

FREE-LIVING NEMATODE ASSEMBLAGES IN  
VARIOUS ENVIRONMENTALLY  
IMPACTED MARINE SITES

CENTRE FOR NEWFOUNDLAND STUDIES

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0-612-73653-9

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Free-living Nematode Assemblages in Various Environmentally Impacted Marine Sites

by

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A thesis submitted to the  
School of Graduate Studies  
in partial fulfilment of the  
requirements for the degree of  
Master of Science

Department of Biology

Memorial University of Newfoundland

April 2001

St. John's, Newfoundland

## **Abstract**

This study considers the composition of nematode assemblages in marine sites receiving discharges from an oil refinery in Come-By-Chance, ones supporting aquaculture and ones that are affected by other forms of human activity in Bay d'Espoir. Changes in nematode population were recorded with respect to season, and in the case of the aquaculture sites comparisons were drawn with non-aquaculture locations. The Maturity Index was calculated together with the Shannon-Weaver, Simpson, Evenness and Species Richness Indices. A suite of environmental conditions (sediment size, temperature, depth, pH and redox potential) in the aquaculture sites was compared to that in non-aquaculture ones.

The importance of integrating absolute numbers and genera collected, feeding type analysis, diversity indices, Evenness, Species Richness, Maturity Index (MI) and c-p analysis was indicated at both study locations. The utility of smaller samples or sub-samples of larger samples was demonstrated (Chapter 2), as was the importance of having a complete data set (Chapter 3).

The identity of nematode communities have been established for these areas for the first time. Both sampled sites near the Come-By-Chance Oil Refinery had relatively high numbers of nematodes and many of the species have been previously associated with oil spills (Chapter 2). In comparison, the samples from Bay d'Espoir had relatively fewer nematodes and in general the aquaculture sites had even fewer nematodes per sample than the non-aquaculture sites in the same region. No nematodes were isolated from forty per



cent of the aquaculture samples (Chapter 3).

For the MI calculation fewer nematodes were required which need only be identified to family. That is, an entire sample need not be examined to yield statistically similar MI values. This gets results faster, leaving more time for a more comprehensive analysis. The advantages and disadvantages of all above mentioned indices are discussed (Chapter 4).

In addition, there was one nematode that occurred several times in Bay d'Espoir samples that could not be identified to genus. The nematode belongs to the family *Comesomatidae*.

## **Acknowledgments**

I extend my sincere gratitude and appreciation to Dr. Jean Finney-Crawley, without her this thesis would not be possible. I have been fortunate enough to have had her as a professor, employer and supervisor, and she has constantly provided encouragement, support and understanding. She had a lot of patience with me, and gave freely of her time. Dr. Finney-Crawley has been enormously empathetic to my many personal problems over the last few years, I can not express how much she means to me. I could not have asked for a better supervisor. Dr. Finney-Crawley has been my academic mentor, friend and confidante - she will always hold a special place in my heart.

Special thanks go to Dr. Goverdina Fahraeus-Van Ree and Dr. Roger Pickavance who sat on my Supervisory Committee. I thank you for your time and critical review of my research.

Thanks is sent to Dr. Michael Tlusty and the Newfoundland Salmonid Growers Association for sampling in Bay d'Espoir and providing laboratory supplies. Dr. Gerry Payne, Department of Fisheries and Oceans is also thanked for sampling in Come-By-Chance.

Thanks is sent to Roy Ficken for his photography assistance and Dr. David Schneider for his statistical advice.

It is acknowledged that funding was provided by Atlantic Accord Career Development through the Government of Newfoundland and Labrador.

I must acknowledge the Biology Department and the many smiling faces that

made everyday complete. The people there became a second family for me. Special thanks go to Christine, Gail, Shena, Shirley and Wendy for providing an ear when I needed it, and patience to answer all my questions.

I am grateful to Dr. Michael Collins for providing me the opportunity to assist him with Biology 2040/2041 throughout my graduate program. The knowledge I have gained from him will certainly enhance any future teaching profession.

I am also indebted to my fellow colleagues Sean Clancy, Ian King, Janet Rideout, and Valerie Power for all of their help.

My acknowledgements would not be complete without mentioning my family. They have been incredible throughout my university career. I was blessed to have two wonderful parents who from as early as I can remember encouraged me to do the best and be the best that I could be. They provided constant support and understanding, especially when times got rough. I can only wish that all children could be as fortunate as I and have parents that instill confidence and integrity in them, while providing security. I consider myself an extremely lucky person. I also thank my sisters, brothers, in-laws, nieces and nephews for just being there when I need you. I thank my Grandmother and Guardian Angel, Hannah Lawlor, for watching over me, protecting and guiding me along the way. At last I thank my niece, Erin, who has shown me what true courage and strength really is!

Thank You

Jennifer

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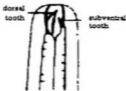
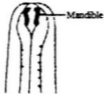
## Chapter 1: Background

Nematodes are a diverse group of organisms which live in a variety of habitats. There are both parasitic and free-living species. Parasitic forms are found in both plants and animals, including humans. They are serious pests in the agricultural industry, vectors of soil-borne viruses (Heip *et al.*, 1985), and possible biological control agents for other pests. Consequently, most nematode research has focussed on economically important parasitic species and one free-living species, *Caenorhabditis elegans*, which has been vital in genetic studies. In comparison, free-living species, especially those in the marine environment, are relatively unstudied (Heip *et al.*, 1985).

Free-living nematodes inhabit soil, freshwater, marine, and estuarine environments. Within the marine environment nematodes play a major role in sediment economy (Platt *et al.*, 1984). They absorb dissolved organic compounds, consume fungi and other organisms, parasitize higher plants, regenerate nutrients, influence sediment texture by their mucus secretion, improve gas diffusion and serve as food for other organisms including predatory nematodes (Bongers and Van de Haar, 1990).

The feeding behaviour and ecology of free-living aquatic nematodes has been studied by Wieser (1959)(Figure 1). He identified four feeding categories: 97 genera of selective deposit feeders (1A), characterized by the lack of or a very small buccal cavity, which feed on bacteria and other finely suspended particles; 73 genera of unselective deposit feeders (1B), characterized by cup-shaped, conical or wide cylindrical buccal cavities and an absence of teeth, capable of feeding on diatoms, organic matter or other

Figure 1: Feeding types according to Wieser (1959).

Feeding Type		Description
1A		Without or with a minute buccal cavity; Selective deposit feeders (Bacteria, diatoms, etc)
1B		With unarmed buccal cavity; Non-selective deposit feeders (Organic detritus)
2A		With small teeth or denticles; Epigrowth feeders (Algae, bacterial mats)
2B		With powerful dentition and musculature; Omnivores / predators (Polychetes, other nematodes)

large particles; 104 genera of epigrowth feeders (2A), with buccal cavities containing teeth, rods or plates, which scrape food from surfaces or puncturing algal cells, and 87 genera of omnivores/predators (2B), with large buccal cavities containing powerful teeth or plates that either swallow prey whole or puncture it and suck the contents (Wieser, 1959). Epigrowth feeders reach their maximum density during spring and summer, while deposit feeders and omnivores reach their maximum densities during fall and winter (Tietjen, 1969).

Nematode faunal assemblages offer prospects for indicating disturbance and assessing the quality of sediments for the following reasons. Nematodes are abundant and often have high species diversity (Bongers and Van de Haar, 1990). Nematode density, for example, can range from  $0.82 \times 10^6$  to  $4.8 \times 10^6$  per square metre of sediment, with 41 to over 100 species (Ferris and Ferris, 1979). Nematode dominance was illustrated in Buzzards Bay, Massachusetts, where nematodes dominated all the samples taken, constituting 89 to 99 % of each sample by dry weight (Wieser, 1960). Nematodes are found under all environmental conditions that can support metazoan organisms. They are permanent members of the benthos and therefore unable to escape from bottom pollution effects (Bongers and Van de Haar, 1990). In particular, nematodes offer a potential means of assessing sediment quality in the frequent cases where the pollution level in water and sediment is not evenly balanced (Bongers *et al.*, 1991).

Under stable conditions the reproductive strategy of nematodes results in a stable population, therefore any changes in a nematode community can be attributed to



environmental disturbance (Platt *et al.*, 1984). Nematodes have a relatively short generation time, and can therefore react rapidly to disturbance. In comparison to other potential biological indicators, nematodes are easy to collect and only small sample sizes are required (Platt *et al.*, 1984).

Free-living aquatic nematodes are a group with diverse biology (Bongers *et al.*, 1991). In general, they have the ability to withstand environmental disturbances better than other fauna, with the result that nematodes are still present after the other macrofauna has disappeared (Bongers *et al.*, 1991). Several species can withstand anaerobic conditions. For example, *Pontonema vulgare*, *Sabatieria* species and *Monhystera* species are tolerant of oxygen stressed conditions (Bongers and Van de Haar, 1990).

Using any organism as an environmental indicator will involve statistical analysis. The diversity measure most used by marine biologists is the Shannon-Wiener (also known as Shannon-Weaver) Index (Platt *et al.*, 1984). The Shannon Index ( $H'$ ) is calculated using the following formula:

$$H' = - \sum_{i=1}^k p_i \log p_i \quad (1)$$

where  $p_i$  is the proportion of observations in category  $i$  or in other words the number of observations in category  $i$  ( $f_i$ ) divided by the sample size ( $n$ ), and  $k$  is the number of

categories (Zar, 1999). Other frequently used measures include the Simpson Diversity Index, Species Richness and Evenness. The Simpson Index is expressed as  $1/D$ , where  $D$  is calculated using the following equation:

$$D = \sum [(n_i (n_i - 1)) / (N(N-1))], \quad (2)$$

where  $n_i$  represents “the number of individuals in the  $i^{\text{th}}$  genus” and  $N$  is “the total number of individuals” (Magurran, 1988). As the value of  $D$  increases, diversity decreases (Magurran, 1988). Evenness is calculated using the following formula:

$$J' = H' / H'_{\max}, \quad (3)$$

where  $H'_{\max} = \log k$  and  $k$  is the number of categories (Zar, 1999). Species Richness is calculated using the following formula, Margalef's Diversity Index ( $D_{mg}$ )

$$D_{mg} = (S-1) / \ln N, \quad (4)$$

where  $S$  is “the number of genera recorded” and  $N$  is “the total number of individuals summed over all  $S$  genera” (Magurran, 1988).

When analysing the significance of any statistical tool both precision and accuracy must be considered. Precision is a measure of how well you can repeat your estimate,

even if your estimate is incorrect (Hellmann and Fowler, 1999). Accuracy is a measure of how close your sample estimates are to the true population value (Hellmann and Fowler, 1999). Hence, a precise index is not necessarily accurate, and an accurate index is not necessarily precise. Other factors to be taken into account when using statistical measures include any bias an index may possess and the usability of that index by non-specialists in mathematics and statistics.

Species Diversity, Species Richness and Evenness have all been utilized in nematological research and some researchers claim that all diversity indices are too insensitive to measure the effects of pollution (Neilson *et al.*, 1996). These indices are pure mathematical functions which do not consider the autecology of the organism (Neilson *et al.*, 1996). For example, whether the dominant species in a sample is a small opportunist with a short generation time or a larger organism with a longer generation time is not part of the index (Bongers *et al.*, 1991). The inclusion of such information as in the calculation of the nematode assemblage Maturity Index (Bongers, 1990) would provide both a broader and a more detailed look at the state of the environment under consideration.

To calculate the MI nematodes must be identified to family or genus and classified on the colonizer (c) to persister (p) scale. Colonizers are nematodes characterized by a rapid increase in numbers under favourable conditions, a short life cycle, a high colonization ability and a tolerance of disturbance. Colonizers are often numerically dominant in samples, show high fluctuations in population densities, have voluminous

gonads, release large numbers of small eggs and are often viviparous (Bongers, 1990). In contrast, persisters have a low reproduction rate, a long life cycle, a low colonization ability and are sensitive to disturbance. Persisters never belong to the dominant species in a sample, hardly fluctuate in number during the year, have few offspring, and have small gonads that produce large eggs (Bongers, 1990).

Pure colonizers (c) and pure persisters (p) are the extremes on a scale from one to five respectively, known as the c-p scale. Nematode taxa (usually families) are assigned whole number values on this scale based on their known biological characters. Tables of c-p values of nematode families are found in Bongers (1990) and Bongers *et al.* (1991), with reclassifications found in Bongers *et al.* (1995). Bongers *et al.* (1991) provide the most complete list including c-p values for nematode genera. The nematode MI is calculated using the following formula:

$$MI = \sum_{i=1}^n v(i) \times f(i), \quad (5)$$

where  $v(i)$  is the c-p value of taxon  $i$  and  $f(i)$  is the frequency of that taxon in a sample (Bongers, 1990). This takes into account not only the c-p value of a given taxon but also the frequency of that taxon in a sample. As such, the MI differs from other indices because it takes into account the biology of the nematode taxon.

The MI has the advantage that only 75 nematodes in a sample need to be identified to family in order to calculate the index which saves a tremendous amount of time.

However, despite this obvious advantage, interpretation of the calculated MI is important. A change in the MI, either increase or decrease, could be the result of a variety of environmental disturbances, such as: pollution, dehydration, recolonization, eutrophication, anaerobic conditions, physical disturbance, increase in salinity, refreshment of marine sediments, temperature effects and increase in decomposition rate (Bongers, 1990).

As for all indices, there may be information lost when using the MI. However, analysis of the c-p frequency distribution of each sample, either in tables or graphs, ensures the retention of information about the distribution of nematode taxa over the constituent c-p groups (de Goede *et al.*, 1993). A graphical view of the c-p frequency can be illustrated in a c-p triangle where the percentage of nematodes with c-p values three, four or five is represented at the triangle base, the percentage of nematodes with a c-p value of one is represented on the left triangle side and the percentage of nematodes with a c-p value of two is represented on the right triangle side. Comparisons of site specific c-p triangles demonstrate relative shifts in the composition of the nematode fauna (de Goede *et al.*, 1993) and are important since sites may have the same MI value but have different nematode communities. Such comparisons would also increase the understanding of ecological change at one site through time, such as the impact of environmental disturbance.

This study considers the nematode assemblages in two different locations in Newfoundland: Come-By-Chance which contains an oil refinery and Bay d'Espoir, an

area of aquaculture development. Nematode assemblages in these areas were determined and compared with those in nearby sites. The nematodes were categorized into feeding types and the Maturity Index was calculated together with the Shannon-Weaver, Simpson, Evenness and Species Richness Indices. The extraction technique was analysed, comparing the utility of smaller or sub-samples of larger samples. An attempt was also made to determine the effect of sediment size, temperature, depth, pH, and redox potential on the nematode assemblages where data was available.

## **Chapter 2: A comparison of Nematode Assemblages at Come-By-Chance and Arnold's Cove, Newfoundland.**

### **Introduction**

An oil refinery was developed in Come-By-Chance, Newfoundland during 1971 by Shaheen Resources Inc. Production began in May 1973 utilizing crude oil shipments from the North Sea, West Africa and the Arabian Gulf. In 1976 the refinery ceased production due to the bankruptcy of Shaheen Resources Inc. Petro Canada purchased the refinery in 1980 but did not re-open it. In 1986 they sold the refinery to Newfoundland Processing Limited which re-opened the refinery. It has operated continuously since then although ownership changed in 1994. The refinery has the capacity to process 105 000 barrels of crude per day. Processing has occasionally exceeded this capacity. For example, 24 910 000 barrels were processed in a four month period in 1989 (Inkster, 1990).

Research on the impact of oil on meiofauna has yielded various results. Fleeger and Chandler (1983) suggested that nematodes have a high tolerance to hydrocarbon and oxygen stress because they dominated all of their experimental samples containing crude oil. DeLauane *et al.*, (1984) attempted to determine the effect of cleaning up an oil spill on the meiofauna. They found that nematode densities were not affected by the removal of oil, but when oil was added to the experimental sediment samples the abundance of all meiofauna significantly increased. Fricke *et al.*, (1981) reported meiofauna results after an oil tanker collision off the coast of South Africa. The number of nematodes isolated

from a nearby sandy beach was not significantly different from those of reference sites throughout the one year study period .

In contrast, Decker and Fleegeer (1984) showed that the total number of nematodes in heavily oil-polluted sediment significantly decreased. Recolonization rates did not appear to be affected and they suggested that nematode community changes due to oil pollution depended on the type of habitat involved as well as the type and quantity of oil present. Elmgren *et al.* (1983) supported this latter suggestion. After studying baseline data collected before the Tsesis oil spill in the Baltic Sea, they showed that nematodes consistently made up 90 % or more of the meiofauna present in all samples, regardless of sampling site or time. After the spill, nematode abundance was lower than normal, but only significantly so at one site.

Martin and Cross (1986) also concluded that nematode abundance in an oiled bay was significantly lower than abundances in four unpolluted bays at Cape Hatt, Northern Baffin Island. Furthermore, five groups of organisms were tested: large protozoans, kinorhynchs, nematodes, mites and small crustaceans. Of these five groups, only nematode abundances were significantly lower than usual. Similar results were obtained near an oil platform in the North Sea. Within 500m of the platform nematode abundances dropped to 103-327 individuals per 10cm<sup>2</sup>, but beyond 1600m from the platform there were from 1918-2439 individuals per 10cm<sup>2</sup> (Moore *et al.*, 1987).

Other studies have suggested that some nematode species are more sensitive to certain forms of pollution than others. For example, *Diplolaimella punicea* and



*Chromadorina germanica* were both shown to be sensitive to polychlorinated biphenyls (PCB), polynuclear aromatic hydrocarbons (PAH) and heavy metals (Tietjen and Lee, 1984). Moore *et al.*, (1987) determined that certain species were resistant to pollution from a nearby oil refinery in the Forth Estuary, Scotland. At heavily polluted sites *Sabatieria pulchra* and *Rhabditis marina* dominated the nematode community. At a second site with similar pollutant composition *Diplolaimella ocellata* dominated the samples. *Daptonema setosum* had the highest density in samples taken nearest the chemical outflow.

This chapter will describe the nematode communities present in July and November 1998 in Come-By-Chance and Arnold's Cove, two towns one on each side of the oil refinery. The nematodes will be categorized into feeding types and the Maturity Index calculated together with the Shannon-Weaver, Simpson, Evenness and Species Richness Indices. In addition, an attempt will be made to ascertain whether or not using a portion of the nematodes extracted from a sample (Bongers, 1990) will yield statistically similar results to those extracted from a complete sample, thus reducing a time consuming component of this work.

## **Materials and Methods**

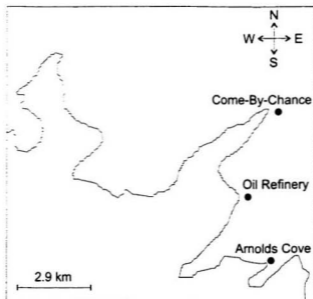
### **Description of Study Area**

The oil refinery and outflow is located between the communities of Come-by-Chance and Arnold's Cove, along the northeast shore of Placentia Bay (Figure 2). The oil refinery outflow, also known as the 'Waste Disposal Site', was used as an unregulated dump for the disposal of construction waste and domestic refuse during the initial construction of the refinery (Anon., 1987). A 1987 study analysed soil and water samples for oil and grease, phenolics, zinc, lead, copper, nickel, chromium and cadmium (Anon., 1987). In general, the soil contained more of all these than the water. Soil samples had concentrations of nickel and zinc that exceeded the removal criteria, and groundwater samples exceeded drinking water criteria for cadmium, chromium, copper and zinc concentrations (Anon., 1987).

### **Sampling of Nematodes**

Triplicate sediment samples were taken in July and November 1998 at a depth of 18-20ft from Come-By-Chance and Arnold's Cove (Figure 2) by Dr. Jerry Payne and colleagues, funded by the Department of Fisheries and Oceans. Each sample was collected in a one litre glass Mason jar by placing the mouth of the jar within the top few centimetres of the sediment and pushing it forward until approximately two-thirds of the jar was filled with sediment and water. The sediment was kept cool on ice in containers during transportation back to the Biology Department where fixative Formaldehyde

Figure 2: Location of sample sites in Come-By-Chance and Arnold's Cove, Newfoundland.



Acetic Acid 4:1, (Goodey, 1963) was added for preservation until extraction.

### **Extraction of Nematodes**

See Appendix A for a flow chart. Prior to extraction any liquid (fixative and/or sea-water) was filtered from each sample to recover nematodes (referred to as “Top” portion of the sample). Because of the large sample size, the samples were sub-divided into 250ml centrifuge bottles, to fill each to a depth of 2.5cm. Extraction was a modified version of Schwinghamer *et al.*, (1991). Samples were centrifuged at 3000 RPM for five minutes to separate the supernatant from the sediment. The supernatant (referred to as “Extraction 1”) was retained for suction filtration. The sediment was mixed with a minimum of 40.0ml Colloidal Silica (Nalco 1060), balanced with Colloidal Silica, resuspended and centrifuged at 3000 RPM for 15 minutes. The supernatant (referred to as “Extraction 2”) was retained for suction filtration. The filtrates of Extraction 1 and Extraction 2 were each washed with a sea-water/formalin solution into scintillation vials and stained with Rose Bengal (0.15g/L).

### **Processing , Mounting and Identification of Nematodes**

The contents of each scintillation vial were emptied into a petri dish and examined under a dissecting microscope. Nematodes were picked individually from the petri dish and processed using the glycerol-ethanol method (Seinhorst, 1959). This procedure left the nematodes in pure glycerine, in which they were mounted on glass microscope slides. Glass wool or small pieces of a crushed cover slip was used to support the cover slips (18mm) that were sealed with clear nail polish. Mounted nematodes were identified

using a compound light microscope and keys by Platt and Warwick (1983, 1988) and Tarjan (1980).

### **Enumeration/Statistical Analysis**

Relative abundance (%) of each nematode genus and the relative abundance (%) of each of the four Wieser (1959) feeding types were calculated for each sample. Community diversity was measured using the Shannon Index ( $H'$ )(Equation 1) and Simpson Index ( $1/D$ ) (Equation 2) (Magurran, 1988). Evenness (Equation 3) and Species Richness (Equation 4) were also calculated (Magurran, 1988). The Maturity Index (MI) (Equation 5) was calculated according to Bongers (1990). A minimum of 70 nematodes was required for calculating the MI. It was stipulated by Bongers (1990) that a minimum of 75 nematodes was required for calculating the MI, however, Lawlor (1998) showed that fewer could produce a statistically similar MI value. As well, the relative abundance of nematode genera per c-p value for all samples was calculated.

These indices were used to determine significant differences between nematode communities in Arnolds Cove and Come-By-Chance during July and November, 1998 using the Mann-Whitney U (SPSS Software). As well, differences between each of the three portions of a sample (Top, Extraction 1, and Extraction 2) and the total sample (Top + Extraction 1 + Extraction 2) were tested for significance with the Wilcoxon Signed Rank Test (SPSS Software).

## Results

Table 1 gives a complete list of genera including c-p values, feeding types and total nematode counts for all samples.

### Arnold's Cove

The total number of nematodes from all three July samples was 5920 from 28 genera and values of individual samples ranged from 628 to 2685 (Table 1). The most abundant nematode genus was *Metachromadora* (37 % of the nematode community). The second and third most abundant genera were *Chromadora* and *Prochromadorella* (16 % and 12 % of the total nematode community, respectively).

The total number of nematodes from all three November samples was 3400 from 42 genera and values of individual samples ranged from 1050 to 1243 (Table 1). The most abundant nematode genus was *Metalinhomoeus* (21 % of the total nematode community). The second and third most abundant genera were *Metachromadora* and *Axonolaimus* (10 % each of the total nematode community).

*Anticoma*, *Notochaetosoma*, *Pontonema* and *Rynchonema* were found only in the July samples and at relatively low abundance. Likewise, *Ammotheristus*, *Comesa*, *Daptonema*, *Eleutherolaimus*, *Enoploides*, *Eumorpholaimus*, *Leptolaimus*, *Mesacanthion*, *Metalinhomoeus*, *Nemanema*, *Paralinhomoeus*, *Promonhystera*, *Terschellingia*, *Theristus*, and *Thoracostomopsis* were found only in the November samples at varying abundances (Table 1). *Amphimonhystera*, *Belbolla*, and *Setosabatieria*, were extremely

Table 1: Summary of nematode composition of each sample, including c-p values (Bongers *et al.*, 1991), feeding types (Wieser, 1959), total number of nematodes and genera for all samples collected at Arnold's Cove and Come-By-Chance during July and November 1998.

Site	Month	Replicate	c-p value	Feeding Type	Arnold's Cove						Come-By-Chance					
					July			November			July			November		
					1	2	3	1	2	3	1	2	3	1	2	3
<i>Actinonema</i>			3	2A	0	0	4	11	5	5	0	4	3	0	0	0
<i>Amnotheristus</i>			2	1B	0	0	0	2	4	3	0	0	0	0	0	0
<i>Amphionkystera</i>			2	1B	0	0	0	0	1	0	0	0	0	0	0	0
<i>Anticona</i>			2	1A	71	73	13	0	0	0	0	0	0	0	0	0
<i>Aracolaimus</i>			3	1A	1	2	0	3	6	6	20	15	21	0	0	0
<i>Axonolaimus</i>			2	2A	5	7	0	82	133	117	0	0	1	0	0	1
<i>Bathylaimus</i>			2	1B	1	0	0	0	2	0	0	1	1	0	0	0
<i>Belbolla</i>			4	2B	0	0	0	0	1	0	0	0	0	0	0	0
<i>Chromadora</i>			3	2A	444	402	70	20	33	11	611	387	316	3	1	3
<i>Comesa</i>			3	1B	0	0	0	3	0	0	0	0	0	0	0	0
<i>Cyartocema</i>			3	1A	0	0	0	0	0	0	0	0	0	0	0	1
<i>Diptoniema</i>			2	1B	0	0	0	1	1	0	0	0	0	0	0	0
<i>Desmodora</i>			2	2A	0	0	1	0	0	1	1	4	67	1	6	3
<i>Eleutherolaimus</i>			2	1B	0	0	0	35	33	50	8	6	16	12	150	231
<i>Enoploides</i>			2	2B	0	0	0	3	15	15	32	23	52	8	24	31



Table 1 Continued

Site	Month	Replicate	c-p value	Feeding Type	Arnold's Cove						Come-By-Chance					
					July			November			July			November		
					1	2	3	1	2	3	1	2	3	1	2	3
	<i>Enoplus</i>		2	2B	3	5	6	0	0	1	17	31	29	12	6	12
	<i>Epacanthion</i>		5	2B	75	57	75	13	28	52	31	27	42	3	0	3
	<i>Eumorpholaimus</i>		2	1B	0	0	0	0	0	5	0	0	0	0	0	0
	<i>Gammarinema</i>		3	2A	0	0	0	0	0	0	2	2	7	1	1	3
	<i>Halalaimus</i>		4	1A	18	13	1	0	7	1	0	3	4	0	1	0
	<i>Leptolaimus</i>		2	1B	0	0	0	3	4	1	0	0	4	0	0	0
	<i>Mesacanthion</i>		3	2B	0	0	0	4	3	1	7	1	0	0	3	0
	<i>Metachromadora</i>		2	2A	1117	885	186	115	132	94	7	74	30	0	6	1
	<i>Metatrinhomoeus</i>		2	1B	0	0	0	340	167	210	0	0	0	0	4	1
	<i>Microalaimus</i>		2	2A	1	0	0	10	34	21	0	0	3	0	1	2
	<i>Monoposthia</i>		3	2A	133	255	11	6	6	6	0	1	0	0	1	7
	<i>Nemaneia</i>		4	1A	0	0	0	0	2	0	0	0	0	0	0	0
	<i>Neochromadora</i>		2	2A	251	274	68	92	121	62	97	149	137	0	4	26
	<i>Notochaetosoma</i>		4	1A	102	61	25	0	0	0	44	61	244	4	5	6
	<i>Odontophora</i>		2	1B	14	37	5	14	34	20	2	1	3	0	0	39
	<i>Oncholaimus</i>		4	2B	14	7	1	6	35	32	14	15	25	2	6	4

Table 1 Continued

Site	Month	Replicate	c-p value	Feeding Type	Arnold's Cove						Come-By-Chance					
					July			November			July			November		
					1	2	3	1	2	3	1	2	3	1	2	3
			4	1A	12	19	2	4	2	0	1	2	1	0	0	3
			2	2A	50	100	31	18	21	14	125	383	219	4	32	53
			2	1B	0	0	0	85	85	68	0	0	0	0	0	0
			2	2B	7	3	0	0	8	0	0	1	0	0	0	0
			4	1A	0	0	5	0	7	3	20	38	65	2	4	3
			4	2A	0	0	0	0	0	0	2	2	2	0	0	0
			4	2B	12	20	11	0	0	0	7	4	8	1	2	6
			2	2A	321	322	94	74	91	41	499	2937	1719	35	51	60
			2	1B	0	0	0	0	11	0	0	0	0	0	0	0
			2	1B	0	0	0	0	0	0	0	0	2	0	0	0
			3	1B	0	0	0	0	0	0	0	0	1	0	0	0
			3	1B	11	19	14	0	0	0	44	22	59	2	8	16
			2	1B	0	1	0	93	89	86	0	1	0	0	0	5
			2	1B	0	0	0	0	0	1	0	0	0	0	0	1
			3	2A	1	1	0	9	8	12	16	30	83	1	3	7
			3	1B	0	1	0	21	37	23	0	0	0	0	2	0

Table 1 Continued

Site	Month	Replicate	c-p value	Feeding Type	Arnold's Cove						Come-By-Chance							
					July			November			July			November				
					1	2	3	1	2	3	1	2	3	1	2	3		
			5	2A	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			3	1A	0	0	0	3	0	6	0	0	0	0	0	0	0	0
			2	1B	0	0	0	0	1	2	0	0	0	0	0	0	0	0
			2	2A	0	0	0	1	13	13	0	0	0	0	0	0	0	0
			3	2B	21	43	5	36	63	67	84	189	131	3	0	14		
					2685	2607	628	1107	1243	1050	1691	4414	3296	94	321	542		
					23	23	20	29	36	33	23	29	31	16	22	27		

rare as evidenced by the recovery of only one specimen during November (Table 1).

In November, *Actinonema*, *Araeolaimus*, *Axonolaimus*, *Microlaimus*, *Odontophora*, *Oncholaimus*, *Parodontophora*, *Phanodemella*, *Sabatieria*, *Spirinia*, *Subsphaerolaimus*, and *Viscosia* had all increased in relative abundance. However, *Chromadora*, *Epacanthion*, *Metachromadora*, *Monoposthia*, *Neochromadora*, *Oxystomina*, *Paracanthocheilus*, and *Prochromadorella* all decreased in relative abundance during the same time. The abundance of *Bathylaimus*, *Desmodora*, and *Enoplus* remained relatively stable over both sampling times.

Results from community diversity, Evenness and Species Richness are summarized in Table 2. The Shannon, Simpson and Species Richness indices all increased in November. In contrast, the Maturity Index decreased slightly and the Evenness measure remained the same over the sampling period.

Nematode genera with a c-p value of two were most abundant during both sampling times, and the relative abundance increased in November. In contrast, nematode genera with c-p values of three, four, and five all decreased in abundance. There were no nematodes present at Arnold's Cove with a c-p value of one (Table 3).

Results from feeding type analysis are summarized in Table 4. In July samples, nematode feeding type 2A made up 85.0 % of the community and there was low abundance of each of the other three feeding types. However, in November the nematodes of feeding type 1B increased in abundance to 45.1 % of the total community. Nematodes of feeding type 2A decreased to 42.1 % of the community in November.

Table 2: Summary of the mean values and standard deviations of the Shannon Index ( $H'$ ), Evenness ( $E$ ), Simpson Index ( $1/D$ ), Species Richness ( $D_{mg}$ ) and Maturity Index ( $MI$ ) values for all samples collected at Arnold's Cove and Come-By-Chance during July and November 1998.

Site	Arnold's Cove		Come-By-Chance	
Month	July	November	July	November
$H'$	2.07±0.14	2.70±0.22	1.73±0.32	2.08±0.13
$E$	0.77±0.11	0.77±0.04	0.52±0.11	0.68±0.08
$1/D$	5.50±1.09	10.8±3.25	3.29±1.08	4.70±0.89
$D_{mg}$	2.85±0.09	4.50±0.47	3.33±0.37	3.69±0.42
$MI$	2.36±0.02	2.19±0.05	2.42±0.19	2.38±0.26

Table 3: Summary of the Relative Abundance (%) of nematode genera per c-p value for all samples collected at Arnold's Cove during July and November 1998. Mean was calculated as the weighted average across samples.

Month	July				November			
Sample	1	2	3	Mean	1	2	3	Mean
c-p value								
1	0	0	0	<b>0</b>	0	0	0	<b>0</b>
2	71.2	67.5	75.3	<b>70.0</b>	88.9	82.7	83.4	<b>84.9</b>
3	22.8	27.7	15.9	<b>24.3</b>	9.2	12.6	12.6	<b>11.5</b>
4	5.9	4.6	7.8	<b>5.5</b>	1.9	4.7	3.9	<b>3.6</b>
5	1.1	0.2	1.0	<b>0.2</b>	0	0	0.1	<b>&lt;0.1</b>

Table 4: Relative Abundance (%) of Wieser (1959) feeding types (1A, 1B, 2A, 2B) for all samples collected at Arnold's Cove during July and November 1998. Mean was calculated as the weighted average across samples.

<b>Month</b>	<b>July</b>				<b>November</b>			
<b>Sample</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>Mean</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>Mean</b>
<b>Feeding Type</b>								
<b>1A</b>	7.6	6.4	7.3	<b>7.1</b>	0.9	1.9	1.5	<b>1.5</b>
<b>1B</b>	1.0	2.2	3.0	<b>1.7</b>	53.9	37.8	44.7	<b>45.1</b>
<b>2A</b>	86.5	86.2	74.0	<b>85.0</b>	39.6	48.0	37.8	<b>42.1</b>
<b>2B</b>	4.9	5.2	15.7	<b>6.2</b>	5.6	12.3	16.0	<b>11.3</b>

Nematodes of feeding type 1A decreased from 7.1 % in July to 1.5 % in November, and nematodes of feeding type 2B increased from 6.2 % in July to 11.3 % in November.

### **Come-By-Chance**

The total number of nematodes from all three July samples was 9401 from 35 genera and values of individual samples ranged from 1691 to 4414 (Table 1). The most abundant genus was *Prochromadorella* (55 % of the total nematode community). The second and third most abundant genera were *Chromadora* and *Paracanthochus* (14 % and 8 % of the total nematode community, respectively).

The total number of nematodes from all three November samples was 957 from 30 genera and individual samples ranged from 94 to 542 (Table 1). The most abundant genus was *Chromadora* (41 % of the total nematode community). The second and third most abundant genera were *Prochromadorella* and *Paracanthochus* (15 % and 9 % of the total nematode community, respectively).

*Actinonema*, *Araeolaimus*, *Bathylaimus*, *Parodontophora*, *Prorynchonema*, *Pterygonema* and *Synonchus* were found only in July samples at relatively low abundance (Table 1). *Desmodora*, *Enoploides*, *Enoplus*, *Gammanema*, *Halalaimus*, *Mesacanthion*, *Microlaimus*, *Monoposthia*, *Odontophora*, *Oxystomina*, *Pontonema*, *Rynchonema*, and *Sabatieria* all increased slightly in abundance in November. In contrast, *Chromadora*, *Epacanthion*, *Metachromadora*, *Neochromadora*, *Notochaetosoma*, *Oncholaimus*, *Phanodemella*, *Spirinia* and *Viscosia* all decreased in abundance. *Metalinhomoeus*, and

*Subsphaerolaimus* were found only in November samples in low abundance.

*Axonolaimus*, *Cyartonema*, and *Setosabatieria* were extremely rare as evidenced by the recovery of only one specimen in November (Table 1).

The Shannon, Simpson, Species Richness Indices and the Evenness measure all increased in November. The MI slightly decreased in November (Table 2). During both July and November nematodes with a c-p value of two were most abundant, making up 71.3 % and 83.4 % of the total community respectively. The abundance of nematodes with c-p values of two and five increased in November. However, the abundance of nematodes with c-p values of three and four decreased. There were no nematodes with a c-p value of one at this site (Table 5).

Feeding type analysis is summarized in Table 6 . During July nematodes of feeding type 2A made up 84.3 % of the total nematode community. There was low abundance of each of the other three feeding types. In November, nematodes of feeding type 1B had increased from 1.8 % to 49.1 % abundance, thus dominating the community. Nematodes of 2A feeding type decreased from 84.3 % to 33.4 % of the community, and 1A feeding type decreased from 5.7 % to 3.0 % of the community. There was a small increase in abundance of nematodes of feeding type 2B, from 8.2 % abundance to 14.5 % abundance.

### **Pooled Data**

Non-parametric analyses were employed due to a lack of statistical normality and independence of the study variables. Within Come-By-Chance there was no significant



Table 5: Summary of the Relative Abundance (%) of nematode genera per c-p value for all samples collected at Come-By-Chance during July and November 1998. Mean was calculated as the weighted average across samples.

Month	July				November			
Sample	1	2	3	Mean	1	2	3	Mean
c-p value								
1	0	0	0	<b>0</b>	0	0	0	<b>0</b>
2	47.4	81.6	69.7	<b>71.3</b>	67.0	86.6	84.3	<b>83.4</b>
3	46.4	14.8	18.8	<b>21.8</b>	10.6	5.9	9.4	<b>8.4</b>
4	5.2	2.9	10.7	<b>6.1</b>	9.6	5.6	4.1	<b>5.1</b>
5	1.0	0.7	0.8	<b>0.8</b>	12.8	1.9	2.2	<b>3.1</b>

Table 6: Relative Abundance (%) of Wieser (1959) feeding types (1A, 1B, 2A, 2B) for all samples collected at Come-By-Chance during July and November 1998. Mean was calculated as the weighted average across samples.

Month	July				November			
Sample	1	2	3	Mean	1	2	3	Mean
Feeding Type								
1A	5.0	2.7	10.2	<b>5.7</b>	6.4	3.1	2.4	<b>3.0</b>
1B	3.2	0.7	2.6	<b>1.8</b>	14.9	51.1	54.1	<b>49.1</b>
2A	80.4	90.0	78.5	<b>84.3</b>	47.9	33.0	30.6	<b>33.4</b>
2B	11.4	6.6	8.7	<b>8.2</b>	30.8	12.8	12.9	<b>14.5</b>

difference between months for all seven measures (all p-values  $>0.100$ ). Within Arnold's Cove there was also no significant difference between months for all seven measures (all p-values  $>0.100$ ). Within the sampling months of both July and November there was no significant difference between Arnold's Cove and Come-By-Chance for all seven measures analysed (all p-values  $>0.100$ ).

Because there were no significant differences results could be pooled. The Mann-Whitney U was used to determine significant differences between months and sites using the pooled data. A significant effect of sampling time on the total number of nematodes (p-value = 0.015), Shannon Index (p-value = 0.026) and Species Richness (p-value = 0.015) was demonstrated. The Maturity and Simpson Indices both had a p-value of 0.093, not significant at the 95 % confidence interval but significant at a 90 % confidence interval. The total number of genera (p-value = 0.589) and Evenness (p-value = 0.310) were not influenced by sampling time.

Tests showed a significant effect of sampling site on Evenness (p-value = 0.015) and Simpson (p-value = 0.015) Indices. The Shannon Index had a p-value of 0.065, not significant at the 95 % confidence interval but significant at a 90 % confidence interval. Total number of nematodes (p-value = 0.818), total number of genera (p-value = 0.485), Species Richness (p-value = 0.937) and the Maturity Index (p-value = 0.180) were not influenced by sampling site.

### **Sub-Sample Analysis**

Further analysis of all samples was conducted to determine if any part of the total

sample would yield the same results as the total sample with respect to the Shannon, Evenness, Simpson, Species Richness and Maturity Indices. Three parts of each sample were tested: Top, Extraction 1 and Extraction 2. The combination of these three was the total sample. A complete list of values for each sample part, together with the total value for all measures are provided in Appendices B through E. The samples collected at Come-By-Chance in November were omitted from this analysis because one of the samples had only 94 nematodes in total, so that when this was subdivided too few nematodes were present in each sub-sample to calculate viable index values. The Signed Rank Test showed there was no significant difference between the Top, Extraction 1, Extraction 2 and the total sample for the Shannon, Evenness, Simpson, Species Richness and Maturity Indices (all p-values were  $> 0.100$ ). A complete list of p-values for each test conducted is provided in Table 7.

Table 7: Summary of p-values comparing the Shannon Index ( $H'$ ), Evenness ( $E$ ), Simpson Index ( $1/D$ ), Species Richness ( $D_{ng}$ ) and Maturity Index ( $MI$ ) values of each of the 3 parts of the sample (Top, Extraction 1, Extraction 2) with the Index values of the complete sample, for all samples collected at Arnold's Cove during July and November 1998 and at Come-By-Chance during July 1998.

Site	Month	Index	Top	Extraction 1	Extraction 2
Arnold's Cove	July	$H'$	0.109	0.285	0.109
		$E$	1.000	0.285	1.000
		$1/D$	0.593	0.285	0.109
		$D_{ng}$	0.109	0.285	0.180
		$MI$	0.109	1.000	0.109
	November	$H'$	0.109	0.109	0.109
		$E$	0.102	0.109	0.102
		$1/D$	0.285	0.593	0.109
		$D_{ng}$	0.109	0.285	0.109
		$MI$	0.655	0.109	0.109
Come-By-Chance	July	$H'$	0.285	0.109	0.109
		$E$	0.414	0.285	0.109
		$1/D$	0.285	0.109	0.593
		$D_{ng}$	0.109	0.109	0.593
		$MI$	0.593	0.109	1.000

## Discussion

One of the most infamous oil spills was that from the *Amoco Cadiz* in Brittany, France in March 1978. Much research was conducted in an attempt to determine any significant changes in nematode numbers after the oil spill. Boucher (1980) found that nematodes were numerically dominant (88 % to 97%) in all the meiofauna samples collected before and after pollution. Although total numbers were not significantly different at these sampling times, there were significant differences in the Shannon Index and Evenness measures. A change in nematode species composition was also detected. Some species dominant in the nematode community before the spill were either suppressed or totally absent from samples taken after the oil spill. These included *Ixonema sordidum*, *Monoposthia mirabilis*, *Rhynchonema ceramatos*, *Chromadorita mucrocaudata*, *Xyala striata*, *Viscosia franzii* and *Rhynchonema megamphidum* (Boucher, 1980). However, there were significant increases in the abundance of *Anticoma ecotronis*, *Sabatieria celtica*, *Paracyatholaimus occultus* and *Microlaimus conspicuus* in the samples after the spill (Boucher, 1980).

The site of that oil spill has undergone extensive long term monitoring since 1978. Boucher (1985) observed that *Metachromadora vivipara* dominated all nematode samples for two years after the incident. During the five year study period there were also dramatic decreases in the nematode communities. Both of these changes in nematode assemblage were assumed to be caused by the oil spill.

Other long term monitoring studies of the same area noted slightly different nematode compositions after the spill. *Sabatieria pulchra*, *Terschellingia communis*, *T. longicaudata*, *Spirinia parasitifera*, *Metalinhomoeus bififormis*, *Sabatieria celtica*, *Neotonchus meeki*, and *Comesa* sp. dominated the various stations analysed (Gourbault, 1987). Seasonal changes were also noted in that diversity was consistently higher during the summer months compared with the autumn months (Gourbault, 1987).

Seven of the nematode genera found to be dominating the *Amoco Cadiz* oil spill site were found in both July and November samples from Arnold's Cove. Five of the nematode genera found to be dominating the *Amoco Cadiz* oil spill site were found in both July and November samples from Come-By-Chance. Another similarity is the dominance of *Metachromadora* at Arnold's Cove during July. Together with *Metalinhomoeus*, these two nematode genera again dominated the November samples. Of the nematode genera found to significantly decrease after the *Amoco Cadiz* oil spill, only *Rhynchonema*, *Monoposthia* and *Viscosia* were found at Arnold's Cove and Come-By-Chance in relatively low abundance.

Seasonal changes were also observed at Arnold's Cove and Come-By-Chance. Pooled statistical analysis indicated a higher total number of nematodes and greater taxon diversity during July. These findings agree with previous work, including the *Amoco Cadiz* studies (Hellmann and Fowler, 1999). However, pooled statistical analysis indicated a higher Species Richness during November. The calculation of Species Richness is dependent on sample size: as size of the sample increases the calculated value

of Species Richness becomes more accurate (Hellmann and Fowler, 1999). Therefore the actual Species Richness of the studied areas may be closer to the July values because of the larger number of nematodes collected.

The MI mean values at both sites decreased slightly in November but were not significantly different from those calculated in July. C-p analysis indicates an overwhelming dominance of nematodes with a c-p value of two, constituting 70 to 84.9 % of the total nematode communities. de Goede *et al.*, (1993) concluded that under stressed conditions, where bacterial activity is also restrained, the c-p two group reaches a high dominance. The suggestion that Arnold's Cove and Come-By-Chance have little bacterial activity is also supported by the complete absence of nematodes of the c-p one group. Nematodes of this group form dauerlarvae (protective coat around the larvae) as soon as bacterial production decreases below a threshold level (de Goede *et al.*, 1993). The sampling and extraction techniques used would have missed any dauerlarvae present. All these observations indicate that Arnold's Cove and Come-By-Chance are environmentally disturbed areas.

At both sites seasonal changes in feeding type were also evident. Nematodes of feeding type 2A dominated all samples during July. In November the percentage abundance of both 1A and 2A feeding types decreased by more than half. In contrast, nematodes of feeding types 1B and 2B increased significantly in November. These observations are in accordance with previous findings of Tietjen (1969), who found epigrowth feeding types (2A) reach their maximum numerical density during spring and

summer, while deposit (1A, 1B) and omnivorous feeding (2B) species reach maximum density during the fall and winter.

It has also been suggested that feeding types alter the MI value. Bongers *et al.* (1991) determined that the mean MI for type 1A is 3.7, for 1B 2.1, and for 2A and 2B about 3.0. With the dominance of type 2A during July at both sites, one would expect a MI value near 3.0, however, the overall MI value for Arnold's Cove was 2.36 and Come-By-Chance was 2.42. Although these values are slightly higher than the November MI values of 2.19 and 2.38 respectively, they are not as high as one would expect in conjunction with the feeding type analysis. These results may be related to the choice of c-p value for each individual genus. For example if MI value were based entirely on family c-p values then *Bolbalaimus*, *Ixonema* and *Microlaimus* would all have the c-p value of three of their family Microlaimidae. However, if genus-level c-p values were used *Bolbalaimus* would be three, *Ixonema* four and *Microlaimus* two (Bongers *et al.*, 1991). Hence, depending on the c-p value chosen for each nematode, and the taxonomic level assigned, two different MI values could result. This will be discussed in Chapter 4.

One of the deterrents to nematological research is that it is labour intensive. As part of this study all samples were analysed in three parts (Top, Extraction 1, Extraction 2) and as a whole (Top + Extraction 1 + Extraction 2). The results from each of the three separate parts of a sample were not significantly different from those of the combined total. Similar findings have been reported elsewhere (Lawlor, 1998). Using a portion of a sample has several advantages over a complete sample. The most obvious advantage is



fewer nematodes, particularly in the Top and Extraction 1 portions of a sample. These two parts of a sample are clearer than the supernatant of Extraction 2, which results in faster filtration during the extraction procedure. These advantages result in easier nematode picking and decreased processing time. With fewer nematodes less time is required for identification and data analysis. Therefore, more samples could be processed in a given time and greater areas and/or longer periods of time could be studied. This increase in the spatial and temporal scope of a sampling programme would be preferable for biomonitoring purposes to studying all nematodes in one sample, at one site, at one particular time.

This study has provided an ecological profile of the nematode assemblages in the marine ecosystems of an oil refinery and has shown that it resembles that of an oil-polluted ecosystem. In addition, it has provided baseline nematode data and demonstrated seasonal changes in nematode communities. These data provide a useful basis for further research in the Come-By-Chance region. This study has also shown the importance of integration of absolute numbers of nematodes and genera collected with feeding type analysis, diversity indices, Evenness, Species Richness, and MI and c-p analysis, particularly when attempting to use nematodes as biological indicators. The utility of smaller samples or sub-samples of larger samples was also shown.

### **Chapter 3: A comparison of nematode assemblages at Aquaculture and Reference Sites, Bay d'Espoir, Newfoundland.**

#### **Introduction**

Aquaculture is becoming economically very important worldwide and will continue to be so in the future. Consumers are purchasing more seafood as they become aware of its nutritional value (NRC, 1992) and are demanding a higher quality finished product which is not always available by traditional commercial fishing (Barnabé, 1994a). Aquaculture can take some pressure off the commercial fishery, providing time for stocks to replenish.

Apart from the benefits, aquaculture has two fundamental problems. The first is the possibility of environmental disturbance due to the production of organic pollutants during aquaculture. The second related problem is that of maintaining environmental conditions suitable for the growth of the species under cultivation. There are three primary sources of organic pollutants in aquaculture: uneaten food, faeces and urinary excretions (Beveridge, 1996). Food is normally given to fish in commercially-made pellets (Heen *et al.*, 1993). Fish food can accumulate both inside and outside the net pens. The pellets sink rapidly in comparison to fish faeces, and normally accumulate directly under the net pens (Heen *et al.*, 1993). It is estimated that 80-90% of a farm's total organic contribution to its environment is deposited directly under the cages (Wallace, 1993). However, accumulation will occur outside the net pens if the water flow is strong enough to sweep away the pellets prior to the fish eating them or after the pellets

have already sank to the bottom of the net pen (Beveridge, 1996).

Fish faeces sink slower than fish pellets, disintegrating and dispersing as they sink (Wallace, 1993). Faeces then become essentially particulate matter (NRC, 1992) and do not have as much energy as food pellets (Wallace, 1993). The primary excretion product is ammonia (NRC, 1992), however, carbon dioxide and excess nutrients in urine are also excreted (Beveridge, 1996). In contrast to food pellets and fish faeces, soluble urinary excretions are more widely dispersed via water currents (Beveridge, 1996).

Increased amounts of wasted food pellets and faeces increase microbial growth, which then alters the chemistry, structure and function of the sediment (Beveridge, 1996). The biological oxygen demand (BOD) increases (NRC, 1992), anaerobic sediments develop (Beveridge, 1996) and toxic hydrogen sulfide gas is produced (Wallace, 1993). Usually, the benthic area affected ranges from 20 to 50 metres from the net pen, but effects have been detected up to 150 metres (Beveridge, 1996).

The optimum environmental conditions of temperature, pH, redox potential and depth are essential for aquaculture and vary for the species under cultivation. Temperature is the most important of these because it affects the rates of nearly all metabolic processes (Wallace, 1993) and influences other environmental factors. For example, the steelhead trout, *Oncorhynchus mykiss*, has an optimum temperature of 14-15°C and a maximum lethal temperature of 23°C (Barnabé, 1994b). When temperature increases, dissolved oxygen decreases (Barnabé, 1994b). Oxygen depletion can therefore be a problem during higher summer temperatures. Oxygen requirements vary for each

species: salmonids, for example, require a mean dissolved oxygen level of 9 mg per litre (Barnabé, 1994b).

Another critical environmental variable is pH. Ammonia, an excreted product of protein digestion, in excess amounts is toxic to most living organisms (Pillay, 1992). The equilibrium between dissolved ammonia gas, ammonium hydroxide and the ammonium ion is pH dependent (Barnabé, 1994b). As the pH increases and the concentration of hydroxide ions increases, ammonium hydroxide is produced from the interaction of hydroxide ions with ammonium ions. Ammonium hydroxide is toxic to fish and most other organisms (Barnabé, 1994b). As pH decreases and the concentration of hydrogen ions increases, the equilibrium shifts so that ammonia gas is produced. Ammonia gas, although not as toxic as ammonium hydroxide, is still toxic to fish (Barnabé, 1994b). The redox potential of the water is affected by pH, and also slightly affected by both temperature and oxygen concentration (Wetzel, 1983). Over the last few decades redox potentials have become a rapid measure of the impact of increased input of organic material to a marine system because as organic matter increased the redox values decreased (Pearson and Stanley, 1979).

A fourth important environmental variable is depth. As depth increases nutrients become more abundant (Barnabé, 1994c). The above four variables as well as particle size will be considered in this thesis, although there are of course several other factors which influence fish aquaculture, including dissolved oxygen concentration, hardness, carbon dioxide concentration (Wallace, 1993), turbidity and hydrogen sulfide

concentration (Pillay, 1992).

This chapter will describe the general nematode community in Bay d'Espoir during July, August and November 1998. The differences between nematode communities under net pens and at non-aquaculture reference sites will be considered using total number of nematodes and genera, indices, feeding types and c-p analysis. In addition, the pH, redox potential, sediment temperature, depth of water above the sediment and particle size will also be used to compare these sites.

## **Materials and Methods**

### Description of Study Area

The sampling sites were located in Bay d'Espoir, Newfoundland, a 250 km<sup>2</sup> estuarine fjord which produces approximately 90% of the total aquaculture product in Newfoundland (Tlusty *et al.*, 1999). The aquaculture sites contained only steelhead trout, *Oncorhynchus mykiss*, kept in net pens (Figure 3) (15 000 fish/cage) and fed 25kg of food (Moore Clark or Shur-gain brand names) per cage every second day during the winter. Throughout the summer months the amount of food was changed to 1% of the fish's body weight. The fish food is 92% organic matter and the approximately 10% of food not eaten settles to the ocean floor.

### Sampling of Nematodes

One sample was taken from each of five aquaculture sites (A1-A5) and 11 reference sites (R1-R11) during July, August and November 1998 (Figures 4 and 5). Roti Bay (2 660 000m<sup>2</sup>) is only used from November to May and is therefore referred to as a Winter Site. As such there were no fish present in the net pens during the sampling period. There are four aquaculture sites in Roti Bay - Hardy Cove (A1), Long Island Resources (LIR) Ltd (A2), Conne River (A3) and SCB Fisheries Ltd (A4) (Figure 5) and all have been used as Winter Sites for seven consecutive years (1992-1999). Northwest Cove (A5)(500 000m<sup>2</sup>) (Figure 4) has been used for two and one half years (1995-1998). Samples were taken from Site A5 which had fish present during July and August but in

Figure 3: Photograph of a single net pen (bottom) and net pen alignment (top) in Bay d'Espoir.





Figure 4: Location of sampled sites in Bay d'Espoir, Newfoundland.

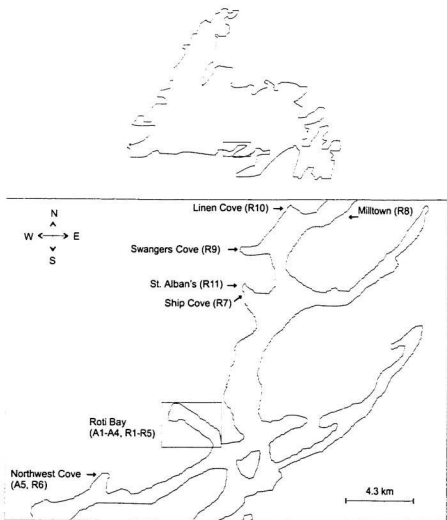
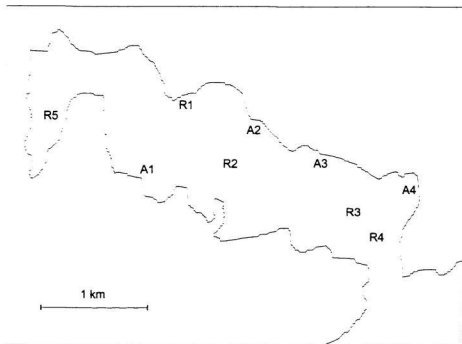


Figure 5: Location of sampled sites within Roti Bay, Newfoundland.



#### Site Key

A1 = Hardy Cove Aquaculture Site

A2 = LIR Aquaculture Site

A3 = Conne River Aquaculture Site (CRA)

A4 = SCB Aquaculture Site

R1, R2, R3, R4 = Reference Sites

R5 = Inner Basin Reference Sites

November the net cages and fish were moved to avoid ice damage.

The 11 reference (non-aquaculture) sites were all located at a minimum of 50m away from any aquaculture site (Beveridge, 1996). Roti Bay had five reference sites (R1 - R5, Figure 5). There was one reference site at each of Northwest Cove (R6), Ship Cove (R7), Milltown (R8), Swanger Cove (R9), Linen Cove (R10) and St. Albans (R11) (Figure 4). R8 had a saw mill, as did site R10. There was also a small farm at site R10 with chicken and cattle; it was unknown how much effluent ran into the water system. R11 was near a Fish Processing Plant which processed steelhead trout, *Oncorhynchus mykiss*, brook trout, *Salvelinus fontinalis*, and atlantic salmon, *Salmo salar*. During the sampling time the plant discharged, untreated, into the water anything that was not marketable, including the frame, trimmings, kidneys and blood.

Samples were collected as part of a comprehensive evaluation of Bay d'Espoir aquaculture sites and surrounding areas by Dr. Michael Tlusty and colleagues. Funding was provided by the Newfoundland Salmonid Growers Association. Each sample was collected from a boat using an Ekman Grab Sampler, which was hoisted out of the water and then placed in a tub. Water was pipetted off the top of the sediment in the grab and the top few centimetres of sediment (approximately 50-100ml) were skimmed off with a scoop and placed in a jar. The sediment sample was kept cool on ice until delivered to the lab at MUN when fixative, Formaldehyde Acetic Acid 4:1 in sea-water, was added (Goodey, 1963).

## Measurement of Physical and Chemical Variables

Redox potential (mV), pH and temperature (°C) of the top few centimetres of sediment samples were measured at the time of sampling using an Accumet combination pH-temperature electrode and an Orion solid state Redox electrode which was interfaced to an Accumet AP-25 dual channel meter. The depth at which the samples were taken was also measured. These measurements were recorded by Dr. Michael Tlusty and colleagues in the field.

Particle size was measured in a laboratory at the Biology Department. Particles large enough to be measured by the naked eye were individually measured for each sample using a millimetre ruler. The range of smaller particles was determined by measuring with a micrometer 20 randomly chosen particles, including the smallest and largest particles, in the field of view of a compound light microscope at 400X. Sediment classification was adapted from West (1991).

## Extraction of Nematodes

The procedure described in Chapter 2 (pp 16) was attempted unsuccessfully three separate times on these sediment samples. Centrifugation speed and time were both increased in an attempt to solve this problem, but both failed. In addition, filtering the Colloidal Silica prior to usage did not improve the extraction procedure. The problem appeared to be that the particle size of the sediment was too fine to form a pellet during centrifugation. Therefore all sediment samples had to be diluted with filtered sea-water

and nematodes individually hand-picked.

### Processing, Mounting and Identification of Nematodes

The procedures used for processing, mounting and identification of nematodes were the same as described in Chapter 2 (pp 16-17).

### Enumeration/Statistical Analysis

Procedures described in Chapter 2 (pp 17) were followed. However, as the total number of nematodes per sample was low, relative abundances (%) and indices were calculated only on samples containing a minimum of 70 nematodes.

## Results

### Aquaculture Sites (A1 - A5)

The total numbers of nematodes and genera for samples A1 - A5, including c-p values and feeding types are presented in Table 8. No nematodes were detected in samples taken from A3 in July, A1, A2, A4 or A5 in August or from A2 and A4 in November. The total number of nematodes collected in all three months from Hardy Cove (A1) was eight, SCB (A4) was five and Northwest Cove (A5) was 14, too few to allow calculation of any indices and so these are not considered below.

#### **LIR Aquaculture Site A2**

The number of nematodes collected from all samples taken at the LIR aquaculture site (A2) totalled 167 from 21 genera. During July, 74 individuals were identified from 14 genera. The most abundant genus was *Anticoma* (54 %) and the second most abundant was *Microlaimus* (11 %). *Adoncholaimus*, *Chromadora macrolaima*, *Chromaspirinia*, *Halalaimus*, *Monoposthia*, *Nemanema*, *Odontophora lituifera*, *Oncholaimus*, *Paracanthocheilus*, *Paradontophora*, *Sphaerolaimus* and *Steinaria* all occurred in low abundance (less than 7 % each) (Table 8). August had the largest community of 93 individuals from 7 genera. The most abundant genus was *Sabatieria* (34 %) and the second most abundant genus was *Linhystra* (29 %). *Laimella longicaudata*, *Oxystomina*, *Paramonhystra*, *Spiliphora* and *Theristus* all occurred in low abundance (less than 15 % each). None of the genera found in July were found in August samples.



Table 8: Summary of nematode composition of each sample, including c-p values (Bongers *et al.*, 1991), feeding types (Wieser, 1959), total number of nematodes and genera for all samples collected at Aquaculture Sites (A1-A5) in Bay d'Espoir during July (J), August (A) and November (N) 1998.

Genus	Month	c-p value	Feeding Type	A1		A2		A3		A4		A5				
				J	A	J	A	J	A	J	A	J	A	J	A	
				N		N		N		N		N		N		
<i>Aldrovandaimus</i>		4	2B	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Annuloheteritis</i>		2	1B	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Anticoma</i>		2	1A	0	0	0	40	0	0	0	11	0	0	0	0	0
<i>Atomolaimus</i>		2	2A	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Belbulla</i>		4	2B	0	0	0	0	0	0	7	0	0	0	0	0	0
<i>Chromadora</i>		3	2A	0	0	0	5	0	0	0	0	0	0	0	0	0
<i>Chromospirina</i>		4	2B	0	0	0	3	0	0	1	0	0	0	0	0	0
<i>Crenopharynx</i>		4	1A	0	0	0	0	0	0	8	0	0	0	0	0	0
<i>Demodora</i>		2	2A	0	0	1	0	0	0	0	37	0	0	0	7	0
<i>Diplocephala</i>		3	1B	0	0	0	0	0	0	2	0	1	0	0	0	0
<i>Dolicholaimus</i>		2	2B	0	0	0	0	0	0	3	0	0	0	0	0	0
<i>Eiweherolaimus</i>		2	1B	0	0	0	0	0	0	9	0	0	0	0	0	0
<i>Engelb</i>		5	2B	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Halidaimus</i>		4	1A	0	0	0	3	0	0	1	0	0	0	0	0	0
<i>Laimella</i>		2	2A	0	0	0	14	0	0	0	24	0	0	0	0	0
<i>Lepidolaimus</i>		2	1B	0	0	0	0	0	0	2	0	0	0	0	0	0
<i>Lindhyera</i>		2	1A	0	0	0	27	0	0	0	0	0	0	0	0	0
<i>Mesacanthion</i>		3	2B	0	0	0	0	0	0	4	0	0	0	0	0	0

Table 8 Continued

Genus	Month	cp value	Feeding Type	A1			A2			A3			A4			A5		
				J	A	N	J	A	N	J	A	N	J	A	N	J	A	N
<i>Micromelanimus</i>		2	2A	0	0	5	8	0	0	0	0	0	0	0	0	0	0	0
<i>Manihystera</i>		2	1B	1	0	0	0	0	0	0	0	0	0	0	0	0	0	7
<i>Monopertitia</i>		3	2A	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
<i>Nonnialimnides</i>		3	2A	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
<i>Nemaneu</i>		4	1A	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
<i>Neochromadora</i>		2	2A	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Obolospira</i>		2	1B	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
<i>Ochulolima</i>		4	2B	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Oxytomina</i>		4	1A	0	0	0	0	4	0	0	26	0	0	0	0	0	0	0
<i>Paracymblocheus</i>		2	2A	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0
<i>Paramanihystera</i>		2	1B	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0
<i>Parodontophora</i>		2	2B	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Pomposena</i>		3	2B	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
<i>Rhabdoclomania</i>		4	1B	0	0	0	0	0	0	1	0	3	0	0	0	0	0	0
<i>Subuliferia</i>		2	1B	0	0	1	0	32	0	4	7	0	0	0	0	0	0	0
<i>Sphaerolaimus</i>		3	2B	0	0	0	1	0	0	0	9	1	0	0	0	0	0	0
<i>Spilopora</i>		3	2A	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
<i>Spirinia</i>		3	2A	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0
<i>Srinertia</i>		2	1B	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0

Table 8 Continued

Genus	Month	c-p value	Feeding Type	A1			A2			A3			A4			A5		
				J	A	N	J	A	N	J	A	N	J	A	N	J	A	N
<i>Subsphaerosulimus</i>	1B	3		0	0	0	0	0	0	0	8	0	0	0	0	0	0	0
<i>Tetrachlingia</i>	1A	3		0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Theritus</i>	1B	2		0	0	0	0	8	0	0	5	82	0	0	0	0	0	0
<i>Troglita</i>	1A	4		0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Unknown Genus 1	1B	2		0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Fluxista</i>	2B	3		0	0	0	0	0	0	0	14	0	0	0	0	0	0	0
<b>Total Number of Nematodes</b>				1*	0*	7*	74	93	0*	0*	184	116	5*	0*	7*	0*	7*	0*
<b>Total Number of Genera</b>				1	0	3	14	7	0	0	28	6	3	0	0	1	0	1

\* = 100 few nematodes for calculation of indices

Table 9 shows that the Shannon, Species Richness and Maturity Indices all increased.

Table 10 shows that nematode genera with a c-p value of 2 were most common at this site during both July and August. There were no nematodes with the c-p values 1 or 5 identified at this site. The percentage of nematodes with a c-p value of 2 increased from July to August, whereas the abundance of nematodes with a c-p value of 3 decreased by a factor of 4.3, and nematodes with a c-p value of 4 slightly decreased.

Table 11 indicates an abundance of selective deposit feeders (1A) during July. By August this abundance had decreased by half, and the non-selective deposit feeders (1B) had increased in abundance by a factor of 5. The percentage of epigrowth feeders (2A) slightly decreased. Omnivores/predators (2B) were present in July but absent in August.

#### **Conne River Aquaculture Site A3**

The total number of nematodes collected at the Conne River aquaculture site (A3) was 300 from 31 genera (Table 8). Of this, 184 nematodes from 28 genera were isolated from the August sample. The most abundant genus was *Desmodora* (20 %) followed by *Oxytomina* (14 %). During August *Anticoma*, *Spirinia* and *Viscosia* were present at 6 to 7 % abundance. Present at less than 6 % each abundance were: *Ammotheristus*, *Axonolaimus*, *Belbolla*, *Chromaspirina*, *Crenopharynx*, *Diplopeltula*, *Dolicholaimus*, *Eleutherolaimus*, *Enoplus*, *Halalaimus*, *Leptolaimus*, *Mesacanthion*, *Nannolaimoides*, *Paracanthochus*, *Pomponema*, *Rhabdodemia*, *Sabatieria*, *Sphaerolaimus*, *Steineria*, *Subsphaerolaimus*, *Terschellingia*, *Theristus* and *Trefusia* (Table 8).

There were 116 nematodes from six genera isolated from the November sample.

Table 9: Summary of the Shannon Index (H'), Evenness (E), Simpson Index (1/D), Species Richness ( $D_{ng}$ ) and Maturity Index (MI) values for samples containing a minimum of 70 nematodes collected at the Aquaculture (A) Sites in Bay d'Espoir during July, August and November 1998.

Site	Month	H'	E	1/D	$D_{ng}$	MI
A2	July	1.75	0.66	3.24	3.02	2.36
	August	1.62	0.83	4.33	1.32	2.11
A3	August	2.80	0.84	12.0	5.18	2.88
	November	0.86	0.48	1.84	1.05	2.01

Table 10: Relative Abundance (%) of nematode genera per c-p value for samples containing a minimum of 70 nematodes collected at the Aquaculture (A) Sites in Bay d'Espoir during July, August and November 1998.

Site	Month	c-p value				
		1	2	3	4	5
A2	July	0	77.0	9.5	13.5	0
	August	0	84.9	2.2	12.9	0
A3	August	0	42.4	28.3	28.8	0.5
	November	0	99.1	0.9	0	0

Table 11: Relative Abundance (%) of Wieser (1959) feeding types (1A, 1B, 2A, 2B) for samples containing a minimum of 70 nematodes collected at the Aquaculture (A) Sites in Bay d'Espoir during July, August and November 1998.

Site	Month	Feeding Types			
		1A	1B	2A	2B
A2	July	60.8	9.5	20.2	9.5
	August	33.3	49.5	17.2	0
A3	August	26.7	17.9	30.4	25.0
	November	0	77.6	21.6	0.8

The most abundant genus was *Theristus* (71 %) and the second most abundant was *Laimella* (21 %). *Theristus*, *Sabatieria* and *Sphaerolaimus* were the only three genera isolated from both August and November samples. During November *Laimella*, *Neochromadora* and Unknown Genus 1 (family *Comesomatidae*) (Appendices F and G) appeared for the first time at this site.

Table 9 shows that the Shannon Index, Evenness, Simpson Index, Species Richness and Maturity Index all decreased from August to November at the A3 site. Table 10 shows that both August and November samples were dominated by nematodes with the c-p value of 2. During November they made up almost the entire sample, with only a small percentage of nematodes with a c-p value of 3.

Table 11 shows that during August all four feeding types were well represented in the A3 sample, with a slight dominance of epigrowth feeders (2A). However, during November 77.6 % of the nematode population were non-selective deposit feeders (1B). There were no selective deposit feeders (1A), a decrease in the abundance of epigrowth feeders (2A) and very few omnivores/predators (2B).

### **Comparison of Sites A2 and A3**

During August both A2 and A3 sites were sampled. Table 9 shows that all indices were higher at site A3 than at A2. However, evenness differed by only 0.01. Table 10 indicates that both sites had an abundance of nematodes with a c-p value of 2, and only site A3 had nematodes with a c-p value of 5. Both sites, however, had relatively similar percentages of nematodes of the 1A feeding type. Site A2 had a higher percentage of

non-selective deposit feeders, where as site A3 had higher percentages of both epigrowth feeders and omnivores/predators (Table 11).

#### **Summary of Physical and Chemical Variables**

All aquaculture samples were taken at depths greater than 12.0 m, down to a maximum of 44.5 m. The pH ranged from 6.0 to 7.6 and the redox potential ranged from -227.9 to 7.0. The temperatures decreased from July to November, with a temperature range of 2.6 °C to 9.4°C (Table 12). Table 13 indicates that all samples taken at the aquaculture sites, except A1 during August and A3 during July, contained clay. Ten samples contained silt, and six of these also had sand. Only five samples contained gravel and two contained cobble.

Table 12: Physical and chemical variables of all Aquaculture Sites (A1-A5) investigated in Bay d'Espoir during 1998.

Variable	Month	A1	A2	A3	A4	A5
Depth (m)	July	30.5	19.0	15.9	27.8	44.5
	August	26.8	25.7	18.9	30.3	13.5
	November	31.1	22.6	18.1	33.8	12.8
pH	July	7.5	NT	NT	7.3	NT
	August	NT	NT	NT	NT	NT
	November	7.4	7.6	7.3	6.0	7.1
Redox Potential (mV)	July	7.0	NT	NT	-227.9	NT
	August	NT	NT	NT	NT	NT
	November	-219.0	-202.0	-50.0	-194.0	-160.0
Temperature (°C)	July	9.4	NT	NT	7.2	NT
	August	NT	NT	NT	NT	NT
	November	2.9	2.6	5.6	4.3	6.9

Key: NT = Data Not Taken



Table 13: Particle Size Analysis of all Aquaculture Sites (A1 - A5) investigated in Bay d'Espoir during 1998.

Month	Sediment Type	A1	A2	A3	A4	A5
July	Cobble		X			
	Gravel		X	X		
	Sand		X			X
	Silt	X	X		X	X
	Clay	X	X		X	X
August	Cobble	X				
	Gravel			X		
	Sand			X		X
	Silt			X		X
	Clay		X	X	X	X
November	Cobble					
	Gravel			X		X
	Sand			X		X
	Silt	X	X	X		X
	Clay	X	X	X	X	X

Key: X = Present In Sample

Cobble = 60-200 mm, Gravel = 2-60 mm, Sand = 0.06-2 mm, Silt = 0.002-0.06 mm,  
Clay = < 0.002 mm

## **Reference Sites (R1 - R11)**

Numbers of nematodes and genera for samples R1 - R11, including c-p values and feeding types are presented in Table 14. No nematodes were detected in samples taken from R2 and R5 in July, or R4 in November. The total number of nematodes collected during July from site R3 was 17, R4 was one, R8 was three. The total number of nematodes collected during August from site R2 was 46, R3 was 23, R5 was 12, and R9 was three. The total number of nematodes collected during November from site R2 was 46, R3 was 13, R4 was 67, R6 was 34, and R7 was 69. All of these numbers were too low to allow calculation of indices and will not be considered further below.

### **Roti Bay Reference Site R1**

There was a total of 1022 nematodes from 38 genera collected at site R1 and numbers per sample ranged from 95 to 500 (Table 14). During July, 95 nematodes were identified from eight genera. The most abundant genus was *Monhystera* (70 %) and the second most abundant was *Terschellingia* (16 %). *Anticoma* was present at 8 % abundance while *Coninckia*, *Crenopharynx*, *Halichoanolaimus*, *Monoposthia* and *Paracanthochus* were present at less than 2 % each (Table 14).

In August 427 individuals from 32 genera were found of which the most abundant genus was *Setosabatieria* (24 %) and the second most abundant was *Richtersia* (19 %). *Crenopharynx*, *Monoposthia* and *Paracanthochus* were present in low abundance, as they had been in the July sample. In addition to these genera, *Anoplostoma*, *Axonolaimus*, *Bathylaimus*, *Chromadora macrolaima*, *Didelta*, *Eleutherolaimus*,



Table 14 Continued

Genus	Month	e-p value	Feeding Type	R1			R2			R3			R4			R5			R6		
				J	A	N	J	A	N	J	A	N	J	A	N	J	A	N	J	A	N
<i>Daldia</i>		2	1B	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diplocephala</i>		3	1B	0	0	3	0	1	1	0	0	1	0	0	0	0	2	2	9	0	0
<i>Dicorema</i>		2	1A	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0
<i>Dolicholaimus</i>		2	2B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	46	3	0
<i>Epeurotelimus</i>		2	1B	0	6	0	0	0	0	0	1	0	0	0	0	0	0	0	1	8	0
<i>Enopliolaimus</i>		2	2B	0	0	0	3	1	0	0	0	0	0	0	0	2	19	2	0	0	0
<i>Enoplia</i>		5	2B	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
<i>Groefbeila</i>		4	1A	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Haldolaimus</i>		4	1A	0	2	2	0	0	0	0	0	0	0	0	0	1	0	8	2	0	0
<i>Holanoechus</i>		4	1B	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Halticosolaimus</i>		3	2B	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0
<i>Laimella</i>		2	2A	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepidolaimus</i>		2	1B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
<i>Merylynella</i>		3	2A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0
<i>Mrazcanthion</i>		3	2B	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Mentakmonera</i>		2	1B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
<i>Metapharodolimus</i>		3	2B	0	0	0	0	0	0	0	3	0	0	0	0	0	0	6	36	0	0
<i>Microlaimus</i>		2	2A	0	3	414	0	0	0	0	0	0	0	0	0	0	0	2	17	16	0
<i>Monkystra</i>		2	1B	66	0	0	0	0	2	1	0	0	0	0	0	0	0	0	9	0	1



Table 14 Continued

	e-p value	Feeding Type	R1			R2			R3			R4			R5			R6			
			J	A	N	J	A	N	J	A	N	J	A	N	J	A	N	J	A	N	
<i>Genus</i>																					
<i>Month</i>																					
<i>Protonotus</i>	3	2H	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhuklitis</i>	1	1A	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhuklemonia</i>	4	1B	0	10	0	0	7	10	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>Richesia</i>	3	1A	0	81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Subutaria</i>	2	1B	0	37	16	0	18	10	0	1	8	0	0	11	0	0	63	2	8	2	
<i>Seroubacteria</i>	2	1B	0	106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
<i>Sphaeroidimus</i>	3	2B	0	0	0	0	0	0	0	0	1	0	0	5	0	0	0	0	0	20	4
<i>Spilphera</i>	3	2A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
<i>Steieria</i>	2	1B	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0
<i>Sphaerulimus</i>	4	1B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
<i>Subsphaerulimus</i>	3	1B	0	1	0	0	1	0	0	7	0	0	0	0	0	0	0	0	0	8	0
<i>Synochella</i>	3	2A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
<i>Tetrachlingia</i>	3	1A	15	2	1	0	0	2	1	0	0	0	0	1	0	0	0	0	3	5	1
<i>Theristas</i>	2	1B	0	6	38	0	8	8	0	2	0	0	0	0	0	0	0	0	8	6	1
<i>Troglusia</i>	4	1A	0	0	0	0	0	3	3	0	0	0	0	0	0	0	0	0	0	1	0
Unknown Genus 1	2	1B	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
<i>Ficostia</i>	3	2B	0	7	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	8	5
<b>Total Number of Nematodes</b>			<b>95</b>	<b>427</b>	<b>500</b>	<b>0*</b>	<b>46*</b>	<b>46*</b>	<b>46*</b>	<b>17*</b>	<b>23*</b>	<b>13*</b>	<b>1*</b>	<b>0*</b>	<b>67*</b>	<b>0*</b>	<b>12*</b>	<b>112</b>	<b>186</b>	<b>356</b>	<b>34*</b>
<b>Total Number of Genera</b>			<b>8</b>	<b>32</b>	<b>12</b>	<b>0</b>	<b>12</b>	<b>13</b>	<b>13</b>	<b>8</b>	<b>9</b>	<b>6</b>	<b>1</b>	<b>0</b>	<b>8</b>	<b>0</b>	<b>5</b>	<b>17</b>	<b>34</b>	<b>33</b>	<b>15</b>

Table 14 Continued

Genus	Month	c-p value	Feeding Type	R7			R8			R9			R10			R11				
				J	A	N	J	A	N	J	A	N	J	A	N	J	A	N		
<i>Actinonema</i>		3	2A	0	0	0	0	0	0	0	0	0	0	0	60	2	0	0	0	0
<i>Annatheridina</i>		2	1B	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
<i>Angulostoma</i>		2	1B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Anticoma</i>		2	1A	0	0	0	0	27	4	8	0	1	31	0	2	1	3	10		
<i>Anticyanobus</i>		2	1B	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0
<i>Arcolimus</i>		2	2B	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Atomolimus</i>		2	2A	1	0	0	0	4	8	0	0	9	148	11	5	1	0	0	0	0
<i>Bathylolimus</i>		2	1B	0	0	0	10	1	0	1	1	32	1	11	6	1	1			
<i>Bethyla</i>		4	2B	0	0	0	0	8	1	0	0	7	2	0	3	0	0	0	0	0
<i>Bethylolimus</i>		3	2A	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0
<i>Campylimus</i>		3	1A	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0
<i>Chromadora</i>		3	2A	26	3	21	3	13	0	4	0	18	230	2	2	1047	11	1		
<i>Chromadorita</i>		3	2A	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
<i>Chromosyrinx</i>		4	2B	0	0	0	0	5	0	1	0	0	3	5	0	2	0	5		
<i>Cinctella</i>	ND		1A	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
<i>Ctenopharynx</i>		4	1A	0	0	0	0	3	1	0	0	5	0	0	4	1	0	8		
<i>Cyrtosoma</i>		3	1A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
<i>Dermadora</i>		2	2A	0	0	3	0	0	1	0	0	0	1	0	0	61	18	5		







Table 14 Continued

Genus	Month	c-p value	Feeding Type	K7			K8			K9			K10			K11		
				J	A	N	J	A	N	J	A	N	J	A	N	J	A	N
<i>Paraphacerosinus</i>		3	2B	0	0	0	0	11	6	0	0	3	1	0	0	31	53	27
<i>Paradictyophora</i>		2	2B	0	0	0	0	1	0	0	0	1	16	1	0	2	0	0
<i>Phaeodroma</i>		4	2A	0	0	0	0	1	1	0	0	0	0	0	0	1	2	0
<i>Polygastrophora</i>		4	2B	0	0	1	0	0	0	0	0	0	4	0	0	0	0	0
<i>Pomponema</i>		3	2B	0	0	0	0	19	2	6	0	0	0	2	24	9	9	
<i>Prochromadora</i>		3	2B	0	0	0	2	0	0	0	0	0	0	0	0	0	0	
<i>Prochromadorella</i>		2	2A	0	0	11	0	0	0	0	0	8	101	2	0	0	2	
<i>Prionema</i>		3	1A	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0
<i>Quadricona</i>		4	1A	0	0	0	0	0	0	0	0	0	0	0	2	0	0	
<i>Rhuhalemania</i>		4	1B	0	0	0	0	0	1	0	0	4	0	0	4	0	0	
<i>Subertia</i>		2	1B	0	0	2	0	79	0	1	0	5	1	0	5	49	8	4
<i>Sesoubacteria</i>		2	1B	0	0	2	0	6	2	0	0	12	0	0	7	15	0	1
<i>Sphaeroidium</i>		3	1A	0	0	0	0	5	0	0	0	2	0	0	0	0	0	1
<i>Sphaeroidium</i>		3	2B	0	0	1	0	4	22	0	0	7	0	0	2	35	14	14
<i>Spirinia</i>		3	2A	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
<i>Steieria</i>		2	1B	0	0	0	0	1	0	0	0	2	0	0	3	22	2	7
<i>Subphacerosinus</i>		3	1B	7	0	0	0	7	0	224	0	11	15	2	7	4	0	1
<i>Terschellgia</i>		3	1A	0	0	0	0	0	0	94	0	0	0	2	11	45	0	0
<i>Theriazus</i>		2	1B	19	0	4	0	0	6	87	1	3	3	0	2	29	0	1

Table 14 Continued

Genus	c-p value	Feeding Type	R7			R8			R9			R10			R11			
			J	A	N	J	A	N	J	A	N	J	A	N	J	A	N	
			Month	Month	Month	Month	Month	Month	Month	Month	Month	Month	Month	Month	Month	Month	Month	Month
<i>Tricoma</i>	4	1A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Tryplosides</i>	2	1B	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0	
Unknown Genus 1	2	1B	0	0	0	8	11	0	0	0	0	0	0	0	4	48	88	77
<i>Plicatula</i>	3	2B	0	0	14	0	2	3	19	0	2	16	3	4	46	2	19	
Total Number of Nematodes			87	86	69*	3*	272	119	734	3*	293	918	181	131	2383	284	264	
Total Number of Genera			9	3	13	1	30	26	22	3	27	36	16	31	46	21	18	

(\* = too few nematodes for calculation of indices)

*Enoplus*, *Halalaimus*, *Mesacanthion*, *Microlaimus*, *Nemanema*, *Odontophora lituifera*, *Oxyonchus*, *Parasphaerolaimus*, *Polygastrophora*, *Pseudonchus*, *Subsphaerolaimus*, *Theristus* and *Viscosia* were all present, each at less than 2 % abundance (Table 14).

In the November sample there were 500 individuals from 12 genera. The most abundant genus was *Microlaimus* (83 %) followed by *Theristus* (8 %). Both genera had increased in abundance from August to November. Of the remaining 30 genera found in August, only *Axonolaimus*, *Halalaimus*, *Nemanema*, *Sabatieria* and *Terschellingia* were found again in November at less than 3 % each. *Diplopeltula*, *Greeffiella*, *Laimella*, *Oxystomina* and *Paramonhystera* were present only in November at less than 3 % each (Table 14).

At site R1 the Shannon, Evenness, Simpson, Species Richness and Maturity Indices all increased from July to August and decreased from August to November (Table 15). All indices were at their lowest values during November, except Species Richness which was slightly lower in July. Nematode genera with the c-p value 2 dominated this site during all three sampling months (Table 16). There were no nematodes with a c-p value of 1 identified. The abundance of nematodes with the c-p value 2 decreased from July to August, and increased by November. Nematodes with c-p values 3 and 4 increased from July to August, and decreased by November. There was only one genus identified with a c-p value of 5 during July, constituting 0.5 % of that sample. Feeding type analysis indicated an abundance of non-selective deposit feeders (1B) during both July and August (Table 17). The percentage of selective deposit feeders (1A) remained

Table 15: Summary of the Shannon Index ( $H'$ ), Evenness ( $E$ ), Simpson Index ( $1/D$ ), Species Richness ( $D_{ng}$ ) and Maturity Index ( $MI$ ) values for samples containing a minimum of 70 nematodes collected at the Reference (R) Sites in Bay d'Espoir during July, August and November 1998.

Site	Month	$H'$	$E$	$1/D$	$D_{ng}$	$MI$
R1	July	1.03	0.50	1.96	1.54	2.19
	August	2.57	0.74	8.02	5.12	2.39
	November	0.76	0.31	1.44	1.77	2.05
R5	November	1.78	0.63	3.04	3.39	2.33
R6	July	3.01	0.85	14.24	6.31	2.60
	August	2.92	0.84	12.6	5.45	2.45
R7	July	1.72	0.78	4.78	1.79	2.70
	August	0.21	0.19	1.10	0.45	2.06
R8	August	2.73	0.81	8.86	5.17	2.42
	November	2.82	0.87	13.32	5.23	2.66
R9	July	2.04	0.66	5.77	3.18	2.64
	November	2.18	0.66	4.05	4.58	2.35
R10	July	2.49	0.69	8.03	5.13	2.72
	August	1.37	0.49	2.14	2.89	2.31
	November	3.14	0.91	20.04	6.16	2.60
R11	July	2.25	0.59	4.40	5.79	2.82
	August	2.23	0.73	6.17	3.54	2.59
	November	2.72	0.77	8.66	6.10	2.59

Table 16: Relative Abundance (%) of nematode genera per c-p value for samples containing a minimum of 70 nematodes collected at the Reference (R) Sites in Bay d'Espoir during July, August and November 1998.

Site	Month	c-p value				
		1	2	3	4	5
R1	July	0	79.8	19.1	1.1	0.5
	August		67.4	26.9	5.2	
	November		97.2	0.8	2.0	
R5	November	0	75.9	15.2	8.9	0
R6	July	0	44.8	35.8	18.8	0.6
	August		66.0	23.3	10.7	
R7	July	0	32.2	65.5	2.3	0
	August		95.3	3.5	1.2	
R8	August	0	64.7	28.3	7.0	0
	November		47.1	40.3	12.6	
R9	July	0	44.3	47.8	7.9	1
	November		72.3	21.2	5.5	
R10	July	0	48.3	32.0	19.5	0.2
	August		58.0	26.7	13.0	2.3
	November		76.3	17.1	5.5	1.1
R11	July	0	53.4	35.2	9.9	1.5
	August		48.2	45.8	4.9	1.1
	November		27.8	62.5	9.6	0.1

Table 17: Relative Abundance (%) of Wieser (1959) feeding types (1A, 1B, 2A, 2B) for samples containing a minimum of 70 nematodes collected at the Reference (R) Sites in Bay d'Espoir during July, August and November 1998.

Site	Month	1A	1B	2A	2B
R1	July	26.3	51.1	17.1	7.3
	August	24.6	69.5	2.1	2.1
	November	2.2	13.8	84.0	0
R5	November	6.3	71.4	5.4	16.9
R6	July	13.4	28.5	19.4	38.7
	August	14.6	12.9	44.1	28.4
R7	July	2.3	34.5	63.2	0
	August	1.2		98.8	
R8	August	13.7	42.3	17.3	26.7
	November	10.9	30.3	17.6	41.2
R9	July	20.8	72.2	2.9	4.1
	November	2.7	14.3	73.7	9.3
R10	July	4.6	10.7	79.2	5.5
	August	1.1	2.2	88.4	8.3
	November	16.8	39.7	22.9	20.6
R11	July	11.1	9.3	70.8	8.8
	August	2.1	35.6	29.6	32.7
	November	8.7	37.1	14.8	39.4

constant during July and August but decreased in November. The omnivores/predators (2B) decreased from July to August. During November epigrowth feeders (2A) dominated the sample. The percentage of both types of deposit feeders (1A and 1B) decreased, and there were no omnivores/predators (2B) identified.

#### **Roti Bay Reference Site R5**

At Roti Bay site R5 an overall total of 124 nematodes from 20 genera were isolated with individual sample sizes ranging from 0 to 112 (Table 14). During July there were no nematodes isolated. There were 12 nematodes from five genera isolated during August. The most abundant genus was *Viscosia* (67%). *Anticyathus*, *Axonolaimus*, *Halalaimus* and *Monoposthia* were equally abundant (8%). During November 112 nematodes from 17 genera were isolated. The most abundant genus was *Sabatieria* (56%). The relative abundance of *Axonolaimus* and *Viscosia* decreased from August to November. *Anticoma*, *Belbolla*, *Camacolaimus*, *Diplopeltula*, *Enoplolaimus*, *Metasphaerolaimus*, *Microlaimus*, *Oncholaimus*, *Phanoderma*, *Pomponema*, *Steineria*, *Theristus* and *Stephanolaimus* were all isolated only during November at less than 8% each (Table 14).

The calculated indices, relative abundance of c-p values and feeding types are shown in Tables 15, 16 and 17, respectively. Nematodes with a c-p value of 2 dominated the sample. There was low abundance of nematodes with c-p values of 3 and 4 (Table 16). There were no nematodes identified at this site with c-p values of 1 or 5. Non-selective deposit feeders (1B) dominated this sample. There was low abundance of



selective deposit feeders (1A), epigrowth feeders (2A) and omnivores/predators (2B) (Table 17).

#### **Northwest Cove Reference Site R6**

There was a total of 576 nematodes from 54 genera collected at Northwest Cove and values per sample ranged from 34 to 356 (Table 14). The July sample contained 186 individuals from 34 genera. The most abundant genus was *Metasphaerolaimus* (19 %) and *Enoplolaimus* was the second most abundant (10 %). The August sample had the largest nematode community of 356 individuals from 33 genera. The most abundant genus was *Axonolaimus* (20 %) and *Dolicholaimus* was the second most abundant (13 %). The November sample had 34 nematodes from 15 genera. The most abundant nematode was Unknown Genus 1 (29 %) and the second most abundant was *Sphaerolaimus* (12 %). *Anticoma*, *Crenopharynx*, *Sabatieria*, *Terschellingia*, and *Theristus* were isolated during each of the three sampling months. *Axonolaimus*, *Belbolla*, *Diplopeltula*, *Eleutherolaimus*, *Enoplolaimus*, *Halalaimus*, *Microlaimus*, *Odontophora*, *Paracanthionchus*, *Pomponema* and *Viscosia* were present during July and August at less than 10 % each. *Enoplus* and *Monhystera* were present during July and November at less than 5 % each. *Dolicholaimus*, *Halichoanolaimus*, *Neochromadora* and *Sphaerolaimus* were present during August and November at less than 13 % each.

*Actinonema*, *Ammotheristus*, *Bathylaimus*, *Campylaimus*, *Chromadorita*, *Disconema*, *Leptolaimus*, *Mesacanthion*, *Metalinhomoeus*, *Metasphaerolaimus*, *Odontophora*, *Pandolaimus*, *Praeacanthionchus*, *Steineria*, *Subsphaerolaimus*,

*Synonchiella*, and *Trefusia* were isolated only during July, each at less than 19 % (Table 14). *Anticyathus*, *Choniolaimus papillatus*, *Desmodora*, *Marylynnia*, *Monoposthia*, *Nemanema*, *Oxyonchus*, *Oxystomina*, *Parasphaerolaimus*, *Parodontophora*, *Phanoderma*, *Setosabacteria* and *Spiliphera* were only isolated in the August sample, each at less than 5 % (Table, 14). *Acanthonchus*, *Chromaspirina*, and *Prochromadorella* were isolated only during November at less than 6 % each.

At site R6 the Shannon, Evenness, Simpson, Species Richness and Maturity Indices all decreased from July to August (Table 15). The abundance of nematodes with a c-p value of 2 increased from July to August, and dominated both samples (Table 16). The abundance of nematodes with c-p values of 3 and 4 decreased from July to August. There was one genus isolated during July with a c-p value of 5, constituting 0.6 % of the population. There were no genera with a c-p value of 1 isolated at this location (Table 16). Feeding type analysis indicated a dominance of omnivores/predators (2B) during July, but with representatives of each all feeding categories (Table 17). By August, however, epigrowth feeders (2A) dominated the sample, with decreases in the abundance of non-selective deposit feeders (1B) and omnivores/predators (2B). There was a slight increase in the abundance of selective deposit feeders (1A).

#### **Ship Cove Reference Site R7**

The total number of nematodes collected at Ship Cove site R7 was 242 from 20 genera and values per sample ranged from 69 to 87 (Table 14). During July 87 nematodes from nine genera were isolated. The three most abundant genera were *Chromadora*

*macrolaima*, *Innocuonema* and *Theristus* composing 30 %, 26 % and 22 % of the total community respectively. During August 86 nematodes from three genera were isolated, *Dichromadora hyalocheile* made up 95 % of the total community. During November 69 nematodes from 13 genera were isolated. *Chromadora macrolaima* and *Viscosia* made up 30 % and 20 % of the total community, respectively.

*Chromadora macrolaima* was the only species that occurred in all three sampling months. *Oxystomina* was present during July and August at less than 3 %, *Monoposthia*, and *Theristus* were present during July and November, each at less than 22 %. *Axonolaimus*, *Gnomoxyala*, *Innocuonema*, *Microlaimus* and *Subsphaerolaimus* were isolated only during July, each at less than 27 %. *Dichromadora* was only isolated in August (95 %). *Desmodora*, *Enoplus*, *Mesacanthion*, *Odontophora*, *Polygastrophora*, *Prochromadorella*, *Sabatieria*, *Setosabatieria*, *Sphaerolaimus*, and *Viscosia* were isolated only during November, each at less than 16 %.

The Shannon, Evenness, Simpson, Species Richness and Maturity Indices all decreased from July to August (Table 15). There was a dominance of nematodes with a c-p value of 3 during July, but by August nematodes with a c-p value of 2 dominated the sample, with only small percentages of nematodes with c-p values of 3 and 4. There were no nematodes isolated from this site with c-p values of 1 and 5 (Table 16). Feeding type analysis indicated an abundance of epigrowth feeders (2A) during July and August. There were no omnivores/predators (2B) isolated at this site. During November there were also no non-selective deposit feeders (1B) isolated (Table 17).

### Milltown Reference Site R8

The overall total number of nematodes collected at Milltown Basin site R8 was 395 from 41 genera and values per sample ranged from 4 to 272 (Table 14). During July only four nematodes were isolated. One was an unidentifiable larval stage and the other three were *Chromadora macrolaima*. August had the largest nematode assemblage of 272 individuals from 30 genera. *Sabatieria* was the most abundant genus (29 %), the second most abundant was *Anticoma* (10 %). Also present in decreasing abundance (7 to 3 %) were *Pomponema*, *Paracanthochus*, *Chromadora macrolaima*, *Metasphaerolaimus*, *Parasphaerolaimus* and *Bathylaimus tenuicaudatus*. *Axonolaimus*, *Belbolla*, *Chromaspirina*, *Crenopharynx*, *Laimella longicaudata*, *Metachromadora*, *Metalinhomoeus*, *Monoposthia*, *Nemanema*, *Oxyonchus*, *Oxystomina*, *Parodontophora*, *Paramonhystera*, *Phanoderma*, *Prochromadorella*, *Setosabatieria*, *Siphonolaimus*, *Sphaerolaimus*, *Steineria*, *Subsphaerolaimus*, Unknown Genus 1 and *Viscosia* were all present at less than 3 % each (Table 14).

During November 119 nematodes from 26 genera were isolated. The most abundant genus was *Sphaerolaimus* (19 %) and *Anticyathus* was the second most abundant (13 %). *Anticoma*, *Bathylaimus*, *Paracanthochus* and *Pomponema* all decreased in abundance from August to November, while *Axonolaimus*, *Monoposthia*, *Parasphaerolaimus*, *Sphaerolaimus* and Unknown Genus 1 increased in abundance. *Belbolla*, *Crenopharynx*, *Nemanema*, *Phanoderma*, *Pomponema*, *Setosabatieria* and *Viscosia* remained present at less than 3 % each. *Mesacanthion*, *Theristus*, *Halalaimus*

*Desmodora*, *Desmolorenzenia*, *Enoplolaimus*, *Eurystomina*, *Microlaimus*, *Onyx* and *Rhabdodemania* were all present only during November at less than 8 % each (Table 14).

The Shannon, Evenness, Simpson, Species Richness and Maturity indices all increased from August to November (Table 15). Nematode genera with a c-p value of 2 formed the largest percentage at this site during both sampling months. The percentage of nematodes with a c-p value of 3 increased from August to November, while the percentage of nematodes with a c-p value of 4 decreased during the same time. There were no nematodes with c-p values 1 or 5 isolated from this site (Table 16). There were representatives of all four feeding types isolated during both months. Non-selective deposit feeders (1B) dominated during August, while omnivores/predators (2B) dominated during November (Table 17).

#### **Swangers Cove Reference Site R9**

The total number of nematodes collected from all samples taken at Swangers Cove totalled 1030 from 41 genera and values per sample ranged from 3 to 734 (Table 14). July had the largest nematode community of 734 individuals from 22 genera. The most abundant genus was *Subsphaerolaimus* (31 %) and the second most abundant was *Paralinhomoeus* (19 %). The August sample had only three nematodes, one each of *Bathylaimus*, *Metalinhomoeus* and *Theristus*. November had a nematode assemblage of 293 individuals from 27 different genera. The most abundant genus was *Paracanthochnus* (48 %) and the second most abundant was *Neochromadora* (9 %).

*Theristus* was the only genus isolated during all three sampling months. *Anticomma*,

*Chromadora*, *Diplopeltula*, *Sabatieria*, *Subsphaerolaimus* and *Viscosia* were present during both July and November. *Metalinhomoeus* was present during July and August, decreasing in abundance. *Bathylaimus* was present during August and November, increasing in abundance. *Chromaspirina*, *Dichromadora*, *Didelta*, *Eleutherolaimus*, *Laimella*, *Leptolaimus*, *Microlaimus*, *Nemanema*, *Oxystomina*, *Paralinhomoeus*, *Paramonhystera*, *Pomponema*, and *Terschellingia* were all present only during July. *Axonolaimus*, *Belbolla*, *Crenopharynx*, *Enoplus*, *Euchromadora*, *Mesacanthion*, *Monoposthia*, *Odontophora*, *Parasphaerolaimus*, *Parodontophora*, *Prochromadorella*, *Rhabdodemia*, *Setosabatieria*, *Siphonolaimus*, *Sphaerolaimus*, and *Steineria* were all present only during November, each at less than 5 % (Table 14).

The Shannon and Species Richness Indices increased from July to November, while the Simpson and Maturity Indices decreased during the same time. The Evenness value stayed the same during both sampling months (Table 15). Nematodes with a c-p value of 3 dominated the July sample, followed closely by nematodes with a c-p value of 2. The remaining July community comprised nematodes with a c-p value of 4. The November community was dominated by nematodes of c-p value 2. There was a decrease in the percentage of nematodes with c-p values 3 and 4 from July to November. There were no nematodes isolated from this site with a c-p value of 1. There was only one nematode isolated with a c-p value of 5 (Table 16). There were representatives of all four feeding types isolated during both July and November (Table 17). The July community was dominated by non-selective deposit feeders (1B), while the November community

was dominated by epigrowth feeders (2A).

#### **Linen Cove Reference Site R10**

The number of nematodes from all samples taken at Linen Cove site R10 totalled 1230 from 51 genera and values per sample ranged from 131 to 918 (Table 14). July had the largest nematode community of 918 individuals from 36 genera. The most abundant genus was *Chromadora* (25 %) and *Axonolaimus* was the second most abundant (16 %). August had a total of 181 individual nematodes from 16 genera. The most abundant genus was *Paracanthochus* (67 %) and *Monoposthia* was the second most abundant (10 %). November's sample had 131 individuals identified from 31 genera. The most abundant genus was *Paracanthochus* (15 %) and the second most abundant were *Bathylaimus* and *Terschellingia* (8 % each).

*Axonolaimus*, *Bathylaimus*, *Chromadora*, *Enoplus*, *Metasphaerolaimus*, *Monoposthia*, and *Paracanthochus* were isolated during all three sampling months. *Actinonema*, *Chromaspirina*, *Hypodontolaimus*, *Parodontophora* and *Prochromadorella* were present only during July and August. *Anticoma*, *Belbolla*, *Enoplolaimus*, *Halalaimus*, *Neochromadora*, *Sabatieria*, and *Theristus* were present during July and November. *Metalinhomoeus* and *Terschellingia* were present during August and November.

*Ascolaimus*, *Chromadorita*, *Desmodora*, *Desmolorenzenia*, *Dichromadora*, *Diplopettula*, *Leptolaimus*, *Mesacanthion*, *Microlaimus*, *Odontophora lituifera*, *Oxystomina aestosa*, *Parasphaerolaimus*, *Polygastrophora*, *Tricoma*, and *Tryploidis*

were all present during July only, each at less than 4 % (Table 14). *Ammotheristus*, *Crenopharynx*, *Eleutherolaimus*, *Halichoanolaimus*, *Linhystera*, *Nemanema*, *Pomponema*, *Rhabdodemia*, *Setosabatieria*, *Sphaerolaimus*, *Spirinia*, *Steineria*, and Unknown Genus 1 were present during November only, each at less than 6 % (Table 14).

The Shannon, Evenness, Simpson, Species Richness and Maturity Indices all decreased from July to August, but then increased and peaked during November (Table 15). Genera with a c-p value of 2 were numerically dominant at this site. The percentage of nematodes with a c-p value of 2 increased over the sampling time, whereas the percentage of nematodes with c-p values of 3 and 4 decreased over the same time period. There were no genera with a c-p value of 1 isolated at this site. There was one genus isolated during each sampling time with a c-p value of 5 making up 0.2 % of the community during July, 2.3 % during August and 1.1 % during November (Table 16). Feeding type analysis showed an abundance of epigrowth feeders (2A) during both July and August (Table 17). There was low abundance of each of the other three feeding types present during the summer sampling period. In November, however, the abundance of each of the four feeding types was more evenly distributed with a slight dominance of non-selective deposit feeders (1B).

#### **Fish Processing Plant Reference Site R11**

The number of nematodes collected from all samples taken at the Fish Processing Plant site R11 totalled 2931 from 55 genera and values per sample ranged from 264 to 2383 (Table 14). July had the largest nematode community of 2383 individuals from 46



genera. The most abundant species was *Chromadora macrolaima* (44 %) and the second most abundant was *Microlaimus* (14 %). During August 284 individuals were identified from 21 genera. The most abundant nematode was the Unknown Genus 1 (31 %) and the second most abundant was *Parasphaerolaimus* (19 %). During November 264 individuals were identified from 18 genera. The most abundant nematode was Unknown Genus 1 (29 %) and the second most abundant was *Parasphaerolaimus* (10 %).

*Anticoma*, *Bathylaimus*, *Chromadora*, *Desmodora*, *Eleutherolaimus*, *Enoplolaimus*, *Enoplus*, *Halalaimus*, *Metasphaerolaimus*, *Monoposthia*, *Paracanthochnus*, *Parasphaerolaimus*, *Pomponema*, *Sabatieria*, *Steineria*, Unknown Genus 1 and *Viscosia* were present during each of the three sampling months. The abundance of *Chromadora* and *Halalaimus* decreased from July to November. In contrast *Anticoma*, *Bathylaimus*, *Eleutherolaimus*, *Enoplolaimus*, *Enoplus*, *Paracanthochnus*, and *Pomponema* all increased in abundance from July to November. *Desmodora*, *Metasphaerolaimus*, *Monoposthia*, *Sabatieria*, *Parasphaerolaimus* and Unknown Genus 1 all peaked in abundance during August. *Steineria* and *Viscosia* decreased in abundance from July to August but increased in November.

*Dolicholaimus*, *Mesacanthion*, and *Phanoderma* were isolated during July and August. *Chromaspirina*, *Crenopharynx*, *Halichoanolaimus*, *Metalinhomoeus*, *Oxystomina*, *Setosabatieria*, *Subsphaerolaimus*, and *Theristus* were isolated during July and November. *Axonolaimus*, *Bolbolaimus*, *Campylaimus*, *Desmolorenzenia*, *Dichromadora*, *Diplopeltula*, *Enoploides*, *Leptolaimus*, *Metachromadora*, *Nemanema*,

*Parodontophora*, *Pselionema*, *Quadricoma*, *Spirinia* and *Terschellingia* were present only during July, each at less than 1 %. *Anoplostoma*, *Cyartonema*, *Epacanthion*, *Greeffiella*, *Monhystera*, *Oncholaimus*, *Prochromadorella*, and *Siphonolaimus* were present only during November, each at less than 1 %.

The Evenness and Simpson Indices increased from July to November. The Shannon and Species Richness Indices decreased slightly from July to August but increased by November. The Maturity Index decreased from July to August and then remained stable during November (Table 15). During July and August the community was numerically dominated by nematodes of c-p 2, followed closely by nematodes with c-p 3. Also present were nematodes with c-p values of 4 and 5 (Table 16). During November, however, the community was dominated with nematodes of c-p value 3. Also present were nematodes with c-p values of 2, 4 and 5. There were no nematodes with a c-p value of 1 isolated at this site (Table 16).

Feeding type analysis indicated a numerical dominance of epigrowth feeders (2A) during July, with low abundance of each of the other three feeding types (Table 17). The August sample had approximately the same numbers of non-selective deposit feeders (1B), epigrowth feeders (2A) and omnivores/predators (2B), with low abundance of selective deposit feeders (1A). During November, however, there was a dominance of omnivores/predators (2B), followed closely by non-selective deposit feeders (1B). There was a low abundance of selective deposit feeders (1A) and epigrowth feeders (2A) (Table 15).

### **Summary of Physical and Chemical Variables**

Table 18 shows the physical and chemical variables, and Table 19 the particle size analysis. Samples were taken at a wide range of depths from 4.2 m to 38.7 m. The pH ranged from 6.7 to 8.0. The redox potential showed the greatest range from -348.4 mV to 173.0 mV, with 14 measures being negative and seven measures being positive. The temperature generally decreased over the sampling period at sites R2, R4, R6, and R11. The temperature at sites R3 and R5 peaked during August. With the exception of the July R1 sample (cobble and gravel), all samples contained clay. Five samples contained only clay and 20 samples contained clay and silt. Of those 20, four also contained sand and one contained cobble.

Table 18: Physical and chemical variables of all Reference Sites (R1-R11) investigated in Bay d'Espoir during 1998.

Variable	Month	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11
Depth (m)	July	36.9	34.1	32.0	34.1	11.4	14.6	NT	NT	NT	NT	9.0
	August	6.6	22.7	32.0	38.7	6.8	14.8	4.2	6.7	5.2	4.7	9.1
	November	33.2	24.7	32.0	27.9	9.3	14.4	5.1	11.5	5.8	7.8	8.7
pH	July	NT	7.5	7.5	8.0	7.6	6.7	NT	NT	NT	NT	7.4
	August	7.2	7.4	7.3	7.4	7.3	7.7	7.6	7.4	7.2	NT	7.5
	November	7.9	7.4	7.3	7.1	7.0	7.1	7.0	7.2	7.1	7.1	7.1
Redox Potential (mV)	July	NT	-89.0	-144.0	-209.6	-159.5	-348.4	NT	NT	NT	NT	-66.6
	August	NT	NT	NT	NT	NT	NT	-225.0	<-1.0	-90.0	NT	110.0
	November	-156.0	90.0	117.0	76.0	-26.0	-14.0	-20.0	173.0	173.0	15.0	-50.0
Temperature (°C)	July	NT	6.8	5.8	7.4	8.3	12.4	NT	NT	NT	NT	11.7
	August	11.2	4.5	6.7	4.6	12.4	10.3	13.2	NT	NT	NT	9.0
	November	2.9	2.3	4.9	5.1	6.6	6.4	7.7	6.0	6.9	6.1	7.0

Key: NT = Data Not Taken

Table 19: Particle Size Analysis of all Reference Sites (R1 - R11) investigated in Bay d'Espoir during 1998.

Month	Sediment Type	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11
July	Cobble	X			NT			NT	NT	NT	NT	
	Gravel	X			NT			NT	NT	NT	NT	
	Sand				NT			NT	NT	NT	NT	
	Silt		X	X	NT	X	X	NT	NT	NT	NT	X
	Clay		X	X	NT	X	X	NT	NT	NT	NT	X
August	Cobble				NT							
	Gravel				NT							
	Sand				NT		X	X			X	X
	Silt	X	X	X	NT	X	X	X		X	X	X
	Clay	X	X	X	NT	X	X	X	X	X	X	X
November	Cobble					NT	X					
	Gravel					NT						
	Sand					NT						
	Silt				X	NT	X	X	X	X		X
	Clay	X	X	X	X	NT	X	X	X	X	X	X

Key: NT = Data Not Taken      X = Present In Sample

Cobble = 60-200 mm, Gravel = 2-60 mm, Sand = 0.06-2 mm, Silt = 0.002-0.06 mm, Clay = < 0.002 mm

## Discussion

When analysing large amounts of data there are two types of error to consider - measurement and confounding. Measurement error, error due to the measurement of specific variables, is usually detected statistically. Unfortunately the data from Bay d'Espoir could not be studied using any statistical technique because of missing data: not all sites were sampled during each month, and physical and chemical variables were not always measured.

In comparison, confounding error, error due to a lack of statistical control of experimental variables that decreases the ability to determine which variables are causing the results, can be avoided by choosing one of three experimental models. The first experimental model operates by controlling the impact on the experimental sites; for example the experimenter could standardize the amount of organic pollutants entering a water system. The second model would include the random selection of reference sites. The third model would be a time series, whereby both treatment and reference sites would be studied over a long period of time. The first two experimental designs could not be used because I had no control over the amount of organic material entering aquaculture sites or control of the selection of sampled sites (aquaculture or non-aquaculture reference). The third design could not be considered because the time period involved was not long enough to be considered a time series. Confounding error can be explained by biological or environmental factors and their interactions, and will be the focus of the Bay d'Espoir data analysis.

To detect differences between the aquaculture and non-aquaculture reference sites, the total number of individual nematodes and the total number of genera collected were considered. In general there were fewer total nematodes and genera at the aquaculture sites than the reference sites. There were either no nematodes or too few nematodes present for calculation of indices in the majority of aquaculture samples. In comparison, most samples from reference sites had an abundance of nematodes. For example, there were no nematodes found in samples from A3, R2 and R5 in July, A1, A2, A4, and A5 in August, A2, A4, and R4 in November. Reference sites R10 and R11 had extremely high nematode numbers even in comparison to other reference sites. Both of these sites are known to be organically polluted which would account for the abundance of nematodes. In addition, there was also more variation for both the total number of nematodes and genera at the reference sites than the aquaculture sites. If reference site R11 was removed from this analysis, the variance would be even greater.

Recently there has been an increase in nematode research at aquaculture sites but results are controversial. A comparison of a farm site with a control site 1 km away in the Mediterranean indicated a significant difference between the total number of individual nematodes at the farm and control sites (Mazzola *et al.*, 1999). In any sample collected at the farm site the total number of individuals did not exceed 1000, whereas the total number of individuals at control sites did not fall below 1000, and mostly fell between 1500 and 2500 (Mazzola *et al.*, 1999). A study in Washington (Weston, 1990) found the opposite. The largest nematode community was at the farm site, and the total number of

individuals decreased in samples taken up to 150m away from the farm site. At 450m, the nematode population began to increase again.

The mean of the Shannon Index ( $H'$ ), Evenness (E), Simpson Index ( $D_{mq}$ ), Species Richness (SR) and MI was also determined. Aquaculture sites' mean values were: ( $H'$ ) 2.05, (E) 0.75, ( $D_{mq}$ ) 7.31, (SR) 3.27 and (MI) 2.52. Non-aquaculture reference site mean values were ( $H'$ ) 2.05, (E) 0.67, ( $D_{mq}$ ) 6.98, (SR) 3.89, and (MI) 2.45. The mean values are not very different for the two data groups, and if variance was considered the values would overlap, indicating no difference. For example, the  $H'$  for aquaculture sites ranged from 0.86 to 2.80 (Table 9), and for reference sites from 0.21 to 3.14 (Table 15). The Evenness at aquaculture sites ranged from 0.48 to 0.89 (Table 9), and at reference sites from 0.19 to 0.91 (Table 15). The Simpson Index values at aquaculture sites ranged from 1.84 to 13.34 (Table 9), and at reference sites from 1.10 to 20.04 (Table 15). The Species Richness at aquaculture sites ranged from 1.05 to 5.18 (Table 9), and at reference sites from 0.45 to 6.31 (Table 15). The MI at aquaculture sites ranged from 2.01 to 2.96 (Table 9), and at reference sites from 2.00 to 2.82 (Table 15). Variance was not considered because within site and among sampling time differences could not be assessed.

To retrieve data overlooked from calculating the MI, c-p analysis was conducted. The percentage of nematodes with a c-p value of 2 dominated four of the aquaculture samples (Table 10). These four were located in Roti Bay. There was also a numerical dominance of nematodes with a c-p value of 2 in 15 of the non-aquaculture reference samples. The remaining three were numerically dominated by nematodes of a c-p value



of 3. Two of these were measured during July at Ship Cove (R7) and Swangers Cove (R9), the remaining one was measured during November at the Fish Processing Plant (R11) in St. Alban's (Table 16). Nematodes with a c-p value of 2 survive environmental pollution the longest and under stressed conditions where bacterial activity is also constrained, the c-p 2 group reaches a high dominance (de Goede *et al.*, 1993). The suggestion that sites studied in Bay d'Espoir had decreased bacterial production is supported by the lack of nematodes with a c-p value of 1.

The feeding type distributions were much more varied than the c-p distributions. Results are more site specific, showing seasonal changes within a site. Epigrowth feeders reach their maximum numerical density during spring and summer, while deposit feeders and omnivores/predators reach their maxima during the fall and winter in accordance with the findings of Tietjen (1969). Similar observations were made at Roti Bay Sites A3, R5, Northwest Cove Site R6, Ship Cove Site R7, Milltown Site R8 (November only), and at Linen Cove Site R10. At Roti Bay Sites A2, R1, Milltown Site R8, Swangers Cove Site R9 and the Fish Processing Plant Site R11 deposit feeders peaked in abundance during July and August. Also at Roti Bay Site R1 and Swangers Cove Site R9 the epigrowth feeders peaked in abundance during November, the exact opposite of what was expected. There were no observed differences between the aquaculture and reference sites with respect to feeding type.

In addition to seasonal effects, there are several factors that can affect a nematode assemblage. It has been well documented that nematode communities are affected by

sediment particle size. Wieser's (1960) study in Buzzards Bay established that certain genera are characteristic of certain substrates. For example, *Synonchiella*, *Latronema*, *Pomponema*, *Leptonemella*, *Laimella*, *Anticoma* and *Odontophora* are all characteristic of sandy substrate. *Terschellingia longicaudata*, *Odontophora loffleria*, *Sabatieria*, *Sphaerolaimus* and *Metalinhomoeus* are characteristic of subtidal silt or mud. Some nematodes were able to thrive in both habitats, for example, *Dorylaimopsis metatypicus* (Wieser, 1960). In Chile, *Sabatieria* species increased in number as depth of sediment increased, while *Terschellingia* species decreased in number (Wieser, 1960).

Since then it has been established that nematode abundance is higher in finer marine sediment, but the diversity is higher in coarse sediments (Bongers and Van de Haar, 1990). In general, fine sediment species are short in length, while coarse sand species are either very small or elongate and thin (Heip *et al.*, 1985). Tietjen (1980) concluded that the families Chromadoridae and Desmodoridae were most abundant in medium and coarse sands while the family Comesomatidae was most characteristic of silty and fine sands.

Clearly, sediment analysis should be taken into account when studying the nematode assemblage at each site. For example, the July sample from Roti Bay reference site R1 consisted of gravel and cobble, whereas the August sample from the same site consisted of silt and clay (Table 19). Because of such differences in sediment size, different nematode communities might be expected. The July sample of larger sized particles contained 1022 individuals. The August sample of smaller sized particles

contained fewer total individuals, yet the Shannon, Simpson and Maturity Indices, as well as Evenness and Species Richness measures were all higher. These findings are exactly opposite to those of previous work (Bongers and Van de Haar, 1990).

However, the findings at site A2 are similar to those of Bongers and Van de Haar (1990) in that there was a complete shift in nematode community between the July and August samples, as none of the genera found in July were found in August. The July sample contained a variety of particles ranging from clay to cobble, whereas the August sample consisted only of clay. In contrast to the July sample the August sample, of finer particles, contained a higher number of nematodes and lower Shannon, Species Richness and MI values.

Redox potential has long been used as an indicator of environmental disturbance, and is particularly useful in studying organic pollution (Gowen *et al.*, 1991). A redox potential of zero indicates the boundary between aerobic and anaerobic processes, a positive measure indicates aerobic activity and a negative measure anaerobic activity (Gowen *et al.*, 1991). Aquaculture farms can produce highly reduced sediment (Beveridge 1996; Wu *et al.*, 1994; Gowen, 1990; Weston, 1990; Brown *et al.*, 1987). For example, Beveridge (1996) found all redox potentials were negative at a heavily disturbed aquaculture area but redox potentials taken 25 m north and south of the farmed site, as well as a control site were all positive.

Measurements at the aquaculture sites at Bay d'Espoir ranged from -227.9 mV to 7.0 mV (Table 12), with only one positive measurement. In comparison, the reference

sites ranged from -348.4 mV to 173.0 mV (Table 18), with seven positive measurements and 14 negative measurements. There is also a relationship between sediment depth and redox potential. As measurements are taken both deeper in the sediment and closer to the farmed site, the redox potential will be more negative (Beveridge, 1996; Gowen *et al.*, 1991). Hence, it is important to measure how deep in the sediment the redox potential is being measured.

Power *et al.*, (1998) studied nematode assemblages in the Bay d'Espoir region during April, May and June 1998, prior to the commencement of this research. MI was the only mathematical tool used to analyse the nematode communities. They only had redox potential, pH, depth and temperature measurements for some sites. Power *et al.*, (1998) suggested that the low redox potential readings, created by the aquaculture sites, were the reason for low nematode numbers and hence low MI values. In that study, similar genera were isolated, but the total number of nematodes was low compared with the present study. In the present study, analysis beyond total nematode counts was restricted to samples containing a minimum of 70 individuals. However, only one site (Swangers Cove) had more than 70 nematodes during May. Because of this, MI values reported for April, May and June are not reliable. Other than Power *et al.*, (1998), a MI of zero has not been reported previously. The reason for choosing the minimum number of 70 individual nematodes is the suggestion by Bongers *et al.*, (1991) that identification of 75 specimens should give a satisfactory estimate of the MI. Seventy nematodes were chosen instead of 75 because Lawlor (1998) has shown that fewer specimens could

produce a statistically similar MI value and a minimum of 75 nematodes would have further reduced the numerical analysis of the present work.

This study has pointed out the complexity of analysing aquaculture sites. Many interconnected factors have to be considered. In this study, temperature, depth, pH, redox potential, particle size and nematode assemblages were all studied in relation to aquaculture development. Other factors which could have been incorporated into the study include wind/wave exposure, tidal currents, salinity, dissolved oxygen concentration, turbidity and organic content.

The methodology of sample collection is an important factor. When the Ekman grab sampler is being raised to the water surface, mud and organisms are often lost out of the top of the grab (Chandler and Hasler, 1979). Another problem is the shape of the Ekman grab sampler. The bottom of the sampler is arc-shaped, and as such does not evenly penetrate the sediment. This is a problem when studying organisms that change in relation to depth within the sediment (Chandler and Hasler, 1979). Nematodes are known to concentrate in the top few centimetres of sediment. For nematode analysis collecting samples by scraping along the sediment surface or using cores would be much more suitable.

This study has clearly indicated the importance of a complete data set. In addition it has provided baseline data for future monitoring programs in the Bay d'Espoir region. The identity of nematode communities has been established from the area for the first time. It is also clear that such an analysis should have been conducted prior to the

installation of the aquaculture farms. Ideally the areas should have been studied for one complete year before the aquaculture farms were installed, with replicate samples and measurements taken every month. The same analysis then would be conducted one year after installation, with a long term monitoring program in place.

## **Chapter 4: General Discussion**

This study was designed to provide baseline data on the community structures of nematode assemblages in marine habitats in which human activity such as oil refineries and aquaculture is an issue. Some conclusions on the affects of aquaculture practices on nematode communities were drawn based on feeding types, the MI and four other indices.

It has long been known that pollution results in decrease in diversity within the biotic community (Green, 1979). A wide variety of indices has been developed and analysed to interpret communities which can be used as bioindicators and monitors. The Shannon-Wiener (also known as Shannon-Weaver or Shannon) Index and Simpson Index are the most commonly used diversity indices. In general, if a community has a high diversity, then the species present are equally or nearly equally abundant. The community would also be assumed to be more complex with more species interaction than a community of low diversity. Such a community is either composed of very few species or only a few species are abundant (Brower and Zar, 1977).

The Shannon Index ( $H'$ ) was separately developed by both Shannon and Weaver during 1949. The  $H'$  makes two assumptions: individuals are randomly sampled from an 'indefinitely large' population and all species are represented in the sample (Magurran, 1988). The largest source of error occurs when all species in a community are not represented in a sample (Magurran, 1988). For example, it has been suggested that the true number of species is not known if in a sample there are many species with few individuals (Poole, 1974). The actual  $H'$  value normally ranges from 1.5 to 3.5, rarely

exceeding 4.5 (Magurran, 1988). The  $H'$  value also considers the 'Evenness' of each species. For example, the more evenly distributed species are in a community, the higher the  $H'$  will be.  $H'$  underestimates the diversity in the sampled community, but as the sample size increases this bias decreases (Zar, 1999). It has been well documented that the  $H'$  is most biased towards Species Richness when compared with other diversity indices (Neilson *et al.*, 1996; Platt *et al.*, 1984). Thus, among the diversity indices available Species Richness affects the  $H'$  value the most.

The Simpson Index or Dominance Measure ( $1/D$ ) was proposed in 1949 and represents the probability that two randomly selected individuals will not belong to the same species (Green, 1979). The Simpson Index is biased towards abundance rather than Evenness or Species Richness (Platt *et al.*, 1984). As such, it is affected by the absolute number of individuals in a community and is not affected by the number of species in a community or how evenly distributed species are within a community. As the value of  $D$  increases, diversity decreases. The actual value calculated ( $1/D$ ) is weighted towards the abundance of the commonest species (Magurran, 1988).

Diversity measures using different formulae might be expected to yield, if not necessarily the same values, then at least the same conclusions. This, however, is not the case especially when comparing the Shannon-Weaver and Simpson Indices. If graphical analysis of dominance curves results in an intersection, then the values of  $H'$  and  $1/D$  cannot be relied upon (Platt *et al.*, 1984). Platt *et al.*, (1984) showed that the Shannon-Weaver Index decreased from 1.95 to 1.86 from January to July, in contrast with the



Simpson Index which increased from 0.65 to 0.72 from January to July. It has therefore been suggested by Platt *et al.*, (1984) that calculating species diversity should be replaced by plotting dominance curves, or at least have these curves plotted and analysed prior to calculating species diversity.

In addition to the Shannon-Weaver and Simpson Indices, the Maximum Diversity ( $H'_{max}$ ) can be calculated and subsequently used to determine Evenness (E) of a sample. Evenness (E) is also known as heterogeneity or relative diversity (Zar, 1999) and is defined as how evenly individuals are apportioned among the species (Platt *et al.*, 1984). Evenness is also a biased statistic because it overestimates the Evenness of a community when the number of species is underestimated, which is a rather common situation (Zar, 1999). It is also assumed when calculating Evenness that all species in the community are accounted for in a sample (Magurran, 1988). The Evenness value (E) ranges from 0 to 1.0. A value of 1.0 would indicate that  $H'$  is equivalent to  $H'_{max}$  ( $H' = \ln S = H'_{max}$ ) and is interpreted as all species being equally abundant (Magurran, 1988).

Another frequently utilized statistical tool in environmental monitoring is Species Richness which can be simply defined as the number of species in a community (Hellmann and Fowler, 1999). There are a variety of formulae to calculate Species Richness. One of the problems with Species Richness is that regardless of the chosen formula, the resulting value is always dependent on sample size. The actual richness of the community is therefore underestimated. However, as sample size increases the calculated value becomes more accurate (Hellmann and Fowler, 1999). Some researchers

find Species Richness formulas inadequate because they do not allow us to differentiate between the diversities of different communities having the same number of species and the same total number of organisms (Brower and Zar, 1977).

It has been proposed that there is no valid connection between high diversity and good environmental health, primarily because there are many other factors that affect diversity in addition to the environmental state (Green, 1979). For example, latitudinal, trophic, seasonal and other temporal factors, as well as spatial variation have all been shown to affect diversity values (Green, 1979). Therefore it has been suggested that the total number of species in a sample and Species Richness are more biologically meaningful, less ambiguous and often more informative than diversity indices (Green, 1979). It has been claimed that the number of species is the only true objective measure of diversity and is actually more appropriate than any diversity index in many situations (Poole, 1974).

The Maturity Index (MI) was proposed by Bongers (1990), specifically to interpret nematode communities. Identification of nematodes to family level and identification of only 75 nematodes were noted as advantages of using the MI (Bongers *et al.*, 1991). Another important aspect of the MI is the incorporation of autecological information into the calculation. The more traditional indices of Species Diversity, Richness and Evenness are mathematical functions which do not take into account the biology of the organism.

Application of the MI has been studied using old and new data. Using previously analysed data it was determined that MI values decrease because of pollution (sewage

waste, oil, heavy metal) but increase during colonisation (Bongers *et al.*, 1991).

Examples of the application of the MI include a study in Brittany, France before and after the *Amoco Cadiz* oil spill of March 1978 (Bongers *et al.*, 1991). MI values were calculated in 1991 from species lists published in 1980. Data before the spill were collected in 1972-73 and after the spill data in 1978-79. The average MI before the spill was 2.81 and afterwards 2.40. The Mann-Whitney U test showed a significant difference between the MI values (Bongers *et al.*, 1991). Another study in the Tay Estuary, Scotland (Neilson *et al.*, 1996) compared MI values at increasing distances from a sewage outfall. Sediment size, nematode density and heavy metal presence did not affect the MI value, but the number of species present, distance from sewage outfall and proportion of nematode feeding groups did significantly affect the MI value. The same conclusions can be drawn using MI and H' values. Gyedu-Ababio *et al.*, (1999) analysed nematode communities in relation to heavy metal concentrations. The lowest values of both indices were found at the same sites, and were considered indications of stress.

Throughout the present study many concerns arose from using the Maturity Index. Although incorporating biology into an index is a good idea, the most problems with the MI revolve around the c-p values. The allocation of marine nematode taxa to particular c-p values has been based on established knowledge of body size, generation time, dominance in samples and sensitivity towards disturbance (Bongers, 1999). For example, members of the subclass Secernmentia are generally less sensitive to pollutants and other disturbances than members of the subclass Adenophorea; Dorylaimina are particularly

sensitive to site disturbances and can be considered k-strategists; Rhabditidae are considered extreme 'r strategists'; tylenchids are intermediate; the suborder Dorylaimina is very sensitive to lead pollution; omnivorous nematodes are sensitive to disturbance; Rhabditidae and Diplogasteridae are the first nematodes to colonize cow dung; Rhabditidae are indicative of organic enrichment and k-strategists are generally more sensitive to disturbance in marine meiobenthos than are r-strategists (Bongers, 1999). Such observations are very general and subjective. Therefore more quantified information about individual nematode taxa is required for more precise c-p scaling.

As research continues on nematodes, more controversy arises. The family Oncholaimidae comprises large nematodes with a long generation time but which are at the same time stress-tolerant (Bongers, 1999). Oncholaimids are attracted to and migrate to decomposing organic matter. As such, their numerical dominance in an area is not necessarily the result of a high reproduction rate in a food-rich environment (Bongers, 1999). These observations are controversial in that these nematodes possess characteristics of both persisters and colonizers.

Another example is the Monhysteridae which were originally classified as opportunists and given a c-p value of one based on their tolerance to pollutants and survival under extreme conditions (Bongers *et al.*, 1995). Since then opportunists have been separated into enrichment opportunists and general opportunists. Enrichment opportunists are given a c-p value of one, develop under food-rich conditions and form dauerlarvae as soon as the microbial activity decreases (Bongers, 1999). General

opportunists are given a c-p value of two, can live under food-poor conditions and are unable to form dauerlarvae (Bongers, 1999). Under these new c-p descriptions, the Monhysteridae were changed to a c-p value of two. This change would affect studies where monhysterids dominated the nematode community. For example, in Humber Arm Newfoundland 96.5 % of the nematode community was *Monhystera* with a MI value of 1.05 (Lawlor, 1998). Another study in the same area (Yeow *et al.*, 1999) showed 86.5 % *Monhystera* at 0m depth and 98.4 % *Monhystera* at 8-10m depth, with MI values of 1.09 and 1.00. These MI values increased to 2.09 and 2.00 respectively when the new c-p values were applied.

One of the biological characteristics used to determine a c-p value is reproductive potential. Colonizers, with c-p values of one or two, have voluminous gonads and release large numbers of small eggs (Bongers, 1990). In contrast, persisters, with c-p values of four or five, have few offspring, small gonads but produce large eggs (Bongers, 1990). It was noted in Arnold's Cove and Come-By-Chance samples that *Metachromadora* and *Neochromadora* females had no more than two large eggs present at any one time and the majority of *Prochromadorella* females had either two or four large eggs present, with a maximum of six eggs in a few females. In comparison to other nematode eggs, these were relatively few and relatively large. The conventional c-p value assigned to these three nematode genera is two, but their corresponding family c-p value is three in all cases. From the observations noted here, the c-p value of two for *Metachromadora*, *Neochromadora* and *Prochromadorella* is questioned, and it is

suggested that family level c-p value three would be more appropriate.

Another problem with c-p classification is that all genera within a family have been assigned the family c-p value. A family consists of closely related genera with morphological and ecological similarities which tend to occupy the same well defined niche and it has been assumed highly unlikely that a family would contain both colonizer and persister genera (Bongers, 1990). However, this is not the case. The family Chromadoridae, for example has a c-p value of three. Within that family there are four genera assigned a c-p value of two, six genera assigned a c-p value of three, three genera assigned a c-p value of four and the 11 other genera have no specific c-p value and are automatically assigned the family c-p value of three (Bongers *et al.*, 1991). The family Desmodoridae has a c-p value of three, with two genera of a c-p value of two, six genera with a c-p value of three, two genera with a c-p value of four and seven genera with no assigned c-p value. This discrepancy between familial and generic c-p values has not been discussed in literature and would be highly significant in situations where the most abundant genera in a community have c-p values different from their family c-p values. In the present work, *Metachromadora* in Arnold's Cove made up 37.0 % of the community. The MI value was 2.36 using the genus c-p value of two. Had the family c-p value of three for *Metachromadora* been used the MI value would have been closer to 3.0.

A final problem with the c-p classification is that not all nematode families have been assigned a c-p value, for example, Pandolaimidae, Lauratonematidae, Tarvaiidae, Paramicroloaimidae, Meyliidae and Coninckiidae. *Pandolaimus* and *Coninikia* were

present at low abundance at some sites in Bay d'Espoir and were excluded from the MI calculations because of no c-p value. Their low abundance means that even if included the MI value would not differ much. But if these nematodes had been in high abundance, and were not included in the MI calculation because of no c-p value then the results would be inaccurate.

In general, using nematodes as biological indicators and/or monitors presents some obvious disadvantages. They are small in size, making extraction from the samples, enumeration and identification, both difficult and time consuming. There are few experts in nematode taxonomy. Spatial and temporal factors can influence the nematode fauna, in addition to environmental disturbance. Some nematodes are resistant to certain types of pollution, so that total nematode numbers or total numbers of genera may not change in response to environmental change. A more detailed analysis may be required (Kennedy and Jacoby, 1999). Many of these problems were encountered in the present study.

The largest problem from the Bay d'Espoir samples and associated sediment data was the lack of a continuous sample set. Samples were collected with an Ekman grab sampler, which was of questionable utility. Collecting the samples by hand at Arnold's Cove and Come-By-Chance may have provided a more accurate representation of the nematode fauna. Extraction was a problem with the Bay d'Espoir samples because of small particle size. Therefore samples had to be hand picked, which was labour intensive. Identification was not a significant problem given the work of Platt and Warwick (1983, 1988), as well as Tarjan (1980). There was only one nematode taxon that was present in

high abundance in Bay d'Espoir samples that could not be identified to genus.

On the other hand, as previously discussed, nematodes offer several advantages as biological indicators and/or monitors. Their small size means that generally small samples are required to yield enough individuals for statistical analysis. Nematodes are ubiquitous, and can be found in areas where few other organisms can exist. They have a rapid generation time which makes the impact of disturbance readily detectable. They are relatively sessile, and so can be used to study a particular site (Kennedy and Jacoby, 1999). They are simple, transparent organisms, so their internal structures can easily be studied without dissection.

It is well documented that nematodes respond rapidly to disturbance and enrichment. They have a permeable cuticle which puts them in direct contact with their surrounding environment and makes them respond rapidly to environmental changes. For example several species can survive oxygen stress and will therefore survive in situations where other organism will perish (Bongers and Ferris, 1999). An increase in microbial activity always produces an increase in bacterial feeding nematodes. It is relatively easy to determine feeding type of a nematode because feeding type is based on the easily observed morphology of the mouth cavity and pharynx (Figure 1) (Bongers and Ferris, 1999).

The present study analysed the nematode assemblages in two separate regions of Newfoundland with different types of environmental disturbance. The analysis included total nematode numbers, total genera numbers, Species Richness, Diversity and Evenness,



feeding type analysis, calculation of Maturity Index and corresponding c-p value analysis. It was shown that to fully understand the nematode assemblage at a given location, such a complete analysis is required. All information collected will serve as important baseline data for future research in these areas.

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**Appendix A: Flow Chart of Nematode Extraction**

Use suction filtration to remove excess liquid (fixative and/or water) from each sample

Wash filtrate into scintillation vials with a sea-water/formalin solution

Referred to as 'Top'

Fill 250 ml centrifuge bottles to a depth of 2.5 cm

Centrifuge at 3000 RPM for five minutes → Pour off Supernatant

Use suction filtration to isolate nematodes

Wash filtrate into scintillation vials with a sea-water/formalin solution

Referred to as 'Extraction 1'

Retain sediment and mix with 40 ml of Colloidal Silica

Add appropriate amounts of Colloidal Silica to balance centrifuge bottles

Resuspend sediment by shaking the centrifuge bottles by hand

Centrifuge at 3000 RPM for fifteen minutes → Pour off Supernatant

Use suction filtration to isolate nematodes

Wash filtrate into scintillation vials with a sea-water/formalin solution

Referred to as 'Extraction 2'

**Appendix B:** Summary of the Total Nematode Counts (N), Total Number of Genera (S), Shannon Index (H'), Evenness (E), Simpson Index (1/D), Species Richness (D<sub>mg</sub>) and Maturity Index (MI) values for all samples collected at Arnold's Cove during July 1998.

	<b>Top</b>				<b>Extraction 1</b>				<b>Extraction 2</b>				<b>Total</b>			
	<b>1</b>	<b>2</b>	<b>3</b>	<b>Avg</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>Avg</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>Avg</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>Avg</b>
<b>N</b>	667	262	267	<b>399</b>	369	556	170	<b>365</b>	1649	1791	191	<b>1210</b>	2685	2607	628	<b>1973</b>
<b>S</b>	16	14	16	<b>15</b>	18	17	13	<b>16</b>	21	22	16	<b>20</b>	23	23	20	<b>22</b>
<b>H'</b>	1.62	2.08	2.07	<b>1.92</b>	1.96	1.80	1.80	<b>1.85</b>	1.99	2.11	2.23	<b>2.11</b>	1.92	2.10	2.21	<b>2.08</b>
<b>E</b>	0.58	0.79	0.75	<b>0.71</b>	0.68	0.64	0.75	<b>0.69</b>	0.65	0.68	0.80	<b>0.71</b>	0.89	0.67	0.74	<b>0.77</b>
<b>1/D</b>	3.36	6.29	6.07	<b>5.24</b>	4.78	3.98	4.61	<b>4.46</b>	4.73	5.82	7.23	<b>5.93</b>	4.36	5.61	6.53	<b>5.50</b>
<b>D<sub>mg</sub></b>	2.31	2.33	2.68	<b>2.44</b>	2.88	2.53	2.34	<b>2.58</b>	2.70	2.80	2.86	<b>2.79</b>	2.79	2.80	2.95	<b>2.85</b>
<b>MI</b>	2.29	2.32	2.27	<b>2.29</b>	2.42	2.32	2.32	<b>2.35</b>	2.36	2.40	2.47	<b>2.41</b>	2.35	2.38	2.34	<b>2.36</b>

**Appendix C:** Summary of the Total Nematode Counts (N), Total Number of Genera (S), Shannon Index (H'), Evenness (E), Simpson Index (1/D), Species Richness ( $D_{mg}$ ) and Maturity Index (MI) values for all samples collected at Arnold's Cove during November 1998.

	Top			Extraction 1			Extraction 2			Total						
	1	2	3	Avg	1	2	3	Avg	1	2	3	Avg				
<b>N</b>	559	442	629	<b>543</b>	206	139	207	<b>184</b>	348	662	214	<b>408</b>	1107	1243	1050	<b>1133</b>
<b>S</b>	24	26	28	<b>26</b>	21	22	26	<b>23</b>	22	29	17	<b>23</b>	29	36	33	<b>33</b>
<b>H'</b>	2.40	2.72	2.69	<b>2.60</b>	2.38	2.74	2.74	<b>2.62</b>	2.30	2.84	2.36	<b>2.50</b>	2.45	2.88	2.77	<b>2.70</b>
<b>E</b>	0.76	0.83	0.81	<b>0.80</b>	0.75	0.89	0.84	<b>0.83</b>	0.74	0.84	0.83	<b>0.80</b>	0.73	0.80	0.79	<b>0.77</b>
<b>1/D</b>	7.67	11.8	10.8	<b>10.1</b>	6.88	12.8	11.9	<b>10.5</b>	6.32	13.6	8.20	<b>9.37</b>	7.25	13.7	11.4	<b>10.8</b>
<b><math>D_{mg}</math></b>	3.64	4.10	4.19	<b>3.98</b>	3.75	4.26	4.69	<b>4.23</b>	3.59	4.31	2.98	<b>3.63</b>	3.99	4.91	4.60	<b>4.5</b>
<b>MI</b>	2.12	2.24	2.21	<b>2.19</b>	2.19	2.25	2.25	<b>2.23</b>	2.11	2.20	2.15	<b>2.15</b>	2.13	2.22	2.21	<b>2.19</b>



**Appendix D:** Summary of the Total Nematode Counts (N), Total Number of Genera (S), Shannon Index (H'), Evenness (E), Simpson Index (1/D), Species Richness (D<sub>mg</sub>) and Maturity Index (MI) values for all samples collected at Come-By-Chance during July 1998.

	Top				Extraction 1				Extraction 2				Total			
	1	2	3	Avg	1	2	3	Avg	1	2	3	Avg	1	2	3	Avg
<b>N</b>	674	604	896	<b>725</b>	290	1744	413	<b>816</b>	727	2066	1987	<b>1593</b>	1691	4414	3296	<b>3134</b>
<b>S</b>	16	19	18	<b>18</b>	13	18	17	<b>16</b>	20	24	30	<b>25</b>	23	29	31	<b>28</b>
<b>H'</b>	1.04	1.54	1.24	<b>1.27</b>	1.80	1.13	1.64	<b>1.52</b>	1.94	1.45	2.12	<b>1.84</b>	1.93	1.36	1.90	<b>1.73</b>
<b>E</b>	0.38	0.52	0.43	<b>0.44</b>	0.70	0.39	0.58	<b>1.67</b>	0.65	0.46	0.62	<b>0.58</b>	0.62	0.40	0.55	<b>0.52</b>
<b>1/D</b>	1.67	2.82	2.00	<b>2.16</b>	3.97	1.86	2.65	<b>2.83</b>	3.65	2.28	4.55	<b>3.49</b>	4.32	2.17	3.37	<b>3.29</b>
<b>D<sub>mg</sub></b>	2.30	2.81	2.51	<b>2.54</b>	2.12	2.28	2.66	<b>2.35</b>	2.88	3.01	3.82	<b>3.24</b>	2.96	3.34	3.70	<b>3.33</b>
<b>MI</b>	2.90	2.32	2.20	<b>2.47</b>	2.40	2.15	2.29	<b>2.28</b>	2.40	2.26	2.56	<b>2.41</b>	2.60	2.23	2.43	<b>2.42</b>

**Appendix E:** Summary of the Total Nematode Counts (N), Total Number of Genera (S), Shannon Index (H'), Evenness (E), Simpson Index (1/D), Species Richness (D<sub>mg</sub>) and Maturity Index (MI) values for all samples collected at Come-By-Chance during November 1998.

	Top				Extraction 1				Extraction 2				Total			
	1	2	3	Avg	1	2	3	Avg	1	2	3	Avg	1	2	3	Avg
<b>N</b>	4	109	200	<b>104</b>	33	51	91	<b>58</b>	60	161	251	<b>157</b>	94	321	542	<b>319</b>
<b>S</b>	3	12	12	<b>9</b>	10	15	16	<b>14</b>	14	17	22	<b>18</b>	16	22	27	<b>22</b>
<b>H'</b>	-	1.45	1.58	<b>1.52</b>	-	2.23	1.94	<b>2.09</b>	-	1.88	2.25	<b>2.07</b>	2.14	1.93	2.16	<b>2.08</b>
<b>E</b>	-	0.58	0.64	<b>0.61</b>	-	0.82	0.70	<b>0.76</b>	-	0.66	0.73	<b>0.70</b>	0.77	0.62	0.66	<b>0.68</b>
<b>1/D</b>	-	2.70	3.18	<b>2.94</b>	-	6.93	4.38	<b>5.66</b>	-	4.15	5.86	<b>5.01</b>	5.61	3.84	4.64	<b>4.70</b>
<b>D<sub>mg</sub></b>	-	2.34	2.08	<b>2.21</b>	-	3.56	3.33	<b>3.45</b>	-	3.15	3.80	<b>3.48</b>	3.30	3.64	4.13	<b>3.69</b>
<b>MI</b>	-	2.15	2.19	<b>2.17</b>	-	2.39	2.34	<b>2.37</b>	-	2.23	2.25	<b>2.24</b>	2.68	2.23	2.24	<b>2.38</b>

**Appendix F:** Comparison of Unknown Genus 1 (family Comesomatidae) with known genera within the same family, based on TL, total body length to the nearest 5µm, De Man ratio 'a' (total body length ÷ maximum body diameter); Hd, head diameter as percentage of posterior oesophagus body diameter; A%, amphid diameter as percentage of corresponding body diameter; At, number of turns of amphid; R3, R3 sensilla length as percentage of head diameter; Cs, number of cervical setae in each sublateral row; Spic, spicule cord length as proportion of cloacal body diameter; Ps, number of precloacal supplements; T, tail length measured in cloacal body diameters (N = 20).

Species	TL	a	Hd	A%	At	R3	Cs	Spic	Ps	T
<b>Unknown Genus 1</b>	<b>2880</b> (2300-3180)	<b>20.4</b> (15.9-27.2)	<b>17.4</b> (12.9-23.8)	<b>44.3</b> (40-60)	<b>3-4</b>	<b>33-60</b>	<b>1-13</b>	<b>1.0-1.7</b>	<b>12-22</b>	<b>3.5</b> (2.8-4.2)
<b>Setosabateria species</b>										
<i>Setosabateria fibulata</i>	1400	34	40	90	5	90	3-4	1.7	17	3-3.3
<i>Setosabateria hilarula</i>	1140-2400	25-38	31-47	48-76	4	80-150	3-21	1.3-1.7	11-16	4-6
<b>Sabateria praedatrix group</b>										
<i>Sabateria alata</i>	3070-3220	36-53	32	55	-	30	-	1.6-1.8	21	5.5
<i>Sabateria ancudiana</i>	1610-1850	42-65	46	70-73	-	60-70	-	1.5	16	3.5
<i>Sabateria conicauda</i>	950-1050	30-35	38	75	-	20-25	-	1.2	4-8	2.1-2.5
<i>Sabateria demani</i>	8000	63	30	65	-	90	-	2.0	14	3.5
<i>Sabateria dodecaspapillata</i>	2430-2525	29-36	20	60	-	50	-	1.6-1.8	12	4.0-4.3
<i>Sabateria falcifer</i>	1750-2430	28-36	-	60	-	40	-	1.5	10	3.0-3.3
<i>Sabateria granifer</i>	1765-2620	26-40	26-30	55-68	-	20-50	-	1.5-1.6	13-17	3.3-4.8
<i>Sabateria intermissa</i>	2110-2550	40-48	40	73	-	70-90	-	2.0	16	3.5
<i>Sabateria lawsi</i>	2060-2400	32-35	26-27	50-66	-	32-44	-	1.6-1.7	17	3.1-3.8

Appendix F Continued

Species	TL	n	Hid	A%	AI	R3	Cs	Spic	Ps	T
<b>Unknown Genus 1</b>	<b>2880</b> <b>(2300-3180)</b>	<b>20.4</b> <b>(15.9-27.2)</b>	<b>17.4</b> <b>(12.9-23.8)</b>	<b>44.3</b> <b>(40-60)</b>	<b>3-4</b>	<b>33-60</b>	<b>1-13</b>	<b>1.0-1.7</b>	<b>12-22</b>	<b>3.5</b> <b>(2.8-4.2)</b>
<i>Subatieria lyonesse</i>	3920	70	46	40	-	50	-	2.1	26	2.1
<i>Subatieria parabyssalis</i>	1470-1830	31-32	30	90	-	70	-	1.3	15-20	3.8-4.3
<i>Subatieria paracupida</i>	1700-1850	36	34	64-70	-	71-77	-	1.7-1.8	19-22	4.0
<i>Subatieria paradosa</i>	1460-1660	35-38	30-32	54	-	38-46	-	1.7-1.8	17-19	4.0
<i>Subatieria praedatrix</i>	1760-2900	38-55	40	50-63	-	40-42	-	1.5-1.8	13-17	4.0-4.5
<i>Subatieria stekhoveni</i>	1550-1700	35-38	32-39	63-72	-	50-60	-	1.3-1.5	8-11	4.0-4.2
<i>Subatieria triplex</i>	2850	57	30	66	-	50	-	1.3	20	3.7
<i>Subatieria vasicola</i>	2020-2550	27-32	29-33	55-57	-	30-40	-	1.3-1.4	19	3.1-4.0
<b>Subatieria armata group</b>										
<i>Subatieria armata</i>	2095-2455	28-82	40	60	-	250	-	1.2	9	5.0-6.0
<i>Subatieria elongata</i>	3430-3575	90-92	48	70-72	-	170-180	-	0.9	13-17	4.2-4.5
<i>Subatieria longispinosa</i>	2150-2650	104-114	50-55	90	-	220-280	-	1.2-1.4	5-6	4.5-5.0
<i>Subatieria migrans</i>	2330-3285	48-68	37	59-72	-	200	-	1.4-1.7	20-30	5.2-6.5
<i>Subatieria supplicans</i>	2000	100	53	80	-	280	-	2	-	8.0
<b>Subatieria pulchra group</b>										
<i>Subatieria breviseta</i>	1105-1220	33-39	38-41	75-85	-	30	-	1.2-1.3	6	3.6-4.0

Appendix F Continued

Species	TL	n	Hd	A%	AI	R3	Cs	Spic	Ps	T
<b>Unknown Genus 1</b>	<b>2880</b> <b>(2300-3180)</b>	<b>20.4</b> <b>(15.9-27.2)</b>	<b>17.4</b> <b>(12.9-23.8)</b>	<b>44.3</b> <b>(40-60)</b>	<b>3-4</b>	<b>33-60</b>	<b>1-13</b>	<b>1.0-1.7</b>	<b>12-22</b>	<b>3.5</b> <b>(2.8-4.2)</b>
<i>Sabatieria mortenseni</i>	1900	47	50	80	-	80	-	1.0	6	3.2
<i>Sabatieria pissina</i>	655	27	44	82-87	-	22	-	1.3	-	2.3-2.6
<i>Sabatieria propissina</i>	670-710	30-35	30	77	-	43-44	-	1.3-1.4	3	3.2-3.7
<i>Sabatieria pulchra</i>	1270-2500	28-45	25-40	55-67	-	33-50	-	1.0-1.3	6-9	3.3-3.4
<i>Sabatieria punctata</i>	990-1350	33-38	30-35	70-75	-	35-40	-	0.9-1.1	5-8	3.2-3.9
<b><i>Sabatieria celtica</i> group</b>										
<i>Sabatieria celtica</i>	1770-3150	31-61	33-42	58-80	-	45-120	-	1.2-1.5	15-22	3.1-4.1
<i>Sabatieria furcillata</i>	1570-2700	34-59	30-40	75	-	50	-	1.7	17-20	4.3-4.6
<i>Sabatieria kelleri</i>	2525-3095	31-40	26-33	60-70	-	42-47	-	1.0-1.2	21-27	3.6-4.3
<i>Sabatieria strigosa</i>	1660-2120	58-69	-	73-87	-	100-180	-	1.1	9-12	5.0
<b><i>Sabatieria ornata</i> group</b>										
<i>Sabatieria abyssalis</i>	1650-1850	25-33	22	80	-	50-65	-	1.1-1.3	10	4.5
<i>Sabatieria longicauda</i>	1310-1585	42-46	40	90	-	100	-	1.2-1.3	13-14	3.7-4.5
<i>Sabatieria macramphix</i>	1700-2120	52-61	-	84-93	-	140-170	-	1.0	18-21	4.5
<i>Sabatieria ornata</i>	1350-2200	35-47	27	78-98	-	40-57	-	1.0-1.3	10-16	4.6-5.9
<b><i>Sabatieria inquirendae</i> group</b>										

Appendix F Continued

Species	TL	n	Hfd	A%	AI	R3	Cs	Spic	Ps	T
<b>Unknown Genus 1</b>	<b>2880</b> <b>(2300-3180)</b>	<b>20.4</b> <b>(15.9-27.2)</b>	<b>17.4</b> <b>(12.9-23.8)</b>	<b>44.3</b> <b>(40-60)</b>	<b>3-4</b>	<b>33-60</b>	<b>1-13</b>	<b>1.0-1.7</b>	<b>12-22</b>	<b>3.5</b> <b>(2.8-4.2)</b>
<i>Subatieria aspera</i>	3290	39	-	52	-	36	-	1.6	14	3.9
<i>Subatieria efflata</i>	3040	76	-	73	-	50	-	2.1	-	4.6
<i>Subatieria possjetica</i>	1305-1620	29-38	-	80	-	40	-	1.2	0	3.4
<i>Subatieria praebosporica</i>	3165-3375	31-40	-	50	-	35	-	1.3	12	3.0
<i>Subatieria quadripapillata</i>	1200-1250	30	-	50	-	30	-	1.2-1.5	4	3.0-3.5
<i>Subatieria rota</i>	1650	46	-	70	-	35	-	1.0	6	4.0
<i>Subatieria sarcina</i>	1295	38	-	70	-	55	-	1.3	-	2.3
<i>Subatieria wieseri</i>	1790-2430	26-38	-	57-60	-	50	-	1.6	15-17	3.8

Known data were extracted from Platt (1985).

**Appendix G:** Photographs of Unknown Genus 1 representatives identified from samples taken at Bay d-Esprit, Newfoundland during 1998.

Figure 6: A, Female; B, Female showing eggs (E); C, Male; Male posterior end showing spicules (S).

Figure 7: A, Female anterior end showing the amphid (A) and cervical setae (C); B, Male anterior end showing the amphid (A) and cervical setae (C).

