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FUNCTIONAL ROLE OF MACROBENTHOS IN ESTUARINE SEDIMENT DYNAMICS

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Author's declaration

The author states that she afforded a major contribution to the conceptual design and technical execution of the work, interpretation of the results and manuscript preparation of the published articles included in this dissertation, according to the *nº2 of art 8º do Decreto-Lei 388-70.*

Sandra Cristina dos Reis Borges Fernandes

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ABSTRACT

Estuaries are areas of high sediment dynamics. Particles in suspension are an important vehicle of several biochemical substances and contaminants. Therefore, the knowledge on the processes ruling sediment dynamics is needed to develop tools for estuarine water quality management. Presently, few numerical models for sediment dynamics incorporate biological interactions with sediment dynamics.

The aim of this study is to gain understanding of the macrobenthic influences on cohesive sediment dynamics. The research was focused on the effects of two species of macrobenthos; 1) the cockle *Cerastoderma edule* (densities of 280 to 1000 ind m^{-2}). Work on this species focused on sediment transport and deposition, by studying the hydrodynamic effect on the sediment removal activity (filtration) and its effects on topography and on the current velocities at the boundary layer 2) The ragworm *Nereis diversicolor* (densities of 320 to 1200 ind m^{-2}). This work focused on sediment erodability and consolidation by studying the bioturbation effect on changes in the sediment properties, and 3) the effect of contamination (3 nmol Cu g^{-1} dw) on the bioturbation activity of *N. diversicolor* and on sediment dynamics.

The results from experiments performed in a racetrack and in an annular flume showed that increasing density of *C. edule* is proportional to increasing sediment topography and related to reduced current velocities near the bed and increased shear velocity (u_*), hydrodynamic roughness length and turbulence kinetic energy (TKE). The higher TKE values were related to the presence of active filtering *C. edule*, producing additional turbulence to the TKE produced by topography. The effect of filtration activity on turbulence is most pronounced at low velocities ($u_* < 1.5$ cm s^{-1}), in agreement with a unimodal response to increased velocities. Increasing densities of *N. diversicolor* are related to increased sediment shear strength (SS), increased biodiffusion coefficients (Db) and increased erosion rates (ER). This antagonistic effect of increase SS and ER is explained by erosion of bigger aggregates resulting from biological bound sediments.

This study provides some evidence that copper contaminated sediments are more stable, as a consequence of decrease in biological response to toxicity, observed in lower values of shear strength and erosion rates. In addition, new methodologies for the determination of some of the parameters involved with this research field are suggested.

Key-words : Benthic boundary layer, bioturbation, biodiffusion coefficients, *Cerastoderma edule*, copper, erosion rates, estuarine cohesive sediments, filtration activity, flumes, *Nereis diversicolor*, sediment dynamics, topography, turbulence

SUMÁRIO

Os estuários são áreas de grande dinâmica de sedimentos. As partículas em suspensão são um importante veículo de substâncias bioquímicas e contaminantes. Por essa razão, o conhecimento dos processos que regem a dinâmica de sedimentos é necessário para desenvolver ferramentas de gestão da qualidade da água do estuário. Presentemente poucos modelos numéricos incorporam as interacções biológicas com a dinâmica de sedimentos.

O objectivo deste estudo é o de contribuir para o conhecimento das interacções entre o macrobentos e a dinâmica de sedimentos coesivos. A investigação focou-se no efeito de duas espécies funcionais; 1) do berbigão *Cerastoderma edule* (densidades de 280 a 1000 ind m^{-2}) no transporte de sedimentos, através do estudo do efeito hidrodinâmico na actividade de remoção de sedimentos (filtração) e o seu efeito na topografia e nas correntes junto ao fundo, 2) da minhoca-do-mar *Nereis diversicolor* (densidades de 320 a 1200 ind m^{-2}) na erodibilidade e coesão do sedimento, através do estudo do efeito da bioturbação nas alterações das propriedades do sedimento, e 3) o efeito da contaminação (3 nmol Cu g^{-1} ps) na bioturbação de *N. diversicolor* e na dinâmica de sedimentos.

Os resultados experimentais, desenvolvidos em canais oval e anular, mostraram que o aumento de densidade de *C. edule* é proporcional ao aumento da topografia e está associado a velocidades reduzidas e com o aumento da velocidade de corte e energia de turbulência cinética (TKE). Os valores mais elevados de TKE relacionam-se com a filtração activa de *C. edule*, que produz uma turbulência adicional à produzida pela topografia. O efeito da actividade filtradora na turbulência é mais pronunciado nas velocidades mais baixas ($u_*<1.5$ cm s^{-1}), de acordo com a resposta unimodal da filtração a velocidades crescentes. Crescentes densidades de *N. diversicolor* estão relacionadas com o aumento da força de corte do sedimento (SS), dos coeficientes de biodifusão (Db) e das taxas de erosão (ER). Este efeito antagónico entre o aumento simultâneo da SS e da ER é explicado pela erosão de agregados maiores em sedimentos ligados biologicamente.

Este estudo fornece evidências de que os sedimentos contaminados com cobre são mais estáveis, como resultado de atenuação da actividade biológica em resposta á toxicidade, sendo observados valores de força de corte do sedimento e taxas de erosão mais baixas. Novas metodologias para a determinação de alguns parâmetros envolvidos neste campo de investigação são sugeridas.

Palavras-chave: bioturbação, camada limite bentónica, *Cerastoderma edule*, cobre, coeficientes de biodifusão, dinâmica de sedimentos, filtração, flumes, *Nereis diversicolor*, sedimentos estuarinos coesivos, velocidade, taxas de erosão, topografia, turbulência

ABREVIATIONS

A	area (m^2)
ADV	accoustic doppler velocimeter
BBL	benthic boundary layer
β	local parameter on Eq. [3]
C	suspended sediment/particle concentration
C_0	initial suspended particle concentration
C_D	entrainment drag coefficient
CFD	computational fluid dynamics
Chl a	Chlorophyll a
CR	clearance rates (L h^{-1})
CR_{ind}	individual clearance rates (L h^{-1} ind^{-1})
CR_{280}	clearance rates for a population of 280 ind m^{-2}
C_t	suspended particle concentration at time t
c_v	consolidation coefficient in vertical direction (m^2 s^{-1})
Cu	copper
Db	biodiffusion coefficients
D_f	flock maximum dimension
d_j	exhalant siphon diameter (m^2)
E_d	eroded depth (m)
E_m	eroded matter (kg m^{-2})
EPS	extracellular polymeric substances
ETDC	erosion, transport, deposition and consolidation cycle of sediment dynamics
ER	erosion rates (kg m^{-2} s^{-1})
FP7	European Commission 7th Framework Programme (2007-2013)
ϕ_s	volumetric solid concentration (kg m^{-3})
$\phi_{s,0}$	in-situ volume concentration (-)
g	gravitational field strength (acceleration) (m s^{-2})
γ	vertical gradient of the critical bed shear stress on Eq. [3]
h	water column height (m)
κ	von Karman constant (~ 0.40 or 0.41)
K	dispersion coefficient (m^2 s^{-1})
k	topographic roughness element height
k_{cell}	exponential decrease in algal concentration

M	empirical erosion coefficient on Eq. [2]
M_E	erosion parameter (m/Pa^{-1}s) on Eq. [4, 5]
m_v	compressibility (m^2 N^{-1})
μ	dynamic viscosity (kg m^{-1} s^{-1})
n	exponent for ER empirical calculations on Eq. [2]
NLI	non-locality index
p	permeability (m s^{-1})
ρ	density (kg m^{-3})
ρ_d	sediment particles dry density (kg m^{-3})
ρ_s	sediment particles density (kg m^{-3})
ρ_w	water density (kg m^{-3})
S	smooth surface
SS	sediment shear strength (Pa)
t	time (h)
τ_b	bed shear stress (Pa)
τ_d	critical shear stress for deposition (Pa)
τ_e	critical shear stress for erosion (Pa)
TKE	turbulent kinetic energy (m s^{-2})
TKE_U	turbulent kinetic energy normalised with free stream velocity
U	free stream current velocity (m s^{-1})
U_{crit}	critical erosion free-stream velocity (m s^{-1})
$\bar{u}$	vertically averaged velocity (m s^{-1})
u_*	shear velocity (m s^{-1})
u_{*crit}	critical erosion shear velocity (m s^{-1})
u_j	siphon in/exhalant currents/jets (m s^{-1})
υ_s	settling mud flock velocity (m s^{-1})
V	volume of water (l)
W_d	settling velocity (m s^{-1})
Y_e	uncorrected weights
Y_s	standardised and weight specific rates
z	depth, distance above the bed (m)
z_0	hydrodynamic roughness length (m)
z_m	jet-in-cross-flow length-scale (m)
$10d_{50}$	median floc size (m)

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PART I

GENERAL INTRODUCTION

Chapter 1

Introduction

INTRODUCTION

There is a present international concern about ecosystem water quality as well as sea-level rise and erosion of coastal margins, caused by increase of rainfall and storm episodes due to global climatic change. Coastal zone management has gained more attention and has been included in several international commitments (UN Framework Convention on Climate Change, UN Convention on Biological Diversity, UN Convention on Combating Desertification, International Strategy for Natural Disaster Reduction), the Kyoto and Montreal protocols, initiatives such as World Summit on Sustainable Development, Global Earth Observation System of Systems initiative (GEOSS), Intergovernmental Panel on Climate Change (IPCC).

One of the main objectives of the FP7- Specific Program for Environment (including Global Changes)[1] is to promote sustainable management of the natural and human environment and its resources by advancing our knowledge on the interactions between the biosphere, ecosystems and human activities, and developing new technologies, tools and services for monitoring, prevention and mitigation of environmental pressures and risks including on health and for the sustainability of the natural and man-made environment, in order to address in an integrated way global environmental issues. Douvere and Ehler (2009) focus on the growing awareness of the need to establish marine spatial planning (as in land use management) and reviewed the international and legal framework relevant to the development of an ecosystem-base sea use management.

Estuaries (semi-enclosed coastal bodies of water) are areas of high biological productivity and have been attractive areas for population establishment for centuries. The consequent pressure of unsustainable and uncontrolled industrial and anthropogenic development creates conflicts with natural processes that sustain the health and productivity of coastal areas and results in situations of contamination and eutrophication and situations of loss or accretion of margins due to erosion and sedimentation.

Estuaries are often characterized by high sediment dynamics. Estuarine sediments are composed of mud and sand and when they have a relevant percent of mud, they exhibit cohesive properties. Sedimentation establishes mudflats. Estuary boundaries are often delineated by mudflats and can be separated in three distinct zones: the lower, the middle and

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[1] *European Commission C(2008)4598 of 28 August 2008*

the upper flats according to water inundation during the tidal cycle. The typology of intertidal mudflats can be characterised in terms of tidal range, wave energy, sediment supply, steepness, bed forms, organic content and biology (Dyer, 1998).

Intertidal mudflats are areas of high production of flora and fauna. They support large populations of birds and form nurseries and feeding areas for coastal fisheries. They are also known as places of high organic matter mineralization (Middelburg et al., 1996; Guarini et al., 2000; Heip et al., 2001), and they constitute an important component in global biogeochemical cycles. Additionally, these zones of high sedimentation are a potential trap for solid phase contaminants. The sedimentation of contaminated sediments, chemical reactions and dissolution under anaerobic conditions (observed in few mm depths in some cases) and resuspension events (leading to recontamination events) are processes that need to be clarified.

The increasing anthropogenic pressures on the environmental systems and the need for sustainable management tools, led to an extensive research focusing on contaminated sediments. Toxicity towards the benthos is another important issue considering their importance at the lower levels of the trophic chain, and considering the contamination amplification to higher levels of the food web and ultimately to human consumption.

1.1 Cohesive sediments

Estuarine sediments are composed of sand (non-cohesive sediments) and mud (cohesive sediments). The non-cohesive sediments can be characterised by being inert material whose transport/erosion/deposition behaviour is mainly determined by physical properties such as gain size distribution (Berlamont et al., 1993). In this case, particle adhesion is due to water-film surface tension. The cohesive sediment particles are characterised for being chemically very reactive. Due to its wide variability of origins and composition the prediction of behaviour, transport and fate of cohesive sediments is much more complex than the non-cohesive.

Cohesive sediments, as encountered in the environment, is a mixture of clay, silt, fine sand, organic material, water and sometimes gas (Winterwerp and van Kestereren, 2004). The mineral composition of cohesive sediments comprises silicates (quartz, feldspar and clay minerals) and non-silicate minerals that are precipitations of salts, carbonates, sulfates, sulfides, phosphates, oxides and hydroxides, depending on chemical conditions (Winterwerp and van Kestereren, 2004).

The granular size and composition are of prime importance for the mechanical behaviour of sediments (in transport, flocculation, erosion, deposition and consolidation). The textural classification of gravel-free muddy sediments was revised by Flemming (2000), who proposed the most recent and more precise diagram using the sand/silt/clay ratio.

The chemical properties of organic matter (organic polymers, colloids or dissolved organic carbon) in the liquid phase are also very important for the cohesive behaviour of sediments by influencing the formation of aggregates and flocs. In general, the amount of organic matter is well correlated and proportional to the clay content (Winterwerp and van Kestereren, 2004). According to these authors, there is a group of organic substances that can be regarded as flocculants (polysaccharides, proteins composed of peptides and amino acids) a second group that is neutral (lipids, hydrocarbons like cellulose, lignin composed of aliphatic and aromatic hydrocarbons), and a third group that are dispergents or deflocculants (humic acids). These polymers occur as charged, or as neutral particles and can adsorb to clay particles through van der Waals forces, bipolar forces or hydrogen bonding (Winterwerp and van Kestereren, 2004).

1.2 Sediment dynamics

The cycle of sediment dynamics, also known by the ETDC cycle (erosion, transport, deposition and consolidation) is a present concern considering that the pollutant dynamics of an estuary is closely linked to the distribution of particulate mater. Therefore, the knowledge of the processes governing sediment dynamics is essential to a better prediction and management.

The sediment particles along with seston are transported in the flowing water. The trajectory of each particle follows laminar flows and turbulent eddies. In their trajectories particles collide with other particles and form aggregates with subsequent adherence among particles of cohesive sediment. These aggregates, or flocs, can collide with each other causing floc break-up.

The sediments are transported in suspension in the water column as long as current velocities exceed critical values for deposition. The critical velocity for deposition is dependent on the shape and the height of the floc, i.e. the flocs in suspension do not deposit at the same time or with the same settling velocity.

Particles with a larger deposition velocity can overtake those with a smaller deposition velocity and collision between those particles may result in further aggregation and floc break-up. The lower the current velocities, the higher deposition rates are observed. Once the particles or flocs are deposited on the seabed, they can either be buried by subsequent deposited sediments or they can be resuspended if current velocity increases. Underneath the deposited sediments there is an increasing pressure that pushes the sediment down and promotes the expulsion of interstitial water as the consolidation process take place. When the current velocities increase and exceed the critical erosion velocity, the consolidated sediments are eroded and brought back in to the water column where they become exposed to turbulent eddies, floc break-up and further aggregation.

Because of their reactivity, mobility and nutritional value, suspended particles have an important role in biogeochemical cycles in estuaries as transfer vehicles of chemical constituents between water column, bed sediment and food chain (Fig. 1.1).

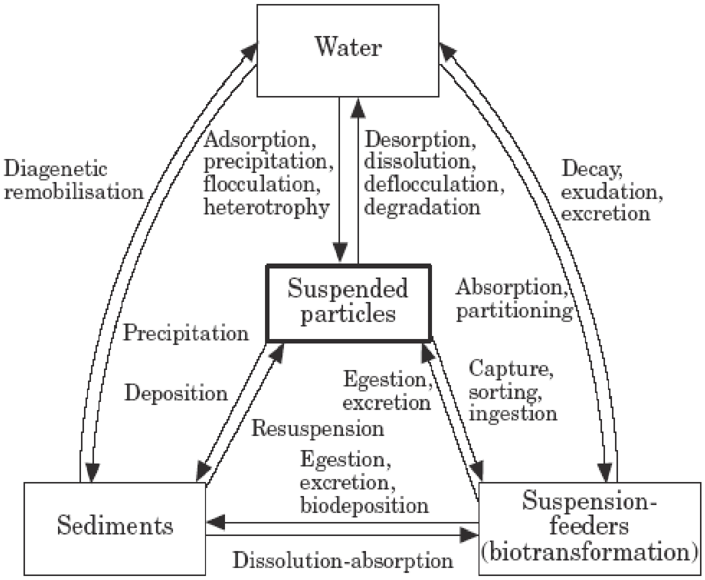

Figure 1.1 - Schematic representation of the role of suspended sediments in estuarine biogeochemical processes. Boxes represent compartments hosting materials and chemical constituents, and arrows denote physical and biogeochemical processes responsible for the transfer of such between compartments (Turner and Millwards, 2002).

Great effort has been taken to develop a more integrated description of the processes in sediment dynamics. While physical (Teisson et al., 1993; Uncles, 2002; Cappucci, et al. 2004) and biogeochemical processes (Correl et al., 1992; Chen et al., 2000; Azevedo et al., 2008) are relatively well understood, the modelling approaches incorporating the biological effects are scarce (Borsje et al., 2008). The models integrating the effect of suspension feeders on sediment and nutrient dynamics have but a description of suspension-feeding activity and other ecophysiological variables (Sohma et al., 2001, 2008; Turner and Millwards, 2002). Due to the multidisciplinary range of processes and their interactions, the clear relevance of each process (within the scale of observation) on sediment dynamics is still not clear.

1.2.1 Transport

The physical processes that dominate sediment transport in aquatic ecosystems are relatively well defined (e.g. Mehta et al., 1989; Houwing, 1999; Gleizon et al., 2003; Dyer et al., 2004). Sediment transport is governed by an advection-dispersion equation for the mass conservation of suspended sediments:

$$\frac{\partial C}{\partial t} + \underbrace{U(\nabla C)}_{\text{advection}} = \underbrace{\nabla(K\nabla C)}_{\text{diffusion}} \qquad [1]$$

where C is the suspended sediment concentration (kg m^{-3}) and t is time (s). $\frac{\partial C}{\partial t}$ expresses the accumulation rate in time and space. U is the free stream current velocity (m s^{-1}) and K is the dispersion coefficient (m^2 s^{-1}).

The description of transport is only completed after considering bottom exchanges by erosion and deposition rates. Erosion and deposition are sources and sinks that started to be taken in to account recently (Brenon and Le Hir, 1999; Cancino and Neves, 1999; Cugier and Le Hir, 2000). These two processes will be discussed in detail later.

The structure of the flow is characterised by shear flow near the bed (Fig. 1.2). At the bottom, there is a benthic boundary layer (BBL) characterise by a velocity gradient developed between U in the water column and the boundary, where no slip- condition applies (i.e. at z=0, U=0). Within the part of the turbulent boundary layer, the velocity profile is logarithmic and may be described by the Prandtl von Karman equation or “law of the wall”:

$$U(z) = \frac{u_*}{\kappa}\ln\left(\frac{z}{z_0}\right) \qquad [2]$$

where U(z) is the velocity at height z above the bed, κ is the von Karman’s constant (~ 0.40), z_0 is roughness length and u_* is the shear velocity. u_* is generally determined from the slope of the logarithmic velocity profile (u (z) versus ln (z)) and z_0 is estimated from the intercept between the relation (u(z) versus ln (z)) and the Y-axis.

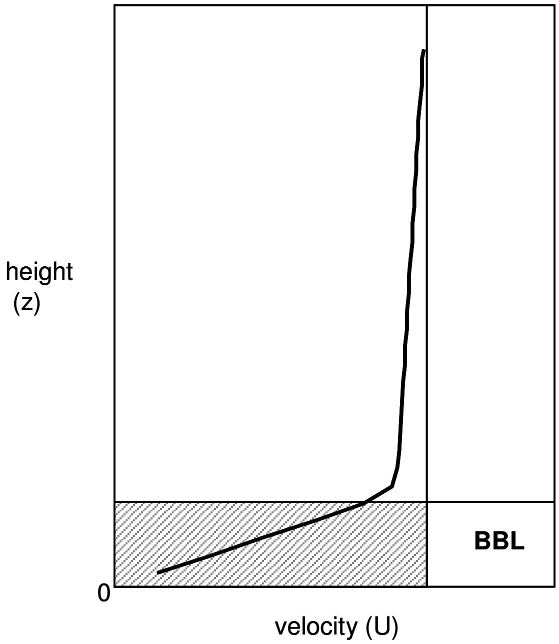

Figure 1.2 – Schematic drawing of a benthic boundary layer (BBL) in parallel flow at zero incidence.

Shearing stresses within the benthic boundary layer of a flow ($\partial u/\partial z$) are a manifestation of the drag force at the bed and the flux of momentum per unit area per unit time is known as the bed shear stress (τ, Pa). The modelling approaches used to estimate erosion and deposition rates are dependent on τ and its critical values at which erosion or deposition start (Houwing, 1999). Therefore, τ is one of the most important parameters used in the modelling of sediment transport in the benthic boundary layer and in evaluation of the benthic flux of material.

Most numerical models using hydrodynamic parameters are based on empirical formulas assuming τ as a quadratic function of shear velocity (u_*, m s^{-1}) $\tau \propto u_*^2$ (Winterwerp et al., 2006), or using the Manning roughness coefficient (Dias and Lopes, 2006) or the Nikuradse roughness coefficient to express topographical roughness (Houwing and Van Rijn, 1998; Lumborg, 2005, Zimmermen et al., 2008).

The estimation of τ is normally performed using indirect approaches and the properties of the sea bed are frequently disregarded, which reduces the accuracy of its determination and the accuracy of hydrodynamic models. Therefore the knowledge on the parameters of the BBL over natural structures needs to be developed.

Some efforts have been taken by El Ganaoui et al. (2004) to discriminate the erosion and deposition behaviour of the top "fluff layer" and of the deeper sediment. They have showed that multi-class models, using two critical shear stresses for erosion and deposition dependent on sediment characteristics, have a better fit to observed data.

1.2.2 Erosion

Sediment erosion is an important component of sediment dynamics. It produces a source of sediment to the water column, but also nutrients, organic matter and contaminants. From the theoretical point of view, the mechanical processes of sediment erosion are well described. A review of the calculation methods to estimate erosion rates was performed by Mehta et al. (1982). The formulations for erosion rates (ER, kg m^{-2} s^{-1}) are based on the concept of critical bed shear stress. Erosion happens when $\tau_b > \tau_e$, which means that erosion occurs as long as the applied bed shear stress (τ_b, Pa) is larger than the critical bed shear stress for erosion (τ_e, Pa). ER is then described as:

$$ER = M(z)(\tau_b - \tau_e(z))^n \qquad [3]$$

where M(z) is an empirical erosion coefficient with its dimension depending on an exponent *n*, $\tau_e(z)$ is the critical bed shear stress for erosion that may vary with z.

Sanford and Maa (2001) revised the mathematical formulation for erosion and proposed a suitable assumption that neglected M and τ_e while setting n = 1, and derived the previous formulation [2] to:

$$ER = \rho_d(z)\beta(\tau_b - \tau_{e0})e^{-\gamma\beta(t-t_0)} \qquad [4]$$

where $\rho_d(z)$ is dry bulk density at sediment depth z, β is a local parameter, τ_{e0} is critical bed shear stress when τ_b is first applied at t = t_0, t is time (h), and $\gamma = d\tau_e / dz$ is the vertical gradient of the critical bed shear stress.

ER has been generally based on critical shear stress (Sanford and Maa, 2001; Wang, 2003; Lumborg, 2005). However, the determination of ER from in situ measurements is not straightforward because both parameters M and τ_e and a variety of physical, chemical and biological factors influencing them, are an unknown function of sediment depth (Aberle et al., 2004).

There are known linear relationships between ER and sediment characteristics such as dry bulk density, water content, mud/sand content, organic content, colloidal carbohydrate (Houwing, 1999, 2000; Friend et al., 2003; Aberle et al., 2004). Friend et al. (2003) verified that the use of Chlorophyll *a* sediment content as a proxy for τ_e is related to seasonal variability for intertidal silty sand sediments of the Ria Formosa. This relationship would only be suitably used for late summer/early autumn.

In 2004 Winterwerp and Van Kesteren proposed a formulation for ER that would incorporate some mechanical sediment characteristics:

$$ER = M_E(\tau_b - \tau_e)\rho_d \qquad [5]$$

and

$$M_E = \frac{c_v \phi_{s,0}}{10 d_{50} SS} \qquad [6]$$

M_E (m/Pa^{-1}s) is an erosion parameter, c_v (m^2 s^{-1}) is the consolidation coefficient in vertical direction,$\phi_{s,0}$ (-) is sediment concentration by volume at onset of swell (i.e. the in-situ volume concentration), $10d_{50}$ (μm) median floc size, SS (Pa) undrained shear strength. c_v (m^2 s^{-1}) is a function of permeability and compressibility of the skeleton:

$$c_v = \frac{p}{m_v}\frac{1}{\rho_w g} \qquad [7]$$

where p is permeability (m s^{-1}), m_v (m^2 N^{-1}) is the compressibility, ρ_w (kg m^{-3}) the water density and g gravitational acceleration (m s^{-2}).

Although some efforts have been taken to include properties of the sediment on the description of erosion mechanisms, the relationships that are likely to influence the quantity of eroded matter, are still poorly described. The proper formulation of ER would imply the numerical descriptions of known physical (shear and critical velocities), chemical and biological functions, involved in sediment biogeochemical properties.

Quite recently Tolhurst et al. (2009) provided an overview of the studies on cohesive sediment erosion describing the response of settled mud beds to fluid stress which can be tracked back to the mid-1950s. They have highlighted the works of three professors who have dominated the stage: R. Krone, A. Mehta, and E. Partheniades. They also provide an overview of the knowledge gained from these and other works, approaching both advances and shortcomings related to mud sampling and the use of benthic erosion devices.

1.2.3 Deposition

The deposition component of sediment dynamics has received attention from many science fields: in geology, because of the depositional record; in geochemistry areas focusing chemically less permanent deposits and interactions with previously deposited constituents and in biology areas focusing settlement of larvae and food particles to benthic fauna.

Deposition is the gross flux of cohesive sediment flocs on the seabed and sedimentation is the net increase in bed level (Winterwerp and van Kestereren, 2004). Thus, the sedimentation rate is the deposition rate minus the erosion rate.

By definition, sediment is formed of granular material that can settle in water by gravity. Settling velocity of an individual particle (W_d, m s^{-1}) in still water corresponds to the constant velocity at which particles settle through a static fluid when the resistance of the fluid exactly equals the downward force of gravity acting on the particle. It is dependent on particle density, shape, size, and surface texture, and on the density and viscosity of the fluid. According to the Stokes Law, W_d can be estimated as

$$W_d = \frac{(\rho_s - \rho_w) g 10 d_{50}^2}{18\mu} \qquad [8]$$

where ρ_s is the density of sediment particles (kg m^{-3}), ρ_w is the density of water (kg m^{-3}), g is the gravitational field strength (m s^{-2}), $10d_{50}$ is the floc size (m) and μ is the dynamic molecular viscosity of water (kg m^{-1} s^{-1}). Observations from previous studies show relationships between W_d and salinity (van Leussen, 1999). A comparison between W_d observed on natural sediments and a model prediction using Stokes law show that natural sediments have W_d two orders of magnitude larger (McCool and Parsons, 2004). Techniques to measure settling velocities of cohesive sediment aggregates were recently reviewed by Mantovanelli and Ridd (2006). They briefly describe a wide variety of devices emphasizing logistical and scientific implications of its use and measuring purposes.

The first particles to deposit are the largest (e.g. aggregates), most dense or closest to the bottom. During deposition, flocs will have high probability of aggregation and collision with other flocs with different W_d. The processes of flocculation (Winterwerp, 2002; Winterwerp et al., 2006), aggregation (McAnally & Mehta, 2002), floc strength and breakage

(Jarvis et al., 2005) are a function of shear stress at steady state. Flocculation plays an important role on the vertical transport of sediments towards the bed (Lick et al., 1992) and is important to understand the partitioning between contaminants and the sediment, organic and water phase (Ongley et al., 1992).

Recent approaches using numerical analysis have indicate correlations between the median floc size and sediment concentration (Xu et al., 2008) and provided a new heuristic formula for turbulence induced flocculation of cohesive sediments (Winterwerp at al., 2006).

The present descriptions of deposition in estuaries are mostly based on the characteristics of the hydrodynamics and suspended matter. Realistic numerical simulations of these phenomena's are complex due to the variety of particle characteristics in time in the water column and the definition of critical shears for deposition of different sediment classes.

In addition, there are biologic activities that may influence sediment deposition. For example, the suspended sediment that is removed from the water column by the benthic organisms is transformed and deposited as faeces, i.e. larger aggregates of suspended material (Turner and Millward, 2002).

1.2.4 Consolidation

As mud flocs accumulate on the bed, the flocs that arrived first are squeezed by the ones on the top and suffer a vertical pressure gradient. Pore water is driven out of the flocs and out of the space between the flocs. This process is known as self-weighting consolidation (Winterwerp and van Kestereren, 2004). In the consolidation phase, flocs are mainly supported by particle interactions and are described by the Gibson equation, which is a simple wave equation in vertical direction z, over time t:

$$\frac{\partial \phi_s}{\partial t} + \frac{\partial}{\partial z}\left(v_s \phi_s\right) = 0 \qquad [9]$$

where ϕ_s is the volumetric solids concentration (kg m^{-3}) and v_s is the settling velocity of the mud flocs (m s^{-1}) relative to a fixed reference plane. The consolidation of cohesive sediments can be expressed as c_v, the consolidation coefficient (see section 1.2.2, Equation [7]), dependent on sediment permeability and compressibility.

In general, consolidation is approached as a mechanical sediment property based on sediment composition and texture, sediment bulk density, porosity, water content (Winterwerp and van Kestereren, 2004; Bartetzko and Kopf, 2007; Jacobs et al, 2007), usually assessed through measurement of the critical erosion threshold (Teisson et al, 1993; Houwing, 1999) and shear strength (Watts et al., 2003). The relationships between some of these parameters are not clear. The expected trend of increasing shear strength and decreasing porosity with depth was only observed in 31% of drill sites from the entire suite of expeditions from the OceanDrilling Program (Bartetzko and Kopf, 2007).

Although the activity of burrowed organisms is not usually taken into account in the processes of consolidation, there are evidences of the effects of the crab *Mictyris longicarpus* (Webb and Eyre, 2004) and the bivalve *Yoldia limatula (*Ingalls et al., 2000) on sediment porosity and irrigation.

1.2.5 Sediment stability

Sediment stability is not by itself a component of what is understood to be the dynamic cycle of estuarine sediments. The concept of sediment stability refers roughly to the resistance of cohesive sediments to erosion. It should be understood as a general term, which can be characterised in terms of the properties of the sediment: sediment shear strength, critical erosion shear stress, porosity, grain size, and in terms of the response to shear velocities: erosion rates, critical shear stress.

The effect of biological processes on sediment stability is important in the transport of microbenthos, nutrients, organic matter and contaminants to the water column (Rasmussen et al., 1998; Mazik and Elliot, 2000). In addition, benthic organisms are known to affect sediment stability and other properties such as their effect on sediment strength and erodability (Baillie and Welsh, 1980; Kornman and de Deckere, 1998; Widdows et al., 2000; Blanchard et al., 2001).

The description of sediment stability and the interactions among the sediment physical, geochemical and biological properties demands a multidisciplinary approach able to recognize multi-side effects and couple interactions taking place at different scales. The efforts taken to develop this subject are illustrated by the increasing precision of measurement equipments and the use of flume devices (Jonsson et al., 2006), which allowed small-scale observations of estuarine sediment dynamics processes. Because the description of sediment stability gives more emphasis to sediment properties, which are much more variable and complex to describe than the hydrodynamic conditions, it is not very popular among the more theoretical scientists.

1.2.6 Contaminated sediments

Heavy metals bind to clay mineral particles follow the same transport and deposition pattern than other particles, having a negligible transport towards the open sea (Sondi et al., 2008). A recent study focusing on the distribution of contaminants in three shelf areas from the Portuguese coast was published by Mil Homens et al. (2006). They observed that the character of the contaminated sediments transported to these shelf areas is further influenced by grain-size sorting as well as by dilution with other marine sediments.

The effect of floods is an important issue because it is a source of material derived from the runoff of the basin soils, increasing suspended sediments, nutrients and pollutants. Zonta et al. (2005) highlighted the effect of floods on the transport of suspended sediments and contaminants in the estuary of the Dese River (Venice Lagoon). They concluded that the transport of most of the analysed heavy metals was driven by the suspended particulate matter concentration.

There are some studies focusing the effect that macrofauna have on contaminant distribution in the sediments and their input to the water column (Rasmussen et al., 1998; Ciarelli et al., 2000; Delmotte et al., 2007; French and Turner, 2008), but the effect of contaminated sediments on the bioturbation activity of macrofauna was never investigated.

In terms of sediment dynamics, contaminated sediments are normally approached as an object (to be transported, deposited or resuspended), and not as an influencing factor on sediments dynamics. In order to predict the response of mudflats to environmental and anthropogenic pressures, a greater understanding on the multidisciplinary processes that affects sediment dynamics with associated contaminants is needed. So far, the effect of contaminate sediments on sediment stability has not been investigate.

1.3 Biologic interactions with sediment dynamics

A wide diversity of organisms, from bacteria to benthic invertebrates and marsh plants that inhabit the sediment bottom performs a variety of activities that influence sediment physical structure, biogeochemical properties, and species distribution (Hewitt et al., 2006; Rabaut et al., 2007).

Considering the effect that some species have on their habitat, a new concept of ecosystem engineering emerged in 1994 (Jones et al, 1994): ecosystem engineers, directly or indirectly modulate the availability of resources (other than themselves) to other species, by causing physical state changes in biotic and abiotic materials. In so doing they modify, maintain and/or create habitats. The distinction between “ecosystem engineers” and “key-stone species”, one whose effect is large and disproportionately large relative to its biomass and abundance (Power et al., 1996) has been widely discussed (Jones et al., 1997; Crain and Bertness, 2006; Wright & Jones 2006 and references within).

The concept of estuarine ecosystem engineers is more applied to plants due to their importance on the processes of fine particles retention and in the promotion of refuge for other macrofauna species influencing the habitat and the distribution of the surrounding community (Brusati and Grosholz, 2006; Hewitt et al., 2006; Neira et al., 2007; Rabaut et al., 2007).

The benthic communities influence sediment dynamics by affecting some components of the ETDC cycle and sediment stability. They are characterized according to size; micro (< 32 μm), meso (>32 μm and < 1 mm) and macrobenthos (> 1 mm) and according to location; hyperbenthos (organisms living above the sediments), epibenthos (living on top of the sediment) and endobenthos (living inside the sediment). They change sediment characteristics and stability, influencing biogeochemistry, bottom topography and velocities near the bed, sediment deposition and in the case of suspension feeders, constitute an additional removal of suspended particles. Although this is a known fact, the quantification of these influences is not properly addressed.

1.3.1 Sediment stabilisers and destabilisers

At the sediment surface, the microphytobenthos produce and live in a biofilm that is known to increase sediment stability. The microphytobenthic community dominated by diatoms (Underwood and Smith, 1998, de Brouwer et al., 2002), have a stabilising effect that derives from the production of extracellular polymeric substances, EPS (Paterson, 1989; Yallop et al., 1994; Miller et al., 1996; de Brouwer et al. 2003), resulting in increased sediment adhesion, higher resistance to resuspension by tidal action (de Jonge and Van den Bergs, 1987; Johnson and Azetsu-Scott, 1995; Blanchard et al., 1997; Austen et al., 1999; Shimeta et al., 2002) and raised erosion thresholds (Self et al., 1989; Tolhurst et al., 1999; Watts et al., 2003). Using a cross-shore morphodynamic model of an intertidal mudflat in which the equilibrium profile of the intertidal flat under tide and wave forcing is simulated, Le Hir et al. (2007) suggested that local stabilisation by the microphytobenthos does not have a significant long-term effect. These conclusions are dependent, of course, on the forcing functions description and the question of whether the simplification of the description of certain parameters influenced the results remains.

The biofilm is confined to the sediment surface layer, and once it is eroded, the sediment resistance is governed by processes occurring below the surface such as the characteristics of deeper sediment layers and the activity of the macrofauna.

Different species of the macrofauna affect sediment stability differently (see Graf and Rosenberg, 1997). Some burrowing species such as crabs, gastropods, polychaetes and amphipods are known to change the properties of the sediment an their stability (Jones and Jago, 1993; Botto and Iribarne, 2000; Volkenborn et al, 2007), affecting sediment shear strength (Meadows and Tait, 1989; Meadows and Hariri, 1991; Widdows et al., 1998) whilst others affect sediment erosion (Davies, 1993; Davey and Partridge, 1998; Rowden et al., 1998; Widdows et al., 1998, 2000; Andersen et al., 2005; Orvain et al., 2006). Le Hir et al. (2007) provide a literature review of bioturbation by deposit feeders in stabilizing and destabilising cases. A recent study at the North Sea (Borsje et al., 2008) suggests the stabilizing organisms are mainly responsible for the seasonal variation in suspended sediment concentrations, while the destabilizing organisms are mainly responsible for the spatial variation in fine sediment on the bed.

There are few studies concerning the interaction of contaminants and the effects of macrofauna on sediment dynamics (Mazik and Elliot, 2000), but in the cases where toxicity is known to reduce performance, bioturbation activity is certainly affected.

1.3.2 Topography

Bottom topography has an important effect on near bed velocities and on the structure of the BBL. The shear stresses at the bottom (see section 1.2.1) are a manifestation of the drag form. Therefore, it is important to define the drag force felt by the mean flow where the bed forms acts as a roughness element. It is also of interest for predictions of sediment transport to determine what is the portion of the total drag that is form drag and what portion is viscous drag, because only the viscous drag gives origin to movement. Large-scale sea bed topography is well known, but a more detailed description of microtopography for small-scale approaches is presently needed.

The effect of bed forms can only be parameterized in numerical models via the drag coefficient (directly related to τ), which most of the cases is calculate through empirical methods. Some studies quantified the effect of topographic roughness on hydrodynamic parameters, concerning different shapes and densities of roughness elements (Wooding et al., 1973) or beds with different median grain sizes and patches (Lawless and Robert, 2001; Thompson et al., 2004) and hydrodynamic parameters (Camenen et al., 2009). There is a recent review by Camenen et al. (2009) reviewing and comparing the different existing predictive formulas to estimate bed roughness where a new relationship based in the common knowledge is proposed. In this study, the commonly used relationship involving the grain size and Shields parameter appeared to be insufficient to characterize roughness height. Although there is an attempt to include natural phenomena in hydrodynamic models, such as movable bed roughness (Zhang et al., 2004), present hydrodynamic models are inaccurate in simulating flow over biogenic structures.

Measurements over natural beds are still few, and most of the present hydrodynamic models are not sufficiently calibrated for certain local parameters such as multi-scale physical roughness, interaction with animal generated flow or with flexile structures. All of these will have an impact on boundary layer properties, such as shear velocity, and ultimately on particle dynamics in the near-bed layer.

In many boundary layers over biogenic structures the relationship between topographical roughness (roughness height) and hydrodynamic roughness (the one estimated from velocity profiles characteristics) is still ill defined (see Chapter 3).

Some studies focused on the effect of epibenthic bivalves on the BBL, either by changes in topography or by increasing turbulence through their continuous filter pumping activity. The patches of organisms protruding from the sediment surface, thus changing surface roughness, produce regions of lower mean longitudinal velocities and more turbulence, as it was observed for the mussels *Atrina zelandica* (Miller et al., 2002; Nikora et al., 2002) and *Mytilus edulis* (Butman et al., 1994, van Duren et al., 2006). Beyond a certain critical density, the shells within these patches produce a skimming flow with a corresponding decrease in the drag coefficient and an uplift of the boundary layer surface (Green et al., 1998). The effect of epibentic organisms in microtopography has never been approached.

1.3.3 Filtration activity

The filtration activity of bivalves, measured as clearance rates (Coughlan, 1969) has a known impact on phytoplankton (Kamermans, 1993). Filter-feeders can promote a direct transport of suspended particles from the water column to the sediment, which is also an important process in sediment dynamics. Suspended particles are removed from the water column, by filtration activity, at velocities much higher than the velocities to allow mechanical deposition. The effect of bivalves' filtration activity on sediment removal is not dissociated from the production of faeces, which are generally thrown back to the environment in a different form and composition than the earlier loose suspended form.

Many authors have observed that the feeding physiology of bivalves such as the cockle *Cerastoderma edule* is affected by the quantity and quality of suspended matter (Navarro and Widdows, 1997, Iglesias et al., 1996; Hawkins et al., 1998; Ibarrola et al., 2000; Velasco and Navarro, 2005). Other works focused on the effect of increasing currents on clearance rates of bivalves (Wildish and Miyares, 1990; Ackerman, 1999; Sobral and Widdows, 2000; Newell et al. 2001; Widdows et al., 2002), showing that clearance rates have an unimodal response to current velocity.

While current velocity influences clearance rates of bivalves, the inverse is also true: the presence of bivalves influences local hydrodynamics. This has been shown in particular for epibenthic species like the blue mussel *Mytilus edulis* (Butman et al., 1994; van Duren et al., 2006) and the horse mussels *Atrina zelandica* (Green et al., 1998; Nikora et al., 2002). The presence of bivalves generally causes flow reduction and increased turbulence. The increase in turbulence is often related to increased bed roughness, produced by the shells protruding in the BBL, and a consequence of the interaction of the exhalent jets with the overlying flow (Monismith et al., 1990; O'Riordan et al., 1995). The effect of endobenthic bivalves (with a relatively low impact on the surface roughness) on the BBL has recently been studied by Ciutat et al. (2007) but the effect of clearance activity on near bed current velocities has never been approached.

1.3.4 Sediment reworking

Biological activities, such as food foraging and gallery/burrow building, increase the transport of solutes and influence the biogeochemical processes in cohesive sediments (François *et al.*, 1997; Hansen and Kristensen, 1998), a process known by bio-irrigation (Aller, 2001) and strongly enhance the transport of particles, a process known as bioturbation in the sediment in depth. These activities can also change geotechnical properties, such as grain size distribution (Giangrande et al., 2002) and influence microbial activity (Aller and Aller, 1998; Goñi-Urriza et al., 1999; Mulsow et al., 2002; Ferro et al., 2003), creating a patchy mosaic of anoxic microhabitats (Fenchel, 1996; Kristensen, 2000), and hence, will alter the diagenetic conditions within the sediment (Aller and Aller, 1998; Forster et al., 1999; Mortimer et al., 1999; Nielsen et al., 2004). Overall, macrofauna bioturbation and bio-irrigation have the potential to strongly influence the production, mobilisation and accumulation of certain contaminants (Rasmussen et al., 1998; Hammerschmidt and Fitzgerald, 2004).

In 1997, François and colleagues proposed a new approach for modelling sediment reworking with four functional groups:

1) Biodiffusers, organisms whose activities result in a constant diffusive transport of sediments in the bioturbated zone, from the interface to the maximum depth of reworking, e.g.: the clam *Ruditapes decussatus*, *C. edule,* amphipods;
2) Upward conveyors, head-down vertically oriented species which ingest material in depth and expel it at the sediment water interface, promoting an "active" transport of the sediment through the gut, ex: *Arenicola marina*;
3) Downward conveyors, head-up vertically orientated species causing an active transport of sediment through their gut from the sediment water interface to their egestion depth, ex: sipunculids and annelis;
4) Regenerator, digging organisms which transfer sediment from depth to the surface where it is washed away and replaced by other sediment. This behaviour has two effects. One effect is the biodiffusional mixing with a high output of sediment to the water column during digging, and the other effect is a net movement of the surficial sediment to the bottom of the burrow after it has been deserted, ex: fiddler crabs.

Bioturbation activity is characterised by both an intensity coefficient (Db, the biodiffusion coefficient) and a depth over which it occurs (mixing depth). It is commonly described using the classical one-dimensional biodiffusion model (Boudreau, 1986), which is governed by:

$$Db\frac{\partial^2 C}{\partial z^2} - \lambda C = 0 \qquad [10]$$

where C is the concentration of tracers and λ is a rate (decay) constant (time^{-1}).

In the past, experimental bioturbation studies have focused on community bioturbation in relation to differences in feeding types (Gerino et al., 1995, 1999; Flach et al., 1998; Turnewitsch et al., 2000; Green et al., 2002), scaling of bioturbation to the size of the organisms (Swift et al., 1996) and the presence of burrows (Dellapenna et al., 1998). Other studies have followed a more theoretical approach, relating Db to biological parameters in simplified models of biological activity (Wheatcroft et al., 1990; François et al., 2002; Meysman et al., 2003; Mugnai et al., 2003; Solan et al., 2004).

Yet, with regard to the interaction between bioturbation and contaminants, our knowledge remains sparse. Some studies have measured the effect of particular organisms (Gilbert et al., 1996, 2003; Kure *et al.*, 1997; Petersen *et al.*, 1998; Rasmussen et al., 1998) or natural communities (Swift et al., 1996) on the distribution of contaminants in the sediment in depth or concerning their transport to the water column, but only few take into account the effect of contaminants on the mixing activity of the biota (Mulsow et al., 2002).

1.4 Species studied

1.4.1 The cockle *Cerastoderma edule*

The cockle *Cerastoderma edule* (Fig. 1.3) is an endofaunal bivalve, widely distributed on European coasts, showing high tolerance to substrate type, salinity, temperature and current velocities (Boyden and Russel, 1972; Ysebaert et al. 2002). It occurs in densities up to 1750 ind m^{-2} (Ysebaert and Herman, 2002).

According to Ysebaert et al. (2002), *C. edule* occurs in environments with flow velocities of up to 80 cm s^{-1}, having a maximum probability of 0.35 to occur at flow velocities around 35 cm s^{-1}. Kamermans (1993) and Lenihan et al. (1996) found that cockles show increased growth with increasing flow velocities (0 – 7 cm s^{-1}) under laboratory and more clearly under field situations.

C. edule is a suspension feeder that feeds on phytoplankton and resuspended microphytobenthos (Sauriau and Kang, 2000; Rossi et al., 2004), and in the process changes the gradient and composition of suspended matter passing over the cockle beds (Smaal and Haas, 1997). The ecophysiology of *C. edule* is relatively well understood regarding the clearance rates, food absorption efficiency and faeces production, according to the quantity aaan quality of suspended matter (Iglesias et al., 1996; Navarro and Widdows, 1997; Ibarrola et al., 2000; Rueda et al., 2005; Widdows and Navarro, 2007).

The effect of *C.edule* on sediment dynamics has been studied recently. The effect of *C. edule* on biodeposit production is an important issue in what concerns sediment deposition. The cockle actively transports and transforms particles present in the water column, producing faeces that are deposited at the surface.

Figure 1.3 – The cockle *Cerastoderma edule*

Widdows and Navarro (2007) focused on the erosion threshold of pseudofaeces and faecal pellets. Loose mucus bound pseudofaeces have a lower erosion threshold of 15 cm s^{-1} compared to 25 cm s^{-1} for faecal pellets. Recent studies showed that the presence of *C. edule* is related to lower near bed currents and that the increasing density of *C. edule* (up to 312 ind m^{-2}) is related to increased erosion rates of sediments from the Tamar estuary Ciutat et al. (2007). The reduction effect of cockles in the BBL is certainly related to inhalant current produced during feeding. Troost et al. (2009), using particle image velocimetry and model calculations, estimated that the average jet speed of *C. edule* was 20.8 cm s^{-1}.

Cockles on their own are not a roughness element, as they are buried in the sediment with the siphons opening levelled with the sediment surface. The roughness they produce is a result of their adjustment to the sediment surface and increasing near bed current velocities. Burrowing behaviour is commonly affected by parasitism and bacterial load (Blanchet et al., 2003). The entrainment drag coefficient (C_D) of cockles varied from 0.43 to 0.56 (estimated using particle image velocimetry) and the fall velocity and settling drag coefficient were constant and estimated to be of 0.36 m s^{-1} and 1.08, respectively (Peña et al., 2008). The description of the effect of *C. edule* on the sediment topography was never performed and the explanation of how *C. edule* affects the near-bed currents is still not clear (see Chapter 2).

1.4.2 The ragworm *Nereis diversicolor*

The ragworm *Nereis diversicolor* (Fig. 1.4), an endobenthic species, is widespread in brackish water environments throughout north and south-western Europe, showing a high tolerance to temperature, salinity and occurring in sand or mud habitats. Typical densities are in the range of 1000-2000 ind m^{-2} for adult populations, but can reach up to 16000 ind m^{-2} for juvenile populations in spring (Davey, 1994; Fidalgo e Costa et al., 1998). In Portugal densities range from 430 to 16000 ind m^{-2} (Fidalgo e Costa et al., 1998; Abrantes et al., 1999).

N. diversicolor builds and lives in mucus-lined galleries with considerable structural complexity (Meadows and Meadows, 1991), which provides refuge from predators. The gallery walls are covered by mucus secreted from epidermis glands covering the entire surface of the body. These secretions are rubbed and pushed against the walls of the burrow to consolidate the walls (Meadows and Tait, 1989; Meadows *et al.*, 1990). Complex gallery networks can extend down to 30 cm depth, and are related to body length, although organisms > 10 cm long can be found in the upper 2 to 3 cm of the sediment (Anderson and Meadows, 1978; Esselink and Zwarts, 1989).

Davey and Partridge (1998) observed that only 20% of the population was found below 8 cm depth. Galleries are known to increase sediment permeability, de-watering, and provide conduits for overburden-driven drainage, thereby stepping up sediment consolidation (Meadows and Tait, 1989; Murray et al., 2002). All these activities result in increased sediment shear strength (Meadows and Tait, 1989; Meadows and Hariri, 1991).

N. diversicolor is an opportunistic species that uses different feeding modes such as filter and deposit feeding, scavenging and predation on other macrofauna (Esselink and Zwarts, 1989 and references within; Vedel et al., 1994). It is known to be resistant to high levels of pollution and is a suitable bioindicator for toxicity (Bryan and Hummerstone, 1971; Ozoh, 1994; Bernds et al., 1998).

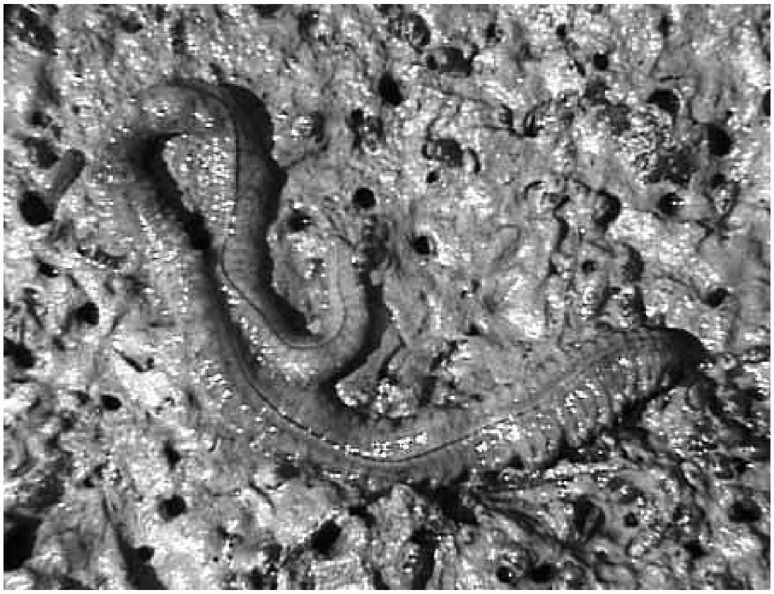

Figure 1.4 – The ragworm *Nereis diversicolor*

Some pioneer studies on this species have concentrated upon the bioturbation effect of burrowing activity (Trevor, 1977; Davey, 1994) and on sediment reworking (Gerino, 1991; François et al., 2002). François et al., (2002) classified *N. diversicolor* as being a “gallery-diffusor” in terms of sediment mixing, distinct from “biodiffusors” and “downward conveyers”. Gallery diffusors display a combination of bio-advective (fast unidirectional downward transport) and bio-diffusive mechanisms (bi-directional mixing) (François et al., 2002). The bioturbation activity of *N. diversicolor* is greatly enhanced by food supply (Nogaro et al., 2008).

There is some controversy about the stabilising effect that *N. diversicolor* has on sediments. Some early works considered this species as a stabiliser, judging from its influence on the shear strength properties of the sediment (Meadows and Tait, 1989; Meadows et al., 1990). On the other hand, this species has a destabilising effect due to its negative influence on the colonisation of the sediments by benthic diatoms (Underwood and Paterson, 1993; Engelsen and Pihl, 2008). Although some literature (Eckman et al., 1981; Davey, 1994) refers to the direct effect of macrofauna density on bioturbation, the quantification of this activity is required, and the relative importance of this species on sediment stability needs to be assessed.

1. 5 General objectives and structure of the thesis

The main objective of this work is to contribute to the knowledge of the biological benthic interactions with estuarine sediment dynamics, using a small-scale study approach. The work presented in this thesis focuses on the behaviour and interactions of two estuarine species, from different functional groups, with sediment dynamics. The effect of the cockle *C. edule* on transport and removal of suspended matter from the water column and the effect of the ragworm *N. diversicolor* on sediment erodability, consolidation and remixing, is investigated taking into account density dependent effects. Each species affects sediment dynamics distinctively by acting on different components of the ETDC cycle. This thesis furthermore assesses the interactive effect of copper (Cu) contaminated sediments on the behaviour of *N. diversicolor* and the combined effect of bioturbation and copper on sediment stability.

This thesis is divided in 6 sections that include chapters corresponding to articles published, in publication or submitted.

PART I – General introduction

A general introduction on sediment dynamics, hydrodynamic processes and biological activity and their interactions is given.

PART II – Interaction between *Cerastoderma edule* and sediment dynamics: hydrodynamics in the benthic boundary layer

Chapter 2 – Fernandes, S., Sobral, P., van Duren L.A. 2007. Clearance rates of *Cerastoderma edule* under increasing current velocity, measured in two different flumes: estimation methods and interrelation with hydrodynamics. Continental Shelf Research 27, 1104-1115

Chapter 3 - Fernandes, S., van Duren, L.A., van der Wal, D., Orvain, F., Herman, P.M.J. How cockles change the benthic boundary layer: density effects on topography and turbulence. *In preparation.*

Chapter 2 deals with methodological issues (flume design) related to bio-fluid dynamic research and aims to address the environmental interaction between local hydrodynamic regime and cockle feeding behaviour. The effects cockles have on fluid dynamics may be the result of topographical differences and of interaction of the feeding currents with the flow. A subsequent study attempted to separate these two processes. These results are included in **Chapter 3**. The objective of this study is to assess the effects of filtration activity of *C. edule* on the microtopography and on the BBL by comparing the effects of artificial (with no filtration activity) and natural (with organisms actively filtering) beds while accessing the influence of density. This study was performed using two densities of organisms and the effect due to the organisms was assessed by quantification of the additional effect of animal pumping activity on the bottom turbulence, by comparing treatments with and without animals, where microtopography is identical.

PART III – Interaction between *Nereis diversicolor* and sediment dynamics: sediment biogeochemical properties and erodability

Chapter 4 – Fernandes, S., Sobral, P., Costa, M.H. 2006. *Nereis diversicolor* effect on the stability of cohesive intertidal sediments. *Aquatic Ecology* 40: 567 – 579

Chapter 5 – Measuring bioturbation of *Nereis diversicolor* with luminophores: experimental and modeling artifacts.

The ragworm *N. diversicolor* inhabits a network of galleries and feeds at the sediment surface. With the aim to characterise the effect of bioturbation by *N. diversicolor* on sediment dynamics, erodability (erosion rates, critical velocity) and sediment shear strength were measured. The effect of organism density on sediment dynamics was also analysed. This study is described in **Chapter 4**.

Other objective of this work is to characterise and quantify the bioturbation activity of *N. diversicolor* in terms of biodiffusion coefficients (Db), while focusing on the methodological procedures and evaluating the effect of sampling time with two different methods detailed in **Chapter 5**. Two different biodiffusion modelling procedures were used, in order to examine if and how artifacts are created by applying an exclusive bio-diffusive model to bioturbation, which is characterized by a mixture of diffusive and advective aspects.

PART IV – Interaction between contaminated sediments and biological activity: *Nereis diversicolor* case study

Chapter 6 - Fernandes, S., Alcântara, F., Sobral, P. 2009. The effect of *Nereis diversicolor* on shear strength and erodability of cohesive sediments: the influence of copper contamination. Estuarine Costal and Shelf Research (in press).

Chapter 7 – Fernandes, S., Meysman, F.J.R., Sobral, P. 2006. The influence of Cu Contamination on *Nereis diversicolor* bioturbation. *Marine Chemistry* 102, 148-158

The transport and deposition of contaminated sediments is an emergent issue with impacts on ecosystem quality. One objective of this work is to quantify the effect of copper (Cu) contaminated sediments on the activity of *N. diversicolor*. This was analysed in terms of sediment stability, by determination of erosion rates and sediment shear strength. These results are in detailed in **Chapter 6**. The effect of Cu on the bioturbation activity of *N. diversicolor* was also assessed in terms of Db (**Chapter 7**). In this study, the effect of Cu contamination on the sediment mixing activity of *N. diversicolor* was evaluated using two different biodiffusion modelling procedures.

PART V – General conclusions

In this section, the major conclusions of each section are summarised and discussed in a more general context of faunal effects on sediment dynamics. Some considerations are drawn regarding the present status of estuarine sediment dynamics research and practical applications.

PART VI – References

This section compiles the references from all chapters.

1.6 References

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PART II

INTERACCTIONS BETWEEN *Cerastoderma edule* AND SEDIMENT DYNAMICS

Chapter 2

Clearance rates of *Cerastoderma edule* under increasing current velocity, measured in two different flumes and interrelation with hydrodynamics

CLEARANCE RATES OF *CERASTODERMA EDULE* UNDER INCREASING CURRENT VELOCITY, MEASURED IN TWO DIFFERENT FLUMES AND INTERRELATION WITH HYDRODYNAMICS

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Abstract

Estimations of clearance rates (CR) of *Cerastoderma edule* (300 ind m^{-2}) as a function of free-stream current velocity (U) (from 5 to 40 cm s^{-1}) are compared between a small annular (60 l) and a large racetrack (8850 l) flume with different hydrodynamic conditions. Results showed that the flumes differ considerably in their hydrodynamic characteristics. The relationship between clearance rate and U is different in the two flume tanks, however, there appears to be a straightforward unimodal trend between CR and shear velocity (u_*). The cockles themselves were found to influence boundary layer (BBL) characteristics, with steeper velocity gradients and increased mixing over the cockle bed compared to bare sediment. This brings new evidence on how endobenthic organisms can affect the BBL. However, the influence of CR on u_* could not be quantified because these parameters have interactive effects that cannot be dissociated.

Key-words: Benthic boundary layer, *Cerastoderma edule*, Clearance rates, Flumes, Velocity

2.1 Introduction

The clearance rate of bivalves has a known impact on the phytoplankton concentrations in aquatic ecosystems (Kamermans, 1993). It is also an important process on sediment dynamics. Filter-feeders can promote a direct transport of suspended particles from the water column to the sediment surface. Accurate establishment of clearance rates of bivalves has been and is the subject of extensive research and discussion (Riisgård, 2001). Many authors have observed that clearance rates increase with quantity and quality of suspended matter up to a threshold, after which clearance and absorption efficiency is negatively influenced by increasing quantity (Navarro and Widdows, 1997) and quality (Iglesias et al., 1996; Hawkins et al., 1998; Ibarrola et al., 2000).

Later works focus on the effect of increasing currents on clearance rates of bivalves (Wildish and Miyares, 1990; Ackerman, 1999; Sobral and Widdows, 2000; Newell et al. 2001; Widdows et al., 2002), showing that low current velocities have a positive effect on clearance rates, but as velocity increases, clearance rates are reduced having an unimodal response to current velocity.

While current velocity influences clearance rates of bivalves, the inverse is also true: the presence of bivalves influences local hydrodynamics. This has been shown particularly for epibenthic species (Butman et al., 1994; Green et al., 1998; Nikora et al., 2002). Their presence is related with regions of increased drag coefficients, more energetic turbulence, and consequently reduced flow speed. The increase in turbulence is often related to the effect of increased roughness, produced by the shells protruding in the BBL, and a consequence of the interaction of the exhalent jets with the overlying flow (Monismith et al., 1990; O'Riordan et al., 1995). The effect of endobenthic bivalves, with a relatively low impact on the surface roughness, on the benthic boundary layer and the effect of clearance activity on current velocities has never been studied.

The cockle *Cerastoderma edule* is a widely distributed bivalve from European coasts that occurs in densities up to 1750 ind m^{-2} in the Scheldt estuary (Ysebaert and Herman, 2002). It is an endobenthic suspension feeder that feeds on phytoplankton and resuspended microphytobenthos (Sauriau and Kang, 2000; Rossi et al., 2004), and in the process changes the gradient and composition of suspended matter passing over the cockle beds (Smaal and Haas, 1997). According to Ysebaert et al. (2002) *C. edule* occurs in environments with flow velocities of up to 80 cm s^{-1}, having a maximum probability of 0.35 to occur at flow velocities around 35 cm s^{-1}. Kamermans (1993) and Lenihan et al. (1996) found that cockles show increased growth with increasing flow velocities (0 – 7 cm s^{-1}) under laboratory and more clearly under field situations.

The reason why clearance activity of bivalves is influenced by water flow is twofold: firstly, the flow determines the rate at which food particles are transported towards the animals, and secondly, because the water exerts a force on the bed and hence on the feeding apparatus of the animals. It is therefore not only the flow velocity that is important, but also the structure of the boundary layer and the shear forces caused by the velocity gradient. Experimental research on the effect of flow effects on bivalves feeding is normally carried out in a flume tank. The boundary layer structure is related to flume design (straight, racetrack, annular and with several dimensions). This can lead to difficulties for comparisons or misinterpretation of results obtained in different laboratory devices. This was recently observed by Amos et al (2004) in a comparative study on sediment stability measurements in different flumes.

Some larger flumes, with a straight working section provide hydrodynamic conditions that closely resemble the boundary layer in the field. However, the large volume of water, relative to a small area of cockle bed can introduce large measurement errors on the estimations of clearance rates. Small annular flumes have the advantage of being easier to operate. There are no entrance and exit conditions to take into account and the relatively small test section surface-to-volume ratio makes it easy to detect reductions in algal concentrations. However, the curvature of the flume walls produces a centrifugal force that generates secondary flows and radial variability in bed stress (Amos et al., 1992; Yang et al., 2000), producing a boundary layer structure very different from the field. Cross-channel variation in bed stress variability is also reported for long straight flumes, but it is considered to be less than stress gradients encountered in annular flume designs (Black and Cramp, 1995).

In this work, we compare the effect of current velocity on clearance rates of *C. edule* in two different flumes: a small annular flume (60 l) and a big racetrack flume (8850 l). We also analyse the effect of *C. edule* on the boundary layer characteristics, by comparing it with the hydrodynamic characteristics over a bare sediment and over a smooth surface.

2.2 Material and methods

2.2.1 Organisms collection and experimental set-up and procedures

The experiments took place in November-December 2002 at the Instituto do Mar-Universidade Nova de Lisboa (IMAR-FCTUNL) and at the Netherlands Institute of Ecology (NIOO) in June-July 2003.

Organisms were collected (see details in Table 2.1a), brought to the laboratory and allowed to acclimatise, for a minimum of 5 days, in seawater aquaria under continuous aeration, temperature and salinity similar to the experimental conditions (see Table 2.1b), prior to introduction into the flume for experimental measurements (see details of flumes below).

In the IMAR flume, fine sand (< 500 μm) was used as an experimental substrate. Observations at that time revealed that the resuspension of very fine grains would interfere with cell counts and produced error in the estimation of clearance rates. For this reason, in the NIOO flume, coarser sand (average grain diameter 750 μm) was used.

On both flumes, each group of organisms (see Table 2.1a) was introduced into the flume and left overnight under a free-stream current velocity of about 2 cm s^{-1}. The next day, the organisms were exposed to increasing free-stream current velocities (from 5 to 40 cm s^{-1}). Before each velocity run, care was taken to substitute dead or inactive organisms. The treatments with *C. edule* will be designated as Ck and the control treatments as C. Later we will refer treatment S, concerning the velocity measurements over a smooth surface, the channel PVC surface of the NIOO flume.

In the beginning of each current velocity run, microalgae (see Table 2.1b) were added to the water in order to obtain a concentration of 16000 cells ml^{-1}. Algal cells were allowed to distribute homogeneously in the water column. Subsequently, water samples were taken at five heights (2, 7, 12, 17 and 22 cm above the bed) at fixed time intervals (see Table 1d) for the determination of suspended cell depletion over time. Cell counts were performed in Coulter Counter® Mod II and Multisizer II. Water samples were also taken in the beginning and at the end of each velocity run and filtered onto Whatman GF/C filters for determination of total particulate suspended matter.

2.2.2 Clearance activity estimations

Clearance rates (CR) were estimated on C and Ck treatments in the two flumes, by the cell depletion method (Coughlan, 1969). It measures the volume of water cleared of suspended particles per unit of time. It has proved to be a reliable method in many clearance studies on bivalves, although it has the disadvantage that cell concentrations decline during the experiments (Riisgård, 2001). This artefact was minimised in this experiment by assuring a minimum concentration of cells in the flume. In the end of all velocity runs in both experiments, cell concentrations were above 5000 cells ml^{-1}. Therefore reduced clearance activity due to low food concentration presumably did not occur.

Table 2.1 – Characteristics for IMAR and NIOO experiments in terms of a) organisms, b) experimental conditions, c) flume characteristics and d) sampling conditions.

Table 2.1a: *Cerastoderma edule*

	IMAR	**NIOO**
Collection site	Mitrena, Sado estuary, Portugal	Paulina Polder, Scheldt estuary, the Netherlands
Shell length (mm)	34 ± 1	26 ± 2
Dry weight (g)	0.40 ± 0.03	0.16 ± 0.01
Experimental density (ind m^{-2})	280	280
Organisms per group in the flume	15	260
Groups	4 + 1 control	3 + 1 control

Table 2.1b: Experimental conditions

	IMAR	**NIOO**
Temperature (T^o)	16 ± 1	15 ± 1
Salinity	34	29
Seston concentration (mg L^{-1})	6 ± 2	18 ± 2
Food type	*Phaeodactylum tricornutum* (live culture)	*Isochrysis galbana* (algal paste, Reed Mariculture)

Table 2.1c: Flume characteristics

	IMAR	**NIOO**
Flume type	Annular	Racetrack
Flume area (m^2)	0.21	21.2
Flume diameter (m)	0.6	-
Length straight section (m)	-	11.7
Width of experimental section (m)	0.15	0.6
Experimental section area (m^2)	0.05	0.9
Current velocity range (cm s^{-1})	0 - 40	0 - 40
Sediment depth (cm)	8	14
Water volume (l)	60	8850
Water column (cm)	30	40

Table 2.1d: Sampling conditions

	IMAR	**NIOO**
Cells homogenisation time (min)	2	10
Time between sampling (min)	20	intervals increasing form 20 to 30 to 45 minutes
Total sampling time (min)	80	240

The numbers of cells for each sample collected over the experiment were weighted according to sampling heights in order to estimate the concentration of cells in the water column. The exponential decrease in algal concentration in the flume (k_{cell}) as a function of time (t) was calculated with STATISTICA® software using a general linear model as

$$k_{cell} = \frac{\ln\left(C_0 / C_t\right)}{t}$$

where C_0 and C_t are the particle concentration at time 0 and time t. To account the flume filtration, i.e. cell attachment onto walls or deposition of cells, the exponent decline of cells measured in the control (k_C) was subtracted from the one observed in the cockle treatments (k_{CK}). To determine the clearance rate in the flume, both k's should be multiplied by the volume of water in the flume (V). Individual clearance rates are obtained by dividing it by the number of organism in the flume (n) as:

$$CR = \frac{V}{n}\left(k_{CK} - k_C\right)$$

The cockles used in the NIOO experiment were considerably smaller than those in the IMAR experiment (Table 1a). Clearance rates do not scale linearly with body weight, but according to a power law. To remove the effect of body weight, the data were standardised and weight specific rates (Y_s) were calculated using the formula:

$$Y_s = Y_e\left(\frac{W_d}{W_e}\right)^b$$

where , Y_e is the uncorrected rate, W_d is the weight to be corrected for (1g) , W_e is the average dry weight of the respective cockles group and b is the weight power (0.70, Møhlenberg and Riisgård, 1978).

2.2.3 Flume calibration

The IMAR flume (see Table 2.1c) is an annular flume with 15 cm channel width, built after Widdows et al. (1998), where current velocities are driven by a rotating drive plate of adjustable height. In the IMAR flume, it was only possible to measure free-stream velocities but no method for accurate velocity measurements in the boundary layer was available. For this reason, a model (F15) was developed using a computational fluid dynamics (CFD) commercial software, CFX 5.7, to simulate hydrodynamic conditions in the IMAR flume, given the geometry of the flume and different driving forces on the rotating plate to produce the different free-stream velocities that were actually measured. Good boundary layer measurements were available from a similar flume, with the same external dimensions, but with a narrower channel width of 10 cm. This flume (IMAR10) is fitted with a Sontek micro-ADV Lab and velocity profiles were available for the free-stream current velocities of 5, 10, 20 and 40 cm s^{-1}. To calibrate the F15 model, we first developed a CFD model for the IMAR10 flume (F10). When this model proved capable of accurately reproducing velocity profiles in the narrower flume, this was used as a basis for the F15 model, only changing the channel width. F15 and F10 were based on the corresponding geometry and were meshed using tetrahedral elements. The walls and the bottom of the flume were modelled as smooth walls with no-slip conditions. Water flow in the model was driven from the top of the flume. A previously determined relationship was used, between the drive plate and the free-stream velocity in the IMAR flume (Fernandes, 2001), based on time measurements of complete rotations of neutrally buoyant particles in the flume and of the driving plate.

In the NIOO-CEME flume the water is circulated around the flume via a motor-driven conveyor belt with vertical paddles. Turning vanes are positioned in each of the two bends of the racetrack flume to reduce secondary circulation and stacks of pvc pipes act as collimators. In the NIOO flume, profiles with a vertical resolution of 8 mm were measured with a Nortek ADV mounted on an automated 3D positioning system, over a smooth surface. To cope with horizontal variability, due to e.g. effect of exhalent jets near the bed, at each height 8 points at different horizontal locations within a surface area of 3 cm^2 were measured. Each individual measurement lasted 5 seconds. The temporal and spatial averages at each height provided the measurements for each profile. The same velocity measurements were repeated during the clearance activity experiments, in the front (5 cm from the leading edge of the cockle bed) and in the back part (60 cm further) of the cockle bed. Subsequent analysis showed significant changes of the boundary layer structure between the front and the back of the cockle bed. This is presumably due to the effect of the cockles on the near-bed flow. For this reason, hydrodynamic comparison between treatments was based on the measurements performed in the back part of the cockle beds.

2.2.4 Hydrodynamic parameters estimations

Shear velocity (u_*) and roughness length (z_0) were determined from the logarithmic velocity profile using Karman-Prandtl Law of the Wall equation:

$$U = \frac{U_*}{\kappa} \ln\left(\frac{z}{z_0}\right)$$

where U is the free-stream velocity, z is the height above the bed and κ is the Von Karman constant (0.40). Comparisons of fluid dynamics between flumes are based on CFX simulations and measurements (NIOO) over a plastic smooth surface (S). Comparisons of fluids dynamics between treatments (in the NIOO experiments) were based on measurements for the respective treatment.

2.3. Results

2.3.1 Flume hydrodynamics

Good agreement was observed between F10 estimates and ADV velocity measurements in the 10 cm flume channel, except for depths below 0.2 cm (Fig. 2.1). The model was not designed to resolve the viscous sub-layer and ADV measurements so close to a solid surface are known to be unreliable. Therefore these values were neglected for further calculations.

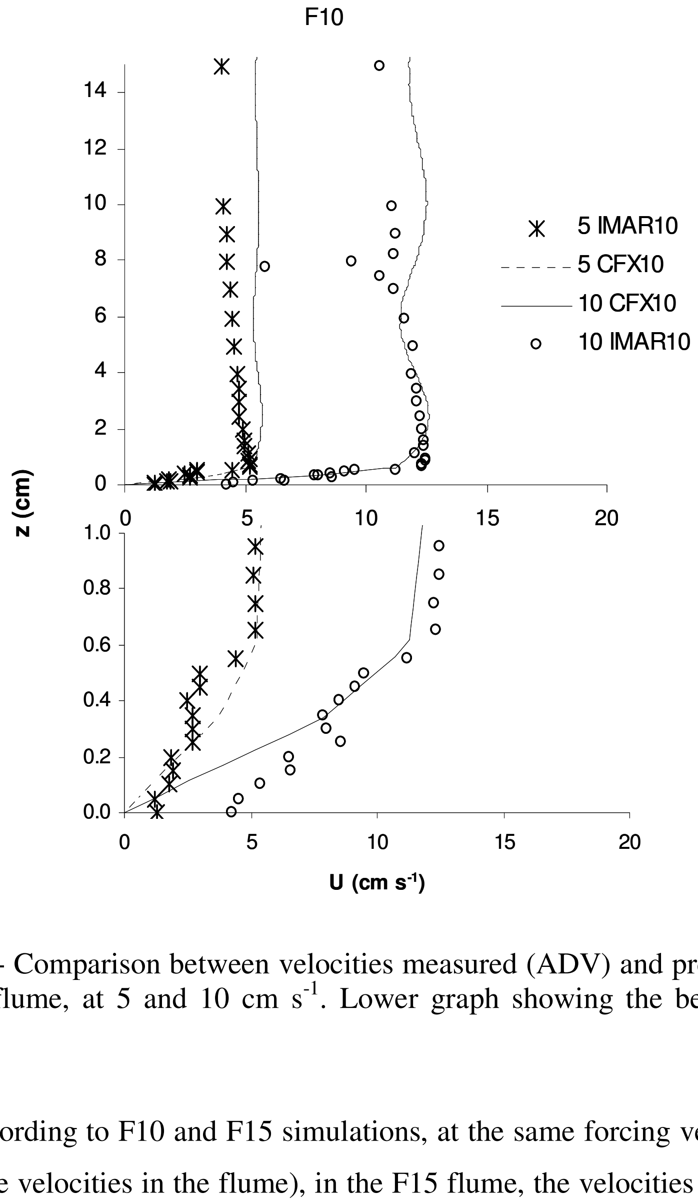

Figure 2.1 - Comparison between velocities measured (ADV) and predicted (CFX) for the 10 cm width flume, at 5 and 10 cm s^{-1}. Lower graph showing the benthic boundary layer in detail.

According to F10 and F15 simulations, at the same forcing velocity (given as input to generate the velocities in the flume), in the F15 flume, the velocities would be slightly higher and the thickness of the benthic boundary layer (0.6 cm in F10) was 0.1 cm higher in F15. Velocity profiles estimated by F15 are shown in Fig. 2.2.

The velocity profiles measured over a smooth surface in the NIOO flume are expressed in Fig. 2.2a. These profiles show a relatively thick, fully developed logarithmic boundary layer in contrast to the thin, steep linear profile measured in the IMAR flume (Fig. 2.2b).

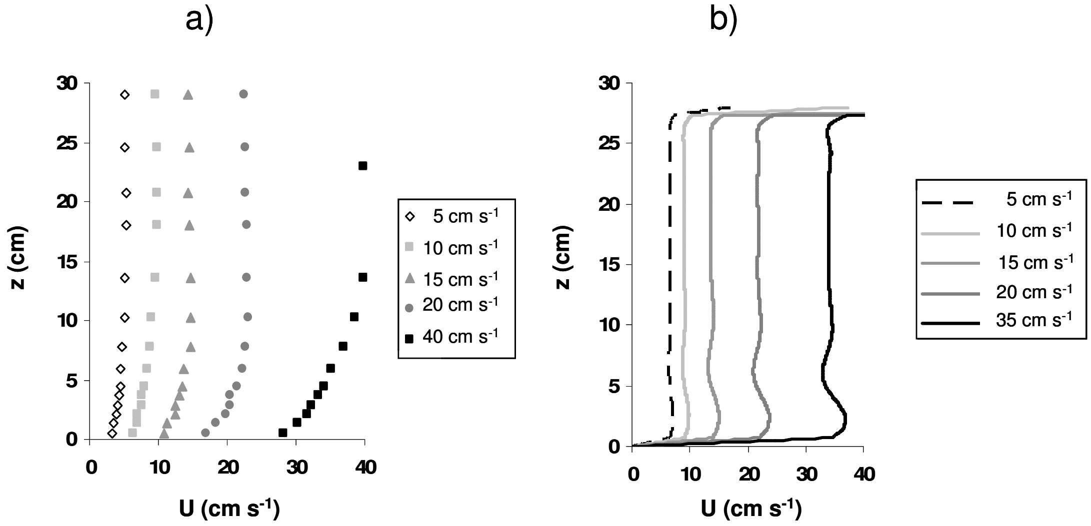

Figure 2.2 - Hydrodynamic conditions in the flumes a) measurements in the NIOO flume with ADV; b) CFX simulations for the IMAR flume.

The two flumes show a distinct difference in the relationship between u_* and free-stream velocity (Fig. 2.3). In the NIOO flume u_* reached 1 cm s^{-1} at 40 cm s^{-1} free-stream current velocity, while in the IMAR flume, at the same current velocity u_* was 8 times higher. Roughness length was estimated to be 1.50 mm in the IMAR flume, and much lower in the NIOO flume (0.001 mm).

2.3.2 Effect of cockles on hydrodynamics

The analysis of the velocity profiles show that cockles reduce the velocity in the first 4 cm above the surface (Fig. 2.4). Considering the average of U at these depths, cockles reduced near-bed velocities up to 12% at 5 cm s^{-1}, and at 40 cm s^{-1} they had a reduction effect of 17% compared to the sediment without cockles (C).

Comparisons of the U profiles measured over the flat flume bottom (S) and over sediment without cockles (C) show that the sediment surface produces almost no effect at low currents but reduces near-bed velocities up to 9% at 40 cm s^{-1} (Fig. 2.4).

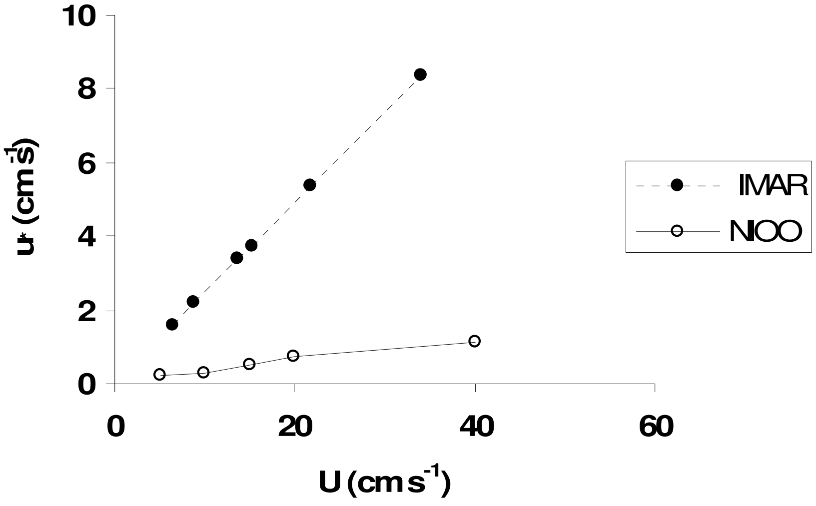

Figure 2.3 - Relationship between shear velocity u* estimations (using LW method) and current velocity (U), in the IMAR and NIOO flumes corresponding to the following linear regressions ($u_* = 0.2462U+0.0141$, $R^2=1.0$) and ($u_* = 0.0267U+0.1008$, $R^2=0.96$).

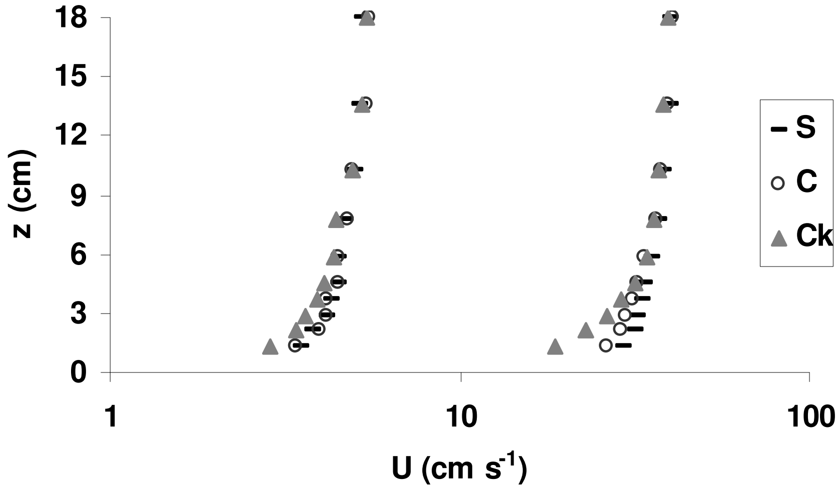

Figure 2.4 - Velocity profiles at 5 and 40 cm s^{-1} measured over a smooth surface (S), the control sediments (C) and cockles bed (Ck) in the NIOO flume.

The flow reduction in the near-bed layers results in a steeper velocity gradient. Consequently we found that u_* estimations where higher in the presence of cockles (Fig. 2.5). On the cockle treatment u_* values were 16% higher at 5 cm s^{-1} and 74% higher at 40 cm s^{-1} (in comparison with bare sediment, C), while u_* on sediment, at 40 cm s^{-1}, was 65% higher than over a smooth surface. As a consequence, the relationships found between u_* and U depend on the presence of sediment and cockles and differ from measurements over a smooth surface (Fig. 2.5).

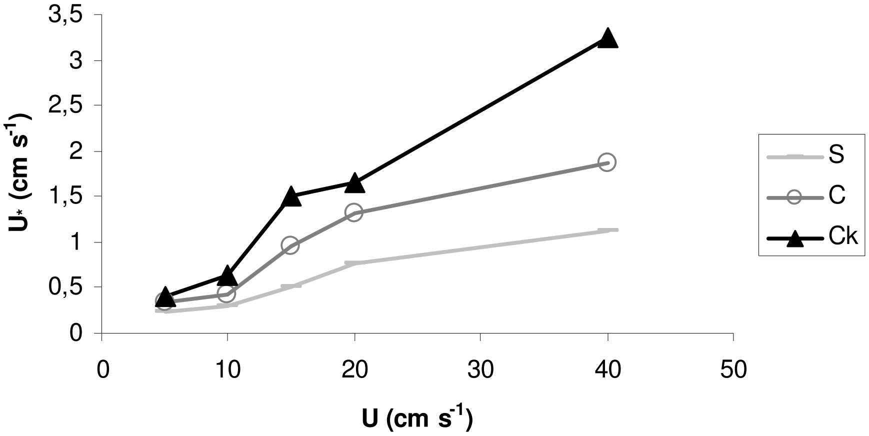

Figure 2.5 - Relationship between current velocity (U) and shear velocity (u_*) for a smooth surface (S), control sediments (C) and cockles bed (Ck) in the NIOO flume.

In comparisons with a smooth surface, *C. edule* and the sediment had a clear effect on z_0 (Fig. 2.6). *C. edule* had an increasing effect over z_0 of 286% at 5 cm s^{-1} and 4681% at 40 cm s^{-1} when compared to C. This large increase in roughness length did not coincide with a conspicuous visual increase of physical bed roughness, although this was not quantified.

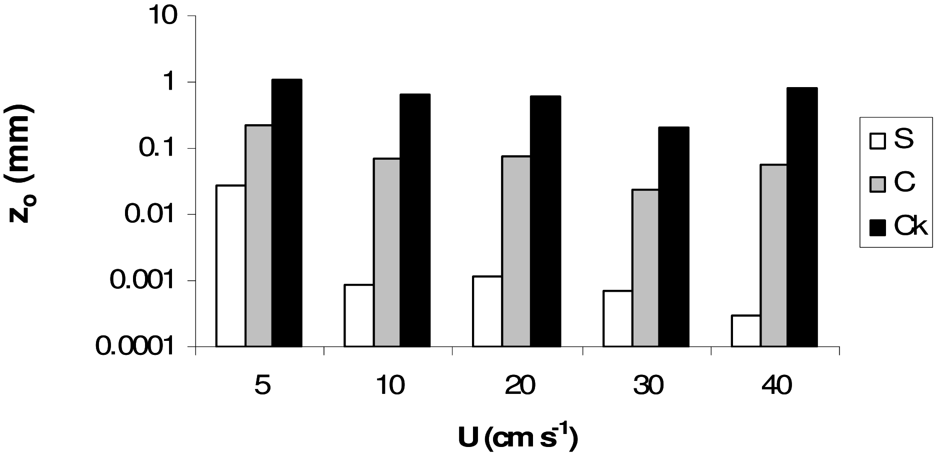

Figure 2.6 - Roughness length (z_0) over increasing U in a smooth surface (S), over the control sediments (C) and cockle bed (Ck) in the NIOO flume. z_0 axis is in a logarithmic scale for a better visualisation.

2.3.3 Visual observations on sediments with cockles

In the IMAR experiments, at U of 15 cm s^{-1}, bed load transport was observed and the flat surface topography started to become rippled. Sand erosion started at 30 cm s^{-1}. At this current velocity it was possible to observe the organisms using their feet to actively burrow in the sand, while aggregating in groups of 3-4 individuals. In an annular flume, it was possible to observe the erosion and the corresponding deposition in further points of the channel. The cockles appeared to be able to cope with considerable sediment deposition. Although covered by sand, the cockles actively crawl up to the surface, and kept feeding. At 40 cm s^{-1}, some organisms were carried away with the current but with their feet, they were able to settle on further ahead ripples. In the NIOO experiments, bed load transport started at U of 20 cm s^{-1} but still at 40 cm s^{-1} there was almost no resuspension. The organisms appear to cope with this bed load transport, which seemed not to interfere with their clearance activity.

2.3.4 The effect of hydrodynamics on clearance activity

The analysis of microalgal profiles showed that the concentration of cell in the water column had a faster reduction over time in the IMAR flume in comparison with the NIOO flume (presumably due to the smaller ratio of volume of water by number of animals in the flume), but no obvious depletion layer was observed. Cell reduction in the control treatments was negligible in the IMAR flume (Fig. 2.7).

In the NIOO flume the control experiments showed a significant cell reduction that was inversely related to flow velocity (Fig. 2.7). At the lowest velocity cell reduction in the control was of the same order of magnitude as in the cockle treatment. At the highest velocity there was hardly any loss at all in control treatments.

The effect of free-stream current velocity on clearance rates is different in both flumes. Statistical analysis (two-way ANOVA) showed that clearance rates were significantly different in the two flumes ($p< 0.001$) and over increasing free-stream current velocity ($p< 0.01$) (Fig. 2.8). The cockles in the NIOO flume increased their clearance rates with velocity. The maximum clearance rates in the NIOO flume were found at 30 and 40 cm s^{-1}. These range between 2.3 and 3.6 l h^{-1} ind^{-1}, corresponding to a weight specific rate of 6.3 – 11.2 l h^{-1} g^{-1}. In the IMAR flume, the highest clearance rates were found at about 15 cm s^{-1} (3.7 l h^{-1} ind^{-1} ± 0.7, corresponding to a weight specific rate of 7.0 l h^{-1} g^{-1} ± 1.3), after which they declined, stopping at 40 cm s^{-1}.

Whereas the relationship between CR and U observed in the two different flumes is different, the one between CR and u* (based in the relationships shown in Fig. 2.3) appears to show a maximum (Fig. 2.9). The analysis of CR based on u* (Fig. 2.9) show that low velocities increase clearance rates up to a point (approximately 3.8 cm s^{-1}), after which they start to decline.

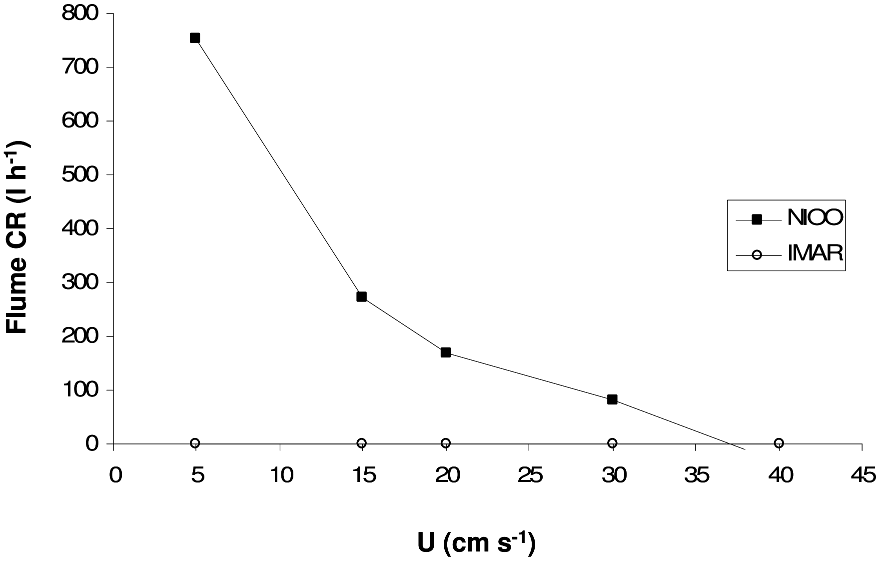

Figure 2.7 – Flume clearance rates (CR) over increasing free-stream current velocities (U) for the control treatments in the NIOO and IMAR flume.

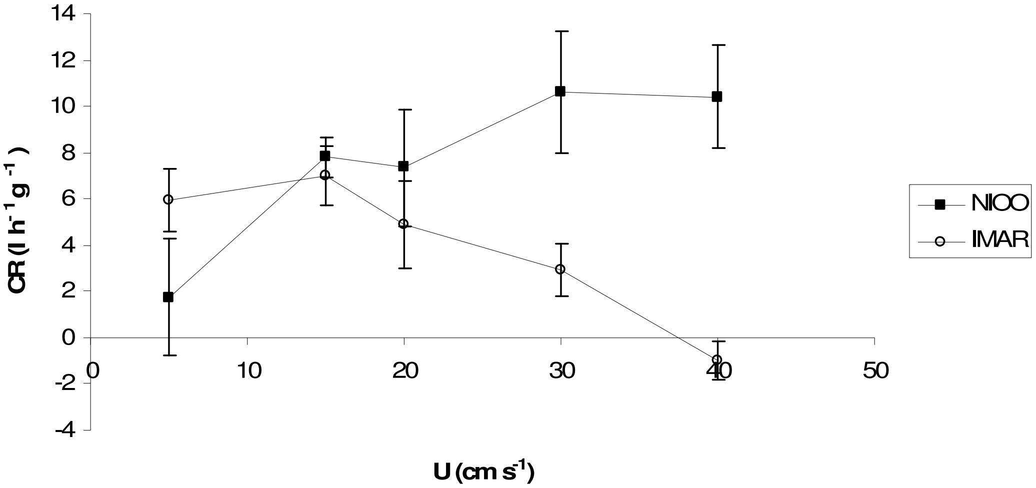

Figure 2.8 – Weight specific clearance rates (estimates with CD method) over increasing free-stream current velocities (U) in the IMAR and NIOO flume. (± Standard deviation)

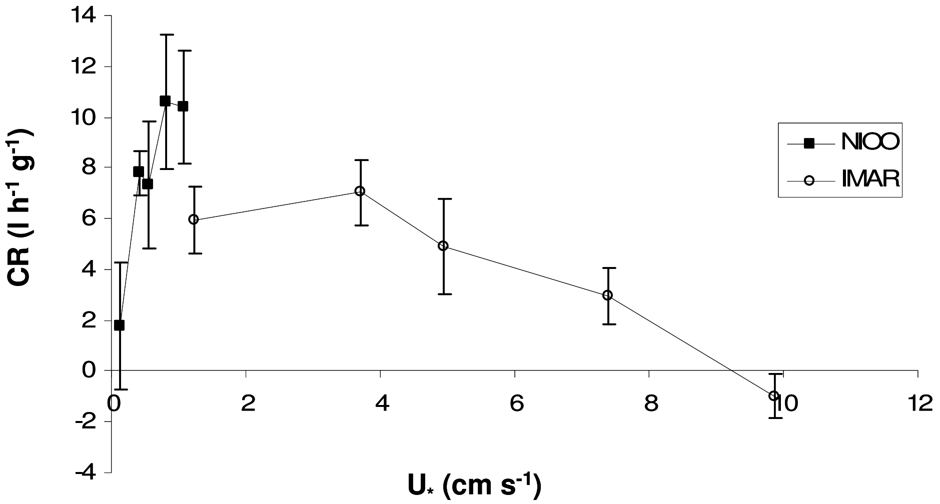

Figure 2.9 - Weight specific clearance rates (estimates with CD method) over increasing shear velocities (u*) in the IMAR and NIOO flume. (± Standard deviation).

2.4 Discussion

2.4.1 Comparisons between flumes

The hydrodynamic conditions in the two flumes presented different characteristics. In the NIOO racetrack flume the hydrodynamic conditions are closer to the observed in natural conditions (Finelli et al., 1999). However, the clearance observed in the NIOO control (Fig. 2.7) indicates that there are some losses of material under low current velocities which compromises optimal laboratory conditions. Due to the very large volume of the flume, relative to the number of filtering cockles, the error of measurement in the NIOO flume is relatively large. Another source of error in this set-up is the relatively large “consumption rate” of the flume itself at low velocities. At the lowest velocity the “flume consumption” even out-competes the consumption by the cockles. This is probably primarily due to turbulence reducing structures, such as collimators, where algae can get trapped. These structures will also promote the break down of any possible vertical gradient. Such structures are absent in the IMAR flume. The higher velocity gradients in the IMAR flume will also prevent sedimentation of the algae.

The hydrodynamic conditions in the IMAR annular flume are characterised by a compressed log layer, as observed in other flumes (Fukuda and Lick, 1980; Thompson et al. 2004), which might only represent few natural situations (e.g. meandering shallow streams). In the IMAR flume, it is likely that once erosion started uncovering the organisms and ripples formed, the shells protruded from the thin boundary layer (considered to be 0.7 cm high), increasing turbulence.

In comparison to the NIOO flume, the IMAR flume presented advantages in terms of the quantity of material needed to perform the experiments (organisms, sediment and algae culture) and the time needed for measurable cell depletion. Although no physical obstacles were presented in the water column, the high shear velocities and turbulent mixing observed in this flume, especially at the higher velocities, prevented depletion of the near-bed layers by the clearance activity of *C. edule*.

Although both flumes presented some limitations regarding natural conditions reproducibility, the trend in the data in both flumes is consistent enough to have confidence in the response of cockles to changes in hydrodynamics.

2.4.2 Influence of velocity on clearance rates

Weight-specific CR in the NIOO experiments, varying from 6 to 11 l^{-1} h^{-1} g^{-1}, were higher than the ones found in the IMAR experiments that varied from 0 to 7 l^{-1} h^{-1} g^{-1}). This is probably due to an overestimation caused by the standardisation to weight-specific rates of the organisms used in the NIOO experiment, with half the weight of the organisms used in IMAR, as the rates for individual organisms were under the same magnitude, varying from 0.5 to 3.0 l^{-1} h^{-1} ind^{-1} in the NIOO and from 0 to 3.7 l^{-1} h^{-1} ind^{-1} in the IMAR experiment. Nevertheless, standardisation has to be performed to allow comparisons between the two different populations. The different rates can also be related with differences in the origin and collection time of the organisms and by differences in the seston concentration and the food type used in the two experiments.

In general, the clearance rates found in this work are in agreement with the rates found by Riisgård et al. (2003) (average clearance rates 4.8 to 6.6 l h^{-1} g^{-1}) at a range of temperature from 5 to 10 ºC. The rates were slightly higher than the ones found by Karlsson et al. (2003) (about 3 l $h^{-1}g^{-1}$) under a current velocity range from 4 to 6 cm s^{-1}, but they specifically investigated particle sizes of 60 μm and larger. Navarro and Widdows (1997) reported that clearance rates decrease with increasing seston concentration. They report maximum clearance rates of 7.1 l h^{-1} g^{-1} for very low seston concentrations, and of 3.7 l h^{-1} g^{-1} for seston concentrations ranging from 10 to 20 mg l^{-1}, which is the range of the concentrations used in the present work. All of the rates from the literature mentioned above were measured on individual animals. The rates determined in the present flume experiments are bed averages.

Normally one would expect these to be somewhat lower than maximum individual rates. However, all the animals in our experiments were hand-selected, healthy and active, which will have increased the average clearance rates, in comparison to natural beds. The clearance rates in the two flumes appeared to give a different relationship with free-stream velocity. In the NIOO flume clearance increased with velocity, in the IMAR flume clearance rates started to decrease at velocities of 20 cm s^{-1}. This was almost certainly due to the fact that the two flumes have very different boundary layers, with a different velocity gradient. Although the experimental free-stream velocities in the flumes overlapped, there was little overlap in the ranges of u_*. If we consider the trend in the responses from both flumes, it appears that clearance rates are primarily influenced by shear velocity, rather than velocity per se.

The relationship between clearance rates and increasing velocities (Fig. 9) is unimodal, as observed for other bivalve species (Lenihan et al., 1996; Ackerman, 1999; Sobral and Widdows, 2000). Under low velocities CR seems to be enhanced. Previous measurements of CR from *C. edule* in the IMAR flume (Fernandes and Sobral, 2001), under shear velocities from 0 to 4.4 cm s^{-1} (according to the conversion between U and u_* used in the present work), showed an increment of CR (from 2.1 to 2.8 l h^{-1} ind^{-1} corresponding to 5.5 and 7.2 l h^{-1} g^{-1}). However, *C. edule* clearance activity is reduced at high shear velocities. According to our results, the critical u_* value from where CR reduction lies between 3.5 and 5.3 cm s^{-1}.

At low velocities, some bivalves seem to increase their clearance rates, by orientating themselves into the flow, thus reducing pumping cost (Englund and Heino, 1996). Others report that they orient both their siphons perpendicularly to the principal component of current direction, hence, reducing interactions between siphon flows and minimizing the risk of refiltration of seawater (Vincent et al., 1988). Low velocities are also known to increase the encounter efficiency with frontal cilia, promoting particle retention (Ward et al., 1998).

Other observations under current velocities between 10 and 30 cm s^{-1} showed that water velocity has a significant negative linear effect on mussel exhalant siphon area, although no significant effect on valve gape was found (Newell et al., 2001).

As velocity increases, there is a critical point at which the organisms have to deal with abrasion due to saltating particles and increased ballistic momentum flux (Amos et al., 2000) at the siphons level. This may be an additional factor in the difference of the clearance rates between the two flumes.

In the annular flume finer sediment was used that had a lower erosion threshold than that in the racetrack flume experiment. The resuspension observed at the higher velocities in the IMAR flume might be related with the decrease on clearance rates. The decline in clearance activity as a consequence of resuspension and therefore, increase in suspended matter was already mentioned by Sobral and Widdows (2000). In addition, under higher current velocities it is possible that bivalves reduce their ability to orientate their body or siphons in relation to the current or might even loose the ability of the ciliary pump to function effectively in removing food particles (Wildish and Miyares, 1990).

Reduced algal concentrations near the bottom over dense cockle beds have been reported from the field (Smaal and Haas, 1997). Near-bed depletion layers have also been observed in a preliminary experiment in the annular flume with similar densities (Fernandes and Sobral, 2001), where reduced concentrations were observed at about 12 cm from the bed and at velocities of about 11 cm s^{-1} (u_*~2.8 cm s^{-1}). In a similar annular flume, Sobral and Widdows (2000) observed a depleted layer at about 10 cm height in an experiment with similar densities of the clam *Ruditapes decussatus,* an endobenthic bivalve with long separated siphons. This effect was marked at low current velocities (3 cm s^{-1}) and still detectable at 17 cm s^{-1}. In the present study we did not find any reduction in algal concentration in the near-bed layers. For depletion to occur, clearance rates per unit area must out-compete vertical mixing rates.

In the IMAR flume the high shear velocities and turbulent mixing are likely to reduce depletion in the water column. The lower shear velocities and mixing rates in the racetrack flume would make the occurrence of depletion layers more likely. However, after leaving the test section with the cockles the water in this flume is completely mixed again at the drive belt. Flume structures, such as the paddles and collimators will promote the break down of any possible vertical gradient. In the racetrack flume the water samples were taken about 60 cm from the leading edge. Even at a low velocity of e.g. 10 cm s^{-1}, the cockles would have only 6 seconds to significantly reduce the concentration in the lower water layers.

2.4.3 Influence of clearance activity on velocity parameters

The shear velocity values could only be estimated for boundary layers over a smooth surface, due to the fact that for calibration of the IMAR flume only measurements over the flat bottom were available. The results for the NIOO-flume show that the actual values of u_* over the cockles were 70% to 190% higher than over a smooth surface (Fig. 2.5). The fact that u_* over bare sediment was increased in comparison to the flat flume bottom is certainly due to increased bottom roughness (Fig. 2.6). The cockles may have increased the physical roughness slightly, but visual inspection of the sediment did not reveal very clear differences with the bare sediment. The increased shear velocity and the highly increased estimates for z_0, are almost certainly due to the effect of the clearance activity of the cockles. André et al. (1993) have shown that a free-stream velocity of 15 cms^{-1} caused a deflection of *C. edule* excurrent that reached a height of less than 1cm above the sediment, 10 cm downstream of the siphons. The in/exhalent jets interacting with the flow, create additional roughness elements and turbulence (Ertman and Jumars, 1988). Van Duren et al. (2005) found similar effects in mussels, with relatively larger effects at higher flow velocities.

The positive relationship between CR and u_* ($F_{1,9}=7.4780$; $p<0.02$), can not be understood as the effect of CR on u_*, as it reflects both the effect of CR on u_* and the effect of velocity on CR. Therefore, the effect of each parameter on the other is interrelated and can not be dissociated in these experiments. Maybe, the use of inactive cockle-beds (containing shells without organisms, in order to prevent clearance) could be a way to access the additional effect of clearance activity.

2.5 Conclusions

Comparisons of results obtained in different flumes should not be based on current velocity estimations because the structure of the BBL may vary considerably, as observed in the two flumes used in this work. For this purpose it is better to base comparisons on shear velocity, which is a better indicator of the BBL structure.

Both flumes showed limitations at different levels. We recommend for future studies that the choice of the flume type should be based on the objectives of the intended observations. The hydrodynamic conditions in the annular flumes are rather different from the field situation, but these flumes are logistically much easier to work with. Provided that the hydrodynamic conditions are well characterised, the conclusions regarding effects from different hydrodynamic treatments should still be valid. However, for an assessment of the effects that cockles have on boundary layer hydrodynamics, it is desirable to use a flume with boundary layer conditions that resemble the field more closely. For such research a relatively large flume with a straight working section is preferable.

As observed for other bivalves, the clearance activity of *C. edule* showed a unimodal response to increased shear velocities, with a turning point between 3.5 and 5.3 cm s^{-1}.

The reduction of flow speed in the benthic boundary layer was already reported for mussels (Butman et al., 1994; Green et al., 1998; Nikora et al., 2002). It is important to stress that the effect of epibenthic organisms in the boundary layer is dominated by roughness produced by the protuberance of their shells, and it is different from the effect of endobenthic organisms. Although endobenthic organisms do not produce considerable physical roughness, it was shown in this work that they are able to influence the velocities near the bed. This effect of *C. edule* should be clarified in order to understand the direct effect of clearance on turbulence and its indirect effect on roughness.

This work suggests that *C. edule* has two types of interactions with sediments dynamics. Firstly, cockles reduce the velocities near the bed, with possible consequences in the particle erosion/deposition pattern. The cockle density used in this work is low in relation to densities found in the Wadden Sea and in the Westerschelde estuary (Kamermans, 1993; Ysebaert and Herman, 2002). Presumably, in higher densities the reduction effect would be even more significant. Secondly, cockles transform loose suspended particles into particles packed as faeces or aggregated as pseudofaeces. Faecal material from molluscs tends to have very different erosion thresholds from sediment particles (Orvain et al., 2003). In addition, *C. edule* is also able to remove from the water column and ingest particles up to 500 µm size (Karlsson et al., 2003), thereby influencing the near-bed composition of suspended material.

The interrelation between bivalves and hydrodynamics needs further developments in order to understand water transport processes. CR are not only influenced by hydrodynamics but also by other parameters such as the quality and quantity of suspended matter and it is important to integrate these effects in order to comprehend the effect of cockle beds in sediment dynamics.

2.6 Acknowledgments

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PART II

INTERACCTIONS BETWEEN *Cerastoderma edule* AND SEDIMENT DYNAMICS

Chapter 3

How cockles change the benthic boundary layer: density effects on topography and turbulence

HOW COCKLES CHANGE THE BENTHIC BOUNDARY LAYER: DENSITY EFFECTS ON TOPOGRAPHY AND TURBULENCE

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Abstract

Cockle beds reduce current velocities in the benthic boundary layer (BBL). This may result from their influence on microtopography or from siphonal feeding currents. To discriminate between and to quantify these two effects, two experiments were performed in a racetrack flume under free-stream current velocities (U) from 5 to 40 cm s^{-1}. The first focused on the effect of *Cerastoderma edule* on the BBL, using two densities (500 and 1000 ind m^{-2}) of live and artificial cockle beds with similar topographic roughness. The second focused the effect of feeding activity of live cockle beds (280 ind m-2) in the BBL parameters. Microtopography, estimate as roughness height (k), was quantified using laser beam projections on the sediment surface and subsequent image analysis. BBL parameters such as U, shear velocity (u_*), hydrodynamic roughness length (z_0) and turbulent kinetic energy (TKE) were calculated from ADV measurements of velocity profiles. Feeding activity was quantified as clearance rates (CR). Increasing density of cockles had an increasing effect on topographic element height (k). Hydraulic roughness was predominantly influenced by k. TKE, in contrast, was not related to topographic or hydrodynamic roughness but was higher in treatments with live cockles. The effect of live cockles on TKE was positively related to CR. Exhalent jets had more pronounced effects at low velocities ($u^* < 1$ cm s^{-1}), as observed in the first experiment. In conclusion, cockle beds reduce velocities in the BBL by increasing topographic roughness and in addition, by increasing turbulence through production of siphonal currents.

Key-words: bedforms, *Cerastoderma edule*, filtration activity, flumes, hydrodynamics, roughness, shear velocity, turbulence kinetic energy.

3.1 Introduction

The hydrodynamic processes in the benthic boundary layer (BBL) for static non-living systems are relatively well known. The passage of flow over the bed surface produces a velocity gradient reflecting the loss of momentum from the flow to the bed. The seabed incurs two types of drag on the flow: friction drag, resulting from the fact that viscosity cause water to resist deformation, and form drag. The latter only occurs over bed structures which are sufficiently large to cause wake formation behind the structures.

According to Prandtl von Karman equation or "law of the wall" the BBL is characterized by a vertical gradient of velocity (u) dependent on shear velocity (u_*) as following:

$$\frac{\partial u}{\partial z} = \frac{u_*}{\kappa}\frac{1}{z} \qquad [1]$$

where u is the current velocity, κ is the von Karman's constant (≈0.4) and z is the distance above the bed. The description of u_* for a well developed boundary layer only formed by friction drag is obtained after integration of Eq. [1] as:

$$u_z = \frac{u_*}{\kappa}\ln\frac{z}{z_0} \qquad [2]$$

where u_z is the velocity at height z, and z_0 the hydrodynamic roughness length. In theory, the square of the shear velocity $(u_*)^2$ is directly proportional to momentum transfer in the boundary layer and to turbulence production (Kim et al 2000).

Although there is an attempt to include natural phenomena such as topographic variability in hydrodynamic models, present hydrodynamic models are inaccurate in simulating flow over biogenic structures (Zhang et al. 2004, Pilditch & Miller 2007). Some studies quantified the effect of topographic roughness on hydrodynamic parameters, concerning different shapes and densities of roughness elements (Wooding et al. 1973) or beds with different median grain sizes and patches (Lawless & Robert 2001; Thompson et al. 2004).

Measurements over natural beds are still few, and most of the present hydrodynamic models are not sufficiently calibrated for certain local parameters such as multi-scale physical roughness, interaction with animal generated flow or flexible structures. All of these will have an impact on boundary layer properties, such as shear velocity, and ultimately on particle dynamics in the near-bed layer. In many boundary layers over biogenic structures the relationship between topographical roughness and hydrodynamic roughness is still ill defined.

Some studies focused on the effect of epibenthic bivalves, protruding from the sediment surface, thus changing surface roughness and the structure of the BBL (Butman et al. 1994, Nikora et al. 2002). Their effect is characterised by decreasing the drag coefficient and uplifting the BBL (Green et al. 1998). Monismith et al. (1990) and O'Riordan et al. (1993) studied the effect of siphonal incurrent-excurrent flow fields on the properties of a turbulent boundary layer in relation to the depletion of phytoplankton. However, these studies were performed with artificial siphons, or based on numerical simulations and did not include live organisms, nor the effect of current velocity on the filtration activity and the consequent production of siphonal currents. The study by Van Duren (2006) measured flow profiles over a flat bed, inactive mussels and actively filtering mussels. This study indicated that the siphonal currents, combined with the very rough structure of the bed appeared to cause a decoupling between the theoretical fixed relationship between u_*, TKE and Reynolds stress ($\overline{u'w'}$).

Little is known about the effect of endobenthic bivalves in the BBL. Although the cockle *Cerastoderma edule* live inside the sediment, and therefore has limited effect on topographical roughness, it is known to reduce current velocities near the bed (Ciutat et al. 2007, Fernandes et al. 2007). *C. edule* exists in average densities of up to 1000 ind m^{-2} and at maximum ebb current velocity of up to 60 cm s^{-1} in the Schelde Estuary (Ysebaert and Herman, 2002). Has the highest probability of occurrence at ambient flow velocities of about 30 to 40 cm s^{-1} (Ysebaert et al. 2002), which corresponds to u_* of 2 to 3 cm s^{-1} in the NIOO flume, where the present experiments were performed. Increasing density of *C. edule* (up to 312 ind m^{-2}) is related to increased erosion rates of muddy sediments from the Tamar estuary (Ciutat et al. 2007). In Ciutat et al. (2007) and in a previous study on the effect of current velocity on the filtration activity (using clearance rates as proxy) of *C. edule* (Fernandes et al. 2007), we observed in the presence of cockles, reduced current velocities near the bed that were related to higher values of roughness length, shear velocity and turbulence.

It was not clear from the these experiments whether the observed reductions in current velocity near the bed and higher turbulence profiles were due to *C. edule* effects on topography, due to their filtration activity, or both. In order to differentiate between these two effects, a first experiment was performed to compare the hydrodynamic conditions between natural cockle beds and artificial beds. More precisely, turbulence profiles were compared between natural beds with live cockles and artificial cockle beds, with different topographic roughness levels. Considering that there is no effect of filtration in the latter case, effects on artificial beds can only be due to increasing topographical roughness in the BBL. A second experiment exploring the relationship between pumping activity (using clearance rates as a proxy) and additional turbulence was performed.

3.2 Materials and methods

3.2.1 Experiment 1: Comparison of the effect of live and artificial cockle beds on microtopography and turbulence

3.2.1.1 Sediment and organisms collection

The cockle *C. edule* and intertidal estuarine cohesive sediments (top first 2 cm) were collected in Paulinapolder (Westerschelde estuary, Netherlands) during low tide in April 2004. The organisms (average size and weight: 2.9 cm ± 0.2 (± standard deviation) and 0.18 g ± 0.04, n = 30) were brought to the laboratory and allowed to acclimate for about 2 weeks in an outdoor tank (area of about 8 m^2) with daily renewed seawater from the Oosterschelde (filtrated on a 0.5 mm mesh) before being introduced in the experimental sediment.

Sediment was defaunated by manually removing bivalves, polychaetes and larger shell fragments. The grain size was measured with a laser particle sizer (Malvern 6000 Hydro 200G Mastersize I). The < 63 µm fraction accounted for 38 % of total particles and median grain size was 143 ± 4 µm. Sediment was characterised as 1.3 % organic matter ± 0.2; 28.4 % water ± 5.6 and sediment dry bulk density of 2.0 g cm^{-3} ± 0.3. These values were obtained on sediment samples dried at 60 °C for 96 h and later incinerated at 450 °C for 5h.

3.2.1.2 Experimental design and flume runs

Flume boxes (1 m length x 0.23 m weight x 0.14 m height) were filled with sediment. 3 treatments (in duplicate) were prepared with a medium density of *C. edule* (M, 500 ind m^{-2}), another with a high density (H, 1000 ind m^{-2}) and a control with sediment and without organisms (C). All sediment boxes were left to stabilise for a period of 10 days in the outdoor tank.

Artificial cockle beds were prepared using empty cockle shells that were randomly glued on PVC boards (same surface area as the flume boxes) using 500 ind m^{-2} and 1000 ind m^{-2} densities. As the emergence of live cockles in natural treatments was not controlled, the artificial treatments were prepared in order to obtain a wide range of roughness that would include the one in the live treatments (M and H). These boards were introduced in the flume boxes and successively covered with sediment in order to obtain 3 different roughness levels (T2, T3, T4), each accounting as a new treatment (Figure 3.1).

Each flume box was introduced individually in the NIOO racetrack flume test section that is placed in the last 2 m of one of the straight sections (Table 3.1). To avoid edge effects on bed roughness, especially where the sediment adjoined the upstream and downstream sides of the flume test section, care was taken to ensure that the sediment surface was exactly flush with the surrounding surface. The flume was filled with filtered water from the Oosterschelde estuary with a salinity of 29. Each treatment was then exposed to stepwise increasing current velocities (from 5 to 40 cm s^{-1}).

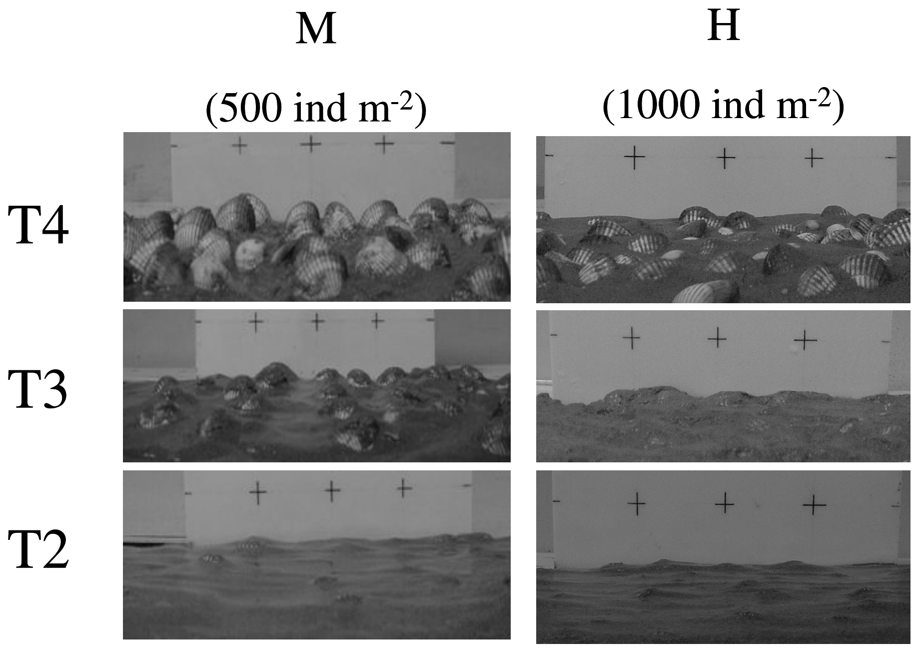

Figure 3.1 – Side-view of the artificial cockle beds using the two density of shells M (500 ind m^{-2}) and H (1000 ind m^{-2}) and the three roughness levels, T4 being the roughest and T2 the smoothest. The distance between two crosses of the white board is 5 cm.

Table 3.1 - Flume characteristics

Flume type	Racetrack
Flume area (m^2)	21.2
Length straight section (m)	11.7
Width of experimental section (m)	0.6
Experimental section area (m^2)	0.9
Current velocity range (cm s^{-1})	0 - 40
Sediment depth (cm)	14
Water volume (l)	8850
Water column (cm)	40

3.2.1.3 Topography analysis

The measurements of topographical roughness were based on image analysis of pictures from laser beam projections in the sediment. At the end of each velocity step, the carriage where the ADV was mounted was driven out of the test section and the flow was stopped to allow still water conditions and to avoid water movement interferences in the picture capture of the bottom. Laser beams were then projected onto the sediment surface in the test section using a construction laser level, placed parallel to the flume length and reaching the full length of the test section. A thin scaled board (5 cm mesh) was previously placed around the test section for subsequent georeferencing of the pictures. The inclination angle was adjusted in order to obtain different projection lines separated by a distance of about 5 cm. Several pictures were then taken from a fixed camera mounted on an upper platform, set perpendicularly to the laser projections.

The images from the laser lines were analysed with image enhancement techniques to reinforce the red colour of the laser profiles and exclude other colours in the images. Using the scaled board placed in the bottom of the test section, the images were georeferenced using Arc View software. Topographic profiles, with a value of height every 0.5 mm along the transect were then extracted. Each profile was detrended by subtracting a best fit second order polynomial, followed by a conversion to actual height, based on a calibration using objects with a known (measured) height. The data were smoothed using a five points moving median filter. The resulting topographic profiles are expressed in Fig. 3.2.

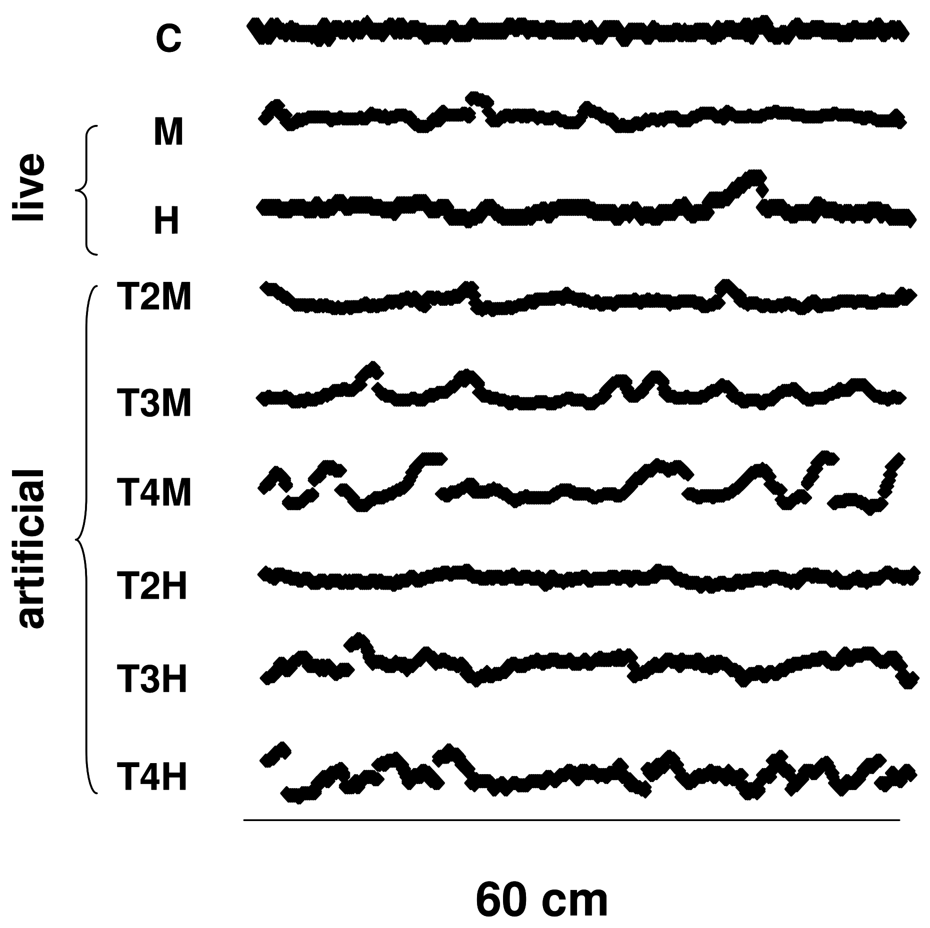

Figure 3.2 - Topographic profiles obtained in the natural an artificial treatments for the two density of C. edule: M (500 ind m^{-2}) and H (1000 ind m^{-2}) and the three roughness levels, T4 being the roughest and T2 the smoothest.

A topographical line can be considered as a succession of elements, resulting from unevenness of the sediment and from the effect of (buried) cockles. A Fast Fourier Transform (FFT) allows decomposing a waveform into sinusoids of different frequencies (Legendre & Legendre, 1998). The technique was applied to cut the topographic line into short segments, with one segment separating two elements:

$$X(f) = \sum_{n=1}^{N} x(n).e^{\left(\frac{-j.2.\pi}{N}(f-1)(n-1)\right)}, \quad 1 \leq f \leq N \qquad [3]$$

The variable x(n) is the topographic height at a distance and the transformed Fourier of this series will be denoted X(f) with N samples. In order to recover all Fourier components of a periodic waveform, it is necessary to use a sampling rate at least twice the highest waveform frequency. The Nyquist frequency, is the highest frequency that can be coded at a given sampling rate in order to be able to fully reconstruct the signal. The period ($1/f_{niquist}$) gives a good estimate of the distance *d* between each element. The amplitude of the topography within each segment of distance *d* was calculated and the averaged amplitude of all peaks within a topographical line was estimated. The element height (k) of each line was estimated as half of the average amplitude. The average k of each treatment and under different current velocities (only for C, M and H) was estimated from about 10 topographical profiles.

When considering the topographical profiles measured in all treatments, the standard deviation of all values of height of each topographical profile (root-mean-square height, RMS) was positively correlated to k (Pearson product moment correlation (PPMC), $n = 192$, $r = 0.843$, $p < 0.001$). Because the characterisation of surface roughness is more meaningful as a measure of element height, rather than the RMS, topographical roughness is therefore based on the element height (k).

3.2.1.4 Hydrodynamic analyses

Hydrodynamic measurements were performed with a Nortek ADV (Acoustic Doppler velocimeter, measuring at 25 Hz, sample volume at 5 cm below the transmit beam), mounted on an automated 3D positioning system, on a carriage, which can be positioned anywhere along the working section of the flume. After flow stabilisation at each velocity step (20 min), 8 velocity profiles (within a surface area of 8 cm^2) were measured 60 cm downward the test section, For each velocity profile, 12 velocity measurements were taken from 0.5 cm above the surface, through 24 cm of water height, with a vertical resolution of 0.8 cm. Each measurement lasted 5 seconds. Measurements done at 5 mm height were eliminated because they clearly deviated from the linear (ln transformed) relationship between flow velocity and distance from the bottom and some of these measurements suggested interference due to the proximity to the bed.

u_* was determined according to Eq. [2], from the slope of the logarithmic velocity profile (u (z) versus ln (z)). Average values of u_* were calculated from 8 sub-profiles. z_0 can be estimated from the intercept between the relation (u(z) versus ln (z)) and the Y-axis (Eq. [2]). However, considerable errors arise with the application of this procedure because the value of the intercept is extremely sensitive to the velocity gradient (Thompson et al. 2003; Pope et al. 2006).

When integrated over the entire water depth, Eq. [1] can be integrated as following:

$$\bar{u} = \frac{u_*}{\kappa} \ln\left(\frac{h}{e.z_0}\right) \qquad [4]$$

where $\bar{u}$ is the vertically averaged velocity, h is the water column height and $\bar{u} = \frac{1}{h}\int_{z_0}^{h} u$.

After transformation, this can be written as:

$$z_0 = \frac{h}{e^{\left(\frac{\bar{u}}{u_*}.\kappa\right)}.e^1} \qquad [5]$$

The ratio $\bar{u}/u_*$ is equal to a, the slope of the function $\bar{u} = a.u_* + b$, considering that the constant b = 0. Because it is based on the calculation of a slope instead of an intercept with Y-axis, this method yields a more robust estimation of roughness length. For each bed roughness and for each velocity step, z_0 was determined with the average values (from 8 sub-profiles) of $\bar{u}$ and u_*.

Turbulent kinetic energy (TKE) or the intensity of velocity fluctuations was calculated from the average standard deviation (‘) of the three components of velocity (u, v, and w) as:

$$TKE = \frac{1}{2}\left(u'^2 + v'^2 + w'^2\right) \qquad [6]$$

In order to compare the values of TKE obtained over the column height, to other parameters, TKE was averaged from the first 6 cm above the bed (measurements at 6 heights) for each sub-profile. The TKE of each treatment was based on averages from 8 sub-profiles. In order to remove the effect of current velocity on TKE, TKE was normalised as $TKE_{U2} = TKE / U^2$, being U the free-stream current velocity at 18 cm above the bed. TKE_{U2} is undimensional.

3.2.2 Experiment 2: Relationship between filtration activity and turbulence

The measurements of filtration activity were not possible during experiment 1, because resuspended muddy sediments and particles within would bias the cell counting for further calculation of clearance rates. Exp. 2 is a re-analysis of data published in Fernandes et al. 2007, where experimental details are given. Clearance rates (CR) were measured in the same flume during another experimental series using sandy sediments (grain diameter = 750 µm). Full details of the experiment are given by Fernandes et al. (2007) where CR were compared as a function of current velocity in two different flumes. Three flume runs were performed using 3 groups of organisms (n = 260 on each group) from a population of cockles (280 ind m^{-2}, average shell length and weight of 26 cm ± 2 and 0.16 g ± 0.01) from the same collection area.

Each flume run was done under 5 steps of increasing velocity from 5 to 40 cm s^{-1}. Velocity measurements were taken following the same methodology as described before, but velocity steps were increased every 4 hours (to allow filtration activity measurements) instead of 1 hour for experiment 1. Velocity parameter calculations followed the methodology described in experiment 1. The seawater temperature varied between 15 and 16 °C and salinity was of 29. A culture of *Isocrysis galbana* was added, (about 16000 cell ml^{-1}) corresponding to initial concentrations of 0.831 ± 0.319 mg Chlorophyll *a* m^{-3}. The filtration activity of the bed was measured as the clearance rates of a population of 280 ind m^{-2} (CR_{280}). It was estimated using the cell depletion method (Coughlan 1969) based on the measurement of the volume of water cleared of suspended microalgae per unit of time (see details in Fernandes et al. 2007) and subtraction of the CR observed in a control treatment without organisms. Individual clearance rates (CR_{ind}) were then estimated by dividing the CR for the population by the 260 individuals.

The current speed of in/exhalant currents/jets (u_j, cm s^{-1}) was estimated as $u_j = CR_{ind} / A$, considering C_{ind} (ml s^{-1} = cm^3 s^{-1}) and A the area of the siphons (cm^2). According to the relationship between the diameter of the siphon of *C. edule* and the organism shell width (Riisgård et al. 2003) the diameter of the siphons of the organism used in that experiments averaged 0.338 cm (for the exhalant) and 0.416 cm (for the inhalant) which correspond to a siphon area of 0.090 cm^2 and 0.136 cm^2 respectively. Jet-in-cross-flow length scale (z_m) was defined by List (1982) as the relative influence of the exhalant jets on the crossflow. According to List (1982): $z_m = u_j d_j / U$, where d_j is the exhalant siphon diameter. This provides an estimation of the ratio between the exhalent jet and the longitudinal flow.

3.3. Results

3.3.1. Experiment 1: Comparison of live and artificial cockle beds on microtopography and turbulence

3.3.1.1. Effects of *C. edule* on topographical roughness

In comparison to control treatments (C), the density of live cockle beds (M and H) produced significant changes on the microtopography (Analysis of Covariance (ANCOVA),

$F_{2.134} = 29.4$; $p < 0.001$, all statistical analysis was performed using STATISTICA 6.1 software ($\alpha = 0.05$)). Topographic roughness (k) was significantly higher in the treatments with the highest density of live *C. edule* (H) compared to the medium density (M, $p < 0.001$) but there was no difference between M and the control C (Table 3.2). The increase in current velocity had no significant effect on k, which remained almost constant over the increasing velocities (Fig. 3.3). The overall averages of k in each treatment were 0.21 cm in C, 0.25 cm in M and 0.34 cm in H (Table 3.2).

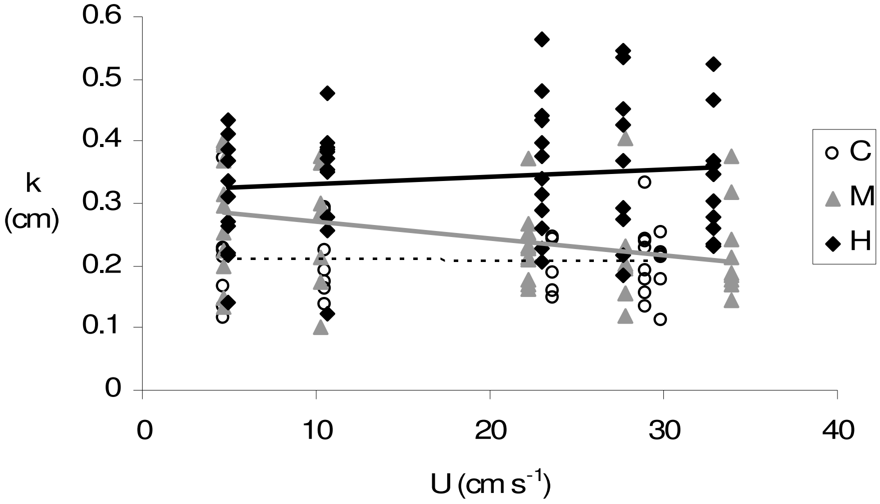

Figure 3.3 – *Cerastoderma edule* changes in topographical roughness, measured as element height (k, cm), over increasing free-stream velocities (u_*, cm s^{-1}) for the natural treatments. Lines refer to linear regressions for each treatment as: black broken line (C, control), solid grey line (M, 500 ind m^{-2}) and solid black line (H, 1000 in m^{-2}).

Topographic roughness in live treatments (M and H) was similar to some of the artificial treatments (T treatments, Table 3.2). ANCOVA analysis of k in all treatments showed that according to homogeneous grouping (Tukey test), M is statistically comparable to T2M and T2H and H to T2M and T3H. Since the objective of this study was to access the additional effects of filtration activity on turbulence, the comparison between live and artificial (with no filtration activity) treatments only considered sediments with comparable topographical roughness (M, H, C, T2M, T2H, T3H).

Table 3.2 – Topography characterised by the elements height (k, cm, ± standard deviation, n ~10) and by roughness length (z_0, cm) in all treatments (C for control (n=10), treatments with live *C. edule* (n=10): M for 500 ind m^{-2} density and H 1000 ind m^{-2} and for the artificial treatments (n=5), the highest roughness level T4 until the lower roughness level T2 for densities M and H). Relationships between hydrodynamic parameters: shear velocity (u_*, cm s^{-1}), free-stream velocity (U, cm s^{-1}) and turbulence kinetic energy (TKE, cm^2 s^{-2}) (n=10 in C, M and H and n=5 in T treatments) and normalised TKE (TKE_U).

Exp. 1	k	z_0	$u_*=aU^b$			$TKE=aU^b$			TKE_{U2}
			a	*b*	R^2	*a*	*b*	R^2	
C	**0.21 ± 0.06**	**0.02 ± 0.01**	**0.0513**	**1.0065**	**0.99**	**0.0109**	**1.7310**	**0.99**	**0.005 ± 0.002**
T2 H	0.21 ± 0.06	0.15 ± 0.09	0.1201	0.8478	0.97	0.0136	1.7041	0.99	0.006 ± 0.002
M	**0.25 ± 0.09**	**0.06 ± 0.03**	**0.0814**	**0.9070**	**1.00**	**0.0206**	**1.7022**	**0.97**	**0.009 ± 0.003**
T2 M	0.31 ± 0.06	0.10 ± 0.03	0.0932	0.8894	1.00	0.0173	1.6695	0.99	0.007 ± 0.002
H	**0.34 ± 0.10**	**0.10 ± 0.11**	**0.0687**	**0.9910**	**0.92**	**0.0280**	**1.5918**	**0.97**	**0.010 ± 0.004**
T3 H	0.41 ± 0.05	0.27 ± 0.08	0.0913	0.9987	0.99	0.0056	2.0454	1.00	0.006 ± 0.000
T3 M	0.47 ± 0.15	0.31 ± 0.09	0.1066	0.9469	0.99	0.0130	1.8432	1.00	0.009 ± 0.001
T4 H	0.55 ± 0.17	0.20 ± 0.10	0.0814	0.9070	1.00	0.0316	1.6010	0.99	0.011 ± 0.005
T4 M	0.57 ± 0.13	0.40 ± 0.15	0.1250	0.9250	0.99	0.0075	2.1005	0.99	0.010 ± 0.001
Exp. 2									
Ct		**0.03 ± 0.01**	**0.0591**	**0.9976**	**1.00**	**0.0131**	**1.6952**	**1.00**	**0.006 ± 0.001**
L		**0.12 ± 0.12**	**0.0752**	**0.9567**	**0.87**	**0.0284**	**1.6372**	**0.94**	**0.011 ± 0.006**

Legends:

Experiment 1: **C** for control (n = 10), treatments with live *C. edule* (n = 10), **M** for 500 ind m^{-2} density and **H** 1000 ind m^{-2} and for the artificial treatments (n = 5). The lowest roughness level T2 until the highest roughness level T4 for densities M and H.

Experiment 2: **Ct** for control (n = 5) and **L** for 280 ind m^{-2} density (n = 10).

3.3.1.2 Effects of *C. edule* on hydrodynamic roughness

The hydrodynamic profiles measured in the treatments with live *C. edule* (M and H), systematically showed lower current velocities near the bed in comparison to the controls. As an example a comparison of profiles for the natural treatments at free-stream current velocity of ~10 cm s^{-1} is provided (Fig. 3.4). Similarly, in the artificial treatments reduced velocities near the bottom were related to increasing roughness. The reduction of velocity in the lower layers for treatments with increasing roughness resulted in higher shear velocities at the bottom.

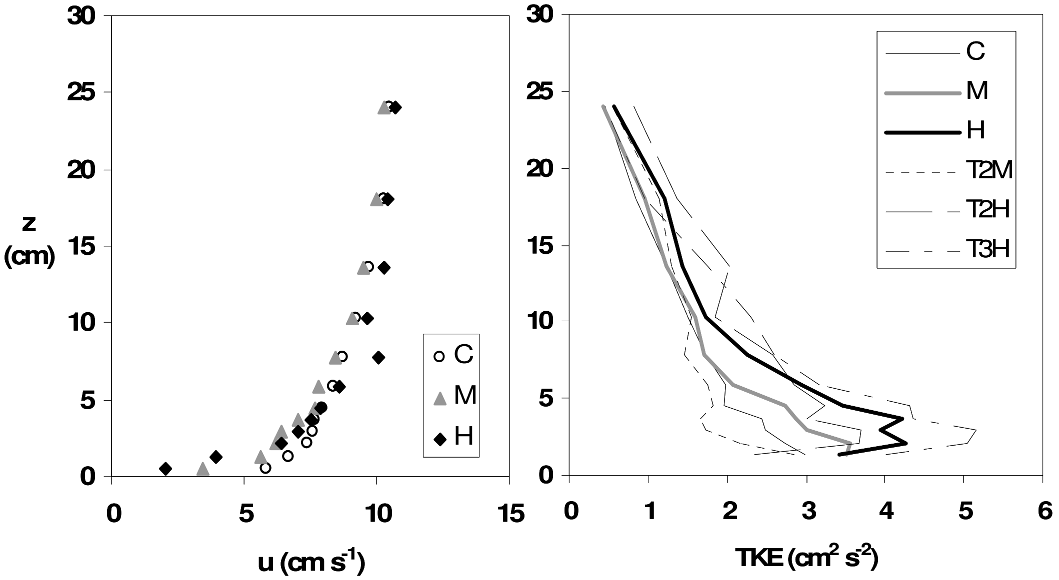

Figure 3.4 – *Cerastoderma edule* effect in the BBL. Average velocity profiles (u, cm s^{-1}, U of ~ 10 cm s^{-1}) and turbulent kinetic energy profiles (TKE, cm^2 s^{-2}, at U of ~ 25 cm s^{-1}). Each profile is an average of 8 individual sub-profiles. Legends as in Fig. 3.3.

The relationship between u_* and U (Fig. 3.5) was significantly affected by live *C. edule* density in comparison to C (ANCOVA, $F_{2.27} = 5.338$, $p < 0.05$). Analysis among comparable treatments (C, M, H, T2H, T2M and T3H, see Table 2) showed a positive relationship between the slope of the linear relationship u_*/U and k (Regression, $F_{(1,7)} = 11.114$, $R^2 = 0.61$, $p < 0.01$).

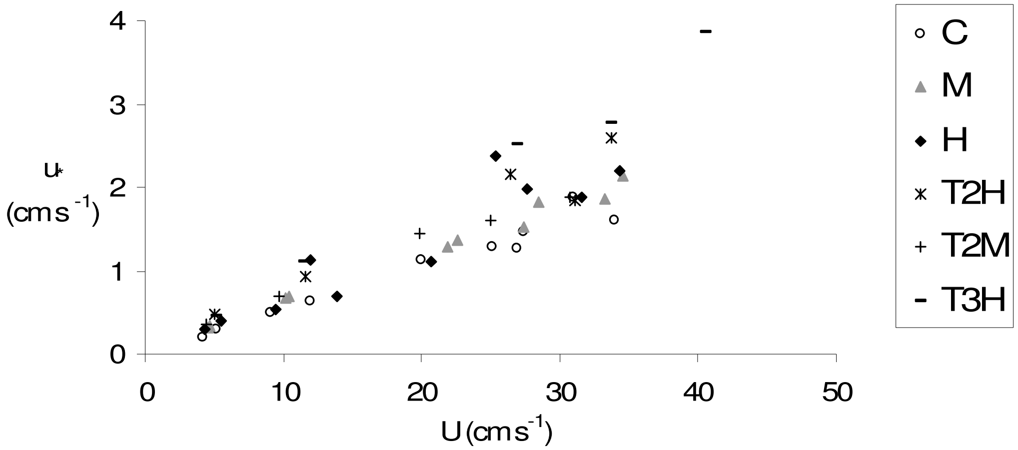

Figure 3.5 – *Cerastoderma edule* effect in the relationships between shear velocity (u_*) and free-stream velocity (U) for comparable treatments. Individual points represent averages of 8 sub-profiles. Legends and functions of the relationships can be found in Table 3.2.

In most cases z_0 was constant with increasing current velocity. As expected, there was a positive correlation between topographical (averaged k) and hydrodynamic roughness (averaged z_0) (PPMC, n = 60, r = 0.717, $p < 0.001$) when considering all treatments (Table 3.2). For methodology calibration purposes, the values of z_o estimated using Eq. [2] (most commonly used) and Eq. [5] were compared. The calculation of z_0 using the direct application of average values of $\bar{u}$ and u_* (Eq. [5]) or the coefficient a (the slope of u (z) versus *ln* z, Eq. [2]) shows a negative correlation (PPMC, n = 60 r = - 0.620, $p < 0.001$). Calculations of z_0 using Eq. [2] show a negative correlation with k (PPMC, r = - 0.466, $p < 0.001$,) and produces more variability of values, which were more representative of the increasing topographies and more precise using Eq. [5].

3.3.1.3 Effects of *C. edule* on turbulence

In general, the profiles of TKE showed consistently higher values on the first 6 cm from the bed, as the example shown at free stream velocity of ~ 25 cm s^{-1} (Fig. 3.4). TKE averaged from the first 6 cm above the bed is higher in the presence of live cockles (M and H), which is reflected by higher slopes (a) in the relationship between TKE and U (Tab. 3.2). Statistical analysis of these relationships showed differences among treatments (ANCOVA, $F_{5.334}$ = 20.172, $p < 0.001$). In M, the relationship between averaged TKE and U was different from C and T2H ($p < 0.001$) and T2M ($p < 0.01$) but comparable to H and T3H ($p > 0.05$), even if k was significantly lower on M than H or T3H (Tab. 2). H was different from C, T2M and T3H ($p < 0.001$).

As expected, there is a strong correlation between TKE (averaged from the first 6 cm above the bed) and current velocities: U (PPMC, $n = 45$, $r = 0.909$, $p < 0.001$) and u_* ($r = 0.882$, $p < 0.001$), reflecting the dependence of the latter two. There were very weak correlations between TKE and the roughness parameters k (PPMC, $n = 45$, $r = 0.1689$, $p > 0.05$) and z_0 ($r = -0.0018$, $p > 0.05$). This shows that TKE is related to current velocity but does not have a clear relationship with roughness parameters.

The results of the comparisons between two groups: live (M and H) and others (C, T2H, T2M, and T3H) showed that the average TKE values were higher in the live treatments (ANCOVA, $F_{1.43} = 10.731$, $p < 0.01$), while considering the effect of U. These results show that turbulence is better explained by the presence of cockles than on a topographic basis alone.

The relationship between TKE and U^2 (Fig. 3.6) showed good linear fit (Linear regression, $R^2 = 0.88$ for M, $R^2 = 0.96$ for H and $R^2 > 0.98$ for the other treatments without live cockles). To remove the effect of current velocity TKE was normalised by U^2. The relationship between TKE_{U2} and u_* (Fig. 3.7) within the treatments was almost constant over increasing currents, although values were slightly higher under low velocities ($u_* < 1$ cm s^{-1}) for the treatments with *C. edule* (M and H). Average values of TKE_{U2} varied from 0.005 to 0.007 for the C and T treatments and were higher in the presence of *C. edule* varying from 0.009 to 0.010 for M and H.

Treatments were organised in two groups: live (M and H) and artificial (C and T treatments) to evaluate the effect of live cockles, who have a significant increasing effect on additional turbulence (One-way ANOVA, $F_{1.159}=6.408$, $p < 0.01$). TKE_{U2} was also compared to k using the live/artificial groups (Fig. 3.8).

The higher values of TKE_{U2} are better related to the live treatments (ANCOVA, $F_{1.43} = 16.384$, $p < 0.001$) than to k (ANCOVA, $F_{1.43} = 0.892$, $p > 0.05$). These observations strongly suggests that there is an additional source of turbulence, which is not related to increasing velocity or to topographical roughness but more with an effect produced by live cockles.

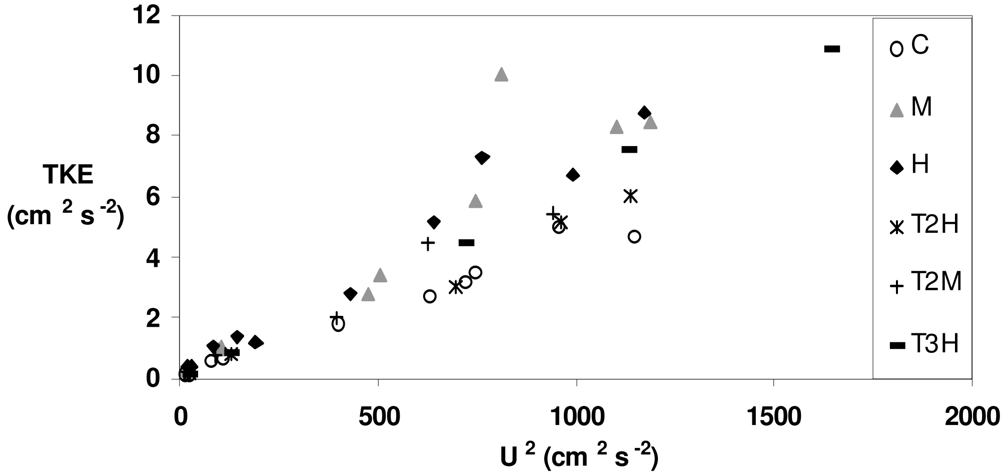

Figure 3.6 – *Cerastoderma edule* effect in the relationships between turbulence kinetic energy (TKE, cm^2 s^{-2}) and the square of free-stream velocity (U^2) for comparable treatments. Individual points represent averages of 8 sub-profiles. Legends and functions of the relationships can be found in Table 3.2.

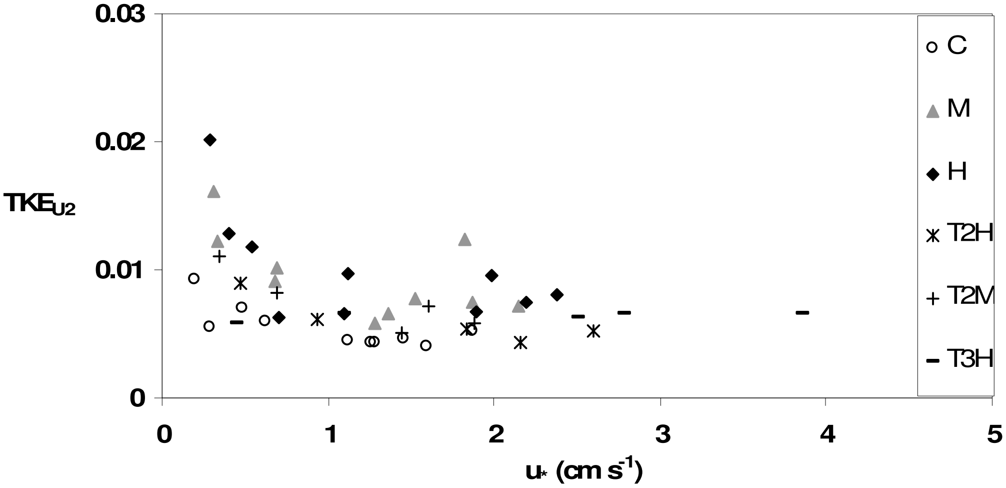

Figure 3.7 – *Cerastoderma edule* effect in the relationships between normalised turbulence kinetic energy (TKE$_{U2}$) and shear velocity (u$_*$) for comparable treatments. Individual points represent averages of 8 sub-profiles. Legends as in Table 3.2.

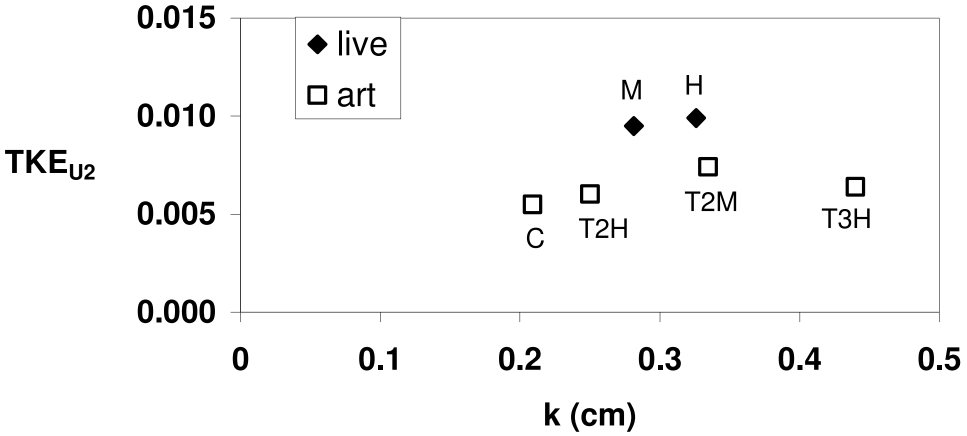

Figure 3.8 - *Cerastoderma edule* effect in the relationships of normalised TKE (TKE$_{U2}$) as a function of roughness or element height (k). Treatments are gathered in two groups: one with live *C. edule* (M and H, ♦) and the second with the treatments without live organisms (C and T treatments, ☐).

3.3.2. Experiment 2: Relationship between filtration activity and turbulence

The second experiment performed in the same flume and with organisms from the same site produced similar and comparable results from Exp.1 (Table 3.2). The averaged TKE$_{U2}$ was higher in the presence of cockles (One-way ANOVA, $F_{1.135} = 34.836$, $p < 0.001$) in comparison to control treatments. The relationship between TKE and CR$_{ind}$ (Fig. 3.9) measured in the flume for a density of 280 ind m^{-2} (a population of 260 organisms), showed that clearance rates has a significant effect on turbulence (Regression analysis, $R^2 = 0.53$, $F_{1.8} = 8.83$, $p < 0.05$, n = 10).

The analysis of individual siphonal ex/inhalant currents produced in the filtration activity experiment can provide further insight in the effect of clearance rates in turbulence. Clearance rates increased with shear velocity up to 1.3 cm s^{-1}, after which they stabilised (Fig. 3.10). Individual clearance rates vary from 0.99 to 3.66 L h^{-1} ind^{-1} and exhalant currents varies from 3.06 to 11.31 cm s^{-1}. The difference between the exhalant and inhalant current resulted in an upward velocity that is 34% of the exhalant currents. The jet-in-cross-flow length scale (z_m) estimated using the values of exhalent currents varies from 0.23 to 0.07 cm under shear velocities up to 1.3 cm s^{-1}, after which it stabilises (Fig. 3.10). When z_m drops below 0.1 cm, the effect of siphonal currents can no longer compete with hydrodynamic currents and the additional effect of cockles becomes less relevant.

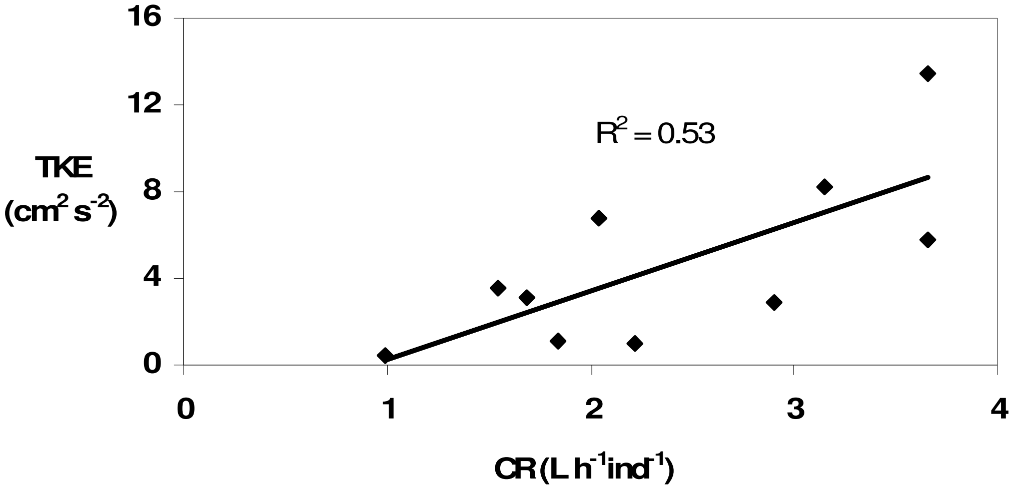

Figure 3.9 - *Cerastoderma edule* effect on turbulence (TKE averaged in the first 6 cm from the bed) as a function of individual clearance rate (CR) of a population of 280 ind m^{-2}, average shell length and weight of 26 mm ± 2 and 0.16g ± 0.01. Linear regression presented in the figure.

On both experiments the averaged TKE_{U2} measured over increasing velocities in the control treatments (C for Exp.1 and Ct for Exp.2) were the lowest and statistically comparable (0.005 ± 0.002 for C and 0.006 ± 0.001 for Ct, $p>0.05$, Tukey post-hoc test) (Fig. 3.11). The averaged TKE_{U2} was higher in the treatments with 280 ind m^{-2} (0.011 ± 0.006) in comparison to treatments with 500 ind m^{-2} (0.009 ± 0.003) and 1000 ind m^{-2} (0.010 ± 0.004).

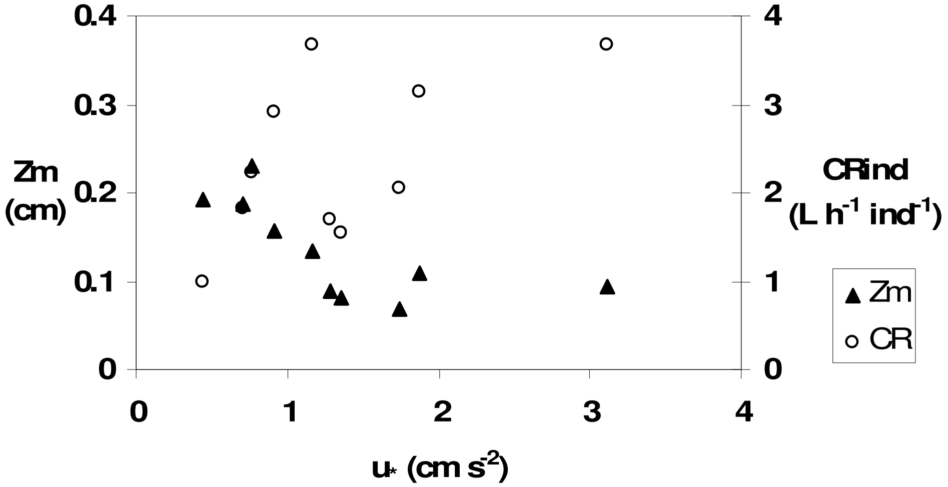

Figure 3.10 - *Cerastoderma edule* jet length scale (z_m, cm) and clearance rates (CR, L h^{-1} ind^{-1}) as a function of shear velocity (u_*) for a population of cockles with 280 ind m^{-2}.

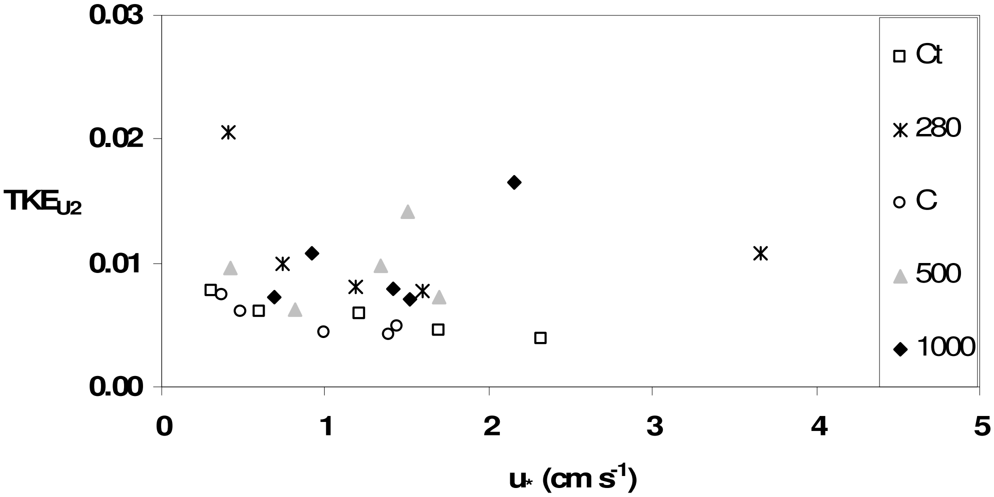

Figure 3.11- *Cerastoderma edule* effect in the relationships of normalised TKE (TKE_{U2}) as a function shear velocity (u_*) for the control treatments of Exp. 1 (C, ○) and of Exp. 2 (Ct, □) and the lowest density of *C. edule* (280 ind m^{-2}, L, *), medium (500 ind m^{-2}, M, ▲) and the highest density (1000 ind m-2, H, ◆).

3.4 Discussion

3.4.1 The effect of *C. edule* on topographical roughness

C. edule is known to prefer ambient flow velocities of about 25 – 30 cm s^{-1} (Ysebaert et al. 2002), which corresponds to u_* of 1.8 to 2.2 cm s^{-1} in the NIOO flume. Previous studies found changes in behaviour of cockles above a certain threshold shear velocity (Fernandes et al. 2007). This behaviour has a direct effect on topography (promoting deposition, resuspension and erosion). The behaviour also appears to be linked to the onset of bed erosion. Previous observations at higher shear velocities up to u_* 8 cm s^{-1} (Fernandes et al. 2007) showed an effect of current velocities on topography, both as a consequence of erosion, and as a consequence of *C. edule* burrowing behaviour (using its foot) possibly in an effort to remain buried during erosion. In these experiments *C. edule* significantly changed bottom topography and increased roughness which is related to increasing density of live cockles. Increasing shear velocities (up to 4 cm s^{-1}) did not affect the topographical roughness nor did it change the behaviour of cockles. In the present study the velocity threshold for the onset of burrowing behaviour was apparently not exceeded.

The positive relation between topographical roughness and the density of organisms is certainly due to increasing surface interaction with sediment topography and increasing probability for organisms to knock against each other. Under low current velocities, at low densities cockles produce little variation in bed height, living buried in the sediment and placing the top of the siphons flush with the sediment surface. Under low current velocities the cockles themselves are not a roughness element, but they change surface roughness as a result of their adaptation to the sediment surface, density, feeding activity and ultimately to current velocity.

3.4.2 The effect of *C. edule* on hydrodynamic roughness

The reduction of velocity near the bottom is directly related to increasing roughness. The presence of cockles increased roughness 0.2 times for 500 ind m^{-2} and 0.6 times for 1000 ind m^{-2}, in comparison to the control treatment. Although these increases in bed roughness are small in comparison to epibenthic fauna, it is sufficient to affect hydrodynamic parameters such as u_* and z_0. This is in agreement with previous observations (Ciutat et al. 2007, Fernandes et al. 2007). This relationship is well-known for epibenthic organisms like mussels. These increase the drag of the bed on the water column (Green et al. 1998, Nikora et al. 2002, van Duren et al. 2006) each organism being a roughness element large enough to incur form drag. *C. edule*, an endobenthic species, is also able to affect the BBL. Its effect in the BBL is characterised by increasing microtopography resulting in lower velocities near the bed and increased velocity gradients in the BBL. These changes in roughness are not large enough to cause form drag and the influence on boundary layer shear must be the result of friction drag alone.

The presence of *C. edule* reduced current velocities in the first 6 cm from the bed (Fig. 3.4). These observations differ from Ciutat's et al. (2007), who refer to an effect in the first 2 cm from the bed. These observations are probably related to the conditions of the annular flume used by Ciutat et al., where the curvature of the flume walls produces a centrifugal force that generates secondary flows and a compressed logarithmic layer.

The values of z_0 produced using Eq. [5], are better correlated to topographical roughness, are least biased and with a smaller variation than the values obtain by direct application of the Law of the Wall method (Eq. [2]). Calculations with Eq. [2] are very sensitive to the exact height of the ADV above the bed (Pope et al. 2006) and in the present study did not reflect increasing topographies.

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3.4.3 Effects of *C. edule* on turbulence

TKE is only directly related to topography when live cockles are not present. In the presence of active *C. edule* the values of TKE are much higher.

The relationship between turbulence and topographic roughness for the artificial treatments is shown in Fig. 3.8. The same relationship obtained in the presence of live organisms, actively filtering the water column showed higher values. Live cockle beds with a fairly low topographic roughness produced turbulence levels in the boundary layer similar to artificial beds with much higher topographic roughness (see Tab. 3.2).

The positive relationship between filtration activity and TKE observed in Exp. 2 (Fig. 3.9) supports the idea that filtration activity and the production of siphonal currents are responsible for higher levels of turbulence near the bed.

The effect of filtration activity on turbulence is most pronounced at low velocities ($u_* <$ 1 cm s^{-1}, Fig. 9). The effect of exhalant currents, measured here as z_m decreases with increasing velocity (Fig. 3.10). This decrease is mainly generated by the bending effect of the trajectory of vertical currents produced by increased overlying cross-flow (Monismith et al.

1990). This is probably why above a certain velocity the exhalant currents can no longer protrude higher in the water column.

In the present work shear velocities showed a positive relationship with filtration activity. However, at $u_* > 4$ cm s^{-1} the effect of siphonal current not only decreases due to decreasing of the ratio siphonal currents/ cross flow velocity, but also due to the reduction effect of higher shear velocities on filtration activity as observed in previous experiments (Fernandes et al. 2007). Therefore, the effect of filtration activity on turbulence will only be significant under relatively low shear velocities (up to 4 cm s^{-1}).

The averaged values of TKE_{U2} were slightly higher in Exp. 2 in comparison to Exp. 1 (Tab. 2 and Fig. 3.11). This is possibly due to the fact that on Exp. 2 courser sand was used and additional food was supplied to the cockles. The courser sand is an additional source of friction drag. The higher values of TKE_{U2} in the live cockle treatment of Exp. 2 might be related to feeding stimulation by addition of *Isocrysis galbana*.

The two densities used in the first experiment produced very similar additional turbulence (Fig. 3.6). This is surprising since the highest density has twice the number of organisms of the medium density and therefore higher filtration activity and higher levels of turbulence were expected on H. The higher values of TKE_{U2} observed at low velocities (Fig. 3.7) are probably related to the values of siphonal currents jet-length scale that are higher under lower velocities). Under increasing shear velocity the jets can no longer compete with the cross-flow, even if additional turbulence is being produced.

This study may have some consequences for the classical description of mixing coefficients based on topography calculations. The effect of live *C. edule* was not detected in the analysis of topographical roughness, shear velocity or roughness length, but only in turbulence. This means that in hydrodynamic models, turbulence and vertical mixing above filter-feeders beds should be discriminated and should be not be considered purely as a function of topographic roughness.

Generally, turbulence is calculated after the drag coefficient (C_D) that expresses the drag the bed imposes on the fluid. C_D is calculated according to the quadratic stress law as $C_D = \tau / \rho \overline{u}^2$ while $\tau = \rho u_*^2$. But, it could also be calculated using the TKE method as $\tau = C_1 TKE$ (Soulsby & Dyer 1981; Kim et al. 2000) being C_1 a constant that expresses the ratio of TKE to u_*. Pope at al. (2006) found a good agreement between τ calculations based on log-profile and TKE and Ciutat et al. (2007) used the TKE method to describe bed roughness in terms of shear stress. Calculation of τ using Ciutat's model resulted in values about ten times higher than the τ calculated with the measured TKE averaged and C_1=0.19.

The adoption of the TKE approach to estimate τ reduces the problems associated with precision on measurements elevation from the bottom and does not suffer the problems associated with roughness length estimation using the log-profile method (Pope et al. 2006). However, there is some ongoing discussion about the value of C_1. Some authors consider it to be of 0.19 (Pope et al., 2006, Thompson et al. 2003, 2004) while other consider it as 0.21 (Kim et al. 2000). In the present work the ratio of TKE to u_* increased with increasing U and it is not possible to follow the assumption of a fixed relationship between TKE and u_*, necessary to the use of the TKE method. This study and a few others (Van Duren et al. 2006) suggest that over beds of filter feeders these relationships may differ considerably. The precise dependence of turbulence and drag on bed roughness in the presence of filter feeders needs to be assessed further.

3.5 Conclusions

This study shows that living organisms modify fluids dynamics in a different way that is generally address by engineering. Density of *C. edule* affects boundary layer parameters such as hydrodynamic roughness length, resulting in reduced current velocities near the sediment surface, as a consequence of changes in topographic roughness. In addition to the effect of *C. edule* on the topographical roughness, the filtration activity produces additional turbulence. The effect of filtration activity is only important under shear velocities lower than ~1.5 cm s^{-1}. At high shear velocities, siphonal currents can no longer compete with the longitudinal currents and lose relevance. For the velocities at which filtration activity is relevant, the cockles produce an additional turbulence in the BBL, comparable to the effect produced by the highest topographic roughness tested in this work.

The effect of filtration activity on the BBL should be combined with the effect of current velocity on filtration activity, as well as the effect of quantity and quality of suspended matter on clearance rates. The effect of cockle beds in sediment dynamics is not only restricted to changes in the BBL, but they also play an important role in sediment stability, suspended matter depletion in the water column and further production of biodeposits, with different transport/resuspension/deposition characteristics than the earlier suspended matter. This study aims to be a contribution for more realistic numerical modelling simulations, a valuable tool for ecosystem management.

3.6 Acknowledgments

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PART III

INTERACCTIONS BETWEEN *Nereis diversicolor* AND SEDIMENT DYNAMICS

Chapter 4

Nereis diversicolor effect on the stability of cohesive intertidal sediments

NEREIS DIVERSICOLOR EFFECT ON THE STABILITY OF COHESIVE INTERTIDAL SEDIMENTS

S. Fernandes, P. Sobral and M. H. Costa

Aquatic Ecology 40, 567 – 579, 2006

Abstract

The effect of the polychaete *Nereis diversicolor* on the stability of natural cohesive sediments was investigated in the laboratory. Three densities (450, 600 and 1200 individuals m^{-2}) of *N. diversicolor* were used. Sediment shear strength was measured using a cone penetrometer. Sediment erodability was assessed using an annular flume (current velocities from 5 to 55 cm s^{-1}) in which flow velocity was increased incrementally, and water sampled to quantify suspended material in order to derive critical erosion velocity and erosion rates. At low current velocities (< 25 cm s^{-1}), we found *N. diversicolor* to have a stabilising effect, reflected by an increase of up to 20% in the critical erosion velocity. This is related to an enhancement of ~ 50% in shear strength, due probably to gallery building activities, responsible for the promotion of lateral compaction, an increase in the area of the sediment-water interface, and enhanced microphytobenthos production. Once the sediment began to erode, the stabilising effect of *N. diversicolor* reversed, leading to an increase of up to 40% in eroded matter due to compaction, which resulted in the erosion of larger aggregates. The balance between the effect of *N. diversicolor* on herbivory and microphytobenthos production due to the presence of galleries is discussed. Our results indicate that neither chlorophyll *a*, nor shear strength nor critical erosion velocity are good indicators of erodability. This underlines the need to include biogeochemical processes in any realistic sediment transport model.

Key-words: bioturbation, critical erosion velocity, erosion rates, resuspension, shear strength

4.1 Introduction

The physical processes that dominate sediment transport in aquatic ecosystems have been relatively well studied (e.g. Mehta et al., 1988; Houwing, 1999; Dyer et al., 2004). However, in most numerical models, the benthic variables are given only as boundary conditions. Some studies focus upon the biochemical exchanges at the sediment water interface (e.g. Rutgers van der Loeff and Boudreau, 1997; Sohma et al., 2001), but less importance is given to the biological processes that interact in this exchange. The erosive behaviour of intertidal deposits which act as sources and sinks for sediments in suspension, are critical to understand the ETDC (erosion, transport, deposition and consolidation) cycle. Furthermore, no models exist which predict adequately the erosion, transport and deposition of sediment in estuaries. Recently, it has been emphasised that the study of biological processes affecting sediment stability, as well as the contribution of microphytobenthic biomass, nutrients, organic matter and contaminants to the water column (Rasmussen et al., 1998; Mazik and Elliot, 2000), are important in the understanding of sediment strength and erodability (Kornman and de Deckere, 1998; Widdows et al., 2000; Blanchard et al., 2001).

Much recent work has focussed upon sediment stabilisation by the microphytobenthos, and their effect on sediment adhesion and resuspension by tidal action (de Jonge and Van den Bergs, 1987; Blanchard et al., 1997; Austen et al., 1999; Shimeta et al., 2002). However, less attention has been given to macroinvertebrates and their interaction with the hydrodynamic and physical properties of the sediment (Davies, 1993; Davey and Partridge, 1998; Widdows et al., 2000). Different species of the macrofauna affect sediment stability differently (see Graf and Rosenberg, 1997). Some burrowing species are known to change sediment stability by changing the properties of the sediment (Jones and Jago, 1993; Botto and Iribarne, 2000) or affecting sediment shear strength (Meadows and Hariri, 1991; Widdows et al., 1998a), whilst others have direct or indirect effects upon sediment resuspension (Davies, 1993; Rowden et al., 1998a; Widdows et al., 1998a).

The ragworm *Nereis diversicolor* is a widely distributed polychaete, occurring in sand or mud habitats in Portugal with densities ranging from 430 to 16000 ind m^{-2} (Fidalgo e Costa et al., 1998; Abrantes et al., 1999). *N. diversicolor* live in semi-permanent burrows which provide refuge from predators, and they have a wide array of different feeding techniques such as filter and deposit feeding, scavenging and predation on other macrofauna (Esselink and Zwarts, 1989 and references within; Vedel et al., 1994). They build complex gallery networks that can extend down to 30 cm depth, and which are related to body length, although organisms > 10 cm long can be found in the upper 2 to 3 cm (Anderson and Meadows, 1978; Esselink and Zwarts, 1989; Davey and Partridge, 1998). Some studies have concentrated upon the bioturbation effect of this species on burrowing activity (Trevor, 1977; Davey, 1994) and on sediment reworking (Gerino, 1991; François et al., 2002). There is some controversy about the stabilising effect of *N. diversicolor*. Some authors considered this species as a stabiliser, judging from its influence on the shear strength properties of the sediment (Meadows and

Tait, 1989; Meadows et al., 1990). On the other hand, Underwood and Paterson (1993) referred to the destabilising effect of *N. diversicolor* due to its negative influence on the colonisation of benthic diatoms. Although some literature (Eckman et al., 1981; Davey, 1994) refers to the proportional effect of macrofauna density on bioturbation, the quantification of this activity is required, and the relative importance of the effect of this species on sediment stability needs to be assessed.

The objective of our study is to understand the effect of (1) *N. diversicolor* on sediment stability; and (2) density (ind m^{-2}) on bioturbation activity. To achieve this, we quantified the species influence on sediment stability parameters, e.g. shear strength, critical erosion velocity, as well as erosion rates and resuspension of particulate organic matter and chl *a*.

4.2 Material and methods

4.2.1 Experimental set-up

Intertidal estuarine sediments from Ponta da Erva, Tejo estuary, Portugal (29SN NC 030 984) were collected in November 2000 and March 2001, corresponding to the first (NOV) and second experiments (MAR). The sediments were collected from the top-most 2 cm and maintained at 4ºC to minimise biological activity, until the experiments commenced (7days). Subsequently, the macrofauna present (occasional small shells, small crabs, *N. diversicolor*) was defaunated manually to minimise sediment disturbance, and the sediment was thoroughly homogenised to reduce variability between treatments. Sediment cores were taken to determine sediment dry bulk density (d_s) and organic content by drying at 90ºC (48h) and incinerating at 450ºC (4h). The sediment comprised ~ 95% silt (Lima, 2002), with an average d_s of 0.75 g dry weight (DW) cm^{-3} (SD = ± 0.15, n=12) and organic-matter content of 7.7 % (± 1.6; n =12). Experiment microcosms were prepared using 8-cm high flume boxes (area, 0.053 m^2) representing a quarter of the total flume channel area (see below). For both experiments, microcosms were replicated 3X for each density treatment, as well as a control treatment on each experiment (C NOV and C MAR). In total, three treatments were carried out for NOV and MAR experiments with different densities of *N. diversicolor.* Organisms were collected from the upper 10 cm of sediment from Barreiro (Tejo estuary) on both occasions. After acclimatisation to laboratory (~ 4 days), the organisms were introduced to the flume box microcosms. For the first experiment (NOV), a density of 450 ind m^{-2} was used, corresponding to 23 ind $microcosm^{-1}$ (average length (AL) of 77 mm ± 11, and 0.11 g DW ±

0.03, n=20 for all treatments). In the second experiment (MAR), two densities treatments with 600 ind m^{-2} (32 ind $microcosm^{-1}$ with 102 mm AL ± 32 and 0.09 g DW ± 0.02) and 1200 ind m^{-2} (64 ind $microcosm^{-1}$ with 78 mm AL ± 18 and 0.05 g DW ± 0.01) were used. After introduction of the organisms, microcosms incubated under a water column of ~ 2 cm, under continuous flow-through (3 l h^{-1}) and controlled temperature (15ºC ± 1), salinity (36 ppt) and light (57 μmol m^{-2} s^{-1} ± 11), and with a 12h photo-period for 20 days.

4.2.2 Shear strength measurements

After the 20 days of incubation, sediment geotechnical shear strength (SS) was measured before (s_b) and after (s_a) erosion runs, with a Geonor® falling-cone penetrometer (described in Watts et al., 2003). This device consisted of a 10g inverted cone dropped on the sediment surface. Cone penetration depth was converted into undrained shear strength using Hansbo (1957) conversion tables. Measurements were triplicated, for each experimental microcosm, both before and after resuspension runs.

4.2.3 Erosion measurements

For the sediment erosion tests, an annular flume similar to that described in Widdows et al., 1998a was used (Fig. 4.1). The annular flume is 60 cm in diameter, and has a channel width of 15 cm, area of 0.21 m^2 and a capacity of 60 l with a water column height of 30 cm. It has seven sampling ports at 5-cm intervals from the base. Current velocities are generated by a rotating drive plate of adjustable height. The drive plate rotation was calibrated previously (Fernandes, 2001) and could generate free stream current velocities of up to 55 cm s^{-1}. After the incubation period, each microcosm was introduced carefully into the flume. Since the experimental microcosms were tested individually, three empty inverted flume boxes were introduced to the flume to complete and level the sediment surface. For each erosion run in the flume, current velocity was increased at 15-min increments from 5 to 10, 15, 20, 25, 40 and to 50 cm s^{-1}. Water samples were collected at 2, 12 and 22 cm above the sediment bed, to obtain an homogeneous quantification of the eroded matter at the end of each velocity step, and to determine suspended particulate mater (SPM), particulate organic matter (POM) and chl *a*. At the end of the erosion runs, the microcosms were defaunated by hand. The organisms were stored in 70% alcohol for size, weight and final density determinations. As the initial density would not have represented the density over the incubation period and the final density would have underestimated the total numbers of organisms in the experiment, experimental density was determined as the average of the initial and the final density.

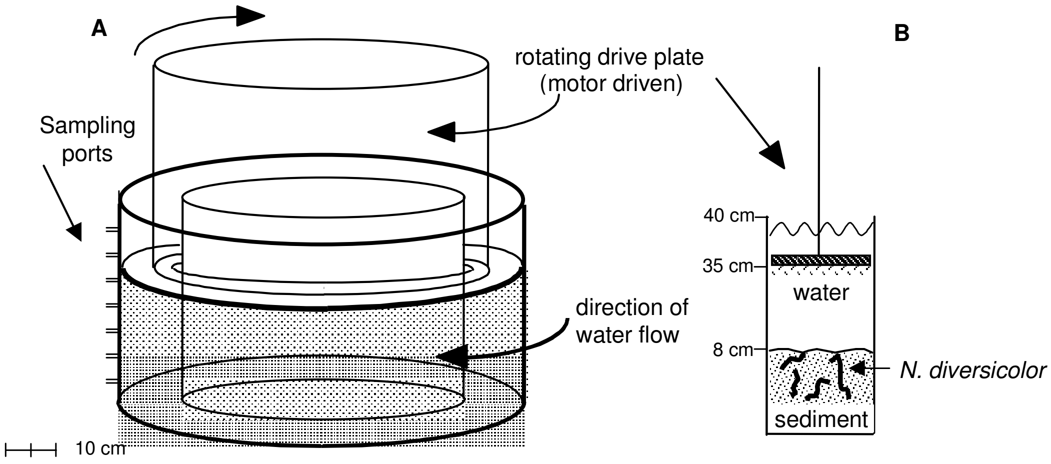

Figure 4.1 - A: Annular flume used to study the effects of bioturbation by *Nereis diversicolor* on the resuspension of sediment exposed to different current velocities. B: Experimental setup (view across the flume channel). (adapted from Sobral & Widdows, 2000).

Water samples were filtered onto washed and pre-weighed glass fibre Whatman GF/C filters (c. 1.2 µm mesh size). SPM and POM were determined gravimetrically after oven drying at 90 ºC (48h), and after combustion at 450 ºC (1 h) respectively. For chl *a* determination, filters were frozen immediately until spectrophotometric analysis. Total chl *a* and phaeopigments were calculated according to the equation of Lorenzen (1967). The chl *a* content of the eroded matter was estimated using SPM standardisation and the quality of eroded chl *a* was determined using an index % chl *a* (chl *a*/ phaeopigments * 100) (Lucas et al., 2000).

Critical current velocities (U_{crit}) were derived according to Widdows et al. (1998a,b), based on a regression of log SPM against current velocity. U_{crit} was defined as the free stream current velocity inducing an increase in SPM concentration above the threshold value of 50 mg l^{-1}. Eroded matter, *Em* (g m^{-2}) was determined using *Em* = (SPM * V)/ A, where V is the volume of water in the flume and A the area of the test section. Erosion rates (g m^{-2} s^{-1}) were quantified as [*Em* (t+1) - *Em* (t)]/ t, where t is the time interval (s) between samplings.

4.2.4 Bioturbation effect (Be)

Bioturbation was described using an index of additional cumulative effect (Blanchard et al., 1997) (B_e, %), where B_e= [(X with *N. diversicolor* - X with zero *N. diversicolor*) / X with zero *N. diversicolor*]* 100, and where X is the value of each parameter.

4.2.5 Statistical analysis

The data were analysed statistically with a multivariate bifactorial ANOVA for $\alpha = 0.5$, and with a Tukey post-hoc HSD (Honest Significant Difference) probability test using STATISTICA (version 6.0) software. Sample independence was assured through experimental design, and homogeneity of variance was verified using Levene's test.

4.3 Results

4.3.1 Visual observations

After the 20-days incubation period, a surface biofilm had developed in the microcosms (Fig. 4.2 a and b). During the incubation period, the presence of natural meiofauna was observed in all treatments. Although a detailed taxonomic analysis was not done, nematodes, harpacticoid copepods and ciliate protozoa were identified. The meiofauna was comparable in all treatments, and hence changes in sediment properties were attributed specifically to *N. diversicolor.*

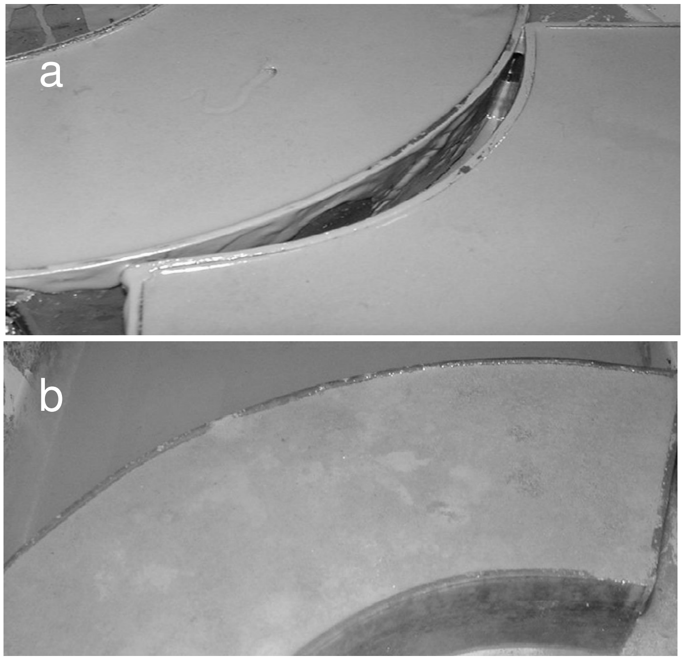

Figure 4.2 - a) Aspects of the sediment surface at the beginning of the incubation period (day 0). Note, on the upper microcosms, the trace of a *N. diversicolor* burrowing. b) Sediment surface at day 20, in the control treatment. It is possible to observe the developed biofilm; lighter shaded areas on the surface correspond to sediment from lower layers, transported to the surface by the meiofauna. (photos from experiment MAR).

After the erodability runs and *N. diversicolor* defaunation, a mortality rate of 43 % (± 2) in NOV for 450 ind m^{-2} was estimated, and 25 % (± 6) and 69 % (± 9) in MAR with densities of 600 and 1200 ind m^{-2}, respectively. Average densities during the runs were: N350 for 450 ind m^{-2}, N530 for 600 ind m^{-2} and N790 for 1200 ind m^{-2}.

4.3.2 Sediment shear strength

Controls (C) and *N. diversicolor* treatments in both experiments did not differ significantly in SS, before erosion runs (s_b) (Table 4.1). However, in NOV, after resuspension runs (s_a) SS increased significantly ($F=18.00$, $p<0.05$) by about 63% (see Table 4.2) in the N350 treatment, although *Em* was similar to the control (1271 g m^{-2} ± 247 and 1139 g m^{-2} ± 299, respectively). In the second experiment (MAR), prior to the erosion runs, surface sediment SS was about 38% higher in the presence of *N. diversicolor* (Table 4.2). After erosion runs, SS differed significantly between N790 and CMAR, and N790 and N530 ($p<0.01$ and $p<0.05$ respectively), depending on the erosion depth at which SS was measured. Nevertheless, SS was always higher in *N. diversicolor* treatments.

4.3.3 Sediment erodability

4.3.3.1 Critical current velocity

The U_{crit} ranged between 27 cm s^{-1} and 35 cm s^{-1} (Table 4.3). Significant differences were observed only in NOV, between CNOV and N350 ($p<0.05$). For both experiments, the presence of *N. diversicolor* had an increasing effect on U_{crit} of up to 22% compared with controls (Table 4.2). U_{crit} was positively correlated with % eroded OM measured before erosion ($R^2= 0.46$, $F= 10.04$, $p< 0.01$).

4.3.3.2 Eroded material

In NOV, no significant differences were apparent in *Em* (g m^{-2}) (Fig. 4.3A) and even at the highest velocity (~ 50 cm s^{-1}), *Em* values were similar (see Table 4.1). However, for the range of velocities below U_{crit}, *Em* values were significantly higher in CNOV ($F=11.95$, $p<0.01$). At current velocities <25 cm s^{-1}, *Em* was lower in N350 (9 to 14 g m^{-2}) compared with CNOV (15 to 41 g m^{-2}).

Table 4.1 - Changes in the shear strength *s* (± SD) of the surface sediment after the 20-day incubation period (s_b), and after erosion of the sediment surface (s_a) for the experiments NOV (CNOV and N350) and MAR (CMAR , N 530 and N 790). Also shown are values of SPM, *Em*, eroded OM, eroded chl *a* and erosion rates at the maximum current velocity tested (~50 cm s^{-1}).

	Sediment shear strength (kPa)		SPM ($g\ l^{-1}$)	*Em* ($g\ m^{-2}$)	Eroded OM ($g\ m^{-2}$)	Eroded chl *a* ($mg\ m^{-2}$)	Erosion rates ($g\ m^{-2}\ s^{-1}$)
NOV	S_b	S_a	at ~50 cm s^{-1}	at ~50 cm s^{-1}	at ~50 cm s^{-1}	at ~50 cm s^{-1}	at ~50 cm s^{-1}
CNOV	0.10 ± 0.00	0.21 ± 0.05	1.01 ± 0.26	1139 ± 299	101 ± 28	54 ± 12	0.516 ± 0.141
N350	0.10 ± 0.00	0.34 ± 0.02	1.12 ± 0.22	1271 ± 247	87 ± 4	22 ± 6	0.668 ± 0.499
MAR							
CMAR	0.13 ± 0.06	0.23 ± 0.08	0.48 ± 0.12	544 ± 130	55 ± 12	35 ± 13	0.272 ± 0.052
N530	0.18 ± 0.03	0.53 ± 0.15	0.60 ± 0.06	681 ± 64	60 ± 5	22 ± 7	0.407 ± 0.011
N790	0.18 ± 0.03	1.02 ± 0.28	0.67 ± 0.11	759 ± 127	75 ± 11	43 ± 5	0.453 ± 0.201

Table 4.2 - Additional cumulative effect of bioturbation (Be, %) on: sediment shear strength (kPa) before (s_b) and after (s_a) resuspension; critical erosion velocity (U_{crit},); *Em*; erosion rate; eroded chl *a* and µg chl *a* g^{-1} DW for the highest experimental velocity (~50 cm s^{-1}) for the three *N. diversicolor* average densities (N350, N530 and N790 ind m^{-2}).

	Be (%)		
ind m^{-2}	**N350**	**N530**	**N790**
Shear strength			
S_b	0	38	38
S_a	63	128	335
Erodability			
U_{crit}	22	3	8
Em	12	25	39
Erosion rate	30	50	67
chl *a*			
Eroded chl *a*	-60	-37	22
µg chl *a* g^{-1} DW	- 65	- 48	- 9

Table 4.3 - Exponential regressions of SPM (y) (mg l^{-1}) on current velocity (x) (cm s^{-1}). Also shown are critical current velocities (U_{crit}) required to increase the SPM above a threshold of 50 mg l^{-1} (± SD).

NOV	Equation	R^2	U_{crit} (cm s^{-1})
CNOV	$y = 2.896e^{0.102x}$	0.85	27.1 ± 1.6
N350	$y = 0.530e^{0.136x}$	0.78	33.1 ± 0.8
MAR			
CMAR	$y = 1.333e^{0.109x}$	0.73	32.7 ± 1.8
N530	$y = 0.337e^{0.154x}$	0.95	33.8 ± 3.1
N790	$y = 0.588e^{0.124x}$	0.76	35.4 ± 1.3

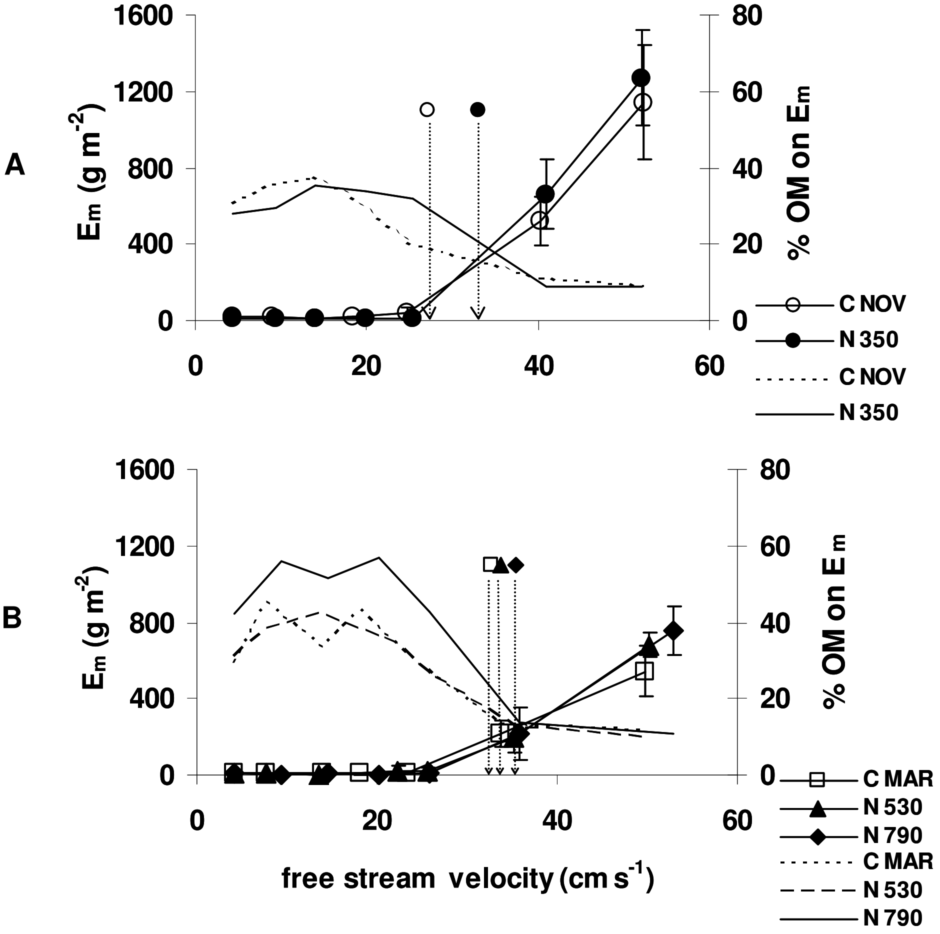

Figure 4.3 - Effect of free stream velocity (cm s^{-1}) on eroded matter (E_m) (g m^{-2})(± SD) (symbols) and on the content of OM on E_m (lines) for experiments NOV (A) and MAR (B), for the controls (CNOV and CMAR) and the treatments with *N. diversicolor* (N350, N530 and N790). Symbols with arrows represent the average U_{crit} (cm s^{-1}) of each treatment.

These results agree with the differences found in % eroded OM, where an earlier decline in CNOV related with the dilution effect of bulk erosion was observed (Fig. 4.3A). Additionally, U_{crit} was lower in CNOV (see Table 4.3). Hence, these results indicate stabilisation or alteration due to the presence of *N. diversicolor*. After resuspension, erosion rates were always higher in N350 (0.54 g m^{-2} s^{-1} ± 0.15 at 40 cm s^{-1} and 0.66 g m^{-2} s^{-1} ± 0.49 at 52 cm s^{-1}), compared to CNOV (0.39 g m^{-2} s^{-1} ± 0.12 at 40 cm s^{-1} and 0.51 g m^{-2} s^{-1} ± 0.14 at 52 cm s^{-1}).

In MAR experiment, *Em* and erosion rates were always higher in *N. diversicolor* treatments (Table 4.1). However, no significant differences were observed after U_{crit} was reached (Fig. 4.3B). Before U_{crit} was reached (velocities up to 25 cm s^{-1}) in CMAR, *Em* was higher and differed significantly from N790 ($p<0.01$) with highest OM contents of eroded matter (varying from 5 to 12 g m^{-2} and 42 to 57 % OM; and 2 to10 g m^{-2} and 29 to 45 % OM, respectively) (Fig. 4.3B). The additional cumulative effect of bioturbation (B_e) (Table 4.2) showed a concomitant increase with *Em* and *N. diversicolor* density, and *Em* and eroded OM were highly correlated ($R^2 = 0.98$) in both experiments (Table 4.1).

The effect of *N. diversicolor* on the input of chl *a* to the water column (Fig. 4.4) was not significantly different between controls and N treatments before erosion. In NOV, however, it differed significantly after erosion (F=16.88, $p<0.01$).

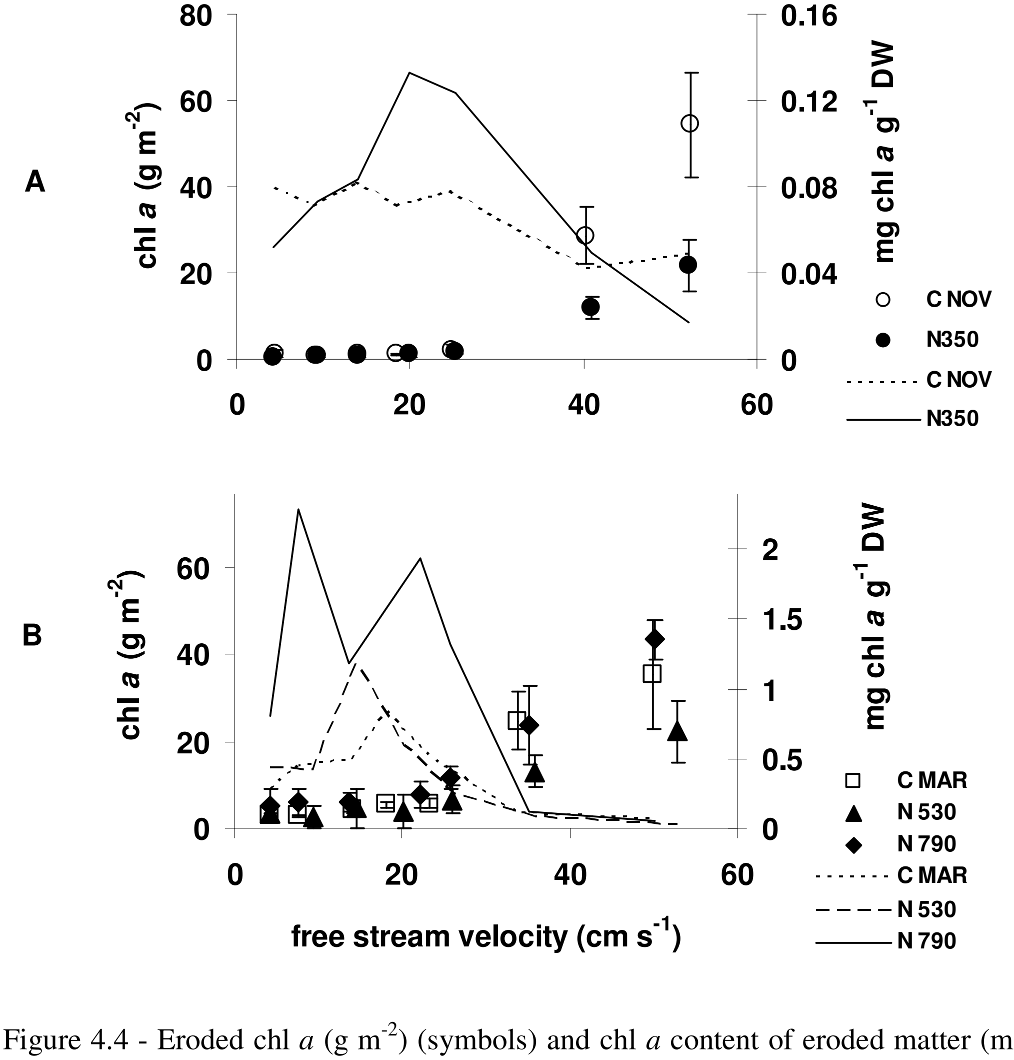

Figure 4.4 - Eroded chl *a* (g m^{-2}) (symbols) and chl *a* content of eroded matter (mg chl *a* g^{-1} dw) (lines), as a function of the free stream velocity, for experiments NOV (A) and MAR (B). Legends as Fig 4.3.

As seen from *Em*, chl *a* resuspension occurred earlier in CNOV before U_{crit} was established, whilst at the highest velocity, eroded chl *a* decreased by 60% in N350 (Table 4.2). In MAR, eroded chl *a* differed significantly between N530 and N790, before ($F=4.70$, $p<0.05$) and after erosion ($F=8.81$, $p<0.01$), and between CMAR and N530 after erosion ($F = 6.88$, $p<0.05$) (Fig. 4B). Higher chl *a* resuspension occurred in N790 at 50 cm s^{-1} (43 mg chl *a* m^{-2} ± 5) whilst values for N530 were lower (22 mg chl *a* m^{-2} ± 7) at 53 cm s^{-1} than CMAR (35 mg chl *a* m^{-2} ± 13) at 50 cm s^{-1}. The B_e index for chl *a* contents (see Table 4.2) shows the negative effect of bioturbation on eroded chl *a*.

To eliminate the effect of bulk *Em* on chl *a* resuspension, the data were standardised to *Em* (i.e. mg chl *a* g^{-1} DW) and the B_e index was even higher (-65%). For NOV, at velocities of 50 cm s^{-1}, the chl *a* content of eroded matter was higher in CNOV (0.05 mg chl *a* g^{-1} DW ± 0.01) than in N350 (0.02 mg chl *a* g^{-1} DW ± 0.00). In contrast, for the velocities below U_{crit}, chl *a* content was higher in N350 (Fig. 4.4A). In MAR, for velocities below U_{crit}, N530 and N790 treatments had higher chl *a* contents than the control (Fig. 4.4B). The quality of chl *a* was higher in the control treatments in NOV (ranging from 8 to 14 % chl *a* in CNOV and from 3 to 11 % chl *a* in N350) and MAR (ranging from 8 to 18 % chl *a* in CMAR, 7 to 11 % chl *a* in N530 and 6 to 16 % chl *a* in N790), implying that *N. diversicolor* had decreased the quality of eroded chl *a*. For the highest velocity tested, CMAR and N790 showed the same weight-specific chl *a* concentration (0.06 mg chl *a* g^{-1} DW ± 0.01), but these values were 48 % lower for N530 (0.03 mg chl *a* g^{-1} DW ± 0.01).

4.3.4 Correlations with sediment shear strength

A positive relation was found between surface SS before erosion and U_{crit} (Fig. 4.5). The significance of the correlation is lowered by the NOV treatments that showed similar values of SS corresponding to different U_{crit}. In MAR, the positive relationship between these two parameters is clearer, suggesting that *N. diversicolor* affect sediment SS.

The interpretation of the relationship between E_m at the highest velocity tested and surface SS after erosion (Fig. 4.6) should take into account that the s_a was measured at different depths, according to the different E_m found. The correlation between E_m and s_a was positive for MAR, but absent for NOV. The average s_a of the control treatments (CNOV and

CMAR) was always lower (0.2 kPa) than for *N. diversicolor* treated samples (0.3 to 1.0 kPa) (Fig. 4.6).

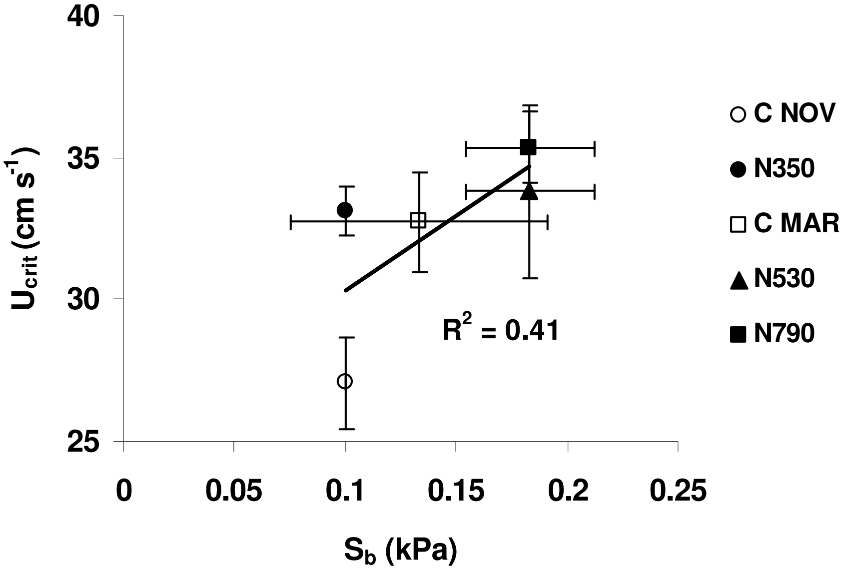

Figure 4.5 - Variation of U_{crit} (cm s^{-1}) and sediment shear strength before erosion s_b (kPa) for both experiments NOV and MAR. Linear regression is based on all data points (F= 8.16, $p<0.01$, n=15). Legends as Fig. 6.3.

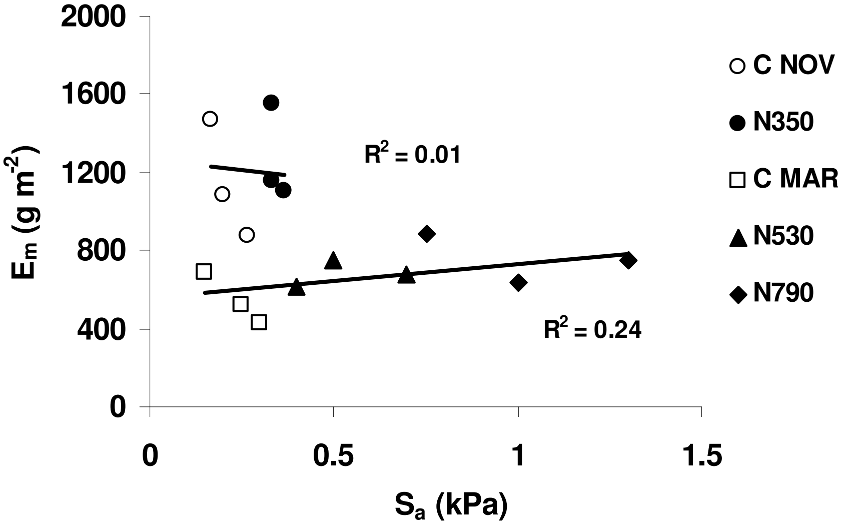

Figure 4.6 - Variation of E_m (g m^{-2}) and sediment shear strength after erosion s_a (kPa) for both experiments NOV and MAR. Lines indicate linear regression tendency for each experiment. Legends as Fig. 6.3.

However, these differences in s_a did not affect E_m to a large extent. Although similar E_m concentrations were found in NOV, the treatments with *N. diversicolor* (N350) exhibited SS values 1.6 times higher than for the control (CNOV). In MAR, higher sediment SS measured after erosion is related to the higher *N. diversicolor* densities, with increases of 2.3 and 4.4 times the SS values for the control for N530 and N790, respectively. This increment in SS may be related to the higher chl *a* content of eroded matter observed in *N. diversicolor* treatments in MAR (see Fig.3). Chl *a* content of eroded matter was positively correlated with both s_b and s_a (R^2=0.35, F=5.44, $p<0.05$ and R^2=0.72, F=27.57, $p<0.001$, respectively). The SS (s_b and s_a) values found for the *N. diversicolor* treatments showed a concomitant increase with average chl *a* content of eroded matter (see Table 4.1 and Fig. 4.4). The overall effect of *N. diversicolor* on sediment SS was larger than its effect on sediment erodability.

4.4 Discussion

4.4.1 *N. diversicolor* effects below critical erosion velocity

The mortality rates reported by us are related not only to real mortality, but also to the methodology used, i.e. we may have missed the organisms during sediment defaunation, or they escaped from the microcosms during the incubation period. Thus, mortality rates are over estimated. The high mortality in MAR (1200 ind m^{-2}) is due probably to stress under high population density or the poor condition of the organisms following the unusually rainy winter of 2001. Nevertheless, for the same experiment and 600 ind m^{-2}, a much lower mortality was observed.

The presence of *N. diversicolor* enhanced the SS of the surface sediments by up to 38% (Tables 4.1 and 4.2), which has also been reported by Meadows and Tait (1989) and Meadows et al. (1990), who found increased sediment SS, which ranged from 37% to 67% for densities from 1000 to 3000 ind m^{-2}. The values obtained by these authors in the laboratory are not inconsistent with the surface SS values found at mid tide level for the inner Clyde estuary (UK), where *N. diversicolor* were abundant (Meadows and Tufail, 1986). The increase in SS is probably related to lateral compaction caused by gallery building activity. The gallery walls of *N. diversicolor* are covered by mucus secreted from glands covering the entire surface of the epidermis. These secretions are pushed against the walls of the burrow, and consolidate the walls (Meadows and Tait, 1989). Galleries are known to increase: (1) sediment permeability; (2) de-watering; and (3) provide conduits for overburden-driven drainage, thereby stepping up sediment consolidation (Meadows and Tait, 1989; Murray et al., 2002).

N. diversicolor increasingly affected not only sediment SS, but also U_{crit}, which was always higher in the presence of *N. diversicolor* (see Table 4.3). This agrees with significantly lower suspended matter concentrations found in the presence of *N. diversicolor* under low velocities (< 25 cm s^{-1}). The lower organic matter contribution of the controls at about 25 cm s^{-1} (Fig. 4.3) is related also to a dilution effect of eroded matter that is particularly clear for the controls of NOV (C Nov, Fig. 4.3A). This effect was observed also for chl *a* content at 25 cm s^{-1} (Fig. 4.4). The lower critical erosion velocities for the controls may be related to earlier resuspension (Type I erosion, Amos et al., 1997) of diatoms and protists, known to have lower critical erosion velocities (Blanchard et al., 1997; Lucas et al., 2000; Shimeta et al., 2002).

Increase in critical erosion velocities in the presence of *N. diversicolor* may be also caused by creation of skimming flow by protruding galleries with associated reduction of shear velocities (Eckman and Nowell, 1984; Friedrichs et al., 2000). However, before erosion occurred, the surface of the treatments with *N. diversicolor* was similar visually to the biofilm surface of the controls, and galleries protruded only after erosion started to occur. These observations suggest that the increasing effect of *N. diversicolor* on U_{crit} is not related to skimming flow.

The increasing effect of *N. diversicolor* on U_{crit} is probably related more to their effect on sediment properties, i.e. compaction, increasing sediment SS, rather than their physical effect on the shear stress velocities, as reported for other burrowing invertebrates (Jones and Jago,1993). The relationship between initial sediment SS and critical erosion velocities observed by us supports this explanation (Fig. 4.5) as well as agrees with Blanchard et al. (1997), who observed that the more cohesive the sediment, the greater is the tidal current velocity required to resuspend an equal quantity of matter. Also, this agrees with the proportionality relationship between critical shear stress measured with a CSM and SS measured with a fall-cone (Watts et al., 2003).

The relationship observed by us between U_{crit} and *N. diversicolor* density (Table 4.2) is related strongly to the fact that sediments and organisms were collected on different occasions, which may have influenced sediment properties and microphytobenthos composition. Nevertheless, critical velocities were always higher in the presence of *N. diversicolor*. Hence, for velocities up to U_{crit}, the presence of *N. diversicolor* causes additional biostabilisation of the surface sediments.

4.4.2 *N. diversicolor* effect above critical erosion velocity

In this study, a positive relationship between sediment *Em* (as well as erosion rates) and organism density was observed (Table 4.1 and Figure 4.3). Destabilisation of the sediment bed did not clear differ in the low density treatments (same erodability as the control) (see Table 4.1 and Fig. 4.3). Hence, a *N. diversicolor* density of 350 ind m^{-2} is considered to be too low to produce measurable sediment erodability effects. In fact, this density is low compared with the natural mean densities found by Fidalgo e Costa et al. (1998) on the south-west coast of Portugal (430 to 16 000 ind m^{-2}). For higher *N. diversicolor* densities we observed a proportional increase in sediment erodability.

Increased erosion rates have been reported for other macrofauna species, e.g. the polychaetes *Diopatra cuprea* (Luckenbach, 1986) and *Owenia fusiformis* (Eckman et al., 1981); the bivalves *Cerastoderma edule* and *Macoma balthica* (Widdows et al., 1998a; 2000); the amphipod *Corophium volutator* (Grant and Daborn, 1994) and the gastropod *Hydrobia ulvae* (Andersen et al., 2002). Davies (1993) studied the effect of several deposit feeder species on direct bioresuspension (Graf and Rosenberg, 1997). These works show that deposit feeders increase resuspension rates due to a tendency to physically eject sediment into the overlying water, thereby disrupting the cohesive structure of the sediment and increasing its water content.

We also observed contradictory effect between an increase in the U_{crit} (reflecting higher sediment stability) coincidental with increased *Em*. According to Luckenbach (1986), once the surface sediment is disrupted, erosion proceeds quicker for dislodged aggregates in mucus-stabilised sediments than for erosion in unbound sediments. Although critical velocities may be higher in the presence of *N. diversicolor*, erosion rates may actually be higher immediately after the onset of erosion.

Pelletisation is known to decrease erosion thresholds and increase suspended sediment concentration (Maa et al., 1998; Andersen, 2001). Although we did not measure pelletisation, *N. diversicolor* feeds on the sediment surface but faeces are deposited in the lower layer of the burrows, which will decrease the effect of faecal pellets production at the sediment surface. However, since U_{crit} was higher in the presence of *N. diversicolor*, it indicated the lack of pelletisation effect.

The effect of *N. diversicolor* on erosion rates can also be related to its deposit feeding activity, which results in sediment reworking at the surface and a reduction of the biofilm. In search for food, the polychaetes will disrupt the biofilm, not only due to predation, but also due to body movements over the sediment surface. Sediment chl *a* was not analysed. However, estimations of total eroded chl *a* suggest that *N. diversicolor* had a decreasing effect on algal biomass. The values measured for the two highest densities tested at the highest velocities are over estimated since (in agreement with the data on *Em* observed in this work) they correspond to deeper sediment layers. The lower quality of chl *a* (% chl *a*) in the treatments with *N. diversicolor* can also indicate herbivory of *Nereis*. Considering that the *Em* is representative of the eroded layer characteristics, it can be assumed that the algal mat decreased due to removal by *N. diversicolor*.

Assuming that chl *a* is correlated with EPS (colloidal carbohydrate) fractions in the sediment (Underwood and Paterson, 1993; Blanchard et al., 2001; Friend et al., 2003), and that chl *a* is a good indicator of sediment stability (Underwood and Smith, 1998), grazing by *N. diversicolor* on the biofilm can be an important factor in decreasing in sediment stability. This agrees with laboratory and field experiments (Peletier, 1996; Smith et al., 1996), which suggest that *N. diversicolor* has a markedly reducing effect on the abundance of benthic diatoms mainly due to deposit-feeding. In addition, they may alter the specific composition of benthic diatom assemblages. Other studies on the microphytobenthos community have reported the effect of macrofauna grazing on the reduction of surface biofilm and on sediment stability (Miller et al., 1996; Austen et al., 1999).

We observed a concomitant increase of *N. diversicolor* density with chl *a* content in eroded matter, e.g. for N790, and biofilm development. High mortality observed in this treatment may have lead to a reduction in grazing activity. Furthermore, N790 was associated with the highest organic matter content (Fig.4.3). This was probably related to a higher decomposition and mineralisation, which may have increased microalgal production. Both Mouritsen et al. (1998) and de Deckere et al. (2001), found that sediment stability and microphytobenthic production increased after mass mortality of the macrofauna. Furthermore, simultaneous increase of *N. diversicolor* density and chl *a* content can also be due to the higher density of burrows. During defaunation of the microcosms, we found gallery walls to appear lighter in colour, with the same visual appearance as the well-oxygenated sediment surface. The burrow walls of *N. diversicolor* are known to provide an additional sediment/water interface, increasing oxygenation, chemical exchanges and chl *a* content in the sediment (Anderson and Meadows, 1978; Gerino, 1991; Davey, 1994; Davey and Partridge, 1998). These facts suggest that high burrow densities may attenuate or contradict the grazing effect.

Our data reveals that for low *N. diversicolor* densities, although chl *a* content was reduced by ~60% in the eroded sediment, there was no corresponding effect on sediment stability (Table 2). Conversely, for the highest *N. diversicolor* densities (where increased chl *a* was recorded) a greater effect on sediment erodability was found. Thus, *N. diversicolor* grazing impact might have no consequences at the sediment stability level. On the other hand, an inverse relationship between chl *a* and U_{crit}, erosion rates as well as sediment SS was found. Although, we did not quantify the sediment chl *a* that limits our conclusions to some extent, our results suggest that chl *a* is probably not a good indicator of stability in the presence of macrofauna. Although this parameter reflects characteristics of the biofilm, it does not reflect the erosion behaviour or erosion rates of further sediment layers. Riethmüller et al. (2000) suggests caution in using chl *a* as an index of stability citing the fact that chl *a* concentrations in the sediments of the tidal flats of the German and Danish Wadden Sea were not always related to critical erosion shear stresses, and that this relationship was found to be highly site-specific.

4.4.3 Interaction between sediment stability parameters

Sediment SS values measured by us after erosion are directly proportional to polychaete density (see also Meadows and Hariri, 1991). These surface measurements are influenced by the eroded depth at which they were measured and it is known that SS generally increases with depth (Amos et al., 1988; Underwood and Paterson, 1993). Although we measured at different depths, the *N. diversicolor* effect on *Em* is much lower compared with their effect on sediment SS (Fig. 4.6). The higher SS values found at the highest density treatments are also related to the high mortality, and consequently effect the microbial production as discussed previously. The relationship between *Em* and sediment SS after erosion, is not very clear in the NOV experiment, particularly due to similar values of *Em* on both treatments. In MAR, sediments with higher SS were related to higher *Em* and erosion rates. In addition, erodability of sediments with similar SS is variable.

Our findings are in contradiction with the classical assumption that sediments with high SS exhibit lower erosion rates. This assumption was recently verified by Watts et al. (2003) on intertidal sediments at Tollesbury (Essex, UK); they used both Fall-Cone and CSM devices, and erosion rate calculations from the s_i index. For our study we can take into account that biologically-mediated sediments have a higher strengths, and that higher SS is related to a higher compaction (Underwood and Paterson, 1993). In addition, such sediments may have a higher probability of erosion as aggregates, rather than as loose material (Luckenbach, 1986).

Although we found a relationship between SS and U_{crit} , these parameters were not related to erosion rates. These parameters are, therefore, not good indicators of erodability. The lack of relationship between erosion rates and erosion thresholds has been mentioned by several workers (Grant and Daburn, 1994; Blanchard et al., 1997; Sutherland et al., 1998), suggesting that erosion rates are a better indicator of sediment stability than erosion threshold. Houwing (1999) considered critical erosion threshold to be inadequate for characterising erodability, since it is restricted to the upper layers of the bed only. Although critical erosion threshold is related to the surface resistance against erosion, erosion rates are related more to the resistance of the subsurface layers. In addition, Andersen (2001) found a large variation in erosion rates for samples with similar erosion thresholds. There is still a strong tendency to relate erosion rates with velocity parameters such as shear stress (Houwing, 1999; Wang, 2003). These relationships completely neglect the influence of the sediment properties that are more likely to influence the quantity of eroded matter.

Although some studies use benthic fluff layers to predict erosion (Wang, 2003) and positive relationships have been found between faecal pellets and erosion rates (Andersen, 2001), this seems to be a weak indicator for erodability, since there is almost no benthic fluff after consecutive resuspension events (Jago et al., 2002). For gastropod *Hydrobia ulvae*, Andersen et al. (2002) observed that low erosion thresholds were related to high faecal pellet contents whereas both high and low thresholds were found at sites with low faecal pellet contents. On the other hand, the relationship between erosion rates and faecal pellets can be related to the presence of destabilising macrofauna rather than the presence of faecal pellets itself.

The correlation between erosion rates or erosion thresholds is also difficult to obtain from sediment features such as water content, grain size or sediment fractions, as well as biochemical measurements such as moisture and organic content, chl *a* and colloidal carbohydrate concentrations (Underwood and Paterson, 1993; Houwing, 1999; Paterson et al., 2000; Andersen, 2001).

The precision of sediment stability proxies, is related not only to the biofilm variability factors such as emersion, seasonality, light intensity or day-night variations (Paterson et al., 2000; de Brouwer and Stal, 2001; Friend et al., 2003), but also to macrofauna activity that can be increased in the summer months (e.g. Rowden et al., 1998b).

4.5 Concluding remarks

This work contributes to clarifying the role of *N. diversicolor* on sediment stabilisation, through the modification of sediment properties and an increase of U_{crit}. The stabilising effect of *N. diversicolor* reverses once U_{crit} is established. *N. diversicolor* enhancement of SS is likely to enhance aggregate erosion rates. Our results contribute towards an understanding of the effect bioturbating macrofauna may have on the long-term sediment dynamics of intertidal cohesive sediments. They confirm the importance of biological processes on sediment transport and stress the need for further study in order to understand their relative importance and interactions. Bioturbation processes can cause large changes in property of sediment stability, and should be integrated in any management or conservation models. We also demonstrate the need: (1) to clarify and obtain a better understanding of factors that regulate sediment SS with depth in bioturbated sediments; (2) to comprehend the effect of bioturbation activities on bed topography and on the velocities near the surface; (3) to understand the influence of the biofilm on bioturbated sediment stability; and (4) to examine further the relationships between SS and erodability in bioturbated cohesive sediments. In particular, the relative importance of direct and indirect bioturbation effects on sediment stability should be assessed.

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PART III

INTERACCTIONS BETWEEN *Nereis diversicolor* AND SEDIMENT DYNAMICS

Chapter 5

Measuring bioturbation of *Nereis diversicolor* with luminophores: experimental and modeling artifacts

MEASURING BIOTURBATION OF *NEREIS DIVERSICOLOR* WITH LUMINOPHORES: EXPERIMENTAL AND MODELING ARTIFACTS

Abstract

Bioturbation activities are very important for mineralization at the sediment water-interface and also for changes in the sediment properties. The aim of this work is to quantify the bioturbation of three densities of *N. diversicolor* (320, 450 and 570 ind m^{-2}). Three experiments were conducted on cohesive sediments over 21 days. Bioturbation was measured as biodiffusion coefficients (Db), using luminophores as tracers. Db were estimated using two different calculation methods: M1, the most common biodiffusion model; M2, a model giving more emphasis to the concentrations in the lower layers. The Db´s observed in this work were not proportional to density, which is probably related to the different experimental mesocosms used in the different experiments. Db estimations using M1 and M2 varied over time. The M2 procedure showed to have better fit to the tracer concentration in the deeper layers. These findings brought up some questions regarding the experimental procedure. Herein, the source of misinterpretations observed in this work is discussed and some recommendations are provided.

Key-words: cohesive sediments, bioturbation, *Nereis diversicolor*, luminophores, biodiffusion model.

5.1 Introduction

Biological activities influence the biogeochemical fluxes of solutes and reaction rates over the sediment-water interface, stimulating aerobic decomposition in the sediment creating a mosaic of microenvironments (Aller and Aller, 1992; Fenchel, 1996; Kristensen, 2000). This regeneration enhancement is particularly important in the upper centimetres of oceanic sediments, where molecular diffusion in the upper layers is the dominant mode of transport to the water column (Middelburg et al., 1997; Berg et al., 2001)

These activities, such as food search and gallery/burrow building, result in redistribution of particles, enhancement of pore water transport and improve irrigation conditions and oxygen concentrations within the sediments (Aller, 1983; Aller and Aller, 1998; Forster et al., 1999; Mortimer et al., 1999; Kristensen, 2000: Nielsen et al., 2004). The effect of biological activity increases microbial degradation of matter along the oxic walls of infaunal burrows, enhancing the decomposition of organic and inorganic matter, nutrients and pollutants (Aller, 1983; Andersen and Kristensen, 1992; Davey and Watson, 1995; Aller and Aller, 1998; Gerino et al., 1999; Hansen et al., 1999; Kristensen, 2000; Hammerschmidt and Fitzgerald, 2004).

The majority of these works focused in the effect of bioturbation on solutes, but few focus on particles distribution. Although bioturbation have a significantly smaller effect on solids than on solutes (15 to 20 times smaller) (Berg et al., 2001), the particle mixing activity is also important for its influences on the recruitment of planktonic populations by generating the burial and the return of buried diapause eggs and cysts to the sediment surface. Particle redistribution increases the opportunities for these eggs to hatch as they repeatedly cycle past the water-sediment interface (Marcus and Schmitd-Gengenbach, 1986), but can also play a negative role on the viability of cysts that have passed through polychaetes' gut (Giangrande et al., 2002).

To quantify these bioturbation activities several tracers are used to access sediment mixing, such as particle-reactive radionuclides (Sandnes et al.,2000a,b; Berg et al., 2001; Shull and Mayer, 2002; Dellapenna et al., 2003), chlorophyll a (Boon and Duineveld, 1998; Green et al., 2002), glass beads (Marcus and Schmitd-Gengenbach, 1986; Berg et al., 2001) and luminophores (Caredec et al., 2004; Ouellette etal., 2004; Solan et al., 2004). Some of them being more accurate than others (Middelburg et al., 1997; Gerino et al., 1998; Berg et al., 2001). The luminophores method showed good agreement between field and laboratory estimations and predict similar orders of magnitude of biodiffusive mixing estimated using ^{234}Thorium and Chl *a* (Gerino et al., 1998).

Several *in situ* studies focus on the effect of the community bioturbation in relation to the present feeding types (Gerino et al., 1995, 1999; Flach et al., 1998; Turnewitsch et al., 2000; Green et al., 2002), scales of bioturbation according to the relation height-width of the present organisms (Swift et al., 1996), or the presence of burrows (Dellapenna et al., 1998). Although the bioturbation found in these works is related with the present communities, they include a high heterogeneity of species composition and not only is difficult to compare them, as it is not possible to evaluate the effect of individual species that are known to have different sediment mixing modes and turnover rates, feeding strategies and mobility (François et al., 1997 (and references within), 2002; Kristensen, 2000; Sandness et al., 2000a).

The estimation of the mixing bioturbation activity in the sediment is normally expressed as biodiffusion coefficients, Db. This incorporates all animal activities affecting the sediment, such as local passive transport (mechanical sediment mixing) and non-local active transport (through the digestive/gut way), into a single coefficient. This parameter can be estimated individually for solids and solutes (Sandness et al., 2000b: Berg et al., 2001) and it is known to be influenced by total community biomass (Sandnes et al., 2000a), the density of organisms (Retraubun et al., 1996), temperature (Aller and Aller, 1992; Gerino et al., 1998; Ouellette et al., 2004), body weight (Giangrande et al., 2001), sediment porosity (Aller and Aller, 1992; Mulsow et al., 1998) and availability of organic matter (Gerino et al., 1998).

Due to the variety of models to estimate Db and the specification of model estimation for each tracer, the comparisons among results becomes complex (Swift et al., 1996; Dellapenna et al., 1998; Gerino et al., 1998; Turnewitsch et al., 2000; Green et al., 2002). In addition, only few works compare tracers distribution obtained in the presence of macrofauna with controls with the absence of macrofauna (Sandnes et al., 2000a; Ulsow et al., 2002; Ouellette et al., 2004). Most of the studies which employ Db estimations based on tracer distribution do not refer the values for control situations (Gerino et al., 1998; Hansen et al., 1999; Caradec et al., 2004). The lack of control measurements is sometimes linked to the fact that defaunating methods would alter the sediment structure (Berg et al., 2001), although was reported that defaunation using microwaves do not affect sediment porosity (Aller and Aller, 1992).

Some authors use experimental mesocosms with small areas (30 to 100 cm^2) and considerable depths (up to 30 cm) (Marcus and Schmitd-Gengenbach, 1986; Gerino et al., 1994; Berg et al., 2001; François et al., 2002; Ouiellette et al., 2004) to assess the effect of macrofauna in the distribution of tracers. In these works, although the number of organisms used per mesocosms is proportional to the tested densities, the small horizontal area at which the organisms are exposed forces them to move in a limited space, increasing reworking activity into a certain extent. To avoid this artefact, the present study was performed in a larger area, employing core sampling to access tracers distribution in depth (Sandnes et al., 2000a).

The present work, examines the effect of the ragworm *Nereis diversicolor* on sediment reworking. This species is a well studied gallery-diffusor (François et al., 2002), being one of the most abundant infaunal benthic organisms in Europe, who lives in complex gallery networks that can extend down to 30 cm depth (according to body length), in burrows diameters from 0.5 to 4 mm (Andersen and Kristensen, 1992; Webster, 1992; Kristensen, 2000) and mean burrow surface ares of 1, 10 and 25 cm^2 for small, medium and large worms (Davey, 1994). Its burrow lines increase the surface area from 0 to 420% in densities <100 to

13100 ind m^{-2} (Mortimer et al., 1999). François et al. (2002) measured D_b of 3.18 cm^2 y^{-1} in the presence of a population of 800 *N. diversicolor* m^{-2}, based on Gerino's experiments (Gerino and Stora, 1991; Gerino, 1992, in François et al., 2002).

The aim of this work is to understand how estimations of Db are affected by: 1) the density of organisms, 2) sampling incubation time for measurements, 3) the calculation procedures.

5.2 Materials and methods

5.2.1 Organisms, sediment and water collection

Three experiments were performed in November 2000, March 2001 and January 2002 using three densities of *N. diversicolor*. In all occasions, organisms (10 to 15 cm length) were collected in Rosarinho (Tagus estuary, Portugal), brought to the laboratory and allowed to stabilize under the experimental conditions for a period of about 5 days before introduction in the experimental mesocosms. Typical densities are in the range of 1000-2000 ind m^{-2} for adult populations, but can reach up to 16000 ind m^{-2} for juvenile populations in spring (Davey, 1994; Fidalgo e Costa et al., 1998).

Intertidal estuarine sediments from Ponta da Erva (Tejo estuary) were scooped from the top 1 cm during low tide, brought to the laboratory where they were kept at 4^{0}C (to reduce biological activity) until mesocosms set-up. Recent grain size analysis at Ponta da Erva (using a Malvern®Mastersizer 2000, n=3) showed that sediment is mostly composed by silts and clays (75.4 ± 5.4 % and 22.9 ± 6.2 respectively) and median grain size ($10d_{50}$) is 9.8 ± 2.0 µm. Besides the fine fraction only a small percentage of very fine sand was present, 1.7 ± 0.9 % (Soares & Sobral, unpublished data).

Seawater used at IMAR-FCTUNL facilities is collected on the coast, 30 Km west of Lisbon, and supplied periodically to our major tank by the navy. The seawater is kept in the laboratory in a continuous recirculation aerated system and under temperature controlled conditions.

5.2.2 Mesocosm set-up

Sediments were hand defaunated for macrofauna, after which they were homogenised and introduced in the mesocosms. The number of mesocosms was prepared according to the number of sampling days (one per sampling day). On the following day, *N. diversicolor* were introduced in the mesocosms and allow to burry in the sediment. All the experimental mesocosms were 8 cm depth, which is the depth at which most of *N. diversicolor* are found, given the fact that they burrow deeper in sand than in mud (Anderson and Meadows, 1978; Esselink and Zwarts, 1989; Davey, 1994).

The activity of *N. diversicolor* was accessed using fluorescent particles (luminophores, 80 – 180 µm) (Mahaut and Graf, 1987; Gerino et al., 1998) that were added in a fine layer into the sediment surface, enough to assure that vertical transport rate would not be influenced by the lack of tracers in the top of the sediment (Gerino et al., 1994). The mesocosms were placed in tanks that were carefully filled with sea water, in order to avoid resuspension of the luminophores. The mesocosms were then allowed to stabilise under experimental conditions with a water column of 2 cm, under a continuous flow-through system (~ 3 L h^{-1}) with constant aeration. At each sampling day 3 cores (area of 3.5 cm^2) were taken from each mesocosms and immediately frozen until analysis. Mesocosms were defaunated in order to estimate the final density. As the final density was related with mortality, organisms escaping during incubation or killed during core sampling, an average density was estimated from the initial and the final density.

Experiment 1 In November 2000, 40 organisms (0.11 g dry weight (DW) ind^{-1}, n=20) were introduced in mesocosms with an area of 0.0875 m^2 and 8 cm height (a density of 450 ind m^{-2}). Sampling was performed after 20 days of stabilisation under experimental conditions (temperature $15^0C \pm 1$; salinity 36 and light 57µmol m^{-2} s^{-1} ± 11 with a 12 hour photoperiod). After the experiment average density was estimated to be 320 ind m^{-2}.

Experiment 2 In March 2001, 17 organisms (0.09 g DW ind^{-1}, n=20) were introduced in each mesocosms with an area of 0.0270 m^2 and 8 cm height (a density of 600 ind m^{-2}). Sampling was done at days 3, 7, 14, 21 and 28. Experimental conditions were the same as Exp.1. After the experiment average density was estimated to be 450 ind m^{-2}.

Experiment 3 In January 2002, 24 organisms (0.08 g dry weight (DW) ind^{-1}, n=22) were introduced in each mesocosms with an area of 0.0270 m^2 and 8 cm height (a density of 900 ind m^{-2}). Sampling was done at days 2,7,15 and 21 days of stabilisation under experimental conditions (temperature $17^0C \pm 1$; salinity 36 and light 54 µmol m^{-2} s^{-1} ± 23 with a 12 hour photoperiod). After the experiments average density was estimated to be 570 ind m^{-2}. The results of this experiment were already mentioned in a work about the bioturbation effect of *N. diversicolor* under the effect of copper spiked sediments (Fernandes et al., 2006).

5.2.3 Luminophores analysis

Frozen cores were sliced in 0.5 cm layers for the top first cm and in 1 cm layers for the layers bellow. Each sample was dried (90ºC, 48h) and homogenised (Mahaut and Graf, 1987). Within each layer, sub samples of 0.25g were taken for counting luminophores in an epifluorescence microscope (Olympus BX60). From this, the number of luminophores per layer (n) and the total number in the profile (N we obtained, the fraction (n/N) of luminophores per layer could be determined. The concentration of luminophores was estimated as $\mathrm{C} = \mathrm{n}/(\mathrm{z} * \mathrm{A} * \mathrm{N})$, where z (cm) is the thickness of sampling layer and A the core area.

5.2.4 Db estimations

Bioturbation was estimated in terms of biodiffusion coefficients (Db) that were estimated using the classical one-dimensional biodiffusion model (Boudreau, 1986; Wheatcroft et al., 1990; Meysman et al., 2003), which is governed by

$$\frac{\partial C(x,t)}{\partial t} = D_b \frac{\partial^2 C(x,t)}{\partial x^2}. \qquad [1]$$

Initially, it is assumed that the luminophores are located in a very narrow layer at the water-sediment interface, as idealized by following initial conditions

$$C(x,0)=\delta(\mathrm{x}). \qquad [2]$$

where $\delta(\mathrm{x})$ represents the Dirac delta function. Over the infinite interval, the initial-value problem [1]-[2] has the solution (Crank, 1975)

$$C(x,t) = \frac{N}{A\sqrt{\pi D_b t}} \exp\left(-\frac{x^2}{4D_b t}\right) \qquad [3]$$

where N is the number of luminophores originally spread at the sediment surface , and A represents the surface area. Because concentrations are expressed relative to the total number of luminophores found in the profile, a value of $N = 1$ should be substituted.

Values for Db were obtained by matching expression [3] to the observed data profiles. For this purpose, two separate procedures were employed. In a first approach (M1), the untransformed data was used. Accordingly, the biodiffusion coefficient D_b was estimated using the least-squares method, i.e. by minimizing the cost function

$$R_1 = \sum_{i=1}^{N} (C_i^{\text{model}} - C_i^{\text{data}})^2 \qquad [4]$$

In a second procedure (M2), the data were log-transformed, and the alternative cost function was minimized

$$R_2 = \sum_{i=1}^{N} (\ln C_i^{\text{model}} - \ln C_i^{\text{data}})^2 \qquad [5]$$

to estimate a second value for the biodiffusion coefficient D_b.

Effectively, both procedures use a different weighting of the data when calculating their "optimal" Db values. The M1 procedure emphasizes the large luminophores concentrations near to the surface, and thus fits the concentration data in the top layers best. In contrast, the M2 procedure emphasizes the difference between model and data at low concentrations values, and thus will provide a better fit to the concentration data in the deeper layers.

Theory predicts that if the actual bioturbation mechanism is truly diffusive, i.e. if biological activity generates effectively isotropic, small-scale particle displacements (Meysman et al., 2003), then both procedures should generate the Db same value. However, if the underlying macrofauna activity is not generating diffusive particle displacements, then Db values generated by the M1 and M2 procedures can show large deviations (see below). In particular, when non-local transport mixes particles faster down than expected, one can expect large discrepancies between the Db estimates. Accordingly, we propose the following non-locality index (NLI) based on the D_b values obtained using M1 and M2 procedures:

$$\text{NLI} = \frac{\left| D_b[M_2] - D_b[M_1] \right|}{D_b[M_1]} \qquad [8]$$

As required, this non-locality index NLI takes the zero value when Db estimates from M1 and M2 have the same value.

The temporal scales of measurements varies from hours (Sandnes et al., 2000a; Berg et al., 2001; Solan et al., 2004) to months (Marcus and Schmitd-Gengenbach, 1986; Gerino et al., 1994; Gilbert et al., 2003) and this lack of consistency might produce decrease in estimation accuracy. To evaluate the effect of sampling time on the estimation of Db, the distribution of luminophores was monitored over a period of up to 28 days.

5.2.5 Statistical analysis

Data was analyzed through one -way and 2-way ANOVA, and Tukey HSD post-hoc comparisons test using the STATISTICA® 6.1 software. Some of the data profiles could not be transformed to match the homogeneity assumption (Levene's test). According to Underwood (1997) the validity and power of the ANOVA test and the probabilities associated with the F-ratio distribution are not much affected by the violations of this assumption and therefore we decided to proceed with ANOVA for all the data, though extreme care was taken on the interpretation of low levels of significance.

5.3 Results and discussion

5.3.1 Control interpretation

The comparison of the luminophores concentration, on Exp. 3, between 570 and the respective control (Fig. 5.1a), after 21 days of incubation, shows similar values, with no statistical significance, although the control treatment shows lower values bellow 3 cm depth. The percentage of standard deviations over the respective average (Fig. 5.1b) indicates that the variability of results is higher in the controls. This counteracts the hypothesis that higher standard deviations are to be expected when non-local transport due to animal activity can take place.

The results found in the controls greatly compromise the interpretation of data, since the transport of particles to lower layers attributed to *N. diversicolor* happens in the controls where these organisms are not present.

Considering the effect of coring artefacts and subtracting the control values, concentrations of luminophores in lower layers due to the activity of *N. diversicolor* become negative in the upper 2cm (Fig. 5.1c). Bellow 2 cm, values become in average 36% lower than the values without control subtraction.

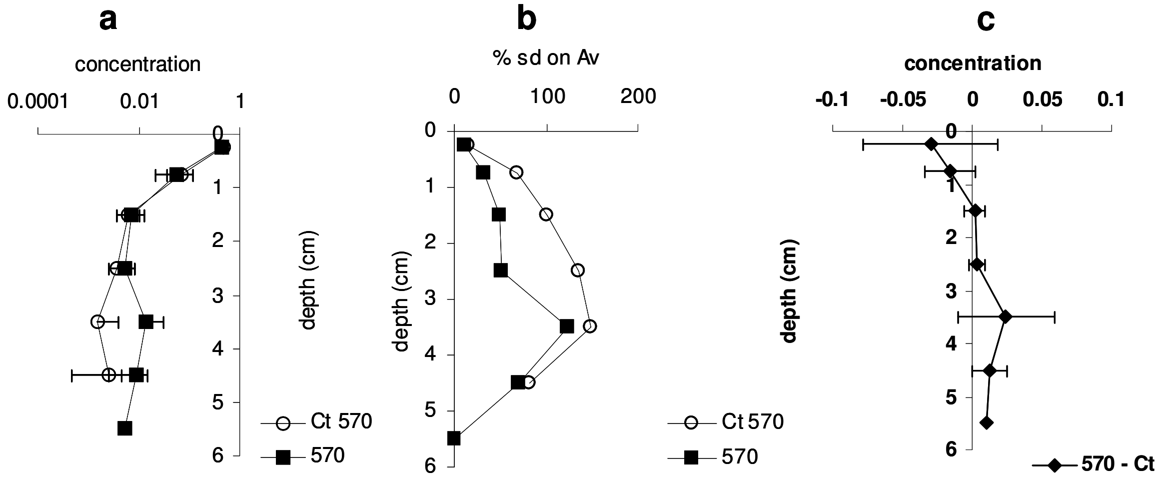

Figure 5.1 - Comparison between the 570 density of *N. diversicolor* (570) and respective control (Ct 570) for the luminophores concentration profiles found after 21 incubation days, in terms of a) luminophores concentration, b) the percentage of standard deviation over the respective average, and c) 570 concentrations with respective control subtraction.

It is possible that the transport observed in the controls is a result of core artefact during core sampling rather the activity of *N. diversicolor*. During penetration, the core produces a compression of the sediment at the top most portions and sediment is pushed aside rather than entering the tube and in addition there is also the effect of internal friction in the areas near the walls (Crusius and Anderson, 1991; Wright, Jr., 1993). This can result into a shorter core with low water content (Blonqvist, 1991). Core shortening is the difference between the depth of sediment recovered and the depth of core penetration and core compression is just a mechanism that could account for core shortening (Cumming et al., 1993).

It is known that diffusion of particles in control sediments is observed until 1.25 cm depth (Sandnes et al., 2000) (defaunated with a solution of 30% NaCl) and until 3 - 4 cm (Marcus and Schmitd-Gengenbach, 1986; Webb and Montagna, 1993) in defaunated sediments. Rusch and Huettel (2000) found a penetration of microbeads (1 – 30 μm) up to 5 cm depth in fine sand sediments containing no macrofauna. It is not clear if the tracers distribution in the depth is due to hydrodynamic disturbance at the surface, due to mechanical diffusion or due to micro and meiobenthos activity. Defaunation was avoided by Berg et al. (2001) who considered that defaunation methods would alter the sediment structure.

The same similarity between Db in controls and treatments was found by Mulsow et al. (2002) in an experiment with *Heteromastus filiformis*. In both cases (Berg et al., 2001; Mulsow et al., 2002), there was no coring artefact, since the tracers were added to the cores after coring collection, and the fauna present in the sediments was mostly meiofauna comprising foraminifers (63%), nematodes (27%), copepods (5%) and annelids (5%). Also Ouelette et al. (2004) report penetration of luminophores down to 2 cm on control sediments sieved from macrofauna (1mm mesh) that were not subjected to coring. They explain these results by the density differences between luminophores and sediments, inducing a little particle displacement towards deeper sediments, while reducing the importance of meiofauna in this deep tracers burial by concluding that the penetration of tracers caused by meiofauna would increased in time, which was not the case for the controls between day 5 and 30.

Furthermore, the core sampling in the present work was performed in zones with high concentration of luminophores in order to assure that vertical transport rate would not be influenced by the lack of tracers in the top of the sediment (Gerino et al., 1994). This might have introduced another sampling artefact in the Db estimations, since zones of lower luminophores concentration at the surface, might result in higher luminophores burrowing. The sampling method used in the present work, might have forced the collection of cores in zones with low tracer transport. In addition, sampling on areas with high concentrations of luminophores increases the probability for luminophores smearing during coring.

Another artefact can be generated during core slicing, where possible leakage between the pistons and the tube or accidental mixing caused by a small stones or shells can allow the pore water to drain down through the sediment making the tracers appear to extend deeper into the sediment than their true position and producing an overestimation of Db (Berg et al., 2001). The possibility of core slicing artefacts was reduced in the present work since sediment was frozen and muddy sediments have a reduced drainage (Berg et al., 2001).

The penetration of particles up to 4 cm depth gives a strong indication of a sampling artefact, however, some considerations bring some doubts of whether the controls are really true controls, and if, by taking them into account we are just introducing an interpretation artefact. In the present work, the sediment in the controls was not defaunated for micro or meiofauna (just for macrofauna) and the transport observed in the controls, can be also a result of meiofauna bioturbation. Meiofauna are known to increase solute transport coefficients in surface sediments by ~ 1.3 – 2 times molecular diffusion (at the same porosity), but tends to be less than macrofauna who typically produce elevations of 2-10 times in molecular diffusion (Aller and Aller, 1992). It is also possible that the meiofauna population in the controls is enhanced, as a consequence of predation by *N. diversicolor*. Considering this assumption, the results indicate that *N. diversicolor* has a larger influence in the sediment bellow 2 cm depth.

On a parallel experiment, about the effect of *N. diversicolor* on sediment erodability (Fernandes et al, 2009), it was observed that this species alters the characteristics of the sediment by increasing sediment shear strength and thus, changing the conditions for advection diffusion. Similar observations by Meadows and Tait (1989) indicate that *N. diversicolor* increases sediment permeability and shear strength. In a work using Br^- and Cl^- tracers to measure solute transport, Aller and Aller (1992) found lower diffusion coefficients in defaunated controls than on treatments containing meiofauna (nematodes, ostracodes, copepods, foraminiferans) and they concluded that 20 – 40 % of the increased transport was due to increased porosity. Likewise, the sediments are not in the same conditions and comparisons with controls might lead into an interpretation artefact.

Another problem present in this work was the fact that the luminophores counting was done manually. Meaning that due to the lack of a large number of particles in the controls, the smaller particles would have the same weight as big particles observed in the 570 treatment, where smaller particles like luminophores "dust" would be neglected. In this way, the numbers of particles counted in the controls are overestimated.

Considering that controls without macrofauna do not reflect the same physical properties of the sediment (Aller and Aller, 1992; Berg et al., 2001) we shall neglect them in our interpretations, even though they give an indication of possible sampling artefact, that limits our conclusions into a certain extent.

5.3.2 Luminophore concentrations

The comparisons of the luminophore profiles measured on different densities and on different sampling days, showed similar results (Fig. 5.2). There was no statistical significance among densities, except for day 20/21, bellow 1 cm layer, between 450 and 320 ($p< 0.01$) according to Levene's post-hoc test.

The concentration of luminophores showed no variation over time, in opposition to the observation of Francois et al. (2002), who found deeper concentrations of luminophores after 30 days (20 cm deep) than after 15 days (15 cm deep) in sediments bioturbated by *N. diversicolor*. Similar results were observed by Ouellette et al. (2004) for *Neanthes virens*.

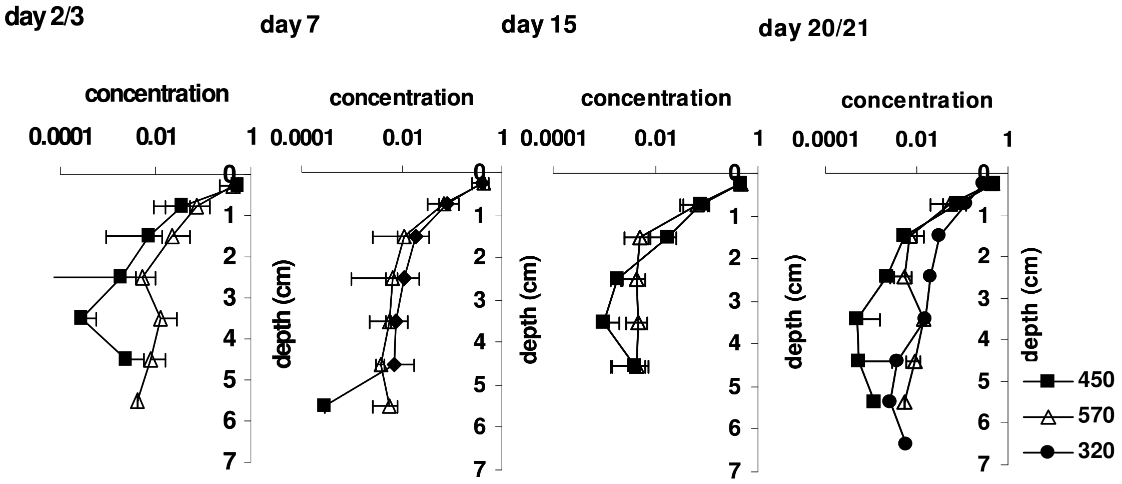

Figure 5.2 – Profiles of luminophores concentration in depth estimated over time after bioturbation of *N. diversicolor* for densities of 320, 450 and 570 ind m^{-2}.

5.3.3 Db estimations

Db estimation using two different procedures for all the densities and over the sampling times are expressed on Table 5.1 and on Figure 5.3.

Table 5.1 – Biodiffusion coefficient values (cm^2 y^{-1}) estimated with the two different estimation methods (M1 and M2) and the NLI (non-locality index) for the three densities tested, 320, 450 and 570 ind m^{-2}.

Density	Sampling days	M1	M2	NLI
320	20	1.93 ± 0.74	73 ± 28	48 ± 40
450	3	3.90 ± 0.08	94 ± 63	23 + 16
	7	3.81 ± 3.51	101 ± 63	31 ± 8
	14	0.98 ± 0.21	33 ± 9	33 ± 7
	21	0.60 ± 0.03	21 ± 4	34 ± 6
	28	0.44 ± 0.02	19 ± 2	43 ± 2
570	2	4.52 ± 2.30	493 ± 141	160 ± 152
	7	1.79 ± 0.05	142 ± 52	79 ± 30
	15	0.84 ± 0.08	48 ± 19	57 ± 27
	21	0.59 ± 0.02	56 ± 18	94 ± 28
570 Ct	21	0.64 ± 0.10	21 ± 10	32 ± 10

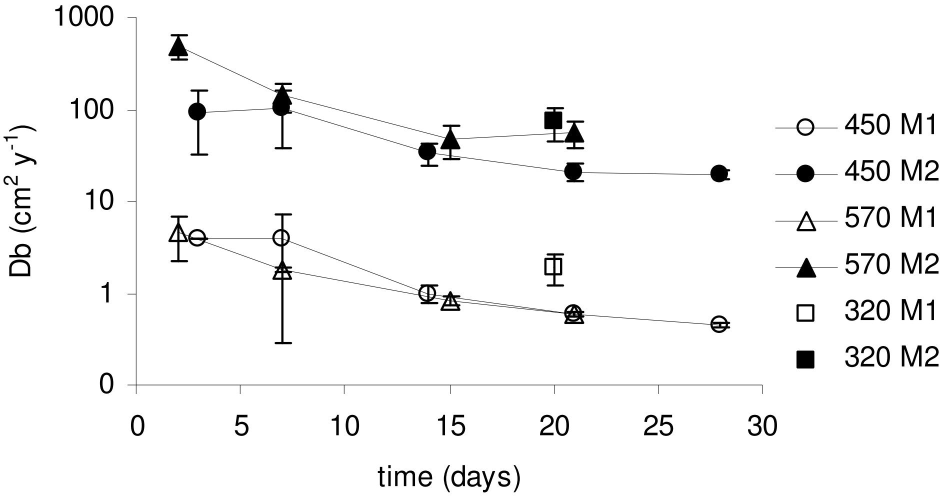

Figure 5.3 - Biodiffusion coefficients over the bioturbation period for a population of *N. diversicolor* of 320, 450 and 570 ind m^{-2} (± standard deviation) estimated using M1 and M2 methods.

Db estimations based on the two procedures showed quite different scales. Db ranged from 0.4 to 7.9 using M1 procedure and from 16.1 to 504.0 using M2 procedure. Db values over time, were slightly higher in 570 density, than on 450, but there was no statistical significance between the two treatments.

A comparison of Db estimates after 20 days (for 320 treatment) and 21 days (for 450 and 570) revealed no relationship between Db and density (Fig. 5.4). The treatments with comparable areas (270 cm^2, 450 and 570) showed higher Db for 570, using M2 procedure, but similar values were observed using M1 procedure (Fig. 5.4). A small area limits the activity of the organisms and increases the probability of one individual to pass over the same spot, and consequently promotes the interpretation of higher reworking activity and higher Db. On most of the works with a smaller surface area the vertical area is also deep, and it is also possible that penetration depths are enhanced by an artefact caused by horizontal space limitation. On the other hand, the possibility of encountering organisms or obstacles, that is higher in smaller cores, might reduce the organisms activity, as observed by Hansen et al. (1999) on a study with *Capitella* sp. I, using ^{51}Cr as tracer, where they suggest that worm density may act as a feed-back mechanisms on particle mixing process and that feeding rate might be inversely related with density.

The Db values estimated in the present work using procedure M1 were within the range of values estimated for macrofauna bioturbation. Francois et al. (2002) measured slightly higher Db that ranged from 2.37 to 4 cm^2 y^{-1}. However, these experiments were performed in mesocosms (8 cm diameter and 30 cm depth) that might have increased organism activity in depth due to its special limitation. In addition, the higher luminophores concentration in deeper sediment might have had an increasing effect on Db.

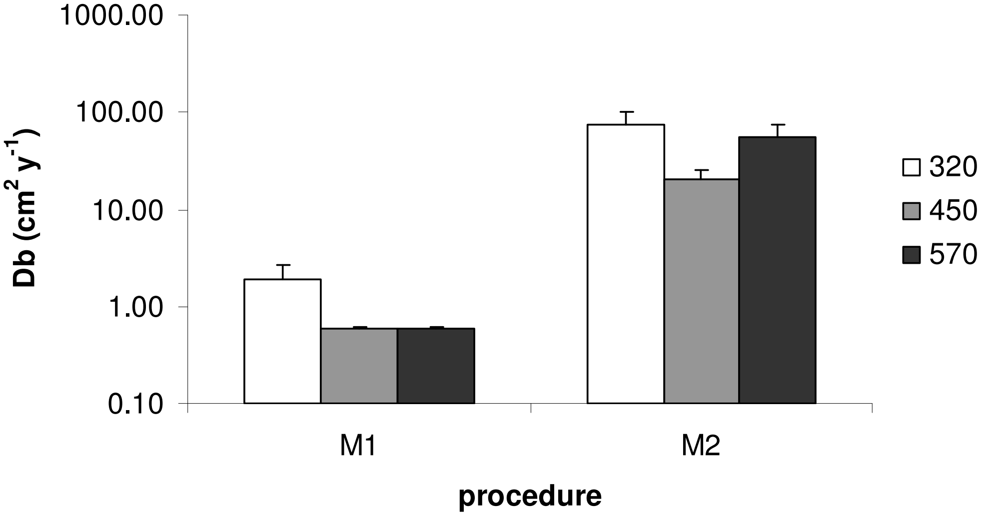

Figure 5.4 – Biodiffusion coefficients measured on day 20 (Exp. 1) and day 21 (Exp. 2 and Exp. 3) for the different densities of *N. diversicolor* (320, 450 and 570 ind m^{-2}) (± standard deviation).

Other models give Db estimations under the range of the values estimated in the present work, using M1. Caradec et al. (2004) found Db ranging from 0.53 to 2.99 cm^2 y^{-1} in four macrofauna species. These values are within the range of values found by Gerino et al. (1994) in sediments from the Rhone river, on *in situ* sediment containing natural communities, where Db was 4.3 ± 2.8 cm^2 y^{-1}. Slightly higher Db were estimated by Solan et al., (2004) using luminophores and profile-imaging system (f-SPI) to determine the bioturbation activity of the brachyuran crab *Hyas araneus* (126 cm^2 y^{-1}, after) and by Ouellette et al., (2004) on a study about sediment reworking by *Neanthes virnes* (13.2 cm^2 y^{-1}, measured after 30 days under temperatures ranging from 16 to 18^{0}C).

On both M1 and M2 procedures Db decreases with increasing sampling time. The values found after 14 days were statistically different (p<0.01) from the ones found on the first 7 days.

The decrease of Db with time, can be related to 1) intense activity and colonisation behaviour during the first days, when organisms are introduced in inhabited sediments, and once the gallery network is established their building activities decrease, or 2) a model artefact due to defectiveness of the biodiffusion model.

Although it is not possible to test the colonisation behaviour of the organisms, it is possible to test the defectiveness of the models used. Considering that the same concentrations of luminophores found in the 570 Ct (after 21 days of incubation) were obtained on different sampling days, one would expect that Db estimation would be the same if there would be no artefact from the models. On the other hand, if we assume that the concentration profiles obtained for 570 treatment over the 21 sampling days (for 2, 7, 15 and 21 days) were taken at the same day (3 or 21), we would expect the same decreasing of Db in time (as in figure 5.3) if there would be a higher bioturbation intensity on the first days, or constant values over time, if we are in the presence of a model artefact.

Results form these exercises are expressed on Fig. 5.5, and show that the estimations of Db from the models are affected by the sampling time (Fig. 5.5a) and that the higher values of Db on the first sampling days (Fig. 5.3) are not a result of higher activity during colonisation, but a consequence of a model defectiveness, produced by time and present on both M1 and M2 estimations. It also shows that estimations for a smaller time interval (3 days) produce higher Db values than estimation for a longer period (21 days), using both M1 and M2 procedures.

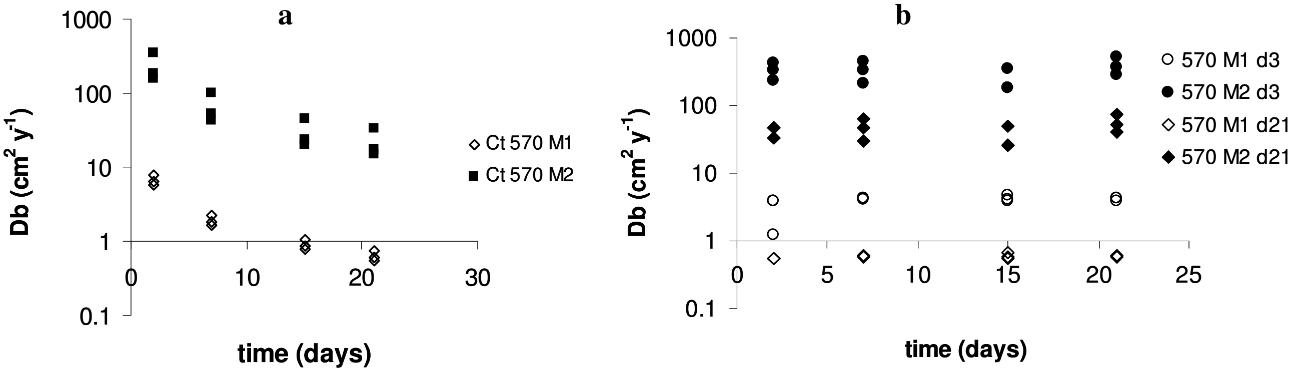

Figure 5.5 - a) Calculations of Db for the 570 Ct treatment using the same concentrations but different sampling time. b) Calculations of Db for 570 treatment using the profile concentrations measured over the sampling period, but assuming these profiles were taken at a fixed time of 3 days (3d) and 21 days (21d). Estimation of Db by M1 and M2 procedure.

Although colonisation behaviour is expected on inhabited sediments, the findings on Fig. 5.5, indicate that if an intense activity exists, then it is not measurable, or it takes place on the first hours and reaches an equilibrium before 3 days of incubation. In order to establish the time at which Db would stabilise and time would have a minimum effect as an artefact, some calculations of the reduction of Db estimations as a result of the model defectiveness were performed using M1 and M2 procedures. Based on data from 450 and 570 treatments, an exponential function describing the daily reduction of Db in time was used to estimate the time at which Db would stabilise according to different reduction thresholds (Table 5.2). From these calculations, a time step of 64 days (using M1 procedure) and 114 days (using M2 procedure) would be needed in order to obtain satisfactory estimates that would not be influenced by the defectiveness of the model.

Table 5.2 – Stabilisation time (days) for Db estimations (with procedure M1 and M2) according to different thresholds.

Reduction threshold	**time (days)**	
	M1	**M2**
5		12
1		32
0.5		40
0.1	14	59
0.05	22	68
0.01	39	87
0.001	64	114

Estimations of Db using M1 or M2 procedures produced results on different range scales. The comparison of the predicted values using the two distinct procedures and the observed data for all luminophore profiles (for each replicate) at day 21 (day 20 for 320 treatment) are expressed on Figure 5.6.

For all profiles, the predictions by M2 procedure are closer to the observed concentrations of luminophores than the predictions by M1 procedure. This should be expected, as M1 procedure generates a diffusive particle displacement, neglecting biological diffusion or non-local transport and emphasizing the high concentrations near the surface, producing an exponential distribution that become zero for infinite depth. In opposition to an exponential diffusion, the observed data is better explained by M2 procedure which provides a better fit to the concentration data in the deeper layers.

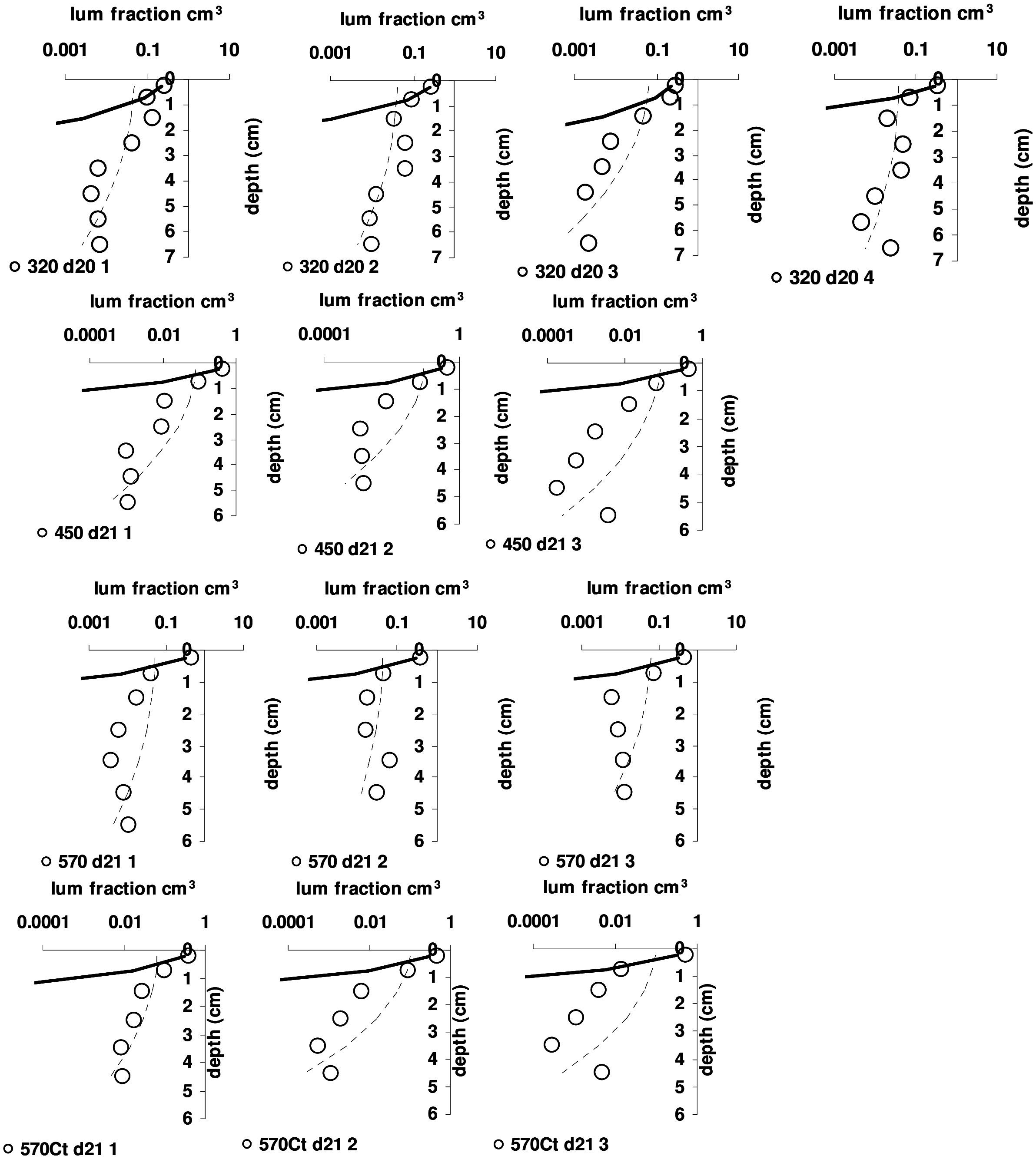

Figure 5.6 – Comparison between the observed profiles of luminophores (o) and predictions from method M1 (bold line) and M2 (dashed line) for each replicate taken on day 21 for 450 and 570 ind m^{-2} and 570Ct and for day 20 to 320 ind m^{-2}.

Some authors report good fits between observed and predicted data (Sandnes et al., 2000), while others report bad fits. The ^{51}Cr distribution on sediments bioturbated by Capitella sp. I, (Hansen et al., 1999) showed poor fits to the simple biodiffusion model proposed by Crank (1975) and they pointed out that the assumption of homogeneous mixing becomes particularly critical when working with deposit-feeders who may ingest and defecate sediment non-randomly with depth.

On a work focusing seabed mixing, Dellapenna et al. (2003) classified the profiles obtained using ^{137}Cs tracers, in 3 types: 1) uniform, 2) stepped-stirs profiles; that result primarily from physical and sediment mixing (Dellapenna et al., 1998) and 3)consistent slope profiles; that reflect either sediment accretion or bioturbation (Dellapenna et al., 2003). In the present work, the profiles found in 570Ct is better described by type 1, while 570 is clearly a consistent slope profile.

5.3.4 The NLI index

The NLI index is an indication of the deviation from the observed data and the theoretical diffusive transport and is expressed on Figure 5.7 for all treatments after 21 or 20 days. The analysis of NLI showed that values are much bigger in the 570 than in the respective control, meaning that the profiles measured in the control have a distribution closer to the theoretical diffusivity, and the profiles measured on 570 have the influence of a non-local transport. The comparison between LNI of 570Ct and treatments 320 and 450 has little sense, since the physical, chemical and biological properties of the sediment are probably not the same.

NLI index also showed to have no relation to density or time. Although there was no correlation between NLI and Db estimated from M1 procedure, a positive significant correlation was found between NLI and Db estimations using M2 procedure ($F_{1.31}$=5.21, $p<0.05$, R^2=0.14, n=33).

The higher values of NLI found on 320 and 570 (Fig. 5.7) are probably related with the concentration peaks found at 2 – 4 cm layers for 320 treatment (replicates 2 and 4 , Fig. 5) and 3 – 5 cm layers for 570 treatments (all replicates, Fig. 5), where deviations from the theoretical diffusion is more evident.

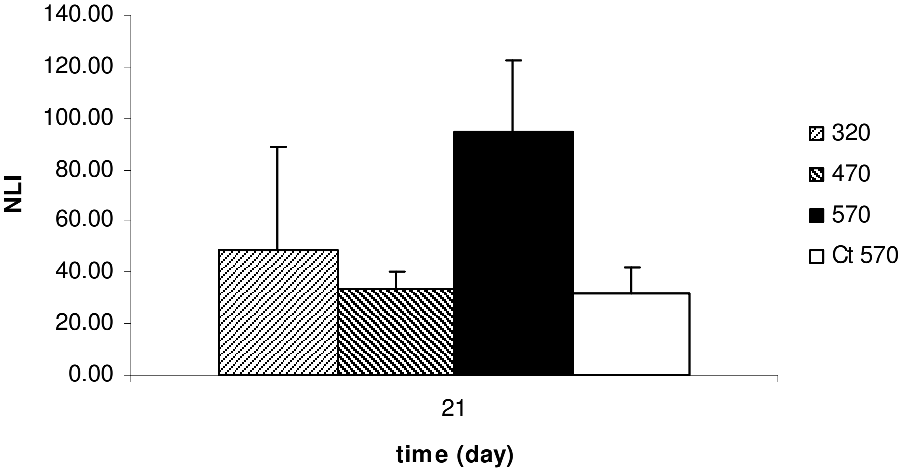

Figure 5.7 - Non-locality index measured after 21 days (20 days for 320 density) in the three densities tested (and control for 570 density) and 450 and 320 density.

5.4 Conclusions

This study highlights some concerns related to the estimation of Db using luminophores. It is concluded from this study that the estimation of Db using M2 provides a better approximation to the bioturbation occurring in the surface sediment layers. The Db estimations are dependent on the sampling time. According to the analysis performed in this study Db values would only reach a constant value after 64 and 114 days of incubation using M1 an M2 estimation models, respectively. The area of the mesocosm where incubations are conducted is also another issue that may influence the estimations of Db. Due to problems associated to core sampling, such as core shortening, compression and internal friction we recommend the use of sharpened cores edges, the use of core with larger diameters. Core freezing before sampling and the discard of the sediment in contact with the core is recommended to avoid the luminophores transport due to coring. The question of whether controls can be true controls, regarding the predation effect of macrofauna on meiofauna is raised.

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PART IV

INTERACTIONS BETWEEN CONTAMINATED SEDIMENTS AND BIOLOGICAL ACTIVITY

Chapter 6

The effect of *Nereis diversicolor* on shear strength and erodability of cohesive sediments: the influence of copper contamination

NEREIS DIVERSICOLOR AND COPPER CONTAMINATION EFFECT ON THE EROSION OF COHESIVE SEDIMENTS: A FLUME EXPERIMENT

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Estuarine, Coastal and Shelf Science, in press

Abstract

The effect of bioturbation on the erodability of natural and manipulated copper spiked sediments (3 μmol Cu g^{-1} dw) was investigated using sediments collected in the Tagus estuary and N*ereis diversicolor* (900 ind m^{-2}). The input of particulate matter and Cu into the water column as a result of erosion was quantified in an annular flume at 7 shear velocities (1 to 13 cm s^{-1}). The biogeochemical characteristics of the sediment were analysed in depth down to 8 cm. Cu contamination elicited lower levels of eroded matter and lower shear strength profiles. Eroded matter and sediment shear strength values were higher (up to 1.7 Kg m^{-2}) in the presence of *N. diversicolor*, whose effect was less pronounced under contamination. Sediment erodability was not only related to hydrodynamics but was highly affected by the biogeochemical characteristics and contamination of the sediments.

Key-words: bioturbation, shear strength, erosion, copper, flumes, *Nereis diversicolor*.

6.1 Introduction

Sediment stability, i.e., resistance against erosion, is an important component of sediment dynamics, not only in terms of the erosion and accretion of margins, but also because resuspended matter is an important source of food and also, in the case of sediment contamination, of particulate contaminants to the system. At the sediment surface, the microphytobenthos community dominated by diatoms (Underwood and Smith, 1998, de Brouwer et al., 2002), has a stabilising effect that derives from the production of extracellular polymeric substances, EPS (Paterson, 1989; Miller et al., 1996; Sutherland et al., 1998; de Brouwer et al. 2003, 2005), resulting in increased grain to grain adhesion (Johnson and Azetsu-Scott, 1995) and raised erosion thresholds (Tolhurst et al., 1999; Watts et al., 2003). However, this biofilm is confined to a surface layer of a few millimeters, below which the biogeochemical properties of the sediment result in part from the activities of other functional groups, such as the macrofauna. Different macrofauna species are known to have either stabilising or destabilising effects on sediments with different effects on erosion thresholds (Widdows et al., 1998a; Austen et al., 1999). Sediment mixing and irrigation by macrofauna are known to influence the biogeochemical processes in the sediment (François et al., 1997; Hansen and Kristensen, 1998). Some studies have been focused on the bioturbation effects of macrofauna on the distribution of contaminants in the sediments, concerning both their transport in depth and to the water column (Petersen et al., 1998; Rasmussen et al., 1998). Mazik and Elliot (2000) showed that pollution reduced the potential for sediment transport as a result of the toxic effect on the bioturbation activity and of the reduced grazing pressure.

It is then essential to understand the effect of pollution on the biological activities of specific functional groups that change the sediment-water interface properties. This knowledge leads to a better description of phenomena of estuarine sediment erosion and transport. In this context, the polychaete *N. diversicolor* (O.F. Müller, 1776), is known to be a suitable bioindicator (Bryan and Hummerstone, 1971; Ozoh, 1994). It is an opportunistic species, very abundant in estuarine and coastal systems, that uses different feeding modes such as suspension and deposit feeding, predation and scavenging. It builds and lives in mucus-lined galleries with a considerable structural complexity (Meadows and Meadows, 1991). During burrowing, mucus secretions are released from glands covering the entire surface of the epidermis. These secretions are pushed against the walls of the burrow, consolidating it in this way (Meadows and Tait, 1989).

In a previous study with natural sediments (Fernandes et al., 2006a), *N. diversicolor* contributed to sediment stability, increasing sediment shear strength and critical erosion velocity. However, when exposed to high shear velocities (up to 13 cm s^{-1}), strong erosion was observed and these effects increased with higher densities of these organisms. The present study is focused on the effect of *N. diversicolor* on contaminated and non-contaminated sediment stability.

The main objectives are directed to the quantification of the effects of: 1) *N. diversicolor* bioturbation activity on the properties of natural and copper spiked sediments (biogeochemical and shear strength) and the resulting input of materials (SPM, Chl *a* and Cu) into the water column, 2) the effect of Cu on the bioturbation activity of *N. diversicolor* and 3) the overall effect of Cu and *N. diversicolor* on sediment stability.

6.2 Materials and methods

6.2.1. Sediment and organisms

Cohesive sediments were collected in Ponta da Erva (Tagus estuary, 38º50'N, 8º58'W, water salinity of about 22, Fig. 6.1) during low tide. Mud from mid-tidal level was scooped from the top first 2 cm into buckets, brought to the laboratory and kept at 4 ºC to reduce biological activity and then acclimated for 1 week maximum. Recent grain size analysis at Ponta da Erva (using a Malvern®Mastersizer 2000, n=3) showed that sediment is mostly composed by silts and clays (75.4 ± 5.4 % and 22.9 ± 6.2 respectively) and median grain size ($10d_{50}$) is 9.8 ± 2.0 µm.

Besides the fine fraction only a small percentage of very fine sand was present, 1.7 ± 0.9 % (Soares & Sobral, unpublished data). Sediment was characterised as 9 ± 1 % of organic matter, 68 ± 1 % water content and Cu content of 0.49 ± 0.05 µmol Cu g^{-1} dry weight (mean ± standard deviation, n=12).

N. diversicolor were collected in Barreiro (Tagus estuary, 38º39'N, 9º05'W, water salinity of about 33, Fig. 6.1), immediately brought to the laboratory and allowed to acclimate to the experimental conditions for 5 days before being added to the sediment. Body size ranged from 10 to 15 cm (first setiger L_3=2.24 ± 0.36 µm, n=22) and biomass was 0.075 ± 0.003 g dw ind^{-1} (n=25).

Seawater used at IMAR-FCTUNL facilities is collected on the coast, 30 Km west of Lisbon, and supplied periodically to our major tank by the navy. The seawater is kept in the laboratory in a continuous recirculation aerated system and under temperature controlled conditions.

6.2.2 Experimental set-up

All macrofauna (*N. diversicolor*, other smaller polychaetes and small crabs) and larger shell fragments that could be visually detected upon close inspection were manually removed from the sediment. This defaunation technique was preferred over wet sieving to avoid dilution of the sediment. Meiofauna (nematodes, harpacticoid copepods and ciliates) was therefore not excluded. In order to decompose the effect of bioturbation and copper a 2-block experiment was designed, using a control for the presence of *N. diversicolor* and another for copper contamination. Two batches of homogenised sediment were prepared. One was spiked with a solution of copper chloride ($CuCl_2$) in order to obtain a final concentration of 3 µmol

Cu g^{-1} dw, the other was used as control. From each batch, two groups of sediments were prepared in triplicate in flume boxes that are an exact quarter section of the flume channel (8 cm height and base of 0.053 m^2), to be later inserted in the flume. One group contained added *N. diversicolor* (~ 900 ind m^{-2}, 48 ind per flume box) and the other, not added, was used as the control. The four sediments obtained were designated as B and CB (blank sediment control and copper spiked sediment control, respectively), and N and CN (non-contaminated sediment and copper spiked sediment, respectively, both with added *N. diversicolor*). An extra box of each of the four types of sediments was prepared in the same conditions and used for sediment properties characterisation before the erosion runs. All the prepared sediments were stabilised in a flow-through system (3 L h^{-1}), under controlled temperature (17 ± 1 ºC), salinity (36) and light (54 ± 23 μ E m^{-2} s^{-1} with a 12 h photoperiod) for 21 days.

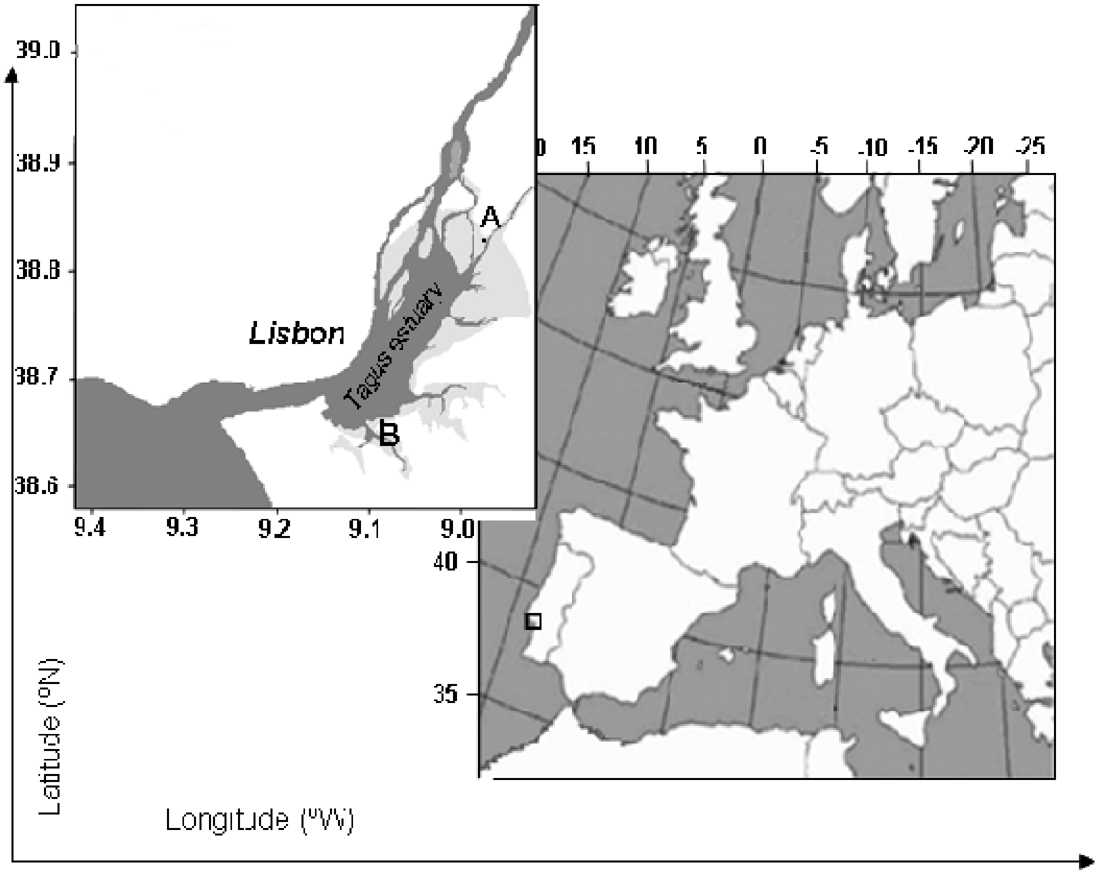

Figure 6.1- Map of Tagus estuary (Portugal). A. Sediment collection; B. organisms collection.

6.2.3. Sediment properties

Cores were taken for the characterisation of the sediment before the erosion runs using a special corer made of 8 one-centimetre rings, allowing the sediment to be sliced into 1 cm layers. Analysis of sediment properties was performed in 1 cm layers from the surface down to 5 cm with the exception of chlorophyll *a* (Chl *a*) which was sampled on 2 layers (0-1 and 1-3 cm). Dry sediment bulk density (d_s) and organic matter content (OM) were determined in oven-dried (90 ºC for 48 h) and incinerated (450 ºC for 4 h) sediment.

Geotechnical shear strength (*SS,* KPa) was measured with a penetrometer, Geonor® - Fall-cone Apparatus (Hansbo, 1957, described in Watts et al., 2003 and also used by Meadows and Hariri, 1991 and Widdows et al., 1998a) with a 10g inverted cone.

Chl *a* was quantified fluorimetrically (Yentsch and Menzel, 1963) in a Jasco FP-777 spectrofluorimeter. Samples were extracted in 10 ml of 90 % acetone in the dark and in the cold (4 ºC). The extracts were centrifuged at 5000 rpm and the fluorescence read at 440nm excitation and 660nm emission. A pre-determined calibration factor was used to convert the readings in Chl *a* concentrations that were then converted in concentration per gram of dry weight sediment. Copper in the sediment was determined following the methods described in section 6.2.6.

6.2.4. Erosion runs

Sediment erodability was determined using an annular flume similar to the one described in Widdows et al. (1998b, 2007). The flume has an outer diameter of 60 cm, a channel of 40 cm high and 15 cm wide, generating a bottom area of 0.21 m^2 and a capacity of ~60 L (water column of 30 cm). It has seven sampling ports distributed every 5 cm in height. The flume has a motor-driven top rotating plate that can generate free-stream velocities (U) up to 53 cm s^{-1}, corresponding to shear velocities (u_*) up to 13 cm s^{-1}. The flume has been previously calibrated using computational fluid dynamics (CFD) modelling and velocity profile measurements over smooth sediment surface (Fernandes et al., 2007). Shear velocities were determined from the logarithmic velocity profile, using the Karman–Prandtl Law of the Wall equation. The relationship between U and u_* in this flume is described by $u_* = 0.2462\ U + 0.0141$ (R^2=1). This type of bench annular flumes is characterised by a compressed boundary layer profile with shear velocities higher than the ones found in the field for the same current velocities. Because this study approaches the sediment bottom conditions, hydrodynamic conditions are characterised by u_*.

For each sediment type (B, CB, N, and CN) a flume box with the manipulated sediment (in triplicate) was introduced into the flume upstream of the sampling ports. The flume bed was then completed with three other flume section boxes in an inverted position, levelled to the sediment surface and tape sealed to avoid inter-scouring between boxes and edge effects.

Shear velocities were increased every 15 min to 1, 3, 4, 5, 6, 10 and 13 cm s^{-1}. Triplicated water samples (100 ml, collected at 2, 2 and 22 cm for from the bed) for determination of suspended particulate mater (SPM), particulate organic matter (POM), were taken for each velocity. Samples for Chl *a*, particulate and dissolved copper concentrations (pCu and dCu) were taken twice before erosion (at u_* 1 and 3 cm s^{-1}) and twice after erosion at (at u_* 10 and 13 cm s^{-1}). Water samples were immediately filtered through washed, ashed and pre-weighed Whatman GF/C filters. The filters were oven-dried at 90 ºC (48h) for calculation of SPM and then incinerated (450 ºC, 1 h) for the calculation of POM. Chl *a* on filters was extracted as described above. Copper concentrations were determined according to the methods described in section 2.6. After each run, the water was carefully drained, sediment was defaunated and *N. diversicolor* density was determined.

6.2.5. Critical erosion velocity and eroded matter

Critical shear velocity (u_{*crit}) was determined after Widdows et al. (1998b) and defined as the shear velocity required to increase SPM concentration above a threshold of 100 mg L^{-1}. It was estimated from the regression of log SPM against shear velocity.

Eroded matter (g m^{-2}) was determined from SPM concentrations and the sediment bed (0.053 m^2). Erosion rates (g m^{-2} s^{-1}) were determined using the difference in eroded matter between two velocity steps.

As suspended sediments result from erosion of a known area, erosion depth (E_d, cm) was estimated from bulk density using the expression: $E_d = \frac{d_w * V}{d_s * A}$, where d_w is the concentration of sediment in the water column at a given current velocity (SPM, g L^{-1}), V is the volume of water in the flume (L); d_s is the sediment dry bulk density (g dw cm^{-3}) and A the area of the bed (cm^{-2}).

6.2.6. Copper analysis

All the materials used in the processing of samples for copper analysis were previously decontaminated overnight in HNO_3 (1:10). The water samples were immediately filtered onto decontaminated Nuclepore® polycarbonate filters (0.4 µm pore size) for pCu and dried at 60 °C in plastic Petri dishes. Samples were processed as in Grasshof et al. (1999). Samples of filtered water in HDPE bottles were acidified with HNO_3 and kept cool until dCu analysis. Extraction was performed with purified 1,1,2-trichloro-1,2,2-trifluoroethane (freon), an extracting mixture of ammonium pyrrolidine dithiocarbamate (APDC), diethylammonium-N,N-diethyldithiocarbaminat (DDDC) and 2M acetate buffer (acetic acid and ammonia).

The sediment, the organisms and the pCu filters were dried at 60ºC for 48h, after which the sediment and organisms were ground into fine powder in a planetary agate ball mill (Retsch® S100). All the samples were individually kept in plastic Petri dishes, in a dry place, until analysis. Samples of the sediment and pCu filters were processed as in Rantala and Loring (1975) and the organisms as in Sobral and Widdows (1997). Wet digestions were performed in tightly closed Teflon (PTFE) containers in a covered water-bath at 100°C. Sediment and pCu samples were digested in *aquæ regia* and fluorhydric acid (HF) for 90 min and 60 min respectively. The organisms were digested in HNO_3 for 4 h, plus 1 h after the addition of hydrogen peroxide (H_2O_2). Copper contents were analysed by FAAS with a Perkin-Elmer® A Analyst spectrophotometer.

Certified reference materials (estuarine water BCR CRM 505, mussel tissue BCR CRM 278R and marine sediment NRC MESS-3) were used in this analysis. Values for copper in the reference water and mussel tissue were 13.5 and 20.6 % lower than the certified values, and so the input of copper from the sediment to the water and accumulation by *N. diversicolor* may have been underestimated. Values for the sediment were within 0.5 % of the certified values.

6.2.7. Statistical analysis

The effects of *N. diversicolor* and copper on the sediment properties (dry bulk density, organic content, Chl *a* and *SS*) were analysed with a multivariate bifactorial ANOVA; eroded matter and input of copper to the water column under increasing shear velocities were analysed with ANCOVA, for α=0.5 in both cases, using STATISTICA (version 6.0) software. Comparisons of different treatments were evaluated using Tukey HSD post-hoc probability test. Independence between samples was assured through the experimental design and homogeneity of variances was verified through Levene's test.

6.3. Results and Discussion

6.3.1. Effects of bioturbation and copper contamination on sediment properties

6.3.1.1. Sediment shear strength

Fall-cone measurements of *SS* at the sediment surface, before erosion runs (after 21 incubation days), did not reveal eventual effects due to bioturbation or contamination (Table 6.1).

Table 6.1 - Biogeochemical characteristics of the sediment before erosion: Control (B), sediment with *N. diversicolor* (N), copper spiked control (CB) and copper spiked sediment with *N. diversicolor* (CN). mean ± SD, (*n,* number of samples).

	Sediment types			
	B	N	CB	CN
Shear strength (KPa)				
0-1cm (*3*)	0.22 ± 0.04	0.45 ± 0.14	0.22 ± 0.06	0.38 ± 0.05
1-5cm (*12*)	0.67 ± 0.18	1.19 ± 0.30	0.33 ± 0.05	0.69 ± 0.30
Bulk density (Kgm^{-3})				
0-1 cm (*3*)	204 ± 48	224 ± 64	175 ± 72	178 ± 75
1-5 cm (*12*)	420 ± 124	436 ± 106	364 ± 175	337 ± 111
Organic matter content (%)				
0-1 cm (*3*)	9.0 ± 0.6	8.3 ± 0.5	9.5 ± 0.7	9.6 ± 0.6
1-5 cm (*12*)	8.8 ± 0.6	8.6 ± 0.6	8.6 ± 1.0	8.7 ± 1.3
Chl a (ugg^{-1})				
0-1 cm (*3*)	9.9 ± 1.3	8.0 ± 0.4	14.1 ± 1.4	8.4 ± 0.5
1-3 cm (*6*)	12.0 ± 0.6	5.6 ± 5.5	8.8 ± 1.3	5.8 ± 0.6
Cu (µmol g^{-1})				
0-1 cm (*3*)	0.49 ± 0.05*	0.49 ± 0.05*	2.95 ± 0.11	2.91 ± 0.13
1-5 cm (*12*)	-	-	3.02 ± 0.08	2.92 ± 0.09

*Cu in the sediment from Ponta da Erva, previous studies

However, the comparison of *SS* measured in depth (Fig. 6.2) showed that bioturbation produced significantly higher *SS* in the deeper sediment layers (one-way ANOVA $F_{1.10}$=42.5936, p<0.001) resulting in a 76 % increase in the non contaminated sediments (B and N, Tukey, p<0.001) and 125 % under copper-spiked sediments (CB and CN, Tukey, p<0.001). Similar observations were done by Meadows and Tait (1989) and Meadows et al. (1990), who found an increase in shear strength of about 40 % under non-contaminated conditions. Higher *SS* in depth may be the result of improved adhesion of particles through mucus production, as well as of compaction of the sediment associated to gallery building and sediment remixing activities (Meadows et al., 1990; Fernandes et al., 2006a).

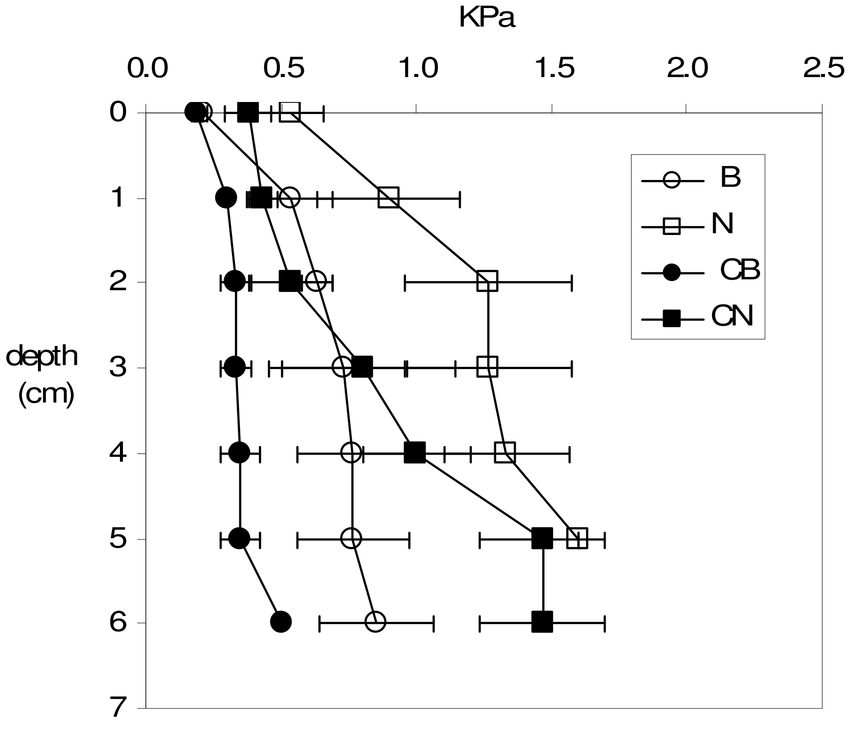

Figure 6.2. - Shear strength (KPa) profiles for each sediment before erosion runs: control (B), sediment with *N. diversicolor* (N), copper spiked control (CB) and copper spiked sediment with *N. diversicolor* (CN). Mean ± SD. n=3. Significant differences among the four sediments (two-way ANOVA $F_{5.88}$=19.0410, p<0.001) and sediment layers (two-way ANOVA $F_{3.90}$=19.9465, p<0.001).

Copper spiked sediments had significant lower *SS* values, than the non-contaminated sediments (Fig. 6.2), 45 % lower in control sediments (B and CB, Tukey, p<0.01) and 40 % in the sediments with *N. diversicolor* (N and CN, Tukey, p<0.001).

In related experiments, Fernandes et al. (2006b) reported that the mixing activity of *N. diversicolor*, quantified as biodiffusion coefficients (Db) in experiments with luminophores, was lower when under Cu contamination on the first days of incubation. Yet, after 14 days of incubation the Db observed in N and CN experiments were similar and this suggests that sediment shear strength profiles observed in Fig. 6.2 are the result of bioturbation activity in the first days when the organisms start to colonize the sediment.

The analysis of bacterial activity in the same batch of sediments used in the present study revealed decreased levels of bacterial abundance and significant low levels of biomass production in comparison to non-contaminated sediments (Almeida et al., 2007). This led to lower bacterial mucus production and to decreased sediment adhesion. The toxic effect of copper on the biota decreases their activity, resulting in lower sediment *SS*.

6.3.1.2. Sediment dry bulk density and organic matter

Surface values of dry bulk densities were ~50 % lower than the depth values in all the sediments (Table 6.1). *N. diversicolor* bioturbation had little effect on d_s but Cu spiked sediments showed significantly lower depth averaged d_s (one-way ANOVA, $F_{1.196}=17.241$, $p<0.001$). These values of d_s are lower than the one found for the Tollesbury estuary (500 – 700 Kg m^{-3}, Watts et al., 2003), the Dutch Wadden Sea (Houwing, 1999) and the Northern Wadden Sea (Austen et al., 1999). The percentage of organic matter ranged from 8 to 10% (average values of 8.8 ± 0.9%, n=84) and was similar in all the sediments (Table 6.1).

As density measurements of cohesive mud are closely related to water content, the lower sediment bulk density in this experiment seems to be a consequence of the continuous immersion of the sediment and also of the contamination procedure. Therefore the comparison between these sediments and the ones described for other field studies in estuaries should be done with caution.

6.3.1.3. Chlorophyll *a* in the sediment

The presence of *N. diversicolor* resulted in lower Chl *a* sediment content (Table 6.1) regardless of copper contamination (two-way ANOVA, $F_{1.22}=19.5396$, $p<0.001$). This reduction was of 20 % in the control and 40 % in the copper contaminated sediments. This effect on Chl *a* has been previously observed (Smith et al., 1996; Fernandes et al., 2006a) and is related to grazing by *N. diversicolor* on the biofilm, since non-selective sediment ingestion is the predominant feeding mode of *N. diversicolor*. A recent work from Engelsen and Pihl

(2008) showed that *N. diversicolor* was able to prevent initial algal growth, affect growth capacity and also partly reduce full-grown algal mats.

Many studies refer the toxic effect of copper on the biota (Morelli and Scarano, 2004, Gillis et al., 2006) but surprisingly the Chl *a* content in the contaminated sediment without *N. diversicolor* was 42 % higher than in the control. To a certain extent high copper concentrations may not be inhibitory to the biofilm community development as tolerant species may replace sensitive ones. Copper concentrations that affect the growth of microalgae are in fact largely variable and depend on the cellular defences of the species assayed and on environmental conditions, especially when sediments sequester toxic metals and reduce their bioavailability. Real et al. (2003) found significantly increased Chl *a* contents in periphyton under copper exposure to 44 μg L^{-1} regardless of the presence of grazers. Cid et al. (1995) have reported that growth of microalgae can be more affected by copper than photosynthesis suggesting that the two processes may be uncoupled. Toxicity of copper towards the meiofauna has also been reported by Hagopian-Schlek, et al. (2001) who found LC50 96-h of 4.4 μmol Cu g^{-1} for harpacticoid copepods and by Fleeger et al (2006) who found that nematode abundance decreased by 50% when under copper concentrations of 2.4 to 6 nmol Cu g^{-1}).

6.3.2. Copper in the sediment and accumulation by *N. diversicolor*

Bioturbation by *N. diversicolor* had no significant effects on the vertical distribution of copper in the sediment (Table 6.1). This was not surprising since copper was added directly and homogeneously to the bulk sediment. Copper values at the surface layer were lower than in the deeper layers. Profiles obtained from sediments with *N. diversicolor* were less variable and showed lower copper contents than the controls. The adsorption of copper to the mucus secreted by *N. diversicolor* to cement the gallery walls reduced its bioavailability, while irrigation through galleries increased the oxygenation of sediments (Ferro et al., 2003), and facilitated copper transport to the water column. In addition, bioturbation by *N. diversicolor* might have promoted resuspension of particles before the onset of erosion. Lower Cu might be a result of sediment ingestion and accumulation of copper in the animal tissues.

N. diversicolor had an initial (day 0 of the experiment) copper content of 0.28 ± 0.08 μmol g^{-1} dw. After 21 days under laboratory condition, in the non-contaminated sediments, there was evidence of depuration and a decrease of 23 % (copper content of 0.22 ± 0.03 μmol g^{-1}). Mortality rates were 13 ± 6 % and related to death during core sampling. In the copper contaminated sediments, mortality rates were higher, 23 ± 7 %. Accumulation took place at a rate of 0.01 μmol Cu g^{-1} d^{-1} leading to a significant increase of 78 % in tissue copper content (0.50 ± 0.08 μmol g^{-1}) (one-way ANOVA, $F_{1.12}$=5.718004, p<0.05). Therefore, before erosion runs the differences in copper content between animals in the contaminated and in the non-contaminated sediments were highly significant (one-way ANOVA, $F_{1.16}$=90.5960, p<0.001).

When exposed to contaminated sediments *N. diversicolor* accumulated copper in the tissues up to 0.63 μmol g^{-1} dw, in the range of the contents found for this species in several polluted estuaries (Bryan and Hummerstone, 1971; Rainbow et al., 2004; Saiz-Salinas and Francés-Zubillaga, 1997). The known ability of *N. diversicolor* to cope, to a certain extent, with copper contamination arises from the production of detoxified metal- rich-granules in both the tegument and intestine walls (Rainbow et al., 2004), from the existence of regulatory systems for copper excretion (Bryan and Hummerstone, 1971), and also from active protection provided by their mucus-lined galleries against contaminated particles in the sediment (Saiz-Salinas and Francés-Zubillaga, 1997). Sediment reworking by *N. diversicolor* (François et al., 1997; Fernandes et al. 2006b) promotes further complexation of copper.

6.3.3. Effects of bioturbation and copper contamination on erosion

6.3.3.1. Eroded matter and critical shear velocities

Eroded matter and erosion rates were higher in the sediments with *N. diversicolor* and lower in contaminated sediments (Table 6. 2).

Table 6.2 - Parameters of the fitted equation $E=a.e^{b.u*}$ for eroded matter, E_m, (g m^{-2}) and erosion rates, E_r, (g m^{-2} s^{-1}) as a function of shear velocity, u_* (cm s^{-1}), and critical shear velocities ($u_{*crit,}$ cm s^{-1}, mean ± SD, n=3), in the control sediment (B), sediment with *N. diversicolor* (N), copper spiked control (CB) and copper spiked sediment with *N. diversicolor* (CN). (R^2, n=24).

	E_m			E_r			u_{*crit}
Sediment types	*a*	*b*	R^2	*a*	*b*	R^2	
B	3.3866	0.3785	0.71	0.001	0.5005	0.73	7.7 ± 0.3
N	3.6661	0.4689	0.81	0.0008	0.5714	0.82	7.5 ± 0.1
CB	1.7598	0.4393	0.84	0.0012	0.4607	0.87	6.7 ± 0.3
CN	1.7639	0.4825	0.85	0.0009	0.5072	0.82	7.1 ± 0.5

The relationship between E_m and shear velocity (Fig. 6.3) was different among sediments (ANCOVA, $F_{3.92}$=3.2, p<0.05) and among velocities (ANCOVA, $F_{1.92}$=154.6, p<0.001). Below 6 cm s^{-1} E_m varied from 4 to 23 g m^{-2}, and was higher in the presence of *N. diversicolor* whose activities clearly displaced particles into the near-bottom water (Fig. 6.3). At the maximum velocity tested, bioturbated sediments eroded 1716 ± 54 g m^{-2} in the control (N) and only 1036 ± 137g m^{-2} in the copper-spiked sediments (CN). In the sediments without bioturbation by *N. diversicolor* (B and CB) the presence of copper reduced eroded matter by ~12% (765 ± 183 g m^{-2} in B and 676 ± 30 g m^{-2} in CB).

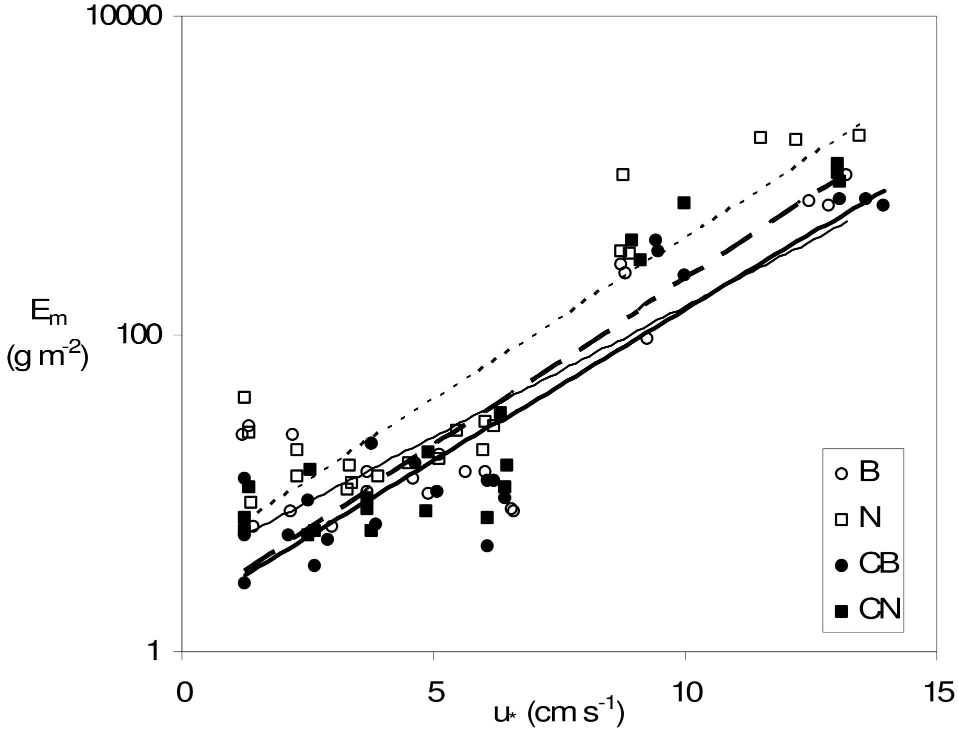

Figure 6.3 - Eroded matter (E_m, g m^{-2}) in a log scale as a function of shear velocities (u_*, cm s^{-1}). Lines represent the exponential relationships (expressed on Table 2) in each sediments: control (B ——), sediment with *N. diversicolor* (N- - - -), copper spiked control (CB – –) and copper spiked sediment with *N. diversicolor* (CN ——). n=3.

Bioturbation by *N. diversicolor* increased eroded matter both in the non-contaminated sediments (124 %) and under copper exposure (53 %). This increasing effect has been previously reported (Fernandes et al., 2006a). *N. diversicolor* disturbs the surface sediment and reduces the biofilm and this results in lower Chl *a* concentrations (Table 6.1). Grazing reduces sediment stability and facilitates erosion, as has been shown by Underwood and Smith (1998) and de Brouwer et al. (2003). A similar effect was observed by Austen et al. (1999), who found that *Hydrobia ulvae* could regulate the biostabilisation process through reduction of the biomass of microalgae on the surface sediment.

Digging burrows and building galleries modify the geotechnical properties of the sediment (Meadows and Meadows, 1991). Once erosion starts, and with increasing shear velocity, the areas around the openings and above the near surface tubes erode more rapidly, as observed in earlier studies (Luckenbach, 1986; Grant and Daborn, 1994). Once the burrows start to become prominent, the generation of eddies within these structures results in a higher transfer of turbulent kinetic energy to the bed, thus, leading to increased shear velocities (Eckman et al., 1981 and Eckman, 1983) and further erosion.

Lower E_m was found for contaminated sediments (Fig. 6.3), especially in the presence of *N. diversicolor* (reduction of 36 %), suggesting that copper in the sediment attenuated the activity of the biota present.

Though we lack direct measurements of activity, copper toxicity towards the biota has been addressed quite extensively (Gillis et al., 2006; Real et al., 2003; Sobral and Widdows, 1997). In a study of the effect of chemical pollution on the bioturbation potential of estuarine intertidal mudflats, Mazik and Elliot (2000) found that increased levels of pollution reduced bioturbation, average burrow depth and volume, as a result of decreasing species diversity and community biomass, as well as diversity of feeding and bioturbation groups. Biosynthesis of EPS is a known mechanism of metal sequestration, used by several bacteria and microphytobenthos, which offer a protective barrier to cells against environmental stress. Some Cu-resistant bacteria are known to be growth stimulated by Cu contamination (on a growth medium of up to 1000 mg $Cu^{2+}l^{-1}$) and to increase EPS production according to the need of metal cation binding onto the EPS (Kazy et al. 2002). However, this is not supported by the lower bacterial activity observed in the contaminated sediments without *N. diversicolor* (Almeida et al., 2007).

Critical shear velocities were similar in all treatments, but slightly lower in the contaminated sediments (Table 6.2). The similarity among treatments can be understood considering the observations of Bale et al. (2006) who found significant correlations between critical erosion threshold and silt content, bulk density and water content in sediments from the Tamar Estuary.

6.3.3.2. Input of organic matter, chlorophyll a and copper to the water column

Bioturbation by *N. diversicolor* was responsible for higher values of eroded mass, and thus increased the transport of materials from the sediment surface to the water column, and the effect was more pronounced in the non contaminated sediments.

As expected, the organic content of eroded matter (E_{POM}) was highly correlated to total eroded matter (E_m) in all sediments (E_{POM}=0.1040.E_m+2.3926, R^2=0.98, n=96, regression analysis, $F_{1.94}$=3832.2, p<0.001) and was similar among sediments. At velocities below u_{*crit} ($u_* < 6$ cm s^{-1}) organic matter content varied between 25 % and 45 % and decreased to 7 % to 20 % when bulk erosion started.

As *N. diversicolor* decreased the chlorophyll content at the sediment surface (Table 6.1) the input of chlorophyll into the water column was ~24 % lower in its presence (Fig. 6.4). At velocities above u_{*crit}, the input of Chl *a* to the water column showed a 10-fold increase. When compared to the controls (B and CB) sediments without *N. diversicolor* contributed with less 20 to 30% Chl *a*.

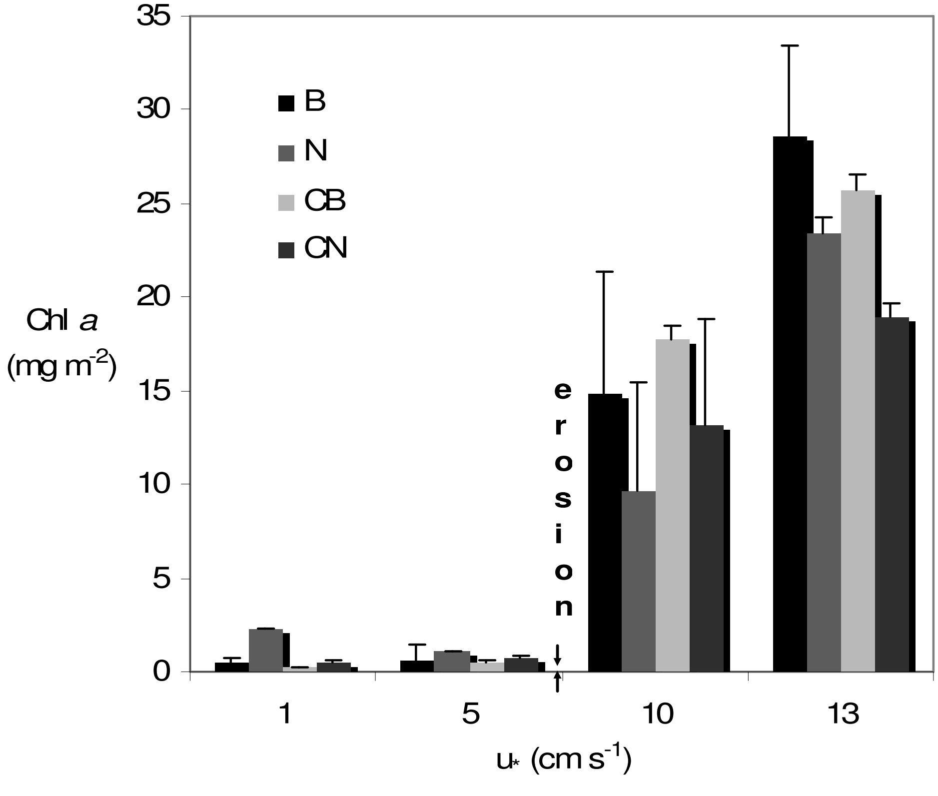

Figure 6.4. Input of chlorophyll *a* (mg m^{-2}) to the water column at selected shear velocities (u* cm s^{-1}) for control sediment (B), sediment with *N. diversicolor* (N), copper spiked control (CB) and copper spiked sediment with *N. diversicolor* (CN). Mean ± SD. n=3.

Sediment bioturbation by *N. diversicolor* had no effect on the concentrations of dissolved copper in the water column (Fig. 6.5), which was.directly related to increasing shear velocities (ANCOVA, $F_{3.61}$=9.8524, p<0.0001), first as a consequence of (shear stress) pumping of interstitial water out of the sediment, and later due to erosion.

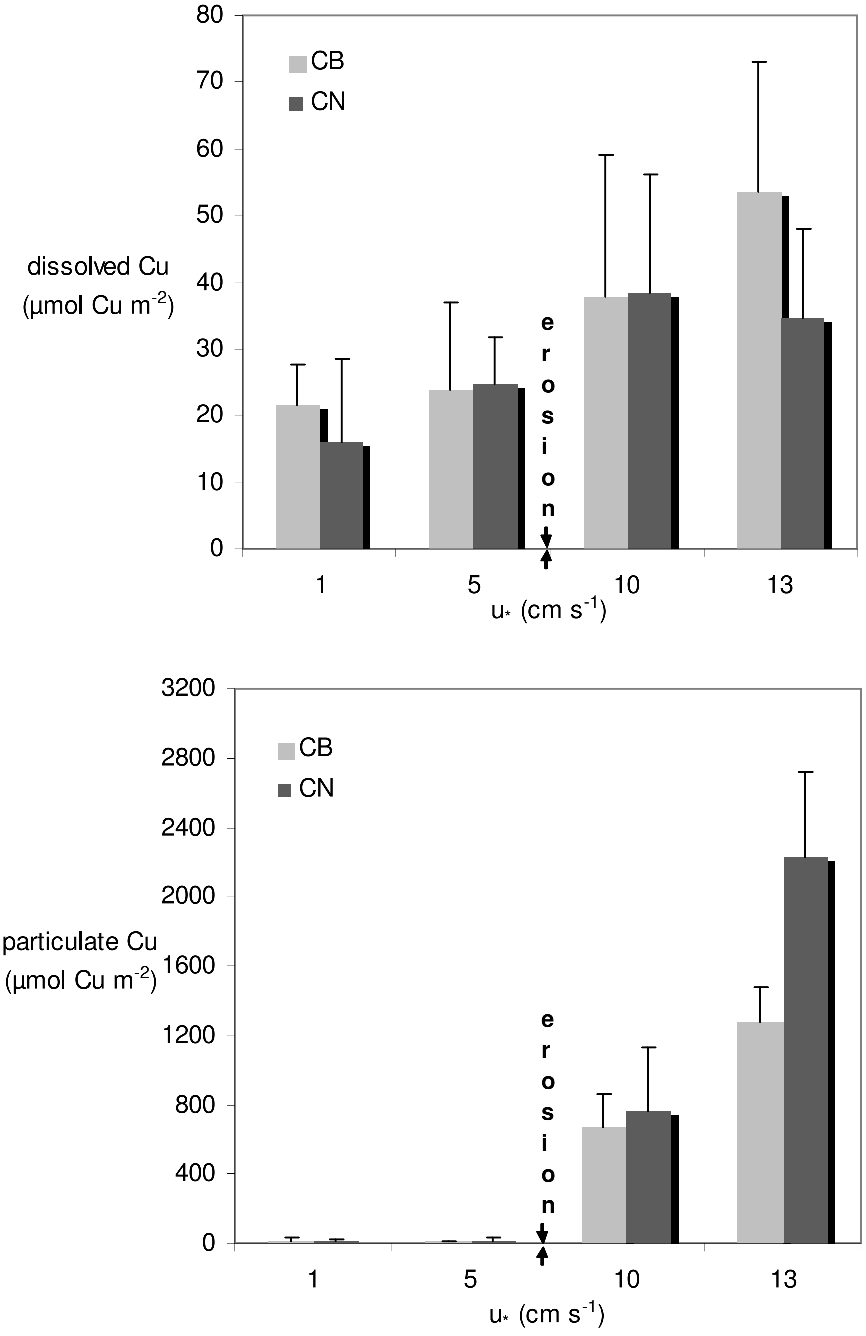

Figure 6.5 - Input of dissolved and particulate copper (dCu and pCu, µmol m^{-2}) to the water column at selected shear velocities (u_* cm s^{-1}) for copper spiked control sediment (CB) and copper spiked sediment with *N. diversicolor* (CN). Mean ± SD. n=3.

Significantly different dissolved copper concentrations (Tukey, $p<0.01$) were found before (15.9 to 24.7 µmol Cu m^{-2}) and after erosion (34.6 to 53.5 µmol Cu m^{-2}).

Concentrations of particulate copper in the water before the onset of erosion were only slightly higher when in the presence of *N. diversicolor* and increased sharply with shear velocity (Fig. 6.5) resulting in a ~200 times increase at the highest velocity tested. Particulate Cu content in the water column increased with E_m as expected and was therefore significantly different below and beyond u_{*crit} (ANCOVA, $F_{3,31}=47.28$, $p<0.0001$). As bioturbation promoted increased values of eroded mass, particulate copper input to the water column was significantly higher in the presence of *N. diversicolor* (ANCOVA, $F_{1,31}=5.27$, $p<0.05$). Strong interaction between shear velocities and the presence of *N. diversicolor* was also found (ANCOVA, $F_{1,31}=9.26$, $p<0.01$). Mixing and reworking of the surface sediment promoted suspension of particles and facilitated the input of organic materials and particulate copper to the water column.

6.3.4 Eroded depth and sediment shear strength

Depth of erosion was calculated from the amount of eroded matter. Erosion depths (Fig. 6.6) were significantly different among sediments (one-way ANOVA, $F_{1.4}=33.47$, $p<0.001$) and the sediments without *N. diversicolor* were clearly lower than in bioturbated sediments. Bioturbation by *N. diversicolor* had a significant impact on this variable increasing it by 2.2 times in the sediments without contamination (1.7 ± 0.4 mm in B and 3.8 ± 0.1 mm in N) and 1.6 times in the copper spiked sediments (1.8 ± 0.01 mm in CB and 2.9 ± 0.4 mm in CN) (Tukey, $p < 0.001$ and $p < 0.01$, respectively). Copper contamination had no effect on erosion depth of the control sediments (B and CB), but bioturbation in the copper spiked sediments significantly increased erosion depth by 22 % (for N and CN, Tukey, $p < 0.01$). As expected, a significant positive correlation ($R^2=0.84$, $F_{1,10}=54.0$, $p<0.001$, $n=12$) was found between erosion depth and *SS* measured after erosion (Fig. 6.6) as the more compact subsurface layers became exposed.

Sediments with higher *SS* are expected to be more resistant to erosion, as was recently verified by Watts et al. (2003) on intertidal sediments of Tollesbury (Essex) using both Fall-Cone and CSM devices and erosion rates calculations from the s_i index. In the present study we found a different relationship between *SS* after erosion and E_d (Fig. 6.6).

SS of 0.2 KPa was enough to prevent further erosion on the sediments without *N. diversicolor*, whereas in the sediments bioturbated by *N. diversicolor* higher values of *SS* were needed (0.3-0.7 KPa). This relation is supported by observations by Grant and Daborn, (1994) who also found positive relationships between *SS* and E_m, which is in this work directly related to E_d. The effect of bioturbation by *N. diversicolor* upon E_m could be explained by increasing particle adhesion and aggregation by mucus production, especially in response to copper toxicity, and by increasing sediment compaction due to sediment reworking and gallery building. Biologically bound sediments, on one hand, lead to higher *SS*, but above u_{*crit} promote erosion of sediment aggregates (Luckenbach, 1986), instead of the gradual erosion observed in sediments with lower shear strength and lower biological activity (as in the absence of *N. diversicolor*).

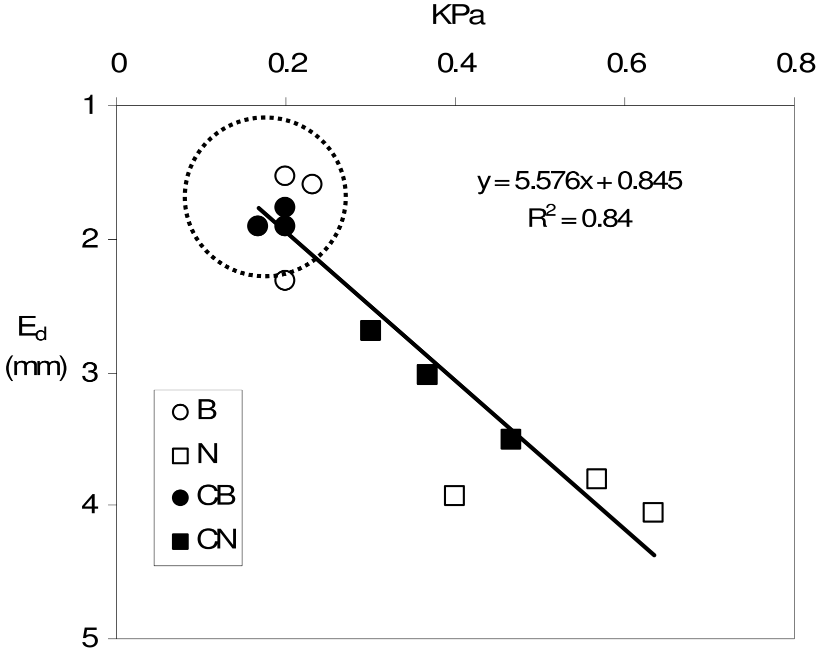

Figure 6.6 - Relation between erosion depth (E_d, mm) and sediment shear strength (KPa) measured after erosion. Circle indicates sediments without *N. diversicolor*. Trend line was built combining data from each sediment ($F_{(1.10)}$=53.98. $p < 0.001$), control (B), copper spiked control (CB), sediment with *N. diversicolor* (N) and copper spiked sediment with *N. diversicolor* (CN).

The results found in this laboratory study are not consistent with field observations that showed a direct relationship between *SS* and Chl *a* (Underwoood and Paterson, 1993; Mazik and Elliot, 2000) using a CSM® and a Geonor® H-10 field inspection vane. Although we could find in this experimental study an inverse relationship between Chl *a* concentration in the sediment and E_m, we also observed that different values of Chl *a* corresponded to the same initial values of *SS* at the surface, as measured with the fall-cone device. This is not surprising since fall-cone measurements do not reflect the changes in the characteristics or abundance of the biofilm, but measure instead the geotechnical strength related to other properties of the sediment, such as physical resistance. Also, sediments in this study were kept immersed and therefore will respond differently from the tidal sediments measured by the different authors. In addition, patchiness and the complex interaction among biogeochemical properties of the sediment, such as grain size, water content, sediment bulk density, organic matter and the biota, contribute to varied responses that are not always clear (Houwing, 1999; Widdows et al.*,* 2000 a,b).

Eroded matter (E_m) and erosion rates (E_r) of cohesive sediments are commonly related to shear stress (Sanford and Maa, 2001; Wang, 2003) but a high variability is generally recognized. In the present study, different E_m and E_r were observed for similar shear velocities, confirming that these parameters do not depend solely on hydrodynamic conditions and are related to sediment characteristics (as shear strength), which in turn are influenced by the biota.

6.4 Conclusions

This study provides evidence that *N. diversicolor* not only disrupts the stability of surface sediment, making it more prone to erosion, but it also modifies sediment shear strength profiles, increasing the resistance to erosion of subsurficial layers.

Copper contamination partially counteracted the effects of bioturbation. Contaminated sediments are more stable as a consequence of biological responses to toxicity such as lower bioturbating activity and the production of mucus that binds sediment particles. Contaminated sediments will have therefore longer residence times and may act as contamination sources during storm events. This issue should be addressed carefully as natural contaminated sediments are complex mixtures of various interacting pollutants, eliciting different defensive responses that may lead ultimately to the death of the biota. In this event, the biological responses that promote stability would no longer be acting. Biological activity of *N. diversicolor* and biogeochemical processes at the sediment water interface modify sediment properties and thus influence the transport of materials to the water column, increasing food availability but also releasing contaminants. This coupling of the benthic and pelagic processes must be taken into account to develop realistic approaches of sediment transport models for the better management of effluent disposal of coastal and estuarine systems.

6.5 Acknowledgements

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PART IV

INTERACTIONS BETWEEN CONTAMINATED SEDIMENTS AND BIOLOGICAL ACTIVITY

Chapter 7

The influence of Cu Contamination on *Nereis diversicolor* bioturbation

THE INFLUENCE OF CU CONTAMINATION ON *NEREIS DIVERSICOLOR* BIOTURBATION

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Abstract

Aquatic sediments feature a two-way interaction between contaminants and benthic macrofauna. The effect of the macrofauna community on the transport of contaminants in sediment has received considerable attention. Yet, few studies have investigated the reverse effect, i.e. the influence of contaminants on bioturbation activity. To this end, we performed laboratory experiments to investigate the effect of copper contamination on sediment reworking activity of the ragworm *Nereis diversicolor*. A density of 570 ind m^2 was introduced in mesocosms containing natural and copper spiked (2.99 nmol Cu g^{-1}) cohesive intertidal sediments from Ponta da Erva (Tejo estuary, Portugal). Luminophore tracers were used to quantify sediment reworking activity over time (2, 7, 14 and 21 days). Bioturbation was quantified via biodiffusion coefficients (Db) using two different parameter estimation routines. In the first days, luminophore concentrations at depth were significantly lower under Cu contamination. Yet, after 14 days, profiles showed no longer differences, indicating that *N. diversicolor* is able to maintain a normal activity under the applied Cu contamination. Bioturbation activity of *N. diversicolor* shows a clear non-local signature, generating a faster penetration of tracer particles at depth than expected by a diffusive mechanism. We show that for this mode of bioturbation, Db values become highly dependent on the actual optimisation algorithm used in the parameter estimation procedure. This puts into question the appropriateness of the biodiffusion model for *N. diversicolor* bioturbation and may have important repercussions for the comparison of Db values between studies.

Key-words: cohesive sediments, bioturbation, *Nereis diversicolor*, copper, luminophores, biodiffusion model.

7.1 Introduction

Intertidal sediments are known to be places of high organic matter mineralization, and hence, constitute an important component in global biogeochemical cycles (Middelburg et al., 1996; Heip et al., 2001). At the same time, these environments are also zones of high sedimentation, and thus, they can act as a potential trap for solid phase contaminants (James, 2002; Aldridge et al., 2003). In order to assess the fate and dynamics of such contaminants in the environment, one needs a proper understanding of the reactive transport of these contaminants (Boudreau, 1997). One crucial aspect in this regard is the two-way interaction between contaminants and the macrofauna community of intertidal environments.

Benthic macrofauna exert a strong influence upon the chemical constituents within the sediment in several ways (Gerino et al., 1995; Gilbert et al., 1996; Flach et al., 1998). Biological activities, such as food foraging and gallery/burrow building, strongly enhance the transport of solutes, a process known as bio-irrigation (Aller, 2001), and induce considerable mixing of solids, a process commonly referred to as bioturbation (Meysman et al., 2003). These activities can also change geotechnical properties, such as grain size distribution (Giangrande et al., 2002), sediment cohesion, and erodability (Widdows et al., 1998, 2000; Fernandes et al., submitted). These factors combined strongly influence microbial activity (Aller and Aller, 1998; Goñi-Urriza et al., 1999; Mulsow et al., 2002; Ferro et al., 2003), creating a patchy mosaic of oxic and anoxic microhabitats (Fenchel, 1996; Kristensen, 2000), and hence, will alter the diagenetic conditions within the sediment (Aller and Aller, 1998; Forster et al., 1999; Mortimer et al., 1999; Nielsen et al., 2004). Overall, macrofauna bioturbation and bio-irrigation have the potential to strongly influence the production, mobilisation and accumulation of certain contaminants (Rasmussen et al., 1998; Hammerschmidt and Fitzgerald, 2004).

Biological mixing is the dominant transport process for solid contaminants in aquatic sediments (Olsen et al., 1982; Weatcroft et al., 1990), thus it is worthwhile to study the effects of contamination on bioturbation rates. In the past, experimental bioturbation studies have focused on community bioturbation in relation to differences in feeding types (Gerino et al., 1995, 1999; Flach et al., 1998; Turnewitsch et al., 2000; Green et al., 2002), scaling of bioturbation to the size of the organisms (Swift et al., 1996) and the presence of burrows (Dellapenna et al., 1998). Other studies have followed a more theoretical approach, relating biodiffusion coefficients (Db) to biological parameters in simplified models of biological activity (Wheatcroft et al., 1990; François et al., 2002; Meysman et al., 2003; Mugnai et al., 2003; Solan et al., 2004). Yet, with regard to the interaction between bioturbation and contaminants, our knowledge remains sparse. Some studies have measured the effect of particular organisms (Gilbert et al., 1996, 2003; Rasmussen et al., 1998) or natural communities (Swift et al., 1996) on the distribution of contaminants, but only few take into account the effect of contaminants on the mixing activity of the biota (Mulsow et al., 2002).

Here, our aim is to evaluate the effect of contaminated sediments on the bioturbation activity of estuarine benthos. We selected the ragworm, *Nereis diversicolor,* as the subject of our study.

This endobenthic species is widespread in brackish water environments throughout north-western Europe, shows a high tolerance to temperature, salinity and lives in muddy to sandy habitats on the southern-western coast of Portugal (Fidalgo e Costa et al., 1998). Typical densities are in the range of 1000-2000 ind m^{-2} for adult populations, but can reach up to 16000 ind m^{-2} for juvenile populations in spring (Davey, 1994; Fidalgo e Costa et al., 1998). It also appears very tolerant to contaminants (Bryan and Hummerstone, 1971; Bernds et al., 1998) and is an important prey for birds and fishes, representing a link for the transfer of contaminants in the food chain.

N. diversicolor inhabits a more-or-less permanent and complex gallery network that can extend down to 30 cm depth, (Anderson and Meadows, 1978; Esselink and Zwarts, 1989; Davey, 1994). However, in a study on the Humber estuary, Davey and Partridge (1998) observed that only 20% of the population was found below 8 cm depth. François et al., (2002) classified it as having a “gallery-diffusor” sediment mixing mode, distinct from “biodiffusors” and “downward conveyers”. Gallery diffusors display a combination of bio-advective (fast unidirectional downward transport) and bio-diffusive mechanisms (bi-directional mixing) (François et al., 2002).

In this study, we evaluate the effect of Cu contamination on the sediment mixing activity of *N. diversicolor* using two different biodiffussive modelling procedures. Besides the contaminant aspect, our aim is also to examine if and how artifacts are created by applying an exclusive bio-diffusive model to a bioturbation mode, which is characterized by a mixture of diffusive and advective aspects.

7.2. Materials and methods

7.2.1 Mesocosms set-up

In January 2002, *N. diversicolor* specimen (10 to 15 cm length) were collected in Rosarinho (Tejo estuary, Portugal), brought to the laboratory and allowed to acclimatize to experimental conditions (temperature 17 ± 1 ^{0}C (± standard deviation); salinity 36 and light 54 ± 23 μmol m^{-2} s^{-1} with a 12 hour photoperiod) for a period of 5 days before being added to the mesocosms.

Fine muddy sediments from Ponta da Erva (Tejo estuary) (~ 8% organic matter and ~ 95% silt) were scooped from the top 2 cm during low tide, and brought to the laboratory, where they were kept at 4 ^{0}C to reduce biological activity until mesocosms were set-up. Macrofauna (*N. diversicolor*, small crabs) and larger shell fragments that could be visually detected upon close inspection were manually removed from the sediment. This defaunation technique was preferred over sieving to avoid disturbance of the sediment structure texture by water dilution. Subsequently, the sediment was thoroughly mixed to ensure homogenisation. Note that this defaunation method does not exclude meiofauna and although a taxonomic analysis was not performed, nematodes, harpacticoid copepods and ciliates amongst others were identified.

Two batches of sediments were prepared, one batch referred to as “natural sediment” to which no contaminant is added, and another one that was spiked with a solution of copper chloride up to a final concentration of 3.15 nmol Cu g^{-1} dw (dry weight) (details in Fernandes et al., submitted). From each sediment batch, five separate mesocosms with an area of 270 cm^2 and a sediment height of 8 cm were prepared. From these, four were allocated for inoculation with organisms, to be sampled on days 2, 7, 14 and 21. The remaining mesocosm was a control (i.e. without organisms) only to be sampled on day 21. The day after mesocosm set-up, 24 organisms (0.08 g DW ind^{-1}, n=22) were introduced resulting in an initial density of 900 ind m^{-2}, which is a typical density for an adult *N. diversicolor* population under natural conditions (Davey, 1994).

The activity of *N. diversicolor* was assessed using luminophores (80 – 180 μm), i.e. inert fluorescent particles (Mahaut and Graf, 1987; Gerino et al., 1998). The luminophores method generally produces good agreement between field and laboratory studies, and predicts similar biodiffussive mixing coefficient as when using ^{234}Thorium and Chl *a* (Gerino et al., 1998). Luminophores were added in a fine layer (~ 1 mm) onto the sediment surface, enough to assure that the vertical transport rate would not be influenced by the lack of particles tracers in the top layer of the sediment (Gerino et al., 1994). The mesocosms were carefully filled with sea water in order to avoid resuspension of the luminophores. The sediment in the mesocosms was covered by a water column of ~ 2 cm, in a flow-through system (~ 3 L h^{-1}) with constant aeration.

Destructive sampling took place after days 2, 7, 15 and 21 days under experimental conditions. At each sampling time, 3 sediment cores (area 3.5 cm^2) were taken from each mesocosm and immediately frozen until analysis. The remaining sediment in the mesocosms was hand defaunated in order to estimate the *N. diversicolor* density at the end of the experiment. Mortality rates were of 13 ± 6 % in N (*N. diversicolor* alone treatment) and 27 ±

7 % in CN (Cu + *N. diversicolor* treatment) after 21 days. The difference between initial (900 ind m^{-2}) and final densities are attributed to natural mortality, organisms escaping during incubation, losses in post-experiment defaunation, or elimination during core sampling. The average density during the 21-day experiment was estimated to be 570 ind m^{-2}.

7.2.2 Luminophores analysis

Frozen cores were sliced in 0.5 cm layers for the top first cm and in 1 cm intervals for the layers below. Each sample was dried (90ºC, 48h) and homogenized (as described in Mahaut and Graf, 1987). Within each layer, subsamples of 0.25 g were taken for counting luminophores under an epifluorescence microscope (Olympus BX60). From this, the number of luminophores per layer (n) and the total number in the profile (N) were obtained, and hence, the fraction (n/N) of luminophores per layer could be determined. The concentration of luminophores was estimated as $\mathrm{C} = \mathrm{n} / (\mathrm{z} * \mathrm{A} * \mathrm{N})$, where z (cm) is the thickness of the sampling layer and A the core area.

7.2.3 Db estimations

The biodiffusion coefficient Db was estimated using the classical one-dimensional biodiffusion model (Boudreau, 1986; Wheatcroft et al., 1990; Meysman et al., 2003), which is governed by

$$\partial C(x,t) = D_b \frac{\partial^2 C(x,t)}{\partial x^2} \qquad [1]$$

Initially, it is assumed that the luminophores are located in a very narrow layer at the water-sediment interface, as idealized by the initial condition

$$C(x,0) = \partial(x) \qquad [2]$$

where δ(x) represents the Dirac delta function. Over the infinite interval, the initial-value problem [1]-[2] has the solution (Crank, 1975)

$$C(x,t) = \frac{N}{A\sqrt{\pi D_b t}} \exp\left(- \frac{x^2}{4 D_b t} \right) \qquad [3]$$

where N is the number of luminophores originally spread at the sediment surface, and A represents the surface area. Because concentrations are expressed relative to the total number of luminophores found in the profile, a value of $N = 1$ should be substituted.

Values for Db were obtained by matching expression [3] to the observed data profiles. For this purpose, two separate procedures were employed. In a first approach (M1), the untransformed data was used. Accordingly, the biodiffusion coefficient D_b was estimated by minimizing the cost function

$$R_1 = \sum_{i=1}^{N} (C_i^{\mathrm{mod}\,el} - C_i^{data})^2 \qquad [4]$$

In a second procedure (M2), the data were first log-transformed, and subsequently, a second value for the biodiffusion coefficient D_b was estimated by minimizing the alternative cost function

$$R_1 = \sum_{i=1}^{N} (\ln C_i^{\mathrm{mod}\,el} - \ln C_i^{data})^2 \qquad [5]$$

Effectively, both procedures use a different weighting of the data when calculating their "optimal" Db values. The M1 procedure emphasizes the large luminophore concentrations near to the surface, and thus fits the concentration data in the top layers best. In contrast, the M2 procedure emphasizes the difference between model and data at low concentration values, and thus will provide a better fit to the concentration data in the deeper layers.

Theory predicts that if the actual bioturbation mechanism is truly diffusive, i.e. if biological activity generates effectively isotropic, small-scale particle displacements (Meysman et al., 2003), then both procedures should generate the Db same value. However, if the underlying macrofauna activity is not generating diffusive particle displacements, then the Db values generated by the M1 and M2 procedures can show large deviations (see below). In particular, when non-local transport mixes particles faster down as expected, one can expect large discrepancies between the Db estimates. To quantify any such deviations, we propose the following non-locality index (NLI) based on the D_b values obtained using the M1 and M2 procedures:

$$NLI = \frac{|D_b[M_2] - D_b[M_1]|}{\sqrt{D_b[M_1] * D_b[M_2]}} \qquad [6]$$

As required, this non-locality index NLI takes a zero value when Db estimates from M1 and M2 have the same value, i.e. when biological transport is truly diffusive. Conversely, for strong non-local transport, the obtained Db values will differ significantly, i.e. Db[M2] will be large while Db[M1] will be small. Under these conditions, the NLI index [6] tends to infinity, indicative of a large "non-local" character of the biological transport considered.

7.2.4 Additional cumulative effect of bioturbation and copper

The effects of bioturbation and copper were quantified after Blanchard et al. (1997), using an index of the additional cumulative effect (ACE, %) of a tested parameter (X), where ACE = $[(X_{treat} - X_{no\ treat})/ X_{no\ treat}]* 100$. The treatments in our case are the presence of *N. diversicolor* and Cu respectively, while the three tested parameters are the biodiffusion coefficients Db derived from M1 and M2, and the NLI.

7.2.5 Statistical analysis

Statistical analysis was performed using two-way ANOVA (α=0.05) and Bonferroni's post-hoc comparison test (STATISTICA® 6.1 software) after data was log-transformed to assure the homogeneity assumption (verified with Levene's test), in order to test . We tested for the significance of the effects of treatment (N x NC, Ct x N, Ct x C and C x CN) and time (2, 7 15 and 21 days) on the Db estimates. With regard to the luminophore profiles in the sediment, because depth intervals within cores are not independent, the primary assumption of ANOVA would be violated. To enable ANOVA analysis, core identity was introduced as a new parameter that was treated as a random factor, nested within the treatment*time interaction. Significance of the treatment, time, treatment*time interaction was tested over the mean square between cores within treatment*time. Significance of depth, and all interaction terms involving depth, was tested over the error mean square.

7.3. Results

7.3.1 Luminophores profiles

Statistical analysis of the profiles of luminophores concentration showed significant differences between treatments (p=0.0014), which are predominantly caused by differences in the profiles after the first two days (Fig. 7.1).

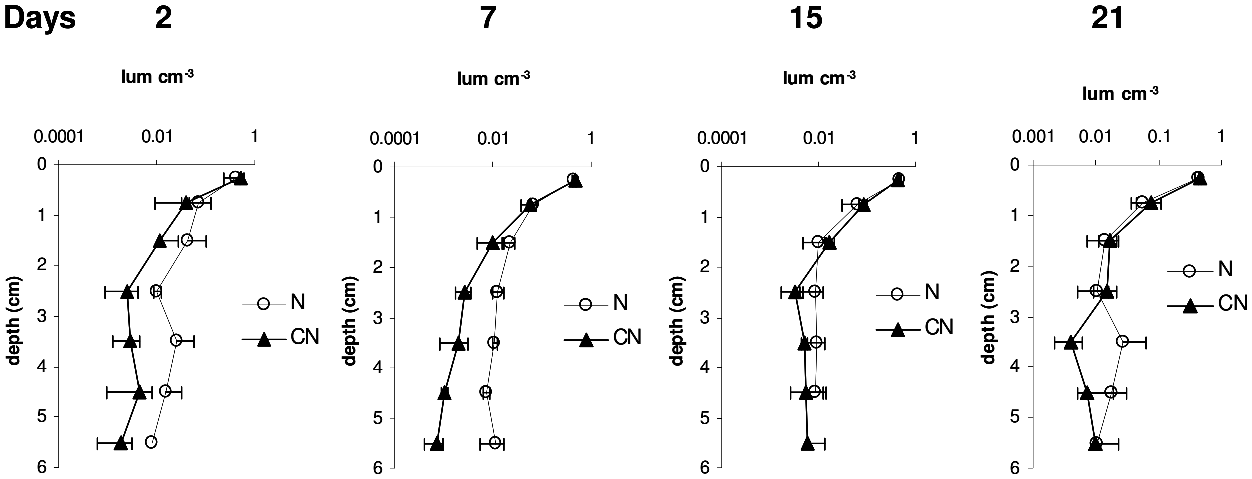

Figure 7.1 – Profiles of luminophores concentration over a period of 21 days for a *N. diversicolor* density of 570 ind m^{-2}. The "N" treatment represents *N. diversicolor* in natural sediment, while "CN" refers to *N. diversicolor* in Cu contaminated sediment. (Bars denote ± standard deviation).

7.3.2 Db estimates

Estimates of Db were highly dependent on the optimisation procedure that was used. Db values ranged from 0.6 to 5.9 cm^2 y^{-1} using the M1 procedure and from 13 to 493 cm^2 y^{-1} using the M2 procedure (Table 7.1; Fig. 7.2,).

Using the M1 procedure, there was no significant effect of Cu addition on Db values, which were similar in the *N. diversicolor* alone (N) and the Cu + *N. diversicolor* treatment (CN). In contrast, using the M2 procedure, Db values were significantly higher (p=0.0081) in the *N. diversicolor* alone (N) as compared to the Cu + *N. diversicolor* treatment (CN).

Time showed to have a highly significant effect on the Db values estimated by both procedures (p<0.0001 for both M1 and M2). This is particularly due to day 2 where Db values were significantly higher than the ones observed in the following days (p<0.001). The decline in the Db value with time is very similar in both procedures (M1 and M2), and amounts to a reduction of 89% between day 2 and day 21.

Table 7.1 – Db (cm^2 y^{-1}) estimates obtained by M1 and M2 procedures and non-locality index (LNI), over a period of 21 days for different mesocosm set-ups. Treatment codes refer to natural sediment with *N. diversicolor* (N), contaminated sediment with *N. diversicolor* (CN), the control without organisms under natural conditions (Ct) and control for contaminated sediment (C). (± Standard deviation).

D_b (cm^2 y^{-1})

		Treatments			
Days	**method**	**Ct**	**C**	**N**	**CN**
2	*M1*			4.52 ± 2.30	5.87 ± 0.06
	M2			493.39 ± 141.36	305.71 ± 55.18
	NLI			11.68 ± 5.78	7.05 ± 0.66
7	*M1*			1.79 ± 0.05	1.72 ± 0.06
	M2			142.13 ± 52.02	65.93 ± 1.01
	NLI			8.69 ± 1.76	6.02 ± 0.13
15	*M1*			0.84 ± 0.08	0.87 ± 0.05
	M2			47.67 ± 19.41	48.09 ± 9.85
	NLI			7.35 ± 1.75	7.26 ± 0.37
21	*M1*	0.64 ± 0.10	0.55 ± 0.00	0.59 ± 0.02	0.62 ± 0.06
	M2	21.42 ± 9.85	12.77 ± 4.34	56.34 ± 17.53	34.81 ± 15.62
	NLI	5.51 ± 0.91	4.57 ± 0.84	9.60 ± 1.44	7.27 ± 2.18

7.3.3 Non-locality index

The non-locality index (NLI) varied in general from 4 to 18 indicating strong non-local effects (Fig 7.3). Time had no statistical effect on the NLI, but treatment produced significant differences. Higher NLI values were obtained in the N treatment as compared to the CN treatment at all sample intervals (p=0.0263).

Comparison at day 21 of the sediments with organisms with the corresponding controls shows that addition of organisms has a significant effect on NLI (p=0.0124). In both the natural and contaminated sediment, the addition of *N. diversicolor* results in a higher NLI.

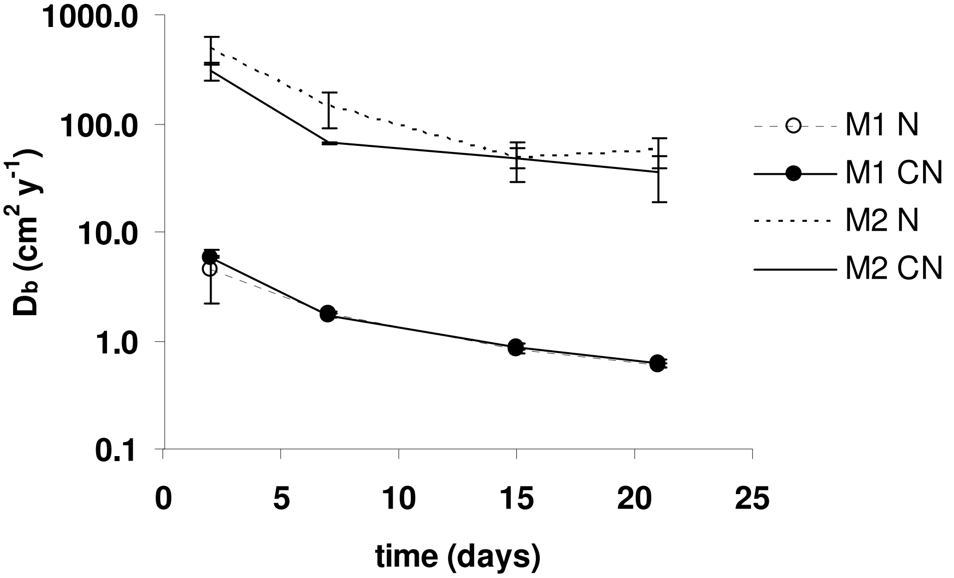

Figure 7.2 – Evolution of the biodiffusion coefficient over a period of 21 days. “N” represents *N. diversicolor* in natural sediment, while “CN” refers to *N. diversicolor* in Cu contaminated sediment. M1 and M2 refer to the different procedures of Db estimation. (Bars represent ± standard deviation).

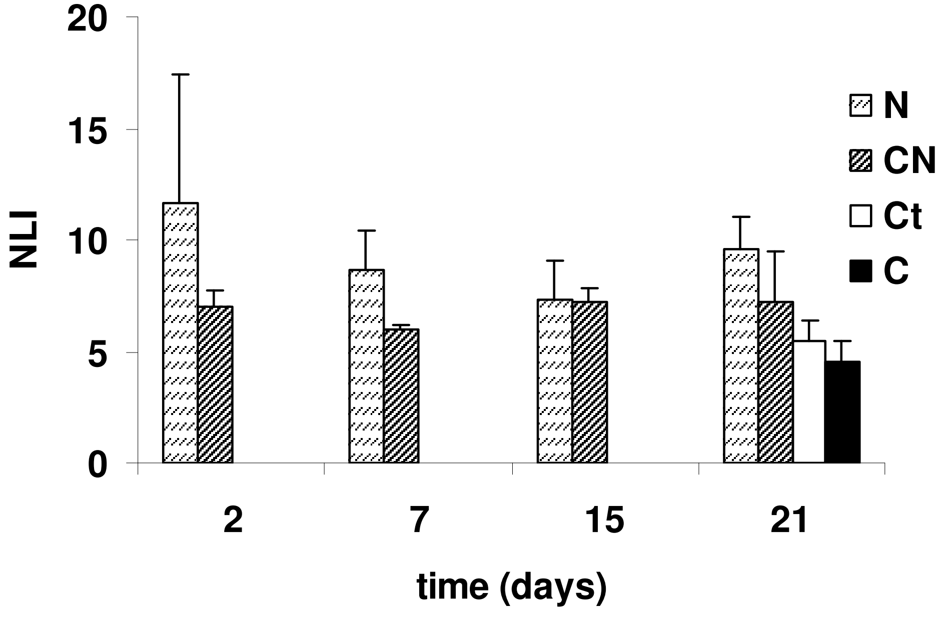

Figure 7.3 – Non-locality index estimated over time for a population of *N. diversicolor* under natural (N) and contaminated sediments (CN). (± Standard deviation).

7.3.4 Comparisons with controls

The Ct control mesocosm (i.e. natural sediment without *N. diversicolor)* showed a Db of 0.64 ± 0.10 cm^2 y^{-1} (M1) and 21.42 ± 9.85 cm^2 y^{-1} (M2), while the copper alone treatment (C) resulted in Db values of 0.55 ± 0.01 cm^2 y^{-1} (M1) and 12.77 ± 4.34 cm^2 y^{-1} (M2) (Table 7.1). The addition of organisms had no significant effect on the M1 estimates, but a significant effect on the M2 estimates (p=0.0156) that are especially due to differences between both controls (C and Ct) and N. The concentration profiles at day 21 (Fig. 7.4) showed no statistically significant differences among treatments. Nevertheless post-hoc analysis indicates significant differences between N and Ct (p=0.0240) and N and C (p=0.0128).

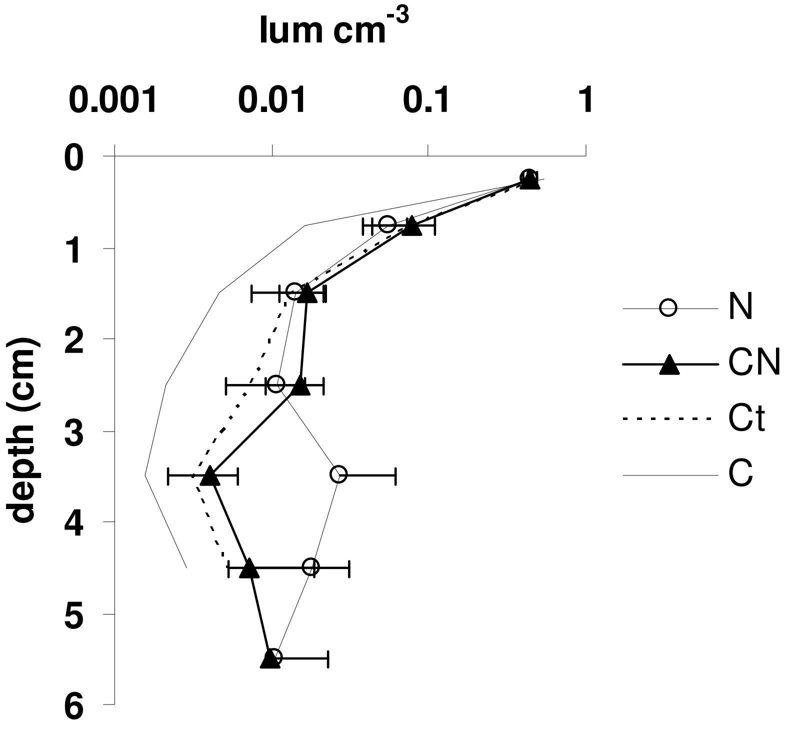

Figure 7.4 – Depth profiles of luminophore concentration after 21 days for a *N. diversicolor* density of 570 ind m^{-2}. Four treatments: *N. diversicolor* (N), Cu and *N. diversicolor* (CN), Cu alone (C), Control (Ct).

7.3.5 Additional cumulative effects

Values for the additional cumulative effect (ACE) due to *N. diversicolor* and Cu treatments are presented in Table 7.2. As shown, these values are highly dependent on the procedure that is used to estimate the Db. The ACE indexes based on the M1 procedure are contradictory with those produced by the M2 procedure. According to the M1 values, *N. diversicolor* produced almost no effect on Db, slightly decreasing Db under natural conditions (-7%), while slightly increasing Db (+13%) in contaminated sediments. In contrast, M2 values showed that *N. diversicolor* is responsible for an increase of Db in both natural and contaminated sediments (163% and 172% respectively).

The same ACE analysis for the NLI index results in no effect (+1%) under natural conditions, i.e. uncontaminated sediments display the same degree of non-locality with and without *N. diversicolor*. However, under contaminated conditions, the NLI index increased about 60% as a result of the presence of *N. diversicolor*.

The effect of Cu on bioturbation activity was also highly dependent on the estimation procedure used to determine Db values. According to M1, the effect of Cu was negligible (ACE's from 0 to +5%), while based on M2, Cu reduced mixing by about 40%. The NLI index was lower in the presence of Cu with or without *N. diversicolor* (ACE's about -20%), suggesting that Cu reduces the non-local character of the particle mixing.

Table 7.2 - Additional cumulative effect (%) due to bioturbation by *N. diversicolor* and copper addition. Calculations are based on the Db estimates for day 21 using the M1 and M2 procedures and the non-linearity index (NLI).

Effect	**Treatment**	**M1**	**M2**	**NLI**
bioturbation	natural	-7	163	1
	contaminated	13	172	59
Cu	*N. diversicolor*	5	-38	-24
	Control	0	-40	-17

7.4 Discussion

7.4.1 Divergent model results

Strongly differing values for the biodiffusion coefficient are obtained when applying either M1 or M2 procedures. These divergent Db's then lead to divergent NLI and ACE values. The nature and quality of the resulting Db/NLI/ACE values can be assessed by inspection of the luminophore profiles (Fig. 7.4). These profiles show hardly any distinction in the top centimetres, while they diverge at depth. The first 0.5 cm virtually contains only particles that have not been displaced, and accordingly, this top layer does not effectively embed information on the mechanism of bioturbation. In contrast, differences in bioturbation activity are only revealed by the data points in the deeper layer (i.e. those particles that have been actually displaced). As a consequence, the Db/NLI/ACE values derived from M2-Db's (based on concentration data in the deeper layers) will be more informative than the ACE values based on M1-Db's (predominantly based on information in the top layer). Therefore, it is not surprising that the ACE values based on M2 engender a more consistent and logical interpretation: the presence of *N. diversicolor* increases the Db, the presence of Cu decreases the Db. In contrast, the ACE values based on M1 suggest that after 21 days the intensity of reworking is about the same in *N. diversicolor* inhabited sediments as in the controls without *N. diversicolor* (Table 7.1). This awkward conclusion is however refuted by inspection of the luminophores profiles (Fig.7.4): these profiles show a clear signal (i.e. elevated luminophore concentrations) at depth due to the presence of *N. diversicolor*. Due to the distinct data-weighting of the two DB-estimation procedures, this signal is not picked by M1, but is clearly exposed by M2.

7.4.2 Interpretation of Db values and non-local effects

The high non-locality index (Fig 7.3) shows that the type of particle transport induced by *N. diversicolor* is not well explained by a simple diffusion description. The high discrepancy between the M1 and M2 estimates for the Db of the same data profile is mainly caused by a small number of particles, which are transported much faster than would be expected by the biodiffusion model. The basic assumption of the diffusion model is that particle displacements are small-scale, and so it requires a considerable number of bioturbation events (and hence considerable time) for a particle to penetrate to deeper layers. Therefore, the finding of luminophores particles at depth after short periods of time indicates that the bioturbation mechanism of *N. diversicolor* is typified by a true non-local mechanism of particle displacement. The M2 is heavily biased to this small number of fast-travelling particles, thus generating high Db values. Conversely, the M1 procedure neglects this "minority" of fast-travelling particles altogether and instead, focuses on the bulk of the luminophores, i.e. those near the surface that move slower, and thus generate far smaller Db values. There are other indications of non-local bioturbation. Figs. 7.1 and 7.4 show a peak in the luminophore concentration at 3-4 cm depth, both at 2 days and 21 days. Such concentration peaks are a clear sign of non-local "injection" of tracer at that depth. In contrast, the biodiffusion model always generates monotonically decreasing concentration profiles, prohibiting the occurrence of local maxima in the concentration profile. Concentration maxima at depth might result from the gallery building, deposit feeding and passive sloping of luminophore particle through the burrows of *N. diversicolor*.

Fig. 7.2 also shows drastic decreases of the biodiffusion coefficient with time (the same trend is obtained using the M1 and M2 procedure). A pertinent question then is whether this decrease is (1) a true feature of the bioturbation mechanism (bioturbation activity effectively decreases with time), (2) a model artifact (due to the defectiveness of the biodiffusion model), or (3) a sampling artifact. Effectively, these high initial Db's originate because, even for these short bioturbation periods, luminophore particles are found in deeper layers. This fast occurrence of particles at deeper layers can be caused by (1) increased non-local transport related to colonisation behaviour of the organisms (i.e. higher gallery building activities immediately after introduction in an uninhabited environment), or (2) by smearing of luminophores during core retrieval. The latter situation can be corrected for by subtracting the profile found in an appropriate control set-up.

In our case, this situation could not be checked because no control sample was taken after 2 days. However, subtracting the control profile at 21 days (and assuming this is only generated by core retrieval), we find that some luminophores still reach depths of 2-5 cm within 2 days. This indicates that non-local transport by *N. diversicolor* has effectively moved particles to these depths. Accordingly, applying a conventional biodiffusion model, these deep penetrating particles will lead to high Db values, when this penetration occurs over short time-scale. Effectively, the shorter the time-scale, the higher the estimated Db-value will be. This is because the time period t in model equation [3] will critically influence the magnitude of the estimated Db value. One can check this model artifact by applying the M2 procedure to same data profile (e.g. the N profile on day 2 in Fig 7.1) for increasing values of t. The resulting Db values will show the same strong decrease with time as observed in Fig. 7.2 (results not shown). Based on this, we conclude that the observed decrease of the Db in Fig. 7.2 is mostly due to a model artifact, i.e. the biodiffusion model [3] does not appropriately represent the factual particle movement of *N. diversicolor*. Although there might well be an increased biological particle transport due to gallery building activities immediately after introduction, in the case of *N. diversicolor* this increased activity can not be explicited by the diffusion model [3]. More in general, when bioturbation is dominated by non-local transport mechanisms, Db values estimated on the first experimental days seem unrealistic to characterize the activity of organisms.

7.4.3 The effect of Cu on Db values

Concentration profiles of luminophores measured after 7 days did show a significant effect of Cu on bioturbation (Figs. 7.1-7.2). This depressed bioturbation activity over the first days of the experiment may result from shock acclimation to copper. Nevertheless, our observations show that after 14 days, the profiles of luminophores become similar, probably as a result of *N. diversicolor* adaptation to contamination. These observations agree with data obtained on Cu bio-accumulation from a parallel experiment (Fernandes et al., submitted). At the start of the experiment, the same batch of organisms had initial Cu tissue contents of 0.283 ± 0.094 nmol Cu g^{-1} dw before being introduced in the contaminated sediments. After 2 days, a bioaccumulation of 0.965 ± 0.076 nmol Cu g^{-1} dw was observed, after which Cu content was gradually reduced to 0.653 ± 0.019 nmol Cu g^{-1} dw after 7 days and 0.472 ± 0.005 nmol Cu g^{-1} dw at day 21. The reduction of the Cu content in the tissues of *N. diversicolor* with time agrees with observations under pyrene contamination. In pyrene contaminated sediments (0.4 ppm), the concentration of water-soluble and insoluble pyrene metabolites increases in the tissues of *N. diversicolor* during the first four days, after which they reach a steady-state level (Christensen et al., 2002). Probably, the negligible effect on Db from Cu contamination is related with the low Cu bioaccumulation. In a study about the ecological impact of Cu bioaccumulation on *N. diversicolor* Grant et al. (1989) concluded that effects only become significant at copper levels above 0.009 nmol Cu g^{-1} dw, which is one order of magnitude smaller than the accumulation values observed in our experiments.

On the one hand, copper is known to promote the loss of muscle co-ordination and balance in juveniles of *N. diversicolor* when exposed to 7.9 to 9.4 nmol Cu L^{-1} (Ozoh, 1992), as well as inducing changes in embryogenesis such as non-motility and reduced ciliary beatings of larvae (Ozoh and Jones, 1988). On the other hand, *N. diversicolor* is able to regulate heavy metal bioaccumulation by excretion and sequestration in secreted mucus (Bryan and Hummerstone, 1971; Sze and Lee, 1995; Saiz-Salinas and Francés-Zubillaga, 1997). The lower NLI index in the presence of Cu (Fig. 4) suggests a change in type of bioturbation activity. One explanation for this might be that Cu reduces the feeding activity of *N. diversicolor* A reduction in feeding activity was also observed by Christensen et al. (2002) and Mulsow et al. (2002). The latter authors observed that after the exposure to DDT contaminated sediments, the polychaete *Heteromastus filiformis* reduced its feeding rate, and only mixed the sediment diffusively, conveying only limited amounts of ingested sediment to the surface. These observations support the idea that through various adaptations, *N. diversicolor* has a high tolerance to Cu contamination and is able to maintain a living in Cu contaminated sediments.

7.4.4 Db estimation on control treatments

The values of Db measured in the control mesocosms were surprisingly high (10-20 cm^2 yr^{-1}, Table 7.1) when compared to the Db values estimated in the presence of *N. diversicolor* (30-60 cm^2 yr^{-1}, Table 7.1). Equally, the controls show a deep penetration of particles up to 4-5 cm deep after 21 days. The observed transport of particles can be due to (1) a coring artifact caused by friction near the core liner walls (Crusius and Anderson, 1991) or (2) due to bioturbation of meiobenthos that were present in the sediments. Meiofauna abundance can extend up to 10 cm in the sediment (Meira et al., 2001). Similar relatively deep penetration of luminophores has been observed in the controls from other studies (> 2 cm; Mugnai et al., 2003; Ouellette et al., 2004). In the latter case, there was no meiofauna defaunation and no coring artifact, since the tracers were added directly in the cores (Ouelette et al., 2004). Considering that meiobenthos were not defaunated from the sediment, the lower Db and NLI values found under Cu contamination might be interpreted as a negative effect of Cu on the activity of the meiofauna.

This also brings up the question whether our controls are true controls. Although the sediments were the same, it is highly probable that *N. diversicolor* has a negative impact on the meiobenthic fauna through predation. If this would be the case, the reproducibility of experimental conditions would be affected. Further studies on macrofauna bioturbation could benefit from a characterisation of the sediment meiofauna (e.g. species composition, biomass, density with depth). This might help to clarify the effect and interactions between meiofauna and macrofauna. In a similar fashion one could ask to what extent the abiotic properties of the sediment are influenced by the presence of *N. diversicolor* and Cu. A parallel study (Fernandes et al., in press) showed that *N. diversicolor* significantly increases sediment shear strength by 70% under natural conditions and by 90% under Cu contaminated conditions. In other words, *N. diversicolor* greatly affects the compaction or cohesion of the sediment, influencing the overall transport properties of constituents within the sediment. The same study revealed that Cu has a significant reduction effect on sediment bulk density (g sediment cm^3).

7.5 Conclusions

The results of this work show that *N. diversicolor* is largely resistant to contamination of 3.15 nmol Cu g^{-1} dw sediment. Bioturbation activity, estimated via biodiffusion coefficients, did not appreciably differ between uncontaminated and contaminated mesocosm set-ups when observed over a sufficiently long period of 3 weeks. However, a small distinction is noticeably when observed over a shorter time-scale. Bioturbation activity of *N. diversicolor* was lower in the days immediately following Cu contamination as compared to the uncontaminated mesocosm. After a sufficient acclimation period (in the order of less than two weeks), the polychaete was apparently able to adapt to the contaminated sediments (presumably by regulating its internal levels of contamination), and reached an activity level that was comparable to that under non-contaminated conditions.

Our work also reveals some intricate problems connected to the model interpretation of luminophores tracer profiles. Here we show that when macrofauna activity has a clear non-local signature, the biodiffusion model becomes inadequate, as estimated values of the biodiffusion coefficient become greatly dependent on the actual optimisation algorithm used in the parameter estimation procedure. In addition, non-local transport induces an apparent decrease of the estimated biodiffusion coefficient for longer incubation periods. We believe this has important repercussions for the comparison of Db values between studies.

7.6 Acknowledgments

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PART V

GENERAL CONCLUSIONS

Chapter 8

General conclusions

GENERAL CONCLUSIONS

8.1 The effect of *Cerastoderma edule* on sediment transport

The results presented in this work have demonstrated that the presence and the density of *C. edule* affects boundary layer parameters. The presence of cockles causes higher surface topography (roughness), higher turbulence conditions at the bottom (u_*, z_0 and TKE) and thus, lower current velocity (Chapter 2 and 3). Apart from the effect that *C. edule* has on increasing surface roughness, the filtration activity is in itself, an additional source of turbulence (Chapter 3).

The effect of *C. edule* on sediment transport cannot be dissociated from the effect of suspended sediment removal by filtration column at velocities far higher than the critical velocities for deposition. However, the sediment removal effect of *C.edule* on sediment dynamics sediment was not studied

8.1.1 Topography and hydrodynamic conditions in the BBL

C. edule significantly increased bottom topography and hydrodynamic roughness (Chapter 3). This effect is associated with increasing density of live cockles, which reduced current velocities in the first 6 cm near the bed. The reduction of velocity near the bottom is directly related to the presence of the cockles which increased roughness by 0.3 times for 500 ind m^{-2} and 0.6 times for 1000 ind m^{-2}, when compared to the control treatment, and consequently increased other hydrodynamic parameters directly related to topography, such as shear velocity and hydrodynamic roughness.

Live cockle beds with a fairly low topographic roughness produced turbulence levels in the boundary layer similar to artificial beds with much higher topographic roughness. The higher turbulence values were observed when *C. edule* was actively filtering.

The analysis of u_* and z_0 over a flat surface and increasing roughness bed presented in this work clearly show that topographic roughness and its relationship with hydrodynamic parameters found in natural beds are different from (theoretical) relationships over smooth surfaces. Moreover, the increasing effect of *C. edule* on the turbulence near the sediment surface can be important for the settlement and redistribution of larvae, which are known to be driven by chemical cues but their probabilities of hitting the bed and remaining on the bed are also driven by local turbulence.

8.1.2 Filtration activity

In addition to the effect that the presence of *C. edule* has on topography and consequently on u_* and z_0, the filtration activity was well correlated with higher levels of turbulence. The positive relationship between CR and TKE (Fig. 3.9) supports the idea that filtration activity and the production of siphonal currents are responsible for higher levels of turbulence near the bed (Fig. 3.7 and 3.10), specially at low velocities ($u_* < 4$ cm $^{-2}$) and maximum at $u_* < 1$ cm $^{-2}$.

There is a dynamic interaction between filtration by *C. edule* and hydrodynamics. The filtration activity of *C. edule* showed a unimodal response to increased shear velocities with a critical u_* value (between 3.5 and 5.3 cm s^{-1}) after which filtration rate starts to decline (Fig. 2.9).

The observed effect of filtration activity on turbulence is more pronounced under low shear velocities ($u_* < 1.5$ cm s^{-1}). The effect of exhalant currents, measured here as z_m decreases at u_* higher than the critical value, because the exhalant currents can no longer compete with cross-flow and no longer protrude higher in the water column (Figs. 3.7 and 3.10).

8.2 The effect of *N. diversicolor* on sediment stability and biodiffusion

N. diversicolor has a dualistic effect on sediment stability. On one hand it acts as a biostabilizer, increasing sediment shear strength and critical velocity for erosion. On the other hand over a tidal cycle, once the critical erosion velocity is established, eroded mass was higher in the presence of *N. diversicolor*, classifying it as a biodestabilizer.

In addition to the increasing effect of *N. diversicolor* on erosion, bioturbation increases sediment remixing in depth promoting biodiffusion. This second aspect suggests that this polychaete affects sediment transport by increasing suspended matter but also the transport of particles from the surface to deeper sediment layers.

The effect of *N. diversicolor* on the topography and hydrodynamic conditions in the BBL was not measured because the ragworm does not cause measurable changes on the sediment surface.

8.2.1 Sediment shear strength

The presence of *N. diversicolor* enhanced the SS of the surface sediments by up to 38% (Tables 4.1 and 4.2), and the SS of sub-surface layers up to 70% (Fig. 6.2). The enhancement of SS can be explained by increasing particle adhesion and aggregation by mucus production, as well as gallery building and sediment remixing activities that result in lateral compaction of the surrounding sediment, especially in a confined flume channel. Considering results from the same main experiment (Chapter 6 and 7), the average of SS in depth (Fig. 6.2) was correlated (Pearson Product Moment Correlation Distribution, R^2=0.92, $p<0.05$, n=4) with Db, the index to estimate bioturbation activity (estimated using M2, Table 7.1), the more appropriate to express bioturbation below 1 cm depth (Fig. 5.6). This is a good indication that the higher SS are related to higher bioturbation activity.

This study shows that low values of SS (of 0.2 KPa) are related to lower erosion (eroded depth of 2 mm), whereas sediments with higher SS (of 0.4 KPa) are related with higher erosion (eroded depth of 4 mm, Fig. 6.6). This might be an indication that macrofauna cohesiveness mediation can produce different effects than the physical gravitational consolidation.

8.2.2 Erodability: critical velocity for erosion and erosion rates

Critical erosion velocities were higher in the sediments bioturbated by *N. diversicolor* (up to 22%, Tables 4.2, 4.3 and 6.2), meaning that the sediments would erode at higher velocities. The higher U_{crit} observed in the presence of *N. diversicolor* are in agreement with the higher values of sediment shear strength measured after erosion (Table 4.1, 4.3, 6.1 and Fig. 6.6). A regression analysis performed using average values of U_{crit} and SS measured after erosion in the experiments of Chapter 4 and 6 shows a positive relationship (Pearson Product Moment Correlation Distribution, R^2=0.44, p=0.0533, n=9).

There was a positive relationship between sediment erosion rates and *N. diversicolor* density. The effect of bioturbation on erosion rates varied from 30% to 67% in densities ranging from 350 to 790 ind m^{-2} (Table 4.2). The effect of *N. diversicolor* on erosion rates is related to bioturbation activities and the disruption of the biofilm due to grazing (deposit feeding) resulting in a decrease of surface sediment stability.

8.2.3 Interaction between sediment stability parameters

Sediments with higher shear strength are expected to be more resistant to erosion. However, once the sediment starts to erode, erosion rates were higher in the sediments with higher SS in depth and U_{crit}. There was also a positive correlation between SS measured at the surface after erosion and erosion depth (Fig. 6.6) showing that in the sediments without *N. diversicolor,* shear strength of 0.2 KPa was enough to prevent erosion, while in the sediments with *N. diversicolor* a shear strength of 0.4 KPa was not enough to do so. This relationship can be understood considering that once the surface sediment is disrupted, erosion proceeds quicker for aggregates in mucus-stabilised sediments than for erosion in unbound sediments.

8.2.4 Sediment mixing

The bioturbation of *N. diversicolor* in the top 6 cm, measured in terms of biodiffusion coefficients (Db) using luminophores after 21 days of activity, ranged from 1.9 to 0.6 cm y^{-1} for organisms densities from 320 to 570 ind m^{-2} (Chapter 5). This figure was determined using the most common procedure (M1) and gives more weight to the large concentration of luminophores remaining at the sediment surface (the particles that were not mixed), and thus, fits the concentration data on the top layers best. To deal with this bias, a complementary procedure for Db estimation (M2) is suggested based on log transformed data (luminophores concentration in depth), which provides a better fit to the distribution of the transported particle tracers in the lower sediment layers, the focus of our observations. The use of M1 and M2 procedures resulted in very different Db estimations, but Db obtained with procedure M2 is considered to be a better indication of bioturbation because differences in bioturbation of *N. diversicolor* are only revealed with the data points at deeper layers. Thus, Db obtained with M2 procedure is more informative than with M1 (the most commonly used).

The non-locality index (NLI index), which expresses the deviations between M1 and M2, indicates that in comparison to the control without large bioturbators, relocation of sediment particles by *N. diversicolor* is not a diffusive process (Chapter 5 and 7). This active transport of tracers may result from gallery building, deposit feeding and passive fall of tracer particles through the burrows.

The estimations of Db were different over time, Db measured after 2 days were higher than the Db measured after 21 days. This can be either an artefact of the model used or an indication that there is an increased biological activity and particle transport due to gallery building activities immediately after addition of *N. diversicolor* to the sediments, i.e. in the first days of colonisation.

Average Db (after 21 days, using procedure M2) was 73 cm y^{-1} for 320 ind m^{-2}, 21 cm y^{-1} for 450 ind m^{-2} and 56 cm y^{-1} for 570 ind m^{-2} (Table 5.1). No proportionality between organism density and Db was found, probably due to the use of different area mesocosms in the different experiments.

8.3 The effect of Cu contamination in *N. diversicolor* bioturbation and estuarine sediment stability

This work provides evidence that the bioturbation of *N. diversicolor* and sediment stability parameters are affected by copper contamination. In copper contaminated sediments bioturbation activity and erodability was lowered as observed for the lower Db (Table 7.1), lower SS (up to 40%, Fig. 6.2) and lower E_m (Fig. 6.3). Critical velocities for erosion were slightly lower for the contaminated sediments, in agreement with lower values of SS (Fig. 6.6). The lower erosion rates found for contaminated sediments, with or without *N. diversicolor* (Chapter 6) suggest that copper toxic effect attenuates the activity of the biota present in the sediment. The control treatments used in this experiment incorporated meso and microbiological activity and therefore the direct effect of Cu on sediment binding was not assessed.

Under Cu contamination the values of Db (using M2) were slightly lower in the first days in comparison to the uncontaminated situation (Fig. 7.2). However, after 14 days Db values became similar on both situations, probably due to *N. diversicolor* recovery/adaptation to contamination.

8.4 Measuring techniques and estimation methodologies: some considerations

A common problem to empirical and experimental studies, with repercussions for numerical simulation predictions, is the lack of calibration among measuring techniques, which limits the interpretation and consolidation of results. This was a constant concern over the course of the studies presented in this thesis. This work contributes to the intercalibration of the methodologies used on quantification procedures, namely:

1) in the inter comparison of hydrodynamic characteristics of different flumes and interpretation of clearance rates measured under each situation (Chapter 2);

2) in the comparison of surface roughness parameters such as hydrodynamic roughness and topographical roughness using different estimations methods (Chapter 3).

3) in the comparison of different parameters to estimate sediment stability (Chapter 4 and 6) such as quantification of erosion rates, critical erosion velocity, sediment shear strength, and other biogeochemical sediment properties (bulk density, organic content, Chl a, copper);

4) in the comparison of two different parameter estimation routines used to quantify the biodiffusion coefficients (Db) and consideration about their meaning and sampling time dependence concerning species behaviour (Chapter 5 and 7).

8.5 Concluding remarks

This thesis is a contribution to the understanding on the effect of macrofauna on sediment dynamics, showing clear evidence on how the presence of *C. edule* affect turbulence, topography and other hydrodynamic parameters in the BBL and how the presence of *N. diversicolor* affects physical and biogeochemical properties of the sediments and their erodability. This study provides evidence that copper contaminated sediments are more stable, as a consequence of decrease in biological response to toxicity, observed in lower values of shear strength and erosion rates.

Laboratory studies like these, performed under controlled conditions, are required to understand small-scale processes and analyse basic principles of sediment dynamics. The parameterisation of numerical environmental models demands the use of empirical relationships to describe hydrodynamic parameters, erosion coefficients, sediment characteristics and biodiffusivity coefficients. The relationships described in this study provide indications on how to include biological interactions in environmental models.

The relationships between sediment properties and erodability parameters found in this work, reinforces the need to understand erosion phenomena's as a function of sediment properties (physical, chemical and biological) and not only a function of physical forcing (shear strength or velocity). The observations and parameters relationships provided in this study do not completely reproduce field conditions, because they were performed under laboratory conditions. Only larger scale field measurement studies would clarify the relevance of the biological processes described in this work.

If the effect of macrobenthos observed in this work has an effect in a larger scale, then this knowledge should be used with an engineering perspective, by means of using the organisms functionality in ecosystem management. This concept is not innovative, but it is still a biological technology that is not taken very seriously.

For example, the role of filter feeders on sediment dynamics should be further investigated for application in places with excess suspended material. In addition to the effect they have on the characteristics of the BBL studied in this work, they have an important effect on suspended sediment removal (by filtration) at velocities far higher than the critical velocities for deposition. They also have an important role on matter transformation considering that the suspended removed particles are transformed in faeces, with different transport/resuspension/deposition characteristics, with a higher probability for deposition. Also, the knowledge about bioturbation activity of sediment reworking could be use as a recycling solution for high organic sediments.

PART VI

REFERENCES

Chapter 9

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