Input, distribution and effects of regulated and emerging organic pollutants in a shallow Mediterranean coastal lagoon using stir bar sorptive extraction, passive sampling and bioanalysis

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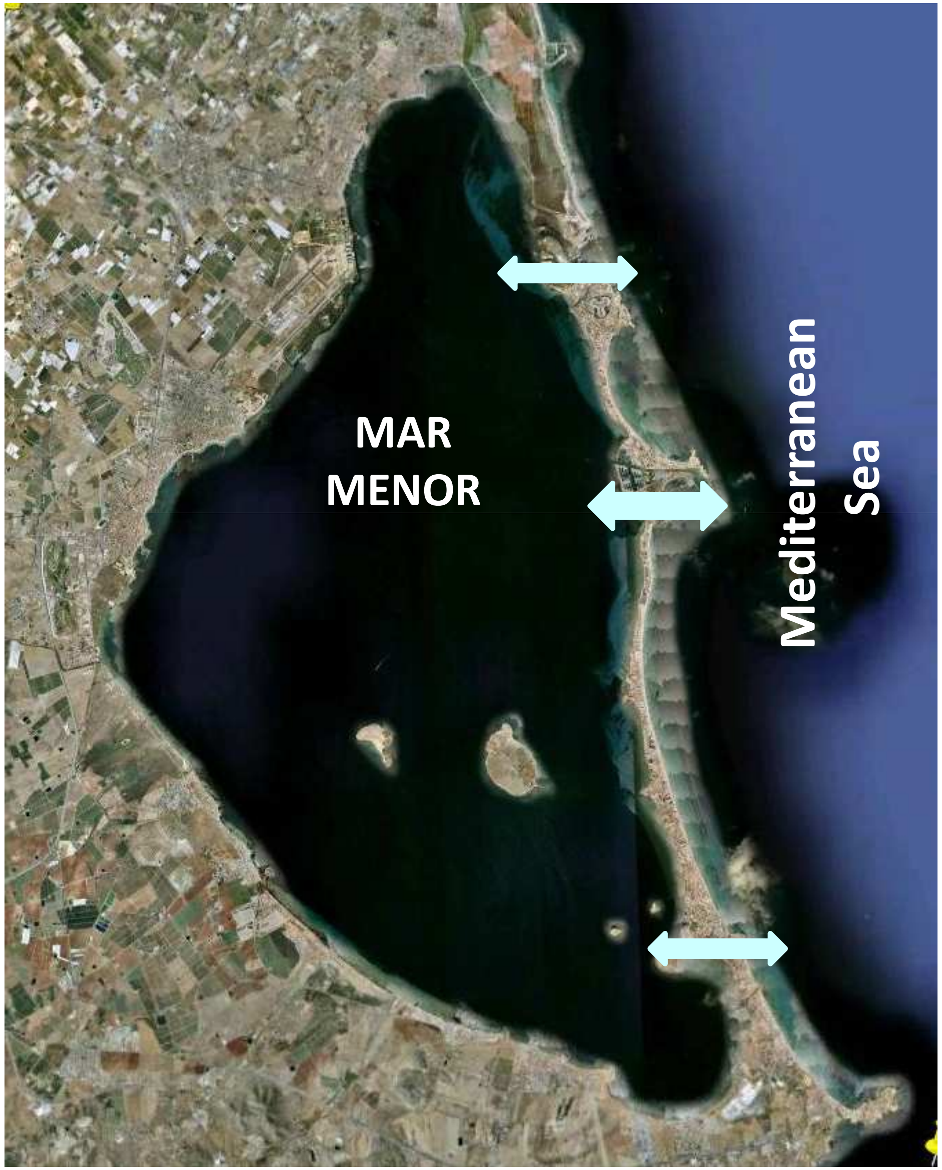
BIOMARO Project

ECOMAR Project

Study Area: Mar Menor lagoon (SE Spain)

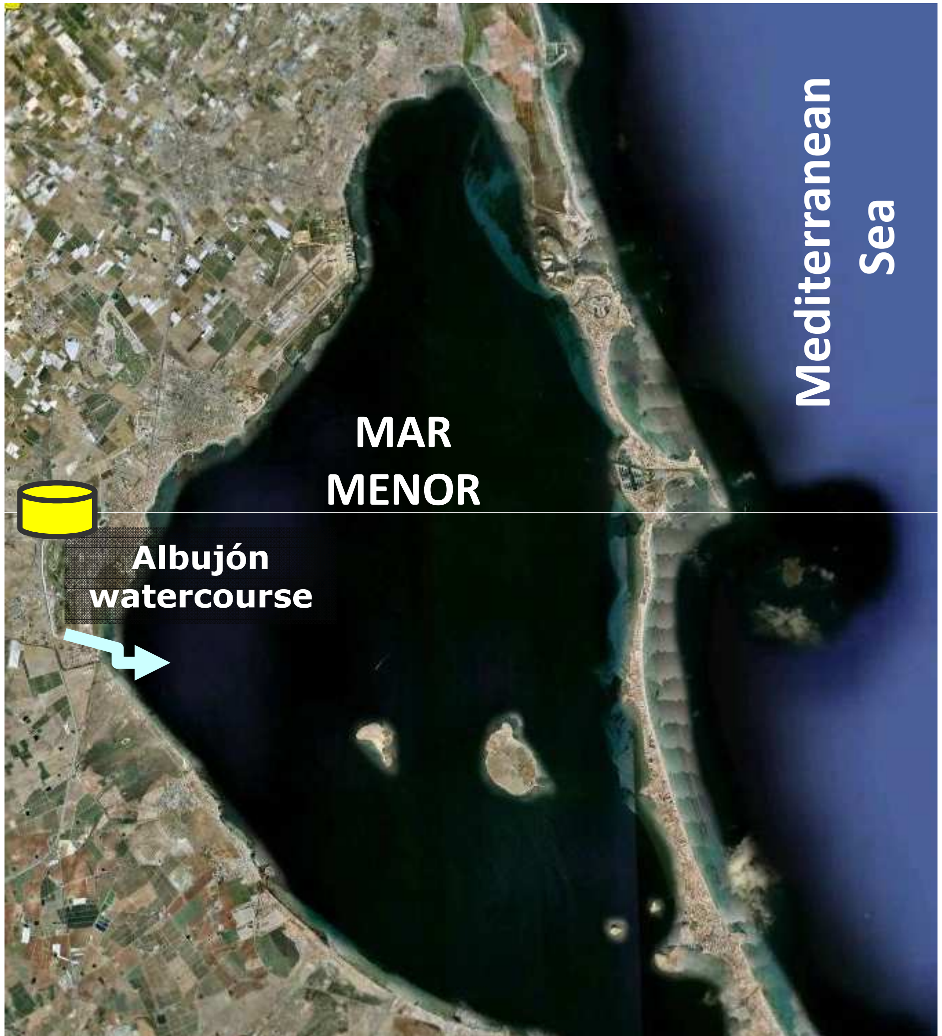
- ✓Biggest in Europe (135.2 Km^2)
- ✓Salinity from 40 to 47 psu
- ✓Mean depth: 3-4m (maximum 6m)
- ✓Water temperature 10-32ºC
- ✓Residence time about 10 months
- ✓Protected area



Study Area: Mar Menor lagoon (SE Spain)

✓One of the main intensive horticulture growing areas in Europe

✓Albujón watercourse: most important trap of the Cartagena field basin



Pérez-Ruzafa et al., 2000
Serrano and Sironi, 2009

I. Analysis of organic contaminants by SBSE/GC/MS

• Input of pollutants from watercourse freshwaters

• Distribution of pollutants in lagoon seawaters

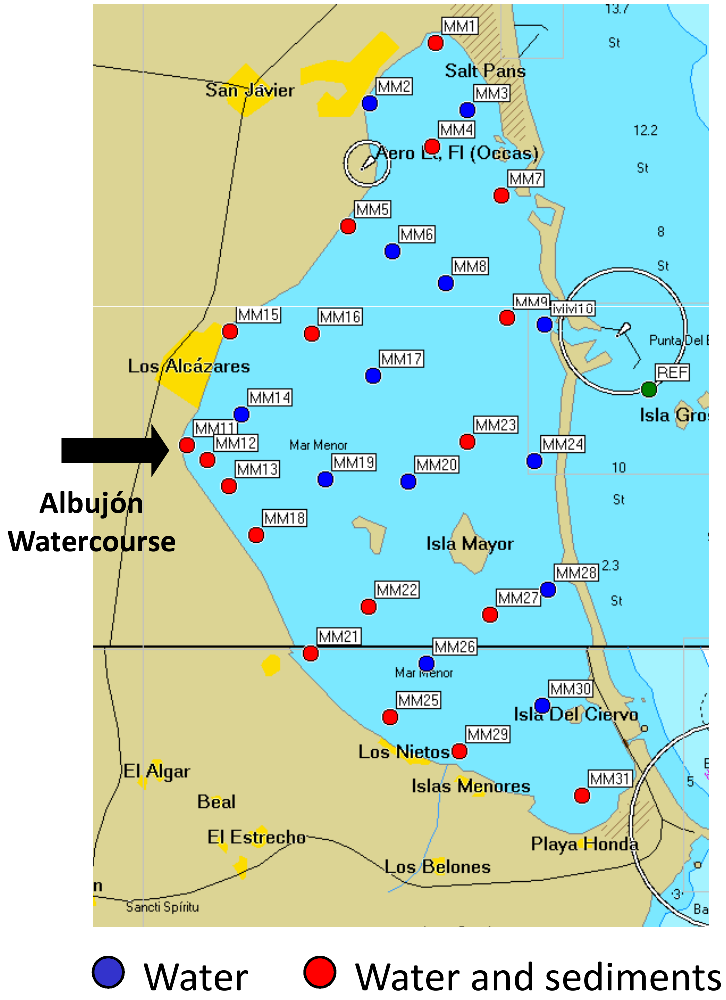
Seasonal input from watercourse freshwaters

Daily evolution in El Albujón watercourse:
2-3 samples per day during 1 week
(once in each season)

Seasonal distribution in Mar Menor
Sampling period: 2009-2010

Every 3 months:

✓Seawater: 32 sampling points



I. Analysis of organic contaminants by SBSE/GC/MS

Sorption stir bar
(2 cm)

+

100 mL WS
(with NaCl)

Extraction time: 22 h

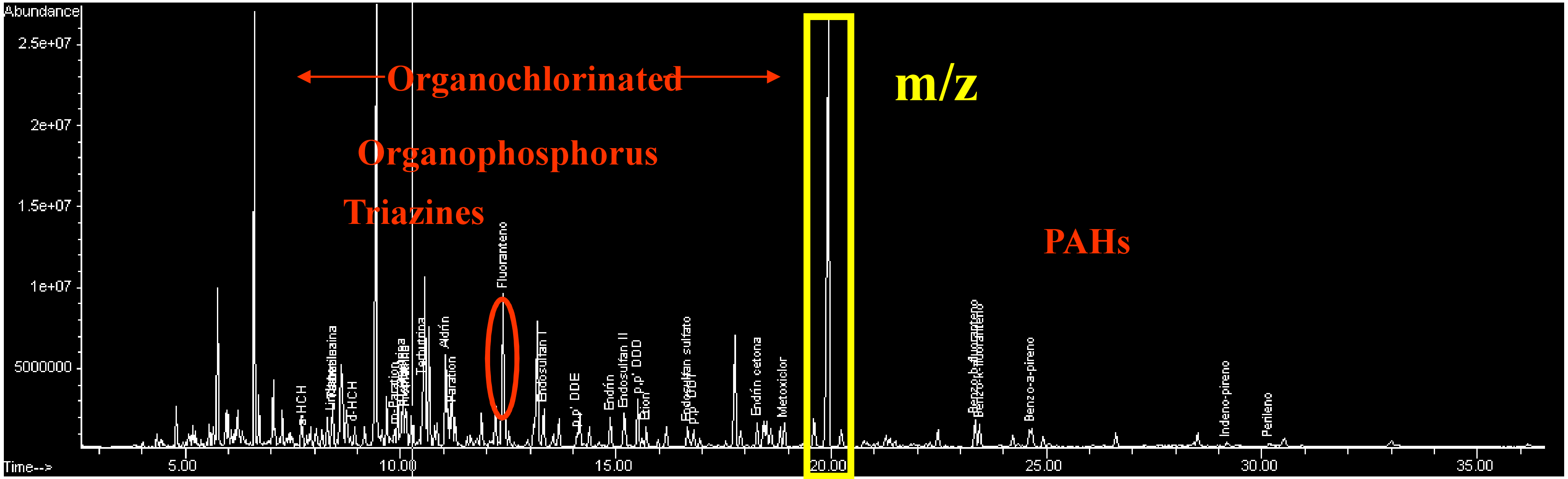
Thermodesorption: 12 min
(Max. Temp. 280ºC)

Cryofocussing on PTV inyector at 40ºC
Temp. ramp GC column: 60-290 ºC

Mass Spectrometry: **Full Scan Mode**

Freshwater:
- León et al., (2006). J.Chrom.A
- More analytes Moreno-González et al., (2012).Chemophere (accepted)

Seawater:
- Perez-Carrera et al., (2007). J.Chrom.A



I. Environmental samples: Screening by SBSE/GC/MS

Pollutant group	Compounds	
Polycyclic Aromatic Hydrocarbons	Acenaphtene, acenaphthylene, naphthalene, anthracene, fluorene, phenanthrene, benzo-a-anthracene, fluoranthene, crysene, pyrene, benzo-b-fluoranthene, benzo-k-fluoranthene, benzo-a-pyrene, benzo-e-pyrene, indeno-(1,2,3-cd)-pyrene, dibenzo-anthracene, benzo(ghi)perylene	**17**
Organochlorinated Pesticides	α-HCH, ß-HCH, lindane, α-HCH, aldrin, dieldrin, endrin, endrin aldehide, ß-endosulfan, α-endosulfan, heptachlor epoxide, heptachlor, endosulfan sulfate, p,p'-DDE, p,p'-DDD, p,p'-DDT, o,p-DDT, hexachlorobencene	**18**
Polychlorinated Biphenyls	PCBs (28, 52,101, 118, 138, 153, 180)	**7**
Triazines	Ametryn, simazine, atrazine, prometryn, prometon, propazine, terbutryn, terbuthylazine, trietazine, simetryn, atraton, secbumeton, terbumeton, terbuthylazine desethyl	**14**
Organophosphorus pesticides	Diazinon, metil-parathion, ethion, malathion, chlorpyrifos-ethyl, dichlorvos, fenitrotion, fention, sulprofos, etoprofos, fenchlorfos, mevinfos, disulfoton, protiofos, tricloronat, merfos, tetrachlorvinfos, chlorpyrifos-methyl,cyanofos, chlorfenvinfos, fensulfotion, sulfotep	**22**
Fungicides and other herbicides	Fenvalerate, flutolanil, propizamide, pendimethalin, myclobutanil, oxyfluorfen, chlortal-dimethyl, cyprodinil, procymidone	**9**
Other compounds	Nonylphenol, pyperonil butoxide	**2**

TOTAL = 89

I. LOD and LOQ in seawater samples by SBSE/GC/MS

	LOD (ng/L)	LOQ (ng/L)
Organochlorinated compounds		
aHCH	1.9	6.3
HCB	1.8	5.9
bHCH	5.1	17.1
lindane	3.5	11.7
d-HCH	2.0	6.7
CB 28	0.8	2.6
heptachlor	2.1	7.1
CB 52	0.4	1.5
Aldrin	3.2	10.7
Heptachlor epoxide	3.0	10.0
CB 101	1.0	3.2
a-endosulfan	3.3	11.1
p,p-DDE	0.3	1.0
dieldrin	2.4	7.9
endrin	2.7	9.1
CB 118	1.0	3.4
b-Endosulfan	1.9	6.3
pp DDD	0.3	1.1
CB 153	0.8	2.5
Endosulfan sulfate	3.8	12.5
pp DDT	1.1	3.8
CB 138	0.6	1.9
Alachlor	1.0	3.3
CB 180	0.6	2.1
Polycyclic Aromatic Hydrocarbons		
Naphthalene	1.5	4.9
Acenaphthylene	0.5	1.7
Acenaphthene	0.3	1.0
Fluorene	0.3	0.9
Phenanthrene	0.2	0.7
Anthracene	0.3	0.9
Fluoranthene	0.2	0.5
Pyrene	0.2	0.6
Benzo(a)anthracene	0.6	1.8
Chrysene	0.5	1.6
Benzo(e)pyrene	0.2	0.8
Benzo(b)fluoranthene	0.3	1.1
Benzo(k)fluoranthene	0.4	1.2
Benzo(a)pyrene	0.4	1.2
Benzo(ghi)perylene	0.9	2.9
Dibenzoanthracene	0.9	3.0
Indeno-pyrene	1.2	4.0

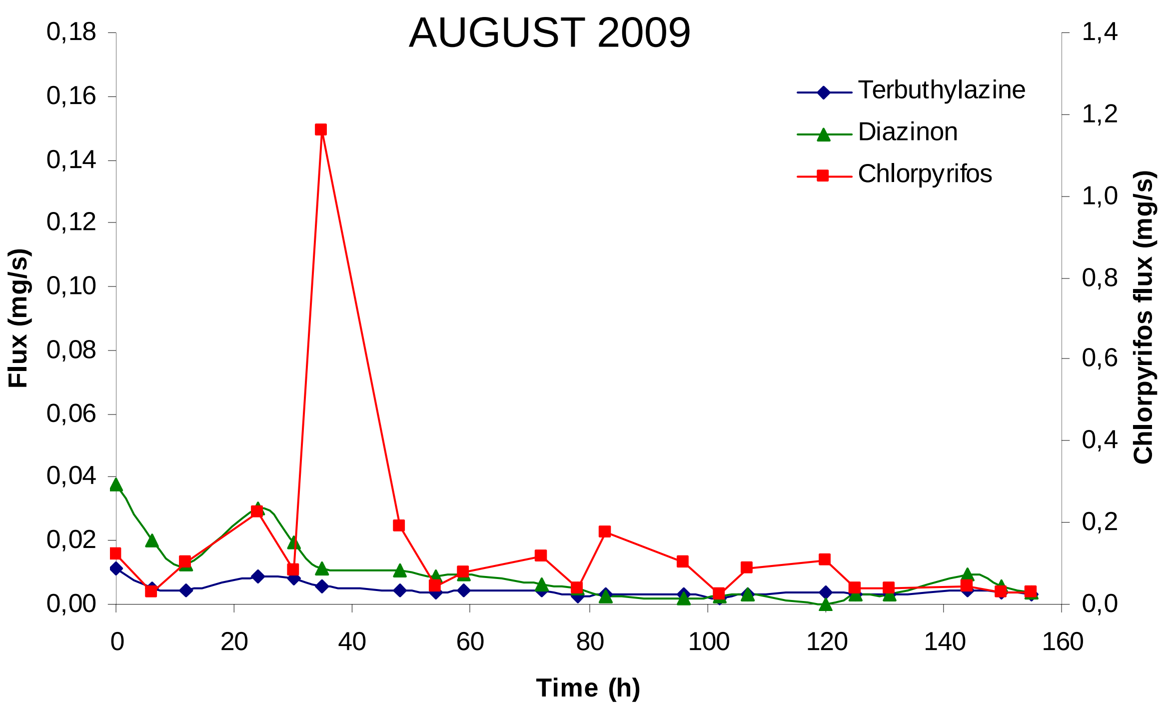
	LOD (ng/L)	LOQ (ng/L)
Triazines		
Simazine	4.3	14.3
Atraton	1.4	4.5
Secbumeton	0.3	0.9
Propazine	0.4	1.2
Atrazine	1.6	5.4
Prometryn	0.3	0.9
Terbutryn	0.3	0.9
Prometon	0.8	2.8
Simetryn	0.5	1.8
Terbutilazine	0.5	1.6
Terbumeton	0.6	2.1
Ametryn	0.8	2.6
Terbuthylazine-desethy	3.5	11.6
Organophosphorus pesticides		
Demeton-S	1.4	4.6
Diazinon	0.3	0.8
Sulprofos	0.1	0.3
Ethoprofos	0.6	2.0
Chlorpirifos	0.1	0.4
Fenclorfos	0.1	0.3
Disulfoton	0.8	2.5
Fenthion	0.1	0.4
Forate	1.5	5.2
Tokuthion	0.5	1.6
Trichloronate	0.2	0.6
Tetrachlorvinfos	0.2	0.5
m-parathion	0.2	0.5
Fensulfothion	0.9	2.9
Chlorfenvinfos	1.6	5.4
Chlorpyrifos-methyl	1.0	3.3
Other pesticides		
Flutolanil	0.4	1.3
Tributhylphosphate	0.3	1.0
Propizamide	0.9	3.1
Pendimethalin	0.7	2.2
Benalaxyl	1.0	3.3
Boscalid	1.1	3.7
Myclobutanil	1.7	5.6
Oxyfluorfen	1.1	3.5
Chlorthal-dimethyl	0.4	1.2
Cyprodinil	0.4	1.2
Procymidone	2.4	7.8
Other compounds		
Piperonyl butoxide	0.7	2.5
4-n-Nonylphenol	0.6	1.9

Seawaters
LOQ < 10 ng/L

(only 6 exceptions LOQ<18 ng/L)

Similar LOQ for freshwaters

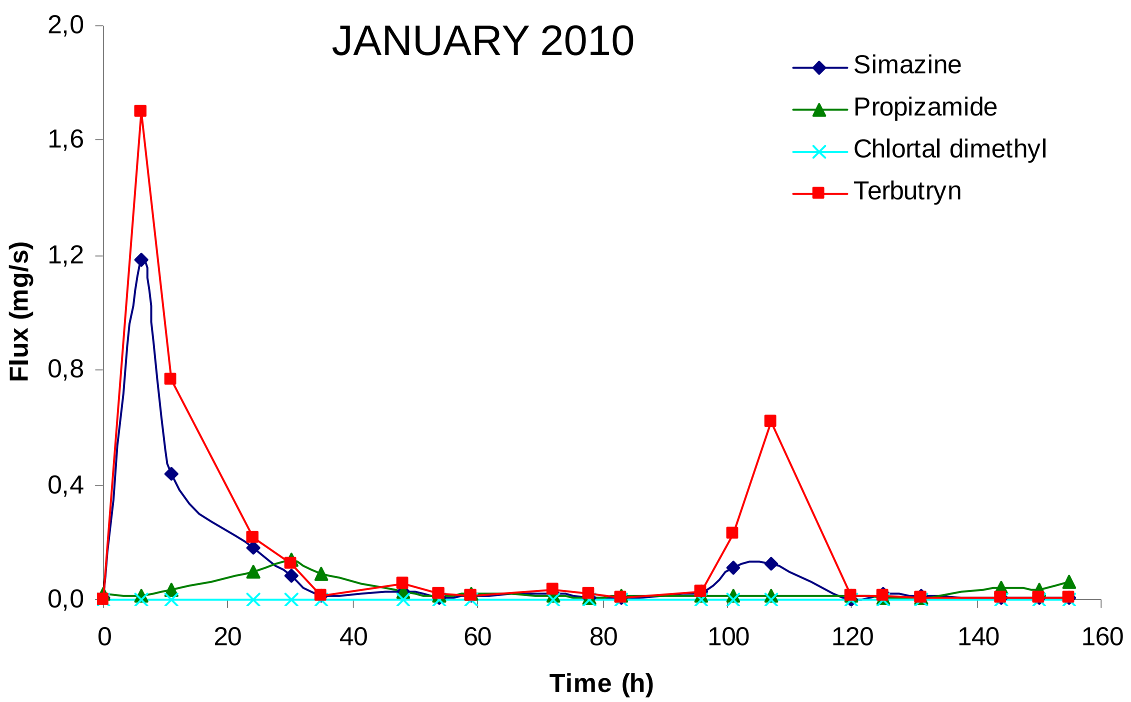
I. Input of organic contaminants through the Albujón watercourse



Total input / week in August 2009 into Mar Menor lagoon:

Terbuthylazine:	**2.50 g**
Diazinon:	**5.10 g**
Chlorpyrifos:	**87.20 g**

I. Input of organic contaminants through the Albujón watercourse



Total input / week in August 2009 into Mar Menor lagoon:

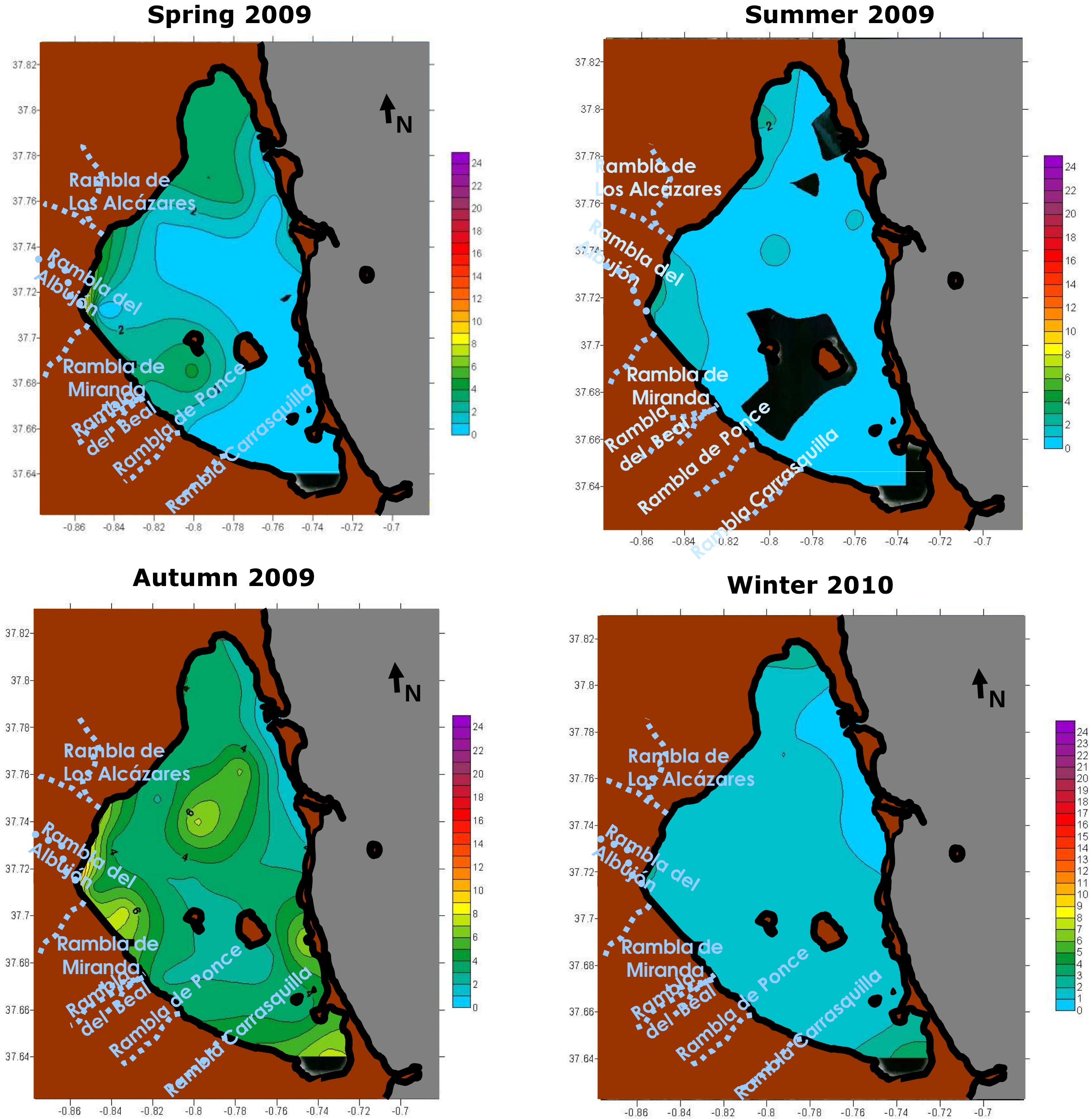
Terbuthylazine:	**2.50 g**
Diazinon:	**5.10 g**
Chlorpyrifos:	**87.20 g**

Total input /week in January 2010 into Mar Menor lagoon:

Terbuthylazine:	**23.50 g**
Diazinon:	**0.00 g**
Chlorpyrifos:	**0.89 g**
Terbutryn:	**101.30 g**
Simazine:	**56.99 g**
Propizamide:	**18.54 g**
Chlorthal dimethyl:	**0.46 g**

I. Distribution and temporal variations of Triazines in Mar Menor

Terbuthylazine in Surface waters (ng/L)



MAIN CONCLUSIONS I: Inputs and distribution

SBSE/GC/MS ADEQUATE FOR WATER ANALYSIS (WFD, MSFD):

More than 20 different compounds included in the EU Priority List of hazardous substances

CONTINUOUS INPUT OF PESTICIDES FROM ALBUJON WATERCOURSE

Significant seasonal and daily variations

Summer: insecticides   Winter: herbicides

Especially chlorpyrifos and terbuthylazine

HETEROGENEUS DISTRIBUTION IN MAR MENOR LAGOON

Higher concentrations close to Albujón watercourse mouth and to other sources (groundwaters).

Spring and Autumn: periods with higher concentrations and analytes

II. Efficiency of water integrative samplers for OPs

SEMIPERMEABLE MEMBRANE DEVICE (SPMD)

SPMD: one of the most developed devices for semivolatile organic pollutants.

Sorbent: **LPDE** (low density polyethylene) filled with triolein.

PRC: **Chrysene-D12, fluorene-D10 y fluoranthene-D12**.

CONTINUOUS FLOW INTEGRATIVE SAMPLER

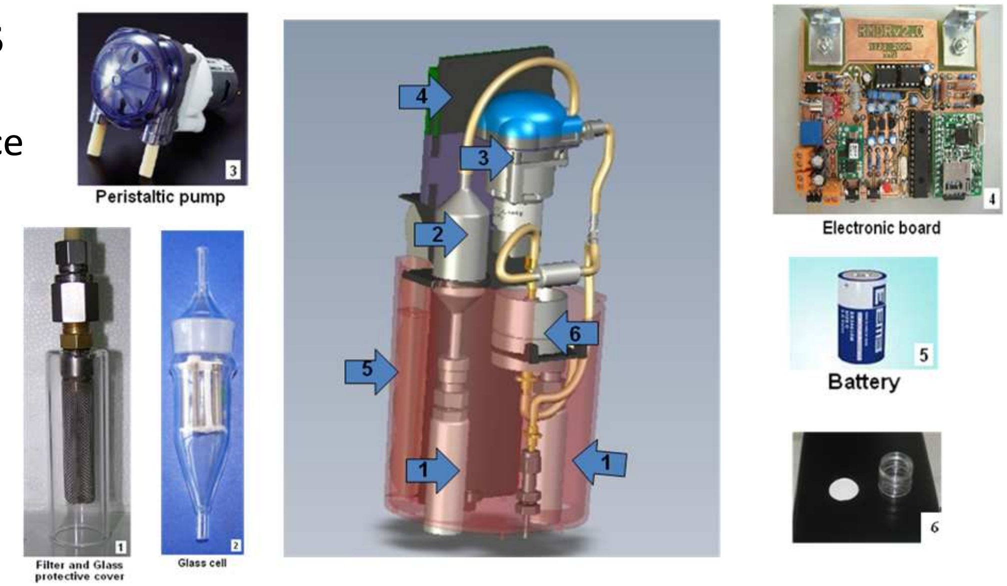
PDMS: polydimethylsiloxane used as sorbent in **CFIS**

Sampling rates independent of external turbulence (flow control)

Continuous measures and controlled sampling period

PRCs: **Fluorene D-10 y Chrysene D-12.**

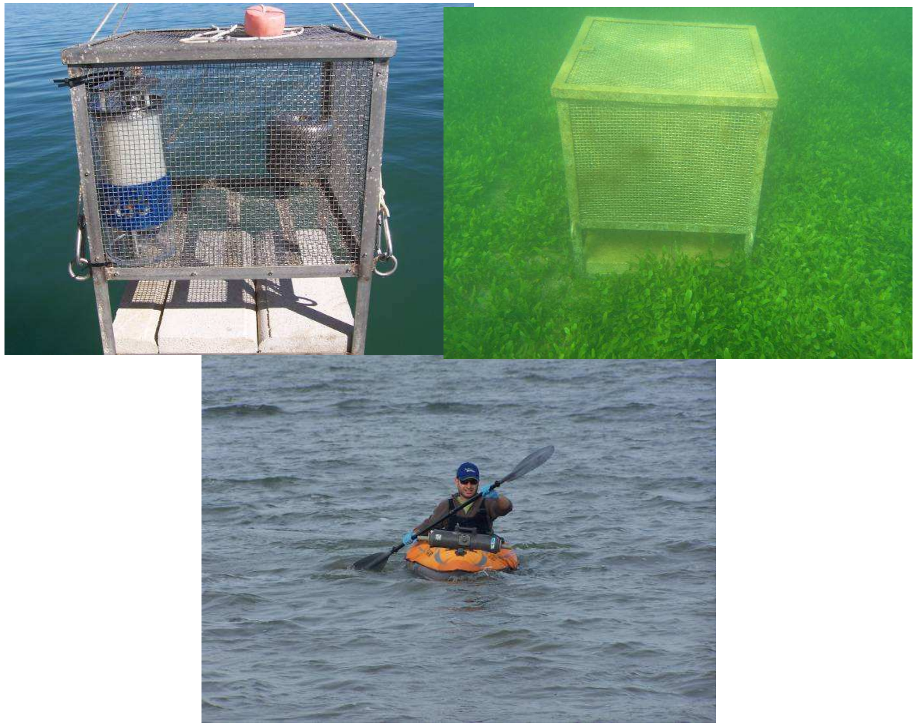
CFIS® components



II. Organic contaminants in seawater: Integrative samplers

Simultaneous exposure of samplers

✓ inmersion 7 days (spring and autumn)



Concentration of dissolved pollutants in surrounding seawater

✓ 2 samples/day during 7 days

II. Seawaters: Mean Concentrations using SBSE/GC/MS

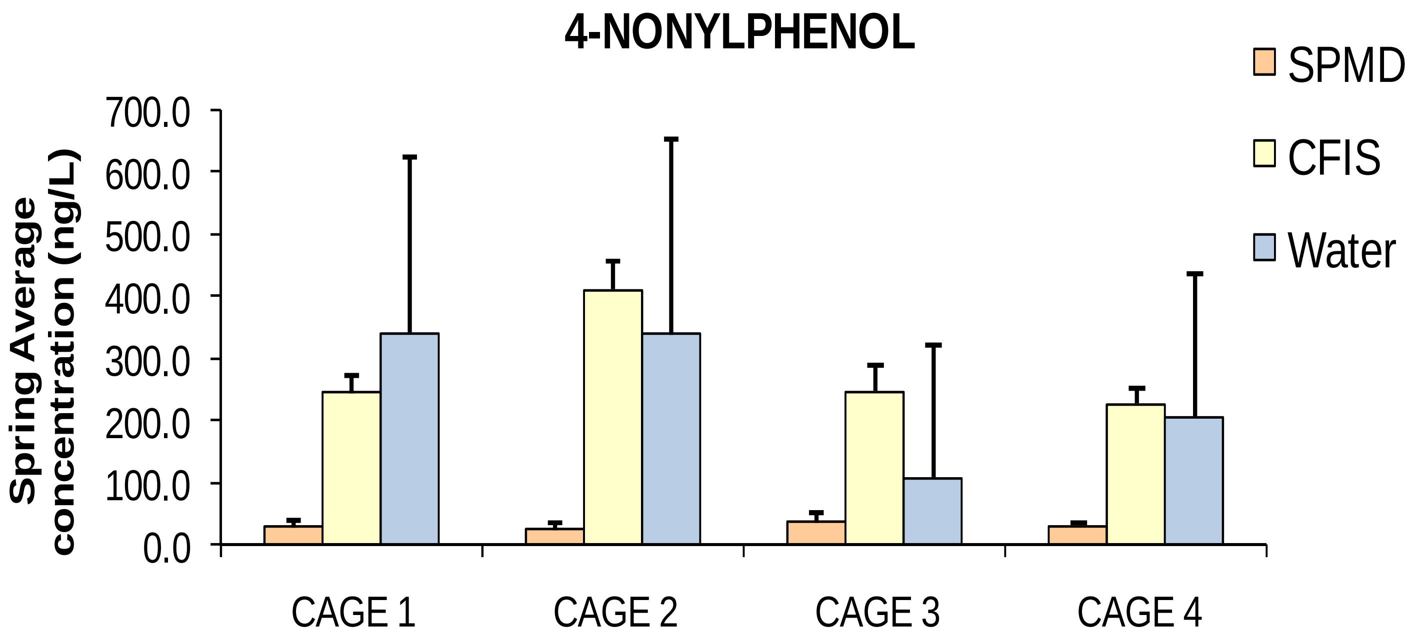
GC-MS (ng/L)	CAGE1	CAGE2	CAGE3	CAGE4
	SPRING 2010			
Anthracene	6.06	6.69	5.50	9.72
Naphthlene	8.54	6.63	14.74	12.63
Fluoranthene	9.03	4.81	1.58	3.92
Benzo-b-fluoranthene	2.81	2.25	2.14	2.47
Benzo-k-fluoranthene	2.81	2.25	1.80	2.36
Benzo-a-anthracene	3.36	2.69	0.73	2.15
Benzoperylene	1.81	1.82	0.61	1.15
Indenopyrene	1.70	0.60	1.39	1.72
Phenantrene	13.50	9.39	8.24	14.48
Di(2-ethylhexyl) phthalate	25.85	19.81	19.81	25.89
Simazine	16.78	7.19	0.00	1.67
Terbuthylazine	8.04	7.96	16.67	13.78
Diazinon	7.56	6.59	19.89	15.67
Pendimethalin	14.04	10.33	20.44	20.67
4-Nonylphenol C.A.S. 104-40-5	347.78	341.48	117.78	209.33
Chlorpyriphos	6.85	6.63	14.74	12.63

GC-MS (ng/L)	CAGE1	CAGE2	CAGE3	CAGE4
	AUTUMN 2010			
Acenaphthene			2.58	
Acenaphthylene	6.04			
Anthracene	5.94	2.43	2.91	7.44
Benzo-b-fluoranthene	2.64	0.17	0.53	0.13
Benzo-k-fluoranthene	0.67	0.36	1.96	2.52
Benzo-a-anthracene		0.39	2.47	1.20
Phenanthrene	15.19	19.80	7.93	13.67
Fluoranthene	15.22	1.17	1.20	6.16
Fluorene	1.67		1.20	
Naphthalene		2.05	2.46	8.11
Pyrene	5.13	0.60	0.44	6.94
Chlorpyriphos	16.34	13.91	63.52	14.73
Pentachlorobenzene	0.23	0.51	0.24	1.52
Monoethoxylated nonylphenol	121.27	51.69	47.07	80.51
4-Nonylphenol(C.A.S. 104-40-5)		121.49		
Terbuthylazine	29.89	45.52	34.63	23.01
Propyzamide	28.62	20.53	28.91	25.29
Ametryne			2.79	
Prometryn	4.56	13.11	8.66	11.15
Pendimethalin	44.17	34.57	108.47	27.91

Highest concentrations of 4-Nonylphenol

Higher concentrations of
4-Nonylphenols and pesticides

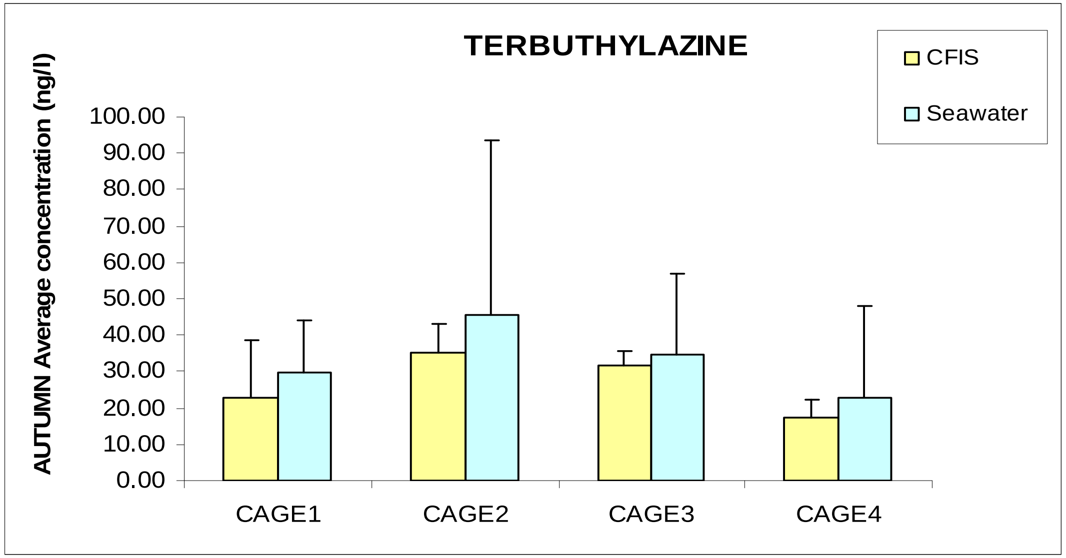
II. Comparison Sampler concentration vs Seawater concentration

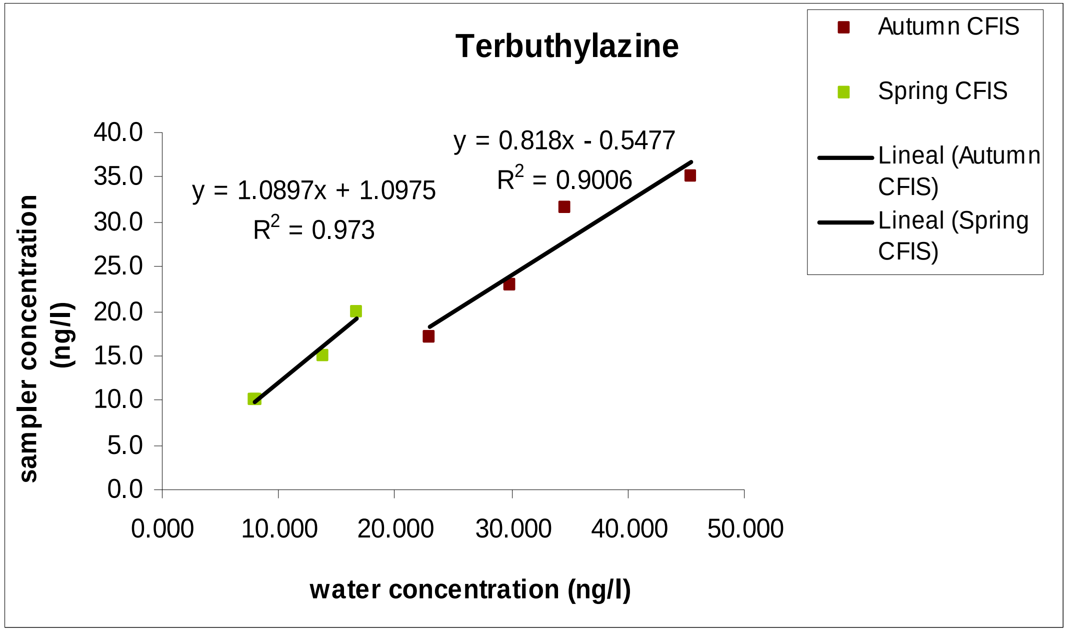


CFIS: Good correspondence with seawater concentration

SPMD: Direct relation, but less effective concentration than CFIS

II. Comparison Sampler concentration vs Seawater concentration





Triazines: only CFIS detected in both seasons. Not detected in SPMD.

CFIS: Good correspondence and efficiency with seawater concentration.

II. Comparison Sampler concentration vs Seawater concentration

Concentration differences

Green	Less than one magnitude order
Yellow	One magnitude order
Red	Two or more magnitude orders

II. Comparison Sampler concentration vs Seawater concentration

SPRING

AUTUMN

Compounds		SPMD				CFIS			
ANALITE	Season	CAGE 1	CAGE 2	CAGE 3	CAGE 4	CAGE 1	CAGE 2	CAGE 3	CAGE 4
Anthracene	SPRING	3.2	30.5	105.4	9.4	5.5	5.2	5.5	5.1
Anthracene	AUTUMN	1.5	0.8	1.0	1.9	3.7	2.1	2.9	5.6
Naphthlene	SPRING	4.7	4.8		8.8	6.9	9.6		3.3
Naphthlene	AUTUMN		2.0	4.8	9.4		12.0	60.6	67.4
Benzo-b-fluoranthene	SPRING	0.6	0.3	1.8	1.3	2.0	0.2	0.2	0.5
Benzo-b-fluoranthene	AUTUMN		0.1					0.2	0.2
Benzo-k-fluoranthene	SPRING	0.5	0.2	1.5	1.1	1.9	0.3	0.1	0.5
Benzo-k-fluoranthene	AUTUMN							0.2	0.2
Benzo-a-anthracene	SPRING	0.4	0.2	0.8	2.2	1.2	1.0	0.5	0.3
Benzo-a-anthracene	AUTUMN						0.3		
Phenanthrene	SPRING	3.9	4.5	1.5	1.1	7.5	3.9	7.2	2.3
Phenanthrene	AUTUMN	1.2	0.7	0.8	1.8	39.5	30.3	55.8	42.4
Fluoranthene	SPRING	0.6	0.7	0.3	0.5	2.9	0.7	0.3	0.4
Fluoranthene	AUTUMN	0.5	0.2	0.3	0.4	1.8	0.8	1.8	1.3
Fluorene	AUTUMN	0.6		0.2		5.1		6.2	
Benzoperylene	SPRING	0.2	0.1	1.6	2.7	1.2	0.8	0.3	0.7
Indenopyrene	SPRING	0.2	0.2	1.3	1.2	1.5	0.6	0.3	0.6
Pyrene	AUTUMN	0.4	0.2	0.3	0.4	2.9	1.2	1.6	2.4
Chlorpyriphos	SPRING	4.8	64.2	130.8	32.7	17.3	13.9	4.7	3.0
Chlorpyriphos	AUTUMN					2.1	4.1	18.2	15.3
Terbuthylazine	SPRING					10.0	10.0	20.0	15.0
Terbuthylazine	AUTUMN					22.9	35.0	31.6	17.1
Pendimethalin	SPRING					18.0	18.0	14.0	14.0
Pendimethalin	AUTUMN					0.5		1.1	
4-Nonylphenol(C.A.S. 104-40-5)	SPRING	26.7	23.3	36.7	30.7	244.0	410.0	247.0	225.0
4-Nonylphenol(C.A.S. 104-40-5)	AUTUMN						181.8		
Diazinon	SPRING					10.0	10.0	15.0	10.0
Simazine	SPRING					15.0	15.0		20.0
Di(2-ethylhexyl) phthalate	SPRING					3.5	2.8	5.2	10.2
Monoethoxylated nonylphenol	AUTUMN					135.2	90.8	110.7	157.5
Propyzamide	AUTUMN					6.9	9.6	9.9	4.5
Ametrina	AUTUMN							55.4	
Prometrina	AUTUMN					2.2	2.2	1.9	1.5
Pentachlorobenzene	AUTUMN					2.8	3.7	1.1	0.7

MAIN CONCLUSIONS II: Water integrative samplers

SPMD IN SEAWATERS:

Adequate for naphthalene, anthracene, benzo-b-fluoranthene, indeno-pyrene, benzo-a-anthracene,benzo-k-fluoranthene and benzo(g,h,i)perylene

CFIS IN SEAWATERS:

Adequate for the majority of PAHs detected, terbuthylazine, prometryn, pendimethalin, chlorpyriphos and nonylphenol

CFIS→ More versatile at different environmental conditions, and useful for more groups of pollutants.

III. Biological effects: Biomarkers and bioassays

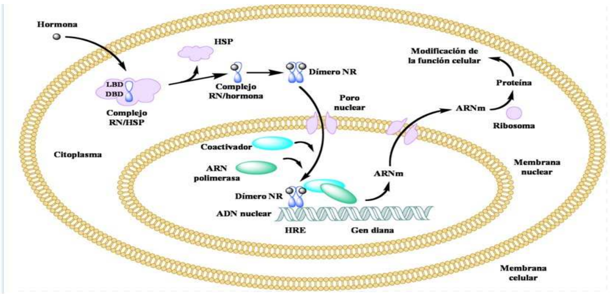
Biliary OH-PAHs and alkylphenols

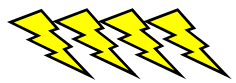
ER-LUC assay

Exposure to estrogenic compounds
Toxicity potency and contribution to response

- **Antioxidant enzymes**
- **EROD activity**
- **Micronuclei frecuency**
- **AChE activity**
- **Metallothioneins**
- **Ala-D**

ER-LUC: Estrogen Responsive Chemical Activated Luciferase Gene Expression

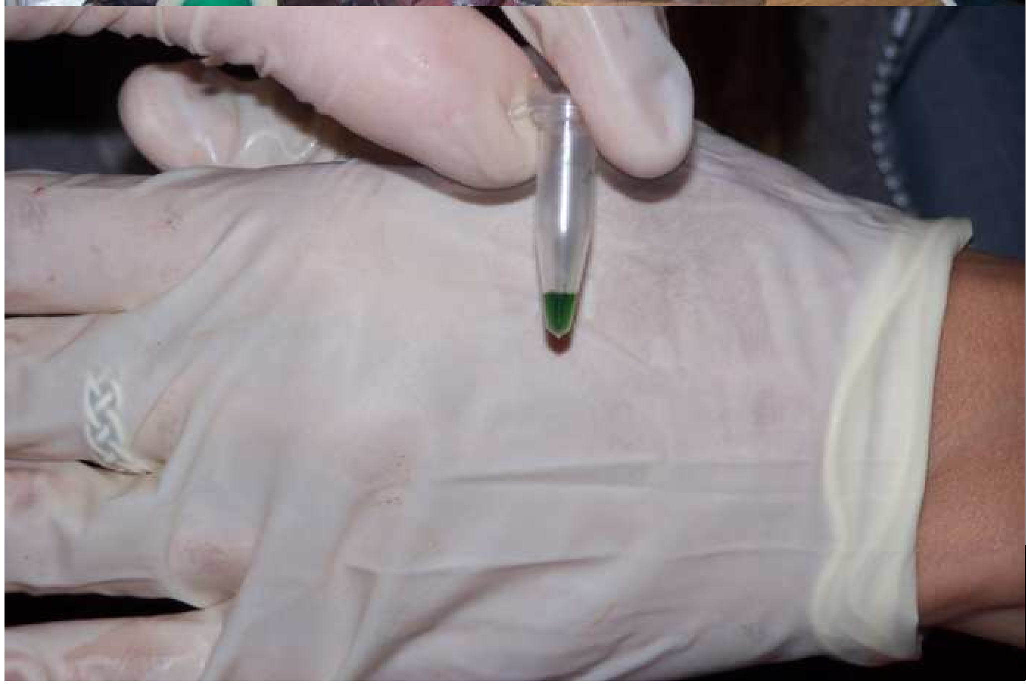


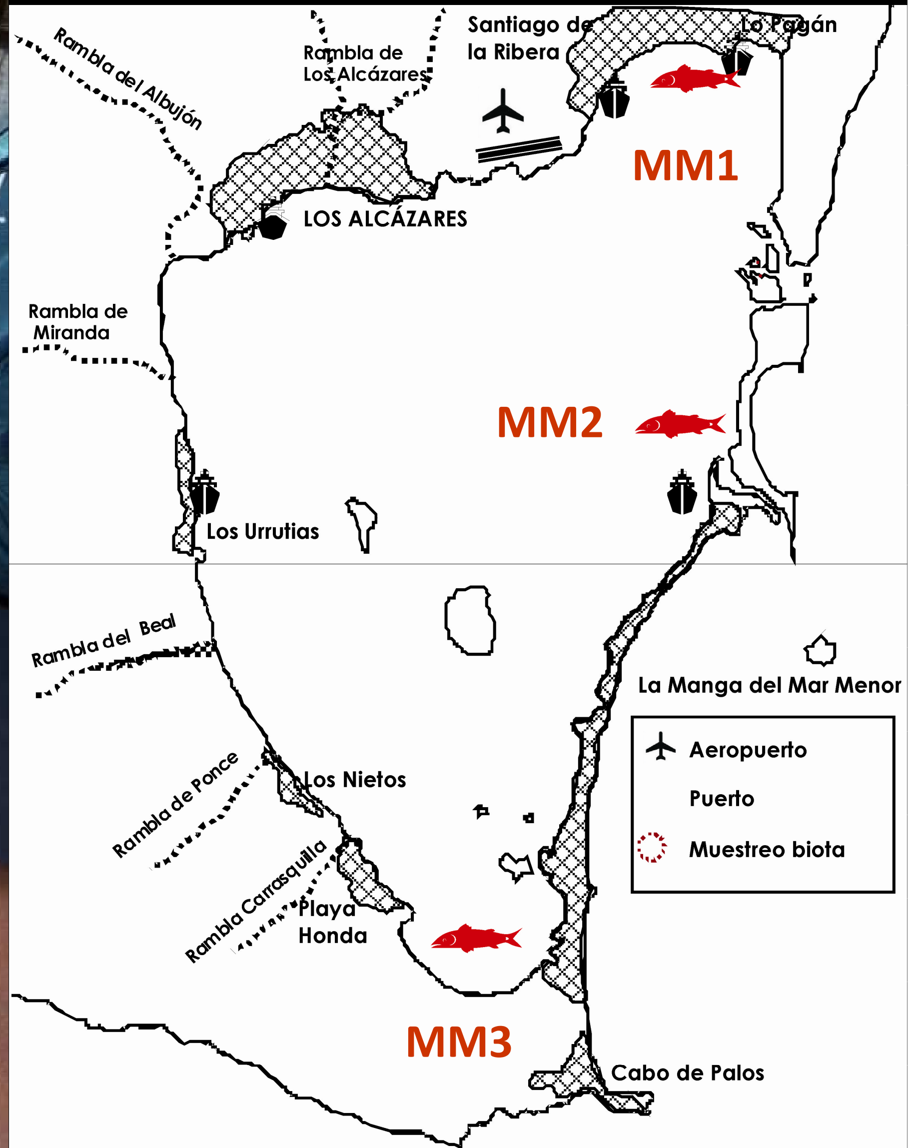
Luciferine + O_2 → Oxoluciferine + 

Luciferase

Stably transfected **BG1luc4E2** human ovarian cancer cells (containing a luciferase reporter gene under transcriptional control of an estrogen-responsive element (ERE))
(Rogers & Denison, 2000).


Energizer




Rambla del Albujón
Rambla de Los Alcázares
Santiago de la Ribera
Lo Pagán
MM1
LOS ALCÁZARES
Rambla de Miranda
MM2
Los Urrutias
Rambla del Beal
La Manga del Mar Menor
Aeropuerto
Puerto
Muestreo biota
Rambla de Ponce
Los Nietos
Rambla Carrasquilla
Playa Honda
MM3
Cabo de Palos

III. Exposure to PAHs and alkylphenols

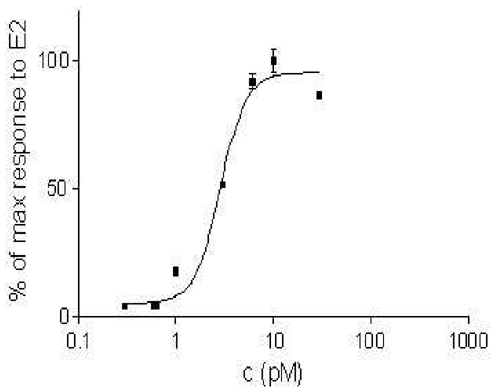
De-conjugation →Internal deuterated standard → Liquid-liquid extraction → Derivatization→**Quantification by GC-EI-MS** (Fernández et al., 2008)

Biliary concentrations (ng/g bile)							
Study area	Palos Cape	Barcelona	Delta Ebro	Valencia	Mar Menor MM1	Mar Menor MM2	Mar Menor MM3
N	1	1	1	2	3	5	3
1-pyrenol	4.4	**50.8**	29.7	13.0 ± 0.3	28.6 ± 6.4	**34.8± 5.6**	25.38± 2.3
9-phenantrol	n.d.	35.8	n.d.	n.d.	73.9 ± 25.9	**154.24 ± 31.00**	**118.5 ± 16.2**
9-fluorenol	n.d.	24.3	11.2	14.1 ± 4.7	32.6 ± 12.6	**34.63 ± 5.58**	**33.3 ± 6.9**
1OH-BaP	<12.1	<12.5	<17.0	<19.4	<12.4	<20.0	n.d.
3OH-BaP	n.d.	n.d.	n.d.	n.d.	n.d.	**80.2**	**68.3**
1-naphtol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ΣOH-PAHs	4.4	110.9	40.9	27.2	135.2	303.9	245.6
4-tert-Octylphenol	26.7	36.4	**44.6**	**63.1 ± 1.8**	25.3 ± 5.18	18.8 ± 5.6	23.9 ± 6.3
4-n-Nonylphenol	358.4	**923.3**	441.5	408.5 ± 30.9	592.4 ± 115.3	**800.9 ± 146.3**	637.7 ± 37.2

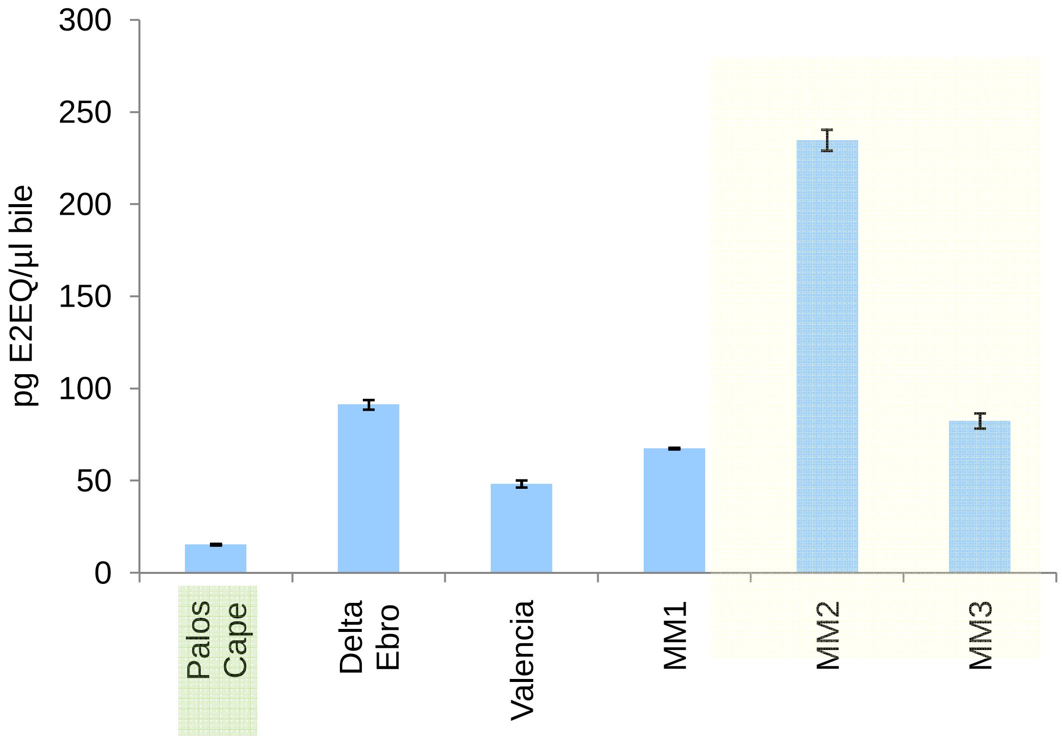
III. Estrogenic activity in bile extracts of males fish: ER-LUC assay

De-conjugation → Liquid-liquid extraction → Culture and Exposure of cells → **Quantification of response by Luminometer**

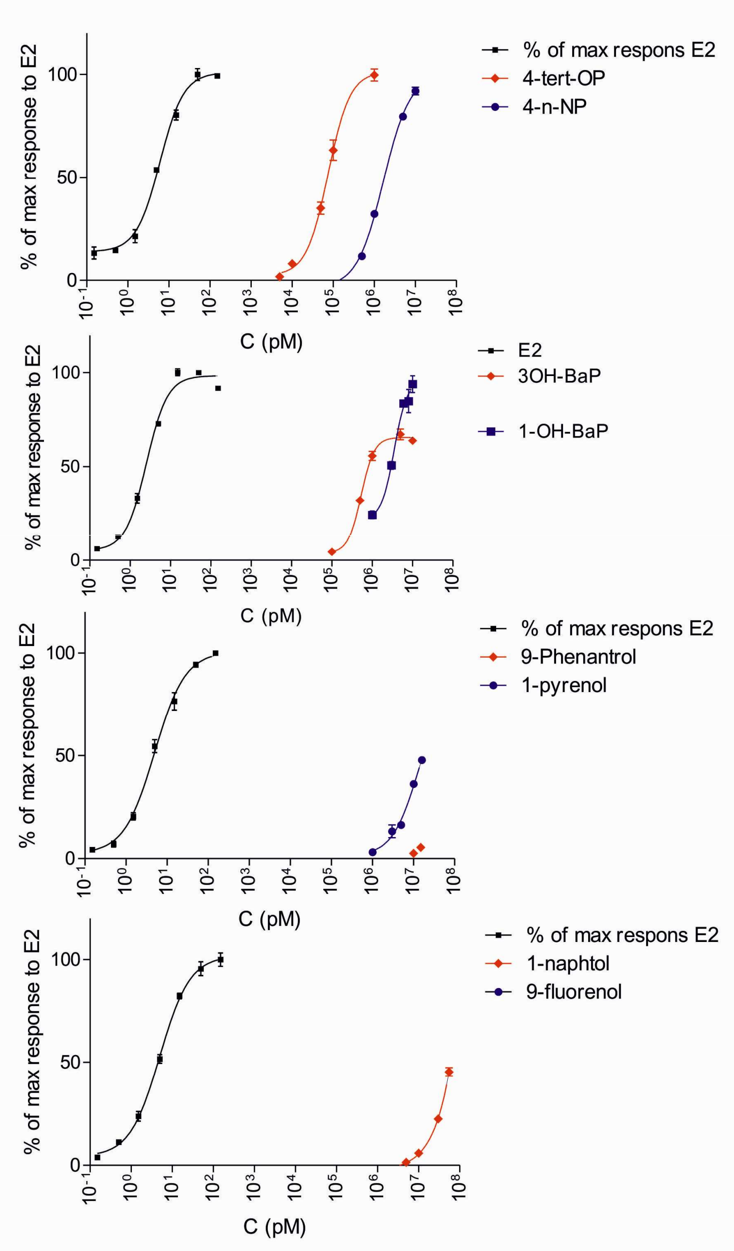
Houtman et al (2004) and Legler et al (2002),



pgE2EQ/ microL bile



III. Estrogenic potency of OH-PAH and APs



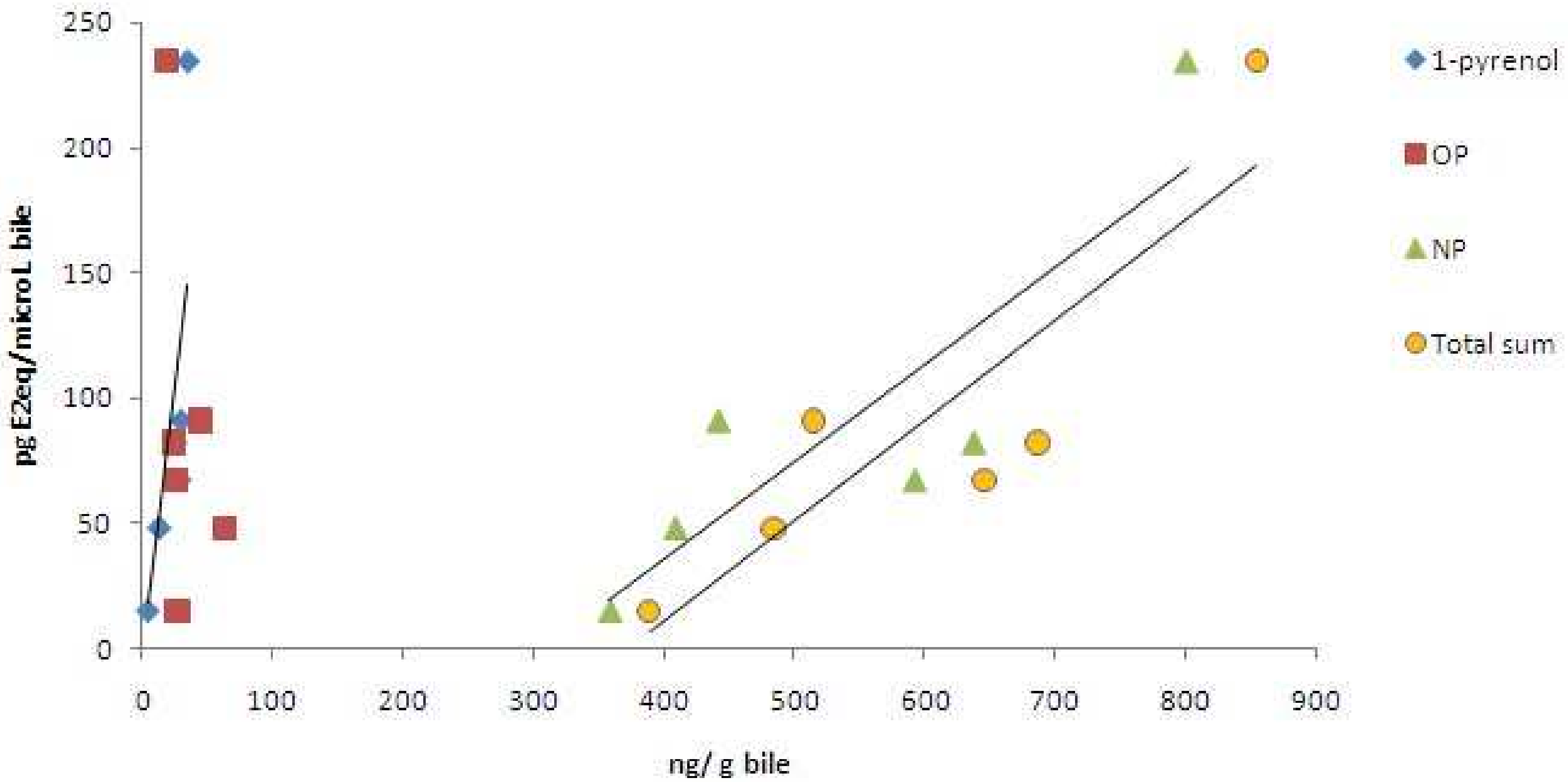
EEFx= EC50E2/EC50x

Compound	% Maximum Response to E2	EC $_{50}$ (pM) ± SEM (pM)	EEF
4-t-OP	**99.7**	**$5.9 \times 10^4 \pm 5.5 \times 10^3$**	**7.2×10^{-5}**
4-n-NP	92.3	$1.7 \times 10^6 \pm 2.8 \times 10^5$	3.6×10^{-6}
3-OH-BaP	67.0	$5.3 \times 10^5 \pm 3.8 \times 10^4$	4.7×10^{-6}
1-OH-BaP	68.0	$9.1 \times 10^6 \pm 6.3 \times 10^6$	5.2×10^{-7}
1-pyrenol	47.9	$1.7 \times 10^7 \pm 1.4 \times 10^6$	2.9×10^{-7}
1-napthol	45.3	$6.2 \times 10^7 \pm 2.3 \times 10^6$	7.8×10^{-8}
9-fluorenol	0.0	n.a.	
9-phenantrol	5.0	n.a.	

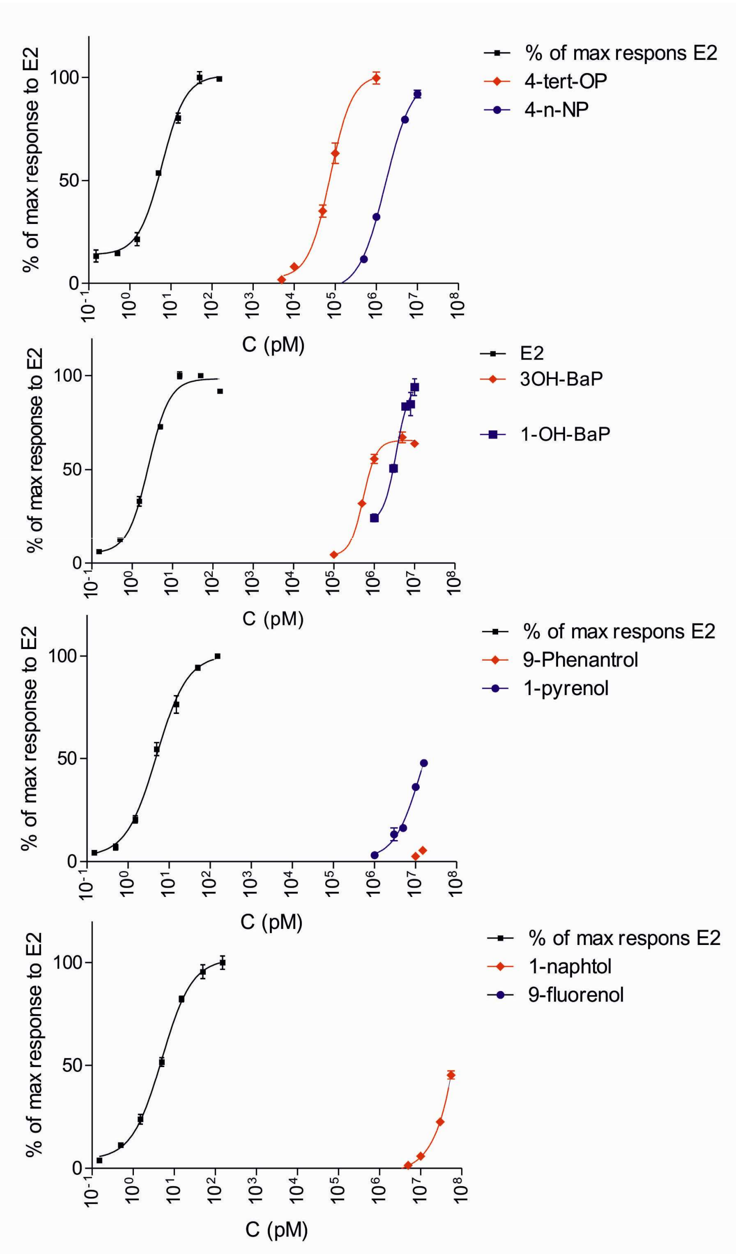
Houtman et al., 2004; Zhao et al., 2010; Van Lipzig, 2011

III. Relationships estrogenic activity vs and estrogenic metabolite concentrations

1-pyrenol	Spearman Correlation Coeff = 0.943 **
4-n-nonylphenol	Spearman Correlation Coeff = 0.829 *
Total Sum	Spearman Correlation Coeff = 0.829*



III. Estrogenic potency of OH-PAH and APs



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Houtman et al., 2004; Zhao et al., 2010; Van Lipzig, 2011

E2EQ = [X] * EEF

Contribution to biliary estrogenic activity in males < 0.02 %

MAIN CONCLUSIONS III: Biological effects

- ✓ MODERATE EXPOSURE: Fish from the Mar Menor Lagoon have a higher exposure to PAHs and alkylphenols in comparison to reference areas
- ✓ Highest bile estrogenic activity found in male red mullet was comparable to highest activity found in male breams from Dutch rivers, associated with high levels of plasma VTG and gonadal intersex (Houtman et al., 2004).
- ✓ Contribution of OH-Pyr and APs to the total estrogenic activity observed in bile fish was negligible
- ✓ Although not included in the analysis, natural and pharmaceutical estrogens may be playing a significant role in the observed estrogenic activities in males

Future research in Mar Menor Lagoon

❖ Development and use of SBSE/GC/MS technique with new matrices (sediment, biota extracts)

Ultrasonication (sediment)+ SBSE extraction (preliminary study)

❖ Quantification of natural and pharmacological hormones concentrations in sea water/bile extracts

❖ Use of more "mechanism-based assays" in sediment extracts for toxicity profiling

Acknowledgments

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