LIMNOFISH-Journal of Limnology and Freshwater Fisheries Research 7(1): 14-23 (2021)

Journal of Limnology and Freshwater Fisheries Research

http://www.limnofish.org

The Comparative Gut Content Analysis of Some Chironomidae Larvae Living in the Freshwaters at Northern Thrace Region of Turkey

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A B S T R A C T

The roles of larval chironomids in the food chain of both the lotic and the lentic ecosystems are very important. On the one hand, chironomid larvae feeding on algae, diatoms, rotting organic matter, plant, and animal residues also play an important role in these systems as a source of food for other carnivores and omnivorous organisms. In this study, the gut contents of *Cryptochironomus defectus* (Kieffer, 1913), *Cladotanytarsus mancus* (Walker, 1856), *Polypedilum scalaenum* (Schrank, 1803), *Tanypus kraatzi* (Kieffer, 1912) collected from the freshwater ecosystems located in the northern parts of the Thrace region of Turkey were analyzed to compare their feeding habits. As a result of the analysis, it was determined that while plant fragments were dominant in *C. defectus* species in the gut content (44.3%), algae were dominant for *C. mancus* (44.7%), *P. scalaenum* (63.5%), *T. kraatzi* (65%). According to the results of the Shannon-Wiener (H') index species, diversity of the *P. scalaenum* was found to be the highest among the larvae (H'= 1.345). Also, according to the Bray-Curtis similarity index, the most similar types of gut contents were *P. scalaenum* and *C. defectus* (38%). This low rate indicated that the species have different food preferences.

Keywords: Chironomidae, gut content, food chain, Turkish Thrace

A R T I C L E I N F O

RESEARCH ARTICLE

Received : 23.05.2020
Revised : 16.09.2020
Accepted : 08.10.2020
Published : 29.04.2021

DOI:10.17216/LimnoFish.741780

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Türkiye'nin Kuzey Trakya Bölgesi'ndeki Tatlı Sularda Yaşayan Bazı Chironomidae Larvalarının Karşılaştırmalı Mide İçerik Analizi

Öz: Gerek lotik gerekse lentik ekosistemlerin besin zincirinde larval chironomidlerin rolü oldukça büyüktür. Ayrıca, algler, diyatomlar, çürüyen organik madde, bitki ve hayvan kalıntıları ile beslenen chironomid larvaları, bu sistemlerde diğer karnivor ve omnivor organizmalar için bir besin kaynağı olarak da önemli bir rol oynamaktadır. Bu çalışmada, Trakya Bölgesi'nin kuzey bölgelerinde bulunan tatlı su ekosistemlerinden toplanan *Cryptochironomus defectus* (Kieffer, 1913), *Cladotanytarsus mancus* (Walker, 1856), *Polypedilum scalaenum* (Schrank, 1803), *Tanypus kraatzi* (Kieffer, 1912) türlerinin mide içerikleri, bu türlerin beslenme alışkanlıklarını karşılaştırmak için analiz edildi. Analiz sonucunda *C. defectus*'un mide içeriğinde (%44,3) bitki parçalarının baskın olduğu, *C. mancus* (%44,7), *P. scalaenum* (%63,5), *T kraatzi* (%65) için alglerin baskın olduğu belirlendi. Shannon-Wiener (H ') indeksi sonuçlarına göre, *P. scalaenum*'un mide içeriğinin çeşitliliğinin larvalar arasında en yüksek olduğu bulundu (H' = 1,345). Ayrıca, Bray-Curtis benzerlik endeksine göre, mide içeriği en benzer *P. scalaenum* ve *C. defectus* olduğu saptandı (%38). Bu düşük oran, türlerin farklı besin tercihlerine sahip olduğunu gösterdi.

Anahtar kelimeler: Chironomidae, mide içeriği, besin zinciri, Türkiye Trakya'sı

How to Cite
Aydın GB, Öterler B, Çamur Elipek B, Güher H. 2021. The Comparative Gut Content Analysis of Some Chironomidae Larvae Living in the Freshwaters at Northern Thrace Region of Turkey. LimnoFish. 7(1): 14-23. doi: 10.17216/LimnoFish.741780

Introduction

Gut content analysis of chironomid larvae provides significant data on their ecological and biological structure, importance in the food chain, habitat use, and modes of feed (Armitage et al. 1995; Manko 2016). In an aquatic ecosystem, chironomid larvae have an important place since they are the most abundant organisms and have an important role in the food chain by being the food of fish, other aquatic invertebrates and by feeding on algae, detritus, associated microorganisms, fungi, woody debris, macrophytes, other aquatic invertebrates (Armitage et al. 1995; Epler 2001; Butakka et al. 2016). Therefore, it can be said that chironomid larvae are an important connection point of the food web in an aquatic ecosystem by being a bridge between producers and consumers (Silva et al. 2008). The gut content studies of chironomid larvae provide important insights into the modes of feeding and according to based on larval feeding modes chironomids can be grouped into collector-gatherers, collector-filterers, scrapers, shredders, engulfers, and piercers (Armitage et al. 1995). But it is important to know that most chironomid larvae are not limited to a single mode of feeding and it is based on larval size, food size and quality, sediment composition (Armitage et al. 1995; Kornijo´w et al. 2019). The other factor on the chironomids feeding is swallowing food non-selectively and selectively in accordance with food availability, type, and size (Armatige et al. 1995). Studies of chironomid feeding show that the diet of chironomids constitutes of detritus and associated microorganisms, macrophytes, invertebrates, and especially algae (Baker and Mclachlan 1979; Armitage et al. 1995; Sanseverino and Nessimian 2008; Butakka et al. 2016; Öterler et al. 2018; Kornijo´w et al. 2019).

In this study, it was aimed to analyze the gut contents of the four species (*Cryptochironomus defectus, Cladotanytarsus mancus, Polypedilum scalaenum,* and *Tanypus kraatzi*) collected from freshwater resources at the Northern Thrace Region of Turkey and make comparisons about the feeding modes and feeding similarities of the species.

Materials and Methods

Sampling

The Thrace Region is the European Part of Turkey and was surrounded by Bulgaria, Greece, and the Black Sea (Figure 1). The material of the study was collected at ten different freshwater resources located in the northern parts of the Thrace region between June 2012 and August 2012. The locality names, habitat features, and coordinates were given in Table 1. The sediment samples were taken by a hand mud scoop net and the obtained material was washed through mesh nets then preserved in 250 cc plastic bottles containing 70% ethanol. In the laboratory, chironomid larvae were selected from the sediment using a binocular stereomicroscope.

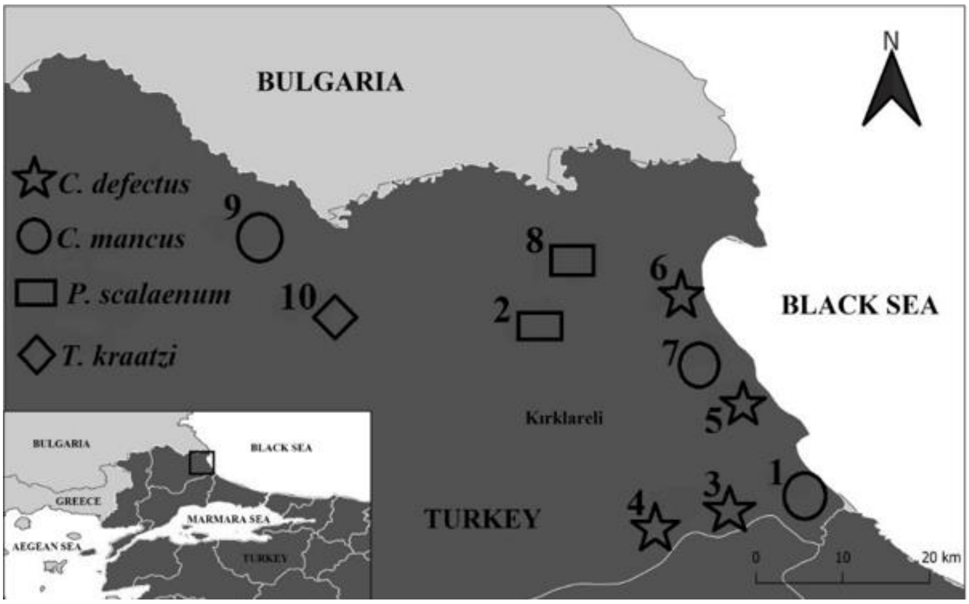

Figure 1. Locations of sampling sites of the chironomid larvae.

Analyses

For the larval identification, Saether (1980), Fittkau and Roback (1983), Pinder and Reiss (1983), Epler (2001), and Vallenduuk and Morozova (2005) were followed. The fourth instar stage of the samples was selected among the material for the gut content analysis. Ten specimens for each species were used to analyze the gut contents. Each chosen specimen was placed into petri dishes containing 1 ml of 70% ethanol and dissected to reveal the gut contents by using a needle under a binocular stereo-microscope. The diffused material in the petri dish was infused into a Sedgewick-Rafter count chamber with a 1 ml volume. All of the organic and inorganic materials in the gut contents were counted. Algal bio-volume was estimated from the abundancedata and measurements of specific cell volumes by approximating geometric shapes of the cells (Hillebrand et al. 1999; Sun and Liu 2003). Identifications were performed at 1000x magnification under immersion oil, and identification of the taxa was based on the literature (Huber-Pestalozzi 1982; John et al. 2002; Krammer and Lange-Bertalot 1986-2004; Round et al. 1990;

Komárek and Anagnostidis 2005; Hindak 2008; Kristiansen and Preisig 2011). All of the determined species were confirmed using AlgaeBase, an electronic database of algae information hosted by the National University of Ireland (Guiry and Guiry 2020).

Table 1. The features of the locations where the larvae were collected.

Locations	Locality Name	Habitat	Coordinates
Loc.1	Kazandere Brook	Stream	41°37′57″N, 28°05′12″E
Loc.2	Kömürköy Brook	Stream	41°40′18″N, 26°33′40″E
Loc.3	Kazandere Dam Lake	Lake	41°37′49″N, 28°05′14″E
Loc.4	Pabuçdere/Kıyıköy	Stream	41°39′55″N, 27°57′30″E
Loc.5	Pabuçdere/Hamidiye	Stream	41°39′55″N, 27°57′30″E
Loc.6	Saka Brook	Stream	41°48′42″N, 27°57′02″E
Loc.7	Saka Lake	Lake	41°48′00″N, 27°59′40″E
Loc.8	Pedina Lake	Lake	41°50′01″N, 27°56′05″E
Loc.9	Dereköy Brook	Stream	41°55′48″N, 27°22′14″E
Loc.10	Armağan Village	Pond	41°52′31″N, 27°25′43″E

Data Analysis

Shannon-Wiener index was used for the evaluation of the species diversity of the larval gut contents. The Bray-Curtis and Correspondence index analyses were used to determine the similarities of the gut contents of the species. Analyses were carried out with the XLSTAT-ADA statistical package program (Addinsoft 2015) and Graphpad PRISM software, trial version (Intuitive Software for Science, San Diego, CA).

Results

Four species were determined in the studied area (*Cryptochironomus defectus*, *Cladotanytarsus mancus*, *Polypedilum scalaenum*, *Tanypus kraatzi)*. While a total of 81 algal species were determined in the gut contents of the larvae, it was observed that the other food fragments belonged to plants, animals, and fungi. The analyses showed that the gut contents of the larvae consisted of algae (52.7%) followed by plant fragments (27.8%), animal fragments (10.2%), and fungi spores (9.3%) (Figure 2). These proportional data belonged to only approximately 50-70% of the alimentary tract of larvae. Because the data in our study included gut content data other than detritus. It is known that detrital components (non-living organic and inorganic matter, microorganisms, bacteria), constitute about 50% of the gut contents and some larvae could complete the larval stage on the detrital components (Rodina 1971).

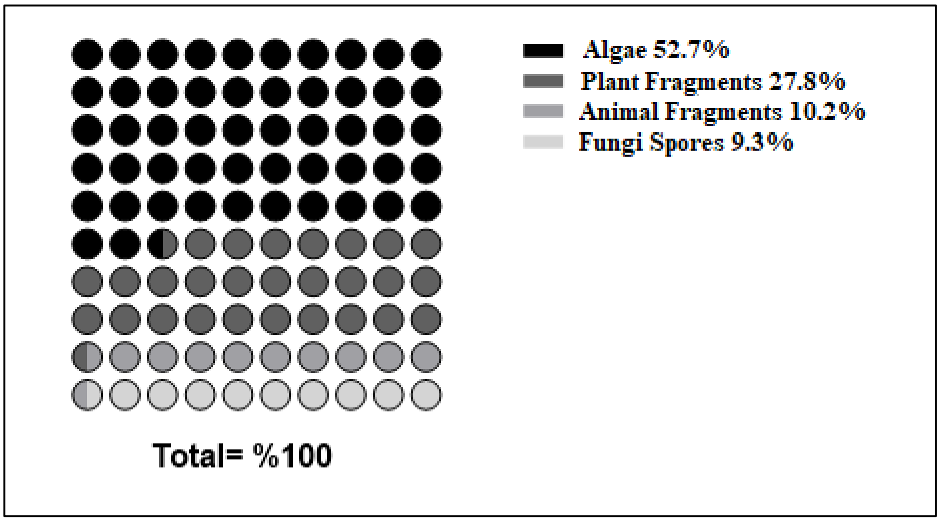

Figure 2. The composition of food items observed in gut contents of the chironomid larvae.

The results of the analyses showed that plant fragments were the dominant diet of the gut content of *C. defectus* (44.3%) followed by algae (37.3%), fungi spores (12.7%), and animal fragments (5.7%). Algae were the dominant diet of the gut content of *P. scalaenum* (63.5%) followed by fungi spores (19%), plant fragments (16.5%), and animal fragments (1%). In the gut content of *C. mancus,* algae were dominant (44.7%) and were followed by plant fragments (32%), animal fragments (18.7%), and fungi spores (4.6%). The gut content of *T. kraatzi* consisted of algae (65%), plant fragments (18.5%), animal fragments (15.5%), and fungi spores (1%) (Figure 3).

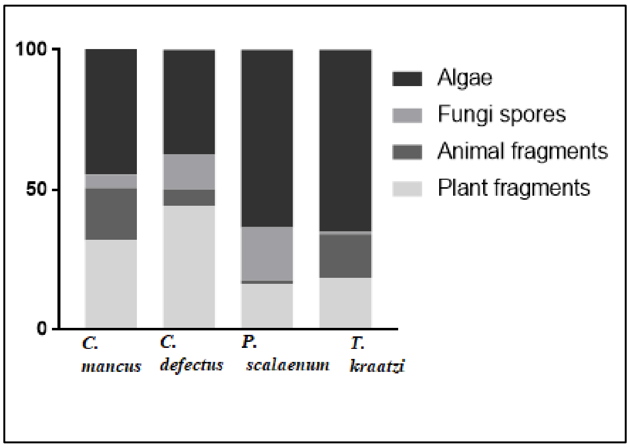

Figure 3. The gut contents of the determined chironomid larvae.

A total of 81 taxa belonging to 5 algal divisions were found in the gut contents of the four chironomid larvae (Table 2). They were Bacillariophyta (with 57 taxa), Euglenophyta (with 1 taxon), Chlorophyta (with 9 taxa), Cyanophyta (with 7 taxa), and Charophyta (with 7 taxa). Based on the data of the algal composition of the larvae, diatoms were dominant for all species (Figure 4). However, euglenoids were found only on the gut content of *T. kraatzi* (Figure 4). Several gut content studies reporting benthic algae as the basic food source for chironomids showed that diatoms especially constitute most of the diet (Tarkowska-Kukuryk 2013; Butakka et al. 2016; Kornijo´w et al. 2019). Members of the subfamily Chironominae, known as non-predatory fed primarily on algae especially diatoms, and in this study the algal diet of *C. defectus*, *C. mancus* and *P. scalaenum* (members of subfamily Chironominae) consisted of mostly diatoms (Figure 4). However, the diatom preference is not restricted to taxa considered to be non-predators. Although subfamily Tanypodinae is known as a predator, as determined in our study members of *Tanypus* genus can preference diatoms as a major food source (Armitage et al. 1995; Galizzi et al. 2012).

Table 2. The composition of gut contents of chironomid larvae.

	C. mancus	*C. defectus*	*P. scalaenum*	*T. kraatzi*
Kingdom: Chromista				
Phylum: Bacillariophyta				
Class: Coscinodiscophyceae				
Melosira varians C.Agardh 1827		+		
Class: Mediophyceae				
Cyclotella meneghiniana Kützing 1844	+	+	+	+
Stephanodiscus hantzschii Grunow 1880			+	
Class: Bacillariophyceae				
Lemnicola exigua Grunow 1880		+		
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot 1999	+		+	
Achnanthes armillaris (O.F.Müller) Guiry 2019			+	
Achnanthidium minutissimum (Kützing) Czarnecki 1994	+		+	+
Achnanthidium sp.		+		
Amphora ovalis (Kützing) Kützing 1844		+		
Caloneis sp.				+
Caloneis amphisbaena (Bory) Cleve 1894				+
Cocconeis sp.				+
Cocconeis placentula Ehrenberg 1838	+	+	+	
Craticula sp.			+	
Craticula cuspidata (Kutzing) D.G.Mann 1990				+
Cymbella sp.			+	
Cymbella affinis Kützing; 1844			+	+
Cymbella cistula (Ehrenberg) O.Kirchner 1878	+			+

Table 2. Continued.

	C. mancus	*C. defectus*	*P. scalaenum*	*T. kraatzi*
Cymbella excisa Kützing 1844	+			
Brebissonia lanceolata (C.Agardh) Mahoney & Reimer, 1986				+
Cymbopleura naviculiformis (Auerswald ex Heiberg) Krammer 2003	+	+		
Denticula sp.	+			
Denticula elegans Kützing 1844		+		
Diatoma vulgaris Bory 1824			+	
Encyonema minutum Cholnoky) D.B.Czarnecki 1994			+	
Encyonema silesiacum (Bleisch) D.G.Mann in Round, R.M.Crawford & D.G.Mann 1990	+			
Epithemia sp.		+		
Epithemia sorex Kützing 1844				+
Epithemia turgida Kützing 1844	+	+		
Epithemia adnata (Kützing) Brébisson 1838	+			
Fragilaria capucina (Kützing) Lange-Bertalot 1980				+
Ulnaria ulna (Nitzsch) Compère; 2001	+	+	+	+
Gomphonema sp.				+
Gomphonema acuminatum Ehrenberg 1832				+
Gomphonema gracile Ehrenberg 1838	+			
Gomphonema parvulum (Kützing) Kützing 1849	+	+	+	+
Gomphonema truncatum Ehrenberg 1832				+
Hannaea sp.		+		
Hannaea arcus (Ehrenberg) R.M.Patrick 1966	+			
Meridion circulare (Greville) C.Agardh 1831		+	+	+
Navicula sp.	+	+	+	+
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski 1996	+	+	+	+
Navicula cryptocephala Kützing 1844			+	+
Navicula lanceolata Ehrenberg 1838			+	
Navicula tripunctata (O.F.Müller) Bory 1822	+			+
Navicula viridula (Kützing) Ehrenberg 1836	+	+		
Neidium affine (Ehrenberg) Pfitzer 1871		+		+
Nitzschia sp.	+	+	+	+
Nitzschia denticula Grunow 1880				+
Nitzschia palea (Kützing) W.Smith 1856	+	+		
Nitzschia recta Hantzsch ex Rabenhorst 1862	+			+
Nitzschia sigmoidea (Nitzsch) W.Smith 1853				+
Pinnularia sp.			+	+
Pinnularia viridis (Nitzsch) Ehrenberg 1843				+
Rhoicosphenia curvata (Kützing) Grunow 1860			+	
Surirella brebissonii Krammer and Lange-Bert. 1987	+			
Tabellaria flocculosa (Roth) Kützing 1844		+		

Table 2. Continued.

	C. mancus	*C. defectus*	*P. scalaenum*	*T. kraatzi*
Kingdom: Plantae				
Phylum: Chlorophyta				
Class: Chlorophyceae				
Tetraëdron minimum (A.Braun) Hansgirg 1889			+	
Desmodesmus opoliensis (P.G.Richter) E.Hegewald 2000			+	
Tetradesmus dimorphus (Turpin) M.J.Wynne 2016		+		
Tetradesmus lagerheimii M.J.Wynne & Guiry 2016	+			
Desmodesmus intermedius (Chodat) E.Hegewald 2000	+			
Scenedesmus quadricauda (Turpin) Brébisson in Brébisson & Godey 1835			+	
Kirchneriella lunaris (Kirchner) Möbius 1894	+			
Chlamydomonas sp.	+			
Class: Trebouxiophyceae				
Oocystis sp.				+
Phylum: Charophyta				
Class: Zygnematophyceae				
Closterium littorale F.Gay 1884				+
Closterium lunula Ehrenberg & Hemprich ex Ralfs 1848				+
Closterium sp.				+
Cosmarium sp.				+
Spirogyra sp.				+
Class: Ulvophyceae				
Cladophora glomerata (Linnaeus) Kützing 1843				+
Cladophora sp.	+		+	+
Kingdom: Protozoa				
Phylum: Euglenozoa				
Class: Euglenophyceae				
Trachelomonas volvocina (Ehrenberg) Ehrenberg 1834				+
Kingdom: Eubacteria				
Phylum: Cyanobacteria				
Class: Cyanophyceae				
Anabaena sp.	+			
Chroococcus sp.		+		
Chroococcus minimus (Keissler) Lemmermann 1904	+			
Kamptonema formosum (Bory de Saint-Vincent ex Gomont, 1892) Strunecky et al., 2014		+		
Leptolyngbya boryana (Gomont) Anagnostidis & Komárek 1988		+		
Lyngbya majuscula Harvey ex Gomont 1892			+	
Oscillatoria princeps Vaucher ex Gomont 1892	+	+	+	
Plant Fragments	+	+	+	+

Table 2. Continued.

	C. mancus	*C. defectus*	*P. scalaenum*	*T. kraatzi*
Animal Fragments	+	+	+	+
Fungi Spores	+	+	+	+
Fungi Hyphal		+	+	
Pollen	+	+	+	+
Protozoan	+			+

According to the Shannon-Wiener index results, *P. scalaenum* has the richest diversity (H'= 1.345) followed by *C. defectus* (H'= 1.303), *C. mancus* (H'= 1.063), and *T. kraatzi* (H'= 0.834) (Figure 5). According to the Bray-Curtis similarity index, while the most similar types of gut contents were *P. scalaenum* and *C. defectus* (38%), *T. kraatzi* had different gut content from the other species (Figure 6). These results were supported by Correspondence analysis (Figure 7).

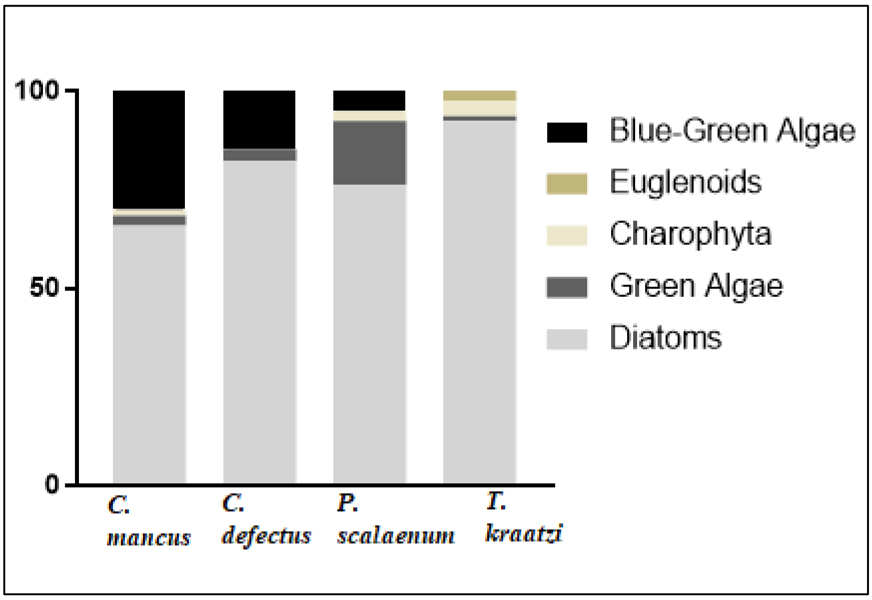

Figure 4. The algal composition of the gut contents of the determined chironomid larvae.

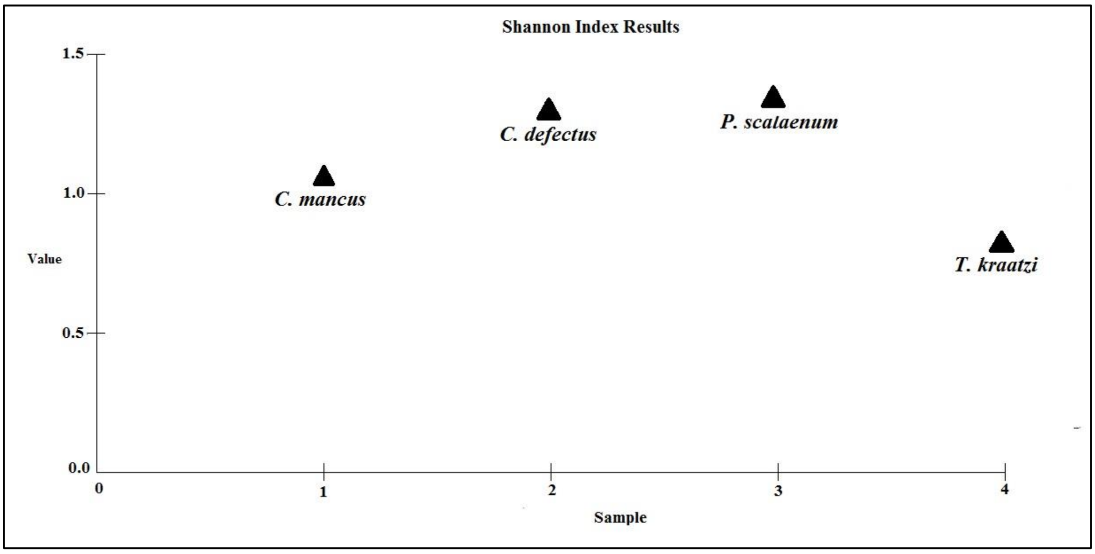

Figure 5. Species diversity (values of the Shannon-Wiener Diversity Index) of gut contents of the chironomid larvae.

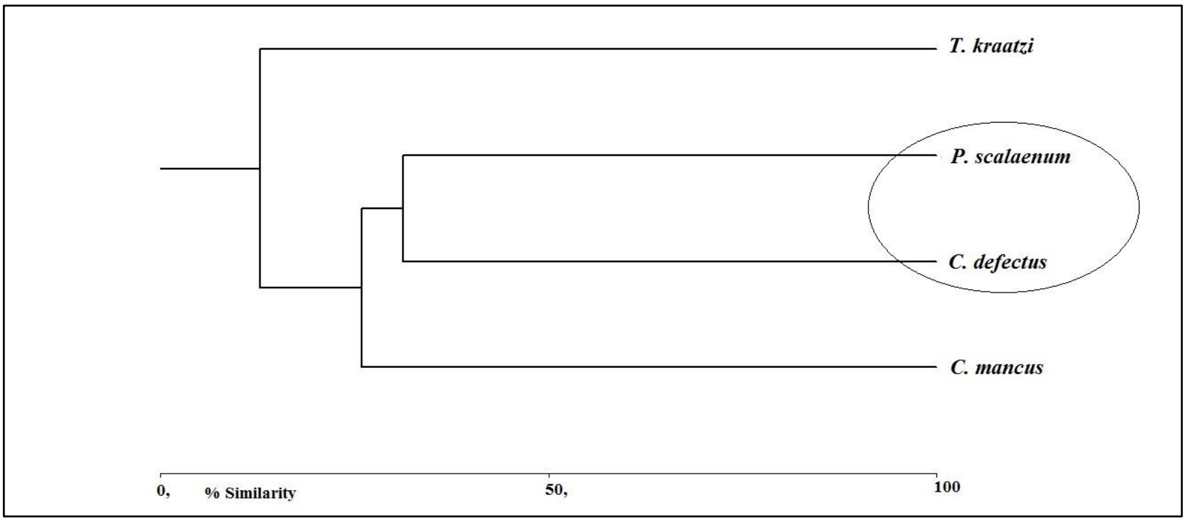

Figure 6. The gut contents similarity (based on the Bray-Curtis Similarity Index) of the chironomid larvae.

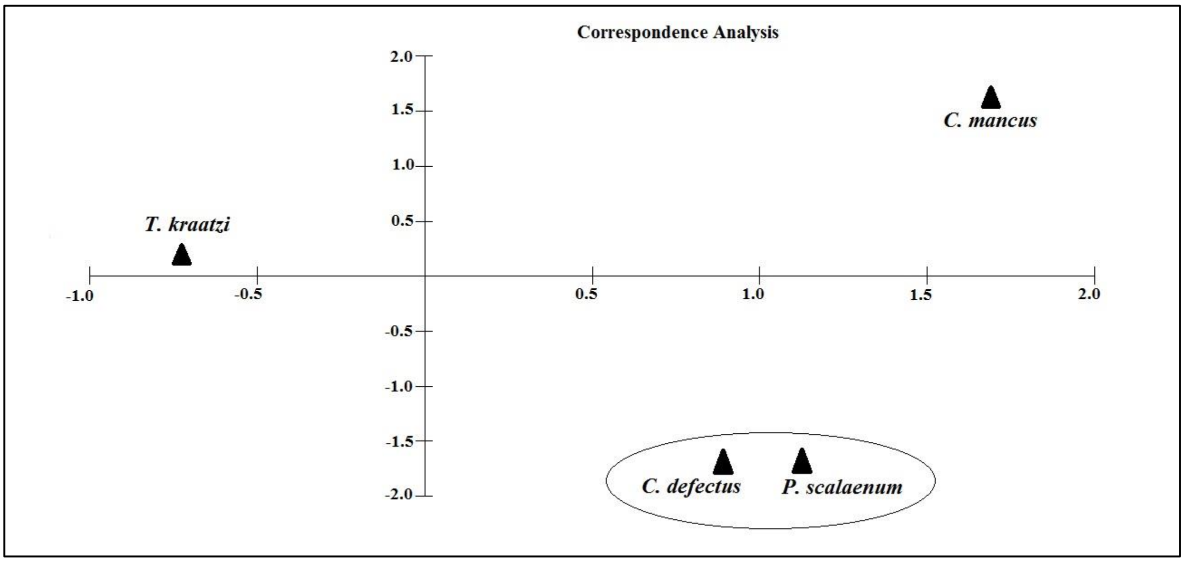

Figure 7. Correspondence analysis of the gut contents of the chironomid larvae.

Discussion

In this study, it was compared to the gut content of *C. defectus*, *C. mancus*, *P. scalaenum*, and *T. kraatzi* collected from the freshwater ecosystems in the Northern Thrace Region of Turkey. The main food component of the alimentary tract of Chironomidae larvae is detritus (Berg 1995; Dukowska et al. 1999; Kornijo´w et al. 2019). But in our study, the data included gut content data other than detritus. As a result of our gut content study, Algae was the dominant component as in other studies (Tarkowska-Kukuryk 2013; Butakka et al. 2016; Kornijo´w et al. 2019). According to Kornijo´w et al. (2019) food of animal origin is a less involved component than plant fragment as in our study. According to the algal composition of the gut contents of the determined chironomid species, diatoms were dominant as in the many other studies (Cattaneo 1983; Tokeshi 1986; Tarkowska-Kukuryk 2013). Although *Tanypus* genus is known as a predator, members of *Tanypus* genus can preference diatoms as a major food source (Armitage et al. 1995; Galizzi et al. 2012) and is probably the result of larval size, food size, and quality, sediment composition (Armitage et al. 1995). Also, diatoms are rich in protein and essential fatty acids and this situation makes them preferred and high-quality food for growing larvae (Tarkowska-Kukuryk 2013).

According to the Bray-Curtis similarity index results, the most similar types of gut contents were *P. scalaenum* and *C. defectus*. It could say that *T. kraatzi* had different gut content among the other three species. The members of the subfamily Tanypodinae (including *T. kraatzi*) generally feed on other organisms as predators and so the results made thought us that the food spectrum of *C. defectus*, *C. mancus*, and *P. scalaenum* (subfamily Chironominae) was broader than *T. kraatzi*.

Gut content studies result in attention to the importance of chironomid larvae and their energy flow role in aquatic ecosystems. Gut content studies provide important insights into the trophic level and food sources of aquatic ecosystems. It is known that many chironomids are grazer on epiphytes and macrophytes and because of the high abundance of chironomids the plant food sources of an aquatic ecosystem could reduce. Thanks to the gut content studies it will be possible to know where it must suppress chironomid populations. We think that the studies examining the gut content of chironomid larvae could provide a good data set to speculate on the structure of an aquatic ecosystem.

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