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DIVERSITY PATTERNS AND STRESS RESISTANCE OF A MARINE NEMATODE COMMUNITY FROM A HETEROGENEOUS SANDY BEACH IN THE UPPER GULF OF CALIFORNIA, MEXICO

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PATRONES DE DIVERSIDAD Y RESISTENCIA AL ESTRÉS DE UNA COMUNIDAD DE NEMÁTODOS MARINOS DE UNA PLAYA ARENOSA HETEROGENEA EN EL ALTO GOLFO DE CALIFORNIA, MÉXICO

Resumen aprobado por:

_____

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Director de Tesis

Entre los problemas centrales que permanecen por resolver en ecología de comunidades destacan las causas que determinan el número de especies de una comunidad y las consecuencias de diferentes niveles de diversidad. En esta investigación doctoral sometimos a prueba hipótesis fundamentales acerca de estos dos temas. Como modelo de comunidad elegimos a los nemátodos de vida libre del intermareal de una playa arenosa heterogénea en el Alto Golfo de California, México; dicha playa se caracteriza por presentar barras de arena y canales paralelos a la costa. Los nemátodos de vida libre del intermareal son organismos modelo muy adecuados en el estudio de ecología de comunidades, ya que presentan una diversidad muy alta a pequeñas escalas.

La primera parte de esta investigación consiste en el estudio de la influencia de la heterogeneidad del hábitat en la estructura de la comunidad. Nuestros resultados muestran tres grandes grupos biológicos asociados a diferencias en el tamaño de grano y en la concentración de clorofila-*a* a lo largo del gradiente estudiado. Estos resultados resaltan la predominancia de los gradientes medio ambientales en el establecimiento de la zonación intermareal. Sin embargo los canales se caracterizan por presentar altos niveles de diversidad taxonómica y funcional, muchos géneros únicos, y la comunidad se diferencia de aquella que se encuentra en el límite del submareal. Este resultado resalta el papel que juegan los canales como micro-hábitats distintos. La nematofauna de la playa heterogénea fue más diversa que aquella de una playa cercana estructuralmente menos compleja, lo cual demuestra la importancia de los micro-hábitats en la evaluación de la biodiversidad. La segunda parte de esta investigación consistió en estudiar los patrones de distribución en dos micro-hábitats diferentes. Los resultados muestran que en los canales existe una gran heterogeneidad en la distribución espacial y que un mayor número de taxa se encuentra distribuido en forma de parches. Este patrón se debe posiblemente a la predominancia de un desplazamiento activo bajo condiciones tranquilas y por la cohesión del sedimento por parte de las algas. Los resultados demuestran que los regímenes

hidrodinámicos contrastantes en diferentes micro-hábitats influencían significativamente la distribución de los nemátodos, lo cual resulta en diferentes patrones espaciales en una misma playa.

La tercera parte de esta investigación consistió de un experimento de microcosmos para evaluar el papel de la diversidad en la resistencia de una comunidad al estrés. Nuestro sitio de estudio, el Golfo de California, un lugar de alta diversidad y endemismo, es extremamente vulnerable a futuros cambios de temperatura. A nosotros nos interesó saber si una comunidad de alta diversidad resiste mejor al estrés térmico como lo predice la Hipótesis del Seguro (IH por sus siglas en inglés). Nuestros resultados no están en concordancia con la IH, pero indican que cada especie contribuye al funcionamiento como lo sugiere el Modelo de Remache. Aunque las dos comunidades de alta y baja diversidad perdieron especies debido a la alta temperatura, la comunidad de alta diversidad sufrió un impacto más grande ya que perdió el grupo funcional de los depredadores y omnívoros, lo cual puede resultar en consecuencias importantes para la red trófica bentónica. La comunidad de baja diversidad consistió de un grupo original de especies resistentes a altas temperaturas, probablemente debido al hecho que se colectaron en una parte más expuesta de la playa. Esto indica que más que la diversidad en sí, la identidad de la especie es fundamental para la resistencia al estrés.

Palabras clave: biodiversidad, ecología de comunidades, nematodos marinos de vida libre, ecología de bentos, diversidad taxonómica y funcional, resistencia al estrés, Golfo de California

ABSTRACT of the thesis presented by **Ruth Gingold** as a partial requirement to obtain the DOCTOR OF SCIENCE degree in Marine Ecology. Ensenada, Baja California, Mexico. June 2010.

DIVERSITY PATTERNS AND STRESS RESISTANCE OF A MARINE NEMATODE COMMUNITY FROM A HETEROGENEOUS SANDY BEACH IN THE UPPER GULF OF CALIFORNIA, MEXICO

The causes that determine the number of species in a community and the consequences of different diversity levels remain among the unresolved central problems of community ecology. In this PhD research, we set out to test corner-stone hypotheses regarding these two fundamental subjects. As a model community we chose intertidal free-living nematodes of a heterogeneous sandy beach featuring intertidal runnels and sandbars in the Upper Gulf of California, Mexico. Intertidal free-living marine nematodes are very suitable model organisms for research in community ecology, as they are usually highly diverse on very small scales.

The first field study of this research addressed the question whether habitat heterogeneity influenced the community structure. Our results revealed a predominance of environmental gradients in establishing intertidal zonation. Three major faunal assemblages along the exposure gradient matched differences in mean grain size and chlorophyll *a*. However, runnels featured higher levels of taxonomic and functional diversity, many unique genera, and the community differed from the assemblage at the limit to the subtidal zone, stressing their role as distinct microhabitats. The nematofauna of the structurally complex beach was more diverse than the one from a structurally less complex beach nearby, highlighting the importance of microhabitats in the assessment of biodiversity.

The second field study addressed distribution patterns in the two different microhabitats. A more heterogeneous spatial distribution, and more patchily distributed taxa were found in the runnel, presumably owing to a predominance of active displacement under calmer conditions and sediment cohesion by algal films. The results show that different hydrodynamic regimes in contrasting intertidal microhabitats significantly influenced the nematofaunal distribution, resulting in different spatial patterns next of one another in the same beach.

The third part of this research consisted in a microcosm experiment evaluating the role of diversity in community stress resistance. Our study site, the Gulf of California, a marine biodiversity and endemism hotspot, is extremely vulnerable to future temperature changes. We were therefore interested whether high diversity would confer higher stress resistance to a community as predicted by the Insurance Hypothesis (IH). Our results do not support the IH but rather suggest that each species contributes to the functioning of the system according to the Rivets model. Although both, high and low diversity assemblages lost species due to the high temperature, the high diversity assemblage suffered the larger impact on ecosystem functioning by loosing the trophic group of large predators and omnivores, which may have important consequences for the benthic food web.

The low diversity assemblage consisted of an original species pool of stress-resistant species, presumably due to the fact that it stemmed from a more exposed part of the beach. This indicates, that species identity rather than diversity *per se* may play an important role for stress resistance.

Keywords: biodiversity, community ecology, free-living marine nematodes, benthic ecology, taxonomic and functional diversity, stress resistance, Gulf of California

Dedication:

To our planet Earth, the one and only we have,
and its endlessly beautiful nature,
whose majesty and misteries have always been the reason
for my inspiration and motivation.

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I can't believe it – I have arrived at the very end of my PhD!! Yeeii! :o) These 4.5 years have been a very important, unforgettable and pathbreaking part of my journey through life, and I would not have been able to bring it to such a good and satisfactory end without the help of many people. I would like to acknowledge some special persons who have contributed in one or many ways to this work.

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"There is a tide in the affairs of men. Which taken at the flood, leads on to fortune; Omitted, all the voyage of their life is bound in shallows and in miseries." Julius Caesar quote by William Shakespeare

“Es gibt Gezeiten auch für unser Tun. Nimmt man die Flut wahr, führet sie zum Glück; versäumt man sie, so muss die ganze Reise des Lebens sich durch Not und Klippen winden”

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Chapter I

General Introduction

I. 1. RATIONALE

I. 1. 1. Biodiversity and the community concept

The conservation of biodiversity has officially been recognized as an international priority since 1992, when the United Nations ratified the Convention of Biological Diversity (CBD[1]), which states: "(…) Determined to conserve and sustainably use biological diversity for the benefit of present and future generations, (…) the objectives of this Convention (…) are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, (…)". Although scientists had extensively studied biodiversity before, the newly gained international (political) prestige conferred to it a new importance and meaning. Almost two decades later, the United Nations declared 2010 the International Year of Biodiversity with the slogan "Biodiversity is life, biodiversity is *our* life". So, if biodiversity is our life – what makes it so important to earth – and to us? How is biodiversity generated and maintained? What are the potential benefits of biodiversity and its conservation? These questions, albeit apparently simple are still among the central topics of the scientific discipline called "Community Ecology" (Morin 1999). Given the current rapid loss of diversity (Dirzo & Raven 2003, Humphrey et al. 2008) there is an urgent need to understand these fundamental issues. The global importance of this "hot topic" combined with a personal genuine

[1] http://www.cbd.int/

interest and never ending admiration for the dazzling array of Earth's creatures, provided the background of interest to develop the present PhD thesis.

The term "biodiversity" is colloquially used as a synonym for the "number of species". Actually, a species assemblage interacting with its environment (in the broadest sense) represents a community, of which the seminal concept dates back to the early 20th century (Clements 1936, Gleason 1939). There are many definitions of community, broader ones like: "A biological community is a collection of organisms in their environment" (Emlen 1977), or: "The organisms that interact in a given area" (Price 1984), and more specific ones such as: " (…) an assemblage of populations of plants, animals, bacteria and fungi that live in an environment and interact with one another, forming together a distinctive living system with its own composition, structure, environmental relations, development, and function" (Whittaker 1975). Whilst being different, they all concur in that a community involves organisms from more than one species that interact with each other and their environment. As such, community ecology has to be placed between population ecology (which focuses mainly on single-species patterns and processes) and ecosystem ecology (which encompasses one or several communities in an abiotic environment, focusing on fluxes and cycles of components such as nutrients). The limits between these disciplines are fluent and – to some extent – artificial. One of the major problems of community ecology is that it is filled with single case studies, but lacks laws and robust generalizations (Loreau 2010). For example, in other research areas such as population genetics, well-established parameters such as effective population size (N_e, which is the number of breeding individuals) and allele frequencies, allow the test of null-models, granting it predictive power for all kind of species, from nematodes to elephants. Currently there is a new tendency to integrate community ecology, ecosystem ecology and evolutionary ecology into one discipline in order to establish powerful unifying theories and predictive models (Loreau 2010).

The properties to characterize a community are abundance (*i.e.*, the number of individuals), species richness (*i.e.*, the number of species), diversity (*i.e.*, relative

abundances of the different species), and – if possible – some measure of functional diversity. To assess species richness and diversity, the taxonomic knowledge of the species involved is fundamental. Ecology began as a descriptive science identifying and listing species, yet currently there are still less than 2% (1.8 million [IUCN]) species described of an estimated 100 million extant species (Blaxter 2003). Traditional taxonomy (*i.e.*, species description based on morphological characters) as a science faces a crisis, because it does not yield the credit of hypothesis driven science, indicated for example by the lower impact factor of the journals, which publish descriptions of new species. Although modern molecular and genetic techniques such as barcoding are useful complementary tools (De Ley et al. 2005, Bhadury et al. 2006, 2008) classical species descriptions are still vital to modern community ecology, because they also yield information about, *e.g.*, functional aspects that can be implied from morphological structures. To explore processes underlying community patterns, complete and accurate species lists are essential especially for small and microscopic organisms, as they are often used as experimental models.

I. 1. 2. Processes influencing communities

Different processes, operating on evolutionary and ecological timescales, influence the number and identity of species in communities (Fig. 1). The creation of new geological features, *e.g.*, by tectonic disruption (separation of a peninsula from the continent), is linked with evolutionary processes that determine the creation of the regional species pool. If geological processes create a physical barrier and/or new environmental conditions or gradients, it may lead to species extinction, or to speciation due to adaptation and genetic drift. The regional species pool is thus a result of the "evolutionary age" of the taxon as well as the geological history of the place. The local community consists of a fraction of the regional species pool. Either, organisms actively migrate and choose a suitable

habitat where they establish viable populations, or they are passively transported (*e.g.*, by the currents) and populations develop where they can survive long enough under suitable conditions. As soon as more than one species is present at a given place, ecological processes may take place, although in the absence of a physical barrier, speciation may also result from intraspecific competition leading to two different species that are adapted to use a given resource differently, or through mutants that either replace or co-exist with their parent form (*e.g.*, Loreau 2010).

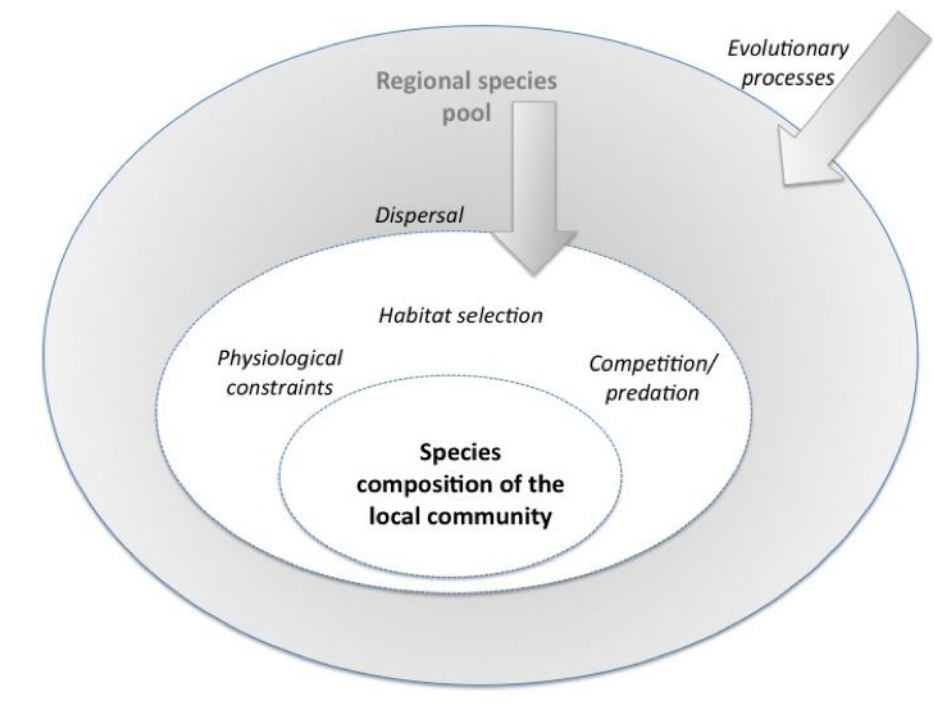

Figure 1. Processes influencing the species composition of a local community. Evolutionary processes such as speciation following geological changes determine the regional species pool. The local species composition reflects a subset of the regional species pool after being shaped by habitat selection. Modified from "Community Ecology" (Morin 1999).

Among other factors, physiological constraints (*e.g.*, temperature tolerance) will determine the potential habitat of a species. Further, inter-specific interactions such as competition and predation (biological interactions) add to the formation of the community. The limitation of resources is crucial, leading to manifold sorts of competition (*e.g.*, direct competitive behavior, allelopathy) and niche differentiation, since no two species can co-exist stably over time when competing for the same resource (Competitive Exclusion Hypothesis, Gause 1934). Based on this concept, each species occupies a different niche, *i.e.*, is "adapted to a multidimensional array of biological and abiotic parameters" (Hutchinson 1957), and plays a different role in a community.

I. 1. 3. Causes and consequences of diversity

The causes that determine the number of species in a community and the consequences of different diversity levels remain among the unresolved central problems of community ecology. For example one of the most striking diversity patterns remains essentially unexplained: the increase in species richness with decreasing latitude. At least ten different hypotheses have been suggested to explain this pattern (Pianka 1966, 1989). Among others, it has been suggested that higher diversity is due to higher productivity (Connell & Orias 1964), however, extensive research on this topic suggests a more complicated relationship, with highest diversity at intermediate productivity (Rosenzweig 1971, Huston 1994). In general, it seems most likely that the latitudinal patterns reflect interactions of several underlying mechanisms. However, several hypotheses have proven to be useful attempts to explain species richness at smaller scales. This is the case of the "Habitat Heterogeneity Hypothesis" (HHH) and the "Intermediate Disturbance Hypothesis" (IDH, Huston, 1979). The HHH states that increased spatial complexity leads to niche diversification allowing a higher number of species to co-exist (MacArthur & Wilson 1967). Again, as it often occurs in community ecology,

some studies found supporting evidence (Kerr & Packer 1997, Hauser et al. 2006), whereas others could not reject the null hypothesis of no difference between hetero- and homogeneous habitats (Davidowitz & Rosenzweig 1998, Cramer & Willig 2005). The IDH, on the other hand, posits that a stochastic, intermediate (partial) elimination of resources by disturbance leads to species-specific mortality. This allows for the co-existence of competitively inferior and/or functionally similar species leading to higher diversity (Huston, 1979). High and low disturbance, by contrast, would lead to competitive advantage of either opportunistic species with short generation times or low-diversity communities of highly specialized species respectively. Highest diversity is therefore to be found at places of high spatial and intermediate temporal heterogeneity. Again, observational and experimental studies do not provide consistent results (Aronson & Precht 1995, Floder & Sommer 1999, Huxham et al. 2000). Because of their strong theoretical background, and their debate due to the lack of uniform empirical results, these hypotheses still build cornerstones of modern ecology.

Exploring consequences of biodiversity inevitably entails crossing the (artificial) border between community and ecosystem ecology. Increasing evidence indicates that diversity has a significant positive effect on ecosystem functions. Two of the most important experimental studies on a large temporal and geographical scale showed that increased species richness yields higher primary production, the maintenance of higher diversity over time, higher overall stability, resistance to and recovery from stress (Tilman & Downing 1994, Tilman et al. 1997, Hector et al. 1999, van Ruijven & Berendse 2010). Similarly, microbial as well as various marine communities exhibit positive diversity – ecosystem functioning relationships (Naeem & Li 1997, McGrady-Steed et al. 1997, Wohl et al. 2004, Cardinale et al. 2006, Worm et al. 2006, Stachowicz et al. 2008). The Insurance Hypothesis is a theoretical model that predicts increased stress resistance with higher species richness (Yachi & Loreau 1999). Its concept is based on the functional overlap or redundancy of species, *i.e.*, if a community loses some species during stressful events, it maintains its ecosystem functionality

as other species remain to carry out the function of the lost species.

In order to explore community patterns and underlying processes, ecologists are often confronted with logistic obstacles, since many observed patterns are experimentally intractable because of the size and habitat range of the target species. Manipulative experiments on "model communities", logistically doable within a limited spatial and temporal frame, are one of the tools ecologists employ (Bulling et al. 2006). As mentioned above, many of the most important studies on the subject were undertaken on terrestrial grasslands, and although they have contributed substantially to the present knowledge, they suffer from the disadvantage of representing only one trophic level – primary producers. Further, they do not meet concerns about the rapid biodiversity loss in marine systems (Humphrey et al. 2008). Similar studies on marine communities are therefore still needed.

In this PhD, we aim at studying fundamental aspects of community ecology that are closely linked to the causes and consequences of diversity, undertaken on intertidal free-living nematodes of a sandy beach. Intertidal free-living marine nematodes are very suitable model organisms for research in community ecology, as they are usually highly diverse on very small scales (Heip et al. 1985, Lambshead 1993) and represent different trophic groups and levels (Wieser 1953, Moens & Vincx 1997, Moens et al. 2005, 2006). The small size of the organisms does not preclude their vast importance as nutrient recyclers, which makes them representative for an entire and fundamental part in the ecosystem of intertidal beaches. The knowledge about their trophic function and life cycle of some selected species have allowed to study fundamental ecological questions about, *e.g.*, colonizing processes (Gallucci et al. 2008), trophic interactions among- and within trophic levels (Moens et al. 2000, De Mesel et al. 2004, 2006) and physiological limits (Moens & Vincx 2000a, b). The research undertaken during this PhD contributes 1) to the understanding of possible mechanisms leading to the high diversity in intertidal ecosystems, and 2) to the understanding of potential consequences of diversity in light of a changing environment.

I. 2. BACKGROUND

I. 2. 1. Sandy beach ecology - Physical aspects and classification of sandy beaches

Sandy beaches cover about two thirds of the world's ice-free coastline, occurring across all latitudes and continents of the world (McLachlan & Brown 2006). They are formed at the interface between land and sea by sediment erosion and deposition by hydrodynamic forces, *i.e.*, tides and waves carry off sands during storms and move them onshore during calm conditions. Beaches are among the most variable, dynamic and resilient ecosystems. Their morphologic classification is based on the defining components: sediment, waves and tides.

Sediments originate from land and the sea. Quartz or silica sands are mainly transported by winds and water (rivers and runoff) from inland, whereas biogenic sediments (mainly consisting of carbonates from animal skeletons and corals) but also mineral fractions such as cliff erosion are washed onshore from the sea (McLachlan & Brown 2006). The most important feature of sediments is their grain size. It is usually expressed according to the Wentworth scale in Phi (Φ) units, the $-\log_2$ of the grain diameter (mm). Sediments of sandy beaches are usually between 0 and 4 Φ (*i.e.*, 1 – 0.0625 mm). Grain size determines porosity and permeability of sediments, the former being the total pore space in a given volume of sand, and the latter the rate of water flow through the sand. Both are important components for the biota as they determine chemical properties and gradients.

Waves transfer energy from the wind at sea to the coastal zone. When a wave approaches the coast, its speed decreases and it changes direction, aligning with the contours of the coastline. When they reach shallower sites, they break. The breaker height is an important variable determining the slope of a beach. The more exposed a beach, the higher the energy and the breaker height, and the steeper the slope of the beach. The more sheltered a beach, the lower the energy

and breaker height and the more gentle the beach slope. When wave energy becomes very high during storms, the resulting breakdown can cause damage on headlands and exposed coasts, therefore beaches are important buffer zones for coastal villages and cities.

Tides are the third important component for beach morphology. Tides are periodical rises and falls of the sea level, generated by the gravitational force of the moon and the sun on the oceans. In most places the sea level rises and falls twice a day (two high and two low tides). In one lunar cycle (27.5 days), two extremely high low tides occur (spring tide and neap tide). The tidal range combined with the wave regime has an important influence on the shape of the beach. The tidal sea level rise can be less pronounced than wave action, leading to beaches that are mainly governed by waves (microtidal beaches). However, it can also exceed the waves, in beaches governed mainly by tidal energy (macrotidal beaches). The three components – sediments, waves and tides – are the main factors responsible for the physical and geomorphological beach processes.

Masselink & Short (1993) designed a conceptual beach model based on three main factors: sediments, waves and tides (Fig. 2). It consists of the relative tide range (RTR) on one hand and on the dimensionless fall velocity or Dean's parameter (Ω) on the other. RTR is calculated as the ratio of the mean springtide range (MSR) over the wave breaker height (H_b). A low RTR means that the beach is wave dominated (*i.e.*, microtidal), whereas tide dominated (*i.e.*, macrotidal) beaches have a high RTR. Ω is calculated as $H_b / w_S T$, where w_S is the sediment's fall velocity, which is linearly dependent on grain size (Stoke's law); H_b is the wave breaker height and T the wave period. A low value of Ω indicates a steep beach slope, due to high hydrodynamic energy, characterized by coarse sand ("reflective beach"). A high value of Ω indicates a gentle slope due to low energy, and fine sand ("dissipative beach").

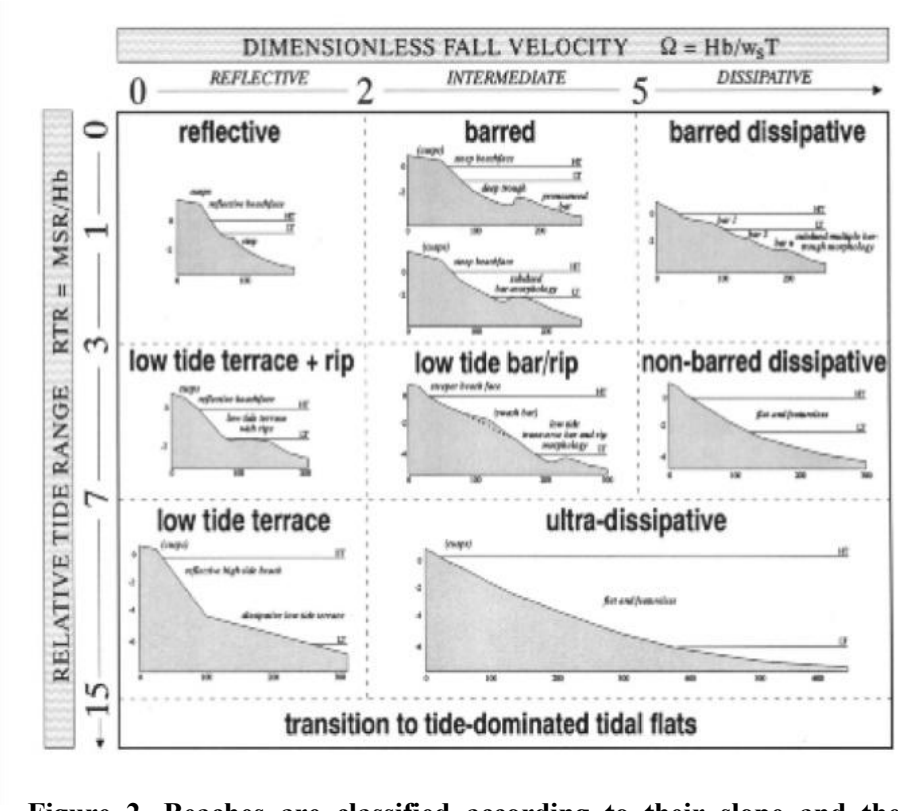

Figure 2. Beaches are classified according to their slope and the relative influence from tides and waves (© Masselink & Short 1993).

Intermediate beaches with high (>1m) tidal amplitude tend to have a complex morphology exhibiting sandbars, runnels, terraces, and other heterogeneous forms. The origin, formation and stability of these forms are only partly understood. Intertidal bars in general have not received much attention so far. There are three main forms of intertidal sandbars: "slip-face bars", "low–amplitude ridges", and "sand waves" differing in their steepness and height (Masselink et al. 2006); "Slip-face bars" are the most pronounced and "sand waves" the least noticeable features. "Low-amplitude ridges" are in between these two extremes, and are characteristic of intermediate beaches with intermediate wave energy and macrotidal regimes (Masselink & Short 1993, Masselink et al. 2006). One sandbar and its respective runnel are called an "intertidal bar system" (Masselink et al. 2006).

Our study site *El Tornillal* in the Upper Gulf of California, is a dissipative ridge-and-runnel beach, featuring "low-amplitude ridges". At our sampling site, sandbars lay almost parallel to the coastline, whereas further southeastward they bend toward the sea (Fig. 3a). The sampling transect for our first study (white bar in Fig. 3a) included four intertidal bar systems, and ranges from the lower limit of low spring tide to the upper limit of high spring tide (Fig. 3b). The sandbars are massive and around 100 m wide (Fig. 3b and c), whereas the runnels are narrow channels connected to the sea (Fig. 3b and d). Similar beaches are found on the northern European coast, *e.g.*, in Belgium (Gheskiere et al. 2004) and France (Anthony et al. 2005). The extent to which these morphological features influence the composition of the inhabiting meiobenthic community has remained unexplored.

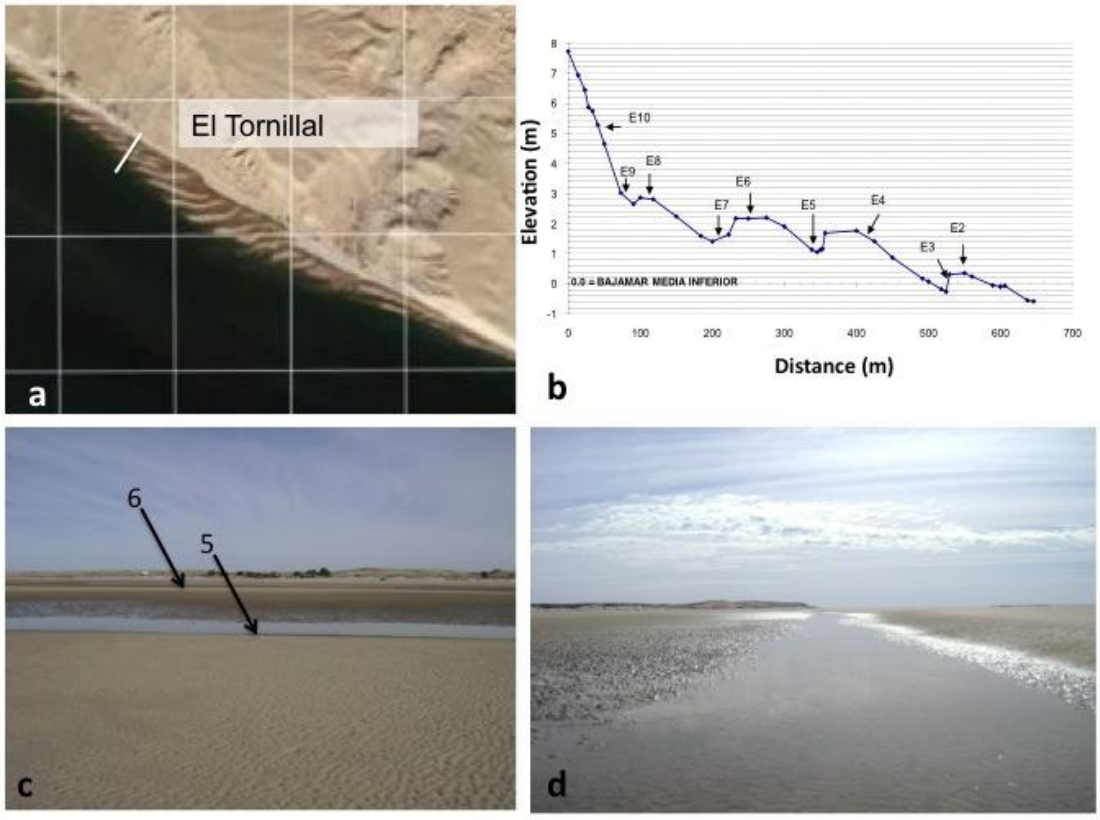

Figure 3: Beach morphology at the study site "El Tornillal" in the Upper Gulf of California. a) High resolution satellite image showing the intertidal bars at the coastline and the approximate position of the sampling transect of the first study for this research and for the calculation of the beach profile. b) Beach profile from the lower limit of low spring tide to the upper limit of high spring tide. Numbers indicate the sampling stations. c) View from station 4 (sandbar) toward the mainland. Arrows indicate positions of the runnel station 5 and the sandbar station 6. d) View from runnel station 5 towards east. The pictures were taken at low tide.

I. 2. 2. Threats for sandy beaches

Different kinds of disturbance, such as sea level rise, increased storm frequency and intensity, as well as direct anthropogenic impacts such as pollution, uncontrolled urban development, physical disturbance induced by recreational activities, affect sandy beaches all over the planet (Brown & McLachlan 2002).

Enhanced storm frequency and intensity, and uncontrolled urban development are among the main causes of beach morphological changes (Fig. 4a). Storms will be especially problematic in the future, as sea level rise will compound their impact. Numerical models indicate that on complex-shaped coastlines, the changing storm- and wave patterns may induce variation in shoreline retreat rates an order of magnitude higher than the baseline retreat rates expected from sea-level rise alone (Slott et al. 2006). Approximately 70% of the beaches worldwide are subject to erosion and receding (Schlacher et al. 2008). Increasing sea level at a rate of 2 mm per year and more severe storms will induce coastal flooding (Miller & Douglas 2006). Increased flood risk will cause not only environmental damage, but also significant economic challenges (Hall et al. 2006). Pollution resulting from agricultural and urban run-off causes severe impacts in terms of eutrophication (Paez-Osuna et al. 1999, Glibert et al. 2006). Chronic oil pollution occurs at beaches near oil platforms and terminals. Toxic components (*e.g.*, polycyclic aromatic hydrocarbons [PAH]) induce mortality of benthic fauna and residues clog delicate filter feeding organisms (Brown & McLachlan 2002). Rapid human population growth results in uncontrolled urban development leading sand transport disruption and erosion (Lizarraga-Arciniega et al. 2001, Brown & McLachlan 2002). Side effects of touristic activities at the beach, such as trampling, littering and the impact of off-road-vehicles induce additional erosion, pollution and mortality of the fauna (Gormsen 1997, Gheskiere et al. 2005).

Figure 4: Threats for sandy beaches: (a) Increased storm frequency and intensity, (b) Unsustainable urban development, (c) Inorganic waste, littered by people indulging leisure time at the beach or washed onshore by tides and waves, (d) industrial pollution by heavy metals and crude oil, and (e) physical disturbance by off-road vehicles

In addition to local threats, an exponential increase in green house gas emissions by anthropogenic activities induces a global climate change, submitting ecosystems to additional and combined environmental stress. The International Panel of Climate Change (IPCC) has identified climate change as "(…) changes in the mean and/or variability of its properties, and that persist for an extended period, typically decades or longer". One of the main consequences of climate change that is expected in the future is a significant increase of average sea and air temperature (IPCC 2007). Prospective models predict an average increase of global average sea surface temperature between 1 and 6.4˚C in the next 90 years (IPCC 2007). Our study site, the Gulf of California, recognized as a marine biodiversity and endemism hotspot (Roberts et al. 2002) is extremely vulnerable to future temperature changes, as temperature has risen about 8˚C over the past

century (Julliet-Leclerc et al. 1991). Moreover, the northern part of the Gulf has been converted to an inverse estuary (Lavín et al. 1998) since numerous dams in the US preclude fresh water discharge of the Colorado River. These changes have had a significant impact on the flora and fauna in general, and especially on benthic organisms (Rodriguez et al. 2001, Stillman 2003).

Temperature is fundamental for organisms, as it directly influences metabolism, respiration (Moens & Vincx 2000a, Hubas et al. 2007a) and population growth (Moens & Vincx 2000b). Also it has indirect effects by affecting other components of the environment, for example by altering bacterial growth (Hubas et al. 2007b, Hoikkala et al. 2009). An individual organism has basically two possibilities to react to temperature changes: avoidance or adaptation. If the species is highly motile, it can move away from suboptimal temperatures; these changes occur on ecological time scales and may lead to shifts in migration patterns, biogeographical shifts of populations as well as range extensions and contractions (Lehikoinen et al. 2004; Ford & Chintala 2006; Helmuth et al. 2006). If a species has limited movement capacity, it must adapt to the new conditions in order to survive. As an immediate response, this usually happens at the expense of other metabolic functions. This, in turn, bears the consequence that species already impacted by and adapted to higher temperatures, may be even more vulnerable to future changes (Stillman 2003). In the long term, selection will favor physiological adaptations, which then become fixed by genetic modifications. This occurs over generations on an evolutionary time scale. If the population that is forced to move or physiologically adapt does not have the capacity to do so (either because there is no other optimal place close enough or because of physiological limits) this can lead to population extirpation and eventually to species extinction. Climate change is among the main factors responsible for the past species decline (Jokiel & Brown 2004, Walker et al. 2006, Waycott et al. 2009, Brierley & Kingsford 2009, Gedan & Bertness 2009).

Given the variety of environmental factors that influence life, and given that changes in one or several of these factors may lead to complex, synergistic effects

that are difficult to trace, explain and predict (Harley et al. 2006), science faces a multidimensional challenge. The uncertainty of future environmental alterations demands the creation of integrative models combining theoretical, experimental and empirical-observational approaches in order to elucidate the role of biodiversity in the resistance to stressful conditions.

I. 2. 3. Benthic fauna

I. 2. 3. 1. Size classes and trophic links

Beaches are vivid ecosystems acting as buffer zones between the terrestrial and marine biomes, and host a high diversity of species. Benthic fauna has been classified according to size (Fig. 5): macrofauna (retained by a sieve of 1mm mesh size, *i.e.*, > 1mm in size), meiofauna (passing 1 mm, retained by mesh size of 45µm) and microfauna (all organisms passing through a 45 µm mesh). Most of the benthic infauna (mostly invertebrates) resides permanently in the sediments. On the other hand, larger vertebrates may use the beach environments for brief periods of their life cycle only, or almost permanently. Sea turtles, for example, use the beach to nest during the breeding season but spend the rest of the year at sea, whereas shore birds live permanently at the beach and adjacent dunes. They feed on invertebrates close to the waterline, and roost and breed in the shelter of the dunes. The (trophic) interactions among the micro-, meio-, and macrofauna result in a complex network (Fig. 6).

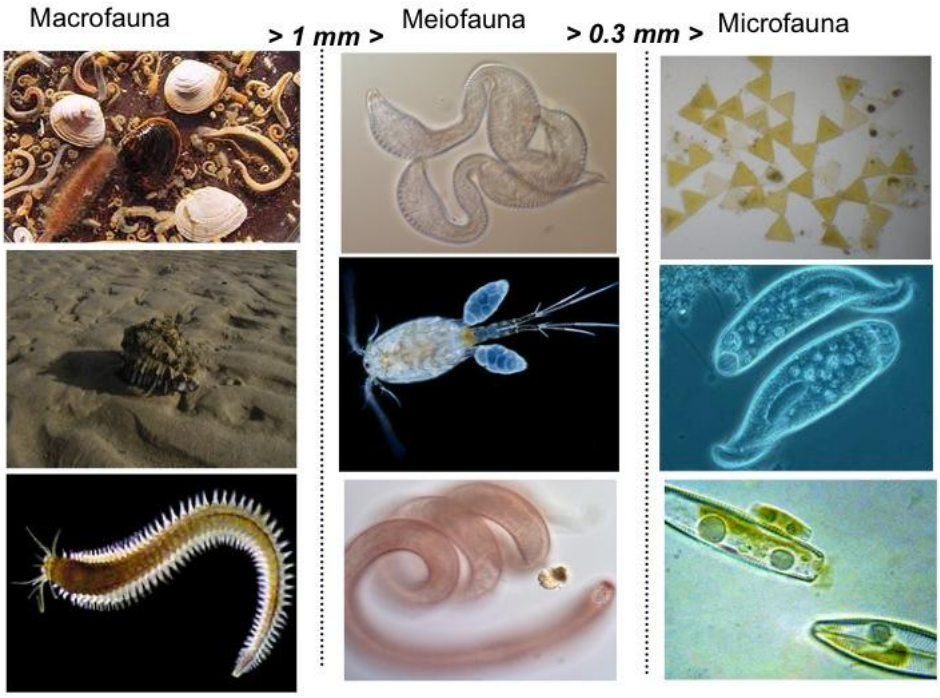

Figure 5: Beach biota is classified into three groups of different size: The macrofauna, which includes all organisms retained by a 1 mm sieve (examples: mollusks, polychaetes), the meiofauna, which includes organisms passing the 1 mm, retained by a 45 µm sieve (examples: nematodes and copepods), and the microfauna, including all organisms smaller than 45 µm (examples: diatoms, flagellates)

The complexity and strength among trophic guilds depend on the physical environment and thus on the beach type. On exposed beaches, the macrofauna is part of a larger, separate food web, whereas the interstitial fauna forms a discrete food web in the sand (McLachlan & Brown 2006). On sheltered beaches, however, there are significant links between the macro- and micro-benthic food webs. Food input at sheltered, wide beaches comes from three main sources: 1) the sea in the form of carrion, particulate and dissolved organic matter, 2) the beach itself in form of dead and live benthic microflora and –fauna and 3) the dunes, in the form of organic detritus (Fig. 6). In its simplest form, the microbenthic food web consists of three parts: bacteria, protozoan and meiofauna. Bacteria feed on exogenous

particulate and dissolved organic matter (POM and DOM respectively) carried in by seawater or generated *in situ* (recycled) by members of the microscopic food web on the beach. Protozoa, *i.e.* small, unicellular organisms prey on bacteria, and members of the meiofauna prey on them, as well as on bacteria directly (Fig. 6). Meiofaunal organisms also feed on phytobenthos on sheltered beaches, where they can grow due to their longer residence time. This microscopic food web represents an important food resource for the macrofauna.

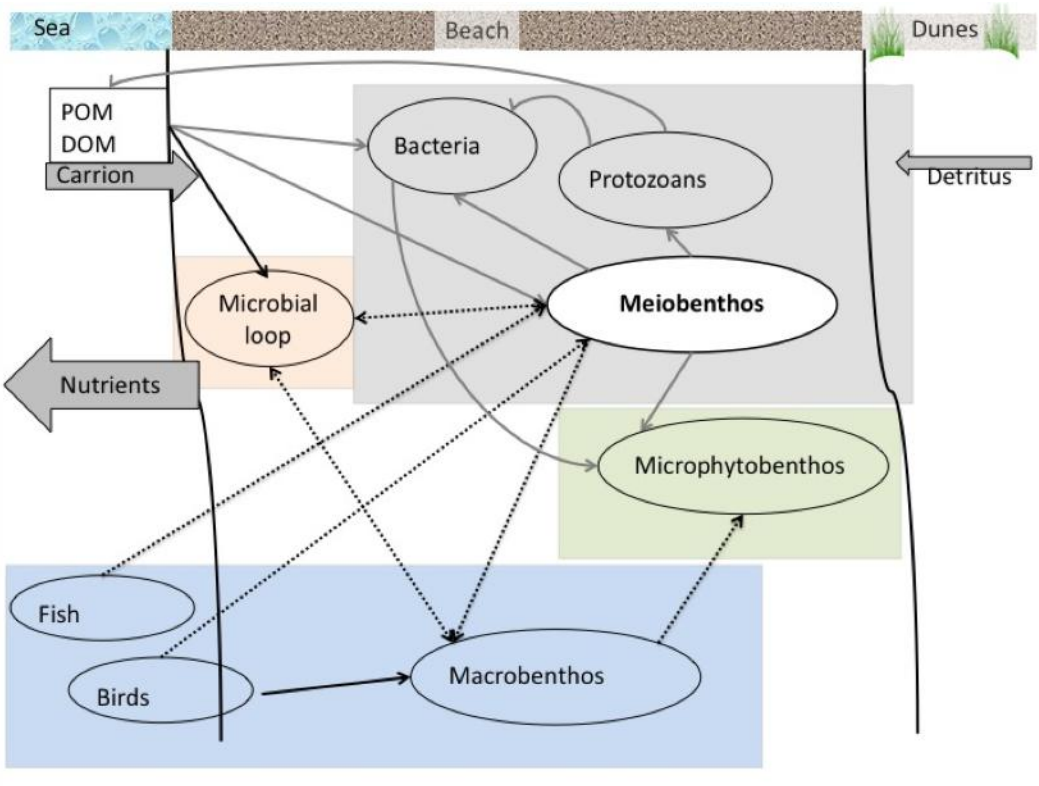

Figure 6: Schematic beach food web, consisting of four main components: the microscopic heterotrophs (grey), primary production (green), the macrofauna (blue) and the microbial loop (orange). The microscopic food web consists of bacteria, protozoan and meiobenthic organisms, linked to the autotrophic microphytobenthos (grey arrows). The macrobenthos feeds on organisms of the microbenthic web (black arrows), although it is not always clear, to which extent the two components are linked (dashed arrows). The microbial loop forms an additional component being linked to the micro- as well as the macrofaunal food web.

Meiofauna plays a key role as a trophic link between the micro- and macrobenthos and has an important impact in the carbon flow through the benthic food web (Li et al. 1997). Free-living marine nematodes, usually the bulk part (in abundance and diversity) of meiofauna, feed on the microbenthic organisms like for example ciliates (Hamels et al. 2001). They themselves serve as food for larger organisms of higher trophic levels (Hamerlynck & Vanreusel 1993, Coull et al. 1995). The interstitial system is essential for nutrient recycling. The pathway from POM and DOM to bacteria and then to meiofauna forms a major mineralization mechanism. Laboratory experiments have shown that 35 to 100% of organic nitrogen is mineralized by the interstitial biota (McLachlan & Brown 2006) and are washed back to the sea. Therefore, beaches are important filtering systems and play a significant role in global nutrient recycling.

I. 2. 3. 2. Free–living marine nematodes

Free-living nematodes are the most abundant metazoans on earth. Their importance and omnipresence is maybe best expressed by a citation from Cobb (1914), one of the pioneers of nematology: "If all matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, valleys, rivers, lakes and oceans represented by a film of nematodes". Nematodes uasually dominate the meiofauna in abundance as well as diversity in inter- and subtidal regions (Lambshead 1993, Heip et al. 1985). Their densities vary between 10^5 and 10^7 individuals per m^2 (Heip et al. 1985). It has been estimated that around one million species might exist, and only about 4000 have been described, most of them from northern Europe (Lambshead 1993). In extreme environments such as the deep sea, we are only about to discover an unexpected high diversity, leading to the hypothesis that nematodes maybe a hyperdiverse taxon (Lambshead 1993, but see Lambshead & Boucher 2003).

Given their high functional diversity, marine nematodes have been classified in many ways. The first and still widely applied categorization is Wieser's trophic groups (Wieser, 1953). It involves four groups (1A, 1B, 2A, 2B) based on the buccal morphology. Groups 1A and 1B represent selective and unselective deposit and bacteria feeders with unarmed small and large buccal cavities respectively. Groups 2A and 2B have small and large armed buccal cavities respectively, representing epistrate feeders and predators/omnivores. Deposit and epistrate feeding species ingest their food differently, the former ingest their prey entire and the latter break the cells with their teeth and suck out the cell contents (Jensen 1987). Although the subdivision of group 1 in selective (A) and non-selective (B) deposit feeders has been criticized and suggested to be omitted (Jensen, 1987) it has found empirical support (Moens & Vincx 1997). Moens & Vincx (1997) suggested a further subdivision of the group 2B in facultative and true predators. Based on their observations on live nematodes, they proposed six feeding guilds in total: two predator groups, microvores, ciliate feeders, deposit feeders and epigrowth feeders (Moens & Vincx 1997). This classification, however, is not widely applied, mainly due to the limited number of included species. Although Wieser's classification system has continuously been modified and improved, it is still the most widely used.

I. 3. HYPOTHESES AND OBJECTIVES

This doctoral research was conducted on a sandy beach in the Upper Gulf of California (for more detailed description see "Background"). Our model organisms were the free-living marine nematodes of this beach. Based on the conceptual framework of community ecology (elaborated under "rationale"), the hypotheses of this research were the following:

I. Given that each species is adapted (and limited) to an array of environmental conditions, we hypothesize that the environmental conditions of different micro-habitats (*e.g.*, runnels and bars) of a sandy beach influence the taxonomic and functional composition of the inhabiting community (*i.e.*, that the species assemblages of runnels and sandbars are different). Based on the HHH, we hypothesize that the environmental heterogeneity will lead to higher species richness compared to a geographically close, but less structurally complex beach.

II. Given that physical conditions create a cross-shore gradient with extreme conditions at each end, and intermediate conditions in the middle, we hypothesize that, according to the IDH, species richness will be highest around the mid-intertidal.

III. In view of the fact that local species richness is influenced by dispersal and the capacity to actively choose a habitat, we hypothesized, that differing hydrodynamic regimes in both micro-habitats (*i.e.*, runnels and sandbars) would lead to different distribution patterns.

IV. Provided that ecosystem functions are influenced by species diversity, we hypothesize that a community of high diversity maintains its functionality in the face of environmental stress in comparison with a low diversity community.

The general objective of this PhD was to test corner-stone hypotheses in community ecology related to the causes and consequences of biodiversity, using the model community of free-living marine nematodes.

The specific objectives were manifold. The first was to relate the attributes of the nematode community of *El Tornillal* (*i.e.*, abundance, taxonomic and functional diversity) to environmental parameters (*i.e.*, sediment grain size, organic matter and chlorophyll as a proxy for microphytobenthos) across shore and between runnels and sandbars. The comparison of nematode species richness and

community composition at *El Tornillal* with those found in a geographically close, structurally less complex beach was the second objective. The third objective was the assessment of the spatial aggregation patterns of a) the community and b) each single species in contrasting micro-habitats (runnels and sandbars). The fourth objective was to compare the response of nematode communities with different diversity to thermal stress.

I. 4. OUTLINE

After the general introduction, which includes the rationale and background of this research (**Chapter I**), the thesis is divided in two sections. The first consists of two chapters (Chapters II and III). **Chapter II** includes a study of the influence of habitat heterogeneity (in the form of intertidal runnels and sandbars) on the nematofaunal community composition. In particular, we studied the effect on zonation patterns and on local and regional diversity. This chapter has been accepted for publication as: Gingold R., Mundo-Ocampo M., Holovachov O. and Rocha-Olivares A. (2010) "The role of habitat heterogeneity in structuring the community of intertidal free-living marine nematodes" in the journal *Marine Biology*. In **Chapter III** we analyze the spatial coherence of the nematofaunal community resulting from microhabitat variation. Specifically, we expected the hydrodynamically less energetic habitat (runnels) to harbor a community exhibiting a patchier distribution, whereas the community of the more energetic sandbars was expected to be more spatially homogeneous. This chapter has been accepted for publication in the *Journal of the Marine Biological Association of the United Kingdom* as: Gingold R., Ibarra Obando S. E. and Rocha-Olivares A. (2010) "Spatial aggregation patterns of free-living marine nematodes in contrasting sandy beach micro-habitats". The second section of the thesis, consisting of one chapter (**Chapter IV**), refers to a microcosm study investigating the role of diversity in stress-resistance. Specifically, we set out to test the hypothesis of functional

redundancy conveying stress resistance to a community of marine nematodes. We achieved that goal by exposing intertidal nematode communities of differing diversities to thermal stress in microcosm setups. This study will be presented in July 2010 at the 14th International Meiofauna Conference in Ghent, Belgium, and then submitted for publication as: Gingold R., Moens T. and Rocha-Olivares A. "Is high diversity an insurance against thermal stress? Assessing the response of a meiofaunal community in a microcosm experiment". Finally, an inclusive general discussion and an outlook on future research are presented in **Chapter V**. The cited literature for all chapters is listed at the end of the thesis (**VI. References**). **Appendix 1** lists supplementary material that has been generated for online publication, while **Appendix 2** represents a short CV and the publication list of the author.

Chapter II

The role of habitat heterogeneity in structuring the community of intertidal free-living marine nematodes

II. 1. ABSTRACT

The role of habitat complexity has been widely neglected in the study of meiofaunal community patterns. We studied the intertidal nematode community of a structurally complex macrotidal beach exhibiting contrasting microhabitats (sandbars and runnels) to understand the influence of environmental gradients and habitat heterogeneity in the community structure. We tested whether topographical complexity affected (1) the zonation pattern in terms of abundance and diversity, and (2) local diversity by promoting compartmentalization into distinct faunal groups. Our analyses revealed three major faunal assemblages along the exposure gradient associated to differences in mean grain size and chlorophyll *a*. Diversity patterns involved a mid-intertidal peak, consistent with the intermediate disturbance hypothesis, and another peak at the limit with the subtidal region, consistent with the transition zone. These results highlight the predominance of environmental gradients in establishing intertidal zonation. However, microhabitats differed in environmental conditions and possessed significantly distinct nematofaunal communities. Runnels featured higher levels of taxonomic and functional diversity, many unique genera, and the community differed from the assemblage at the limit to the subtidal, stressing their role as distinct microhabitats. The nematofauna of the structurally complex beach was more diverse than the one from a homogeneous beach nearby, supporting the hypothesis that structural heterogeneity promotes diversity by compartmentalization and highlighting the importance of microhabitats in the assessment of biodiversity. Contrary to previous

predictions, our results indicate potentially high regional marine nematode diversity in the Upper Gulf of California.

II. 2. INTRODUCTION

The intermediate disturbance hypothesis (IDH), the dynamic equilibrium hypothesis (DEH), and the habitat heterogeneity hypothesis (HHH), are building blocks of modern community ecology and relate ecological processes to the generation and maintenance of diversity and community functioning. The IDH posits that species diversity will be maximal in habitats subject to intermediate levels of disturbance because stochastic, intermediate (partial) elimination of resources by disturbance leads to species-specific mortality allowing the co-existence of competitively inferior species (Huston 1979). Moreover, according to the DEH, the combination of intermediate disturbance with intermediate productivity levels, predicts a peak in species richness due not only to periodic decreases of competitively dominant species but also to increased niche packing (Huston 1994). Finally, the HHH states that structurally complex environments provide more niches thereby increasing species diversity (MacArthur & Wilson 1967). The majority of empirical studies find increased diversity at intermediate disturbance (Aronson & Precht 1995, Flöder & Sommer 1999; but see Huxham et al. 2000) and a positive relationship between habitat complexity and species diversity (Kerr & Packer 1997, Davidowitz & Rosenzweig 1998, French & Picozzi 2002, Tews et al. 2004, Hendrickx et al. 2007, but see Cramer & Willig 2005). Intertidal sandy beaches may appear homogeneous, but horizontal and vertical physical, chemical, and biological gradients create a spatially and temporally heterogeneous environment for the inhabiting fauna (Rodil et al. 2006). Swash and surf processes, tidal exposure (submergence) as well as related chemical gradients dominate the environmentally extreme upper and lower beaches, respectively, whereas the combination of those factors creates an environment of intermediate disturbance in the mid-intertidal (McLachlan & Brown 2006). Whereas

species richness and abundance tend to increase toward the lower intertidal in macrofauna (*i.e.* organisms retained by a sieve of 1mm pore size), since their feeding activity is directly dependent on tidal submergence (McLachlan & Jaramillo 1995, Armonies & Reise 2000), meiofauna (*i.e.* organisms passing through a 1-mm mesh and retained by a 0.04-mm mesh) exhibit a peak in species richness around the mid-intertidal. Meiofauna is usually dominated by the highly diverse free-living marine nematodes (Lambshead 1993), and is more likely to respond to the three-dimensional complex interaction between chemical, physical, and biological factors rather than to any single factor or process alone (Rodriguez et al. 2001). Cross-shore meiofaunal distribution patterns consist in species assemblages corresponding to different intertidal levels (Rodriguez 2004, Gheskiere et al. 2004, 2005). Although diversity patterns do not always parallel abundance, both tend to increase with increasing distance from the waterline, often with a peak around the mid-intertidal (Armonies & Reise 2000, Rodriguez et al. 2001, Gheskiere et al. 2004). The mid-intertidal maxima in species richness have been attributed to optimal combinations of physical and chemical conditions (Armonies & Reise 2000; Gheskiere et al. 2004). Environmental gradients as well as individual environmental factors have received foremost attention in our understanding of intertidal community patterns, whereas the role of habitat complexity has been widely neglected or even avoided (Gheskiere et al. 2004; Mundo-Ocampo et al. 2007). However, complexity is the hallmark of some beach types, such as macrotidal (*i.e.* tide governed) or intermediate beaches featuring tidal sandbars and runnels providing additional temporal and spatial heterogeneity. On dissipative macrotidal ridge-and-runnel beaches (*sensu* Masselink & Short 1993), such as the one studied here, several intertidal bar systems are located parallel to the shoreline (Masselink et al. 2006). Runnels are less exposed than the intervening sandbars, since they remain partially submerged during low tide and partly protected against cross-shore currents. Consequently, environmental factors such as humidity, temperature, sediment characteristics, and organic matter content may differ considerably between runnels and sandbars. As a result,

morphodynamically complex beaches have been hypothesized to harbor higher species diversity than other beach types (McLachlan & Turner 1994). The role of local structural complexity in determining meiofaunal community structure, in general, and of tidal sandbars and runnels, in particular, remains unexplored.

In this study, we test relevant hypotheses about the role of environmental gradients and habitat heterogeneity in the community structure of meiobenthic marine nematodes. First, we tested whether the topographical complexity of a beach affects the general community structure in terms of zonation and abundance/diversity patterns. In particular, we predict that (1) the prevalent tidal regime exerts a major influence on the community, leading to distinct faunal assemblages associated to their tidal level, and (2) diversity patterns are consistent with the IDH and DEH, exhibiting a peak in diversity around the mid-intertidal level. Second, we address for the first time the issue of whether beach structural complexity leads to higher nematofaunal diversity. In particular, we predict that (3) runnels and sandbars feature distinct environmental conditions and function as microhabitats harboring distinct communities, and (4) in accordance with the HHH, a morphologically heterogeneous beach will harbor a more diverse fauna than a structurally less complex beach.

II. 3. MATERIAL AND METHODS

II. 3. 1. Study site and sampling design

El Tornillal beach is a pristine beach located far from direct urban sewage outfalls or industrial and agricultural runoff in the northern Gulf of California in the Biosphere Reserve of the Upper Gulf of California (UGC) and Colorado River Delta (31°33ʾN, 114°17ʾW; Fig. 7). The Gulf of California is a marine biodiversity hotspot worldwide and one of the greatest reservoirs of marine species (Enríquez-Andrade et al. 2005). Morphodynamically, *El Tornillal* is a dissipative macrotidal ridge-and-

runnel beach (*sensu* Masselink & Short 1993), featuring an intertidal area >600-m wide and a tidal range reaching 7 m during spring tides (Lluch-Cota et al. 2007). Intertidal runnels are oriented almost parallel to the waterline retaining seawater during low tide. The temporal and spatial dynamics of sandbars and runnels have not been studied in the Gulf of California. However, in topographically similar beaches in northern Europe, sandbars tend to remain stationary across shore for up to 17 months (King 1972), whereas longshore currents as well as tidal and wave action cause longshore migration of medium-sized bedforms (<100m) due to advection and re-suspension of sand (Anthony et al. 2005). Sea surface temperatures are 30–32°C from June to September and range from 16 to 18°C from November to April. Southeastern currents and relatively low productivity prevail during summer and the pattern reverses with northwestern currents and high productivity during winter (Lluch-Cota et al. 2007). No macroalgal wrack deposits are found across the beach.

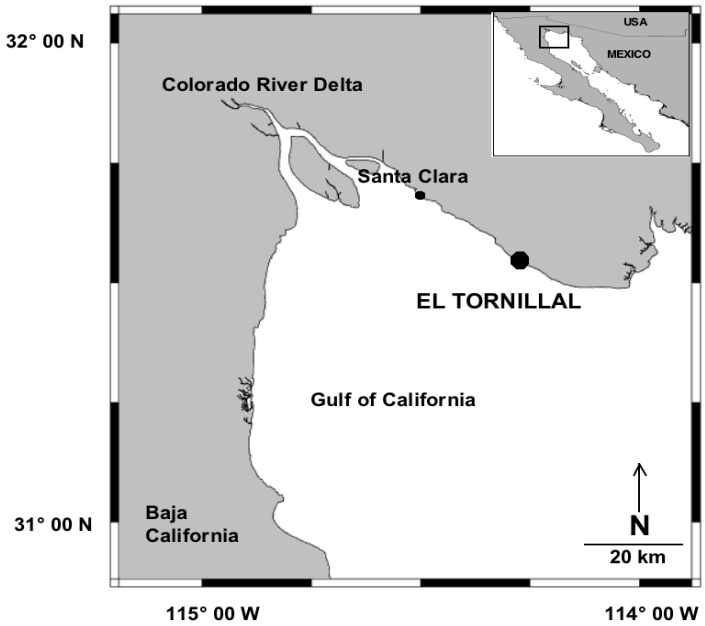

Figure 7. Location of the study site (El Tornillal beach) on the east coast of the Upper Gulf of California, state of Sonora, Mexico

Sampling took place in contrasting seasons: September (27/09/07) and March (08/03/08), during the highest spring tide of the month. For each season, ten stations were placed along a transect perpendicular to the shore ranging from the waterline (station 1) to the high tide mark (station 10). Stations were placed such that they alternated runnels (odd-numbered) and sandbars (even-numbered; Fig. 8a). The locations of sampling stations between seasons were at most 100m away from each other, as indicated by their GPS position. At each station, sediment cores were taken at random within a $1m^2$ area using a PVC corer. Triplicate samples were taken for each of the following analyses (1) meiofauna, (2) granulometry, (3) organic matter, and (4) microphytobenthos. Core size for meiofauna, granulometry and organic matter was 9.8-cm long by 2.9 cm in diameter, and for microphytobenthos 1-cm long by 1cm in diameter. Samples for meiofaunal analyses were fixed immediately in 5% formaldehyde. Organic matter samples were kept under ice in the field, and then frozen at -20°C until processed. Chlorophyll samples were kept in dark tubes under ice in the field, and then stored at -40°C until processed.

II. 3. 2. Faunal analyses

In the laboratory, formalin was rinsed off sediment samples with freshwater using a 45-µm sieve. Bulk extractions of meiofauna from the sediment cores involved suspension in colloidal silica (LUDOX™, specific density 1.15) following De Jonge & Bouwman (1977). Extracted organisms were stored in 5% formalin and five aliquots of 5ml (25 ml in total per sample) were used to quantify nematodes using a counting dish under a Leica Zoom 2000 stereoscope. Nematode density (ind. $10cm^{-2}$) in each core was calculated by the mean abundance of the five aliquots and extrapolated to total abundance based on the fraction of the volume of each aliquot relative to that of the fixed bulk extraction, which varied between 25 and 35%. Aliquots were transferred to a 5% glycerol solution and slowly evaporated on a heating plate. The first 50 randomly picked

nematodes were mounted on permanent slides for identification. Nematodes were identified to the generic level when possible, using both pictorial (Platt & Warwick 1983, 1988; Warwick et al. 1998) and online (http://nemamex.ucr.edu) taxonomic keys with an OLYMPUS BX51 compound microscope with differential interference contrast optics. In cases where generic identification was not possible (*e.g.*, for juveniles or females lacking unequivocal male counterparts) specimens were identified to family level and included in statistical analyses as such. If more than one species could be distinguished among congeners, they were labeled sp1, sp2, and treated separately in statistical analyses, except in calculations of genus richness.

II. 3. 3. Habitat characterization

Granulometric analyses included first treating samples with 30% peroxide (H_2O_2) to oxidize organic matter. After rinsing gently with distilled water and drying at 60°C they were sieved through a stack of Wentworth grade sieves and the dry weight of each fraction was obtained (Bale & Kenny 2005). Mean grain size was calculated as Φ ($-\log_2$ [grain diameter]) with the program SysGran 2.4. Organic matter content was determined after treating samples with 10% HCl to dissolve inorganic carbonates (mainly $CaCO_3$), rinsing them thoroughly with fresh water, freeze-drying and then combusting them at 550°C for 24h (Dean 1974, Froelich 1980). Organic matter was computed as the difference in dry weight before and after combustion and standardized to percentage of total dry weight before combustion. Phytobenthic chlorophyll was extracted by grinding sediment samples in 90% acetone, extracting for 24h in the dark and then centrifuging at 3,000 rpm for 10 min. Absorbance of the supernatant was measured at 665 and 750nm before and after acidification with a few drops of 10% HCl (Spectrophotometer Ely-2000, Elyptica, Ensenada, BC, Mexico). Chlorophyll density was calculated following Lorenzen (1967) and Colijn and Dijkema (1981) and expressed as mgm^{-2}.

II. 3. 4. Data analyses

To understand the faunal and environmental spatial structure across the intertidal zone (*i.e.* the grouping of similar samples), cluster analysis and non-metric multidimensional scaling (MDS) were applied to similarity and distance matrices. Faunal analyses were carried out with Bray-Curtis similarity matrices (Clarke & Warwick 1994). Environmental analyses were based on Euclidean distances after normalization (x-mean/SD). Clusters were constructed using a hierarchical agglomerative method with group average linkage (Clarke & Warwick 1994). Similarity profiles were used a posteriori to determine the statistical significance of each split in the dendrogram using a permutation technique under the null hypothesis of no inherent structure among samples (Clarke et al. 2008). To assess the relationships between multivariate environmental (*i.e.* mean grain size, organic matter and chlorophyll density) and biotic (*i.e.* genus abundance) data, we used RELATE analysis, which conducts a Spearman's ranked correlation between the two similarity matrices (biotic and abiotic). To determine if environmental variables changed gradually across shore, we correlated them with station numbers, as a proxy for position along the intertidal. To evaluate which environmental variables were defining community structure in different regions of the intertidal, we used the linkage tree (LINKTREE) routine, which maximizes the *R* statistic at each split of the community matrix in concordance with differences in underlying environmental parameters (Clarke & Warwick 1994, Clarke et al. 2008). To explore abundance and community structure in terms of taxonomic and functional diversity we calculated the genus richness (*S*), the Shannon Wiener (*H'*) index and the Index of Trophic Diversity (presented as ITD^{-1} henceforth) modified from Heip et al. (1985), applying the formula $1/\theta^2$, where θ is the fraction of each of the four functional groups. It ranges from 1 (when one functional group contributes 100% and functional diversity is lowest) to 4 (when each functional group contributes 25% and functional diversity is highest). The ITD is based on Wieser's (1953) classification. Nematode genera are grouped into four feeding types: 1A Selective deposit and bacteria feeders with unarmed, small buccal cavity, 1B non-

selective deposit feeders with unarmed wide buccal cavity, 2A Epistratum feeders, herbivorous and bacterivorous species with lightly armed small buccal cavity, and 2B carnivores and omnivores with wide armed buccal cavities.

To test for differences in abundance as well as taxonomic and functional diversity between runnels and sandbars, student's *t* tests were performed after verifying homoscedasticity with Bartlett's test (Sokal & Rohlf 1995). If the data exhibited heteroscedasticity, Welch's approximate *t* tests were performed (Zar 1984). Analyses of similarites (ANOSIM) were applied to multivariate data. ANOSIM is conceptually comparable to ANOVA, yet makes no assumptions about the data distribution. The test statistic *R* equals 1 if all replicates within groups are more similar to each other than to any replicate from different groups and is approximately 0 if similarities within and among groups are the same on average. In order to determine which genera and functional groups contributed most to the similarity within each assemblage, we performed similarity percentage analyses (SIMPER).

To compare the potential local genus richness at *El Tornillal* with a comparable beach studied near Santa Clara, 25km further north by Mundo-Ocampo et al. (2007), we estimated total genus richness at *El Tornillal* by plotting a species accumulation curve (SAC) and computing non-parametric genus richness estimators. The SAC was constructed by plotting the cumulative number of genera against number of samples applying the Ugland index (Ugland et al. 2003) with the program EstimateS (Colwell 2005). The Morgan-Mercer-Flodin (MMF) Model:

$$y=(ab+cx^d)/(b+x^d) \qquad (1)$$

was fitted to the SAC (Morgan et al. 1975) using the software Curve Expert[2]. The estimated maximum genus richness is represented by the asymptote (parameter

[2] (http://curveexpert.webhop.net)

c) of the model. As a complementary method, we computed non-parametric genus richness estimators. Among all possible estimators, we chose the Incidence-Based Coverage Estimator (ICE, Chazdon et al. 1998), which allowed a direct comparison with the study by Mundo-Ocampo et al. (2007). The Second-Order Jackknife Estimator (Jack2, Burnham & Overton 1979) was also chosen as it yielded the best estimation compared to the SAC. The ICE is based on the proportion of infrequent genera that are not unique, whereas the Jack2 is based on the frequency of unique and duplicate genera. To assess the estimation error associated with our sampling effort, we calculated the estimation error of Jack2 using the equation:

$$y=100-(A/E)*100 \qquad (2)$$

where y is the estimation error (in percent), A is the asymptote of the SAC (parameter c of the MMF Model) and E is the estimated genus richness by the Jack 2 estimator (Canning-Clode et al. 2008). To calculate the number of samples at which the error associated with the estimation of taxonomic richness would be 0 or <5%, we fitted different models to the plot of the relative estimation error against number of samples (Canning-Clode et al. 2008).

Multivariate analyses were conducted with the program PRIMER version 6 (Clarke & Warwick 1994, Clarke & Gorley 2001). Univariate analyses were performed with the program STATISTICA (Statsoft 1993).

II. 4. RESULTS

II. 4. 1. Zonation patterns

Even tough the same five dominant families (*Epsilonematidae*, *Xyalidae*, *Desmodoridae*, *Cyatholaimidae*, *Chromadoridae*) accounted for >75% of nematodes in both seasons, faunal groups differed slightly but significantly (ANOSIM, R=0.099, p=0.004), therefore subsequent analyses were conducted separately. The nematode community structure at *El Tornillal* was spatially heterogeneous across the intertidal and featured distinct species assemblages. Cluster analyses revealed at least three major groups (p<0.05) at a level of 18.26% similarity in September 2007 and 18.25% similarity in March 2008 (Fig. 9a). In September 2007, groups clearly matched sample position in the intertidal: lower (stations 1– 3), middle (stations 4–9), and high beach (station 10; Fig. 9a). In March 2008, the pattern recurred, except that stations 5 and 7 (both runnels) clustered with stations 1–3 (Fig. 9a). MDS plots revealed the same groups with low stress values (September 2007: 0.1; March 2008: 0.12; Fig. 9b).

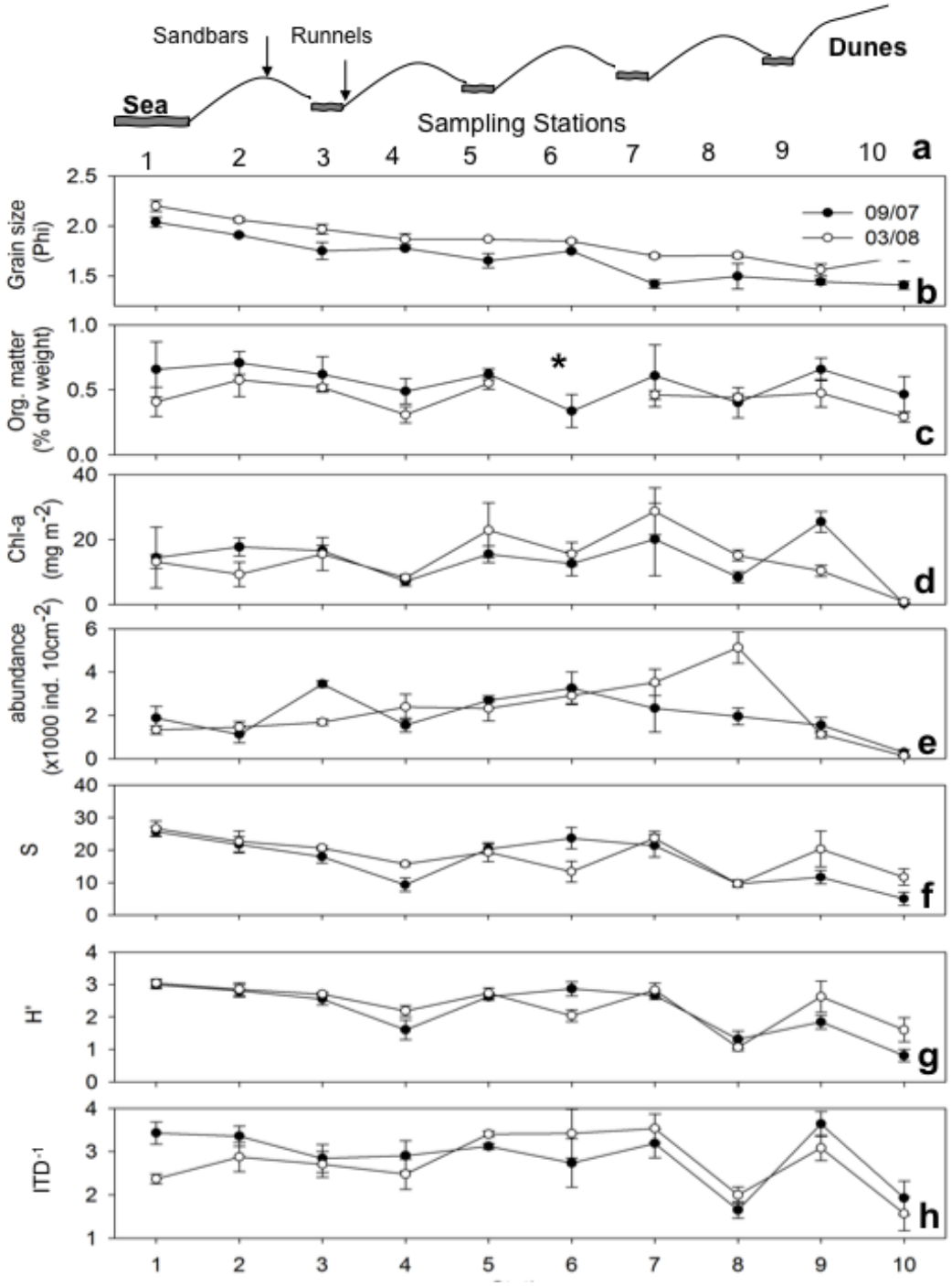

Figure 8. Environmental and biological variables across the intertidal in September 2007 (*black circles*) and March 2008 (*open circles*). a) Schematic illustration of stations along a transect; means and standard deviations of b) mean grain size (in Φ); c) organic matter content (% dry weight); d) chlorophyll *a* density (mgm^{-2}); e) nematode abundances (individuals 10cm^{-2}); f) genus richness (*S*); g) taxonomic diversity (Shannon Wiener *H*□) and h) trophic diversity (ITD^{-1}). *Asterisks* denote missing data

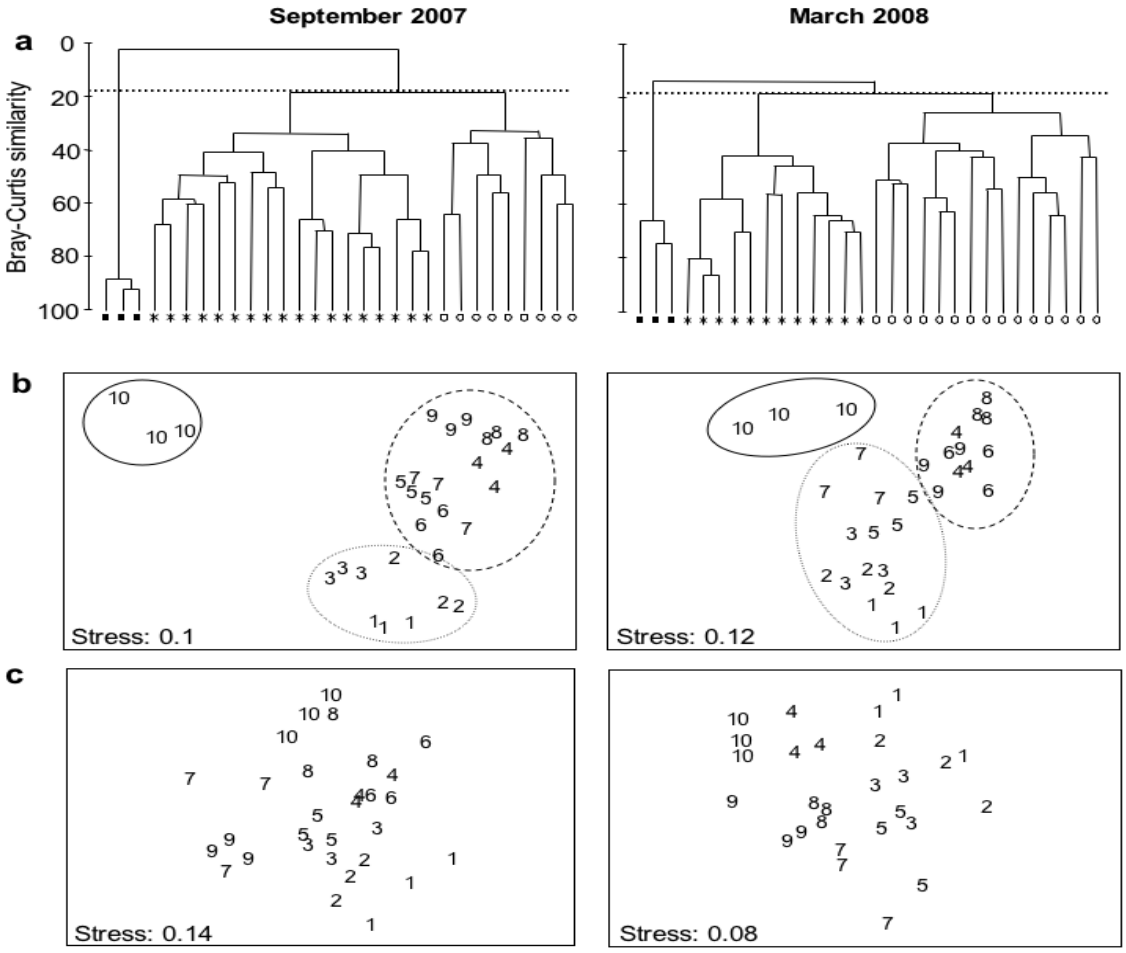

Figure 9. Multivariate analyses of community structure. a Cluster analyses based on Bray-Curtis similarities resulting in three main groups at 18.26% similarity in September 2007 and 18.25% in March 2008, indicated by the *dotted lines*. *Symbols* represent intertidal regions: *open circles* lower beach (September 2007 stations 1–3; March 2008 stations 1–3, 5, 7), *asterisks* middle beach (September 2007 stations 4–9; March 08 stations 4, 6, 8, 9), *black squares* upper beach (station 10). b Non- metric multidimensional scaling (MDS) of genus abundance for both sampling seasons. Replicate samples are identified by their station number. *Lines* reflect the same three main groups that resulted from the cluster analyses: *continuous line* station 10, *dashed line* stations 4–9 in September 2007 and stations 4, 6, 8, 9 in March 2008, *dotted line* stations 1–3 in September 2007 and stations 1–3, 5, 7 in March 2008. c Non-metric multidimensional scaling (MDS) of environmental variables (mean grain size, chlorophyll *a* and organic matter). Replicate samples are identified by their station number.

Unlike nematodes, environmental variables were not spatially clustered. Instead, the position of replicate samples in MDS plots suggested the presence of

an environmental gradient (stress values September 2007: 0.14; March 2008: 0.08; Fig. 9c), corroborated by the significant correlation of environmental variables with station number, as a proxy of position in the intertidal (RELATE September 2007: ρ=0.472, *p*=0.001; March 2008: ρ=0.515, *p*=0.001).

Table I. Percentage contribution of the top 50% discriminating genera for each assemblage and their feeding strategy (FS)

	Genus	FS	
RUNNEL			
September 2007	*Epsilonema*	1A	25.68%
	Xyala sp 2	1B	33.17%
	Chromadorita	2A	38.94%
	Xyala sp 1	1B	44.41%
	Richtersia	1B	49.08%
	Metachromadora	2B	53.13%
March 2008	*Xyalidae gen.*	1B	15.73%
	Chromadorina	2A	25.30%
	Chromadorita	2A	34.31%
	Epsilonema	1A	41.17%
	Pomponema	2A	46.37%
	Metachromadora	2B	51.15%
SAND			
September 2007	*Epsilonema*	1A	36.34%
	Praeacanthonchus	2A	44.98%
	Desmodora sp 1	2A	50.94%
March 2008	*Epsilonema*	1A	38.75%
	Chromadorina	2A	55.53%

Transitions between intertidal faunal groups were consistently associated with shifts in environmental conditions in both seasons. In September, the high beach community (station 10) was set apart from the rest due to very low to zero chlorophyll *a* values (LINKTREE September 2007: *R*=0.97, *B*%=99). Further grouping led to a division into two main groups differentiated by mean grain size: stations 1, 2 and one replicate of station 3 versus the rest of replicates from stations 3–9 (*R*=0.63, *B*%=63). In March, the pattern was similar but ranked differently: One replicate of station 10 was set apart due to a zero chlorophyll *a* value (*R*=0.58, *B*%=80). The next division was due to mean grain size and separated stations 1–3 from the rest (*R*=0.64, *B*%=67). Finally, the two remaining station 10 replicates were set apart from stations 4 to 9 due to lower chlorophyll *a* values (*R*=0.65, *B*%=61). These results stress the significance of chlorophyll levels in structuring the faunal assemblages in the high intertidal and of mean grain size in the middle and lower beach, which is also consistent with the gradual trend of increasing grain size (*i.e.* decreasing Φ values) with increasing distance from the sea (Fig. 8b).

II. 4. 2. Local abundance and diversity patterns

The mean density of nematodes over the entire intertidal was strikingly similar between contrasting seasons (September 2007: 2,001 ±1,007 ind. $10cm^{-2}$, March 2008: 2,194 ±1,407). In September, there was no discernible spatial pattern, with highest abundance (3,445 ind. $10cm^{-2}$) at station 3, and a second peak at station 6 (3,247 ind. $10cm^{-2}$; Fig. 8e). In March, however, there was a gradual increase in abundance with increasing distance from the waterline, with a peak at station 8 (5,124 ind. $10cm^{-2}$). Lowest abundance was found at station 10 for both seasons (September 2007: 277 ind. $10cm^{-2}$; March 2008: 121 ind. $10cm^{-2}$; Fig. 8e).

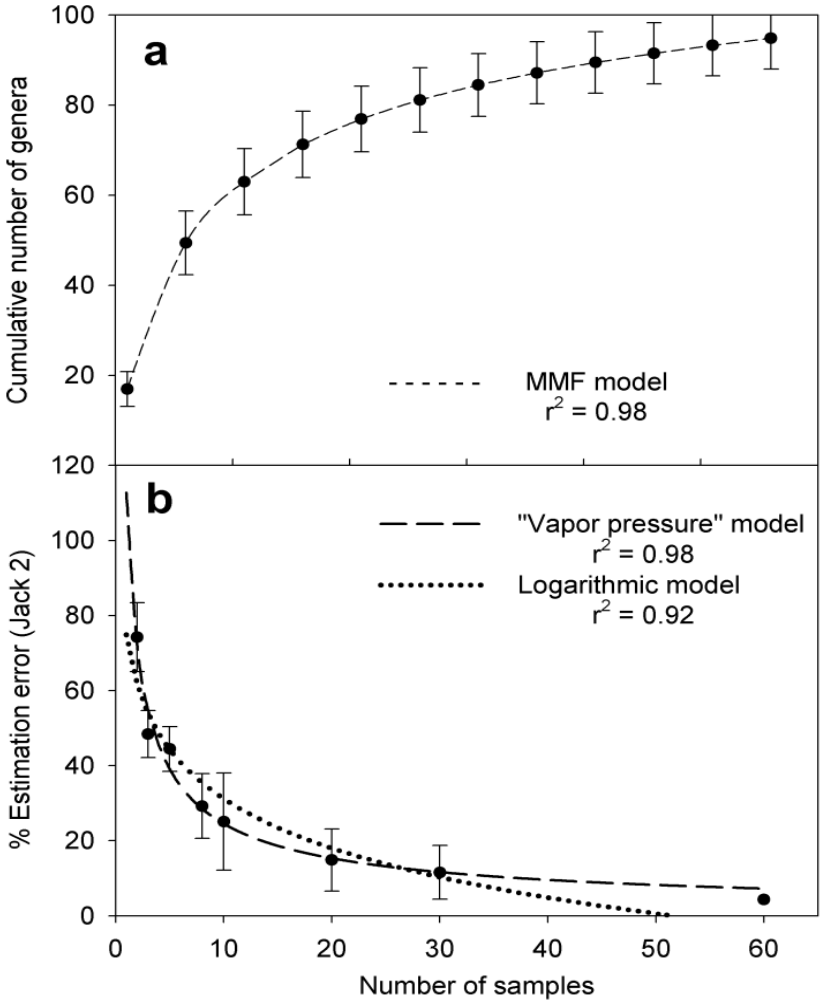

Figure 10. Local diversity and error estimations. a) Species accumulation curve using the Ugland index for the combined datasets of September 2007 and March 2008 (*black diamonds*). The *line* indicates the adjusted MMF Model $y=(ab+cx^d)/(b+x^d)$, where the model parameters are: a= -7.97, b=4.36, c=125.03 and d=0.67. b Relative estimation error (mean and standard deviation) of the second-order jacknife richness estimator (Jack 2) for different samplesizes (*black circles*). Adjusted models: logarithmic model $y=a+b\ \ln(x)$ with the model parameters: a=74.91, b= -18.99 (*dotted line*) and "vapor pressure model" $y=e^{a+bx+c\ \ln x}$ with the model parameters: a=4.78, b= 0.05 and c= -0.68 (*dashed line*). r coeffcient of determination

A total of 96 genera belonging to 25 families were recorded among 3,000 individuals (September 2007: 23 families and 74 genera; March 2008: 23 families and 87 genera; see Appendix 1). Highest genus richness and diversity (Shannon

Wiener *H'*) occurred at station 1 [mean (±SD); September 2007: 25.67 (±1.53) genera, *H'*=2.99 (± 0.12); March 2008: 26.67 (± 2.31) genera, *H'*=3.04 (± 0.12)] followed by station 2 (September 2007: 21.67 (± 2.52) genera, *H'*=2.8 (± 0.19); March 2008: 22.67 (± 3.21) genera, *H'*=2.85 (± 0.2); Fig. 8f, g). A second peak in genus richness and diversity was found at station 6 in September [23.67 (± 3.21) genera, *H'*=2.86 (± 0.21)] and station 7 in March [23.67 (± 2.08) genera, *H'*=2.83 (± 0.22); Fig. 8f, g).

II. 4. 3. Community structure of runnels and sandbars

Nematode assemblages from runnels were significantly different from sandbars (ANOSIM, September 2007: R=0.103, p=0.027; March 2008: R=0.228, p=0.01). Accordingly, they differed in most community attributes: abundance was higher in runnels in September 2007 (t=2.111, p=0.04) but not in March 2008 (t= -0.78, p>0.2; Fig. 8e). Higher taxonomic and functional diversities were found in runnels in both seasons (number of genera in runnels vs. sandbars: September 2007: 67 vs. 56, March 2008: 79 vs. 60; Genus richness S: September 2007: t=2.276, p=0.03; March 2008: t=4.6, p<0.0001; Fig. 8f; Shannon Wiener *H'*: September 2007: t=2.636, p<0.05; March 08: t=4.665, p<0.001; Fig. 8g; ITD^{-1}: September 2007: t=2.44, p<0.05; March 2008: t=3.49, p<0.01; Fig. 8h). SIMPER analyses pointed in the same direction: in sandbars only two (March 2008) or three (September 2007) genera accounted for 50% of the cumulative similarity (Table I), whereas in runnels there were at least twice as many (September 2007: 6; March 2008: 6, Table I). In runnels, the discriminating genera revealed by SIMPER represented all four feeding groups, whereas in sandbars, there were only two, namely 2A (epistrate feeders) and 1B (non-selective deposit feeders; Table I). More than a fifth (21.87%) of all genera were exclusive of runnels, whereas fewer genera were exclusive of sandbars (8.3%). Environmental variables had a significant influence on the structure of the nematode community, indicated by

significant correlations between faunal and environmental variables (RELATE analysis: September 2007: ρ=0.42, *p*=0.001; March 2008: ρ=0.464, *p*=0.001). Accordingly, organic matter content was higher in runnels; however, the difference was significant only in September (2007: *t*=2.785, *p*=0.0095; March 2008: *t*=1.878, *p*=0.072; Fig. 8c). Chlorophyll *a* content was significantly higher in runnels in both seasons (September 2007: *t*=3.749, *p*=0.0008; March 2008: *t*=3.133, *p*=0.004; Fig. 8d). On the other hand, there was no significant difference in mean grain size between runnels and sandbars (September 2007: *t*= -0.079, *p*=0.94; March 2008: *t*=0.344, *p*=0.73; Fig. 8b).

Intertidal runnels possessed a different faunal composition from station 1, based on SIMPER analyses between station 1 and higher intertidal runnels. Station 1 is located at the transition line between the sub- and intertidal region. Higher intertidal runnels comprised a large number of genera that did not contribute to the similarity within station 1 (September 2007: 14/28 [50%]; March 2008: 19/34 [56%]). The contribution of the top discriminating genera for each runnel suggested a “dilution effect”, in which typical nematodes of station 1 were replaced by new taxa toward the higher beach. This is reflected in the higher dissimilarity values of runnels relative to station 1 with increasing distance from the sea (Table II).

Table II. Presence of the top 90% discriminating genera across the intertidal

September 2007

	Station 1	Runnel 3	Runnel 5	Runnel 7	Runnel 9
Dissimilarity[1]	**--**	**66.67**	**77.11**	**82**	**95.33**
Subtidal	Marylynnia				
	Cobbia	X[2]			
	Pomponema		X		
	Dichromadora	X		X	
	Chromadorita	X		X	
	Actinonema				
	Ceramonema sp 2		X		
	Xyalidae Gen.				
	Nannolaimoides				
	Richtersia	X		X	
	Latronema				
	Paracomesoma				
	Xyala sp 2	X	X	X	
	Catanema sp 1		X		
New[3]		Desmodora sp 1	X		
		Tricoma	X	X	
		Xyala sp 1	X		
		Rhynchonema	X	X	
			Epsilonema	X	X
			Metachromadora	X	
			Oncholaimidae Gen.	X	
				Desmodora sp 2	X
				Chromaspirinia	
				Ceramonema sp 3	
				Enoploides	
				Gammanema	X
					Theristus sp 2
					Microlaimus sp 2

March 2008

Dissimilarity	**--**	**63.66**	**75.85**	**85.37**	**86.26**
Subtidal	Pomponema sp 1	X	X		
	Xyalidae Gen.	X	X		X
	Cobbia	X			
	Dichromadora				
	Marylynnia	X			
	Promonhystera				
	Ceramonema sp 2				
	Daptonema	X			
	Cyartonema				

Table II (continued). Presence of the top 90% discriminating genera across the intertidal

March 2008

Dissimilarity	**--**	**63.66**	**75.85**	**85.37**	**86.26**
Subtidal	Nannolaimoides				
	Pomponema sp 2				
	Stylotheristus				
	Prochromadorella		X	X	
	Richtersia	X		X	
	Viscosia				
New		Chromadorita	X	X	
		Xyala sp 1			
		Metachromadora	X	X	
		Desmodora sp 1			X
			Chromadorina	X	X
			Epsilonema	X	X
			Odontophora	X	
			Tricoma	X	X
			Enoploides		X
			Neochromadora		
				Chromaspirinia	
				Epacanthion	
				Chromadoridae gen.	
				Rhynchonema	
					Microlaimus sp 2
					Xyala sp 1
					Theristus sp 2
					Metoncholaimus
					Oxyonchus

[1]Dissimilarity = Result from SIMPER analyses between station 1 and the respective runnel

[2]X = Contributing genus that has been listed before

[3]New = Appearance of a new discriminating genus

II. 4. 4. Local and regional diversity estimations

Based on data from both sampling seasons, the estimated asymptotic number of genera was 125 (MMF model adjusted to the SAC, r^2=0.98; Fig. 10a). Non-parametric estimations yielded maxima of 106 (ICE) and 120 genera (Jack 2), with an estimated error of 4.3% with 60 samples for the latter (Fig. 10b). Of the two models tested, the so-called "vapor pressure model" gave a better fit ($y=e^{a+bx+c\ \ln x}$, r^2=0.98) than the logarithmic ($y=a+b\ \ln(x)$, r^2=0.92). Error estimates ranged from zero with 52 samples, for the latter, and 7.3% with 60 and <5% with 104 samples, for the former (Fig. 10b).

The ICE and the observed number of genera were approximately 1.5–1.7 times higher in *El Tornillal* (ICE=106 for a total of 96 observed genera) than in Santa Clara Beach (ICE=72 for a total of 55 observed genera), a homogeneous beach located 25 km north from our study site (Mundo-Ocampo et al. 2007). At Santa Clara, sampling involved a 30 x 60-m grid at the low intertidal and the number of identified nematodes was ca. 600. Given the different sampling efforts, comparison of that study with the lower intertidal of *El Tornillal*, *i.e.* stations 1, 2, and 3, is more meaningful. Genus richness at Santa Clara beach was 55 (Mundo-Ocampo et al. 2007) and at the lower intertidal of *El Tornillal* 73. The two combined host a total of 87 genera, with almost half of them being shared (41/87). More than one-third (32/87) were found exclusively at the structurally complex *El Tornillal* beach, whereas only less than half of that (14/87) were unique to the featureless beach at Santa Clara.

II. 5. DISCUSSION

II. 5. 1. Intertidal three-tiered zonation dominates over structural complexity

One of the goals of this study was to test whether meiofaunal intertidal cross-shore distribution patterns were dominant over structural complexity. To our

knowledge, the influence of topographical heterogeneity in the structure of a nematofaunal community has never been addressed. In many beaches, three different assemblages have been discerned (Rodriguez 2004, Gheskiere et al. 2005). Gheskiere et al. (2004) found that the nematofaunal zonation of a topographically comparable beach (De Panne, Belgium) involved three assemblages across the intertidal, with a fourth, distinct assemblage at the driftline. However, sampling was restricted to sandbars, since the authors hypothesized that meiofauna in runnels consisted mostly of subtidal organisms. Consequently, the eVect of runnels on intertidal zonation patterns remained unexplored. Our results show that the presence of topographical complexity, in the form of runnels and sandbars, did not alter the expected zonation pattern involving three major assemblages of nematodes.

At *El Tornillal*, these assemblages correlated with changes in chlorophyll *a* and mean grain size. The nematode assemblage in the uppermost limit of the intertidal zone (station 10) was consistently unique and survived in very low to undetectable chlorophyll *a* levels. Juvenile *Praeacanthonchus* accounted for 82% of nematodes and were exclusive of this station in September, whereas adult *Praeacanthonchus* appeared in the middle intertidal 6months later, at which time *Trichotheristus* dominated the community at station 10. As adults, *Praeacanthonchus* are herbivores (Moens et al. 2005), and their high abundance at station 10 in September is surprising given the low chlorophyll *a* levels. Furthermore, osmotic and temperature stress may reach levels limiting survival, reproduction, and maturation time as well as assimilation and respiration (Moens & Vincx 2000a, b). Given the extreme environmental conditions at station 10, other advantages may be responsible for enhanced juvenile survival rates, such as low intra- and interspecific competition due to decreased nematode abundance and diversity.

The rest of the intertidal community was grouped in two assemblages associated with different mean grain sizes, reflecting the gradient of tidal and wave energy across shore. Grain size is an important factor structuring nematode

communities (Gheskiere et al. 2004, 2005), given their interstitial abode during their entire life cycle. The zonation pattern at *El Tornillal* was temporally variable. In September, lower (station 1–3) and middle (station 4–9) nematode assemblages were clear-cut and did not overlap. In March, nematodes from middle runnels (stations 5 and 7) clustered with those from the lower intertidal (stations 1–3), reflecting a greater biological similarity presumably favored by enhanced passive transport induced by stronger wave action and hibernal winds (Lluch-Cota et al. 2007).

The existence of specific taxa characteristic of particular intertidal levels is better documented in macrofauna (McLachlan & Jaramillo 1995) than in meiofauna. The fact that some nematode genera consistently dominated certain intertidal horizons in contrasting seasons suggests that a substantial part of the community may be spatially constrained to complete their life cycles. Gheskiere et al. (2005) hypothesized the existence of isocommunities to refer to specific species assemblages resulting from community convergence at given intertidal levels among geographically separated beaches with similar morphodynamics. Comparison of our typical genera in each intertidal level with their analogs at De Panne does not support the isocommunity hypothesis. At *El Tornillal*, *Pomponema*, *Marylynnia*, and *Cobbia* were among the top five discriminating genera in the lower beach in both seasons, whereas *Epsilonema*, *Microlaimus,* and *Tricoma* were the same in the middle beach. These genera differ from those discriminating at De Panne (Gheskiere et al. 2004).

II. 5. 2. Across-shore abundance and diversity patterns are consistent with IDH and DEH

Meiofaunal abundance patterns are spatially and temporally heterogeneous in the intertidal, but many studies have documented a peak in meiobenthic diversity around the mid-intertidal of sandy beaches in response to intermediate

disturbance levels (Armonies & Reise 2000, Gheskiere et al. 2004). Although the abundance pattern at *El Tornillal* was not temporally consistent, the existence of two diversity peaks at different intertidal levels at our study site points to the presence of two environmental optima, which may relate to the interaction of different mechanisms (IDH and DEH).

Mean nematode abundances at *El Tornillal* and Santa Clara were of comparable magnitude (Mundo-Ocampo et al. 2007) and fall within the range of other studies (Rodriguez et al. 2001, Gheskiere et al. 2004). At *El Tornillal*, abundance peaked at the middle beach (station 7 in September 3,247 ind. $10cm^{-2}$ and station 8 in March 5,124 ind. $10cm^{-2}$), whereas in the morphodynamically similar macrotidal beach at De Panne highest abundance occurred at the lower beach (2,784 ind. $10cm^{-2}$). A pattern of increasing meiofaunal abundance with increasing distance from the sea has been reported for many beaches (Nicholas & Hodda 1999, Rodriguez et al. 2001, Gheskiere et al. 2005).

At *El Tornillal*, a peak in genus richness occurred in the mid-intertidal, in concert with the pattern described in other studies (Armonies & Reise 2000, Gheskiere et al. 2004). According to the IDH, intermediate disturbance allows for the coexistence of more species because, on the one hand, it mediates periodic reductions of competitive dominant species precluding competitive exclusion, and on the other, disturbance is not as extreme as to reset ecological succession in favor of opportunistic and competitively inferior *r*-selected species (Huston 1979). Intermediate levels of disturbance in the middle beach result from gradients of disturbance produced by surf and swash processes and aerial exposure. At *El Tornillal*, sediment mean grain size indicates a cross-shore gradient of hydrodynamic energy, indicating that disturbance levels at the mid-intertidal is intermediate. Also, desiccation due to tidal exposure and related temperature fluctuations are intermediate. These factors create a more extreme environment in the upper intertidal (high desiccation and temperature fluctuation) and more stable conditions in the lower intertidal (predominantly submerged, thus experiencing moderate temperature fluctuations).

The high intertidal (station 10) is characterized by consistent low diversity levels. Intermediate levels of disturbance are thus reasonable explanations for the mid-intertidal peak in species richness. The lower intertidal limit (station 1), exhibited a second peak and overall maximum in genus richness, which suggests the existence of another set of optimal conditions favoring diversity. Given the different processes prevailing in the lower intertidal, this optimum likely involves other factors than those responsible for the middle intertidal peak.

The DEH predicts maximum species richness under conditions of intermediate productivity and disturbance, since high productivity promotes a positive relationship between diversity and disturbance whereas low productivity reverses it (Huston 1994). Intermediate productivity in the lower intertidal is suggested by the levels of organic matter and chlorophyll *a* (Fig. 10c, d). Macrofauna and megafauna may act as sources of intermediate disturbance; since high macrofaunal species richness and abundance generally occur in the lower intertidal of sandy beaches as is also the case in the UGC (McLachlan & Jaramillo 1995, Avila-Serrano et al. 2006). Macrofauna may affect meiofaunal community structure through complex interactions involving predation and bioturbation (Austen et al. 1998). In addition, wading birds congregate close to the waterline to roost and feed, disturbing superficial sediments (personal observations). Moreover, excreta and feces from birds may be a source of organic matter to the infaunal community (Palomo et al. 1999). Finally, the maximum in species richness found at the limit between the sub- and intertidal also reflects the transitional nature of this zone.

II. 5. 3. Intertidal runnels harbor distinct communities augmenting regional diversity estimates

A major contribution of this study is the comparative analysis of the nematofauna from two microhabitats (intertidal runnels and sandbars). Former

studies have limited their scope to analyzing the influence of environmental factors on the structure of meiofaunal communities, largely neglecting the role of habitat heterogeneity. Our results revealed major differences in environmental parameters and in the nematofauna inhabiting intertidal runnels and sandbars. Chlorophyll *a* and organic matter levels revealed contrasting availability of food sources between them. Both were consistently and significantly higher in runnels (except for the organic matter in winter), presumably owing to the presence of conspicuous benthic algal mats, as well as detritus and microbial biomass. Runnels and sandbars represent thus microhabitats with contrasting environmental conditions and distinct resource provisions.

Previous studies assumed that nematodes found in runnels represent a subset of subtidal fauna (Gheskiere et al. 2004). According to this, we would have expected that runnels host a fraction of the genera found at station 1, which was located at the transition to the subtidal. However, the runnels did not represent a subset of station 1 but a distinct community from it. Its distinctiveness was best reflected by the dilution of taxa typical for station 1 away from the sea and concomitant increase in the number of runnel–restricted genera. More than a fifth of the overall taxonomic richness (21 genera out of 96) was exclusive for runnels, and they would have been missed had we only focused on sandbars. This high number (21) of genera unique to runnels was more than double that of sandbar-specific genera (8). The high taxonomic diversity may be due to the relatively high hydrodynamic stability provided by being sheltered from cross-shore currents and by superficial sediment cohesion produced by phytobenthic algal films (Sutherland et al. 1998). This allows nematodes to control their spatial distribution by actively selecting their environment (Ullberg & Olafsson 2003), burrowing and attaching themselves to sediment particles (Chandler & Fleeger 1983).

Not only taxonomic, but also functional diversity was higher in runnels, where the top 50% of discriminating genera represented all feeding groups, indicating that they provide resources for herbivores, bacterivores, organic matter users, as well as predators. On the other hand, only genera from feeding groups 1A

(selective deposit feeders) and 2A (epistrate feeders) were among the major taxa in sandbars. The relative stability of the runnel environment, and the high abundance of microphytobenthos and organic matter may promote favorable conditions for the coexistence of more functional groups.

Our study highlights the importance of habitat heterogeneity in determining nematofaunal community structure and diversity in the intertidal. This stands in line with the HHH and many other studies reporting on the importance of habitat heterogeneity for diversity (Tews et al. 2004, O'Dea et al. 2006). Comparison of the lower intertidal of a structurally heterogeneous (*El Tornillal*) with a nearby featureless beach (Santa Clara Beach) revealed that 33% of the regional taxon richness was unique to the former, whereas only 16% was restricted to the latter. The high taxon turnover between sites (only 47% of the genera were shared) may be unexpected, given the proximity of the beaches and the potential for passive transport. It suggests a high level of regional diversity on the east coast of the UGC. More reliable estimates of regional taxon richness can be achieved by an approach that integrates extrapolation to a bigger area in the presence of habitat heterogeneity. Ugland et al. (2003) proposed a Total-Species Curve (T-S) constructed by joining the endpoints of SACs constructed for each different habitat. The T-S can then be extrapolated to a bigger area and usually yields much higher estimates than non-parametric estimators (Ugland et al. 2003). Many beaches along the coasts of the UGC are heterogeneous featuring microhabitats such as the ones described here (sandbars and runnels), but also rocky outcrops, vegetation, freshwater inputs and others. Our results point to the need for a thorough survey of these habitats in the UGC, a region where the marine nematofauna has only begun to be described (Holovachov et al. 2008a, b, 2009), and for the use of improved models to estimate regional species richness.

II. 6. ACKNOWLEDGEMENTS

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Chapter III

Spatial aggregation patterns of free-living marine nematodes in constrasting sandy beach micro-habitats

III. 1. ABSTRACT

In the absence of chemical or physical gradients, random displacement of organisms can result in unpredictable distribution patterns. In spite of a limited locomotive capability, marine nematodes may choose where to settle after re-suspension and may maintain their position in the sediment under calm conditions, leading to small-scale (<1 m) spatial variability. However, in more energetic environments, nematodes become re-suspended with sediments and re-distributed at distances dependent on prevalent hydrodynamic regimes, from meter- to decameter-scale or more. In this study, we tested the hypothesis that micro-habitats (*i.e.* runnels and sandbars) in a macrotidal sandy beach influence the distribution patterns of free-living marine nematodes by exhibiting contrasting hydrodynamic regimes. Specifically, we predicted patchier distributions in the calmer environment (runnel). We sampled nematodes in each habitat from <1m to decameter scales. Our results show more heterogeneous spatial distributions in the runnel, presumably owing to a predominance of active displacement under calmer conditions and sediment cohesion by algal films. Biological similarity among runnel replicates was low, whereas replicates from the sandbar exhibited higher similarity, presumably because of homogenization of the sediment and inhabiting fauna by tidal currents. A significant negative correlation between biological similarity and sampling distance was found in the runnel, but not in the sandbar. The most similar samples were the closest in the runnel and the most distant in the sandbar. More patchily distributed taxa were found in the runnel and

a larger fraction of homogeneously or randomly distributed taxa in the sandbar. We conclude that different hydrodynamic regimes in contrasting intertidal microhabitats significantly influenced the nematofaunal distribution, resulting in different spatial patterns next of one another in the same beach. This has significant implications for sampling and monitoring designs and begs the need for detailed studies about the physical and biological processes governing meiobenthic communities.

III. 2. INTRODUCTION

The distribution of benthic meiofauna in seemingly homogeneous sediments has been recognized as "almost chaotic or certainly unpredictable" (Fleeger & Decho 1987). The absence of directional movements triggered by chemical or physical gradients results in unpredictable and stochastic distribution patterns with important implications for population dynamics (Byers 2001). For instance, organism aggregations, which may result primarily from the patchiness of food sources, may confer diminished predation risks (Moody et al. 1996, Hines et al. 2009). Understanding the scales and patterns of aggregations is essential to comprehend trophic links, inter-specific interactions and other biological and environmental processes governing communities; particularly in organisms not readily observable in their environment, such as the microscopic meiofauna of sandy beaches, often dominated by free-living marine nematodes (Moens et al. 1999, Sandulli & Pinckney 1999, Somerfield et al. 2007, Gallucci et al. 2008).

Hydrodynamic and biological processes determine the structure and spatial scale of aggregation patterns of meiofaunal communities. The microscopic size of nematodes limits the radius of active displacement resulting in competitive interactions and resource partitioning at a scale of 10^{-3} to 10^{-2} m (Findlay 1981, Moens et al. 1999). On the other hand, passive transport in the bed load and water column may lead to dispersal at a scale of 10 to 10^{2} m (Palmer 1988, Depatra & Levin 1989, Sun & Fleeger 1994). Small scale (≤ 1m) meiofaunal aggregations

have been found in semi-exposed tidal flats (Findlay 1981, Blanchard 1990, Somerfield et al. 2007), and shallow open-coast environments (Hogue 1982), with biological similarity decreasing with increasing distance (Hogue 1982, Somerfield et al. 2007). On the other hand, in a high–energy sandy beach in Australia, distant samples (1000 m apart) were more similar than those found closer (200 m), due to strong hydrodynamic forces constantly redistributing the intertidal fauna (Nicholas & Hodda 1999). In open-coast environments, seasonal changes in energy regimes result in different aggregation patterns, with nematodes being randomly distributed during the stormy winter season, and more intensely aggregated during the calmer summer (Hogue 1982).

Prevalent hydrodynamic regimes are crucial for meiobenthic distributional patterns, and may vary between and within sites. Contrasting energy conditions can be found in intermediate ridge-and-runnel macrotidal beaches (*sensu* Masselink & Short 1993) due to their heterogeneous topography. Sandbars exposed during low tide are covered with seawater during the incoming tide, resulting in constant sediment reworking and re-suspension at the marginal and superficial layers. They are massive (10 m to 10^2 m), stationary over time scales of 2 (Anthony et al. 2005) to 17 months (King 1972), and thus function as wave barriers for cross-shore tidal currents, protecting intervening runnels. By contrast, the embedded intertidal runnels are subject to constant, but calmer incoming or outgoing water flow along their main axis. They accumulate detritus, algae and organic matter and are sometimes partially covered by algal mats (personal observation), which may induce superficial sediment cohesion and stabilization (Paterson 1989, Sutherland et al. 1998). These two contrasting microhabitats have a strong influence on the nematofaunal community structure (Gingold et al. 2010).

Given the influence of environmental differences of sandbars and runnels on the inhabiting community, we hypothesize that they also induce different spatial aggregation patterns. We expect that constant sediment suspension of sandbars leads to passive transport and a more homogeneous distribution of meiobenthic organisms, whereas the less energetic runnel environment allows active swimming

and settlement and thus smaller–scale aggregations leading to higher patchiness. Assessing the spatial variation of benthic communities in the absence of environmental gradients is essential to our understanding of ecological patterns and processes and has strong implications for choosing the spatial scale of observations (*i.e.* sampling design). This is even more relevant in contrasting micro-habitats, as they may differ in their scale of spatial variation (Phillips & Fleeger 1985). The present study addresses the need for information about variability of benthic communities in seemingly homogeneous environments (Fleeger & Decho 1987) by comparing the extent of spatial variation of nematofaunal communities from contrasting sandy beach intertidal micro-habitats.

III. 3. MATERIAL AND METHODS

III. 3. 1. Study site and sampling design

El Tornillal beach (31° 33'N, 114° 17'W; Fig. 7) is located in the northern Gulf of California within the Biosphere Reserve of the Upper Gulf of California and Colorado River Delta. It is far from direct urban sewage outfalls, industrial and agricultural runoff, and touristic activities. The closest village is Santa Clara, 25 km north. The intertidal zone at this beach is more than 600 m wide and the tidal range reaches 7 m during spring tides (own unpublished data). Within the intertidal zone, runnels and sandbars are oriented almost parallel to the water line. Sea surface temperatures for the sampling period (summer) are 30 - 32° C. In winter, they range from 16 - 18° C (Lluch-Cota et al. 2007).

Sampling took place August 17 2008 during low spring tide. Five sampling stations were placed along two transects (ten stations in total) parallel to the shore. Each transect was placed in a different micro-habitat (runnel and sandbar), approximately 250 m away from the other (Fig. 11). One sampling station was placed at the reference line (0 m), further stations were placed along each of the

two transects at fixed distances from the reference line: 10, 20, 40 and 80 m. At each sampling station, three replicate sediment cores were taken at random within

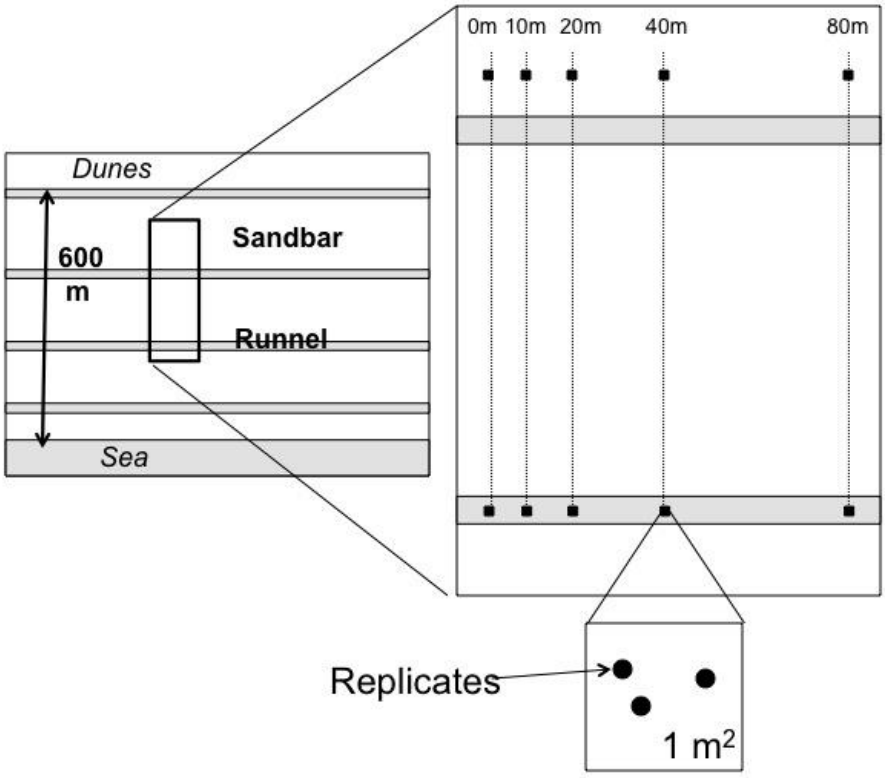

Figure 11. Sampling design. Three replicate samples were taken within a square meter at predefined distances from the reference line (0 m): 10, 20, 40 and 80 m, along a transect in a sandbar and a runnel.

a square meter area using a PVC corer (core size: 9.8 cm long by 2.9 cm internal diameter). The full length of the core was inserted in the sand and dug out as gently and carefully as possible to avoid excessive disturbance of the infaunal community. The entire unsliced sediment core was immediately fixed in 5 % formaldehyde for posterior faunal analyses. Transects were placed in the middle beach zone, which has been shown to host a distinct community from the lower and higher intertidal at <18.3% Bray-Curtis similarity level (Gingold et al. 2010).

III. 3. 2. Faunal analyses

In the laboratory, formalin was rinsed off sediment samples with freshwater over a 45 µm mesh size sieve. Meiofauna was extracted by suspension in colloidal silica (LUDOX^{TM}, specific density 1.15) following De Jonge & Bouwman (1977) and stored in 80 ml 5% formalin. Nematodes were counted in three aliquots of 5 ml under a Leica Zoom 2000 stereoscope, transferred to a 5% glycerol solution and slowly evaporated on a heating plate. We randomly picked 50 nematodes for identification with the help of a gridded dish and pseudo-random numbers generated in a spreadsheet. These organisms were mounted on permanent slides and identified with an OLYMPUS BX51 compound microscope with differential interference contrast (DIC) optics. Where possible, nematodes were identified to generic level, using pictorial (Platt & Warwick 1983, 1988, Warwick et al. 1998) and online (http://nemamex.ucr.edu) taxonomic keys. Juveniles and females lacking unequivocal male counterparts were identified to family level and included as such in statistical analysis. If more than one species could be distinguished among congeners, they were labeled sp 1, sp 2, and treated separately in statistical analyses.

III. 3. 3. Data analyses

To visualize the faunal spatial structure across all distances in the two micro-habitats, non-metric multidimensional scaling (MDS) was constructed from the similarity matrix based on Bray-Curtis index of untransformed data (Clarke & Warwick 1994). To test differences in community composition between predefined groups, (*i.e.*, replicates of sampling stations at different distances), analyses of similarities (ANOSIM) were applied to multivariate data. Analyses of variance (ANOVA) were used to assess variability of abundance and diversity within and between replicates of the different sampling stations (*i.e.*, distances). Diversity was

estimated as genus richness [S], Shannon Wiener's index of diversity [H'], and Hurlbert's expected number of species [$E(S_{30})$], which is less dependent on sample size (Hurlbert 1971). Data normality was verified with the Kolmogorov Smirnov test and homoscedasticy with Bartlett's test (Sokal & Rohlf 1995). If assumptions could not be met, variables were log-transformed.

Similarities between locations were assessed with Similarity Percentage Analyses (SIMPER). Mantel tests were used to test hypotheses that assemblage similarities between samples taken at a given distance were different from similarities between samples at any other distance. We correlated the biotic similarity matrix with a) the matrix of absolute distances between samples, and b) model matrices where the distance of interest was coded as 1 and all other distances as 0. Since there is no *a priori* assumption about the relationship of similarities with respect to distance (*i.e.*, similarities may increase or decrease with increasing distance), two tailed tests were applied with significance values of $p<0.025$ and $p>0.975$.

SIMPER also determined which genera were characteristic of a given assemblage in a given habitat. For these differentiating genera, we calculated the index of dispersion:

$$D = \text{variance/mean} \qquad (3)$$

and the sample-size independent Green's index:

$$C_x = (\text{variance/mean})-1/(n-1) \qquad (4)$$

(Elliot 1971), to evaluate whether they were randomly distributed ($D = 1$ or $C_x = 0$), spatially clumped ($D > 1$ or $C_x > 0$, over-dispersed), or regularly distributed ($D < 1$ or $C_x < 0$, homogeneous distribution). Deviations from the random distribution were calculated by exact permutation tests for D (Clarke et al. 2006). Given the two-tailed test, α was set at 0.025 for significant overdispersion and 0.975 for

significant underdispersion. Significant overdispersion of C_x was computed from its upper value under a null random distribution:

$$C_x,(1-\alpha) = [\chi^2(1-\alpha)/(n-1)]-1/(n\ mean-1) \qquad (5)$$

where χ^2 has n-1 df (Green 1966).

PRIMER version 6 (Clarke & Gorley 2006) was used for multivariate analyses. STATISTICA (Statsoft 1993) was used for univariate analyses.

III. 4. RESULTS

III. 4. 1. Nematodes sampled in different micro-habitats

The nematode communities from the runnel and the sandbar were significantly different (ANOSIM R=0.719, p=0.001). Sandbar samples were more similar to each other than runnel samples, as indicated by their tighter clustering in the MDS plot (Fig. 12). On the other hand, no clear pattern could be discerned in biotic similarity among samples at different distances within each micro-habitat (Fig. 12). Due to the clear biological difference, further analyses were conducted separately on each micro-habitat.

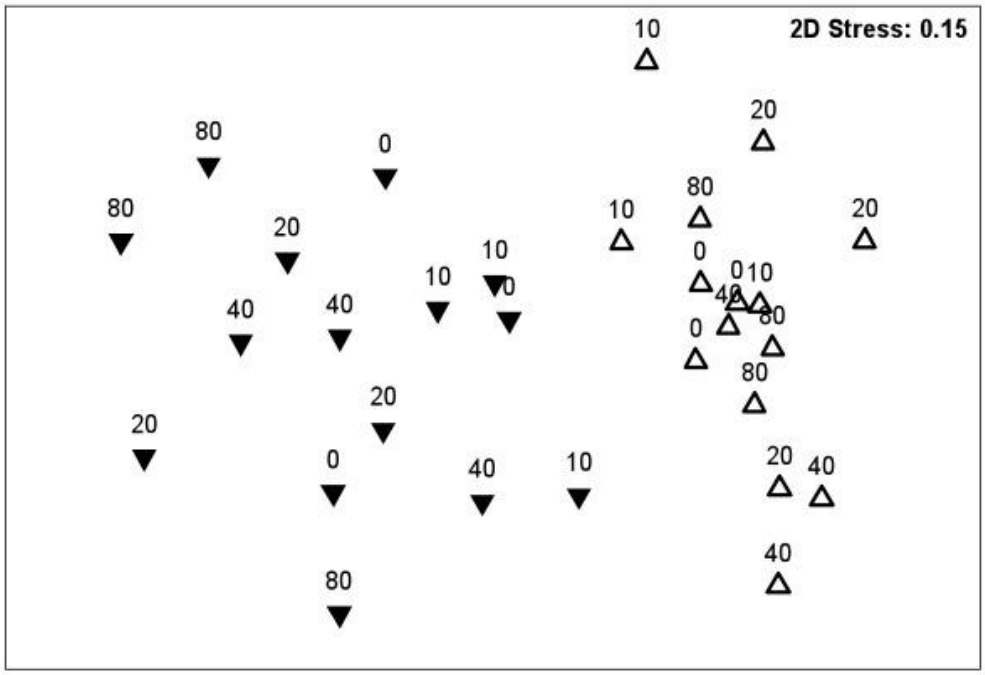

Figure 12. Non-metric multidimensional scaling (MDS) of community structure of the sandbar (open triangles) and the runnel (black triangles). Numbers indicate distances (10, 20, 40, 80 m) at which replicates were taken with respect to the reference line (0 m).

III. 4. 2. Nematodes sampled at different distances

There was no significant difference in community structure (ANOSIM), nor in species richness, diversity and abundance (ANOVA) among stations at spatial scales > 1m in both micro-habitats (Table III). This indicates that the variance of these variables is higher at the replication scale of ≤1 m².

Table III. Tests of spatial homogeneity in nematode communities from intertidal sandbar and runnel. (H_0: $\mu_0 = \mu_{10} = \mu_{20} = \mu_{40} = \mu_{80}$, where m_i is the mean value of the variable at distance i).

	Sandbar		Runnel	
Variable	**Statistic[1]**	**p-value**	**Statistic[1]**	**p-value**
Community structure	0.2	0.06	0.15	0.12
Species richness	2.05	0.16	0.56	0.69
Diversity (*H'*)	1.48	0.28	0.48	0.75
$E(S_{30})$	2.02	0.17	0.38	0.82
Abundance	1.66	0.23	1.07	0.42

[1] Test statistic refers to *R* (ANOSIM) for "Community structure" and *F* (ANOVA) for the other variables

However, there were some important differences in distribution patterns between micro-habitats. Sandbars possessed a more homogeneous nematode community, as revealed by the MDS plot (Fig. 12) and by a higher average similarity among all replicates (sandbar: 50.10; runnel: 44.63). Also, maximum similarity among replicates was higher in the sandbar (boldface in Table IV, "within station"). Congruently, it was in the sandbar where the most similar stations were the more distant from each other, *i.e.*, at the reference line and at 80 m, whereas in the runnel they were the closest, *i.e.*, at the reference line and at 10 m (Table IV, "between station"). These patterns indicate a patchier distribution in the runnel.

Correlations between biotic similarity matrices of nematode assemblages and matrices of physical distance between station pairs are consistent with the previous results. Similarity between assemblages decreased significantly with increasing distance in the runnel, whereas the opposite occurs in the sandbar, although not significantly so (Table V). Nematodes sampled 10 m apart in the runnel were significantly more similar than those separated by any other distance (Table V), whereas in the sandbar, samples 80 m apart were significantly more similar than those separated by any other distance.

Table IV. Biotic similarity of nematode assemblages within and between stations. Similarity within station is the similarity among three replicate samples taken within one square meter. Similarity between stations is evaluated by comparing samples taken at fixed distances (10, 20, 40 and 80 m) from the reference samples. Highest values are in boldface (see results for details).

Similarity within station	Sandbar	Runnel
0	**63.3**	46
10	48.4	**55.3**
20	42.7	45.3
40	60.4	46.7
80	53.3	40.7
Similarity between stations		
0 vs. 10 m	56.3	**50**
0 vs. 20 m	48.4	41.6
0 vs. 40 m	51	47.8
0 vs. 80 m	**60**	41.3

Table V. Matrix correlations between biotic similarity of nematode samples and physical distance between station pairs. Bold values indicate statistically significant results.

	Sandbar		Runnel	
Physical distance matrix	**Rho[1]**	**p[2]**	**Rho**	**p**
True distances[3]	0.132	0.138	**-0.294**	**0.992**
1 m vs. others[4]	0.124	0.054	0.096	0.119
10 m vs. others	-0.089	0.757	**0.233**	**0.027**
20 m vs. others	-0.084	0.724	-0.078	0.754
40 m vs. others	0.044	0.36	0.051	0.35
80 m vs. others	**0.329**	**0.003**	-0.15	0.891

[1] Spearman's rank correlation coefficient

[2] Significance level estimated from 999 permutations. Two-tailed test requires p to be <0.025 when samples are significantly more similar (*i.e.* positive correlation) and >0.975 when samples are significantly more different (*i.e.* negative correlation) relative to samples at any other distance.

[3] "True distances" refer to a matrix of physical metric distances between station pairs, whereas the rest represent model matrices in which the distance of interest is coded as 1 and the others coded as 0.

[4] 1 m scale refers to within 1 m² distances among replicates within a station.

III. 4. 3. Genus specific dispersal

The number of genera found in both micro-habitats was very similar (Appendix 1: sandbar: 66; runnel: 62). However, more genera contributed to the 90% cumulative similarity of the runnel, which indicates higher evenness. Twice the number of genera accounted for 50% of the cumulative similarity in the runnel than in the sandbar (Table VI). Applying C_x, considerably more genera were over-

dispersed in the runnel (19% [12/62] of all taxa or 26% [12/47] of taxa with n≥2) than in the sandbar (11% [7/66] of all taxa or 15% [7/47] of taxa with n≥2). Over-dispersed taxa in the runnel were *Chromadorita*, *Daptonema*, *Elzalia*, *Perepsilonema*, *Metachromadora*, *Odontophora*, *Pomponema*, *Spirinia*, *Tricoma*, *Xyala* sp. 1 and 2, and Xyalidae gen. 1, which accounted for 63% (474/750) of nematodes in the runnel. In the sandbar, *Chromaspirinia*, *Desmodora* sp. 1, *Perepsilonema*, *Microlaimus*, *Spirinia*, *Xyala* sp. 1, and Xyalidae gen. 1 showed significantly contagious distributions and accounted for 58% (432/750) of nematodes in the sandbar. Considering only the typical (*i.e.* top 90%) genera, the contrast between environments becomes more evident: 59% (10/17) over-dispersed genera in the runnel and 36% (4/11) in the sandbar. Among typical genera, *Rhynchonema* and *Microlaimus* showed significant under-dispersion in the runnel, and *Metachromadora* in the sandbar (Table VI, see Appendix 1). The sample size-dependent dispersion index (D) also revealed a higher degree of patchiness in runnels but fewer significantly over-dispersed taxa overall (7 in runnel and 4 in sandbar). The fact that more taxa and nematodes are over-dispersed in runnels underlines the higher patchiness in this micro-habitat.

Table VI. Typical genera (up to 90 % cumulative similarity [Cum.%]) of both the sandbar and runnel micro-habitat, Green's Index (C_x) and dispersal index (D).

		Sandbar	
Genus	**Cum.%**	***C_x***	***D***
Perepsilonema	35.35	0.007*[1]	2.29*[1]
Xyala sp. 1	49.64	0.001*[1]	1.88
Desmodora sp.1	62.1	0.010*[1]	1.70*[1]
Microlaimus	69.87	0.047*[1]	3.97
Pomponema	73.21	-0.015	0.84
Tricoma	76.53	0.006	1.14
Rhynchonema	79.64	0.005	1.11
Enoploides	82.64	0.003	1.06
Xyalidae gen. 2	85.41	0.005	1.10
Gammanema	87.92	-0.008	0.86
Metachromadora	90.05	-0.024	0.68*[2]
		Runnel	
Chromadorita	17.93	0.012*[1]	2.07
Perepsilonema	30.6	0.025*[1]	2.78*[1]
Tricoma	40.8	0.017*[1]	1.90
Pomponema	47.97	0.010*[1]	1.38
Xyala sp 2	54.93	0.059*[1]	4.35*[1]
Desmodora sp.1	60.61	-0.011	0.70
Metachromadora	66.2	0.047*[1]	2.92*[1]
Rhynchonema	71.66	-0.015	0.62*[2]
Xyala sp 1	75.37	0.034*[1]	1.96
Spirinia sp 2	78.96	0.032*[1]	1.84
Richtersia	81.35	0.013	1.21
Microlaimus	83.44	0.000	1.00
Xyalidae gen. 1	84.93	0.117*[1]	3.10*[1]

Table VI (continued). Typical genera (up to 90 % cumulative similarity [Cum.%]) of both the sandbar and runnel micro-habitat, Green's Index (C_x) and dispersal index (D).

	Runnel		
Genus	**Cum.%**	C_x	*D*
Odontophora	86.43	0.086*[1]	2.38
Metoncholaimus	87.67	-0.024	0.79
Actinonema	88.86	0.058	1.76
Ceramonema sp 3	90.06	-0.042	0.67

Significantly (a = 0.05) over-dispersed (*[1]) or underdispersed (*[2]), non-significant values refer to random distributions (see text for details).

III. 5. DISCUSSION

In the absence of physical or chemical gradients, the distribution of meiofauna in sandy environments has been thought to be highly probabilistic and unpredictable (Fleeger & Decho 1987). In this study, we determined the scale at which free-living marine nematodes form aggregations (*i.e.*, "patchiness") in two microhabitats (runnels and sandbars) with contrasting hydrodynamic regimes at scales from meter to tens of meters. We tested the influence of micro-habitats in the spatial patterns of the nematode community at scales commonly used in benthic surveys, which is a topic widely neglected in the literature.

Our findings indicate that scales of long-shore variability vary between microhabitats likely in response to differences in hydrodynamic regimes. Previous work on the community structure of intertidal nematodes at *El Tornillal* allowed us to discard the influence of grain size as the driver of differences between runnels and sandbars (Gingold et al. 2010). As expected, the runnel community exhibits a higher degree of patchiness. This is the result of spatially restricted, presumably active displacement of the single organisms resulting in small-scale aggregations and clumped distributions. Experiments have shown that free-living marine

nematodes can actively select where to settle when descending from the water column and actively migrate towards their preferred food source (Jensen 1981, Ullberg & Olafsson 2003). In addition, individuals may burrow deeper into the sediment as a strategy to avoid being transported passively by the constant alongshore water flow. Nematodes react to increased current speeds of approximately 25 cm s^{-1} 20 cm above the sediment by burrowing deeper into the sediment (Fegley 1987). At *El Tornillal*, current speeds in the runnels at low tide (which was the time of sampling) are ~ 5 - 10 cm s^{-1} (unpublished data), which may allow them to aggregate even in superficial layers. Since runnels are a resource rich micro-habitat (Gingold et al. 2010), nematodes are likely to remain around food patches, limiting unnecessary active dispersal saving on energy expenditure as well as decreasing the risk of predation (Depatra & Levin 1989, Coull 1990). A calm environment abundant in patchy resources is conducive to small-scale displacement, resulting in contagious or over-dispersed spatial distributions at a scale of a few meters or less.

In the sandbar, on the other hand, passive transport is unavoidable when superficial and deeper sediments are re-suspended (Armonies 1994, Commito & Tita 2002). Cross-shore currents on top of sandbars are ≥ 25 cm s^{-1} (10 cm above ground, unpublished data), therefore incoming tides re-suspend sediments deeper than in the sheltered runnels. In addition, food (*i.e.*, organic matter and microbenthic algae) is scarcer in sandbars (Gingold et al. 2010). Meiobenthic copepods have been found to actively swim and ingest planktonic diatoms when they are covered with water, switching to benthic microalgae when there is no water cover (Decho 1986). Nematodes are thought to be rather poor swimmers, but passive transport may enhance the probability of reaching new food patches. They may actively choose where to settle after being suspended in the water column (Ullberg & Olafsson 2003) and are attracted to resource rich sediment patches (Gallucci et al. 2008). The prevalence of passive dispersal in sandbars is reflected in either random or more homogeneous spatial distributions of

nematodes in comparison to the runnel and in the higher similarity of widely separated assemblages.

Our results partly indicate that herbivorous species are more prone to passive transport. According to Wieser's (1953) classification, trophic group 2A nematodes (epistratum feeders) exhibit mainly herbivory as a feeding strategy, but herbivory has also been demonstrated in unselective deposit feeders, group 1B (Nehring et al. 1990, Moens & Vincx 1997). Herbivorous species are rather passively transported, as they need to reside close to the surface to feed (Warwick & Gee 1984, Commito & Tita 2002). *Metachromadora*, mainly feeding on microphytobenthos and residing very close to the surface (Warwick & Gee 1984, Moens et al. 2005), was under-dispersed in the more dynamic (sandbar) and over-dispersed in the calmer (runnel) habitat. It is thus likely that under hydrodynamic harsh conditions prevalent in the sandbar, *Metachromadora* would be passively transported and homogeneously distributed; by contrast, the relatively calm conditions in the runnel may allow the formation of aggregations around algal patches. Probably in response to the same hydro dynamical forcing, genera of the functional group 1B (*Daptonema*, *Elzalia*, *Xyala* and Xyalidae gen. 1), which potentially exhibit herbivory at least as a partial feeding strategy, were over-dispersed in the runnel. Of the latter, *Xyala* sp.1 and Xyalidae gen. 1 were also over-dispersed in the sandbar. However, other herbivorous genera did not follow the same pattern: *Desmodora*, *Microlaimus* and *Spirinia* (all trophic group 2A) were over-dispersed in the sandbar and it is not clear why passive transport would not affect them in the same way.

Perepsilonema was over-dispersed in both habitats, and this may be related to its small size, characteristic of the family Epsilonematidae. Epsilonematids are very small nematodes (0.3-0.5 mm) shaped as the Greek letter Epsilon (Warwick et al. 1998) and have a strong tendency towards clumped distributions (Somerfield et al. 2007); which is consistent with *Perepsilonema* being over-dispersed in both micro-habitats. Previous studies suggest that mainly small nematodes move and settle actively (Ullberg & Olafsson 2003), whereas others indicate that not only

small but also larger species tend to form aggregations (Gallucci et al. 2008). This was the case in our study, in which significantly aggregated distributions were not restricted to particularly small nematodes. *Daptonema*, *Elzalia*, *Xyala* sp. 1 and 2 and Xyalidae gen. 1 (all in the family Xyalidae), range from 0.5 to 2 mm in size (Warwick et al. 1998). The same holds for *Nannolaimoides* (Cyatholaimidae), reaching 1.5 to 2 mm in size. *Desmodora* sp.1, *Metachromadora* and *Spirinia* (all in the family Desmodoridae), can reach sizes of 1 to 4 mm (Platt & Warwick 1988).

Some over-dispersed genera, but not all, possessed anatomical adaptations for locomotion and attachment to sediment grains. Attachment to sediment grains would prevent entrainment of nematodes in flow regimes incapable of sediment re-suspension. In more energetic environments, attachment to sand grains would provide faster settlement rates at shorter dispersal distances after re-suspension, relative to unattached nematodes. *Perepsilonema* exhibits aberrant positions of glandular outlets, attributed to their aberrant locomotion patterns (Raes et al. 2006). Caudal glands are a plesiomorphic character present in almost all aquatic nematodes, and play a fundamental role in active locomotion (Adams & Tyler 1980, Turpeenniemi & Hyvarinen 1996, Raes et al. 2006). Possibly the position of glandular structures and the presence of ambulatory setae in some genera (Platt & Warwick 1988, Raes et al. 2003) make Epsilonematids particularly good competitors adhering to sediments around food patches. Among the other over-dispersed genera, the (most likely new) species Spirinia in our samples exhibited conspicuous large somatic setae, similar to *Spirinia gerlachi* (Luc & De Coninck 1959) or *S. gnaigeri* (Ott, 1977). Such setae or tubes are thought to have an adhesive function in other nematodes (Decraemer et al. 1997); which opens the possibility that in Spirinia they function as anchors between sediment grains. Although *Perepsilonema* and *Spirinia* exhibit anatomical characters clearly relatable to active displacement favoring aggregations, nothing similar was detected in the other over-dispersed genera. The latter may exhibit less conspicuous adaptations facilitating active locomotion and/or adhesion to sediments.

Our results show that along-shore spatial distribution patterns differ in nearby micro-habitats exhibiting distinct hydrodynamic regimes and beg the need for detailed studies to unravel the underlying physical and biological processes. Additional replicate observations from runnel- and ridge-transects will greatly help to establish the generality of these patterns. Nevertheless, our results have important implications for sampling designs addressing cross-shore variability. Although this is a critical issue, very few authors justify the distance among stations in their sampling designs. Station separation along transects has been set to <1 m (Moreno et al. 2006, Gingold et al. 2010), 5 m (Gheskiere et al. 2004), 10 m (Mundo-Ocampo et al. 2007) or > 20 m (Gheskiere et al. 2005) without a clear rationale. Extrapolations from small sampling grids to wider areas (*e.g.*, whole beaches) should be addressed with caution. We therefore suggest pilot studies evaluating the spatial patterns of the community under study, prior to final sampling, as this may help greatly in understanding the main underlying processes and further interpretation of the data.

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Chapter IV

Is high diversity an insurance against thermal stress? Assessing the response of a meiofaunal community in a microcosm experiment

IV. 1. ABSTRACT

Biodiversity has diminished dramatically over the past decades with climate change being among the main responsible factors. Increasing sea surface temperature is one of its consequences, and may put ecological services at risk due to changes of community patterns and to the loss of species. Several associations between biotic diversity and ecosystem function have been proposed, among them the model based on functional redundancy where species' functions overlap, allowing high diversity communities to maintain the functioning of the system in the case of species loss. In this study we assess the response to thermal stress of marine nematode communities exhibiting contrasting levels of taxonomic diversity. According to the Insurance Hypothesis (IH) and the prediction that high diversity may beget stability, we hypothesized that the more diverse community would be more able to maintain its functionality than the less diverse, even though both communities would loose taxonomic richness under stressful conditions. We exposed natural intertidal communities to elevated temperature in a microcosm experiment. In order to separate the real temperature from the enclosure effect, we established two control groups: one at the beginning of the experiment, and one with a normal temperature treatment. To evaluate the function of the system, we assessed functional diversity of the community and biomass as an indirect proxy for secondary production. Our results do not support

the IH but rather suggest that each species contributes to the functioning according to the Rivets model. Although both assemblages lost species due to the high temperature, the high diversity assemblage suffered the larger impact on the functioning by loosing the trophic group of large predators and omnivores, which may have important consequences for the benthic food web. The low diversity assemblage consisted of an original species pool of stress-resistant species, presumably due to the fact that it stemmed from a more exposed part of the beach. This indicates, that species identity rather than diversity *per se* may play an important role for stress resistance. Our results are in concordance with other studies relating benthic diversity with ecosystem functioning, indicating that the relationship is much more complex. We suggest that sophisticated microcosm experiments with meiofaunal communities provide a promising tool for further studies on this highly relevant subject.

IV. 2. INTRODUCTION

Biodiversity has diminished dramatically over the past decades with climate change being among the main factors responsible for it (Jokiel & Brown 2004, Brierley & Kingsford 2009). Increasing sea surface temperature is one of the consequences of climate change, and possibly the most pervasive of present-day impacts on marine systems (IPCC 2007). In intertidal areas, the combination of high water-temperature and long exposure to high air-temperature during spring tides may exceed the tolerance of some intertidal organisms causing local extinctions (Brierley & Kingsford 2009). Ecological services provided by intertidal organisms such as water filtration and nutrient recycling (De Mesel et al. 2006, Ieno et al. 2006) may be at risk due to changes of community patterns and the loss of species.

Several associations between biotic diversity and ecosystem function have been proposed (Peterson et al. 1998) and there is still an ongoing debate about

the question of whether it is taxonomic diversity at one trophic level or complex interactions across trophic levels that are the main drivers of ecosystem functioning (McCann 2000 and references therein, Ieno et al. 2006). Available models can be assigned to three different concepts: The first is based on a linear relationship between species richness and ecosystem functioning, also referred to as the “rivets hypothesis”. Rivets are represented by species holding together a complex machine, whose functioning is impaired as rivets (species) fall out (Darwin 1859, MacArthur 1955, Ehrlich & Ehrlich 1981). The second is based on the idiosyncratic contribution of each species, which implies that ecosystem functioning changes along with diversity, but the magnitude and direction of change are unpredictable and depend on which species are lost and their relative contribution to ecosystem functioning (Lawton 1994, Peterson et al. 1998). Finally, the third is based on functional redundancy, which means that a minimal or threshold level of diversity is necessary for proper ecosystem functioning but beyond that, species are redundant in their roles and therefore expendable. In this case, species loss does not necessarily compromise ecosystem functioning and the two are uncorrelated until that critical threshold is attained (Walker 1992). The compensation potential in the case of functional overlap or redundancy allows the maintenance of ecological processes when species are lost and as such may represent a critical feature of ecosystems (Naaem 1998). This non-linear, positive relationship between diversity and ecosystem functioning and stability is the basis of the "Insurance Hypothesis" (Yachi & Loreau 1999).

Most experimental studies indicate that higher diversity leads to increased ecosystem functioning and higher temporal stability and resilience. Highly diverse microbial communities show higher productivity, maintain higher levels of diversity over time, as well as consistent levels of biomass, density and ecosystem respiration unlike those with lower diversity (Naeem & Li 1997, McGrady-Steed et al. 1997, Wohl et al. 2004). The nonlinear relationship between microbial diversity and ecosystem functioning implies functional redundancy among species (McGrady-Steed et al. 1997). Similar observations on terrestrial plant communities

and a variety of marine ecosystems confirm the positive relationship between species diversity and ecosystem functioning (Cardinale et al. 2006, Tilman et al. 2006, Worm et al. 2006, Stachowicz et al. 2008). However, idiosyncratic contributions of species have also been observed (Emmerson et al. 2001, Bolam et al. 2002, Cardinale et al. 2006, Stachowicz et al. 2008). Not only ecosystem functioning, but also resistance to and recovery from environmental stress (droughts in plants, high temperature in aquatic microbes) increase with increased species richness (Tilman & Downing 1994, Leary & Petchey 2009, van Ruijven & Berendse 2010), although there is also evidence for the contrary (Pfisterer & Schmid 2002, Naeem 2002). On the other hand, contrary to theoretical diversity-stability predictions, recovery from heat stress in marine algal communities depends on the presence of certain species and not on species richness *per se* (Allison 2004). These examples show that diversity effects on the functional aspects of the community are varied and differ among systems, hence the debate is far from settled. Consequently, further empirical evidence on a variety of systems is needed.

Free-living marine nematode communities provide highly suitable research models, as they can be easily manipulated and maintained over considerable time allowing population development and interaction of species (Austen & Warwick 1995, Schratzberger & Warwick 1998, Gallucci et al. 2008, Dos Santos et al. 2009). In intertidal regions they are functionally and taxonomically diverse playing a fundamental role in the benthic ecosystem as nutrient recyclers and as a trophic link between microorganisms and macrofauna (Hamerlynck & Vanreusel 1993, Coull et al. 1995, Li et al. 1997, Hamels et al. 2001, Olafsson 2003). Apparently, many functionally similar or equivalent species co-exist temporally and spatially (De Mesel et al. 2006, Derycke et al. 2006). However, it is unclear to which extent single species are redundant. On the one hand, a general linear positive relationship between taxonomic and functional diversity and experimental results from simplified food webs, suggest a considerable contribution of each species to ecosystem functioning (De Mesel et al. 2006, Schratzberger et al. 2007). On the

other hand, *in situ* decomposition rate of organic matter in a sandy beach did not correlate with nematode diversity in experimental litterbags, but with circumjacent beach diversity, suggesting redundancy in the former, but not in the latter (Urban-Malinga et al. 2008). Understanding the relationship between species richness and their function is particularly important for benthic organisms such as intertidal nematodes, as they are prone to exceptional environmental stress ensuing from increased anthropogenic pressure from both the marine and terrestrial side (Schlacher et al. 2008).

In this study we assess the response to thermal stress of marine nematode communities exhibiting contrasting levels of taxonomic diversity. According to the Insurance Hypothesis and the prediction that high diversity may beget stability, we hypothesized that the more diverse community would be more able to maintain its functionality than the less diverse, even though both communities would loose taxonomic richness under stressful conditions. To test this hypothesis, we exposed natural intertidal communities drawn directly from their environment to elevated temperature in a microcosm experiment. In order to separate the real temperature from the enclosure effect, we established two control groups: one at the beginning of the experiment, and one with a normal temperature treatment. To evaluate the function of the system, we assessed functional diversity of the community and biomass as an indirect proxy for secondary production. Functional diversity was assessed by categorizing species in different trophic groups. This method is straightforward and allows for an approximate assessment of functional overlap when two species belong to the same trophic guild. The communities of the present study originate from a beach of the northern Gulf of California, one of the world's marine endemism and biodiversity hotspots (Roberts et al. 2002). This semi-enclosed sea is exceptionally prone to sea temperature increase (8˚C over the past century, Julliet-Leclerc et al. 1991), putting at stake a high number of marine species (Stillman 2003). Hence, this study addresses at a local scale the globally and highly relevant issue of sea temperature rise (IPCC 2007, Humphrey et al. 2008).

IV. 3. MATERIAL AND METHODS

IV. 3. 1. Sampling site and strategy

Samples were taken September 1st 2008 during low spring tide at *El Tornillal* in the Upper Gulf of California, Mexico (UGC; Fig. 7). *El Tornillal* is a 600 m wide dissipative ridge-and-runnel macrotidal beach (Masselink & Short 1993). We chose two stations fulfilling two important criteria based on previous knowledge of the beach (Gingold et al. 2010): 1) they hosted communities differing in diversity (*i.e.*, species richness and Shannon's diversity index) but not in abundance, and 2) they did not differ in important environmental characteristics such as mean grain size, organic matter content and microphytobenthos. The two stations were located on different sandbars in the upper half of the middle beach (Gingold et al. 2010). The station closer to the waterline was the "high diversity" site (HD henceforth), and the one closer to the dunes the "low diversity" site (LD henceforth; Fig. 13). Differences in taxonomic diversity between sites were verified *in situ* at the time of sampling under a Leica Zoom 2000 stereoscope. Twenty sediment cores were taken to a depth of 10 cm at each of the two sites with a metal corer (10.8 cm in diameter) and carefully transferred to microcosm containers (Fig. 13 a-d). All microcosms were immediately placed in cooler-boxes filled with cool seawater and brought as fast as possible to aquaculture facilities at CICESE.

In addition to the microcosm cores, four sediment samples were taken with a small corer (9.8 cm long by 2.9 cm in diameter) to evaluate the nematode community composition and diversity in the field at the time of sampling. These samples were fixed immediately in 5% formalin. In order to characterize the habitat of both locations, four replicate samples were taken for each of the following analyses: 1) granulometry, 2) organic matter and 3) microphytobenthos. Core size for granulometry and organic matter was 9.8 cm long by 2.9 cm in diameter, and for microphytobenthos 1 cm long by 1 cm in diameter. Granulometry and organic matter samples were kept under ice in the field, and then frozen at – 20°C until

processed. Chlorophyll samples were kept in dark tubes under ice in the field, and then stored at – 40°C until processed. Water temperature at time of sampling was 31°C.

IV. 3. 2. Experimental setup

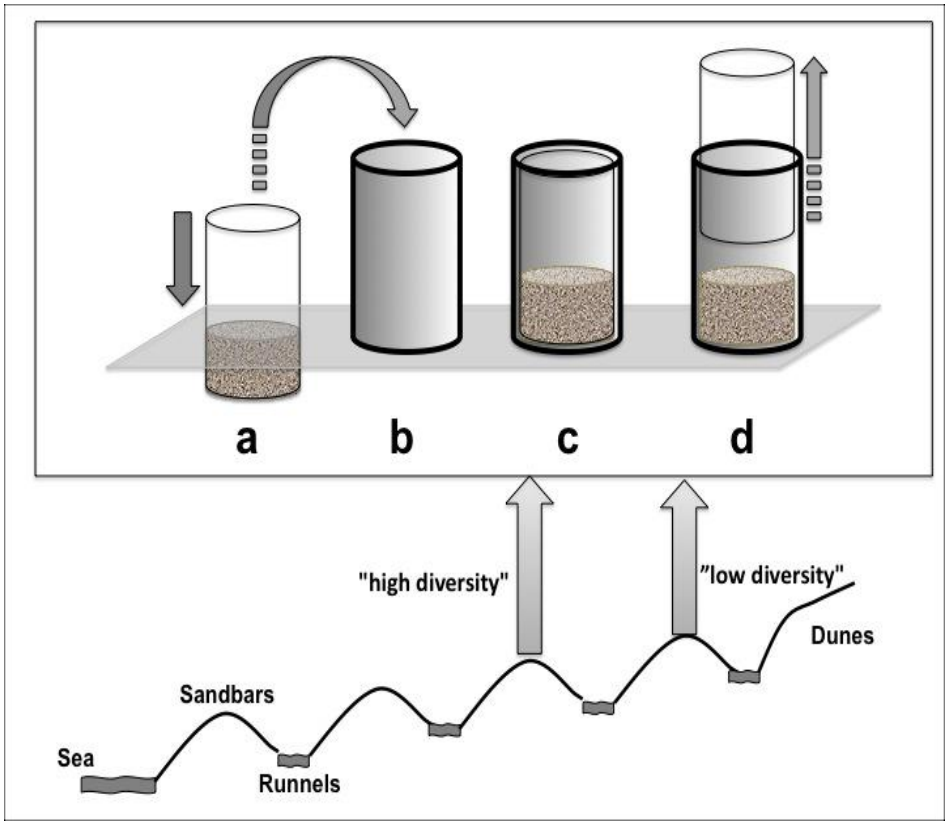

Figure 13. Sampling strategy in the field. 40 sediment cores were sampled at two stations (20 cores each) located on different sandbars of the intertidal, and hosting communities of high or low diversities respectively. (a) Samples were taken with a metal cylinder to 10 cm depth. (b) Entire sediment cores were placed into containers. (c) The sampling corer fitted exactly in the container so that the internal structure of the sediment core remained as intact as possible. (d) The metal corer was carefully removed.

In the laboratory, four microcosms (two HD and two LD) were randomly assigned to ten 100 l tubs. Previously, the tubs had been filled with filtered seawater and maintained at 31°C. All microcosms were acclimated to experimental

conditions at 31˚ for 5 days. Temperature was maintained constant with thermostats (1000 W titanium heater for high, and regular 250 W heaters for the normal temperature), and the water was homogenized with bubbling air stones placed next to the heaters. All microcosm containers were closed with a transparent plastic lid and water oxygenation and circulation inside containers was achieved by bubbling air with an air stone through a hole in the lid.

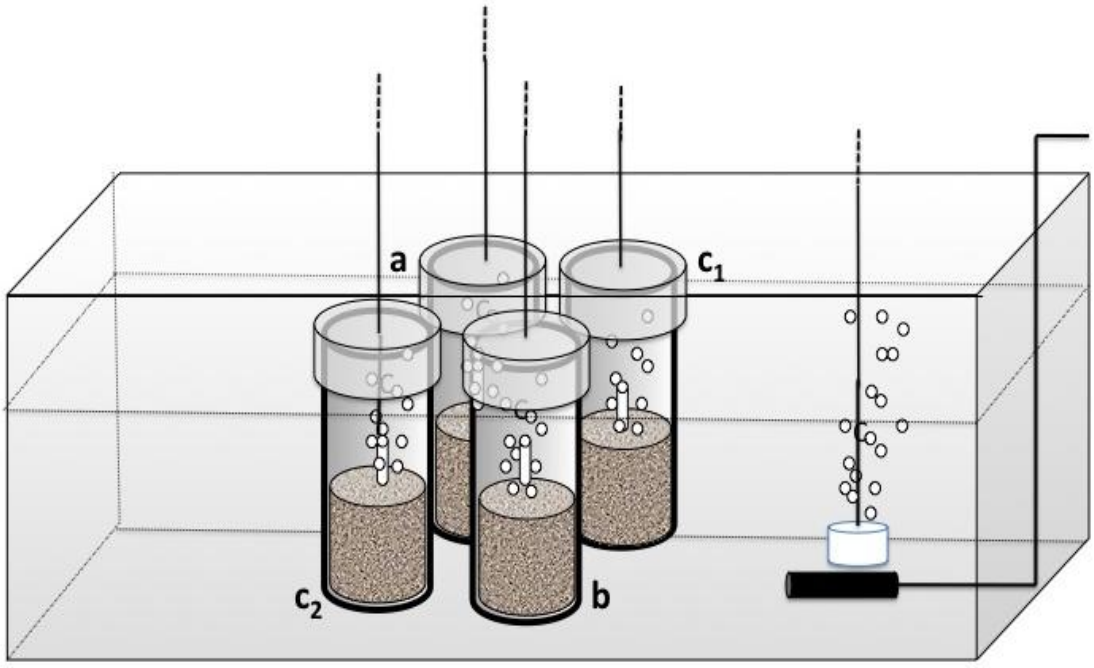

Figure 14. Experimental design: 4 microcosm containers were placed in each of 10 tubs. The live control (a) was used to monitor nematodes throughout the experiment. The $time_0$ control (b) was analyzed at the start of the experimental treatment when temperature was changed in the high temperature treatments. The experimental groups of high (c_1) and low (c_2) diversity were kept at constant temperature throughout the experiment: elevated (36˚C) for the test group and normal (31˚C) for the "temperature control" group. Each experimental group was replicated 5 times.

After the acclimation period, temperature was gradually (24 h) augmented to 36°C in the five randomly assigned high temperature tubs. This temperature is above the highest recorded mean summer temperature (31.1˚) but within the range of future (50-100 years) temperatures that could be reached following linear

extrapolations of *in situ* temperatures over the past 40 years (M. Lavín, pers. comm.). As soon as temperature reached 36˚C, one of the four containers was removed from each tub (five replicates from the HD and five from the LD group respectively). These microcosms represent the "time zero control" (t_0) at the start of the experimental treatment (Fig. 14b). The remaining two microcosms were sampled at the end of the experiment (Fig. 14 c_1 and c_2). The experimental treatment consisted in high temperature incubation (maintained at 36˚C), whereas the experimental control was incubated at field temperature (maintained at 31˚C). Henceforth, abbreviations of the different groups (*i.e.*, diversity levels and treatments) will be used according to Table VII.

Table VII. Abbreviations for experimental groups.

	Low diversity	High diversity
Time zero control [1]	LDt_0	HDt_0
Normal temperature	LD_{31}	HD_{31}
High temperature	LD_{36}	HD_{36}

[1] Samples taken at the time in which high temperature treatments reached their target temperature (36°C).

The experiment was run for 25 days, after 5 days of acclimation and 1 of gradual temperature change. Microcosms were checked daily and salinity was maintained constant (ca. 35‰), compensating evaporation by adding reverse osmosis purified water. Tubs were refilled daily to maintain their water level constant. The experiment was stopped when clear signs of anoxic conditions in the high temperature treatment affected nematode populations. At the end of the experiment, whole microcosms were fixed in 5% formalin.

During the course of the incubation, one of the four microcosms in each tub served as a "live control" to continuously monitor and record activity of nematodes (Fig. 14a). Each of these ten live controls was sub-sampled once at regular

intervals from the acclimation period to the end of the experiment. The microcosms themselves were left in the tubs after sub-sampling. Sampled organisms were immediately checked under a light stereoscope.

IV. 3. 3. Faunal analyses

Fixed samples were rinsed with freshwater over a 45 mm sieve. Meiofauna were extracted by suspension in colloidal silica (LUDOX^{TM}, specific density 1.15) following De Jonge and Bouwman (1977) and stored in 80 ml 5% formalin. Nematodes were counted in five aliquots of 5 ml (25 ml in total per sample) using a counting dish under a Leica Zoom 2000 stereoscope. Nematode density (ind. 10 cm^{-2}) was calculated by the mean abundance of the five aliquots and extrapolated to total abundance based on the fraction (31.25%) of the volume of each aliquot relative to the total sample. All aliquots were transferred to a 5 % glycerol solution and slowly evaporated on a heating plate. The first 100 randomly picked nematodes were mounted on permanent slides for identification using an OLYMPUS BX51 compound microscope with differential interference contrast (DIC) optics. Nematodes were identified to the species or genus level where possible, using pictorial keys (Platt and Warwick 1983; Platt and Warwick 1988; Warwick et al. 1998). In the case where male reproductive organs were essential to determine the genus, juveniles and females were determined in the most conservative way: if possible, they were identified as the genus that resembled most in the other characteristics; alternatively they were identified to family level. If the species could not be determined, they were labeled sp. 1, sp. 2. Pictures were taken of all individuals for morphometric and biomass analyses.

IV. 3. 4. Habitat characterization

IV. 3. 4. 1. Granulometry

Samples were first treated with 30 % peroxide (H_2O_2) to oxidize organic matter. After rinsing gently with distilled water and drying at 60° C they were sieved through a stack of Wentworth grade sieves (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 Φ, where $\Phi = -\log_2$ [grain diameter]) and the dry weight of each fraction was obtained (Bale & Kenny 2005).

IV. 3. 4. 2. Organic matter

Samples were treated with 10 % HCl to dissolve inorganic carbonates (mainly $CaCO_3$; Froelich 1980), rinsed thoroughly with fresh water, freeze-dried and then combusted at 550° C for 24 hours (Dean 1974). Organic matter was computed as the difference in dry weight before and after combustion and standardized to percentage of total dry weight before combustion.

IV. 3. 4. 3. Chlorophyll

Phytobenthic chlorophyll was extracted by grinding sediment samples in 90% acetone, extracting for 24 hrs in the dark and then centrifuging at 3,000 rpm for 10 minutes. Absorbance of the supernatant was measured at 665 and 750 nm before and after acidification with a few drops of 10 % HCl (Spectrophotometer Ely-2000, Elyptica, Ensenada B.C.). Chlorophyll density was calculated following Lorenzen (1967) and Colijn & Dijkema (1981) and is expressed in mg m^{-2}.

IV. 3. 5. Statistical analyses

To analyze differences in nematode assemblages among experimental groups we applied Analyses of Similarity (ANOSIM) on Bray-Curtis similarities. ANOSIM is conceptually similar to ANOVA but makes no assumptions about the distribution of the data. The test statistic R = 1 if all replicates within groups are more similar to each other than to any replicate from different groups, whereas R = 0 if similarities within and among groups are the same on average. Analysis of Similarity Percentage (SIMPER) was applied to assess the species that contributed the most (*i.e.*, were the most "typical") to each of the assemblages.

We used Student's t-tests to assess differences in community and environmental attributes between the two field stations. Differences in community attributes of experimental groups (= response variables, see next paragraph) were tested with ANOVAs. Given that our main predictions related to the independent effect of temperature in each diversity group, we applied 1-way ANOVA separately to test the hypotheses H_0: m-xDt_0 = m-xDt_{31} = m-xDt_{36}, where m is the mean response variable and x refers to L (low diversity) or H (high diversity) (Table VII). Rejection of H_0 was further investigated using Dunnett test for multiple comparisons (Zar 1984), taking xDt_0 as the control group. Rejection of H_0: m-xDt_0 = m-xDt_{36} was interpreted as evidence of significant high temperature effect with or without significant enclosure effect, the latter was assessed by the rejection of H_0: m-xDt_0 = m-xDt_{31}. If both effects were significant, t-tests between xD_{31} and xD_{36} were performed in order to assess whether both effects were additive (in case of significance) or whether the enclosure effect was dominant (in case of non-significance). The independence of temperature (time$_0$, 31˚C and 36˚C) and diversity (high and low) effects on response variables was verified by assessing the non-significance of interaction terms of a 3x2 factorial Type III ANOVA, with temperature as fixed and diversity as random effects. Homoscedasticity was verified with Bartlett's test and normality with the Kolmogorov-Smirnov test (Sokal & Rohlf 1995). In the presence of heteroscedasticity data were log-transformed.

The community attributes (response variables) tested were the number of species (species richness, *S*), taxonomic diversity (Diversity index of Shannon Wiener, *H'*), number of individuals per 10 cm^{-2} (abundance), trophic diversity (Index of trophic diversity, ITD^{-1}) and biomass. The index of Shannon Wiener was calculated as $H' = -\sum_i p_i \ln(p_i)$, where p_i is the proportion of the total count arising from the *i*th species. The Index of trophic diversity was modified from Heip (1985) applying the formula $1/\sum\theta^2$, where θ is the fraction of each of the four functional groups. It is therefore presented as ITD^{-1}. It ranges from 1 (when one functional group contributes 100% and functional diversity is lowest) to 4 (when each functional group contributes 25% and functional diversity is highest). Biomass was calculated following a slightly adapted version of Andrassy's formula (1956):

$$\text{Biomass (in µg wet weight)} = (LW^2/1.7)\, NRd * 10^3 \qquad (6)$$

where *L* = total body length (mm), *W* = body width (average body diameter in mm), *NRd*= relative density, estimated at 1.13 for marine nematodes (Somerfield et al. 2005). Nematodes were measured using the software ImageJ.

To visualize the relative contribution of the characteristic species of each experimental group, we plotted results from SIMPER (90% cumulative percentage) in a doughnut chart; we chose trophic groups as the functional units, according to Wieser (1953): 1A (selective deposit and bacteria feeders with small, unarmed buccal cavities), 1B (non-selective deposit feeders with unarmed wide buccal cavities), 2A (epistrate feeders with lightly armed small buccal cavities) and 2B (carnivores and omnivores with wide armed buccal cavities).

PRIMER version 6 (Clarke & Warwick 1994, Clarke & Gorley 2001) was used for multivariate analyses. STATISTICA (StatSoft 1993) was used for univariate analyses.

IV. 4. RESULTS

IV. 4. 1. Biological and environmental differences between source communities in the field

The two sampled stations hosted different nematode assemblages (ANOSIM, R=1, p=0.029); the HD assemblage had significantly higher species richness, diversity (*H'*), trophic diversity (ITD^{-1}), and abundance (individuals per 10 cm^2) (Table VIII, Fig. 15 a-d).

Table VIII. Student's t-test of community attributes between the two field sampling stations.

	t	p
Species richness	7.44	<0.001
Diversity[1]	11.28	<0.0001
Abundance	5.55	0.002
Trophic diversity[2]	11.04	<0.0001

[1] Shannon Wiener diversity index

[2] Index of trophic diversity ITD^{-1}

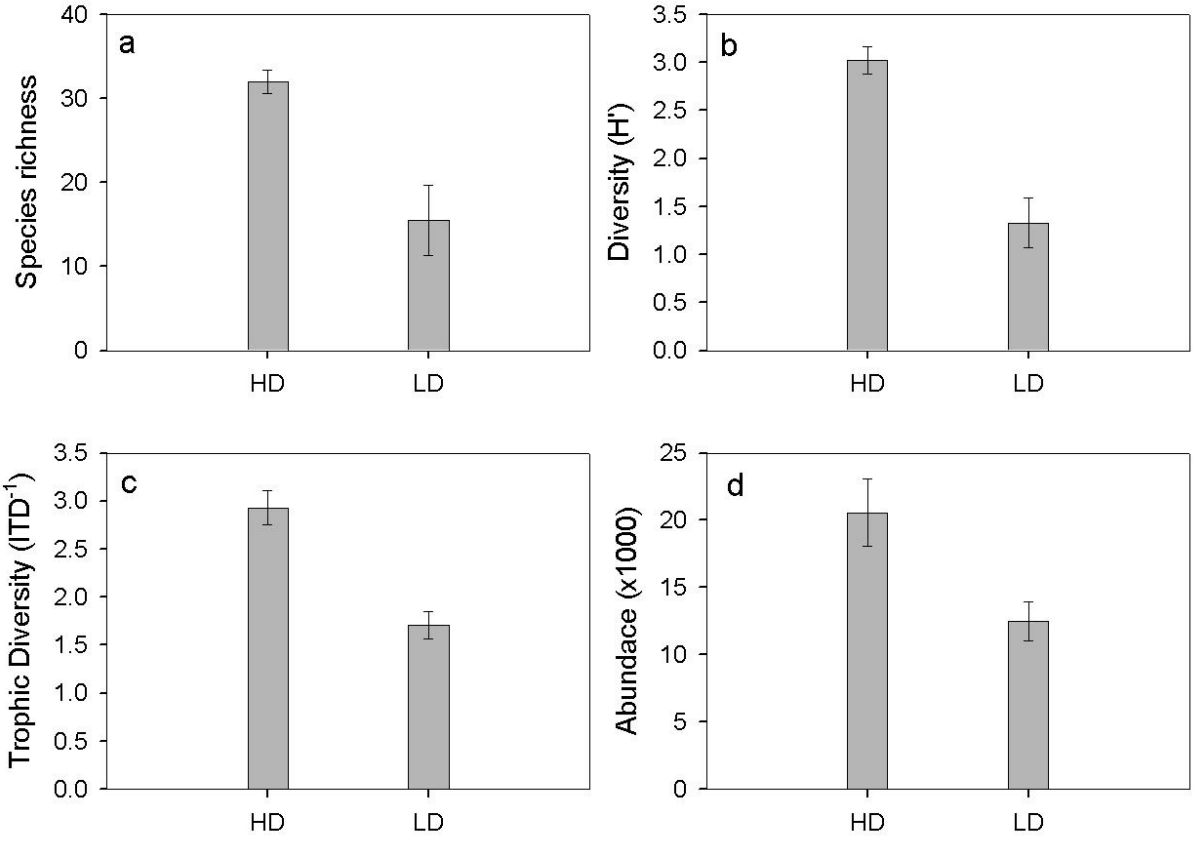

Figure 15. Attributes of the two field assemblages: Mean (± Standard deviation) of (a) species richness (S; numbers of species), (b) diversity (H'; Index of Shannon Wiener), (c) trophic diversity (IDT^{-1}) and (c) abundance (numbers of individuals per 10 cm^2)

SIMPER analysis revealed that the LD assemblage was largely dominated by *Perepsilonema* sp. (83.61%, Fig. 16a, Table IX). Considering all species (and not only the cumulative 90% represented in the SIMPER analysis), the two assemblages shared 20 species. In addition, 12% of all species were unique to the LD whereas 31% were unique to the HD. *Perepsilonema* sp., *Microlaimus* sp. 2, *Metachromadora* sp. 2 and *Chromadorina* sp., making up 90% of the discriminating species of the LD (*i.e.*, being the most typical species), were also

present at the HD with exception of *Chromadorina* sp. (*Microlaimus* sp. 2 was not part of the discriminating species shown in table IX). Thus, the large difference between the two assemblages revealed by ANOSIM is mainly due to a) the dominance of a single species (*Perepsilonema sp.* contributed almost 40% to the dissimilarity between the two assemblages) and b) the large number of unique species in the HD.

Table IX. Percentages contribution (%) of the top 90% discriminating genera of the two field stations and the six experimental groups

FIELD

	HD[1]	**%**		**LD**[2]	**%**
1A[3]	*Tricompa* sp. 1	5.38	**1A**	*Perepsilonema* sp.	83.61
	Perepsilonema sp.	5.1	**2A**	*Microlaimus* sp. 2	4.36
	Ceramonema sp. 2	1.7		*Metachromadora* sp. 2	1.86
1B	*Rhynchonema* sp.	6.23		*Chromadorina* sp.	1.66
	Xyala sp. 2	5.1			
	Xyala sp. 1	4.53			
	Omicronema sp.	2.55			
2A	*Metachromadora* sp. 1	19.83			
	Desmodora sp. 1	7.37			
	Chromadorita sp. 1	6.51			
	Metachromadora sp. 2	6.23			
	Pomponema sp.	6.24			
	Dichromadora sp.	2.55			
	Hypodontolaimus sp.	2.27			
2B	*Epacanthion* sp.	2.83			
	Odontophora sp.	1.99			
	Chromaspirinia sp.	1.98			
	Enoploides sp.	1.69			

EXPERIMENT

Time$_0$	**HD**	**%**		**LD**	**%**
1A	*Tricoma* sp. 1	3.5	**1A**	*Perepsilonema* sp.	50

Table IX (continued). Percentages contribution (%) of the top 90% discriminating genera of the two field stations and the six experimental groups

EXPERIMENT

Time$_0$	**HD**[1]	**%**		**LD**[2]	**%**
1A	*Perepsilonema sp.*	2.53	**1B**	*Rhynchonema sp.*	5
	Calomicrolaimus	1.56		*Theristus sp. 2*	3.23
	Ceramonema sp. 3	1.17		*Theristus sp. 1*	2.58
1B	*Xyala sp. 2*	12.65	**2A**	*Desmodora sp. 1*	8.55
	Xyala sp. 1	6.03		*Microlaimus sp. 2*	5.97
	Xyalidae gen. 1	3.31		*Metachromadora*	3.87
	Richtersia sp.	2.53		*Microlaimus sp. 1*	1.94
	Rhynchonema sp.	2.33		*Pomponema sp.*	1.94
	Omicronema sp.	1.36	**2B**	*Gammanema sp.*	4.68
2A	*Metachromadora sp. 1*	26.85		*Adoncholaimus sp.*	2.42
	Pomponema sp.	8.95			
	Metachromadora sp.2	5.84			
	Desmodora sp.1	4.47			
	Chromadorita sp. 1	1.36			
	Microlaimus sp. 1	1.17			
2B	*Chromaspirinia sp.*	1.95			
	Epacanthion sp.	1.75			
	Enoploides sp.	1.56			

Normal T (31°)	**HD**[1]	**%**		**LD**[2]	**%**
1A	*Tricoma sp. 1*	8.01	**1A**	*Perepsilonema sp.*	**17.77**
	Ceramonema sp. 3	2.98		*Ceramonema sp. 3*	**2.26**
	Calomicrolaimus sp.	2.23	**1B**	*Xyala sp. 1*	**4.53**
	Perepsilonema sp.	1.68		*Rhynchonema sp.*	**4.01**
	Ceramonema sp. 2	1.12		*Theristus sp. 2*	**3.48**

Table IX (continued). Percentages contribution (%) of the top 90% discriminating genera of the two field stations and the six experimental groups

Normal T (31°)	HD[1]	%		LD[2]	%
1B	*Xyala* sp. 1	10.24		*Theristus* sp. 1	2.26
	Xyala sp. 2	10.24	**2A**	*Desmodora* sp.1	32.4
	Rhynchonema	8.19		*Microlaimus* sp. 2	8.01
	Xyalidae gen. 1	4.66		*Microlaimus* sp. 1	4.18
	Richtersia sp.	3.35		*Pomponema* sp.	3.66
	Sabatieria sp.	2.23		*Paracyatholaimus* sp.	2.26
	Omicronema sp.	2.61	**2B**	*Gammanema* sp.	7.32
	Stylotheristus sp.	1.68			
2A	*Desmodora* sp.1	15.27			
	Pomponema sp.	6.33			
	Kraspedonema sp.	2.23			
	Metachromadora sp. 2	1.86			
	Metachromadora sp. 1	1.68			
	Spirinia sp. 1	1.3			
2B	*Chromaspirinia* sp.	2.42			

High T (36°)	HD[1]	%		LD[2]	%
1A	*Perepsilonema* sp.	15.52	**1A**	*Perepsilonema* sp.	43.96
	Tricoma sp. 1	14.48		*Ceramonema* sp. 3	7.25
	Ceramonema sp. 3	3.79	**2A**	*Microlaimus* sp. 1	22.54
1B	*Richtersia* sp.	22.24		*Desmodora* sp.1	8.21
	Xyala sp. 2	3.97	**2B**	*Gammanema* sp.	9.98
	Sabatieria sp.	3.62			
2A	*Desmodora* sp. 1	20.86			
	Metachromadora sp. 1	3.28			
	Microlaimus sp. 1	3.79			

[1]HD=High diversity

[2]LD=Low diversity

[3]1A, 1B, 2A, 2B = Feeding groups according to the classification of Wieser (1953)

Trophic diversity was lower in the LD: nematodes accounting for 90% of the cumulative similarity only comprised two trophic groups, namely selective deposit feeders (1A) and epistrate feeders (2A). The predominance of selective deposit feeders was again due to the predominance of *Perepsilonema sp*. (table IX).

Environmentally, the two stations differed slightly but significantly only in sediment mean grain size (Table X).

Table X. Student's t-test of environmental variables between the two field sampling stations

	t	p
Mean grain size	10.64	<0.0001
Organic matter content	1.95	0.1
Chlorophyll	-0.0006	0.99

IV. 4. 2. Initial conditions of experimental units (*x*Dt_0)

At the start of the experimental treatment, the two assemblages (HDt_0 and LDt_0) were significantly different (ANOSIM R= 0.972, p=0.008). More species (19) contributed to the 90% cumulative similarity of the HDt_0 compared to the LDt_0 (11; Fig. 16b, table IX). The HDt_0 hosted a total of 57 species and more unique species (24) than the LDt_0 (total: 46, unique: 13). However, these differences were less pronounced than those observed in the field and non-significant for species richness, diversity (*H'*), abundance and trophic diversity (ITD^{-1}) (Fig. 17 a-d). A larger fraction of large nematodes in the HD assemblage accounted for the higher biomass (Fig. 17e).

Table XI. F- and p-values of 1-way ANOVAs for each diversity group. The factor (=treatment) consists of three levels: $time_0$, 31˚ and 36˚.

	High diversity		Low diversity	
	F	**P**	**F**	**p**
Species richness	18.13	0.0003	6.88	0.01
H' diversity	8.12	0.007	2.86	0.096
ITD^{-1}	0.08	0.92	0.95	0.41
Biomass	6.35	0.015	0.36	0.71
Abundance	50.41	0.000003	11.94	0.001

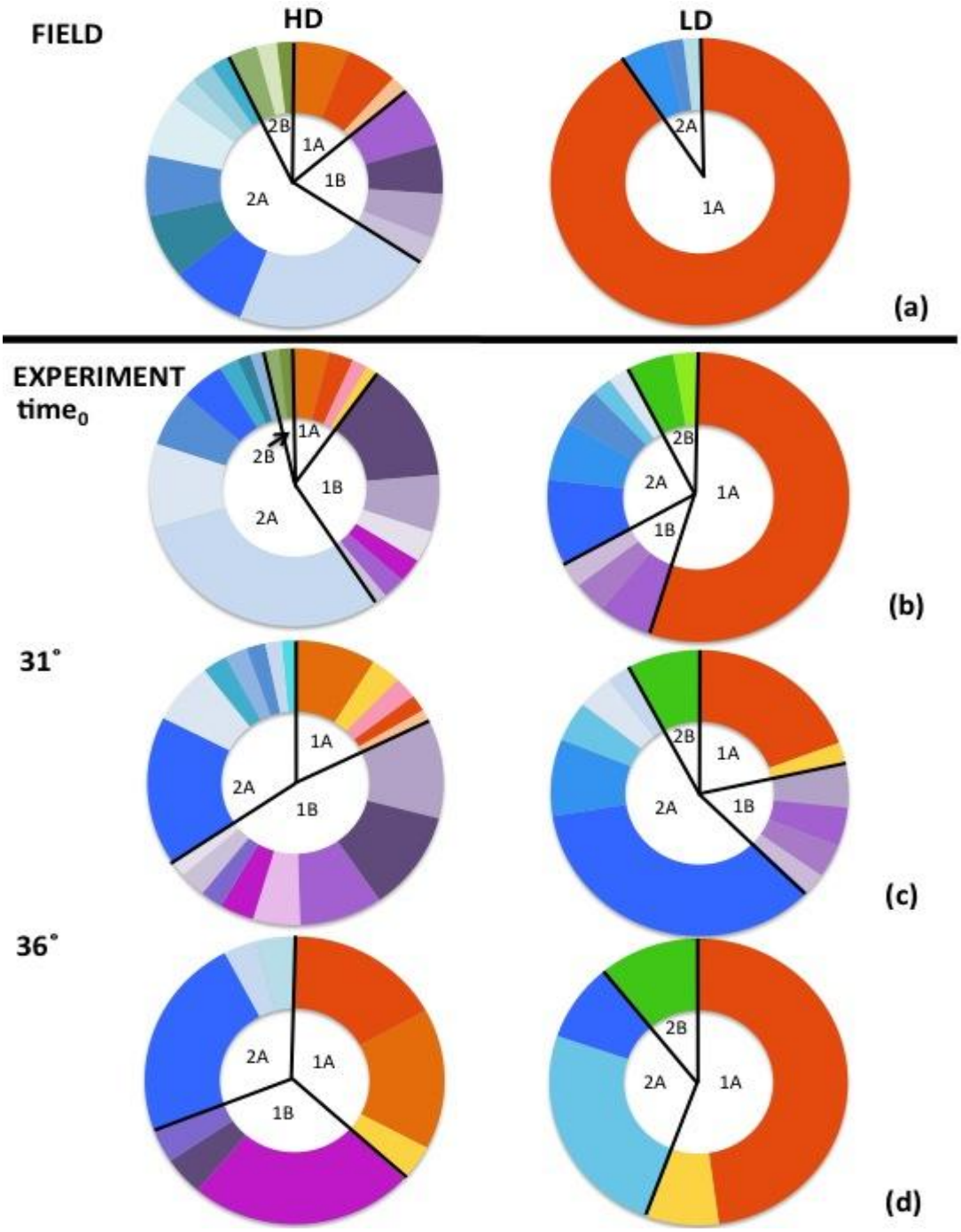

Figure 16. Graphical representation of the 90% contribution of "typical" species of each diversity group, calculated by SIMPER: Species are represented by different colors. The corresponding percentage of contribution is listed in table IX. HD=High diversity, LD=low diversity. (a) the two communities sampled in the field, (b) Experimental control groups (before the start of the experiment), (c) Assemblages exposed to normal temperature and (d) assemblages exposed to high temperatures.

IV. 4. 3. Structural changes in community resulting from high temperature effects: species richness and diversity

Non-significant differences in *S* and *H'* between t_0 and t_{31} microcosms revealed that significant differences between t_0 and t_{36} were attributable to high temperature exposure alone. High temperature had a clear effect in both HD and LD assemblages, but was stronger in the former. Significantly less species survived the high temperature exposure in both LD_{36} and HD_{36} relative to their t_0 controls (Table XI, Fig. 17a). Consequently, these microcosms exhibited decreased levels of diversity (*H'*) at the end of the 25 d incubation, albeit the change was significant only in HD_{36} (Table XI, Fig. 17b). In addition, SIMPER revealed that the dissimilarity between HDt_0 and HD_{36} was much higher (70.08) than the one between LDt_0 and LD_{36} (52.04), which was similar to those associated with the non-significant enclosure effect (HDt_0 – HD_{31}: 53.44; LDt_0 – LD_{31}: 51.48).

A few taxa became more dominant under the stressful treatment (Fig. 16d, table IX). *Perepsilonema* sp., *Tricoma* sp., *Richtersia* sp., Desmodora sp. 1 and *Metachromadora* increased in abundance in HD_{36}. *Sabatieria* sp. also increased in HD_{31} and HD_{36} appearing for the first time among the top 90% discriminating species (Fig. 16 c and d, table IX). *Microlaimus* sp. 1 and *Gammanema* sp. increased in the LD_{36}, and *Ceramonema* sp. 3 appeared in LD_{31} and LD_{36} among the 90% discriminating species (Fig. 15 c and d, table IX).

IV. 4. 4. Functional changes in community resulting from high temperature and enclosure effects: abundance, biomass and trophic diversity

Abundance decreased significantly in both, HD_{36} and LD_{36}, due to the combined effects of temperature and experimental enclosure (Fig. 17, table XI). In both, HD_{36} and LD_{36}, the loss of individuals was species specific, since the high

temperature also caused a significant loss of species richness (in both, HD and LD) and diversity (in HD). By contrast, the loss of individuals in the HD due to the enclosure effect was size specific, since biomass decreased exclusively in response the experimental enclosure effect. Two of the four species lost exclusively due to the enclosure effect, namely *Epacanthion* sp. and *Enoploides* sp., were among the largest found in this study. On the contrary, the loss of 6 species in the LD was not size specific, since biomass remained unaffected The community function of the high diversity microcosms (HD_{36}) was affected to a larger extent as it suffered the loss of an entire trophic level (2B) consisting of large-sized predators and omnivores (Fig. 16d, table IX). In contrast, the decrease in abundance of the LD_{36} microcosms was related to the loss of trophic group 1B consisting of size-unspecific, unselective deposit feeders (Fig. 16d, table IX). Even though both assemblages had lost an entire yet different trophic group, these changes were not reflected in significant differences in ITD^{-1} (Fig. 17, table XI). All three species representing 2B in HDt_0 disappeared, two of them (*Epacanthion* sp. and *Enoploides* sp.) due to the enclosure effect and one (*Chromaspirinia* sp.) due to the temperature effect. This is consistent with the loss of individuals (*i.e.*, decreased abundance) due to the enclosure effect on one and the temperature effect on the other hand. In the low diversity microcosms, of the two species representing 2B in at LDt_0 (*Gammanema* sp. and *Adoncholaimus* sp.), *Gammanema* sp. was temperature tolerant and even increased in relative abundance (Fig. 16 d, table IX). In contrast, the species of trophic group 1B in LDt_0 (*Rhynchonema* sp. and the two *Theristus* species) disappeared in the high temperature treatment (Fig. 16d, table IX). Many of the species representing 1B in the HDt_0 (*Xyala sp. 1* species, *Xyalidae gen.1*, *Rhynchonema* and *Omicronema*) were lost in the high temperature treatment, but *Xyala* sp. 2 and *Richtersia* sp. survived (Fig. 16d, table IX). Functional group 2A (epistrate feeders) were represented by 3 and 2 species in HD_{36} and LD_{36} respectively. In both, *Desmodora* sp. 1 (HD and LD), *Microlaimus* sp. 1 (HD and LD), and *Metachromadora sp. 1* (HD) survived the high temperature exposure (Fig. 16 d, Table IX).

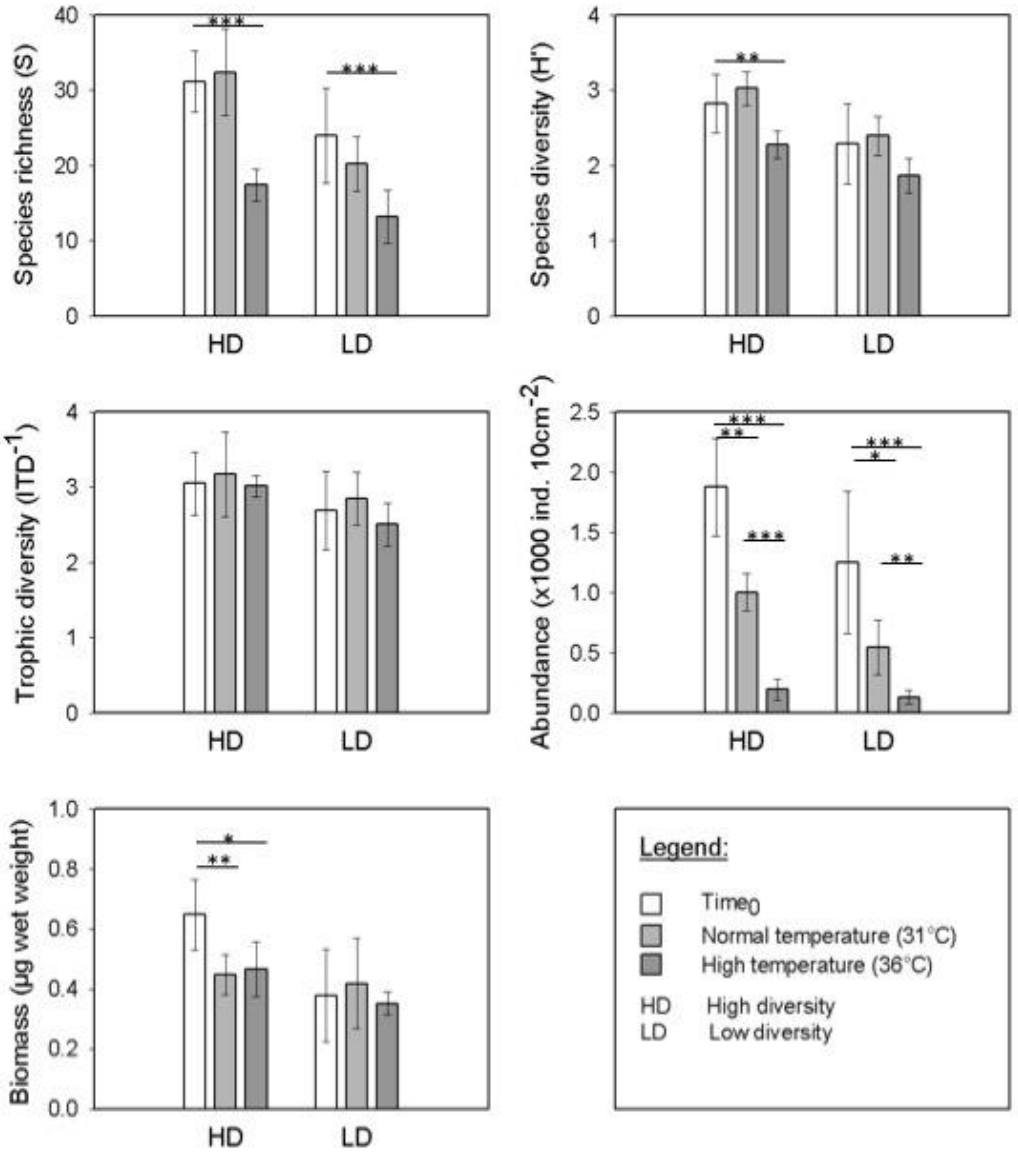

Figure 17. Assemblage attributes of the experimental groups: Mean (± standard deviation) of (a) species richness (*S*; numbers of species), (b) diversity (*H'*; Index of Shannon Wiener), (c) abundance (numbers of individuals per 10 cm-^{2}), (d) trophic diversity (*IDT*$^{-1}$) and (e) biomass (in µg wet weight). Asterisks indicate significance levels after multiple comparisons with Dunnett test between xDt_0 and xD_{31} and xD_{36} respectively. Significance between xD_{31} and xD_{36} were assessed with Student's t-tests. *<0.05, **<0.01, *<0.001.**

IV. 5. DISCUSSION

In this study we set out to test whether high diversity confers resistance to stress in marine nematode assemblages. According to the Insurance Hypothesis (IH), and general predictions that high diversity provides stability, we expected that despite a species loss, a community of high diversity would maintain its functional attributes due to functional redundancy of species, whereas loss of species in a low diversity community would impair its functioning. In order to test this hypothesis we exposed natural nematode assemblages of contrasting diversity levels to a stressful temperature. To our knowledge this is the first microcosm experiment with natural intertidal nematode assemblages addressing the subject of functional redundancy.

Our results do not support the IH but rather suggest that each nematode species contributes to the functioning, as predicted by the Rivets hypothesis (Ehrlich & Ehrlich 1981). Our expectation in the case of functional redundancy would have been that the HD microcosms would maintain their functionality, whereas the LD would have been impaired after a loss of species. In the case of idiosyncrasy, the number and identity of the species lost would not have allowed us to predict the direction and magnitude of change in system functioning. By contrast, the Rivets model predicts that a) a community of high diversity functions better than one of low diversity and b) the loss of functionality is contingent on the loss of species. Both predictions are met with our data: In its original state ($time_0$) HD has a higher biomass indicating that it can meet the energetic demand of big-sized species. This is consistent with general diversity-biomass patterns in nematode communities where high diversity communities tend to have a higher biomass (Duplisea & Hargrave 1996, Abebe et al. 2001, Danovaro & Gambi 2002). Further the HD assemblage suffered a bigger loss of species and consequently experienced a higher impact on its functioning. It lost an entire functional group of big sized predators and omnivores, representing a trophic level that does not overlap with any other of the four trophic guilds. On the other hand, the LD lost the

size-independent functional group of unselective deposit feeders, whose ecological function could overlap –at least partly –, with nematodes from functional groups 1A and 2A (Moens & Vincx 1997).

The loss of two of the biggest species (*Epacanthion* sp. and *Enoploides* sp.) due to the enclosure effect indicates that these species may have died because the rest of the assemblage could not support the high-energy demand of big sized individuals anymore. Predatory nematodes depend on the biomass of other nematodes under natural conditions (Danovaro & Gambi 2002). Experimentally, it has been shown that *Enoploides* (longispiculosus), an obligate predatory nematode, is influenced in its feeding behavior by food availability: with decreasing prey availability, ingestion rate decreased as a functional response, and at a quarter of the maximal ingestion prey density, the ingestion rate dropped to a quarter as well (Moens et al. 2000). The survival of *Chromaspirinia* sp. through the enclosure treatment indicates, that this species is probably an omnivore or scavenger rather than obligate predator. It may also be able to switch from one food source to another depending on availability. The same holds for *Gammanema* sp., the only temperature resistant species representing 2B. For the benthic food web, the loss of large predatory nematodes may have a considerable impact on energy transfer from meio- to macrofaunal trophic levels, since larger nematodes are more prone to predation by hyperbenthos (Hamerlynck & Vanreusel 1993).

The question of how diversity influences ecosystem functioning and what effects diversity loss may have is far from settled. Long-term experiments on terrestrial plant and aquatic microbial communities clearly support the IH, whereas many studies on benthic systems do not. In macrobenthic communities, species identity rather than diversity seems to be essential (Emmerson et al. 2001, Bolam et al. 2002, De Mesel et al. 2006). Species identity influenced nutrient generation and release from the sediment in intertidal invertebrates, where no globally consistent effect of either species richness or functional diversity could be detected (Emmerson et al. 2001). Species richness of macroinvertebrates in a tidal flat had an effect only on biomass and oxygen consumption among many variables

measured as proxies for ecosystem function; moreover, this effect was essentially due to the presence of the largest species in the study (Bolam et al. 2002). The authors hypothesized that diversity-biomass-ecosystem function relationships in soft sediments may be very complex and depend on functional groups rather than species richness (Bolam et al. 2002). Consistent with this hypothesis, functional diversity rather than species richness had significant effects on ecosystem performance (Emmerson & Raffaelli 2000). Similar to the macrobenthic community, no clear diversity-functioning relationship has been experimentally evidenced so far for meiobenthic communities or nematode assemblages. Support for the idiosyncratic model has been obtained in a bacterivorous nematode assemblage, given that species identity was the driver of algal decomposition process, and initial species richness was not a good predictor of this functional aspect of the assemblage (De Mesel et al. 2006). The results of our study point to the same direction: although they are consistent with the Rivets model, species identity was clearly an important factor for stress resistance.

The LD consisted of an initial pool of thermo-tolerant species as it maintained a high similarity with its original state, whereas the HD became very different from its original state. The predominance of resistant species in LD presumably resulted from its higher exposure times in their natural environment. Something similar happened when communities from sandy and muddy intertidal regions were exposed to organic enrichment: the effect was less drastic for the community from the muddy site, as it was originally better adapted to higher loads of organic matter (Schratzberger & Warwick 1998). In our study, four of the five species present in LD_{36} increased in abundance in HD_{36} (*Perepsilonema* sp., *Desmodora* sp. 1, *Ceramonema* sp. 3 and *Microlaimus* sp. 1). We have three not mutually exclusive hypotheses for this: 1) They exhibit high tolerance for high temperature and/or other effects of the temperature treatment (*e.g.*, lower oxygen availability), 2) they benefit from the lower abundance or disappearance of other species, which induces a competitive release and 3) they benefit from the higher bacterial biomass allowing rapid population development. Our experiment only provides

evidence for the first. Consistently, *Epsilonematids*, *Microlaimus* and *Desmodora* have all been observed in extreme places such as oxygen-limited bathyal sediments (Neira et al. 2001, Neira et al. 2005), or tidal mangrove forest with high salinity fluctuations (Olafsson 1995, Olafsson et al. 2000), indicating their potential to adapt to extreme conditions. Especially members of the genus *Perepsilonema* sp., which was by far the most abundant species in the LDt_0 and the one that increased most in abundance in HD_{36}, are known to be cosmopolitans (Decraemer et al. 2001) persisting in a variety of environments due to their high adaptation potential. The potential increase in bacterial biomass may have provided *Perepsilonema* sp. and *Ceramonema* sp. 3 (both members of the feeding guild 1A) a rich food source allowing rapid population development. Such an increase in bacteria feeding species also occurred during experimental enrichment of organic matter, which, as a secondary effect, caused increased bacterial growth (Schratzberger & Warwick 1998). It is, however, not clear why *Microlaimus* sp. 1 and *Desmodora* sp. 1 (both epistrate feeders, 2A) would have benefitted from this, although it has also been hypothesized that bacteria may be part of the food sources of epistrate feeders (Moens & Vincx 1997).

The fact that the LD consisted of an original species pool of stress resistant species is most probably a consequence of sampling natural communities from their natural environment, where adaptation to prevalent, potentially extreme environmental conditions may have occurred. Although high diversity is thought to exhibit higher average stress resistance due to the “sampling effect” (Tilman 1999), our study shows that the opposite can also occur. In our case, the LD contained a smaller, but stress tolerant species pool, whereas the HD was more vulnerable to stress. This problem is inherently linked to the use of natural communities deriving from their natural environment. Finding nematode assemblages that naturally differ in diversity while originating from a similar environment contradicts basic ecological principles, since nematode diversity is dependent on beach morphodynamics (Rodriguez et al. 2001, 2003) and physical and chemical gradients (Gheskiere et al. 2004, Gingold et al. 2010) among other

factors. The field samples of the present study show that the two stations hosted clearly different assemblages while environmentally differing only slightly in mean grain size. However, the predominance of *Perepsilonema* sp. in the LD field samples may also be related to the smaller core size. This may be explained if by chance our small core coincided with clusters of *Perepsilonema* sp., since they have the tendency to aggregate (Somerfield et al. 2007, Gingold et al. 2010). Still, LDt_0 controls showed a difference in diversity with HDt_0 hosting a higher number of species, although the difference was not significant. Whether the difference between the field samples and the $time_0$ control groups is due to an effect of the acclimation under laboratory conditions or the different sampling strategy (small vs. large cores) cannot be evaluated unambiguously.

Another issue inherently linked to the use of microcosms is the enclosure effect *per se.* Although the goal of the experiment was to study the effect of high temperature, the enclosure had an effect on abundance (in both HD and LD) and biomass (in HD). Something similar happened in a mesocosm experiment studying food supply on meiobenthic communities (Austen & Warwick 1995): experimental communities originated from two different sites, one exhibiting 4-10 times higher abundance than the other. Differences between $time_0$ and control samples revealed a clear decrease in abundance in the high abundance community and no difference in the low abundance community after 16 weeks. No decrease in species richness occurred in both communities (Austen & Warwick 1995). On the other hand, Dos Santos et al. (2009) were able to maintain abundance and diversity unaltered during 30 days in small microcosms of 300 ml (100 ml sand). In our study, we set up $time_0$ controls and microcosms at a control temperature of 31˚. We are able to separate the effects arising from the enclosure and the high temperature, and therefore our hypothesis and predictions relating diversity with stress-resistance, are not influenced by it.

Our results are concordant with other diversity-ecosystem functioning studies on intertidal benthic communities, *i.e.*, they do not reveal an unequivocal diversity effect but rather indicate that species identity may be at least as important for

stress resistance and the functioning of the system. Although other studies on other systems have shown clear redundancy effects of high diversity communities, we cannot presently affirm that functional redundancy exists in nematode assemblages at the diversity levels we measured. Instead, we hypothesize that each marine nematode species may occupy its own niche, which is possibly due to the high spatial and temporal heterogeneity characteristic of intertidal areas. Further, our data indicate that especially large nematodes are prone to extinction under environmental stress since their high energy demand may not be met when the rest of the community structure changes. This may have fundamental consequences for the meiobenthic food web on the one hand, and for the energy transfer to the macrofaunal level on the other. Without a doubt, the issue of benthic diversity and its role on ecosystem functioning requires much more attention in the future. In addition to the well-established plant and microbial systems, we believe that microcosm experiments with meiofaunal (and if possible together with macrofaunal) communities are promising experimental tools to study high-diversity communities. More sophisticated methods would further allow disentangling the effects of taxonomic and functional diversity as well as species interactions between and within trophic levels.

IV. 6. ACKNOWLEDGEMENTS

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Chapter V

General discussion

In this doctoral thesis, we set out to study fundamental aspects regarding intertidal meiobenthic diversity, focusing on free-living nematodes of a sandy beach. Of particular interest were the causes responsible for creating and maintaining biotic diversity and consequences resulting from it. We tested corner-stone hypotheses in community ecology by studying a) the role of topographical features (intertidal runnels and sandbars) as a cause for taxonomic and functional community structure and diversity as well as distribution patterns of intertidal nematodes and b) the role of diversity in withstanding stressful conditions (Fig. 18). In this closing chapter we want to discuss some key questions in community and benthic ecology in the light of our results.

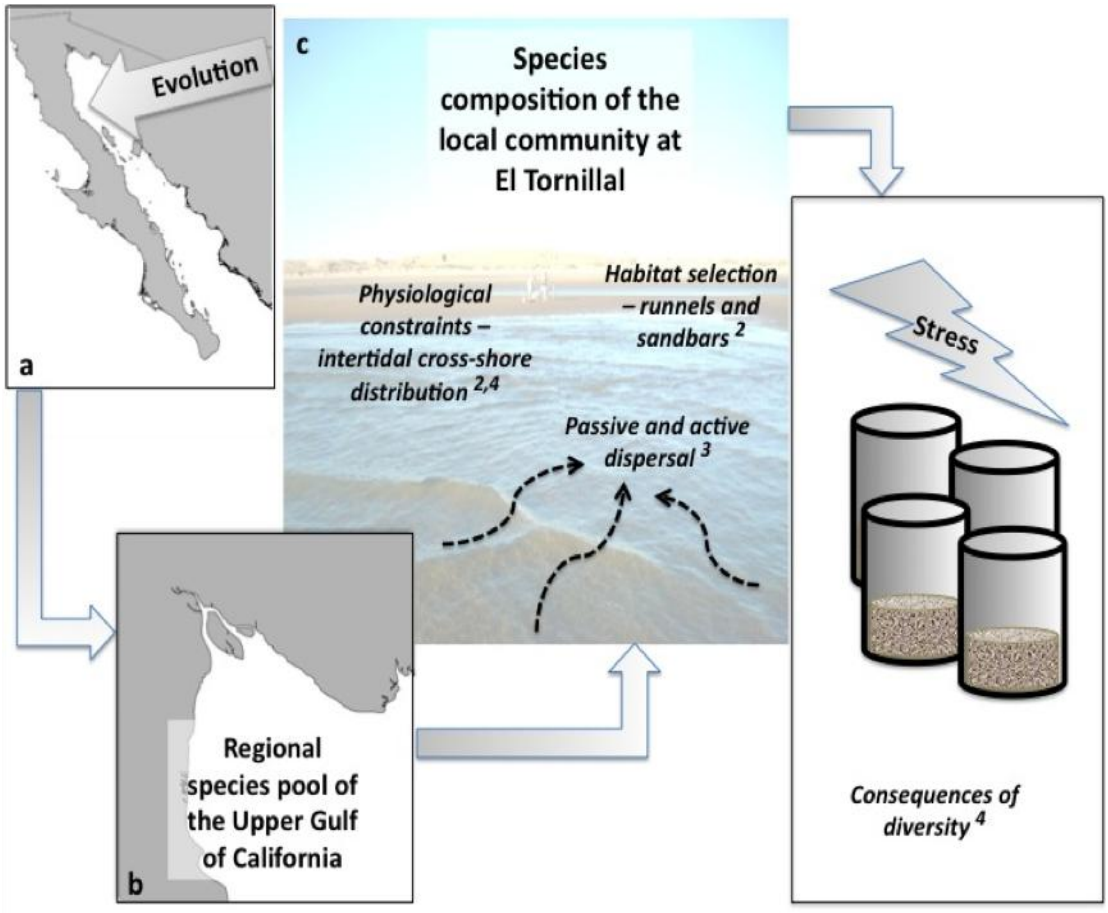

Figure 18. Conceptual model of the causes and consequences of the local community structure at El Tornillal, resulting from various processes at different time scales and their relationship to this research. (a) Separation of the peninsula of Baja California from the mainland was the evolutionary background for the regional species pool of the Gulf of California (b). (c) Further ecological processes at local scales influenced the creation and maintenance of the local community. (d) One of the consequences of diversity is that high diversity communities may exhibit functional redundancy, which, in the case of stress, may buffer adverse effects on the function of the system. Superscripts indicate which thesis chapter addresses the subject mentioned on the picture.

V. 1. WHAT HAVE WE LEARNED ABOUT THE CAUSES OF DIVERSITY IN FREE-LIVING MARINE NEMATODES?

Our results are the first to show that intertidal habitat heterogeneity on sandy beaches plays a fundamental role in structuring the inhabiting nematofaunal community. We showed that runnels and sandbars hosted significantly different

communities reflecting environmental conditions, with different distribution patterns in concordance with the hydrodynamic environment. In agreement with previous studies in other locations, we found that the intertidal environmental gradient is responsible for a three-tiered cross-shore grouping of the community, a pattern that predominated over the differences between runnels and sandbars. The importance of habitat heterogeneity in generating and maintaining biodiversity was evidenced by the comparison of *El Tornillal*, a structurally complex beach, with a geographically close but structurally less complex beach.

The causes that determine the number of species in a community remains among the unresolved central problems of community ecology. **Dispersal, habitat selection, physiological constraints and competition/predation** have been hypothesized to be the main ecological processes determining the local species composition of a specific site. In the following section we discuss the possible influence of one or more of these processes in the nematofaunal community of *El Tornillal*.

Physiological constraints may be one of the main causes for intertidal cross-shore distribution at *El Tornillal*. The results of our microcosm experiment showed clearly, that extreme temperatures (36°C) are beyond the tolerance limits of some species. In some species, the tolerance (or susceptibility) is in concordance with their cross-shore distribution on the beach. This is especially interesting for the case of two pairs of congeneric species, one in the genus *Xyala* and the other in *Ceramonema*. When subjected to chronic exposure to high temperature stress (cf. Chapter 4), *Xyala sp. 1*, *Ceramonema sp. 2* disappeared, whereas *Xyala sp. 2*, *Ceramonema sp. 3* endured. In the field, we observed that *Xyala sp. 2* and *Ceramonema sp. 3* could be found on exposed sandbars and in the uppermost intertidal, places with high environmental fluctuations, whereas *Xyala sp. 1* and *Ceramonema sp. 2* mainly occurred on the lower intertidal and in the more stable runnels. The observed field distribution in combination with the experimentally tested differential tolerance to thermal stress indicates that physiological constraints may play a significant role in intertidal distribution

patterns. Differential temperature tolerance has been reported for closely related species: cryptic species of the nematode *Pellioditis marina* exhibit differential seasonal and geographical distribution patterns (Derycke et al. 2006), and experimental tests evidenced their different temperature tolerance (own unpublished data). The two *Xyala* and two *Ceramonema* species are new to science (Holovachov 2008 a, b, 2009; King 2010) and may be endemic to the Upper Gulf of California. Hypothetically, differential temperature tolerance ranges among closely related species could be the result of disruptive selection and local adaptation in response to the environmental cross-shore gradient on sandy beaches, which could lead to speciation.

Active displacement and habitat selection may be among the main processes underlying the conspicuous sandbar-runnel community pattern at *El Tornillal*. Two results of the present research indicate that the hydrodynamic environment seems to be calm enough to allow certain nematode species to settle and actively choose their habitat. First, the distribution in the runnels was patchier than in the sandbar with lower similarity among assemblages from closely positioned samples (see Chapter 3). And second, the dissimilarity between runnel nematode assemblages and that of station 1 at the limit to the subtidal, increased with increasing distance from the sea (see Chapter 2). Until now, it has been hypothesized that the nematode community of intertidal runnels may represent a fraction of the subtidal species pool (Gheskiere et al. 2004), because runnels are constantly connected to the sea (at least the ones of the lower and middle intertidal). However, the existence of distinct communities as shown in this study indicate that the continuity between subtidal and runnel environments may not be as large as previously expected. Especially the upper part of the beach environments are separated by a relatively long distance (600 m), which could hypothetically act as a micro-geographic allopatry, leading to speciation, given the distinct environmental conditions and the relatively short life-cycles of nematodes. However, further research is needed to determine a) how many and which

nematodes are in the water column and brought in regularly with waves and tides and b) the active displacement capacity in the field of certain nematode species.

Our study was not designed to directly assess interactions among species, therefore we could not show that predation or competition play a significant role in structuring the community at *El Tornillal*, although they most probably do. In the rocky intertidal, it has been experimentally shown that the lower intertidal community is structured due to biotic interactions such as inter-specific competition (Connell, 1961). For meiofaunal communities of intertidal or shallow sandy areas, experimental work in laboratory mesocosm studies showed the predatory/disturbance effects of macrofauna (heart urchins) on the meiofaunal community structure (Austen & Widdicombe 1998). However, studies comparable to Connell's seminal field experiments are among the missing pieces in soft bottom community research.

V. 2. WHAT HAVE WE LEARNED ABOUT THE CONSEQUENCES OF DIVERSITY IN FREE-LIVING MARINE NEMATODES, AND WHAT DO OUR FINDINGS IMPLY FOR BIODIVERSITY CONSERVATION?

The results of our experiment were not consistent with studies in other systems that provided evidence for functional redundancy of species in highly diverse communities. Instead, our results support the Rivets Model, where each species contributes to ecosystem, and the impairment of this functioning is contingent on the loss of species. They also clearly show that stress resistance does not necessarily improve with species richness, given that our high diversity assemblage suffered a bigger loss of species and functionality. Rather, species identity was crucial for stress resistance, as the low diversity assemblage consisted of a pool of naturally stress-resistant species.

Following the results of our microcosm study, we would opt for a strategy that biodiversity should be protected where a) most species are preserved in order to

maintain ecosystem processes and b) species show a high probability to adapt to (or resist) future (different) environmental conditions. The former is in concordance with Myers (1988), who pioneered the term “hotspot” of diversity. Hotspots are those places “featuring exceptional concentrations of species with exceptional levels of endemism, and facing exceptional degrees of threat.” In this sense, the term “hotspot” has been applied by others (Myers et al. 2000, Roberts et al. 2002) or, more generally, as an area with either one or a combination of the following assets: a) particularly high species richness, b) particularly high levels of endemism, c) numbers of rare or threatened species and c) intensity of threat (Reid 1998). The identification of hotspots should be a strategy to optimally invest the limited resources in order to protect the highest number of species on the smallest possible area (Myers et al. 2000, Roberts et al. 2002). The latter (*i.e.*, probability to adapt to (or resist) future (different) environmental conditions) is, to our knowledge, not integrated in any biodiversity conservation plan. Although it would be a logical thing to do, two main reasons may impede its application: first, we have a limited capability to forecast future environmental conditions, and second, we can predict even less which species would be able to adapt to or resist them. So, at present, the “hotspot-policy” probably is still the best option for most effective biodiversity conservation, although we think, that in addition to the four assets listed above, productivity and ecosystem services should be additional criteria that should be integrated in the evaluation as well.

V. 3. ARE NEMATODE ASSEMBLAGES AN IDEAL MODEL FOR COMMUNITY ECOLOGY RESEARCH?

Although microcosm experiments can only be an approximation to the natural environment, they are currently probably the best compromise between field experimentation and completely controlled laboratory experiments, having shown their value in rigorous ecological experimentation (Benton et al. 2007). However,

working with nematode assemblages in microcosm experiments implies to deal with at least two fundamental methodological issues: first, the identification of nematodes requires great expertise and often it is not possible to identify individuals to species level. And second, the setup of microcosm experiments requires some crucial decisions about the number of replicates, the type of substrata and the (natural or artificial, *i.e.*, random) composition of the community.

V. 3. 1. Nematode identification

Nematodes are very difficult to identify for several reasons: 1) they are microscopic, 2) many species lack obvious unequivocal distinctive morphological features and to distinguish between two genera it is often necessary to have male individuals (which are not always available), 3) many species are new to science, especially in previously unexplored places such as the Gulf of California where nematology is still in its infancy and 4) some species descriptions (especially old ones) are often based on very few (sometimes even only one!) individuals, and are therefore inadequate to allow positive identification in the face of natural morphological variation. To date, about 5,000 species have been described; most of these descriptions correspond to species found in the north Atlantic (Platt & Warwick 1983, 1988, Warwick et al. 1998). Today there are two complementary ways of identifying nematodes: the classical, based on phenotypic species descriptions, and the molecular, called DNA-barcoding, which has been suggested as a possible solution to enhance biodiversity research in nematodes (Blaxter 2003, Bhadury et al. 2006). DNA-barcoding is based on the concept that each species possesses a standard region in the genome like a fingerprint. It provides a method to identify high numbers of individuals rapidly and accurately. This is useful in many cases, like for example when a fast screening for an estimation of extant diversity is necessary. It has also proven highly useful to distinguish morphologically very similar, but genetically distinct species (*i.e.* cryptic species)

(Derycke et al. 2006). In our case, DNA-barcoding would provide information about the “real” (including cryptic) diversity of *El Tornillal*, but it would not yield information about functional diversity, which was a fundamental aspect of our research. Ideally, the two methods would be applied complementarily.

V. 3. 2. Experimental issues

Two main issues are to be considered when using nematode species assemblages as a model community to study effects of biodiversity on ecosystem functioning: 1) How should communities of different diversity levels be obtained? and 2) What degree of "artificiality" is necessary to standardize, but at the same time maintain the system close enough to its natural state allowing “normal” species interactions and population development?

The first can be approached by choosing from purely natural to purely artificial conditions. One possibility is to experiment on a sample of natural communities and its natural environment, as we did in the present microcosm study (see Chapter 4). In our case, not only diversity, but also abundance was different between the two communities. Further, grain size and maybe other environmental variables we did not measure differed between the two sites and were, together with the organisms, bottled in the microcosms. Further, our study revealed that the LD community contained a pool of stress resistant species, presumably because of the higher exposure time they experience in their natural environment. All these issues, among others, may influence an experiment and need adequate interpretation.

Another possibility, not entirely natural or artificial, is to extract the organisms from their natural environment and place them in standardized and defaunated sediment. Dos Santos et al. (2009) sampled communities from a reflective (low diversity) and a dissipative (high diversity) beach. They extracted the nematodes from the original sediment and placed a standardized number of them in

microcosms prepared with uniform and defaunated sediment of intermediate grain size. Depending on the focus of the study, the replacement of natural by artificially sorted and cleaned sediments may indeed be the best option, but it may also have the consequence, that a) one of the communities (or both) will have to cope with an inadequate grain size which may affect their reaction to the experimental treatment and b) if the sediment is defaunated, the lack of important food sources may selectively influence the survival and abundance of some species, which is an undesired and uncontrolled effect of the experimental setup.

Finally, species can be randomly assigned from a species pool (Naeem & Li 1997, Tilman et al. 2006). For experiments with nematodes this implies that many nematode species need to be cultivated previously under standardized conditions. This is possible (Moens & Vincx 1998), but needs a high level of expertise and is very time-consuming. The effects in the experiment may then be unnatural species interactions but, on the other hand, it allows disentangling the diversity effect from interactions among species that co-evolved.

Given all the options, a trade-off must be found between the entirely natural and the entirely artificial community and environment. Almost any variant is possible when using nematode assemblages as "model" communities to study controlled diversity effects on ecosystem functioning in marine systems. No other marine metazoan community is represented by such high abundance and taxonomic diversity (Lambshead 1993). Given their accessibility and manipulability, which makes high numbers of replicates affordable for increased statistical power, we believe that it currently represents one of the most promising options to study diversity related subjects. Rigorous experimentally controlled research on the relationship of taxonomic diversity and ecosystem functioning in pelagic and benthic systems is virtually absent (Duffy & Stachowicz 2006), but experimentation is necessary to allow an integrative understanding of processes and mechanisms acting at broader scales, in combination with field observations and mathematical models (Bulling et al. 2006).

V. 4. Suggestions for future research

With each of our studies, we were able to shed light in some aspects of nematode communities and to identify new scientific questions.

To have a generally more complete picture about the diversity at *El Tornillal* and the Upper Gulf of California in general, we suggest the combined approach of morphological ("classical") identification and DNA-barcoding. We also suggest sampling other beaches along the coast, and especially heterogeneous places. As mentioned in the second chapter, the Ugland index could then give a much more accurate estimation of the regional species diversity.

To understand the underlying processes responsible for creating the patterns we described in the second chapter, we suggest experimental approaches. Physiological constraints may be evaluated by exposing different species to, for example, different temperature and salinity levels. Predation rates by macrofauna may be investigated by setting up mesocosms with different density levels of meio- and macrofaunal species, and analyzing gut-contents of the macrofauna. Dispersal could be better evaluated by setting up traps at different depths in the water column on the one hand, and by repeated high-resolution cross-shore sampling on the other. It would be interesting to evaluate the same dispersal patterns at different nearby beaches to understand the connectivity between these places.

To further study the relationship between species richness, functional diversity and the functioning of the benthic system, we suggest implementing further microcosm experiments in combination with field experiments. In addition to the suggested improvements above relating to experimental microcosm issues, we suggest measuring an increased number of proxies for ecosystem functioning such as decomposition of organic matter and nutrient regeneration.

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VI. 2. Picture credits and internet addresses

Internet addresses are reported as they had been found in January 2010.

Figure 3:

Satellite image (a) and beach profile (b): © L.G. Álvarez Sánchez, Dept. Physical Oceanography, CICESE, Ensenada, Mexico.

Photos (c and d): © R. Gingold

Figure 4:

Storm at coast (a): © Lyne Kennedy

Urban development at Miami beach (b): © www.condo-southflorida.com

Inorganic waste (c) and off-road vehicles (d) at Ensenada beach: © R. Gingold

Oil pollution on a beach (d): © FOE Europe

Figure 5:

Mollusk: © R. Gingold.

Polychaete: www.handbook.unsw.edu.au

Copepode: www.reefkorea.org

Nematodes: © R. Gingold

Benthic diatoms (upper picture): © Peter Brueggeman

Protozoa: http://starcentral.mbl.edu

Diatoms (lower picture):

http://people.westminstercollege.edu/faculty/thrrison/emigraion/2_diatoms.gif

Appendix 1 (supplementary material that has been published online)

SUPPLEMENTARY MATERIAL OF CHAPTER 2:

	September 2007		March 2008	
	RUNNEL	**SAND**	**RUNNEL**	**SAND**
GENUS				
Actinonema	x	x	x	x
Ammotheristus			x	
Axonolaimidae gen.			x	
Bathylaimus	x	x	x	x
Calyptronema			x	
Camacolaimus	x	x	x	x
Campylaimus				x
Catanema	x	x	x	
Ceramonema	x	x	x	x
Chromadorella			x	
Chromadoridae gen.	x	x	x	x
Chromadorina	x	x	x	x
Chromadorita	x	x	x	x
Chromaspirinia	x	x	x	x
Cobbia	x		x	x
Comesoma			x	
Cyartonema		x	x	x
Cyatholaimus				x
Cyatholaimidae gen.	x	x	x	x
Daptonema	x		x	x
Dasynemoides	x	x	x	x
Desmodora	x	x	x	x
Desmodoridae gen.	x	x	x	
Desmoscolex	x	x	x	x
Dichromadora	x	x	x	x
Diplopeltoides				x
Elzalia	x	x	x	
Enoploides	x	x	x	
Enoplolaimus				x

	September 2007		March 2008	
	RUNNEL	**SAND**	**RUNNEL**	**SAND**
GENUS				
Epacanthion	x	x	x	x
Epsilonema	x	x	x	x
Ethmolaimidae gen.		x		
Familiy n.i. gen.	x			
Filitonchus-Nannolaimus			x	
Gammanema	x	x	x	x
Halalaimus	x		x	
Hypodontolaimus		x	x	
Kraspedonema	x	x	x	
Latronema	x	x	x	x
Leptolaimidae gen.				x
Leptonemella	x	x	x	x
Linhomoeus	x	x	x	
Marylynnia	x	x	x	x
Mesancanthion			x	x
Mesacanthoides	x	x	x	x
Metachromadora	x	x	x	x
Metadasynemella			x	
Metadasynemoides	x	x	x	x
Metoncholaimus	x	x	x	x
Meylia	x	x		x
Microlaimus	x	x	x	x
Nannolaimoides	x		x	x
Neochromadora			x	x
Odontophora	x	x	x	x
Omicronema	x		x	x
Oncholaimellus	x		x	
Oncholaimidae gen.	x	x	x	x
Oncholaimus				x
Oxyonchus			x	
Oxystomina			x	x
Paracanthonchus	x		x	x
Paracomesoma	x	x	x	
Paracyatholaimoides	x			
Parodontophora	x	x		
Paramonhystera	x			

	September 2007		March 2008	
	RUNNEL	**SAND**	**RUNNEL**	**SAND**
GENUS				
Polygastropoda	x			
Pomponema	x	x	x	x
Praeacanthonchus		x	x	x
Prochromadora	x	x		
Prochromadorella			x	x
Promonhystera	x	x	x	
Prooncholaimus			x	
Pselionema	x		x	
Pseudosteineria	x	x	x	x
Ptycholaimellus	x		x	x
Rhabdodemania		x	x	x
Rhips	x			
Rhynchonema	x	x	x	x
Richtersia	x	x	x	x
Sabatieria			x	
Scaptrella			x	
Spilophorella	x	x	x	x
Spirinia	x	x		
Stylotheristus	x	x	x	
Synonchiella	x		x	x
Tarvaia			x	
Theristus	x	x	x	x
Thoracostomopsidae gen.	x		x	x
Trichotheristus		x		x
Tricoma	x	x	x	x
Tripyloides	x		x	
Trissonchulus		x	x	x
Viscosia	x	x	x	
Xyala	x	x	x	x
Xyalidae gen. 2	x		x	x
Xyalidae gen. 1	x	x	x	x

ELECTRONIC SUPPLEMENTAL MATERIAL OF CHAPTER 3:

Indices of dispersion [D = (variance/mean) and Green´s C_x = (variance/mean)-1/(n-1)] for the genera of free-living marine nematodes identified in this study from two intertidal micro-habitats (runnel and sandbar) from *El Tornillal*, a sandy beach in the Upper Gulf of California, Mexico.

SAND	C_x	D	**p-value**
Acanthonchus	0.36	2.43	0.04
Actinonema	0.29	1.57	0.318
Axonolaimidae gen.	n.d.	1.00	1
Calyptronema	n.d.	1.00	1
Camacolaimus	0.04	1.14	0.779
Catanema sp. 1	-0.07	0.93	1
Ceramonema sp. 3	-0.04	0.67	0.912
Chaetonema	n.d.	1.00	1
Chromadora	n.d.	1.00	1
Chromadoridae gen.	n.d.	1.00	1
Chromadorina	-0.07	0.86	1
Chromadorita	-0.07	0.79	1
Chromaspirinia	**0.093**	2.21	0.142
Comesoma	0.286	1.57	0.339
Cyartonema	-0.071	0.93	1
Cyatholaimidae gen.	0.133	1.80	0.37
Desmodora sp.1	**0.010**	1.70	**0.013**
Desmodoridae gen.	0.071	1.36	0.358
Desmoscolex	0.143	1.71	0.486
Enoploides	0.003	1.06	0.653
Epacanthion	0.046	1.60	0.241
Eubostrichus	-0.071	0.93	1
Euchromadora	-0.071	0.93	1
Gammanema	-0.008	0.86	0.318
Gerlachius	n.d.	1.00	1

SAND	C_x	*D*	p-value
Hypodontolaimus	n.d.	1.00	1
Kraspedonema	0.048	1.43	0.393
Leptolaimidae gen.	n.d.	1.00	1
Leptonemella	0.214	2.07	0.159
Leptosomatidae gen.	n.d.	1.00	1
Marylynnia	n.d.	1.00	1
Mesancanthoides	n.d.	1.00	1
Metachromadora	-0.024	0.68	**0.988**
Metadasynemoides	n.d.	1.00	1
Metoncholaimus	1.000	2.00	0.334
Microlaimus	**0.047**	3.97	0.042
Nasobema	1.000	5.00	1
Nannolaimoides	-0.071	0.93	**0.011**
Odontophora	-0.071	0.71	1
Oncholaimidae gen.	-0.071	0.43	1
Oxyonchus	n.d.	1.00	1
Paracanthonchus	0.464	2.39	0.099
Paracomesoma	n.d.	1.00	1
Perepsilonema	**0.007**	2.29	**0.003**
Pomponema	-0.015	0.69	0.622
Praeacanthonchus	n.d.	1.00	1
Pselionema	1.000	2.00	1
Pterygonema	-0.071	0.86	0.342
Rhabdodemania	0.214	2.07	0.16
Rhips	0.036	1.14	0.324
Rhynchonema	0.005	1.11	0.099
Richtersia	0.107	1.32	0.792
Sabatieria	n.d.	1.00	1
Siphonolaimus	0.143	1.71	0.126
Spilophorella	n.d.	1.00	1
Spirinia	**0.123**	2.36	**0.021**
Theristus sp. 1	0.286	1.57	0.786
Theristus sp. 2	0.036	1.14	0.332
Thoracostomopsidae gen.	n.d.	1.00	1

SAND	C_x	***D***	**p-value**
Tricoma	0.006	1.14	0.178
Trissonchulus	-0.071	0.86	1
Viscosia	0.286	1.57	0.346
Xyala sp. 1	**0.011**	1.88	0.114
Xyala sp. 2	n.d.	1.00	1
Xyalidae gen. 1	**0.176**	3.11	0.305
Xyalidae gen. 2	0.005	1.10	0.944
RUNNEL	C_x	**D**	**p-value**
Actinonema	0.058	1.76	0.086
Axonolaimidae gen.	n.d.	1	1
Bathylaimus	-0.071	0.93	1
Camacolaimus	-0.071	0.86	1
Ceramonema sp. 1	n.d.	1	1
Ceramonema sp, 2	0.031	1.18	0.338
Ceramonema sp. 3	-0.042	0.67	0.887
Chromadoridae gen.	0.036	1.14	0.546
Chromadorina	n.d.	1	1
Chromadorita	**0.012**	2.07	0.042
Chromaspirinia	0.000	1	0.384
Cobbia	-0.071	0.93	1
Comesomatidae gen.	n.d.	1	1
Cyartonema	n.d.	1	1
Daptonema	**0.220**	4.52	**0**
Dasynemoides	n.d.	1	1
Desmodora sp.1	-0.011	0.70	0.874
Desmodoridae gen.	-0.071	0.86	1
Desmoscolex	-0.071	0.93	1
Dichromadora	-0.071	0.86	1
Diplolaimelloides	n.d.	1	1
Elzalia	**1.000**	6	**0.004**
Enoploides	0.000	1	0.759
Epacanthion	0.042	1.46	0.312
Eubostrichus	0.167	2.33	0.4
Euchromadora	0.018	1.14	0.369

RUNNEL	C_x	D	p-value
Gammanema	-0.071	0.86	1
Halalaimus	0.077	1.62	0.639
Hypodontolaimus	0.286	1.57	0.312
Kraspedonema	n.d.	1	1
Latronema	-0.071	0.93	1
Leptonemella	0.075	1.82	0.119
Marylynnia	0.000	1	0.853
Mesancanthoides	0.286	1.57	0.321
Metachromadora	**0.047**	2.92	**0.013**
Metoncholaimus	-0.024	0.79	0.816
Microlaimus	0.000	1	1
Nasobema	-0.071	0.93	1
Neochromadora	n.d.	1	1
Odontophora	**0.086**	2.38	0.08
Oncholaimidae gen.	-0.071	0.50	1
Paramonhystera	1.000	2	0.339
Perepsilonema	**0.025**	2.78	**0**
Pomponema	**0.010**	1.38	0.04
Praeacanthonchus	n.d.	1	1
Prochromadora	1.000	2	0.321
Prochromadorella	-0.071	0.93	1
Pterygonema	n.d.	1	1
Rhips	-0.071	0.93	1
Rhynchonema	-0.015	0.62	**0.985**
Richtersia	0.013	1.21	0.295
Sabatieria	n.d.	1	1
Spilophorella	n.d.	1	1
Spirinia	**0.032**	1.84	0.053
Stylotheristus	n.d.	1	1
Thalassolaimus	n.d.	1	1
Theristus sp. 2	0.036	1.14	0.335
Tricoma	**0.017**	1.90	0.4
Xyala sp. 1	**0.034**	1.96	0.426
Xyala sp. 2	**0.059**	4.35	**0**
Xyalidae gen. 1	**0.117**	3.10	**0.002**

RUNNEL	C_x	D	p-value
Xyalidae gen. 2	0.000	1	0.849

Boldface indicate significant ($\alpha = 0.05$) C_x or D p-values (2-tailed test)
n.d. indicates C_x is not defined for taxa with $n = 1$

Appendix 2 (CV and publication list of the author)

Curriculum vitae:

Name: Ruth Gingold

Birthday: 3rd of August 1975

Nationality: Swiss

Education:

2006-2010 **PhD in Marine Ecology**

Department of Biological Oceanography, CICESE, Ensenada, Mexico
Supervisor: Dr. Axayácatl Rocha-Olivares
Thesis committee: Dr. M. Mundo-Ocampo (UCR), Dr. S. Ibarra-Obando (CICESE), Dr. M. Lavín-Peregrina (CICESE)
Thesis title: Diversity patterns and stress resistance of a marine nematode community from a heterogeneous sandy beach in the Upper Gulf of California, Mexico.
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2004-2006 **Scientist/qualifying training at the Department of Conservation Biology, University of Berne.**
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1999-2000 **Master of Science in Zoology with major in Ethology**

Department of Ethology, University of Berne/CH and NIOO in Heteren/NL,
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1995-1999 Studies in Biology with major in Zoology, University of Berne, Switzerland

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Publication list:

Gingold R, Rocha-Olivares A. and Moens T. (2010). Is high species diversity a security against thermal stress? The Insurance Hypothesis tested on a community of free-living marine nematodes. *In preparation.*

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