for this study

| Fullness category | Description |
|---|---|
| Empty stomach | Stomach barely bloated with no or/and few small prey |
| Semi-empty stomach | Stomach slightly bloated with few or/and considerably small prey |
| Semi-full stomach | Stomach sufficiently bloated with considerable amounts of small or a few large prey |
| Full stomach | Stomach immensely bloated with large amounts of small prey or a few large prey |

Percentage numerical abundance ($C_i$) : $C_i = n_i / \sum_{i=1}^{m} n_i$

where $n_i$ is the number of $i$th food item and $m$ the number of food items.

The Shannon index ($H'$) was employed to measured trophic diversity as:

$$H' = -\sum_{i=1}^{m} C_i \cdot \log_e(C_i).$$

The relative importance of a food item on the diet of sand smelt was assessed by the Weighted Resultant Index (%Rn; Mohan and Sankaran, 1988),

$$\%\mathrm{Rn}_i = \frac{Q\sqrt{C_i^2 + F_{pi}^2}}{\sum_{i=1}^{m} Q\sqrt{C_i^2 + F_{pi}^2}} \times 100,$$

where $\%\mathrm{Rn}_i$ the Weighted Resultant Index of the $i$th food item and

$$Q = 1 - \frac{|4 \cdot \tan^{-1}(C_i/F_{pi}) - \pi|}{\pi}.$$

Statistical differences in the diet composition with respect to length class, season and sex were assessed by one-way analysis of variance by ranks (Kruskal–Wallis test) of the arcsine-root transformed numerical abundance of a given food item. Effects of length class and season on the mean number of prey items per stomach ($N_m$) were also tested by Kruskal–Wallis test. The variation in fullness category was also tested by a chi-square test ($\chi^2$) over a contingency table of the number of stomachs (Zar, 1999). In addition, to examine similarities on the Weighted Resultant Index among the months a simple clustering technique based on the Bray–Curtis dissimilarity index was used. From all analyses the unimportant prey (overall %Rn < 0.05) were excluded.

## Results

The Kruskal–Wallis test showed that the mean TL was not significantly different among months ($H = 16.1$, d.f. = 11, $P > 0.05$). However, lower mean TLs were observed during the period of June to December (Fig. 2).

### Feeding intensity

Table 2 shows statistical parameters of the sand smelt stomach according to the fullness category. The number of prey showed

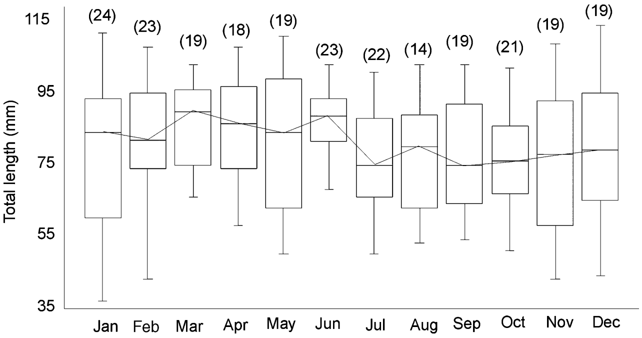

Fig. 2. Monthly total length frequency distribution of sand smelt from randomly selected samples. Within brackets = number of selected samples

Table 2
Statistical parameters of sand smelt stomach fullness categories ($N_m$: average number of prey per stomach; SD ($N_m$): standard deviation of $N_m$; n: total number of prey; N: number of stomachs)

| Fullness category | $N_m$ | SD ($N_m$) | Range of prey numbers | n | N |
|---|---|---|---|---|---|
| Empty stomach | 4.5 | 10.2 | 0–52 | 239 | 53 |
| Semi-empty stomach | 25.4 | 19.6 | 2–92 | 1119 | 44 |
| Semi-full stomach | 234.7 | 231.3 | 2–1179 | 20 421 | 87 |
| Full stomach | 1223.5 | 981.1 | 18–5182 | 68 516 | 56 |
| Total | 376.3 | 709.6 | 0–5182 | 90 295 | 240 |

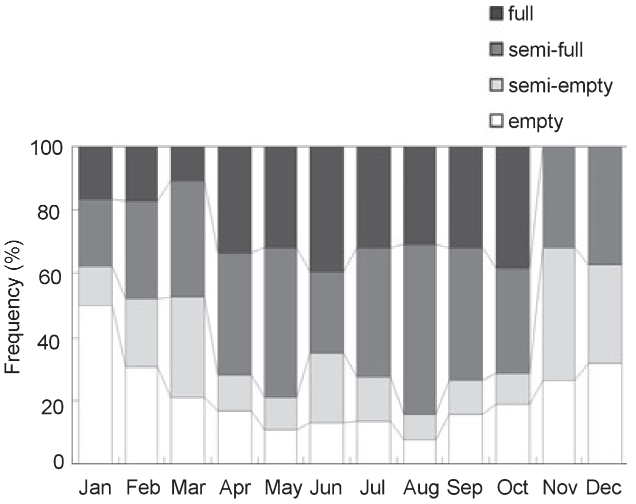

Fig. 3. Seasonal variation frequency of sand smelt fullness categories in Trichonis Lake

an exponential increase ($R^2 = 0.95$) per stomach ($N_m$) from empty to full. Of the 240 stomachs examined, 53 (22.1%) were empty. The monthly proportion of empty stomachs varied significantly over the year ($\chi^2 = 21.3$, d.f. = 11, $P < 0.05$), with a maximum in January (50%) and lower value in August (7.6%; Fig. 3). The proportion of empty stomachs among size classes ranged from 15% (72.1–84 mm size class) to 32% (84.1–95 mm size class) and did not differ statistically ($\chi^2 = 4.8$, d.f. = 4, $P > 0.05$).

### Diet composition

Analyses of prey in the stomachs identified 15 important items (%Rn > 0.05) belonging to six major groups: crustacean (copepods, cladocera), mollusca (bivalve: larvae), insects (larvae), cestode worms and teleostei (fry and eggs). The most important prey were the bivalve larvae, copepods and cladocera (%Rn > 20). Fish eggs, finfish fry, the Chironomid larvae and the cestodes showed low importance in the *A. boyeri* diet in Lake Trichonis (%Rn: 0.05–0.88).

Various prey belonging to phytoplankton, crustacean (copepods, cladocera, harpacticoida, amphipoda, and decapoda), nematoda worms and other plant items also showed very low importance in the sand smelt diet of Lake Trichonis (%Rn < 0.05; Table 3).

At the species level three species were dominant in the sand smelt diet: larvae of bivalves *Dreissena polymorpha* (%Rn = 33.8), copepods *Eudiadomus drieschi* (%Rn = 26.4) with their copepodites (%Rn = 0.20), and the cladocera *Diaphanosoma brachyurum* (%Rn = 24.2). Supplementary prey of sand smelt were the copepods *Cyclops* sp. (%Rn = 1.1), the cladocera *Bosmina longirostris* (%Rn = 5.3), *Daphnia longispina* (%Rn = 1.5), *Leptodora kindtii* (%Rn = 1.15) and the *D. hyalina* (%Rn = 0.1; Table 3).

Table 3
Overall diet composition of Trichonis Lake sand smelt ranked by Weighted Resultant Index (%Rn)

| | Food item | n | $N_1$ | $C_i$ | $F_{pi}$ | Rn | %Rn |
|---|---|---|---|---|---|---|---|
| Bivalves (larvae) | *Dreissena polymorpha* | 30 606 | 172 | 0.34 | 0.92 | 0.44 | 33.90 |
| Copepods | *Eudiatomus drieschi* | 23 991 | 152 | 0.27 | 0.81 | 0.34 | 26.46 |
| | Unidentified copepods | 4781 | 161 | 0.05 | 0.86 | 0.07 | 5.19 |
| | *Cyclops* sp. | 1053 | 97 | 0.01 | 0.52 | 0.01 | 1.14 |
| | Copipodides | 194 | 35 | 0.00 | 0.19 | 0.00 | 0.21 |
| | Total copepods | 30 019 | ** | 0.33 | ** | ** | 33.00 |
| Cladocera | *Diaphanosoma brachyurum* | 21 733 | 104 | 0.24 | 0.56 | 0.32 | 24.24 |
| | *Bosmina longirostris* | 4903 | 108 | 0.05 | 0.58 | 0.07 | 5.33 |
| | *Daphnia longispina* | 1411 | 53 | 0.02 | 0.28 | 0.02 | 1.53 |
| | *Daphnia* sp. | 205 | 45 | 0.00 | 0.24 | 0.00 | 0.22 |
| | *Leptodora kindtii* | 136 | 32 | 0.00 | 0.17 | 0.00 | 0.15 |
| | *Daphnia hyalina* | 98 | 14 | 0.00 | 0.07 | 0.00 | 0.11 |
| | Total cladocera | 28 486 | ** | 0.32 | ** | ** | 31.58 |
| Fish | fish eggs | 811 | 69 | 0.01 | 0.37 | 0.01 | 0.88 |
| | Finfish fry | 164 | 36 | 0.00 | 0.19 | 0.00 | 0.18 |
| Insects (larvae) | Chironomidae | 93 | 7 | 0.00 | 0.04 | 0.00 | 0.10 |
| Worms | Cestoda | 50 | 16 | 0.00 | 0.09 | 0.00 | 0.05 |
| | Others | 66 | ** | 0.00 | ** | ** | 0.07 |
| Amphipods | *Gammarus* sp. | 22 | 15 | 0.00 | 0.08 | 0.00 | 0.02 |
| Plants | Diatoms | 14 | 12 | 0.00 | 0.06 | 0.00 | 0.02 |
| | Unidentified plant parts | 6 | 5 | 0.00 | 0.03 | 0.00 | 0.01 |
| Worms | Nematoda | 6 | 6 | 0.00 | 0.03 | 0.00 | 0.01 |
| Copepods | Nauplii | 5 | 4 | 0.00 | 0.02 | 0.00 | 0.01 |
| Worms | Unidentifed worms | 4 | 4 | 0.00 | 0.02 | 0.00 | 0.00 |
| Harpacticoida | *Harpacticoida* sp. | 2 | 2 | 0.00 | 0.01 | 0.00 | 0.00 |

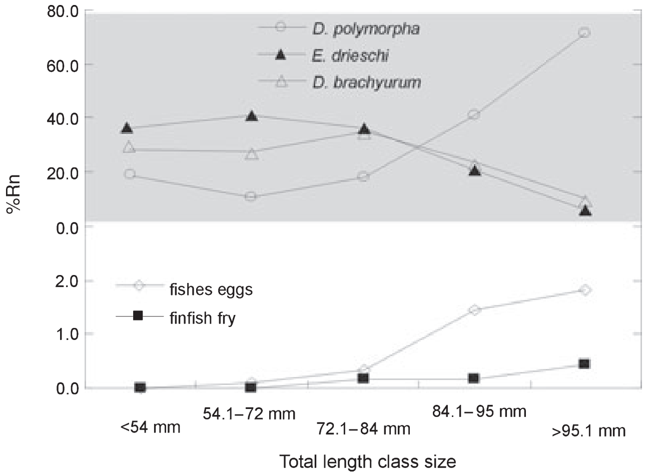

Fig. 4. Variation in prey Weighted Resultant Index (%Rn) according to length class size. (Figure shows prey where numerical abundance differed significantly among class sizes; Kruskal–Wallis test, $P < 0.05$)

### Food in relation to fish size

The Kruskal–Wallis test based on the individually arcsine-root transformed numerical abundance of a given food item showed that the dominant prey items (*D. polymorpha, E. drieschi*, and *D. brachyurum)* as well as finfish fry and fish eggs differed significantly among the length class sizes ($H > 13.7$, d.f. $= 4$, $P < 0.05$). The importance of cladocera and copepods decreased with increasing size, while at the same time the importance of bivalve larvae, fish eggs and finfish fry increased (Fig. 4). However, the mean number of prey items was not significantly different among the size classes (Kruskal–Wallis test, $H = 0.6$, d.f. $= 4$, $P > 0.05$). The diversity index among the size classes was rather constant ($H'$: 1.48 for class size $L_1$ to 1.58 for class size $L_4$).

### Seasonal variation in diet composition

The monthly numerical abundance of the majority of food items showed seasonal differences (Kruskal–Wallis test, $P < 0.05$). Bray-Curtis clustering based on the monthly Weighted Resultant Index (%Rn) showed higher similarity ($<50\%$), which occurred between January to May (cluster I) and from June to December (apart from August; cluster II), while August formed cluster III (Fig. 5). Cluster I was characterized by the high importance of *D. polymorpha* larvae (%Rn $= 66.8$) and the low importance of the cladocera *D. brachyurum* (%Rn $= 0.1$), while cluster II was characterized by the rather high importance of the cladocera *D. brachyurum* (%Rn $= 33.3$) and the rather low importance of *D. polymorpha* larvae (%Rn $= 7.4$). During August the cladocera *D. brachyurum* showed high importance (%Rn $= 78.6$). Furthermore, cluster II is also characterized by the higher diversity ($H' = 1.5$) of food items (Fig. 6).

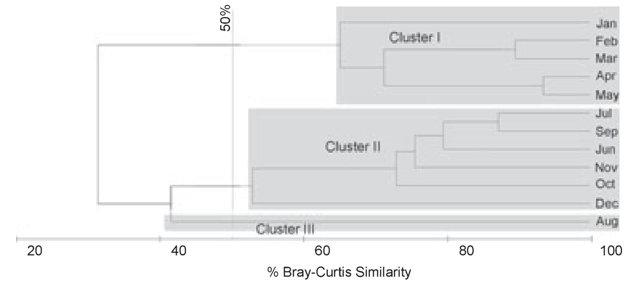

Fig. 5. Bray–Curtis clustering scheme based on monthly Weighted Resultant Index (%Rn)

The Kruskal–Wallis test based on the log transformed data showed that the mean number of prey items was significantly different among months ($H = 32.1$, d.f. $= 11$, $P < 0.05$). Lower values were shown from November to February, whereas the higher numbers were shown in August. The number of *E. drieschi* per stomach showed rather low variations among months. *D. polymorpha* and *D. brachyurum* showed strong supplemental seasonal patterns, with high number values per stomach from January to May and June to December, respectively (Fig. 7). Finally, no significant differences were found in the number of empty stomachs ($\chi^2$, d.f. $= 1$, $P > 0.05$) or in the number of prey per stomach of females or males (Kruskal–Wallis test, $H = 0.38$, d.f. $= 1$, $P > 0.05$).

### Discussion

Feeding habits and strategies of sand smelt have been studied in various areas using stomach content analyses (Moretti et al., 1959; Castel et al., 1977; Marfin, 1981; Ferrari and Rossi, 1983–84; Gon and Ben-Tuvia, 1983; Mantilacci et al., 1990; Danilova, 1991; Rosecchi and Grivelli, 1992; Trabelsi et al., 1994; Bartulovic et al., 2004); these studies have revealed an opportunistic feeding behavior and marked preferences for small crustaceans (Castel et al., 1977; Gon and Ben-Tuvia, 1983; Bartulovic et al., 2004). Although considered a planktonivorous species, it is well known that *A. boyeri* shift exclusively to benthic prey when zooplankton are scarce (Trabelsi et al., 1994; Bartulovic et al., 2004).

In general, in freshwater bodies the diet composition of sand smelt is dominated by copepods and cladocerans (Moretti

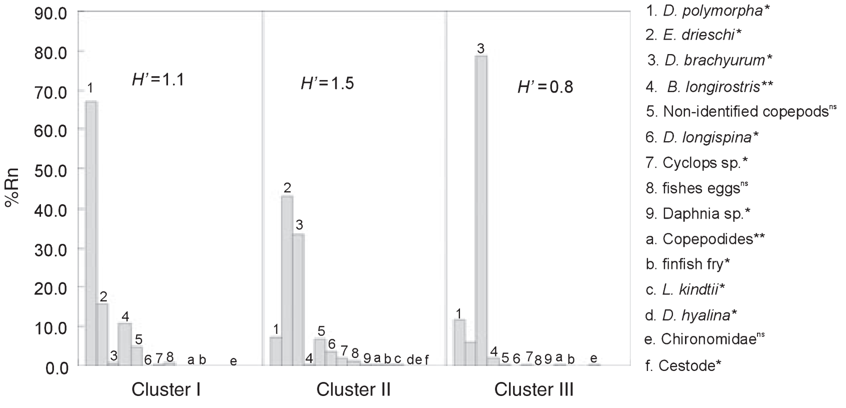

Fig. 6. Prey Weighted Resultant Index (%Rn) distribution according to each cluster resulting from Bray–Curtis similarity. (Similarity of monthly Weighted Resultant Index in each cluster was $>50\%$; $H'$, Shannon Index; *numerical abundance differed significantly among clusters I and II at level 0.05; **numerical abundance differed significantly among clusters I and II at level 0.1, $^{ns}$; numerical abundance not significantly different among clusters I and II – Kruskal–Wallis test)

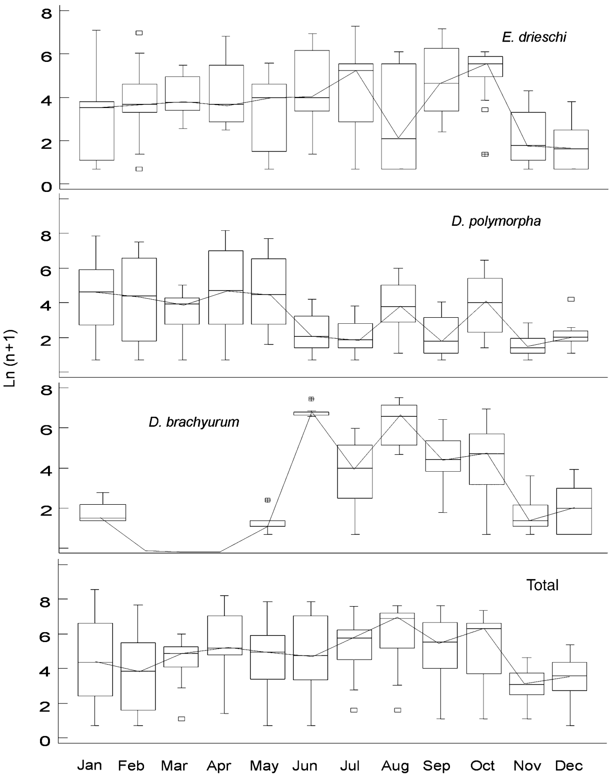

Fig. 7. Seasonal variation of dominant prey items and total prey number per stomach

et al., 1959; Mantilacci et al., 1990; Rosecchi and Grivelli, 1992). However, there are marked differences at the species level. For example, in Trasimeno Lake (Italy) as to cladocerans the *B. longirostris*, *Daphnia cucullata*, *Daphnia galeata* and *L. kindtii* were the most important prey (Moretti et al., 1959; Mantilacci et al., 1990), while in Lake Trichonis the most important cladocera was *D. brachyurum*, with a low importance of *B. longirostris*. On the other hand, although rotifers are abundant during the spring in Lake Trichonis (Doulka et al., 2005), they have not been observed as prey for *A. boyeri* in Trasimeno Lake (Mantilacci et al., 1990). Because rotifers are a dominant prey of young sand smelt (TL < 30 mm; Castel et al., 1977), the above difference can be explained by the fact that the Trasimeno Lake results were obtained with smaller specimens (TL > 20 mm) than those examined in the present study (TL > 34 mm).

According to the foraging theory, the probability of prey capture is a function of prey density, size, total visibility and motion (Lazzaro, 1987). The seasonal composition of sand smelt stomach contents followed the seasonal composition of the zooplankton community in Trichonis Lake. Copepods were the dominant group followed by the cladocerans, the planktonic larvae of *D. polymorpha* and rotifers, while the total abundance of zooplankton was higher (3–55 ind $L^{-1}$; Kehayias et al., 2004; Doulka et al., 2005). The total zooplankton abundance is higher in summer and autumn than in spring and winter. For summer and autumn, copepods are the dominant group in the zooplankton community, whereas for winter and spring the dominant group is *D. polymorpha* and rotifers, respectively (Doulka et al., 2005). These indicate that *A. boyeri* are following an opportunistic feeding behavior, each time targeting the more available prey items. However, the species could be selecting the most efficient prey, because during the spring feeding period it feeds on *D. polymorpha* larvae despite the higher abundance of rotifers. Vertical distribution of zooplankton in Trichonis Lake also showed high abundance in the 0–10 m depth layer (Kehayias et al., 2004); possibly this indicates that the feeding activity of the species occurs on the surface layers of the lake.

The present study found that food habits change considerably as fish grow. Preference for size selection by species has been suggested by other authors (Rosecchi and Grivelli, 1992; Bartulovic et al., 2004). However, in the present study, this size-selective preference may be related to seasonality of the prey availability because this coincided with the seasonality of total length distribution of the samples (Fig. 2).

According to Markevich (1977), sand smelt feeding ceases at temperatures below 8°C. In Trichonis Lake the lowest seasonal temperature is higher, thus the feeding activity of species is dependent on availability as well as suitability of prey items. Indeed, the feeding activity of *A. boyeri* as shown from the proportion of each fullness category per season (Fig. 3) as well as the number of prey per stomach (Fig. 7) follows the seasonal abundance of prey in the lake (Kehayias et al., 2004; Doulka et al., 2005). In contrast to the findings of our study, in Trasimeno Lake as well as in the estuary of Mala Neretva River the higher values of stomach vacuity were observed during the summer to autumn period (Mantilacci et al., 1990) and summer (empty stomachs = 36% of total: Bartulovic et al., 2004), respectively. In all cases the food items of *A. boyeri* during summer and autumn periods are composed of cladocera and copepods, but of different species. It seems that in Trichonis Lake the *E. drieschi* (copepods) and *D. brachyurum* (cladocera) comprise a high qualitative and quantitative prey for sand smelt. It should be noted that *D. brachyurum* have been considered as a new species of zooplankton fauna in Trichonis Lake (Kehayias et al., 2004). Thus the preference of sand smelt for this species indicates an efficient adaptation to the new trophic status of the lake.

In some sand smelt stomachs parasites (worms) were found (%Rn = 1.09), mainly belonging to cestodes (%Rn = 1.04) and nematodes (%Rn = 0.28; Table 1). These items were referred to as the stomach contents of sand smelt by others authors (Moretti et al., 1959; Gon and Ben-Tuvia, 1983). In the present study cestodes were mainly found in stomachs of sand smelts that had consumed fry of fishes (data not present here). Probably, these fry were the first hosts of the cestodes. As regards nematodes, it is not clear if these are actually parasites of sand smelt (Gon and Ben-Tuvia, 1983) or if they are food for the fish (Fouda, 1995). However, it has been clarified that some species of nematodes are sand smelt parasites and others are food for the fish (Danilova, 1991).

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