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**The project is co-funded by the European Union**  
Instrument for Pre-Accession Assistance



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Project:  
**Ballast Water Management System  
For Adriatic Sea Protection**







# *Monitoring for Ballast Water Management – from pros and cons to yes or no?*



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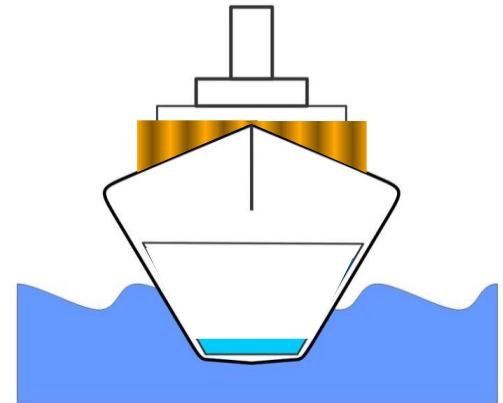
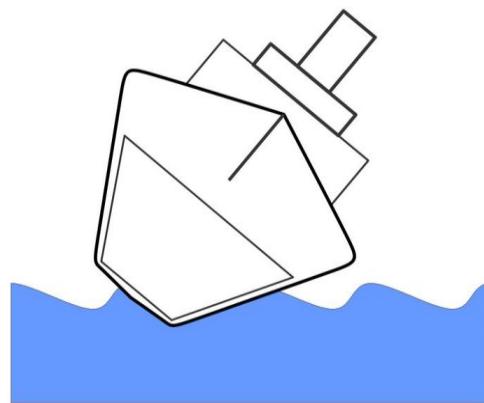
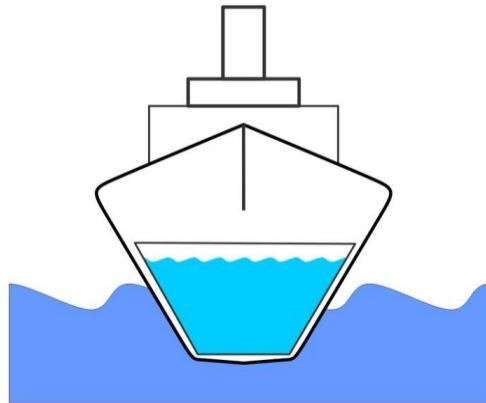


## Ballast Water Management System for Adriatic Sea Protection





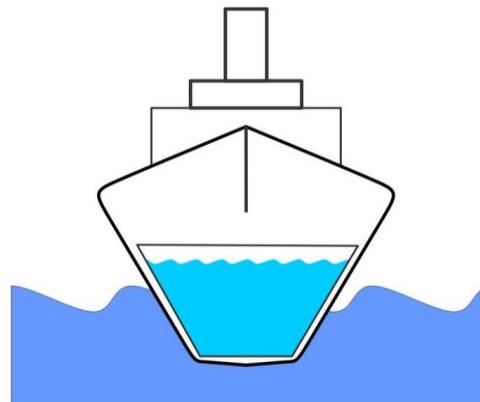
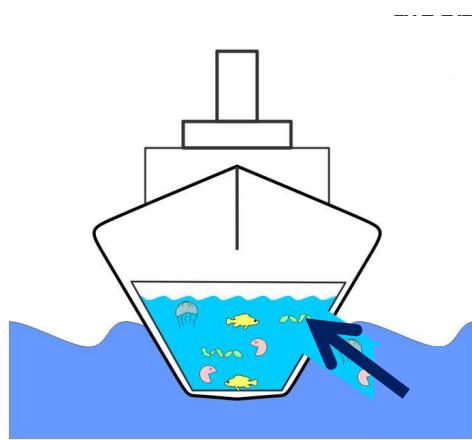
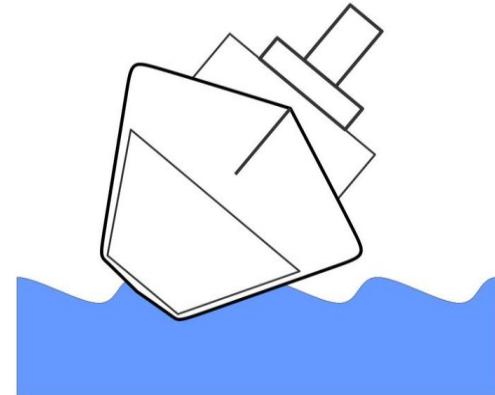
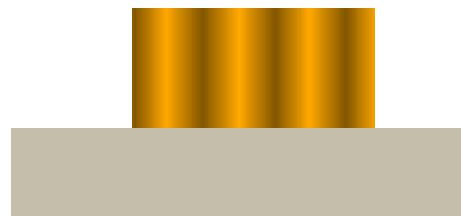
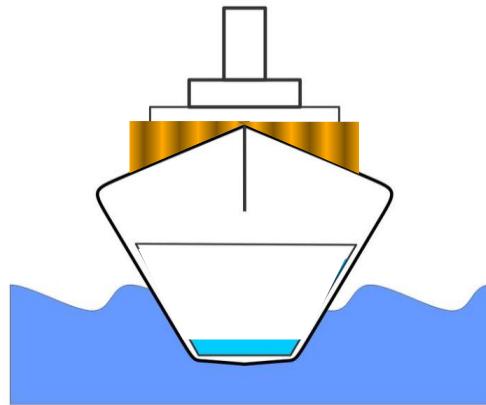
Transfer of harmful aquatic organisms and pathogens (HAOP) / invasive aquatic species (IAS) across natural barriers is recognized as one of greatest pressures to the sea realm, imposing threat to the environment, human health and economy.



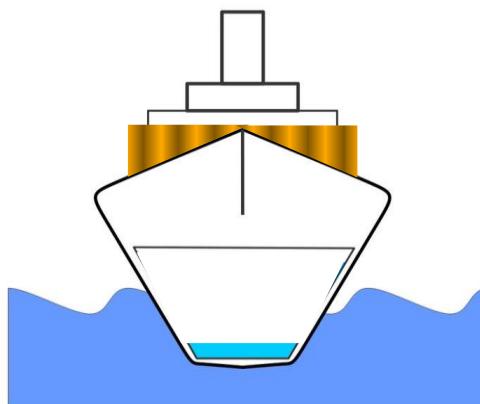
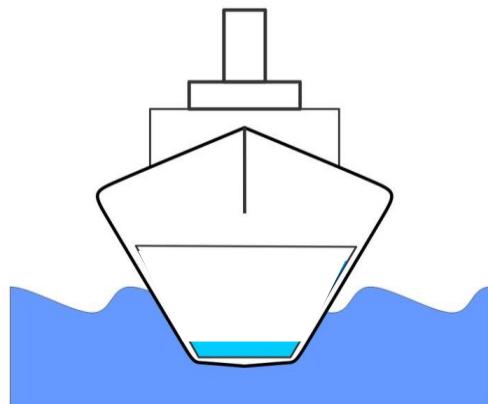
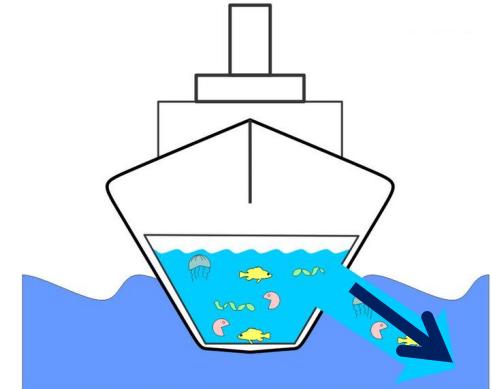
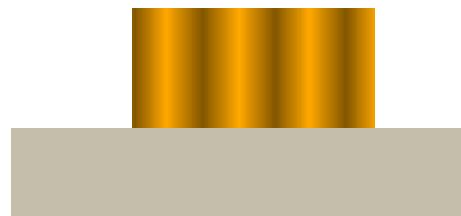
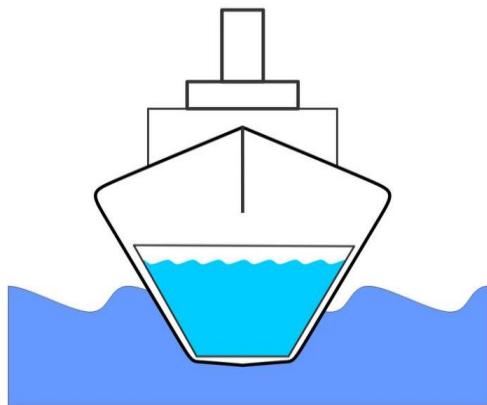
Prominent vector = ballast waters (BW)  
(transferred by vessels engaged in the international shipping of goods)



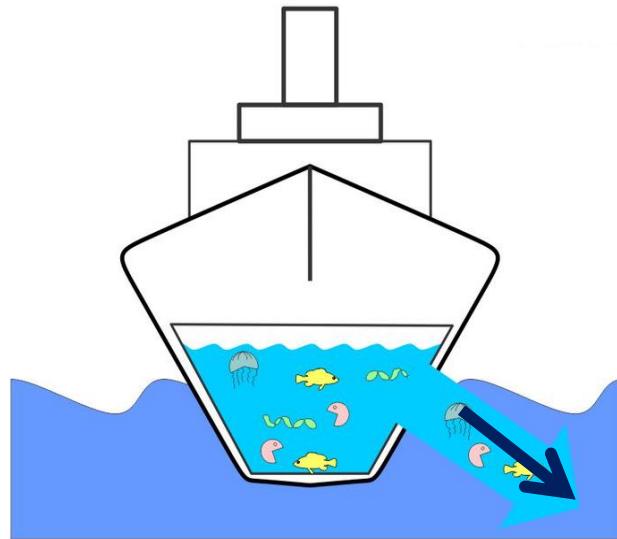
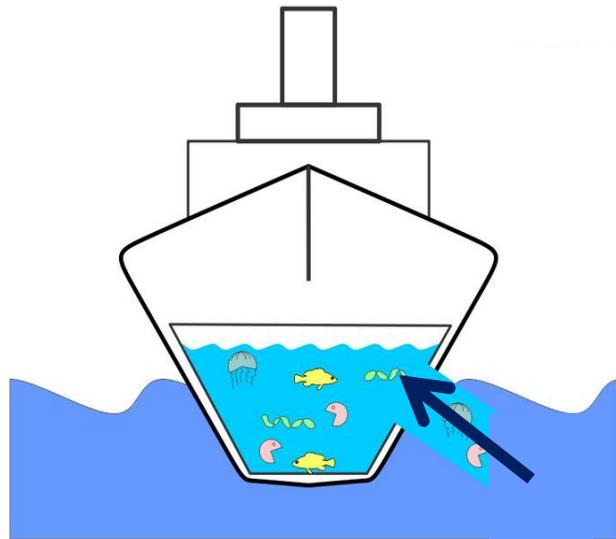
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## Ballast waters



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# International Convention for the Control and Management of Ship's Ballast Water and Sediments, 2004 (BWM Convention) includes **implementation of monitoring**



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International Convention for  
the Control and Management of Ship's Ballast Water  
and Sediments, 2004 (BWM Convention)  
includes **implementation of monitoring...**

W h y ?



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# International Convention for the Control and Management of Ship's Ballast Water and Sediments, 2004 (BWM Convention)

includes **implementation of monitoring**

as vital part of **early detection of introduction of HAOP/IAS in ports**  
in order to **enable rapid response**

with available methods of eradication, control or containment  
for an identified HAOP/IAS species.

Also, to **prevent** ships from loading seawater containing HAOP.



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## Monitoring... what?

**Provisional list of harmful aquatic organisms relevant for warning ships and environmental authorities**, invasiveness, relevance to ballast waters uptake and discharge, demonstrated impact type and information reference.

Colors refer to the **impact category level**

red=strong

orange=medium

yellow=low

green=absent

white = unknown

Abbreviations:

**Means of arrival:** A=Aquaculture; AQ=aquarium; F=bio Fouling; L=live imports; SM=Suez canal migration; BW=ships ballast water; U=unknown.

NIS= Non Indigenous Species; C=Cryptogenic species; HN=Harmful native species; IAS=Invasive Alien Species.

Groups	Categories levels					
	Total	Strong	Medium	Low	Absent	Unknown
Viruses*	1	1	0	0	0	0
Bacteria*	12	12	0	0	0	0
MICROALGAE	113	113	0	0	0	0
MACROPHYTES	32	2	4	13	0	13
Foraminiferans	2	0	0	2	0	0
Poriferans	1	0	0	1	0	0
CNIDARIANS	34	19	2	1	0	12
Ctenophores	1	1	0	0	0	0
GASTROPODS	11	0	2	8	0	1
BIVALVES	20	1	1	9	1	8
Cephalopods	1	0	0	0	0	1
POLYCHAETES	32	0	3	15	0	14
Copepods	3	0	1	1	0	1
DECAPODS	17	0	1	11	0	5
Amphipods	5	0	0	0	1	4
Isopods	1	0	0	0	0	1
Tanaids	1	0	0	0	0	1
Mysid	1	0	0	0	0	1
Stomatopods	1	0	0	1	0	0
Pycnogonids	1	0	0	0	0	1
Bryozoans	2	0	0	1	0	1
Echinoderms	2	0	0	1	0	1
Ascidians	7	0	2	3	0	2
FISHES	21	4	2	13	1	1

Group	Scientific Name	Means of Arrival	NIS/C/IAS/H N	Relevance for BW uptake	Impact type	Impact description**	Reference
microalgae	<i>Alexandrium catenella</i> (Whedon & Kofoid) Balech, 1985			yes			
microalgae	<i>Alexandrium andersonii</i> Balech, 1990			yes			
microalgae	<i>Alexandrium balechii</i> (Steidinger) F.J.R. Taylor, 1979			yes			
microalgae	<i>Alexandrium catenella</i> (Whedon & Kofoid) Balech, 1985	A, BW	IAS	yes	ecosystem, health and social, economic impacts	red tides; paralytic shellfish poisoning (PSP) toxins-producing species responsible for human illnesses and deaths after consumption of infected shellfish; economic damage to aquaculture and the shellfish harvest	DAISIE
microalgae	<i>Alexandrium cohorticula</i> (Balech) Balech, 1985			yes			
microalgae	<i>Alexandrium fundyense</i> Balech, 1985			yes			
microalgae	<i>Alexandrium hiranoi</i> Kita & Fukuyo, 1988			yes			
microalgae	<i>Alexandrium minutum</i> Halim, 1960			yes	economic	economic losses to aquaculture	Nehring S (1998) Archive of Fishery and Marine Research 46(3): 181–194
microalgae	<i>Alexandrium monilatum</i> (J.F. Howell) Balech, 1995			yes			
microalgae	<i>Alexandrium ostenfeldii</i> (Paulsen) Balech & Tangen, 1985			yes			
microalgae	<i>Alexandrium pseudogonyaulax</i> (Biecheler) Horiguchi ex Kita & Fukuyo, 1992			yes			

## Before monitoring...

### **PBS (Port Baseline Study)**

#### **PARAMETERS (frequency)**

**Environmental data** (temp, psal, nutrients: 0, 5, 10 m & bottom; CTD, Secchi disc; seasonally)

**Human pathogens** – *Escherichia coli*, enterococci & *Vibrio cholerae* strains 01 and 0139 in seawater (seasonally) and in sediments (twice/y)

**Phytoplankton** – qualitative assessment: vertical & horizontal tow (seasonally)  
– quantitative assessment: 0 m, 5 m, 10 m & bottom (seasonally)

**Zooplankton** – 1 vertical tow (seasonally)

**Ichtyoplankton** – 3 vertical tows (seasonally)

**Dinoflagellate cysts** – 4 replicates (3 fixated, 1 raw for germination; twice/y)

**Mobile epifauna** – traps, visual searches by divers (twice/y)

**Fish community** – trammel net (twice/y)

**Flora & fauna along vertical transects** – 3 sampling frames at 3 depths (0,5 m, 3 m, 7 m;  
twice/y)

**Flora & fauna along horizontal transects** – 3 inner and 3 outer cores (twice/y)

Optional parameters:

Meiofauna – 1 sampling

Circulation pattern – (geostrophic currents; seasonally)

ADCP with CTD – 1 year long (2x 6 months)

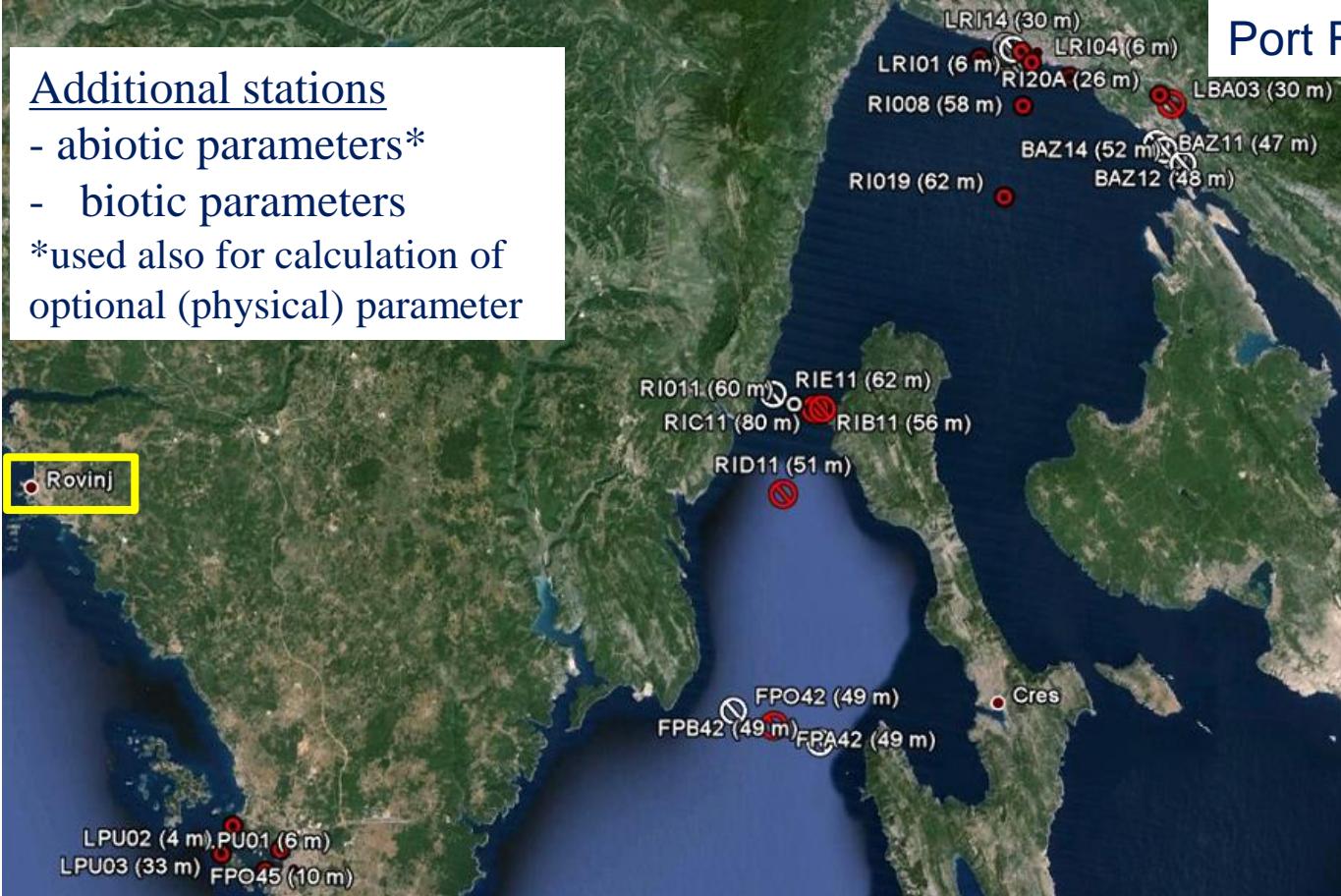
## Port RIJEKA

### Additional stations

- abiotic parameters\*

- biotic parameters

\*used also for calculation of optional (physical) parameter



## Port PULA



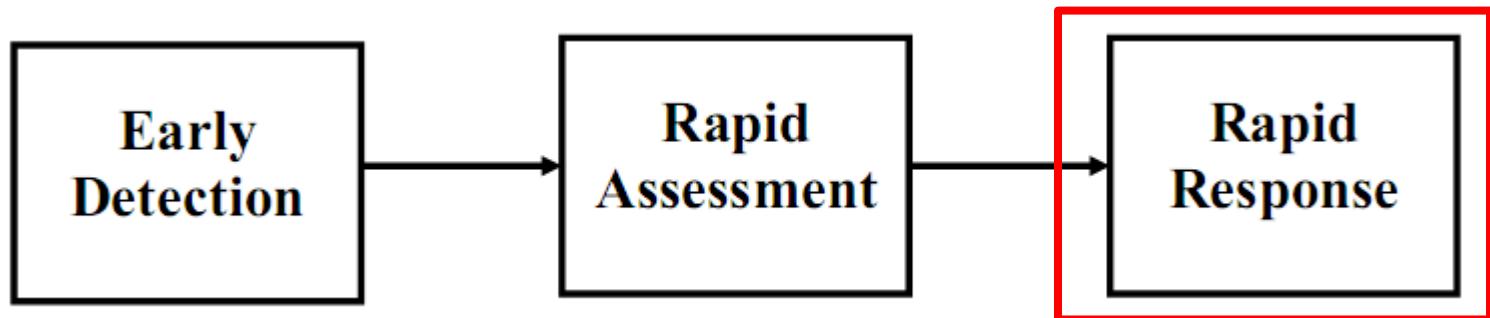
# CHOICE OF SAMPLING SITES

Port	Station	PORT AREA		P r i o r i t y
		Commercial shipping facilities	Adjacent areas outside port	
Pula	LPU02	active berth		1
	LPU01	active berth		1
	FPO45	channel marker		1
	LPU03	nearby natural habitats		2
Rijeka	LRI01	active berth		1
	LRI04	active berth		1
	JPG28	channel marker		1
	LRI05	nearby natural habitats		2





## Schematics of the basic approach against IAS introductions in the host environment





*Remedial Actions may be envisaged when the species' impacts are classified as '**strong**'. When the detected species belongs to an impact category classified as '**medium**', we may intensify the monitoring efforts in ports and adjacent areas, while continuing routine monitoring activities will be required when species belong to the **moderate to low** impact category.*

*The presence of a **human pathogen** or of **indicator microbes** at concentrations above those of Regulation D-2 of the IMO BWM Convention will have to be promptly communicated to the Health Authorities.*



## Response strategies and methods

### Four main strategies:

**Eradication** is the most desirable, but often the most difficult approach. Once the establishment of an alien species is accepted as irreversible, control can be divided into **containment**, i.e., keeping species within regional barriers, and **control** in a stricter sense, i.e., suppressing population levels of alien species to below an acceptable threshold. Last option is **no action**.



## Response strategies and methods

### **Response methods:**

**Mechanical control** can be carried out by directly removing individuals of the target species either by hand or using tools.

**Chemical** treatment offers one of the few options for control of marine invasive species, although its potential is limited. Development, testing and registration of a new compound is a very expensive process, and few products are likely to be developed specifically to address environmental targets.

**Biological** control is the intentional use of populations of upper trophic level organisms commonly referred to as natural enemies, or naturally synthesized substances against pest species to suppress pest populations (introduction of natural enemies, augmentation of enemies, enhance populations of native predators and parasitoids).



## HAB species

L=lab test; F=field test

*Alexandrium minutum* (L)

*Alexandrium tamarens* (L)

*Alexandrium* sp. (L)

*Amphidinium carterae* (L)

*Aureococcus anophagefferens* (L)

*Chattonella subsalsa* (L)

*Chattonella marina* (L)

*Chlorella* sp. (L)

*Cochlodinium polykrikoides* (L+F)

*Eutreptiella gymnastica* (L)

*Gonyostomum semen* (L)

*Gymnodinium breve* (*Karenia brevis*) (L)

*Gymnodinium catenatum* (L)

*Gymnodinium sanguineum* (L)

*Gymnodinium* spp. (L)

*Heterocapsa triquetra* (L)

*Heterosigma akashiwo* (L)

*Lingulodinium polyedrum* (L)

*Microcystis aeruginosa* (L)

*Nitzschia pungens* (L)

*Noctiluca scintillans* (L)

*Prorocentrum micans* (L)

*Prorocentrum minimum* (L)

*Pseudonitzschia pungens* f. *multiseries* (L)

*Pseudonitzschia* sp. (L)

*Pyrodinium bahamense* var *compressum* (L)

*Pyrophacus horologium* (L)

*Scrippsiella trochoidea* (L)

*Skeletonema costatum* (L)

small coccoid cyanobacterium (L)



## HAB species

L=lab test; F=field test

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*Gonyostomum semen* (L)  
*Gymnodinium breve* (*Karenia brevis*) (L)  
*Gymnodinium catenatum* (L)  
*Gymnodinium sanguineum* (L)  
*Gymnodinium* spp. (L)  
*Heterocapsa triquetra* (L)

## Red species in the HAO list

*Heterosigma akashiwo* (L)  
*Lingulodinium polyedrum* (L)  
*Microcystis aeruginosa* (L)  
*Nitzschia pungens* (L)  
*Noctiluca scintillans* (L)  
*Prorocentrum micans* (L)  
*Prorocentrum minimum* (L)  
*Pseudonitzschia pungens f. *multiseries** (L)  
*Pseudonitzschia sp. (L)*  
*Pyrodinium bahamense var *compressum** (L)  
*Pyrophacus horologium* (L)  
*Scrippsiella trochoidea* (L)  
*Skeletonema costatum* (L)  
small coccoid cyanobacterium (L)



## HAB species

L=lab test; F=field test

*Alexandrium minutum* (L)

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*Amphidinium carterae* (L)

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*Chattonella subsalsa* (L)

*Chattonella marina* (L)

*Chlorella* sp. (L)

**Cochlodinium polykrikoides** (L+F)

*Eutreptiella gymnastica* (L)

*Gonyostomum semen* (L)

**Gymnodinium breve (Karenia brevis)** (L)

**Gymnodinium catenatum** (L)

*Gymnodinium sanguineum* (L)

*Gymnodinium* spp. (L)

*Heterocapsa triquetra* (L)

**Red species** in WP6.3 list of HAO

**Red species** with available mitigation measure

**Heterosigma akashiwo** (L)

**Lingulodinium polyedrum** (L)

**Microcystis aeruginosa** (L)

**Nitzschia pungens** (L)

**Noctiluca scintillans** (L)

**Prorocentrum micans** (L)

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**Pseudonitzschia pungens f. multiseries** (L)

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small coccoid cyanobacterium (L)



## HAB species

*Cochlodinium polykrikoides* (L+F)

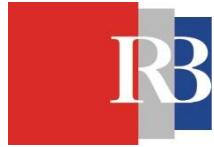
Chemical method – sophorolipid-yellow clay mixture with removal efficiency of 95 % after 30 minutes in field test

(*sophorolipid* = biosurfactant produced by yeast *Candida bombicola*)

→ can be applied on other HAB



Clay scattering on the area affected by HABs in Korea.



## MACROPHYTES (8 species)

*Ascophyllum nodosum* (F) - mechanical (physically removed, successful eradication)

### *Caulerpa taxifolia* (L+F)

- mechanical (pumping, limited success, effecting non-target organisms)
- chemical
  - chlorine – successful, effecting non-target organisms
  - copper – poor efficacy
  - freshwater – fair efficacy



## CNIDARIA – jellyfish

Mechanical methods – using Pelican boats (400 removed in a day)

Physical destruction – cutting nets use (jellyfish are transported by currents and pushed against these nets and get destroyed then)

Chemical methods *in research* – for polyps destruction (stages which are „seeds” for jellyfish blooms: active substances which induce resistance in target species, impacting also potential predator

Biological methods – introduction of predator, e.g., ctenophore *Beroe ovata* to control *Mnemiopsis leidyi* (becoming pest itself), butterfish *Peprilus triacanthus* (good results) – altering ecosystem

Robot – *jellyfish terminator* suck up specimens into submerged nets



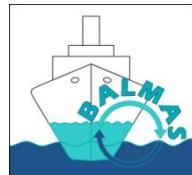
## FISH

Mechanical methods – selective removal by electric fishing and netting methods, successive in suppressing abundance and reducing recruitment

Biological methods – introduction of natural predator

Chemical methods *in research* – pheromones which mediate social behaviour (anti-predator, social and reproductive impacts)

Port	SAMPLES	Date	Station	<i>E. coli</i>	Enterococci	<i>Vibrio cholerae</i>		Detected species ( <i>Vibrio</i> = V., <i>Aeromonas</i> = A.)	Compliance with Croatian legal regulations for BW
				<250/100mL	<100/100mL	strain 01	strain 0139		
Pula	Seawater	9.9.2014	LPU01	61	390	<1	<1	<i>V. parahaemolyticus</i> and <i>V. alginolyticus</i>	NO
			LPU02	0	4	<1	<1	<i>V. parahaemolyticus</i> and <i>V. alginolyticus</i>	YES
			LPU03	0	0	<1	<1	<i>V. alginolyticus</i>	YES
			FP045	0	14	<1	<1	<i>V. parahaemolyticus</i> and <i>V. alginolyticus</i>	YES
	Seawater	18.12.2014.	LPU01	454	1540	<1	<1	<i>V. alginolyticus</i> (52/100 mL)	NO
			LPU02	0	226	<1	<1	<i>V. alginolyticus</i> (3/100 mL)	NO
			LPU03	0	126	<1	<1	<i>V. alginolyticus</i> (1/100 mL)	NO
			FP045	0	216	<1	<1		NO
	Sediment	19.12.2014	LPU01	<1	1	<1	<1		Regulative for sediment in Croatia is missing
			LPU02	<1	4	<1	<1	<i>Vibrio alginolyticus</i> or <i>V. metschnikovii</i>	
			LPU03	<1	<1	<1	<1		
			FP045	<1	<1	<1	<1		
Rijeka	Seawater	10.9.2014	LRI05	8500	2500	<1	<1	<i>V. alginolyticus</i>	NO
			LRI01	400	1100	<1	<1		NO
		11.9.2014	JPG28	28	7	<1	<1	<i>V. fluvialis</i>	YES
			LRI04	1400	600	<1	<1	<i>V. cholerae</i> non-O1/non-O139	NO
	Seawater	19.12.2014	LRI05	9	14	<1	<1		NO
			LRI01	14500	2600	<1	<1	<i>Vibrio metschnikovii</i>	NO
		20.12.2014	JPG28	350	97	<1	<1	<i>Aeromonas hydrophilla</i>	NO
			LRI04	1800	560	<1	<1	<i>Aeromonas hydrophilla</i>	NO
	Sediment	19.12.2014	LRI05	<1	<1	<1	<1		Regulative for sediment in Croatia is missing
		20.12.2014	LRI01	1	4	<1	<1		
			LRI04	5	28	<1	<1		
		12.02.2015.	LRI05	460	160	<1	<1		
	Seawater		LRI01	13000	3200	<1	<1	<i>Aeromonas hydrophilla</i>	NO
			JPG28	410	100	<1	<1		NO
		13.02.2015.	LRI04	7500	1800	<1	<1		NO



## Light microscopy

### Microscope



### Electron microscopy



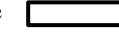
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## Sampling of phytoplankton by vertical tow with plankton net in Port Pula



# Phytoplankton species observed in vertical and horizontal net

Pula & Rijeka on white



Pula only on yellow



Rijeka only on cyan



Species categorised as strongly relevant (WP6.3) on grey

## Phylum CHRYSOPHYTA

### Class Chrysophyceae

*Dictyocha fibula* Ehrenberg

*Dictyocha octonaria* Ehrenberg

*Dictyocha speculum* Ehrenberg

*Meringosphaera mediterranea* Lohmann

### Class Prymnesiophyceae (sin. Haptophyceae)

*Cocco* sp.

*Anaplosolenia brasiliensis* (Lohman) De flandre

*Calciopappus caudatus* K.R. Gaarder & Ramsfjell

*Calciosolenia murrayi* Gran

*Rhabdosphaera tinguifera* Schiller

*Syracosphaera pulchra* Lohmann

### Class Bacillariophyceae (sin. Diatomae)

*Amphora* sp.

*Asteromphalus heptactis* (Brébisson) Ralfs

*Bacteriaptron biconicum* Pavillard

*Bacteriaptron delicatulum* Cleve

*Bacteriaptron furcation* Shadolt

*Bacteriaptron hyalinum* Lauder

*Bacteriaptron jadranum* Godrijan, Marie & Phannkuchen

*Bacteriaptron mediterraneum* Pavillard

*Bacteriaptron* sp.

*Bleakeleyanotata* (Grunow) Round

*Cerataulina pelagica* (Cleve) Hendey

*Chaetoceros affinis* Lauder

*Chaetoceros anastomosans* Grunow

*Chaetoceros borealis* Bailey

*Chaetoceros brevis* Schütt

*Chaetoceros circinalis* (Meunier) K.G. Jensen & Moestrup

*Chaetoceros compressus* Lauder

*Chaetoceros constrictus* Gran

*Chaetoceros contortus* F. Schütt

*Chaetoceros curvisetus* Cleve

*Chaetoceros costatus* Pavillard

*Chaetoceros danicus* Cleve

*Chaetoceros decipiens* Cleve

*Chaetoceros didymus* Ehrenberg

*Chaetoceros diversus* Cleve

*Chaetoceros eibenii* Grunow

*Chaetoceros lauderii* Ralfs

*Chaetoceros messanensis* Castracane

*Chaetoceros peruvianus* Brightwell

*Chaetoceros pseudocurvisetus* Mangin

*Chaetoceros rostratus* Lauder

*Chaetoceros* sp.

*Chaetoceros socialis* Lauder

*Chaetoceros simplex* Ostendorf

*Chaetoceros tenuissimus* Mettner

*Chaetoceros tortissimus* Gran

*Cyclotella* sp.

*Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin

*Cylindrotheca fusiformis* Reimann & J.C. Lewin

*Dactyliosolen fragilissimus* (Bergon) Hasle

*Dactyliosolen mediterraneus* (Peragallo) Peragallo

*Dactyliosolen phuketensis* (B.G. Sundström) G.R. Hasle

*Detonula punila* (Castracane) Gran

*Entomoneis pulchra* (Bailey) Reimer

*Eucampia cornuta* (Cleve) Grunow

*Guinardia flaccida* (Castracane) Peragallo

*Guinardia striata* (Stolterfoth) Hasle

*Hemiaulus hauckii* Grunow

*Hemiaulus sinensis* Greville

*Leptocylindrus danicus* Cleve

*Leptocylindrus mediterraneus* Peragallo

*Leptocylindrus minimus* Gran

*Leptocylindrus* sp.

*Licmophora* sp.

*Lioioma pacificum* (Cupp) Hasle

*Neocalyptrella robusta* (Norman ex Ralfs) Hemández-Becerril et Meave del Castillo

*Nitzschia longissima* (Breb.) Ralfs in Pritch.

*Nitzschia sigma* (Kützing) W. Smith

*Nitzchia* sp.

*Penate* sp.

*Pleurosigma* sp.

*Proboscia alata* (Brightwell) Sundström

*Pseudo-nitzschia delicatissima* (Cleve) Heiden (SEM)

*Pseudo-nitzschia fraudulenta* (Cleve) H. Peragallo

*Pseudo-nitzschia* spp.

*Pseudosolenia calcar-avis* (Schultze) Sundström

*Rhizosolenia imbricata* Brightwell

*Rhizosolenia styliflora* Brightwell

*Skeletonema* sp.

*Tabellaria fenestrata* (Lyngbye) Kützing

*Thalassionemanitzschioidea* (Grunow) Mereschkowsky

*Thalasiosira* sp.

## Phylum DINOPHYTA

### Class Desmophyceae

*Prorocentrum compression* (Bailey) Abé ex Dodge

*Prorocentrum micans* Ehrenberg

*Prorocentrum minimum* (Pavillard) Schiller

*Prorocentrum triestinum* Schiller

### Class Dinophyceae

*Alexandrium* sp.

*Ceratium arietinum* Cleve

*Ceratium candelabrum* (Ehrenberg) Stein

*Ceratium furca* (Ehrenberg) Claparède et Lachmann

*Ceratium fusus* (Ehrenberg) Dujardin

*Ceratium hexacanthum* Gourret

*Ceratium horridum* (Cleve) Gran

*Ceratium kofoidii* Jørgensen

*Ceratium macroceras* Schrank

*Ceratium macroceras* (Ehrenberg) VanHoeffen

*Ceratium massiliense* (Gourret) Jørgensen

*Ceratium minutum* Jørgensen

*Ceratium pulchellum* Schröder

*Ceratium setaceum* Jørgensen

*Ceratium* sp.

*Ceratium trichoceros* (Ehrenberg) Kofoid

*Dinophysis caudata* Seville-Kent

*Dinophysis fortii* Pavillard

*Dinophysis tripos* Gourret

*Diplopsalis* complex

*Gonyaulax* sp.

*Gonyaulax spinifera* (Claparède et Lachmann) Diesing

*Gymnodinium cucumis* Schütt

*Gymnodinium* sp.

*Ostreopsis ovata* Fukuyo

*Oxytoxum sceptrum* (F. Stein) Schröder

*Oxytoxum sphaeroidum* Stein

*Protorperidinium brochii* (Kofoid et Swezy) Balech

*Protorperidinium depressum* (Bailey) Balech

*Protorperidinium diabolus* (Cleve) Balech

*Protorperidinium divergens* (Ehrenberg) Balech

*Protorperidinium fatulipes* (Kofoid) Balech

*Protorperidinium globulatum* (Stein) Balech

*Protorperidinium oceanicum* (VanHoeffen) Balech

*Protorperidinium ovum* (Schiller) Balech

*Protorperidinium pediculatum* (Schütt) Balech

*Protorperidinium* sp.

*Protorperidinium steinii* (Joergensen) Balech

*Protorperidinium tubum* (Schiller) Balech

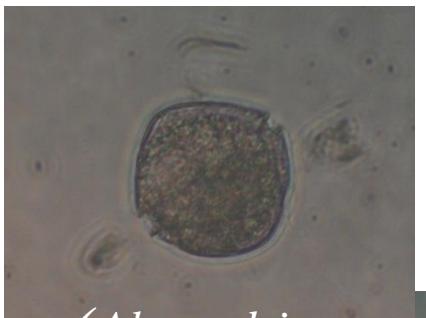
*Pseliodinium vaubani* Soumia

*Pyrophacus horologicum* Stein

*Pyrophacus* sp.

*Scrippsiella* sp.

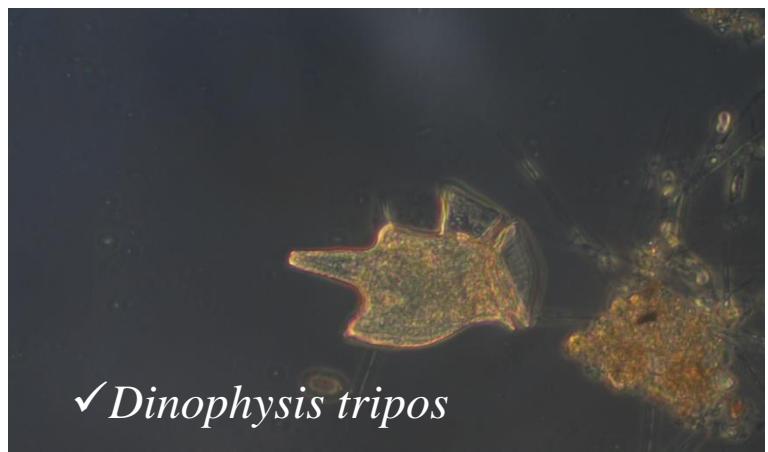
- ✓ *Pseudo-nitzschia delicatissima* (SEM)
- ✓ *Pseudo-nitzschia fraudulenta* (SEM)
- ✓ *Pseudo-nitzschia* spp.
- ✓ *Prorocentrum micans*
- ✓ *Alexandrium* sp.
- ✓ *Dinophysis caudata*
- ✓ *Dinophysis fortii*
- ✓ *Dinophysis tripos*
- ✓ *Gonyaulax spinifera*
- ✓ *Ostreopsis ovata*



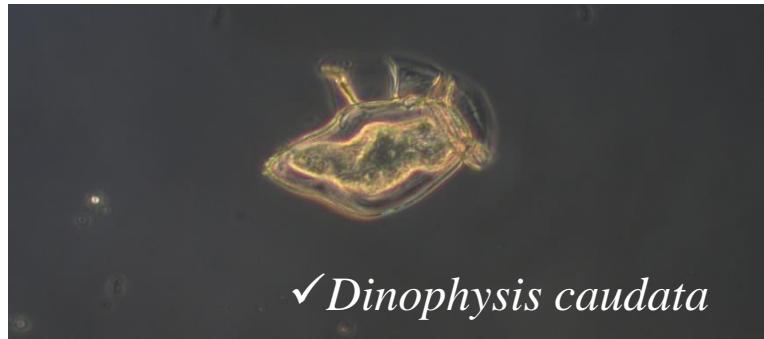
✓ *Alexandrium* sp.



✓ *Dinophysis fortii*



✓ *Dinophysis tripos*



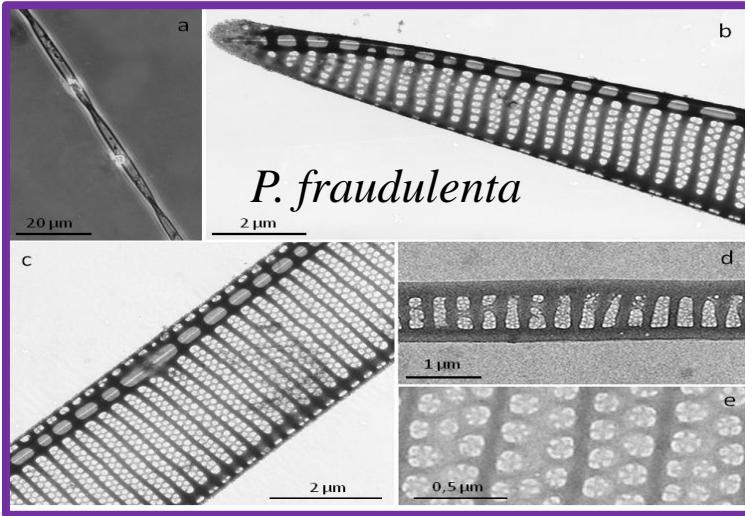
✓ *Dinophysis caudata*

Phytoplankton species observed in ports  
Pula and/or Rijeka in summer samples &  
categorised as strongly relevant

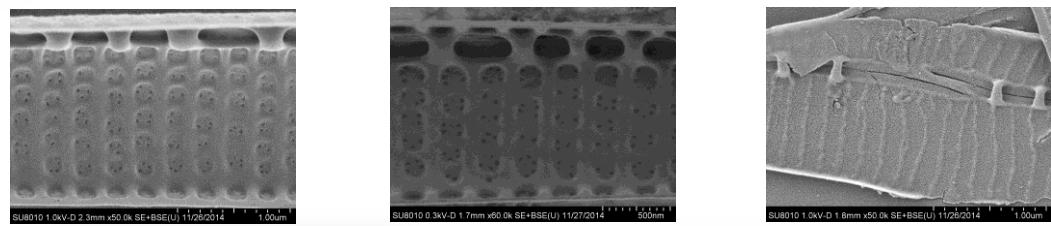
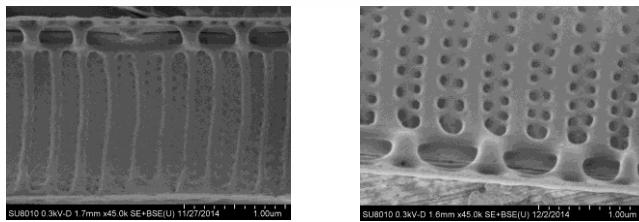
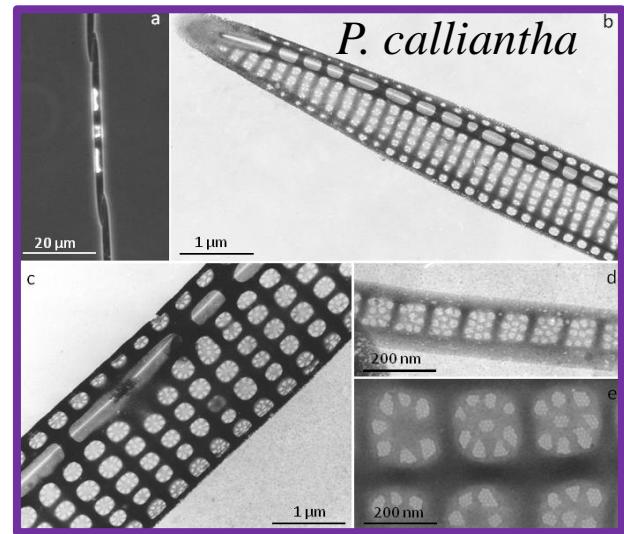
- ✓ *Pseudo-nitzschia delicatissima* (SEM)
- ✓ *Pseudo-nitzschia fraudulenta* (SEM)
- ✓ *Pseudo-nitzschia* spp.
- ✓ *Prorocentrum micans*
- ✓ *Alexandrium* sp.
- ✓ *Dinophysis caudata*
- ✓ *Dinophysis fortii*
- ✓ *Dinophysis tripos*
- ✓ *Gonyaulax spinifera*
- ✓ *Ostreopsis ovata*

List of *Alexandrium* and *Pseudo-nitzschia*  
species categorised as  
strongly relevant in the list

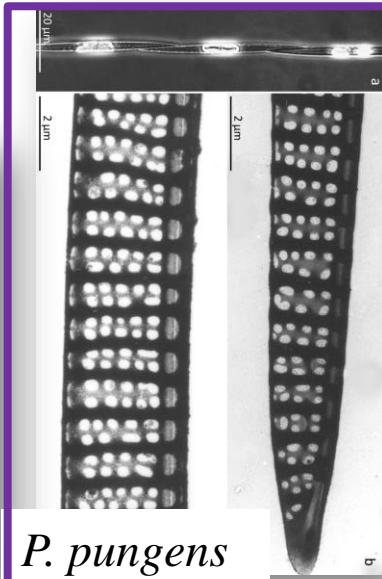
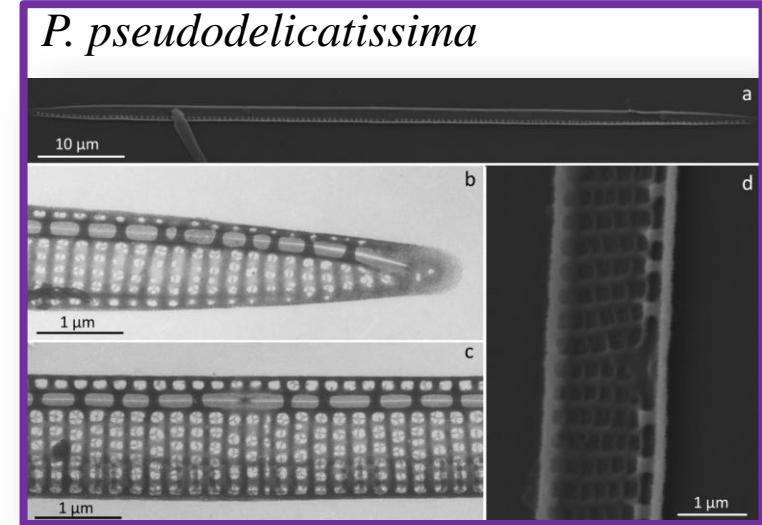
- ❖ *Alexandrium acatenella*
- ❖ *Alexandrium andersonii*
- ❖ *Alexandrium balechii*
- ❖ *Alexandrium catenella*
- ❖ *Alexandrium cohorticula*
- ❖ *Alexandrium fundyense*
- ❖ *Alexandrium hiranoi*
- ❖ *Alexandrium minutum*
- ❖ *Alexandrium monilatum*
- ❖ *Alexandrium ostenfeldii*
- ❖ *Alexandrium pseudogonyaulax*
- ❖ *Alexandrium tamarense*
- ❖ *Alexandrium tamiyavanichii*
- ❖ *Alexandrium taylori*
- ❖ *Pseudo-nitzschia australis*
- ❖ *Pseudo-nitzschia calliantha*
- ❖ *Pseudo-nitzschia cuspidata*
- ❖ *Pseudo-nitzschia delicatissima*
- ❖ *Pseudo-nitzschia fraudulenta*
- ❖ *Pseudo-nitzschia galaxiae*
- ❖ *Pseudo-nitzschia multiseries*
- ❖ *Pseudo-nitzschia multistriata*
- ❖ *Pseudo-nitzschia pseudodelicatissima*
- ❖ *Pseudo-nitzschia pungens*
- ❖ *Pseudo-nitzschia seriata*
- ❖ *Pseudo-nitzschia turgidula*



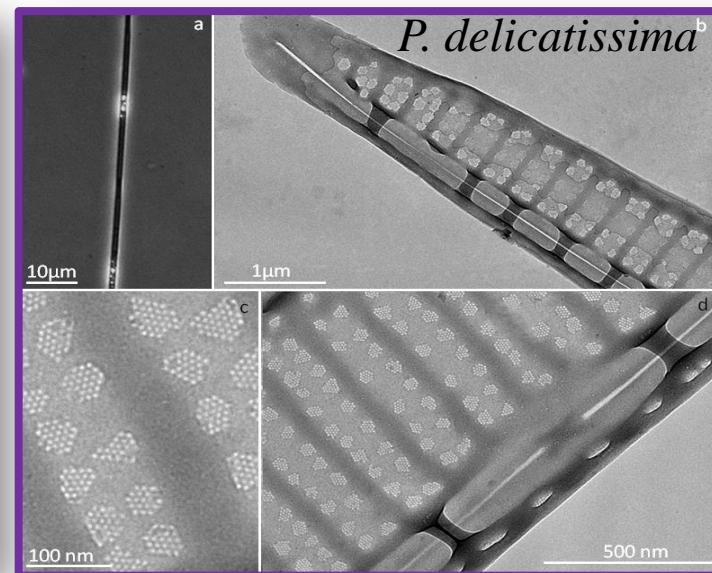
## Pseudo-nitzschia spp.



## *P. pseudodelicatissima*



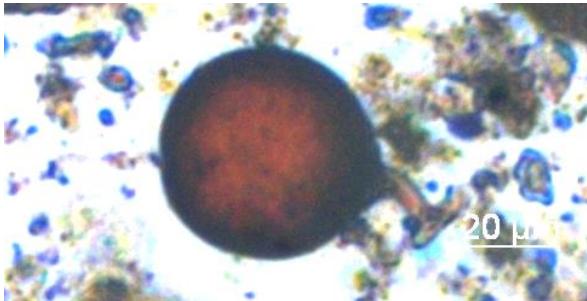
## *P. pungens*



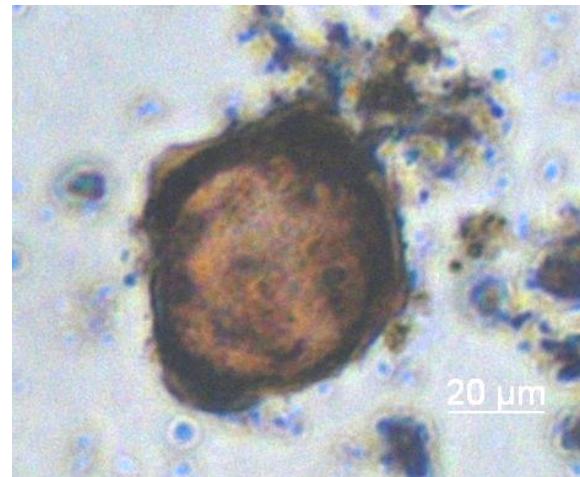
## *P. delicatissima*

# Dinoflagellate cysts observed during Rijeka PBS in 2011 (species identification has to be confirmed)

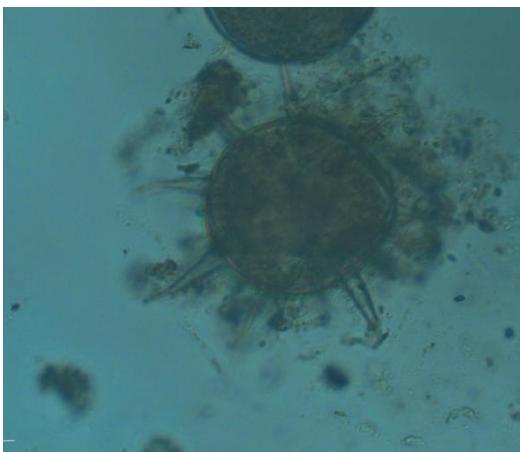
Rijeka



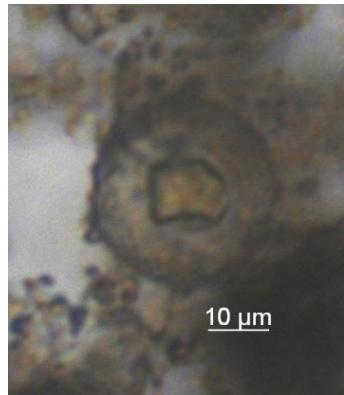
*Gymnodinium catenatum* H. W. Graham



*Protoperdinium americanum*  
(Gran & Braarud) Balech



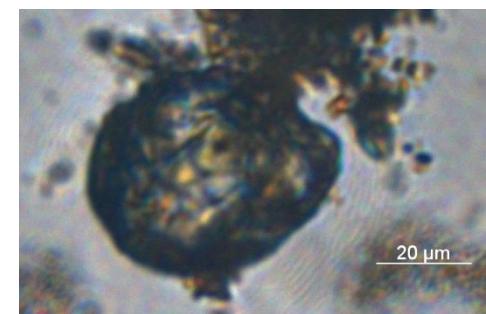
*Linguloidinium polyedrum* (F. Stein) J. D. Dodge



*Protoperdinium conicoides*  
(Paulsen) Balech

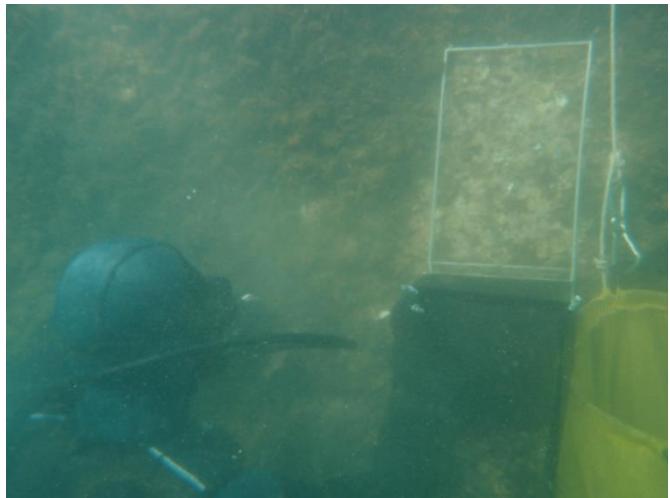


*Protoperdinium minutum* (Kofoid) Loeblich III

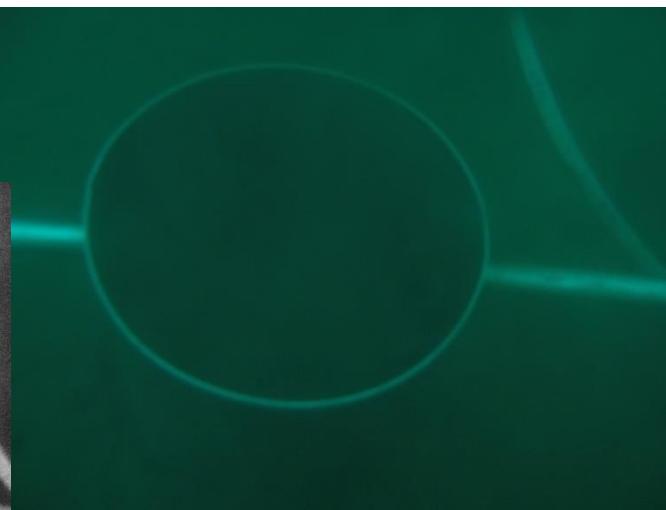
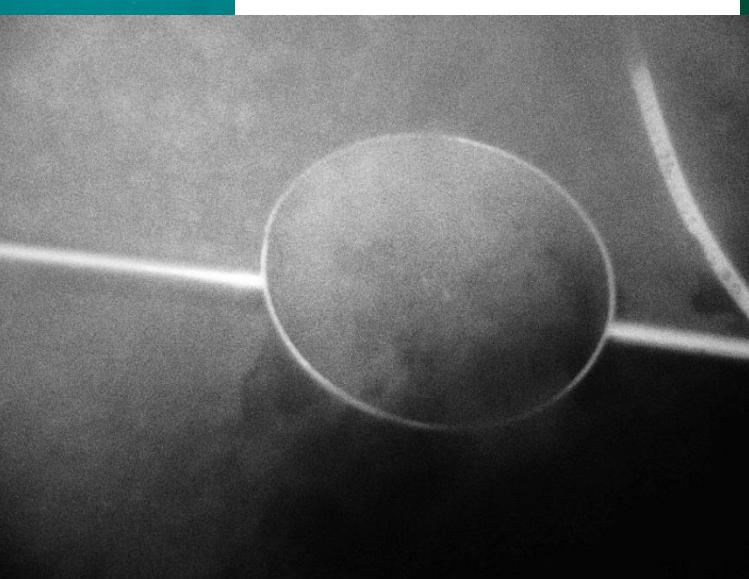
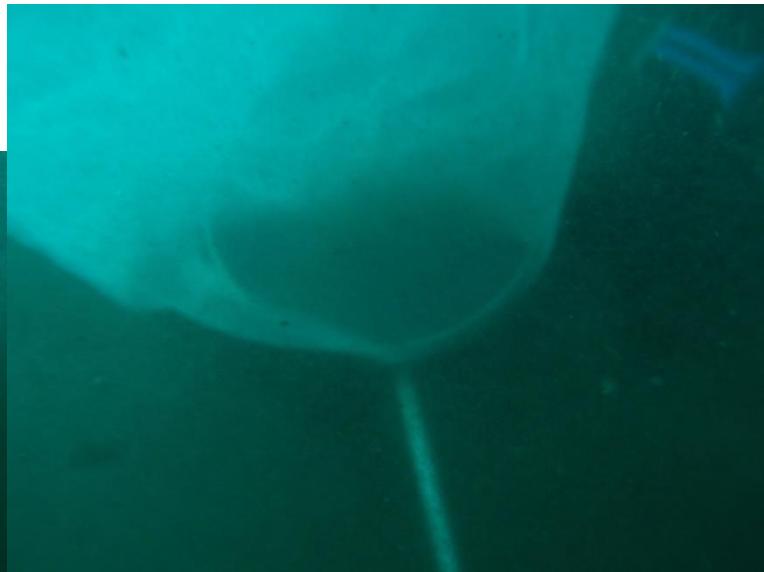
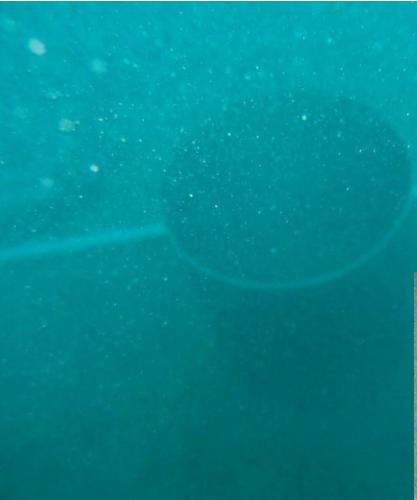


*Scrippsiella crystallina* J. Lewis

## Sampling flora and fauna from the vertical transect



# Sampling flora and fauna from the horizontal transect



## Working hours for separation flora & fauna at H&V transects

Station	Samples processed / n	Sampling time
LPU02	7/12	1 morning
LPU01	3/12	1 morning
LPU03	3/9	1 morning
LRI01	3/12	1 morning
LRI04	3/12	1 morning
LRI05	3/9	1 morning

Sampling time for 2 ports, autumnal samples: 6 mornings

## Working hours for separation flora & fauna at H&V transects

Station	Samples processed / n	Sampling time	Separation from detritus
LPU02	7/12	1 morning	Several hours
LPU01	3/12	1 morning	Several hours
LPU03	3/9	1 morning	Several hours
LRI01	3/12	1 morning	Several hours
LRI04	3/12	1 morning	Several hours
LRI05	3/9	1 morning	Several hours

Sampling time for 2 ports, autumnal samples: 6 working days

## Working hours for separation flora & fauna at H&V transects

- Varia indet.
- Polyplacophora
- Anthozoa
- Cirripedia
- Euphausiacea
- Echinoidea
- Isopoda
- Porifera
- Sipuncula
- Tanaidacea
- Cumacea
- Holothuroidea
- Amphipoda
- Decapoda
- Ophiuroidea
- Scaphopoda
- Bivalvia
- Gastropoda
- Polychaeta

### Flora & fauna along horizontal transects

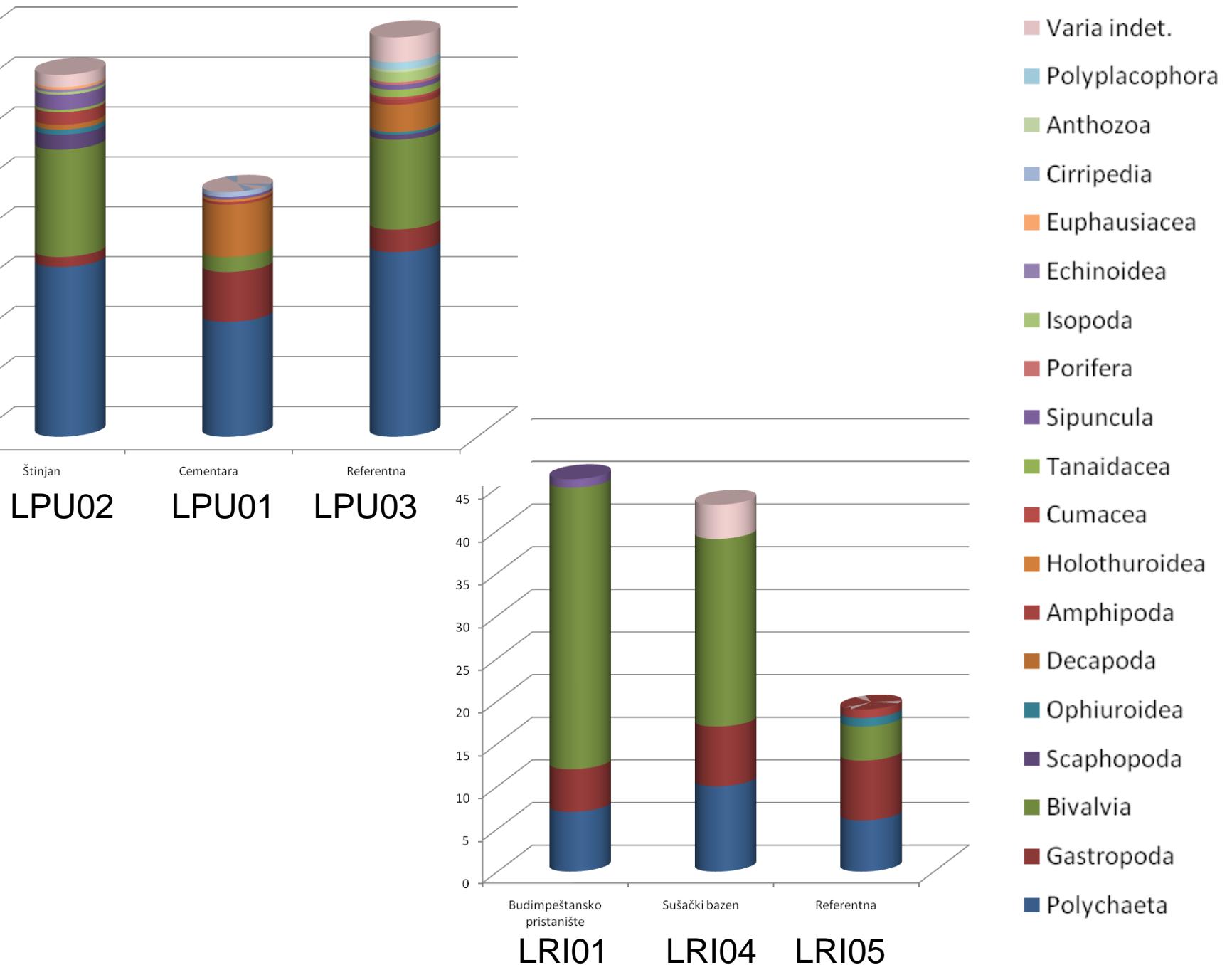
3 inner and 3 outer cores (twice/y)

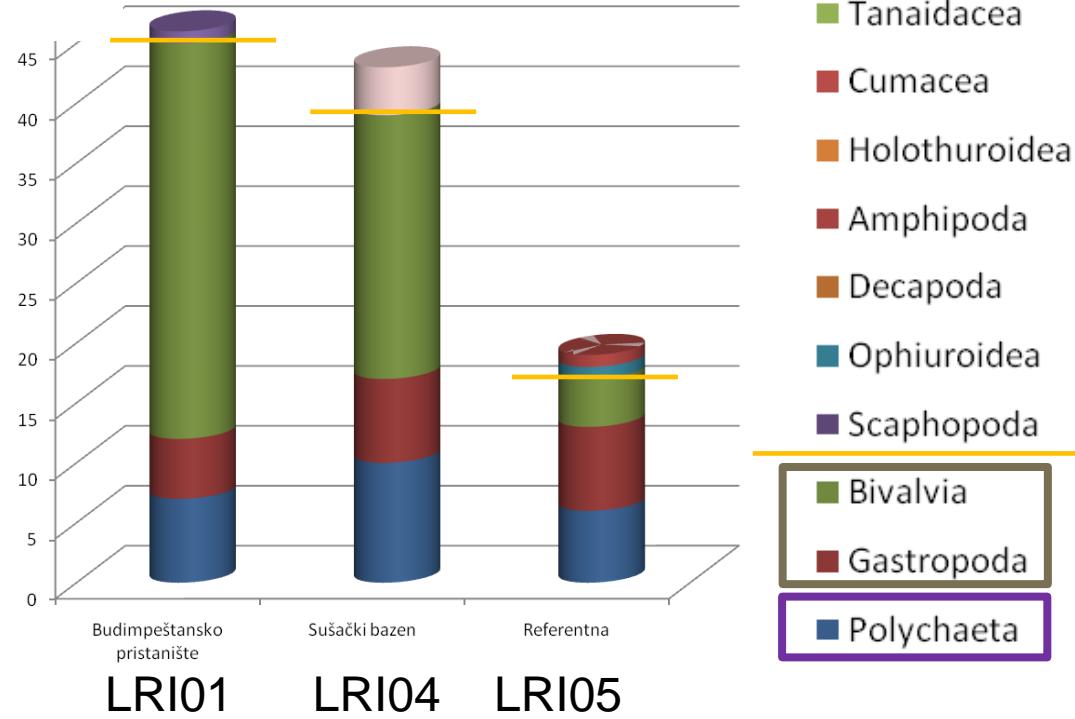
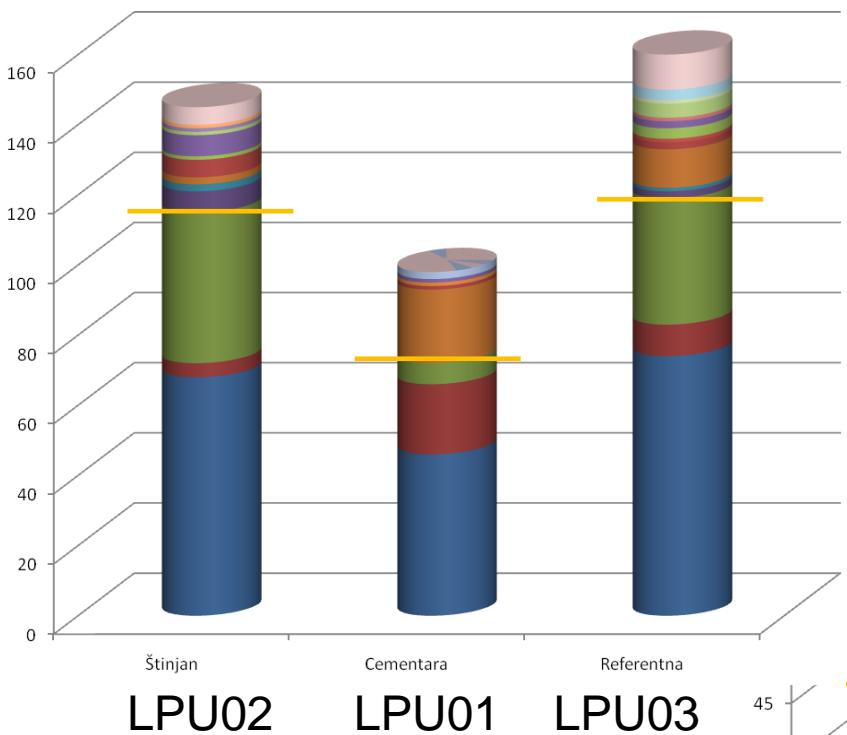
separation time for 2 ports, autumnal samples: 2 months

### Flora & fauna along vertical transects

3 sampling frames at 3 depths (0,5 m, 3 m, 7 m; twice/y)

separation time for 2 ports, autumnal samples: 6 months





Taxon observed

TAXON

FB11 expert +

Taxon NOT observed

TAXON

FB11 expert -

Taxon/Impact		strong	medium	low	absent	unknown	total
	<b>FORAMINIFERA</b>	-	-	2	-	-	2
1	<b>PORIFERA</b>	-	-	1	-	-	1
2	<b>CNIDARIA</b>	19	2	1	-	12	34
	<b>CTENOPHORA</b>	1	-	-	-	-	1
3	<b>GASTROPODA</b>	-	2	8	-	1	11
	<b>BIVALVIA</b>	1	1	9	1	8	20
	<b>CEPHALOPODA</b>	-	-	-	-	1	1
4	<b>POLYCHAETA</b>	-	3	15	-	14	32
5	<b>COPEPODA</b>	-	1	1	-	1	3
	<b>DECAPODA</b>	-	1	11	-	5	17
	<b>AMPHIPODA</b>	-	-	-	1	4	5
	<b>ISPOPODA</b>	-	-	-	-	1	1
	<b>TANAIDACEA</b>	-	-	-	-	1	1
	<b>MYSIDACEA</b>	-	-	-	-	1	1
	<b>STOMATOPODA</b>	-	-	1	-	-	1
	<b>PYCNOGONIDA</b>	-	-	1	-	1	2
6	<b>BRYOZOA</b>	-	-	1	-	1	2
7	<b>ECHINODERMATA</b>	-	-	1	-	1	2
	<b>ASCIIDIACEA</b>	-	2	3	-	2	7
	<b>TOTAL</b>	<b>21</b>	<b>12</b>	<b>55</b>	<b>2</b>	<b>54</b>	<b>144</b>

Taxon/Impact		strong	medium	low	absent	unknown	total
	<b>FORAMINIFERA</b>	-	-	2	-	-	2
1	<b>PORIFERA</b>	-	-	1	-	-	1
2	<b>CNIDARIA</b>	19	2	1	-	12	34
	<b>CTENOPHORA</b>	1	-	-	-	-	1
	<b>GASTROPODA</b>	-	2	8	-	1	11
3	<b>BIVALVIA</b>	1	1	9	1	8	20
	<b>CEPHALOPODA</b>	-	-	-	-	1	1
4	<b>POLYCHAETA</b>	-	3	15	-	14	32
	<b>COPEPODA</b>	-	1	1	-	1	3
	<b>DECAPODA</b>	-	1	11	-	5	17
	<b>AMPHIPODA</b>	-	-	-	1	4	5
5	<b>ISPOPODA</b>	-	-	-	-	1	1
	<b>TANAIDACEA</b>	-	-	-	-	1	1
	<b>MYSIDACEA</b>	-	-	-	-	1	1
	<b>STOMATOPODA</b>	-	-	1	-	-	1
	<b>PYCGNOGONIDA</b>	-	-	1	-	1	2
6	<b>BRYOZOA</b>	-	-	1	-	1	2
7	<b>ECHINODERMATA</b>	-	-	1	-	1	2
	<b>ASCIDIACEA</b>	-	2	3	-	2	7
	<b>TOTAL</b>	<b>21</b>	<b>12</b>	<b>55</b>	<b>2</b>	<b>54</b>	<b>144</b>

<b>PULA</b>	<b>RIJEKA</b>	<b>PULA &amp; RIJEKA</b>
<b>Gastropoda (8)</b>	<b>Gastropoda (10)</b>	<b>Gastropoda (17)</b>
<i>Antalis dentalis</i>	<i>Acteon tornatilis</i>	<i>Acteon tornatilis</i>
<i>Fustiaria rubescens</i>	<i>Alvania discors</i>	<i>Alvania discors</i>
<i>Calyptaea chinensis</i>	<i>Bela fuscata</i>	<i>Antalis dentalis</i>
<i>Hyla vitrea</i>	<i>Bittium reticulatum</i>	<i>Bela fuscata</i>
<i>Natica dillwynii</i>	<i>Crisilla depicta</i>	<i>Bittium reticulatum</i>
<i>Nassarius incrassatus</i>	<i>Epitonium clathrus</i>	<i>Calyptaea chinensis</i>
<i>Nassarius reticulatus</i>	<i>Gibbula adansoni</i>	<i>Crisilla depicta</i>
<i>Turritella communis</i>	<i>Nassarius incrassatus</i>	<i>Epitonium clathrus</i>
	<i>Raphitoma linearis</i>	<i>Fustiaria rubescens</i>
	<i>Runcina sp.</i>	<i>Gibbula adansoni</i>
		<i>Hyala vitrea</i>
		<i>Nassarius incrassatus</i>
		<i>Nassarius reticulatus</i>
		<i>Natica dillwynii</i>
		<i>Raphitoma linearis</i>
		<i>Runcina sp.</i>
		<i>Turritella communis</i>

# List of harmful aquatic organisms relevant for warning ships and environmental authorities:

*Pintada radiata*

Taxon/Impact	strong	medium	low	absent	unknown	total
<b>FORAMINIFERA</b>	-	-	2	-	-	2
1 <b>PORIFERA</b>	-	-	1	-	-	1
2 <b>CNIDARIA</b>	19	2	1	-	12	34
<b>CTENOPHORA</b>	1	-	-	-	-	1
<b>GASTROPODA</b>	-	2	8	-	1	11
3 <b>BIVALVIA</b>	1	1	9	1	8	20
<b>CEPHALOPODA</b>	-	-	-	-	1	1
4 <b>POLYCHAETA</b>	-	3	15	-	14	32
<b>COPEPODA</b>	-	1	1	-	1	3
<b>DECAPODA</b>	-	1	11	-	5	17
<b>AMPHIPODA</b>	-	-	-	1	4	5
<b>ISPOPODA</b>	-	-	-	-	1	1
<b>TANAIDACEA</b>	-	-	-	-	1	1
<b>MYSIDACEA</b>	-	-	-	-	1	1
<b>STOMATOPODA</b>	-	-	1	-	-	1
<b>PYCGONOGONIDA</b>	-	-	1	-	1	2
6 <b>BRYOZOA</b>	-	-	1	-	1	2
7 <b>ECHINODERMATA</b>	-	-	1	-	1	2
<b>ASCIDIACEA</b>	-	2	3	-	2	7
<b>TOTAL</b>	<b>21</b>	<b>12</b>	<b>55</b>	<b>2</b>	<b>54</b>	<b>144</b>

## PULA Bivalvia (13)

*Acanthocardia deshayesii*

*Axiinulus croulinensis*

*Corbula gibba*

*Dosinia lupinus*

*Gari fervens*

*Kurtiella bidentata*

*Mysia undata*

*Pitar rudis*

*Plagioocardium papillosum*

*Tapes (Tapes) rhomboides*

*Tellina distorta*

*Tellina donacina*

*Thyasira flexuosa*

## RIJEKA Bivalvia (45)

*Abra alba*

*Abra nitida*

*Abra tenuis*

*Acanthocardia echinata*

*Acanthocardia sp.*

*Anomia ephippium*

***Axiinulus croulinensis***

*Azorinus chamasolen*

*Bornia sebetta*

*Bryopa aperta*

*Clavisinella fasciata*

*Corbula gibba*

*Flexopecten hyalinus*

*Gari fervens*

*Gouldia minima*

*Hiatella arctica*

*Hiatella rugosa*

*Kellia suborbicularis*

*Kurriella bidentata*

*Laevicardium crassum*

*Laevicardium oblongum*

*Loripes lacteus*

*Lucinoma borealis*

*Modiolarca subpicta*

*Modiolus barbatus*

*Mytila spinifera*

*Mysia undata*

*Mytilus galloprovincialis*

*Nucula nitidosa*

*Nuculana illirica*

*Nuculana pellata*

*Parvicardium ovale*

*Phaxas pellucidus*

*Pitar rudis*

*Plagioocardium papillosum*

*Pseudochama gryphina*

*Roccellaria dubia*

*Saxicavella jeffreysi*

*Sphenia binghami*

*Tapes (Tapes) rhomboides*

*Tellina distorta*

*Tellina donacina*

*Tellina serrata*

*Thyasira flexuosa*

*Timoclea ovata*

## PULA & RIJEKA Bivalvia (49)

*Acanthocardia deshayesii*

*Abra alba*

*Abra nitida*

*Abra tenuis*

*Acanthocardia echinata*

*Acanthocardia sp.*

*Anomia ephippium*

***Axiinulus croulinensis***

*Azorinus chamasolen*

*Bornia sebetta*

*Bryopa aperta*

*Clavisinella fasciata*

*Corbula gibba*

*Flexopecten hyalinus*

*Gari gibba*

*Gouldia minima*

*Hiatella arctica*

*Hiatella rugosa*

*Kellia suborbicularis*

*Kurriella bidentata*

*Laevicardium crassum*

*Laevicardium oblongum*

*Loripes lacteus*

*Lucinoma borealis*

*Modiolarca subpicta*

*Modiolus barbatus*

*Mytila spinifera*

*Mysia undata*

*Modiolus barbatus*

*Mytilus galloprovincialis*

*Nucula nitidosa*

*Nuculana illirica*

*Nuculana pellata*

*Parvicardium ovale*

*Phaxas pellucidus*

*Pitar rudis*

*Plagioocardium papillosum*

*Pseudochama gryphina*

*Roccellaria dubia*

*Saxicavella jeffreysi*

*Sphenia binghami*

*Tapes (Tapes) rhomboides*

*Tellina distorta*

*Tellina donacina*

*Tellina serrata*

*Thyasira flexuosa*

*Timoclea ovata*

## List of harmful aquatic organisms relevant for warning ships and environmental authorities:

*Hydroides dianthus*  
*Hydroides elegans*  
*Leiochrides australis*

## Not on List:

## *Phycopomatus enigmaticus*

Taxon/Impact		strong	medium	low	absent	unknown	total
	<b>FORAMINIFERA</b>	-	-	2	-	-	2
1	<b>PORIFERA</b>	-	-	1	-	-	1
2	<b>CNIDARIA</b>	19	2	1	-	12	34
	<b>CTENOPHORA</b>	1	-	-	-	-	1
3	<b>GASTROPODA</b>	-	2	8	-	1	11
	<b>BIVALVIA</b>	1	1	9	1	8	20
	<b>CEPHALOPODA</b>	-	-	-	-	1	1
4	<b>POLYCHAETA</b>	-	3	15	-	14	32
	<b>COPEPODA</b>	-	1	1	-	1	3
	<b>DECAPODA</b>	-	1	11	-	5	17
	<b>AMPHIPODA</b>	-	-	-	1	4	5
5	<b>ISPOPODA</b>	-	-	-	-	1	1
	<b>TANAIDACEA</b>	-	-	-	-	1	1
	<b>MYSIDACEA</b>	-	-	-	-	1	1
	<b>STOMATOPODA</b>	-	-	1	-	-	1
	<b>PYCNOGONIDA</b>	-	-	1	-	1	2
6	<b>BRYOZOA</b>	-	-	1	-	1	2
7	<b>ECHINODERMATA</b>	-	-	1	-	1	2
	<b>ASCIIDIACEA</b>	-	2	3	-	2	7
	<b>TOTAL</b>	<b>21</b>	<b>12</b>	<b>55</b>	<b>2</b>	<b>54</b>	<b>144</b>

**PULA**      **RIJEKA**  
**Polychaeta (63)**      **Polychaeta (48)**

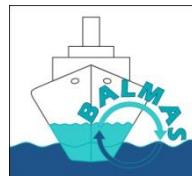
## PULA & RIJEKA Polychaeta (103)



# Monitoring requirements suggestions for BW in the Adriatic



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**Table 2**

Suggested sampling frequency requirements for monitoring of presence-absence and population dynamics (abundance and/or biomass) of NIS of different taxonomic groups and varying life cycle lengths.

Organism group	Presence/ absence	Population dynamics
Pathogens and other disease agents	Seasonal	Variable
Phytoplankton	Seasonal	Frequent, depending on biosecurity requirements
Zooplankton	Seasonal	Monthly (bi-weekly)
Benthic vegetation	Seasonal/ annual	Seasonal/annual
Zoobenthos	Annual	Annual
Fish	Annual	Annual at specific times (e.g. reproduction)

From: **Dose of truth—Monitoring marine non-indigenous species to serve legislative requirements** by Lehtiniemi, M., Ojaveer, H., David, D., Galil, B., Gollasch, S., McKenzie, C., Minchin, D., Occhipinti-Ambrogi, A., Olenin, S., Pederson, J. Marine Policy 54 (2015) 26–35.



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## **Recommendations for monitoring frequency would be as follows:**

- A. For **microorganisms** (viruses, bacteria, microalgae) – on **weekly** basis (also in accordance with BW Control and Management in developing countries recommendations).
- B. For **macroflora** and **fauna** (i.e., all other groups) – **seasonally** or at least **twice a year** (as suggested for monitoring of IAS in Mediterranean Marine Protected Areas).



Groups	Categories levels						Taxonomist present in at least 1 country
	Total	Strong	Medium	Low	Absent	Unknown	
Viruses*	1	1	0	0	0	0	
Bacteria*	12	12	0	0	0	0	
MICROALGAE							+
MACROPHYTES							+
Foraminiferans							
Poriferans							
CNIDARIANS							
Ctenophores							
GASTROPODS							+
BIVALVES							+
Cephalopods							
POLYCHAETES							
Copepods							
DECAPODS							
Amphipods							
Isopods							
Tanaids							
Mysid							
Stomatopods							
Pycnogonids							
Bryozoans	2	0	0	1	0	1	
Echinoderms	2	0	0	1	0	1	
Ascidians	7	0	2	3	0	2	
FISHES	21	4	2	13	1	1	

## MICROORGANISMS – plankton

- relatively fast results (days – weeks)
  - experts often available
    - available methods
  - but no/poor remedy measures

therefore...

Monitoring?  
Frequency? Purpose?

Groups	Categories levels						Taxonomist present in at least 1 country
	Total	Strong	Medium	Low	Absent	Unknown	
Viruses*	1	1	0	0	0	0	
Bacteria*	12	12	0	0	0	0	
MICROALGAE	113	113	0	0	0	0	+
MACROPHYTES	22	20	2	12	0	12	+
Foraminiferans							
Poriferans							
CNIDARIANS							
Ctenophores							
GASTROPODS							+
BIVALVES							+
Cephalopods							
POLYCHAETES							
Copepods							
DECAPODS							
Amphipods							
Isopods							
Tanaids							
Mysid							
Stomatopods							
Pycnogonids							
Bryozoans	2	0	0	1	0	1	
Echinoderms	2	0	0	1	0	1	
Ascidians	7	0	2	3	0	2	
FISHES	21	4	2	13	1	1	

## MACRO FLORA and FAUNA

- long separation period
- various experts
- no efficient remedy measures

therefore...

Monitoring?  
Frequency? Purpose?



**In addition to detecting IAS/HAB species, a method which should be highly advised to be included in standard monitoring efforts is a rapid bacterial bioluminescence assay with *Vibrio fisheri* (Microtox standardised test)**

a useful and rapid bacterial bioluminescence assay with *Vibrio fisheri* (Microtox standardised test)

Determination of seawater toxicity in Shuaiba Industrial Area, Arabian Gulf, Bay of Brest, France, Bay of Mexico, Albufera National Park, Spain, Aegean Sea, Greece, as well as at 24 sampling sites along Adriatic coast, Croatia, during 12 years of continuous monitoring.



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