

MEIOFAUNA TRAVERSING THE TIDE LINE OF AN OCEAN BEACH IN SOUTH-EASTERN AUSTRALIA, WITH SPECIAL REFERENCE TO NEMATODA

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ABSTRACT

The meiofauna was investigated on a sandy ocean beach, usually exposed to strong surf, in southeast Australia. Samples were taken from high tide level to 5 metres below low tide level at a beach previously damaged by a storm and re-established by natural wave action. The meiofauna were counted to major taxa, with nematodes identified to genus or species. Differences were observed between the subtidal and intertidal samples. Some species or higher taxa are restricted to either the subtidal or the intertidal zone while others extend across both zones. A majority of the genera are cosmopolitan, but species appear to be unique to Australia. The results suggest that when a beach is rebuilt with fresh sand following a storm, re-colonization is a taxonomically selective process. However, the mechanisms of selection are currently unknown.

Keywords: marine nematodes; beach meiofauna; surf; intertidal; subtidal

INTRODUCTION

In this paper we describe the distribution of the beach meiofauna, with special focus on nematodes, from a sandy, steep, reflective ocean beach in southeast Australia. At the sample site this beach had earlier been removed by a storm and rebuilt through natural wave action. Dolphin Beach, 240 km south of Sydney, at 35° 55'S, 150°

09'E, is a typical surf beach with a tidal range of 1.6 metres. It comprises a flat, sandy supra-littoral zone with Marram grass (*Ammophila arenaria*) and Sea Rocket (*Cakile maritima*) that is immersed at infrequent intervals when high tides combine with storm surges. Inland from the supra-littoral the beach is bordered by a short, steep ridge topped by trees and bushes. Direct human interference is minimal and the study area is in the most remote part of the beach.

In previous work on nematodes at this beach, samples were taken over a two-year period at the high, mid and low tide marks to a depth of 1 m of sand, and along a 2 km horizontal transect at mid tide level (Nicholas & Hodda 1999). A total of 58 nematode species from 48 genera were found, many from as deep as 1 m in the sand. It appeared that strong surf repeatedly reshuffles the nematode fauna within the top metre. Bimonthly samples from the same stretch of beach later showed that nematode populations increase in the warmer months but that juveniles and adults of both sexes of the more abundant species are present throughout the year (Nicholas 2001).

In August 1998, storms and unusually high tides swept away the supra-littoral on a section of this beach as far as the tree line, replacing the sand with a channel of sea water. The intertidal profile and supra-littoral platform were re-built over a period of about two

months. Some nematode species not present in July 1998 were found in the newly reconstituted beach during September of that year but they did not persist until the following January (Nicholas 2001). It was hypothesized that nematodes re-colonise the re-constituted intertidal sand by being carried passively with subtidal sand that replaced the lost beach. It seemed less likely that nematodes from deeper in the sand would re-colonise the shallow layers because genera such as *Perepsiloma* and *Tricoma*, common below 20 cm, rarely occurred in the top 20 cm of the re-colonised intertidal region.

For the present study, in order to further investigate the distribution of meiofauna at this beach, samples were taken from the top 10 cms of sand along transects from the high tide mark to 5 m below low tide mark. The distributions permit some conclusions about the ecological preferences of certain species and higher taxa, and also about the pattern of beach re-colonization following physical disruption of the beach.

PRIOR LITERATURE

There have been several papers on the ecology of subtidal nematodes from sandy habitats, notably from the southern bight of the North Sea (Vincx *et al.* 1990), but very few samples have been taken close in-shore and studies have not focused on the interface between the subtidal and intertidal zones. One reason for this is very probably the difficulty of sampling in the surf zone, even in calm weather. Similarly, there have been numerous studies of intertidal nematodes from sandy ocean beaches but these studies usually do not extend to the surf zone. An exception that makes an interesting comparison with the Dolphin Beach studies by Nicholas & Hodda (1999) and Nicholas (2001) is a 10-year study by McIntyre & Murison (1973) of the

meiofauna inhabiting a wave swept sandy beach on the Atlantic coast of Scotland. In that study samples were taken from high water to 10 m below low water. At higher taxonomic levels the fauna was very like that at Dolphin Beach. Nematodes were the most abundant taxon, with different species but many of the same genera as at Dolphin Beach. No sharp discontinuity was reported between the intertidal and subtidal faunas.

The nematode faunas from wave-dominated beaches in the Baltic and Mediterranean seas have been described (Gheskiere *et al.* 2005). However, these are microtidal habitats and even the most exposed of these beaches are much less subject to wave action than the Scottish or Australian ocean beaches. Neither the physical environments nor the nematode assemblages have much in common with Australian surf beaches.

Kotwicki *et al.* (2005) reported that nematodes are the most abundant intertidal meiofaunal taxon on sandy beaches in the southern North Sea, but that study did not sample the subtidal fauna. Rodriguez *et al.* (2003) described the intertidal meiofauna of 10 sandy beaches in northern Spain, but again not the subtidal fauna. Those beaches increased in meiofaunal species richness as wave exposure and grain size increased. The ecology of sandy beaches in South Africa has been discussed by McLachlan *et al.* (1981) but concentrating on the macrofauna.

Hourston *et al.* (2009) have studied the nematode fauna of shallow subtidal (<2m deep) reaches of the Swan River estuary in Western Australia, a microtidal habitat. Nematode densities were generally greatest in spring and close to the mouth of the river. Densities were influenced by the abundance of food resources for particular feeding groups and by

physical and chemical features of the environment.

MATERIAL AND METHODS

Sampling took place on an unusually calm day (April 15, 2007) beginning at the time of low water (noon), and was completed in about 2 h. The water temperature was 24°C. Samples were taken by driving corers into the sand along three transect lines extending from the high tide line of the previous high tide across the intertidal and subtidal zones to a depth of 5 m below extreme low water. These transect lines were about 50 m apart, perpendicular to the beach profile and aligned by reference to markers on shore. The offshore ends were about 40 m from the low tide line. Intertidal sampling was done on foot. A scuba diver took the subtidal samples, depths were measured by a wrist depth meter.

Corers consisted of 10 cm lengths of PVC tubing of 5.4 cm internal diameter with PVC caps to close each end. An additional cap, modified by inserting a steel rod projecting on each side, enabled the diver to force the corer into the sand. This modified cap was replaced in situ by an unmodified cap and the corer was angled sufficiently for a cap to be placed over the bottom end. Corers were brought back to the laboratory where nematodes and other organisms were extracted. Each corer held about 200 ml of sand, although some sand was lost in fitting the caps.

A core samples an area of 23 cm². Each core was stirred in 5 litres of fresh water and, after allowing it to stand for the sand to settle, this water was passed through a nylon sieve with mesh of 50 µm and the residue back-washed into a beaker. This procedure was repeated twice with the same water and material retained on the sieve was pooled, concentrated by aspiration to 10 ml and transferred to a screw-capped glass

tube. The contents were fixed by adding a few drops of Lugol's iodine (Sigma), sufficient to stain the liquid a pale yellow colour (see Appendix for an explanation and justification of this novel fixing method). The fauna was counted in three replicate 1 ml volume subsamples, taken from the tube, under a binocular microscope on a Hawksley counting slide. The contents of each tube then were tipped into a petri dish. When detritus obscured the fauna a few grains of Rose Bengal Stain were used to stain the meiofauna bright scarlet. Nematodes were picked up with a fine glass pipette and transferred to 5% aqueous glycerol that had been lightly coloured with cotton blue stain, in watch glasses which then were placed in an oven at 40°C to let the water evaporate. After 48 h, nematodes were individually picked up with an eye-brow hair glued to a wooden spill and transferred to anhydrous glycerol on a microscope slide. About 30 nematodes, the number that could be individually identified when spread out, were placed on each slide. The cover slip was supported by glass beads (Ballotini) and its edges sealed with nail varnish to prevent movement of the cover slip when viewing under an oil immersion objective, which is necessary for many nematode identifications. Nematodes were identified and counted until about 100 had been counted from each sample tube, except that for several samples fewer than 100 were successfully mounted. Estimates of the number of nematodes per unit volume of sand are based on the number counted, the proportion of each sample mounted, and the volume of sand in the sample. Nematode density is reported here as the number of individuals per 10 cm² surface area, as is conventional in nematode survey. The samples correspond to the top 10 cm of sand but, in this high-energy system, nematodes frequently may be redistributed to as much as 1 m depth by wave action (Nicholas and Hodda, 1999), and the

numbers we report can equally be interpreted as counts per 10 cm³ of the well-oxygenated upper part of the sand. Counts were corrected for a recovery rate of 78%, following Nicholas & Hodda's (1999) estimate that this method of extracting nematodes from sand cores recovers approximately 78% of nematodes present.

Sand from each sample was washed in fresh water, air dried and weighed into an approximately 100g lot which was passed through a set of sieves. The fractions retained by each sieve were weighed and the distribution of grain size was analysed using the granulometric spreadsheet program GRADISTAT (Blott & Pye 2001).

Species counts from the three transects (A, B, C) at six sampling points per transect (high, mid and low tide, and 1 m., 3 m., and 5 m. below low tide level) were summarized and compared. Two-dimensional non-metric multidimensional scaling (MDS) was used to visualise the composition of samples by nematode species across the sampling points. For this purpose the specimen counts were first converted to proportions because the absolute counts reflect an arbitrary stopping point of the counting process rather than anything inherent to the sites. MDS calculations were done with the software program PAST (Hammer *et al.* 2001) using the well-known Bray-Curtis similarity index. This measure, unlike alternative diversity measures, can handle real-valued data and so can be used to compare proportions.

Species richness statistics at each of the six tide levels were calculated in the program EstimateS 8.0 (Colwell 2006). Conventional sample-based measures of species richness rely either on equal-effort sampling or on an equal number of individuals being sampled from each site (Chao 2005). Although our

intention had been to sample an equal number of individuals, about 100 from each tube, the actual numbers identified ranged from 104 to only 37 per tube and the final counts per tide level ranged from 297 to only 210. This may cause unquantifiable biases in the comparison of sample-based species richness measures across the six tide levels. The main alternative method for estimating species richness is to compute a species accumulation curve from the sample data and infer its asymptote by extending beyond the data. This too can be problematic when applied to a set of non equal-effort, non equal-number samples, because different asymptotes could be estimated or the precision could differ due to some curves being extrapolated further beyond the data than others. Our interest is in the relative species richness of the nematode fauna at each of the six tide levels. Since no method can guarantee equal bias or no bias across tide levels, given our samples, we report a range of species richness measures and compare the means and confidence intervals (where available) against a null hypothesis that the six tide levels do not differ in species richness.

Individual species distributions were compared as between the intertidal and the subtidal sampling points. Since our raw counts typically are small and the distributions across sample points cannot be assumed to approximate a normal distribution, we used a non-parametric Mann-Whitney U-test for difference in the median to assess each species against a null hypothesis of equal relative abundance in the intertidal and subtidal samples.

RESULTS

Granulometry

Sieving showed that the offshore sandy sediments, like those of the onshore

beach, contained some shell fragments but was predominately fine sand, although rather variable in proportion between grain sizes 425-250 μm and 250-106 μm . Virtually nothing passed the 106 μm sieve. A mean grain size of 225-230 μm was recorded in every intertidal sample. Mean grain sizes for sites at 3 m and 5 m fell within the range 250-260 μm , but at 1 m depth the mean grain size was 300 μm owing entirely to an excess of shelly grit at transect A. At transect C the mean grain size of the 1 m sample was 225 μm (matching the intertidal samples) while at transect B it was 263 μm , (matching the subtidal samples).

Overview of the Meiofauna

The most abundant taxonomic group was the nematodes (Table 1). The next

in abundance were copepods, more numerous below low tide mark than in the intertidal zone. Gastrotrichs were abundant except close to high tide mark, with species of *Heteroxenotrichula* and *Tetranchyroderma australiense* the most common. Tardigrada were common but only below low tide mark. Turbellaria, of several families, occurred in samples from high tide to 5 m depth. Ostracods and Collembola were confined to the intertidal samples, the latter occurring only at the low tide mark where they may accumulate by wind or wave drift and be restricted to the surface rather than distributed through the body of the sand. Oligochaeta and Polychaeta were common in intertidal samples. The archannelid *Protodrillus* was present at depths of 3 m and 5 m.

Table 1: The major meiofaunal components. Estimated numbers of individuals per 10 cm² of sand. Results marked * are approximate, based on a small number of individuals counted; p = present in low numbers.

	High tide	Mid tide	Low tide	1 metre	3 metres	5 metres
Nematoda	1710	2492	2246	1629	2264	2173
Copepoda	258	350	622	575	857	622
Gastrotricha	-	136	442	122	173	738
Tardigrada*	-	-	-	70	70	20
Turbellaria*	30	50	20	60	30	70
Ostracoda*	30	40	30	-	-	-
Collembola*	-	-	30	-	-	-
Annelida*	p	p	p	p	p	p
Archeannelida*	-	-	-	-	p	p

Nematodes

The nematodes from each transect were identified and counted. The estimated density of nematodes is shown in Table 1. A total of 65 species were identified although many could only be named to a genus (Tables 2, 3, 4). In *Onyx* one species has been named and a second, undescribed species is cited as *Onyx b*. A total of 11 species were found only in intertidal samples and 22 species only in

subtidal samples, while 32 occurred in both zones. However, due to small sample sizes and high variances only four species could be confirmed by Mann-Whitney U-test as more common in the intertidal than in subtidal samples, and 15 as more common in subtidal than in intertidal samples. Species with a higher mean abundance in intertidal samples are (with intertidal and subtidal counts in brackets) *Microloaimus* (97, 16), *Theristus* (59, 8),

Table 2: Nematode fauna along Transect A

Species	High	Mid	Low	1 m	3 m	5 m
<i>Atrochromadora</i>	-	2	-	-	-	3
<i>Actinonema</i>	-	-	-	2	-	-
<i>Steineridora</i>	-	-	-	4	-	-
<i>Cromadorina</i>	4	-	3	-	-	-
<i>Dichromadora</i>	-	1	-	10	12	4
<i>Ptycholaimellus</i>	-	-	-	2	4	1
<i>Pomponema</i>	-	-	-	-	-	1
<i>Paracyatholaimoides</i>	34	-	1	-	-	17
<i>Phyllolaimus</i>	-	-	-	-	2	-
<i>Onyx macramphis</i>	-	-	-	16	17	-
<i>Onyx b</i>	6	33	4	12	12	12
<i>Acanthomicrolaimus jenseni</i>	-	-	-	2	7	2
<i>Microlaimus</i>	6	12	15	2	-	3
<i>Nudora</i>	-	-	-	1	10	10
<i>Cyartonema</i>	-	-	-	1	-	-
<i>Cynura</i>	4	1	-	-	-	-
<i>Haliplectus bickneri</i>	10	--	2	1	-	-
<i>Ceramonema carinatum</i>	-	-	8	2	-	3
<i>Metadasynemoides cristatus</i>	-	-	8	1	-	3
<i>Pselionema</i>	-	-	-	-	-	1
<i>Daptonema</i>	-	-	-	2	-	5
<i>Gonionchus australis</i>	-	-	4	2	1	8
<i>Goniochus alastairi</i>	-	-	8	-	-	-
<i>Gullanema fragilis</i>	1	15	-	2	2	-
<i>Omicronema australis</i>	-	6	-	11	4	9
<i>Theristus</i>	5	1	3	-	-	3
<i>Xyala brevibucca</i>	-	-	-	-	4	2
<i>Prorhynchonema goubaultae</i>	-	-	-	2	-	-
<i>Rhynchonema tomakinense</i>	-	-	1	1	1	7
<i>Eleutherolaimus</i>	3	-	3	-	-	-
<i>Nicascolaimus punctatus</i>	1	9	-	1	-	-
<i>Odontophora</i>	-	-	2	-	-	-
<i>Trileptium</i>	-	-	-	1	1	-
<i>Enoplus</i>	1	6	3	-	-	-
<i>Enoploides</i>	-	-	10	2	-	-
<i>Enoplolaimus</i>	-	-	-	2	-	-
<i>Fleuronema robusta</i>	-	2	3	2	-	-
<i>Epacanthion exploratoris</i>	-	-	1	1	-	-
<i>Fenestrolaimus</i>	-	-	2	-	2	-
<i>Mesacanthion</i>	-	-	-	1	2	3
<i>Oxyonchus evelynae</i>	-	1	2	12	8	3
<i>Oxyonchus longisetosus</i>	-	-	1	-	2	1
<i>Anoplostoma</i>	2	-	-	-	-	-
<i>Chaetonema</i>	-	1	-	1	3	-
<i>Pheronous ogdeni</i>	-	1	-	-	-	-
<i>Oncholaimus</i>	-	1	-	-	-	-
<i>Viscosia</i>	-	1	-	2	2	-
<i>Bathylaimus capacosus</i>	-	-	-	1	-	-
<i>Bathylaimus stenolaimus</i>	2	10	-	-	-	-
<i>unknown genus</i>	-	-	-	-	3	3
TOTAL	79	103	84	102	99	104

Table 3: Nematode fauna along Transect B

Species	High	Mid	Low	1 m	3 m	5 m
<i>Atrochromadora</i>	-	-	-	-	2	-
<i>Actinonema</i>	-	1	2	-	1	-
<i>Steineridora</i>	-	-	2	7	8	-
<i>Cromadorina</i>	15	8	-	-	-	-
<i>Dichromadora</i>	5	1	3	14	4	5
<i>Ptycholaimellus</i>	-	-	-	2	3	1
<i>Paracyatholaimoides</i>	21	27	-	-	1	-
<i>Phyllolaimus</i>	-	-	-	-	-	1
<i>Gammanema</i>	-	-	-	-	-	1
<i>Chromaspirina</i>	-	-	1	1	-	2
<i>Onyx macramphis</i>	-	-	4	9	1	1
<i>Onyx b</i>	1	2	4	17	8	6
<i>Acanthomicrolaimus jenseni</i>	-	-	-	3	2	1
<i>Microlaimus</i>	8	14	7	1	-	1
<i>Nudora</i>	-	-	-	-	3	24
<i>Leptolaimus</i>	-	-	-	3	-	-
<i>Haliplectus bickneri</i>	1	3	-	-	-	-
<i>Ceramonema carinatum</i>	-	1	-	-	2	-
<i>Metadasynemoides cristatus</i>	-	-	-	-	-	2
<i>Daptonema</i>	-	-	-	-	1	-
<i>Gonionchus australis</i>	-	-	1	6	7	18
<i>Gullanema fragilis</i>	-	-	-	1	-	-
<i>Omicronema australis</i>	-	1	2	7	6	3
<i>Theristus</i>	10	-	-	2	2	-
<i>Xyala brevibucca</i>	-	-	4	2	3	2
<i>Prorhynchonema goubaultae</i>	-	-	-	-	4	5
<i>Rhynchonema tomakinense</i>	-	-	-	1	3	3
<i>Eleutherolaimus</i>	-	-	1	-	1	-
<i>Nicascolaimus punctatus</i>	11	7	-	4	2	-
<i>Enoplus</i>	7	10	-	-	-	-
<i>Enoploides</i>	-	-	15	1	1	-
<i>Enoplolaimus</i>	2	1	-	-	2	-
<i>Fleuronema robusta</i>	-	-	3	-	-	-
<i>Epacanthion exploratoris</i>	-	-	-	1	1	2
<i>Mesacanthion</i>	2	1	-	9	6	1
<i>Okranema eileenae</i>	1	1	-	-	-	-
<i>Oxyonchus evelynae</i>	-	-	5	4	12	4
<i>Anoplostoma</i>	2	1	-	-	-	-
<i>Chaetonema</i>	-	-	-	4	5	2
<i>Pheronous ogdeni</i>	4	1	-	-	-	-
<i>Eurystomina</i>	1	1	-	-	-	-
<i>Ditlevsenella</i>	-	-	-	-	1	1
<i>Oncholaimus</i>	-	1	1	-	-	-
<i>Viscosia</i>	-	-	-	-	4	5
<i>Symplocostoma</i>	-	-	-	1	-	2
<i>Bathylaimus capacosus</i>	-	1	1	-	-	1
<i>Bathylaimus stenolaimus</i>	7	10	-	-	-	-
<i>Eurystomina</i>	1	-	-	-	-	-
TOTAL	99	93	56	100	96	94

Table 4: Nematode fauna along Transect C

Species	High	Mid	Low	1 m	3 m	5 m
<i>Actinonema</i>	1	3	4	2	-	1
<i>Steineridora</i>	-	-	-	1	-	9
<i>Cromadorina</i>	12	-	-	-	-	-
<i>Dichromadora</i>	-	-	1	9	4	-
<i>Ptycholaimellus</i>	-	-	-	1	1	1
<i>Gomphonema</i>	-	-	-	2	-	-
<i>Paracyatholaimoides</i>	15	-	-	-	-	-
<i>Praeacanthonchus</i>	-	-	-	4	4	1
<i>Phyllolaimus</i>	-	-	-	1	-	4
<i>Halichoanolaimus</i>	-	-	-	-	1	-
<i>Chromaspirina</i>	-	2	3	1	5	1
<i>Pseudonchus</i>	-	-	-	-	-	1
<i>Onyx macramphis</i>	-	-	2	6	11	7
<i>Onyx b</i>	3	5	9	8	2	9
<i>Acanthomicrolaimus jenseni</i>	-	-	-	2	3	2
<i>Microlaimus</i>	12	11	12	5	1	3
<i>Nudora</i>	-	-	-	11	14	10
<i>Leptolaimus</i>	-	-	-	1	-	-
<i>Cyartonema</i>	-	-	-	-	1	-
<i>Cynura</i>	3	-	-	-	-	-
<i>Haliplectus bickneri</i>	3	-	-	-	-	-
<i>Ceramonema carinatum</i>	1	-	-	4	-	-
<i>Dasynemoides</i>	-	-	-	-	4	-
<i>Daptonema</i>	-	-	-	2	7	4
<i>Gonionchus australis</i>	-	1	4	3	-	12
<i>Gullanema fragilis</i>	-	-	3	-	-	-
<i>Omicronema australis</i>	2	-	1	7	2	9
<i>Theristus</i>	31	3	6	-	1	-
<i>Xyala brevibucca</i>	-	-	-	6	5	6
<i>Prorhynchonema goubaultae</i>	-	-	-	3	18	4
<i>Rhynchonema tomakinense</i>	-	-	1	1	4	6
<i>Nicascolaimus punctatus</i>	3	-	6	-	-	-
<i>Odontophora</i>	-	-	-	-	1	-
<i>Tricoma</i>	-	-	-	-	-	1
<i>Enoplus</i>	4	1	1	-	-	-
<i>Enoploides</i>	-	1	1	-	1	-
<i>Enoplolaimus</i>	-	-	1	-	-	-
<i>Fleuronema robusta</i>	-	1	-	3	-	-
<i>Epacanthion exploratoris</i>	-	-	-	1	2	1
<i>Fenestrolaimus</i>	-	-	-	-	1	-
<i>Mesacanthion</i>	-	-	-	1	-	2
<i>Oxyonchus evelynae</i>	-	1	4	-	-	1
<i>Anoplostoma</i>	5	-	-	-	-	-
<i>Chaetonema</i>	-	-	1	7	1	1
<i>Syringolaimus</i>	-	-	-	-	-	1
<i>Pheronous ogdeni</i>	1	-	-	-	-	-
<i>Oncholaimus</i>	-	-	1	-	-	-
<i>Viscosia</i>	-	-	-	1	-	1
<i>Symplocostoma</i>	-	-	-	-	-	1
<i>Bathylaimus stenolaimus</i>	2	8	9	-	-	-
<i>unknown genus</i>	-	-	-	1	4	-
TOTAL	98	37	70	94	98	99

Enoplus (33, 0), and *Bathylaimus stenolaimus* (48, 0). Species with higher abundance in subtidal samples are *Dichromadora* (11, 62), *Ptycholaimellus* (0, 11), *Onyx macramphis* (6, 68), *Acanthomicrolaimus jenseni* (0, 24), *Nudora* (0, 83), *Daptonema* (0, 21), *Gonionchus australis* (10, 57), *Omicronema australis* (12, 58), *Xyala brevibucca* (4, 30), *Prorhynchonema goubaultae* (0, 36), *Rynchonema tomakinense* (2, 27), *Epacanthion exploratoris* (1, 9), *Mesacanthion* (3, 25), *Chaetonema* (2, 24), and *Viscosia* (1, 15). Only two species were found to extend across both the intertidal and subtidal zone in reasonable and roughly equal numbers: *Onyx sp. b* (67, 86), and *Ceramonema carinatum* (10, 11).

Twelve of the named species have previously been described from specimens collected at Dolphin Beach or an adjacent beach:

Acanthomicrolaimus jenseni Stewart & Nicholas 1987; *Fleuronema robusta*, *Epacanthion exploratoris* and *Okranema eileenae* Greenslade & Nicholas 1991; *Gonionchus alastairi*, *G. australis*, *Omicronema australis*, and *Xyala brevibucca* Stewart & Nicholas 1994; *Gullanema fragilis* Nicholas & Stewart 1995; *Oxyonchus evelynae* and *O. longisetosus* Nicholas 2004; *Nicascolaimus punctatus* Riemann 1986. None of the above species have been recorded from elsewhere. *Onyx macramphis* Blome and Riemann 1994 was described from elsewhere in south eastern Australia. In contrast, some species found at Dolphin Beach are cosmopolitan species with a wide geographical distribution. For example, *Ceramonema carinatum* and *Oxyonchus culcitatus* are known also

from Puget Sound, Washington State, USA (Wieser 1959); *Haliplectus bickneri* from the Maldives in the Indian Ocean (Gerlach 1963); *Metadasynemoides cristatus* from Brazil (Haspelslagh 1973); and *Perepsilonma ritae* from Kenya (Verschelde & Vincx 1994).

Non-metric multi-dimensional scaling on the Bray-Curtis measure of site similarity shows a clear separation between the inter-tidal and sub-tidal sites (Figure 1), with a greater separation between the inter-tidal stations than the sub-tidal stations. In the corresponding Shepard plot (not shown) all points cluster along the 45 degree line and the stress statistic is 0.1404 (low). A minimal spanning tree (not shown) also draws the major line of demarcation (longest branch) between intertidal and subtidal sites.

Species richness estimates are given in Table 5 together with the number of specimens counted and identified at each tide level. Although mean richness estimates vary according to the method used, and the Chao 1 richness measure has a very high variance at 5 m depth due to a large number of singleton species, the various resampling and extrapolation results largely agree and mean estimates fall within the 95% confidence limits of other estimators where confidence limits exist. The overall pattern is of approximately constant species richness from mid tide level to 5 m depth, but lower species richness at the high tide mark. Since this pattern does not correlate with the specimen count (the lowest count, 210 identified specimens, is for the low tide samples) we take this lower richness at high tide as a real effect.

Table 5: Species richness estimates from shallow samples for each of the six tide levels

	high tide	mid tide	low tide	1metre	3metres	5metres
Specimen count	276	233	210	296	293	297
Species observed	23	31	34	38	40	38
Abundance-based coverage estimator (ACE species richness)	30	44	38	43	47	49
Chao1 species richness (95% confidence limits)	27 (24, 45)	40 (33, 67)	36 (34, 47)	42 (39, 60)	44 (41, 59)	93 (55, 211)
Chao2 species richness (95% confidence limits)	35 (26, 74)	41 (34, 64)	42 (36, 61)	40 (39, 50)	52 (44, 75)	45 (40, 61)
Michaelis-Menton species richness	30	58	59	50	61	54
Summary (range of estimator mean values)	27-35	40-58	36-59	40-50	44-61	45-93

DISCUSSION

The intertidal meiofauna, especially the nematodes, of Dolphin Beach have been previously described from samples taken over several years (Nicholas & Hodda 1999, Nicholas 2001), 58 nematode species being recorded. This study extends sampling into the subtidal zone to a depth of 5 m and extends the species record by 17 species. Many species are observed to occur in both intertidal and subtidal samples but others show a preference for one or the other zone. The relative rarity of some species, resulting in low counts, has prevented some of the new-found subtidal species from being classified as definitively subtidal only, but 15 species are shown statistically to be more abundant in the subtidal than the intertidal zone, and four are more abundant in the intertidal zone. Comparison against the larger samples in Nicholas and Hodda (1999) indicates that all species newly recognised here from Dolphin Beach are confined to the low tide level or the subtidal zone.

The wider fauna of Dolphin Beach is similar to that reported from beaches in other parts of the world, and in many ways resembles that of the Scottish Atlantic beach described by McIntyre & Murison (1973). Ostracods are restricted to the intertidal samples and oligochaetes almost so. Gastrotrichs and a variety of turbellarians occur both intertidally and subtidally, but tardigrades were both numerous and confined to the subtidal samples. This is interesting because in 12 years of collecting at Dolphin Beach, tardigrades have never been found in intertidal samples. A striking difference in the nematodes of Dolphin Beach compared to the Scottish beach is the presence of 10 species of Thoracostomopsidae at Dolphin Beach compared with only one from the Scottish beach. Otherwise the fauna lists are rather similar at and above genus level.

Nematode species richness as estimated from the present samples shows no clear trend across the tide levels other than that the richness at high tide level appears to be depressed. In contrast,

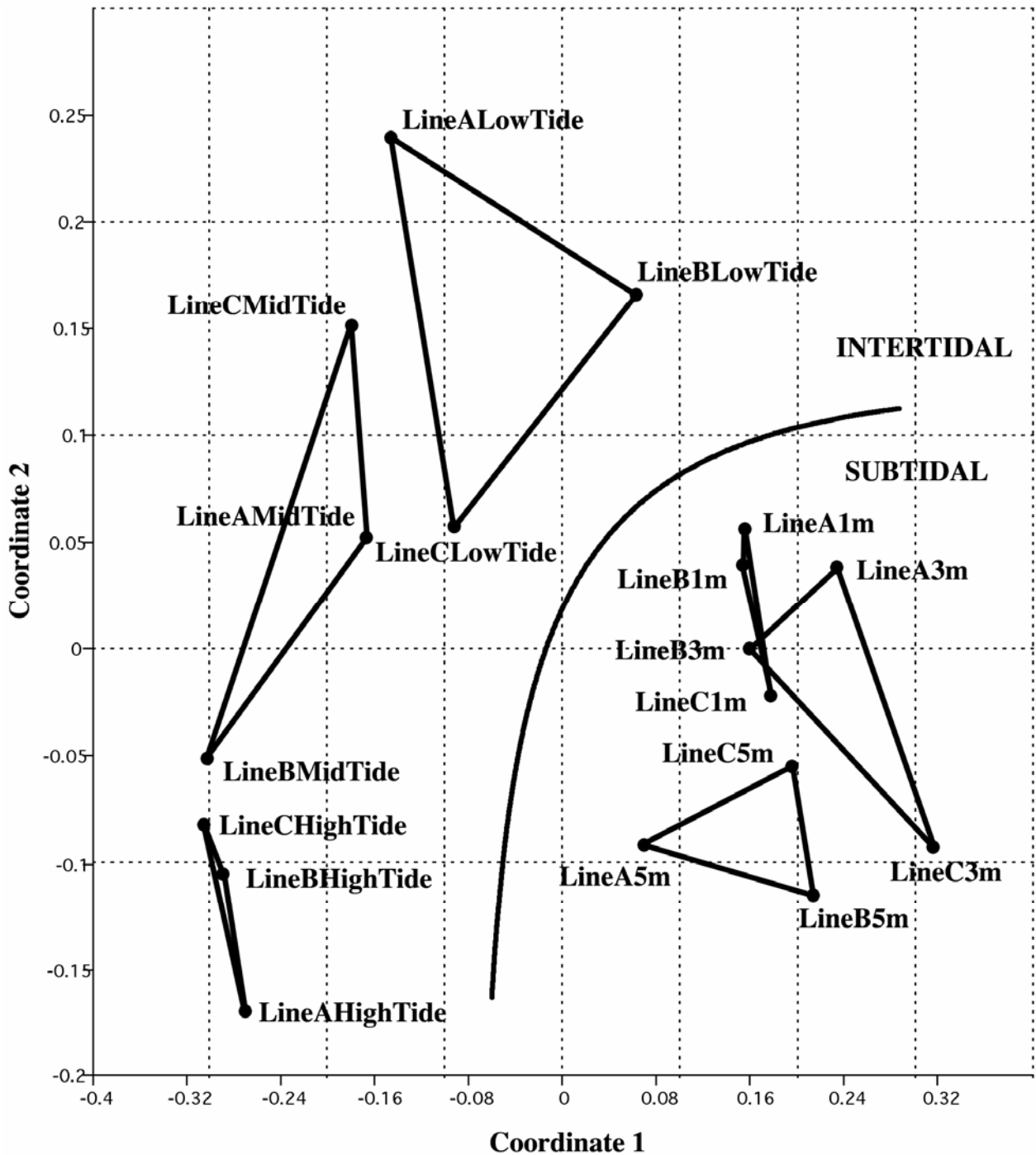


Figure 1. Nonmetric multidimensional scaling of the nematode fauna across all transects, using Bray-Curtis distances

Nicholas and Hodda (1999) reported no statistical difference in species richness across high, mid and low tide levels at this beach. A possible explanation is that here we have sampled only the top 10 cms of sand, whereas Nicholas and Hodda sampled to a depth of 1 metre.

Nicholas and Hodda reported additional species from deep in the sand, especially at the high tide level. Those deep-living species suffice to bring the total species count at high tide to approximately the same as the other tide levels. It is not known

whether additional deep-living species are to be found in the subtidal zone.

It is clear that the intertidal beach fauna at Dolphin Beach is not an unmodified continuation of the subtidal fauna, since there are significant differences in species composition. It follows that nematodes colonizing a reconstituted beach are not the same as those of the adjacent subtidal fauna. Various mechanisms may be involved in selective colonization. Large numbers of nematodes are suspended in the swash zone of breaking waves as the tide comes in and nematodes are easily collected from buckets of water scooped from the swash zone of breaking waves. Probably there is selection in which nematodes become suspended, and it would be interesting to find out which intertidal species are represented in the suspended population. Site selection behaviour also may play a small part by determining the spatial distribution of nematodes coming out of suspension. Ullberg & Olafsson (2003) have shown that marine nematodes may actively select a settling site under still-water conditions. However, the opportunity for active choice of settling site on an open surf beach must be limited and this mechanism may play only a very small role. McIntyre & Murison (1973) found a tendency for many elements of the meiofauna, including nematodes, to migrate vertically down into the sand as the tide advanced over them, and nematodes may migrate into the sand to avoid passive transportation but be transported along with the sand if intertidal sand is swept or blown on to or into the reconstituting beach.

The fact that so many of the species reported here were first described from Dolphin Beach should not be interpreted as indicating a restricted geographical distribution for these species. Rather, it reflects the very limited collections from sandy beaches

in Australia and the absence of collections from New Zealand and South East Asia. Much more work has been done on nematode collections from mangroves, coral reefs and lagoons than from exposed sandy beaches.

The use of Logol's iodine as a fixative for marine nematodes, as reported in the Appendix, provides a safe alternative to fixatives conventionally used in this application and may encourage further work on this fascinating and little-studied system.

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APPENDIX: Lugol's iodine as a fixative for marine nematodes

Lugol's iodine (lugol) (Sigma Chemical Co. St Louis, USA) was used instead of the invariably recommended formalin as a fixative because the Australian National University has banned the use of formalin as being a carcinogen. Some alternative fixative had to be found that was non-carcinogenic. Eventually, lugol was found to be satisfactory. In addition to being non-carcinogenic it is non volatile and pleasant to work with.

Lugol is made up by dissolving 1 g. of iodine in 15 ml. water to which is added 300 ml. of water containing 2 g. potassium iodide. To collections of nematodes in glass tubes or bottles in sea water a few drops of lugol, sufficient to give the solution a pale yellow colour, should be used. More will overstain specimens making

identification impossible. Nematode specimens fixed in lugol and transferred to anhydrous glycerol by the usual steps remain as well preserved as formalin fixed specimens for at least a year. The fixative is not satisfactory for long term preservation of bulk samples which fairly rapidly deteriorate. It will not prove an acceptable substitute for the almost universally accepted formalin for type specimens or museum collections.

Figure 3 compares photomicrographs of glycerol mounts of *Enoploides* and *Xyala* after one year storage. Temporary sea water mounts of lugol fixed specimens are useful for identification and counting meiofauna on microscope slides. Both measurements of morphological features and staining of internal organs remain undistorted by the fixative.

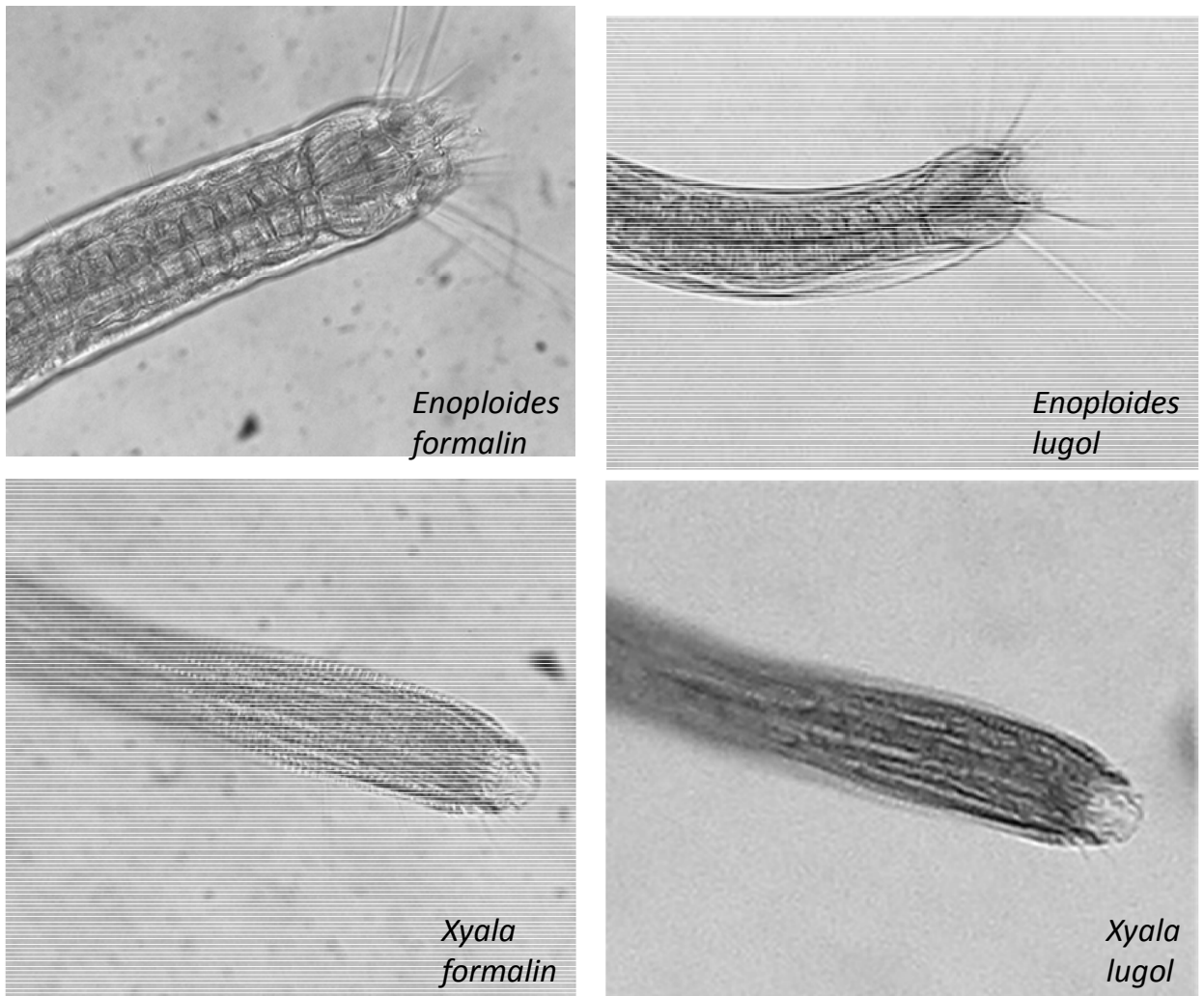


Figure 2. Comparisons of formalin fixed and lugol fixed *Enoploides* sp. and *Xyala brevivucca* after more than one year mounted in glycerol.