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Nematode community response to fish-farm impact in the western Mediterranean

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"Capsule": *Nematode analysis in terms of k-dominance, maturity index and genus composition provides a sensible tool for describing the environmental impact due to fish-farm biodeposition in the Mediterranean.*

Abstract

A previous investigation on fish-farm biodeposition effects on benthos, carried out in the Gaeta Gulf (northwestern Mediterranean Sea), revealed a strong impact on meiofaunal assemblages. This study implements these findings by examining in detail the nematode assemblage and its response to organic enrichment from the start of a fish farm activity to the conclusion of the fish rearing cycle. Density, community structure and individual size were utilised for univariate (genus and trophic diversity and abundance patterns) and multivariate analyses (MDS) in order to identify the best descriptors of impact and the response of the nematode assemblages. Nematodes displayed significantly reduced densities, diversity and richness in sediments beneath fish farms. The impact of biodeposition was evident not only from structural community parameters but also in terms of functional indices. Forty-five days after starting fish farming, an increase of the nematode individual biomass was observed. MDS ordination pointed out the presence of two different nematode communities in disturbed sediments and in the control site. These results were substantiated by the analysis of the temporal changes of k-dominance curves, the maturity index and, to a lesser extent, by the index of trophic diversity. Some nematode genera were highly sensitive to biodeposition (*Setosabatieria, Latronema* and *Elzalia*) and disappeared almost completely in farm sediments, whereas other genera largely increased their dominance (*Sabatieria*, *Dorylaimopsis* and *Oxystomina*). This study indicates that nematodes are very sensitive to this kind of environmental disturbance. The use of simple tools, such as the k-dominance analysis and maturity index, are recommended for monitoring of aquaculture impact.

Keywords: Fish-farm impact; Nematode community; Trophic groups; Mediterranean Sea

1. Introduction

Fish-farm biodeposition results in the organic matter accumulation on bottom sediments, causing a strong modification of the physical and chemical characteristics of the benthic environment (GESAMP, 1990; Holmer, 1991; Wu, 1995; Handerson et al., 1997; Karakassis et al., 1998). It has been recently demonstrated that such changes have a strong impact on the structure and characteristics of benthic assemblages (Brown et al., 1987; Tutsumi et al., 1991; Pocklington et al., 1994; Wu et al., 1994), and particularly on meiofauna (Vincx and Heip, 1987; Duplisea and Hargrave, 1996; Mazzola et al., 1999; Mazzola et al., 2000). It is generally expected that benthic assemblages respond to organic disturbance in terms of: (1) decreased species richness, due to a selection of a few opportunistic species (Ritz et al., 1989; Weston, 1990); (2) reduced density and biomass (Frid and Mercer, 1989; Weston, 1990), partially offset by the increased abundance of opportunistic species; (3) shift in the relative importance of the different trophic guilds (Pearson and Rosenberg, 1978) and in the size of benthic organisms (Tsujino, 1998); decrease of the maturity index (Bongers et al., 1991).

Among benthic metazoans, meiofauna, being characterised by short generation time, lack of larval dispersion

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and a life entirely spent into the sediment, are the most sensitive assemblages (Heip et al., 1988; Higgins and Thiel, 1988; Warwick, 1993). Nematodes, the dominant meiofaunal taxon, have been largely utilised as indicators of organic disturbance because of their ubiquity, high densities and high taxonomic diversity (Higgins and Thiel, 1988; Bongers and Ferris, 1999). Previous studies have shown that nematodes are sensitive to hydrocarbons (Boucher, 1980; Bonsdorf, 1981; Elmgren et al., 1983; Danovaro et al., 1995; Montagna and Li, 1997), different kinds of organic contamination (Fricke et al., 1981; Hodda and Nicholas, 1986; Gee et al., 1992; Lampadariou et al., 1997; Essink and Keidel, 1998), but also display short resilience times after disturbance ends (Wormald, 1976; Giere, 1979; Danovaro et al., 1995; Colangelo et al., 1996; Mazzola et al., 2000). However, detailed investigations on the effect of fish-farm biodeposition on nematode assemblages are, to our knowledge, almost completely lacking (Duplisea and Hargrave 1996; Porter et al., 1996).

This study completes a previous investigation on the effects of fish farm impact on total meiofaunal assemblages (Mazzola et al., 1999), by examining in detail the nematode assemblage analysed in terms of density, community structure and individual size. Univariate (genus and trophic diversity, maturity index and abundance patterns) and multivariate techniques (MDS) were applied to impacted and control sites to describe the ecological effects of fish-farm biodeposition.

2. Materials and methods

2.1. Study site and sampling

This study was conducted from July 1997 to February 1998 in the Gaeta Gulf (Tyrrhenian Sea, northwestern Mediterranean Sea, Lat. 41°21′ N; Long. 13° 60′ E). The fish farm is composed of 20 floating cages, that produce 400 t year^{-1} of *Sparus aurata, Dicentrarchus labrax*, and *Diplodus puntazzo*. Salinity and bottom temperature ranged from 35.3 to 39.1 and from 14 to 25.3°C, respectively, during the entire sampling period. The area has microtidal regime (about 30 cm) and a dominant currents flow in a southeast northwest direction, following the cyclonic circulation of the Tyrrhenian Sea. The study area is sheltered and has sandy-muddy sediments.

In July of 1997 a new cage (Farmocean, 2000 m^3) was placed in a non-impacted area of the Gulf. In July, the fish farm contained about 120 000 specimens of *Dicentrarchus labrax* (equivalent to a biomass of 9 kg m^{-3}) reared till November (reaching a biomass of 18 kg m^{-3}). In December fish biomass was harvested, and the cage was filled again with about 150 000 specimens of *Sparus aurata* (equivalent to a biomass of 12 kg m^{-3}). The fish were fed daily automatically (using dry pellet composed of 46% proteins, 18% carbohydrates, 22% lipids and 14% ash/cellulose) with a final food conversion coefficient of 1.7%. No major cleaning operations were carried out during the period of investigation.

Previous surveys were carried out in the Gulf of Gaeta (La Rosa et al., 2000; Mazzola et al., 2000) to assess the spatial extent of the fish-farm biodeposition (i.e. during its maximum carrying capacity). After a preliminary analysis of the bottom characteristics and hydrological features (Mirto, 1998) beneath the cage, the control station was identified at ca. 1 km distance from the farm. From a grid sampling during different surveys, two stations were selected on the basis of similar abiotic characteristics but representative of opposite conditions: the cage station (in the area visually impacted by farm biodeposition) and the control (southern from the cage, in an area not affected by the aquaculture). Both stations were located at a depth of 10 m, and displayed a very similar sediment texture; the silt-clay fraction accounted for 13.2% at the control station and 13.9% at the cage station. The remaining fraction was sand with 30% carbonate (Mazzola et al., 1999). The Gulf of Gaeta is an organic rich area and the redox potential discontinuity depth (RPD) ranged from 1.4 to 2.9 cm in the control and from 0 to 1.1 cm in the fish farm site. Sediment samples were collected on a monthly basis manually by scuba divers at two stations 15 days after starting fish rearing.

Meiofaunal samples were collected in three replicates cores (diameter 3.7 cm, 10.7 cm^2 surface area) down to a depth of 10 cm (Mazzola et al., 1999). Sediment cores were sectioned into layers (0–1, 1–5 and 5–10 cm) but only the top 1 cm is considered here for nematode analysis, as differences between impacted and control nematode assemblages are assumed to be more evident in surface than in deeper sediment layers. All environmental parameters have been previously reported by Mazzola et al. (1999) and summarised in Table 1.

2.2. Nematode analysis

Sediment samples were fixed with 4% buffered formaldehyde in filtered (0.4 μm) seawater solution. Sediments were sieved through 1000 and 37 μm mesh nets. The fraction remaining on the 37 μm sieve was centrifuged three times with Ludox HS (density 1.18 g cm^3) as described by Heip et al. (1985). After staining with Rose Bengal (0.5 g l^{-1}), all nematodes were counted.

From each sampling replicate a sub-sample (1:4 of the total, after splitting) was taken and all subsamples of the three replicates were pooled together. All nematodes were then identified to genus level (always $n > 100$) according to Platt and Warwick (1983) and classified per trophic group according to Wieser's (1953) original groupings as follows: (1A) buccal cavity absent or fine and tubular-selective bacterial feeders; (1B) large but

Table 1
Environmental parameters[a]

	RPD				Water content				CPE				BPC			
	Control		Cage		Control		Cage		Control		Cage		Control		Cage	
	cm	±S.D.[b]	cm	±S.D.[b]	%	±S.D.[b]	%	±S.D.[b]	μg g^{-1}	±S.D.[a]	μg g^{-1}	±S.D.[b]	μg g^{-1}	±S.D.[b]	μg g^{-1}	±S.D.[b]
July	2.9	0.5	1.1	0.8	41.6	0.8	64.9	6.8	14.7	2.1	30.9	5.7	3399.6	568.3	6275.4	800.0
August	1.4	0.2	0.0	0.0	58.8	2.9	56.6	2.3	12.8	0.9	8.9	0.5	3173.7	780.1	2458.2	166.4
September	1.6	0.4	0.1	0.2	52.6	1.7	44.4	2.9	12.5	0.9	9.8	1.8	2648.5	319.0	2443.0	356.7
October	1.7	0.3	0.3	0.4	69.4	11.6	48.1	1.6	10.7	0.3	9.9	1.3	2753.8	831.7	1709.4	293.6
November	1.5	0.4	0.3	0.3	25.5	0.1	61.9	14.7	10.4	0.6	23.5	7.5	2201.2	1502.6	2345.8	477.9
December	1.4	1.0	0.3	0.4	55.2	2.3	44.4	1.7	10.8	0.5	14.0	0.7	1743.5	330.1	2804.0	631.0
January	1.8	0.8	0.0	0.0	35.4	3.0	33.1	1.9	12.8	1.1	3.3	0.3	1447.0	267.1	3749.5	520.9
February	2.0	0.5	0.0	0.0	45.0	3.4	40.2	15.9	7.8	1.3	9.4	1.5	2685.9	270.7	4444.2	399.0
Avg±S.E.[c]	1.8	0.1	0.3	0.1	47.9	1.3	49.2	2.1	11.6	0.2	13.7	1.0	2506.6	150.7	3278.7	70.3

[a] RPD, redox potential discontinuity layer depth; CPE, chloroplastic pigment equivalents; BPC, biopolymeric carbon (data are relative to the top 1 cm of the sediment).
[b] S.D., standard deviation.
[c] S.E., standard error.

unarmed buccal cavity-non-selective deposit feeders; (2A) buccal cavity with scraping tooth or teeth-epistrate (diatom) feeders; (2B) buccal cavity with large jaws-predators/omnivores. Analysis of the nematode genus composition and trophic groups was carried out in the first 4 months of the sampling period (July, August, September, and October) and at the end of this investigation (February 1998). These periods were selected to check the initial and medium terms' effects of the fish-farm impact.

Nematode biomass was calculated from biovolume estimates according to Feller and Warwick (1988), and was determined as the sum of the product of individual body weight (expressed as μg C) and total nematode density, and expressed as μg C 10 cm^{-2}.

2.3. Statistical analyses and ecological indexes

Numerical analyses performed on the nematode genera abundance data include various multivariate procedures. A similarity matrix was constructed using the Bray–Curtis measure of similarity on untransformed and 4th root transformed data. An ordination analysis using group average sorting was carried out with multidimensional scaling ordination (MDS) and the SIMPER test (Clarke and Green, 1988). Only those specimens representing more than 3% of the total number of individuals in any one sample were utilised.

To test the hypothesis of high similarities between the two stations, mean dissimilarities among replicates and among stations were carried out. Comparisons were based on Bray–Curtis similarity values (SBC) calculated on all genera within each replicate (PRIMER, Clarke, 1993). Differences between the two stations were represented by non-metric multidimensional scaling ordinations (MDS), considering all the replicates for the three stations separately, but representing graphically only a month's centroids (the mean abundance of each taxon from the three replicates). SIMPER was used to identify "important" taxa. These had abundance that contributed for more than 10% to similarity within the two stations and/or that accounted for at least 5% of the average dissimilarity among them (Clarke 1993).

Univariate techniques included the calculation of the Shannon-Wiener diversity index (H′), evenness as Pielou's (J), richness as Margalef's index (D) and Hill's diversity numbers (Hill, 1973). All these analyses were performed on nematodes at the genus level. For univariate and multivariate analyses programme PRIMER (Plymouth Marine Laboratory) was utilised.

The diversity trophic index (ITD) was calculated as follows:

$$\mathrm{ITD} = \Sigma\theta^2$$

where θ is the percentage of each trophic group (Heip et al., 1985).

The maturity index (MI) was calculated according as the weighted mean of the individual taxon scores Bongers et al. (1991):

$$\mathrm{MI} = \Sigma\nu(i) \times f(i)$$

where ν is the colonisers – persisters (c–p) value of taxon i (as given in Appendix by Bongers et al. 1991) and f is the frequency of that taxon in a sample

3. Results

3.1. Meiofaunal abundance and community structure

Meiofaunal density in the top 1 cm of the sediments ranged from 546±131 to 1829±474 ind. 10 cm^{-2} (in

December and July, respectively) in the control, whilst in the cage station meiofaunal density ranged from 294±201 to 662±164 ind. 10 cm^{-2} (in September and November, respectively, Table 2). Meiofaunal density beneath the cage during the 8 months of sampling was significantly lower than in the control (*t*-test, $P<0.01$). Nematodes accounted for 75% of the total density in the top 1 cm in both stations.

Nematode abundance in farm sediment ranged from 223±29 ind. 10 cm^{-2} to 519±188 ind. 10 cm^{-2} (in January and November, respectively) and were significantly lower values (*t*-test, $P<0.01$) than in the control site, where nematode abundance ranged from 436±131 ind. 10 cm^{-2} to 1328±349 ind. 10 cm^{-2} (in December and September, respectively).

Nematode body weight at the control site ranged from 0.07±0.01 μg C to 0.16±0.03 μg C (in October and November, respectively) but was significantly higher at the cage station during the entire study period (*t*-test, $P<0.01$), ranging from 0.10±0.03 μg C in December to 0.25±0.04 μg C in October (Table 2). Nematodes biomass in the control station displayed lowest values in December (39.2±11.8 μg C 10 cm^{-2}) and maximum values in September (159.4±41.8 μg C 10 cm^{-2}), but in farm sediments nematodes biomass ranged from 42.9±7.8 μg C 10 cm^{-2} to 88.2±32.0 μg C 10 cm^{-2} (in December and November, respectively) (Table 2).

Nematodes genera identified in both sampling stations are reported in the Appendix. Control sediments displayed the dominance of the following 10 genera: (1) *Setosabatieria* (14.0% of the total nematode density on average across the entire sampling period); (2) *Pierrickia* (9.9%); (3) *Ptycholaimellus* (5.3%); (4) *Comesomoides* (3.9%); (5) *Latronema* (3.9%); (6) *Neochromadora* (3.2%); (7) *Oncholaimellus* (3.2%); (8) *Elzalia* (3.0%); (9) *Leptolaimus* (3.0%); and (10) *Sabatieria* (3.0%). Cage sediments displayed the dominance of the following 10 genera: (1) *Pierrickia* (13.1%); (2) *Dorylaimopsis* (11.9%); (3) *Sabatieria* (8.9%); (4) *Oncholaimellus* (6.2%); (5) *Oxystomina* (6.0%); (6) *Ptycholaimellus* (3.7%); (7) *Comesomoides* (3.2%); (8) *Daptonema* (3.0%); (9) *Setosabatieria* (3.0%); and (10) *Polysigma* (2.5%). Four of the most dominant genera encountered in cage sediments are almost negligible (<1.5% of total nematode density) in control sediments.

SIMPER identified *Setosabateria* characterising the control station and *Pierrickia* characterising the cage station. Their contribution, expressed as a percentage of the average similarity, was 9.4% for *Setosabateria* within the control station that displayed an average Bray–Curtis similarity, within each group of samples, of 42.7%. Conversely, *Pierrickia* contributed to the average similarity of the cage station for 12.9% and displayed an average Bray–Curtis similarity, within each group of samples, of 36.6%.

Table 2
Meiofaunal parameters in the top 1 cm of the sediment

	Meiofauna abundance			Nematode abundance				Nematode body weight				Total Nematode biomass			
	Control			Control		Cage		Control		Cage		Control		Cage	
	Ind. 10 cm^{-2}	±S.D.[a]	Ind. 10 cm^{-2}	±S.D.[a]	Ind. 10 cm^{-2}	±S.D.[a]	μg C	±S.D.[a]	μg C	±S.D.[a]	μg C 10 cm^{-2}	±S.D.[a]	μg C 10 cm^{-2}	±S.D.[a]
July	1829	474	493	334	980	182	366	255	0.09	0.01	0.12	0.01	88.2	16.4	43.9	30.7
August	1058	157	452	275	741	119	288	260	0.08	0.02	0.20	0.03	59.3	9.6	57.7	51.9
September	1656	395	294	201	1328	349	278	198	0.12	0.02	0.18	0.02	159.4	41.8	50.1	35.6
October	1110	169	422	63	888	145	295	94	0.07	0.01	0.25	0.04	62.2	10.2	73.7	23.5
November	1077	58	662	164	920	69	519	188	0.16	0.03	0.17	0.03	147.1	11.1	88.2	32.0
December	546	131	639	206	436	131	429	78	0.09	0.01	0.10	0.03	39.2	11.8	42.9	7.8
January	1052	531	304	15	811	480	223	29	0.08	0.02	0.20	0.06	64.9	38.4	44.5	5.8
February	1351	314	335	84	973	266	270	70	0.07	0.01	0.18	0.02	68.1	18.6	48.5	12.6
Avg±S.E.[b]	1210	62	450	38	885	49	333	32	0.10	0.00	0.18	0.01	86.0	4.6	56.2	5.6

[a] S.D., standard deviation.
[b] S.E., standard error.

The SIMPER analysis also indicated an average dissimilarity between the two stations of 63.6%. The main genera responsible such differences were *Setosabateria*, *Latronema*, *Dorylaimopsis, Oncholaimellus, Comesomoides* and *Oxystomina*.

The analysis of the trophic groups revealed that control site sediments were dominated by group 1A (31.9% of total nematode density), followed by group 1B (31.4%), group 2A (25.5%) and group 2B (11.2%), while the most abundant trophic group encountered in farm sediments were: 1B (30.7%), 1A (28.4%), 2A (27.6%) and 2B (14.2%).

3.2. Ecological indices

Genus diversity, evenness and richness are reported in Table 3. The temporal changes in the Index of Trophic Diversity (ITD) are reported in Fig. 2a. Temporal changes in the significance of the different trophic groups are reported in Fig. 1b–e. No clear changes in ITD values were observed in both stations. However, the ITD at the cage station increased at the end of our study (i.e. 8 months after cage deployment), due to the increase of the relative importance of the non-selective deposit-feeder nematodes (1B, Fig. 2c).

The results of the analysis of the maturity index are reported in Fig. 3. The MI value of the nematode assemblage from fish-farm sediments are similar at the beginning of the fish-farm activity to decrease notably from August to September. In October and February MI values of the two assemblages were indistinguishable.

Curves of k-dominance were constructed to compare nematode community structure in the control and cage station. Fifteen days after fish farm deployment (i.e. in July sampling, Fig. 4a), nematode community structure was very similar in control an cage stations, but in the next 3 months k-dominance curves of cage nematodes were clearly above the control nematode curves (Figs. 4b–d). Finally, 225 days (i.e. 8 months) after cage deployment, cage and control curves returned to the initial conditions.

3.3. Multivariate analyses

MDS ordination analysis (4th root transformation) applied to nematodes identified to genus level clearly indicates the presence of three groups of stations: the first on the left side includes only July samplings; at the beginning of the farming activity (i.e. in July) nematode assemblages in cage and control sediments were very similar. The central pool of stations represents non-impacted assemblages (only control samples are grouped here) and finally the pool of samples on the right side of the MDS square represents impacted assemblages (i.e. where only cage samples are grouped; Fig. 5).

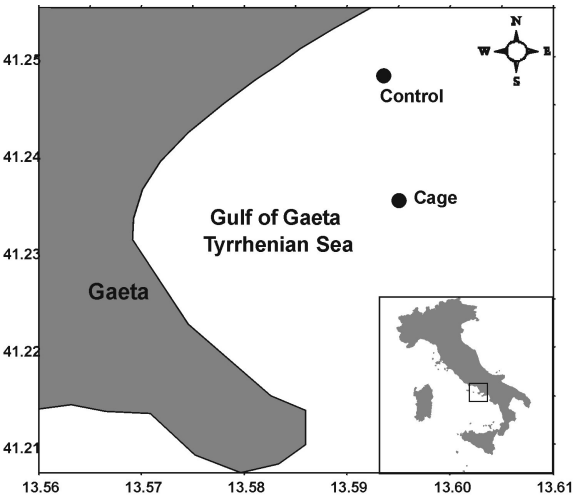

Fig. 1. Sampling area and station location.

Table 3
Univariate measures for nematodes[a]

	G	D	H′	J	S	N1	N2	Ninf	N10	N10′	N21	N21′
Control												
July	25	3.72	3.98	0.86	0.09	15.70	11.40	5.50	0.63	0.61	0.73	0.71
August	32	4.79	4.64	0.93	0.05	25.00	20.40	11.10	0.78	0.77	0.81	0.81
September	31	4.71	4.48	0.90	0.06	22.30	16.80	8.30	0.72	0.71	0.76	0.75
October	39	5.74	4.31	0.82	0.11	19.80	8.75	3.27	0.51	0.49	0.44	0.41
February	22	3.34	3.58	0.80	0.13	12.00	7.68	3.90	0.55	0.52	0.64	0.61
Cage												
July	33	4.99	4.50	0.89	0.06	22.60	15.60	6.54	0.69	0.68	0.69	0.68
August	22	3.20	3.76	0.84	0.10	13.60	9.91	5.22	0.62	0.60	0.73	0.71
September	18	2.68	3.27	0.78	0.15	9.62	6.48	3.38	0.53	0.51	0.67	0.64
October	25	3.73	3.67	0.79	0.12	12.80	8.64	5.06	0.51	0.49	0.68	0.65
February	33	4.93	4.46	0.89	0.06	22.10	15.80	6.92	0.67	0.66	0.71	0.70

[a] G, total number of genera; D, Margalef's richness index; H′, Shannon-Wiener diversity index; J, Pielou's evenness index; S, Simpson's dominance index; N and N′, Hill's (1973) diversity and evenness numbers.

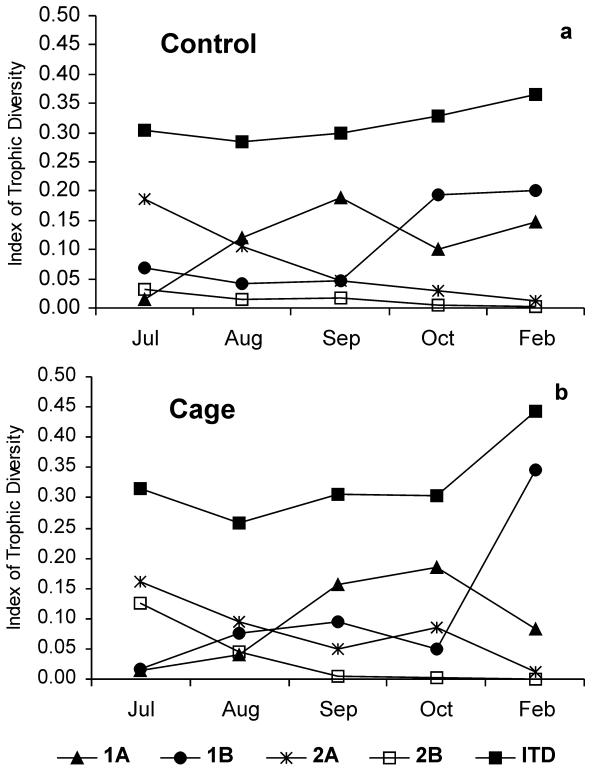

Fig. 2. Trophic composition of the nematode assemblage: (a) index of trophic diversity (ITD); (b) the contribution (expressed in %) of the trophic group 1A; (c) 1B; (d) 2A and (e) 2B.

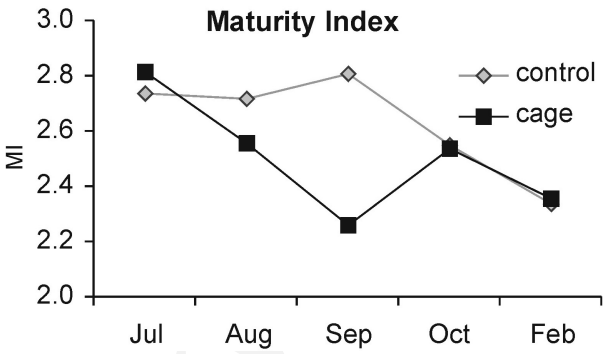

Fig. 3. Temporal trend of the Maturity Index calculated on nematodes from control and fish-farm sediments.

4. Discussion

Previous studies demonstrated the clear impact of fish-farm biodeposition on the benthic environment (Ritz et al., 1989; Wu et al., 1994; Duplisea and Hargrave, 1996; Karakassis et al., 1998; Mazzola et al., 2000). It is now widely accepted that the deployment of fish farms in a non-impacted area provokes immediate and evident changes in the sediment characteristics (also described for the investigated area), that can be summarised as follow (Table 1): (1) creation of reducing conditions under the cage, as suggested by the strong reduction of the depth of oxygen penetration into the sediment (RPD depth ranging from 0 to 1.1 cm; Mazzola et al., 1999); (2) eutrophication of bottom cage sediments, as pointed out by the strong accumulation of chloropigment concentrations (likely due to *Beggiatoa*-like assemblages; La Rosa et al., 2000); (3) evident reduction of meiofaunal densities (Mazzola et al., 1999); (4) changes in the structure of both microbial and meiofaunal assemblages (La Rosa et al., 2000).

This study confirms these findings also from the point of view of the community structure, trophic composition, individual size of nematodes and maturity index. In particular, this investigation revealed that nematode densities were significantly reduced under the cage, when compared to the control site (t-test, $P<0.01$), confirming the results of similar studies carried out on salmon fish cultures in the Bay of Fundy (Duplisea and Hargrave, 1996). These results indicate that nematodes are also sensitive to fish farm pollution in the warm, micro-tidal Mediterranean Sea.

In addition, this study indicates that the effects of biodeposition might be evident also in terms of body size. Nematodes had significantly higher body weights in organic enriched sediments beneath the cage, than in the non-impacted site (t-test, $P<0.01$). Studies conducted so far of fish-biodeposition effects on nematode size provided conflicting results. Our results are consistent with previous studies that reported meiofaunal biomass of organic enriched environments became increasingly dominated by large specimens, when compared to non-enriched sites (Moore and Bett, 1989). Also, Tsujino (1998) and Porter et al. (1996) reported the presence of large size nematodes in organic impacted sediments. Prein (1998) and Lorenzen et al. (1987) reported that the large Oncholaimidae *Pontonema vulgare* (12.3–14.9 mm in body length; Platt and Warwick, 1983) accumulated in organically polluted fjords. In contrast, Duplisea and Hargrave (1996) did not find differences in nematode individual biomass comparing fish-farm and control sediments. Nematode body size is still not a universally accepted parameter for detecting organic pollution; for instance, Monhysterids are small and tolerant and Enoploids are large and sensitive (Heip et al., 1982). Nonetheless, our study indicates that after an initial significant increase of the individual biomass, from November to December (when the fish were harvested and consequently biodeposition strongly reduced), average body weight of nematodes beneath the cage became immediately indistinguishable from control values (Table 2).

Immediately after fish-farm deployment, despite the increased individual size, the strong reduction of nematode density beneath the cage determined a decrease of the total nematode biomass, that remained significantly higher at the control station throughout the study period (t-test, $P<0.05$).

The analysis of nematodes to genus level proved to be highly efficient for describing changes occurring in

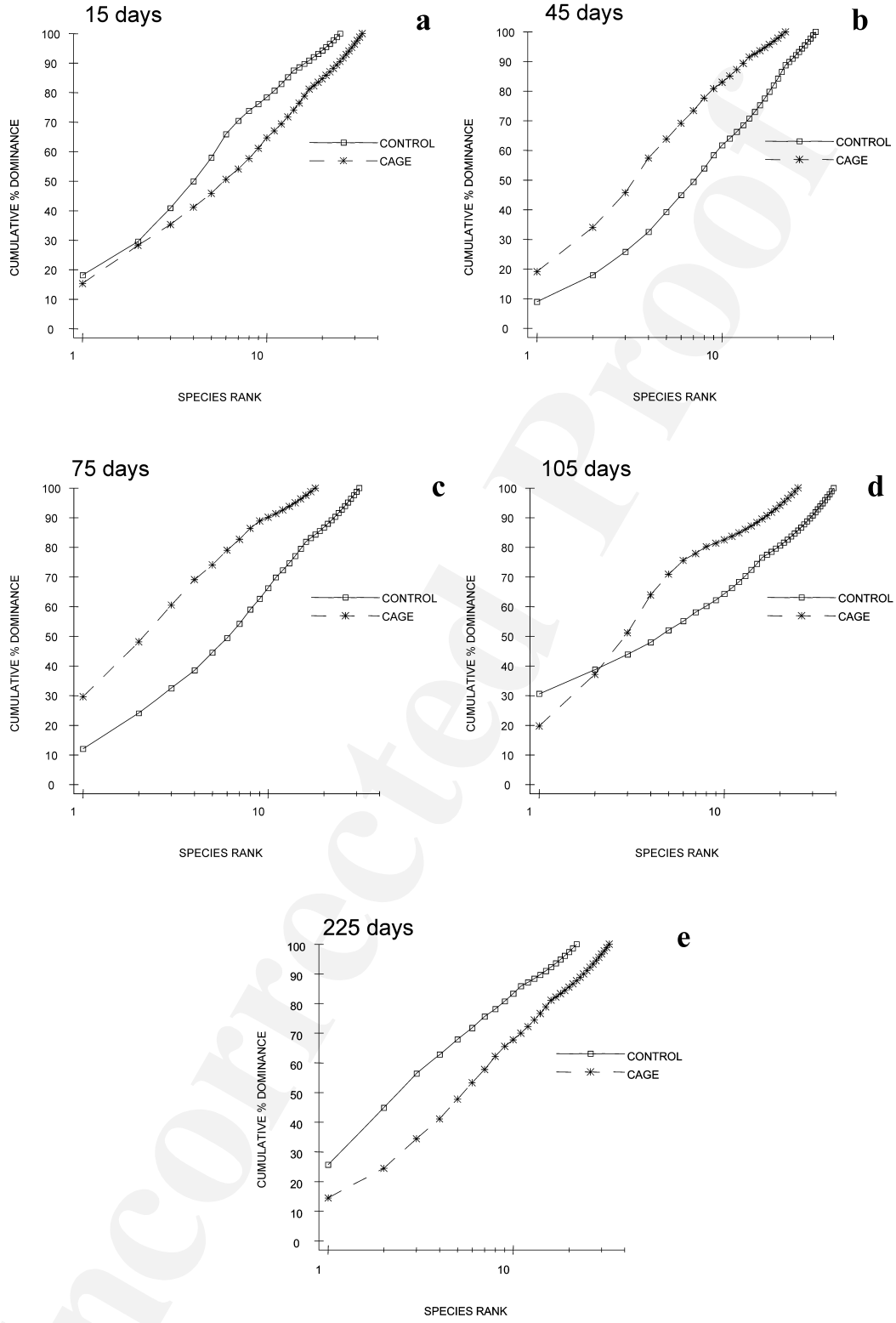

Fig. 4. Nematode genera dominance. Computer constructed k-dominance curves: (a) nematodes immediately after fish farm deployment; (b) after 45 days; (c) after 75 days; (d) after 105 days; (e) after 225 days. Each curve compared nematode structure in control and cage sites synoptically.

sediments beneath the cages due to organic enrichment (Essink and Keidel, 1998; Bongers and Ferris, 1999). Nematode assemblages in disturbed sediments appeared clearly different from control nematode assemblages. SIMPER analysis of dissimilarity proved a clear overall difference of the nematode genera (63.6%). MDS ordination revealed clear differences between July sampling and other sampling periods. Moreover, MDS output

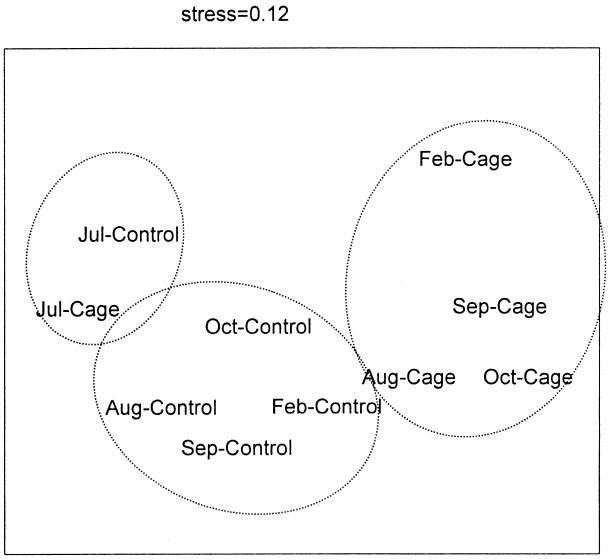

Fig. 5. MDS plot based on nematode genera in the sediments of the Gaeta Gulf. The sample grouping was based on a Bray–Curtis clustering from on 4th root transformed abundance data (not shown). Stress coefficient was 0.12.

(Fig. 5) clearly separated the assemblages of cage and control samples, but did not detect any community resilience.

The analysis of the Maturity Index also provided evidence of a similarity of nematode assemblages in July and a clear drop of the MI in August and September when highest impact occurred. MI values also indicated a rapid resilience of the nematode assemblage, which was indistinguishable from control from October to the end of the sampling period. In this regard, MI analysis appears to be sensitive to detect the resilience of nematode assemblages.

K-dominance curves plotted at each sampling time clearly illustrated temporal changes of the impact on nematode assemblages. While no clear differences were noted 15 days after cage deployment, after 45–75 days the difference between impacted and control assemblages was clearly evident (Fig. 4). A first sign of reassessment of equilibrium conditions (that cannot be defined here as "resilience" because the nematode community was different from pre-pollution conditions) was noticed 105 days after cage deployment, and after 225 days, k-dominance curves of cage and control resembled the k-dominance curve of July (i.e. before the impact was evident).

Similar results were obtained from the analysis of species richness and diversity, which both declined in impacted sediments (Table 3). As for k-dominance curves, nematode response to biodeposition impact was evident 45 days after cage deployment, and differences between cage and control were evident until October. Only after 8-months (i.e. in February), both H′ and evenness (Pielou's J) clearly increased. These results are in contrast with other investigations on organic pollution, which showed much longer recovery periods for hydrocarbon pollution (more than 2.5 years, Bodin and Boucher, 1983; 2 years, Elmgren et al., 1983; more than 1 year, Wormald, 1976).

The clear impact on nematodes beneath the cage, described above, was not equally evident from the analysis of the Index of Trophic Diversity (here utilised as a measure of functional diversity). The lack of significant differences between control and cage sediments (t-test, $P = 0.4$), would suggest that the impact was not selective towards specific trophic groups, but non-selective deposit feeding nematodes (1B) strongly increased in organic enriched sediments after 225 days (Fig. 2c). However, recently the Wieser's classification has been largely revisited and modified (Moens and Vincx, 1997). Therefore it is possible that the adopted classification did not reflect the actual trophic structure of nematode assemblages (Moens et al., 1999). Unfortunately, in our case, the use of the Moens and Vincx's classification was hampered by the limited number of nematode genera classified from a trophic point of view.

Some genera were apparently highly sensitive to organic disturbance: *Setosabatieria* was found to be the dominant genus in control sediments and disappeared after cage deployment. SIMPER analysis indicated that this species was mainly responsible for the dissimilarity between assemblages of pristine vs organic impacted sediments. In this regard, Danovaro et al. (1995) and Heip et al. (1982) also reported that *Setosabatieria* was very sensitive to hydrocarbon and organic pollution. *Latronema,* and *Elzalia* disappeared almost completely in farm sediments. In contrast, other nematode genera were tolerant to biodeposition and took advantage on the new conditions. The SIMPER analysis confirmed that *Dorylaimopsis, Sabatieria*, and *Oxystomina* increased in dominance in polluted conditions, being responsible for the dissimilarity between impacted and non-impacted sites. *Sabatieria* can be considered a genus indicator of organic enrichment, being dominant in sub-oxic sediments (Vanreusel, 1990; Vincx et al., 1990; Lampadariou et al., 1997). Among dominant genera only *Pierrickia* and *Ptycholaimellus* did not show differences between control and cage sediments.

Results reported here are promising and indicate that nematode assemblage structure and genus composition are sensitive tools for describing the environmental impact due to fish farming in the Gulf of Gaeta. We recommend, for future monitoring studies, the use of k-dominance curves and the maturity index on putative impacted stations vs control sites. Further investigations are needed in other Mediterranean and non-Mediterranean areas before these results can achieve a more universal value for utilisation as bio-indicators of biodeposition impact.

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Appendix. Identity and abundance (as percentage) of nematodes in the Gulf of Gaeta. Nomenclature as in Platt and Warwick (1983)

Appendix

Nematode genera	Trophic group	July		August		September		October		February	
		Control	Cage	Control	Cage	Control	Cage	Control	Cage	Control	Cage
Actarjania	1B	0.00	0.00	0.00	0.00	1.20	0.00	0.00	1.16	2.56	1.11
Aegialoalaimus	1A	1.14	1.18	1.12	0.00	0.00	0.00	1.02	0.00	0.00	0.00
Amphimonhystrella	1B	0.00	1.18	0.00	0.00	0.00	0.00	0.00	0.00	2.56	0.00
Aponema	2A	0.00	0.00	0.00	0.00	0.00	3.70	0.00	1.16	0.00	2.22
Calligyrus	1A	0.00	0.00	0.00	0.00	1.2	0.00	0.00	0.00	0.00	0.00
Campylaimus	1B	0.00	0.00	3.37	1.06	0.00	1.23	2.04	0.00	1.28	0.00
Cervonema	1A	2.27	1.18	0.00	0.00	3.61	1.23	1.02	1.16	1.28	2.22
Chitwoodia	1A	0.00	0.00	0.00	0.00	0.00	0.00	1.02	0.00	0.00	0.00
Chromadorita	2A	2.27	1.18	4.49	0.00	0.00	0.00	1.02	0.00	0.00	0.00
Chromaspirina	2B	0.00	2.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Comesoma	1B	0.00	0.00	0.00	0.00	0.00	0.00	2.04	0.00	0.00	4.44
Comesomoides	1B	0.00	0.00	0.00	6.38	1.20	8.64	1.02	0.00	19.23	1.11
Coninckia	1A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11
Crenopharinx	1A	0.00	0.00	0.00	2.13	0.00	0.00	0.00	0.00	0.00	0.00
Cyartonema	1A	0.00	0.00	1.12	0.00	0.00	0.00	2.04	1.16	0.00	0.00
Daptonema	1B	4.55	0.00	2.2	0.00	0.00	0.00	0.00	0.00	1.28	14.44
Desmolaimus	1B	0.00	0.00	0.00	0.00	0.00	1.23	0.00	0.00	0.00	1.11
Desmoscolex	1A	0.00	1.18	4.49	0.00	8.43	0.00	0.00	0.00	0.00	0.00
Didelta	1B	0.00	1.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diplopeltoides	1A	0.00	3.53	0.00	0.00	2.41	0.00	8.16	0.00	0.00	0.00
Disconema	1A	0.00	0.00	2.25	1.06	1.20	0.00	1.02	0.00	1.28	0.00
Dolicholaimus	2B	0.00	0.00	0.00	2.13	0.00	4.94	0.00	2.33	0.00	0.00
Dorylaimopsis	2A	0.00	3.53	2.25	19.15	0.00	12.35	1.02	19.77	2.56	0.00
Elzalia	1B	0.00	2.35	7.87	0.00	2.41	0.00	3.06	1.16	1.28	0.00
Eumorpholaimus	1B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.28	0.00
Filoncholaimus	1B	0.00	0.00	1.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Graphonema	2A	0.00	0.00	1.12	0.00	0.00	0.00	0.00	0.00	0.00	1.11
Halalaimus	1A	1.14	0.00	4.49	1.06	4.82	0.00	1.02	0.00	1.28	1.11
Halanoncus	1B	0.00	0.00	0.00	1.06	0.00	0.00	0.00	0.00	0.00	0.00
Halaphanolaimus	1A	0.00	0.00	0.00	2.13	0.00	0.00	0.00	2.33	5.13	1.11
Halichoanolaimus	2B	0.00	0.00	0.00	0.00	1.20	0.00	0.00	0.00	0.00	0.00
Hopperia	2A	0.00	0.00	0.00	0.00	1.20	0.00	1.02	0.00	0.00	0.00
Innocuonema	2A	9.09	0.00	2.25	0.00	0.00	0.00	1.02	0.00	0.00	1.11
Kraspedonema	2A	0.00	4.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

(Appendix continued on next page)

Appendix (*continued*)

Nematode genera	Trophic group	July		August		September		October		February	
		Control	Cage	Control	Cage	Control	Cage	Control	Cage	Control	Cage
Latronema	2B	1.14	0.00	8.99	0.00	6.02	0.00	0.00	0.00	3.85	0.00
Leptolaimoides	1A	1.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leptolaimus	1A	2.27	1.18	5.62	0.00	0.00	3.70	4.08	1.16	2.56	2.22
Linhystera	1B	1.14	0.00	0.00	0.00	1.20	0.00	1.02	0.00	0.00	2.22
Litinium	1A	0.00	0.00	2.25	0.00	0.00	0.00	1.02	0.00	0.00	0.00
Marylynnia	2A	0.00	0.00	0.00	0.00	0.00	0.00	1.02	0.00	0.00	0.00
Metachromadora	2B	1.14	3.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Metacomesoma	1B	0.00	0.00	2.25	3.19	0.00	1.23	2.04	0.00	0.00	0.00
Metadesmolaimus	1B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.56
Metalinhomoeus	1B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11
Metasphaerolaimus	2B	0.00	0.00	1.12	0.00	2.41	0.00	0.00	0.00	0.00	0.00
Meyersia	2B	0.00	2.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Micoletzkya	1A	1.14	0.00	0.00	0.00	0.00	0.00	0.00	1.16	0.00	0.00
Microlaimus	2A	0.00	0.00	1.12	0.00	3.61	0.00	1.02	0.00	0.00	0.00
Minolaimus	2A	0.00	0.00	1.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Molgolaimus	1A	0.00	0.00	0.00	0.00	1.20	0.00	0.00	0.00	0.00	0.00
Monoposthia	2A	0.00	0.00	2.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Monhystera	1B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.98	0.00	2.22
Neochromadora	2A	7.95	4.71	2.25	1.06	0.00	0.00	5.10	1.16	0.00	0.00
Neothoncus	2A	0.00	1.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Oncholaimellus	2B	11.36	15.29	0.00	14.89	1.20	0.00	2.04	0.00	1.28	0.00
Oxystomina	1A	2.27	0.00	0.00	1.06	1.20	4.94	3.06	17.44	0.00	6.67
Pandolaimus	2B	0.00	1.18	0.00	0.00	1.20	0.00	0.00	1.16	0.00	0.00
Paracanthonchus	2A	0.00	1.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Parachromadorita	2A	2.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Paracomesoma	2A	0.00	1.18	0.00	0.00	0.00	1.23	0.00	0.00	0.00	2.22
Paralinhomoeus	1B	0.00	0.00	0.00	0.00	0.00	0.00	1.02	0.00	0.00	0.00
Paralongicyatholaimus	2A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11
Paramesonchium	2A	0.00	0.00	0.00	0.00	0.00	1.23	0.00	0.00	1.28	0.00
Paramonohystera	1B	1.14	0.00	0.00	0.00	1.20	0.00	0.00	0.00	0.00	3.33
Paraxystomina	1A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.16	0.00	0.00
Parironus	2A	0.00	0.00	0.00	5.32	0.00	2.47	2.04	0.00	6.41	2.22
Phanodermopsis	2A	7.95	0.00	2.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pheronus	2B	0.00	0.00	0.00	2.13	0.00	0.00	0.00	0.00	0.00	0.00
Pierrickia	1A	1.14	1.18	8.99	11.70	12.05	29.63	4.08	13.95	25.64	10.00
Polysigma	2A	2.27	12.94	0.00	0.00	4.82	0.00	0.00	0.00	0.00	0.00
Prochaetosoma	1A	0.00	0.00	0.00	1.06	2.41	0.00	1.02	0.00	0.00	0.00
Promonhystera	1B	1.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00
Prooncholaimus	2B	1.14	1.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pselionema	1A	0.00	1.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ptycholaimellus	2A	11.36	7.06	6.74	4.26	6.02	1.23	1.02	4.65	1.28	1.11
Quadricoma	1A	0.00	0.00	1.12	0.00	3.61	0.00	0.00	0.00	0.00	0.00
Sabatieria	1B	0.00	5.88	1.12	4.26	2.41	18.52	1.02	12.79	11.54	4.44
Setoplectus	1A	0.00	0.00	2.25	0.00	0.00	0.00	0.00	0.00	0.00	1.11
Setosabatieria	1B	18.18	2.35	2.25	11.70	12.05	0.00	30.61	0.00	3.85	0.00
Sigmophoranema	2B	0.00	2.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Southernia	1A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11
Southerniella	1A	0.00	0.00	0.00	0.00	0.00	0.00	1.02	0.00	0.00	1.11
Sphaerolaimus	2B	3.41	3.53	2.25	0.00	0.00	0.00	2.04	0.00	0.00	0.00
Spilophorella	2A	0.00	0.00	0.00	0.00	1.20	0.00	2.04	0.00	0.00	0.00
Steineria	1B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11

Appendix (*continued*)

Nematode genera	Trophic group	July		August		September		October		February	
		Control	Cage	Control	Cage	Control	Cage	Control	Cage	Control	Cage
Steineridora	2A	0.00	2.35	6.74	1.06	4.82	0.00	1.02	1.16	0.00	0.00
Stylosteristus	1B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.67
Symplocostoma	2B	0.00	1.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Syringolaimus	2B	0.00	0.00	0.00	0.00	0.00	1.23	0.00	0.00	0.00	0.00
Terschellingia	1A	0.00	0.00	0.00	0.00	0.00	0.00	1.02	0.00	0.00	1.11
Thalassironus	2B	0.00	0.00	0.00	2.13	1.20	1.23	2.04	1.16	0.00	1.11
Tricoma	1A	0.00	1.18	1.12	0.00	0.00	0.00	0.00	1.16	0.00	0.00
Trissonchulus	2B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.16	0.00	0.00
Trochamus	2A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.16	0.00	0.00
Vasostoma	1A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.16	1.28	0.00
Viscosia	2B	0.00	2.35	0.00	0.00	0.00	0.00	1.02	0.00	0.00	0.00
Wieseria	1A	0.00	0.00	0.00	0.00	1.20	0.00	1.02	1.16	0.00	0.00

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