FINAL REPORT

EXTREMIS – Effects of extreme freshwater input fluctuations on the ecological status assessment in estuarine ecosystems - the benthic perspective
(Contract number: III/36/2008)

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1. EXECUTIVE SUMMARY

The Project EXTREMIS – Effects of extreme freshwater input fluctuations on the ecological status assessment in estuarine ecosystems - the benthic perspective, had as participant institutions the IMAR-CMA and the CO-FCL. Researchers from different research lines of IMAR-CMA have worked together, always with a fruitful interdisciplinary perspective.

The project main objective was to identify changes in benthic communities related to natural stress caused by freshwater discharges in different habitat types, by comparing two Portuguese estuaries (Mondego and Mira) with strong seasonal changes in water flow and different anthropogenic impacts. Complementarily, the comparison between the Northern and Southern branches of the Mondego estuary could provide some evidence on the differences of benthic communities related to distinct flow regimes and salinities.

Within the project framework, five tasks were foreseen:
Task 1. Data compilation
Task 2. Data collection
Task 3. Laboratorial and *in situ* experiments
Task 4. Ecological modelling
Task 5. Data integration and assessment of differences between natural and human stress

The results obtained within this project confirmed earlier findings and gave important new contributions on some aspects of the ecology of benthic invertebrates, mainly the osmotic tolerance of characteristic species of different habitat types and their responses to physical stress impacts. Sampling locations included the upper regions of the estuaries, which are in general poorly known in respect to the dynamics of benthic communities (responses) relatively to environmental changes and the influence of freshwater discharges. But, due to the good regional representation of channel shaped estuaries with strong seasonal changes, provided by the Mondego and Mira systems (Portugal), and due to the worldwide distribution these estuarine features have, the achieved results gave also an important contribution at a regional, national and international level in the development of assessment tools (see list of deliverables).

Understanding the seasonal dynamics of benthic communities clarified their importance on the overall ecosystem functioning, since benthic invertebrates give an important contribution to the high productivity of estuaries. The determination of time variations on benthic communities is crucial for management purposes, mainly concerning the data interpretation and the importance of monitoring activities recently given to European aquatic systems. Since benthic macroinvertebrates are included in the list of biological elements to be used in the ecological quality status classification aimed by the Water Framework Directive (2000/60/CE), it is important to understand what are natural effects (derived from differences in the hydrological regimes along European waters) and what are human induced impacts, when developing classification systems. Furthermore, the adopted laboratory and *in situ* testing methodologies, together with the ecological models, generated valuable hints to rapid and cost-effective monitoring tools, based on the response of estuarine characteristic species to environmental variations, able to provide more sounded and integrated measures of ecological quality. No ethical clearance was required to perform the experimental work.

In our opinion, the implementation of the project supported environmental and social benefits.

1.1. RESEARCH OBJECTIVES

Main research objectives per task:
TASK 1. DATA COMPILATION: to carry out an exhaustive search of all the relevant information on benthic communities of estuaries presenting seasonal changes, published in scientific journals or available as grey literature. The accomplishment of this task would provide a database of environmental variables and benthic communities of the Mondego and Mira estuaries, which basic information should support the research on the effects that physical stress variables have on benthic invertebrate communities.

TASK 2. DATA COLLECTION: to sample the subtidal soft-bottom macrobenthic assemblages and assess the water quality, seasonally during the project's first year, covering the salinity stretches previously identified in the Mira and Mondego estuaries. The new biological and physicochemical collected during the EXTREMIS project would complement the already existent long-term datasets. The amount of data to be produced on the benthic composition, abundance and biomass, should provide a good characterisation of the spatial and seasonal patterns of both estuaries, and allow relating short-term changes on the benthic structure to the observed environmental changes.

TASK 3. LABORATORIAL AND *IN SITU* EXPERIMENTS: to adapt short-term laboratory and *in situ* testing methodologies, developed for estuarine/marine groups of organisms, to the environmental conditions of the Mondego and Mira estuaries, to assess the influence of salinity variations independently of other factors (contaminant/environmental) and, in this way, to complement field data. The testing methodologies should be adapted to the different feeding strategies of selected key species, which comprise the variability in the functioning of estuaries and cover the entire range of habitat preferences in terms of salinity (from euhaline to oligohaline), namely: 1) polychaete *Hediste diversicolor* - epibenthic omnivorous, 2) decapod *Carcinus maenas* – benthic omnivorous, 3) gastropod *Hydrobia ulvae* - benthic surface deposit feeder herbivore/detritivore, and 4) amphipod *Corophium multisetosum* - epibenthic surface deposit feeder detritivore.

TASK 4. ECOLOGICAL MODELLING: to predict medium- and long-term variations on the system’s productivity caused by extreme weather events, namely, significant floods and droughts, estimating (experimentally) DEB parameters of selected species of key-estuarine macroinvertebrates, to develop a general DEB model capable of predicting the energetic budget of organisms under different conditions. The introduction of species-specific parameters within the general DEB model would allow making predictions at the species and/or population level.

TASK 5. DATA INTEGRATION AND ASSESSMENT OF DIFFERENCES BETWEEN NATURAL AND HUMAN STRESS: to determine differences in ecological data and laboratory/*in situ* tests, and patterns over time and between sites of the Mondego and Mira estuaries located within the same habitat; to verify if changes caused by the flow and salinity fluctuations influence the sites ecological status classification; to support suggestions on sampling designs and methodologies adequate for estuaries with strong seasonal freshwater flow variation.

1.2. ACTIVITIES

Main research activities per task:

TASK 1. DATA COMPILATION:

1. Compilation of all relevant references on the Mondego and Mira estuaries and references regarding the influence of freshwater flows and extreme climatic events on macrobenthic communities, published in scientific journals or available as grey literature.

TASK 2. DATA COLLECTION:

1. Compilation and description of already existing datasets regarding macrofauna and water quality parameters for Mira and Mondego estuary;
2. Seasonal field campaigns (4 times during the project's first year) of subtidal soft-bottom macrobenthic assemblages and water quality parameters (depth, transparency, salinity, temperature, pH, dissolved oxygen, ammonia, nitrates, nitrites, phosphate, silica, chlorophyll a, TSS, POM, sediment granulometry and organic matter content), covering the salinity stretches previously identified in the Mondego estuary;
3. Laboratory procedures to process macroinvertebrates samples (sieving, sorting, preservation, identification, counting, biomass determination) from the Mondego and Mira estuaries;
4. Laboratory and chemical procedures to analyse water and sediment samples;
5. Matrices construction: macroinvertebrates and water/sediment physico-chemical parameters

TASK 3. LABORATORIAL AND *IN SITU* EXPERIMENTS:

1. Development of an approach based on the postexposure feeding of *H. ulvae* to measure the potential effects of stressors (environmental and contaminants) on the functioning of estuaries, i.e., on grazing on biofilms and organic matter decomposition;
2. Development of a model (under laboratory conditions) to assess the effects of salinity, alone and in combination with other environmental variables, namely temperature and sediment grain size, on the postexposure feeding of *H. ulvae*, and its validation *in situ* under more realistic exposure scenarios at reference and contaminated sites, to be able to discriminate effects due to environmental variables from those due to contaminants.
3. Confronting the hypothesis that *H. ulvae* may have a key role in preventing macroalgal blooms.

TASK 4. ECOLOGICAL MODELLING:

1. Simulating the impacts at the system-level by upscaling a model developed for primary producers' productivity (macroalgae at m^2) to the whole system of the Mondego estuary, through coupling modelling techniques with GIS.
2. Predicting the variations on two important system consumers (*H. ulvae* and *Carcinus maenas*) caused by productivity variations on primary producers.
3. Carrying out field and laboratory work to determine the key parameters for the consumers: *H. ulvae* and *C. maenas*.
4. Developing energetic models for *H. ulvae* and *C. maenas* and coupling them to the system-level model.

TASK 5. DATA INTEGRATION AND ASSESSMENT OF DIFFERENCES BETWEEN NATURAL AND HUMAN STRESS:

1. Integration of present and past biological, physicochemical and morphological informations regarding the Mondego and the Mira estuaries (temporal and spatial variations);
2. Assessment of the effects of natural vs. anthropogenic variation in benthic communities of Portuguese estuaries;

3. Evaluation of the ecological quality of two estuaries, based on benthic macroinvertebrate communities;
4. Suggestion on sampling designs and methodologies adequate for estuaries with strong seasonal freshwater flow variation.

1.3. DELIVERABLES

Globally, the EXTREMIS project has fulfilled and surpasses all expected deliverables (Table 1, all pdf files are given as supplementary material).

For Ecology-based projects, 2 years of duration is a short time span to complete all tasks and publish the results. Nevertheless, the privileged way to communicate the outcome of the project was the production of several papers published in international scientific indexed journals. At the end of the project, 6 papers have completed the publication process, 4 manuscripts are submitted to SCI journals and 4 other papers are under preparation to be submitted. In addition, results were also presented at international meetings, through 12 oral communications and 4 posters. The EXTREMIS project has made viable the elaboration and conclusion of 9 MSc Thesis.

Table 1. Indicators/deliverables: expected and achieved.

	Expected	Achieved
Publications in international SCI journals	**7**	**6 published + 4 submitted**
Publications in national journals	0	0
Communications in international meetings	**5**	**12+4**
Communications in national meetings	0	0
Organization of scientific and technical meetings	0	0
Books	0	0
Book Chapters	0	0
M.Sc. Thesis	**3**	**9**
Ph.D. Thesis	0	0
Patents	0	0
Prototypes	0	0
Reports	**2**	**2**

Regarding the submitted and in preparation papers, summaries, but not the entire manuscript, are provided. The full version will be further added once the manuscript is accepted for publication.

1.4. COMMENTS ON THE FINANCIAL EXECUTION

List of changes regarding de budget execution originally foreseen:

1st change: In order to start on time the short-term *in situ* experiments to assess the effects of salinity and other stressors (contaminants) on *H. ulvae*, we had to anticipate the “construction” of the assay chambers. For this purpose, we had to allocate 812 € from Services acquisition (2nd year) to Services acquisition (1st year).

2nd change: Funds originally foreseen to cover fieldwork (missions-field) expenditures were allocated, throughout the project duration, to other items. The reason for this change was the fact that field work expenditures are extremely difficult to be accepted as eligible in the public administration and, therefore, this item has to be cover by other sources in the scope of the IMAR-CMA framework. Therefore, during the first year, 790 € from Missions-field were allocated to Current Expenses (500€) to by laboratorial material and to Equipment (290€) to buy a compressor needed for the short-term laboratory experiments.

3rd change: In order to complete experiments from Task 3, we had to pay extra months of grant to Liliana Saro, therefore, we have allocated funds from Missions-field and Services acquisition to Human Resources.

4th change: During the 2nd year, in order to buy necessary laboratorial material to complete Tasks 2, 3 and 4 we have allocated funds from several items to Current Expenses.

At the end of the project, Liliana Saro presented 752, 20 € of SSV that were not initially accounted for, due to this, IIIUC gave us the permission to use 162.99 € of extra budget and we have cancelled the acquisition of two micropipettes.

Apart from these changes, the financial execution progressed as initially foreseen and the project budget was fully utilized.

1.5. COMMENTS AND EXPLANATIONS OF ANY DEVIATIONS /MODIFICATIONS TO THE OBJECTIVES APPROVED

TASK 1. DATA COMPILATION: All objectives were fulfilled as initially planned.

TASK 2. DATA COLLECTION: The scientific objectives were fulfilled; nevertheless, some logistical adjustments had to be done. Studies on benthic macroinvertebrates are extremely time-consuming. From field samples collection, throughout sorting and identification tasks, the demand on time and human resources are extremely high. For the present project, due to several order constrains and research interests (human, logistic, data availability), it was decided to:

1) sample the subtidal soft-bottom macrobenthic assemblages and assess the water quality, seasonally during the project's first year, covering the salinity stretches previously identified only in the Mondego estuary.
2) work out the Mira's previously collected biological samples (2006) to totally complete existent data series instead of processing new ones and leave gaps on data series. In a very similar way as the predicted for the present project, and covering coincident salinity stretches as the ones required for this study, subtidal soft-bottom macrobenthic assemblages were already sampled in the Mira (and simultaneously in the Mondego). As those Mira samples were not completely processed at the starting moment of EXTREMIS, resources were conducted to complete data series on its full extension without leaving Information gaps. For the objectives of the project, comparisons between macrobenthic communities from the Mondego and the Mira were not compromised since data from the Mondego estuary were also collected, coincidently in time and under the same climatic conditions as those from the Mira.

TASK 3. LABORATORIAL AND *IN SITU* EXPERIMENTS: Task 3 intended to adapt to key estuarine species short-term laboratory and *in situ* testing methodologies to assess the influence of salinity variations, independently of other factors (contaminant/environmental), in the functioning of estuaries. With this goal in mind four estuarine key species were originally proposed as potentially important species in the functioning of the Mira and Mondego estuaries while covering the entire range of habitat preferences in terms of salinity (from euhaline to oligohaline), namely: (1) the polychaete *Hediste diversicolor* - epibenthic omnivorous, (2) the decapod *C. maenas* - benthic omnivorous, the (3) gastropod *H. ulvae* - benthic surface deposit feeder herbivore/detritivore, and 4) the amphipod *Corophium multisetosum* - epibenthic surface deposit feeder detritivore. However, as a representative of the amphipods, a literature review conducted at the start of the project revealed that using the species *Gammarus locusta* would be more valuable than to use *C. multisetosum*. This was because the species *G. locusta* had already been shown to be tolerant to certain environmental variables and sensitive to contaminants, factors that support its adaptation to laboratory and in situ methodologies. As both amphipod species have a sporadic occurrence in the Mondego and Mira estuaries, this replacement was considered not to affect the objectives of Task 3. Thus, with the ultimate goal of working out an assay based on the feeding response of amphipods after exposure to stressors, research to establish a standardised method to quantify the feeding rate of *G. locusta* was conducted during the project. Yet, the establishment of sound experimental designs and thus the achievement of reliable results were both highly compromised by the limited occurrence of this species at the reference sites, i.e., by the low number of organisms available to conduct experiments. Besides, some information on the salinity tolerance, alone and in combination with other environmental variables (e.g. sediment grain size, food availability) on the polychaete *H. diversicolor* and the decapod *C. maenas* had been previously explored by the research team.

Given the above, research during the EXTREMIS project focused mainly on the species *H. ulvae*. The latter species is known not only to be tolerant to wide variations in salinity (from around 4 to 40), and thus representative of a wide range of habitats in terms of salinity preferences, and also temperature (from around 5 to 35°C), but also to play an important role in European estuarine food webs, both as a decomposer and a secondary consumer, being thus selected as a valuable candidate to fulfil the main project objectives.

TASK 4. ECOLOGICAL MODELLING: Throughout the development of this task some modifications were undertaken in relation to the initial objectives, namely, it was considered more accurate to assess the impacts of extreme weather events by using a system-level model than by using DEB models of the selected consumers. For that, we have used a previously developed model (Martins et al. 2007), which was specifically run with external conditions of extreme weather events (drought versus flood). This model predicts variations on the dominant macroalgae species (*Ulva intestinalis*) at the system-level. Subsequently, by coupling the energetic models of *H. ulvae* and *C. maenas* to the core model, the impacts of extreme weather events on consumers are predicted. Due to the difficulties and uncertainties related to the obtaining of experimental parameters for the DEB models, traditional energetics models were developed instead.

TASK 5. DATA INTEGRATION AND ASSESSMENT OF DIFFERENCES BETWEEN NATURAL AND HUMAN STRESS: All objectives were fulfilled as initially planned.

2. PROJECT DESCRIPTIVE MEMORY

2.1. OBJECTIVES, RESEARCH WORK, RESULTS AND MAIN CONCLUSIONS BY TASK

TASK 1 – Data compilation

1 – OBJECTIVES

According with the project proposal, this task was finished after 6 months of work. The accomplishment of this task provided a database of environmental variables and benthic communities of the Mondego and Mira estuaries, which basic information supported the research on the effects that physical stress variables have on benthic invertebrate communities.

2 – DESCRIPTION OF THE RESEARCH WORK, RESULTS AND MAIN CONCLUSIONS

An exhaustive search was carried out during the first months of the project to compile all the relevant information on benthic communities of estuaries presenting seasonal changes, published in scientific journals or available as grey literature.

The deliverables produced within this task may be listed as (see Annexes):

i) List of references regarding the Mondego estuary;
ii) List of references regarding the Mira estuary;
iii) List of references regarding the influence of freshwater flows and extreme climatic events on macrobenthic communities;
iv) List of physical-chemical and biological parameters produced by other studies regarding the Mondego estuary;
v) List of physical-chemical and biological parameters produced by other studies regarding the Mira estuary;

TASK 2 – Data collection

1 – OBJECTIVES

To sample the subtidal soft-bottom macrobenthic assemblages and assess the water quality, seasonally during the project's first year, covering the salinity stretches previously identified in the Mira and Mondego estuaries. The new biological and physicochemical data collected during the EXTREMIS project should complement the already existent long-term datasets. The amount of data to be produced on the benthic composition, abundance and biomass, should provide a good characterisation of the spatial and seasonal patterns of both estuaries, and allow to relate these short-term changes on the benthic structure to the observed environmental changes.

2 – DESCRIPTION OF THE RESEARCH WORK, RESULTS AND MAIN CONCLUSIONS

MACROFAUNA

Database description

MONDEGO: Previous studies carried out during the last two decades on the Mondego estuary, provided comprehensive long-term data sets, namely for benthic macroinvertebrate communities. These data cover the macrofaunal community structure (species composition and abundance – numbers and biomass) and functioning issues.

Subtidal soft-bottom macrobenthic assemblages were first sampled in 1989/1990. Ever since, quantitative samples were regularly collected seasonally in 1990 and 1992, and in springtime for 1998, 2000 and 2002 at 14 sampling stations located at the lower Mondego estuary, covering its last 8 km. From 2003 onwards 11 new sampling stations were added covering the upper parts of this estuary (Mesohaline and Oligohaline zones). These 25 sampling stations were seasonally sampled in winter, spring, summer and autumn until spring 2007.
From summer 2007 on, due to logistic demands, a new reduced subtidal monitoring network (Figure 1) was implemented in the estuary:

Complete subtidal monitoring network - 25 sampling stations, placed as to cover the whole estuarine area, start to be sampled annually only in springtime so that the spring data temporal series already existent to this estuary could be maintained;

Reduced subtidal monitoring network - from the previous 25 sampling stations were chosen the most representative 15 subtidal sampling stations covering all saline stretches and some particular habitats previously identified in this estuary (Teixeira et al., 2008). The samples were taken in the remaining seasons (winter, summer and autumn) extended over the following sampling stations: St1, St2, St3, St4, St6, St7, St8, St9, St12, St14, St18, St19, St21, St23, and St25

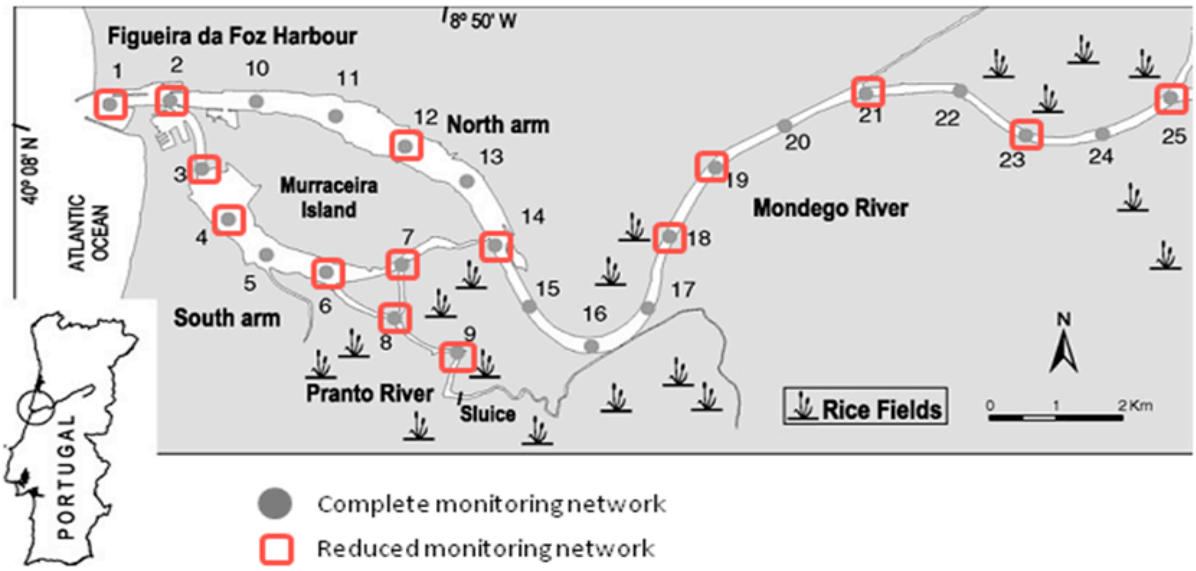


Figure 1. Mondego estuary. Location of the subtidal sampling stations in the complete monitoring network (25 sampling stations) and in the reduced monitoring network (15 sampling stations)

MIRA - Biological data series from the Mira estuary are not abundant and were mostly provided by punctual (Andrade, 1986; Campos & Fonseca, 1985; Bruxelas et al., 1985) and short term ecological/biological studies (Chainho *et al.*, 2008). An earlier project aiming to assess quality changes related to environmental oscillations, where part of the present research team participated, developed a seasonal sampling survey (4 times a year) covering all the salinity stretches previously identified for the system (Chainho et al., 2006). This study was conducted during a full annual cycle, and each of the 10 selected sampling stations

(Figure 2) were characterised by 3 benthic macroinvertebrates replicates, collected subtidally by a modified van Veen grab (0.05 m^2).

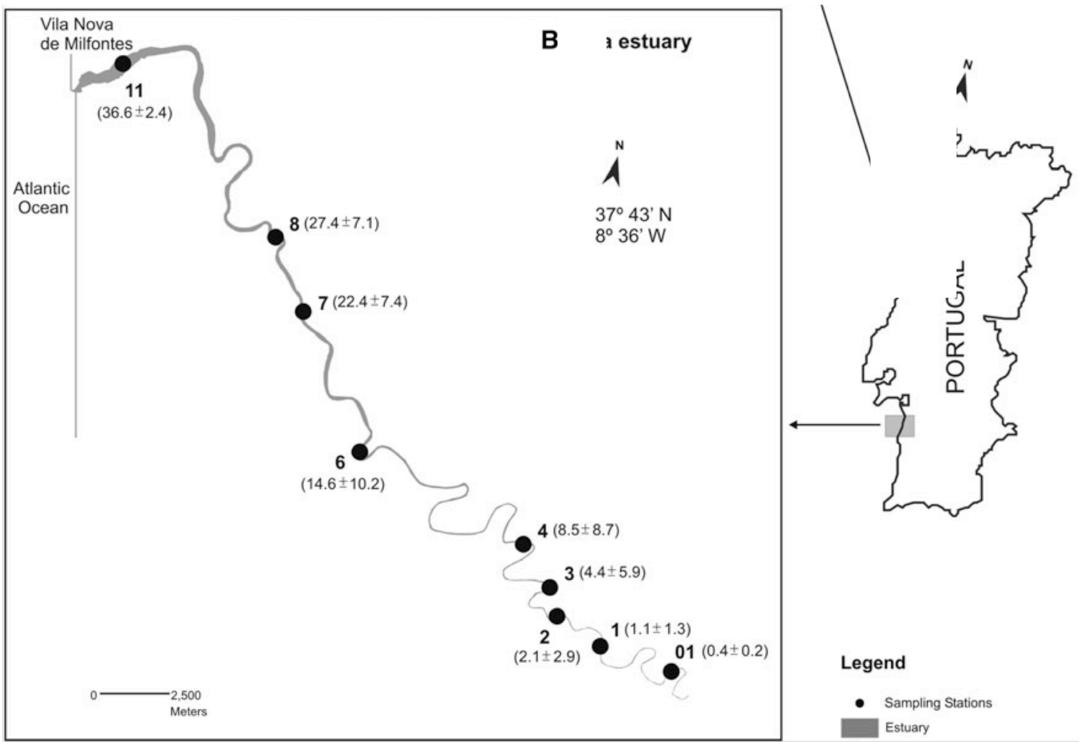


Figure 2. Mira estuary. Location of the subtidal sampling stations and bottom salinity range (in brackets).

Data collection

MONDEGO - Subtidal soft-bottom macrobenthic assemblages were sampled seasonally during the project's first year, covering the salinity stretches previously identified in the estuary.

To characterise the subtidal communities, samples were taken quarterly in soft-bottom substrates during high tide. Biological samples were collected using a LMG model of a Van Veen grab with 0.078 sq m area, gathering randomly three replicates each time at each station.

In the laboratory, samples were sieved through a 0.05 mm mesh size and fixed with 4% buffered formalin solution. Afterwards, macroinvertebrates were sorted and preserved in 70% ethanol for subsequent identification and counting. Whenever possible the organisms were identified up to species level (or lowest reliable taxonomic level) and the biomass (g AFDW m^{-2}) determined. The organisms were quantified in terms of abundance and biomass. Sediment samples were also collected for granulometric measurement and for organic matter content determination at each sampling station.

MIRA – Studies on benthic macroinvertebrates are extremely time consuming. From field samples collection, throughout sorting and identification tasks, the demand on time and human resources are extremely high. For the present project, due to several order constrains and research interests (human, logistic, data availability), it was decided to work out the Mira's previously collected biological samples (2006) to totally complete existent data series instead of processing new ones and leave gaps on data series. In a very similar way as the predicted for the present project, and covering coincident salinity stretches as the ones required for this study, subtidal soft-bottom macrobenthic assemblages were already sampled in the Mira (and simultaneously in the Mondego). As those Mira samples were not completely processed at the starting moment of EXTREMIS, resources were conducted to complete data series on its full extension without leaving Information gaps. For the objectives of the project, comparisons between macrobenthic communities from the Mondego and the Mira were not compromised since data from the Mondego estuary were also collected, coincidently in time and under the same climatic conditions as those from the Mira.

During 2006, samples were taken quarterly in soft-bottom substrates. Biological samples were collected using a LMG model of a Van Veen grab with 0.05 sq m area, gathering randomly three replicates each time at each station. In the laboratory, samples were sieved through a 0.05 mm mesh size and organisms fixed in 4% buffered formalin solution.

During EXTREMIS project first year, after sorting, macroinvertebrates were preserved in 70% ethanol for subsequent identification and counting. Whenever possible the organisms were identified up to species level (or lowest reliable taxonomic level) and the biomass (g AFDW m^{-2}) determined. The organisms were quantified in terms of abundance and biomass. Sediment samples were also collected for granulometric measurement and for organic matter content determination at each sampling station.

Field campaigns and laboratorial procedures: current status

MONDEGO - The new macrofauna data collected during the EXTREMIS project complemented the already existent long-term dataset on the benthic communities. A synopsis for the status relatively to field campaigns carried out and laboratorial procedures (sorting and taxonomic identification) is shown in Table 2.

Table 2. Processing status of Mondego's estuary subtidal macrobenthic data: √ - samples processed; x - samples not processed yet. Wi - Winter; Sp - Spring; Su - Summer; Au - Autumn.

Year	**Field campaigns** (Data collected)	**Laboratorial procedure of subtidal samples**	
		Sorting	**Taxonomic identification**
1990 to summer 2008	Study previously undertaken in the scope of IMAR-CMA research activities; Data already existent.		
2008	Au	√	√
2009	Wi	x	x
	Sp	x	x
	Su	x	x

MIRA - Macrofaunal data collected previously to the EXTREMIS project was completely identified to species level in order to incorporate the already existent dataset on the benthic communities. A synopsis for the status of the task is shown in Table 3.

Table 3. Processing status of Mira's estuary subtidal macrobenthic data: √ - samples processed; x - samples not processed yet. Wi - Winter; Sp - Spring; Su - Summer; Au - Autumn.

Year	**Field campaigns** (Data collected)	**Laboratorial procedure of subtidal samples**	
		Sorting	**Taxonomic identification**

1984 (punctual); autumn 2003; winter 2004	Study previously undertaken in the scope of research activities; Data already existent.		
2006 campaign	Wi, Sp, Su, Au	√	√

PHYSICOCHEMICAL PARAMETERS

Database description

MONDEGO - The subtidal dataset comprises information gathered in 1990 and 1992 (winter, spring, summer and autumn), in the springs of 1998, 2000 and 2002, at 14 stations, covering the estuary terminal part (Figure 1). This initial database has been regularly expanded since 2003 thanks to several projects that sponsored the survey activities in the estuary and to private investments from IMAR. Since 2003, the physicochemical characterization has been done along 25 sampling stations, covering the entire estuary (Figure 1), from the river mouth to the Pranto sluice (through the Southern arm), and till the Arunca river discharge point (in the Northern arm). All salinity stretches (from euhaline until the oligohaline area) are included in the sampling program.

The database includes information regarding the following physicochemical factors: water depth, transparency, salinity, temperature, pH, dissolved oxygen, ammonia, nitrates, nitrites, phosphate, silica, chlorophyll a level, TSS and POM. Sediment granulometry and organic matter content are also included.

MIRA – The water column dataset comprises information gathered in June 1984, October 2003, March 2004, seasonally in 1997/98, February and September of 2002 and 2003, September 2004, and seasonally in 2006, from an irregular sampling stations' network. Physicochemical parameters registered in water samples (although not the full list of parameters in all sampling occasions) were the water depth (m) and transparency (m, Secchi disk), and the water temperature (ºC), salinity, pH, Oxygen (%, mg L^{-1}), silica (mg L^{-1}), Particulate Organic Matter (POM), Total Suspended Solids (TSS), and Chlorophyll a. Sediment was also collected and determined its grain size distribution and organic matter content. Database includes information regarding all salinity stretches, from euhaline until the oligohaline area.

Data collection

MONDEGO and MIRA - The water quality in the estuary was characterised based on water samples collected at the subtidal areas, during high tide (subsurface and bottom).

a. Environmental parameters measured *in situ*.

Whenever possible, water column environmental parameters were measured *in situ* using portable analytical instruments. Measurements were taken monthly, at high tide situation, from subsurface and bottom water samples.

Depth data were always registered from the deepest part of the channel by using a measuring rope. Water transparency was determined by Secchi Disk. Temperature and salinity were registered by using a portable probe LF 330/ Set WTW. Oxygen and pH were also measured using, respectively, pH 330i/ Set WTW and Oxi 330i/ Set WTW portable devices. Subsurface water samples were collected in a bottle submerged 0.3 - 0.5m, and bottom samples by a sampling bottle (2 L). Bottom water sampler was open near the bottom, closed on the same depth, and pulled back to the surface with the opening closed (to avoid the water to get mixed with water column water). Water samples were transported to the laboratory on cooling boxes.

b. Parameters determined in the laboratory

The subsurface and bottom water samples, collected for *in situ* determination of physicochemical parameters, were filtered (Whatman GF/C) to remove suspended materials and frozen (-18ºC). The concentration of nutrients, ammonia-nitrogen (mg L^{-1} $N\text{-}NH_4^+$), nitrate-nitrogen (mg L^{-1} $N\text{-}NO_3^-$), nitrite-nitrogen (mg L^{-1} $N\text{-}NO_2^-$) phosphorus from orthophosphates (mg L^{-1} $P\text{-}PO_4^{3-}$) and silica were determined following standard procedures (APHA 1980, Strickland & Parsons 1972). Filters were used to determine total suspended solids, particulate organic matter and chlorophyll *a* concentration.

Sediment was sampled at same time as benthic macroinvertebrates survey. Sediment samples were also taken using a Van Veen grab LMG model, according to the procedures described for benthic macroinvertebrates sampling. Sediment organic matter content was quantified by weight difference between sediment after oven drying at 60 8C for 72 h and after combustion at 450 8C for 8 h, and expressed as a percentage of total sample weight. Grain size analysis was carried out by mechanical separation through a column of sieves with different mesh sizes. Sediments were classified as coarse sand (0.5 mm), fine + medium sand (>0.063 and <0.5 mm), silt (>0.038 and <0.063 mm) and clay (0.038 mm), adapted scale from Brown and McLachland (1990). Grain composition was expressed in percentage of total sample weight.

Field campaigns and laboratorial procedures: current status

MONDEGO - The new physicochemical data collected during the EXTREMIS project complemented the already existent long-term dataset on environmental parameters. A synopsis for the current status relatively to field campaigns carried out and laboratorial analysis is shown in Table 4.

Table 4. Summary of the current status of Mondego estuary physicochemical data processing. √**:** Samples processed. x: Samples not processed yet.

Year	**Field campaigns** (Data collected)	**Laboratorial analysis**	
1990 to August 2008	Study previously undertaken in the scope of IMAR-CMA research activities; Data already existent.		
2008	September to December	Salinity, temperature, depth, transparency, dissolved oxygen, pH, nutrients, silica, TSS, POM, sediment organic matter and granulometry	√
2009	January to August	Salinity, temperature, depth, transparency, dissolved oxygen, pH, nutrients, silica, TSS, POM, sediment organic matter and granulometry	√

MIRA - The physicochemical data collected before the EXTREMIS project will be used on the work (Table 5).

Table 5. Physicochemical data processing status of Mira estuary. √**:** samples processed. x: samples not processed yet.

Year	Field campaigns (Data collected by)	Laboratorial analysis	
June 1984 (punctual)	Andrade, 1986; Campos & Fonseca, 1985; Bruxelas, Ferreira & Albergaria, 1985	Salinity, temperature, depth, transparency, dissolved oxygen, sediment's grain size distribution	√
February-October 1989	Ferreira et al., 2003	Temperature, salinity and chlorophyll *a*	√
Seasonal 1997/98	IMAR	Temperature, salinity, dissolved oxygen and water transparency	√
February and September 2002/03; September 2004	INAG	Salinity, temperature, dissolved oxygen, pH, TSS, nutrients (NO_3, NO_2, NH_4, P, Si), chlorophyll (*a, b, c*), TOC, and sediment's grain size distribution	√
October 2003; March 2004	Chainho *et al*., 2008	Salinity, temperature, depth, dissolved oxygen, nutrients (NO_3, NO_2, NH_4, P), TSS, sediment organic matter and granulometry	√
Seasonal 2006	IMAR and CO (FCUL)	Salinity, temperature, depth, transparency, dissolved oxygen, nutrients (NO_3, NO_2, NH_4, P, Si), TSS, sediment organic matter and granulometry	√

TASK 3 – Laboratorial and *in situ* experiments

1 – OBJECTIVES

Within the framework of the main goal of the EXTREMIS project - to assess the effects of freshwater discharges and associated salinity variations on the benthic communities of two small Portuguese estuaries (Mondego and Mira) - the specific objective of Task 3 was to adapt short-term laboratory and *in situ* testing methodologies, developed for estuarine/marine groups of organisms, to the environmental conditions of the Mondego and Mira estuaries, to assess the influence of salinity variations independently of other factors (contaminant/environmental).

Task 3 intended to adapt such methodologies to key species in the functioning of the estuaries. Four species were originally proposed as potentially important species in the functioning of the Mira and Mondego estuaries while covering the entire range of habitat preferences in terms of salinity (from euhaline to oligohaline), namely: (1) the polychaete *Hediste diversicolor* - epibenthic omnivorous, (2) the decapod *Carcinus maenas* - benthic omnivorous, the (3) gastropod *Hydrobia ulvae* - benthic surface deposit feeder herbivore/detritivore, and 4) the amphipod *Corophium multisetosum* - epibenthic surface deposit feeder detritivore. As a representative of the amphipods, a literature review conducted at the start of the project revealed that using the species *Gammarus locusta* would be more valuable than to use C. *multisetosum*. This was because the species *G. locusta* had already been shown to be tolerant to certain environmental variables and sensitive to contaminants, factors that support its adaptation to laboratory and in situ methodologies. As both amphipod species have a sporadic occurrence in the Mondego and Mira estuaries, this replacement was considered not to affect the objectives of Task 3. Thus, with the ultimate goal of working out an assay based on the feeding response of amphipods after exposure to stressors, research to establish a standardised method to quantify the feeding rate of *G. locusta* was conducted during the project. However, the establishment of sound experimental designs and thus the achievement of reliable results were both highly compromised by the limited occurrence of this species at the reference sites, i.e., by the low number of organisms available to conduct experiments. Besides, some information on the salinity tolerance, alone and in combination with other environmental variables (e.g. sediment grain size, food availability) on the polychaete *H. diversicolor* and the decapod *C. maenas* had been previously explored by the research team. Thus, research during the EXTREMIS project focused mainly on the species *H. ulvae*. The latter species is known not only to be tolerant to wide variations in salinity (from around 4 to 40) and also temperature (from around 5 to 35°C), but also to play an important role in European estuarine food webs, both as a decomposer and a secondary consumer, being thus a valuable candidate to fulfil the main project objectives.

2 – Description of the Research Work, Results and Main Conclusions

Given the above in what regards the selection of key species in the functioning of the estuaries, research during the EXTREMIS project focused on the adaptation of laboratory and in situ testing methodologies to the gastropod *H. ulvae*, to assess the direct effects of salinity variations on the species performance and the potential ultimate effects of natural salinity variations in the functioning of the estuaries. Like for other groups of estuarine/marine organisms, postexposure feeding rate was selected as the experimental endpoint, because it allows direct measurements of ecosystem functions/processes; an impairment in feeding has direct and immediate effects on ecosystems, by hampering key functions (e.g. organic matter decomposition), long before its effects at the individual level have consequences at successively higher levels of biological organization. Because feeding responses when the organisms are exposed to stress factors usually persist in the period immediately after exposure and due to the difficulty of determining processing rates during exposure in the field, and even in the laboratory, a postexposure feeding technique has been employed in many studies. Moreover, because measuring the feeding rate of *H. ulvae* is difficult, as the amount of food eaten is hard to quantity, egestion rate was used as a surrogate for ingestion rate, i.e., the experimental endpoint was selected based on the premise that an impairment of feeding rate may lead to a subsequent reduction in the egestion rate.

The research conducted with this species can be divided into four subtasks, namely: (1) Adaptation of experiments based on the postexposure feeding of *H. ulvae*, (2) Short-term laboratory experiments to assess the effects of salinity, alone and in combination with other stressors, on *H. ulvae*, (3) Short-term in situ experiments to assess the effects of salinity, alone and in combination with other stressors, on *H. ulvae*, (4) Short-term in situ experiments to assess the effects of stressors on *H. ulvae* independently from other stress factors, and (4) Laboratory experiments on the feeding behaviour of *H. ulvae* under more realistic scenarios of feeding conditions, to gauge the role of *H. ulvae* on the Mondego estuary food webs.

2.1. Adaptation of experiments based on the postexposure feeding of *H. ulvae*

To adapt experiments based on the postexposure feeding of *H. ulvae*, using egestion rate as a surrogate of feeding rates, two sequential research phases were accomplished:

First phase – a standardised method to quantify, after exposure, the egestion rate of the organisms under controlled conditions, with a high level of precision and preventing an eventual physiological recovery from the effects of potential stressors during the exposure period (contaminants/environmental), was established. Experiments conducted combining different conditions and periods during feeding and egestion allowed easily establishing a procedure to determine mean posexposure feeding rates of *H. ulvae* within a postexposure period of two hours and a half with a good level of precision (coefficient of variation ≤ 25%).

Second phase – the sensitivity of the postexposure feeding responses after a 48 hours exposure to stress factors was assessed, as only a responsive measurement allows discriminating effects of stressors. As a model stressor, the reference toxicant copper (as copper sulphate) was selected because it is known to cause adverse effects on biological organisms at low concentrations and with a good level of precision. The sensitivity of the postexposure feeding rate was compared with that of other sublethal (28 days growth) and lethal (96 hours hours mortality) endpoints after exposure of *H. ulvae* to a range of copper concentrations.

The experiments conducted showed the 48 hours postexposure feeding determinations to be three times more sensitive to copper exposure than 96 hours lethality responses, confirming the results of several other studies demonstrating that feeding inhibition on other invertebrate species was a more sensitive endpoint than lethality in determining toxicity to a wide range of chemicals. Results also showed that postexposure feeding was less sensitive than growth. Yet, the observed difference in sensitivity was very close from the factor of 2-fold difference that is acceptable in toxicity tests. Moreover, the use of postexposure feeding as an experimental response has major attractive features compared to the use of growth, especially for in situ studies. Growth measurements require long exposure periods, which involve a huge effort in time and expenses, due to the need for periodic field trips to clean the chambers mesh windows to guarantee water flow, and increases the risks of vandalism.

2.2. Short-term laboratory experiments to assess the effects of salinity, alone and in combination with other stress factors, on *H. ulvae*

To assess the effects of salinity on the postexposure feeding of *H. ulvae*, two sequential research phases were accomplished:

First phase – the effect of salinity as a single stress factor on *H. ulvae* was determined by exposing organisms to a range of salinities (from 0 to 35 units), and in this way complement field data. Experiments conducted revealed that the studied salinity range had no effects on the 96 hours mortality of *H. ulvae*. However, effects were revealed on the postexposure feeding of *H. ulvae*; the lowest salinity for which no effects were observed was 20 units, whereas the highest salinity for which effects were observed was 14 units. Effects were also observed on the growth of *H. ulvae*. Similarly to the effects observed for copper, the growth of the organisms was revealed to be more sensitive that their postexposure feeding, even though the difference was lower than a factor of two.

Second phase – the interaction of salinity with other environmental parameters on the 48 hours postexposure feeding rate of *H. ulvae* was determined by conducting a factorial experiment on the effects of a 48 hours exposure period to different levels of salinity (15 to 35 units), temperature (10 to 25°C) and sediment grain size (silty to sandy) on the feeding rates of *H. ulvae*. The combined influence of temperature, salinity and sediment particle size during a 48 h exposure on the postexposure feeding of *H. ulvae* was determined by a General Linear Model regression with a Poisson distribution (and a logit link function), which showed that postexposure feeding was significantly increased by increases in temperature, from 10 to 25 °C, in salinity, from 15 to 35, and by the interaction effect between salinity and the silt content – the fitted model explained 56% of the deviance observed in the postexposure feeding response. Therefore, these three environmental variables may act as confounding factors during in situ experiments, and thus, this regression model is a valuable tool to reduce uncertainties in the causal relationships between stressors and organism responses.

2.3. Short-term in situ experiments to assess the effects of salinity, alone and in combination with other environmental factors, on *H. ulvae*

The model developed under laboratory-controlled conditions of salinity, temperature and sediment was validated under more realistic exposure scenarios. For this, an in situ experiment, adapting previously developed chambers and procedures, was conducted by deploying organisms at three reference sites in the Mira estuary. Mean postexposure feeding rates observed in the field and predicted by the model at the reference sites were compared. Since no significant differences were detected between observed and predicted values, the model was validated not only to accurately predict the observed values at the three reference sites, but also as a potential tool to distinguishing effects of other stressors from effects associated with the variability in temperature, salinity and sediment grain size across study sites.

2.4. Short-term in situ experiments to assess the effects of contaminants on *H. ulvae* independently from other stress factors

The effectiveness of the methodologies adapted in the previous section to conduct situ experiments with *H. ulvae* and assess the effects of various stress factors on hydrobiids was evaluated by deploying organisms simultaneously at the three reference sites in the Mira estuary and at four contaminated sites in the Sado estuary (since the Mondego estuary is considered to be slightly contaminated). The Sado estuary is the second largest in Portugal with an area of approximately 240 Km^2. Although most of the estuary is classified as a natural reserve, there are many industries, mainly at the Northern margin of the estuary, and, harbour-associated activities, the city of Setúbal and copper mines on the Sado watershed all use the estuary for waste disposal purposes without suitable treatment. Three assay chambers with thirty-five organisms each were deployed at each study site to ease the retrieval at the end of the assay of the 30 organisms required for postexposure feeding quantification, since such density was below the threshold recommended for *H. ulvae*. All chambers were deployed at low tide and ensuring that the tide level would not decrease during exposure, to avoid air exposure of organisms during the assay. Each chamber was pushed into the sediment to a depth of about 14 cm (around 1 cm under the top of the lateral windows), to guarantee the exchange of both overlying water and porewater between the chamber and the surrounding area. After an exposure period of 48 hours, chambers were retrieved from the sediment and transported to the laboratory for postexposure feeding quantifications.

To be able to distinguish potential toxic effects from effects associated with the variability in environmental conditions across study sites, the postexposure feeding responses at the study sites were first adjusted to the same level of temperature, salinity and sediment grain size (environmental factors previously demonstrated to influence the measured organism response and thus to act as confounding factors); the model derived under laboratory conditions in the previous section and validated under field conditions at reference sites was used. The postexposure feeding of *H. ulvae* was significantly inhibited at three of the contaminated sites relatively to the references. As expected, the lowest postexposure feeding was registered at an area previously described as presenting a high risk to biota and where toxic effects on the postexposure feeding of the polychaete *H. diversicolor* have been formerly detected.

The *in situ* experiment here proposed is a valuable tool to be added to the battery of *in situ* assays that has been developed in recent years to assess the toxicity of estuarine sediments. In effect, it constitutes an important addition to the existing battery of test species with a key role in the functioning of estuaries, and thus to increase the ecological relevance of the environmental decisions taken for protecting these very productive habitats.

2.5. Laboratory experiments on the feeding behaviour of *H. ulvae*

To gauge the role of *H. ulvae* on estuarine food webs, at different salinity stretches, a study on the feeding activity of *H. ulvae* on the major primary producers available at the Mondego estuary, not only seagrass beds (*Z. noltii*) and green macroalgae (*Ulva* sp. and *Gracilaria* sp.) but also microphytobenthos, was accomplished in two research phases:

First phase – the ability of *H. ulvae* to feed on the major sources was determined by quantifying the ingestion and assimilation rates (as total organic carbon) and the egestion rates (as total number of faecal pellets produced) of hydrobiids fed separately the following food types: *Z. noltii*, *Gracilaria* sp., and *Ulva* sp. from the central and apical regions of adult stages, all with and without epiphytes. All provided food types were ingested by snails so that none of them can be considered grazing resistant. Moreover, the presence of periphyton did not alter the grazing by *H. ulvae*. Although when fed on *Z. noltii* the hydrobiids showed the highest ingestion and egestion rates, the fact that overall assimilation rates ranged between 50 and 70% indicated low assimilation efficiency toward *Z. noltii*. These findings strongly suggest *H. ulvae* to be a generalist grazer.

Second phase – a study on the feeding behaviour of hydrobiids under a more realistic scenario of feeding conditions was conducted, by performing experiments to evaluate the feeding preferences of hydrobiids given simultaneously the opportunity to choose among the following food sources, provided *ad libitum*: *Z. noltii*, *Ulva* sp. and *Gracilaria* sp., all three with and without the associated perithytes, and sediment rich in microphytobenthos. Each replicated unit comprised the seven feeds (3 sub-replicates of each) randomly distributed, and the hydrobiids were allowed to feed for 24 h. As of the first hour of the experiment, sediment was clearly the preferred food, being the percentage of organisms in this food between 30 and 60%. Yet, *Z. noltii*, either with or without periphytes, was also a preferred food relatively to the remaining four types of food (10 – 20% of organisms). Thus, the present results do not support the hypothesis that *H. ulvae* may have a role in preventing estuarine macroalgal blooms.

TASK 4 – Ecological modelling

1 – Objectives

The aims delineated for this task were achieved through the development of system modelling coupled to energetics models. The system model is based on a macroalgal productivity model coupled to GIS, which can predict opportunistic algal biomass for the whole system under extreme weather scenarios. Energetic models for *H. ulvae* and *C. maenas* should be developed to detect how these consumers assimilate and utilise energy under different scenarios concerning environmental conditions and macroalgal productivity.

2 – Description of the Research Work, Results and Main Conclusions

The research work for this task has been focusing on two components: the modelling component and the experimental component to obtain parameters for the bioenergetics models.

2.1. Modelling components

2.1.1. The system model for macroalgal productivity

The model used to simulate *Ulva intestinalis* productivity at the system level is based on a core model developed by Martins et al. (2007) (Figure 3). The model describes the growth of adult macroalgae, as well as the germination and growth of macroalgal spores per square meter. Subsequently, using bathymetry data, the model is upscaled to the system area and allows estimations of macroalgal productivity and nutrient loading at the whole system level. Because this model has been calibrated for the Mondego estuary, it can be further used for any other data sets of external parameters, namely, the ones corresponding to variations for extreme weather events.

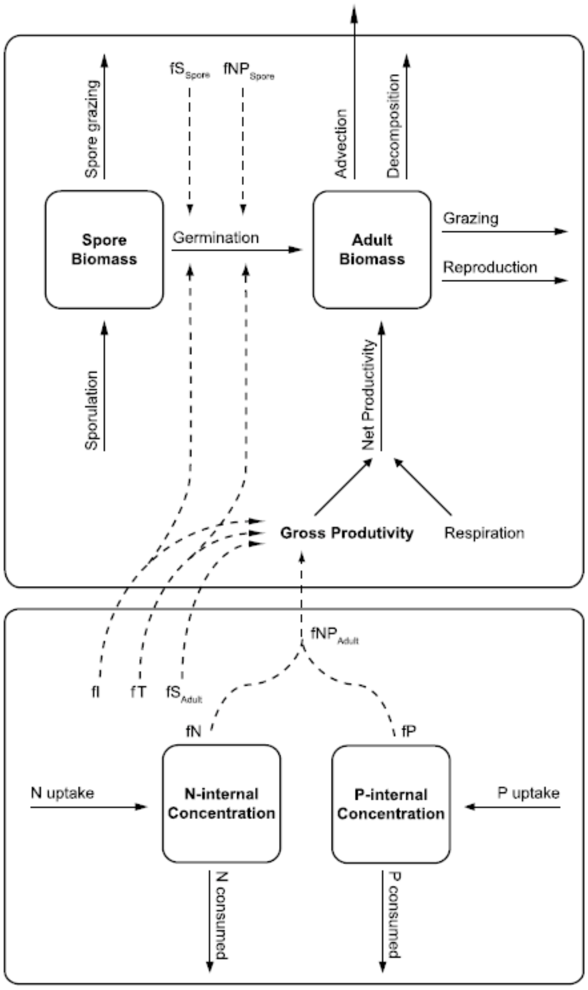


Figure 3. Conceptual diagram of the model used to simulate *Ulva intestinalis* productivity at the system level (from Martins et al., 2007).

2.1.2. Energetics models

The DEB theory assumes that the utilisation of energy by an individual for growth, maintenance and reproduction involves at least three compartments: storage, body volume and reproduction (Bacher & Gangnery, 2006). The relationships between these compartments and their flows follow a number of assumptions that constitute the DEB theory (Kooijman, 2008).

Since February 2009, the researcher responsible for this task has been following a course on DEB theory (http://www.bio.vu.nl/thb/deb/course/), which is being coordinated by the theory's creator: S. A. L. M. Kooijman from the Free University of Amsterdam. The aim is to understand and develop a general DEB model capable of predicting the energetic budget of organisms under different conditions. Then, the introduction of species-specific parameters within the general DEB model will allow making predictions at the species and/or population level.

The DEB models referring to *Hydrobia ulvae*, *Carcinus maenas* and *Hediste diversicolor* (Figures 4, 5 and 6) are in the phase of model testing, with verification, calibration and sensitivity analysis being performed, following an iterative process. The processes used in the model were obtained either experimentally, following experimental protocols specifically developed under this project or extracted from literature.

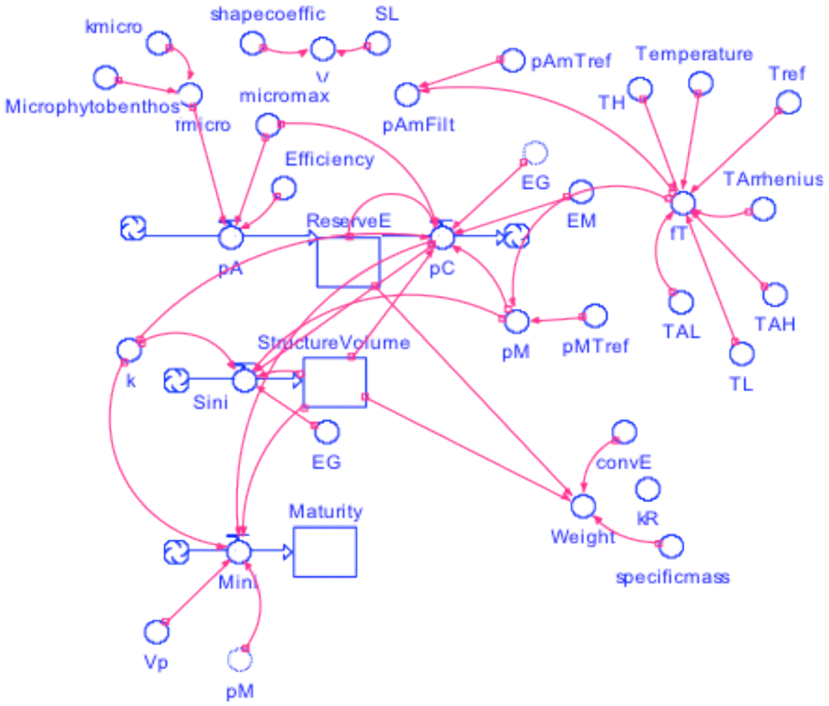


Figure 4. Conceptual diagram of the DEB *Hydrobia ulvae* model.

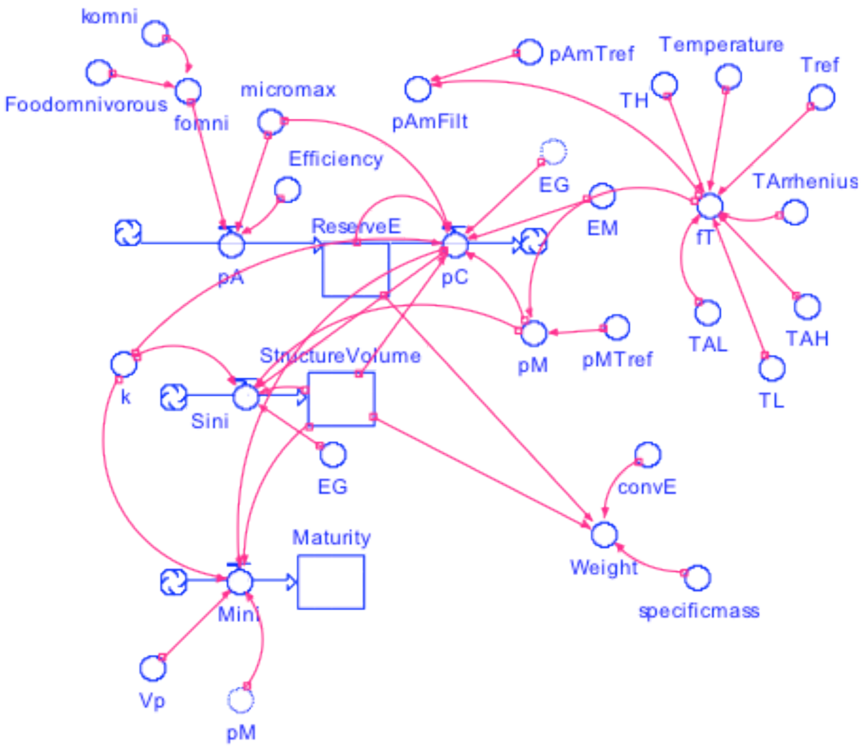


Figure 5. Conceptual diagram of the DEB *Carcinus maenas* model.

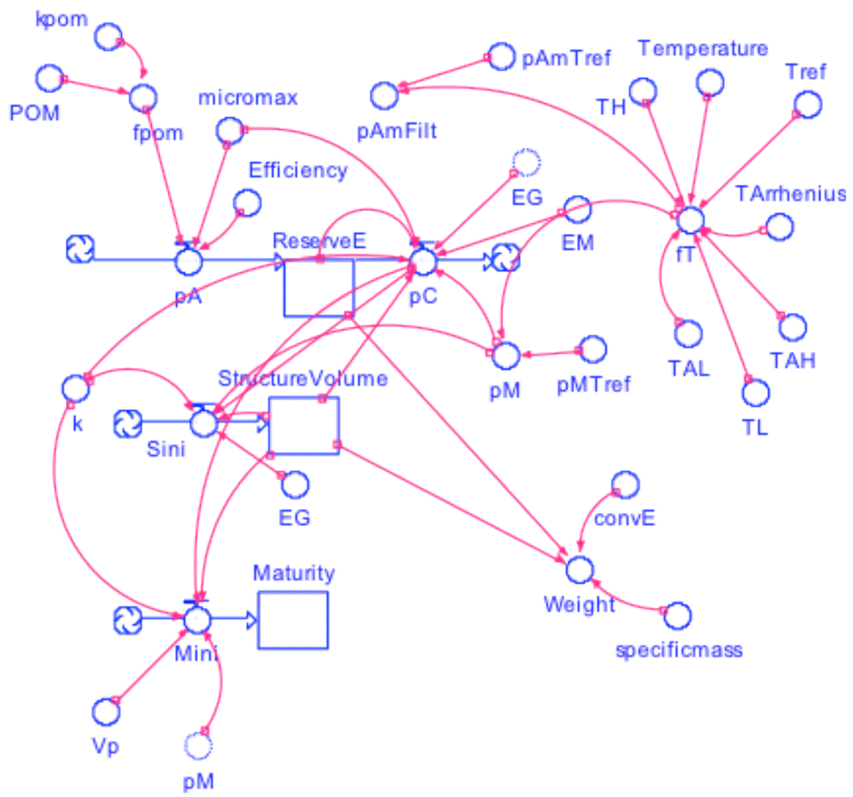


Figure 6. Conceptual diagram of the DEB *Hediste diversicolor* model.

The calibration of DEB models is difficult and time consuming due, in one hand, to the inner complexity of this type of models and, on the other, to the experimental limitations in obtaining some of the parameters. In spite of this, the three DEB models are being further developed with MATLAB software.

2.2. Experimental component to determine DEB parameters

Since May 2009, experimental protocols and laboratorial setups have been developed to ascertain the species' parameters to include within DEB models.

This experimental component included both field and laboratorial work. The fieldwork consisted on the collection of the animals, sediment and water to be used in the experiments. The laboratorial work consisted on animal's acclimatization (approximately 1 day), setting up the conditions for the experiments (e.g. filtering sea water, burning the sediment to exclude all organic matter, cultivate microalgae to feed *H. ulvae*, etc) and finally run the experiments to obtain the required parameters.

The following parameters were assessed for the selected species: maximum food ingestion rates, assimilation efficiency, energy for maintenance, energy for growth, energy for reproduction and structural volume. Furthermore, biometric parameters such as age and length at birth, age and length at puberty, maximum length and weight versus length were measured or obtained from the literature.

Presently, most parameters concerning *C. maenas* are already available from experimental work developed by Nathan Hoefnagel at the NIOZ (The Netherlands) and the experiments with *H. ulvae* and *H. diversicolor* were fully carried out at IMAR-CMA, University of Coimbra.

Experimental work with *H. ulvae* and *C. maenas* allowed obtaining essential parameters to include in DEB models.

Hydrobia ulvae experiments

Experiment 1: Starvation for V, [Em], [pM], z and δ_M
A starvation experiment was performed to ascertain the fraction of the organic weight of the snails that serves as reserves. Seventy individuals of *H. ulvae* within the range of 4 to 6 mm TSL were equally divided over ten experimental recipients, with a volume of 10 ml each. Visible epiphytes attached to the snails' shells were removed, to avoid food supply for the snails during the experiment. Each experimental recipient contained 3 ml of autoclaved and filtered seawater, which were replaced, at least, three times per week to avoid oxygen depletion and contamination of the water by waste products.

The starvation experiment started at the 11th of November 2009 and lasted until the 6th of January 2010 (56 days). Ten individuals were removed from the recipients once every one or two weeks to determine changes in weight. The first sample was taken on day zero (November 11). Each sample consisted of one snail from each experimental recipient to have an average measure. Snails were dried and combusted to determine dry weight and (AFDW). AFDW was plotted against starvation time to analyze the decrease in the organic matter. The difference between the first and the lowest measurement was interpreted as energy reserves; the rest would then be structural body volume. Maintenance rate was calculated from the slope of the decreasing weight over time

Experiment 2: Consumption for {Jxm} and {pAm}
A consumption experiment was carried out to determine the maximum energy intake on the long term by *H. ulvae*. Satiated individuals were used to exclude peak consumption due to starvation prior to the experiment. TSL was measured in a stereo loupe with magnification 4x and a meter with accuracy to 0.08 mm. Twelve size classes between TSL = 3.08 mm and TSL = 6.16 mm were considered. Each size class contained 10 snails which were placed in a 30 ml vial. Snails were fed with the diatom *Phaeodactilum tricornutum*, which is a preferred prey for *H. ulvae* (Lopez-Figueroa and Niell, 1988). Algae concentration was determined before and after each trial to estimate the number of ingested algal cells by *H. ulvae*. Each size class received 10^7 diatom cells in each one of the three trials, lasting between 18 and 20 hours. Algal concentration was determined with a micro liter counting chamber using four replicated counts or more per size class per trial. The difference in cell concentration between initial and final counts was converted into cell consumption per snail, per day, and into joules per snail per day using a conversion factor of $4{\cdot}10^{-7}$ J cell^{-1} (Riisgard and Randlov, 1981). Sometimes there were less than 15% of the algae left in a vial, indicating that food was not *ad libitum* in those cases. These data points were excluded from calculations, since the low food density can constrain the consumption and bias the outcome. Consumption can be expressed in energy per unit of structural area when the structural body volume is known. Assimilation efficiency was set at 0.7, which is the assimilation efficiency of *Hydrobia ventrosa* feeding on diatoms (Kofoed, 1975). This value was used, because it was not possible to collect enough faeces to calculate assimilation effiency with Conover's ratio (Conover, 1966). Therefore, {pAm} is equal to 0.7 · {Jxm}.

Experiment 3: Growth for [EG] and [pM]

Twenty-five individuals of *H. ulvae* with TSL between 3.9 and 4.1 mm were placed individually in 10 ml plastic recipients and each group of five individuals received one of five food regimes for five weeks. These regimes were, respectively, 100, 50, 20, 5 and 1% of the consumption that was previously measured in the consumption experiment. TSL was measured four times during the five weeks of the experiment using a stereo loupe (16 x magnification gives resolution = $5.88 \cdot 10^{-3}$ cm). Increase in structure was based on length data, because AFDW of satiated snails was not available. Thus the increase in *V* was calculated by subtracting the initial *V* from the final *V* as calculated with the previously determined relation of *V* with TSL. The food density when growth is zero can be used to calculate maintenance costs, because the snails use all their energy for maintenance at that point.

DEB model

The obtained data and parameter values were used in a computer model based on the energy fluxes in a living organism. The parameters are surface-area specific ingestion rate {Jxm} in J d^{-1} cm^{-3}, costs for generating a unit of structural volume [EG] in J cm^{-3}, volume specific maintenance rate [pM] in J cm^{-3} d^{-1}, and Energy density, [EM] in J cm^{-3} structure. See Table 2 for parameter values and conversion factors that were obtained from literature.

Structural body, shape coefficient and zoom factor

Structural body *V* was calculated with AFDW of the snails and correlated with TSL^3. The shape coefficient and "zoom factor" can be calculated when the structural body volume is known. These descriptive coefficients make the results comparable with other organisms. The size of an organism can be measured in many ways. The size of a snail can be measured by either the width or the length of the shell. Shape coefficient (δM) converts the applied size measure to structural body volume (V) and the zoom factor z gives *V* relative to a *V* of 1 cm^3. The zoom factor z is given by the maximum volumetric length $V_{max}^{1/3}$.

All these parameters and factors were used to create an energy budget for *H. ulvae*. The DEBtool environment returns a great number of parameters that are calculated based on the input.

Results

Experiment 1: Starvation V, [Em], [pM], z and δM

The data show that AFDW of individuals of *H. ulvae* generally decreases throughout time during starvation (Figure 7). The AFDW increases in the last data point. The lowest value of AFDW occurs at 37 days, with 6.1 mg AFDW left at this point. The initial value of AFDW was 8.2 mg, so the difference can be used as reserves, giving (8.2 – 6.1)/8.2 = 25.3% of the organic weight. The slope of the trend line is used to calculate maintenance costs, expressed in joules per day. The slope is -0.0356 mg AFDW d^{-1}. The energetic value of *H. ulvae* organic material is 24.6 kJ per g (Rumohr, 1987) thus, 0.0356 mg AFDW per day is equivalent to 0.88 J d^{-1} for maintenance. The snails had TSL between 0.4 and 0.6 cm, so the average length was $(0.5 \cdot (0.4^3 + 0.6^3))^{1/3} = 0.52$ cm. This gives AFDW = $2.52 \cdot 10^{-3}$ g and 74.7% of that is $1.88 \cdot 10^{-3}$ g (and also cm^3) for V. This results in [pM] = 468 J d^{-1} cm^{-3}. The energy per unit of *V* is then $(25.3\% \cdot 2.52 \cdot 10^{-3} \cdot 24.6 \cdot 10^3) / 1.88 \cdot 10^{-3} = 8.34 \cdot 10^3$ J cm^{-3}.

The zoom factor and shape coefficient are calculated based on *V*. Maximum TSL is 0.8 cm, so $z = (74.7\% \cdot 1.79 \cdot 10^{-2} \cdot 0.8^3)^{1/3} = 0.19$. The shape coefficient δ_M is given by $V^{1/3}$/ TSL, so $\delta_M = 0.24$.

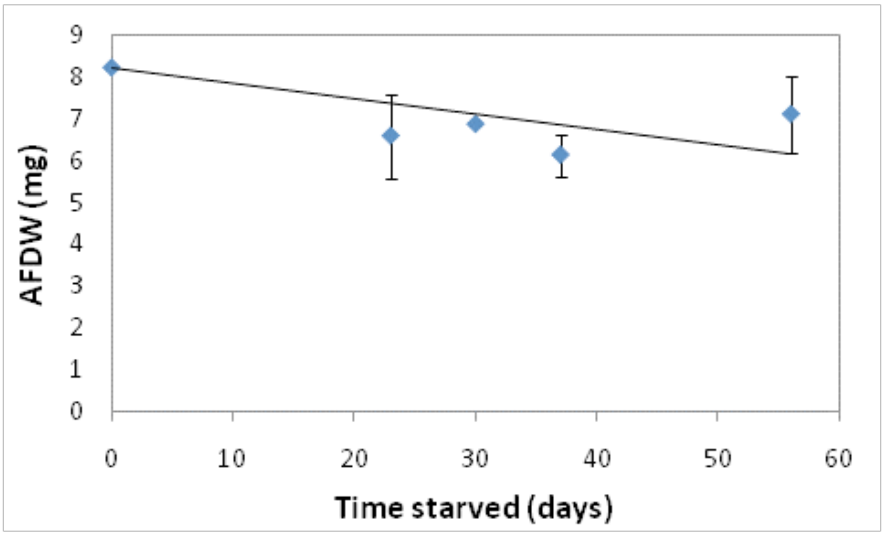


Figure 7. Variation of *H. ulvae*'s ash free dry weight during the starvation experiment. Error bars correspond to standard deviation. The trend line is given by AFDW = -0.0356 · Time + 8.207 ($r^2 = 0.14$)

Experiment 2: Consumption {Jxm} and {pAm}

Consumption generally increases with size (Figure 8), although this increase is not consistent throughout the whole size range. Consumed energy {Jxm} is given by the trend line of the average of the three trials:

{Jxm} = 6.77 · $V^{2/3}$ + 0.294 ($r^2 = 0.70$). The assimilation efficiency was set to 0.7 (Kofoed, 1975), giving that {pAm} = 4.74 · $V^{2/3}$ + 0.206.

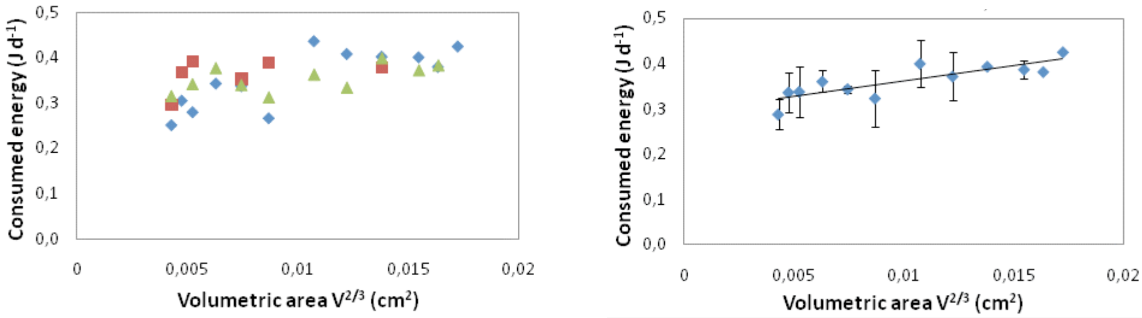


Figure 8. Consumed energy plotted over structural area. Colours denote different trials (A), the average of the three trials is plotted in the right figure (B). Data where more than 85% of the algae was consumed, were left out. Error bars correspond to SD.

Experiment 3: Growth [EG] and [pM]

The growth experiment yields an estimate for energetic costs of structure. Growth reaches a horizontal asymptote at high food levels (Figure 9). This asymptote was reached at $1.02 \cdot 10^{-4}$ cm^3 increase in structure in 35 days.

Snails are able to survive on food densities as low as 1% of previously measured consumption and none of them shrank or died except for one. Costs for growth are estimated

from the relation between energy that is allocated to soma and increase in structure. These data show that the production of one cm^3 of structure costs $43.4 \cdot 10^3$ J (Figure 9).
Four out of five snails that received only 1% of sufficient food survived (they received $3.24 \cdot 10^{-3}$ J d^{-1}), although two of them did not grow. Their initial *V* was $8.28 \cdot 10^{-4}$ cm^3 and this results in [pM] = 3.91 J d^{-1} cm^3.
The snails that did not grow were at the lowest two food densities indicating that the lower food limit for survival is below 5% of the consumption that was measured in the previous experiment.

Energy for reproduction ER and κ
A *H. ulvae* female maximally produces 150-200 eggs per breeding season and they live for 20 months (610 days). 175 eggs for two breeding seasons with $5.9 \cdot 10^{-3}$ J per egg (Van der Veer et al., 2006) gives a total of $3.87 \cdot 10^{-3}$ J d^{-1} on reproduction. This is 1.2% of assimilated energy in a snail of 0.6 cm TSL. Energy not allocated to maintenance and growth contains not only energy for reproduction, but also energy for maturity maintenance. Therefore κ was set at 0.98.

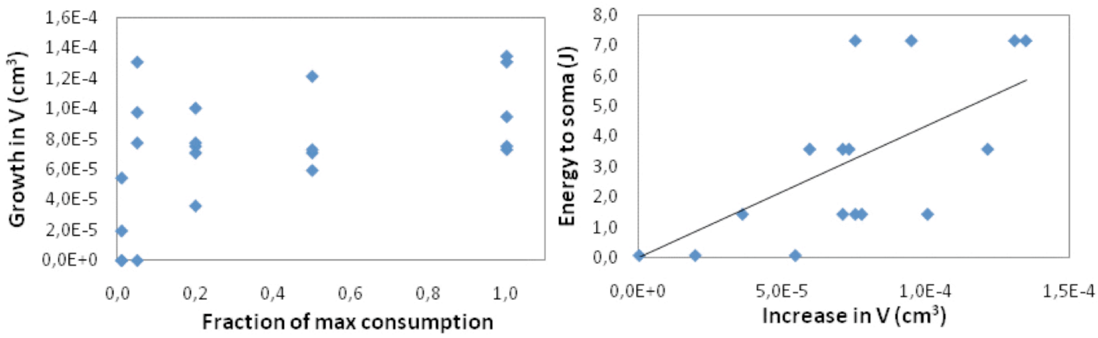


Figure 9. Growth in relation to food density (left) and energy to soma (right).

Increase in structural volume *V* (left, as calculated from initial and ultimate TSL) as a function of consumed energy. The latter is expressed as fraction of maximum consumption as measures in the Consumption experiment. The raw growth data (right). The trend line was forced though the origin and is given by $y = 43.4 \cdot 10^3 \cdot x$ ($r^2 = 0.47$)

Summary of results
All results from the experiments are summarized in Table 6. This table summarizes the results of the experiments in the present study. Correlation coefficient is given for {Jxm} and [EG] because these are calculated from a linear regression through the relevant data.

Table 6. Summary of results of the experiments with *H. ulvae.*

Parameter	Value	Corr. coeff.
V	$1.34 \cdot 10^{-2} \cdot TSL^3$	
[Em]	$8.34 \cdot 10^3$ J cm^{-3}	
z	0.19	
δ_M	0.24	
{Jxm}	$6.77 \cdot V^{2/3} + 0.294$	($r^2 = 0.70$)
AE	0.7	
{pAm}	$5.08 \cdot V^{2/3} + 0.220$	
[pM] (Exp 1)	468 J d^{-1} cm^{-3} (exp 1)	
	3.91 J d^{-1} cm^{-3} (exp 3)	
[EG]	$46.5 \cdot 10^3$ J cm^{-3}	($r^2 = 0.47$)
ER	$3.87 \cdot 10^{-3}$ J d^{-1}	

Discussion

An example of an energy budget

The equations and values as presented in the Results section will be tested for realism. The experiments and results are discussed below the test results.

The test includes only the maximum length and the age at which the maximum length is reached.

For *Hydrobia ulvae*:

Maximum length (Lmax) = 0.8 cm

Age at maximum length (A_Lmax) = 392 d (adult life when life span is 20 months)

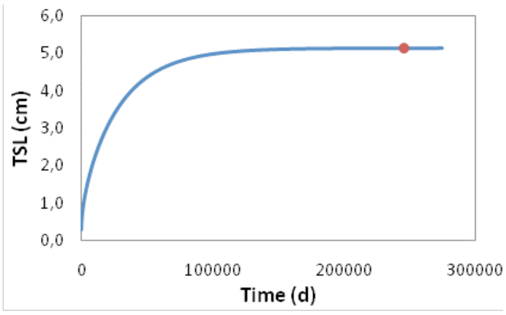
The test model is based on energy fluxes. The ingested (consumed) energy correlates with $V^{2/3}$ and was multiplied by 0.7 to get assimilated energy {pAm}. Energy that is allocated to somatic maintenance [pM] and growth is 98% of {pAm} (κ = 0.98) and increase in *V* is calculated by dividing the energy that is left after paying maintenance [pM] by the costs for growth [EG]. The new *V* is then used in the next iteration. Each run contained 1500 iterations. Every step shows the used parameter values for the run and the outcome is given at the bottom two lines of each table and depicted in the graphs as a red circle. The blue line continues after the red circle, but the life of a *Hydrobia* should stop at the point of the red circle.

Increase in length decreases over time, because at some point all the allocated energy will be required for maintenance and no energy is left for growth. The point at which increase in structure is *V* is first below 1% of the initial increase at t = 1 was taken as time for maximum length. The results of the simulation are given below the thick line in the tables. Only [pM] and [EG] were changed (See tables below) because this was enough to reach the goals of this test. Note that the starting TSL is 0.3 cm, which is the TSL at puberty, because the assimilation rate {pAm} is given for adults only in the experiments that were performed in this study. The simulations below do not consider juvenile time and start at TSL = 0.3 at time = 0. Thus, A_Lmax is actually the time that *Hydrobia* lives as an adult. *Hydrobia* larvae are planktonic for 28 days, before they become benthic (Fish and Fish 1976). After that, they live for 190 days before they start reproducing (Lillebo et al, 1999), thus, A_Lmax is the maximum life span (610 days) minus 28 and 190 = 392 days for adult life.

Step 1: Initial parameter values.

[pM] was extracted from the Growth experiment, because the value from the Starvation experiment was much too high. Energy for reproduction (ER) was used to calculate the fraction of energy that was allocated to maintenance and growth, κ.

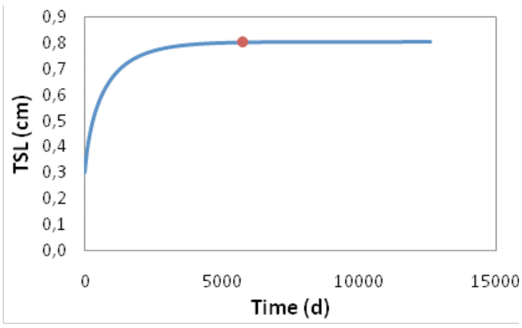
Parameter	Value	Unit
V	$1.34 \cdot 10^{-3} \cdot TSL^3$	cm^3
{pAm}	$4.74 \cdot V^{2/3} + 0.206$	$J\ d^{-1}\ cm^{-2}$
[pM]	3.91	$J\ d^{-1}\ cm^{-3}$
[EG]	43437	$J\ cm^{-3}$
κ	0.98	
Step size	170	d
TSLmax	**5.14**	**cm**
A_Lmax	**245836**	**d**



Both TSLmax and A_Lmax are much too high, because they should be 0.8 cm and 392 days respectively. This deviation can be caused by either [pM] or [EG] being too low or {pAM} is too high. [EG] is already very high and it is hard to lower {pAM} because of its equation, but it was known that [pM] was not certain.

Step 2: Raising [pM]

Parameter	Value	Unit
V	$1.34 \cdot 10^{-3} \cdot TSL^3$	cm^3
{pAm}	$4.74 \cdot V^{2/3} + 0.206$	$J\ d^{-1}\ cm^{-2}$
[pM]	53	$J\ d^{-1}\ cm^{-3}$
[EG]	43437	$J\ cm^{-3}$
κ	0.98	
Step size	9	d
TSLmax	**0.8**	**cm**
A_Lmax	**5743**	**d**

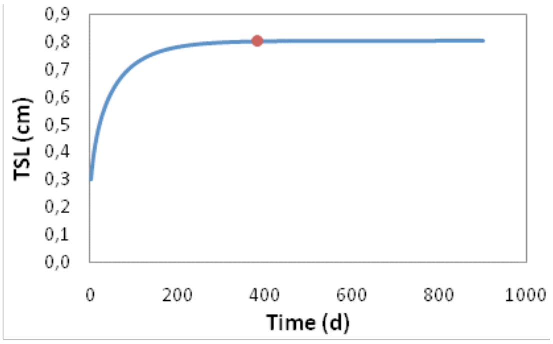


TSLmax is 0.8 cm when Maintenance costs are 57 $J\ d^{-1}\ cm^{-3}$, thus the right TSLmax can be reached with reasonable [pM]. The max age is lowered by increasing [pM], but it is still far too high. Real snails reach their max length much earlier. This can be obtained by lowering [EG] or raising {pAm}. As said before, the value for [EG] is much higher than the standard value of 2800 $J\ cm^{-3}$ as presented by Kooijman (2008), so adjusting the costs for structure is the strategy of choice.

Step 3: Lowering [EG]

It was enough to change [pM] and [EG] because these are the parameters with the strongest relation to the two tested values.

Parameter	Value	Unit
V	$1.34 \cdot 10^{-3} \cdot TSL^3$	cm^3
{pAm}	$4.74 \cdot V^{2/3} + 0.206$	$J\ d^{-1}\ cm^{-2}$
[pM]	57	$J\ d^{-1}\ cm^{-3}$
[EG]	2892	$J\ cm^{-3}$
κ	0.98	
Step size	1	d
TSLmax	0.8	cm
A_Lmax	382	d



Experiment 1: Starvation

The increase in AFD in the past data point must be due to some experimental artefact. The snails only pay maintenance during starvation, so the surviving time is calculated with $[Em]/[pM] = 8.34 \cdot 10^3/57 = 157$ days. The starvation experiment must be prolonged in future research, also because *Lymnaea stagnalis* was found to use up to 70% of its dry weight as reserves during starvation (Zonneveld and Kooijman, 1989).

A previous starvation experiment in this study (results not shown) resulted in increasing AFDW, indicating that the snails are able to consume energy. The water was filtered and autoclaved and refreshed every two days, but still the snails were able to grow. A possible explanation is the consumption of epiphytes that attach to the snail's shell. Although visible algae were removed from the shells, probably, this was not 100% efficient due to algae attachment in its microscopic form. Anyhow, this source does not seem sufficient to provide enough food for almost 60 days. On the other hand, if any living algae would reproduce in the water, they would be removed when the water was changed. Arguing that the slope of decreasing AFDW during starvation should be steeper has great consequences for the estimation of [pM], the maintenance costs. These were estimated at 468 $J\ d^{-1}\ cm^{-3}$ and this is already much higher than the standard value given by Kooijman (2008), although one study fitted a [pM] for the pond snail *L. stagnalis* of 307 $J\ d^{-1}\ cm^{-3}$ to the data (Kooijman, 2009). This high value for [pM] is partly due to the very small structural volume of *Hydrobia*, which makes it easy to make a large mistake. The energy that is allocated daily to maintenance by a snail with TSL = 0.52 cm is 0.88 J, whereas the daily consumption for that same snail would be 0.38 J (A snail with TSL = 0.52 cm has a *V* of $1.88 \times 10^{-3}\ cm^3$ and a structural area of $1.52 \times 10^{-2}\ cm^2$). The calculated values for consumption and maintenance rate are not compatible, since energetic costs for maintenance are higher than what the snail consumes. Moreover, the results from the growth experiment show that *H. ulvae* is able to survive on very low food abundance (Figure 5) and even grow a bit. This means that maintenance costs are around 1% of the consumed energy and no more than 3.91 $J\ d^{-1}\ cm^{-3}$. It should be kept in mind that the explanation for the long survival of the snails in the starvation experiment could also apply to snails with very low food available. [pM] as calculated from the Growth experiment would then be under estimated.

Experiment 2: Consumption

The correlation should be not only linear with squared length, but also cross the horizontal axis at the origin (0,0). The latter is not the case when a best fit is plotted. Any correlation disappears when the trend line is forced through the origin ($r^2 = -9.8$), but the current trend line implies that snails with TSL = 0 mm, would consume 0.29 J per day. Removing data points does not improve the regression coefficient. A possible explanation for this is that juveniles consume more than adults relative to their size. Another explanation is that the bigger size classes did not have enough food and consumed all the present food. This is not sustained by the data, because all data points where more than 85% of the available algae was consumed, were left out of the calculation. This was done for certainty and the trend line hardly changed by this. It is hard to compare these data with literature data, because most articles use number of fecal pellets h^{-1} as measure for ingestion (e.g. Shipp and Grant, 2006; Blanchard et al., 2000). It is not possible to use these data when values for Consumption in J d^{-1} are needed and the required information to transform the data is absent.

Experiment 3: Growth
The high value for costs for growth draws attention, since it is much higher than the value provided by Kooijman (2009), which is $2.8 \cdot 10^3$ J cm^{-3} structure. It is, however, in the order of magnitude of the value for energy per gram of reserves, which is $24.6 \cdot 10^3$ J cm^{-3}. The increase in volume might not be very accurate since both values (before and after) were calculated from the length as measured with the stereo loupe during the experiment. This was done because the trend line for AFDW in the literature, that was supposed to be used to calculate starting AFDW, clearly did not apply to the snails that were used here, because the AFDW of all snails was after the experiment about 50% of the starting value. This means that the variation caused by growth in the experiment was overruled by the difference in correlations between AFDW and TSL. Apparently, the relation between AFDW and length is different from the individuals used by Lillebø et al. (1999).

Food availability was not limiting growth at 100% food, indicating that food in the Consumption experiment was sufficient as well.

It is amazing to see that the snails could survive on less than 5% of the maximum ingestion rate and maintenance costs seem to be underestimated with this method. Still, [pM] has to be very low, since some snails with f = 0.01 still increased in length. Here emerges a disadvantage of using length instead of real AFDW, because snails could be able to grow for a while on reserves when food is not available. In this case, the length increases, but the AFDW decreases. This can only be measured if snails are dried and combusted. On the other hand, snails could be able to increase in biomass without enlarging their shell. Therefore future research must always work with AFDW or shell free dry weight. The quick model test revealed that a [pM] of around 53 J d^{-1} cm^{-3} fits the life history parameters of *Hydrobia* better and this would amount to 15% of the maximum ingestion rate of the snails in the Growth experiment.

Other parameters
Barnes (1994) stated that *H. ulvae* allocates a lot of energy to reproduction. He probably based this statement on the high turnover rate of individuals in *H. ulvae*. Although snails produce many eggs and offspring, the total energy for producing these eggs amounts to less than 2% of the assimilated energy according to the results in the present paper. The energy for egg production is probably right, but the results of the Consumption experiment are less certain. I.e. the order of magnitude is right, but the slope of the trend line is far too flat. It was allowed to use 0.98 for κ and $3.87 \cdot 10^{-3}$ J d^{-1} for reproduction rate.

Recommendations for future research
The outcome of the experiments turned out to be rather delicate and a slight change in one parameter value can have large effects on others. Therefore, the following recommendations must be considered when similar experiments are repeated.

1) The starvation experiment must be prolonged and only be ended when snails die of starvation. This turned out to be especially important, since the values for other parameters depend on V, which is obtained from the Starvation experiment.

2) New correlations between size and weight must be made at the start of each study, because the correlations between e.g. AFDW and TSL may change during the year or over a larger time scale. These correlations are important to calculate increase in V in the Growth experiment.
3) Juvenile snails (TSL <0.3 cm) must be included in the size range for the Consumption experiment, because it was not possible to include the origin (0,0) in the relation between consumption and size. It is important to find out whether this relationship differs between juvenile and adult snails.

Conclusions

The structural body volume (V), which is the basis for all other parameters can be approximated by $V = 1.34 \cdot 10^{-2} \cdot TSL^3$, where TSL is total shell length in cm and V is given in cm^3. This results in a zoom factor z of 0.19 and a shape coefficient δ_M of 0.24. The maximum energy density [*Em*] is $8.34 \cdot 10^3$ J cm^{-3}. All these values are calculated from a Starvation experiment, in which it was shown that *H. ulvae* is able to survive for, at least, 60 days without food and can use more than 25% of its organic weight as reserves. Maintenance rate [pM] was found to be 468 J d^{-1} cm^{-3} in the Starvation experiment and 3.91 J d^{-1} cm^{-3} in the Growth experiment but it was adjusted to 53 J d^{-1} cm^{-3}, to fit the maximum size of *H. ulvae* in nature by running a test of the basic DEB model. Costs for generating a unit of V [*EG*] was calculated as $46.5 \cdot 10^3$ J cm^{-3} from the Growth experiment, but it was adjusted to 2892 J cm^{-3} to fit the age at which the maximum size is reached in nature. Energy that is allocated to reproduction [ER] was $3.87 \cdot 10^{-3}$ J d^{-1} based on lifetime egg production. This is almost 2% of {pAM}, so κ was set at 0.98.

A quick and simple test with some selected parameters showed that the parameter values as acquired from the experiments in the present study did not fit the biological data of *H. ulvae* such as maximum length and age. It is hard to find the right values for the Dynamic Energy Budget model for *H. ulvae* and some adjustments to the experiments are required to obtain better estimates

***Carcinus maenas* experiments**

Experiments with *C. maenas* were carried out by N. Hoefnagel, following the same type of experimental protocols as the ones described above for *H. ulvae*.

Results

Experimental results indicate that energy consumption increases linearly with carapace width, with an average assimilation efficiency of 75% (Figure 10).

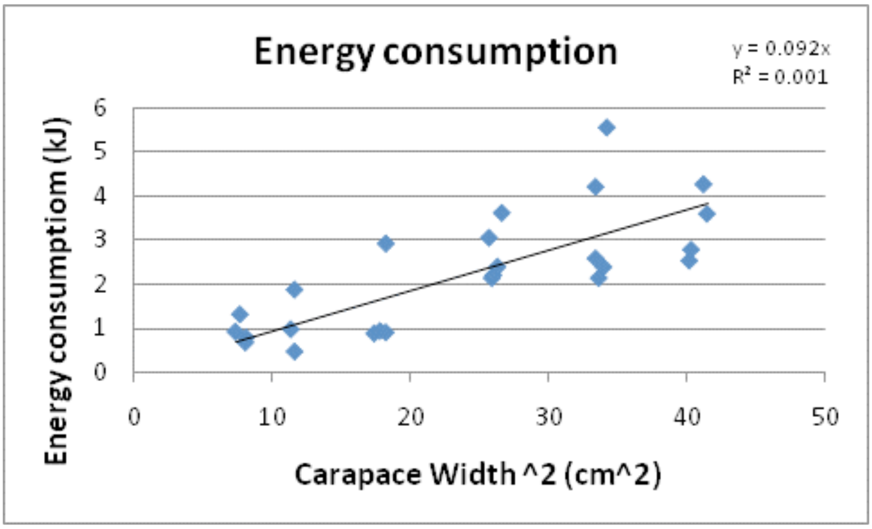


Figure 10. Variation of energy consumption (kJ) with carapace with of *C. Maenas*.

The experiment to assess the variation in weight (as a fraction of W_0 in ash free dry weight) with starvation time (Figure 11) indicates that close to day 80, *C. maenas* reaches the lowest weight, corresponding to approximately 46% of the initial weight, which corresponds to [E]= 29.3 kJ cm^{-3}.

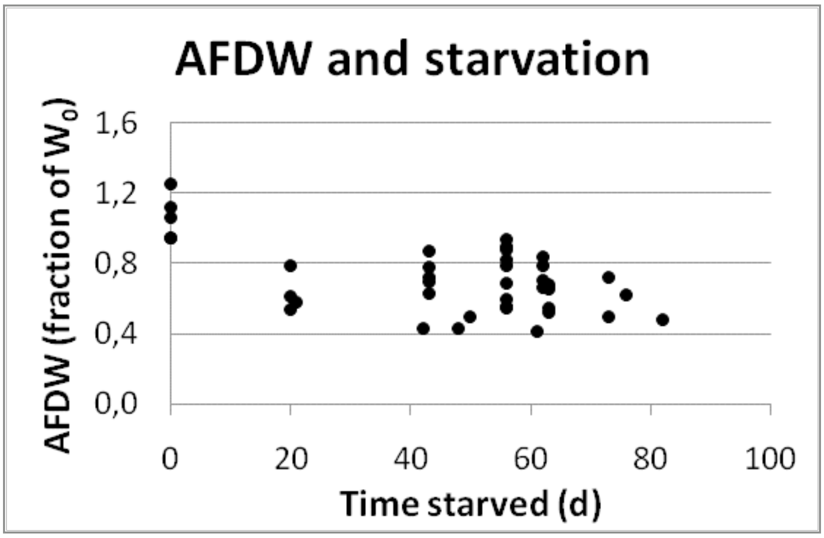


Figure 11. Variation of *C. maenas* weight with starvation time.

The experiment performed to assess the weight variation expressed in grams (AFDW) during starvation time in *C. maenas* with a carapace width= 35 mm (Figure 12), allowed to estimate a cost of maintenance of 181 J d^{-1} cm^{-3}

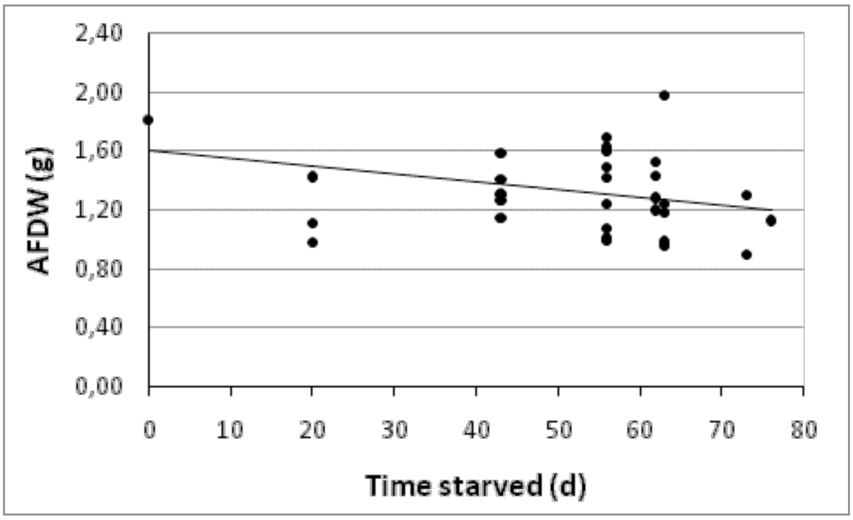


Figure 12. Variation of the average weight of *C. maenas* with 35 mm carapace width with starvation time.

C. maenas experiments of energy intake versus growth (Figure 13) indicate an average cost for growth of 9.388 kJ g^{-1} ± 0.84

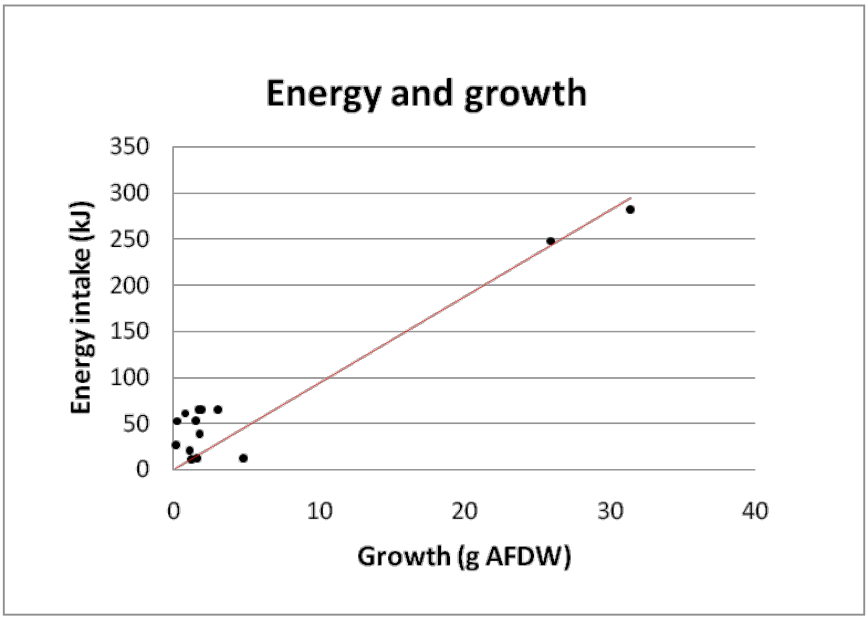


Figure 13. Variation of energy intake versus growth in *C. Maenas.*

2.1.3. DEB models *versus* traditional energetics models

Due to experimental difficulties and numerical inconsistency associated to the measurement of the required parameters, the DEB models were replaced by traditional bioenergetics models. Whenever it was possible, the parameters obtained experimentally in the previous section were used in the bioenergetics models, while missing parameters were obtained from bibliography.

TASK 5 – Data integration and assessment of differences between natural and human stress

In order to explore the results deeply, we have organized the task as two main case studies: 1) The Mondego estuary and 2) The Mira estuary. Afterwards, a general integration regarding the effects of natural vs. anthropogenic variation in benthic communities of Portuguese estuaries was done and suggestions on sampling designs and methodologies for estuaries with strong seasonal freshwater flow variation were given.

1. Mondego estuary case study

1.1. The Mondego estuary morphological characteristics and evolution

The Mondego River basin has approximately 6670 km^2, including a large alluvial plain consisting of good quality agricultural land. The construction of harbour facilities in the river's estuary introduced changes into the system at least since the 1930s, but it was from the 1960s that the Mondego's catchment area suffered large-scale morphological modifications. These involved the construction of stonewalls and water reservoirs in order to regulate the river water flow, improve the uses of water resources in industry and agriculture, to produce electric power, and to expand the harbour facilities. All this had a strong anthropogenic impact on the system, modifying the riverbed's topography and changing hydrodynamics.

The entire Mondego river's catchment area, particularly the direct runoff from 15,000 ha of cultivated land in the lower river valley (mainly rice fields), presently contributes with a considerable loading of nutrients and several chemicals into the estuary (western coast of Portugal; 40º08'N, 8º50'W), which constitute a relatively small (860 ha) warm-temperate polyhaline intertidal system. In addition, the Mondego estuary supports industrial activities, salt-works, and aquaculture farms, being the location of the commercial and fishing harbours of Figueira da Foz, also a centre of seasonal tourism activity.

The estuary is 21 km long and its terminal part, 7 km long and 2-3 km across at its widest section, consists of two arms, North and South, separated by the Murraceira Island. The Northern arm, where the commercial harbour is located, is deeper (5-10 m during high tide) and constitutes the main shipping channel. The Southern arm is shallower (2-4 m during high tide), and is characterised by large areas of intertidal mudflats (almost 75% of the area) exposed during low tide.

In the early 1990s, the South arm was strongly silted up in the upstream areas, causing the river discharge (from 27 m^3 s^{-1} in dry years up to140 m^3 s^{-1} in wet years; mean annual average 79 m^3 s^{-1}) to flow essentially through the Northern arm. Consequently, water circulation in the South arm became mostly dependent on tides and on the reduced freshwater input from the Pranto River, a small tributary artificially controlled by a sluice (Marques et al., 2003). The tidal range varies between 0.35 and 3.3 m depending on site and tide coefficient, while water residence time varies between 2 days, in the Northern arm (Marques et al., 2007), and 5 days, in the Southern arm with a very small water discharge from the Pranto sluice (Neto et al., 2008).

Also during the 1990s, two major hydraulic changes occurred in the estuary:

a) From 1990 to 1994, the North channel was sunk 1.5 to 2 m and the banks filled, narrowing the width of the river bed by about 75% (Figure 14). This reduction would allow the water to flow faster, aiding in preventing the silting that would otherwise normally occur in these parts of the river.

Figure 14. Final interventions at the North arm of the Mondego estuary. Situation in 1994: area between the commercial harbour (at the mouth) and the point of separation of the two arms. Detail of the left margin's land filling process.

As a consequence, the communication between the two arms of the estuary was totally interrupted in the upstream area due to the completion of stonewalls in the North arm banks (Figure 15). In fact, four water pipes with a Ø 0,4 m section were kept aiming at ensuring a minimum communication, but these rapidly became totally silted up and the water stopped running through them.

b) During the period between 1997/98, the following experimental interventions were carried out to decrease eutrophication symptoms (green macroalgae blooms occurred from the early 1990s) and to test ways of ameliorating the system's condition:

- The freshwater discharge of the Pranto river sluice into the South arm was reduced to a minimum to decrease nutrient loading, being instead diverted to the Northern arm by another sluice located further upstream;

- The communication between the North and South arms was re-established, although only for a very limited extent (periods of only 1.5 to 2 hours before and after each high tide peak and through a section of only 1 m^2) to improve water circulation (Figure 16) (Neto et al., 2008; Neto et al., 2010).

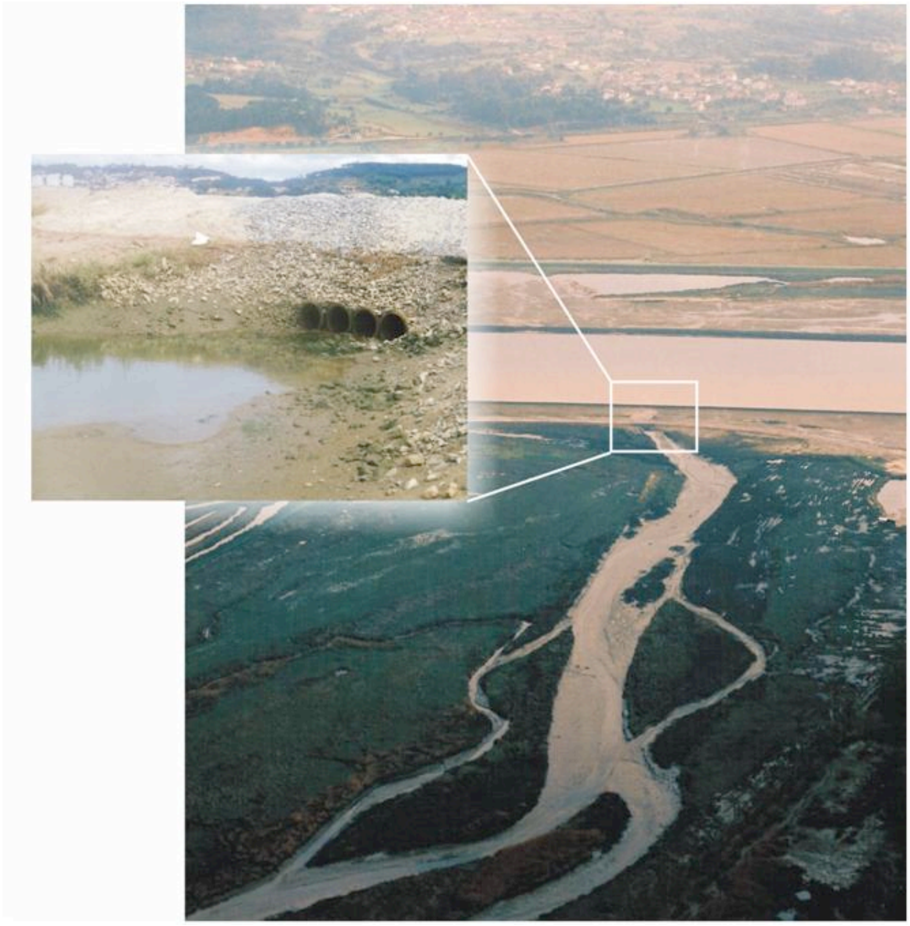


Figure 15. The communication between the two arms of the Mondego estuary after the Northern arm interventions in 1994. Detail of the water pipes.

Figure 16. Detail of the experimental reestablishment of the communication between the two arms of the estuary late in 1997.

From the mid 1990s to 2006, the main pressures harassing the Mondego's Northern arm resulted mainly from harbour facilities and consequent dredging activities, causing physical disturbance of the bottoms.

A long-term study of the Mondego estuary, pursued since the mid 1980's, allowed to follow the system's response, not only in terms of changes in physical conditions but also concerning the occurrence of extreme climatic events. Important quality elements, such as water quality, hydraulics and sediment dynamics, benthic intertidal and subtidal communities, changes in *Zostera noltii* beds and green macroalgae distribution, have been monitored since then. The main alterations (Figure 17) can be roughly summarised as follows:

a) In spite of morphological changes artificially introduced in the natural river course since the 1960s, eutrophication symptoms were not noticeable in the estuary before the early 1990s.

b) Following the interruption of the upstream communication between the two arms, especially from 1991 to 1997, the ecological conditions in the South arm showed a rapid deterioration. The combined effect of increased water residence time and nutrient concentrations became major driving forces behind the emergence of clear eutrophication symptoms. Seasonal blooms of *Ulva* spp. were observed concomitantly with a severe reduction of the area occupied by *Z. noltii* beds, as a function of spatial competition with macroalgae (Marques et al., 2003).

c) The shift in benthic primary producers affected the structure and functioning of the biological communities, and through time such modifications started inducing the emergence of a new selected trophic structure, which has been analysed in abundant literature (e.g. Cabral et al., 1999; Dolbeth et al., 2003; Cardoso et al., 2004a; 2004b; Lopes et al., 2005; 2006; Marques et al., 1997; 2003; Martins et al., 2005; 2007; Patrício et al., 2004; Patrício & Marques, 2006).

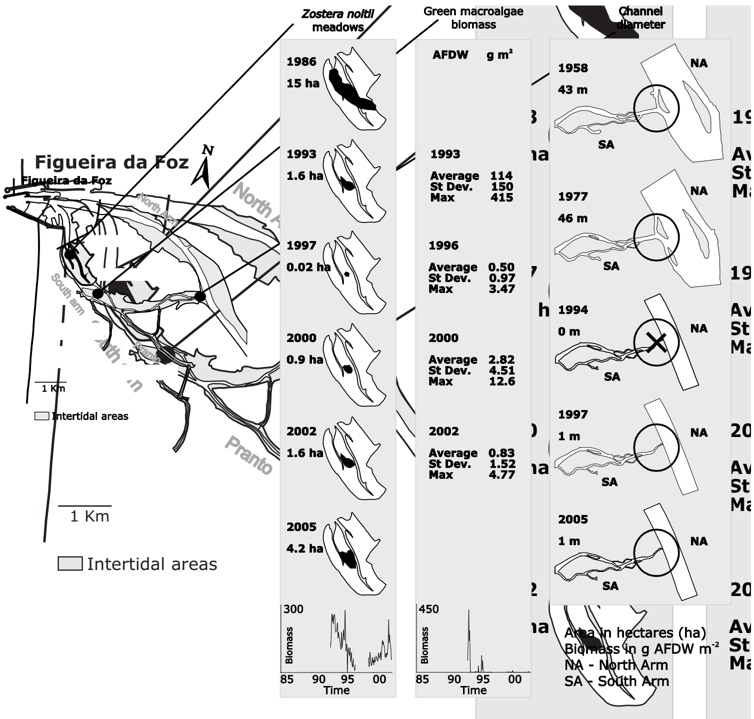


Figure 17. Temporal changes of *Zostera noltii* meadow area (ha) and biomass (g AFWD m^{-2}), green macroalgae biomass (g AFWD m^{-2}) and the connection width between the two estuarine arms. Black areas correspond to *Z. noltii* meadows, eutrophic area and connection between the two arms (from Patrício et al., 2009).

d) From 1998, following the reduction of freshwater discharge proceeding from the Pranto River sluice and the limited re-establishment of the upstream communication between the two arms (1997/98), this trend appeared to reverse to a certain extent. This is suggested by the partial recovery of the area occupied by *Z. noltii*, previously the richest habitat in terms of productivity and biodiversity (Marques et al., 1993), and the cessation of green Ulva spp. blooms (Lillebø et al., 2007; Verdelhos et al., 2005).

The full re-establishment of the communication between the two arms appeared therefore as a suitable way of improving the system's ecological quality (Marques et al., 2005), an intervention that was undertaken during the spring of 2006.

1.2. Temporal and spatial variations of the physicochemical parameters

The physicochemical parameters analyzed were sampled during spring, along the 14 sampling stations located in the terminal part of the estuary (Figure 18), in 1990, 1992, 1998, 2000, 2002, 2004, 2005 and 2006. Some parameters, such as temperature, salinity, and dissolved oxygen, were measured *in situ*, at the surface and bottom of the water column. Afterwards, the sampled waters were analyzed for dissolved nutrient concentrations determination (nitrogen from nitrates, nitrogen from nitrites, nitrogen from ammonia, and phosphorous) (Strickland & Parsons, 1972; APHA, 1995). In the same campaigns, sediment samples were also collected and its organic matter content was estimated at the laboratory.

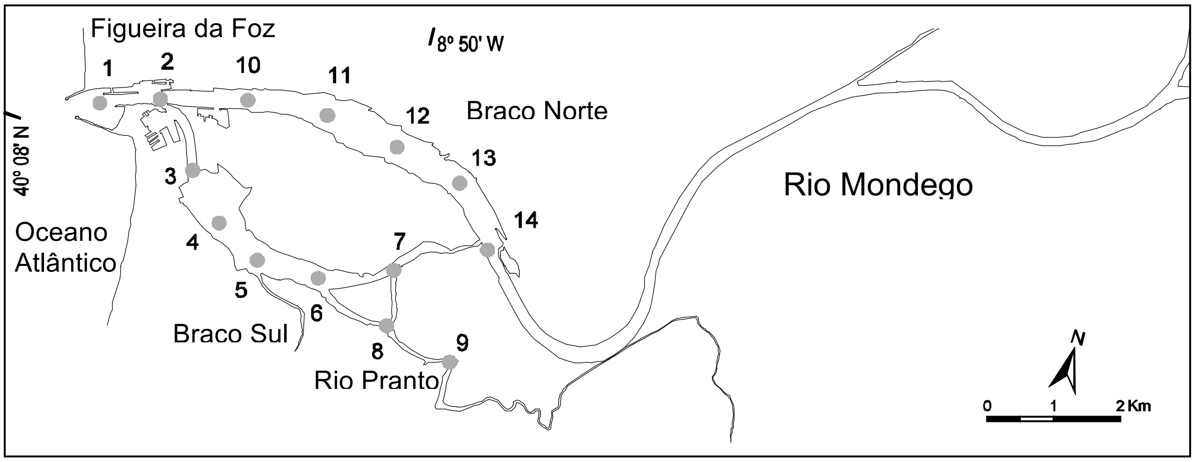


Figure 18. Terminal part of the Mondego estuary: location of the subtidal sampling stations.

From 1990 to 1992, the spatial distribution of the different types of sediments in the estuary remained relatively stable. In a general way, there was a dominance of small sized particles (clay and silt) and a higher organic matter content in the inner stations of the South arm. In the North arm and in the downstream areas of the South arm there was dominance of coarse sand. In the stations closer to the estuarine mouth the dominant fraction was mainly fine sand. From 1992 onwards, the sediment characteristics of the North arm have changed due to the constant dredging activities undertaken to ensure the good navigability of that system. As consequence, the sediment gradients have dramatically changed along the North arm and in the areas near the estuarine mouth. On the contrary, on the inner parts of the South arm the changes were residuals, not being, apparently, affected by that activity.

The water quality, however, has changed. Before the communication interruption, the stations near the estuarine mouth were characterized by higher salinities, while that the middle and inner stations of both arms presented higher nitrogen concentrations. After the interruption, due to the freshwater inflow decrease and residence time increase on the system, there was a decrease in dissolved oxygen and a substantial increase of nitrates and ammonia in the South arm, especially in the inner zones. The salinity has increased in the downstream areas of the South arm. Under these conditions, macroalgae blooms were reported in this area of the estuary (1993 to 1997) (Marques et al., 1997; 2003; Martins et al., 2001). In 2000, after the experimental re-establishment of the communication between the North and South arms, the water quality has suffered a new change. The dissolved oxygen concentration has increased in the inner stations of the South arm, while the nitrates and phosphorous concentrations was relatively similar in all estuary, with exception of the stations close to the estuarine mouth (for more details see Neto et al., 2010).

On the other hand, the occurrence of extreme climatic events has introduced substantial changes, although occasional, in terms of water quality. In Figure 19 is presented the mean annual freshwater inflow (m^3s^{-1}) variation in the Açude-Ponte of Coimbra from 1990 to 2007 (source: INAG, 2008, www.inag.pt). Approximately one year after the Winter flood event in 2000/2001, and as consequence of a long drainage process of the agriculture fields located upstream the Pranto river (Marques et al., 2007), the South arm still presented high silica and chlorophyll a concentrations. Simultaneously, the nitrates concentrations, coming from the river discharges, have increased along the North arm till the estuarine mouth area.

The drought event that occurred between 2004 and 2005 also had dramatic effects upon the estuarine conditions. In the inner stations of the South arm, the concentrations of phosphate (released from the sediment to the water column) and ammonia have substantially increased. In the same way, the salinity values also increased in the South arm areas as well as along the North arm (for more details see Neto et al., 2010).

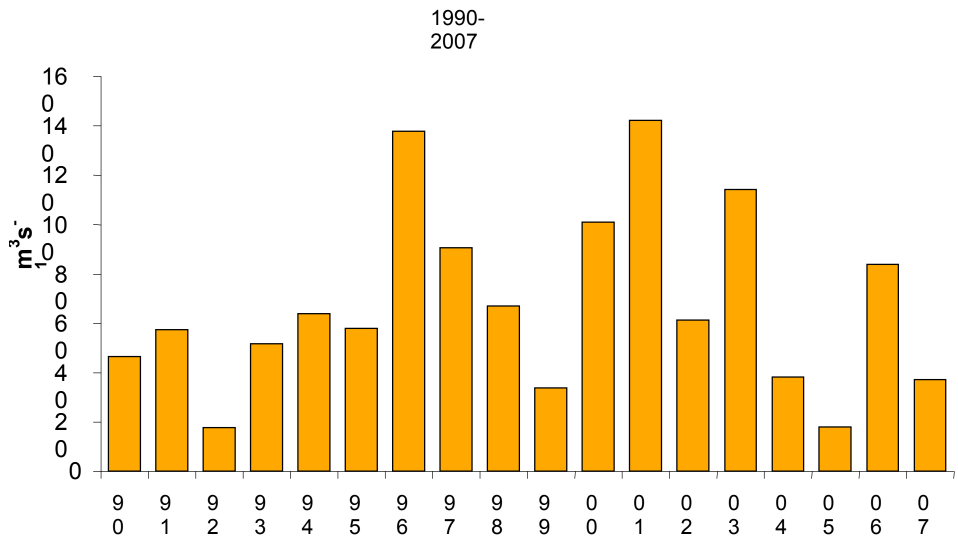


Figure 19. Mean annual freshwater inflow (m^3s^{-1}) in the Açude-Ponte of Coimbra from 1990 to 2007 (source: INAG, 2008, www.inag.pt).

1.3. Spatial and temporal variation in subtidal communities of benthic invertebrates

The benthic macroinvertebrates subtidal communities were regularly monitored, with sampling campaigns during the spring of 1990, 1992, 1998, 2000, 2002, 2004, 2005 and 2006 in 14 stations located on the terminal area of the estuary (Fig. 28). Until 2005, including, 5 replicates of mobile substrate were collected per station but in the spring of 2006 only 3 replicates were processed. A van Veen dredge model LMG was used in all sampling campaigns (until 2003 the dredge had a sampling surface of 0,0496 cm^2 and in the following campaigns the sampling surface was 0,078 cm^2). In the laboratory, a sieve with 1mm mesh size was used and the organisms were identified, if possible, to the species level, counted and had their biomass determined (g AFDW m^{-2}).

The resulting data sets of each spring were analyzed separately so as to reveal potential changes to the ecological conditions throughout time, in association with human activities and the occurrence of extreme climate events (for further details see Neto et al., 2010).

Firstly, a PCA analysis was carried out with the density data sets relative to the macroinvertebrates benthic communities, observed for each spring. The results showed a characteristic drive of those communities, much more influenced by extreme climate events than by anthropogenic pressures (for further notes see Neto et al., 2010). Although human disturbances act on the macroinvertebrates communities level the disturbances caused by extreme climate events seem to have a much larger influence. An upward displacement of typical marine influenced communities or typical freshwater communities in locations near the mouth was observed after long periods of drought or high precipitation, respectively. From this first analysis also resulted the recognition of a group of 4 stations located in the inner South arm (stations 5, 6, 7 and 8) that consistently turned up clustered throughout time, given that they characterize a relatively homogenous area in the system. Thus, the data from these 4 stations were used to track the temporal variations of the subtidal macrobenthic community and its eventual response to the environmental changes that occurred in that section of the South arm between 1990 and 2006. To do so, a new PCA was applied to the density data sets of benthic macroinvertebrates, corresponding to those inner stations of the South arm.

Despite the spatial and temporal changes, the subtidal macrobenthic community structure analysis allowed a consistent depiction of different estuarine areas (groups A, B and C) (Fig. 20 and 21). Although these 3 areas are spatially recognizable, their “limits” have changed during the study period, demonstrating the macrobenthic community’s response to the environmental conditions, namely extreme climate events (for further details see Neto et al., 2010). This type of events may cause dramatic temporary outcomes on the structure and composition of these biological communities to the point that they overlap the anthropogenic effects. This can represent a true difficulty when it comes to environmental quality evaluation and management, as pointed out by Chainho et al., 2007, that obtained different classifications when they estimated a set of biological indicators in different times of the year, during a year with a high intensity flood (200/2001). In fact, it becomes extremely difficult to determine an estuarine system’s ecological status when natural disturbances tend to mask the response of potential ecological indicators (Dauvin, 2007; Elliott & Quintino, 2007). This problem may gain a striking importance in the global climate change scenario that we are currently facing.

Figure 22 shows the variations on the subtidal macroinvertebrates benthic community in the inner area of the South arm (stations 5, 6, 7 and 8). We can observe a drift on the 1990’s and 1998’s community which is probably related to the interruption of the upward connection of both arms of the estuary. However, the centenary flood in the winter of 2000/2001 as well as the severe drought of 2004/2005 seems to have had a much more dramatic consequence over the community structure.

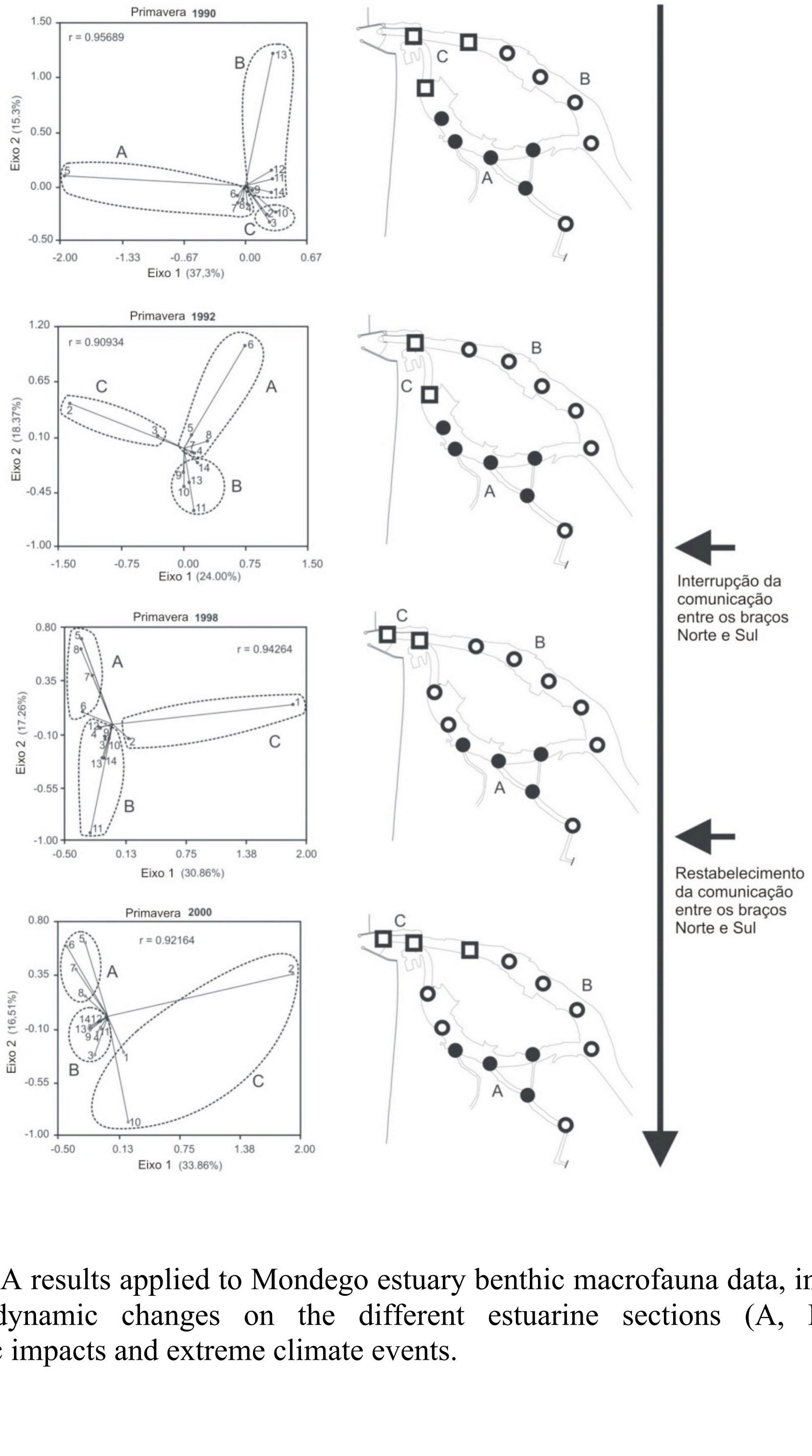


Figure 20. PCA results applied to Mondego estuary benthic macrofauna data, in the spring of 1990-2000; dynamic changes on the different estuarine sections (A, B, C); main anthropogenic impacts and extreme climate events.

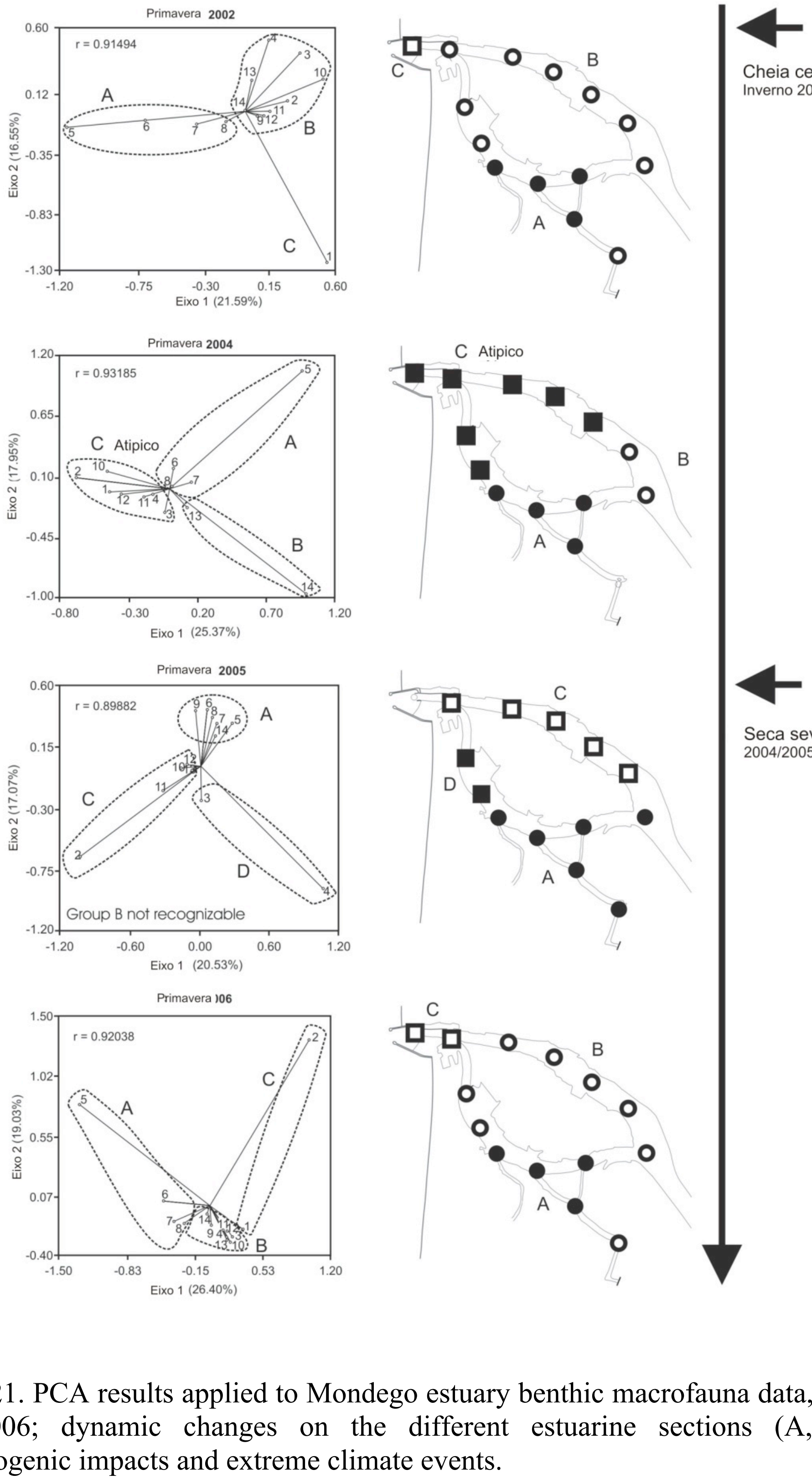


Figure 21. PCA results applied to Mondego estuary benthic macrofauna data, in the spring of 2002-2006; dynamic changes on the different estuarine sections (A, B, C); main anthropogenic impacts and extreme climate events.

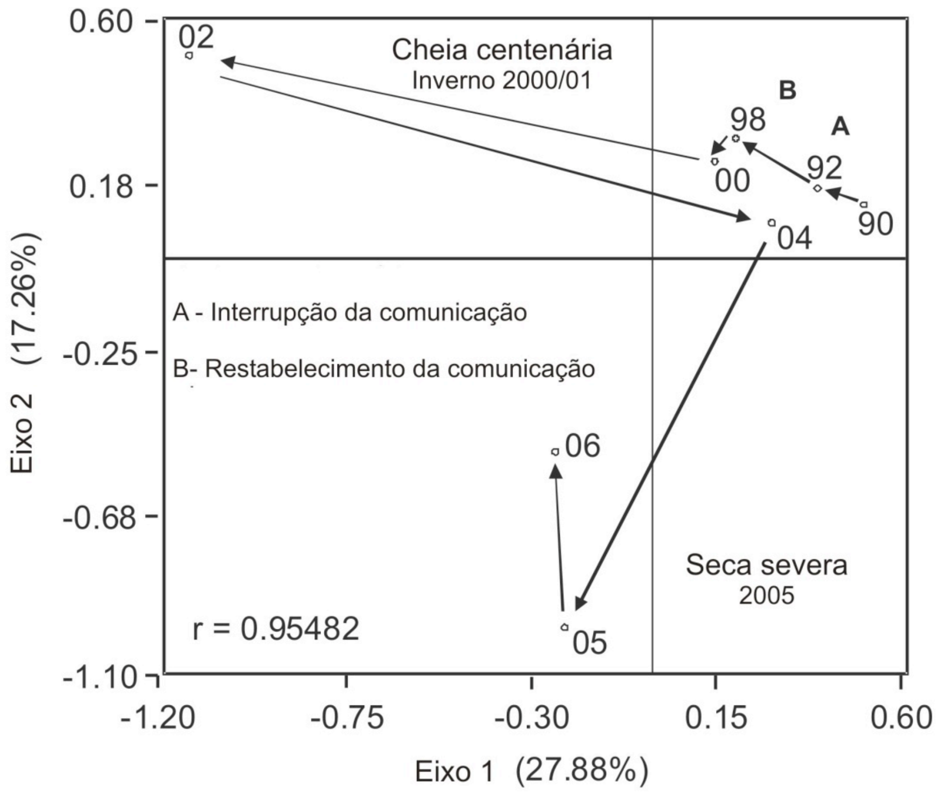


Figure 22. PCA results using subtidal benthic macrofauna data of the South arm's inner stations (stations 5, 6, 7 and 8), here considered as a group. Arrows symbolize the community's temporal sequence and the main anthropogenic impacts and extreme climate events that affected the system are indicated.

As an important result of this study we can infer that human impacts are generally persistent throughout time and even if they are responsible by a small shift on the ecosystem health status, they are, undoubtedly, accountable for continuously impel the deterioration status to a higher level. On the other hand, extreme climate events pose stronger effects but take action in a smaller period of time, which allows for a faster recovery of the macrobenthic communities.

These results prove that we must be very careful when developing and applying water management policies. The effect of different events (natural or anthropogenic) on the ecosystem's ecological status (ex. biological communities or water quality degradation) cannot be underestimated and must be targeted for additional research.

2. Mira estuary case study

2.1. Rio Mira general characteristics

Located in the Southwest of Portugal (37º 20' a 37º 45' N; 8º 05' a 8º 50' W), the Mira's hydrographic basin occupies an approximate area of 1 576 km^2 (Loureiro et al., 1986) and is surrounded at North, east and Southeast by the Sado, Guadiana and Arade systems, respectively, at Southwest by the streams of the barlavento algarvio and at West by the coastline, although at Northwest are a group of small temporary watercourses which drain directly to the Atlantic Ocean (Anonymous, 1995/96).

It is situated in Baixo Alentejo and almost comprises the Odemira municipality. It also includes marginal fringes of other adjacent municipalities, namely local councils of Gomes Aires and Santa Clara-a-Nova in Almodôvar and Santana da Serra in Ourique (Mota et al., 1988).

The Mira River has its origin at an altitude of 470 m (Loureiro et al., 1986) on the North front of Serra do Caldeirão, specifically in the Cansados brow near Almodôvar. The total river course has about 110 km in length and drains approximately at 30 km South of Sines, near Vila Nova de Milfontes (Leitão, 1997). As opposed to most Portuguese rivers and very much like the Sado river, it flows essentially from South to North, more precisely from Southeast to Northwest, although a few direction shifts can be observed in some regions.

This river can be considered as a relatively stationary one with a reasonably slow stream from the source until the mouth. Currently, it is the only watercourse, between the Sado river and the Ria de Alvor, that is permanently exposed to the sea, which grants it an increased value at the regional level.

2.2. Detailed characterization of the Mira estuary

The Mira estuary (Figure 23) is a small brackish water system that comes across the Atlantic Ocean on the Southwest coast of alentejo, near Vila Nova de Milfontes (37º 40' N; 8º 45' W). Although the tidal influence can be felt over 40 km upward (Andrade et al., 1991) until the nearby Porto da Torrinha (Vilela, 1975), the lower limit of the limnetic zone can be found slightly above the Odemira region at 32 km from the sea.

This estuary is characterized by an elongated layout with a gradually increasing width downwards (Bettencourt et al., 1993). During high tide in the Odemira region, the distance between margins is about 25 m whereas in Vila Nova de Milfontes the distance between margins can reach up to 400 m (Leitão, 1997).

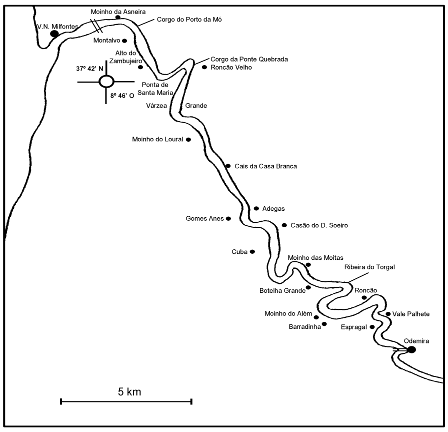


Figure 23. Mira estuary with the main villages and margin structures represented as well as relevant hydrographic and geographic elements for its characterization.

Regarding sediment granulometry, Andrade (1986) presented the following estuarine zonation:

a) The upmost located area of this system is characterized by the presence of an important coarse fraction, corresponding to fluvial gravel.

b) Between Volta da Cuba and Moinho da Asneira, alongside an extension of 15,5 km, the substrate is almost exclusively constituted by silt and clay. However, along the lower 13,5 km of this estuarine section (downwards of Volta de D. Soeiro) sediments with a low sandy component are dominant on the convex margins of slightly intense bending zones.

c) The stream of the estuary's terminal region is mainly constituted by well calibrated marine medium sands

In this brackish body exists a fairly stable longitudinal distribution of bathymetries (Andrade, 1986). Generically, we can observe a gradual decrease of the estuarine stream's mean depth upwards, with minimum values in its upper limit (Guerreiro, 1991), where it is also obvious a sensitive regularization of the bathymetric profile.

If during a wet semester of a normal year the precipitation and freshwater influx to this estuarine system can reach important figures, during the summer season the input of fluvial streams becomes almost none, thus being only influenced by extreme climate events (Figure 24). Thereby the Mira estuary, while always exposed to the sea and having a scarcity regime during dry periods, will be basically determined by the tidal influence during a significant part of the annual cycle (Leitão, 1997). Nevertheless, in high rainfall conditions the estuarine water mass rapidly responds to these sudden changes, namely regarding salinity (Bettencourt et al., 1993).

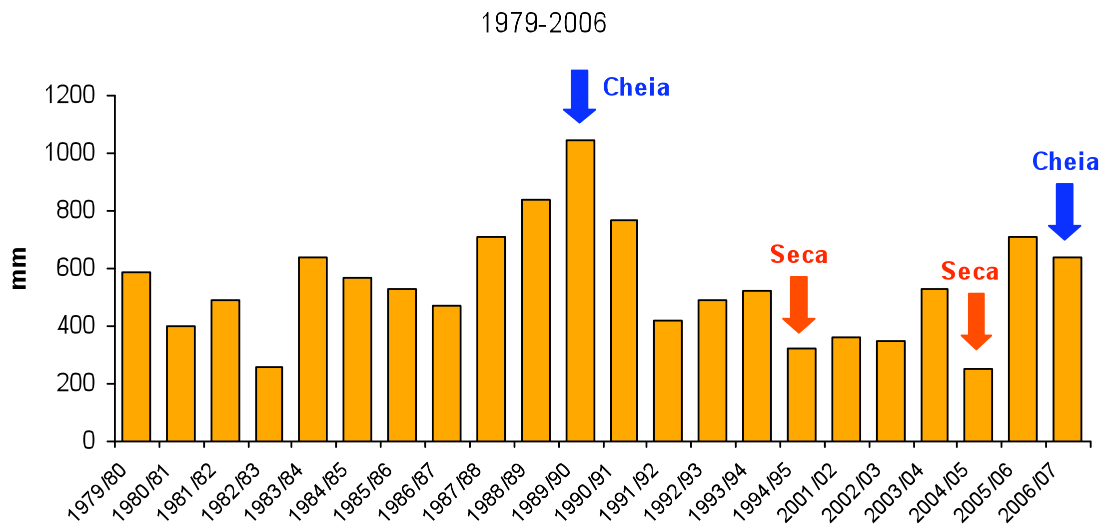


Figure 24. Annual mean precipitation (mm) in the Odemira meteorological station, from 1979 to 2007 (source: INAG, 2008, www.inag.pt)

Leitão (1997) estimated that the tide prism of the Mira estuary will be about 9 x 106 m^3 for a tidal cycle of mean amplitude (2,5m). Regarding this value and the system's general characteristics (weak freshwater flow and reduced stream width and bathymetry) the water mass residence time in the estuary appears to be rather short (Anónimo, 1999). The water

circulation in this brackish body is clearly oriented parallel to the margins, although it always presents a perpendicular component directed from North to South, corresponding to the deviation between the flood and ebb tide's orientations (Andrade, 1986).
According to Andrade (1986) the estuarine gradient is globally characterized downward to upward by:

a) Decreasing salinity
b) Increasing turbidity
c) Reduction of the thermal differential in relation to the atmosphere
d) Decreasing dissolved oxygen

Also according this author, vertically from surface to bottom, it can be observed:

a) Increasing salinity
b) Increment in turbidity
c) Ascent of the thermal differential in relation to the atmosphere
d) Decreasing dissolved oxygen.

2.3. Temporal and spatial variation of the physicochemical parameters

The data gathering regarding the physicochemical parameters in the Mira estuary was not performed in a continuous way over the years, despite the wide coverage along the estuarine gradient. The most recent observations (2003, 2004 and 2006) cover all the saline stretches till the tidal area of the estuary, with seasonal regularity.

Regarding the salinity parameter is possible to see that the estuarine areas upstream Moinho das Moitas present values lower than 5 (Anónimo, 1999). The same author argues that the distribution of the maximum values for this parameter is a reflex of the profound saline intrusion, allied to the presence of water bodies with marine characteristics till the Roncão area. This pattern defines a large intermediate section on the estuary, characterized by important variations of salinity in the aquatic environment (Anónimo, 1999). Along the annual cycle, this parameter is relatively constant near the estuarine mouth, while in the upstream areas the oscillations are much more intense, being directly related with the weather conditions (Guerreiro, 1991). The maximum amplitude values for salinity are observed between the Roncão and Volta da Cuba areas (achieving a variation around 30), starting to decrease from here to the upstream areas, as the marine influence starts to be attenuated (Anónimo, 1999). The saline changes also present great seasonal amplitude, as observed in Figure 25.

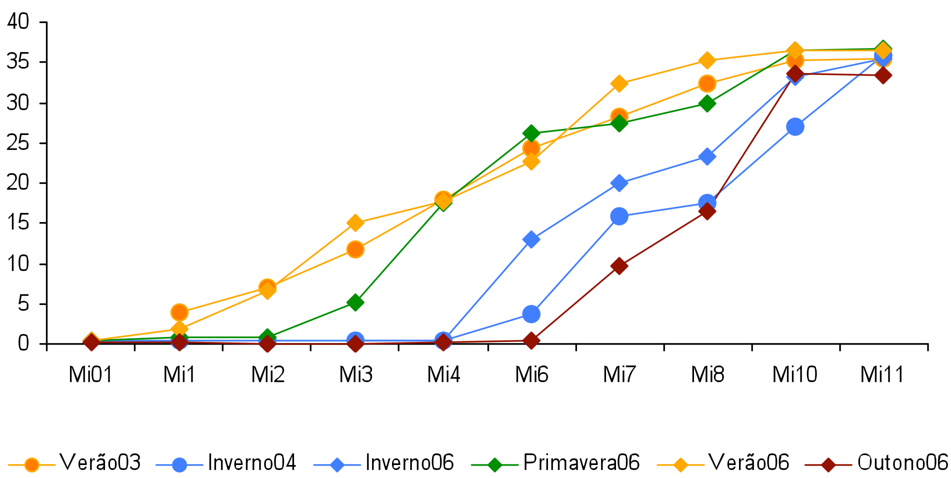


Figure 25. Seasonal salinity measured along the Mira estuary.

The seasonal changes with higher amplitude were observed in the area of Casa Branca (Mi7). It should also be highlighted the effects of a flood event occurred in Autumn 2006, that lead to the occurrence of much lower salinity values in the estuary, comparatively to the other considered seasons.

The water temperature amplitude observed for the Mira estuary follows the inverse pattern of the one exhibited by the atmospheric temperature differential. While the first one increases progressively from downstream to upstream, the second significantly decreases in the same direction (Guerreiro, 1991). The main cause for this pattern lies in the fact that the upstream areas of this system are mainly ruled by the atmospheric temperature regime, while that, in its terminal part, the marine waters play an important moderating role (Andrade, 1986). This way, while in the downstream areas of the estuary the temperature ranges between 12ºC in Winter and 24.5ºC in Summer, in the upstream areas it ranges between 8 and 27ºC, respectively.

The Mira estuary behaves as a well-oxygenized system, especially in the section with salinity ranges similar to the marine environment; which is understandable due to the low residence time of the water bodies, temperature conditions and constant renewal (Andrade, 1986; Anónimo, 1999). However, in the upstream areas the dissolved oxygen concentrations near the bottom can achieve very low values, suffering as well from seasonal changes, as illustrated in Figure 26. Therefore, higher concentration values are observed during Winter conditions, while that during Summer the values can decrease to below 4 mg/L (Chainho et al., 2008; non-published data).

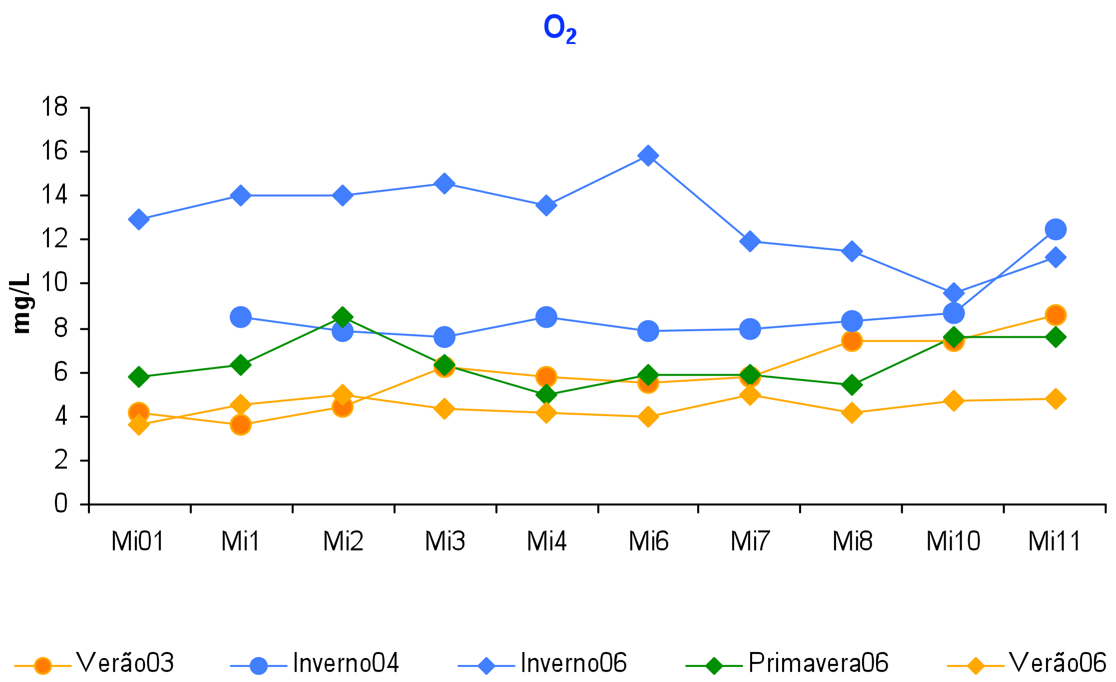


Figure 26. Seasonal dissolved oxygen concentration (O_2) measured along the Mira estuary.

Turbidity values observed in the Mira estuary, present their maximum along the 5 km extension upstream the Moinho da Moita station (Anónimo, 1999), although very high levels may also be observed in the area near Casa Branca as well. These two peaks of turbidity indicate the existence of muddy areas in these spots, suffering from hydrodynamic ressuspension in each tide cycle (Anónimo, 1999). In fact, the system turbidity depends more on the tidal amplitude than on the seasonality, being the maximum values obtained during spring-tide events, when a higher quantity of fine sediments are mobilized and circulate in the water column (Andrade, 1986).

Comparatively with other estuaries, the downstream area of the Mira estuary presents low nutrients concentrations, reason that may lead to its classification as an oligotrophic area (Bettencourt et al., 1993). This fact seems to be related with the relative impoverishment of the system in oligo-elements and in organic matter of the renewal oceanic waters, that move upstream and downstream, along a large extension of the system at each tide (Barata, 1997), having a very short residence time (Anónimo, 1999). Nevertheless, the upstream stations presented higher nutrients concentrations, especially during the 2003/2004 years, coincident with a drought period (Figure 27).

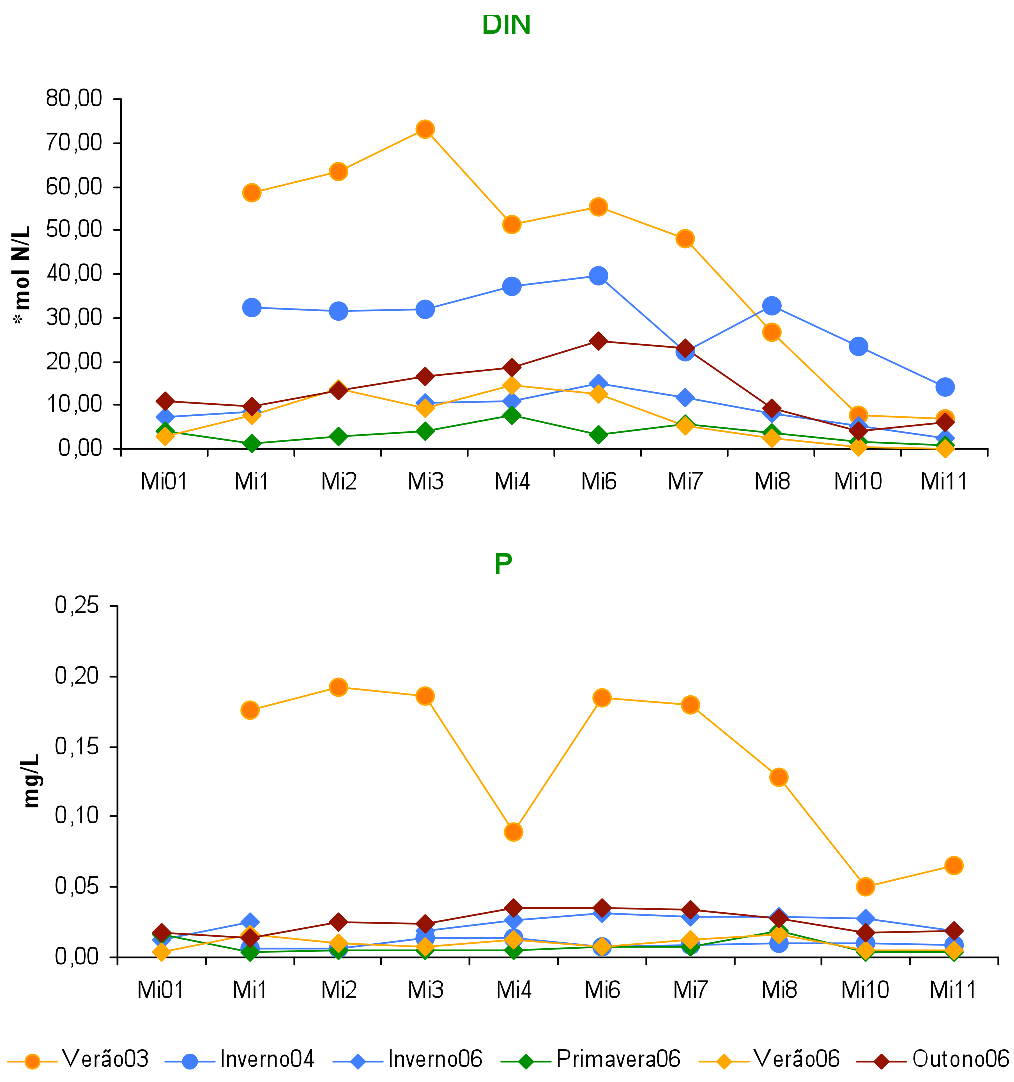


Figure 27. Seasonal concentrations of dissolved inorganic nitrogen (DIN) and phosphorous (P) measured along the Mira estuary.

Contrarily, the vegetation productivity levels of the marsh areas of this estuary are similar to other systems with identical characteristics (Catarino & Serôdio, 1992). This characteristic contributes to the high importance of the Mira marsh communities for the whole system productivity, comparatively to other systems (Bettencourt et al., 1993).

The Mira basin, and its estuary, seems to be well preserved when compared with other similar systems. This is, in part, due to the fact that an important extension of the system (mainly the estuarine area) is inserted in the 'Parque Natural do Sudoeste Alentejano e Costa Vicentina' (PNSACV), which has prevented several anthropogenic activities in the surrounding areas and the installation of infra-structures potentially injurious for the environment. However, there is also the occurrence of important environmental constrains, like the fact that the river bed and estuarine mouth are becoming silted-up. Other aspects that should be highlighted, but that are less important in this moment, are the freshwater inputs reduction, the entrance of contaminants in the aquatic environment, the exploration and introduction of biological resources, and the modifications of the river margins.

2.4. Temporal and spatial variation of the subtidal benthic invertebrates communities

The studies performed regarding the subtidal benthic invertebrates communities of the Mira estuary are somewhat heterogeneous and were not performed in a regular basis, which does not allow for a temporal series dataset. However, the composition of the subtidal benthic communities in the terminal part of the estuary is well documented, where several studies, e.g. Andrade (1984, 1986), Bruxelas et al. (1985), Campos & Fonseca (1985), Almeida (1987, 1988, 1994) and Ferreira (1994), describe these communities in detail in the area between the estuarine mouth and Moinho da Asneira. According to Andrade (1984, 1986) and Almeida (1988), in this estuarine sector may be considered the following types of biotopes, with the respective benthic macrocommunities associated:

a) Coarse sand, with dominance of polychaetes as *Ophelia bicornis*, *Ophelia rathkei* and *Ophelia negleta*;
b) Medium coarse sand, with relatively impoverished communities, where there is the occurrence of bivalves as *Cerastoderma edule* and, with much lower densities, *Nephtys* sp., *Angulus tenuis* and *Echinocardium cordatum*;
c) Muddy areas dominated by *Scrobicularia plana* and *Carcinus maenas*, and, in some areas, a relative abundance of *Cerianthus membranaceus* and *Diopatra neapolitana*;
d) Bottoms of decomposition, as a result of the accumulation of detritus, dominated by the polychaetas *Capitella capitata* and *Scolelepis fuliginosa*;
e) Areas covered by a fixed substrate, almost depleted from benthic communities, probably due to the development of an intense abrasion caused by the sandy material in suspension, allied to the inherent characteristics of the rocky elements.

The macrophytes communities (*Zostera noltii*) were particularly well studied, where much information may be gathered regarding the structure, dynamic, and production of the associated macrofauna (Almeida, 1987; 1988; 1992; 1994; Ferreira, 1994). Therefore, the macrobenthic communities of these areas are dominated by gastropods, both in terms of biomass and number of individuals, being only overruled by the polychaetes and bivalves when considering the specific richness parameter (Ferreira, 1994). According to this author, the taxa that present higher densities values and constancy along the year are the oligochaetes *Tubificoides benedeni* and *Monopylephorus irroratus*, the polychaetes *Mediomastus capensis* and *Capitella* sp., the gastropods *Bittium reticulatum*, *Peringia ulvae* and *Calliostoma zizyphinum*, and the bivalves *Loripes lacteus* and *Abra alba*.

When considering the upstream areas of Moinho da Asneira, the existing studies regarding the macrozoobenthic communities are more rare and less detailed, being the main works from Bruxelas et al. (1985), Campos & Fonseca (1985), Andrade (1986) and Chainho (2008).

According to these authors, is possible to see that, outside the *Zostera* banks, the groups of macroinvertebrates that are better represented in this estuary are the polychaetes, the bivalves and the amphipods. The analysis of these communities allows to infer that in the Alto do Zambujeiro area, around 6 km from the estuarine mouth, that occur the major biological discontinuities, being verified here the transition of communities with marine characteristics to more typical groups of estuarine environments (Bruxelas et al., 1985). From this point to upstream areas, the benthic communities present more homogeneous characteristics, being mainly dominated by tolerant species. The invertebrates' communities are structured according with their estuarine gradient, with a strong salinity influence, as illustrated in Figure 28. Although several sampling seasons are represented (Summer 2003; Winter 2004; Winter, Spring, Summer and Autumn 2006), the grouping of communities is mainly made by salinity classes, regardless the sampling season, with exception of one station sampled in the euhaline area for the Winter period, that presented a high level of impoverishment, becoming more similar to the typical community structure of polihaline areas.

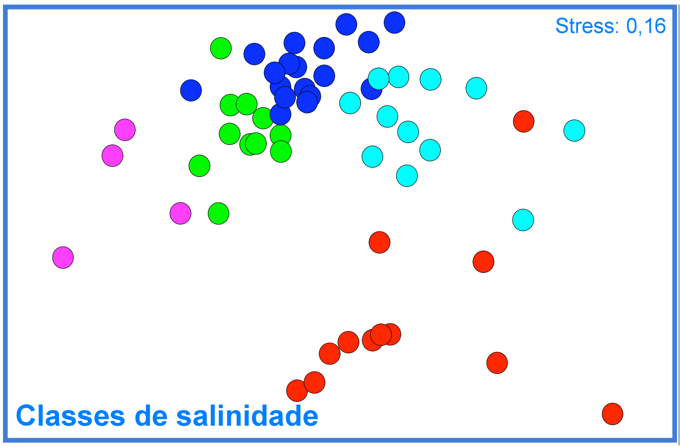


Figure 28. Nonmetric multidimensional scaling (nMDS) analysis of the benthic communities of the Mira estuary identified in 2003, 2004 and 2006 (pink: tidal; green: oligohalino; dark blue: mesohalino; light blue: polihaline and red: euhaline)

According to Chainho (2008; non-published data) and Chainho et al. (2008), the benthic communities' characterization, along the Mira estuarine gradient, can be done as:

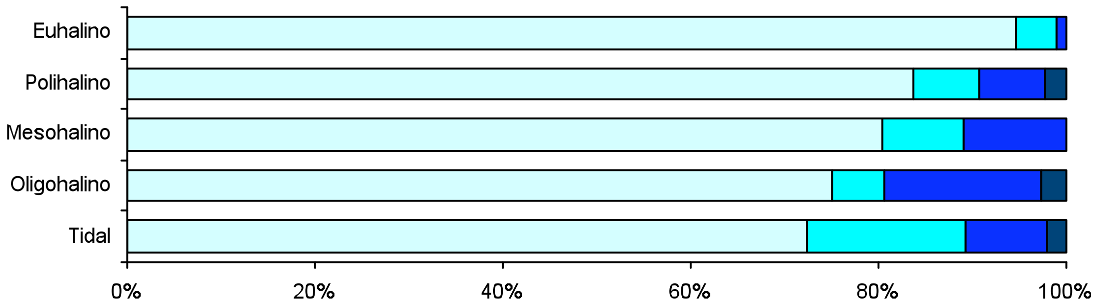
a) Tidal – bottoms with heterogeneous substrate, of low depths, being the communities dominated by insects, some species of amphipods (*Gammarus insensibilis*; *Corophium orientale*) and oligochaetes;
b) Oligohaline – sediments that vary from medium to coarse sand, dominated by species as *Corophium orientale* and *Corbicula fluminea*;
c) Mesohaline – muddy sediments, dominated by the amphipods *Corophium orientale* and *Leptocheirus pillosus*, the polychaetes *Alkmaria romijni* and *Streblospio shrubsolii* and the isopod *Cyathura carinata*;
d) Polyhaline – muddy sediments, where the dominant species are the amphipod *Corophium acherusicum* and a group of tolerant polychaetes as *Heteromastus filiformis*, *Hediste diversicolor* and *Nephtys hombergii*;
e) Euhaline – being composed in its majority by muddy-sand bottoms, the sediments of the terminal area of the estuary are composed by a diverse community, dominated by some opportunistic species, like as oligochaetes and the polychaete *Capitella capitata*, but where, simultaneously, occur several species of polychaetes and bivalves, typical of the marine environment.

Although there is a clear dominance of tolerant species in the intermediate areas of the estuary, there are substantial differences in the communities' structure between the years 2003/2004 and 2006, where a substantially higher number of species were identified in this last year. These results may suggest a direct consequence/effect of the severe drought observed on the system, being the driest year 2005, which may have caused a degradation of the environmental conditions, as described in the previous point.

The seasons variability is highlighted by the constancy index results *(Cij = (nij/nj)*100*, where *nij* is the number of occurrence of species *i* in station *j* and *nj* is the number of stations) calculated for the six seasons of the years for which there are observations for the same sampling stations (Figure 29).

The species were considered as constants (C > 50%), characteristics (100% > C > 67%) or exclusives (C = 100%) (Bachelet et al., 1996). The results show a strong seasonal variation in the taxonomic composition in all the saline stretches of the estuary, where only 3 taxa occur in all the sampled seasons (Chironomidae, *Corophium orientale* and *Heteromastus filiformi*). The total of exclusive, characteristic and constant species represent less than 30% in all the saline areas, and in the euhaline it represents around 5%. All the other species occurred in less than half of the sampling seasons.

Although the Mira estuary is considered as a relatively pristine system (Marques et al., 1993; Carvalho et al., 2005), the benthic invertebrate communities seem to be highly conditioned by the strong natural stress, which results from both seasonal and interannual variations in environmental conditions. The obtained results during a drought period (2003/2004) and a period with higher precipitation are illustrative of those differences, as for example in the total number of observed taxa (68 in 2003/2004 and 278 in 2006).



	Tidal	Oligohalino	Mesohalino	Polihalino	Euhalino
Constantes	*Atyaephyra desmaresti* Ecnomidae Hydracarina Limoniidae *Mediomastus fragilis* Oligochaeta spA Simulidae	*Boccardia redeki* *Gammarus insensibilis*	*Alkmaria romijni* *Boccardia redeki* Chironomidae Oligochaeta	*Cyathura carinata* *Nephtys hombergii* *Streblospio shrubsolii*	*Abra alba* *Ancistrosyllis* sp. *Capitella capitata* *Corbula gibba* *Heteromastus filiformis* *Mediomastus fragilis* *Nephtys hombergii* Oligochaeta
Características	Ceratopogonidae *Corophium orientale* Empididae *Gammarus insensibilis*	Chironomidae *Corbicula fluminea* *Cyathura carinata* *Hediste diversicolor* *Leptocheirus pilosus* Oligochaeta	*Corophium orientale* *Cyathura carinata* *Hediste diversicolor* *Leptocheirus pilosus* *Streblospio shrubsolii*	*Alkmaria romijni* *Corophium orientale* *Hediste diversicolor*	
Exclusivas	Chironomidae	*Corophium orientale*			*Heteromastus filiformis*

Figure 29. Results obtained from the constancy index for the 5 saline classes of the Mira estuary, for the different seasons of 2003, 2004 and 2006. In the figure is presented the list of exclusive, characteristic and constant species.

These differences may have strong consequences in the use of tools that aim to evaluate the ecological quality of a system, especially in the application of indices that use the information relative to the sensibility/tolerance of species regarding anthropogenic stress. As demonstrated by Chainho et al. (2008), although the Mira estuary presents lower levels of anthropogenic stress, when compared with other Portuguese estuaries, the results from the application of biotic indices indicate that it may be placed below the Good Ecological Status required by the Water Framework Directive. This demands further studies to adapt it so that a correct evaluation of this kind of systems is achieved.

3. Effects of natural vs. anthropogenic variations in benthic communities of Portuguese estuaries

3.1. Comparative summary of the two estuaries' characteristics

The Mira and Mondego estuaries are both located on the western coast of Portugal. In the scope of the WFD typologies definition process (Bettencourt et al., 2004) these were classified as type A2 – mesotidal estuaries, well-mixed with irregular freshwater discharges, although presenting distinct hydrological characteristics, as described in Table 7.

Table 7. Main hydrographic characteristics of the Mondego and Mira estuaries. BN – North arm and BS – South arm.

	Mondego	Mira
Area (km^2)	9	3
Volume ($10^6 m^3$)	21	17
Flow (m^3s^{-1})	80	10
Residence time (days)	2 (BN) 3 (BS)	14

The Mondego estuary occupies an area of approximately 9 km^2 while the Mira estuary covers an area of about 3 km^2. The mean annual flow of these two estuaries varies between 10 m^3s^{-1} and 80 m^3s^{-1}, in Mira and Mondego, respectively. The water residence time is higher in the Mira estuary, being necessary 14 days to the total water renewal in the estuary. In the Mondego estuary, the water residence time is lower and varies between 2 and 3 days, in the North and South arms, respectively.

The degree of human pressure varies on the studied systems (Figure 30), with higher population, industry and agricultural levels on the Mondego than what is registered for the Mira estuary (Chainho et al., 2008). In agreement with data from the INAG monitoring programs, eutrophication is considered the main problem in the Mondego whereas in the Mira estuary some areas register levels of heavy metal contamination by metals such as As, Cr, Cu and Ni (Chainho et al., 2008). Furthermore, the Mondego has suffered profound hydromorphological changes while the Mira estuary only suffered minor changes. In both estuaries the freshwater discharges are regulated by sluices located upstream in their hydrographic basins (Chainho et al., 2008).

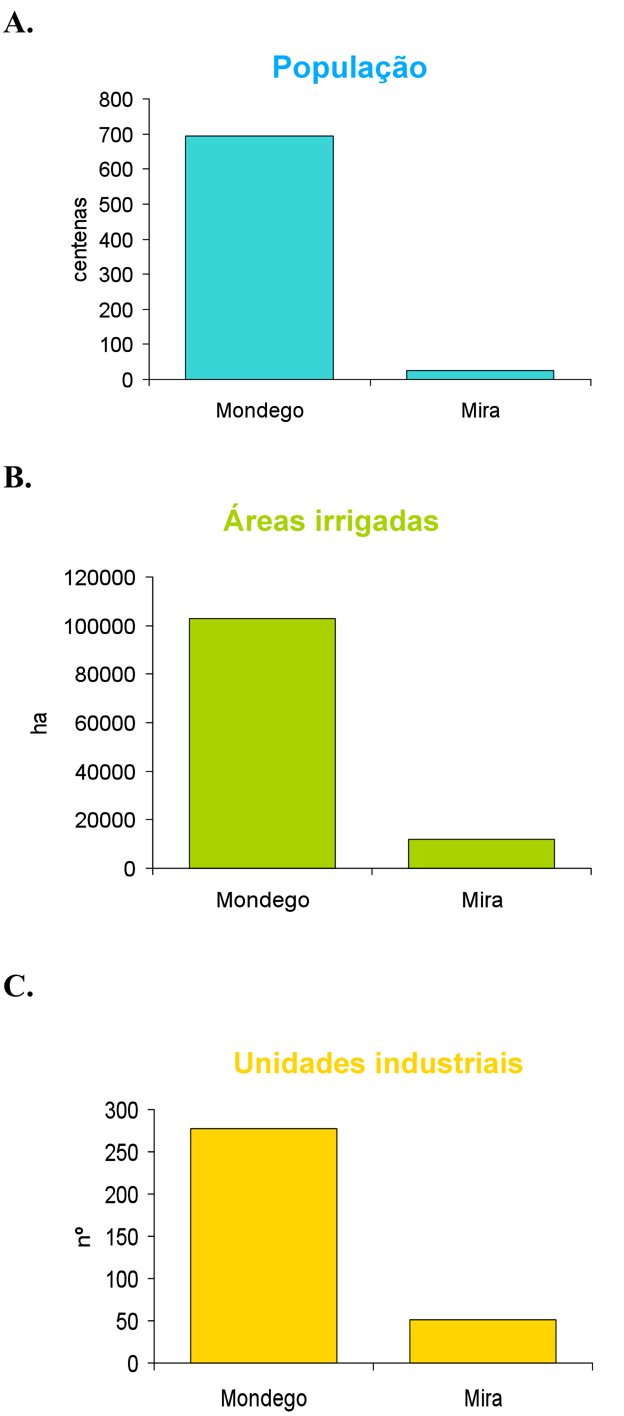
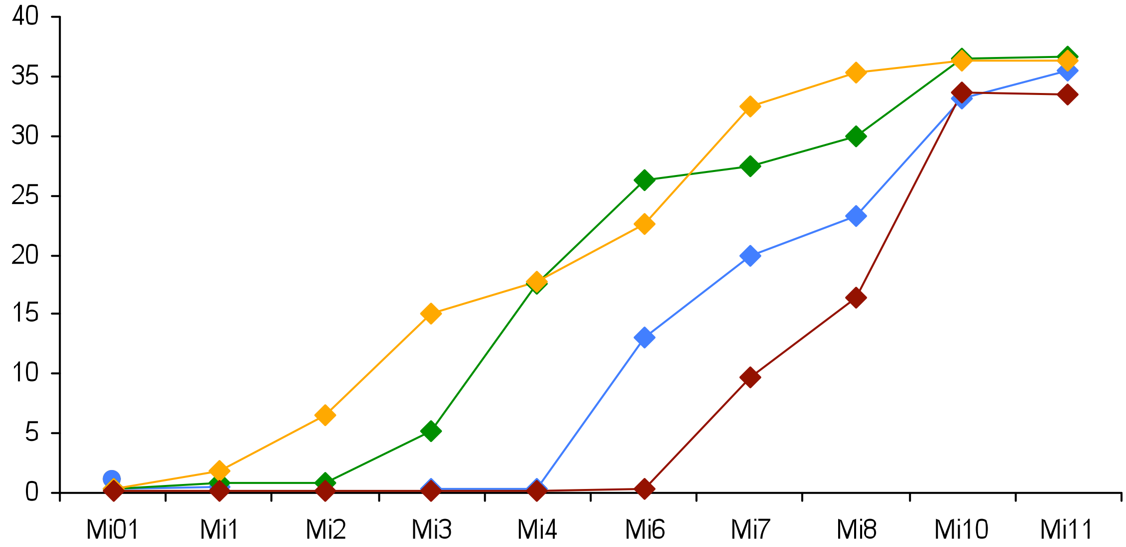


Figure 30. A. Population, B. Irrigation area and C. Number of industries in the Mondego and Mira estuaries (source: INAG, 2006, www.inag.pt).

Both estuaries register strong variations in environmental conditions, not only seasonally but also daily, associated with the tidal cycles. Figure 31 shows the salinity variation along the estuarine gradient in both study systems. In the Mira estuary salinity amplitudes of 26 units were registered among seasons (Figure 31 A) and measurements along the tidal cycle showed variations until 15 units during the 2006 autumn flood (Figure 32 A).

A. Mira



B. Mondego

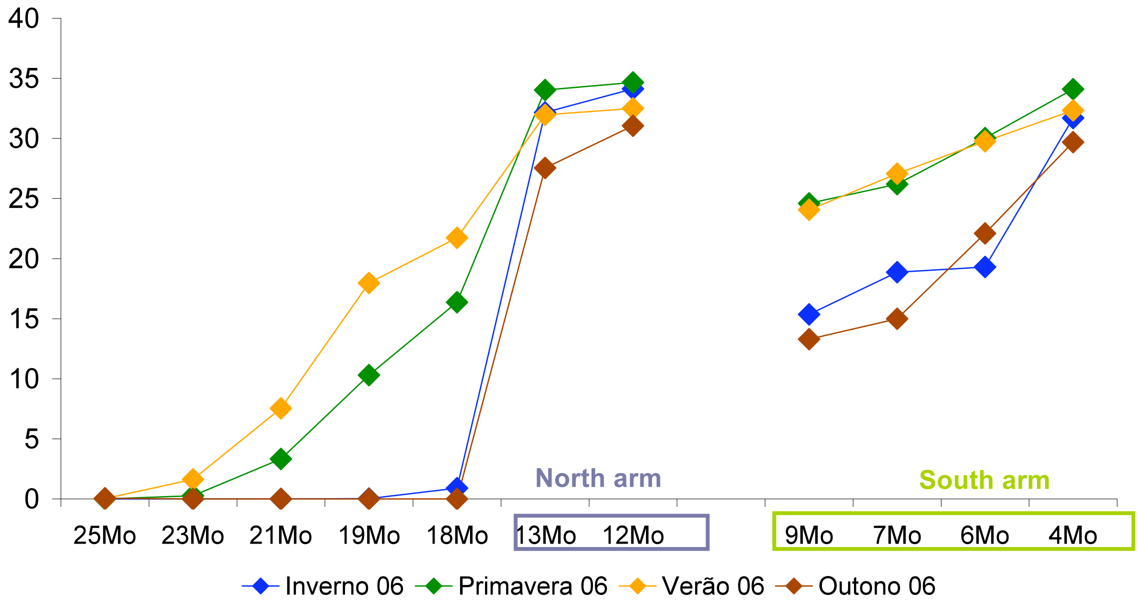
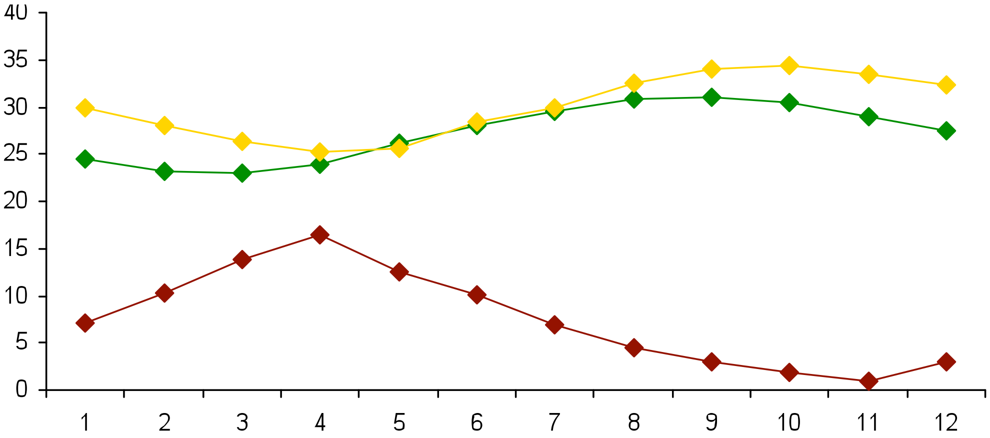


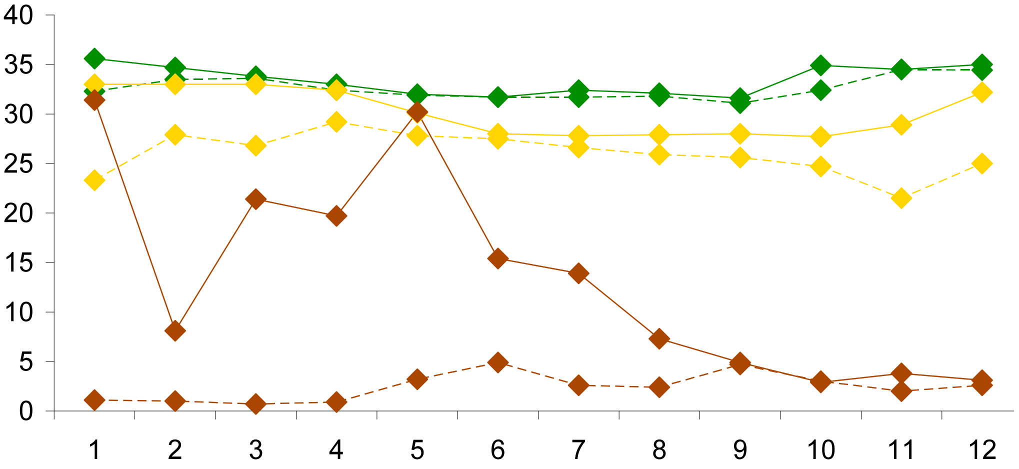
Figure 31. Bottom salinity variation along the two estuaries for each sampling season in 2006. A. Mira and B. Mondego estuary.

In the Mondego estuary were registered salinity amplitudes of 21 units (in the bottom) and 30 units (at surface) among seasons (Figure 31 B) and measurements along the tidal cycle showed variations until 32 units during the 2006 autumn flood (Figure 32 B).

A. Mira



B. Mondego – station 3 (SA)



C. Mondego – station 10 (NA)

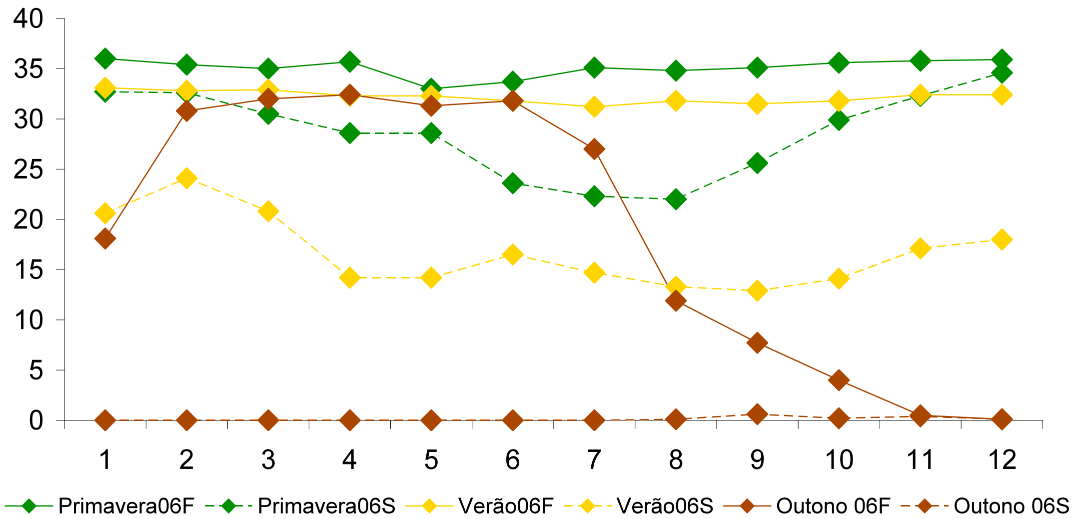


Figure 32. Salinity variation (bottom: solid line and surface: dashed line) along a 12 hours cycle. A. sampling station 7 of the Mira estuary, B. and C. sampling stations 3 and 10 of the Mondego estuary in spring, summer and autumn of 2006.

In both systems, the greater salinity amplitudes were recorded in the mesohaline and oligohaline areas. The higher daily salinity ranges were registered during the flood period in the 2006 autumn. In sum, the communities of these two estuaries are subjected to constant physiological stress being especially pronounced during the flood period.

Both systems have been exposed to extreme climatic events in the last 15 years. Figure 33 shows the average monthly precipitation in the Odemira station from 1990 to 2006, highlighting the winter and autumn 1990 floods, the 2006 autumn flood, as well as, the 2005 drought.

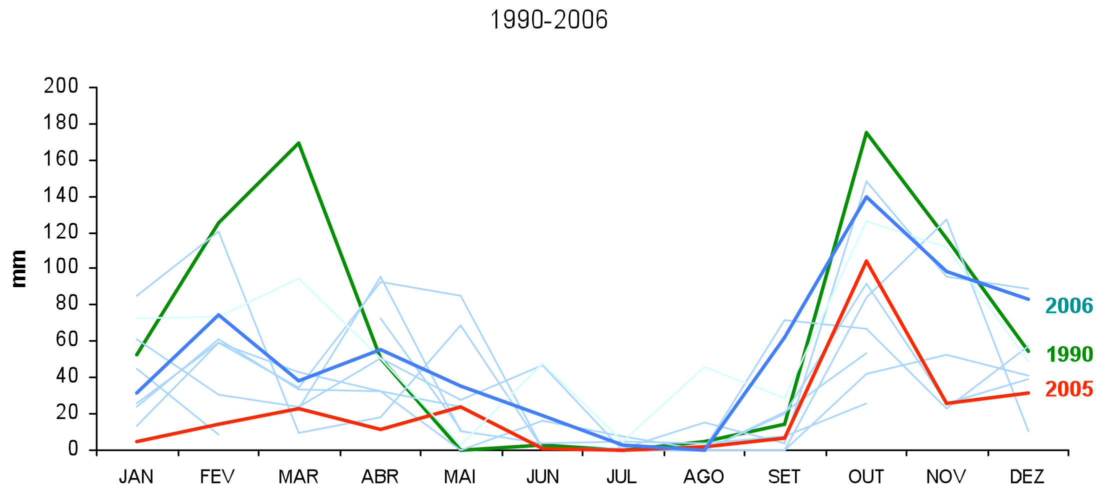


Figure 33. Average monthly precipitation (mm) in Odemira station from 1990 to 2006 (from: INAG, 2008, www.inag.pt).

Figure 34 represents the monthly average daily flow in the Açude-Ponte, Coimbra, from 1990 to 2007. It is clearly visible the centennial flood of the 2000/2001 winter, as well as, the severe drought of 2004/2005 and the 2006 autumn flood.

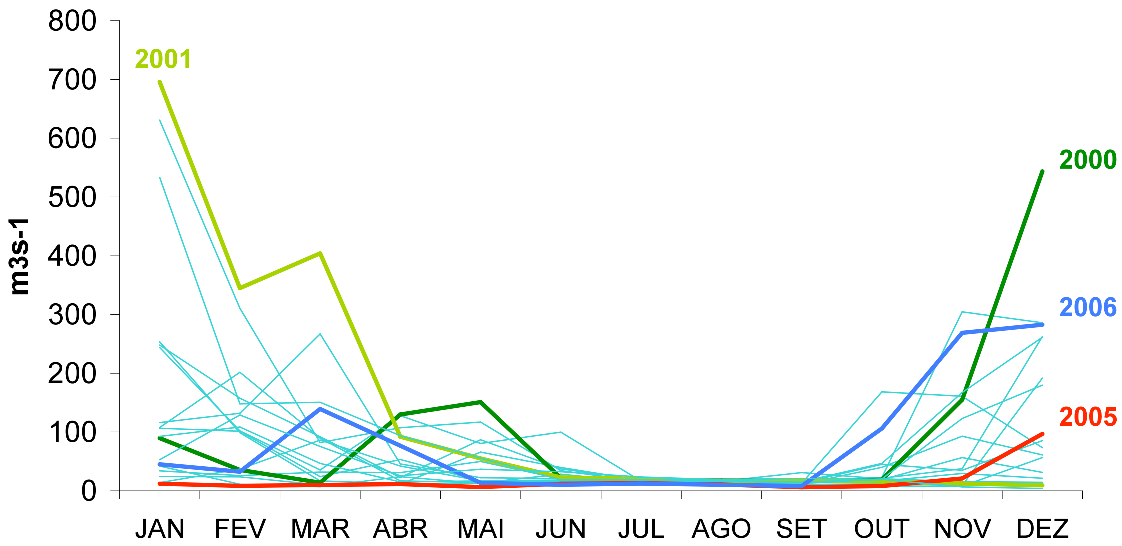


Figure 34. Monthly average daily effluent flow (m3/s) in the Açude-Ponte, Coimbra, from 1990 to 2007 (from: INAG, 2008, www.inag.pt).

3.2. Subtidal macroinvertebrates benthic communities' spatial and temporal variations

The occurrence of seasonal variations in macroinvertebrates communities' composition and abundance is mainly due to most species recruitment peaks during spring and autumn, but also to the occurrence of extreme environmental conditions, such as temperature fluctuations, floods and droughts (Alden et al., 1997; Attrill & Power, 2000; Salen-Picard & Arlhac, 2002).

Considering structural parameters, such as the number of identified *taxa* or the individuals mean density per sampling station (Figure 35), similar values for Mira and Mondego estuary were observed, except for the euhaline stations (Mi10 e Mi11) located in the Mira estuary mouth. In these, the number of registered *taxa* is considerable higher relatively to the number found for other estuarine sections and also to the number found for the Mondego estuary. However, it is important to note that on the Mondego sampling was limited to the polihaline/euhaline transition zone so that there are no records for the areas close to the mouth with higher marine influence and thus, the absence of comparable registers concerning the number of *taxa* per station identified in this section between the two systems. In both systems there was an increase in the number of identified *taxa* per station from upstream towards downstream.

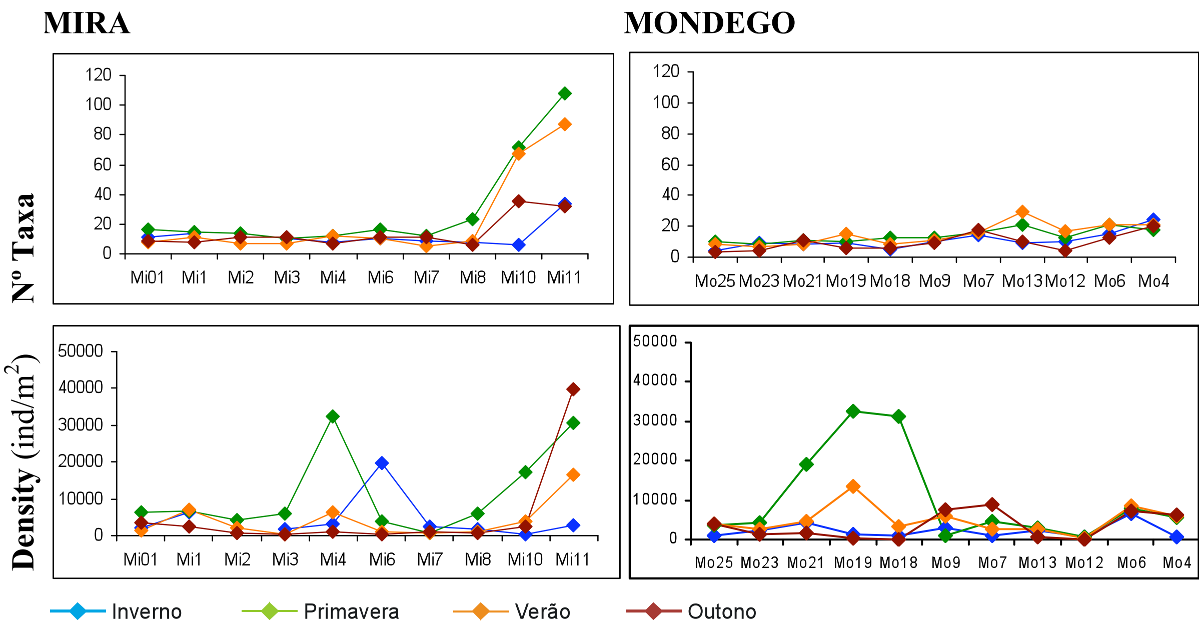


Figure 35. Variation in the number of *taxa* and mean density of benthic macroinvertebrates along the Mira and Mondego estuaries.

As well, in spring and summer periods the number of identified *taxa* per station was high with the exception of the oligohaline/tidal zone (Mi01, Mi1, M12, Mo25, Mo23, Mo21) in the Mira estuary, where the number of *taxa* registered was high in winter and spring. In the Mondego estuary variations in the number of *taxa* throughout the year were unclear. This situation seems to be associated to the fact that upstream sampling stations in the Mira estuary are located in zones where the river bed and banks are already very similar to those of freshwater environments and thus the communities are dominated by *taxa* from the Class Insecta. In this estuarine zone, the flow reduction during the driest months is quite significant and leads to the formation of lentic areas with less connectivity. As indicated by Ward et al. (1999), there is less diversity in areas of lower connectivity than in a watercourse with lotic conditions.

The average density per station was more variable, either during the different seasons or for the different estuarine zones in both systems, reaching peaks in the mesohaline zones mainly in the spring. Although highly variable, the station further downstream of the Mira estuary (Mi11) was the one that showed consistently higher densities during the entire year of 2006.

The macrobenthic communities in the Mondego and Mira estuaries are similarly affected by the occurrence of hydrological variations and thus are dominated by very tolerant species to both natural and anthropogenic stress. Despite the occurrence of such variations, communities' spatial segregation throughout the salinity gradient is quite remarkable in both estuaries and it overlaps the observed seasonal variations within each salinity zone (Figure 36).

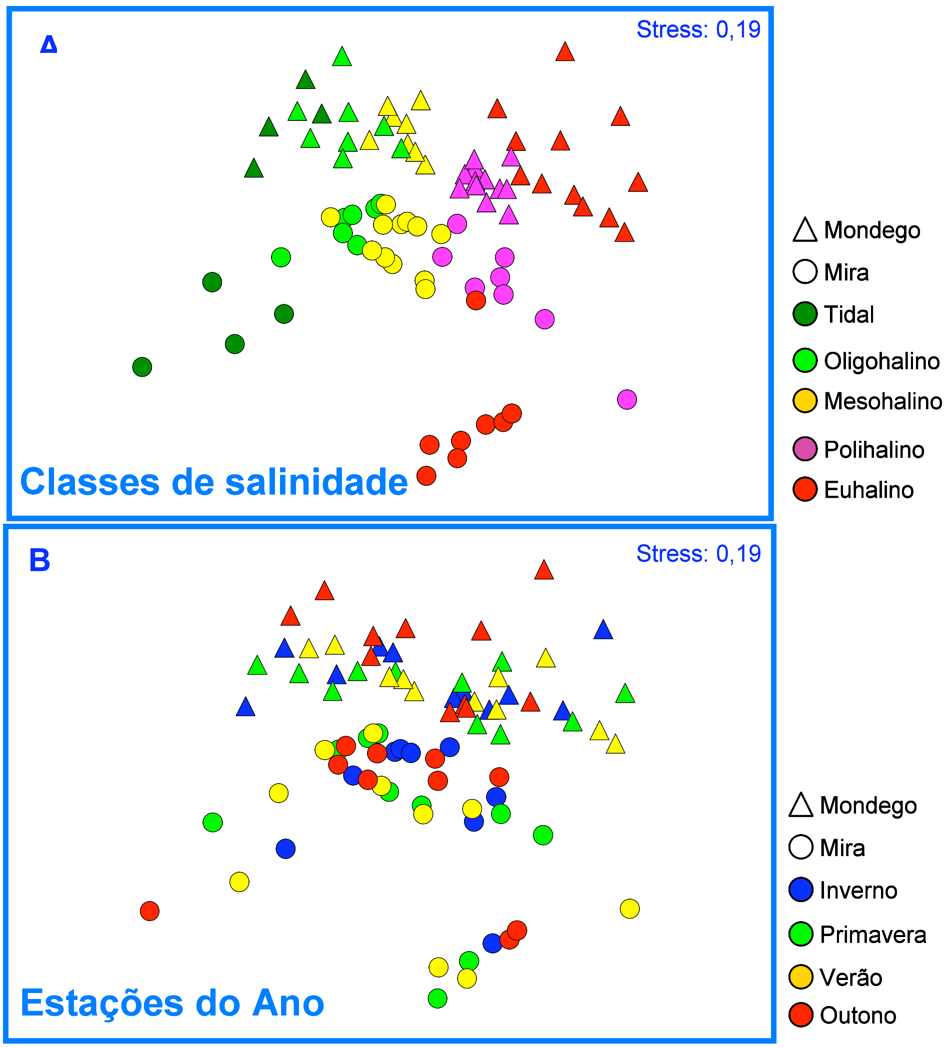


Figure 36. Non-metric multidimensional scaling analysis (nMDS) of benthic macroinvertebrate communities of the Mira and Mondego estuaries identified in the 2006 year.

A similarity analysis performed considering salinity as a factor (*one-way* ANOSIM), allowed to conclude that there are significant differences among the communities belonging to several salinity regions (R=0.476; P=0.001). The pairwise-tests revealed that there are significant differences among all salinity regions (P<0.05). When testing for statistical differences among the several seasons, in general, these are not significant (R=0.008; P=0.27), except when seasons are compared for each salinity region (*two-way* ANOSIM, salinity nested in season; R=0.338; P=0.002). Pairwise tests for the different seasons showed significant differences among all seasons (P<0.05), except between summer and autumn. The ordination shown in Figure 36 also allows concluding a clear separation among the communities of the two estuaries for all the salinity regions (one-way ANOSIM; R=0.309; P=0.001). The communities from the oligohaline, mesohaline and polihaline zones seem to be more similar between estuaries than the tidal and euhaline communities, however significant differences among all salinity regions of both estuaries are observed (*two-way* ANOSIM, salinity nested in estuary; R=0.712; P=0.008). These results seem to reflect the effect of geographic differences between the two estuaries but also can be a result of the different pressure levels registered in each estuary, validating the establishment of reference conditions to ecological quality status assessment in type A2 estuaries. Some of the observed differences may be due to the occurrence of different species but with the same kind of ecological valences, as is the case of amphidods *Corophium multisetosum* that occurs only in the Mondego estuary and *Corophium orientale* only registered in the Mira estuary.

In agreement with the constancy index most of the species occur only one or two times per year in both estuaries and the set of species always present in the different salinity regions throughout the seasons is very reduced (Figure 37).

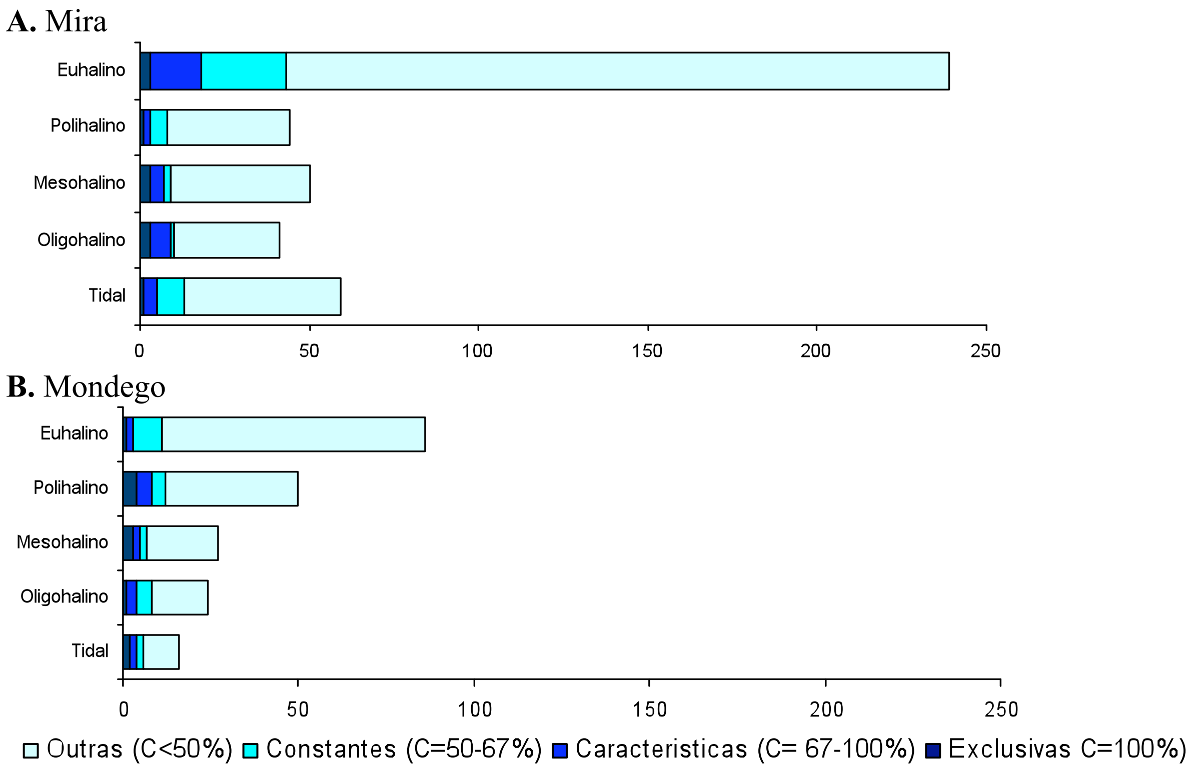


Figure 37. Constancy index results for the 5 salinity classes of the A. Mira and B. Mondego estuary.

The set of key-species in the different salinity regions, in most seasons, seem to indicate a similar ecological valence, particularly the dominance of tolerant and opportunistic species (Table 8).

Table 8. List of exclusive, characteristic and constant species of Mira and Mondego estuaries according to the constancy index results.

	Exclusivas		**Características**		**Constantes**	
	Mira	Mondego	Mira	Mondego	Mira	Mondego
Tidal	Chironomidae	*Corbicula fulminea*	Ceratopogonidae	Chironomidae	Ecnomidae	*Corophium multisetosum*
		Oligochaeta	*Corophium orientale*	Diptera	Gastropoda	Tubificidae
			Empididae		Hydracarina	
			Gammarus insensibilis		Limoniidae	
					Oligochaeta spA	
					Simulidae	
Oligohalino	*Corophium orientale*	*Corbicula fulminea*	Chironomidae	*Corophium multisetosum*	*Boccardia redeki*	*Alkmaria romijni*
	Cyathura carinata		*Gammarus insensibilis*	*Cyathura carinata*		Chironomidae
	Corbicula fulminea		*Streblospio shrubsolii*	Oligochaeta		*Streblospio shrubsolii*
			Hediste diversicolor			*Ephoron virgo*
			Leptocheirus pilosus			
			Oligochaeta			
Mesohalino	*Corophium orientale*	*Corbicula fulminea*	*Streblospio shrubsolii*	*Hediste diversicolor*	*Alkmaria romijni*	Oligochaeta
	Cyathura carinata	*Corophium multisetosum*	*Hediste diversicolor*	*Cyathura carinata*	*Boccardia redeki*	*Hydrobia ulvae*
	Leptocheirus pilosus	*Streblospio shrubsolii*	Chironomidae		*Cyathura carinata*	
			Oligochaeta	Oligochaeta	*Nephtys hombergii*	
Polihalino	*Heteromastus filiformis*	*Cyathura carinata*	*Corophium orientale*	*Alkmaria romijni*	*Streblospio shrubsolii*	*Capitella capitata*
		Hediste diversicolor	*Hediste diversicolor*		*Alkmaria romijni*	*Polydora ligni*
		Scrobicularia plana			*Scrobicularia plana*	*Corophium multisetosum*
		Streblospio shrubsolii				
Euhalino	*Nephtys hombergii*	*Hydrobia ulvae*	*Heteromastus filiformis*	*Cerastoderma edule*	*Glycera alba*	*Cerastoderma glaucum*
	Oligochaeta		*Mediomastus fragilis*	*Streblospio shrubsolii*	*Pseudopolydora paucibranchiata*	*Cyathura carinata*
	Maldanidae		Nemertea		*Venerupis senegalensis*	*Lekanosphaera levii*
			Abra alba		*Prionospio cirrifera*	Oligochaeta
			Ancistrosyllis sp.		*Pholoe inornata*	*Scrobicularia plana*
			Capitella capitata		*Pectinaria koreni*	*Abra alba*
			Melinna palmata		*Notomastus latericeus*	*Tharyx* sp.
			Owenia fusiformis		*Diopatra neapolitana*	
			Lucinoma borealis		*Chamelea gallina*	
			Glycera convoluta		*Streblospio shrubsolii*	
			Aonides oxycephala		*Cerastoderma glaucum*	
			Tellina donacina		*Cerastoderma edule*	
			Spisula subtruncata		Chironomidae	
			Kefersteinia cirrata		*Eunereis longissima*	
			Aphelochaeta sp.		*Spiophanes bombyx*	
					Sphaerosyllis taylori	
					Prionospio sp.	
					Nassarius reticulatus	
					Myriochele heeri	
					Magelona minuta	
					Lanice conchilega	
					Hiatella arctica	
					Digitaria digitaria	
					Corbula gibba	

4. Evaluation of the ecological quality of the two estuaries based on benthic macroinvertebrate communities

The ecological indicators used in this analysis highlight some of the patterns described for the basic structural parameters from the benthic subtidal communities of the Mira and Mondego estuaries (Figure 38). The specific richness, evaluated with Margalef index, tends to increase from upstream to downstream in both estuaries. In the Mira estuary, the increase is less clear throughout the estuarine areas but it becomes clearly higher in the euhaline stations, particularly, in spring and summer. In the Mondego, the gradual increase of the specific richness is more perceptible, as well as the variation of the Margalef index throughout the different seasons.

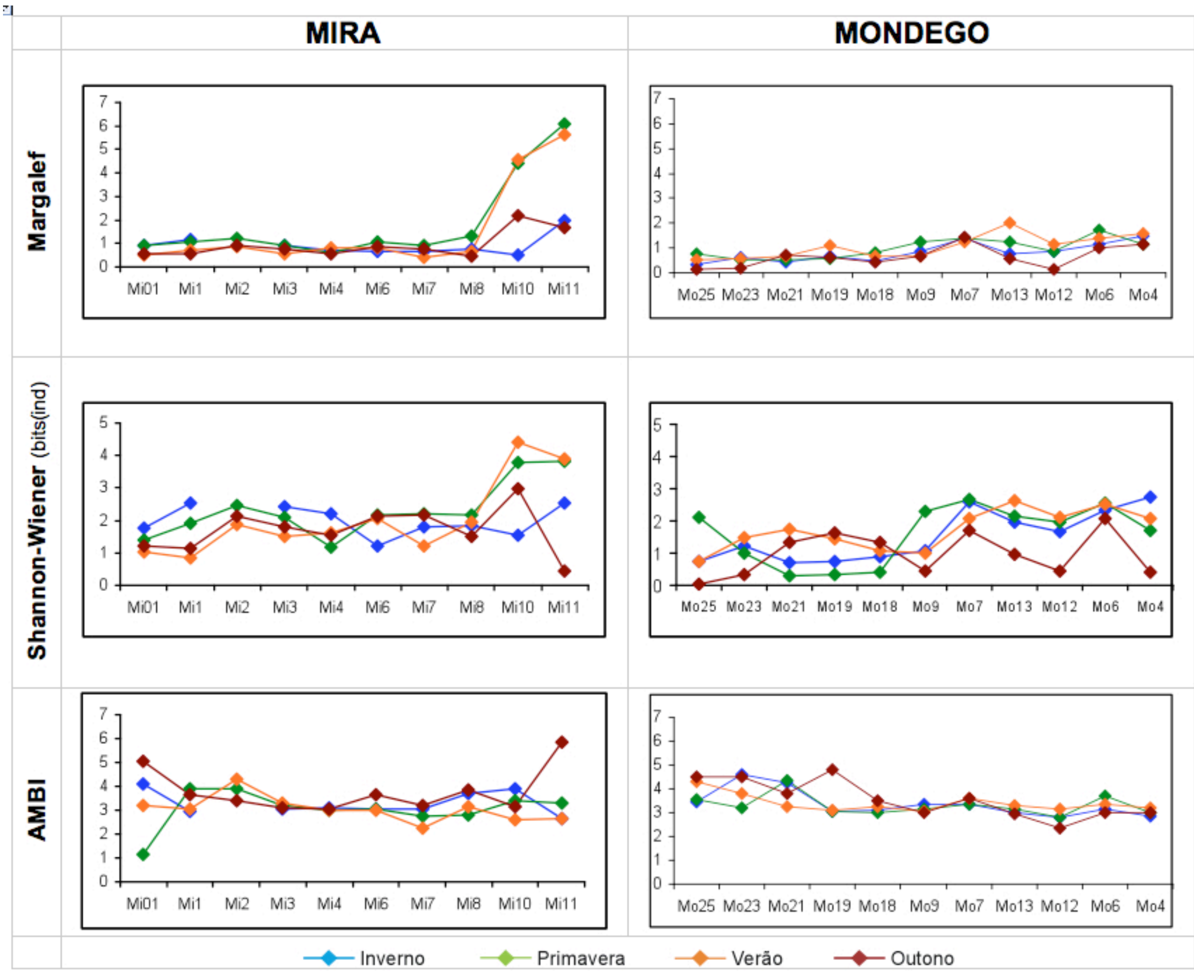


Figure 38. Seasonal variation of Margalef, Shannon-Wiener and AMBI in Mira and Mondego, in 2006.

Shannon-Wiener diversity increases from upstream to downstream, exhibiting higher similarity along the system in the Mira estuary than in the Mondego. Both estuaries exhibited similar values of diversity in the polihaline areas and, again, the euhaline area of the Mira estuary exhibited the highest diversity value for this index, for the entire study period. The values for the upstream areas (mesohaline and oligohaline/tidal) of the Mondego estuary are lower than the values for the same areas of the Mira estuary and, clearly, lower than the values in the downstream areas. In the two estuaries, there are also differences in the variation of this index in the upstream areas for the same seasons. In general, in the Mira estuary, higher values were found in winter and spring, while in the Mondego, the increase in the diversity in the upstream areas was more evident in summer and autumn, probably due to the reasons indicated for the number of taxa. From the three used indices, this is the one that exhibits higher seasonal variations in both estuaries.

Both estuaries have shown similar values for the AMBI index, as well as a similar variation along the different areas, where, in general, the upstream areas exhibited higher values, which indicates a higher number of species indicating some kind of disturbance.

The ecological status (EQS) was evaluated (Figure 39) using the BAT multimetric, developed for benthic subtidal macroinvertebrate communities, in the scope of the WFD, in Portugal (for details see Marques et al., 2007; Pinto et al., 2009; Teixeira et al., 2009; Neto et al., 2010).

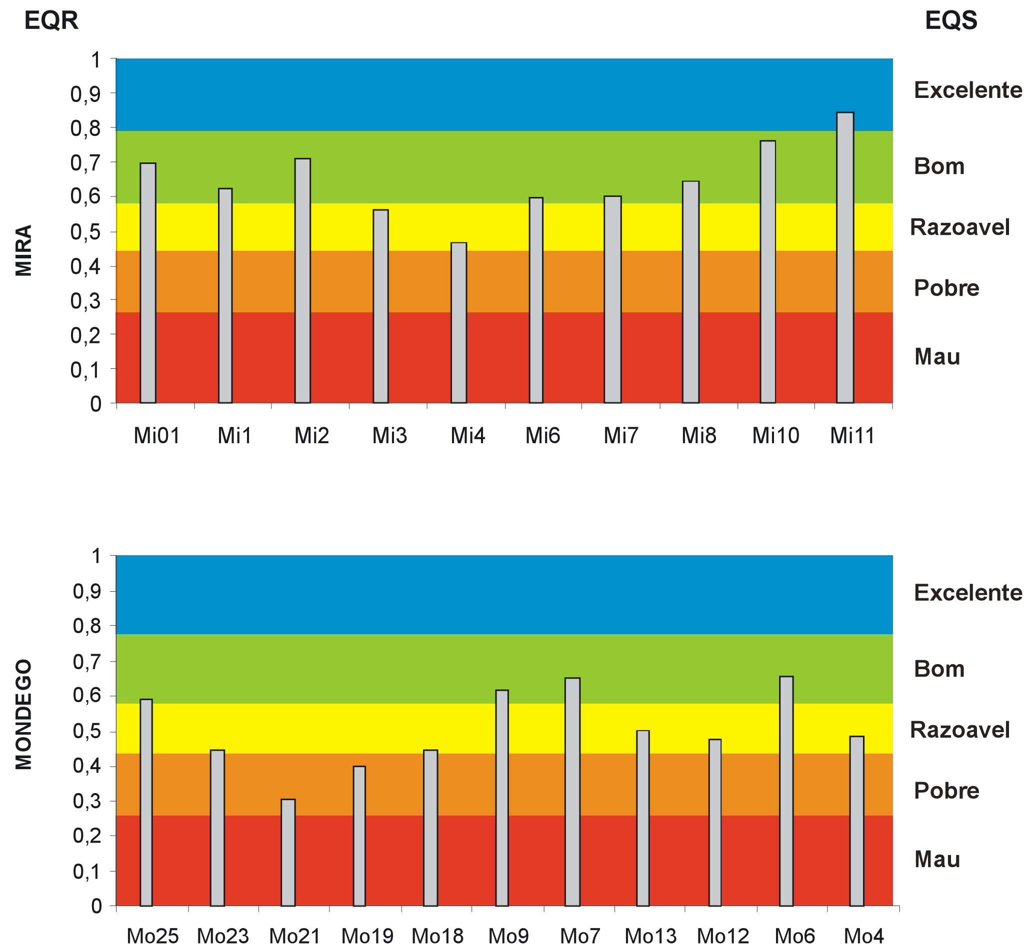


Figure 39. Changes in EQR and EQS at Mira and Mondego estuaries, in 2006 (Spring).

Since both studied systems belong to the same typology (A2) (Bettencourt et al., 2004), according to the criteria adopted for the WFD application, the evaluation of the ecological status was performed based on the reference conditions suggested for the Mondego estuary (Teixeira et al., 2008b). The adopted reference values take in consideration the need to reflect the spatial heterogeneity in determining EQS, as proposed by Teixeira et al. (2008a) e de Paz et al. (2008). Initially, the proposed reference values were calculated based on the average for sampling station (Teixeira et al., 2008b), due to limitations related with the type of available data and the need to consider a long data set, which would allow to ascertain the capacity of the different indicators to capture variations in the system caused by anthropogenic disturbances (Pinto et al., 2009; Teixeira et al., 2008b). However, in order to apply the WFD, the evaluation of the ecological status of benthic communities will be done based on replicates. Consequently, the values of the indices are slightly lower than expected for a calculation based on station, which is reflected on a final EQR value lower than the one that the system could achieve.

Both estuaries show differences related to the ecological quality status (EQS) in their benthic communities. In general, the Mira estuary exhibited a better ecological quality than the Mondego estuary, with 80% of the sampling stations showing the minima criteria demanded by the WFD for the year 2015, the Good ecological status. Stations Mi3 and Mi4 showed a condition of Moderate, which is an ecological quality status lower than the defined aim, which might indicate the existing anthropogenic pressures related with the direct discharge of Odemira effluents to the estuary, due to problems in the functioning of the WWTP. In the Mondego estuary, in 2006, only 36% of the stations presented a Good ecological status for benthic communities, 46% were one class lower than the defined aim and 18% were classified as Poor ecological status. In general, for 2006, the upstream stations in the North arm of the Mondego estuary showed a worst condition than the downstream stations, which may have been caused to impacts related with the structures used in the construction of a new highway, located between stations Mo21 and Mo19. The sampling stations from the South arm showed, in general, a better ecological status, still not all stations have shown the classification of Good.

Besides the determined ecological status based on benthic macroinvertebrates, the evaluation of the final ecological status of the transitional systems integrates other biological and chemical elements. The evaluation of the status of the physico-chemical elements (Lillebø et al., 2007) in this estuary, for the previous years (1936 until 1996) and after the mitigation actions (1999 until 2002) highlight an improvement in the system but also indicate that some nutrients still show higher values, namely, in the North arm, where freshwater inputs initially discharged to the South arm were driven to. Samplings from 2003 to 2006 have applied another index (TRIX) to evaluate the trophic status of the system concerning the nutrient concentration, oxygen saturation and chlorophyll a, which have also indicated still high nutrient concentrations and a higher degradation in the upstream areas of the South arm in relation to the downstream areas (Salas et al., 2008). In parallel, Patrício et al. (2007) have tested the methodologies to evaluate the ecological quality of the system based on opportunistic macroalgae. Results indicate an agreement with the dynamics of this biological element in the Mondego estuary, in the considered period of time. This work contributed to improve the general characterization of the South arm water mass.

2.2. DETAILED DESCRIPTION OF THE INDICATORS ACHIEVED

Globally, the EXTREMIS project has fulfilled and surpasses all expected deliverables (all pdf files are given as supplementary material).

It is important to mention that for Ecology-based projects, 2 years of duration is a short time span to complete all tasks and publish the results. Nevertheless, the privileged way to communicate the outcome of the project was the production of several papers published in international scientific indexed journals. At the end of the project, 6 papers have completed the publication process, 4 manuscripts are submitted to SCI journals and 4 other papers are under preparation to be submitted. In addition, results were also presented at international meetings, through 12 oral communications and 4 posters. The EXTREMIS project has made viable the elaboration and conclusion of 9 MSc Thesis.

The overall production indicators may be summarized/listed as:

PUBLICATIONS SCI

Baeta, A., Niquil, N., Marques, J.C. & Patrício, J. 2011. Modelling the effects of eutrophication, mitigation measures and an extreme flood event on estuarine benthic food webs. Ecological Modelling (doi:10.1016/j.ecolmodel.2010.12.010).

Couto, T., Patrício, J., Neto, J.M., Ceia, F.R., Franco, J. & Marques, J.C. 2010. The influence of mesh size in environmental quality assessment of estuarine macrobenthic communities. Ecological indicators, 10: 1162-1173 (doi:10.1016/j.ecolind.2010.03.019)

Krell, B., Moreira-Santos, M. & Ribeiro, R. Pollution versus organic matter decomposition in estuarine sediments: a mudsnail in situ assay based on postexposure feeding. Environmental Toxicology and Chemistry (accepted with major revisions).

Patrício, J., Adão, H., Neto, J.M., Alves, A.S., Traunspurger, W. & Marques, J.C. 2011. Do nematode and macrofauna assemblages provide similar ecological assessment information? Ecological Indicators (accepted with moderate revisions).

Patrício, J., Neto, J.M., Teixeira, H., Salas, F. & Marques, J.C. 2009. The robustness of ecological indicators to detect long-term changes in the macrobenthos of estuarine systems. Marine Environmental Research, 68: 25–36

Teixeira, H., Neto, J.M., Patrício, J., Veríssimo, H., Pinto, R., Salas, F. & Marques, J.C. 2009. Quality assessment of benthic macroinvertebrates under the scope of WFD using BAT, the Benthic Assessment Tool. Marine Pollution Bulletin, 58: 1477-1486. (doi:10.1016/j.marpolbul.2009.06.006)

Submitted:

Ceia, F.R., Patrício, J., Franco, J., Pinto, R., Fernández-Boo, S., Losi, V., Marques, J.C. & Neto, J.M. Dredging activities: ecological quality status and assessment of estuarine macrobenthic assemblages. Ocean and Coastal Management (submitted)

Pinto, R., de Jonge, V.N., Neto, J.M., Domingos, T., Marques, J.C., Patrício, J. Towards a DPSIR driven integration of ecological value, water uses and ecosystem services for estuarine systems. Ocean and Coastal Management (submitted)

Veríssimo, H., Neto, J., Teixeira, H., Fath, B., Marques, J.C. & Patrício, J. Ability of Benthic indicators to assess ecological quality in estuaries following management. Ecological Indicators (submitted)

Vinagre, P., Patrício, J., Neto, J.M., Teixeira, H., Ceia, F., Franco, J., Marques, J.C. & Neto, J.M. Defining reference conditions for meso- and oligohaline stretches sensus WFD. Adapting the benchmark concept to intercalibrate freshwater and transitional water assessments. Estuarine Coastal and Shelf Science (submitted)

In preparation:

Araújo, C.V.M., Moreira-Santos, M., Patrício, J., Martins, I., Moreno-Garrido, I., Blasco, J., Marques, J.C. & Ribeiro, R. Feeding behaviour of the mudsnail *Hydrobia ulvae* and the prevention of macroalgal blooms in estuaries. Estuarine, Coastal and Shelf Science (under revision to be submitted).

Hoefnagel, N. & Martins, I. Applying DEB theory to the gastropod *Hydrobia ulvae* parameters estimation and preliminary simulations. Ecological Modelling (under revision to be submitted).

Martins, I. et al. 2011. Long-term predictions of flood and drought events in macroalgal and consumer populations from the Mondego estuary. Ecological Modelling (in preparation)

Saro L, Moreira-Santos M and Ribeiro R. Effects of extreme events due to global climate changes on the intertidal mudsnail *Hydrobia ulvae*. Estuarine, Coastal and Shelf Science (in preparation).

ORAL COMMUNICATIONS IN INTERNATIONAL SCIENTIFIC MEETINGS

2011

Martins, I. & Lopes, R. 2011. Long-term predictions of flood and drought events in macroalgal and consumer populations from the Mondego estuary. ECClima – Estuaries in a Changing Climate, 8-5- April, Porto, Portugal.

2010

Ceia, F., Patrício, J., Neto, J.M., Franco, J., Pinto, R., Fernández-Boo, S., Losi, V. & Marques, J.C. 2010. The influence of dredging in macrobenthic assemblages. ECSA47 Symposium, Figueira da Foz, Portugal, 14-19 September.

Couto, T., Patrício, J., Neto, J.M., Ceia, F.R., Franco, J. & Marques, J.C. 2010. The influence of mesh size in environmental quality assessment of estuarine macrobenthic communities. ECSA47 Symposium, Figueira da Foz, Portugal, 14-19 September.

Moreira-Santos, M., Krell, B. & Ribeiro, R. 2010. Assessing estuarine sediment toxicity using an in situ postexposure feeding assay with the mudsnail *Hydrobia ulvae*. VIII Congresso Ibérico, V Iberoamericano de Contaminación y Toxicologia Ambiental, Heredia, Costa Rica (not numbered).

Moreira-Santos, M., Krell, B. & Ribeiro, R. 2010. In situ estuarine sediment toxicity assessments: tools for a multi-functional approach. ECSA47 Symposium, Figueira da Foz, Portugal, 14-19 September, p 80-81.

Pinto, R., de Jonge, V.N., Patrício, J., Neto, J.M., Marques, J.C. 2010. How to assess the brittle connection between biodiversity and the human welfare? A case study. ECSA47 Symposium, Figueira da Foz, Portugal, 14-19 September.

Pinto, R., Patrício, J., Neto, J.M., Domingos, T. & Marques, J.C. 2010. Integration of ecological significance within the water uses and services framework: a DPSIR approach on the Mondego estuary. 12º Encontro Nacional de Ecologia, Porto, Portugal, 12-14 October.

Veríssimo, H., Patrício, J., Neto, J.M., Teixeira, H., Fath, B., Marques, J.C. 2010. Benthic indices performance in capturing ecological changes in transitional systems following a human-induced physical disturbance. ECSA47 Symposium, Figueira da Foz, Portugal, 14-19 September.

Vinagre, P., Patrício, J., Neto, J.M., Teixeira, H., Ceia, F., Franco, J. & Marques, J.C. 2010. The Mondego estuary (Southwestern Europe) mesohaline and oligohaline macrozoobenthic communities. A contribution for their Ecological Quality Status assessment. ECSA47 Symposium, Figueira da Foz, Portugal, 14-19 September.

2009

Couto, T., Patrício, J., Neto, J.M., Ceia, F.R., Franco, J. & Marques, J.C. 2009. The influence of mesh size in environmental quality assessment of estuarine macrobenthic communities. IMMR09, Peniche, Portugal, 16 September.

Patrício, J., Neto, J.M., Teixeira, H., Salas, F. & Marques, J.C. 2009. A multi-year comparison of ecological indicators of benthic macrofaunal community condition in an estuarine system. ASLO 2009, Nice, France, 24-31 January.

Pinto, R., Patrício, J., Neto, J.M., Domingos, T., Baeta, A., Teixeira, Z. & Marques, J.C. 2009. Water uses and ecological cost's evaluation: A DPSIR approach on the Mondego estuary. ECSA 45th meeting, Dublin, Ireland, 30 August-4 September.

POSTER COMMUNICATIONS IN INTERNATIONAL SCIENTIFIC MEETINGS

Araújo, C.V.M., Moreira-Santos, M., Patrício, J., Martins, I., Moreno-Garrido, I., Blasco, J., Marques, J.C. & Ribeiro, R. 2010. Feeding preferences of the mudsnail *Hydrobia ulvae* and the prevention of macroalgal blooms in estuaries. XX Annual Meeting of SETAC-Europe (Society of Environmental Toxicology and Chemistry), Sevilla, Spain, p. 197.

Franco, J.N., Ceia, F.R., Patrício, J., Neto, J.M., Modesto, V. & Marques, J.C. 2010. Spatial intrusion and ecological response of *Corbicula fluminea* (Muller, 1774) in the mesohaline and oligohaline areas of a Southern european estuary. IMMR2010 - International Meeting on Marine Resources, Peniche, Portugal, 16-17 November.

Martins, I. & Colaço, A. 2009. Dynamic energy budget models of the mixotrophic vent mussel *Bathymodiolus azoricus*. DEB Symposium 2009, 19-22 April, Brest, France.

Veríssimo, H., Gamito, S., Patrício, J., Neto, J.M. & Marques, J.C. 2010. Effects of extreme events on estuarine subtidal macroinvertebrates of the Mondego estuary. ECSA47 Symposium, Figueira da Foz, Portugal, 14-19 September.

SEMINARS IN LAB SCIENTIFIC MEETINGS

Hoefnagel, N. 2009. DEBs in Crabs – Allocating energy to different components of life in *Carcinus maenas*. Lab Meeting, IMAR-CMA, September, Coimbra.

Hoefnagel, N. 2010. Snail tales – Allocating energy to different components of life in *Hydrobia ulvae*. Lab Meeting, IMAR-CMA, Janeiro, Coimbra.

REPORTS

First Year Progress Report

Final Report

MASTER THESIS

Ana Eduarda Saraiva Pereira Campos (2010). Influence of hydrodynamics and sediment grain size in environmental quality of the estuarine polyhaline sector. University of Coimbra (Portugal).

Ana Margarida Carriço (2010). Spatial and temporal heterogeneity of macrofauna assemblages in the polyhaline sector of a mesotidal estuary. University of Coimbra (Portugal).

Augusta da Conceição de Jesus Duarte (2010). Variação temporal da comunidade macrobentónica subtidal do sector polihalino do braço Sul do estuário do Mondego. Contributo para a avaliação do estado ecológico. University of Coimbra (Portugal).

Dimitri Vilhena Barroso (2010). Spatial and temporal variability of benthic producers and consumers $\delta^{13}C$ and $\delta^{15}N$ signatures in the Mondego estuary food web. University of Coimbra (Portugal).

João Nuno R. Franco (2010). Population dynamics of *Corbicula fluminea* (Muller, 1774) in the mesohaline and oligohaline areas of the Mondego estuary: spatial intrusion and ecological response. University of Coimbra (Portugal).

Bonny Krell (2009) Assessing estuarine sediment toxicity using an *in situ* postexposure feeding assay with the snail *Hydrobia ulvae*. University of Coimbra, Coimbra, Portugal.

Natan Hoefnagel (2010). Applying DEB theory to the gastropod *Hydrobia ulvae* parameters estimation and preliminary simulations. Department of Ocean Ecosystems, Faculty of Mathematics and Natural Sciences, University of Groningen, The Netherlands.

Pedro de Almeida Bica Lança Vinagre (2009). The Mondego estuary mesohaline and oligohaline macrozoobenthic communities. A contribution for their Ecological Quality Status Assessment. University of Coimbra (Portugal).

Tânia Isabel Jesus Fernandes (2010). Dinâmica da flora macroalgal no estuário do Mondego. Respostas das métricas de avaliação ecológica ao longo do gradiente de salinidade. University of Coimbra (Portugal).

3. CONCLUSIONS

The use of benthic macroinvertebrates communities in the ecological quality evaluation of European estuaries is one of the requirements for the Water Framework Directive implementation. However, the development of tools to achieve this classification is especially difficult in estuaries that present strong seasonal variations, once it becomes even harder to distinguish between natural- and anthropogenic-caused impacts (Borja & Muxika, 2005; Reiss & Kröncke, 2005; Quintino et al., 2006; Chainho et al., 2007; Zettler et al., 2007). The Portuguese estuaries can be characterized by strong seasonal variations in freshwater inputs, presenting low values during the summer period, and very high values during the winter period, when flood events may occur.

This project had as main objective to identify the changes in benthic communities related with the occurrence of extreme events (drought and floods), and to distinguish the effects caused by these events with those derived from human pressure. For that, two estuaries were studied, the Mira and the Mondego. Although both belong to the same WFD typology, these estuaries present very different characteristics, not only in terms of environmental properties but also in terms of pressures occurrence. With this project several other questions were raised, related with the Portuguese estuaries ecological quality evaluation problematic; some of which are somewhat clarified by the project results, while others may be the starting point for future works on this field. In addition to the results presented in the several sections, one of the main contributions of this project is that it allowed proposing some recommendations and challenges, which resulted from the integration of the several studied components and of the gathered knowledge from previous studies of the several teams involved:

1. **Water body's limits** – The WFD implementation represented an additional effort for countries as Portugal that had a limited available dataset regarding its estuarine condition. This was mainly due to the fact that previous monitoring campaigns did not include all the physicochemical and biological elements required by the WFD, and its geographical coverage did not include all the water bodies. The data gathering undertaken with this study highlighted the fact that the Mondego estuary dataset is much more complete than the ones available for the Mira estuary. In both estuaries were identified some missing points when considering the upstream areas (oligohaline and mesohaline zones); being this effort the first consistent data gathering for these saline areas. This data gathering presented two important contributions for the WFD implementation in Portugal:
   a) the characterization of the Mira and Mondego estuaries upstream saline areas, and usage of the Mira data to determine the reference conditions for this system;
   b) the reformulation of the water bodies limits, based on recent physicochemical estimations. For the Mira estuary case-study, this implies the definition of three additional water bodies, mesohaline, oligohaline and tidal, that were left out in the original proposal, where only 3 water bodies were identified, being Odemira the upstream limit (Ferreira et al., 2005);

2. **Typology and Reference Conditions** – the typology and establishment of reference conditions exercises have been conducted by the Member States to guarantee that the temporal and spatial variability are correctly integrated in the ecological quality evaluation assessment. The benthic communities present strong spatial variations along an estuarine gradient, due to the influence of several factors. Benthic communities are composed by eurytopic species, that present a wide distribution, but also by stenotopic species, which habitat is much more limited. The overlapping of distributions results in complex structured communities, for which, in order to

facilitate the monitoring process, is attempted to identify homogeneous patterns. This study allowed two important conclusions regarding the WFD implementation:

a) The saline zones, defined according to the Venice system and the respective alterations proposed by Bald et al. (2005), seem to be a good starting point to identify the homogeneous communities in a estuary, in terms of macrofauna, (Chainho et al., 2007; Teixeira et al., 2008a). The communities keep those spatial arrangements, although seasonal variations may be observed when considering the structure and composition parameters.

b) Regardless their typologies, the different water bodies cannot be classified using the same reference conditions. As so, different reference values are proposed for the homogeneous units identified (Teixeira et al., 2008b). These values were proposed for the Mondego estuary, due to the long dataset available, and, although its application in the Mira estuary seems promising, it still demands for validation with a wider dataset. The reference conditions development for the upstream saline areas are still in open, once that the results obtained with this project allowed for a first characterization, but are not enough to validate the reference values definition.

3. **Natural pressures versus anthropogenic pressures** – the development of biotic indices is based on a set of ecological presupposes regarding the responses that benthic communities may given when faced with anthropogenic stress. It is assumed that in perturbation conditions: (1) the diversity is reduced (e.g. Green, 1979); (2) the opportunistic species are better represented than the species that are sensitive to pollution (e.g. Weisberg et al., 1997; Borja et al., 2000; Paul et al., 2001); (3) the species abundance is dominated by small sized species (e.g. Warwick, 1986); (4) there is a higher detritivore/suspension feeders ratio (e.g. Dauvin et al., 2007), the species living in deeper burrows are less abundant (e.g. Rosenberg, 2001); and (5) there is less filogenetic diversity (Clarke & Warwick, 1998). However, the natural factors occurring on estuaries may induce similar responses in benthic communities, making harder to separate the stress caused by natural factors from the one caused by anthropogenic factors. This study allowed concluding that, although both are subject to a very strong natural stress, which is also reflected in the dominance of tolerant species, there is a clear distinction between the macroinvertebrate communities of both estuaries, which is also reflected in the obtained results for the biotic indices. These findings are very promising for the reference conditions definition, once that the Mira estuary presents very low anthropogenic pressure levels and that seems to be reflected on the evaluation tools used.

4. **Impacts of floods and droughts** – Benthic communities of estuaries subject to floods and droughts are changed because of (1) influxes of organic matter which stimulate an increase in the abundance of opportunistic species during the floods (Salen-Picard & Arlhac, 2002) and (2) changes in water quality, such as higher concentrations of pollutants during the occurrence of prolonged droughts (Attrill & Power, 2000). In general, the consequences of floods are more dramatic than droughts because the former occur during a short period with great intensity and have immediate effects (e.g. mortality) in benthic communities, while droughts extend for longer periods with effects graded on communities (e.g. colonization by marine species). The effects of floods and droughts were recorded in this project, both based on data generated during the course of the project, and either through the analysis of data collected during the compilation phase. It was found that the impact of floods on invertebrate communities is immediate and proportional to the intensity of flood. Chainho et al. (2006; 2007a; 2007b) observed a drastic reduction in species richness, abundance and

biological indices results over the whole estuary, in a year where a flood of great intensity occurred, while in 2006 (year of flood but of lesser intensity) the effects observed in both estuaries had not so obvious consequences on the indices outcome although, following the same general pattern (a reduced number of taxa and abundance). There was also a recovery of the communities sampled after the flood, thus indicating a high resilience of the system. Experiments on the tolerance of some key species in relation to salinity also showed a differential osmotic regulation capacity of the species tested, when subjected to different levels of physiological stress. The models developed under the project scope will allow in the future a better understand of the effects of extreme events in the population dynamics of key species present in each of the estuaries.

5. **Periodicity and frequency of sampling** – EXTREMIS project results have revealed that the composition and structure of benthic invertebrate communities of the Mondego and Mira estuaries varies dramatically throughout the year and from year to year, and that this variation is more intense when there are extreme events such as floods and droughts. Such changes in composition and structure of the communities in the different places may eventually, be reflected in the classification of their ecological status, which may induce significant errors when comparing communities indiscriminately sampled in different seasons and at different hydrological conditions (Chainho et al., 2007). For this reason, it is necessary to adapt the tools developed for assessing the water ecological quality based on benthic communities for the different seasons and also, to avoid that sampling can occur under the influence of extreme climatic events. Taking into account this natural variability of communities, the ideal is that these samples are taken with a seasonal periodicity. Falling to implement such a program, due to logistic and budgetary constraints, it is necessary to select a particular time of year (whose definition calls for further studies) for the completion of sampling and take them on an annual basis. Intervals over one year are liable to be insufficient for proper determination of ecological status of communities, especially if it occurs during a period of flood or drought. Under the scope of this project a first approach has been made to this need for temporal stratification, defining reference conditions for a specific time of year.

6. **Mesh size** – During the first European intercalibration exercise, consensus on using a 1 mm mesh to evaluate the ecological status of coastal waters was reached. However, in most community studies carried out in transitional systems worldwide samples have been screened using a 0.5 mm mesh sieve. Therefore, density, number of species and biomass data of benthic invertebrates are usually related to this mesh size. In the context of mandatory regular monitoring carried out to determine the EQS of transitional waters bodies, the scientific community is faced with the challenge of having to choose between the two meshes in the short-term. In the scope of this project, Pinto et al. (2009) analyzing samples from the Mondego estuary found that the density is significantly higher when using a 0.5 mm mesh (about 60% higher), and the same was observed with the number of identified species. Also, a detailed analysis along the estuarine gradient showed that these differences are most pronounced in the upstream areas. With regard to biomass, the authors found no significant differences between meshes, since only very small individuals are retained in the 0.5 mm sieve. This evidence will likely have an impact on the determination of the EQS in transitional systems, and we may run the risk of assigning different classifications to a given water body depending on the sieve used (Pinto et al., 2009). In parallel, Couto et al. (2010) found that the time taken to sort samples screened using a 0.5 mm mesh is at least, two times greater than the time spent to sort samples retained on the 1 mm

mesh. EXTREMIS project results highlight that the choice of the mesh size may have clear implications in determining the EQS but also important financial consequences.

7. **Biological elements** – This study showed that macrofauna communities reflect the particular conditions associated with the different salinity classes' characteristic of transitional systems. On the one hand, the benthic macroinvertebrate biological elements have been repeatedly chosen to determine the water bodies' ecological quality and there is a vast literature on its ability to integrate episodes of disturbance; on the other, the project results also indicates that other benthic groups may eventually, play a significant role in the classification systems. Macrofauna can provide additional photographs of the system's response to different impacts. In situations of doubt when assigning a classification, having information from both groups can be an asset. It is clear that to characterize the overall ecological status of a transitional water body is essential to know the behavior of the remaining biological and physicochemical elements. At its low anthropogenic impact is also of utmost importance to extend the exercise to the Mira estuary, since this system plays a central role in the comparison of systems belonging to the A2 type.

8. **Taxonomic uncertainty** – Studies where taxonomic diversity is an important component depend on the type of sampling equipment and the mesh sieve size used, but rather on the ability of taxonomists to identify benthic organisms (Stribling et al., 2003). During this project was made an effort to harmonize the taxonomic classifications used in terms of the nomenclature rules adopted and the taxonomic level chosen to the identification of more problematic goups (e.g. oligochaetes). Also, the rules defined under the WFD first intercalibration exercise were followed (Borja et al., 2007). Still, it was found that some of the taxa identified as different taxonomic entities, could actually be the same entity, and that these differences result most from the variable degree in taxonomic knowledge of the researches involved. This problem is general for all the teams working with benthic invertebrates communities, since taxonomy is an area in which the investment is being reduced. This issue was partially resolved in this project and it is recommend that all teams involved in studies to assess the ecological quality make this taxonomic harmonization exercise in light of recent publications for the taxa distribution in Portuguese estuaries.

9. **Biotic indices versus statistical methods** – To assess the water ecological quality from benthic communities several biotic indices are typically employed (some of them including several metrics), which have the advantage of being easily interpreted and are straightforward in their application, as demonstrated in this report. On the contrary, due to its difficult application and worse understanding by decision makers, statistical methodologies are used less often for this purpose. However, these are essential for validating biotic indices and should be used together with them to evaluate the water ecological quality. Nevertheless, its implementation still dependent on obtaining data sets relatively robust (high spatial and temporal coverage and high number of replicates per site/sampling time).

Overall the EXTREMIS Project gave a relevant contribution regarding the ecological quality status assessment of transitional systems with high natural seasonal oscillations, specially and when extreme climatic events (e.g. droughts and floods) occur. Nevertheless, several important issues still need further research:

1) Validation of metrics with independent datasets;
2) Definition of sampling season for transitional systems with high seasonal oscillations;

3) Testing and validation of methodologies in other estuaries with distinct characteristics and included in other typologies;
4) Regular performance of uncertainty and quality control analysis;
5) Validation of the models developed within the scope of the project.

4. MANDATORY SUPLEMENTARY MATERIAL (Annex)

The project deliverables are given as digital annexes (pdf format), organized in nine major files, as follow:

1. List of references regarding the Mondego estuary;
2. List of references regarding the Mira estuary;
3. List of references regarding the influence of freshwater flows and extreme climatic events on macrobenthic communities;
4. List of physical-chemical and biological parameters produced by other studies regarding the Mondego estuary;
5. List of physical-chemical and biological parameters produced by other studies regarding the Mira estuary;
6. Papers SCI
   a) Published
   b) Submitted
   c) In preparation
7. Oral communications
   a) International meetings
   b) Lab seminars
8. Posters
9. MSc Thesis
10. Reports

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