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Qualitative and Quantitative Examination of Algal Flora in the Gut Content of *Tanypus punctipennis* Meigen, 1818

Burak ÖTERLER*1, Gazel Burcu AYDIN1, Belgin ÇAMUR ELİPEK1

[1]Trakya University, Faculty of Science, Department of Biology, 22030, Edirne

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Algal flora

Abstract: Larval chironomids feed on algae, diatomae, detritus, decaying plant and animal fragments. The larvae in turn are food for other carnivore and omnivore animals. Thus, larval chironomids have a very important role in the aquatic food cycle. In this study, the algal flora in the stomachs of *Tanypus punctipennis*, which is known as a very common species in Turkish Thrace, was examined in terms of qualitative and quantitative factors. It was found that Bacillariophyta was the dominant group with 42 taxa in the stomach of *T. punctipennis.* It was followed by Cyanophyta with 3 taxa, Euglenophyta with 6 taxa, Chlorophyta with 4 taxa, Carophyta with 2 taxa, and Rhodophyta with 1 taxa.

Tanypus punctipennis Meigen, 1818'in (Diptera, Chironomidae) Mide İçeriğindeki Alg Florasının Kalitatif ve Kantitatif Açıdan İncelenmesi

Anahtar Kelimeler
Tanypus punctipennis,
Mide içeriği,
Alg Florası

Özet: Chironomid larvaları algler, diatomlar, detritus, bitki ve hayvansal parçalarla beslenir. Chironomid larvaları ayrıca etobur ve omnivore hayvanlar için besindir. Böylece, larval chironomids sudaki besin döngüsünde çok önemli bir role sahip olmaktadır. Bu çalışmada, Türkiye Trakya'sında yaygın olarak bulunan *Tanypus punctipennis* larvalarının mide içeriğindeki alg florası kalitatif ve kantitatif olarak incelenmiştir. Bacillariophyta 42 takson ile *T. punctipennis* larvalarının midesinde hakim grup olarak tespit edilmiştir. Bu grubu 3 takson ile Cyanophyta, 6 takson ile Euglenophyta, 4 takson ile Chlorophyta, 2 takson ile Charophyta ve 1 takson ile Rhodophyta grubu takip etmiştir.

1. Introduction

In recent years, benthic macroinvertebrates have been used for two main aims in scientific research associated with the evaluation of aquatic ecosystems. The first is taxonomical examination, which aims to determine the biodiversity of the biotope. The second one is functional examination, which aims to determine the food chain and energy flows [1]. The studies on the food preferences of zoobenthic organisms in an aquatic ecosystem provide the main knowledge on utilization of a water resource [2]. Species in an ecosystem may have different features and strategies than their competitors for a specific food source [3].

Benthic macroinvertebrates are one of the groups which are affected from modification of aquatic ecosystems. They have a very important role in the food chain, mainly feeding fish, and they are indicator species which are used to determine the water quality, biological trophic level, and ecological features of the ecosystem [4]. Chironomids, which are the most populated family in the order Diptera, live in aquatic environments, such as lakes and ponds. They also live in marshes and running waters in larval and pupa stages. Larval Chironomids ventilate aquatic sediment, causing nutrients to be realised from the sediment, which prevents water purification. Chironomids (in larvae, pupae, and adult stages) are the main food source for invertebrates, fish, amphibians, and birds [5]. They feed on algae, detritus, macrophytes, and other invertebrates. Therefore, they are classified as filters, pickers-spoolers, shredders, scrapers, piercings, and they swallow by their nutrition type. Members of the family Chironomidae are an important bridge between producers and consumers in an aquatic ecosystem [6].

The Chironomidae family also has species which have important roles in determining the trophic status of

*İlgili yazar: burakoterler@trakya.edu.tr

an aquatic biotope. They are commonly used as indicator organisms to determine the trophic level of aquatic environments because of their ability to filter fine particulate organic material (FPOM), their long life as larvae in aquatic environments, and their limited mobility as larvae. The studies on their feeding characteristics have increased in recent years [7].

It is reported that the dominant niches of Tanypodinae, which includes the genus *Tanypus*, are detritus, plants, and bryophytes [8]. Although it is known that the members of the subfamily Chironominae feed on algae and especially diatoms only, it is reported that the members of the subfamily Tanypodinae also feed on larvae of other organisms as predators [9].

In this study, the stomach of the species *Tanypus punctipennis* Meigen, 1818 was examined to determine feeding characteristics. The species *T. punctipennis* is commonly distributed in Europea and Turkish Thrace [10, 11, 12, 13, 14, 15, 16, 17, 18]. Although it has been reported in previous studies that algae are the dominant foods in the stomach of this species, there is no study with qualitative and quantitative examination of *T. punctipennis* [19, 20].

2. Material and Method

2.1. Study area

This study was done on the larvae of *T. punctipennis* which were obtained from five different locations in the Kırklareli provinces of Turkish Thrace (Figure 1). Although larval chironomids were collected in 64 locations in the Kirklareli provinces between the dates of June 2012 and August 2012, specimens of *T. punctipennis* were found in only 5 of these 64 locations. Loc.1, surrounded by trees in the sampling arae and shaded on both sides of brook. Loc. 2, there is no vegetation on the shore and in the pond. Loc. 3, there is only shading on right sides of the brook flowing through agricultural fields. Loc. 4, there is shading on both sides of the brook flowing through agricultural fields. Loc. 5, there is shading on both sides of the brook flowing through agricultural fields. The characteristics of the locations where *T. punctipennis* samples were obtained are presented in Table 1.

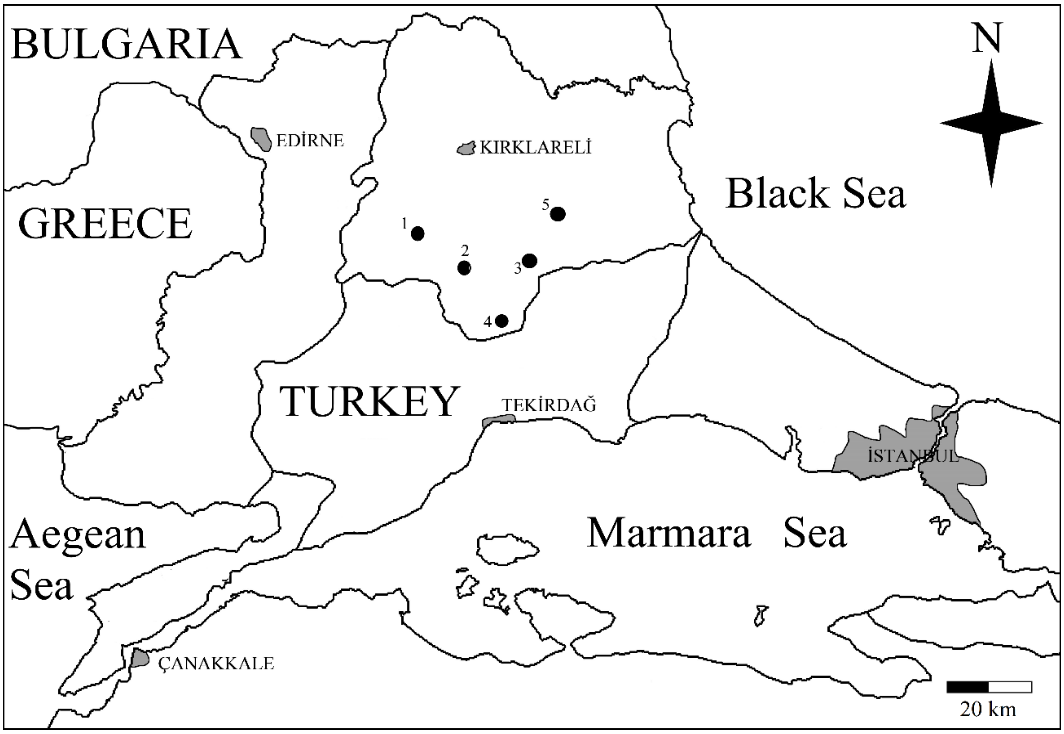

Figure 1. The study area and the locations where larvae were obtained.

Table 1. The features of the locations where larvae of *T. punctipennis* were obtained.

Locations	Locality Name	Habitat	Bottom Features	Coordinates	Major Vegetation
Loc.1	Sakızköy Brook	Stream	Stone, pebble	41°27′21″N 27°28′38″E	*Fraxinus* sp., *Quercus* sp., *Rumex* sp. *Urtica* sp.
Loc.2	Kırıkköy Pond	Pond	pebble, sand	41°26′57″N 27°16′03″E	-
Loc.3	Alacaoğlu Brook	Stream	stone, pebble, mud	41°16′51″N 27°18′36″E	*Quercus cerris, Pyrus communis, Carpunus orientalis, Juncus* sp.
Loc.4	Ahmetbey Brook	Stream	Mud, sand	41°28′21″N 27°34′45″E	*Ulmus minor, Typha* sp., *Rumex* sp.
Loc.5	Üsküpdere Brook	Stream	stone, pebble, sand	41°40′58″N 27°22′02″E	*Ulmus minor, Typha* sp., *Rumex* sp., *Urtica* sp.

2.2. Sampling

Hand-driven mud sampling devices to collect the sediment samplings. The sediment was sieved and the obtained larval material was fixed in plastic bottles containing 70% ethanol for transport to the laboratory.

2.3. Analyses

The *T. punctipennis* larvae were selected from the collected material and counted using a stereomicroscope binocular. Identification of the larvae was based on the literature of [21, 22, 23, 24]. To analyse the stomach contents of the larvae, 10 specimens were chosen from each locaties and placed into petri dishes containing 1 ml of 70% ethanol. Each specimen was dissected to reveal the stomach contents. The diffused material in the petri dish was poured into a Sedgewick-Rafter count chamber with a 1 ml volume. All organic and inorganic materials in the stomach were counted. Algal biovolume was estimated from abundance data and measurements of specific cell volumes by approximating geometric shapes of the cells [25, 26]. Identifications were carried out at 1000x magnification under immersion oil, and identification of taxa was done with the help of related literature [27, 28, 29, 30, 31, 32, 33]. Finally, all species were confirmed using AlgaeBase, an electronic database of algae information hosted by the National University of Ireland [34].

2.4. Data analysis

Analysis using the Shannon-Weaner index was performed in order to evaluate the species diversity of the larval stomach contents. The Bray-Curtis index analysis was then performed in order to determine the similarities of the sampling locations where the larvae of *T. punctipennis* were collected. Species richness was measured as the number of taxa present in each sample, and biological diversity was calculated based on (H´). The analyses were carried out using the XLSTAT-ADA statistical package program [35].

3. Results and Discussion

In this study, it was observed that the stomach contents of *T. punctipennis* larvae primarily contained algae, followed by inorganic materials, animal and plant fragments, pollens, and fungi spores (Figure 2). Although it is known that zoic material is dominant in the diet of Tanypodinae, in our study the algal material was dominant location 3 and 5 as the food resource in the diet of *T. punctipennis* larvae [36, 37].

The results of analyses by location showed the algae was the dominant diet for the larvae in locations 1, 2, and 3, while animal fragments were dominant in location 4 and plant fragments were dominant in location 5 (Figure 3).

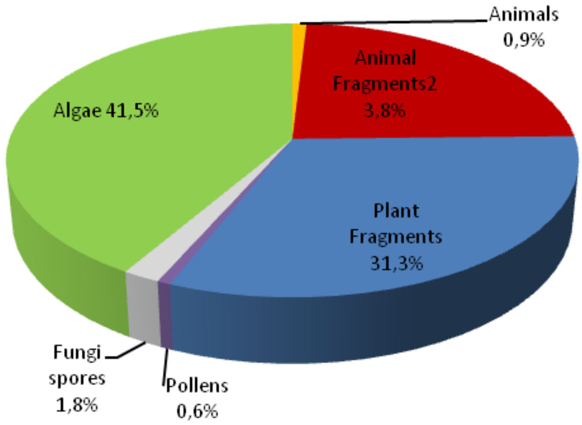

Figure 2. Relative composition of food items observed in gut contents of *T. punctipennis* larvae.

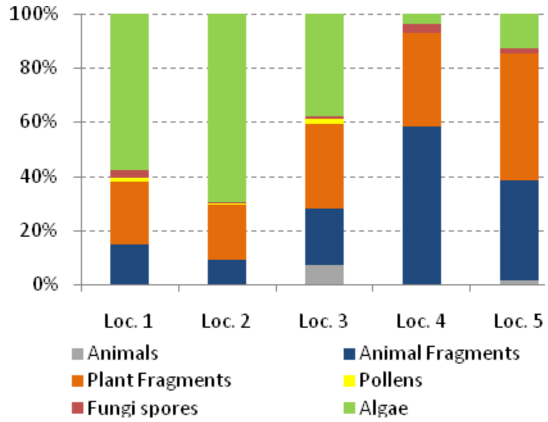

Figure 3. The gut contents and their distributions in the sampling locations.

A total of 58 different taxa belonging to six algal divisions were found in the stomachs of the examined larvae (Table 2). They were represented by Bacillariophyta with 42 taxa, Euglenophyta with 6 taxa, Chlorophyta with 4 taxa, Cyanophyta with 3 taxa, Charophyta with 2 taxa, and Rhodophyta with 1 taxa, respectively.

Based on observations of the algal material in the stomachs of the examined larvae, filamentous blue-green algae represented the dominant group with 35.5% abundance, followed by diatoms with 31.9% abundance and filamentous green algae with 20.8% abundance, respectively. The abundance of the other algal groups in the stomachs of the larvae was observed in very low ratios (Figure 4).

In location 1, members of the filamentous blue-green algae group (especially members of genus *Oscillatoria*) were determined to be the dominant group in both quantity and biovolume. This group was followed by diatoms (especially the species *Craticula cuspidata*).

In location 2, members of the filamentous blue-green algae group (especially members of genus Leptolyngbya) were also determined to be the dominant group in both quantity and biovolume.

Table 2. Algal composition of *T. punctipennis* larvae in gut contents.

	Loc 1	Loc 2	Loc 3	Loc 4	Loc 5
Phylum: Bacillariophyta					
Classis: Bacillariophyceae					
Achnanthes exigua Grun.			+		+
Achnanthidium minutissimum (Kütz.)	+			+	
Amphora ovalis (Kütz.) Kütz.		+			
Caloneis amphisbaena (Bory) Cleve	+	+	+		+
Caloneis silicula (Ehren.) Cleve		+			
Craticula cuspidata (Kütz.) Mann	+		+		
Cymatopleura elliptica (Bréb.) Smith		+			
Cymatopleura solea (Bréb.) Smith		+	+	+	+
Cymbella amphicephala Näegeli			+		
Cymbella cistula (Ehren) Kirch.	+			+	
Cymbella lanceolata (Agardh) Kirc.				+	+
Cymbella sp.		+	+	+	
Diatoma vulgaris Bory					+
Encyonema minutum (Hilse) Mann	+		+	+	
Encyonema silesiacum (Bleisch) Mann			+		
Gomphonema parvulum (Kütz.) Kütz.	+	+			
Gomphonema truncatum Ehren.			+	+	
Gyrosigma acuminatum (Kütz.) Raben.		+	+	+	
Navicula capitata Ehrenberg	+	+			+
Navicula lanceolata Ehrenberg				+	
Navicula meniscus Schumann	+	+			
Navicula sp.	+	+	+	+	+
Navicula tripunctata (Müller) Bory	+	+			+
Navicula viridula (Kütz.) Ehren.		+			+
Nitzschia acicularis (Kützing) Smith			+		+
Nitzschia denticula Grunow	+			+	
Nitzschia dissipata (Kütz.) Raben.	+	+			
Nitzschia flexa Schumann	+	+			
Nitzschia hungarica Grun.	+			+	+
Nitzschia recta Hantzsch	+				
Nitzschia sigma (Kütz.) Smith		+			
Nitzschia sigmoidea (Nitzsch) Smith		+			
Nitzschia sp.	+		+	+	+
Pinnularia sp.		+			
Pinnularia viridis (Nitzsch) Ehren.		+			
Rhopalodia gibba (Ehren.) Müller	+			+	
Sellaphora pupula (Kütz.) Meres.	+				
Surirella brebissonii Kram.&L-Bertalot		+			
Tryblionella gracilis Smith			+	+	
Ulnaria capitata (Ehren.) Compère			+		+
Ulnaria ulna (Nitzsch) Compère	+	+	+		+
Classis: Coscinodiscophyceae					
Melosira varians Agardh		+			
Phylum: Euglenophyta					
Classis: Euglenophyceae					
Euglena sp1.		+	+		
Euglena sp2.			+		+
Euglena sp3.		+			+
Euglena gracilis Klebs			+		+
Phacus acuminatus Stokes	+	+			
Trachelomonas hispida Stein	+	+			
Phylum: Chloropophyta					
Classis: Chlorophyceae					
Scenedesmus acutus Meyen			+		
Classis: Ulvophyceae					
Cladophora glomerata (L) Kütz	+				+
Cladophora sp.		+	+	+	
Ulothrix sp.				+	
Phylum:Cyanophyta					
Classis: Cyanophyceae					
Chroococcus minimus (Keiss.) Lemm.	+		+		
Leptolyngbya sp.	+	+		+	
Oscillatoria sp.	+	+	+	+	
Phylum: Charophyta					
Classis: Conjugatophyceae					
Closterium littorale F.Gay		+		+	
Closterium lunula Ehren.&Hemp.		+	+		
Phylum: Rhodophyta					
Classis: Florideophyceae					
Batrachospermum sp.					+

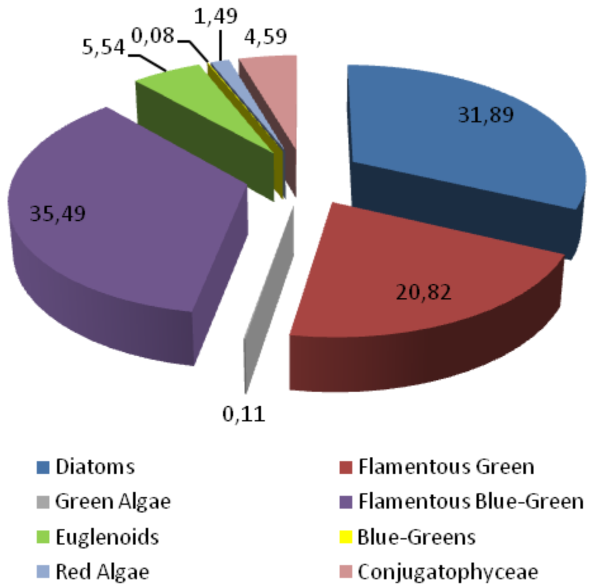

Figure 4. The abundance ratios in the stomach of T. punctipennis larvae (% abundance).

This group was followed by diatoms (*Cymatopleura* solea and members of genus *Navicula*) and euglenoids (*Phacus acuminatus* and members of genus *Euglena*).

In location 3, members of the diatoms group (*Caloneis amphisbaena, Tryblionella gracilis, Encyonema silesiacum, Cymatopleura elliptica, Cymatopleura solea,* and *Nitzschia sigmoidea* especially) were determined to be the dominant group in both quantity and biovolume. This group was followed by *Leptolyngbya* sp., belonging to the filamentous blue-green algae group.

In location 4, members of the diatoms group (*Cymatopleura elliptica, Caloneis amphisbaena, Cymatopleura solea,* and *Craticula cuspidata*), *Closterium lunula* belonging to Conjugatophyceae, and *Cladophora glomerata* belonging to the filamentous green algae group were determined to be the dominant group in both quantity and biovolume.
In location 5, members of the filamentous green algae group (*Cladophora* sp. and *Ulothrix* sp.) were determined to be the dominant group in both quantity and biovolume. Members of diatoms (*Diatoma vulgaris*, *Sellaphora pupula* and *Craticula cuspidata*) were also determined to have high ratios in the stomach of the larvae in location 5 (Figure 5).

According to the Bray-Curtis cluster analysis, similarities were found in very low percentages in the sampling locations for floristic gut contents of *T. punctipennis* larvae. While locations 1 and 2 were determined to be the most similar to each other with 41% similarities, location 5 was determined to be the most different from the others (Figure 6). These low differences may show that there are no special diet preferences of the larvae.

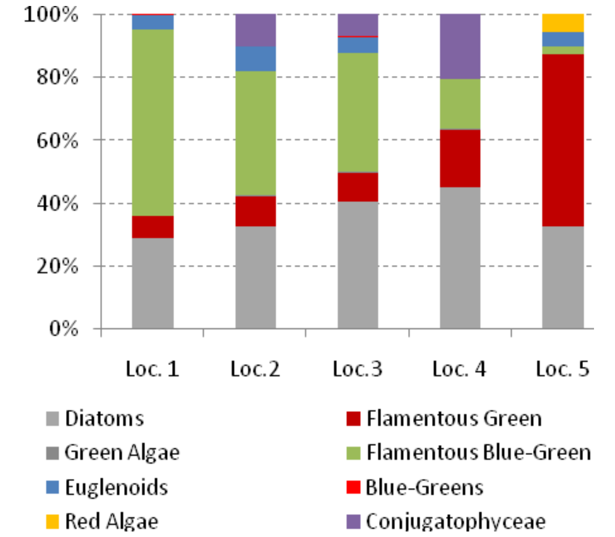

Figure 5. The distribution of stomach contents of T. punctipennis larvae according to sampling locations.

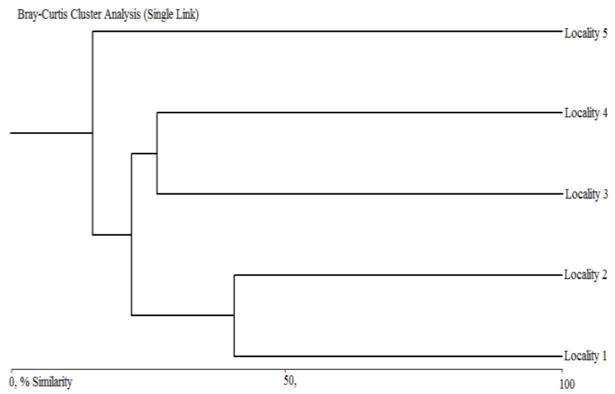

Figure 6. The dendrogram of cluster analyses based on differences in the floristic composition of the gut content of *T. punctipennis* (using the Bray-Curtis similarity).

The algal diversity of gut content fluctuated slightly among the sampling locations. According to analysis using the Shannon index (H′), algal diversity in the stomach of the larvae ranged from 0.3 to 1.0. In general, low values were found during the study period (Figure 7).

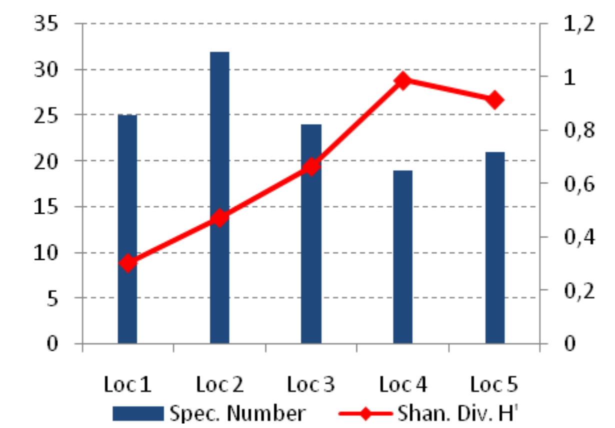

Figure 7. Species diversity of gut contents of *T. punctipennis* larvae (using the Shannon-Wiener diversity index).

According to the species diversity analysis of the contents of larvae stomachs, the algae group was the most dominant group, followed by plant and animal fragments. The individuals of *Oscillatoria* sp., belonging to blue-green algae, and *Caloneis amphisbaena* and *Navicula* sp., belonging to diatoms, were determined to have the highest values for both biovolume and quantity.

Larval chironomids distribute in the aquatic ecosystems randomly [38, 39]. After the first instar stage, the larvae can drift according to water flows and winds [40]. The nutrient contents of an aquatic ecosystem also affect the distribution of species richness and densities of larvae [41, 42].

In hypertrophic conditions, periphytic algae (especially diatoms) are the main food source of the chironomid larvae [42, 43]. In our study, the diatoms belonging to *Caloneis amphisbaena*, *Cymatopleura solea*, *Navicula* sp., and *Encyonema minutum* were found at almost every sampling location. Although diatoms are foods with rich protein, green algae also have rich protein, including essential fat oils that are needed for larvae growth [44, 45, 46]. The role of algae in chironomid diets could be related to the phenology of *T. punctipennis* in the study area.

Pinder (1986), pointed out that the Tanypodinae larvae have quite a diverse diet of animal and plant items [47]. The occurrence of some animal and plant genera in the gut contents of these chironomids is due to its availability in the habitat and can also be related to prey size and the predator's development stage.

In our study, it was determined that the *T. punctipennis* larvae, which are generally known as predators, prefer small detritus particles because the small sizes are able to pass through the larvae mentum. In addition, particles larger than 100 μm are fragmentized by mouth organelles. The size of individual larvae may also affect individual feeding behaviours. The algae which were found in the stomach of the larvae in this study represent the first records of the algal flora in the studied area. We think that further large-scale studies examining the algal flora, plants and trophic level of the region will provide a good data set to contribute to knowledge of the chironomids nutrition of the region as a whole.

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