WP 3 *Ecosystem and contaminants*

IAMC-CNR

Angela Cuttitta, Armeri G.M., Biondo G., Bennici C., Di Natale M., Maneiro I., Masullo T., Monastero L., Musco M., Nicosia A., Patti C., Tagliavia M., Torri M.

In collaboration with: UniPa DiSTEM, IFC-CNR

Mechanisms of toxicity in marine animals, exerted by conventional and emerging pollutants in SINs

Issues:

- ✓ **Dynamic of biodiversity**
- ✓ **Alteration of transcriptional expression of genes in selected species associated to chemical "defensome"**
- ✓ **Transcriptome and epigenetic modifications in response to pollutants**

***In vivo*, *in vitro*, *in silico* assay**

Task 1
Evaluation of the status of biodiversity in Sins

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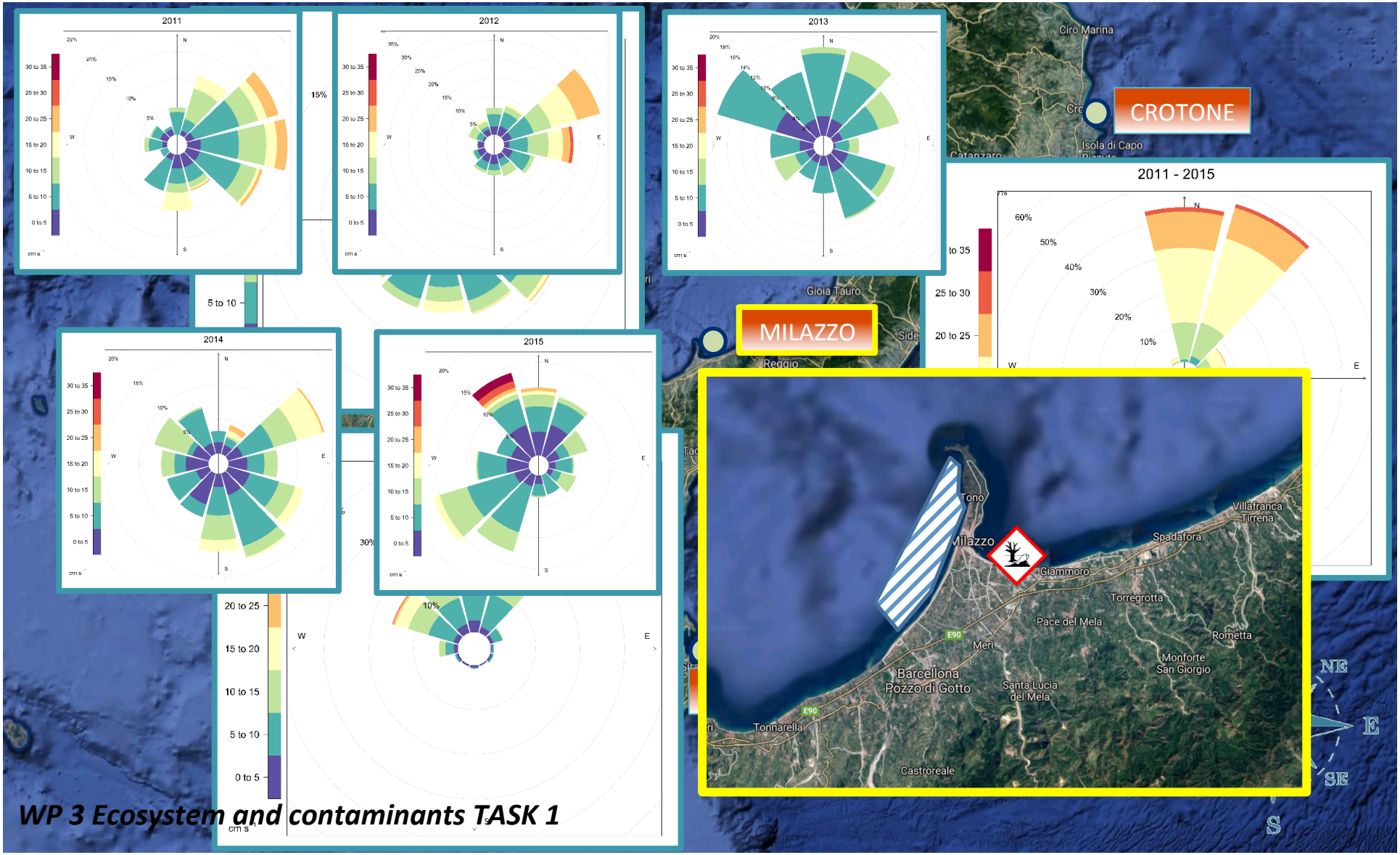
The loss of biodiversity linked to marine and lagoon ecosystem represents a direct effect of pollutants present in the environment. Thus, the evaluation of biodiversity during the 4 seasons will be carried out. The three study areas will be investigated by visual census transects for nekton sampling method for benthos and plankton communities.
Ecological indices: species richness and population density.

Sub Task 3.1 Early surveys, standardization of protocol for biodiversity evaluation

Sub Task 3.2 Ecological surveys during the four seasons

ASSESSMENT OF **CURRENT PATTERNS** IN THE STUDY AREAS TO IDENTIFY **BLANK POINTS**



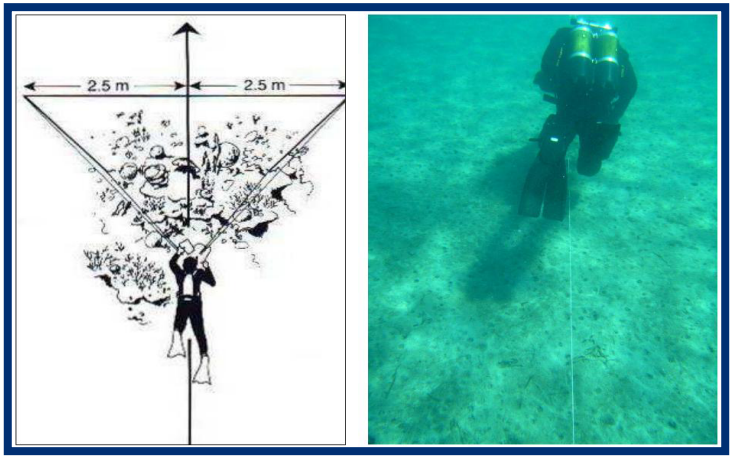
EVALUATION OF THE STATUS OF BIODIVERISTY

ASSESSMENT OF THE **NECTONIC** COMPONENT

Underwater Visual Census (UVC)

HARMELIN-VIVIEN *ET AL.*, 1985

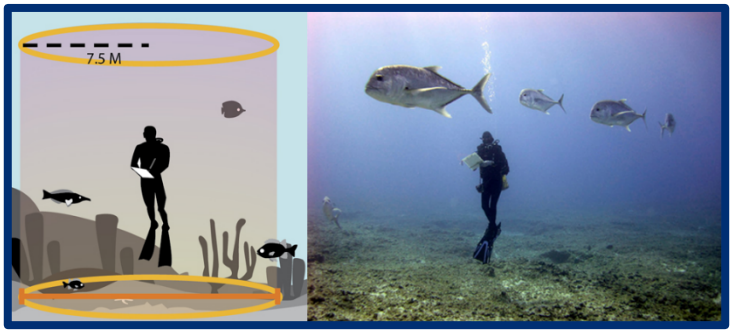
WIDESPREAD AND ACCEPTED METHODOLOGY BY THE SCIENTIFIC COMMUNITY IN THE FRAMEWORK OF FISH POPULATION STUDIES



Belt transect

CHEAP;
FAST AND SIMPLE REPLICABILITY;
NON-INVASIVE METHOD;
POSSIBILITY TO REFER FISH COUNTS TO SURFACE (BELT TRANSECT) OR VOLUME (STATIONARY POINT) UNIT.

OBJECTIVE: COLLECT **ABUNDANCE**, **SIZE CLASS** AND **SPECIES RICHNESS** DATA OF FISH POPULATION



Stationary point

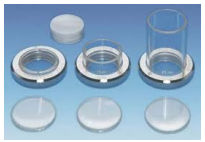
EVALUATION OF THE STATUS OF BIODIVERISTY

ASSESSMENT OF THE **PLANKTON** AND **BENTHIC** COMPONENT

Seasonal biodiversity among SINs and comparative analyses with control areas

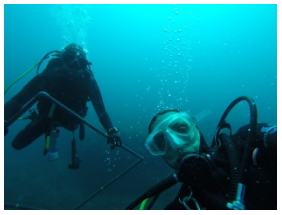
Ordinance 260/2010 “Stato Corpi Idrici Superficiali” (ISPRA “Metodologie Studio Plancton Marino”)
Ordinance 190/2010 208/56/CE Strategies for Marine Environment

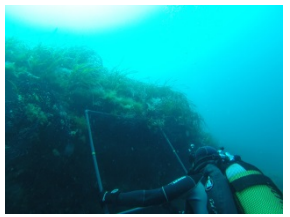
Fitoplankton:
Niskin bottle, 2 m depth
Count on Utermöhl chamber



Zooplankton:
Net WP2 56 µm mesh
flowmeter,
oblique recovery
Count on Bolgorov chamber

Benthos:
Diving on hard and soft bottoms . Video tape footage and photographs, scraping and coring to identify the biotic communities





Task 2

Ecotoxicological and biochemical analyses *in vitro*

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Ecotoxicological and biochemical investigations at molecular and cellular level in fish cell lines in order to identify biomolecular markers useful as sensors and early warning systems.

Sub Task 2.1 In vitro tests on fish cell cultures: antioxidant / pro-oxidant switch, dose-time dependent toxicity, biomarkers of oxidative stress; cell cycle modulation; cell survival/ apoptosis, markers of inflammation and angiogenesis

Sub Task 2.2 biochemical markers in marine "model" organisms: stress proteins modulated by contaminants/pollutants (metallothionein MTs, heat shock protein Hsp, HIFs), oxidative stress markers (ROS, malondialdehyde MDA, HNE,PUFAs), apoptosis - stress-related pathways

Definition of molecular sensors and concentrations for early-warning

Task 3

The use of model organisms to unveil novel toxicity mechanisms

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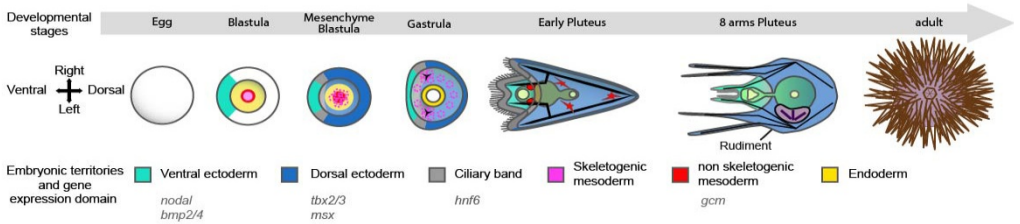
Based on critical issues recognized in the SINs, possible mechanisms involved in emergent diseases will be investigated in aquatic model systems at developmental and molecular level

Sub Task 3.1 Finding and housing of animals and pollutants
Sub Task 3.2 Animal exposure to chemicals in mesocosm
Sub Task 3.3 Animal exposure to sea water from SINs
Sub Task 3.4 Animal housing in SINs
Sub Task 3.5 Molecular analyses

Phenotypical and molecular effects of the exposure to pollutants



P. lividus

D. rerio

D. labrax

Gene Regulatory Networks

Development, Specification, Axes patterning

Exploiting the Gene expression pattern of master regulators

Aberrant embryo development in response to elicitor

		Mytilus spp.	*P. lividus*	*D. rerio*	*O. vulgaris*	*D. labrax*
BTEX			X	X		X
Fluoranthene		X		X	X	X
Benzo(a)pyrene		X		X	X	X
Fluoranthene + Benzo(a)pyrene		X		X	X	X
PBDE 209		X	X	X	X	
PBDE 209 +Pb/Zn		X	X	X	X	
PAHs mix					X	
PAHs+ Cd		X	X		X	
SW Augusta – Priolo		X	X		X	
SW Milazzo		X	X		X	
SW Crotone		X	X		X	
				X	X	X
		X	X		X	
PCB Congener mix		X	X		X	
		X	X		X	
		X	X		X	
Dioxins (TCDD)		X			X	X
Heavy metals	Pb					X
	Cd					X
	Hg					Methyl-Hg
	Cr					
	Zn					
	As		X			X
TiO_2		+Cd			+Cd	
TBT			X			
Total Metal mix		X		X	X	X

Table 1. Arsenic exposure and global DNA methylation.

Model	Arsenical	Global DNA methylation	References
Human cells			
Prostate epithelial cell line RWPE-1	As^{III}	Hypo	Coppin et al. 2008
Prostate epithelial cell line RWPE-1	As^{III}	Hypo	Benbrahim-Tallaa et al. 2005
HaCaT keratinocytes	As^{III}	Hypo	Reichard et al. 2007
Animal cells			
TRL 1215 rat liver epithelial cell line	As^{III}	Hypo	Zhao et al. 1997
V79-Cl3 Chinese hamster cells	As^{III}	Hypo	Sciandrello et al. 2004
Animal studies			
Goldfish	As^{III}	Hypo	Bagnyukova et al. 2007
Fisher 344 rat	As^{III}	Hypo	Uthus and Davis 2005
129/SvJ mice	As^{III}	Hypo	Chen et al. 2004
C3H mice	As^{III}	Hypo	Waalkes et al. 2004
C57BL/6J mice	As^{III}	Hypo	Okoji et al. 2002
Homozygous Tg.AC mice	As^{III}	Hypo	Xie et al. 2004
	As^{V}		
	MMA^{V}		
	DMA^{V}		
Human subjects			
	As^{III}	Hyper	Pilsner et al. 2007; Majumdar et al. 2010
	As^{III}	Hypo (in skin lesion patients)	Pilsner et al. 2009

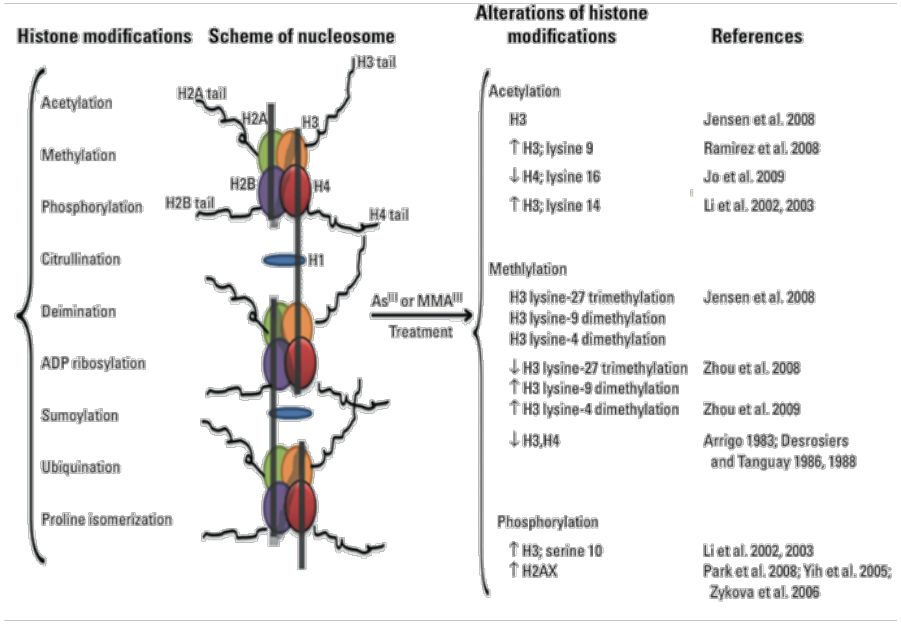


Table 2. Arsenic exposure and gene-specific (promoter) methylation status.

Mode	Arsenical	Dose	Time (weeks)	Genes: Hyper	Genes: Hypo	Reference
Human cells						
UROtsa urothelial cells	As^{III}	1 μM	9	*DBC1, FAM83A, ZSCAN12, C1QTNF6*		Jensen et al. 2008
	MMA^{III}	50 nM				
Uroepithelial SV-HUC-1 cells	As^{III}	2, 4, 10 μM	24 or 52	*DAPK*		Chai et al. 2007
Myeloma cell line U266	As^{III}	1, 2 μM	0.4	*P16*		Fu and Shen 2005
Lung adenocarcinoma A549 cells	As^{III}	0.08–2 μM	0.3	*P53*		Mass and Wang 1997
	As^{V}	30–300 μM	0.3			
Animal cells						
Syrian hamster embryo cells	As^{III}	3–10 μM	0.3		c-*myc*, c-*Ha-ras*	Takahashi et al. 2002
	As^{V}	50–150 μM	0.3			
TRL 1215 rat liver epithelial cells	As^{III}	125–500 nM	8 or 18		c-*myc*	Chen et al. 2001
Animal studies						
C57BL/6J mice	As^{III}	2.6–14.6 μg/g body weight	18.5		c-*Ha-ras*	Okoji et al. 2002
A/J mice	As^{V}	100 ppm	74	*p16, RASSF1*		Cui et al. 2006a
C3H mice	As^{III}	85 ppm	1.4		*ERα*	Waalkes et al. 2004
Human subjects						
	As^{III}	NA	NA	*DAPK*		Chen et al. 2007
	As^{III}	Variable[a]	NA	*p53, P16*		Chanda et al. 2006
	As^{III}	NA	NA	*p16*		Zhang et al. 2007b
	As^{III}	Variable[b]	NA	*RASSF1A, PRSS3*		Marsit et al. 2006b

Task 4

Gene expression pattern analysis and epigenetic modifications in model organisms

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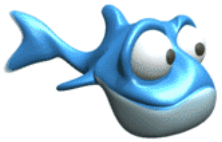
Molecular mechanisms of detoxification in selected organisms , evaluation of chromatin status, chromatin remodelling, transcriptome wide analyses, miRNA charaterization

Sub Task 4.1 mRNA expression patterns of “defensome-related genes
Sub Task 4.2 Identification of Differentially Expressed miRNAs
Sub Task 4.3 Identification of epigenetic status by MS-qPCR
Sub Task 4.4 Chromatin condensation in selected genetic loci and chromatin remodelling
Sub Task 4.5 mRNA expression analysis of genes involved in chromatin remodelling

WP 3 Ecosystem and contaminants



Grazie!!!