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Interactions between free-living nematodes and benthic diatoms: insights from the Gulf of Trieste (northern Adriatic Sea)

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Abstract

From July 2010 to July 2012, free-living nematodes were investigated in terms of abundance, genera and trophic composition at the long term St. C1 (depth 18 m), located in the Gulf of Trieste (northern Adriatic Sea). The integration of these results with environmental (e.g. sediment grain-size, Biopolymeric C and Chl *a*) and biological variables (benthic diatom biomass and composition) collected synoptically with nematodes, allowed the clarification of the linkage between these organisms and another ecosystem component, i.e. benthic diatoms. The observed peaks of nematode abundance in summer samplings were attributable almost exclusively to piercing nematodes that feed on microalgae (Chromadoridae and, among them, *Ptycholaimellus)* and were concomitant with the highest numbers of benthic diatoms. DISTLM outputs further corroborated this interaction by indicating Chl *a*, i.e. a proxy of benthic diatoms, as the only environmental variable that significantly shaped nematode assemblage over the 2-year period. This linkage was not explained only quantitatively (i.e. more diatoms supported more nematodes) but also qualitatively. During winter, in fact, the presence of heavily silicified diatoms co-occurred with nematode minima and the lowest percentage of piercing organisms, suggesting an overall minor ability of the assemblage in using this feeding strategy. In a benthic ecosystem-functioning point of view, the observed seasonal pattern of variation in both nematodes and benthic diatoms suggests that most of the energy flowing to nematodes during the summer derives directly from benthic diatom primary production while in the winter this linkage is less important.

Keywords: Free-living nematodes; benthic diatoms; Chromadoridae; *Ptycholaimellus*; benthic ecosystem functioning; northern Adriatic Sea.

Introduction

In the northern Adriatic Sea, studies on free-living nematodes, the dominant members of meiofauna (i.e. benthic animals with a body size between 30 μm and 1000 μm), are sporadic. Travizi & Vidaković (1997) and Travizi (2010) provided checklists of nematodes inhabiting the offshore sediments especially along the Croatian side of this sub-basin, while in an *in situ* experiment, Taheri *et al.* (2015) investigated the effects of short- and long-term induced anoxia on these organisms in the shallow sediments along the Slovenian coastline. Focusing on the Italian side, nematodes were investigated only once at four shallow sites (1-4 m depth) located within the Marine Protected Area (MPA) of Miramare (Sandulli *et al.,* 2010), that is also the only Specially Protected Area of Mediterranean Importance (SPAMI) in the whole northern Adriatic Sea.

At the outer border of this MPA, there is a strategic long-term station - i.e. St. C1 - where, to the best of our knowledge, free-living nematodes have never been studied. The importance of St. C1 derives from the fact that it is located in the Gulf of Trieste, the northernmost area of the whole Mediterranean Sea. This sampling site is characterized, therefore, by the main oceanographic and environmental peculiarities of the Gulf, a shallow semi-enclosed basin with a wind-driven circulation and a stratification of the water column during summer (Querin *et al.,* 2007 and references therein). Included in the Italian network of Long Term Ecological Research (LTER-Italy) in 2006, St. C1 has been regularly sampled in order to create time series of oceanographic and ecological data that are essential for analyzing the natural variability of the ecosystem and for detecting any long-term deviation linked to anthropogenic and global changes (Giani *et al.,* 2012). Dating back to the ‘70s, the earliest investigations included physical, chemical and biological parameters of the water column, while sampling started to be conducted

on a regular monthly basis in 1986 (Cabrini *et al.,* 1992). Afterwards, in 2002, the investigation was extended to the sediments. More precisely, in the period from 2002 to 2005, studies were mainly focused on microbial autotrophs (i.e. benthic diatoms) in terms of diversity (Cibic *et al.,* 2007a; Cibic *et al.,* 2012) and functioning (Cibic *et al.,* 2008), while other benthic assemblages have attracted less attention (macrozoobenthos, Nasi *et al.,* 2017) or have been ignored (meiofauna).

The study of the benthic domain at St. C1 was implemented in July 2010, extending the investigation to meiofauna and microbial processes, as primary production, secondary production and microbial degradative activities, in order to assess the benthic ecosystem functioning. Focusing on the period 2010-2012, Franzo *et al.,* (2016) reported that at St. C1 the benthic ecosystem tended to shift between two states, a 'source system' in summer and a 'detritus sink system' in winter, and that benthic diatoms were the main element responsible for this switch. In a 'source system' state, their proliferation produces pulsed inputs of fresh organic matter that stimulates microbial heterotrophic activities and enhances the efficiency of the system in conveying re-worked C to higher trophic levels as meiofauna. In winter, the lower availability of fresh organic matter from diatoms entails a minor prokaryotic C reworking and an overall major confinement of C within the benthic microbial loop (Franzo *et al.,* 2016).

Although meiofauna were thus investigated from 2010 to 2012, the level of determination (abundance and composition of main groups) did not allow a full understanding of how this assemblage interacts with the other components of the ecosystem, as microbial autotrophs and prokaryotes. A more comprehensive understanding of the ecosystem functioning could have been achieved by implementing the information gained by Franzo *et al.,* (2016) with the study of free-living nematodes. These organisms, being typically the dominant and the most diverse group of meiofauna (e.g. Balsamo *et al.,* 2010; Appeltans *et al.,* 2012), fulfill a large portion of the ecological role of this community. Meiofauna, and specifically nematodes, act as vertical conveyors within sediments and between sediments and the overlying water by bioturbating sediments and generating bioconstructions (e.g. burrows and mucous spots) (Nehring *et al.,* 1990). These activities influence the structure and the main processes in the sedimentary environment such as the permeability/stability of the sediments, nutrient cycling, biogeochemical fluxes and chemical gradients (Schratzberger & Ingels, 2017 and references therein). Since this community is both a consumer of a range of carbon sources and a food source for secondary consumers at the same time, it occupies a unique position in the benthic food web with repercussions on the ecosystem scale (Schratzberger & Ingels, 2017).

Focusing on their feeding ecology, nematodes consume a variety of food sources, including detritus, bacteria, diatoms and other microalgae, ciliates and other meiofauna (by predation and scavenging). The importance of investigating the trophic relations between these food sources and nematodes encouraged the development of classifications based on the morphology of buccal cavities (Wieser, 1953; Jensen, 1987; Moens & Vinx 1997; Moens *et al.,* 1999) and dedicated indices such as the Index of Trophic Diversity (ITD; Heip *et al.* 1985).

In the present study, the first thorough description of free-living nematodes inhabiting the long-term St. C1 is provided, also in view of producing a time-series of this kind of data at this strategic LTER site in the near future. From July 2010 to July 2012 nematode abundance, genera and trophic composition were studied and interpreted in the light of the benthic ecosystem functioning assessment (Franzo *et al.,* 2016) carried out synoptically with these organisms. We addressed, therefore, the following questions: 1. Does the nematode assemblage change over a time lapse of two years at St. C1? 2. In a benthic ecosystem functioning context, how do nematodes interact with the other ecosystem components and in particular with benthic diatoms?

Materials and Methods

Study site

The Gulf of Trieste, located in the north-western end of the Adriatic Sea, is a shallow basin with an average depth of 17 m and a maximum depth of 25 m (Celio *et al.,* 2002). Almost completely surrounded by land, the basin is isolated from the rest of the Adriatic by a sill (~22 m depth) between Grado and the Salvore peninsula (Ogorelec *et al.*, 1991). Tidal amplitude is about 1.5 m, which is the highest in the Mediterranean Sea (Cardin & Celio, 1997). The Gulf experiences annual fluctuations of temperature (from 5°C to ≥ 24°C at the surface and from 6°C to ≥ 20°C at the bottom) and the water column is usually stratified during summer. Although the water enters the basin from the southeast and surface circulation is predominantly from southeast to northwest, this general pattern may be rapidly modified in response to intense winds and river plumes (Querin *et al.,* 2007; Malačič & Petelin, 2009). The region is, in fact, influenced by Bora, a north-easterly wind characterised by strong intensity, which can mix the entire water column also due to the shallow depth of the basin (Querin *et al.,* 2007). The sediments are mainly sandy mud although soft bottoms can vary from sands with patches of rocks to detrital mud (Brambati & Catani, 1988). Sedimentation is mainly controlled by riverine inputs rather than by marine currents (Brambati & Catani, 1988) and the main terrigenous supply comes from the Isonzo River (Covelli & Fontolan, 1997). The annual average sedimentation rate is about 1 mm yr^{-1} in the middle of the Gulf and increases to 2.5 mm yr^{-1} in front of the Isonzo mouth (Covelli *et al.,* 1999 and references therein).

The study was carried out at the long-term St. C1 (Gulf of Trieste), located ca. 200 m offshore (45°42.05' N, 13°42.60' E) at a depth of around 18 m, near the outer

border of the MPA of Miramare (Fig. 1). This small MPA is divided into two distinct zones: the inner part (30 ha), subjected to a regime of integral protection (i.e. all human activities are banned with the exception of a little corridor for diving) and a surrounding buffer zone (90 ha), for boats and professional fishing.

Sampling

Eight sampling campaigns were carried out from July 2010 to July 2012. During each sampling, bottom water temperature was measured using a Seabird 19 PlusSeacat probe while five virtually undisturbed sediment cores were taken by a KC Haps bottom corer (KC-Denmark, Silkeborg, Denmark) using polycarbonate sample tubes (13.3 cm I.D. resulting in a sampling surface of 127 cm^2). Three pseudo-replicates of meiofauna were subsampled from one of these sediment cores by using cut-off plastic syringes (internal diameter: 2.7 cm, surface area: 5.72 cm^2) and immediately frozen at -20°C. The remaining four cores were dedicated to grain-size, Biopolymeric Carbon-BPC (i.e. proteins, lipids, carbohydrates extracted in H_2O and in EDTA), Chl *a,* Phaeopigments and benthic diatom biomass. The oxic sediment layer was collected from all four cores, homogenized and subsampled differently for the analysis of each variable listed above, following specific protocols as exhaustively described in Franzo *et al.* (2016). These variables were considered in order to provide an overall description of the environmental context over the study period. In particular, BPC, the most labile fraction of the organic matter, was taken into account for testing the importance of detritus as a food source for nematodes, while benthic diatom biomass (BIOM) was used to obtain an indication of the trophic link between nematodes and these microbial autotrophs. To estimate BIOM (expressed as µg cm^{-3}), the biovolume of diatom cells was calculated according to Hillebrand *et al.,* (1999). Afterwards, it was multiplied to the diatom abundance (cell cm^{-3}) and to the carbon content of counted cells using the formula introduced by Menden-Deuer & Lessard (2000).

Focusing on meiofauna, once the three pseudo-replicates were defrosted, the top 10 cm of the sediment samples were extruded, collected and preserved in buffered 4% formaldehyde solution using prefiltered seawater and stained with Rose Bengal (0.5 g L^{-1}). For each pseudo-replicate, metazoans passing through a 1 mm sieve and retained on a 38 µm mesh net were extracted from the sediment by centrifugation with Ludox HS-40 (density 1.15-1.18 g cm^{-3}) as described in Heip *et al.,* (1985).

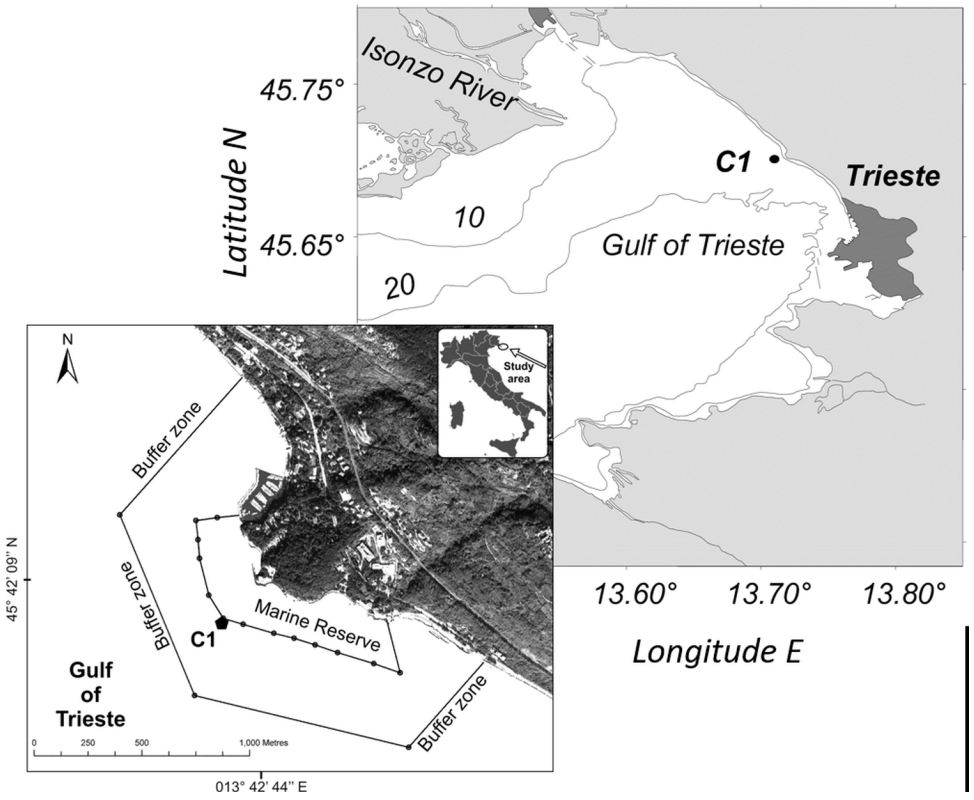

Fig. 1: Location of the study site C1 (45°42.05' N, 13°42.60' E) near the outer border of the Marine Protected Area of Miramare, along the Italian coastline of the Gulf of Trieste (northern Adriatic Sea).

Nematode community

For each pseudo-replicate, extracted meiofaunal organisms were placed on a Delfuss cuvette and all specimens were counted (abundance was expressed in individuals per 10 cm^2) and sorted to the main groups (Higgins & Thiel, 1988) under a stereomicroscope (Olympus SZX12; final magnification of 40 or 80X). In order to ensure randomness during collection, the first 100 nematodes encountered in the cuvette were hand-picked using a fine pin while the remaining ones were only counted. Collected specimens were transferred from formalin to glycerol through a series of ethanol-glycerol solutions and finally mounted on slides in anhydrous glycerin (Seinhorst, 1959). All nematodes on permanent slides were identified at the genus level under a 100x oil immersion objective (Olympus BX51) using the pictorial keys of Platt & Warwick (1983, 1988) and Warwick *et al.* (1998), as well as the original species descriptions and identification keys available through NeMys (Guilini *et al.,* 2017).

The trophic structure of nematode assemblage was studied by assigning each genus to one of the following feeding groups (Wieser, 1953): selective (1A) and non-selective (1B) deposit feeders, epistrate feeders (2A) and predators/omnivores (2B). The Index of Trophic Diversity (ITD) was calculated according to Heip *et al.* (1985): ITD = $\sum\vartheta^2$, where ϑ is the percentage contribution of each feeding type. ITD values range from 0.25 (the highest trophic diversity; i.e. each trophic group accounts for 25% of the whole nematode assemblage) to 1.0 (the lowest trophic diversity; i.e. one feeding type represents 100% of the assemblage).

Statistical analysis

All statistical analyses were performed using the PRIMER v7 software package (Clarke & Warwick, 2001) while linear regression between total benthic diatom biomass and Chl *a* was carried out using Excel software.

The sample size consisted in one fixed factor (i.e. month) with 8 levels multiplied for 3 observations (pseudo-replicates) for all nematode variables (i.e. abundance, genera composition and trophic composition).

To test for temporal differences in the composition of nematodes over the study period, a data matrix based on the abundance of genera of the eight samplings was constructed by applying the Bray-Curtis similarity and square root transformation of data, the latter to scale down densities of highly abundant genera and increase the importance of less abundant ones. The PERMANOVA test was conducted on this matrix using the month of each sampling (July 2010, November 2010, etc.) as a fixed factor and the unrestricted permutation of raw data was performed (9999 permutations). The null hypothesis (i.e. no significant temporal difference among samplings) was rejected when the significance level p was <0.05. The Monte Carlo permutation p was used when the number of permutations was lower than 150. If significant differences were detected, *a posteriori* pair-wise comparisons were performed (unrestricted permutation of raw data, 9999 permutations).

To check for temporal differences of nematode abundance and trophic composition, a one-way PERMANOVA analysis was applied, using the same design described for nematode genera. For this purpose, another data matrix, based on Euclidean-distance similarity, was built.

A non-metric Multidimensional scaling ordination (nMDS) was performed on a Bray-Curtis similarity matrix based on genera abundance in order to visualize any difference among assemblages during the 2-year study period (Clarke & Green, 1988). Similarity profiles (SIMPROF) analysis was used to test significant differences ($p<0.05$) over time. Afterwards, the relative contribution of each genus to the average dissimilarities between these groups was calculated using a one-way similarity percentage procedure (SIMPER, cut-off percentage: 70%).

In order to determine whether the nematode assemblage was influenced by the main environmental variables (Table 1), a distance-based linear model (DISTLM, McArdle & Anderson, 2001) routine was carried out. The step-wise selection procedure and the adjusted R^2 was used as a selection criterion to enable the fitting of the best explanatory environmental variables in the model (Anderson *et al.,* 2008). Prior to analysis, the environmental variables were tested for collinearity (Draftsman plot and Spearman correlation matrix). Sand % was omitted from the analysis because it is tightly correlated with mud % ($r^2>0.90$) and therefore considered redundant. To compensate for skewness, log(X) transformation was performed on Chl *a* while mud % was arcsine-transformed.

Results

During the 2-year study period, free-living nematodes experienced temporal fluctuations for the majority of the environmental variables (Table 1). Bottom water temperature was lower in winter-early spring samplings (February 2011 and March 2012) while warmer conditions were observed during summer (September 2011 and July 2012). Although minima of BPC components (lipids, proteins and carbohydrates) and pigments (Chl *a* and Phaeopigments) did not characterize the same samplings, overall higher values were measured during summer. Similarly, the highest benthic diatom biomass values were reached in June 2011 and July 2012 and a highly significant correlation between this variable and Chl *a* was obtained over the study period ($r = 0.89$, $p<0.01$; Fig. 2). On the contrary, nematodes were not subjected to a noticeable variation of sediment grain-size, which was classified as clayey silt (Shepard, 1954) in all samplings and the sand fraction always resulted as <10%.

Varying between 442.4 ± 205.5 ind. 10 cm^{-2} (November 2010) and 1124.9 ± 159.2 ind. 10 cm^{-2} (June 2011) (Table 2), nematode abundances differed significantly over the 2-year period (Table 3 and Table 4), with peaks of abundances generally in summer samplings (June and

Table 1. Environmental data measured at the bottom water (Temp = temperature) or in the sediment surface layer (top 0-1 cm) over the period July 2010-July 2012. Mud = sum of silt % and clay %; CHO_{EDTA} = EDTA-extractable carbohydrates; CHO_{H2O}= Colloidal carbohydrates extracted in water; Chl *a* = Chlorophyll *a*; Phaeo = Phaeopigments; BIOM = benthic diatom biomass; [1] = data published in Franzo *et al.,* (2016).

	Temp	Sand	Mud	[1]Proteins	[1]Lipids	[1]CHO_{EDTA}	[1]CHO_{H2O}	[1]Chl *a*	[1]Phaeo	BIOM
	°C	%	%	μg C g^{-1}	μg C g^{-1}	μg C g^{-1}	μg C g^{-1}	μg g^{-1}	μg g^{-1}	μg cm^{-3}
July2010	16.5	3.1	96.9	627.8 ± 26.8	1069.4 ± 43.4	156.3 ± 2.5	25.2 ± 1.0	7.2 ± 0.9	41.8 ± 2.6	23.34 ± 1.35
Nov2010	15.5	5.8	94.2	590.2 ± 24.2	937.5 ± 51.8	129.8 ± 3.9	65.5 ± 2.0	1.9 ± 0.2	32.4 ± 2.7	13.32 ± 0.36
Feb2011	8.7	4.2	95.8	426.9 ± 15.7	859.9 ± 33.1	169.7 ± 5.4	39.5 ± 0.1	5.4 ± 0.2	20.7 ± 0.6	32.44 ± 0.36
June2011	15.1	6.1	93.9	957.1 ± 13.3	1192.1 ± 8.8	261.9 ± 15.7	87.3 ± 3.2	26.2 ± 1.5	35.5 ± 1.1	51.27 ± 5.98
Sept2011	19.4	8.7	91.3	888.9 ± 38.1	1328.7 ± 2.1	201.5 ± 5.5	78.3 ± 3.4	12.9 ± 0.7	40.9 ± 1.2	29.85 ± 3.68
Dec2011	12.7	7.9	92.1	881.9 ± 11.3	976.3 ± 23.4	189.9 ± 6.0	55.2 ± 1.4	4.5 ± 0.3	27.5 ± 0.8	12.06 ± 1.70
March2012	7.5	4.6	95.4	1109.6 ± 26.9	835.1 ± 6.1	89.1 ± 4.7	78.2 ± 4.9	6.6 ± 0.2	33.3 ± 0.4	24.43 ± 6.14
July2012	19.1	7.8	92.2	1123.1 ± 27.1	1134.5 ± 54.8	318.8 ± 3.6	78.5 ± 4.8	9.9 ± 0.0	36.2 ± 1.4	33.48 ± 2.88

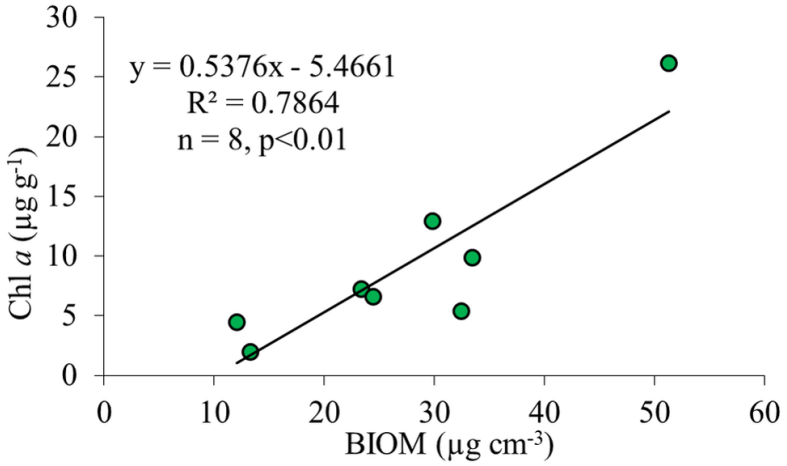

Fig. 2: Relationship between benthic diatom biomass (BIOM) and Chlorophyll *a* (Chl *a*).

September 2011, July 2012) and minima in November 2010 and December 2011.

Overall, 72 genera belonging to 24 families were identified. Chromadoridae and Comesomatidae were the dominant families over the 2-year study and represented, on average, 44.5% and 23.1% of the whole community, respectively. Their Relative Abundance (RA) varied significantly according to the sampling: Chromadoridae (22%) were less abundant than Comesomatidae (24.3%) only in February 2011 and March 2012, while they accounted for >65% of the assemblage in summer, especially in June 2011 and July 2012 (Fig. 3). This difference was mainly ascribable to *Ptycholaimellus,* a chromadorid that was the dominant genus in all samplings except in February 2011 and March 2012, when the assemblage was characterized by the dominance of *Dorylaimopsis* (Comesomatidae) (Table 2). Although PERMANOVA outputs indicated that genera composition significantly differed over the 2-year study (Table 3), the pair-wise comparisons did not highlight pairs of samplings that differed significantly, suggesting a certain variability among pseudo-replicates, sometimes even for those of the same sampling (Table 4). The nMDS ordination plot evidenced this variability graphically (Fig. 4), since group B included, in fact, pseudo-replicates of both summer and winter samplings (i.e. July 2010, December 2011, March and July 2012). Apart from this, the other groups identified by SIMPROF discriminated quite well among samplings periods: A and C gathered the majority of November 2010-December 2011 samplings and of February 2011, respectively, while group D clearly assembled summer samplings, i.e. June and September 2011, July 2012 (Fig. 4). SIMPER analysis pointed out the highest dissimilarity between C and D (50.13%), i.e. the late winter and the

Table 2. Average abundance (ind. 10 cm^{-2}), Relative Abundance (RA %) of nematodes, rank by density (Rk) of nematode genera. Only genera with an average RA >1% are listed. ITD = Index of Trophic Diversity (Heip *et al.,* 1985).

	Average abundance	July2010			Nov2010			Feb2011			June2011			Sept2011			Dec2011			March12			July12		
		%	mean	%	Rk	mean	%	Rk	mean	%	Rk	mean	%	Rk	mean	%	Rk	mean	%	Rk	mean	%	Rk	mean	%	Rk
Ptycholaimellus	227.0	27.5	207.6	28.1	1	117.6	26.6	1	74.7	8.5	3	503.5	44.8	1	274.0	28.2	1	142.9	31.4	1	127.6	13.7	2	383.8	34.9	1
Dorylaimopsis	126.9	15.4	107.8	14.6	2	98.0	22.1	2	168.9	19.1	1	108.5	9.6	2	142.1	14.6	2	65.6	14.4	2	250.9	26.9	1	78.4	7.1	3
Sabatieria	55.9	6.8	90.6	12.3	3	56.2	12.7	3	57.3	6.5	6	19.5	1.7	10	37.8	3.9	4	42.2	9.3	3	111.8	12.0	3	25.8	2.3	9
Prochromadorella	55.7	6.8	35.0	4.7	7	7.5	1.7	13	70.9	8.0	4	52.1	4.6	5	26.1	2.7	11	30.1	6.6	5	32.4	3.5	7	181.4	16.5	2
Sphaerolaimus	46.3	5.6	42.0	5.7	5	25.1	5.7	4	64.1	7.3	5	61.4	5.5	3	36.0	3.7	5	23.9	5.3	7	89.8	9.6	4	24.6	2.2	10
Terschellingia	34.0	4.1	14.9	2.0	9	11.6	2.6	8	78.9	8.9	2	12.1	1.1	13	27.9	2.9	10	24.1	5.3	6	65.6	7.0	5	34.9	3.2	7
Chromadorita	27.4	3.3	35.0	4.7	6	14.3	3.2	6	24.1	2.7	11	28.3	2.5	7	17.1	1.8	14	39.6	8.7	4	23.0	2.5	9	34.4	3.1	8
Halalaimus	19.4	2.4	26.9	3.7	8	7.5	1.7	12	25.9	2.9	10	22.8	2.0	8	30.2	3.1	8	6.7	1.5	12	17.3	1.9	10	21.5	2.0	12
Euchromadora	17.8	2.2	4.0	0.5	19	11.6	2.6	7	4.2	0.5	27	57.9	5.1	4	23.0	2.4	12	10.3	2.3	8	30.9	3.3	8	1.9	0.2	32
Metadesmolaimus	17.7	2.1	4.1	0.6	18	6.6	1.5	14	22.4	2.5	12	10.9	1.0	14	12.2	1.3	19	2.7	0.6	18	44.9	4.8	6	35.7	3.2	6
Spilophorella	16.8	2.0	8.4	1.1	14	5.5	1.2	15	16.6	1.9	13	44.2	3.9	6	35.2	3.6	6	9.3	2.0	9	5.3	0.6	19	15.9	1.4	14
Leptolaimus	15.6	1.9	11.8	1.6	12	2.9	0.7	18	35.9	4.1	8	6.2	0.6	21	28.5	2.9	9	8.8	1.9	10	17.2	1.8	11	17.7	1.6	13
Parodontophora	14.9	1.8	62.5	8.5	4	19.5	4.4	5	1.7	0.2	41	6.7	0.6	20	13.1	1.3	17	1.4	0.3	30	10.9	1.2	14	2.8	0.3	30
Daptonema	14.0	1.7	11.2	1.5	13	7.7	1.7	11	48.4	5.5	7	3.3	0.3	30	21.2	2.2	13	3.1	0.7	17	8.7	0.9	15	11.1	1.0	18
Chromadorina	13.1	1.6	12.7	1.7	11				3.4	0.4	31	10.0	0.9	15	4.1	0.4	35	1.4	0.3	25	3.4	0.4	21	66.9	6.1	4
Other genera	122.1	14.8	63.0	8.5		51.1	11.5		185.2	21.0		177.4	15.8		243.5	25.0		42.9	9.4		92.6	9.9		161.7	14.7	
Average total abundance			737.6 ± 229.1			442.7 ± 205.5			882.6 ± 430.2			1124.9 ± 159.2			971.9 ± 120.8			454.8 ± 37.6			932.4 ± 194.9			1098.4 ± 596.4		
ITD			0.43 ± 0.10			0.48 ± 0.08			0.33 ± 0.02			0.65 ± 0.08			0.43 ± 0.13			0.50 ± 0.06			0.36 ± 0.04			0.59 ± 0.17		

Table 3. Outputs of the one-way PERMANOVA test (based on the month of each sampling as fixed factor). Significant differences (i.e. p<0.05) are indicated in bold.

	Source of variation	Degree of freedom	Sum of squares	Mean squares	Pseudo-F	P(perm)
Total abundance	Period	2	1672.7	238.95	3.3448	0.0236
	Residual	15	1071.6	71.44		
	Total	22	2744.3			
Genera composition	Period	7	11151	1593	2.0166	0.0001
	Residual	15	11849	789.94		
	Total	22	23000			
Trophic composition	Period	7	2914.9	416.41	4.3523	0.0001
	Residual	15	1435.1	95.676		
	Total	22	4350			

Table 4. Outputs of the *a posteriori* pair-wise comparisons according to the factor "month" of each sampling. Significant differences (i.e. p<0.05) are indicated in bold.

Groups	Total abundance	Genera composition	Trophic composition
July2010 vs Nov2010	0.172	0.216	0.108
July2010 vs Feb2011	0.714	0.112	0.200
July2010 vs June2011	0.104	0.154	**0.014**
July2010 vs Sept2011	0.336	0.188	0.188
July2010 vs Dec2011	0.097	0.361	0.051
July2010 vs March2012	0.337	0.209	0.226
July2010 vs July2012	0.420	0.326	0.275
Nov2010 vs Feb2011	0.139	0.065	**0.047**
Nov2010 vs June2011	**0.020**	0.073	**0.004**
Nov2010 vs Sept2011	0.081	0.107	**0.038**
Nov2010 vs Dec2011	0.731	0.264	0.275
Nov2010 vs March2012	**0.046**	0.060	**0.025**
Nov2010 vs July2012	0.091	0.090	0.055
Feb2011 vs June2011	0.314	0.060	**0.015**
Feb2011 vs Sept2011	0.657	0.212	0.446
Feb2011 vs Dec2011	0.094	0.097	**0.029**
Feb2011 vs March2012	0.698	0.159	0.228
Feb2011 vs July2012	0.670	0.122	0.171
June2011 vs Sept2011	0.325	0.426	0.054
June2011 vs Dec2011	**0.001**	0.113	**0.003**
June2011 vs March2012	0.240	0.057	**0.006**
June2011 vs July2012	0.726	0.255	0.302
Sept2011 vs Dec2011	**0.004**	0.186	**0.013**
Sept2011 vs March2012	0.771	0.134	0.200
Sept2011 vs July2012	0.916	0.296	0.806
Dec2011 vs March2012	**0.004**	0.125	**0.007**
Dec2011 vs July2012	0.061	0.225	0.082
March2012 vs July 2012	0.804	0.137	0.211

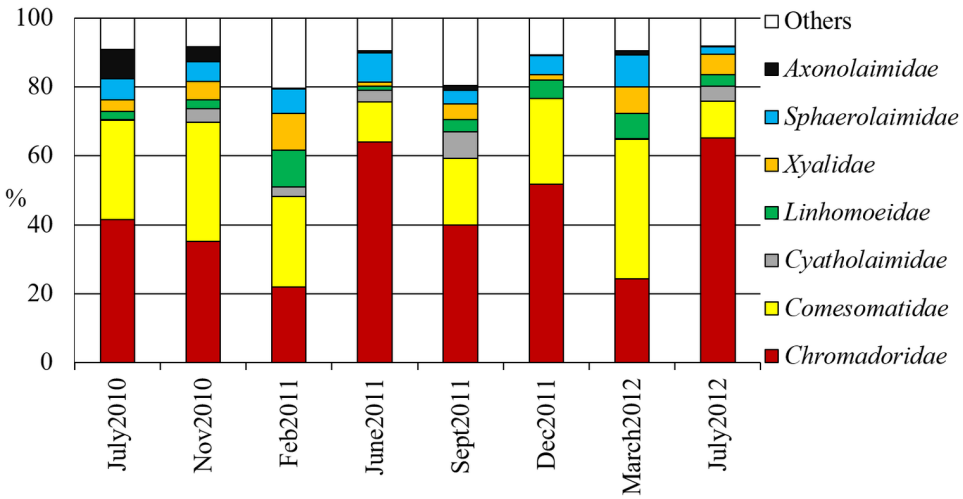

Fig. 3: Relative Abundance (Ra %) of the seven dominant nematode families at St. C1 over the period July 2010-July 2012.

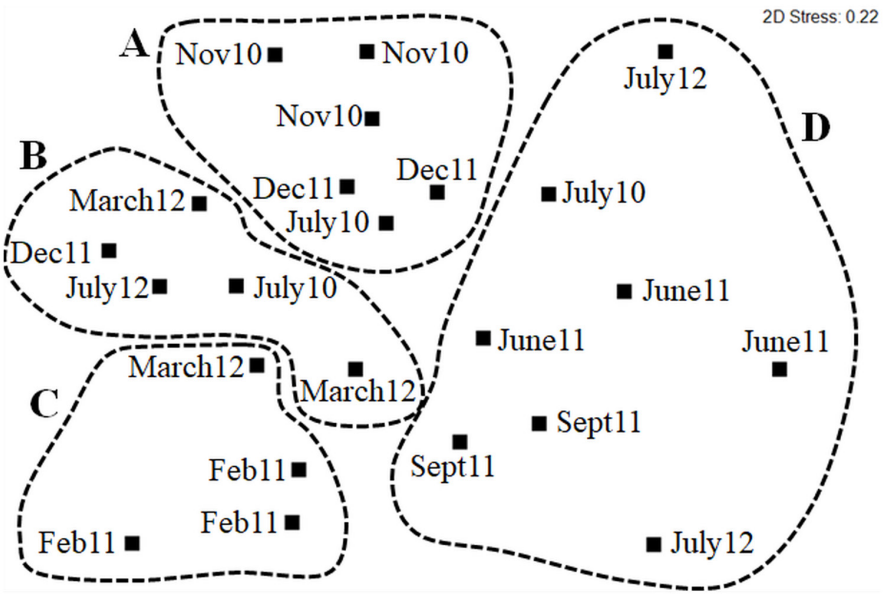

Fig. 4: nMDS ordination based on the abundances of nematode genera. The groups were identified by the SIMPROF test ($p<0.05$).

summer assemblages, with the former mainly characterized by *Molgolaimus*, *Dorylaimopsis*, *Terschellingia* and *Daptonema*, while the latter was clearly dominated by Chromadoridae as *Ptycholaimellus, Prochromadorella* and *Chromadorina* (Appendix 1).

Epistrate feeders (2A) were the most abundant trophic group in all samplings (Fig. 5), accounting for >80% of the community in June 2011 (ascribable mainly to *Ptycholaimellus*). With an RA of ~ 30%, selected deposit feeders (1A) were more abundant in February 2011, due to higher numbers of *Terschellingia* and *Halalaimus*. Overall, these differences were statistically supported by PERMANOVA outputs as shown in Tables 3 and 4. Since higher trophic diversity corresponds to low ITD values, the assemblage was more diverse in February 2011 and March 2012, when ITD minima were calculated (0.33 ± 0.02 and 0.36 ± 0.04, respectively). On the contrary, ITD maxima (0.65 ± 0.08 and 0.59 ± 0.17, in June 2011 and July 2012, respectively) indicated a lower trophic diversity in summer samplings, determined by the dominance of epistrate feeders and in particular by chromadorids (Table 2).

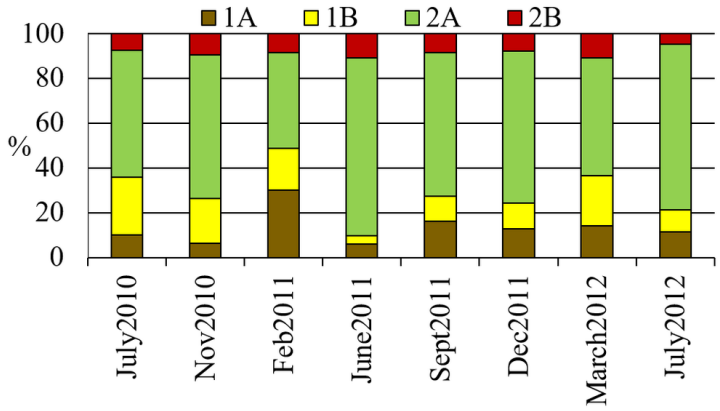

Fig. 5: Relative Abundance (RA %) of the trophic groups according to Wieser (1953). 1A = selective deposit feeders; 1B = non-selective deposit feeders; 2A = epistrate feeders; 2B = predators/omnivores.

In order to ascertain the role of different environmental variables (Table 1) on nematode genera composition, the DISTLM routine was performed. The results of marginal testing (i.e. each environmental variable was considered alone, while the others were ignored) indicated that Chl *a*, lipids and carbohydrates had a significant relationship with the genera-multivariate data cloud, with Chl *a* explaining nearly 25% of the variability, while both lipids and carbohydrates explained ~20% (Table 5). Sequential test outputs indicated that the variability of nematode composition was significantly explained only by Chl *a*, responsible for ~25% of the observed variance (Table 6).

Discussion

Over the period 2010-2012, free-living nematodes were investigated in terms of abundance, genera and trophic composition in order to provide the first thorough description of this assemblage at the long-term St. C1. By integrating these results with synoptically collected environmental and biological variables (Table 1; Appendix 2), these organisms were investigated in relation with other ecosystem components as benthic diatoms.

Nematode abundance and composition were in line with previous studies conducted in the northern Adriatic Sea. The assemblage was composed, in fact, of the same families (Comesomatidae, Linhomoeidae and Sphaerolaimidae) and, to some extent, also of the same genera (*Ptycholaimellus, Dorylaimopsis, Sabatieria, Terschellingia* and *Sphaerolaimus*) already observed along the Croatian side of the northern Adriatic Sea (Travizi, 2010) and considered widespread in this basin (Balsamo *et al.,* 2010).

The presence of a rather homogeneous community in the northern Adriatic Sea was further corroborated by considering the sediment grain-size. Comparable composition of nematodes was observed, in fact, in both the silty-sand area investigated by Travizi (2010) and at the clayey silt at St. C1, suggesting to some extent a limited influence of this environmental variable on the assem-

Table 5. Results of the DISTLM marginal test. Chl *a* = Chlorophyll *a*; CHO = Total carbohydrates (i.e. sum of EDTA-extractable carbohydrates and colloidal carbohydrates extracted in water); LIP= lipids; PROT = proteins; Temp = bottom water temperature (°C); Phaeo = sedimentary phaeopigments; Mud = sum of silt % and clay %; *** = P < 0.001; ** = P < 0.01; * = P < 0.05; n.s. = not significant.

Variable	SS(trace)	Pseudo-F	P	Prop. %
Chl *a*	1160.3	1.9624	***	24.646
LIP	1070	1.7647	*	22.728
CHO	1031.9	1.6844	*	21.919
Temp	850.55	1.3231	n.s.	18.067
Phaeo	650.39	0.9618	n.s.	13.815
PROT	637.81	0.94028	n.s.	13.548
Mud	606.85	0.88789	n.s.	12.891

Table 6. Results of the DISTLM sequential test. SS = mean square; F = F statistic; Chl *a* = Chlorophyll *a*; LIP = lipids; Temp = bottom water temperature *** = P < 0.001; ** = P < 0.01; * = P < 0.05; n.s. = not significant.

Variable	Adj R^2	SS(trace)	Pseudo-F	P	Prop. %	Cumul.
Chl *a*	0.12087	1160.3	1.9624	***	24.646	24.646
LIP	0.23052	762.17	1.4728	n.s.	16.19	40.836
Temp	0.15776	715.28	1.2628	n.s.	15.194	56.03

blage. Furthermore, in the present study the sediment grain-size did not influence nematodes since it did not vary remarkably over the 2-year period (sand % <10% in all samplings) and, in fact, was not indicated by DISTLM as one of the abiotic factors that shaped the composition of nematodes significantly.

Focusing on nematode abundance, the observed peaks in summer are consistent with Vrišer (1997) and Vrišer & Vukovič (1999), who studied the meiofauna/nematodes inhabiting the Slovenian side of the Gulf of Trieste (northern Adriatic Sea). Aside from the summer reproduction patterns of meiofauna, supposed by the authors and that likely affected free-living nematodes also at St. C1, Vrišer (1997) suggested a direct link between meiofauna/nematodes and their food sources, ascribing the abundance peaks that the author observed in July and September to seasonal phytoplankton blooms. Although we agree that phytoplankton represents a potential food source for nematodes, at our sampling site the major phytoplankton bloom generally does not occur during summer but in February-March, as indicated by our 17-year phytoplankton data series (Cibic *et al*., 2018) and by the main findings of a microscopic analysis carried out on settled material collected by a sediment trap (Cibic *et al*., 2007b). In the present study, the maxima of Chl *a* in surface sediments were observed in summer and confirmed the importance of autotrophic organisms. Nevertheless, these high concentrations were ascribable mainly to the benthic diatom biomass that is known to reach its maximum amount from May to August (Cibic *et al*., 2009). This was also supported by the highly significant correlation between these two variables (r = 0.89; p<0.01, Fig. 2) that allowed us to consider Chl *a* as a proxy of benthic diatom standing stock in the DISTLM analysis.

Nematode trophic composition indicated a strong linkage between these organisms and benthic diatoms. The assemblage was clearly dominated, in fact, by epistrate feeders (2A), mainly ascribable to *Ptycholaimellus* and *Dorylaimopsis*, in accordance with Sandulli *et al.* (2010). DISTLM outputs confirmed the role of benthic diatoms in supporting nematodes at St. C1 since Chl *a* was indicated as the environmental variable that significantly shaped the assemblage over the study period. On the other hand, it is worth noting that a major gathering of selective deposit feeders (1A) was observed in February 2011 and March 2012, especially due to *Terschellingia*. Such results suggest that at St. C1 a fraction of free-living nematodes may be able to exploit food sources other than diatoms, such as bacteria and sedimentary organic matter even if the influence of the latter variable (expressed in terms of protein, lipids and carbohydrates) was not clearly evidenced by the DISTLM analysis.

The shift observed in the taxonomic composition of nematodes during the 2-year study period indicated the role of benthic diatoms in supporting specific genera of nematodes during summer. The observed taxonomic changes were mainly ascribed to clearly higher abundances of *Ptycholaimellus* and to a more elevated number of genera belonging to the Chromadoridae family in June-September 2011 (average number of genera = 8) in comparison to November 2010 and February 2011 (average number of genera = 4.6). These genera are known for feeding on diatoms (Moens & Vincx, 1997; Moens *et al.,* 2005), which were particularly abundant in the same samplings (Franzo *et al.,* 2016), by puncturing and emptying microalgae (Moens & Vincx, 1997) in a foraging strategy oriented to the most rewarding cells, i.e. the largest diatoms (Moens *et al.,* 2013). At St. C1, the dominance of chromadorids during summer corresponded to a similar dominance of piercing nematodes, indicating that this feeding strategy was enhanced when benthic diatoms proliferated.

Apart from the cost-benefit balance where the diatom size drives the choice of a nematode, the thickness of the frustule could also play a role. The costs required for puncturing heavily silicified diatoms can be too high when compared to the benefits obtained from the assimilation of the cytoplasmic material, thus influencing the grazing strategy exerted by piercing nematodes. At St. C1, benthic diatoms typically show two distinct assemblages over the year: one dominated by photophilous genera (*Navicula* and *Nitzschia)* and warm-water loving species such as *Gyrosigma fasciola* (Cibic *et al.,* 2007a, 2012) in summer; one characterised by heavily silicified diatoms, such as *Diploneis* (Rogelja *et al.,* 2016), *Pinnularia* and *Paralia* (Cibic *et al.,* 2009), in winter. Since this alternation was also reported during the study (Appendix 2; Franzo *et al.,* 2016), the lower abundances of Chromadoridae in winter samplings were concomitant with the higher numbers of heavily silicified diatoms, indicating a minor ability of these nematodes to graze on microalgae.

Free-living nematodes results pointed out a tight linkage between these organisms and benthic diatoms, as already reported for other ecosystem components such

as macroinfauna (Grippo *et al.,* 2011). As indicated by Franzo *et al.* (2016) in the benthic ecosystem functioning assessment of St. C1, benthic diatoms seem to drive the switch from a 'source system' to a 'detritus sink system'. In a 'source system' state, benthic diatom proliferation supported higher abundances of piercing nematodes, such as *Ptycholaimellus* and more generally Chromadoridae, enhancing the C flow from the sedimentary organic matter to the higher trophic levels. On the contrary, in the 'detritus sink system', the lower number of diatoms during winter together with the dominance of more silicified ones (unfavorable cost-benefit balance), likely hampered nematode proliferation, resulting in a more pronounced confinement of C at the lower trophic levels.

Conclusions

Nematode results (abundance, genera and trophic composition) indicated that the assemblage at the long-term St. C1 changed over the period July 2010-July 2012. Significantly higher abundances were observed in summer samplings and were mainly due to the proliferation of chromadorids and in particular of *Ptycholaimellus*.

A tight interaction between nematodes and benthic diatoms was highlighted. The dominance of epistrate feeders in all samplings suggested that benthic diatoms played an important role in supporting nematode assemblage. Variations in the composition of nematode genera partially revealed the mechanisms behind the nematodes-diatoms linkage. In summer samplings, the higher abundance of diatoms together with the dominance of less silicified taxa likely enhanced the proliferation of piercing nematodes (as chromadorids), resulting in overall higher abundances. On the contrary, the presence of more silicified diatoms during winter seemed to hamper the feeding strategy of piercing nematodes, resulting in lower abundances, especially for chromadorids.

Throughout the 2-year study period, nematodes showed inter-annual variations as indicated by the low abundance and the unusual composition (characterized by the highest % of *Parodontophora*) in July 2010 if compared to the other summer samplings. This anomaly stresses the importance of continuing the study of free-living nematodes at the long-term St. C1 because, as recommended by Vrišer (1997), only by considering a longer time scale would it be possible to detect and explain any actual deviation from the typical temporal patterns.

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Appendixes

Appendix 1. Nematode genera contribution to average dissimilarity between groups identified by the SIMPROF in the nMDS.

A Vs B				
Average dissimilarity = 43.57				
Genus	Av.Diss	Diss/SD	Contrib%	Cum.%
Dorylaimopsis	2.64	1.53	6.06	6.06
Sabatieria	1.98	1.28	4.55	10.62
Parodontophora	1.95	1.25	4.47	15.08
Chromadorita	1.87	1.30	4.28	19.37
Terschellingia	1.85	2.14	4.25	23.62
Sphaerolaimus	1.83	2.04	4.20	27.82
Pierickia	1.73	1.79	3.97	31.79
Actinonema	1.61	1.43	3.68	35.48
Metadesmolaimus	1.58	1.31	3.63	39.11
Spilophorella	1.55	1.87	3.56	42.67
Prochromadorella	1.55	1.52	3.55	46.22
Halalaimus	1.44	1.95	3.31	49.53
Tricoma	1.31	1.62	3.00	52.52
Euchromadora	1.28	1.71	2.94	55.46
Leptolaimus	1.12	1.71	2.56	58.02
Daptonema	1.05	1.30	2.41	60.43
Rhabdodemania	0.97	1.26	2.23	62.65
Anticoma	0.96	1.11	2.21	64.86
Ptycholaimellus	0.83	1.33	1.90	66.76
Metacyatholaimus	0.82	0.93	1.88	68.64
Amphymonhystrella	0.80	0.84	1.83	70.47
A Vs C				
Average dissimilarity = 46.44				
Genus	Av.Diss	Diss/SD	Contrib%	Cum.%
Molgolaimus	2.63	4.28	5.67	5.67
Terschellingia	2.36	3.91	5.09	10.76
Dorylaimopsis	2.28	1.68	4.91	15.67
Daptonema	2.04	1.85	4.40	20.07
Leptolaimus	1.82	3.42	3.92	23.99
Prochromadorella	1.82	1.33	3.91	27.90
Ptycholaimellus	1.72	1.52	3.71	31.61
Sphaerolaimus	1.67	1.60	3.60	35.20
Parodontophora	1.31	1.08	2.82	38.02
Linhystera	1.30	1.54	2.81	40.83
Tricoma	1.29	1.60	2.77	43.60
Chromadorita	1.28	1.35	2.76	46.36
Metalinhomoeus	1.22	0.94	2.62	48.99
Metadesmolaimus	1.19	1.32	2.57	51.56
Sabatieria	1.18	1.30	2.54	54.10

(continued)

Appendix 1 continued

A Vs C				
Average dissimilarity = 46.44				
Genus	Av.Diss	Diss/SD	Contrib%	Cum.%
microlaimus	1.09	1.01	2.34	56.44
Metacyatholaimus	1.04	1.44	2.24	58.68
Halalaimus	1.01	1.77	2.17	60.85
Euchromadora	0.98	1.77	2.11	62.95
Pierickia	0.97	0.91	2.08	65.04
Nannolaimus	0.94	1.55	2.02	67.06
Rhabdodemania	0.78	1.21	1.67	68.73
Oxystomina	0.77	1.04	1.66	70.39
B Vs C				
Average dissimilarity = 42.22				
Genus	Av.Diss	Diss/SD	Contrib%	Cum.%
Molgolaimus	2.14	3.04	5.07	5.07
Dorylaimopsis	1.79	1.29	4.25	9.32
Spilophorella	1.72	8.57	4.08	13.40
Chromadorita	1.63	1.35	3.86	17.26
Halalaimus	1.53	1.50	3.61	20.87
Daptonema	1.52	1.68	3.59	24.47
Sabatieria	1.50	1.29	3.56	28.02
Ptycholaimellus	1.47	1.52	3.48	31.50
Metadesmolaimus	1.46	1.40	3.47	34.97
Parodontophora	1.41	1.02	3.35	38.31
Sphaerolaimus	1.33	1.49	3.15	41.46
Prochromadorella	1.30	1.38	3.08	44.55
Metacyatholaimus	1.15	1.59	2.73	47.28
Euchromadora	1.15	1.32	2.72	50.00
Actinonema	1.10	1.25	2.61	52.61
Metalinhomoeus	1.06	1.14	2.50	55.11
Pierickia	1.02	1.42	2.42	57.53
Linhystera	0.99	1.38	2.34	59.87
Terschellingia	0.97	1.67	2.29	62.17
Nannolaimus	0.91	1.64	2.15	64.32
Microlaimus	0.89	0.90	2.10	66.42
Leptolaimus	0.78	1.63	1.84	68.26
Rhabdodemania	0.77	1.17	1.83	70.10
A Vs D				
Average dissimilarity = 47.35				
Genus	Av.Diss	Diss/SD	Contrib%	Cum.%
Ptycholaimellus	4.26	2.23	8.99	8.99
Prochromadorella	2.67	1.09	5.64	14.63
Chromadorina	2.14	1.34	4.52	19.15
Spilophorella	1.52	1.71	3.21	22.36
Parodontophora	1.48	1.36	3.13	25.48
Dorylaimopsis	1.37	1.28	2.89	28.37
Euchromadora	1.35	1.31	2.84	31.21
Metadesmolaimus	1.32	1.44	2.79	34.00
Anticoma	1.31	1.40	2.77	36.77

(continued)

Appendix 1 continued

A Vs D				
Average dissimilarity = 47.35				
Genus	Av.Diss	Diss/SD	Contrib%	Cum.%
Sphaerolaimus	1.28	1.24	2.71	39.48
Chromadorita	1.24	1.07	2.61	42.10
Chromadora	1.18	0.77	2.49	44.59
Paracanthonchus	1.15	0.86	2.42	47.01
Viscosia	1.12	1.20	2.36	49.37
Metacyatholaimus	1.05	1.11	2.22	51.59
Terschellingia	1.05	1.41	2.21	53.81
Daptonema	1.05	1.35	2.21	56.02
Halalaimus	1.03	1.65	2.17	58.19
Sabatieria	1.02	1.26	2.16	60.35
Marylynnia	1.00	0.99	2.12	62.47
Leptolaimus	0.99	1.38	2.10	64.56
Rhabdodemania	0.96	1.15	2.02	66.59
Actinonema	0.85	0.66	1.79	68.37
Parasphaerolaimus	0.83	1.15	1.74	70.11
B Vs D				
Average dissimilarity = 48.67				
Genus	Av.Diss	Diss/SD	Contrib%	Cum.%
Ptycholaimellus	3.76	2.28	7.72	7.72
Spilophorella	2.16	2.14	4.44	12.15
Dorylaimopsis	2.03	1.39	4.17	16.32
Sabatieria	1.99	1.48	4.09	20.41
Chromadorina	1.85	1.24	3.79	24.20
Prochromadorella	1.83	1.04	3.76	27.96
Metadesmolaimus	1.61	1.38	3.31	31.27
Sphaerolaimus	1.59	1.34	3.28	34.55
Terschellingia	1.56	1.52	3.20	37.76
Halalaimus	1.55	1.56	3.18	40.94
Euchromadora	1.50	1.23	3.08	44.02
Parodontophora	1.43	1.21	2.93	46.95
Chromadorita	1.26	1.21	2.59	49.54
Actinonema	1.25	1.31	2.56	52.10
Pierickia	1.15	1.50	2.36	54.47
Chromadora	1.06	0.76	2.17	56.64
Viscosia	1.05	1.08	2.16	58.81
Paracanthonchus	1.02	0.79	2.09	60.90
Daptonema	0.96	1.34	1.97	62.87
Anticoma	0.95	1.25	1.96	64.83
Rhabdodemania	0.95	1.26	1.95	66.77
Marylynnia	0.92	1.01	1.89	68.66
Tricoma	0.86	1.28	1.78	70.43
C Vs D				
Average dissimilarity = 50.13				
Genus	Av.Diss	Diss/SD	Contrib%	Cum.%
Ptycholaimellus	4.67	2.58	9.31	9.31

(continued)

Appendix 1 continued

C Vs D				
Average dissimilarity = 50.13				
Genus	Av.Diss	Diss/SD	Contrib%	Cum.%
Molgolaimus	2.07	3.40	4.13	13.45
Prochromadorella	1.94	1.21	3.87	17.31
Terschellingia	1.84	1.82	3.66	20.98
Dorylaimopsis	1.77	1.54	3.54	24.51
Daptonema	1.73	1.73	3.45	27.97
Chromadorina	1.60	1.22	3.19	31.16
Sphaerolaimus	1.53	1.33	3.05	34.21
Metadesmolaimus	1.35	1.36	2.69	36.89
Euchromadora	1.31	1.33	2.61	39.50
Chromadorita	1.24	1.31	2.47	41.97
Leptolaimus	1.22	1.88	2.43	44.40
Sabatieria	1.19	1.42	2.37	46.77
Parodontophora	1.11	1.15	2.22	48.99
Linhystera	1.05	1.53	2.10	51.09
Metacyatholaimus	1.01	1.45	2.01	53.10
Spilophorella	0.97	1.90	1.94	55.05
Chromadora	0.96	0.76	1.92	56.97
Metalinhomoeus	0.96	0.99	1.92	58.89
Paracanthonchus	0.95	0.88	1.89	60.78
Anticoma	0.92	1.25	1.84	62.62
Viscosia	0.91	1.17	1.81	64.43

Appendix 2. Biomass of diatom genera, expressed as percentage, at the St.C1 over the study period. For the applied conversion factors see material and methods.

	July 2010	**Nov 2010**	**Feb 2011**	**June 2011**	**Sept 2011**	**Dec 2011**	**March 2012**	**July 2012**
Amphora spp.	5.40	4.73	9.71	1.84	3.17	5.22	1.39	1.88
Asteromphalus sp.	0.00	0.00	2.88	0.00	0.00	0.00	0.00	0.00
Auricula spp.	0.00	0.00	0.31	0.20	0.34	0.00	0.55	0.63
Bacillaria sp.	0.00	0.00	0.00	0.00	1.63	0.00	0.00	0.00
Caloneis sp.	0.09	0.50	0.00	0.09	0.22	0.46	0.15	0.07
Campylodiscus spp.	0.00	0.00	0.00	0.00	1.32	0.00	0.00	0.00
Cylindrotheca sp.	0.00	0.00	0.06	0.00	0.01	0.00	0.00	0.01
Diploneis spp.	0.26	2.87	2.61	0.67	0.78	1.64	2.87	0.38
Entomoneis spp.	3.18	0.00	2.29	0.00	0.00	0.00	5.06	2.22
Fragilaria spp.	0.00	0.00	0.00	0.34	0.00	0.00	0.00	0.00
Grammatophora spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.00
Gyrosigma spp.	20.74	4.77	22.94	16.36	23.89	33.36	27.24	37.52
Licmophora spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00
Mastogloia sp.	0.00	0.00	0.00	0.00	0.00	1.15	0.00	0.00
Melosira sp.	0.00	0.00	0.00	0.00	0.75	0.00	0.00	0.00
Navicula spp.	4.83	1.79	3.67	11.22	1.92	3.31	1.16	15.94

(continued)

Appendix 2 continued

	July 2010	Nov 2010	Feb 2011	June 2011	Sept 2011	Dec 2011	March 2012	July 2012
Nitzschia spp.	30.29	17.31	19.18	27.58	32.15	26.66	15.60	23.20
Paralia sp.	6.96	29.29	3.43	1.99	4.04	6.35	9.57	3.19
Pinnularia spp.	0.97	15.35	14.00	24.37	3.81	7.54	14.57	0.00
Pleurosigma spp.	0.00	1.64	0.00	0.00	0.00	0.90	0.35	0.13
Rhopalodia sp.	0.44	0.00	0.16	0.40	0.52	0.64	0.78	0.62
Surirella spp.	0.00	0.00	1.11	0.00	1.37	0.00	0.49	0.00
Synedra sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.00
Thalassiosira sp.	0.00	0.00	0.28	0.00	0.00	0.00	0.00	0.00
Toxarium sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.36
Tropidoneis sp.	5.76	0.00	0.00	2.62	13.52	5.58	5.51	0.00
undet. Centric diatoms	7.59	8.87	5.46	1.15	0.00	2.45	4.83	1.76
undet. Pennate diatoms	13.49	12.89	11.91	11.16	10.55	4.75	8.79	11.11
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00