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INVESTIGATION OF THE DIET OF *PALINGENIA LONGICAUDA* (OLIVIER, 1791) LARVAE BASED ON DIATOMS

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TISZAVIRÁG [*PALINGENIA LONGICAUDA* (OLIVIER, 1791)] TÁPLÁLKOZÁSÁNAK VIZSGÁLATA KOVAALGÁK SEGÍTSÉGÉVEL

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ABSTRACT: Diatom assemblages derived from gut content of *Palingenia longicauda* larvae, phytobenthos of the clay surface around the burrows of larvae and seston of surface water samples were compared to ascertain the utility of diatom assemblages in order to functional feeding group classification. Furthermore we aimed to unravel whether the larvae of *Palingenia longicauda* belong to active filter-feeder or detritus feeder. According to our results, diatom valves derived from the gut contents of the mayfly larvae remained well preserved and could be used to identify whether valves belong to planktonic or benthic diatom group. Our results based on comparing the species composition of the samples demonstrated that the gut content revealed higher similarity with the seston than with the phytobenthos according to the diatom assemblages. The proportion of the planktonic and benthic diatoms of the gut content was also similar to the seston samples rather than the phytobenthos. These results suggested that *Palingenia longicauda* larvae are mainly belonged to the active filterer Functional Feeding Group.

Key words: *Palingenia longicauda*, Functional Feeding Groups, Diatoms

KIVONAT: Kovaalga együttes vizsgálatokat végeztünk tiszavirág [*Palingenia longicauda* (Olivier, 1791)] lárva béltartalomból, illetve a tiszavirág járatok körüli élőbevonatból, valamint felszíni vízmintából, a tiszavirág táplálkozástípusának vizsgálata céljából. Elsődleges célunk volt, hogy kiderítsük, a lárvák béltartalmában található kovaalga vázak határozhatóak maradnak-e az elfogyasztás és az emésztés után, illetve alkalmasak-e ilyen célú vizsgálat elvégzésére. További célunk volt kideríteni, hogy a tiszavirág lárvák táplálkozása során az aktív szűrő, vagy a detrituszfogyasztó táplálkozástípus a meghatározó. Eredményeink alapján megállapítható, hogy a béltartalomból kinyert kovaalga vázak jól megőrződtek, azok jól határozhatók maradtak. A három, különböző eredetű minta kovaalga együttesének összehasonlítása során megállapíthattuk, hogy a béltartalom kovaalga együttese mind fajkészlet alapján, mind pedig a planktonikus, valamint a bentikus életmódú kovaalgák aránya alapján a szeszton kovaalga együttesével mutat nagyobb hasonlóságot. Ezen eredmények alapján arra következtethetünk, hogy a vizsgált tiszavirág lárvák táplálkozásában az aktív szűrő táplálkozás típus a meghatározó.

Kulcsszavak: Tiszavirág, *Palingenia longicauda*, funkcionális táplálkozási csoportok, kovaalgák

Introduction

Aquatic insects can be classified in Functional Feeding Groups by characteristics of feeding behaviour (CUMMINS 1995; OFENBÖCK et al. 2004). According to the source and the consumption of the food, larvae of the *Palingenia longicauda* (Olivier 1791), as all the other burrowing mayflies of Central Europe, is classified in the active filterer and the detritus feeder functional feeding groups (BAUERNFEIND and HUMPESCH 2001; MOOG 2002). In pursuance of the filter-feeder theory, the larvae generate water current in their U-shaped burrows with the synchronised movement of their gills, and filter the seston particles from the water (LANDOLT et al 1997; WALLACE and MERRIT 1980). The second theory suggests that the larvae consume the deposited organic material from the clay surface, where they live on. Previous studies mentioned the larvae of *Palingenia longicauda* as mud-eaters (SWAMMERDAM 1681; UNGER 1929; SCHOENEMUND 1929). The functional morphology of the ultrastructure of the mouths parts supported the deposit feeder theory (STRENGER 1970, 1979; RUSSEV 1987; ELPERS 1997). However, recent studies regarded the *Palingenia longicauda* larvae as active filter-feeders (HAYBACH 2007; LANDOLT et al. 1995). According to our observations, the larvae of *Palingenia longicauda* can survive longer period in aquarium without circulation, or lighting, which presumably exclude the high rate of seston in laboratory conditions (not planned experiment, not showed data).

Former studies showed that the gut content of larvae contains diatoms beside filamentous algae and particulated organic material, respectively (GRAYL and WARD 1979; LANDOLT et al. 1995). Diatoms can be consumed by both feeding mechanisms, since there are planktonic taxa (mainly ex-Centrales species) and benthic taxa (regularly ex-Pennales species). The origin of the food can be concluded from the silica valvae remaining in identifiable state after the process of feeding and digestion in the gut content.

First of all, the main aims of our study were to find out, whether the valvae of diatoms were identifiable in the larval gut content or not and to identify the feeding type of the *Palingenia longicauda* larvae (active filterer/detritus feeder) based on the diatom assemblage of the gut content (planktonic/benthic ratio; composition).

Thus, the composition of diatom assemblages derived from (i) larval gut contents, (ii) from the seston, and from (iii) the sediment surface were identified and compared.

Materials and methods

Samples were collected from the river Tisza, near the village Cigánd in 2013. (WGS 84 coordinate: 21,92397; 48,24897). Second years old (with 2,1-3 cm body length) *Palingenia longicauda* larvae were collected from burrows. Benthic diatom samples were collected from the clay surface (from two places, close to the burrows), and one surface water sample was also taken. The collected larvae were preserved in 70% ethanol. The gut content was dissected under binocular microscope. The rest of samples were fixed in Lugol's solution on the field, and were kept at 4ºC in dark.

After collection and washing of planktonic (25 ml) and benthic samples (5 ml), diatom valves were processed by the hot hydrogen-peroxide method. Naphrax synthetic resin was used for embedding (MSZ EN 13946:2003). Leica DMRB research microscope and 1000-1600-fold magnification was used for identification of diatom taxa. At least 400 valves were counted (MSZ EN 14407:2004), for diatom identification KRAMMER and LANGE-BERTALOT (1997a, 1997b), KRAMMER and LANGE-BERTALOT (2004a, 2004b), and POTAPOVA and HAMILTON (2007) were used.

The ratio of the planktonic and benthic taxa, and the species compositions of the different assemblages were compared by UPGMA – presence-absence data with Jaccard dissimilarity index and the abundances with Bray-Curtis index. Abundance data were log-transformed where necessary.

Results

In the different samples total of 91 diatom taxa were identified (52 taxa from the phytobenthos, 42 taxa from the seston, and 72 taxa from the gut contents respectively). Among the identified 91 diatom taxa, 81 taxa were determined as benthic form, while the remained 10 taxa were classified as planktonic diatoms (Table 1). The valvae of the diatoms were easily identifiable after the preparation, even in case of gut contents.

The abundances show that the ratio of planktonic diatoms were 36+/-4,5% (mean+/-SE) in the gut contents, 33,6% in the seston sample, and 8+/-0,5% (mean+/-SE) in phytobenthos samples, respectively (Fig. 1). Due to the low number of samples, the statistical comparison was not possible.

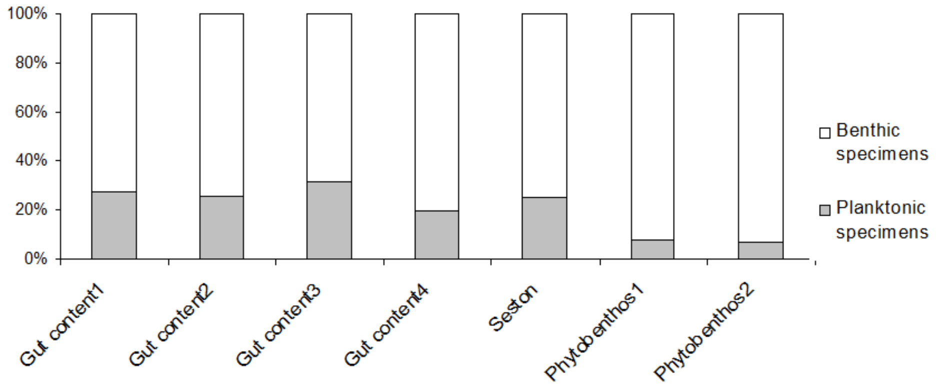

Figure 1. The ratio of the total number of benthic and planktonic diatoms in the different samples

Comparing the diatom assemblages, the highest similarity was calculated between the seston and the gut contents by using the cluster analysis. The calculation of phytobenthos gives totally different result comparing to the others (Fig. 2-3).

Comparing the different samples according to the planktonic (Fig. 4-5) and the benthic (Fig. 6-7) diatom taxa only, the highest similarity was calculated between the seston and the gut contents. The benthic groups separated from other groups by the same condition.

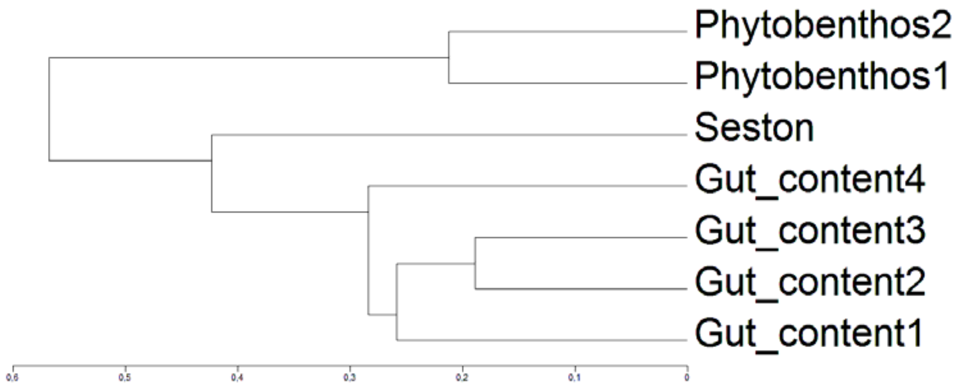

Figure 2. Comparison of the whole diatom assemblages from the different samples according to the species composition (Bray-Curtis dissimilarity, UPGMA)

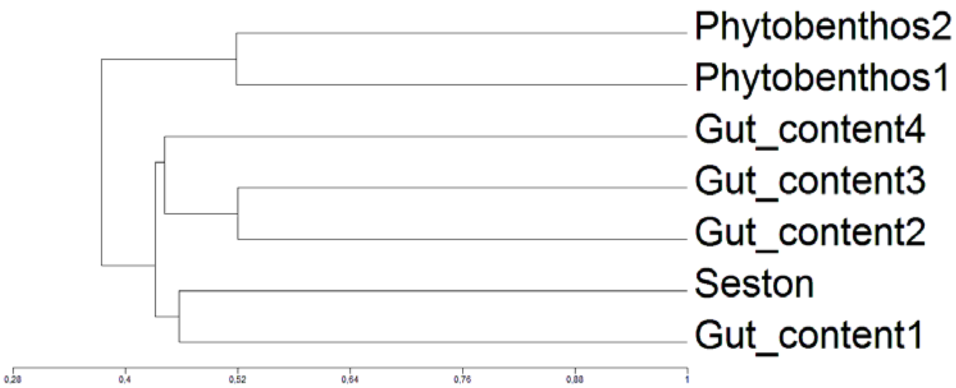

Figure 3. Dissimilarity of the whole diatom assemblages from the different samples according to the abundances (log-transformed data; Jaccard dissimilarity; UPGMA)

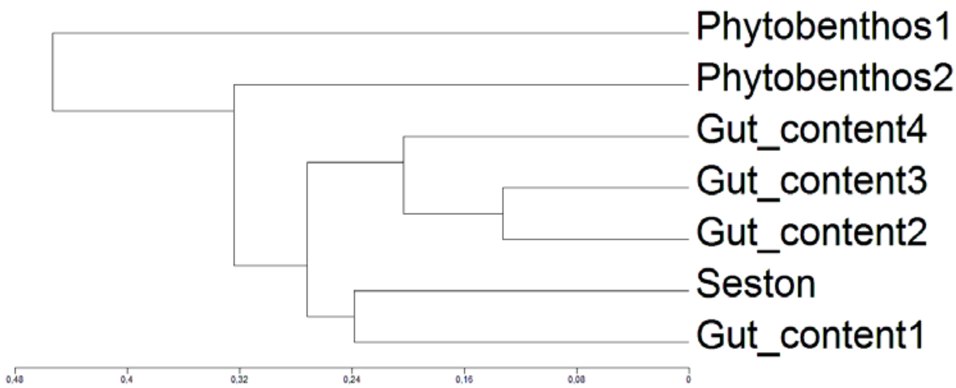

Figure 4. Comparison of the planktonic diatom assemblages from the different samples according to the species composition (Bray-Curtis dissimilarity, UPGMA)

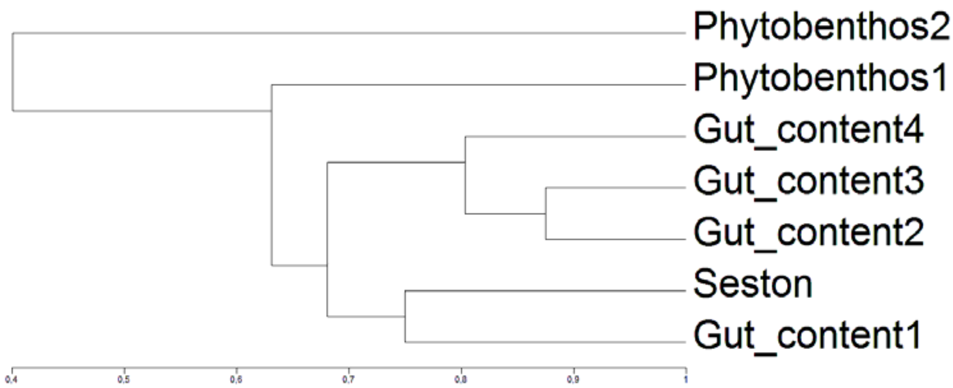

Figure 5. Dissimilarity of the planktonic diatom assemblages from the different samples according to the abundances (lg-transformed data; Jaccard dissimilarity; UPGMA)

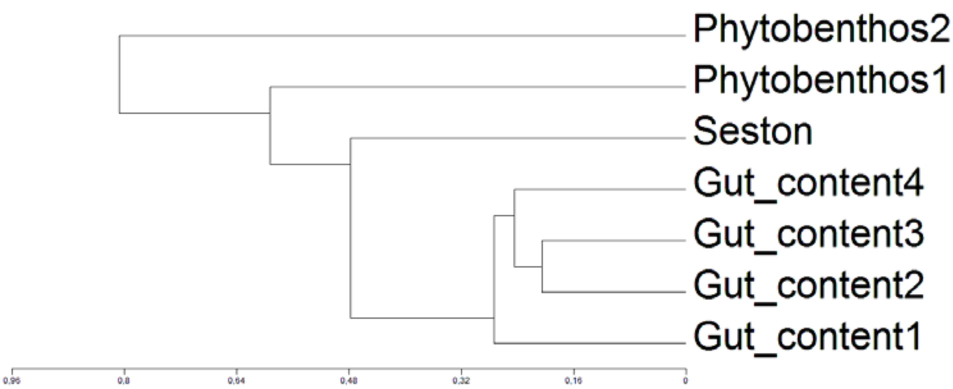

Figure 6. Comparison of the benthic diatom assemblages from the different samples according to the species composition (Bray-Curtis dissimilarity, UPGMA)

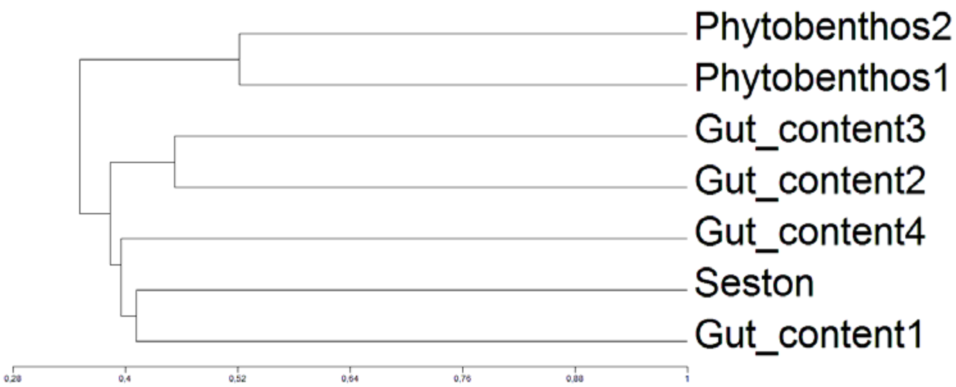

Figure 7. Dissimilarity of the benthic diatom assemblages from the different samples according to the abundances (log-transformed data; Jaccard dissimilarity; UPGMA)

Conclusions

This was the first time comparing similarities and differences in diatom assemblages derived from mayfly gut content, between phytobenthos and seston of surface water samples. The main aim of this study was to ascertain (i) the utility of diatom assemblages in order to functional feeding group classification and (ii) to unravel whether the larvae of *Palingenia longicauda* which functional feeding group belong to.

The high number of intact diatom valves and the high number of the identified diatom taxa (72) from the gut contents of the mayfly larvae suggested that the siliceous cell walls are able to maintain their integrity over the process of consumption and digestion enough to decide whether valva belong to planktonic or benthic diatom group. There was significant overlap in the taxa composition of the samples with different origin, and there were also no striking distinction according to the different forms (planktonic or benthic) in case of the seston or phytobenthos samples.

Comparing the different diatom samples, the results suggested, that the feeding-type of the 2-3-year old larvae could be regarded much more as active filterer than detritus feeder. Namely the diatom assemblages of gut content show higher similarity with diatom assemblage of seston than with phytobenthos.

Nevertheless, the active filter-feeding mechanism based on the premise that the larvae live in the burrows. According to the fact that the younger larvae live in the interstitial, the probability of this feeding mechanism seems to be low. Further studies are required to ascertain, (i) whether the larvae change the feeding type, furthermore in view of the experiences with the *Palingenia longicauda* larvae were kept in aquarium (ii) how obligate is the active filter-feeding mechanism by the older larvae of *Palingenia longicauda*.

Table 1. The list of diatom taxa identified from the different samples

Taxa name	**Benthic/ planktonic**	**Gut content**	**Seston**	**Benthos**
Achnanthidium minutissimum (Kutz.) Czarnecki	Benthic	●	●	●
Amphora libyca Ehr.	Benthic	●		
Amphora montana Krasske	Benthic	●	●	
Amphora ovalis (Kützing) Kützing	Benthic	●		
Amphora pediculus (Kützing) Grunow	Benthic	●		●
Caloneis bacillum (Grunow) Cleve	Benthic	●	●	●
Caloneis silicula (Ehr.)Cleve	Benthic	●		
Cocconeis pediculus Ehrenberg	Benthic	●	●	
Cocconeis placentula Ehrenberg var. *placentula*	Benthic	●		
Cocconeis placentula Ehrenberg var. *lineata* (Ehr.) Van Heurck	Benthic	●		
Craticula buderi (Hustedt) Lange-Bertalot	Benthic	●		
Cymatopleura solea (Brebisson) W.Smith var. *solea*	Benthic	●		●
Cymbella affinis Kützing var.affinis	Benthic	●		
Diatoma ehrenbergii Kützing	Benthic		●	
Diatoma moniliformis Kützing	Benthic	●	●	●
Diatoma vulgaris Bory 1824	Benthic	●	●	●
Encyonema caespitosum Kützing	Benthic	●		
Encyonema silesiacum (Bleisch in Rabh.) D.G. Mann	Benthic	●	●	●
Encyonopsis microcephala (Grunow) Krammer	Benthic	●		●
Eolimna minima (Grunow) Lange-Bertalot	Benthic			●
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	Benthic	●		
Fallacia subhamulata (Grunow in V. Heurck) D.G. Mann	Benthic	●		
Fragilaria capucina Desm. var. *capucina* morphotyp 1 Van de Vijver & al.	Benthic	●	●	●
Fragilaria capucina Desmazieres var. *gracilis* (Oestrup) Hustedt	Benthic	●	●	●
Fragilaria pulchella (Ralfs ex Kutz.) Lange-Bertalot (Ctenophora)	Benthic	●		
Fragilaria sp.	Benthic	●		
Fragilaria ulna (Nitzsch.)Lange-Bertalot var.*acus* (Kutz.)Lange-Bertalot	Benthic	●	●	●
Frustulia vulgaris (Thwaites) De Toni	Benthic			●
Gomphonema angustum Agardh	Benthic	●		
Gomphonema clavatum Ehr.	Benthic	●	●	
Gomphonema grovei M.Schmidt	Benthic	●		●
Gomphonema olivaceum (Hornemann) Brebisson var. *olivaceum*	Benthic	●	●	●
Gomphonema parvulum (Kutzing) Kutzing var. *parvulum* f. *parvulum*	Benthic	●	●	
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	Benthic	●		●
Gomphonema sp.	Benthic	●	●	
Gomphonema tergestinum Fricke	Benthic	●	●	●
Hannaea arcus (Ehr.)Patrick	Benthic		●	
Melosira varians Agardh	Benthic	●		●
Navicula capitatoradiata Germain	Benthic	●	●	●
Navicula cincta (Ehr.) Ralfs in Pritchard	Benthic	●		●

Table 1. (continued)

Taxa name	Benthic/ planktonic	Gut content	Seston	Benthos
Navicula cryptocephala Kutzing	Benthic	●		●
Navicula cryptotenella Lange-Bertalot	Benthic	●		●
Navicula erifuga Lange-Bertalot	Benthic			●
Navicula germainii Wallace	Benthic	●		●
Navicula gregaria Donkin	Benthic	●		●
Navicula lanceolata (Agardh) Ehrenberg	Benthic	●		
Navicula radiosa Kützing	Benthic		●	
Navicula recens (Lange-Bertalot) Lange-Bertalot	Benthic	●	●	●
Navicula schroeteri Meister var. *schroeteri*	Benthic			●
Navicula sp.	Benthic	●		
Navicula submuralis Hustedt	Benthic	●		
Navicula trivialis Lange-Bertalot var. *trivialis*	Benthic		●	
Navicula veneta Kützing	Benthic	●	●	●
Navicula viridula (Kütz.) Ehr. var. *rostellata* (Kütz.) Cleve	Benthic		●	●
Navicula viridula (Kützing) Ehrenberg	Benthic	●	●	
Nitzschia acicularis (Kützing) W.M.Smith	Benthic	●		
Nitzschia capitellata Hustedt in A.Schmidt & al.	Benthic			●
Nitzschia clausii Hantzsch	Benthic			●
Nitzschia dissipata (Kützing) Grunow var. *dissipata*	Benthic	●	●	●
Nitzschia filiformis (W.M.Smith) Van Heurck var. *filiformis*	Benthic		●	●
Nitzschia fonticola Grunow in Cleve et Moller	Benthic	●	●	●
Nitzschia frustulum (Kützing) Grunow var. *frustulum*	Benthic			●
Nitzschia heufleriana Grunow	Benthic			●
Nitzschia inconspicua Grunow	Benthic	●		●
Nitzschia intermedia Hantzsch ex Cleve & Grunow	Benthic		●	
Nitzschia liebetruthii Rabenhorst var. *liebetruthii*	Benthic	●		●
Nitzschia palea (Kützing) W.Smith	Benthic	●	●	●
Nitzschia perminuta (Grunow) M.Peragallo	Benthic	●	●	●
Nitzschia recta Hantzsch in Rabenhorst	Benthic			●
Nitzschia sinuata (Thwaites) Grunow var. *tabellaria* Grunow	Benthic			●
Nitzschia vermicularis (Kützing) Hantzsch	Benthic	●		●
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	Benthic	●	●	
Reimeria sinuata (Gregory) Kociolek & Stoermer	Benthic	●	●	●
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	Benthic	●		
Sellaphora bacillum (Ehrenberg) D.G.Mann	Benthic	●		
Sellaphora pupula (Kützing) Mereschkowksy	Benthic	●		
Staurosirella pinnata (Ehr.) Williams & Round	Benthic	●		
Surirella angusta Kützing	Benthic			●
Surirella brebissonii Krammer & Lange-Bertalot var. *brebissonii*	Benthic	●	●	●
Tryblionella apiculata Gregory	Benthic	●	●	

Table 1. (continued)

Taxa name	Benthic/ planktonic	Gut content	Seston	Benthos
Ulnaria ulna (Nitzsch.) Compere	Benthic	●	●	
Aulacoseira granulata (Ehr.) Simonsen	Planktonic	●	●	●
Cyclostephanos dubius (Fricke) Round	Planktonic	●		●
Cyclotella atomus Hustedt	Planktonic	●	●	●
Cyclotella meneghiniana Kützing	Planktonic	●	●	●
Cyclotella ocellata Pantocsek	Planktonic	●	●	●
Cyclotella sp.	Planktonic	●	●	
Discostella pseudostelligera (Hustedt) Houk et Klee	Planktonic	●	●	●
Fragilaria crotonensis Kitton	Planktonic	●		
Stephanodiscus sp.	Planktonic	●		●
Thalassiosira weissflogii (Grunow) Fryxell & Hasle	Planktonic		●	●

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