Free-living nematode assemblages downstream from a pulp and paper mill in Humber Arm, Newfoundland

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Summary. The composition of benthic nematode populations was studied at two depths at each of three sites 0.8, 2.0 and 4.0 km downstream from a pulp and paper mill situated at the head of the Humber Arm, Corner Brook. Nematodes from 29 genera were identified. Extremely high relative abundance of *Monhystera* was observed at both depths at the site (DeGrouchy Point) closest to the mill. At the other two locations (Church Cove and Birchy Point), population compositions were found to differ both between each location and between depths. Population diversity, evenness and richness values decreased with increasing proximity to the mill. The Maturity Indices at Church Cove and Birchy Point were similar, and both were higher than those at DeGrouchy Point. Indications were that moderate amounts of purely organic detritus associated with sewage outfall had a somewhat positive impact on the population measures such as diversity and richness, while the opposite was true for the mill effluent which contained chemical pollutants in addition to organic waste.
Key words: pulp and paper mill, effluent, sewage, depth, population diversity, evenness, richness, Maturity Index.

Aquatic free-living nematodes constitute a large and very important portion of the benthic fauna, usually comprising upwards of 90% of the total meiofauna in numerical abundance (Heip *et al.*, 1982). However, in the Province of Newfoundland and Labrador only Allgén (1957) has provided information on the benthic nematode populations; 10 species from Newfoundland and 12 from Labrador collected during the Swedish Antarctic Expedition (1901-1903).

Nematodes have the potential of being an important group of environmental indicators (Samoiloff, 1986) because of their nearly ubiquitous distribution and high numerical densities. Even under conditions where environmental disturbance is not a factor, persistent patterns in nematode populations could provide valuable information about the state of the local environment. For example, certain distribution patterns are characteristic of nematode populations along a horizontal gradient. On a scale of a few centimetres, some species aggregation is usually noted but this variability becomes less important with larger scales (Heip *et al.*, 1985). Characteristic patterns are also commonplace with vertical distribution where most nematodes usually occur in the upper 2-3 cm of sediment (Heip *et al.*, 1985). In addition, vertical migration associated with changes in season, temperature, salinity and exposure to wave and tide action produce an interactive relationship between the benthic meiofauna and the environment under normal conditions (Heip *et al.*, 1985). Changes in such characteristic patterns could therefore indicate changes taking place in the environment.

Humber Arm on Newfoundland's west coast is an inlet of brackish water which receives significant amounts of effluent from Corner Brook Pulp and Paper Mill, a thermomechanical sulphite mill located at the head of the Arm in the town of Corner Brook. The situation at Humber Arm could be compared to several other sites like the Saltkallefjord fjordic estuary in Sweden (Leppäkoski, 1968) and the sea-loch system of Lochs Eil and Linhe in Scotland (Pearson, 1971, 1972) where large amounts of organic detritus and toxic chemicals from mills collected in the sediments, resulting in lowered benthic faunal diversity, species evenness and richness values. In this research the population diversity, evenness and richness values and Maturity Indices were compared for populations at two depths and three sites downstream from the pulp and paper mill at Humber Arm.

MATERIALS AND METHODS

Samples were taken in September, 1993, at three locations along the Humber Arm, Corner Brook, one year after the installation of a dissolved air flotation (DAF) clarifier at the pulp and paper mill. For the previous 68 years, the mill had operated without this apparatus. The locations were DeGrouchy Point, 0.8 km downstream from the mill effluent discharge point, Church Cove, 2.0 km downstream from the mill and Birchy Point about 4.0 km downstream (Fig. 1). Apart from the pulp mill, a sewage outfall was present at 8-10 m depth near Birchy Point.

At each location two sediment samples were taken at each of two water depths: 0 m and 8-10 m (except at DeGrouchy Point where only a single sample was collected at 0 m). The sediment was a mixture of sand, gravel and small rocks (Table 1). Large amounts of pulp fibre formed a conspicuous layer on the top of both 8-10 m samples from DeGrouchy Point and to a lesser extent from the same depth at Church Cove. Sewage was apparent in the 8-10 m sample at Birchy Point.

Water speed at all locations was 0.2 m/s or less while all temperatures were 5 °C. All three locations were considered to be very sheltered (Exposure ranking = 1 following the Department of Fisheries & Oceans Coastal Classification Atlas, Canada).

Each sample was collected in a 1 litre glass jar. The mouth of the jar was positioned within the top 2-3 cm of sediment surface and gradually pushed horizontally until one-third of the jar was filled with sediment, then 5% formalin-seawater was added. The volume of sediment in each sample was measured.

The extraction process used was a modified version of that developed by Schwinghamer *et al.*, (1991). Samples were first centrifuged to separate the supernatant from the sediment; the supernatant was saved for further filtration. The sediment was mixed with a predetermined amount (which varied according to the sediment volume of each sample) of Nalco 1060 colloidal suspension and centrifuged again. Both supernatants were then subjected to suction filtration (aperature size 15 μm) and the filtrate washed with a 10% formalin in seawater solution into scintillation vials and stained with Rose-Bengal (0.15g/L)

Nematodes were prepared for mounting according to the glycerol-ethanol method (Seinhorst, 1959) and were identified using keys by Platt & Warwick (1983, 1988) and Tarjan (1980). Nematodes from each sample were counted and assigned to feeding groups (Wieser, 1959). These counts of nematodes per sample were used to calculate relative abundance and common abundance values for faunal similarity (Wieser, 1960). The Shannon Index (H') and the Simpson Index (1/D) were used as measures of diversity (Magurran, 1988). Evenness (E) (Pielou, 1969) and Species Richness (SR) were expressed according to Margalef (Clifford & Stephenson, 1975). The significance of differences between locations and depths were determined by two-way analysis of variance (ANOVA). The Maturity Index (MI) was calculated based on c-p values (Bongers, 1990; Bongers *et al.*, 1991, 1995).

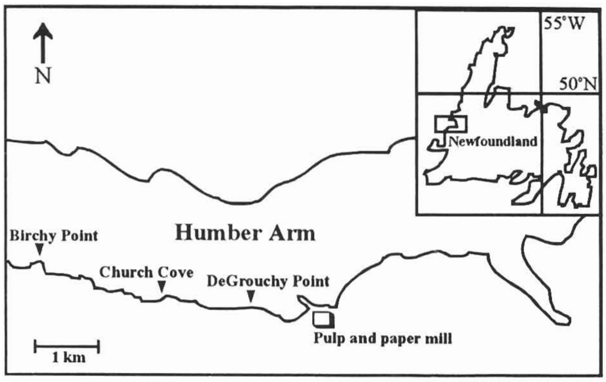

Fig. 1. Locations of the three sampling areas along Humber Arm in relation to Corner Brook Pulp and Paper Mill.

RESULTS

Population counts

The number of nematodes collected from all samples totalled 17 423 and values for individual samples ranged from 409 to 2934 (Table 1). Densities were lowest amongst samples collected at 8-10 m depth near Church Cove and Birchy Point.

Nematode assemblages

Genera. A total of 29 genera were identified from the Humber Arm (Table 2), 17 of which were first-time records for Newfoundland. At 0 m depth at Birchy Point (Fig. 2) the most abundant genera in the nematode fauna collected were members of the family Linhomoeidae particularly *Eleutherolaimus* (21.9%) and *Desmolaimus* (21.4%). Other abundant genera were *Odontophora* (15.8%), *Chromadora* (13.3%) and *Metalinhomoeus* (9.5%). At 8-10 m in the same area (Fig. 2) the nematode fauna consisted largely of *Odontophora* (24.7%), *Enoplolaimus* (12.0%) and *Metalinhomoeus* (10.7%).

At 0 m depth at Church Cove (Fig. 2) the nematode fauna was dominated by the genus *Microlaimus* (34.6%). *Hypodontolaimus* (19.0%), *Odontophora* (16.6%) and *Eleutherolaimus* (11.4%) were

Table 1. Summary of location, sediment type, depth, total number of nematodes per sample, Shannon Index (H'), Evenness (E), Species Richness (SR), Simpson Index (1/D) and Maturity Index (MI) values for all samples collected at Humber Arm.

Location/ sediment	Depth	Total number of nematodes/sample	H'	E	SR	1/D	MI
Birchy Point							
Sand*, gravel**	0 m	1320	2.09	0.69	2.78	6.73	2.23
	0 m	2892	2.09	0.71	2.26	6.30	2.19
Sand, gravel, sewage	8-10 m	639	2.17	0.80	2.17	8.95	2.39
	8-10 m	1126	2.40	0.83	2.42	8.32	2.42
Church Cove							
Sand, gravel	0 m	2934	1.78	0.64	1.88	4.41	2.41
	0 m	1620	2.00	0.68	2.44	5.78	2.42
Sand, gravel, small	8-10 m	409	1.63	0.62	2.16	3.59	2.27
rock, pulp fiber	8-10 m	567	2.03	0.73	2.37	5.99	2.31
DeGrouchy Point							
Sand, gravel	0 m	1549	0.58	0.22	1.63	1.33	2.09
Sand, blanket of pulp	8-10 m	2393	0.07	0.03	0.77	1.02	2.00
fiber	8-10 m	1974	0.14	0.06	1.19	1.05	2.00

* 0.06-1.9 mm; ** 2.0-6.00 mm.

Table 2. List of genera present in samples from Humber Arm (x, present; –, absent from the sample).

Key	Genera	Site					
		Birchy Point		Church Cove		DeGrouchy Point	
		0 m	8-10 m	0 m	8-10 m	0 m	8-10 m
1	*Anticoma*	X	–	–	–	X	X
2	*Axonolaimus**	X	X	X	–	X	X
3	*Bathylaimus*	X	–	–	X	–	–
4	*Chromadora*	X	X	X	X	X	X
5	*Desmolaimus**	X	X	X	X	–	–
6	*Eleutherolaimus**	X	X	X	X	X	X
7	*Enoplolaimus*	X	X	X	–	–	–
8	*Enoplus*	X	X	–	–	–	–
9	*Euchromadora**	–	–	–	X	–	–
10	*Halalaimus**	X	X	X	X	–	–
11	*Hypodontolaimus**	X	X	X	–	–	–
12	*Leptolaimus**	X	X	–	X	–	–
13	*Linhystera**	X	–	X	–	X	X
14	*Metalinhomoeus**	X	X	X	X	–	–
15	*Microlaimus*	–	–	X	X	X	–
16	*Monhystera**	–	X	X	X	X	X
17	*Monoposthia*	X	X	X	X	–	–
18	*Odontophora*	X	X	X	X	X	–
19	*Oncholaimus**	–	–	–	–	X	X
20	*Paracanthonchus*	X	X	X	X	X	X
21	*Parapinnanema**	X	X	X	X	–	X
22	*Platycoma**	–	X	–	–	–	–
23	*Richtersia**	X	–	–	–	–	–
24	*Sabatieria*	X	X	X	X	X	X
25	*Sphaerolaimus**	X	X	X	–	–	–
26	*Terschellingia**	X	X	X	–	–	–
27	*Theristus*	X	X	X	X	X	X
28	*Tripyloides**	–	–	X	X	–	–
29	*Viscosia*	X	–	–	–	X	–

* genera recorded for the first time in Newfoundland from sediment.

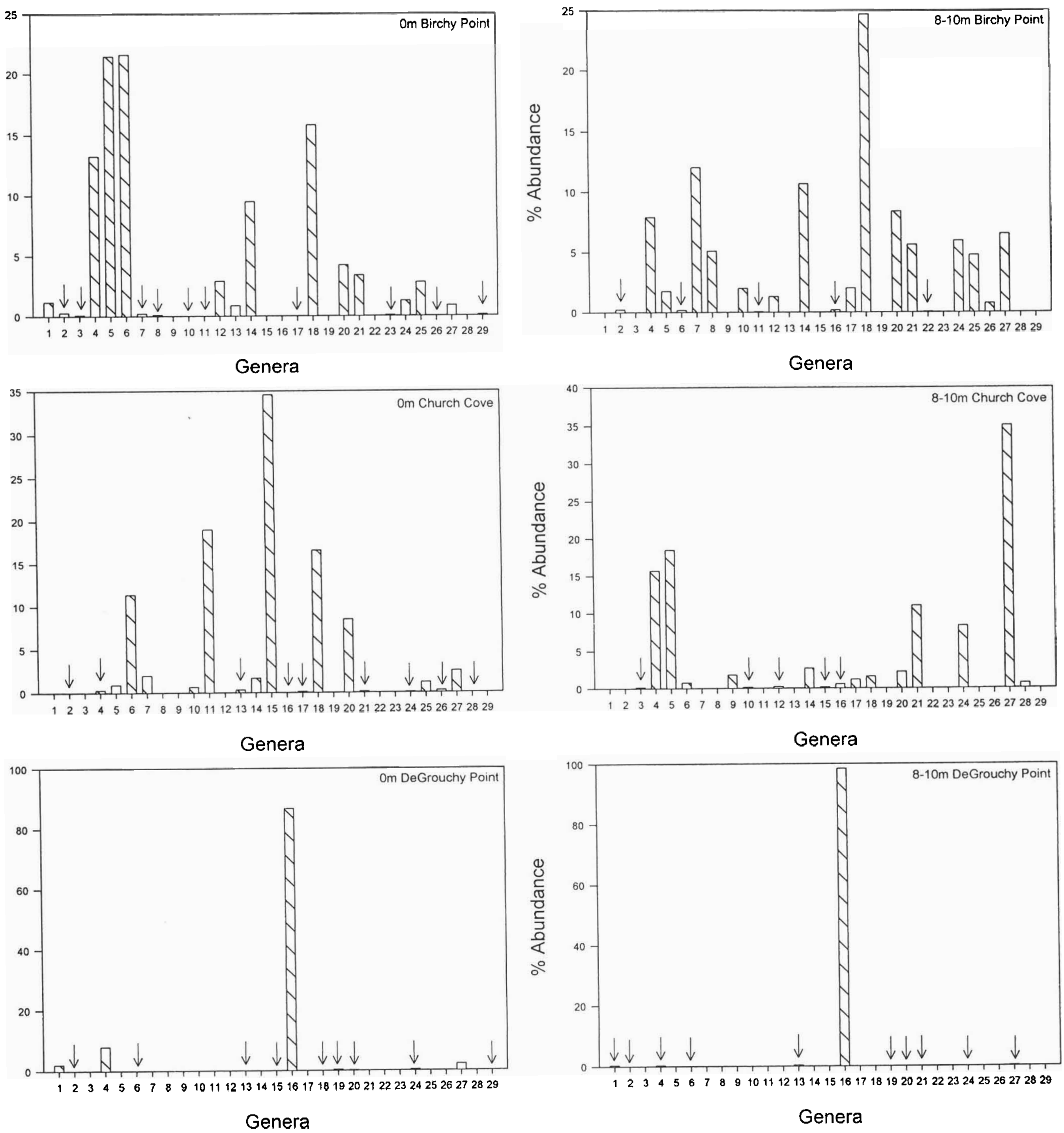

Fig. 2. Relative abundance of different genera from the total population of nematodes at each depth and each site on the Humber Arm. (For key to genera, see Table 2).

also present in abundance. At 8-10 m depth in the same area (Fig. 2), *Theristus* (35.0%) was the dominant genus followed by *Desmolaimus* (18.4%), *Chromadora* (15.7%) and *Parapinnanema* (11.0%).

The nematode fauna at both depths at DeGrouchy Point (Fig. 2) consisted almost entirely of *Monhystera* with 86.5% from the 0 m depth sample and 98.4% from 8-10 m depth samples. Other genera

			Birchy Point				Church Cove				DeGrouchy Point		
			0 m		8-10 m		0 m		8-10 m		0 m	8-10 m	
			1	2	1	2	1	2	1	2	1	1	2
Birchy Point	0 m	1	X	76.1	53.35	37.81	34.92	37.65	47.15	47.83	12.01	0.92	2.07
		2		X	43.15	46.06	36.46	37.39	34.95	48.29	10.26	0.92	1.76
	8-10 m	1			X	68.52	29.11	32.70	26.54	30.87	11.24	0.75	0.40
		2				X	31.58	32.70	34.11	41.77	8.46	0.79	0.70
Church Cove	0 m	1					X	89.95	8.10	10.07	3.14	0.43	0.66
		2						X	9.10	12.99	3.50	0.91	1.04
	8-10 m	1							X	73.17	11.68	1.69	1.63
		2								X	11.01	0.93	0.88
DeGrouchy Point	0 m	1									X	87.43	88.04
	8-10 m	1										X	98.09
		2											X

Fig. 3. Trellis Diagram depicting the degree of fauna similarity between the three sampling areas.
>50% 25-50% <25%

common to both depths here were *Chromadora, Anticoma* and *Theristus.*

Faunal similarity. Faunal similarities were determined according to common abundance values (Wieser, 1960). First, the abundance of each genus was expressed as a percentage of the total number of genera in that sample. The common abundance value was then taken as the smaller of the two values common to each pair of samples. The sum of the common abundance values for each pair was then presented in a "Trellis Diagram" (Fig. 3). Figure 3 shows that Birchy Point and Church Cove had between 25-50% of their fauna in common. However, there was little in common (<25%) between the fauna at DeGrouchy Point with either of the other two areas.

A comparison between depths at each location revealed that depth influenced faunal composition. The fauna at the two depths at Birchy Point showed only moderate (25-50%) similarity, while at Church Cove less than 25% of the fauna was common to the two depths. At DeGrouchy Point there was more homogeneity (>50%) between faunas at the two depths.

Population diversity, Evenness, Species Richness and Maturity Index

Both the Shannon (H') and Simpson (1/D) measures of diversity revealed similar patterns (Table 1). Population diversity was greatest in the samples from Birchy Point and lowest in those from DeGrouchy Point. Two-way ANOVAs were used to test the effects of location, depth and their interaction. These tests revealed the significant influence of location ($p<0.001$ for H' and $p=0.001$ for 1/D) but not depth ($p=0.326$ for H' and $p=0.415$ for 1/D) or interaction ($p=0.127$ for H' and $p=0.202$ for 1/D) on faunal diversity. Residuals indicated normality and acceptability of the model.

Measures of Evenness (E) and Species Richness (SR) followed diversity measures with the greatest values observed for the Birchy Point location followed, in turn, by Church Cove and DeGrouchy Point. Two-way ANOVA tests on Species Richness had results similar to diversity measures with only location having a significant effect ($p=0.012$). Such was not the case however with Evenness where both location ($p<0.001$) and the interaction were significant ($p=0.017$). A plot of residuals again indicated normality for these models.

The Maturity Indices were higher at both depths at Birchy Cove and Church Cove than at 0 m and 8-10 m depths at DeGrouchy Point. At each site the Maturity Indices varied slightly between depths.

DISCUSSION

That local nematodes have been little studied explains why 17 of the 29 genera identified from Humber Arm were first-time records for the Province, Allgén (1957) being the only previous study.

The relatively low values observed for nematode faunal diversity, evenness, richness and MI at both depths at DeGrouchy Point suggest that the environment in this area had been disturbed. The difference as indicated by MI between this area and the other two locations provided further evidence for this. Located a mere 0.8 km from the mill effluent discharge, DeGrouchy Point had been receiving mill wastes for the 69 years since the construction of the mill. It is reasonable to assume that the mill effluent played a crucial role in shaping the local nematode population composition. This is especially true with wood wastes such as pulp fibre which, when present in large quantities, have previously been found to result in low diversity, evenness and richness values in a range of invertebrates (Kathman *et al.*, 1984). The conspicuous blanket of pulp fibre observed on the top of samples from the 8-10 m depth in this area confirmed the impact of such wastes.

In comparison with DeGrouchy Point, the nematode fauna at both depths at Church Cove and Birchy Point had higher diversity, evenness, richness and MI values. This, in addition to the high degree of faunal similarity between Church Cove and Birchy Point, suggested that the pulp mill effluent had less impact on the fauna at these two locations.

The high values for diversity and evenness observed at 8-10 m depth at Birchy Point appear to reflect the sewage outfall in the vicinity. Organic enrichment with moderate amounts of detritus and sewage have been shown to enhance benthic faunal colonization (Pearson, 1978). Although organic matter comprises a major portion of most pulp mill effluent, no such enhancement of fauna was seen at DeGrouchy Point probably because the nature of the chemical discharge, along with organic fibres, offsets the "benefits" of organic enrichment.

Comparison of the population composition between depths at the locations also revealed some patterns. No difference was noted between the nematode populations at 0 m and 8-10 m depths at DeGrouchy Point as shown in Figure 3. The environment in this area has been so greatly disturbed that only a very narrow range of taxa were capable of exploiting this habitat. This was not the case with the other two locations. The faunal compositions differed between depths at these two locations, but this was not reflected in the diversity index values. This shows an important weakness in using diversity indices for ecological surveys and calls for caution when attempting to make inferences from such indices. That the populations at the two depths at Birchy Point had between 25 and 50% of their nematodes in common suggests some degree of faunal variation associated with vertical distribution. Such variation appears to be a common occurrence with nematode fauna especially in relation to seasonal changes and temperature fluctuations (Heip *et al.*, 1982). At Church Cove the striking differences in population composition and in overall numerical density between the two depths might be accounted for by the very different sediment types found at each depth. The composition of nematode populations has been found to differ significantly according to sediment type, particularly according to grain size and silt-clay content (Wieser 1960; Tietjen 1977; Heip *et al.*, 1985). Heip *et al.*, (1985) noted the increased prevalence of certain families with increasing grain size. The abundance of *Theristus, Chromadora* and *Parapinnanema* at 8-10 m depth with large sediment grain size conforms to this idea.

In studies on benthic macrofauna at pulp-mill polluted sites in a Swedish fjordic estuary (Leppäkoski, 1968; Pearson, 1978), a Scottish loch (Pearson, 1971, 1972) and Humber Arm, similarities can be seen between the apparent effects of mill effluent on faunal composition. In all these studies there were sites at various distances from the source of pollutants that included organics like wood pulp fibre and lignins as well as an assortment of chemicals. The nematode populations at DeGrouchy Point are dominated by a few opportunistic species and genera typical of sites in close proximity to the point of effluent discharge.

One example is *Monhystera* which is overwhelmingly dominant at DeGrouchy Point. *Monhystera* is known to thrive under environmentally stressed conditions (Bongers & Van De Haar, 1990), such as those imposed by pulp-mill discharge. The major stress conditions associated with such areas are an increased biological oxygen demand (BOD) and a redox potential discontinuity (RPD) layer that approaches the surface (Pearson, 1978). These stresses are caused by the increased dissolved organic content of the discharge and the suspended solids (Pearson, 1972) as well as the microorganisms associated with the anaerobic decomposition of these organics (Edde, 1984). The result is a reduced oxygen concentration in the water and a reduced number of niches available for benthic fauna. Populations may also be affected by direct toxicity effects from the chemical effluents commonly discharged from a sulphite mill such as the Corner Brook one (prior to 1992 when it was converted from a sulphite to a nonchlorinated thermomechanical pulping mill). These chemicals usually include fungicides and algicides as well as sulphite waste liquor. Many of these have been shown (Pearson, 1972) to have deleterious effects on the physiology and reproduction of fishes and oysters, or are known to have extremely slow degradation rates in the environment. They could possibly build up in the food chain, of which benthic nematodes are an important part.

Apart from *Monhystera,* other nematode genera commonly associated with anaerobic conditions such as *Sabatieria* and *Terschellingia* (Bongers and Van De Haar, 1990) were not present at DeGrouchy Point in significant numbers. *Sabatieria pulchra* (Schneider, 1906), the species of *Sabatieria* found in Humber Arm, is reported in Tietjen's (1980) study of the New York Bight Apex to have been highly abundant in contaminated medium-coarse sands. This species has also been reported to be common in anoxic waters where sulphur reducing bacteria are abundant (Bouwman, 1978). The fact that *S. pulchra* did not make up a significant portion of the fauna at DeGrouchy Point could be related to the occurrence of sulphur reducing bacteria in low abundance. Another possible explanation may be sediment type. This species, although found in contaminated medium-coarse sands by Tietjen (1980), is characteristically a mud endemic species (Tietjen, 1969, 1980; Wieser, 1960) and may therefore not have been present here initially in very high numbers. *Terschellingia communis* (De Man, 1888), another stress-tolerant inhabitant of anaerobic conditions (De Winter, 1988), was not found in Humber Arm, however *T. longissimicaudata* (Timm, 1962) was found, but because little is known about the biology of these two species few if any inferences can be made.

The quantities of organic material deposited in the form of pulp fibre at DeGrouchy Point and sewage input at Birchy Point explain why deposit feeders were dominant at almost all locations. The success of deposit feeders could have come as a result of direct feeding on organic particles or through feeding on the bacteria which thrive on organic enrichment. The observed success may also be attributable in part to the season: samples were collected in the fall, a time when deposit feeders are known to exhibit maximum densities due to increased organic detritus from dead and decaying vegetation from benthic and terrestrial systems (Tietjen, 1969).

The marked difference in genera which represented the dominant deposit feeders at the three locations of the present study might be interpreted as a reflection of their tolerance to pollution. Should this be true, then genera such as *Eleutherolaimus* and *Desmolaimus* which dominated at Birchy Point, but were gradually replaced by genera such as *Theristus* and *Monhystera* at DeGrouchy Point, might be perceived as being less tolerant to high stress.

The reported dominance of the omnivore/predator group at the 8-10 m depth at Birchy Point was due to the large numbers of *Odontophora* spp. These were here labelled omnivores/predators based on the morphology of their buccal cavity, but other authors such as Tietjen (1969) have labelled them as deposit feeders. Wieser (1960) cautioned that some benthic species which have been labelled "predators" may actually act as deposit feeders most of the time. If this is true, then it would appear that the deposit feeders were the dominant feeding group for both depths at Birchy Point.

However, the dominance of the epigrowth feeders at the 0 m depth at Church Cove cannot be similarly explained. An explanation here perhaps might lie in the presence of a richer epifauna (hydrozoa, bryozoa and algae) associated with the large rocks and pebbles found at this depth.

Although it would appear that the effluents from Corner Brook Pulp and Paper Mill and organic material from the sewage outfall near Birchy Point were the major factors, influences other than pollution may have played a significant role in shaping the nematode population composition found in the present samples from Humber Arm. These factors include seasonal fluctuations, temperature, salinity, normal fluctuations in life cycles, relative amounts of exposure to wave and tidal action, horizontal distribution and vertical migration patterns. Therefore, future studies involving marine benthos in relation to environmental perturbation should attempt to assess the role of these factors. Nevertheless, the use of nematodes as environmental indicators remains an important and ever-expanding area with much potential. Nematodes have already been used not only in instances of organic and sewage pollution (Raffaelli & Mason, 1981) but also in cases involving heavy metals (Tietjen, 1977, 1980) and oil pollutants (Samoiloff, 1986).

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Yeow H.-W., Finney-Crawley J.R., Lawlor J., King I. Wm. Сообщества свободноживуших нематод в Хамбер Арм, Ньюфаундленд, ниже по течению от целлюлозно-бумажного комбината.

Резюме. Исследовали состав бентосных нематодных сообществ на двух глубинах, на расстоянии 0.8, 2.0 и 4.0 км от целлюлозно-бумажного комбината (ЦБК) в верхней части эстуария Хамбер Арм в Корнер Брук. Были обнаружены нематоды, относящиеся к 29 видам. Весьма высокая относительная встречаемость видов из рода *Monhystera* наблюдалась на двух глубинах в ближайшей от ЦБК точке (Де Груши Пойнт). На двух других точках (Черч Коув и Берчи Пойнт) состав нематодных сообществ различался как между глубинами, так и между самими точками наблюдений. Значения параметров, характеризующих таксономическое разнообразие и богатство нематодных сообществ, снижались по мере приближения к ЦБК. Индексы зрелости сообщества в Черч Коув и Берчи Пойнт были сходны и превышали таковой в Де Груши Пойнт. Показано, что умеренные количества органического детрита в выбросах ЦБК оказывали определенное положительное воздействие на такие популяционные показатели, как разнообразие и общее богатство нематодных сообществ, тогда как отрицательное воздействие на эти параметры было отмечено при сбросе вместе с органикой химических отходов.