Feeding Ecology of the Endemic
Phoxinellus pseudalepidotus (Cyprinidae)
from Mostarsko Blato (Neretva River Basin, Bosnia and Herzegovina)

Ivana Markotić¹*, Zlatko Mihaljević², Marko Ćaleta³, Branko Glamuzina⁴

¹University of Mostar, Faculty of Science and Education, Department of Biology, Matice hrvatske b. b., 88000 Mostar, Bosnia and Herzegovina
²University of Zagreb, Faculty of Science, Department of Biology, Rooseveltov trg 6, 10000 Zagreb, Croatia
³University of Zagreb, Faculty of Teacher Education, Savska cesta 77, 10000 Zagreb, Croatia
⁴University of Dubrovnik, Department of Aquaculture, Ćira Carića 4, 20000 Dubrovnik, Croatia

*Corresponding Author:
Ivana MARKOTIĆ
University of Mostar, Faculty of Science and Education, Department of Biology,
MOSTAR, BOSNIA AND HERZEGOVINA
E-mail: ivana.markotic@fpmoz.sum.ba

Published: 24 September 2019
Copyright © Markotić et al.

Cite this article: Markotić, I., Mihaljević, Z., Ćaleta, M. & Glamuzina, B. (2019). Feeding Ecology of the Endemic Phoxinellus pseudalepidotus (Cyprinidae) from Mostarsko Blato (Neretva River Basin, Bosnia and Herzegovina). European International Journal of Science and Technology, 8(8), 5-14.
Abstract
Results of the analysis of seasonal diet of the endemic fish species *Phoxinellus pseudalepidotus* from Mostarsko Blato (Neretva River basin, Bosnia and Herzegovina) were presented. Intestinal contents of 96 caught specimens, sampled on a seasonal basis in February, May, August and November 2009, were analyzed. It was found that the diet spectrum consisted of invertebrates from the taxa: insect (Insecta) and snails (Gastropoda); plant material and amorphous mass. Insects were present in diet during the whole year, with representatives of classes: Diptera, Ephemeroptera, Trichoptera, Plecoptera and Coleoptera. Representatives of snails were found in the summer period only. The amorphous mass was detected only in fall, while the plant material was found during summer and fall period. Diet of *P. pseudalepidotus* showcases significant seasonal variability and intensity of feeding is not uniform during the year. According to these results, primary diet for this species is animal-origin food with aquatic insects as the main component of nutrition. In addition, it can be concluded that *P. pseudalepidotus* belongs to the group of omnivores, i.e. subgroup of zoophytophages.

Keywords: feeding strategies, native species, diet composition, seasonal variability, Bosnia and Herzegovina

1. Introduction
*P. pseudalepidotus* (Bogutskaia & Zupančič, 2003) is a small-sized (< 15.0 cm TL) cyprinid endemic freshwater fish distributed in Mostarsko Blato in the Neretva catchment area in Bosnia and Herzegovina. On top of that, it is considered to be distributed more widely in the River Neretva Basin (Bogutskaia and Zupančič, 2003). Some authors considered this fish from Mostarsko Blato as *Phoxinellus alepidotus* because of morphological similarity between *P. alepidotus* and *P. pseudalepidotus* (Heckel and Kner, 1858; Seeley, 1886 (as Paraphoxinus alepidotus); Vuković and Ivanović, 1971 (as Paraphoxinus alepidotus); Kottelat, 1997). Also, Zupančič and Bogutskaia (2002) found an undescribed species close to *P. alepidotus*, which was detected in Mostarsko Blato. *P. pseudalepidotus* inhabits streams or shallow canals with little current and clean water (Crivelli, 2006; Bogutskaia and Zupančič, 2003). During unfavorable periods, it lives in subterranean waters (Markotić et al., 2013; Markotić, 2013). This endemic fish is classified by the IUCN as vulnerable (Crivelly, 2006). It is endangered because of the extremely limited range of distribution, river regulation and influence of non-indigenous species (Mihinjač et al., 2014). The diet of *P. pseudalepidotus* was not familiar prior to this research. No data on qualitative and quantitative composition of food were found in bibliography for this species. This information could prove extremely useful for other small cyprinid species whose feeding habitats are poorly understood.

2. Material and Methods
2.1. Study Area
Mostarsko Blato (43°19’55”N 17°41’53”E) is an enclosed karst field in the Neretva River catchment area, SW Bosnia and Herzegovina. The area of Mostarsko Blato is characterized
by sub-Mediterranean climate, i.e. temperately warm humid climate with hot summers (Cfa), according to Köppen's climatic classification. In general, winters are mild and rainy, while summers are hot and relatively dry (there is no specific minimum like in Csa climatic zones, such as the neighboring littoral and insular part of Southern Croatia). Pluviometric regime is maritime, with most of precipitation concentrated in fall and winter (primary maximum), and in April and May (secondary maximum). The influence of the Adriatic Sea reaches from three sides; southeast, east and south respectively. The strongest inflow of air arrives through the Neretva River valley, and over low Varda ridge, which separates lower alluvial plain of Mostarsko Blato from higher Mostarsko Blato Polje. This inflow relieves the influence of colder air coming from northern mountains. During fall, winter and spring, the most part of Polje is flooded, but during summer almost all water springs dry out (Studija izvodivosti HE Mostarsko blato, EP HZHB, Salzburg, 2000).

The area of Mostarsko Blato, through which the Lištica River flows, is surrounded by limestone hills: Orlovac, Mikuljača, Virača, Trtla and Varda. The field is flooded 5-6 months during the year, on average. In addition to permanent water sources in Mostarsko Blato, significant amounts of periodic waters of Rivers Ugrovača, Orovnik, Mokašnica and a number of small torrents are active during major rainfall seasons. In order to reduce the floods, the tunnel Varda was built in the year of 1947. Besides the Varda tunnel, water from Mostarsko Blato is managed also by the following sinkholes: Krenica, Košina, Renkovača, Kruševća, and the Velika Jama (Great Hole). These sinkholes are important for *P. pseudalepidotus* life cycle (Bogut et al., 2007).

2.2. Sampling

Fish sampling was carried out from Feb. 2009 to Nov. 2009 by gill nets (7 m length and 0.7 m height, with a 7 mm mesh size) and „krtol“, traditional fishing tool in the area of Mostarsko Blato (Neretva River Basin, Bosnia and Herzegovina) during dry season (August) and wet season (November, February and May). In order to analyze the seasonal diet, a total of 96 fish were sampled. All samples were weighed and total lengths were measured to the nearest 0.1 g and 0.1 cm, respectively. The study area with sampling sites is shown in Figure 1.

In the same period, at these locations, water temperature, concentration of dissolved oxygen and water oxygen saturation were measured by using a WTW Oxymeter Oxi 197, pH with a pH-meter WTW Sen Tix 41, and conductivity was measured by using a WTW Conductometer (Cond 197i; probe WTW TetraCon 325).
Figure 1. Map of the study area, showing the location of the sampling sites: 1. Pisak, 2. Međurić, 3. Pološki Gaz

2.3. Diet Analysis

The fish were opened by a longitudinal incision from the anal cavity to the head. The digestive system was separated by a scissors incision at its beginning and end and was extracted by means of dissecting forceps. This was followed by measuring the length of the digestive system and isolating the intestinal contents by making a longitudinal incision through the digestive system. Larger portions of the intestinal contents were stored in Eppendorf tubes with 70% alcohol solution, while smaller portions were washed from the intestines with alcohol and suctioned with a dropper, and then stored in tubes. Each tube was marked with a corresponding serial number. Determination of the intestinal contents was carried out afterwards. Each sample was washed into a Petri dish or on a watch glass and examined under a magnifying glass (Zeiss Stemi 2000-C). The following determination keys were used to determine the intestinal contents: Bertrand (1954), Brauer (1961), Kerovec (1986), Nilsson (1997), Sansoni (1992), Steinmann and Zombori (1984).

Intensity of diet was determined by using the vacuity coefficient %V (Hureau, 1970).
1. The vacuity coefficient (%V) was calculated by using formula \( %V = \frac{E_r}{N} \times 100 \), where \( E_r \) is the number of empty digestive systems and \( N \) is the total number of all analyzed digestive systems.

Quantitative nutrition composition of individual relatives was stated by means of nutrient indexes (Hyslop, 1980; Bowen, 1996):
1. Percentage number (%N) was calculated by using formula \( %N = \frac{n_p}{N_p} \times 100 \), where \( n_p \) is the number of individuals of certain prey, and \( N_p \) is the number of individuals of all found prey.
2. Frequency of occurrence (%F) was calculated by using formula \( %F = \frac{n}{N} \times 100 \), where \( n \) is the number of digestive systems containing certain prey, and \( N \) is the total number of analyzed digestive systems.
3. Results

3.1. Total Length, Weight and Gut Length
A total of 96 specimens were investigated. The values for mean total length, weight, and gut length of the specimens (± standard deviation [with minimum-maximum]) were 6.5±0.6 cm (5.1-7.7), 3.1±0.7 g (1.7-5.4) and 6.1±0.6 cm (4.5-7.4), respectively (Table 1).

Table 1. The mean total length, weight, and gut length of members of both sexes of *P. pseudalepidotus* collected from the area of Mostarsko Blato in 2009.

<table>
<thead>
<tr>
<th></th>
<th>Total length (cm)</th>
<th>Weight (g)</th>
<th>Gut length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female (N=45)</td>
<td>Male (N=49)</td>
<td>Female (N=36)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.5±0.6</td>
<td>6.4±0.5</td>
<td>3.2±0.9</td>
</tr>
<tr>
<td>Min/max</td>
<td>5.1-7.7</td>
<td>5.6-7.7</td>
<td>1.7-5.1</td>
</tr>
</tbody>
</table>

3.2. Environmental Variables
During the research period, at the sampling sites, the water temperature varied from 10.2 °C in winter to 24 °C in summer, pH ranged from 7.35 in summer to 8.02 in winter, and conductivity from 319 μS cm⁻¹ in winter to 532 μS cm⁻¹ in fall. Concentration of dissolved oxygen varied from 5.52 mg L⁻¹ in summer to 10.57 mg L⁻¹ in winter, and water oxygen saturation was within the range from 62.6 % in fall to 104 % in spring.

3.3. Feeding Habits
3.3.1. Feeding Intensity: The %V values for female, male and all fish were 13.04 %, 24 % and 18.75 %, respectively. The %V of *P. pseudalepidotus* specimens changed seasonally (Table 2). In general, the %V was high in summer season 2009 (26.1 %).

Table 2. Seasonal variability of the vacuity coefficient (%V) of individual specimens of *P. pseudalepidotus* collected from the area of Mostarsko Blato (number of specimens was provided in brackets) in 2009.

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Fall</th>
<th>All seasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>5.3 (19)</td>
<td>0 (8)</td>
<td>22.2 (9)</td>
<td>30 (10)</td>
<td>13.04 (46)</td>
</tr>
<tr>
<td>Male</td>
<td>40 (5)</td>
<td>21.4 (14)</td>
<td>28.6 (14)</td>
<td>17.6 (17)</td>
<td>24 (50)</td>
</tr>
<tr>
<td>All fish</td>
<td>12.5 (24)</td>
<td>13.6 (22)</td>
<td>26.1 (23)</td>
<td>22.2 (27)</td>
<td>18.75 (96)</td>
</tr>
</tbody>
</table>

3.3.2. Frequency of Occurrence and Abundance: Eleven food categories were identified in the intestinal contents of *P. pseudalepidotus*. Some of the food categories were found in small numbers. Although the species consumed a large spectrum of food categories, the diet base was primarily insect larvae (F = 60.25 %), and remains of insect larvae (F = 29.48 %) (Table 3). Of all the animal prey items recorded, Ephemeroptera Baetidae larvae and Diptera Chironomidae larvae were the most frequent, occurring in 30.76 % and 17.94 % of the fish, and accounting for 23.72 % and 28.81 % of the abundance (N), respectively (Table 3).
Table 3. Frequency of occurrence (% F) and abundance (% N) of food categories found in the digestive system of *P. pseudalepidotus* in the area of Mostarsko Blato in 2009.

<table>
<thead>
<tr>
<th>Food category</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=24</td>
<td>N=22</td>
<td>N=23</td>
<td>N=27</td>
</tr>
<tr>
<td>Diptera Chironomidae larvae</td>
<td>38.09</td>
<td>56.52</td>
<td>23.53</td>
<td>9.52</td>
</tr>
<tr>
<td>Ephemeroptera larvae</td>
<td>0</td>
<td>31.58</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Ephemeroptera Baetidae larvae</td>
<td>52.38</td>
<td>68.42</td>
<td>66.67</td>
<td>0</td>
</tr>
<tr>
<td>Trichoptera larvae</td>
<td>4.76</td>
<td>2.17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plecoptera larvae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coleoptera larvae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Remains of insect larvae</td>
<td>23.81</td>
<td>10.87</td>
<td>52.94</td>
<td>42.86</td>
</tr>
<tr>
<td>Adult insects</td>
<td>9.52</td>
<td>4.35</td>
<td>11.76</td>
<td>0</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>0</td>
<td>0</td>
<td>5.88</td>
<td>0</td>
</tr>
<tr>
<td>Plant material</td>
<td>0</td>
<td>0</td>
<td>29.41</td>
<td>4.76</td>
</tr>
<tr>
<td>Amorphous mass</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

4. Discussion and Conclusion

The diet of *P. pseudalepidotus* was not familiar prior to this research. No data on qualitative and quantitative composition of food were found in bibliography for this species, as well. The results of the study showcase that the food composition of *P. pseudalepidotus* include the invertebrate groups Insecta and Gastropoda, plant material and amorphous mass. The Insecta class is present throughout all four seasons, and the Gastropoda only in summer. Within the Insecta class, categories of Diptera, Ephemeroptera, Trichoptera, Plecoptera, and Coleoptera are present. Plant material is present in summer and in fall, and amorphous mass only in fall. The diet of *P. pseudalepidotus* shows significant seasonal variability and the intensity of feeding is not uniform throughout the year. The food composition of *P. pseudalepidotus* was also observed according to gender, and it was found that the diet of females was more diverse, i.e. that it had a wider range of prey. According to these results, primary diet for this species is animal-origin food with aquatic insects as the main component of nutrition. In addition, it can be concluded that *P. pseudalepidotus* belongs to the group of omnivores, i.e. subgroup of zoophytophages. The total number of empty digestive systems in *P. pseudalepidotus* was 18.75 %. The lowest count of empty digestive systems was noted in the winter season (12.5 %), and the highest count of empty digestive systems was in the summer (26.1 %). Krivokapić (1997) detected 11.8 % of empty digestive systems for *Telestes montenegrinus*, from the Morača River, and Vuković (1985) established empty digestive systems ranging from 16 % in summer to 44.74 % in spring for *Telestes souffia*, from the Drina River. For *Telestes ukliva*, from the Cetina River, a range of empty digestive systems was determined from 20 % in December, May, and September to 40 % in March (Zanella et al., 2009). *Delminichthys adspersus*, which is prevalent in karst waters of Southern Croatia.
and Bosnia and Herzegovina, feeds on invertebrates, mostly on aquatic insect larvae and crustaceans (Mrakovčić et al., 2006). *Phoxinellus alepidotus*, recorded in the Fields of Livno, Duvanj, Glamoč and Sinj, feeds on insect larvae, crustaceans and other invertebrates (Mrakovčić et al., 2006). *Phoxinellus dalmaticus*, widespread in the Čikola and Krka Rivers, and *Telestes metohiensis*, found in Southern Croatia and Bosnia and Herzegovina, mostly feed on aquatic insects and crustaceans (Mrakovčić et al., 2006). *Delminichthys jadovensis*, recorded in watercourses of Lika, feeds on insect larvae, crustaceans, other invertebrates and dead organic matter (Mrakovčić et al., 2006). *Delminichthys ghetaldi*, recorded in Popovo Polje in Herzegovina, the Buna River in the Neretva River Basin, the Kasindolka stream in the Bosnia River Basin and in the streams and rivers of Southern Croatia, also feeds on crustaceans and other invertebrates (Mrakovčić et al., 2006). *Telestes turskyi*, which inhabits the Čikola River in Croatia and Buško Blato in Bosnia and Herzegovina, feeds on aquatic invertebrates (Mrakovčić et al., 2006). *Telestes ukliva*, from the Cetina River, also feeds on invertebrates, primarily on crustaceans and insect larvae (Mrakovčić et al., 2006). This species is predominantly insectivorous, with water insects as the major component of the diet. Plant material is also present and includes algae which are characteristic of clean water: Chlorophyceae, Cyanophyceae, Xanthophyceae and Diatomeae, as well as fragments of higher plants. Certain seasonal variability in qualitative and quantitative composition of organisms were detected in the diet of this species and the feeding intensity is not uniform throughout the year (Zanella et al., 2009). The results of a study of the *Telestes karsticus* diet from the Sušik creek show that the species is a euryphagous omnivore, whose diet encompasses a plant and animal nutritional component and a tendency for cannibalism. Aquatic invertebrates are the main and most common source of food. The dominant element of diet throughout the year includes groups of organisms from the Diptera category, of which larvae from the Chironomidae family are the most abundant and the most frequent prey in the digestive system of fish. In summer and in spring, larvae of the Chironomidae are the most abundant prey, in fall planktonic shrimps from the Cladocera group and the Ephemeroptera larvae, while in winter the most common groups are Hydracarina, Cladocera and Oligocheta (Sučić, 2012). By analyzing the seasonal diet of *Telestes montenegrinus*, from the Morača River, it was determined that algae are predominantly found in the digestive system during winter and spring, while insects are more common in summer and fall. It was also found that the primary food for this species was of animal origin, and the difference in diet can be explained by the seasonal dynamics of animal forms (Krivokapić, 1992b). Vuković (1985) established for *Telestes souffia* species from the Drina River it was an omnivore, with a significant share of plant material. For this species, uniform diet throughout the year was determined (Vuković, 1985).

A similar spectrum of diet was noted for *Pelasgus epiroticus*, an endemic species widespread in Lake Pamvotis, Greece. Its nutrition consists of several groups of small aquatic invertebrates, the most common of which are shrimp, insect larvae, small insects and algae (Leonardos et al., 2005). The species *Pelasgus stymphalicus*, widespread in Lake Stymphalia, Greece, feeds on invertebrates and plant food, as stated by Kottelat and Freyhof (2007). The predominant diet of *Pseudophoxinus kervillei*, from Lake Agmon in Israel, consists of crustaceans and insects, with presence of algae and higher plants (Gophen et al., 1998). The
species Telestes pleurobipunctatus, prevalent in Southern Albania and Western Greece, feeds primarily on insect larvae, with the addition of other animals and plant material (Kottelat and Freyhof, 2007). Anaeocypris hispanica, found in the Guadiane Basin in Spain and Portugal, feeds on filamentous algae and small invertebrates (Kottelat and Freyhof, 2007). The results of the analysis of the P. pseudalepidotus diet largely coincide with the results obtained for the diet of other minnows and related endemic species.

5. References


