

# **CHAPTER IV**

## **Meiofauna as descriptor of tourism-induced changes at sandy beaches**

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## ABSTRACT

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Tourism has long been considered as a 'clean industry' with almost no negative effects on the environment. This study demonstrated, in two different coastal systems (Mediterranean and Baltic), that tourism related activities are particularly affecting the sandy beach meio- and nematofauna in the upper beach zone, the specific ecotone in which many meiofauna species from both the marine and the terrestrial environment congregate. Tourist upper beaches are characterized by a lower % Total Organic Matter, lower densities, lower diversities (absence of Insecta, Harpacticoida, Oligochaeta, terrestrial and marine Ironidae nematodes) and higher community stress compared to nearby non-tourist locations. The %TOM was found to be the single most important factor for the observed differences in meiofauna assemblage structure at tourist versus non-tourist beaches in both the Mediterranean and the Baltic region. The free-living nematode assemblages from tourist upper zones depart significantly from expectations based on random selections from the regional nematode species pool. Furthermore upper zone assemblages are characterised by a low species diversity consisting of taxonomically closely related nematode species with *r*-strategist features.

Generally, faunal differences between tourist and non-tourist beaches are decreasing towards the lower beach zones.

**KEYWORDS:** meiofauna, free-living nematodes, sandy beach, taxonomic diversity, tourist impacts, Mediterranean, Baltic

## INTRODUCTION

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Sandy beaches are examples of simple ecosystems, principally driven by the physical forces of waves, tides and sediment movements (Short 1999). The simplicity is mainly related to the biodiversity of the system rather than to the adaptation of the organisms which may be very specialized and therefore, because of the highly dynamic environment, very fragile (Brown and McLachlan 1990). Notwithstanding their barren appearance sandy beaches, they support diverse populations of benthic invertebrates, bacteria, diatoms and algae. While the variety of animal life inhabiting sandy beaches is strikingly less than that on the rocky intertidal or shallow tidal flats, the individual species are often highly abundant (Hedgpeth 1957). These species play important roles in the ecological functioning of the beach, as primary producers (diatoms and algae), as decomposers (bacteria), as first line (heterotrophic bacteria and meiobenthos) or as second line consumers (macrobenthos) (Knox 2001). The latter are consumed by juvenile flatfish and wading birds, which can be considered as the top predators in the sandy littoral. Since the beach is considered to be the dynamic natural transition zone between land and sea representatives from both terrestrial and marine origin can be found.

Several authors have noted that perturbed benthic environments are generally kept in an early stage of the successional series (low species diversity and often consisting of taxonomically closely related species), while unperturbed benthic communities, in a late(r) successional stage, often consist of a wider range of taxonomically more distinct species (Warwick and Clarke 1995). Therefore, following Tilman (1996), the taxonomic range of an assemblage may be indicative in maintaining ecosystem stability during natural as well as anthropogenic disturbances. Disturbance is known to represent an important causative factor for spatial heterogeneity, and consequently for the structure and dynamics of natural assemblages (Sousa 1984). As a rule the major stresses on the coastal environment have been linked with overexploitation of natural resources, pollution, industrialisation and erosion (McIntyre 1995; Dronkers and Devries 1999) while tourism and recreational activities have been largely neglected. Gormsen (1997) estimated that 25% of all hotel beds worldwide are located along sandy coastlines. During the last decades, recreational activities have been increasing rapidly in developed countries as people enjoy more leisure time and higher standards of living. Clearly many of these activities are, and will increasingly be, focused on coastal tourism. Partly due to their barren appearance sandy beaches have not been regarded as vulnerable to human disturbance, while sand dunes are widely recognized as very fragile systems, even sensitive to barefoot human traffic (Andersen 1995; Poulson and McClung 1999). Human trampling has long been recognised as a critical factor for many vulnerable land biotas whereas it is only recently considered to play a key role in marine coastal conservation management. Presence of people on the beach and swimming in the surf zone has a marked effect on the activities of macrofauna and the semi-terrestrial crustaceans, inhibiting the intertidal feeding grounds of shorebirds (Andersen 1995). The impact of tourism does not only include the human trampling on the beach itself but also the whole of activities to organise and maintain it (beach management operations). In tourist popular regions dune systems are destroyed by the construction of vacation facilities (Cori 1999; Nordstrom *et al.* 2000) and the upper beaches are cleaned frequently with mechanical beach cleaners. Along with the removal of algae and wrack, almost all macroscopic items are removed from the beach as the upper sand layer is shovelled

up and replaced after sifting. Therefore the % Total Organic Matter (%TOM) can possibly serve as a biological proxy for mechanical beach cleaning. The cleaning may also affect the sand transport, sediment chemistry and fore-dune stabilisation. Physical disturbance by cleaning activities is already known to cause a decrease or disappearance of macrofauna (*e.g.* sandhoppers) as well as the wading birds feeding on them (Brown and McLachlan 1990; Llewellyn and Shackley 1996; Mann 2000). To date, the effects on the interstitial meiofauna (all metazoans between 38  $\mu\text{m}$  and 1mm) are unclear. Owing to the high abundances, high species richness and trophic diversity, meiofauna occupies a significant position in the so-called "small food web" (bacteria, protists, meiofauna) (Kuipers *et al.* 1981) and have many inter-relations with macrofauna (Reise 1979). Nematodes were chosen in this study as a representative group within the sandy beach meiofauna as this group is well suited (overall high abundances, wide spectrum from highly tolerant to non-tolerant species to several kinds of pollution and disturbances, no pelagic life stages, ubiquitous distribution, rapid generation and fast metabolic rates, relatively short life spans) for studying the impacts of different kinds of natural and anthropogenic disturbances in the marine environment (Heip *et al.* 1985; Schratzberger *et al.* 2000).

The aims of this study were to:

- (1) Compare the meiobenthic composition at higher taxon levels between tourist and non-tourist beaches in both the Baltic Sea and the Mediterranean Sea, ( $H_{01}$ : no differences in meiofaunal assemblages between tourist and non-tourist beach zones),
- (2) Assess possible changes in the taxonomic composition of the nematofauna, ( $H_{02}$ : no differences in taxonomic composition between tourist and non-tourist beach zones) and
- (3) Determine the environmental variables that are primarily responsible for potential differences at tourist versus non-tourist beaches.

# MATERIAL AND METHODS

## Study sites

Two micro-tidal beach systems in two different climatic areas were investigated (Figure 1). At each location a tourist beach and a pristine beach were selected. Both tourist sites are characterized by high tourist pressure and are cleaned frequently by means of mechanical beach cleaners. Detailed information on these beaches and their morphodynamics are described in Table 1 and elsewhere (Gheskiere *et al.* 2005a), but generally all beaches belong to the micro-tidal intermediate group *sensu* Short (Short 1999).

(1) The study sites of Hel and Jurata are located on the northern coast of Poland, at the end of the Hel Peninsula. The beach of Hel has been closed to the public entrance for more than 50 years because it was a strategic military area. Since only limited access is available now, human impact can be considered as relatively low. This beach is backed by a well-developed dune system forming the upper limit of storm accumulation. The study site of Jurata is located in front of a hotel complex, constructed in the dunes. As a consequence of the leisure infrastructure a natural dune system is absent in Jurata.

(2) The Mediterranean study sites are located in the Tuscan region. The beach of San Rossore is situated in the San Rossore Massaciuccoli Natural Park (24.000 ha). This park stretches along 30 km of flat coastal strip in Tuscany (Ligurian Sea), bounded by Viareggio in the North and Livorno in the South. The San Rossore beach is, like the beach in Hel, backed by a natural dune system and forests of pine-trees. There is free public access to the Massaciuccoli Natural Park but since access to the littoral area is restricted, this beach is classified as undisturbed. The study site of Viareggio is located just outside the San Rossore Massaciuccoli Natural Park. Viareggio is a primary place of national and international tourism since the 19<sup>th</sup> century and has over 400 bathing facilities constructed in or nearby the dunes.

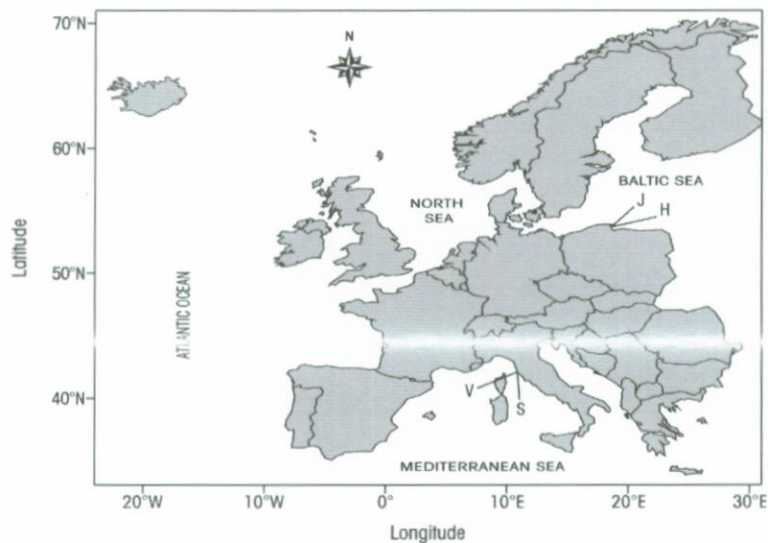


Figure 1: Map showing the tourist and non-tourist beaches studied in each geographic area: Viareggio (V) and San Rossore (S) in Italy and Jurata (J) and Hel (H) in Poland, respectively.

Beach Characteristics Mediterranean	San Rossore	Viareggio
Longitude	10°16'40" E	10°14'39" E
Latitude	43°42'53" N	43°50'56" N
Beach width (m)	45-60	50-60
Breaker type	Spilling-surging	Spilling-surging
Median grain size ( $\mu\text{m}$ )	509 $\pm$ 19	500 $\pm$ 21
Sediment textural group	coarse sand	coarse sand
Sediment sorting ( $\Phi$ )	0.322 $\pm$ 0.004 well sorted	0.342 $\pm$ 0.006 well sorted
Beach exposure	very exposed	very exposed
Dean's parameter ( $\Omega$ )	2.39	2.34
Relative Tidal Range (RTR)	0.5	0.5
Beach type	intermediate/reflective	intermediate/reflective

Beach Characteristics Baltic	Hel	Jurata
Longitude	18°45'37" E	18°43'06" E
Latitude	54°37'04" N	54°41'08" N
Beach width (m)	60-85	60-75
Breaker type	Spilling-plunging	Spilling-plunging
Median grain size ( $\mu\text{m}$ )	385 $\pm$ 14	375 $\pm$ 22
Sediment textural group	medium sand	medium sand
Sediment sorting ( $\Phi$ )	0.309 $\pm$ 0.01 well-sorted	0.315 $\pm$ 0.02 well-sorted
Beach exposure	very exposed	very exposed
Dean's parameter ( $\Omega$ )	5.29	5.20
Relative Tidal Range (RTR)	0.1	0.1
Beach type	intermediate/dissipative	intermediate/dissipative

Table 1: Detailed information on the beaches studied.

## Sampling strategy and sample collection

Sampling was performed in September (Poland) and October (Italy) 2000, just after the end of the tourist summer season. On each of the four beaches, three zones across the beach slope (upper, middle and lower) each with eight randomly positioned replicates were sampled for meiofauna. The tourist and non-tourist beaches in each geographic area are only some kilometres from each other, along the same coastline. Tourism-induced changes were most likely to occur in the surface layers of the sediment and thus only the top 10 cm of sediment were sampled using transparent plexi-cores (sampling surface area 10 cm<sup>2</sup>). Six additional samples per zone were randomly collected for granulometric, %TOM and interstitial salinity analyses. Meiofauna samples were immediately fixed with a heated (70°C) 4% buffered formaldehyde water solution (Heip *et al.* 1985; Vincx 1996).

## Laboratory treatment

In the laboratory, meiofauna samples were rinsed with a gentle jet of freshwater over a 1 mm sieve to exclude macrofauna, decanted over a 38  $\mu\text{m}$  sieve, centrifuged three times with Ludox<sup>®</sup> HS40 (specific density 1.18) and stained with Rose Bengal. Meiofauna was counted and identified at the higher taxon level using a stereomicroscope. Per replicate, the extract was then placed into a beaker, made up to a standard volume with filtered tap-water and homogenized into suspension before a constant proportion (25%) of the sample was taken with a semi-automatic pipette. Per sub-sample all nematodes were picked out, transferred from formalin to glycerol through a series of ethanol-glycerol solutions, mounted in glycerine slides (Vincx 1996) and then identified to the species level and classified according to the phylogenetic system of De Ley and Blaxter (2002, 2003).

Sediment samples were oven-dried at 105°C for 12 h and ashed at 500  $\pm$  50°C for 2 h to determine the %TOM by loss of mass. Sediment particle-size distribution was determined using Coulter LS100<sup>®</sup> particle size analysis equipment. The sediment fractions were defined according to the Wentworth scale (Buchanan 1984); sediment sorting coefficient and other granulometric characteristics were calculated as described by Dyer (1986).

## Data processing

The meiofauna data were analysed by non-metric Multi-Dimensional Scaling (MDS) (Kruskal 1964) and by a Detrended Canonical Analysis (DCA) (Ter Braak 1988). A measurement of the degree to which the MDS-plots correspond to the dissimilarity values is given by the stress or loss function value. Analysis of similarities (ANOSIM, Clarke 1993) was used to test for significant differences between multivariate groups of samples from different zones and from different beaches. The similarity percentages programme (SIMPER, Clarke 1993) was applied to determine the contribution of higher meiofauna taxa and individual nematode species towards the discrimination between the equivalent beach zones. The Index of Multivariate Dispersion (IMD, Warwick and Clarke 1993) has been applied here as measure of community stress. The IMD is a measure of the increase in variability among replicate samples from perturbed versus pristine situations, *i.e.* a Multivariate Stability Index (MSI) *sensu* Warwick *et al.* (2002). All multivariate analyses were performed using square-root-transformed data in order to indicate the responses of highly dominant species but also put some weight on the rare ones (Clarke 1993).

Nematode species abundance data (Ind/10 cm<sup>2</sup>) were used to calculate the diversity as the expected number of species per sample based on 100 individuals ES(100) (Sanders 1968; Hurlbert 1971), Simpson Index (1- $\lambda'$ ) and average taxonomic distinctness based on quantitative ( $\Delta^*$ ) and presence/absence data ( $\Delta'$ ) (Warwick and Clarke 1995). For the calculation of the taxonomic indices equal step-lengths between each taxonomic level were assumed, setting the path length  $\omega$  to 100 for two species connected at the highest (taxonomically coarsest) possible level (Clarke and Warwick 1999). Eight taxonomic levels were used (species, genus, family, super-family, sub-order, order, sub-class and class) and consequently weights are:  $\omega=12.5$  (species in the same genus),  $\omega=25$  (same family but different genus),  $\omega=37.5$  (same super-family but different family),  $\omega=50$  (same sub-order but different super-family),  $\omega=62.5$  (same order but different sub-order),  $\omega=75$  (same sub-class but different order),

$\omega=87.5$  (same class but different sub-class) and  $\omega=100$  (different class), respectively. Calculation of average taxonomic distinctness from simulated sub-samples of different numbers of species  $m$  from the master list ( $\Delta_m^*$ ) were used to produce probability funnels against which distinctness values for all zones were checked. This formally address the question whether these zones have a 'lower than expected' taxonomic spread, assuming a null hypothesis that each sample is a random selection from the regional species pool (Clarke and Warwick 1998). The same procedure was used to produce joint plots of average taxonomic distinctness ( $\Delta^*$ ) and variation in taxonomic distinctness ( $\Delta^*$ ). Taxonomic diversity measures have the distinct advantage of being unbiased by sampling size and have potential for environmental impact assessment studies (Warwick and Clarke 2001). The relationships between multivariate biotic patterns and environmental variables were assessed using the BIO-ENV procedure (Clarke and Ainsworth 1993). Diversity measurements, community analyses and BIO-ENV calculations were performed using the PRIMER v5.2.9 software package (Clarke and Gorley 2001). Differences in univariate community attributes were analysed using a multi-factorial ANOVA with model terms including 'GEOGRAPHIC AREA' (Mediterranean, Baltic), 'TOURIST' (tourist, non-tourist) and 'ZONE' (upper, middle, lower). Residuals from the fitted models were visually assessed for evidence of outliers or of non-normality. Bartlett's and Cochran's tests were used to verify for homoscedasticity of variances prior to the analysis. Tukey multiple comparison tests were performed to investigate any differences between tourist and non-tourist beach zones (Zar 1996). Statistical analyses were performed using the STATISTICA v6.0 software package (StatSoft 2001). A significance level of  $p<0.05$  was used in all tests.



# RESULTS

## The abiotic environment

At both non-tourist sites the %TOM showed a similar decreasing pattern, being noticeably higher at the upper beach zone ( $2.28 \pm 0.10$  and  $1.17 \pm 0.04$  for San Rossore and Hel respectively) coinciding with a visible cover of marine and terrestrial detritus at these zones. The upper beaches from the tourist sites both showed a significantly lower %TOM in contrast to their non-tourist counterparts, confirming the use of this parameter as biological proxy for upper beach cleaning activities (Viareggio:  $0.25 \pm 0.12$  and Jurata:  $0.34 \pm 0.07$ ). Values at the middle and lower tourist zones were comparable with the same zones on the non-tourist sites (Figure 2A).

Generally no significant granulometric differences (grain size, sorting, skewness, granulometric size class distribution) were noted between tourist and non-tourist equivalent beach zones in both regions. Figure 2B shows only median grain size. Median grain size was highest at the middle zone (the swash/breaker zone) of all beaches studied, a known feature caused by the wave-dominated character of this kind of micro-tidal beaches (Short 1999).

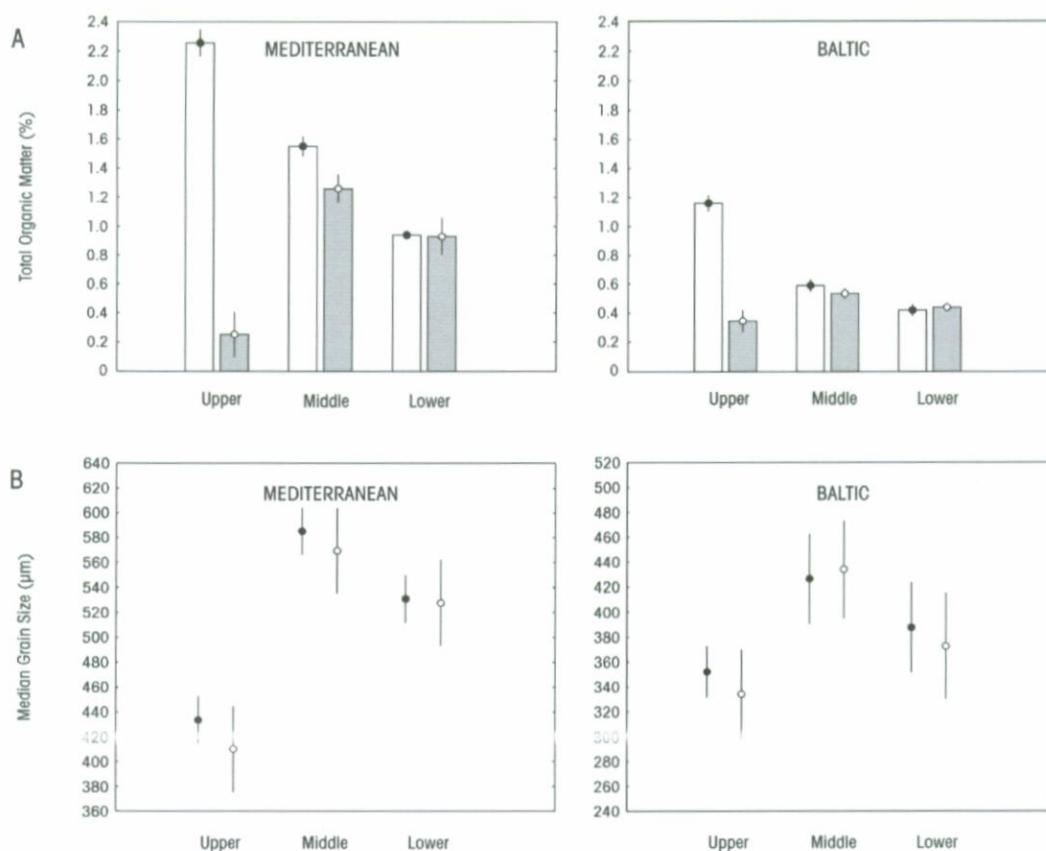


Figure 2: A: Mean % Total Organic Matter ( $\pm$  SD) and B: Mean median grain size ( $\pm$  SD). (All calculated by pooled variance of six replicates per zone and per beach for the Mediterranean and the Baltic area) (Closed symbols=non-tourist, open symbols=tourist)

## Assemblage structure

The two geographic areas differed significantly in higher meiofauna assemblage structure (ANOSIM,  $R=0.319$ ,  $p<0.01$ ). Following the SIMPER-analyses this is in essence caused by higher numbers of turbellarians in the Mediterranean and high numbers of oligochaetes in the Baltic. MDS-plots and DCA-ordinations (not shown) for total meiofauna (including nematode species data) denoted in each area a clear separation between tourist and non-tourist sites and between the different zones sampled (Figure 3). On the Mediterranean beaches, as well as on the Baltic ones the meiofauna of the upper zones is more dissimilar in assemblage composition than middle and lower zones. Results of the ANOSIM tests (Table 2) confirmed this trend on the total meiofauna as well as on the nematode species level. Similarity of percentage analyses (SIMPER) attributed this discrimination in the Mediterranean region mainly to a complete loss of Insecta, Harpacticoida, Oligochaeta, terrestrial Dorylaimida (*Aporcelaimus* sp.1, *Aporcelaimellus* sp.1, *Aporcelaimellus* sp.2, *Mesodorylaimus* sp.1 and *Nygolaimus* sp.1), Haliplectidae (*Haliplectus* sp.) and marine Ironidae (*Trissonchulus oceanus*) on the tourist upper beach. *Epsilonema pustulatum* and *Theristus heterospiculum* both occurred in very high densities on the non-tourist Mediterranean middle beach zone while these species were nearly or complete absent, respectively on the tourist middle beach zone. *T. heterospiculum* seemed to be entirely replaced by *Theristus heterospiculoides* on the tourist beach. In the Baltic region the tourist upper beach was in essence characterised by the absence of Oligochaeta, Tardigrada, Insecta and also terrestrial nematode species (several *Aporcelaimus* and *Aporcelaimellus* species, *Plectus* sp. and *Acrobeles ciliatus*) and the marine Ironidae (*Trissonchulus benepapilosus*).

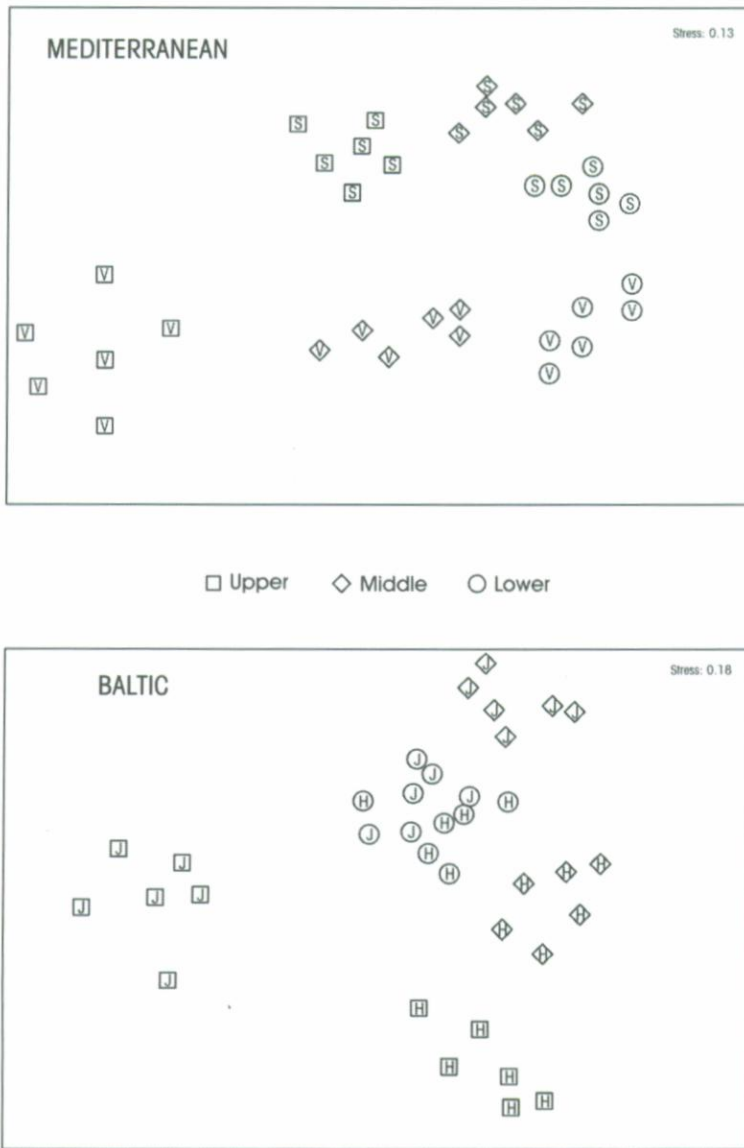


Figure 3: Meiofaunal assemblages (higher taxa and nematode species data combined): Output of non-metric Multi-Dimensional Scaling (MDS) on square-root-transformed abundance data (Six replicates per beach zone). (A: Mediterranean, S= San Rossore, V=Viareggio. B: Baltic, H=Hel, J=Jurata)

	MEDITERRANEAN				BALTIC			
	Meiofauna		Nematodes		Meiofauna		Nematodes	
	R	p	R	p	R	p	R	p
Global test	0.822	<0.001	0.802	<0.001	0.606	0.001	0.530	<0.001
<i>Zones compared</i>								
UPPER	0.998	0.029	0.997	0.029	0.737	0.020	0.863	0.020
MIDDLE	0.760	0.029	0.875	0.029	0.694	0.020	0.330	0.011
LOWER	0.533	0.049	0.646	0.039	0.206	0.078	0.152	0.087

**Table 2:** Results of the ANOSIM and pair-wise tests for difference between non-tourist and tourist equivalent beach zones for both areas on the meiofauna and nematode assemblage structure (R-values and p-values are reported). Analyses performed on square-root transformed data.

The MDS-plots also indicated a higher inter-variability among replicate samples from disturbed upper beaches as the replicates are more scattered in the plots. The latter is reflected in the calculations of the indices of multivariate dispersion (IMD). In equivalent zones, the inter-variability among replicate samples from tourist beaches is higher than for non-tourist beaches. Consequently, when making pair-wise comparisons between equivalent zones the multivariate stability indices (MSI) indicated negative values for total meiofauna and for nematodes separately. (Table 3)

AREA	Zones	Non-tourist	Tourist	MSI
MEDITERRANEAN	All	0.91 (0.90)	1.30 (1.20)	-0.39 (-0.29)
	Upper	1.06 (0.77)	1.44 (0.99)	-0.38 (-0.23)
	Middle	1.17 (1.22)	1.40 (1.42)	-0.23 (-0.21)
	Lower	0.79 (0.76)	0.94 (0.91)	-0.14 (-0.15)
BALTIC	All	0.98 (0.97)	1.13 (1.10)	-0.15 (-0.13)
	Upper	1.19 (0.88)	1.39 (1.10)	-0.20 (-0.22)
	Middle	1.13 (0.99)	1.28 (1.07)	-0.15 (-0.09)
	Lower	0.81 (0.70)	0.90 (0.78)	-0.09 (-0.08)

**Table 3:** Values of multivariate dispersion from both non-tourist and tourist beaches, separately for both study sites and zones on the beach, based on square-root-transformed total meiofauna abundance data and Bray-Curtis similarities, and the resulting Multivariate Stability Index (MSI). Values for nematodes separately are given in brackets.

## Univariate community indices and taxonomic measurements

The results from the three-way ANOVA are presented in Table 4. Figures 4-6 show the graphical summary of means and standard errors of univariate indices for higher meiofauna and nematode assemblages from both tourist and non-tourist beach zones along with the results of Tukey multiple comparison tests. Most indices (except ES(100),  $p < 0.10$  and  $\Delta^*$ ,  $p < 0.22$ ) differed significantly between geographic areas. Total meiofauna density and number of taxa were the only indices not showing a significant difference between tourist and non-tourist samples ( $p < 0.48$  and  $p < 0.35$ ). For all indices (except Simpson dominance) a significant TOURIST x ZONE interaction was noted. Apart from the number of taxa, a significant GEOGRAPHIC AREA x TOURIST interaction was absent for all indices.

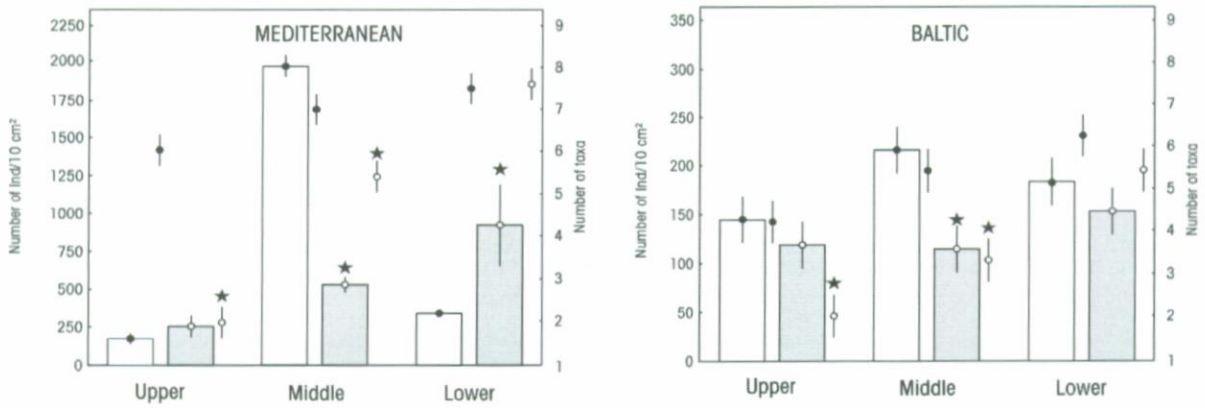
Fourteen higher meiofauna groups were recorded during this study, in overall decreasing order of density: Nematoda, Turbellaria, Oligochaeta, Harpacticoida, Gastrotricha, naupliar larvae, Halacaroidea, Insecta, Amphipoda, Polychaeta, Tardigrada, Kinorhyncha, Gnathostomulida and Acari. Thirteen meiofauna groups were recorded in the Mediterranean region and 12 in the Baltic. Generally, higher average total meiofauna densities were noted in the Mediterranean region: ( $638 \pm 208$  Ind/10 cm<sup>2</sup>) versus ( $161 \pm 23$  Ind/10 cm<sup>2</sup>) in the Baltic. Nematodes numerically dominated all beaches studied (accounting for more than 75% of the total meiofauna). Naupliar larvae and Turbellaria, and Oligochaeta and Turbellaria, were subdominant in Mediterranean and Baltic samples respectively. Significantly higher total meiofauna densities were recorded on the non-tourist middle beach zone ( $1990 \pm 45$  Ind/10 cm<sup>2</sup>) and on the tourist lower beach zone ( $950 \pm 198$  Ind/10 cm<sup>2</sup>) in the Mediterranean. The higher density on the non-tourist middle beach zone is caused by higher numbers of *Epsilonema pustulatum* while naupliar larvae were responsible for the higher density in the tourist lower zone. The nematode abundance is remarkably uniform across both Baltic beaches.

A total of 73 and 68 free-living nematodes species were recorded on the Mediterranean and Baltic beaches respectively. 66 species were recorded in San Rossore, 34 species in Viareggio, 56 species in Hel and 41 species in Jurata. From Figure 5 shows that the most significant differences in univariate measurements (with exception of density) can be found on the upper beach zones. Tourist upper beach zones showed a significant lower expected species diversity ( $3 \pm 2$  for Viareggio and  $4 \pm 1$  for Jurata) and significant higher dominance compared to the non-tourist upper zones.

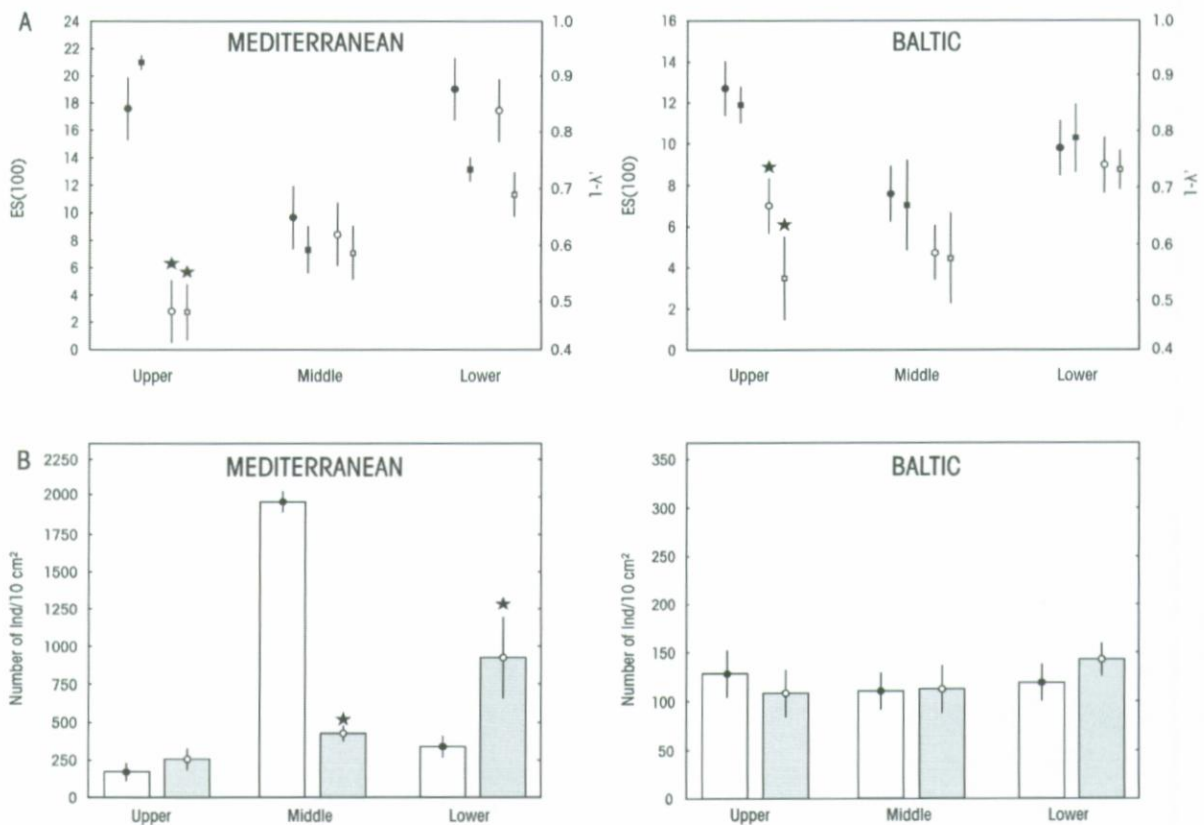
Although middle and lower beach zones from both geographic areas showed similar values, tourist upper beach zones had lower average taxonomic diversity values ( $\Delta^*=60$ ,  $\Delta^*=68$ ) compared to their non-tourist equivalents ( $\Delta^*=78$ ,  $\Delta^*=81$ ) (Figure 6).

	Effect	df	F-value	p-value
N(meio)	GEOGRAPHIC AREA	1	16.50	<0.01 *
	TOURISM	1	0.51	0.48
	ZONE	2	9.03	<0.01 *
	GEOGRAPHIC AREA x TOURISM	1	0.86	0.36
	GEOGRAPHIC AREA x ZONE	2	7.61	<0.01 *
	TOURISM x ZONE	2	4.88	0.01 *
	GEOGRAPHIC AREA x TOURISM x ZONE	2	5.76	0.01 *
Number of Taxa	GEOGRAPHIC AREA	1	37.14	<0.01 *
	TOURISM	1	0.88	0.35
	ZONE	2	59.56	<0.01 *
	GEOGRAPHIC AREA x TOURISM	1	31.65	<0.01 *
	GEOGRAPHIC AREA x ZONE	2	2.69	0.08
	TOURISM x ZONE	2	9.45	<0.01 *
	GEOGRAPHIC AREA x TOURISM x ZONE	2	6.10	<0.01 *
N(nema)	GEOGRAPHIC AREA	1	44.94	<0.01 *
	TOURISM	1	8.67	0.01 *
	ZONE	2	14.20	<0.01 *
	GEOGRAPHIC AREA x TOURISM	1	0.03	0.86
	GEOGRAPHIC AREA x ZONE	2	16.99	<0.01 *
	TOURISM x ZONE	2	7.06	<0.01 *
	GEOGRAPHIC AREA x TOURISM x ZONE	2	4.16	0.02 *
ES(100)	GEOGRAPHIC AREA	1	2.72	0.10
	TOURISM	1	34.82	<0.01 *
	ZONE	2	14.35	<0.01 *
	GEOGRAPHIC AREA x TOURISM	1	0.52	0.47
	GEOGRAPHIC AREA x ZONE	2	7.76	<0.01 *
	TOURISM x ZONE	2	1.44	0.05 *
	GEOGRAPHIC AREA x TOURISM x ZONE	2	1.27	0.29
1-λ'	GEOGRAPHIC AREA	1	6.52	0.01 *
	TOURISM	1	4.32	0.04 *
	ZONE	2	7.29	<0.01 *
	GEOGRAPHIC AREA x TOURISM	1	3.17	0.08
	GEOGRAPHIC AREA x ZONE	2	0.47	0.63
	TOURISM x ZONE	2	1.55	0.22
	GEOGRAPHIC AREA x TOURISM x ZONE	2	0.52	0.60
Δ*	GEOGRAPHIC AREA	1	1.56	0.22
	TOURISM	1	16.15	<0.01 *
	ZONE	2	3.67	0.03 *
	GEOGRAPHIC AREA x TOURISM	1	0.29	0.59
	GEOGRAPHIC AREA x ZONE	2	1.67	0.20
	TOURISM x ZONE	2	9.93	<0.01 *
	GEOGRAPHIC AREA x TOURISM x ZONE	2	0.63	0.54

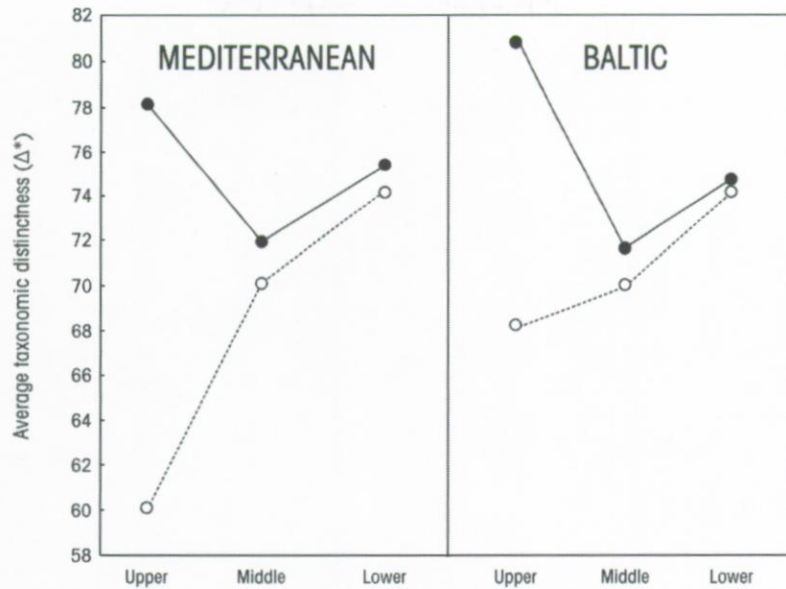
Table 4: Results from three-way ANOVA for univariate community indices.



**Figure 4:** Total meiofauna densities (Ind/10 cm<sup>2</sup>) (bars ± SE, left ordinate) and mean higher taxon richness (circles ± SE, right ordinate) (All calculated by pooled variance of eight replicates per zone and per beach for the Mediterranean and the Baltic area) (Closed circles=non-tourist, open circles=tourist) ★ Indicates significant differences (p<0.05) from non-tourist samples (Tukey multiple comparison test for equal n).



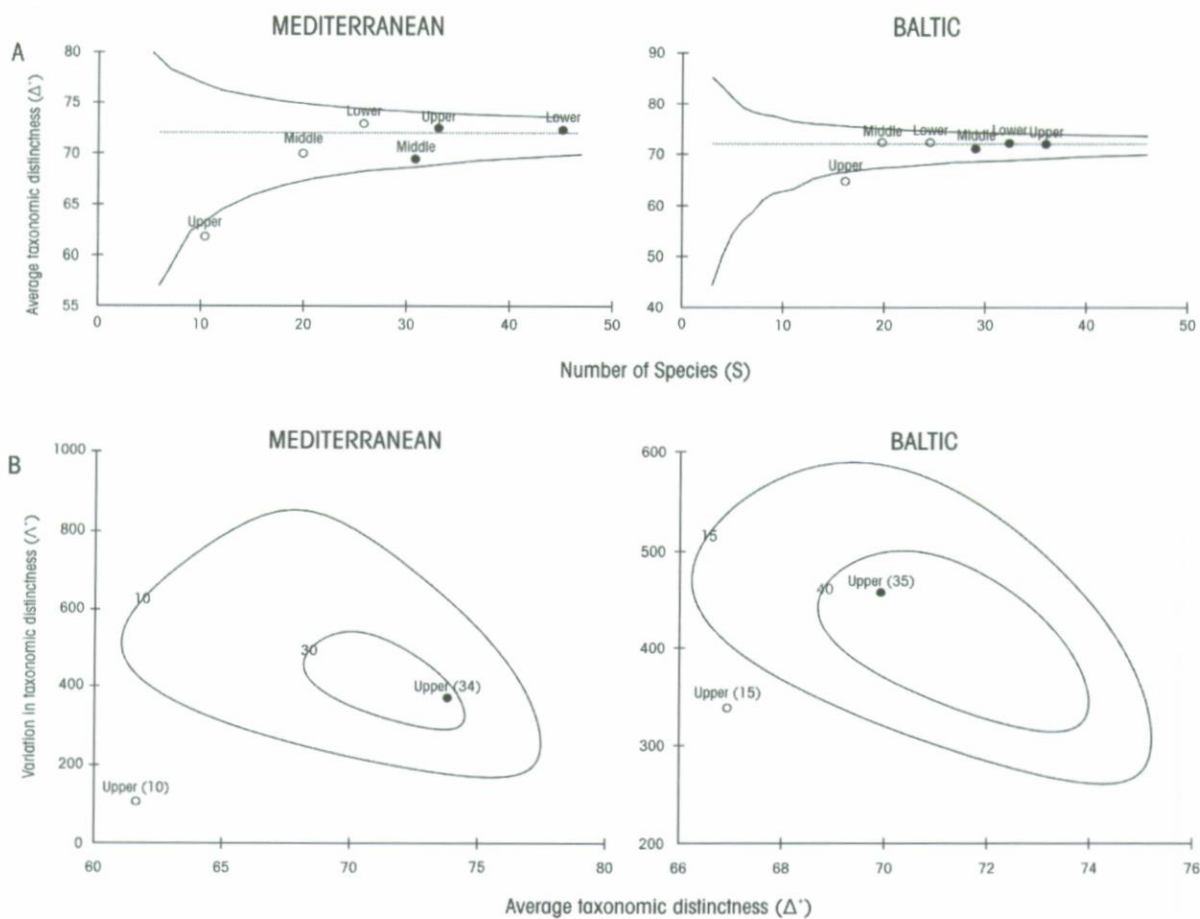
**Figure 5:** A: Expected number of species ES(100) (circles ± SE, left ordinate) and Simpson index (1-λ') (squares ± SE, right ordinate). B: Total nematode densities (Ind/10cm<sup>2</sup>) (bars ± SE). (All calculated by pooled variance of six replicates per zone and per beach for the Mediterranean and the Baltic area) (Closed symbols=non-tourist, open symbols=tourist) ★ Indicates significant differences (p<0.05) from non-tourist samples (Tukey multiple comparison test for equal n)



**Figure 6:** Average taxonomic distinctness values ( $\Delta^*$ ) based on quantitative nematode species data for each beach zone in the Mediterranean and the Baltic area. (Closed symbols=non-tourist, open symbols=tourist)

Figure 7A displays the 95% funnel for the simulated distribution of average taxonomic distinctness ( $\Delta^*$ ) for 15000 random subsets of fixed size  $m$  from the master nematode list for each area (73 species for Italy and 68 species for Poland). Superimposed on this plot are the true values of  $\Delta^*$  generated from species lists for each of the zones across the beaches. Upper beach zones from tourist Mediterranean and Baltic beaches and, to a lesser extent, Mediterranean middle beach zones all have reduced average taxonomic distinctness values, whereas all other zones have  $\Delta^*$  values close to that of their master list suggesting no evidence of reduced taxonomic distinctness for these zones. However, only the decrease in average taxonomic distinctness from both tourist upper beaches is significant ( $p < 0.05$ ). Figure 7B shows a bivariate equivalent of the univariate funnels in which  $\Delta^*$  is considered jointly with the variation in taxonomic distinctness ( $\Lambda^*$ ) to produce probability regions within which 95% of the simulated values fall for a list of sub-listed sizes from random selections from the master nematode list for the upper beach zones from Mediterranean ( $m=10, 30$ ) and Baltic ( $m=15, 40$ ) tourist and non-tourist zones. The observed ( $\Delta^*, \Lambda^*$ ) values for these two upper beach zones are superimposed on their appropriate plot. As can be seen, both tourist upper beaches depart significantly ( $p < 0.05$ ) from expectation as they fall outside the 95% ellipse. All other equivalent tourist and non-tourist beaches zones did not show a departure from the expected probability ellipses (plots are not shown).





**Figure 7 A:** The 95% probability funnels for Average Taxonomic Distinctness ( $\Delta^*$ ) plotted against the number of species for the different nematode assemblages from the non-tourist (closed circles) and tourist (open circles) beach zones in Italy and Poland studied for each sub-listed size. Dashed lines indicates the simulated mean  $\Delta^*$  and  $\Lambda^*$  from 15000 ad random selections from the master list of 73 nematode species (Italy) and 68 nematode species (Poland). **B:** 'Ellipse' plots of 95% probability areas for ( $\Delta^*$ ,  $\Lambda^*$ ) pairs from tourist and non-tourist upper beach zones for a range of sub-list sizes in Italy ( $m=10, 30$ ) and Poland ( $m=15, 40$ ). The observed ( $\Delta^*$ ,  $\Lambda^*$ ) values for each upper beach zone are superimposed on the appropriate plot for their particular species list size (given in brackets).

## Relationship with environmental variables

The results of the Spearman rank correlation analyses between total meiofaunal assemblage structure and environmental variables for each geographic area are shown in Table 5. The BIO-ENV analyses indicated that both %TOM and salinity were important environmental factors in determining meiofaunal assemblage structure,  $\sigma=0.597$ ,  $\sigma=0.444$  for the Mediterranean area and  $\sigma=0.444$ ,  $\sigma=0.396$  for the Baltic area, respectively.

	Mediterranean	Baltic
Median grain size	0.499	0.104
Sorting	0.230	0.395
%TOM	<b>0.597</b>	<b>0.444</b>
Salinity	0.533	0.396

Table 5: Spearman rank correlations ( $\sigma$ ) between total assemblage structure and environmental variables per geographic area.

## DISCUSSION

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Tourism has long been considered as a 'clean industry' with almost no negative effects on the environment worthwhile considering. Man has continued to impose changes upon the sandy environment, partly through ignorance and inability to learn from experience but also in belief that it must be possible to shape nature to his own needs and desires (Brown and McLachlan 1990). Yet, this stand is now outmoded as most parties are aware of the possible negative impacts on the coastal biodiversity and see the need for actions (UNEP 2000). However, on the other hand human populations are still increasingly concentrating in the coastal zone, and dunes and beaches are subjected to ever-expanding pressures from recreational activities.

To date, most tourism-impact studies have been mainly focused on changes in abundance and diversity of large macrofauna (Chandrasekara and Frid 1996), loss of individual species (*e.g. Talitrus saltator*, Weslawski *et al.* 2000) or decreasing populations of shore birds (*e.g. Cornelius et al.* 2001), whereas smaller animals were largely neglected. However, Kennedy and Jacoby (1999) indicated the meiofauna (a total of 23 phyla are represented) as phyletically more diverse than any other marine benthic component and moreover as an excellent indicator of marine environmental quality. Tourist and non-tourist zones were expected to be similar as they were located close to each other (only a few km), had similar exposure and showed negligible differences in granulometry, these (mean grain size, sorting, skewness, different fractions) being basic factors in meiofauna distribution (McIntyre 1969; Fricke and Flemming 1983).

The overall higher meiofauna densities in the Mediterranean are explained by the coarser and therefore more oxygenated sands, in contrast to finer and therefore more sulphidic Baltic sands (Gheskiere *et al.* 2005a). Higher meiofauna density in coarser sediments has been reported frequently (Gray and Rieger 1971; McLachlan 1977a, b; Giere 1993; Rodríguez *et al.* 2003). Lower salinity in the Baltic region (7 PSU) has most likely contributed to the comparatively lower density at those beaches. Benthic communities in brackish water have lower densities and fewer species than either pure marine or pure freshwater communities (Remane 1933; Gerlach 1954; Bouwman 1983). Major biological differences in assemblage structure (univariate, multivariate and taxonomically) between tourist and non-tourist beaches were found at the upper beach zones. In general these differences were more pronounced on the Mediterranean beaches. This is most likely caused by the length and intensity of the tourist period which is almost all year round in the Mediterranean while only in the summer months (Weslawski *et al.* 2000) in the Baltic region. BIO-ENV analyses clearly indicated the %TOM as the single most important factor responsible for the observed differences in assemblage structure at tourist versus non-tourist beaches in both areas. On pristine beaches, the amount of %TOM generally increases towards the upper beach and dune system (Wall *et al.* 2002) which corresponds with our findings. As a result of the intensive use, almost daily (mechanically) cleaning of the tourist upper beaches and destruction of the natural connections to the dune areas by numerous bathing facilities, considerable less marine and terrestrial debris was found. Consequently a significant decrease of interstitial %TOM was noted on the tourist upper beaches. Mechanical beach cleaning not only removes organic matter and anthropogenic waste from the beach but also physically disturbs the sediment, its micro-topography and its inhabitants, therefore creating a uniform habitat with a short durational stability (Van de Velde 2003; Gheskiere

*et al.* SUBMITTED). Low levels of organic matter and lack of suitable niches to support rich nematode assemblages are indicative of a stressed or resource-limited environment (Wall *et al.* 2002). Also the human trampling, primarily taking place at the upper beach, possibly influences the meiobenthic environment. Weslawski *et al.* (2000) calculated that one square meter of a Baltic tourist beach receives more than 100 human steps daily during the peak summer season. This causes reduction in soil macro porosity, air/water permeability, changes in sediment topography and perturbs the sand almost continuously. Wynberg and Branch (1994) demonstrated a reduction of oxygen content through compaction of the sediment. Multivariate analyses (ANOSIM) showed a clear significant discrimination of meio- and nematofauna between equivalent zones from tourist and non-tourist beaches at both study sites and therefore  $H_{01}$  (no differences in meiofaunal assemblages between tourist and non-tourist beach zones) has to be rejected. As noted above these differences were more defined at the upper beaches and decreased downwards the beach. SIMPER-analyses attributed this discrimination in the Mediterranean region mainly to a complete loss of Insecta, Harpacticoida, Oligochaeta, terrestrial nematodes and marine Ironidae nematode species on the tourist upper beach. In the Baltic region the tourist upper beach was in essence characterised by the absence of Oligochaeta, Tardigrada and Insecta and terrestrial nematode species. The absence of terrestrial nematodes on tourist beaches is most likely due to the destruction of the dunes and probably also a disruption of subterranean freshwater connections from the hinterland which terrestrial nematodes use to invade on the beach (Gheskiere *et al.* 2004). Absence of insects (mainly Coleoptera, Diptera larvae) and perhaps also oligochaetes (Inglis 1989) is linked with the absence of marine/terrestrial organic matter. As tourist beaches are cleaned frequently, the amount and presence of terrestrial and marine debris on the upper shore is reduced significantly. This debris is considered to be the primary food source for many upper shore macrofauna (Stenton-Dozey and Griffiths 1983), serves as a refugium (Colombini *et al.* 2000) and attracts terrestrial insects to invade on the beach (Remmert 1960). Many insects use the wind to travel (anemochory) from the dunes to the beach (Remmert 1960; Desender 1996). At tourist sites the fetch (open distance) of winds coming from the dunes is disturbed by buildings and bathing facilities and this might also contribute to the absence of insects. The colonization and breakdown of stranded wrack and debris by different faunal groups are described in detail by Jedrzejczak (1999). However it is still not clear whether the meiofauna use the dissolved organic matter in the interstitial water below the debris as a direct food source or whether the initial utilisation of the interstitial organic matter is by bacteria and that these in turn constitute the food source of the meiofauna (Jedrzejczak 2002a, b). Malm *et al.* (2004) have noted a significant reduction of %TOM and bacterial production and fewer ciliates on mechanically cleaned beaches in contrast to uncleaned ones.

Caswell and Cohen (1991) first hypothesised that disturbance might induce higher spatial variability in assemblages (community stress). Warwick and Clarke (1993) and more recently Fraschetti *et al.* (2001) have also consistently recorded increased variability among replicates from several benthic communities (meio- as well as macrofauna) exposed to increasing disturbance levels. Our calculations of the multivariate dispersion indices indeed positively demonstrated an increase in variability on the impact beaches and support this contention. Multivariate stability indices comparing tourist and non-tourist sites generally increased down the studied beaches indicating higher community stress on the upper zones.

Generally, clear changes in the nematode assemblage structure between tourist and non-tourist beaches were found. Multivariate analyses revealed that changes in assemblage structure were less well defined on middle and

lower beaches than on upper beaches. Average taxonomic distinctness ( $\Delta^+$ ) is a measure of the degree to which species in an assemblage are related taxonomically to each other while the degree to which species from the regional species pool are over- or under-represented is reflected in the variation in taxonomic distinctness ( $\Delta^*$ ). The latter can be seen as the 'evenness' of the distribution of taxa across the nematode taxonomical tree. We have found clear differences in the taxonomic diversity range of nematode assemblages between tourist and non-tourist upper beaches while middle and lower beach zones were more similar. Therefore  $H_{oz}$  (no differences in taxonomic composition between tourist and non-tourist beach zones) can only be rejected partially. The changes in taxonomic range closely mirror the similar patterns in dominance ( $1-\lambda'$ ) and species diversity. Most of the species absent on tourist upper beaches belong to orders of Nematoda (O. Dorylaimida, O. Ironidae and O. Rhabditida) which are relatively species-poor represented in our samples but which cause the high distinctness (*cf.* Dorylaimida are in the different subclassis Dorylaimia), within the upper beach assemblages. On the other hand orders like Monhysterida and Enoplida, containing species-rich, well-represented genera such as *Enoplolaimus* (with *E. attenuatus*, *E. littoralis*, *E. villosus*, *E. balgenis*) and *Theristus* (with *T. heterospiculum*, *T. heterospiculoides*, *T. inermis*, *T. aculeatus*, *T. pictus*) are recorded at both tourist and non-tourist upper beaches. As Dorylaimida are generally thought to have long life-cycles, low colonization abilities and are sensitive to several types of disturbance (Johnson *et al.* 1974; Zullini 1976), their absence on the disturbed upper beaches is not unexpected. Dorylaimid nematodes and especially the Aporcelaimidae are generally known as true *K*-strategists or extreme persisters while Monhysterid and Enoplid nematodes are known as colonizers or *r*-strategists (Bongers *et al.* 1991). These findings correspond well with the suggestion of Clarke and Warwick (2001) that benthic communities which have been perturbed switch to an early successional stage community (colonizers) with low species diversity, and are characterized by the loss of distinctive taxa (reduced  $\Delta^+$ ) which are species-poor (reduced  $\Delta^*$ ), consequently leaving an assemblage of opportunist species with close taxonomic affinities. Changes in sandy sediment nematode assemblages subjected to continuous and spasmodic perturbations in contrast to unperturbed situations were also detected by Schratzberger and Warwick (1999) during microcosm experiments.

## CONCLUSIONS

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This study demonstrated, in two different coastal systems (Mediterranean and Baltic), there is evidence that tourism related activities are: (1) particularly affecting the sandy beach meio-nematofauna, especially in the upper sandy beach zone, the specific ecotone in which many meiofauna species from both the marine and the terrestrial environment congregate and (2) contribute to higher community stress, lower taxonomic range and species diversity of the nematode assemblages compared to nearby pristine locations. The %TOM was found to be an important factor for the observed differences in meiofauna assemblage structure at tourist versus non-tourist beaches in both the Mediterranean and the Baltic region. The tourist upper beach zones are characterized by lower meiofauna diversities and low mature nematode assemblages consisting of taxonomically closely related species with *r*-strategist features.

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