DO PHARMACEUTICALS BIOACCUMULATE IN MARINE MOLLUSCS AND FISHES FROM A COASTAL LAGOON?

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BIOMARO Project

SéNeCa (+)
Agencia de Ciencia y Tecnología
Región de Murcia

IMPACTA

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Nantes 2016
SETAC Europe

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INTRODUCTION

CONTAMINANTS OF EMERGING CONCERN: IMPACTA PROJECT

MAR MENOR LAGOON: PREVIOUS KNOWLEDGE

MATERIAL AND METHODS

PHARMACEUTICALS ANALYSIS IN BIOTA

SAMPLING CAMPAINGS AND SELECTED SPECIES

RESULTS AND DISCUSSION

ADAPTATION OF PHARMACEUTICAL ANALYSIS FOR BIVALVES

PHARMACEUTICAL BIOACCUMULATION IN NATIVE SPECIES

ACTIVE BIOMONITORING (TRANSPLANTED SPECIMENS)

CONCLUSIONS

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EMERGING CONTAMINANTS CONSIDERED

CONTAMINANTS TYPES:

- Triazines,
- Organophosphorus pesticides
- Others CUPs
- Pharmaceuticals
- Personal care products
- Perfluorinated compounds
- Phthalates
- Microplastics, etc

Additional information required (presence, distribution and effects IMPACTA project and previous projects)

ORIGIN

Agriculture y other uses

Different uses (domestic, farming,...)

Human health and veterinary uses

Cosmetics, solar protectors, etc.

Industrial and other uses

Plastics additives and residues

COASTAL LAGOONS: MAR MENOR LAGOON (SE SPAIN)

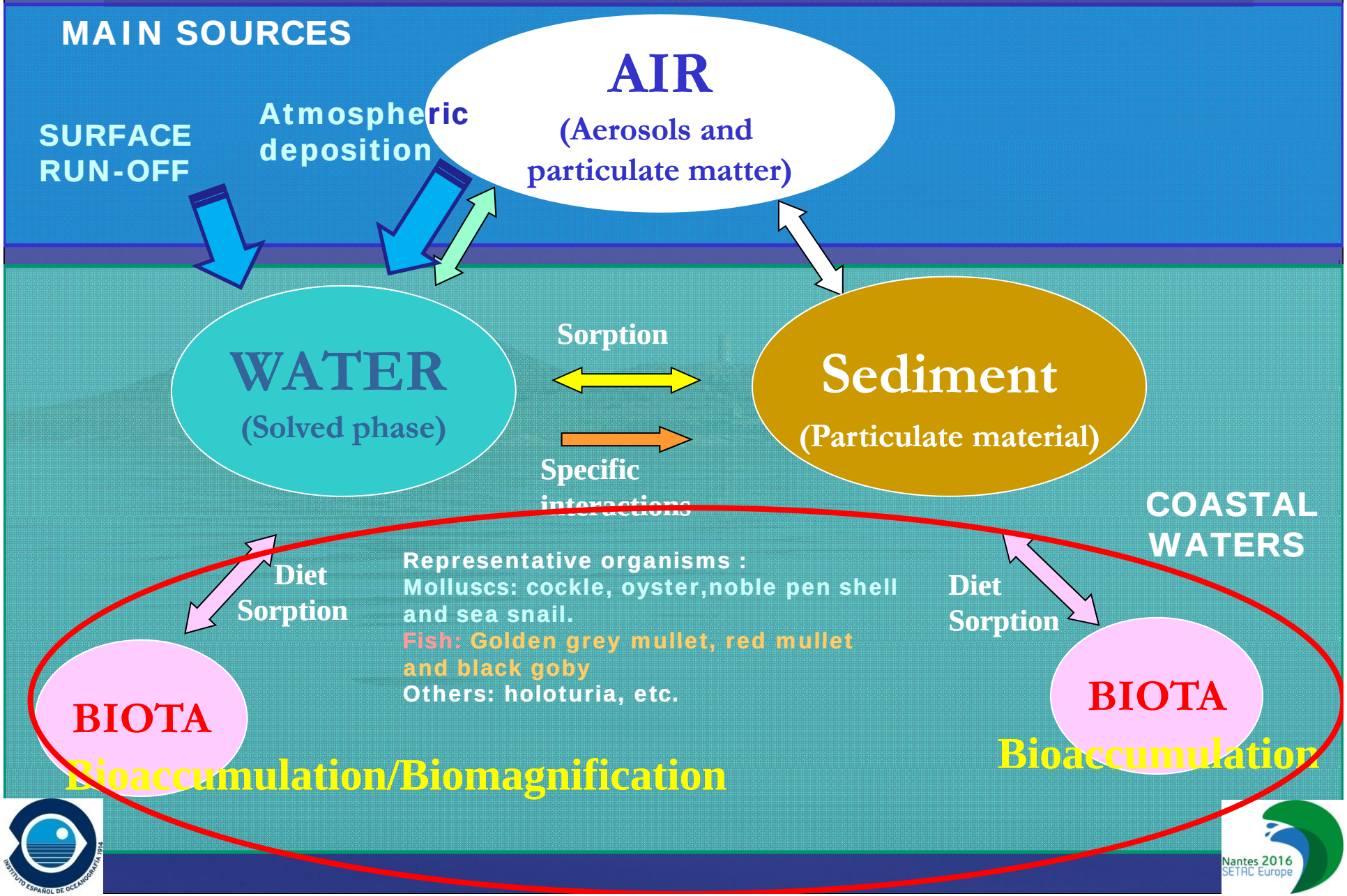
Mar Menor: hypersaline (40 to 44 psu)
Mean depth: 3-4m (maximum 6m)

One of the main intensive horticulture growing areas in Europe.

Albujón watercourse: most important collector of the drainage basin of Cartagena Field area+WWTP effluents

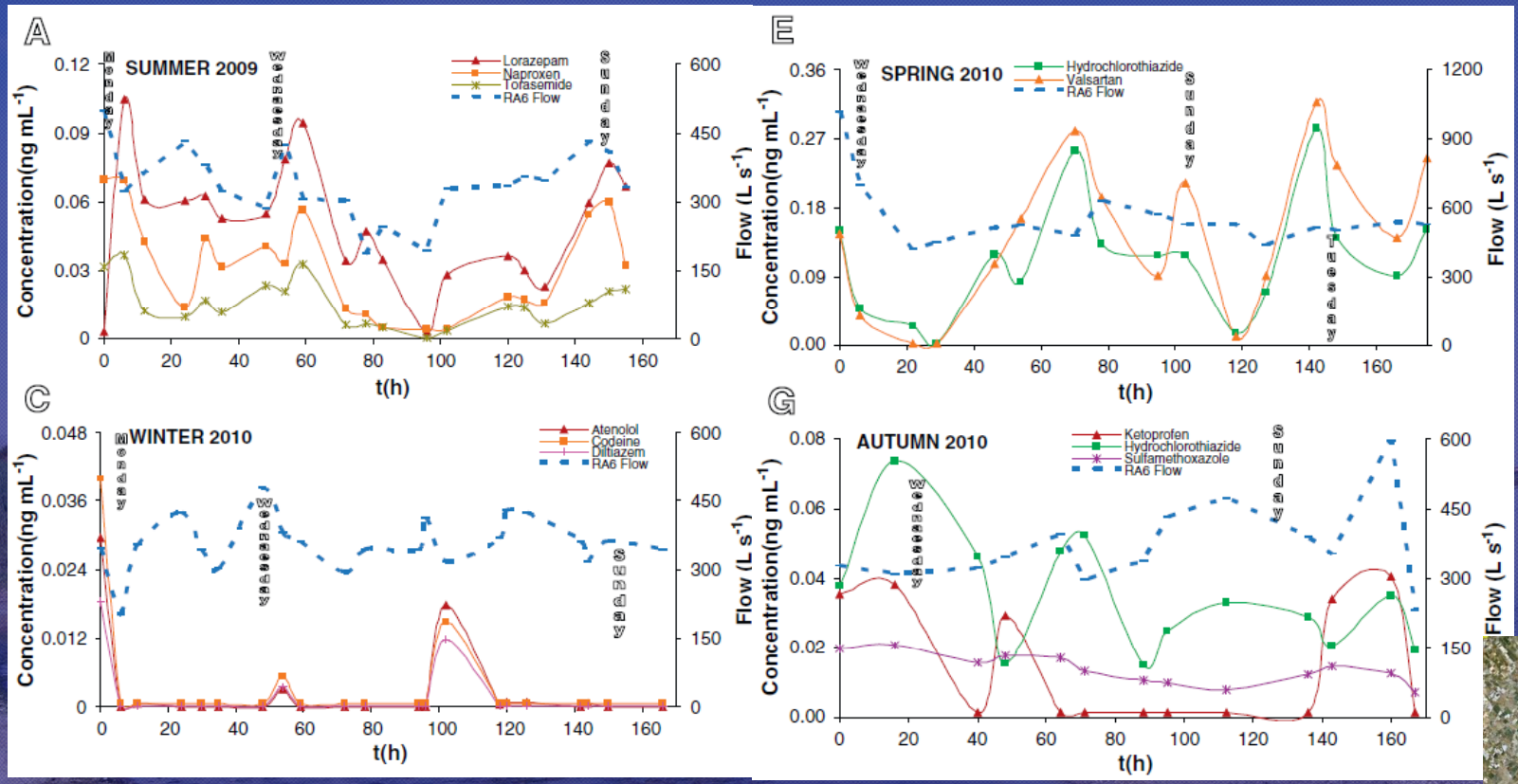
Relevant touristic activity (seasonality)

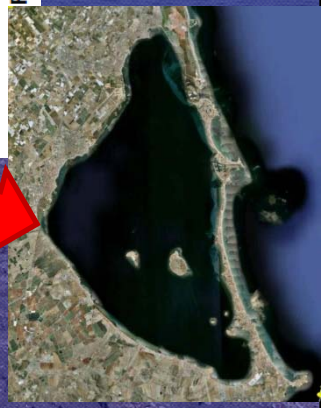
ENVIRONMENTAL COMPARTMENTS



MAIN SURFACE WATER INPUT: EL ALBUJÓN WATERCOURSE

WEEKLY INPUT TO MAR MENOR: PHARMACEUTICALS





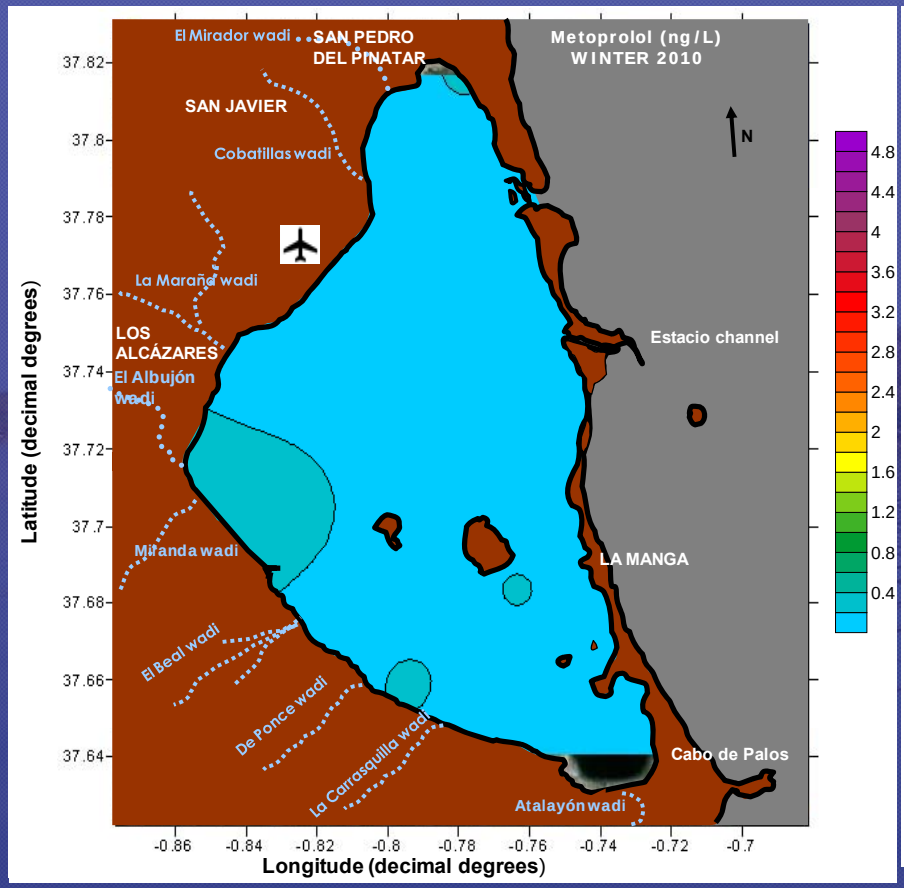
Seasonal variation inputs: predominant antibiotics in spring, psycopharmaceuticals and antihypertensives in summer, etc

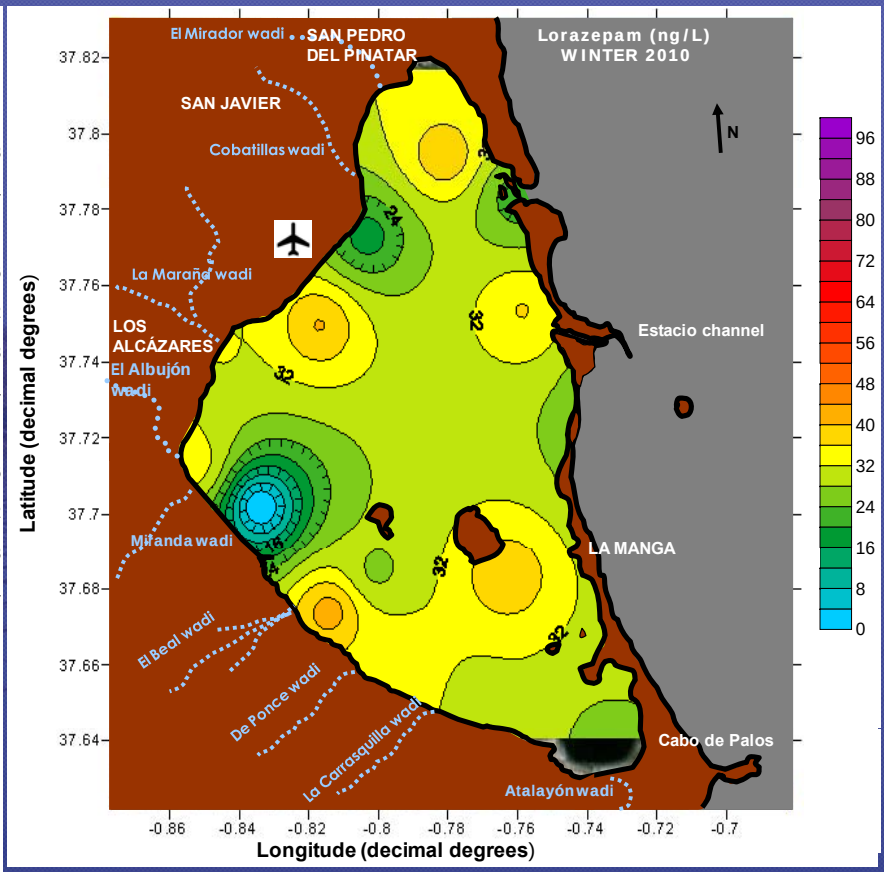
TOTAL INPUT: 11.5 kg/year

(Moreno-González et al.,2014: Sci Tot Environ 490, 59-72)

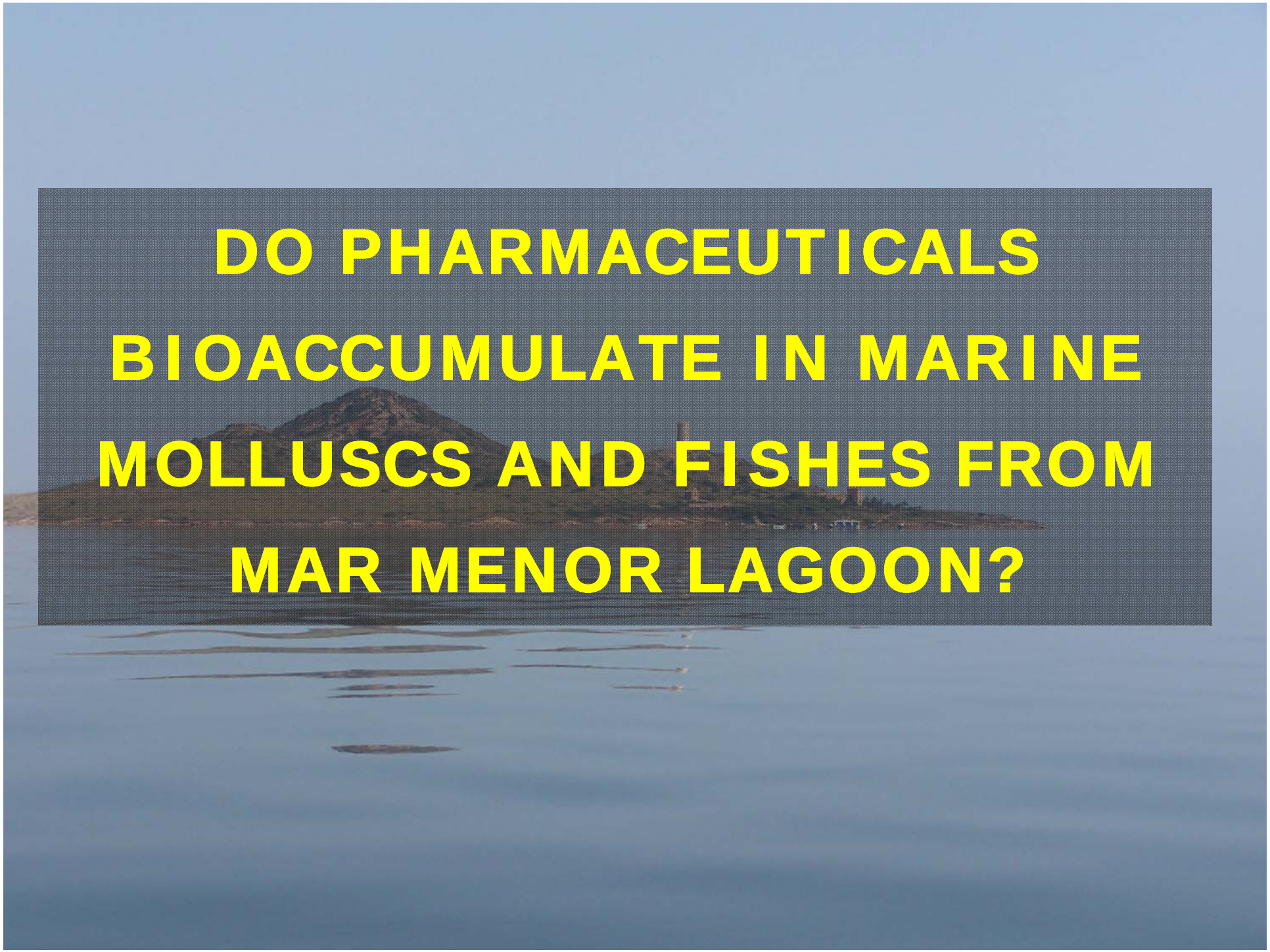
PHARMACEUTICAL DISTRIBUTION IN SEAWATER

Pharmaceuticals seasonal distribution: metropolol and lorazepam





Moreno-González et al., 2015. Environmental Research, 138, 326-344.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SAMPLING BIOMONITORING IN MAR MENOR LAGOON

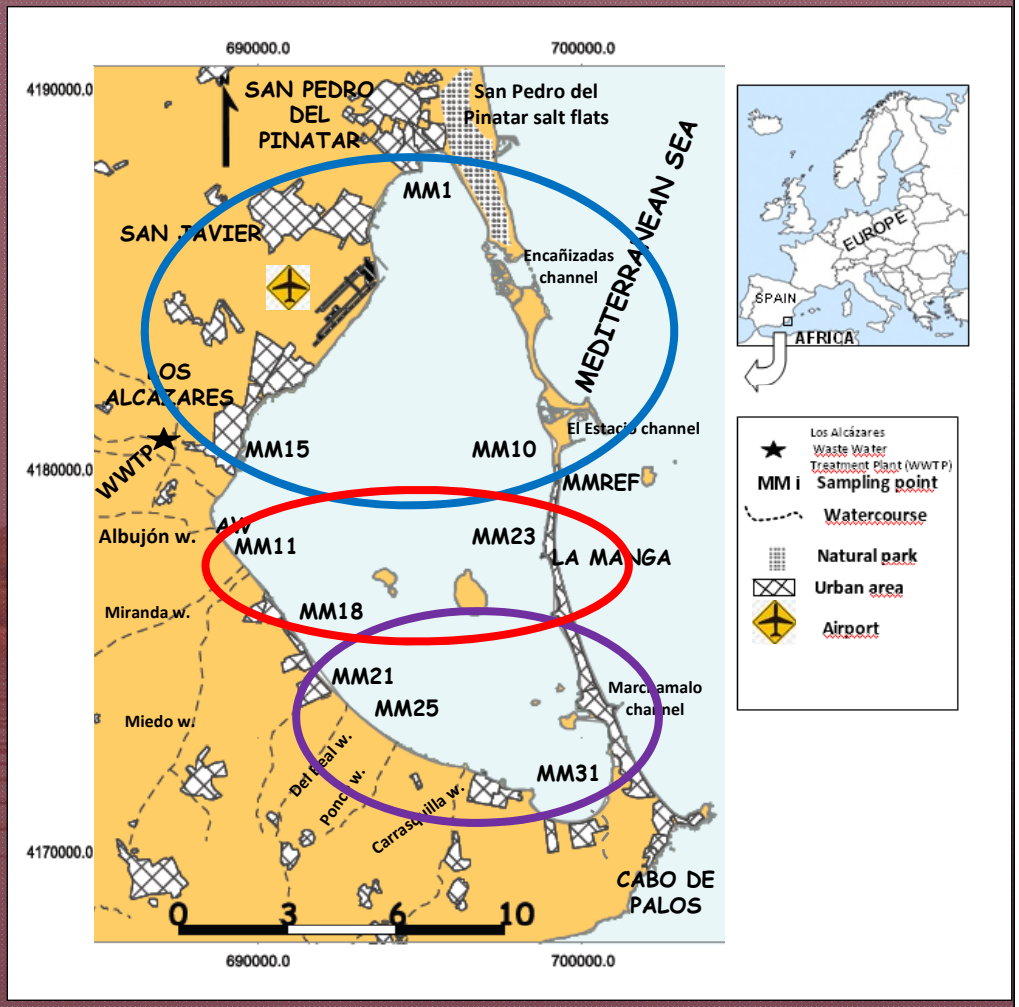
Sampling period: spring and autumn 2010

WILD BIOTA

SPATIAL DISTRIBUTION

9 sampling areas (north,central and south)

+ External reference. (Mediterranean Sea)



Location of sampling areas in the Mar Menor lagoon for wild species (MMi) and for clam field exposure (Si) in spring and autumn.

SAMPLING BIOMONITORING IN MAR MENOR LAGOON

Target species:

Molluscs

Cockle
Cerastoderma glaucum

Whole soft tissues

Noble pen shell
Pinna nobilis

Sea snail
Murex trunculus

Fish

Muscle and liver

Golden grey mullet
Liza aurata

Whole specimens

Black goby
Gobius niger

SAMPLING BIOMONITORING IN MAR MENOR LAGOON

Specimens obtained in spring and autumn sampling campaigns for each species

	Cerastoderma glaucum n= 50-150		*Pinna nobilis* n= 1		*Murex trunculus* n= 10-15		*Liza aurata (muscle)* n= 1-5		*Liza aurata (liver)* n= 1-5		*Gobius niger* n= 8-51	
Code	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
MM1	X*	X*			X	X	X*		X		X	X
MM10	X*		X	X*	X	X	X*	X	X*	X	X	
MM11				X	X	X	X*	X*	X	X	X	X
MM15	X						X	X*	X	X	X	X
MM18	X*	X*				X	X*	X*	X	X	X	
MM21					X		X*		X*			
~~MM23~~			X*	X	X	X	X	X	X	X	X	
MM25	X*	X				X		X*		X		
MM31	X	X			X	X	X	X	X	X	X	
MM REF			X*	X*			X		X			
Albujon Watercourse							X	X	X	X		
Avge. Large length (cm)	2.1	2.3	35.5	36.1	5.3	5.5	16.4	12.3			8.2	8.4
Avge. Small length (cm)	1.7	1.7					25.4	24.7				
Avge. Small lipid content (d.w. %)									20.1	48.5	1.6	1.8
Avge. Large lipid content (d.w. %)			8.8	9	43.4	46.1			29.9	37.8		
Avge. REF Large length (cm)**			43.5	32.5								
Avge. REF Small length (cm)**								19.5				
Avge. REF Large lipid content (d.w. %)**			6.2	8.2								
Avge. REF Small lipid content (d.w. %)**												

SAMPLING BIOMONITORING IN MAR MENOR LAGOON

Sampling period: spring and autumn 2010

WILD BIOTA

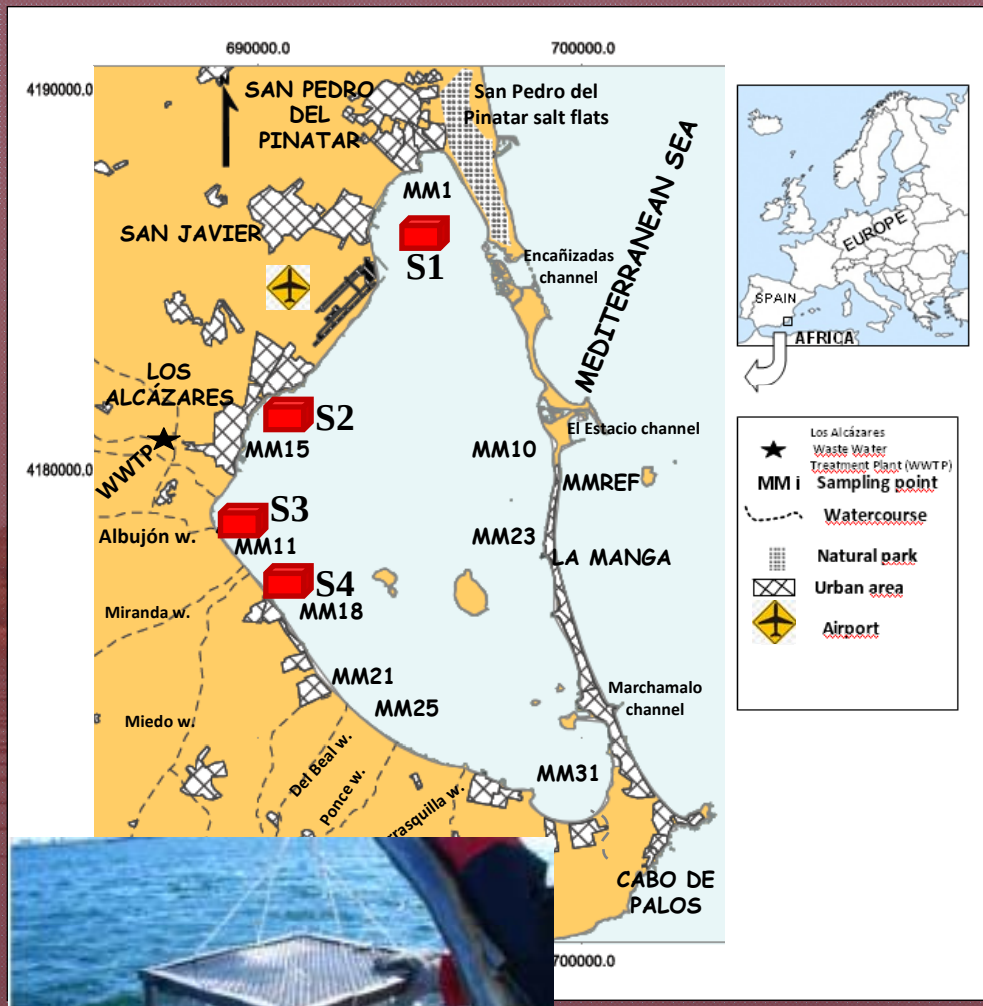
SPATIAL DISTRIBUTION

9 sampling areas (north,central and south)

+ External reference. (Mediterranean Sea)

ACTIVE BIOMONITORING

Clams transplanted from a less polluted area and immersed in stainless steel cages at four sites (S1, S2, S3 and S4).
Concentrations determined at t=0, 7 and 21 days of exposure.



...eas in the Mar Menor (MMi) and for clam ...ing and autumn.

ANALYSIS OF PHARMACEUTICALS: SPE and UPLC/MS/MS

BIOTA EXTRACTION

- Pressurized liquid extraction (PLE).
  - -1g of free-dry biota (muscle and molluscs).
  - -0.5g of free-dry liver.
  - -100% MeOH, Tª 50ºC, 4 cycles of 5min.

CLEAN-UP

- Gel permeation chromatography (GPC).
  - -DCM/MeOH (90:10, $v \cdot v^{-1}$), 5 mL min^{-1} flow rate.

ANALYSIS

- Ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS).
  - -Electrospray Ionization (ESI) positive and negative mode.

Method proposed for 20 pharmaceuticals analysis in fish tissues (Huerta et al., 2013) was adapted for molluscs.

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ANALYSIS OF PHARMACEUTICALS: SPE and UPLC/MS/MS

Molluscs	CLAM		COCKLE	SEA SNAIL		NOBLE PEN SHELL	
	Recovery (%) (n=3)	LOQ (ng g^{-1})	LOQ (ng g^{-1})	Recovery (%) (n=3)	LOQ (ng g^{-1})	Recovery (%) (n=3)	LOQ (ng g^{-1})
β-blockers							
Atenolol	35.0	1	0.5	20.4	1.37	28.7	1.3
Carazolol	77.6	0.2	0.1	<20	1.2	46.1	0.1
Metropolol	49.6	0.1	0.1	21.5	0.9	36.8	0.02
Nadolol	51.8	0.2	0.2	26.5	0.4	48.9	0.2
Propanolol	74.7	0.5	0.6	35.8	4.4	66.2	0.6
Sotalol	57.4	4.1	2.4	<20	1.4	36.4	1
Psychiatric drugs							
Carbamazepine	85.3	0.3	0.1	66.7	0.5	76.9	0.1
Citalopram	34.0	0.3	0.1	<20	0.3	22.9	0.2
Diazepam	68.9	0.1	0.6	63.0	0.7	67.9	0.1
10,11-EpoxyCBZ	36.5	0.3	0.2	30.2	1.2	30.5	0.3
2-HydroxyCBZ	47.9	0.2	0.1	45.9	0.6	47.3	0.2
Lorazepam	<20	4.7	3.6	20.0	0.3	35.5	3.9
Sertraline	88.7	19.9	19.2	40.3	13.4	125.5	19.6
Venlafaxine	66.0	0.1	0.2	40.1	0.99	50.6	0.07
Antiplatelet agent							
Clopidrogel	57.3	0.02	0.3	39.3	0.3	151.9	0.3
Analgesics/anti-inflammatories							
Codeine	41.9	0.04	0.4	27	0.3	28.4	0.04
Diclofenac	<20	0.1	0.2	<20	2.5	20.0	0.3
Diuretic							
Hydrochlorothiazide	89.3	0.001	0.05	83.7	1.1	87.3	0.02
Antihelmintics							
Levamisole	51.2	0.1	0.1	41.5	0.3	31.2	0.1
To treat asthma							
Salbutamol	29.5	0.1	0.3	23.5	0.7	41.5	0.4

Lower extraction for sea snail than for bivalves

Recovery > 50% (many cases) and >30% (low SD also considered)

Moreno-González et al., 2016. Env. Research 146, 282-298

ANALYSIS OF PHARMACEUTICALS: SPE and UPLC/MS/MS

Fish: Golden grey mullet	MUSCLE		LIVER	
	Recovery (n=3) (%)	LOQ (ng g^{-1})	Recovery (n=3) (%)	LOQ (ng g^{-1})
β-blockers				
Atenolol	27.4	0.5	117.4	1
Carazolol	46.9	0.2	192.2	0.7
Metropolol	31.4	0.6	91.0	2.0
Nadolol	37.7	0.3	133.7	0.4
Propanolol	53.6	0.4	44.2	0.4
Sotalol	20.3	0.6	121.3	6.2
Psychiatric drugs				
Carbamazepine	82.6	0.3	43.0	0.3
Citalopram	<20	0.6	136.4	1.7
Diazepam	81.5	0.2	51.2	2.0
10,11-EpoxyCBZ	32.8	0.2	47.0	0.4
2-HydroxyCBZ	59.9	0.2	80.0	0.8
Lorazepam	25.4	2.7	139.7	7.5
Sertraline	103.8	2.6	97.2	22.1
Venlafaxine	22.9	0.09	101.5	0.5
Antiplatelet agent				
Clopidrogel	72.9	0.1	95.6	0.6
Analgesics/anti-inflammatories				
Codeine	26.3	0.3	195.8	2.9
Diclofenac	37.6	0.4	60.0	0.4
Diuretic				
Hydrochlorothiazide	92.1	0.05	20.0	0.2
Antihelmintics				
Levamisole	<20	0.1	200.0	0.5
To treat asthma				
Salbutamol	38.3	0.3	109.8	0.6

Higher recoveries in liver than in muscle

Recoveries higher than 180% due probably to matrix effects

Moreno-González et al., 2016. Env. Research 146, 282-298

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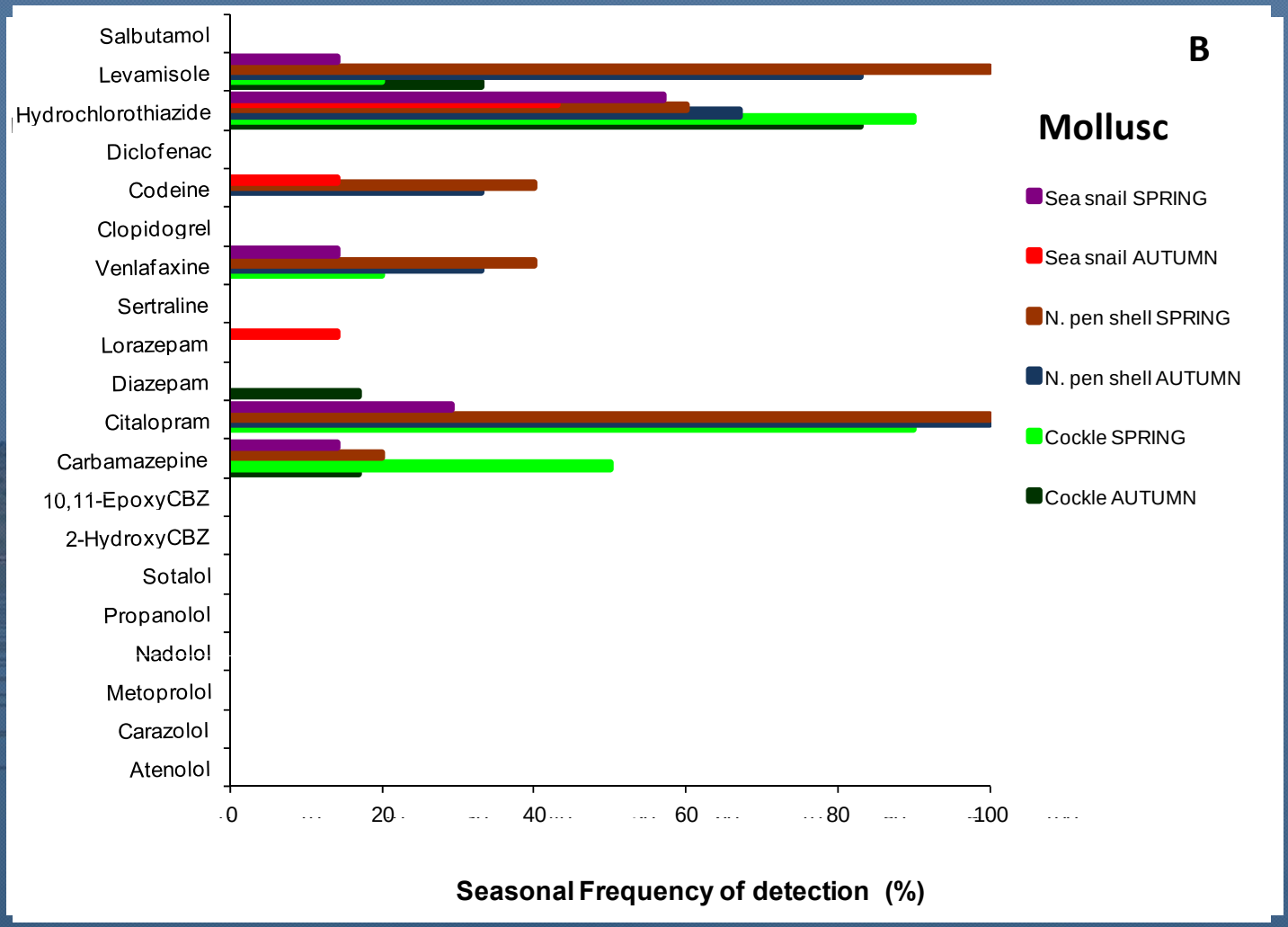
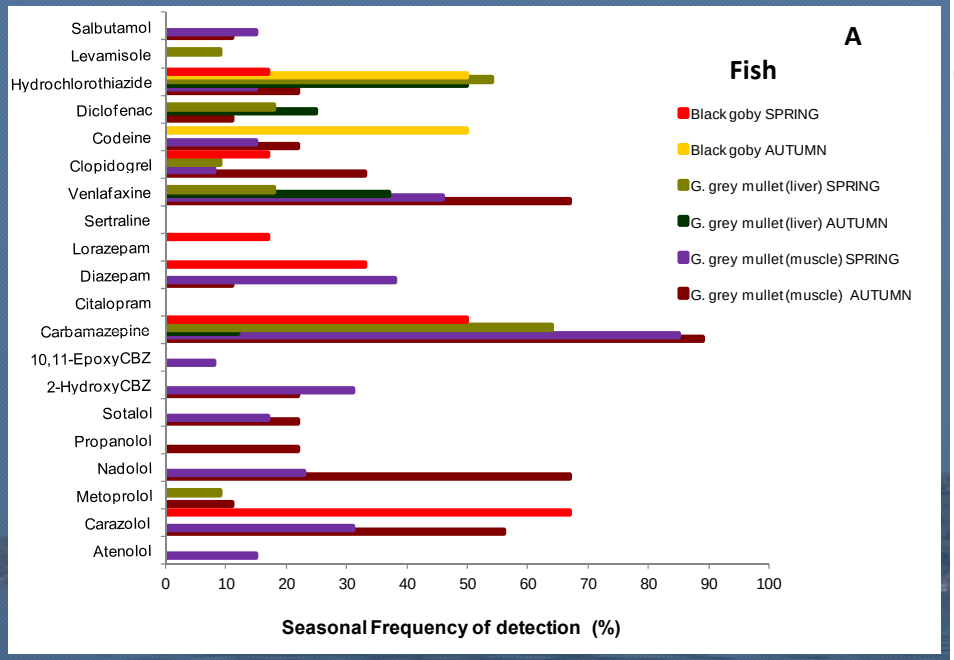
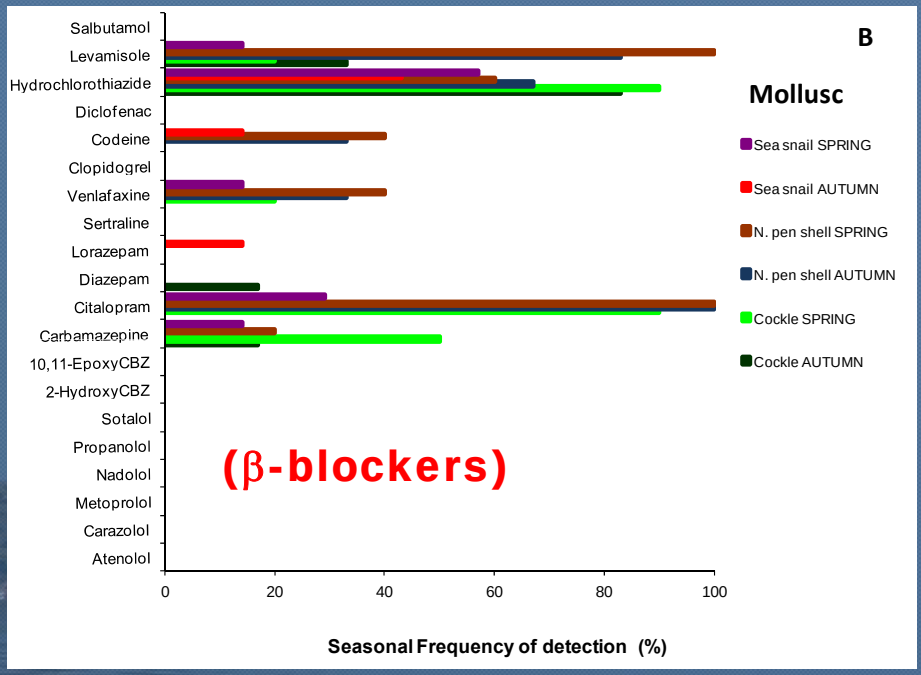
PHARMACEUTICALS DETECTED IN WILD BIOTA



Seasonal frequency of detection (% of total samples)

PHARMACEUTICALS DETECTED IN WILD BIOTA





Seasonal frequency of detection (% of total samples)

18 out of the 20 pharmaceuticals were found at low ng g^{-1} .

Hydrochlorothiazide and carbamazepine were detected in all species considered.

More pharmaceuticals were detected in fish (18) than in wild molluscs (10), particularly in golden grey mullet muscle (16).

PHARMACEUTICALS IN MOLLUSCS

Compound	Caged Clams		Wild Bivalves				Wild Gastropod	
	Clam		Cockle		Noble pen shell		Sea snail	
	LOQ (%)	Maximum	LOQ (%)	Maximum	LOQ%	Maximum	LOQ (%)	Maximum
β-blockers								
Atenolol	0	n.d.	0	n.d.	-	-	0	n.d.
Carazolol	0	b.q.l.	0	n.d.	0	n.d.	-	-
Metropolol	0	n.d.	0	n.d.	0	n.d.	0	n.d.
Nadolol	0	n.d.	0	n.d.	0	n.d.	0	n.d.
Propanolol	5	0.3 (S1)	0	n.d.	0	n.d.	0	n.d.
Sotalol	0	n.d.	0	n.d.	0	n.d.	-	-
Psychiatric drugs								
Carbamazepine	17	0.7 (S2)	15	1.5	10	0.2	7	2.3 (MM11)
Citalopram	17	0.5 (S3)	45	2.3	-	-	-	-
Diazepam	0	n.d.	0	b.q.l.	0	n.d.	0	n.d.
10,11-EpoxyCBZ	0	n.d.	0	n.d.	0	n.d.	0	n.d.
2-HydroxyCBZ	0	n.d.	0	n.d.	0	n.d.	0	n.d.
Lorazepam	-	-	-	-	0	n.d.	-	-
Sertraline	0	n.d.	0	0	0	n.d.	0	n.d.
Venlafaxine	5	0.3 (S2)	22	1.1	37	2.7 (MM9)	7	0.4 (MM11)
Antiplatelet agent								
Clopidrogel	0	n.d.	0	n.d.	0	n.d.	0	n.d.
Analgesics/anti-								
Codeine	0	n.d.	0	n.d.	-	-	-	-
Diclofenac	-	-	-	-	-	-	-	-
Diuretic								
Hydrochlorothiazide	100	1.8 (S3)	87	1.6	63	3.2	36	1.8 (MM1)
Antihelmintics								
Levamisole	0	n.d.	8	0.2	91	2.1 (MM9)	9	0.5 (MM9)
To treat asthma								
Salbutamol	-	-	-	-	0	n.d.	0	n.d.

Moreno-González et al., 2016, Environ. Res. 146, 282-298.

PHARMACEUTICALS IN MOLLUSCS

Detected in all molluscs:

Psychiatric drugs (carbamazepine, c
hydrochlorotiazide (diuretic) and levan

Most ubiquitous compounds:

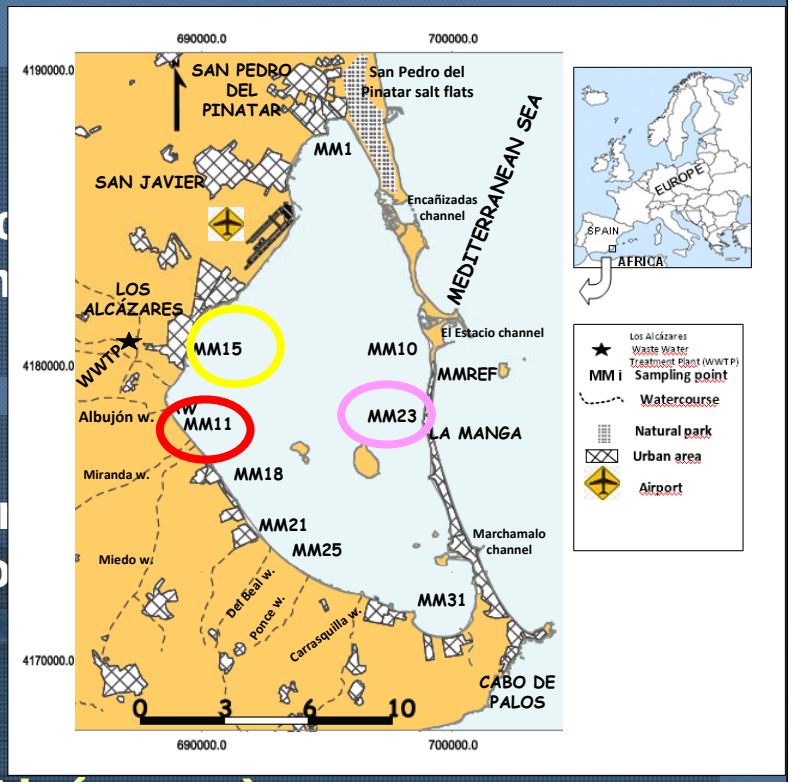
Hydrochlorothiazide in cockle ar
Citalopram and levamisole in no

Highest concentrations:

Citalopram (2.3 ng g^{-1}) in cockle (Los Alcázares)

Hydrochlorotiazide (3.2 ng g^{-1}) in noble pen shell (MM23)

Carbamazepine (2.3 ng g^{-1}) in sea snail (MM11)



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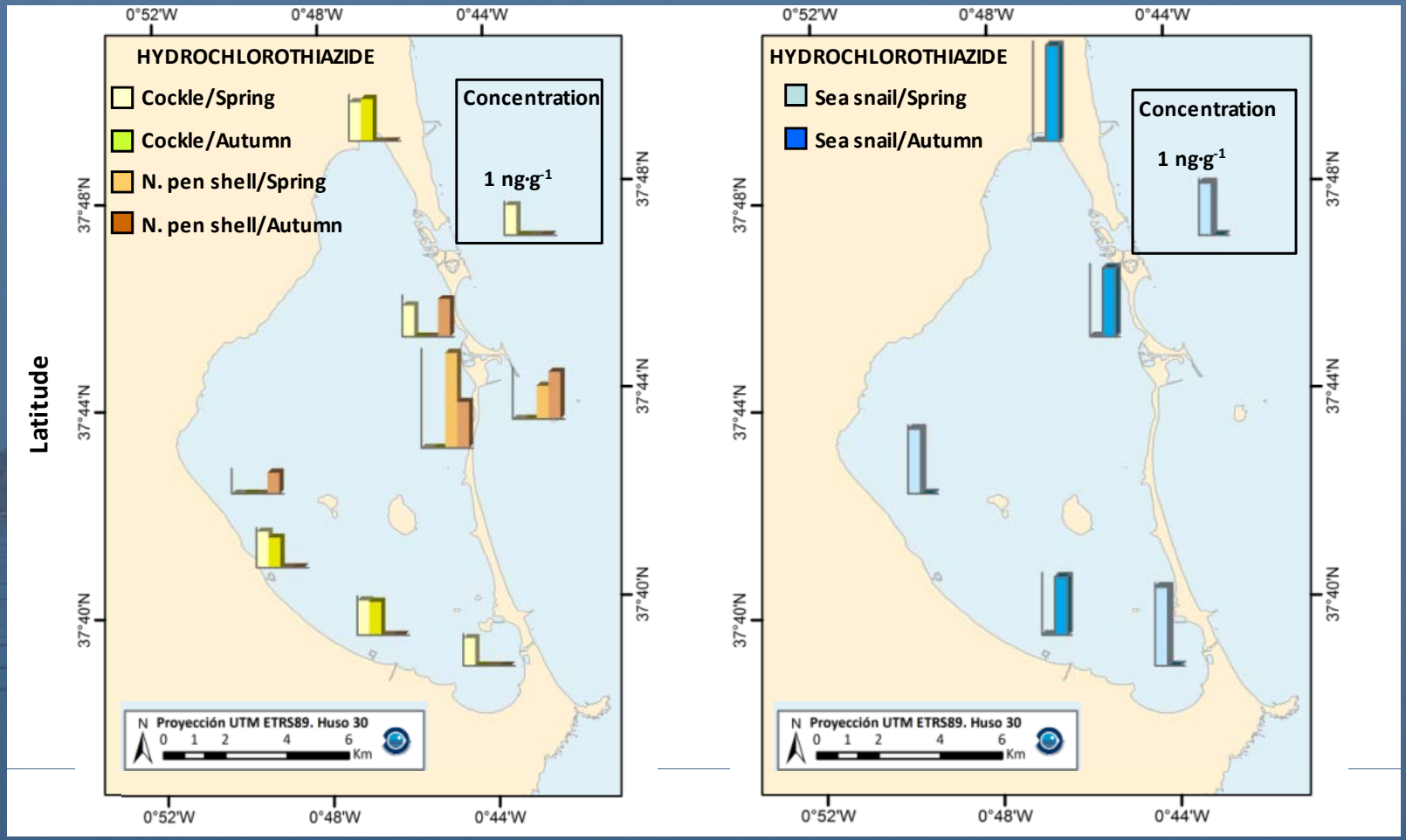
SPATIAL DISTRIBUTION OF PHARMACEUTICALS IN MOLLUSCS



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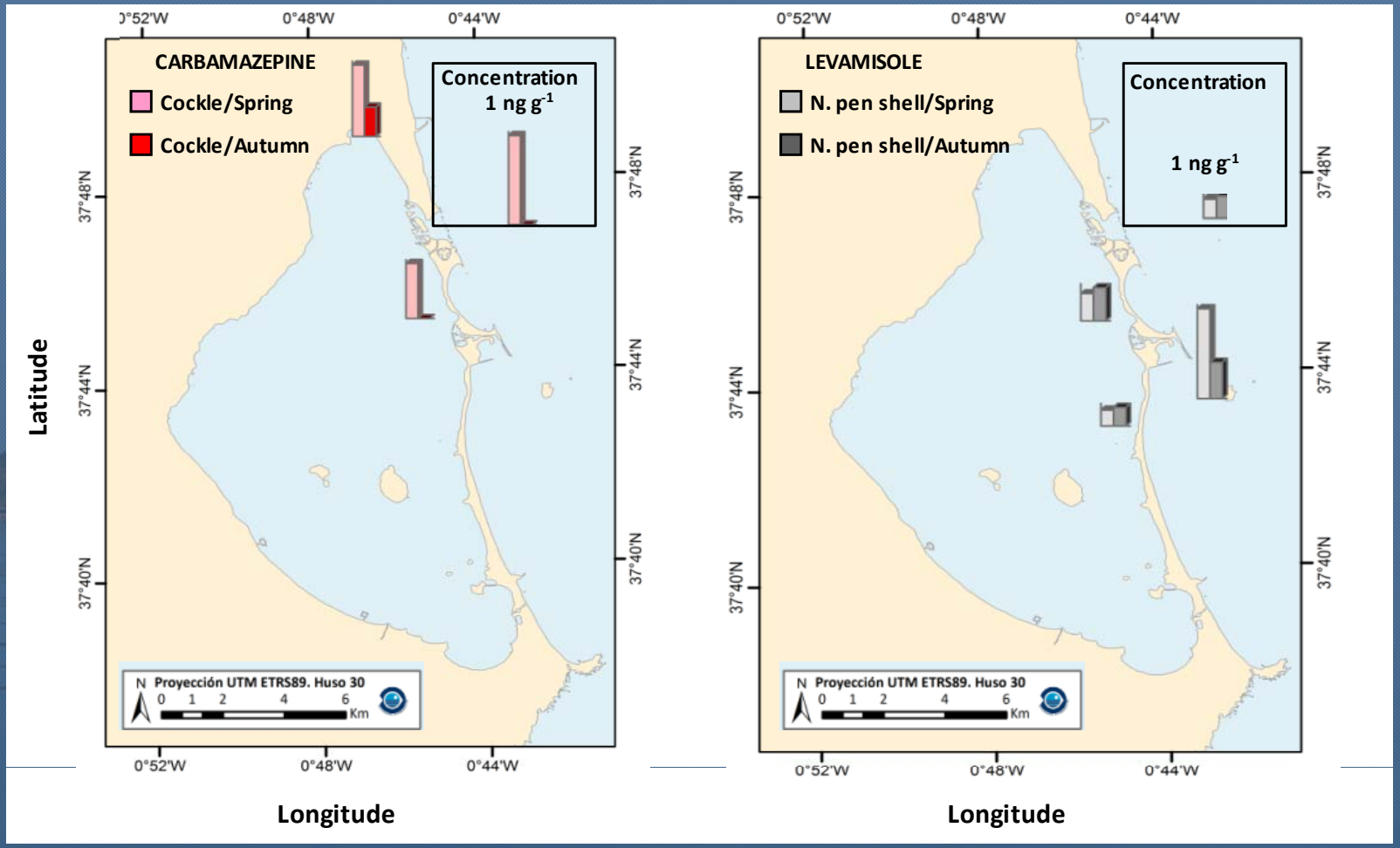


Heterogeneous distribution: several sources also confirmed (more compounds found in north area but higher concentrations in south)

Moreno-González et al., 2016, Environ. Res. 146, 282-298.

PHARMACEUTICALS IN FISH

Compound	Wild Fish					
	G.g. Mullet (liver)		G.g. Mullet (muscle)		Black goby	
Compound	LOQ (%)	Maximum	LOQ (%)	Maximum	LOQ (%)	Maximum
β-blockers						
Atenolol	0	n.d.	-	-	-	-
Carazolol	0	n.d.	22	1.7	0	b.q.l.
Metropolol	0	b.q.l.	5	0.7	0	n.d.
Nadolol	0	n.d.	28	0.6	0	n.d.
Propanolol	0	n.d.	11	0.5	0	n.d.
Sotalol	0	n.d.	-	-	-	-
Psychiatric drugs						
Carbamazepine	33	2.6	83	6.3	1	0.4
Citalopram	0	n.d.	-	-	-	-
Diazepam	0	n.d.	15	1.8	1	3.5
10,11-EpoxyCBZ	0	n.d.	5	0.2	0	n.d.
2-HydroxyCBZ	0	n.d.	4	0.3	0	n.d.
Lorazepam	0	n.d.	-	-	-	-
Sertraline	0	n.d.	0	n.d.	0	n.d.
Venlafaxine	22	3.1	-	-	-	-
Antiplatelet agent						
Clopidrogel	0	b.q.l.	5	0.2	0	n.d.
Analgesics/anti-						
Codeine	0	n.d.	-	-	-	-
Diclofenac	22	2.2	5	1.3	0	n.d.
Diuretic						
Hydrochlorothiazide	-	-	19	10.5	25	3.9
Antihelmintics						
Levamisole	9	0.5	-	-	-	-
To treat asthma						
Salbutamol	0	n.d.	5	0.6	0	n.d.

Higher number of pharmaceuticals in golden grey mullet than in bivalves.

Muscle better tissue than liver (higher concentration but low number of detections)

Mugilids as sentinel organisms for pharmaceuticals.

Moreno-González et al., 2016, Environ. Res. 146, 282-298.

PHARMACEUTICALS IN FISH

Detected in both fish species:

Psychiatric drugs (carbamazepine, citalopram, diazepam), codeine (analgesic), carazolol (β-blockers), hydrochlorotiazide (diuretic) and levamisole (antihelmintic).

Lorazepam was only found in black goby
Atenolol was only found in golden grey mullet

Most ubiquitous compounds:

Carbamazepine and venlafaxine in mugilid muscle

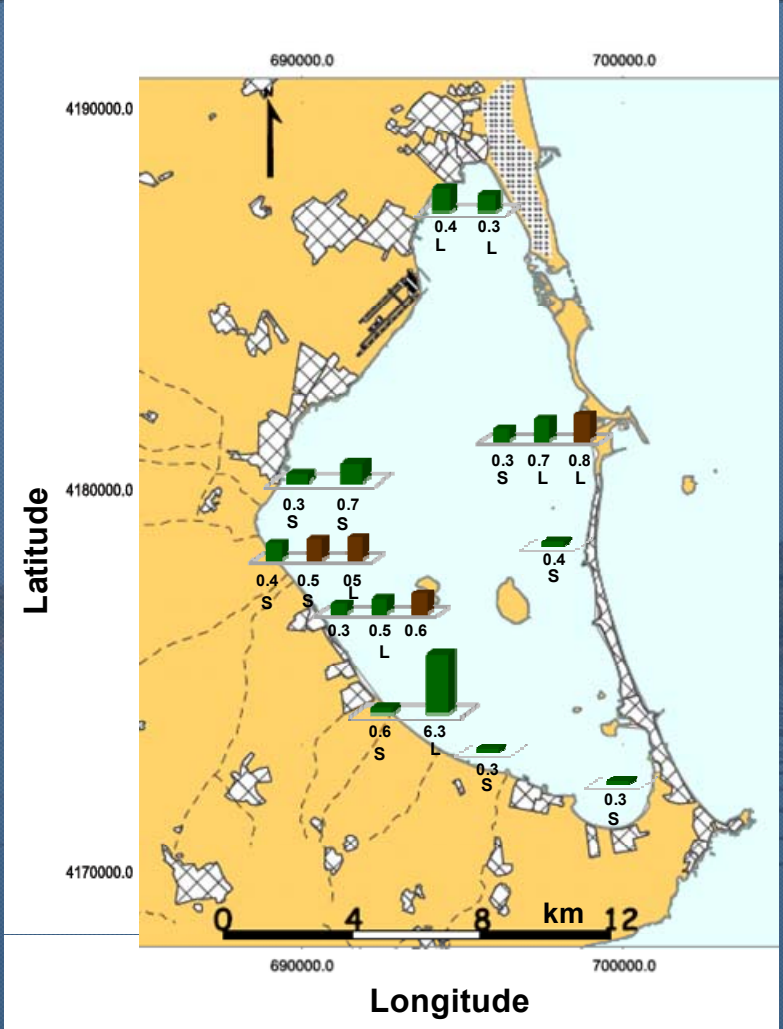
Highest concentrations:

Carbamazepine(6.3 ng g^{-1}) in G.g. mullet muscle (MM21)

Hydrochlorotiazide (3.9 ng g^{-1}) in black goby (MM23)

Moreno-González et al., 2016, Environ. Res. 146, 282-298.

SPATIAL DISTRIBUTION OF PHARMACEUTICALS IN FISH



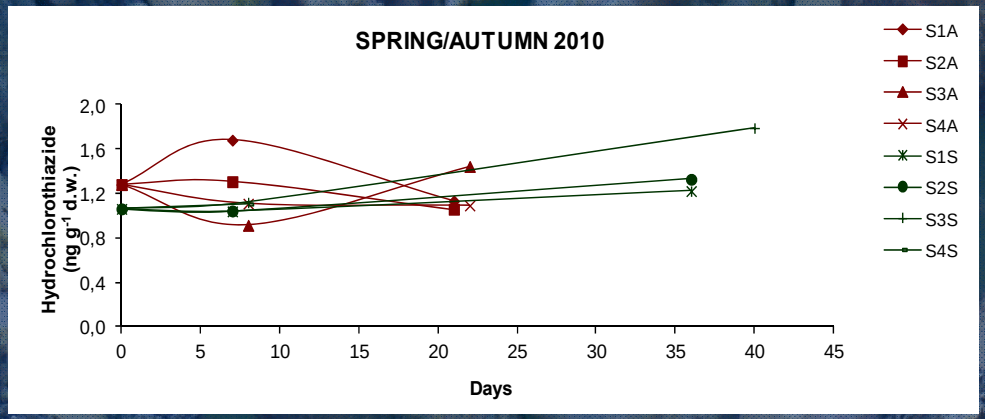
Concentration of carbamazepine in golden grey mullet (μg·kg^{-1} d.w.) in spring and autumn 2010 for small (S) and large specimens (L).

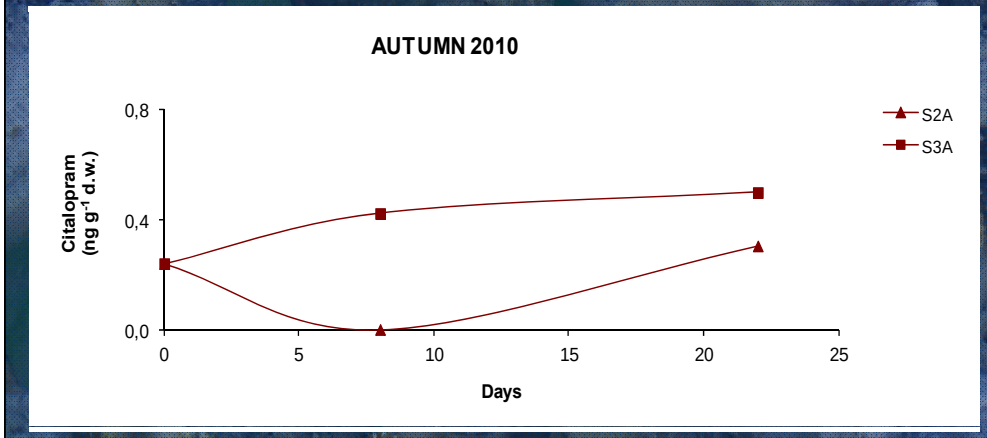
Psychiatric drugs in golden grey mullet were preferentially found in spring.

Concentrations of propanolol and nadolol in muscle were higher in autumn than in spring ($p<0.05$).

Only two pharmaceuticals were found in g.g. mullets from Mediterranean area (lower exposition).

ACTIVE BIOMONITORING: CAGED CLAMS





HPLC

Slight bioaccumulation of hydrochlorothiazide was also confirmed in caged clams in the influence area of El Albujón watercourse (S3/S4).

Longer exposition period was probably required to assess bioaccumulation of pharmaceuticals more properly.

DO PHARMACEUTICALS BIOACCUMULATE IN MARINE MOLLUSCS AND FISHES FROM A COASTAL LAGOON?

INTRODUCTION

CONTAMINANTS OF EMERGING CONCERN: IMPACTA PROJECT

MAR MENOR LAGOON: PREVIOUS KNOWLEDGE

MATERIAL AND METHODS

PHARMACEUTICALS ANALYSIS IN BIOTA

SAMPLING CAMPAINGS AND SELECTED SPECIES

RESULTS AND DISCUSSION

ADAPTATION OF PHARMACEUTICAL ANALYSIS FOR BIVALVES

PHARMACEUTICAL BIOACCUMULATION IN NATIVE SPECIES

ACTIVE BIOMONITORING (TRANSPLANTED SPECIMENS)

CONCLUSIONS

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CONCLUSIONS

-The distribution of pharmaceuticals in wild organisms was heterogeneous in the lagoon, higher concentrations close to wastewater discharges or other pollution sources.

-Psychiatric drugs preferentially bioaccumulated in fish muscle, while citalopram did so in molluscs. Carbamazepine and hydrochlorothiazide were detected in all species in this study.

-The bioaccumulation of pharmaceuticals was lower in sea snail than in bivalves, and in black goby than in golden grey mullet. Bioaccumulation of hydrochlorothiazide was also confirmed in caged clams.

-The psychiatric drugs in all species were preferentially found in spring.

- Those results suggest that mugilids could be used as an indicator of contamination by pharmaceuticals in coastal areas.

Acknowledgements

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IMPACTA

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European Regional Development Fund

Generalitat de Catalunya (Consolidated Research Group: Catalan Institute for Water Research 2014 SGR 291).

Other results (posters): MPs in red mullet stomach(MO073) and toxicity (MOPC13)

CUPs in interstitial water (MO001)

Sediment toxicity (MO190)

http://www.impacta2014.blogspot.com.es/

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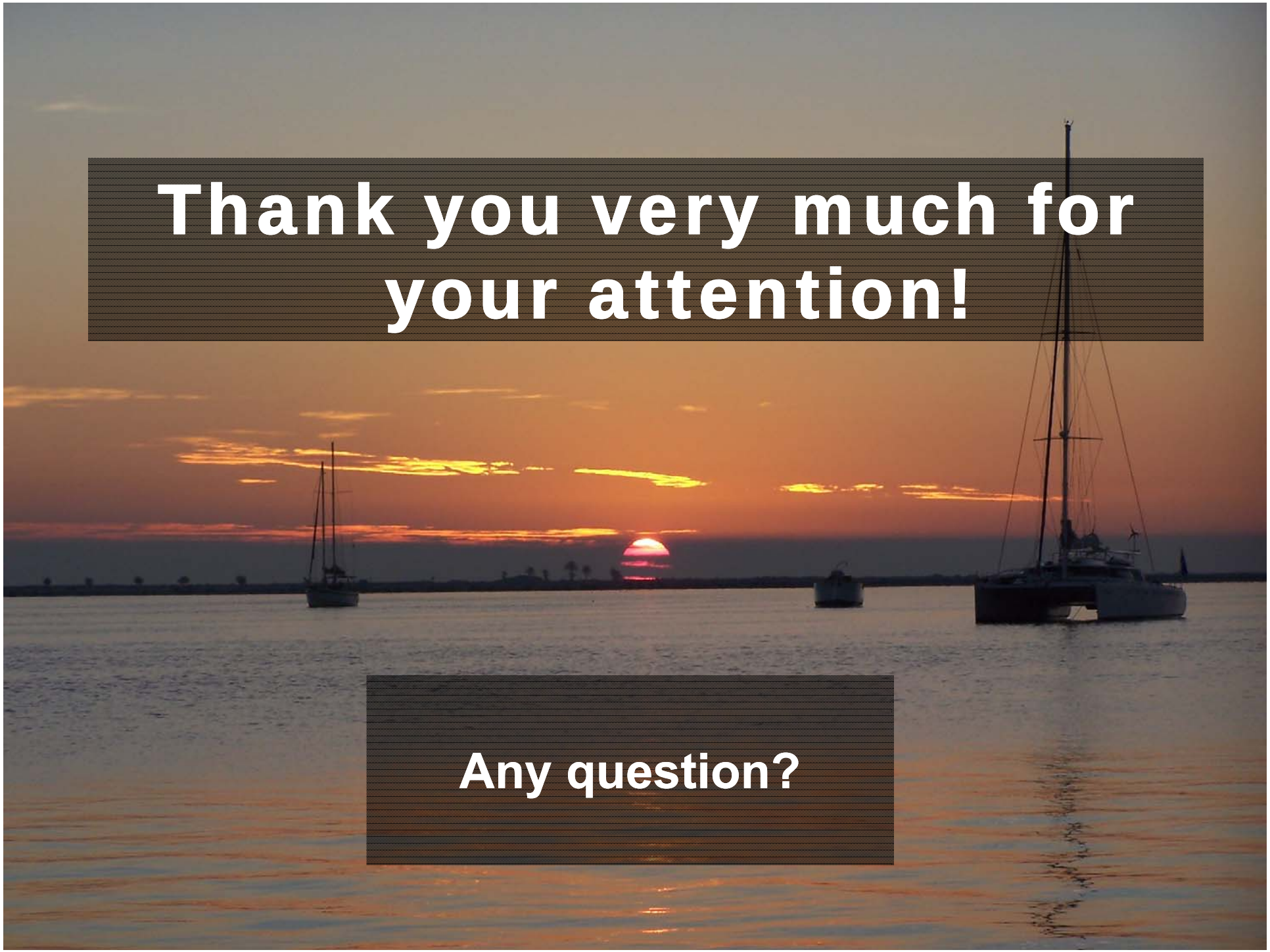
Thank you very much for your attention!
Any question?