MINHO, MONDEGO, AND MIRA ESTUARIES observatory: Long term vaRiation of ECOLOGICAL sTAtus as a response to naturaL and human induced changes. Implications for management and restoration.

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INTRODUCTION

The rationale of proposing an L-TER project focusing on these three estuarine systems is to understand the on-going processes and their evolution under the combined effects of human pressure and climate change, helping in providing answers regarding their sustainable use and management.

This implies dealing with problems such as: a) losses of habitats and species diversity, b) decrease in habitats size and heterogeneity, c) decrease of many species populations size and changes in their dynamics and distribution, d) habitat fragmentation and increased vulnerability of the remaining isolated pockets; e) decrease of economic goods and services naturally provided by ecosystems.

Linking these ecological concepts and the management framework is essential to understand, manipulate and manage estuarine systems, and the data to approach such problematics can only be provided by L-TER programmes. In our case, data comparison, integration, and interpretation, regarding present and past events in the three systems, will be complemented with new data acquisition to provide a) better knowledge on ongoing processes and changes, and b) management recommendations.

Objectives are:

a. Identification of long term-variations in habitats and biological communities;
b. Characterization of long-term variations of ecological status, relating it to natural (e.g. extreme weather events, climate change) and human induced changes;
c. Understand long-terms variations of ecosystems' functioning, taking into account nutrients input and trophic energy flows;
d. Data comparison and integration to provide a basis for building possible evolutive and management scenarios and provide management recommendations for the sustainable use of the selected systems.

1. DESCRIPTION OF THE L-TER SYSTEMS

1.A. Minho

1.1. Literature review and compilation (Milestone 1a)

The Minho River, located in the West Iberian coast, is more than 300 Km long, drains a basin of about 17,080 Km2 and has a mean flow higher than 300 m3s-1. Its estuary is a mesotidal partially mixed one but during high floods it tends towards a salt wedge estuary, with the influence of tides extending for about 40 Km upstream. The estuary has a great socio-economic importance, providing goods and services to about 80,000 of persons. It has also a high ecological value, mainly due to its large diversity of habitats and biodiversity (it is included in NATURA 2000; it is classified as a "Zona de Protecção Especial para Aves" (ZEP), an "Important Bird Area" (IBA) and is a CORINE Biotope).

This estuary is still relatively low impacted by anthropogenic activities. However, in the last decades, the environmental pressures and impacts have been increasing, with the most important at the present being: physical and hydrological alterations mainly due to the construction of dams and other facilities to support human activities (e.g. fluvial transport of people and goods, touristic activities, etc); the presence of non-native (exotic) invasive species that have been contributed to the decline of several native species, including some with high economic and conservational interest; and organic and chemical contamination produced or increased by anthropogenic activities (industrial, urban and agricultural sources) still found in low concentrations in water, sediments and organisms but with a general increasing trend over the years; and alterations resulting from global climate changes.

Table 1 summarises the information published in the scientific literature in relation to the main pressures and impacts affecting biodiversity and ecosystem functioning found in Minho River estuary.

The Minho River estuary (hereafter designed as Minho estuary) has been intensively studied especially in the last two decades, including in relation to hydromorphological characteristics, water quality, communities diversity and functioning, populations health, and pollution, among other aspects (e.g. Antunes et al., 1994; Antunes and Weber, 1996; Cabral et al., 2007; Quintaneiro et al., 2006; Guimarães et al., 2009, 2011; Sousa et al., 2005, 2006, 2007a,b,c,d,e; Vasconcelos et al., 2009). Thus, a considerable amount of historical data is available for integration and comparison with further data obtained in the scope of 3M_RECITAL providing an excellent opportunity to investigate long term alterations. The most relevant literature is summarized in Table 2.

Table 1 – Main types of pressures and impacts on the ecosystem of Minho River estuary and examples of published literature addressing them.

Environmental pressure	Examples of main impacts/effects	Published work or other source of information
Physical and hydrological alterations	- Habitats alteration and degradation; - Challenges to adapt to new and sometimes variable conditions; - Communities alterations with the decline of populations of the less tolerant species;	Baños (1978) Moreno *et al.* (2005a) dados do INAG, ARH, E.P.T.I.S.A., etc.
Exotic species	- Competition with native species often contributing to the decline of native species; - Habitat, nutrients cycling and energy flow alterations, due to the role of some exotic species as ecological engineers; - Opportunities for species able to live in close dependency with exotic species (e.g. feeding on them; taking advantage of shell as refugees and substract, etc) - Landscape occupation and transformation in riparian areas;	Araújo *et al.* (1993) Cerqueira *et al.* (2006) Cigoña and Ferreira (1996b) Ilarri *et al.* (2011, 2012) Rodriguez *et al.* (2009) Sousa *et al*. (2006a,b) Sousa *et al*. (2008a,b,c,d,f)
Environmental contamination by synthetic chemicals produced by anthropogenic activities and natural substances and pathogenic organisms increased as result of them	- Toxic chemicals present in pulses or in a continuous way potentially inducting toxic effects directly in organisms; adversely affecting populations; changing interspecies relationships and ecosystem health and functioning; - Toxic chemicals potentially affecting human health (e.g. through the consumption of contaminated organisms and/or less healthy ones; - Increased production of biotoxins (e.g. cyanobacteria toxins) due to favourable conditions (e.g. increase of nutrients) for species producing them, potentially affecting human and ecosystem health; - Alterations of habitats due to the abiotic effects of chemicals; - Toxics may act in favour of exotic species in their competition with native ones; - Pathogens	Almeida *et al*. (2007) Cairrão *et al.* (2004) Cunha *et al.* (2005) Díez *et al*. (2005) Dioníso *et al.* (2000) Gravato *et al.* (2010) Guimarães *et al.* (2009, 2012) Moreira *et al.* (2010) Moreira *et al.* (2005, 2006a,b,) Monteiro *et al.* (2007) Reis *et al.* (2008) Rubal *et al*. (2009, 2011) Quintaneiro *et al.* (2006) Santillo *et al*. (2006, 2007) Vasconcelos *et al*. (2009)
Alterations due to global climate changes	- Changes in abiotic conditions (e.g. increase of temperature potentially decreasing O2 levels in water) creating additional pressures to organisms; - Some abiotic factors (e.g. salinity, temperature) are stressors by themselves; - Abiotic factors may interact and modify chemicals toxicity; - Increase of the frequency of extreme events (e.g. heath waves); - Indirect changes such as alterations of River flow due to discharges of dams, etc.	Freitas (2011) Guimarães *et al.* (2009, 2012) Ilarri *et al.* (2011) Sousa et al. (2012)

Table 2 – Summary of the main studies performed in the Minho River estuary that are available in the literature.

Sampling time/period	Sampling Time/Frequency	Location or Range covered	Information provided	Reference
1977-78	Seasonal sampling	Mouth - 20km - 30km upstream	Physico-chemical parameters, macrobenthos, inert extraction; physical impacts	Baños (1978)
1978-1997	Annual sampling (1975-1978, 1982, 1986-1997). Data obtained after 1997 exists but it is not yet published.		*Census* of winter aquatic birds (mainly limicolous and anatides)	Rufino (1978, 1982, 1988, 1989, 1990, 1991, 1992), Rufino & Neves (1982) Rufino & Costa (1993), Costa & Rufino (1994, 1996), Costa & Guedes (1994)
Spring and autumn 1980	No standardized observations. Two visits	*Ínsua*	Characterization of the *insua* ornitofauna (mouth of Minho River). Species identification and counts of individuals	Cigoña *et al.* (1981)
1981-1982		Estuary + freshwater tidal area (TFW)	Review, Physico-chemical parameters, inert extraction, fisheries	E.P.T.I.S.A (1982)
November 1981 – April 1982 / November 1982 – April 1983	Monthly sampling	Mouth – 19 Km upstream	Production, biometry pigmentation of the glass eel (*Anguilla anguilla*)	Weber (1986)
January 1981 - December 1981 + 1982	Monthly sampling	Mouth - 18Km upstream	Physico-chemical parameters macrobenthos structure + seston + demersal fish	Weber (1987)
1985	Information from several years.	estuary	Characterization of the estuary ornitofauna do estuário (list of species and distribution by sections of th estuary corresponding to main biotopes. Fenology.	Nuñez (1985)
1987-1989	Monthly sampling	Mouth - 6 Km and 18 km upstream	Production, experimental fishery, biometry, pigmentation status of the glass eel (*Anguilla anguilla*). Abundance and biometry of the yellow eel (*Anguilla anguilla*).	Antunes and Weber (1990)
1987-89	Monthly & seasonal sampling	Estuary + TFW	Fish fauna	Antunes and Weber (1990)
			Fauna piscícola do estuário	Antunes (1990)
June 1990 – July 1991	Monthly sampling	Mouth - 15 Km + 30 km upstream	Morphology, morphometry, microstructure of *Corbicula fluminea*	Araújo *et al.* (1993)
July 1989	Punctual (1x)	All estuary	Granulometry +	Maze *et al.* (1993)

			macrozoobenthos abundance	
1991-1992 / 1991-1993	Monthly sampling	6 Km / 18 km da foz	Production, experimental fishery, biometry, Fulton index, pigmentation status of the glass eel, age analysis (*Anguilla anguilla*)	Antunes (1994a,b)
1981-1982 / 1991-1992	Monthly sampling	18 Km from the mouth	Production, experimental fishery, biometry of the glass eel (*Anguilla anguilla*); by-catch	Antunes and Weber (1996a)
1987-89	Monthly	Estuary + limnetic area	Fauna piscícola	Antunes and Weber (1996b)
Spring 1996	Punctual (1x)	30 Km from the Mouth	Record of the presence of the genus *Cobitis*	Cigoña and Ferreira (1996a)
October 1992	Punctual (1x)	30 Km from the Mouth	Record of the presence of 2 exotic crustaceans	Cigoña and Ferreira (1996b)
April + October 1995	Punctual	30 Km from the Mouth	Quantitative analysis, abundance, diversity of plactonic crustaceans crustáceos planctónicos	Coelho (1996)
1989 / 1991		2 Km from the Mouth	Otoliths – diary increments, larvae and glass eel	Antunes (1997)
1 year	Monthly sampling	estuary	Physico-chemical parameters including nutrients + chlorophyll	Fidalgo (1998)
June1990 – May 1991	Monthly sampling	17Km & 30 km from the mouth	Growth and dynamics of *Pisidium amnicum*	Araújo *et al*. (1999)
Review			Presence of *Acipenser sturio*	Almaça and Elvira (2000)
1999	punctual	V N Cerveira and other sitess	Physico-chemical parameters, benthic macroinvertebrates, cyanobacteria toxicity; phyto and ictyofauna (qualitative) (descriptive results)	Fidalgo *et al.* (2000)
		Estuary	bacterioplankton	Pinheiro and Fidalgo (2000)
		Estuary	Microbiological contaminatiom	Dionísio *et al.* (2000)
Spring & summer 1989 - 1999		Estuary + FTA	phytoplankton	Vasconcelos and Cerqueira (2001)
Review		Estuary	Monitoring recruitment of the glass eel	Antunes (2002)
1998-1999	Monthly sampling	mouth	Conger larvae – biometry and meristics, otoliths diary increments	Correia *et al.* (2002)
1998		Mouth	Conger and eel larvae – otoliths	Antunes and Correia (2003)
April 2000 + April 2001	Annual	Mouth	Conger larvae – biometry and meristics, otoliths diary increments; Sr/Ca microchemistry	Correia *et al.* (2003)
2001	punctual	Estuary (crabs for ectoxicological assays)	Responses of crabs (Carcinus maenas) from the Minho estuary to acute stress of metals and mixtures –	Elumalai *et al.* (2002)

			mortality	
July 2002	1x	Mouth - 10 Km upstream	Sediments granulometry, physico-chemical parameters + quantitative analysis) of foraminifers	Fatela *et al.* (2003)
Spring 2002	Sampling in 3 estuarine areas		Patterns of spatial and temporal use of the estuary by limícolas migradoras. (core data are not available)	Ferreira et al. (2002)
Junho 2003	1x	Marsh + TFW + Coura river	Icthyofauna	Mota (2003)
		estuary	Occurrence and specific congener profile of 40 PBFR	Lacorte *et al.* (2003)
2003	Punctual	Mouth + 3Km upstream	Biomarkers (GST) in Fucus spp – environmental contamination	Cairrão *et al*. (2004)
review		Estuário + TFW	Icthyofauna	Antunes and Rodrigues (2004)
May2001 + February 2002	Monthly sampling	Mouth	Osmotic plasticity of the glass eel	Wilson *et al.* (2004)
Autumn 2004	1 x	Estuary + TFW	Physico-chemical parameters, including nutrients + abundance, diversity and biomass of molluscs	Sousa *et al.* (2005)
October 2003	1x	Marsh	Geochemical analysis of sediments	Moreno *et al.* (2005a)
Abril + Julho 2002	1x (48 st)	Foz – até 10 Km da foz + marsh	Abiotic factors and distribution of foraminefers populations	Moreno *et al.* (2005b)
2004	Punctual (in situ assays)	estuary	In situ assay with *Hedistes diversicolor* for estuarine sediments – feeding inhibition	Moreira *et al*. (2005)
2003-04	punctual	Estuary	Perfluorooctane sulfonate in mussles (*Mytilus galloprovincialis*)	Cunha *et al.* (2005)
2004	Punctual (crabs for ecotoxicological bioassays)	estuary	Responses (biomarkers-reproduction) of crabs (*Carcinus maenas*) from the Minho estuary to metals	Elumalai et al. (2005)
Summer 2005	1x	8 Km from the mouth	Pollutants (PBDE's, HBCD, PCB) in eels	Santillo *et al*. (2005)
2004	Punctual (sampling of fish for lab assays)	estuary	Characterization of *Pomatoschitus microps* cholinesterases for use in biomonitoring studies in with wild populations	Monteiro *et al.* (2005)
		Up limit of the estuary	BT and TBT in water, bivalves and sediments	Díez *et al.* (2005)
2005	Punctual (in situ bioassays)	estuary	In situ post-exposure feeding assay with *Carcinus maenas* for estuarine sediment-overlying water toxicity evaluations; comparison with other estuaries including Mira	Moreira *et al*. (2006a)

			and Mondego	
2005	Punctual (in situ bioassays)	estuary	In situ assay with the algae *Phaeodactylum tricornutum* – development and validation of the assay in several estuaries, including Minho, Mondego and Mira comparison of contamination levels	Moreira *et al*. (2006b)
2005	Punctual collections of shrimps	estuary	Biomarkers responses of *Crangon crangon* to temperature, salinity and handling stress – lab study based on biomarkers	Menezes *et al.* (2006)
2004-2005	Punctual collections of *P. microps* for lab assays	estuary	Effects of 3-4 dichloroaniline on biomarkers and spleen histology of *Pomatoschistus microps* from the Minho estuary	Frasco *et al.* (2006)
Winter 2001/02 to the autumn 2002	seasonal	estuary	Biomonitoring study with *Cragon cragon* to assess the effects of pollution (biomarkers) – comparison with other estuaries	Quintaneiro *et al.* (2006)
2004-05	Punctual (several times) Prawn for lab assays	estuary	Characterization of Palaemon serratus cholinesterases (from the minho estuary) and their responses to organophosphate and carbamate compounds	Frasco *et al.* (2006)
2005-2007	Fecal samples	2 sites of the estuary	Feeding diet (number and relative importance of preys, through the identification of prey bones) of *Phalacrocorax carbo* in the estuary in relation to environmental factors.	Dias (2007)
April 1998	2x	Caminha	Abundance, biomass and growth rates of bacterioplankton	Anne *et al.* (2006)
February 1999	1x	Mouth	Conger larvae - genetics	Correia *et al.* (2006b)
February 1999	1x	Mouth	Conger larvae – otolith increments, growth rate, age	Correia *et al.* (2006a)
Summer 2003	1x	Estuary – saline gradient	Bethic macroinvertebrates, abundance, diversity	Mendes (2006)
Review		Estuary including TFW	Exotic fauna - distribution and impacts	Sousa *et al.* (2006a)
September + December 2005 / March 2006	1x	Marsh	Geochemistry of metals in sediments	Reis *et al*. (2006)
January – December 2005	3-months intervals	0.5 Km – 18 Km from the mouth	Macroinvertebrates in relation to the contamination of	Lyra *et al.* (2006)

			sediments metals and organochlorine pesticides	
March 2005 + March 2006	1x	estuary	Parasites in the gills of eels	Hermida *et al.* (2006)
April + October 2002	2x	Marsh	Distribution and ecology of *Pseudothurammina limnetis*	Moreno *et al.* (2006)
1990-2004	Review	estuary	Environmental conditions, estuary as nursery for flat fish	Cabral *et al.* (2007)
October 2001 – May 2004		Mouth	Glass eel – osmotic regulation	Wilson *et al.* (2007a)
March + April 2004		Mouth	Glass eel – osmotic regulation	Wilson *et al.* (2007b)
2005	Monthly sampling	Estuary – superior limit & TFW	Physico-chemical parameters, including nutrients, MO, abundance, biomass, diversity of molluscs	Sousa *et al.* (2007a)
2005	Punctual (crabs for ecotoxicological bioassays)	estuary	Responses (biomarkers-reproduction) of crabs (*Carcinus maenas*) from the Minho estuary to zinc and mercury	Elumalai *et al.* (2007)
2006	1x	Estuary - TFW	Genetics and morphometry of *Corbicula fluminea*	Sousa *et al.* (2007b)
October 2003 + April 2004	1x	Mouth, marsh, VNCerveira	Qualitative and quantitative analysis of foraminifers	Moreno *et al.* (2007)
April 2002	1x	Caminha + march + V N Cerveira	Influence of salinity in the populations of foraminifers	Fatela *et al.* (2007)
		estuary	Water and sediment – chlorothalonil + PCP + TCP+TeCP	Almeida *et al.* (2007)
October 2005 - March2006 / August 2006 – March 2007	Monthly sampling	18Km + 25Km from the mouth	Diet of *Phalacrocorax carbo* - impact on the icthyofauna	Dias (2007)
January 2006 - December 2005	Seasonal sampling	0.5km - 18Km from the mouth	Macroinvertebrates, pollutants and physico-chemical paramaters in sediments	Lyra (2007)
September 2005 – June 2006	Seasonal sampling	marsh TFW + Coura River	Macroinvertebrates + physico-chemical parameters	Mota (2007)
September 2005 – June 2006	Seasonal sampling	marsh	Bethic macrofauna, sediments	Picanço (2007)
2001- 2002	seasonal	estuary	Biomonitoring study with *Pomatoschistus microps* to assess the effects of pollution (biomarkers) – comparison with other estuaries	Monteiro *et al.* (2007)
2002- 2003	seasonal	estuary	Biomonitoring study with *Fucus* to assess the contamination of wild populations by mercury; comparison with other estuaries	Cairrão *et al.* (2007)

July 2006	1x	All estuary	physico-chemical paramaters, macrozoobethos (spatial analysis, abundance)	Sousa et al. (2008c)
October 2004 + October 2005	1x	Estuary + TFW	Abiotic parameters, abundance, biomass, population structure of, estrutura população *Corbicula fluminea*	Sousa *et al*. (2008b)
January 2005 – August 2006	Monthly sampling	Up estuary, TFW	Abiotic parameters, Abundance, biomass, growth rate, production of *Pisidium amnicum*	Sousa *et al.* (2008f)
October 2004-07	Annual (October)	All estuary	Abiotic parameters, MO, abundance of *Pisidium amnicum*	Sousa *et al.* (2008e)
January 2005 – August 2006	Monthly sampling	Up estuary, TFW	Growth and production of *Corbicula fluminea*	Sousa *et al.* (2008g)
February 2008 – August 2008	Every 2 months	15 Km from the mouth	Assessment of the salubrity degree of *C. fluminea*	Cerqueira *et al.* (2008)
Abril 2005 – Agosto 2008	Seasonal sampling	0.5 Km – 18 Km from the mouth	Feeding ecology of *P. flesus*	Mota *et al.* (2008)
July 2006	1x	Mouth– 20Km upstream	Characterization of aquatic communities (phyto and zooplankton, benthic macroinvertebrates, epifauna) + physic-chemical parameters	Dias *et al.* 2008
July 2007 - April 2008	Seasonal sampling	0.5Km - 18Km from the mouth	Age, growth, IGS, K, sex and migration of *Platichthys flesus*	Capela 2008 (not yet published)
review			invasive success of *Corbicula fluminea*	Sousa (2008a)
January-December 2007	Mothly sampling (census)		Detailed spatial and temporal characterization of the estuarine aquatic avifauna	Rodrigues (2008)
2003-04	Punctual (collection of animals)	estuary	Effects of transport and acclimatization on fish (*Pomatoschistus microps*) biomarkers	Quintaneiro *et al.* (2008)
2007	Punctual (collection of animals for lab assays)	estuary	Single and combined effects of dichlorvos and mercury on Paçlaemon serratus from the Minho estuary	Frasco *et al.* (2008)
2007	Punctual (sampling for ectoxicological bioassays)	estuary	Responses of *Pomatoschistus microps* from Minho estuary to PAHs and oil – biomarkers + behaviour	Vieira *et al.* (2008)
2007-2008	seasonal	estuary	Individual, population and community effects of contaminantion in estuarine meiobenthos	Rubal *et al.* (2009)
May + July 2006		Saline gradient estuary	Bethic epifauna– functional guilds	França *et al.* (2009)
2004 + 2005	Monthly sampling	Caminha -	Physico-chemical	Freitas *et al.*

		VNCerveira	parameters, abundance and distribution of bentic epifauna – temporal and spatial analysis ; population structure of *Platichthys flesus*	(2009)
April 2003	1x	estuary	Environmental constraints of foraminiferal assemblages distribution	Fatela *et al.* (2009)
			eco-geography of the brown shrimp *Crangon crangon*	Campos (2009)
Set2005 - Ago2006	Monthly sampling	6Km - 40Km from the mouth	Population dynamics of *Corbicula fluminea*	Rodríguez (2009)
2007	Punctual (sampling for ectoxicological bioassays)	estuary	Responses of *Pomatoschistus microps* from Minho estuary to Cu and Hg stress – biomarkers + behaviour	Vieira *et al.* (2009)
Summer 2005 – spring 2006	Sesonal sampling	estuary	Health status of yellow eel (*Anguilla anguilla*) – biomarkers, K, HSI index, morphometric parameters + physico-chemical parameters including nutrients in water, sediments concentrations of metals and PAHs; comparison with Lima and Douro estuaries	Guimarães *et al.* (2009)
Winter 2006	Monthy sampling	estuary	effects of pollution on glass and yellow eels (*Anguilla anguilla*) – health status biomarkers, Fulton index, morphometric parameters; PAHs and metals in sediments, comparison with Lima and Douro estuaries	Gravato *et al.* (2010)
January 2008 – November 2010	1x	Estuary + FTA	Prevalence of *A. crassus* in *A. anguilla*	Braga *et al.* (2010)
May 2009 – October 2010	Seasonal sampling	Estuary + FTA	Water quality (physico-chemical and microbiological paramaters	Santos *et al.* (2010)
April – September 2010	Monthly sampling	15 + 30 Km from the mouth	Dynamics of cyanobacteria populations	Moreira *et al.* (2010)
2002-2006	52 st	marsh	Distribution and composition of foraminifera community in relation to the saline gradient	Leorri *et al.* (2010)
November-April 2004-05 / 2005-06	Monthly sampling	mouth	Glass eels: biometry, K, pigmentation status	Iglesias *et al.* (2010)
October 2003 – July 2007	Monthly sampling	500m fo – 18 km from the mouth	Inter-annual comparison, growth rates, production, demersal fish	Dolbeth *et al.* (2010)
2006 - summer	1x	All estuary	Benthic epifauna – spatial analysis, abundance, biomass, diversity, community structure	Costa-Dias *et al. (*2010)
			Marine xxxx (*Petromyzon marinus* L.)	Suissas (2010)
October 2007 – July 2008	Monthly sampling	Caminha + SP da Torre	Chemical analysis of otoliths – migration pattern of	Morais *et al.* (2011)

			Platichthys flesus	
October 2004-09	Once per year	All estuary	Abundance and biomass of *Corbicula fluminea*	Ilarri *et al.* (2011)
			Climate-induced changes in estuarine predator-prey systems	Freitas (2011)
January 2005-August 2006 / October 2004-2010	Monthly sampling & annual sampling	All estuary	physico-chemical parameters, spatial and temporal analysis of abundance and biomass of *Pisidium amnicum*	Sousa *et al.* (2011)
April-August 2009		VNCerveira	Spatial and temporal analysis – biometry, GSI, K of *Alosa alosa*	Mota and Antunes (2011)
May + July 2006	1x	Estuary – salinity gradient	Fish assemblages, comparison with other estuaries	França *et al.* (2011)
2006-2007	Seasonal sampling	Estuary (two sampling sites)	Health status of the common goby (*Pomatoschistus microps*) in relation to pollution and abiotic factors variation; biomarkers, morphometric parameters, K and HPS index, metals and PAHs in sediments and in fish	Guimarães *et al.* (2011)
Out-Dez 2009		TFW area	CPUE, biometry, feeding of *Alosa alosa* and *A. fallax* juveniles	Mota and Antunes (2012)
2010-2011	periodical	estuary	Two strategies to live in low chronic pollution estuaries: the potential role of lifestyle	Rubal *et al.* (2011)
October-March 2005-2006 + 2006-2007	Monthly sampling	12 Km and 25 Km from the mouth	Diet of *Phalacrocorax carbo*, in relation to estuarine hydrodynamics and influence in the ictyofauna	Dias *et al.* (2012)
2009	Seasonal sampling	3 Km from the mouth + TFW	Physico-chemical parameters + assemblages of macrozoobenthos in relation to different densities of *C. fluminea*	Ilarri *et al*. (2012)
2011	Punctual sampling	Estuary (animals for ecotoxicological assays)	Responses of *Pomatoschistus microps* from Minho estuary to acute stress induced by pyrene – biomarkers + behabiour and mortality	Oliveira *et al.* (2012)

A complete list of references covering all scientific research conducted in this estuary over the years is provided in section 6.A of the present report.

1.2. Historical data compilation and banking (Milestone 1b)

The historical compilation of literature and data was started. For complete list of references on the system, please see section 6.1A (Milestone 1a). Data associated with those previous projects and studies in the Minho estuary will be compiled to allow further comparison with current data, in the scope of this LTER project.

1.3. GAP analysis

- More data on environmental chemical contamination is needed, including bioaccumulation and biomagnification (especially regarding edible species and top predators);
- The situation regarding exotic species and their impacts needs further studies;
- More attention should be given to alterations resulting from global climate changes, in particular in relation to extreme events (e.g. heat waves, floods), interactions between pollution, invasive species, and abiotic factors variation;
- More studies are needed on the effects of chemical contaminants (both synthetic and natural agents) on native species, and species interactions;
- Knowledge on plants and microorganisms (especially pathogens and parasits) is lacking;
- More knowledge is needed on the effects of complex mixtures of pollutants in wild populations, communities and ecosystem functioning are needed.

1.4. Elements of study in 3M-RECITAL

- Community of fish
- Health status of selected fish populations (*Pomatoschistus microps* and *Dicentrarchus labrax*) in relation to abiotic variation.
- Zooplankton community variation
- Spatial and temporal variation of the macroinvertebrates bethic community

1.B. Mondego

1.5. Literature review and compilation (Milestone 1a)

The Mondego River drains a basin of approximately 6670 Km2 and its valley is considerably steep in upstream sections, forming a large alluvial plain consisting of good agricultural land in downstream areas. Since the 1960s, the Mondego catchment area underwent a large scale morphological modification, involving the construction of stone walls, to regulate the river water flow and enlarge harbour facilities, and water reservoirs to control floods, improve the uses of water resources, and produce electric power, which modified the riverbed topography and changed the system hydrodynamics. The river contributes presently with a high anthropogenic loading of nutrients and several chemicals into its relatively small estuary (1600 ha, splitting in two arms separated by an island in the terminal part), causing eutrophication. Besides, the estuary - Western coast of Portugal - North Atlantic Ocean Eco-region - supports industrial activities, salt-works, and aquaculture farms, and is the location of Figueira da Foz, a harbour and a centre of seasonal touristic activity.

A comprehensive study on the Mondego estuary environmental quality has been carried out during the last two decades, focusing on water quality, hydraulics and sediments dynamics, plankton communities (phyto, zoo, and ichtyoplankton), the term evolution of the benthic communities (intertidal and subtidal), and the changes in macrophytes' beds (*Zostera noltei*) and green macroalgae distribution in relation to morphological alterations. Illustrative publications addressing the most relevant aspects: Cardoso et al., 2004; Dolbeth et al., 2003; Lopes et al., 2005, Marques et al., 1997; 2003; Martins et al, 2001; Patrício & Marques, 2006; Patrício et al., 2009; Teixeira et al., 2008; 2009).

A complete list of references covering all scientific research conducted in this estuary over the years is provided in section 6.B of the present report.

1.6. Historical data compilation and banking (Milestone 1b)

One of the goals of the 3M_RECITAL project is the recovery and compilation of historical data capable of serving future environmental quality evaluations and tracking of evolutive trends in the ecosystems.

Most of this type of data, though, were spread and have been acquired for different purposes; nevertheless they often constitute the only reliable source of information feeding long-term studies.

These data may help in the definition of reference conditions for this system, while the knowledge of the pressure levels may contribute to clarify the level of anthropogenic perturbation that this system was subjected to, during the last few decades. They also help to interpret the evolution of the water quality according to the evolution of the pressure levels.

1.6.1. Historical data compilation

Data on the Mondego system is being collected for several years now, for physical-chemical, nutrient and biological parameters.

The map bellow (Figure 1) shows part of the stations included in the monitoring network of the Mondego estuary. Until 2003 surveys would only take part until station 14, but from then on a network of 25 stations was established to allow covering the whole extent of the estuarine gradient, from fully marine to tidal fresh waters.

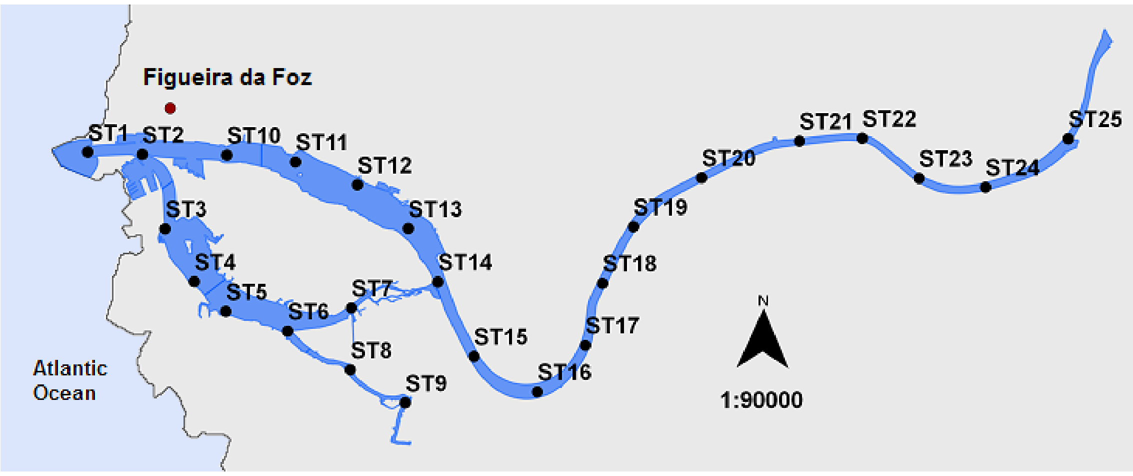


Figure 1 – Subtidal monitoring network on the Mondego estuary, stations covering the whole extent of the estuarine gradient from fully marine to tidal fresh waters.

The following tables (3 to 7) show the type of data collected in the Mondego estuary since it started to be regularly monitored in 1985.

Table 3 – Physical-chemical and nutrient surveys in the Mondego estuary since 1985.

Dates/Period	Data		Nº of Stations	Periodicity
	Physical-chemical	Nutrients		
Winter 1985	yes	yes	14	n.a.
Summer 1986	yes	yes	14	n.a.
1990 and 1992	yes	yes	14	seasonal
Spring 1998, 2000 and 2002		yes	14	n.a.
Jan 2003 to Aug 2011	yes	yes	25	monthly

Table 4 – Phytoplankton surveys in the Mondego estuary.

Dates /period	Nº of Stations	Periodicity
Spring 2009	6	n.a.
Winter 2010	6	n.a.

Table 5 – Meiofauna and nematodes surveys in the Mondego estuary.

Dates/Period	Data			Nº of Stations	Periodicity
	Richness	Composition	Abundance		
Summer 2006	Yes	Yes	yes	11	n.a.

Table 6 – Surveys of macroinvertebrate fauna of intertidal habitats, in the Mondego estuary, since 1985.

Dates/Period	Data			Nº of Stations	Periodicity
	Richness	Composition	Abundance		
Winter 1985	Yes	Yes	yes	2	n.a.
Summer 1986	Yes	Yes	yes	2	n.a.
Jun 1994 to Jan 1997	Yes	Yes	yes	2	Every two weeks
Jan1999 to Dec 2002	Yes	Yes	yes	2	monthly
Jun 2008 to Dec 2009	Yes	Yes	yes	3	monthly
Jan 2010 to Aug 2011	Yes	Yes	yes	4	monthly

Table 7 – Surveys of macroinvertebrate fauna of subtidal habitats, in the Mondego estuary, since 1990.

Dates/Period	Data			Nº of Stations	Periodicity
	Richness	Composition	Abundance		
1990 and 1992	Yes	Yes	yes	14	seasonal
Spring 1998, 2000 and 2002	Yes	Yes	yes	14	n.a.
2003 to 2007	Yes	Yes	yes	25	seasonal
2008 and 2009	Yes	Yes	yes	25 or 15 (depending on the season)	seasonal
2010 and 2011	Yes	Yes	yes	16	winter, spring and summer

***Zostera noltei* Covered Area (%):** The percentage of the intertidal area of the estuary south arm covered with *Zostera noltei* was measured once a year usually in the end of summer (September) in the following years: 1986, 1993, 1997, 2002 and since 2005 to the present.

Sediment Grain Size: Samples for sediment grain size analysis were collected in all stations of the campaigns for Intertidal and Subtidal Macrofauna.

1.6.2 Data banking

Past data on physical-chemical conditions and biological communities in the Mondego estuary system over the past two decades was compiled and banked in order to describe and characterize the main changes occurred regarding hydromorphology, physical-chemical parameters and biological communities.

An integrated information system (Figure 2) was developed in order to store data, as well as field methodologies and laboratory procedures. It comprises twelve registry modules, coupled with a research and statistical module to support enquiries; and with an administration module to guarantee the information system consistency. All information can be exported to spread sheets and/or word processor. The following are the registry modules:

1. Project
2. Team member
3. Bibliographic references
4. Geographic unit
5. Sampling station - general information
6. Element (physical chemical, biological and pressures)
7. Sampling method
8. Laboratory procedures
9. Campaign
10. Sampling station - specific information regarding the campaign
11. List of elements
12. Data registry

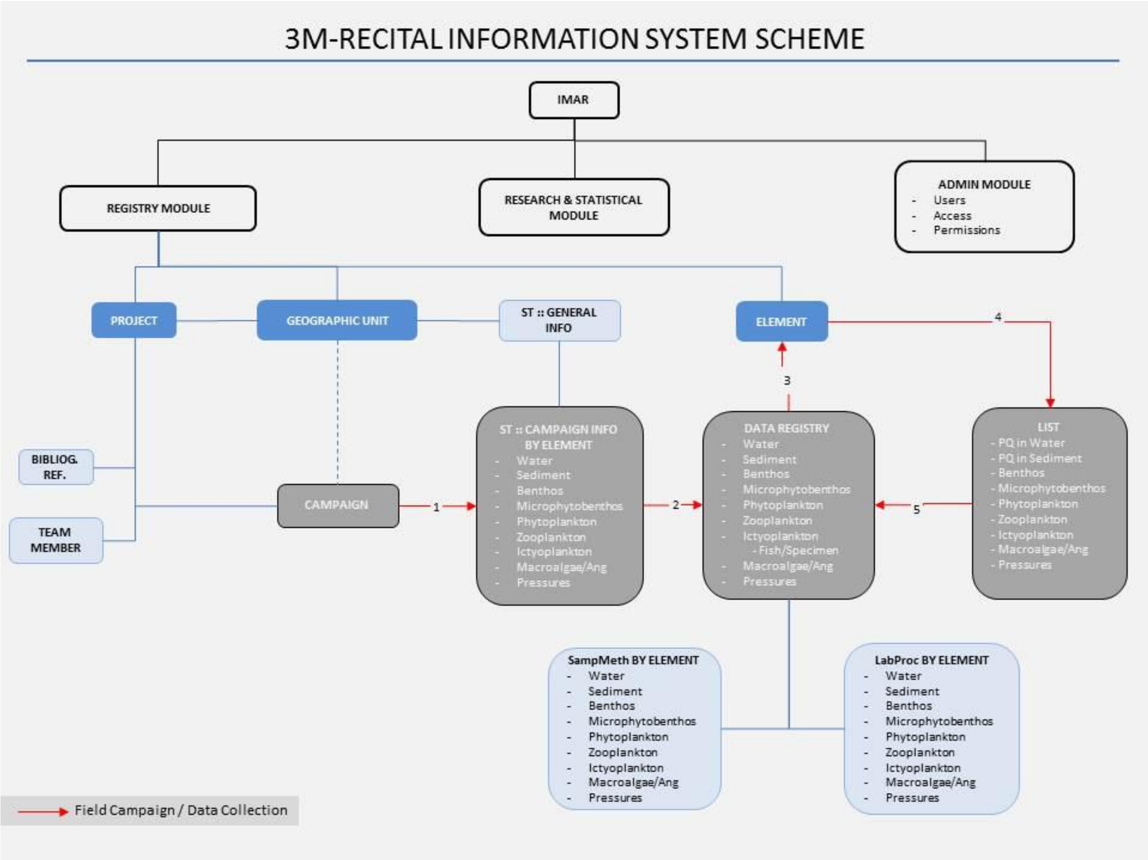


Figure 2 – Scheme of the integrated information system indicating relationships between registry, research, statistical and administration modules.

1.7. GAP analysis

The Mondego is one of the three estuaries that have been selected as targets for this LTER project because it has been monitored for more than two decades, being one of the most comprehensively studied Portuguese transitional waters ecosystems (See literature review section 6.1.B).

Estuarine communities' description, key species identification and role, and population dynamics were the focus of initial basic research in the system. Since then, research evolved naturally to explore topics related to the response of the estuarine communities to natural and anthropogenic stress, integrating therefore information on the supporting environment and local activities on the estuarine area and respective river basin. Several studies attempted to understand the ecological processes involved, namely energy flow in the system, response to specific pressures and long term evolution of estuarine biological communities driven by both natural climatic events and anthropogenic actions.

More recently, the increasing knowledge of the system properties and the amount of data and information gathered through the years has allowed for more complex and integrative approaches, such as species ecological models, hydrological models, and more recently, network analysis approaches and spatially explicit integrated models (combining hydrodynamic, biogeochemical and hydrological models). Due to the long-terms studies on this estuarine system, it was also possible to use the Mondego estuary for developing and testing new frameworks for the establishment of environmental quality monitoring programs with implications at regional, national and european scales.

The established monitoring network is therefore to be largely maintained, contributing to feed the systems' database (check previous section) and allow for higher insights into the ecological processes on such estuarine systems, in particular the Mondego.

A GAP analysis has however pointed the ways in which we can improve the current knowledge on the system, by optimizing the existent monitoring network. It defined three ways:

a. Increasing the **type of biological elements** surveyed;
b. Increasing **the spatial coverage** of the monitoring network;
c. Increasing **the type of information/parameters** measured.

For e.g., some biological elements, such as the phyto and zooplankton, the fish, or the saltmarshes, which have been only punctually or recently regularly surveyed, will be included in the monitoring network. This project will contribute to increase the information on them, but more important will try to identify the best approach to integrate the information gathered across biological elements in the system.

Another major concern is to increase the spatial coverage of the monitoring network, ensuring that distinct habitats in the system are incorporated, allowing not only to continue following temporal variations in the system in previously surveyed areas, but also to allow establishing habitat connection influences in the processes occurring within the system.

Finally, another way to improve the current knowledge will be by diversifying the type of information collected. Here we consider new parameters not only from biological communities, like for e.g. biomass or spatial coverage; but also environmental parameters. We will focus on those related to pressures in the system, such as specific pollutants and anthropogenic activities, and to systems' hydrologic and habitat characteristics. This will improve our capacity to understand changes in the system and establish cause-effect relationships, eventually supporting more efficient management actions.

1.8. Elements of study in 3M-RECITAL

The database on the Mondego system covering the past two decades will be consolidated and complemented with new observations and data collection, providing a sounder basis to help in the design and implementation of adequate policies and management actions by national and regional authorities involved in the project as end users.

In the Mondego estuary all elements considered in the initial proposal will be monitored, with only a few adjustments to the pre-established sampling effort, which will not compromise in any case the objectives proposed for 3M-RECITAL.

In the Mondego estuary, surveys within the scope of this project will focus on the following elements:

- **Water column and sediments physicochemical survey** - will provide data on the temporal and spatial variability of water and sediments quality within each water body of the ecosystem, to relate with changes in Ecological Status (Objective 1);
- **Phytoplankton survey** – will provide data on changes in the temporal and spatial patterns of phytoplankton biomass and abundance distribution, to relate it with the major environmental parameters and stressors. Data comparison will allow relating phytoplankton ecological variability with physical conditions (Objectives 1 and 2);
- **Zooplankton survey** – will provide data on changes in the temporal and spatial patterns of zooplankton composition, abundance, and distribution, to relate it with the major environmental parameters and stressors. Data comparison will allow relating zooplankton assemblages with physical conditions (Objectives 1 and 2);
- **Macroalgae and Macrophytes survey** – will provide information on macroalgae and macrophytes as ecological indicators of ecological status. Historical data will be worked out as measurable attributes (metrics) to help in defining Reference Conditions for the three systems (Objectives 1 and 2);
- **Benthic invertebrate communities survey** – will provide data on macrofauna and meiofauna communities at different spatial and temporal scales, supporting (a) the assessment of long-term trends on benthic ecological status, (b) understanding the influence of local scale and regional scale variations on ecological quality status results and (c) identifying the influence of climate change on ecological status assessment tools and necessary adaptations (Objectives 1 and 2);
- **Fishes survey** – This task will provide data on fish communities at different spatial and temporal scales to a) support the assessment of long-term trends in fish ecological status, b) help understand the influence that local and regional scale variations have on the ecological quality status, and c) identify the influence of climate change on ecological status assessment tools and necessary adaptations (Objectives 1 and 2);

- **Shorebirds and estuarine habitats** – will analyse the role of waders, as main bird communities, in the estuarine foodwebs in relation to long-term variations in ecosystems ecological status and functioning (Objectives 2 and 3).

Details on the field campaigns, sampling methods, sampling effort, for each of the abovementioned elements can be found in Section 2.B. of the present report.

Apart from field surveys of the ecological elements of interest, pressure identification and quantification will also be carried out within the project, to relate with changes in Ecological Status (Objective 1).

1.C. Mira

1.9. Literature review and compilation (Milestone 1a)

The Mira estuary is located in the south-west coast of Portugal (37º40'N, 8º45'W) and extends between the two most important towns in the area, Vila Nova de Milfontes at the mouth, and Odemira at its upper limit. It is a narrow entrenched system, with a length of more than 40 km and a mean depth of about 6 m. Tides have a semi-diurnal cycle and the water column is well mixed during spring tides and partially stratified during neap tides (Andrade, 1986). Water temperature ranges between 8.0 ºC and 27.0 ºC in the upper reaches and between 12.0 ºC and 22.5 ºC in the lower reaches (Costa *et al*., 1994). Saline variability is higher in the middle estuary and sediments are usually dominated by mud and/or fine sand, except in both ends of the system where coarse sediment particles predominate (Costa *et al*., 2008). Near the river mouth this estuary presents ecologically important seagrass beds of *Zostera marina* and *Zostera noltei* in the subtidal and intertidal areas, respectively (Almeida, 1994). In recent years these *Zostera* beds had suffered a serious collapse due to unknown causes.

The Mira estuary is usually considered a well preserved system (Costa *et al*., 2004), mostly because it always presented reduced human population and industry around the entire river basin (although some mining activity was developed during the Roman Empire domination and the 19th century) (Quaresma, 2003). In addition, this system has only minor hydromorphological changes, being the Santa Clara dam (constructed in 1964/1967, 60 km from the river mouth, mainly for irrigation purposes) the only important source of water abstraction from this basin. However, in the lower and middle estuary several fish farms were installed in the last decades (although in recent years they become quite inoperative) and in the upper and middle reaches of the estuary agriculture (mainly rice fields) and forestry activities (mainly eucalyptus plantations) occupy an important extension of the water margins (Costa, 2004). The upstream estuarine areas show a slight contamination by heavy metals (Chainho *et al*., 2009), due to previous mineral extraction activities in the river basin. On the other hand, given the high conservation status of this ecosystem and its species richness and biological diversity (partially due to the subtropical influence), in Portugal the Mira is the only large river with its estuary entirely included in a nature protected area (Parque Natural do Sudoeste Alentejano e Costa Vicentina) (Campos *et al*., 2008). Because of that status, fishing activities are currently not allowed in the Mira estuary. Therefore, in the present the major threat to this ecosystem is the increasing touristic pressure in all the south-western coast of Portugal, which may have serious implications to the estuarine water quality.

Data on phytoplankton and macroalgae in the Mira estuary are scarce (Bettencourt *et al*., 1993; Andrade, 1986) although some new information about these components was recently obtained in the EEMA project - *Assessment of Ecological Status of Coastal and Transitional Water Bodies and of Ecological Potential of Highly Modified Water Bodies*. On the contrary, data about saltmarshes and *Zostera* beds are much more abundant (Almeida, 1988; Bettencourt *et al*., 1993; Ferreira, 1994; Adão, 2003). The Mira estuary presents a high abundance of halophytic species comprising 17 species distributed among 7 taxonomic families, occupying a total salt marsh area of 1.46 km^2. In recent years the important *Zostera* beds located near the estuarine mouth had suffered a serious collapse, which led the IMAR and CO groups to start a monitoring program of these communities, in order to understand the causes and impacts on biodiversity and ecological quality.

The most complete work on the zooplankton of the Mira estuary belongs to Mattos (1995), who studied the group of organisms that make up this community throughout the estuarine gradient over a relatively extended period of time. According to this work, the copepods are the most abundant group, sometimes reaching up more than 80% of the catches. The macrozooplankton presents a small number of taxa (Paula, 1987a), among which stand out the hydromedusae *Blackfordia virginica*, the mysid *Mesopodopsis slabberi* and the bento-pelagic isopod *Paragnathia formica*. The hydromedusae is an exotic invasive species, particularly abundant in the intermediate sector of this brackish water system during the warmer months (Paula, 1987a), although it can survive in a range of salinities much wider (3 ‰ to 35 ‰), as referred by Moore (1987).

The first study of meiofauna and free-living nematodes communities in Portuguese estuaries was carried out in sediments associated with seagrass beds of *Zostera noltei* in the Mira estuary (Adão, 2004). Recently, the spatial distribution of meiofauna and free-living nematodes was studied along the estuarine gradients of this system (e.g. Adão *et al*., 2009). The results obtained revealed, once more, that the meiofauna assemblages provide a high resolution biological tool to detect natural and anthropogenic disturbance and environmental changes.

Benthic macroinvertebrates of the Mira estuary were studied for the first time by Andrade (1986) and Marques & Bellan-Santini (1987) and after that by Almeida (1988) and Ferreira (1994) in *Zostera* beds and Chainho *et al.* (2008) in subtidal habitats. Recently, a two-year data series on the benthic macrofauna was collected in the Mira estuary for the project "*Effects of Natural Stress Generated by Freshwater Discharges in the Benthic Invertebrate Estuarine Communities and its Influence on the Assessment of the Benthic Ecological Status – EFICAS*". Some additional information was also obtained in the EEMA project. Generally, these set of data corroborates the high degree of preservation of the ecosystem.

The fish community of the Mira estuary is particularly well known since the middle of the 1980's due to several studies performed by the Centre of Oceanography all over the system. The first works were conducted by Almeida (1986) in the *Zostera* beds and by Costa *et al*. (1987) out of the *Zostera* beds. After that, this information was updated by two European projects: "*Comparative Studies of Salt Marsh Processes"* and "*The Effects of Environmental Changes on European Salt Marshes: Structure, Functioning and Exchange Potentialities with Marine Coastal Waters*" (see Costa *et al*., 2004); and one national project: *Importance of Estuarine and Coastal Nursery Areas for the Stocks Maintenance of Commercial Valuable Fish Species in Portuguese Coast – NURSERIES*. Recently, some additional information was also obtained in the EEMA project. Despite being a small system, the Mira estuary presents a rich and diverse fish community, including more than 90 species. It has important nursery grounds for several species with high commercial value, like the sole (*Solea vulgaris*), the breams (*Diplodus vulgaris* and *Diplodus sargus*) and the sea bass (*Dicentrarchus labrax*) (Costa *et al*., 1994). However, the Mira estuary fish community is dominated by the top predator Lusitanian toadfish (*H. didactylus*), which regulates the populations of other fishes and crustaceans (Costa, 2004), including that of the eel (*Anguilla anguilla*), a species Critically Endangered (IUCN) all over its distribution area (Costa *et al*., 2008).

A complete list of references covering all scientific research conducted in this estuary over the years is provided in section 6.C of the present report.

1.10. Historical data compilation and banking (Milestone 1b)

Characteristics of historical data available for the Mira estuary are displayed in the Table 8 below.

Table 8 – List of historical data available for the Mira estuary.

Communities	Samples	Biological Parameters
Saltmarshes	2009	Species; Abundance; Area occupied
Seagrasses	Seasonally, 2009 and 2010	Not available yet: biomass, no. shoots
Zooplankton	July 2011	Abundance
Benthic macroinvertebrates	October 2003 and March 2004	Species; Abundance; Biomass
Benthic macroinvertebrates	May 2006	Species; Abundance
Benthic macroinvertebrates	Seasonally: March 2006 and January 2008	Species; Abundance; Biomass
Benthic macroinvertebrates	April 2010	Species; Abundance; Biomass
Fishes	Monthly: 1987	Species; Abundance; Size
Fishes	Monthly:1991-1992	Species; Abundance; Size
Fishes	Seasonally:1992-1993;	Species; Abundance; Size
Fishes	March and September 2005; May and July 2006	Species; Abundance; Size
Fishes	June 2010	Species; Abundance; Size

1.11. GAP analysis

In the Mira estuary little information is available for phytoplankton and macroalgae. On the contrary, data about saltmarshes and seagrass beds are much more abundant. However, the causes of the seagrass beds collapse in recent years remain unknown as well as several aspects of saltmarshes importance for the maintenance of the estuarine ecosystem health. Like for phytoplankton, the knowledge on the Mira estuary zooplankton communities is scarce. Therefore, the ecology and impacts of the hydromedusae exotic invader *Blackfordia virginica* remains largely unknown. Contrary to meiofauna, which was only studied in *Zostera* beds, benthic macroinvertebrates and fishes have been studied in more detail along the entire estuarine gradient. Nevertheless, investigation of modifications in these communities related with climate change, human activities and introduction of exotic species must proceed.

1.12. Elements of study in 3M-RECITAL

Given the knowledge already available for fishes, benthic macroinvertebrates and saltmarshes communities in the Mira estuary, these are the biologic groups that will be studied with more detail in the current project in order to monitor their evolution and assess the hypothetic influence of climatic change and human activities in such evolution. In addition, zooplankton studies will be performed to investigate the impact of the hydromedusae exotic invader *Blackfordia virginica* in the different compartments of the Mira estuary ecosystem.

2. 3M-RECITAL SURVEYS

Despite cuts on the final budget approved for the project, most of the elements considered in the initial proposal will be monitored, with only a few adjustments to the pre-established sampling effort, which will not compromise in any case the objectives proposed for 3M-RECITAL.

A summary of the elements surveyed in each of the three estuaries of the 3M_RECITAL: Minho, Mondego and Mira, is given in Table 9.

Table 9 – Elements surveyed in each of the three estuaries of the 3M_RECITAL: Minho, Mondego and Mira; (X) surveyed; (-) not surveyed.

Elements surveyed	Minho	Mondego	Mira
Pressures	X	X	X
Physical-chemical	X	X	X
Phytoplankton	-	X	-
Zooplankton	X	X	X
Macroalgae	X	X	-
Seagrasses	-	X	-
Saltmarshes	X	-	X
Macroinvertebrates	X	X	X
Fish	X	X	X
Shorebirds	Yet to defined	X	-
Ecotoxicological tests	X	X	X

2.A. Minho

2.2. Physical-chemical elements in 3M-RECITAL

2.2.1. Parameters surveyed and methodological approach description

Table 10 presents the number of sites sampled in each water body, as well as the sampling date and the measured parameters. Water samples were collected at each site in low- and high-tide and at surface and bottom (when depth was higher than 5 meters) and the following parameters were determined:

- Salinity and dissolved oxygen were measured *in situ* with an YSI 650 meter.
- Suspended particulate matter (SPM) was obtained by filtering water through polycarbonate membranes (0.45 µM) and determined gravimetrically (drying at room temperature).
- Water samples for the determination of dissolved inorganic nutrients (nitrate, NO_3^-+NO_2^-; ammonia, NH_4^+; phosphate, PO_4^{3-} and silicate, $Si(OH)_4$) were filtered through MSI Acetate Plus filters and analysis carried out using an autoanalyser TRAACS 2000 (Bran+Luebbe).
- Niquel, Cr, Cu, Pb, Co and Cd were measured in the collected waters using diffusive gradients of thin films (DGT). Total dissolved mercury was quantified in filtered and acidified water samples by cold-vapour atomic fluorescence spectrometry.
- Water samples were filtered and extracted with appropriate solvents for the determination of organic compounds by chromatographic techniques.

Table 10 – Number of sampling sites (by water body - WB) in Minho estuary, the sampling dates and the measured parameters.

System	Water Bodies	Nº of sampling sites at each WB	Sampling date	Measured parameters
Minho	WB1	2	April 2010	. Physical-chemical parameters (salinity, dissolved
	WB2	2		oxygen, suspended particulate matter)
	WB3	2		. Nutrients (ammonium, nitrate, nitrite, phosphate,
	WB4	2		silicate)
	WB5	2		. Dissolved metals (Ni, Cr, Cu, Pb, Co, Cd, Hg)
				. Dissolved organic compounds (nonilfenol, DDD)

2.2.2. Results

2.2.2.1. Physical-Chemical parameters

Figure 3 presents the values of salinity, dissolved oxygen and suspended particulate matter (SPM) in water of the five water bodies (WB). The highest values of salinity and suspended particulate matter were found in water bodies 1 and 5 that are closer to the estuary inlet (Figure 3). Dissolved oxygen was lower in WB1 and WB5.

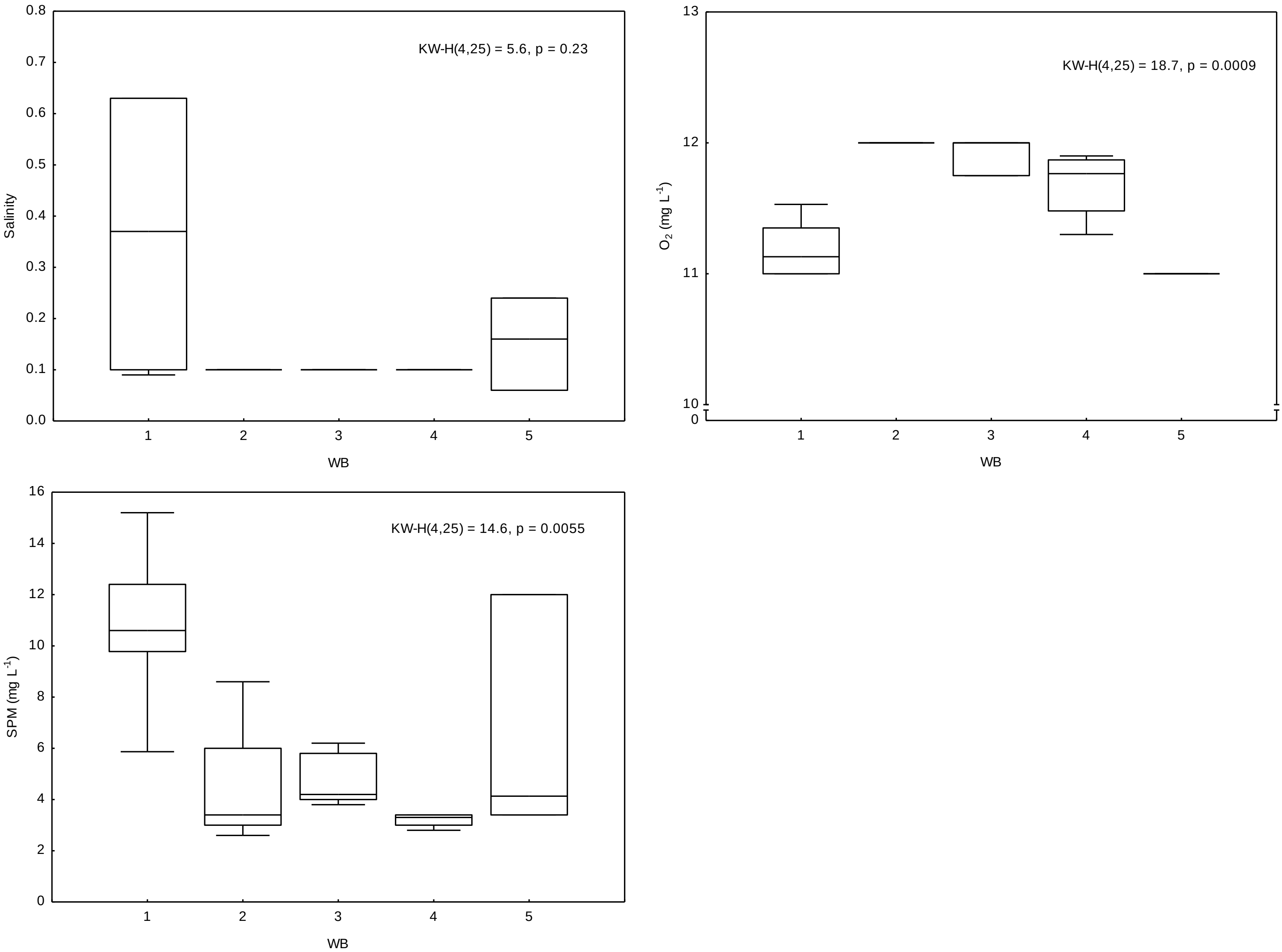
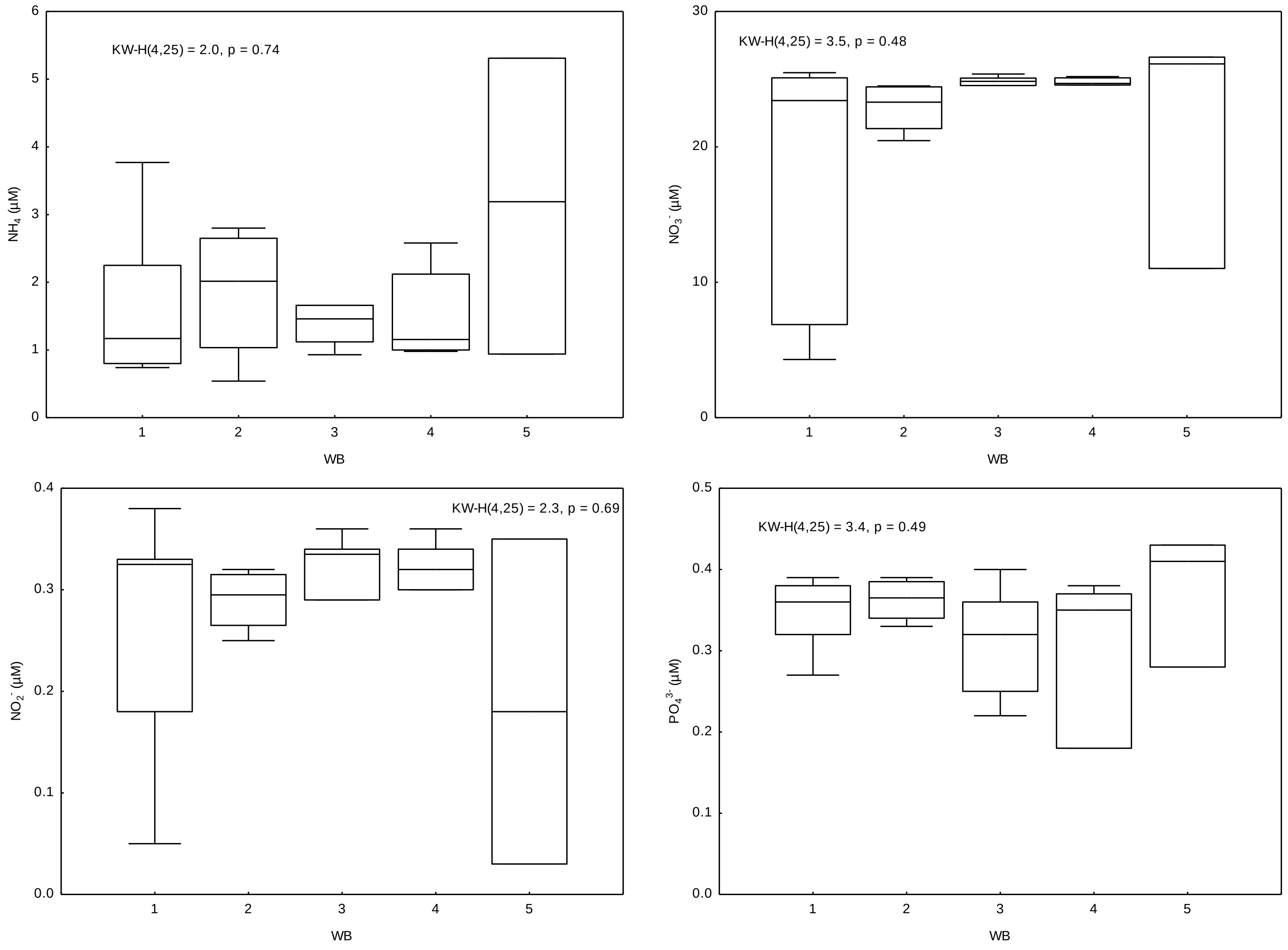


Figure 3 – Salinity, dissolved oxygen and suspended particulate matter (SPM) in five water bodies of the Minho estuary in April 2010. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.2.2.2. Nutrient concentrations in water

Figure 4 presents the concentrations of dissolved nutrients in the five water bodies (WB) of the Minho estuary. In general, WB5 presented the highest dispersion of values and the maximum levels of ammonium, nitrate and phosphate. Both sites of WB5 are located in a salt marsh that is known as a highly productive area. Nevertheless, no significant differences were found among water bodies for dissolved nutrients.



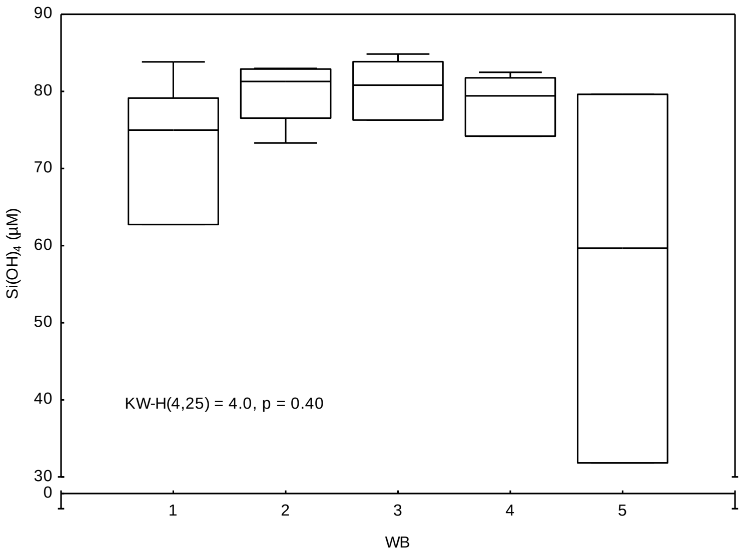
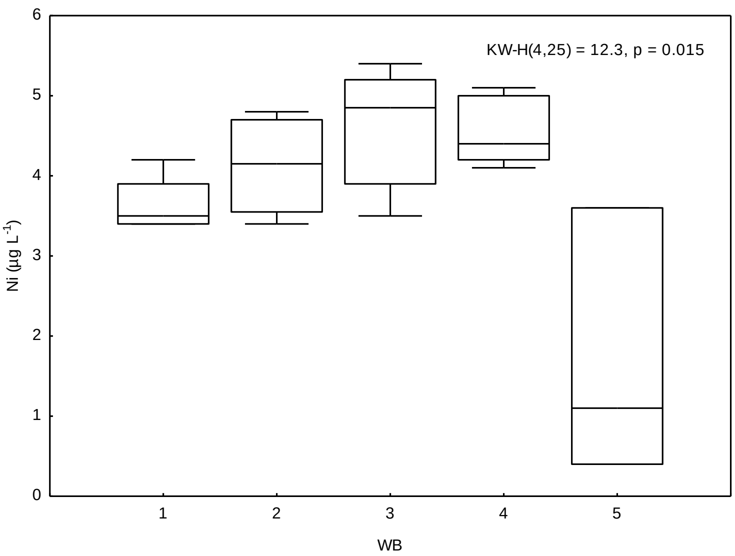
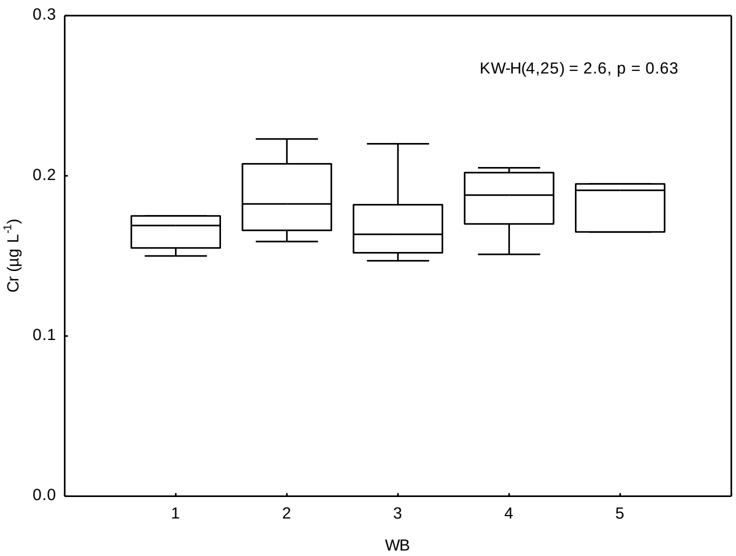


Figure 4 – Dissolved nutrients in five water bodies of the Minho estuary in April 2010. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.2.2.3. Levels of dissolved metals

Figure 5 presents the levels of dissolved metals in the five water bodies (WB) of the Minho estuary. Levels of dissolved Ni and Co were lower in WB5 that in the remaining surveyed areas, while an opposite spatial pattern was obtained for Cd. WB5 is corresponds to the salt marsh area. No significant spatial differences were recorded for Cr, Cu, Pb and Hg.





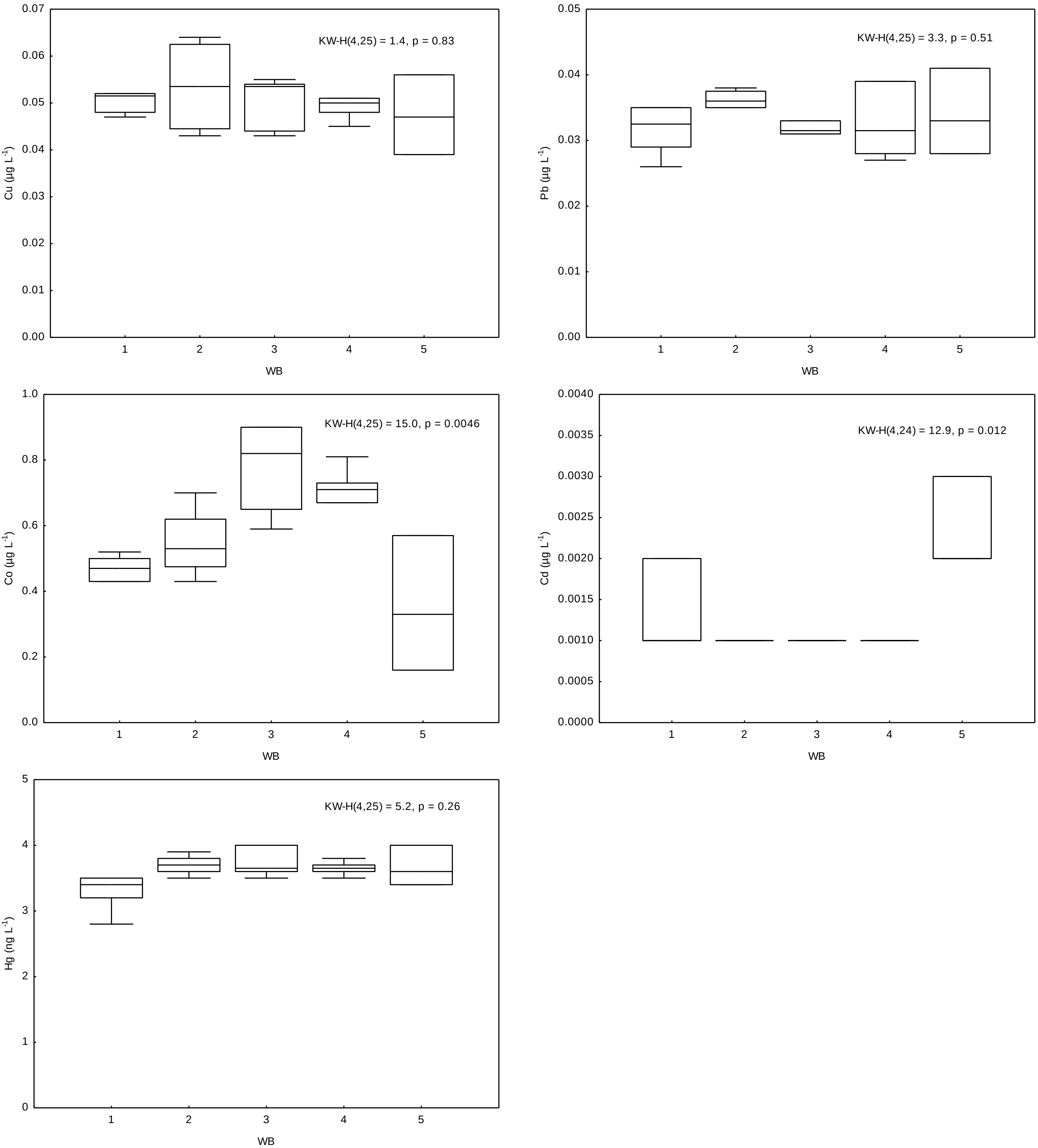


Figure 5 – Dissolved Ni, Cr, Cu, Pb, Co, Cd and Hg in five water bodies of the Minho estuary in April 2010. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.2.2.4. Levels of dissolved nonilfenol and DDD

Figure 6 presents the levels of dissolved nonilfenol and DDD in the five water bodies (WB) of the Minho estuary. Lower levels of nonilfenol were detected in the WB5 while no significant spatial differences were found for DDD.

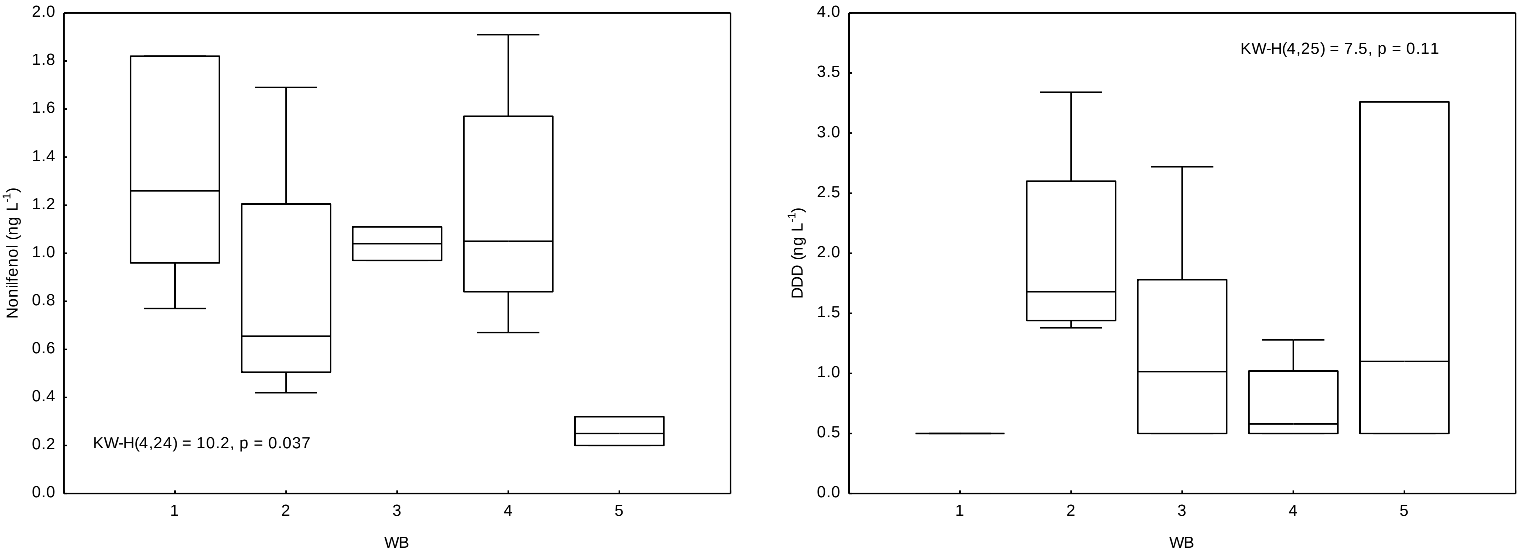


Figure 6 – Dissolved nonilfenol and DDD in five water bodies of the Minho estuary in April 2010. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.3. Biological elements in 3M-RECITAL

Table 11 gives an overview of the biological elements considered for survey under the Minho estuary monitoring network within the 3M_RECITAL project. Details on each of the elements are given on the following sections.

Table 11 – Campaigns planned for the Minho estuary under the scope of 3M_RECITAL project.

Date	Type of campaign	Target	notes
Setembro 2011 a Julho 2012	Campanhas exploratórias pontuais		
Julho de 2012	Missões no âmbito do projecto SIGNAL que contribuem com dados também para o 3M-RECITAL	- análises de isótopos, zooplâncton + peixes - parâmetros físico-químicos	
Setembro/Outubro de 2012	Missões para recolha de macrobentos Missões para recolha de peixes; Missões para recolha de zooplâncton Missões para recolha de amostras para análises químicas na água, sedimento, zooplâncton e peixes	Estudo de comunidades Estudo de comunidades Estudo comunidade	Em concertação com campanhas noutros estuários

2.3.1. Zooplankton

In the Minho estuary, this element will be monitored seasonally in 2013.

Historical data (monthly surveys) will be made available for comparison with campaigns of the current project.

2.3.2. Macroalgae and macrophytes

2.3.2.1. Macroalgae

Only blooming macroalgae will be considered in the Minho Estuary.

Habitats: the macroalgae will be sampled in the Minho system, at the intertidal area.

Sampling periodicity: for opportunistic macroalgae, twice per season (in a total of 8 sampling events) for biomass estimations, and once per year (during summer) for the estimation of mat extents.

Sampling procedures: for biomass quantification, samples will be collected during low tide, by using a manual corer (13.5 cm inner diameter), at four sampling stations in the South Arm. Each time, six replicates will be randomly taken. Samples will be washed *in situ*, with estuarine water, through a 0.5 mm mesh size sieve, for removal of sediment attached to the plants. The mats extent of blooming macroalgae will be surveyed during summer by using aerial photographs (confirmed by field visits) or a GPS to register the size and position of opportunistic macroalgae mats. During field surveys, the extent of the mats is reported to different cover densities (%), assuming the 0-5%, 5-25%, 25-50%, 50-75%, 75-100% as the % cover intervals.

Laboratory procedures: for opportunistic macroalgae, samples are sorted, the taxa identified, and the biomass (g DW m^{-2}) determined for each taxa after dried in a stove until weight stabilization. To determine the mats extent of opportunistic macroalgae, aerial photos are treated in a GIS program, the presence confirmed in the field, and the areas calculated. When a GPS device is used, data are downloaded and treated into the same GIS software to calculate the macroalgal mats extent.

2.3.2.2. Saltmarsh plants

Habitats: the sampling sites were placed at the upper zone of the intertidal area.

Sampling periodicity: Field campaigns programmed to collect data on saltmarshes during the project are yet to be defined.

Sampling procedures: inside every characteristic marsh (e.g., *Spartina* dominated) of each water bodies identified for the Minho (defined in the scope of the WFD implementation in Portugal), it was placed a transect. This transect line was disposed in the marsh in order to cross all the significant vegetation zones from the area. The taxonomic composition and the (%) coverage of the different taxa were registered for every meter by using a 1 m side plastic tube frame.

Laboratory procedures: based on SIG software and on the information collected during fields surveys (taxonomic composition and % coverage of each taxa), different vegetation polygons could be defined from aerial photographs. The deviation from the optimal coverage could also be calculated for each taxa present in the marsh.

2.3.3. Benthic invertebrates

Habitats: Soft-bottom subtidal habitat will be sampled to assess the benthic invertebrate communities.

Sampling periodicity: Two *per* year, Spring and late Summer (September), with possibility of collecting seasonally yet to be confirmed (Table 12).

Sampling procedures: Biological samples will be 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 (covering a network of 10 pre-defined stations along the entire estuarine gradient – 2 stations per salinity stretch). At each station, an extra sediment sample will be collected (150-200ml) to characterize sediment properties (e.g. granulometry and organic matter content). Biological samples will be washed *in situ*, with estuarine water, through a 0.5 mm mesh size sieve.

Laboratory procedures: In the laboratory, samples will be sieved through a 0.5 and 1 mm mesh sizes and fixed with 4% buffered formalin solution. The 0.5 mm fraction will be stored for future studies. Only the 1 mm mesh size fraction will be further processed.

Afterwards, macroinvertebrates will be sorted and preserved in 70% ethanol for subsequent identification and counting. Whenever possible the organisms will be identified up to species level (or lowest reliable taxonomic level) and the biomass (g AFDW m^{-2}) determined. The organisms will be quantified in terms of abundance and biomass (g AFDW m-^{2}).

Table 12 - Campaigns planned for the benthic macroinvertebrates in the Minho estuary under the scope of 3M_RECITAL project.

Habitat	Biological Element	Sampling time	Stations	Methodology	"Status"
	Macroinvertebrates	9/2011 to 7/2012	Exploratory campaigns	-	completed
SUBTIDAL	Macroinvertebrates	September /October2012	10 stations (2 per salinity stretch)	standard	programmed
SUBTIDAL	Macroinvertebrates	Spring	10 stations (2 per salinity stretch)	standard	programmed

2.3.4. Fish

Habitats: the fish communities will be assessed in the open channels, free of obstacles that could represent a difficulty to the selected fishing gear (beam trawl).

Sampling periodicity: seasonal (four times per year), in the last part of the season, to integrate its effect over the fish community.

Sampling procedures: a minimum of 3 tows, 10 minutes each, will be done on each WB or significant salinity gradient zone. Fish captured will be preserved in ice inside thermo-containers and transported into the laboratory for further processing. The water column physicochemical parameters will be registered immediately before or after the fishing event, as well as the boat velocity and the coordinates of the inicial and final point of the tow. Fish sampling is performed at night, during ebbing tide, covering around 300 m in 10 minutes, and in the same direction of the water flow.

Laboratory procedures: samples will be sorted and all fishes captured identified, measured (total length with precision of 1 mm) and weighed (total weight with an accuracy of 0.01 g) individually; in case of large number of catches (more than 50 individuals) a subsampling may be made immediately in the field in which individuals captured in addition to that 50 individuals should be counted and weighed together; all individuals of the accompanying fauna should be identified and counted and weighed together, by species.

2.3.5. Shorebirds

Surveys of this element are programmed for the Minho estuary in the scope of 3M_RECITAL, but details of campaigns are yet to be defined.

2.4. Ecotoxicological tests

Baseline conditions relative to the population growth rate of the unicellular diatom microalgae *Phaedocatylum tricornutum* and feeding rates of the benthic polychaete *Hediste diversicolor* and the epibenthic crab *Carcinus maenas* were previously gathered for a single site at the Minho estuary. By then, differences between the surveyed site and those at the reference Mira estuary were fully explained by variations on environmental factors other than anthropogenic pressures: temperature, salinity, sediment organic matter content and sediment particle size. Therefore, ecotoxicological tests are programmed if, and only if, other surveyed elements dictate so.

2.B. Mondego

2.5. Physical-chemical elements in 3M-RECITAL

In the Mondego Estuary this element will be surveyed under 3 distinct approaches:

a. a common campaign in the three LTER systems using standardized methods undertaken by the IPIMAR project partner for system characterization (details in section 2.4.1);

b. additional data collected together with specific surveys of biological elements, as supporting information for biota (details to be provided with respective biological surveys);

c. and third set of physical-chemical parameters collected by means of an autonomous system (SIMPATICO) for water quality and current monitoring, installed at the mouth of the Mondego Estuary. The system measures in continuous the current velocity and direction, pressure, water temperature, turbidity, dissolved oxygen, salinity and pH. The data are automatically uploaded to a remote server on a daily basis (http://webserver.mohid.com/SIMPATICO/).

2.5.1. Parameters surveyed and methodological approach description

Table 13 presents the number of sites sampled in each water body, as well as the sampling date and the measured parameters. Water samples were collected at each site in low- and high-tide and at surface and bottom (when depth was higher than 5 meters) and the following parameters were determined:

- Salinity and dissolved oxygen were measured *in situ* with an YSI 650 meter.
- Suspended particulate matter (SPM) was obtained by filtering water through polycarbonate membranes (0.45 μM) and determined gravimetrically (drying at room temperature).

- Water samples for the determination of dissolved inorganic nutrients (nitrate, NO_3^-+NO_2^-; ammonia, NH_4^+; phosphate, PO_4^{3-} and silicate, $Si(OH)_4$) were filtered through MSI Acetate Plus filters and analysis carried out using an autoanalyser TRAACS 2000 (Bran+Luebbe).
- Niquel, Cr, Cu, Pb and Cd were measured in the collected waters using diffusive gradients of thin films (DGT). Total dissolved mercury was quantified in filtered and acidified water samples by cold-vapour atomic fluorescence spectrometry.
- Water samples were filtered and extracted with appropriate solvents for the determination of organic compounds by chromatographic techniques.

Table 13 – Number of sampling sites (by water body - WB) in Mondego estuary, the sampling dates and the measured parameters.

System	Water Bodies	Nº of sampling sites at each WB	Sampling date	Sampled parameters
Mondego	WB1 WB2 WB3	2 2 1	June 2009	. Physical-chemical parameters (salinity, dissolved oxygen, suspended particulate matter) . Nutrients (ammonium, nitrate, nitrite, phosphate, silicate) . Dissolved metals (Ni, Cr, Cu, Pb, Cd, Hg) . Dissolved organic compounds (PBDE)

2.5.2. Results

2.5.2.1. Physical-Chemical parameters

Figure 7 presents the values of salinity, dissolved oxygen and suspended particulate matter (SPM) in water of the three water bodies (WB). Water body 3 presented significantly lower salinity in comparison to WB1 and WB2. No significant differences were found among sites for dissolved oxygen and suspended particulate matter (SPM).

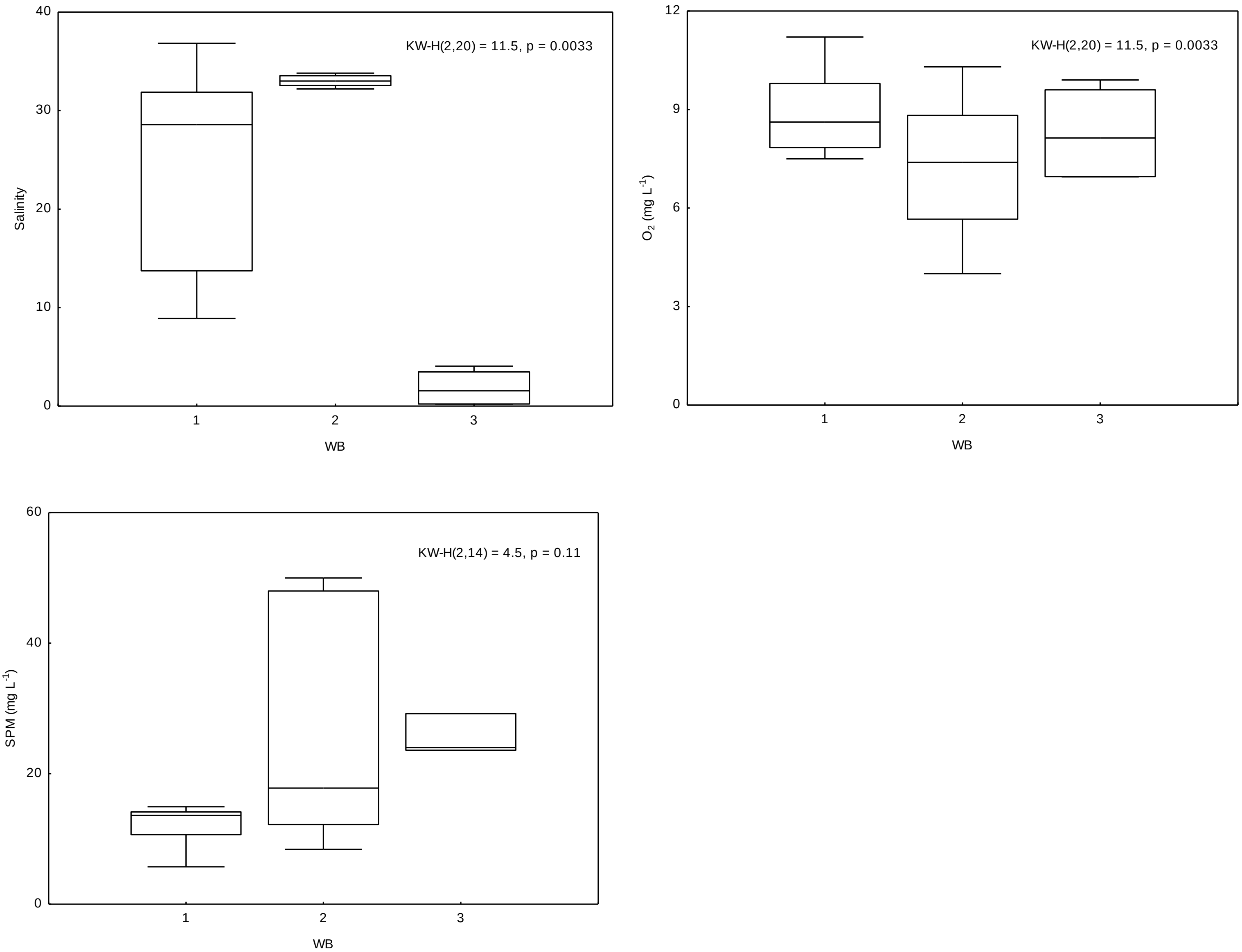


Figure 7 – Salinity, dissolved oxygen and suspended particulate matter (SPM) in three water bodies of the Mondego estuary in June 2009. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.5.2.2. Nutrient concentrations in water

Figure 8 presents the concentrations of dissolved nutrients in the three water bodies (WB) of the Mondego estuary. WB3 that is located in the upstream area presented lower levels of ammonium than the other water bodies. WB2 that is located in the south arm of the estuary presented the highest levels of phosphate and silicate.

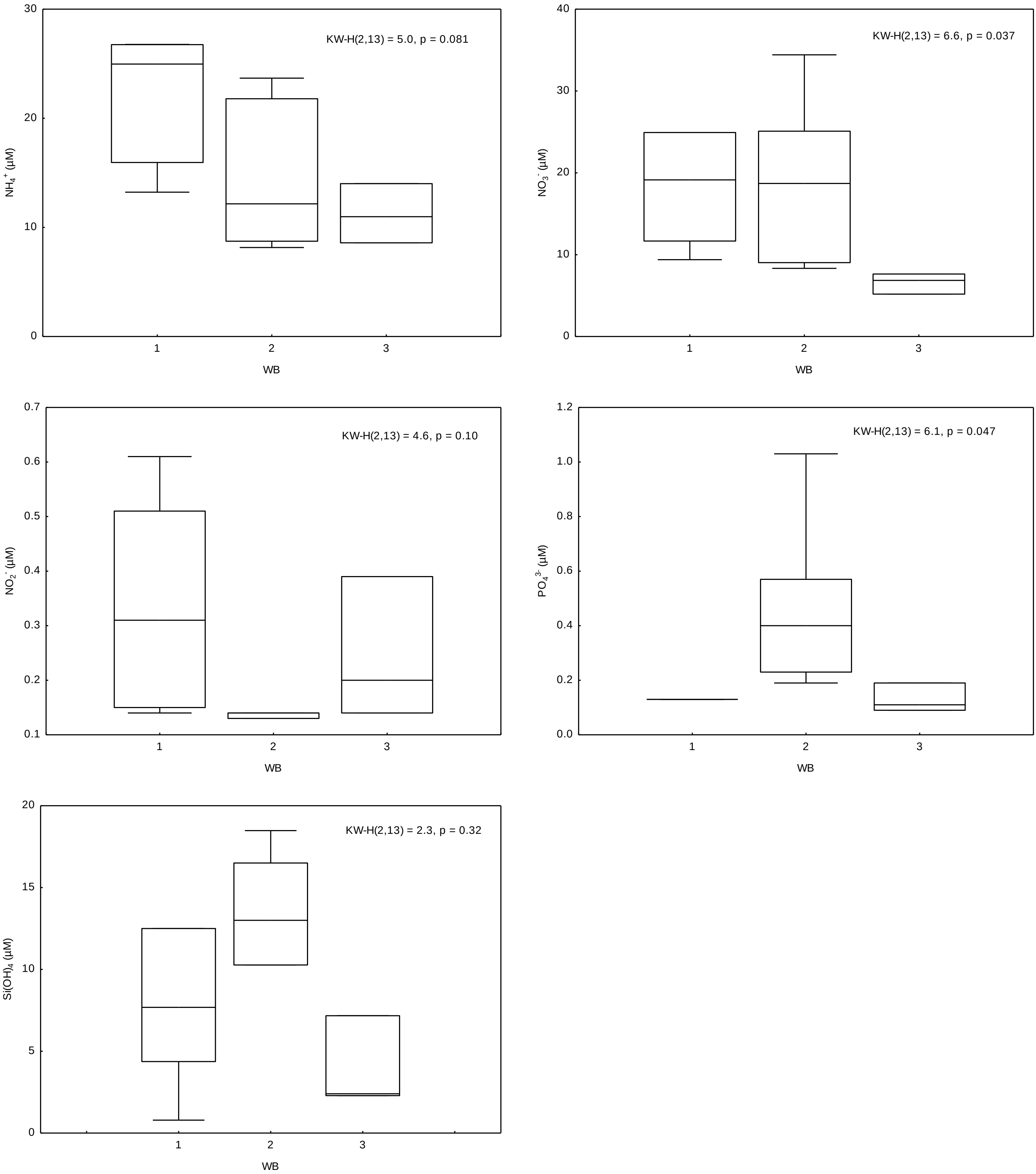
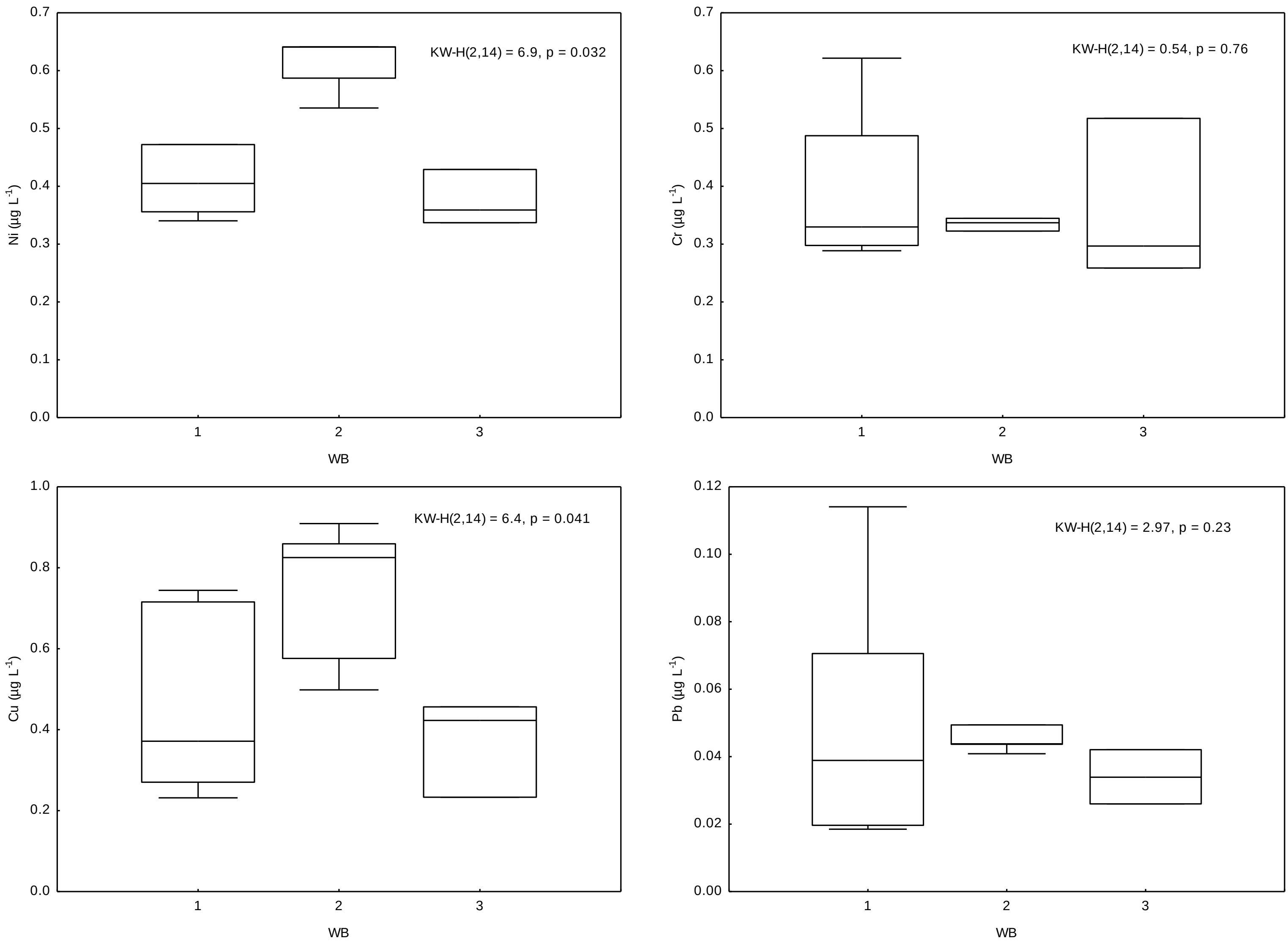


Figure 8 – Dissolved nutrients in three water bodies of the Mondego estuary in June 2009. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.5.2.3. Levels of dissolved metals

Figure 9 presents the levels of dissolved metals in the three water bodies (WB) of the Mondego estuary. WB2 presented the higher levels of dissolved Ni, Cu and Cd than the other areas. This water body is located in the south arm of the estuary that was previously described as an anthropogenic impacted area.



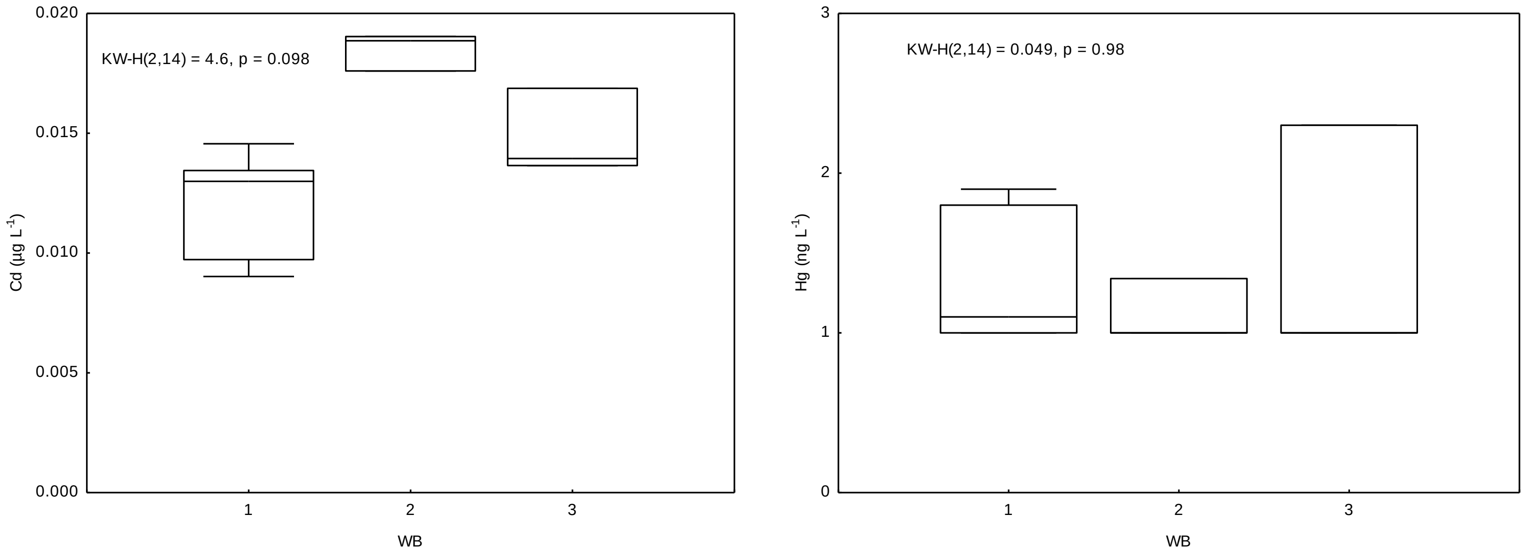


Figure 9 – Dissolved Ni, Cr, Cu, Pb, Cd and Hg in three water bodies of the Mondego estuary in June 2009. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.5.2.4. Levels of dissolved PBDE

Figure 10 presents the levels of PDBE in three water bodies (WB) of the Mondego estuary. No significant differences were found between the three areas.

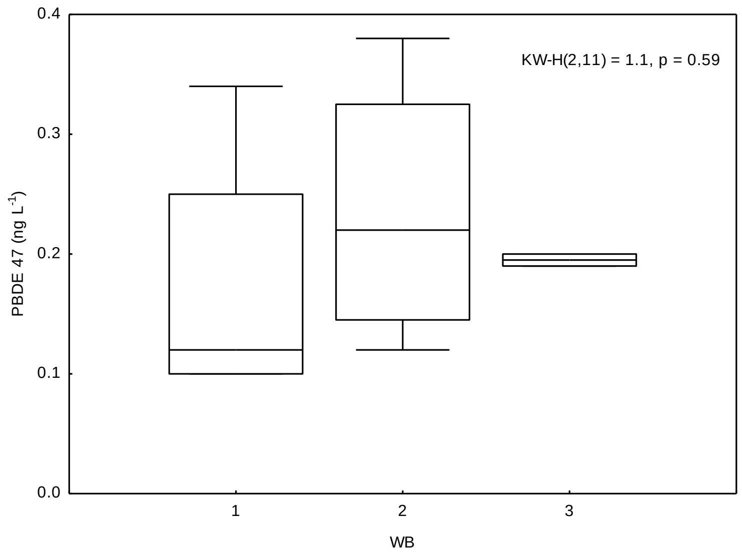


Figure 10 – Dissolved PBDE in three water bodies of the Mondego estuary in June 2009. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.6. Biological elements in 3M-RECITAL

2.6.1. Phytoplankton

This first year report presents the sampling campaigns related to the Phytoplankton Response Survey, Task 3 of the 3M-RECITAL project. Here is presented a section to cover sampling sites, phytoplankton sampling collection and laboratory procedures. The report is an extension to that of the first year and at this stage does not include any data analysis.

Sampling collection and laboratory procedures: Phytoplankton samples are collected monthly in the Mondego estuary, during spring tide, at high tide, at 6 sampling stations (St 2; St 5; St 9; St 12; St 18; St 23) distributed throughout both arms (Figure 11). Phytoplankton is collected with a Niskin bottle at 0.5 m below the surface and at 0.5m above the bottom of the estuarine sites. 300 ml of each water sample is fixed in 1% neutral Lugol solution to preserve phytoplankton for enumeration. The samples are stored in dark glass bottles at ambient temperature.

Additionally, at each site in the estuary, water samples are collected at surface and bottom to determine the concentrations of nitrate (mg/L), nitrite (mg/L), phosphate (mg/L), ammonia (mg/L), total suspended solids (mg/L) and chlorophyll *a* concentration (mg/m^3). Furthermore, hydrological parameters (temperature, salinity, dissolved oxygen, transparency and pH) are measured in *situ*. All water parameters measured and analysed are linked to Water Quality Survey for Task 2, following the same procedures.

Figure 11 – Location of the Mondego estuary on the western coast of Portugal and the 6 sampling stations within the estuary (St 2, mouth station; St 12, St 18 and St 23, stations of the north arm; St 5 and St 9, stations of south arm).

Table 14 summarises the dates of sampling at the 6 stations since the start of Task 3 in May 2012. Are also presented the proposed campaign dates until the end of the year 2012. Usually, the sampling takes place during the first spring tide occurring in the first half of each month.

Table 14 - Dates for phytoplankton sampling campaigns in the Mondego estuary, at high tide, from the surface and the bottom, at the 6 sampling stations, during 2012.

Date (2012)
8th May
4th June
3rd July
1st August
17th September
1st October
31 st October
13th December

2.6.2. Zooplankton

This first year report presents the sampling campaigns related to the Zooplankton Response Survey, Task 4 of the 3M-RECITAL project. The sampling sites, zooplankton sampling collection and laboratory procedures are presented below. This is the first report and at this stage there is no data analysis.

Sampling collection and laboratory procedures: Zooplankton samples are collected seasonally (2 sampling campaigns at each season) in the Mondego estuary, during high tide, at 6 sampling stations: at the mouth (St 2), at the north arm (St 12; St 18; St 23) and at the south arm (St 5 and St 9) (Figure 11). Zooplankton samples are collected by sub-surface tows with a plankton net of 335 µm mesh sized (frame diameter: 0.5 m), the net is fitted with a Hydro-Bios flow meter, to calculate the filtered volume (average 50 m^3). Samples collected are immediately fixed and preserved in 4% formaldehyde buffered in seawater. Additionally, at each site, water samples are collected at surface to determine nutrient concentrations (nitrate, nitrite; phosphate; ammonia and total suspended solids – mg/L; chlorophyll *a* concentration - mg/m^3). Moreover, environmental parameters (temperature, salinity, dissolved oxygen, transparency and pH) are measured in *situ*. All water parameters measured and analysed are linked to Water Quality Survey for Task 2, following the same procedures.

Table 15 summarises the dates of sampling at the 6 stations since the start of Task 4 in May 2012. Campaigns dates are also proposed for the time-period until the end of the year 2012.

Table 15 - Dates for zooplankton sampling campaigns in the Mondego estuary, at high tide during 2012.

Date (2012)
8th May
6th June
6th July
18th September
1st October
31 st October

2.6.3. Macroalgae and macrophytes

Macroalgae and Macrophytes survey – will provide information on macroalgae and macrophytes as ecological indicators of ecological status. Historical data will be worked out as measurable attributes (metrics) to help in defining Reference Conditions for the three systems (Objectives 1 and 2).

2.6.3.1. Macroalgae

Historical data for opportunistic macroalgae (e.g., *Ulva* sp.) in the Mondego report to the parameter biomass (g DW m^{-2}), collected monthly during the periods Jan. 1993 – Dec. 2001 and Jan. 2009 – Jul. 2011. The sampling periodicity and data availability changed over time, depending on the interest of the active projects in this biological element.

Apart from the opportunistic macroalgae, was also surveyed the composition and dynamics of the estuarine macroalgal community. These data were collected for all seasons, from autumn 2008 to winter 2010. Samples were taken during low tide at 6 stations distributed along the estuarine salinity gradient. Six replicates were semi-randomly collected at each sampling station, on a maximum length of 30 m, using a 40 cm side quadrate.

Habitats: the blooming macroalgae are sampled in the South Arm, at the intertidal area. The macroalgal taxonomic composition was performed at the hard substratum from the margins (artificial).

Sampling periodicity: for opportunistic macroalgae, twice per season (in a total of 8 sampling events) for biomass estimations, and once per year (during summer) for the estimation of mat extents. The taxonomic composition was surveyed every season but is no longer programmed to continue during the 3M-Recital project's life time.

Sampling procedures: for biomass quantification, samples will be collected during low tide, by using a manual corer (13.5 cm inner diameter), at four sampling stations in the South Arm. Each time, six replicates will be randomly taken. Samples will be washed *in situ*, with estuarine water, through a 0.5 mm mesh size sieve, for removal of sediment attached to the plants. The mats extent of blooming macroalgae will be surveyed during summer by using aerial photographs (confirmed by field visits) or a GPS to register the size and position of opportunistic macroalgae mats. During field surveys, the extent of the mats is reported to different cover densities (%), assuming the 0-5%, 5-25%, 25-50%, 50-75%, 75-100% as the % cover intervals.

Concerning the taxonomy and dynamics of macroalgae, six replicates were semi-randomly collected at each sampling station (along a maximum length of 30 m), from the upper to lower parts of the intertidal, using a 40 cm side quadrate.

Laboratory procedures: for opportunistic macroalgae, samples are sorted, the taxa identified, and the biomass (g DW m^{-2}) determined for each taxa after dried in a stove until weight stabilization. To determine the mats extent of opportunistic macroalgae, aerial photos are treated in a GIS program, the presence confirmed in the field, and the areas calculated. When a GPS device is used, data are downloaded and treated into the same GIS software to calculate the macroalgal mats extent.

2.6.3.2. Seagrasses - *Zostera* meadows

Historical data for *Zostera* meadows in the Mondego report to the parameter biomass (g DW m^{-2}), monthly campaigns during the periods Jan. 1993 – Dec. 2001 and Jan. 2009 – Jul. 2011, and to punctual estimations registered before the project (2007 – 2011) for the bed extent parameter. The sampling periodicity and data availability changed over time, depending on the interest of the different active projects at the moment.

Habitats: the sampling site is placed in the south arm, at the intertidal area, comprising 3 sampling stations along the seagrass meadows. These sampling stations include an earlier degraded area (Armazéns), where blooms of green macroalgae were observed and seagrass disappeared, but where a recent expansion of *Zostera* is taking place.

Sampling periodicity: campaigns during the 3M-Recital life include monthly surveys, to collect biomass and shoots' density data, in the period Aug. 2011 – Mar. 2012, and a new format that will include 2 sampling events per season (Feb., Mar., May, Jun., Jul., Sep., Nov., Dec.), in a total of 8 samplings events per year, from May 2012 forward. Concerning the estimation of bed extent, an annual sampling event in late summer (September) is programmed to take place, and will enlarge the punctual estimations registered before the project.

Sampling procedures: sampling has been taken through the use of a manual corer (13.5 cm inner diameter) for the quantification of biomass (g DW m^{-2}) and shoot density (ind m^{-2}), and by geo-referencing the meadow with the use of a GPS device (covering the margin of the meadow by foot).

Laboratory procedures: *Zostera noltei* plants are separated, counted the number of shoots for the density estimation (ind m^{-2}), and quantified the dried weight (DW) for the biomass (g DW m^{-2}) present in the sample. The bed extent mapping is based on field observations (GPS to register the field meadows perimeter), vertical photographs and GIS methodology (ArcView GIS version 8.3).

2.6.3.3. Saltmarsh plants

Historical data for saltmarshes in the Mondego are scarce. Taxonomic composition and abundance (%) were surveyed during summer in 2009.

Habitats: the sampling sites were placed at the upper zone of the intertidal area. In 2009 a total of four stations were surveyed; three of them located in the South Arm and one in the North Arm, near the connection point of the two arms.

Sampling periodicity: There are no field campaigns programmed to collect data on saltmarshes during the project.

The previous sampling events were punctual; in 2009 the survey occurred during summer.

Sampling procedures: inside every characteristic marsh (e.g., *Spartina* dominated) of each water bodies identified for the Mondego (defined in the scope of the WFD implementation in Portugal), it was placed a transect. This transect line was disposed in the marsh in order to cross all the significant vegetation zones from the area. The taxonomic composition and the (%) coverage of the different taxa were registered for every meter by using a 1 m side plastic tube frame.

Laboratory procedures: based on SIG software and on the information collected during fields surveys (taxonomic composition and % coverage of each taxa), different vegetation polygons could be defined from aerial photographs. The deviation from the optimal coverage could also be calculated for each taxa present in the marsh.

2.6.4. Benthic invertebrates

Habitats: To monitored and characterised the benthic invertebrate communities, two habitats will be sampled: the soft-bottom subtidal and the soft-bottom intertidal (Table 16).

Sampling periodicity:

INTERTIDAL: twice per season (in a total of 8 sampling occasions).

SUBTIDAL: once per year, in late Summer (September).

Sampling procedures:

INTERTIDAL: Biological samples will be collected during low tide, at four different stations in the South Arm. Each time, six replicate cores will be randomly taken to a depth of 20 cm (13.5 cm inner diameter). At each station, an extra sediment sample will be collected (150-200ml) to characterize sediment properties (e.g. granulometry and organic matter content). Biological samples will be washed *in situ*, with estuarine water, through a 0.5 mm mesh size sieve.

SUBTIDAL: Biological samples will be collected using a LMG model of a Van Veen grab with 0.1 sq m area, gathering randomly three replicates each time at each station (covering a network of 16 pre-defined stations along the entire estuarine gradient). At each station, an extra sediment sample will be collected (150-200ml) to characterize sediment properties (e.g. granulometry and organic matter content). Biological samples will be washed *in situ*, with estuarine water, through a 0.5 mm mesh size sieve.

Laboratory procedures:

INTERTIDAL: In the laboratory, samples will be sieved through a 0.5 mm mesh size and fixed with 4% buffered formalin solution.

SUBTIDAL: In the laboratory, samples will be sieved through a 0.5 and 1 mm mesh sizes and fixed with 4% buffered formalin solution. The 0.05 mm fraction will be stored for future studies. Only the 1 mm mesh size fraction will be further processed.

Afterwards, for both habitat types, macroinvertebrates will be sorted and preserved in 70% ethanol for subsequent identification and counting. Whenever possible the organisms will be identified up to species level (or lowest reliable taxonomic level) and the biomass (g AFDW m^{-2}) determined. The organisms will be quantified in terms of abundance and biomass (g AFDW $m\text{-}^{2}$).

Table 16 - Campaigns completed for the benthic macroinvertebrates in the Mondego estuary under the scope of 3M_RECITAL project.

Habitat	Biological Element	Sampling time	Stations	Methodology	"Status"
SUBTIDAL	Macroinvertebrates	14, 15/9/2011	16 stations (reduced network + st 16)	standard	completed
	Macroinvertebrates	7, 8, 9/3/2012	16 stations (reduced network + st 16)	standard	completed
	Macroinvertebrates	26, 27 /9/2012	16 stations (reduced network + st 16)	standard	completed
INTERTIDAL	Macroinvertebrates	2/9/2011	4 stations (Zo, It, Ar, Mo)	standard	completed
	Macroinvertebrates	13/10/2011	4 stations (Zo, It, Ar, Mo)	standard	completed
	Macroinvertebrates	14/11/2011	4 stations (Zo, It, Ar, Mo)	standard	completed
	Macroinvertebrates	13/12/2011	4 stations (Zo, It, Ar, Mo)	standard	completed
	Macroinvertebrates	13/1/2012	4 stations (Zo, It, Ar, Mo)	standard	completed
	Macroinvertebrates	9/2/2012	4 stations (Zo, It, Ar, Mo)	standard	completed
	Macroinvertebrates	12/3/2012	4 stations (Zo, It, Ar, Mo)	standard	completed
	Macroinvertebrates	9/5/2012	4 stations (Zo, It, Ar, Mo)	standard	completed
	Macroinvertebrates	5/6/2012	4 stations (Zo, It, Ar, Mo)	standard	completed
	Macroinvertebrates	5/7/2012	4 stations (Zo, It, Ar, Mo)	standard	completed
	Macroinvertebrates	3/9/2012	4 stations (Zo, It, Ar, Mo)	standard	completed

Subtidal and Intertidal new campaigns are scheduled for 2012/2013, according with the defined periodicity.

2.6.5. Fish

The fish fauna data from the Mondego report to historical data, collected monthly (~15 events), every two months (~14 events) or two times a year. These events took place in 2003, 2004, 2009, 2010, 2011 and 2012, and tows were made in 5 or 6 different sites selected inside the system, covering the entire salinity gradient (Venice system) and the morphology of the estuarine area of the Mondego.

The future fishing events, during the project's life, are programmed to occur 4 times a year, preferentially, during the last part of the season, in order to capture the season's effect over the community; as outlined below:

Habitats: at sampling stations reported in Figure 11, covering the estuarine entire salinity gradient (Venice system) and the morphology of the estuarine area of the Mondego.

Sampling periodicity: seasonal sampling.

Sampling procedures: tows were made in 5 or 6 different sites (Figure 11) selected inside the system.

2.6.6. Shorebirds

The shorebird data from the Mondego reports to:

a. Time series of winter census (1993-2012) and monthly census (1993-2003) and association with long time series of macroalgal cover area (1996-2003).
b. Time series of shorebird energy intake rates and habitat use (1993-2003)

They cover all the shorebird community, especially the main wintering species, the Dunlin (*Calidris alpina*).

Habitats: Intertidal mudflats and supra tidal habitats (mostly saltpans)

Sampling periodicity: seasonally, but most of the sampling is going to occur during the winter 2012 and 2013.

Sampling procedures: census of estuarine habitats, energy intake rates and energy networks.

2.7. Ecotoxicological tests

Information on the population growth rate of the unicellular diatom microalgae *Phaedocatylum tricornutum* and feeding rates of the benthic polychaete *Hediste diversicolor* and the epibenthic crab *Carcinus maenas* were previously gathered for a single site at the south arm of the Mondego estuary. By then, significantly reduced organism responses at the surveyed site compared to those at the references Minho and Mira estuaries were not fully explained by variations on environmental factors other than anthropogenic pressures: temperature, salinity, sediment organic matter content and sediment particle size.

Therefore, ulterior ecotoxicological tests are found to be pertinent and are programmed within 3M_Recital, though the deployment of a multispecies *in situ* test battery covering key processes on ecosystem functioning: primary production, organic matter decomposition, grazing on microphytobenthos, predation. Ideally, such a battery will be conveyed at the worst-case scenario on the temporal scale and on a pressure gradient on the spatial scale

2.C. Mira

2.8. Physical-chemical elements in 3M-RECITAL

2.8.1. Parameters surveyed and methodological approach description

Table 17 presents the number of sites sampled in each water body, as well as the sampling date and the measured parameters. Water samples were collected at each site in low- and high-tide and at surface and bottom (when depth was higher than 5 meters) and the following parameters were determined:

- Salinity and dissolved oxygen were measured *in situ* with an YSI 650 meter.
- Suspended particulate matter (SPM) was obtained by filtering water through polycarbonate membranes (0.45 µM) and determined gravimetrically (drying at room temperature).
- Water samples for the determination of dissolved inorganic nutrients (nitrate, NO_3^-+NO_2^-; ammonia, NH_4^+; phosphate, PO_4^{3-} and silicate, $Si(OH)_4$) were filtered through MSI Acetate Plus filters and analysis carried out using an autoanalyser TRAACS 2000 (Bran+Luebbe).
- Manganese, Ni, Cu, Pb and Cd were measured in the collected waters using diffusive gradients of thin films (DGT). Total dissolved mercury was quantified in filtered and acidified water samples by cold-vapour atomic fluorescence spectrometry.
- Water samples were filtered and extracted with appropriate solvents for the determination of organic compounds by chromatographic techniques.

Table 17 – Number of sampling sites (by water body - WB) in Mira estuary, the sampling dates and the measured parameters.

	Water Bodies	Nº of sampling sites at each WB	Sampling date	Sampled parameters
Mira	WB1	3	March 2010	. Physical-chemical parameters (salinity, dissolved oxygen, suspended particulate matter)
	WB2	4		. Nutrients (ammonium, nitrate, nitrite, phosphate, silicate)
	WB3	4		. Dissolved metals (Mn, Ni, Cu, Pb, Cd, Hg)
				. Dissolved organic compounds (nonilfenol, phenanthrene, DDD)

2.8.2. Results

2.8.2.1. Physical-Chemical parameters

Figure 12 presents the values of salinity, dissolved oxygen and suspended particulate matter (SPM) in water of the three water bodies (WB). Salinity decreased gradually from downstream to upstream areas, SPM was higher in water bodies 2 and 3 and no statistical differences were found for dissolved oxygen.

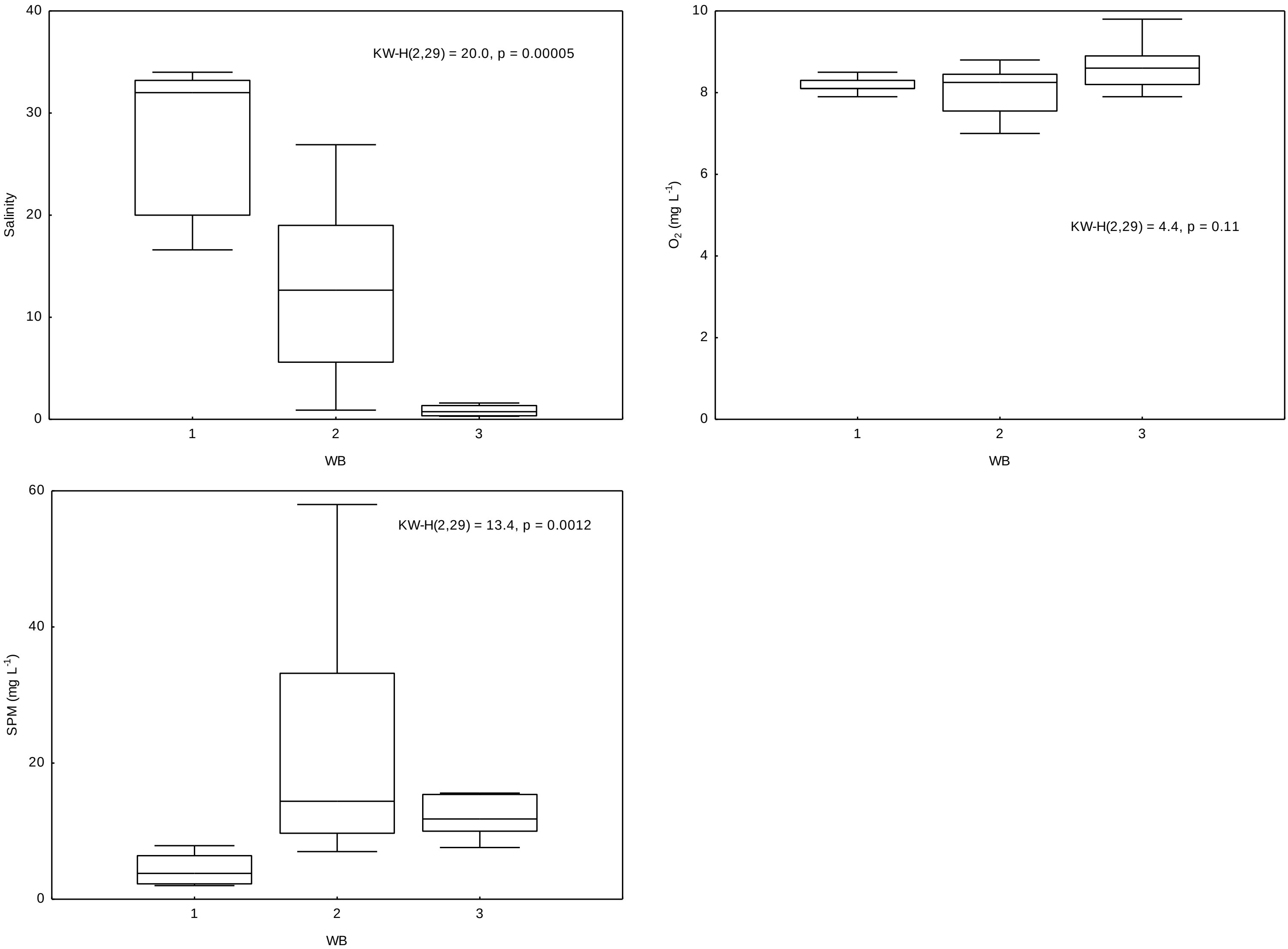


Figure 12 – Salinity, dissolved oxygen and suspended particulate matter (SPM) in three water bodies of the Mira estuary in March 2010. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.8.2.2. Nutrient concentrations in water

Figure 13 presents the concentrations of dissolved nutrients in the three water bodies (WB) of the Mira estuary. Water bodies differed significantly for nitrate, nitrite and silicate. Higher values of nitrite, nitrate and silicate were found in WB2. Additionally, nitrate was also higher in WB3. No statistical differences were found for ammonium and phosphate.

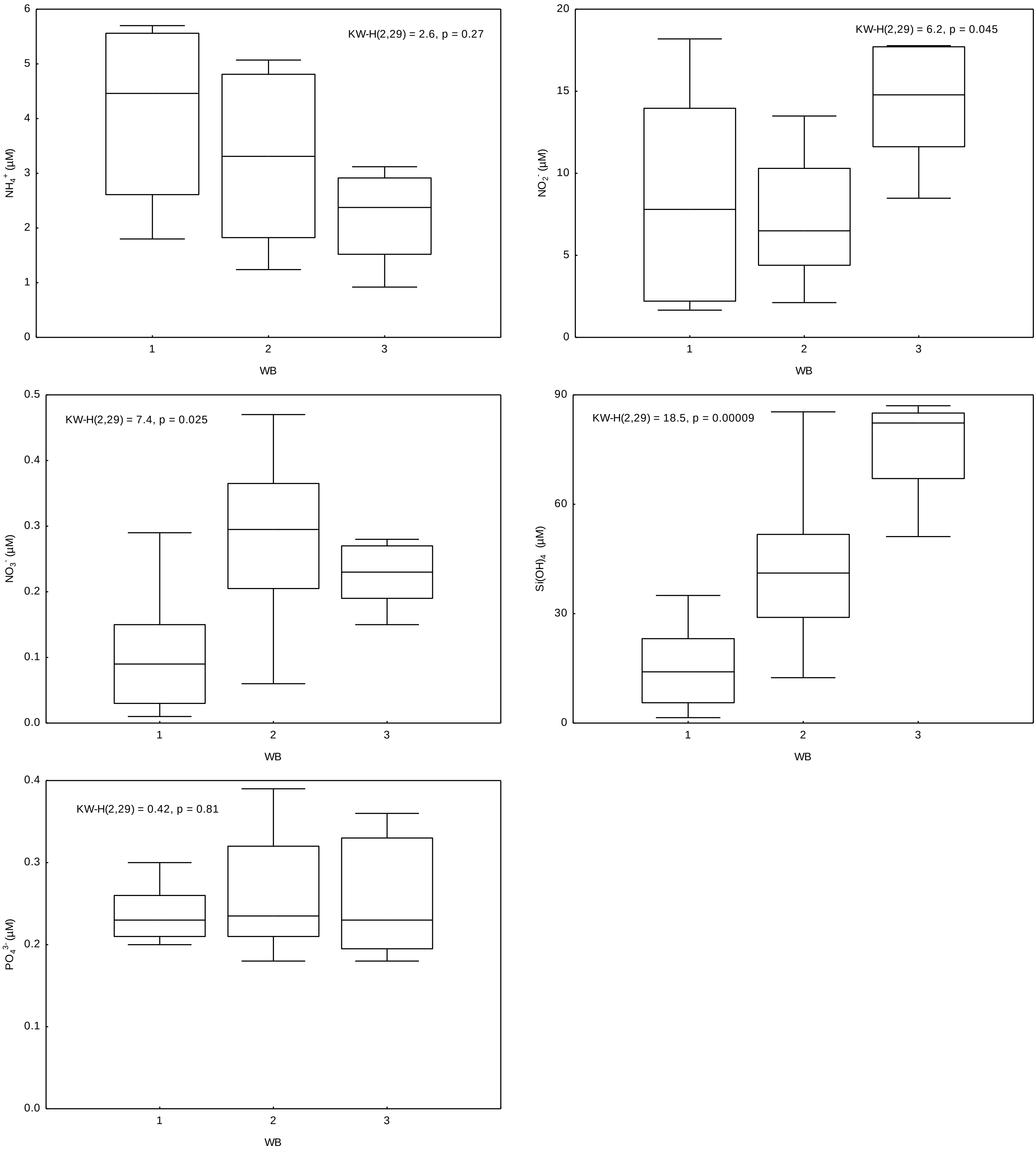
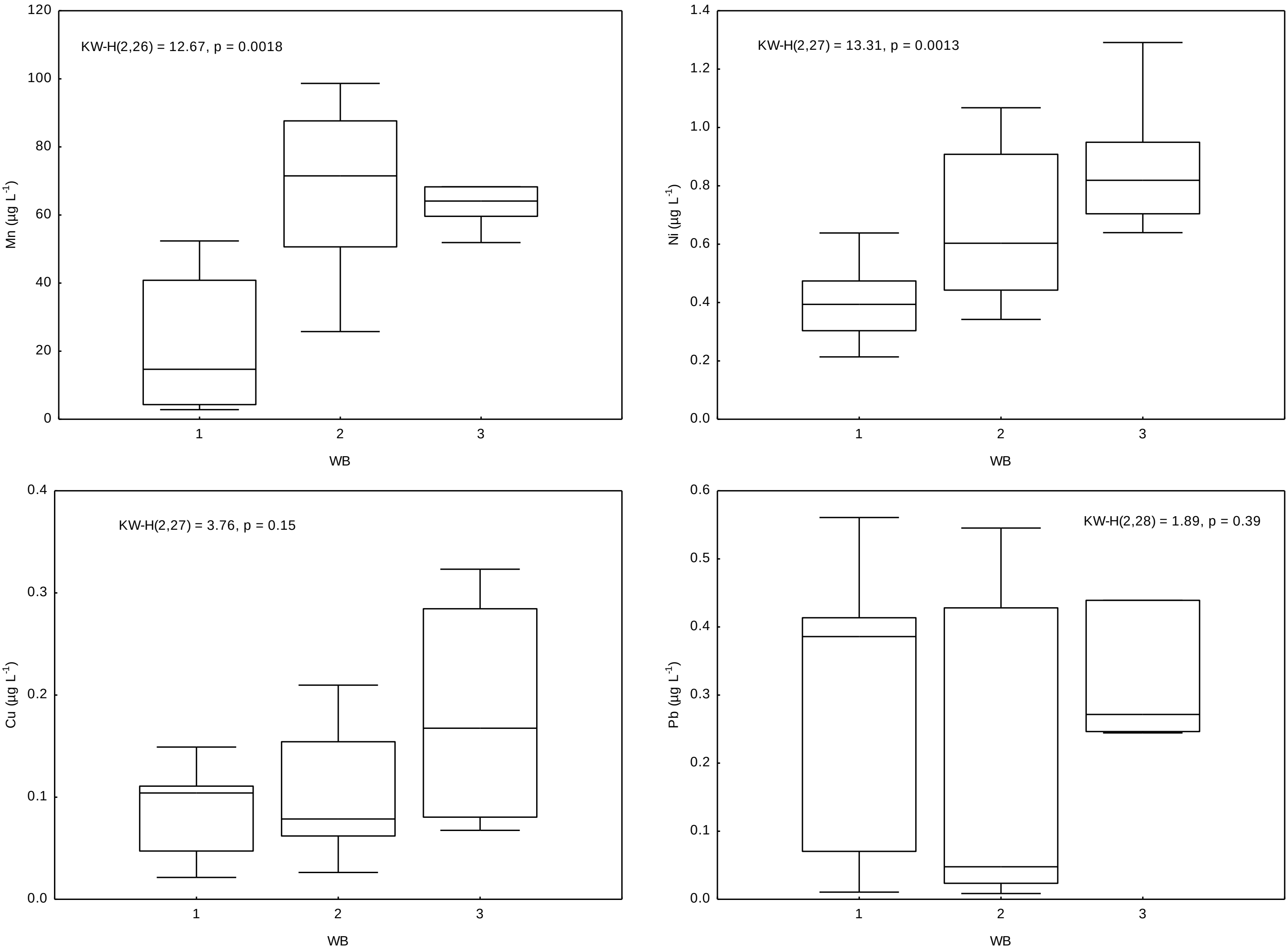
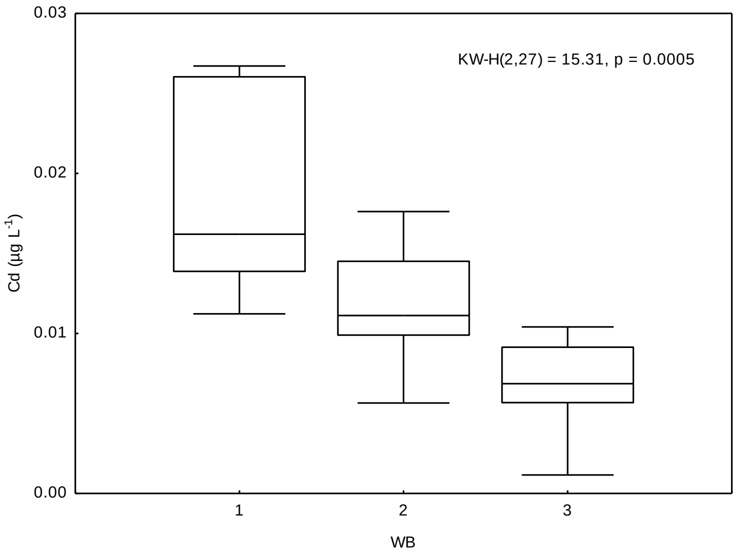


Figure 13 – Dissolved nutrients in three water bodies of the Mira estuary in March 2010. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.8.2.3. Levels of dissolved metals

Figure 14 presents the levels of dissolved metals in the three water bodies (WB) of the Mira estuary. Water bodies 2 and 3 presented significantly higher levels of Mn and Ni than WB1, while an opposite spatial pattern was found for Cd. No spatial differences were recoded for Pb and Hg.





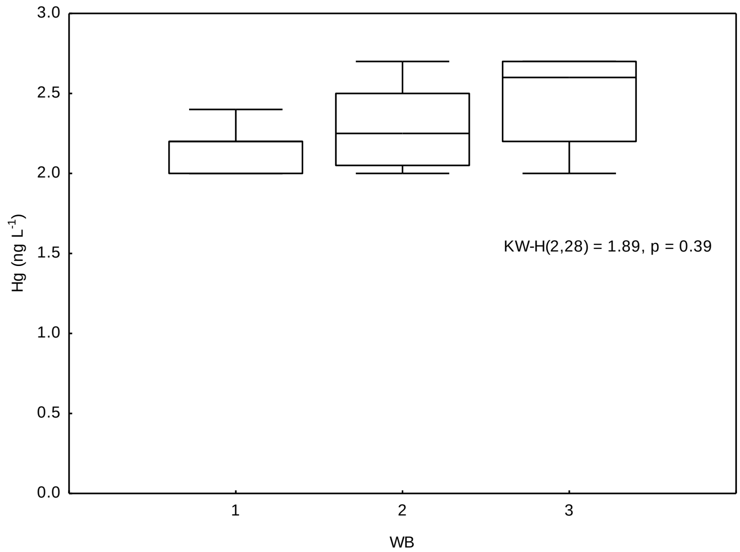


Figure 14 – Dissolved Mn, Ni, Cu, Pb, Cd and Hg in three water bodies of the Mira estuary in March 2010. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.8.2.4. Levels of dissolved nonilfenol, phenanthrene and DDD

Figure 15 presents the levels of the detected organic contaminants in three water bodies (WB) of the Mira estuary. Higher levels of phenanthrene were detected in water bodies 2 and 3.

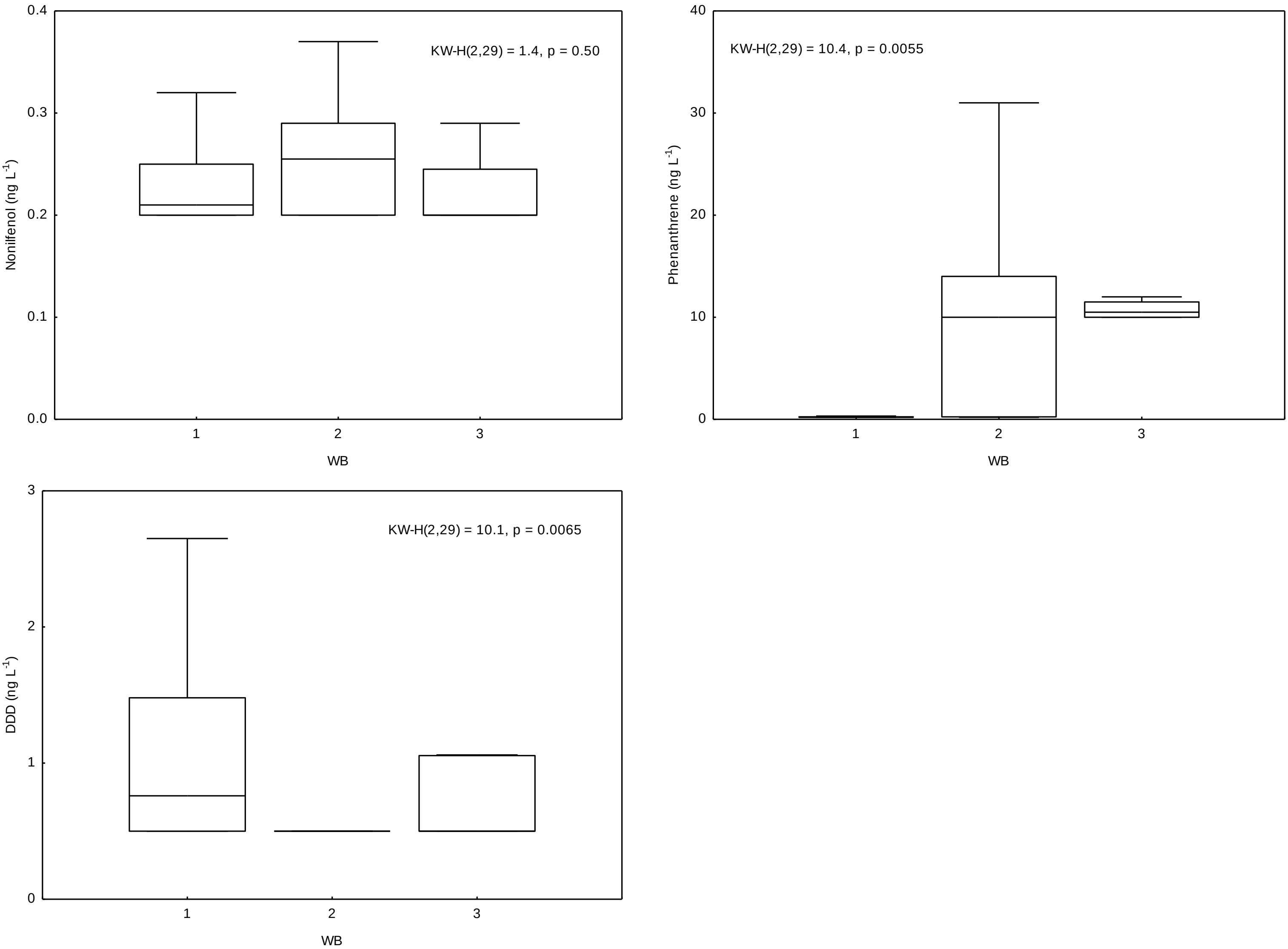


Figure 15 – Dissolved nonilfenol, phenanthrene and DDD in three water bodies of the Mira estuary in March 2010. The median, percentil 25%, percentil 75%, minimum and maximum are presented.

2.9. Biological elements in 3M-RECITAL

2.9.1. Zooplankton

Zooplankton samples, including hydrozoans and their planktonic prey, were collected from eleven sites, located along the salinity gradient of the Mira estuary during the 2011 seasonal bloom period of summer. Collections were made with a horizontally towed conical zooplankton net (500 μm mesh, 0.6 m diameter, 2.5 m length) paired with a General Oceanics flowmeter to determine water volume sampled. Tows were performed during 5 minutes and an average speed of 2 knots, near the surface of the water column (approximately 1m below the surface). Samples were immediately preserved in 4% buffered formalin. All *Blackfordia virginica* specimens collected were counted and its umbrella diameter measured using a stereoscopic magnifying glass. Sampling of this component will continue in the next years in a seasonal basis.

2.9.2. Macroalgae and macrophytes

2.9.2.1. Saltmarsh plants

Historical data for saltmarshes in the Mira are scarce. Taxonomic composition and abundance (%) were surveyed during summer in 2010.

Habitats: the sampling sites were placed at the upper zone of the intertidal area. In 2010 a total of 12 stations, located at the intertidal margins along the first 19 km from the sea, were surveyed.

Sampling periodicity: The field campaigns to collect data on saltmarshes during the project (still to decide) will occur during summer.

Sampling procedures: inside every characteristic marsh (e.g., *Spartina* dominated) of each water bodies identified for the Mira (defined in the scope of the WFD implementation in Portugal), it was placed a transect (12 transects). This transect line was disposed in the marsh in order to cross all the significant vegetation zones from the area. The taxonomic composition and the (%) coverage of the different taxa were registered for every meter by using a 1 m side plastic tube frame.

Laboratory procedures: based on SIG software and on the information collected during fields surveys (taxonomic composition and % coverage of each taxa), different vegetation polygons could be defined from aerial photographs. The deviation from the optimal coverage could also be calculated for each taxa present in the marsh.

2.9.3. Benthic invertebrates

Habitats: Soft-bottom subtidal habitat will be sampled to assess the benthic invertebrate communities.

Sampling periodicity: Once per year, in late Summer (September) (Table 18).

Sampling procedures: Biological samples will be 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 (covering a network of 8 pre-defined stations along the entire estuarine gradient – 2 stations per salinity stretch). At each station, an extra sediment sample will be collected (150-200ml) to characterize sediment properties (e.g. granulometry and organic matter content). Biological samples will be washed *in situ*, with estuarine water, through a 0.5 mm mesh size sieve.

Laboratory procedures: In the laboratory, samples will be sieved through a 0.5 and 1 mm mesh sizes and fixed with 4% buffered formalin solution. The 1 mm fraction will be stored for future studies. Only the 0.5 mm mesh size fraction will be further processed.

Afterwards, macroinvertebrates will be sorted and preserved in 70% ethanol for subsequent identification and counting. Whenever possible the organisms will be identified up to species level (or lowest reliable taxonomic level) and the biomass (g AFDW m^{-2}) determined. The organisms will be quantified in terms of abundance and biomass (g AFDW m-^{2}).

Table 18 - Campaigns completed for the benthic macroinvertebrates in the Mira estuary under the scope of 3M_RECITAL project.

Habitat	Biological Element	Sampling time	Stations	Methodology	"Status"
SUBTIDAL	Macroinvertebrates	28/09/2012	8 stations (2 per salinity stretch)	standard	completed

2.9.4. Fish

Fish communities will be studied at different spatial and temporal scales to: (a) support the assessment of long-term trends in fish ecological status, (b) help understand the influence that local and regional scale variations have on the ecological quality status, and (c) identify the influence of climate change on ecological status assessment tools and necessary adaptations.

Fishes will be sampled according to the methodological protocol described below.

Fishing gear: Beam trawl with the following characteristics: length - 2 m, height - 50 cm; mesh size (45 mm; 5 mm in the cod end); footrope with metal chains.

Sampling Periodicity: Seasonal. Late Winter - 1st half of March; Late spring - 1st half of June; Late Summer - 1st half of September; Late Autumn - 1st half of December.

Sampling strategy: Random sampling; five replicates by water body (defined according the EEMA project) in each estuarine system; tows with a length/duration of 300 m / 10 min.

Fishing conditions: Sweeps should be made in ebb tide periods and overnight.

Measurements of environmental variables: At the beginning or end of each tow the following environmental variables should be measured: water temperature, salinity, dissolved oxygen and depth; measurements should be performed at surface and bottom in areas with depths higher than 4 m.

Processing of samples: All fishes captured should be identified, measured (total length with precision of 1 mm) and weighed (total weight with an accuracy of 0.01 g) individually; in case of large number of catches (more than 50 individuals) a subsampling may be made in which individuals captured in addition to that 50 individuals should be counted and weighed together; all individuals of the accompanying fauna should be identified and counted and weighed together, by species.

2.10. Ecotoxicological tests

Baseline conditions relative to the population growth rate of the unicellular diatom microalgae *Phaedocatylum tricornutum* and feeding rates of the benthic polychaete *Hediste diversicolor*, the epibenthic crab *Carcinus maenas*, the benthic isopod *Cyathura carinata*, and the epibenthic mud snail *Hydrobia ulvae* were previously gathered for several sites at the Mira estuary. By then, differences among study sites within the estuary were fully explained by variations on environmental factors other than anthropogenic pressures: temperature, salinity, sediment organic matter content and sediment particle size. Therefore, ecotoxicological tests are programmed if, and only if, other surveyed elements dictate so.

2.D. Methods standardization

The standardization of the methods used across the three sites that constitute this 3M_RECITAL umbrella project is a crucial step for the optimization of long-term trends comparison among these LTER systems.

Despite some compromise had to be made, to ensure data comparability within systems between newly collected and historical data, some effort was attempted to ensure partial harmonization of methods and parameters surveyed across systems.

Below we mention how this will be tackled within the current project, focusing on the main elements monitored.

2.11. Pressures quantification

A common approach was adopted within the 3M_RECITAL project to identify and quantify anthropogenic pressures across the three estuaries.

Details of the methodology adopted can be found in Section 3 of the present report.

2.12. Physical-Chemical

IPIMAR partner established similar campaigns for the three estuaries, covering a common set of physical-chemical parameters and specific pollutants, in similar periods and using the same methodology. For details see section 2 of Physical-chemical surveys for each system.

Additionally, specific surveys within each system are also available, associated with features of respective monitoring network.

2.13. Ecotoxicological tests

Ecotoxicological testing will be carried out following already available and validated protocols. Nevertheless, optimized methodologies to ensure maximum efficiency are aimed, and, thus, revised and adapted protocols will be made available as soon as the programmed tests are finished.

2.14. Phytoplankton and zooplankton

Common measurements and laboratory procedures description is provided below.

Hydrological parameters measured *in situ* are: water temperature (°C) and salinity (WTW Cond 330i, WTW Wissenschaftlich - Technischewerkstätten, Germany), dissolved oxygen (DO) (WTW OXI 330i, mg/L), pH (WTW pH 330i), and transparency as Secchi-disc depth (m).

Standard methods were followed to determine dissolved inorganic nutrients (nitrate, NO_3^-+NO_2^-; ammonia, NH_4^+; phosphate, PO_4^{3-} and silicate, $Si(OH)_4$) by filtering water samples through MSI Acetate Plus filters and analysis were carried out using an autoanalyser TRAACS 2000 (Bran+Luebbe). Chlorophyll-a concentration was determined by filtering 500–1000 ml of water through GF/C filters, which were then treated with acetone (90%) to extract the Chl-*a* and the pigments measured at 630, 647, 665 and 750 nm (Parsons et al., 1985). Dry weight (TSS) was estimated by filtering 500– 1000 ml water through Whatman GF/C filters, tarred and dried at 60ºC for 72 hours and combustion at 450ºC for 8 hours (APHA, 1995).

Phytoplankton

Phytoplankton samples preserved in Lugol solution are processed for counting using the Utermöhl technique (1958). A known volume of sample, usually 25 ml is placed in a sedimentation chamber for 24 h before identifying and counting phytoplankton with an inverted microscope.

Zooplankton

In order to determine the number of *taxa* and abundances, all zooplankton samples are counted (individuals (ind.)/m^3) and identified to the lowest taxonomic level possible. At each subsample a minimum of 500 individuals are counted (Gonçalves et al. 2010 a,b; 2012 a,b and works therein).

2.15. Macroalgae and macrophytes

Methods standardization is still being established within the project for the different elements that constitute this biological group. Partners are working on common methodological approaches. For the moment common method was agreed for saltmarsh plants, and is described below.

2.15.1 Saltmarsh plants

Habitats: the sampling sites are placed at the upper zone of the intertidal area, covering the pioneer, lower, mid and upper zones from the marsh. Marshes until the oligohaline zone should be considered to survey.

Sampling periodicity: The field campaigns should be during the growing season (spring/summer).

Sampling procedures: inside every characteristic marsh (e.g., *Spartina* dominated area) of each water bodies identified for the systems (defined in the scope of the WFD implementation in Portugal), should be placed a transect representative of the variety of the marsh. These transect lines are disposed in the marsh in order to cross all the significant vegetation zones from that area (e.g., from the upper to the lower zone). Along the transect line, using a 1 m side quadrate (plastic tube frame), the taxonomic composition and the (%) coverage of the different *taxa* are registered for every meter.

Laboratory procedures: based on SIG software and on the information collected during fields surveys (taxonomic composition and % coverage of each *taxa*), different vegetation polygons could be defined from aerial photographs. The deviation from the optimal coverage could also be calculated for each *taxa* present in the marsh.

2.16. Benthic invertebrates

The selection of sampling methods in the Minho, Mondego and Mira estuaries had two major objectives, namely (i) to support a long-term database at each system and (ii) to ensure comparability between systems. Those objectives required the implementation of methods that had some differences between systems but, at the same time, were consistent and comparable. The major adaptations were the following:

Habitats: subtidal habitats will be surveyed in all estuaries while intertidal habitats will be surveyed only in the Mondego estuary, since previous datasets on intertidal communities are available only for that system;

Sampling periodicity: late summer surveys will be conducted in all estuaries, since most historical datasets coincide for this season in the three systems. Spring surveys will also be conducted in the Minho estuary in order to ensure datasets comparable previous data;

Sampling procedures: A van Veen grab with 0.05 sq m area will be used in the Minho and Mira estuaries while a 0.1 sq m area will be used in the Mondego, since those were the devices used in previous surveys within each systems. Nevertheless, datasets will be comparable since data will be quantified in terms of abundance and biomass (g AFDW $m\text{-}^2$);

Laboratory procedures: Different mesh size fractions (0.5 and 1 mm) will be used in different estuaries in order to ensure a consistent long-term dataset. However, both fractions will be preserved and stored for further processing, if needed.

2.17. Fish

Fish communities will be studied at different spatial and temporal scales to: (a) support the assessment of long-term trends in fish ecological status, (b) help understand the influence that local and regional scale variations have on the ecological quality status, and (c) identify the influence of climate change on ecological status assessment tools and necessary adaptations.

Fishes will be sampled according to the methodological protocol described below.

Fishing gear: Beam trawl with the following characteristics: length - 2 m, height - 50 cm; mesh size (45 mm; 5 mm in the cod end); footrope with metal chains.

Sampling Periodicity: Seasonal. Late Winter - 1st half of March; Late spring - 1st half of June; Late Summer - 1st half of September; Late Autumn - 1st half of December.

Sampling strategy: Random sampling; five replicates by water body (defined according the EEMA project) in each estuarine system; tows with a length/duration of 300 m / 10 min.

Fishing conditions: Sweeps should be made in ebb tide periods and overnight.

Measurements of environmental variables: At the beginning or end of each tow the following environmental variables should be measured: water temperature, salinity, dissolved oxygen and depth; measurements should be performed at surface and bottom in areas with depths higher than 4 m.

Processing of samples: All fishes captured should be identified, measured (total length with precision of 1 mm) and weighed (total weight with an accuracy of 0.01 g) individually; in case of large number of catches (more than 50 individuals) a subsampling may be made in which individuals captured in addition to that 50 individuals should be counted and weighed together; all individuals of the accompanying fauna should be identified and counted and weighed together, by species.

Historical data on the fish assemblages in the 3 estuaries will be compiled and analysed together with the new data. Characterization of fish assemblages, for each dataset, will be based on abundance and weight of catches and on species richness, diversity and evenness indices. Species will be assigned to ecological guilds that reflect their trophic habits and habitat use patterns. Several multi-metric approaches, including conservation indices like COPIEF (Branco *et al*., 2008) and biotic integrity indices like EFAI (Cabral et al., 2012), will also be used. Special emphasis will be given to the nursery function of the system and the trophic structure of the community. Abundance and quality (condition and infection by the parasite *Anguillicola crassus*) of eel specimens will be carefully monitored due to the high conservation status of this fish species (Critically Endangered, IUCN).

All these parameters together with the use of multivariate statistical techniques will allow the assessment of the ecological integrity of the 3 estuaries, using fishes as indicators. These analyses will also enable the evaluation of climate change impacts on these communities and contribute to assess the evolution of eel population in Portugal.

3. PRESSURE QUANTIFICATION

Assuming that ecological responses are comparable along equivalent coastal systems, the results produced by the environmental assessment methodologies are expected to produce similar classifications for water bodies (WB) under similar anthropogenic disturbance.

At the European Commission level it has also been encouraged that the MSs use available pressure information as a common derived scale, in order to investigate the relationship between the pressure indicators and the ecological quality status (EQS) derived from the national assessment methods.

In this sense, it is essential to produce pressure data under a standard protocol, to ensure comparability between different systems and, equally important, to guarantee the national homogeneity of compiled data.

3.1. Methodological approach

The following approach derives from lessons learnt during the implementation of the European Water Framework Directive (WFD). The list of pressure indicators hereafter presented updates the one used during the WFD Intercalibration Phase (IC 2), by including other indicators considered important to understand the biological response and trends observed on each WB, and to infer about the modification that took place in the systems along years (e.g., hydromorphological changes).

Raw data are desired in a first step that, afterwards, will be translated through a standard scoring system into a common scale (0 – 9). This scoring system allows the different pressure indicators, with different measuring units, to be summed together and to produce a pressure index value that has in consideration all the indicators quantified.

Additional information is to be provided at the System/WB level (Table 19) to help weighting final score of pressure indicators selected in the current project (presented in Table 20).

Table 19 – Meta-information at the System/Waterbody level, for weighting final pressure score for the 3M-RECITAL systems in the current project.

Meta-information	Minho	Mondego	Mira	...
Country				
WaterCategory				
HydrographicBasin				
NationalType				
EU_Type				
WB_Name				
WB_National_Code				
WB_EU_Code				
WB _TotalArea (m2)				
WB_IntertidalArea (m2)				
WB_Volume (m3)				
MarginsLength (km)				
WB_ResidenceTime (days)				
NitrogenInput (ton N year-1)				
PhosphorusInput (ton N year-1)				
SalinityRange(Euh, Polyh, Mesoh, Oligoh)				
Population				
RiskAssessment(2004)				

Table 20 – Pressure indicators, criteria and scoring system. Biological elements (BE): F – fish; MA – macroalgae/angiosperms; MI - macroinvertebrates.

Pressure category	**Pressure indicator**	**Criteria**	**No change (0)**	**Very low (1)**	**Low (3)**	**Medium (5)**	**High (7)**	**Very high (9)**	BE
Hydromorphological changes	Land Claim (ha)	- area claimed from the sea or estuary. - consider both: mudflats and tidal marshes. - this indicator includes both anthropogenically induced changes (direct or indirectly induced) and natural variations.	No change	<0.5% lost over the last decades	<1%	<5% lost	<10%lost	≥ 10% lost	F, MA, MI
Hydromorphological changes	% Shoreline re-enforcement	- intertidal and subtidal area affected by shoreline structures. - Consider both margins. - values as percentage of WB extension/margin.	No development	<5% of the coastline impacted by industrial or urban activities	<30%	< 60%	< 90%	≥ 90%	F, MA, MI
Resources use change	Maintenance dredging area (ha)	- the area (ha) designated for maintenance dredging in estuaries. - the % in relation to the WB surface area	No dredging	<1% of the surface area dredged	<10%	<30%	<50%	≥ 50%	F, MA, MI
Resources use change	Maintenance dredging volume (tons)	- the amount (ton) of dredged material from estuaries.	no disposal	< 5000 tons deposited annually	<100,000 tons	< 1 million tons	< 4 million tons	≥ 4 million tons	F, MA, MI
Resources use change	Maintenance disposal area (ha)	- the area (ha) where dredged material is dumped in estuaries. - consider both intertidal and subtidal areas.	no disposal	<1% of the subtidal area dredged	<10%	<30%	<50%	≥ 50%	F, MA, MI
Resources use change	Maintenance disposal volume (tons)	- the amount (ton) of dredged material dumped inside estuaries. - consider both intertidal and subtidal dumped volumes.	no disposal	< 5000 tons deposited annually	<100,000 tons	< 1 million tons	< 4 million tons	≥ 4 million tons	F, MA, MI
Resources use change	Other fisheries nearshore disturbance	- % of the length of coast (riverbank) affected by fishery. - % of estuarine (WB) area affected by fishery.	No fishery activities	< 10% of the length of coast (riverbank) affected by fishery	<30%	<60%	<90%	≥ 90%	F, MA, MI
Resources use change	Marina Development	- number of berths in marinas per km2 of WB surface.	No marina	< 100 berths in marina / km2	<150 berths / km2	<300 berths / km2	<500 berths / km2	≥ 500 berths / km2	F, MA, MI
Resources use change	Tourism and recreation	- % of the length of coast (riverbank) affected by tourism and recreation activity. - % of estuarine (WB) area affected by tourism and recreation activity.	None	< 10% of the length of coast (riverbank) or estuarine area affected by activities	<30%	<60%	<90%	≥ 90%	F, MA, MI

Pressure category	Pressure indicator	Criteria	No change (0)	Very low (1)	Low (3)	Medium (5)	High (7)	Very high (9)	BE
Resources use change	Direct discharges	- count the point sources per km of margin	no disposal	<0.2 points / km	<0.4 points / km	<0.8 points / km	<1.2 points / km	>1.2 points / km	MI
Resources use change	Agriculture	- area occupied by agriculture fields in the 1 km stripe of land next to the WB/system; - use CORINE land cover system (2004).	No agriculture	<1% of the intertidal and subtidal area covered	<10%	<30%	<50%	≥ 50%	MI
Resources use change	Aquaculture	- area occupied by aquaculture structures in relation to the intertidal and subtidal areas of the WB/system.	No fishfarming	<1% of the intertidal and subtidal area covered	<10%	<30%	<50%	≥ 50%	F, MI
Resources use change	Port development	- extension of the margin occupied by harbour quays.	No harbour	<500 m of quays	<2 km	<5 km	<10 km	≥ 10 km of quays	F, MI
Resources use change	Area covered by pipelines, cables, gas exploration	- Intertidal and subtidal area occupied by pipelines, cables, and other exploratory structures.	No pipelines or other structures	<1% of the intertidal and subtidal area covered	<10%	<30%	<50%	≥ 50%	MA, MI
Environmental quality and its perception	Water chemical quality	- Compliance with Environmental Quality Standards (EQSs) for List I and List II substances of EU Dangerous Substances Directive (metals, organic compounds, ammonia, drins) are measured to qualify the water chemical quality.	100% compliance of samples with EQSs for all substances	100% compliance of samples with EQSs for all substances	One List II substance fails to comply with EQS AND no significant increase in the concentration of this substance	(i) One List II substance fails to comply with EQS AND significant increase in the concentration of this substance OR (ii) More than one List II substances fail to comply with EQSs AND no significant increase in the concentration of these substances failing the EQS	(i) One or more List II substances fail to comply with EQSs AND significant increase in the concentration of these substances failing the EQS OR (ii) one List I substance fails to comply with EQSs	More than one List I substance fails to comply with EQSs	F, MI

Pressure category	Pressure indicator	Criteria	No change (0)	Very low (1)	Low (3)	Medium (5)	High (7)	Very high (9)	BE
Environmental quality and its perception	Sediment chemical quality	- Measured as the concentration of metals in the sediments.	e.g. low metal concentration	Low concentration for all metals (<2 x National background level)	The concentration for one or more metals is≥ 2 x National background level and < substantially elevated level	The concentration for one or more metals is ≥ substantially elevated level and < Grossly elevated level	The concentration of one metal is ≥ grossly elevated level	The concentration of more than one metal is > grossly elevated level	MI
Environmental quality and its perception	Water quality - biological effect (bioaccumulation and shelfish quality)	- The number of catch interdictions per year due to bioaccumulation of toxic substances in bivalves or in other organisms.	e.g. No interdiction to bivalves catch	one interdiction per year to bivalves catch	two interdictions per year to bivalves catch	four or less interdictions per year to bivalves catch	eight or less interdictions per year to bivalves catch	more than eight interdictions per year to bivalves catch	F, MI
Environmental quality and its perception	Interference with fish migration routes - physical barriers (estuaries)	- Percentage of natural drains and rivers affected by a physical barrier.	No interference	<5% of natural drains and rivers affected by a physical barrier	≥5% and <30%	≥30% and <60%	≥60% and <90%	≥90%	F
Environmental quality and its perception	DO (saturation in the time)	- DO saturation over time; - saturation measured over 95% of sampling events in the year.	No DO problem	DO saturation >80% for 95% of the time	≤80% and >70% for 95% of the time	≤70% and >50% for 95% of the time	≤50% and >20% for 95% of the time	≤20% for 95% of the time	F, MI
Environmental quality and its perception	Nutrients (DIN winter median concentration) (µmol/L)	- TW normalized for 25 salinity; - CW polyhaline normalized for 25 salinity; - CW normalized for 32 salinity.	Winter DIN concentration lower than 6.5 µmol/L	< 10 µmol/L	<30 µmol/L	<60 µmol/L	<90 µmol/L	≥ 90 µmol/L	MA
Environmental quality and its perception	Natural turbidity: secchi disk (m) (mean)	- use same normalization criteria as for DIN (if possible). - use growing period values (May to September).	Secchi depth transparency ≥ 2.5 m	< 2.5 m	< 2 m	< 1.5 m	< 1 m	< 0.5 m	MA

4. SCIENTIFIC CHALLENGES – CORE QUESTIONS IN 3M-RECITAL

Several key-scientific questions in the research field of management and restoration ecology are to be addressed in the scope of this LTER project. The proposed interdisciplinary topics envisage integrating all aspects considered for monitoring under the umbrella of this network of LTER sites.

Below we indicate some core topics guiding research efforts during this project.

*Characterize the **physical-chemical variation** in the studied systems, accounting for the effect of extreme climatic events e consequent river flow alterations.*

*Investigate if the same pressure level, even including different **pressure** indicators in different WB/systems, produces: a) in different systems, a similar response from each single biological element, and b) in the same system, a similar response from the different biological element.*

*Are global changes of **phytoplankton** and **zooplankton** communities affected by the same stressors, at a southern-european temperate estuary?*

Long-term changes on phytoplankton and zooplankton dynamics of a temperate estuary are related to seasonal or superimposed by interannual variability?

Impacts and risk of dispersion of Non-indigenous species The introduction and dispersion of non-indigenous species (NIS) is considered as one of the major threats to biodiversity and is the second cause of global marine biodiversity loss after direct habitat destruction, having adverse environmental, economic and social impacts.

The conditions the Mira estuary provide an optimal scenario to assess NIS impacts once there are few anthropogenic impacts. For instance, the NATURA 2000 classification of the Mira estuary as a protected area imposed several restrictions to human use, including a ban on commercial fishing activities. Additionally, this estuary has minor anthropogenic pressures and a reduced number of NIS currently registered (*i.e. Blackfordia virginica, Corbicula fluminea, Percnon gibbesi, Amphibalanus improvisus* and *Potamopyrgus antipodarum*) validating its use as a case study for impacts and risk assessment of a specific NIS. The occurrence of *B. virginica* in the Mira estuary was selected as a case study within this particular context since the presence of this species is known since 1984 but no specific studies were developed to assess the its abundance, spatial distribution, impacts and risk of dispersion. The major questions to be addressed with the current methodological approach are:

Do spatial and temporal patterns of B. virginica population indicate widespread or located impacts?

Does B. virginica has impacts over zooplankton and fish communities?

What is the dispersion risk of this species to other estuarine systems?

Despite their small size, Minho, Mondego and Mira estuaries present rich and diverse **fish communities**, as well as important nursery grounds for several species with high commercial value. This nursery function can however, change naturally or due to anthropogenic factors. On the other hand, the European eel (*Anguilla anguilla*), a species classified as Critically Endangered (IUCN) all over its distribution area, is an important species in these systems. Therefore, monitoring the European eel population in Minho, Mondego and Mira estuaries can contribute to evaluate the success of the measures implemented within the framework of Regulation 1100/2007, aiming at the recovery of the European eel stock. The major questions to be addressed regarding the fish communities will be:

Are climatic changes modifying fish communities in these three estuaries? If so, are the changes acting differently according to the latitude or human pressures?

Is it possible to separate the influence of natural causes from anthropogenic activities in the spatio-temporal variability of these fish communities?

Does the recruitment of fish species and the estuarine nursery function change from year to year within and between systems?

Is the population of the European eel recovering in the estuaries studied?

The last question will be analysed in more detail in the Minho estuary, where monitoring of European eel population has been conducted in the last years.

5. EXPECTED INDICATORS

5.1. Milestones

Milestone 1 (**M1**) - ***Literature review and data compilation and banking*** had to be completed during the first year of project. This Milestone was divided in two parts, included in the present report:

a. Milestone 1a (*Literature review and compilation*); and

b. Milestone 1b (*Historical data compilation and banking*).

5.2. Publications acknowledging 3M_RECITAL

1. Marques, L., Carriço, A., Bessa, F., Gaspar, R., Neto, J., Patrício, J. In press. Response of intertidal macrobenthic communities and primary producers to mitigation measures in a temperate estuary. Ecological Indicators (http://dx.doi.org/10.1016/j.ecolind.2012.08.022)
2. Alves, A.S., Adão, H., Ferrero, T., Marques, J.C., Patrício, J. 2013. Benthic meiofauna as indicator of ecological changes in estuarine ecosystems: The use of nematodes in ecological quality assessment. Ecological Indicators, 24: 462-475. (doi.org/10.1016/j.ecolind.2012.07.013).
3. Pinto, R., de Jonge, V.N., Marques, J.C., Chainho, P., Medeiros, J.P., Patrício, J. 2013. Temporal stability in estuarine systems: implications for ecosystem services provision. Ecological Indicators, 24: 246-253 (doi.org/10.1016/j.ecolind.2012.06.022)
4. Veríssimo H., Lane M.F., Patrício J., Gamito S., Marques J.C. 2013. Trends in water quality and subtidal benthic communities in a temperate estuary: Is the response to restoration efforts hidden by climate variability and the Estuarine Quality Paradox? Ecological Indicators, 24: 56-67. (doi.org/10.1016/j.ecolind.2012.05.028)
5. Veríssimo, H., Bremner, J., Garcia, C., Patrício, J., van der Linden, P. & Marques, J.C. 2012. Assessment of the subtidal macrobenthic community functioning of a temperate estuary following environmental restoration. Ecological Indicators, 23: 312-322 (doi:10.1016/j.ecolind.2012.04.020)
6. van der Linden, P., Patrício, J., Marchini, A., Cid, N., Neto, J.M. & Marques, J.C. 2012. A biological trait approach to assess the functional composition of subtidal benthic communities in an estuarine ecosystem. Ecological indicators, 20: 121-133. (doi:10.1016/j.ecolind.2012.02.004)
7. Gamito, S., Patrício, J., Neto, J.M., Marques, J.C. & Teixeira, H. 2012. The importance of habitat-type for defining the reference conditions and the ecological quality status based on benthic invertebrates: the Ria Formosa coastal lagoon (southern Portugal) case study. Ecological Indicators, 19: 61-72. (doi:10.1016/j.ecolind.2011.08.004)
8. Gamito, S., Patrício, J., Neto, J.M., Teixeira, H. & Marques, J.C. 2012. Feeding diversity index as complementary information in the assessment of ecological quality status. Ecological Indicators, 19: 73-78. (doi:10.1016/j.ecolind.2011.08.003)

9. Ceia, F.R, Patrício, J., Franco, J.N., Pinto, R., Fernández-Boo, S., Losi, V., Marques, J.C. & Neto, J.M. 2011. Assessment of estuarine macrobenthic assemblages and ecological quality status at a dredging site in a southern Europe estuary. Ocean & Coastal Management (doi:10.1016/j.ocecoaman.2011.07.009).

10. Pinto, R., de Jonge, V.N., Neto, J.M., Domingos, T., Marques, J.C. & Patrício, J. 2011. Towards a DPSIR driven integration of ecological value, water uses and ecosystem services for estuarine systems. Ocean & Coastal Management (doi:10.1016/j.ocecoaman.2011.06.016).

11. Patrício, J., Adão, H., Neto, J.M., Alves, A.S., Traunspurger, W. & Marques, J.C. 2012. Do nematode and macrofauna assemblages provide similar ecological assessment information? Ecological Indicators, 14: 124-137. (doi:10.1016/j.ecolind.2011.06.027)

12. Franco, J.N., Ceia, F.R., Patrício, J., Modesto, V., Thompson, J., Marques, J.C. & Neto, J.M. 2011. Population dynamics of Corbicula fluminea (Müller, 1774) in mesohaline and oligohaline habitats: invasion success in a Southern Europe estuary. Estuarine Coastal and Shelf Science (doi:10.1016/j.ecss.2011.07.014)

13. Veríssimo, H., Neto, J.M., Teixeira, H., Franco, J.N., Fath, B., Marques, J.C. & Patrício, J. 2012. Ability of benthic indicators to assess ecological quality in estuaries following management. Ecological Indicators, 19:130-143. (doi:10.1016/j.ecolind.2011.06.014).

14. 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, 222: 1209-1221 (doi:10.1016/j.ecolmodel.2010.12.010).

15. Pinto, R., Patrício, J., Neto, J.M., Salas, F. & Marques, J.C., 2010. Assessing estuarine quality under the ecosystem services scope: ecological and socioeconomic aspects. Ecological Complexity, 7: 389-402 (doi:10.1016/j.ecocom.2010.05.00199).

16. Neto, J.M., Teixeira, H., Patrício, J., Baeta, A., Veríssimo, H., Pinto, R. & Marques, J.C. 2010. The response of estuarine macrobenthic communities to natural-and human-induced changes: dynamics and ecological quality. Estuaries and Coasts, 33:1327-1339. (doi 10.1007/s12237-010-9326-x)

17. Medeiros, J.P., M.L. Chaves, G. Silva, C. Azeda, J.L. Costa, J.C. Marques, M.J. Costa & P. Chainho. 2012. Benthic condition in low salinity areas of the Mira estuary (Portugal): lessons learnt from freshwater and marine assessment tools. Biological Indicators, 19: 79-88

5.3. Communications

5.3.1 Oral communications

1. Pinto, R., Patrício, J., Abreu, P., Marta-Pedroso, C., Baeta, A., Franco, J., Domingos, T., Marques, J.C. 2012. Ecosystem services and ecological status improvement: Determinants of willingness-to-pay values for water quality and its role for decision-making. EcoSummit 2012, Columbus, Ohio, USA, 30 September - 5 October.

2. van der Linden, P., Patrício, J., Marchini, A., Cid, N., Neto, J.M.; Marques, J.C. 2012. A biological trait approach to assess the functional composition of subtidal benthic

communities in an estuarine ecosystem. XVII Simpósio Ibérico de Estudos de Biologia Marinha (SIEBM), 12-14 September, San Sebastian (Spain).

3. Neto, J.M., Feio, M.J., Teixeira, Serra, S., Patrício, J.,Calapez, R., Franco, J., Constantino, E. 2012. Transitional and freshwater bioassessments: one site - two perspectives. XVII Simpósio Ibérico de Estudos de Biologia Marinha (SIEBM), 12-14 September, San Sebastian (Spain).
4. Neto, J., Feio, M., Teixeira, H., Serra, S., Patrício, J., Calapez, A., Franco, J. & Constantino, E. 2012. Transitional and freshwater bioassessments: is the river continuum discontinued? XVIth Congress of the Iberian Society of Limnology - Limnologia 2012, Guimarães, Portugal, 2-6 July.
5. Alves, A.S., Adão, H., Costa, M.J., Marques, J.C. & Patrício, J. 2012. Do nematode and macrofauna assemblages provide similar ecological assessment information? 2nd International Symposium on Nematodes as Environmental Bioindicators (2ISNEB), Ghent University, Ghent, Belgium, 5-6 July.
6. Pinto, R., Patrício, J. & Marques, J.C. 2012. Integration of ecological significance within the water uses and services framework: a DPSIR approach on the Mondego estuary. XIII Jornadas sobre Conservação da Natureza e Educação Ambiental, Leiria, Portugal, 21-22 April.
7. 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. ISEM 2011, Beijing, China, 20-23 September.
8. Chainho, P. Portuguese update on alien marine species. 2011. ICES Working Group on Introductions and Transfers of Marine Organisms (WGITMO), Nantes, France, 16 - 18 March.
9. Chainho, P., J.L. Costa, J.P. Medeiros & Costa, M.J. 2011. A influência das espécies exóticas na avaliação do estado ecológico com base em comunidades bentónicas estuarinas. VII Congresso Ibérico da Água, Talavera de la Reina, Spain, 16-19 February.

5.3.2 Posters

1. Caetano, A., A.S. Alves, J. Patrício, J.L. Costa & J.C. Marques. 2012. Distribution of intertidal meiofaunal communities along the south arm of the mondego river estuary (Portugal): preliminary results. Second International Symposium on Nematodes as Environmental Bioindicators. 5-6 July, Ghent University, Ghent, Belgium.
2. Chainho, P., A. Amorim, J. Castro, A. Costa, J.L. Costa, T. Cruz, D. Sobral, A. Fernandes, R. Melo, T. Silva, M. Sousa, P. Torres, V. Veloso & M.J. Costa. Introduced marine non-indigenous species in Portuguese estuaries and coastal areas: who, where and how? NEOBIOTA 2012, 12-14 September, Pontevedra, Spain.

5.4. Advanced training

5.4.1 Thesis

1. Ana Vanessa Pinto Modesto. 2011. Spatial and temporal variation of *Corbicula fluminea* (Muller, 1774) in the Mondego estuary. MSc Thesis. Department of Life Sciences, University of Coimbra, 47p. (completed)
2. Pieter Van der Linden. 2011. The functional composition of the benthic invertebrate community of the Mondego estuary: insights within the functioning of this ecosystem. MSc Thesis. Department of Life Sciences, University of Coimbra. (completed)
3. Alexandra Caetano. Intertidal and subtidal meiofauna of a southern european estuary. MSc Thesis. Department of Life Sciences, University of Coimbra. *(ongoing)*

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6.1. Literature compilation (Milestone 1a)

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