ciimar

Interdisciplinary Centre of Marine
and Environmental Research

Challenges in Microbiology

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GEOGRAPHIC LOCATION
1
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4
1 Porto Headquarters
2 Lisboa
3 Açores Archipelago
4 Madeira Archipelago

RESEARCH DOMAINS

3
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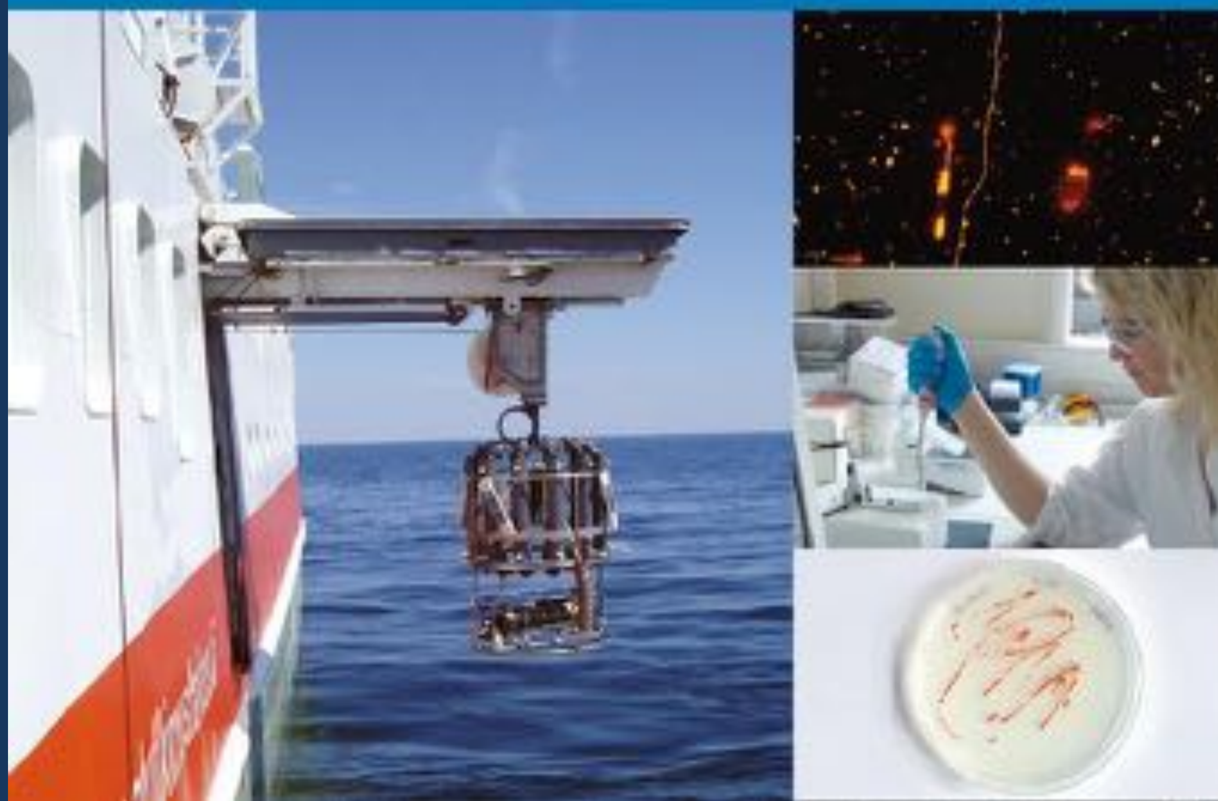
MARINE BOARD

Position Paper 17

Marine Microbial Diversity and its role in Ecosystem Functioning and Environmental Change

May 2012



Marine microbial diversity

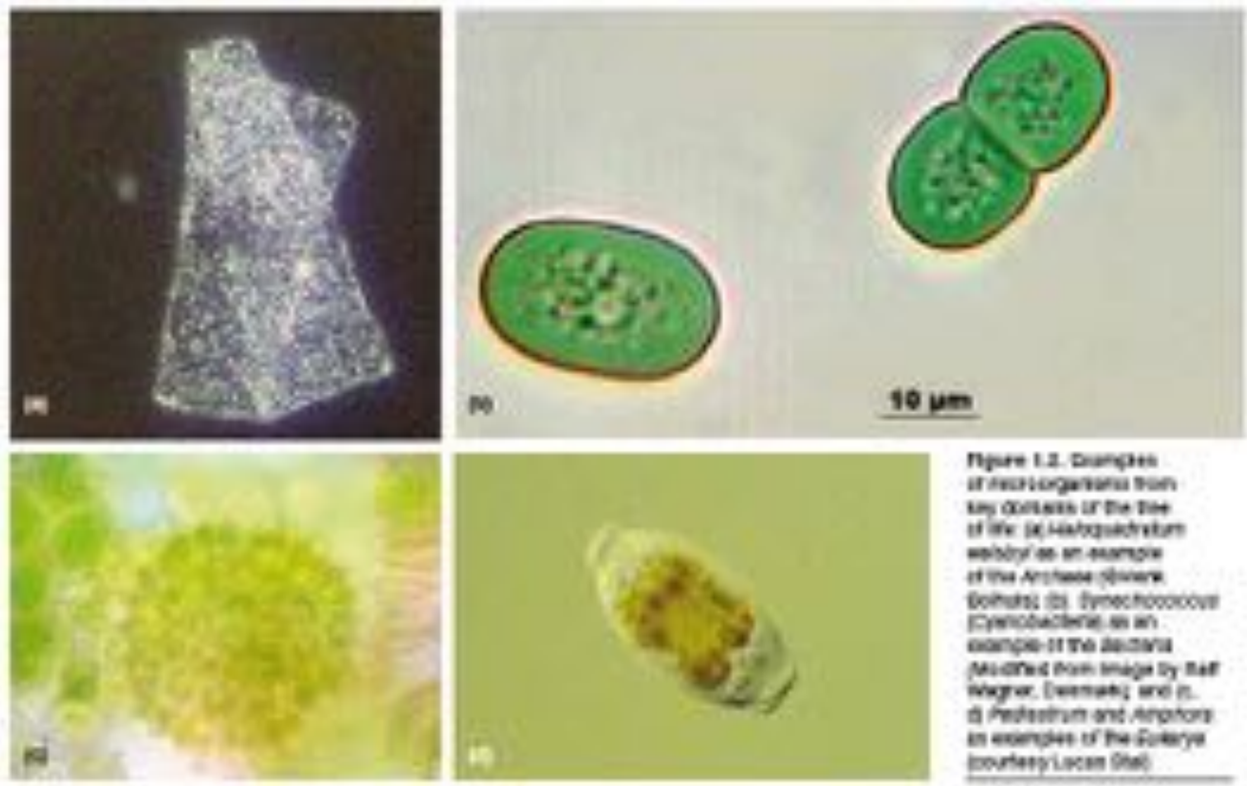


Figure 1.2. Examples of microorganisms from key domains of the tree of life: (a) Haloquadratum walsbyi as an example of the Archaea domain; (b) Synechococcus (Cyanobacteria) as an example of the Bacteria (Modified from image by Ralf Wagner, Denmark) and (c, d) Pediastrum and Amphora as examples of the Eukarya (courtesy Lucas Stal)

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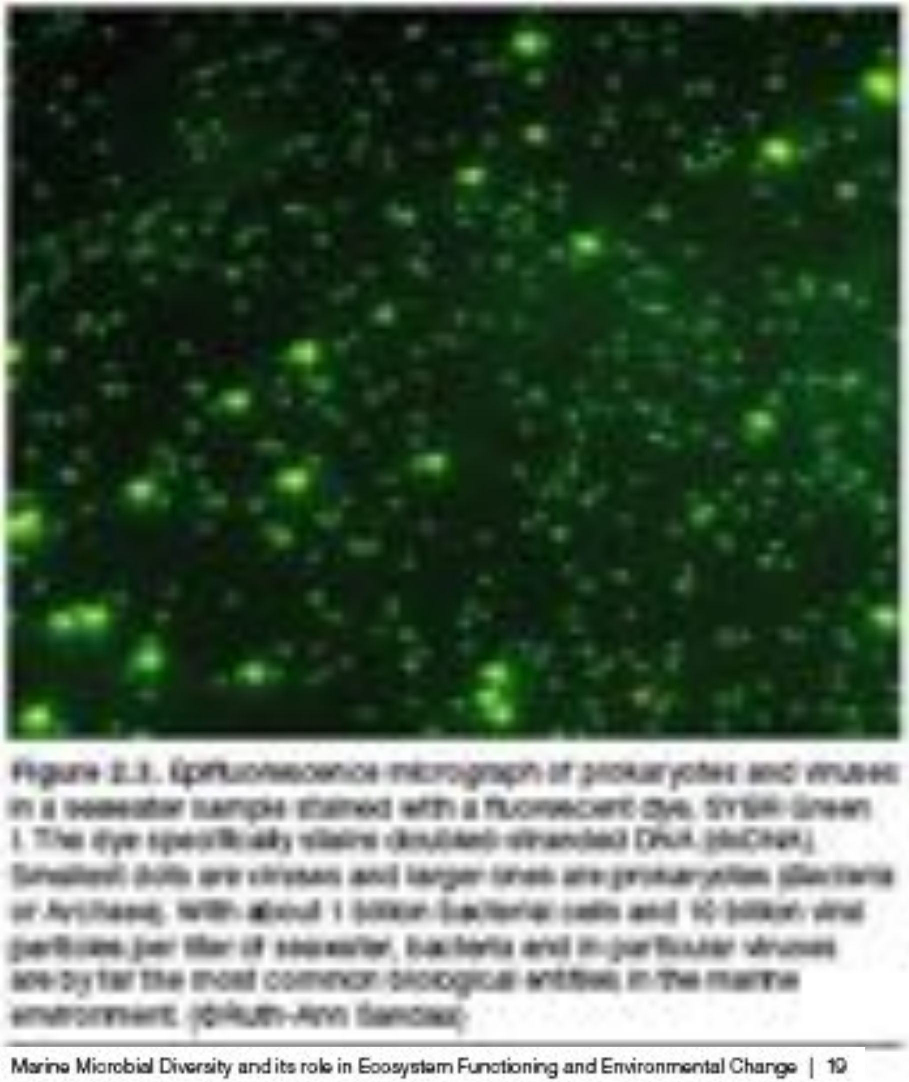


Universe http://purehdwall.com

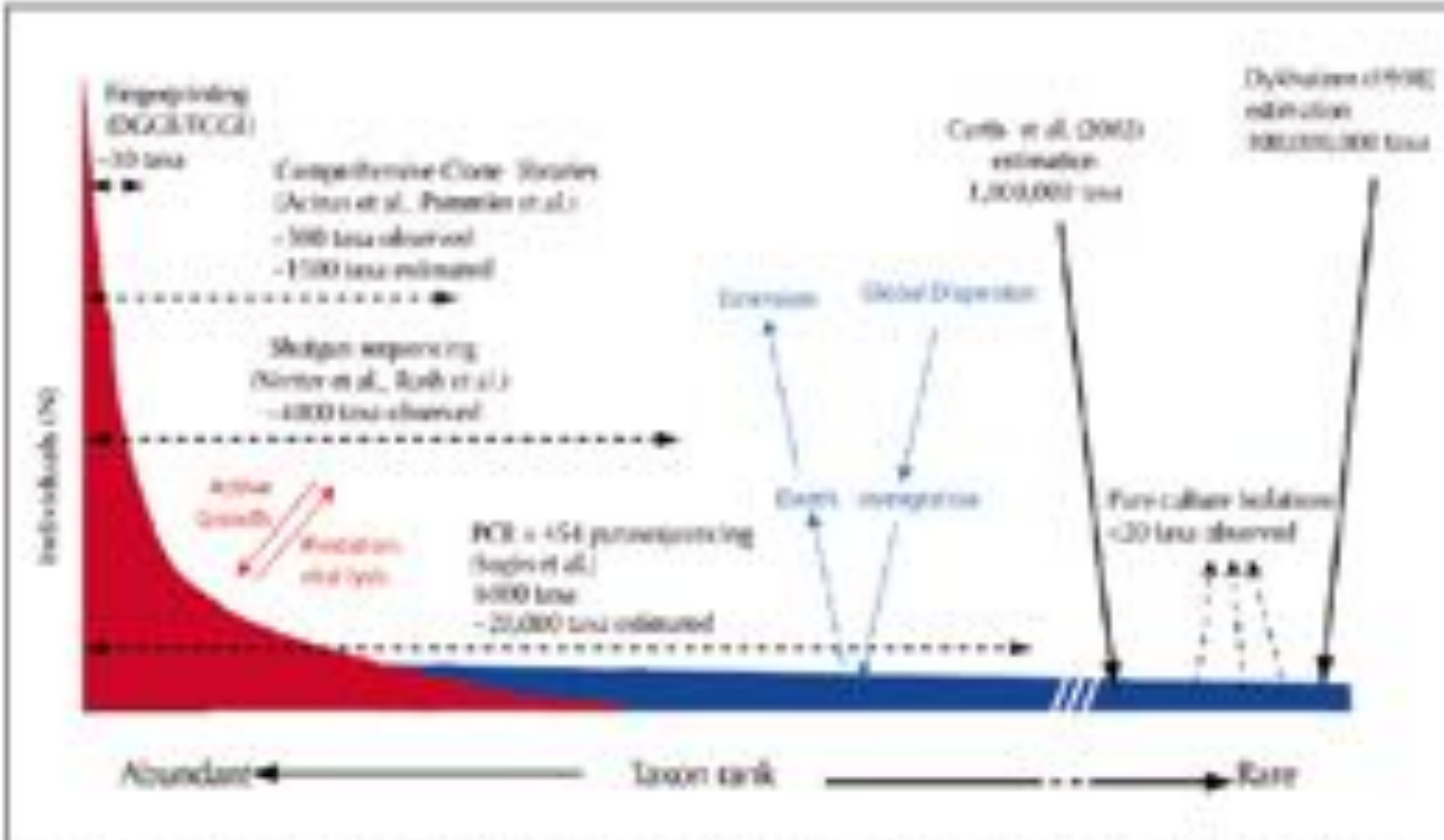
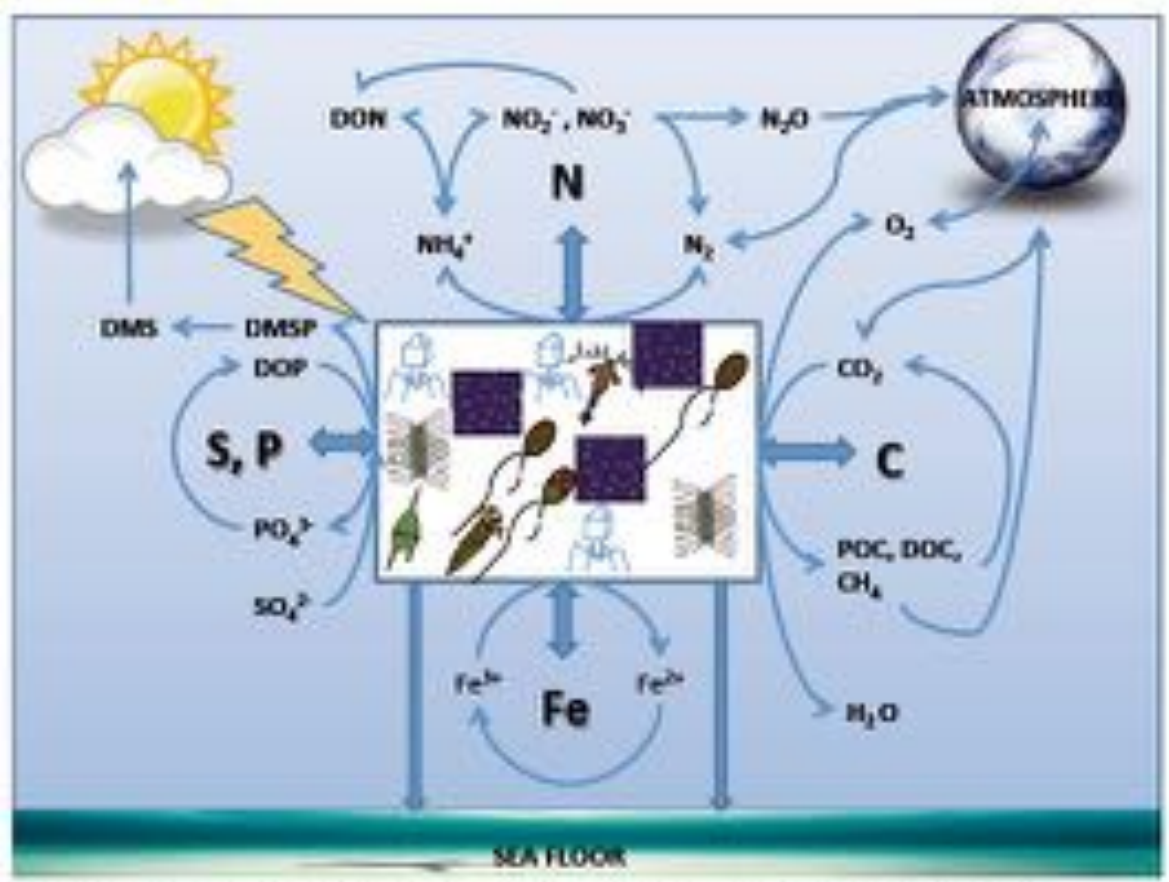


Figure 2.14. A conceptual model of how microbial diversity in the ocean is expressed, i.e. a few organisms are relatively abundant and participate in ecosystem functioning while the majority of microbes await more-optimal growing conditions, as shown above in the large 'tail of biodiversity'. The image also indicates the level of diversity that can be assessed by each current methodology (Adaptation by Thomas Pommier, CNRS, of an original figure by Pedrós-Alió, 2006).

Microbes and ecosystem functioning

- Baseline studies
- Nitrogen and carbon fixation



Microbes and ecosystem functioning

Chlorophyll a distribution

Information Box 6. Cyanobacterial blooms in the Baltic Sea

Eutrophication is one of the fundamental results of human activity in the Baltic Sea. High nutrient loads, predominantly from intensive agriculture or municipal sewage, cause the formation of extensive blooms of primary producers in spring and summer. The spring bloom is dominated by diatoms and dinoflagellates and leads to a decrease of nitrate and phosphorus concentrations in the water. The summer bloom is dominated by the cyanobacteria, *Nodularia spumigena*, *Aphanizomenon flos-aquae*, *Anabaena* sp., and *Synechococcus* spp., of which the first three are often characterized by their ability to fix dinitrogen. Dinitrogen-fixing primary producers become predominant in the summer bloom when nitrogen availability is limited but phosphorus is still available. The general availability of phosphorus in the water column is a specific feature of the central Baltic Sea and is connected to the anoxic nature of the bottom waters of this area as anoxia enhances the release of phosphorus from the sediment. The phosphorus stock in the water column thus increases and feeds surface primary production. As demonstrated by satellite images (Figure 2.15), cyanobacterial blooms may cover wide areas of the whole Baltic Sea and since some Cyanobacteria are potential cyanotoxin producers, the blooms can be hazardous for higher life forms, which in itself reveals the potential socio-economic impact on Baltic riparian countries.

Figure 2.15. Satellite image, acquired on 21 July 2004, by Envisat's Medium Resolution Imaging Spectrometer (MERIS), captures a cyanobacterial bloom in the Baltic Sea (provided by H. Siegel, IOW). (DESA)

Difficulties in studying microbes

Size

Diversity

Systematics

Dynamics

Distribution

Sampling

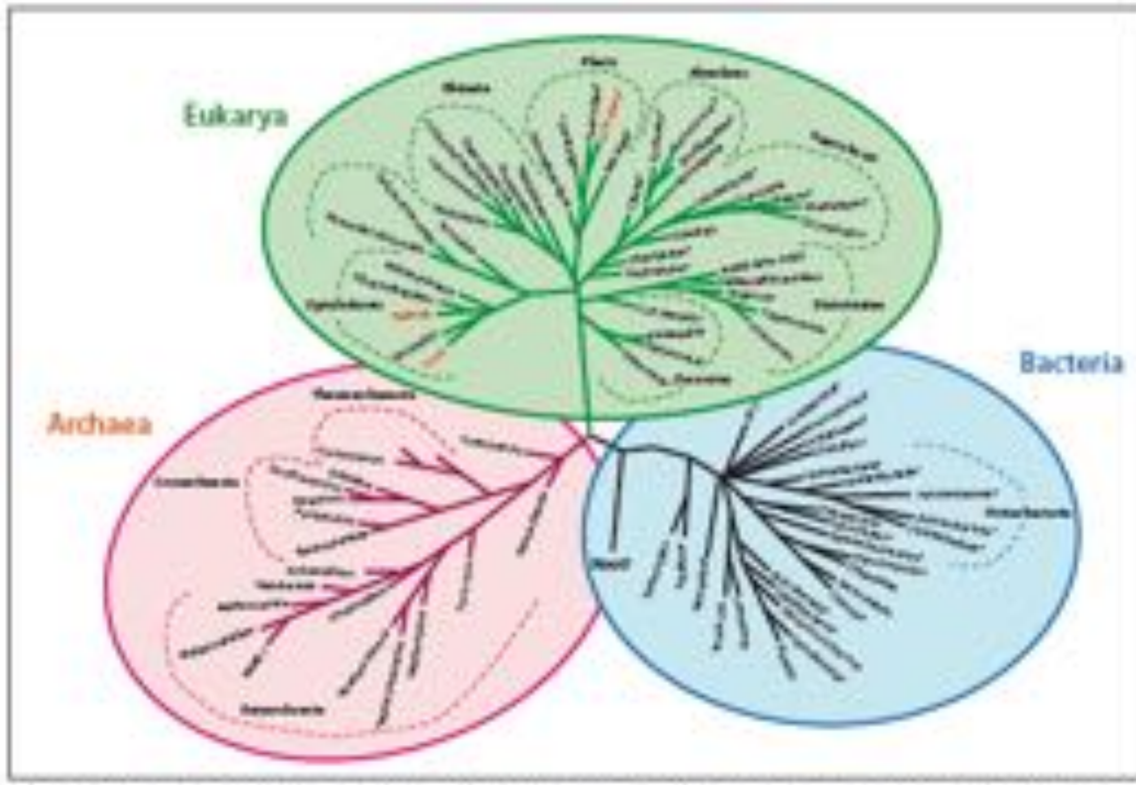


Figure 1.1. A tree of life including Archaea, Bacteria and Eukarya. The groups in black colour are mostly or completely microbial. The groups in red are not. Groups with asterisks are marine, or include a large amount of marine organisms. (Figure adapted from Baldauf 2003 using the colouring scheme of Barton et al. 2007 and the archaeal groups following Brochier-Armanet et al. 2008).

Marine Microbial Diversity and its role in Ecosystem Functioning and Environmental Change | 11

Unbalanced microbe communities

- Contamination (anthropogenic origin)
- Global changes (temperature increase, acidification)
- Invasive species (balast water, aquaria, aquaculture)

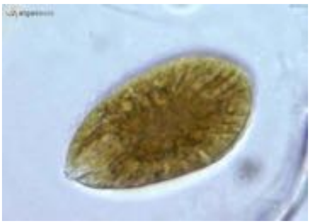
www.marine-aquarium-time-os-9softsia.com

www.aquaculture-product.com

www.sqeacademy.com

Ostreopsis ovata

palytoxin and ovatoxin-a



- Episodes in Europe reported since 2001
- Until recently confined to Mediterranean
- Bloom in 2011 in Algarve (south Portugal)

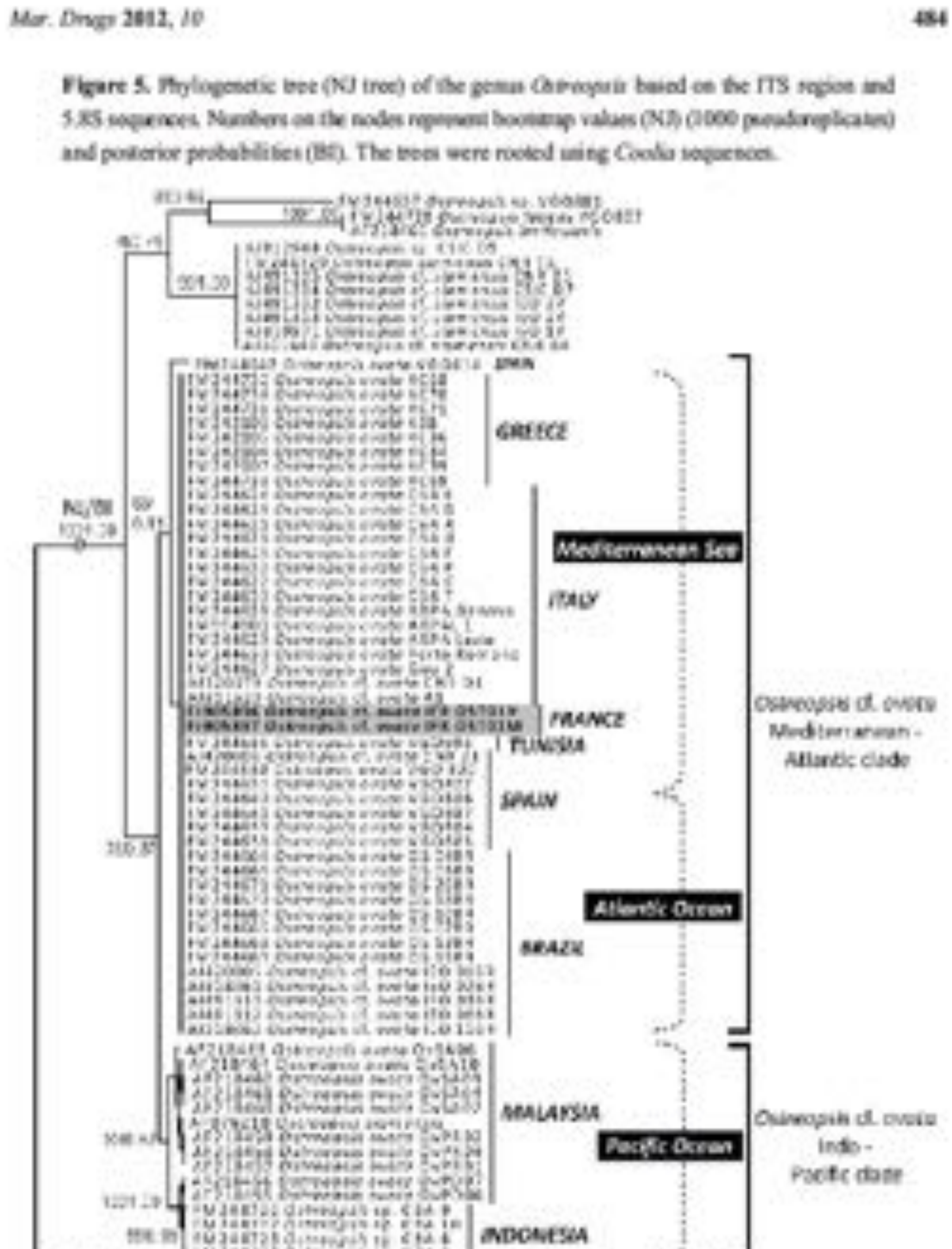


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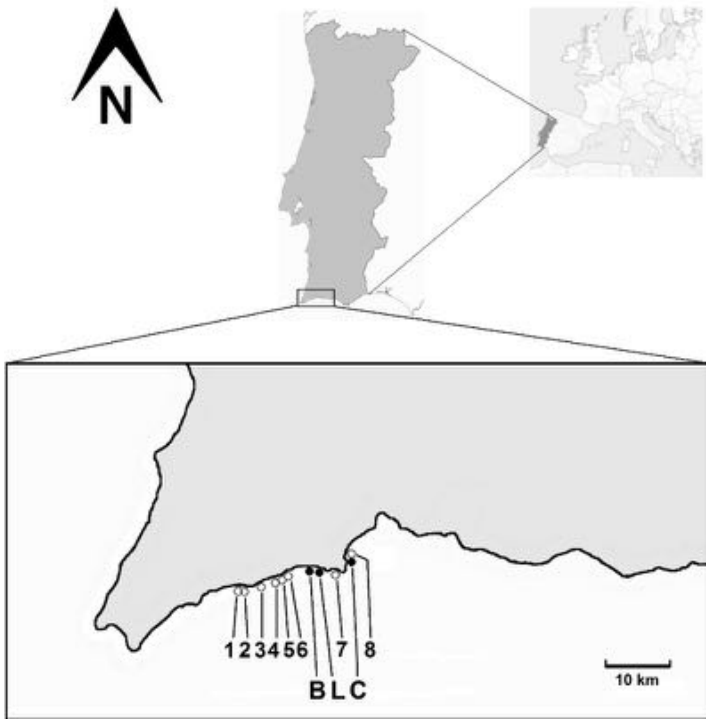
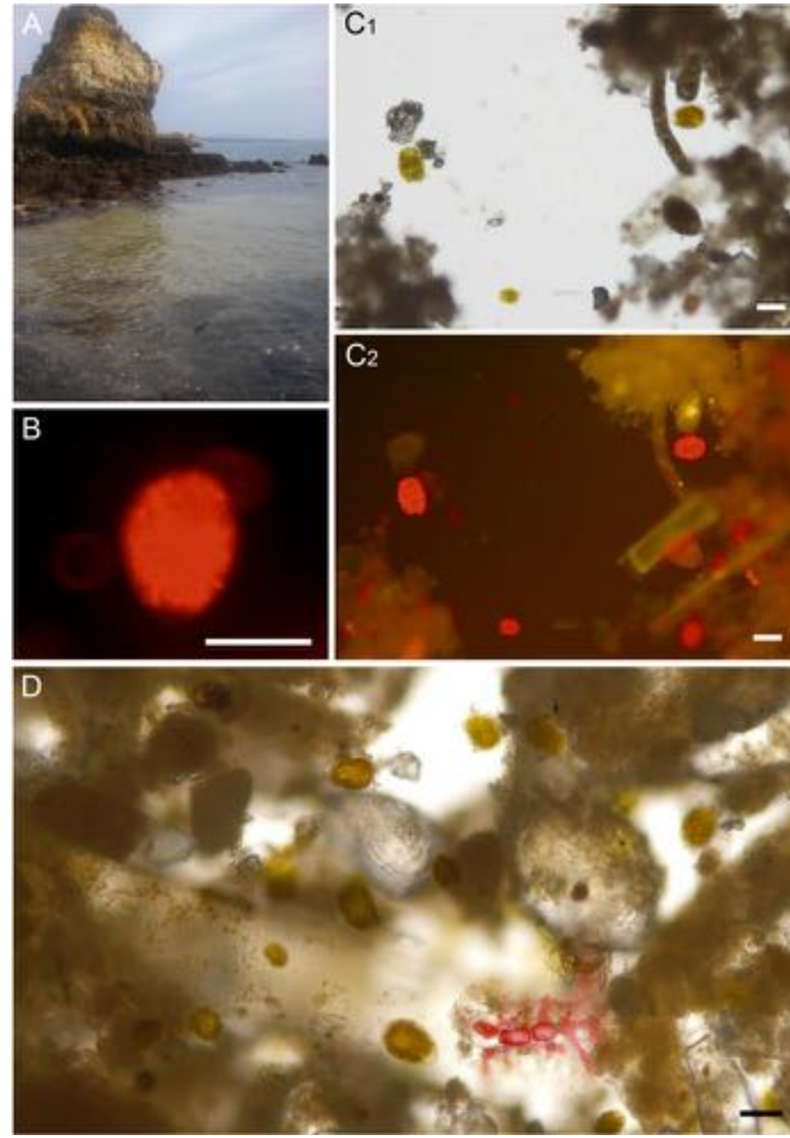
Figure 5. Phylogenetic tree (NJ tree) of the genus *Ostreopsis* based on the ITS region and 5.8S sequences. Numbers on the nodes represent bootstrap values (NJ) (1000 pseudoreplicates) and posterior probabilities (BI). The trees were rooted using *Coolia* sequences.

Emergent risks- “new” red tides

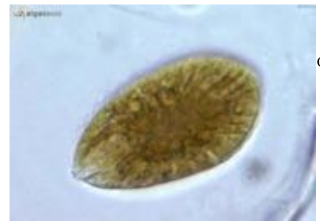
Algarve 2011

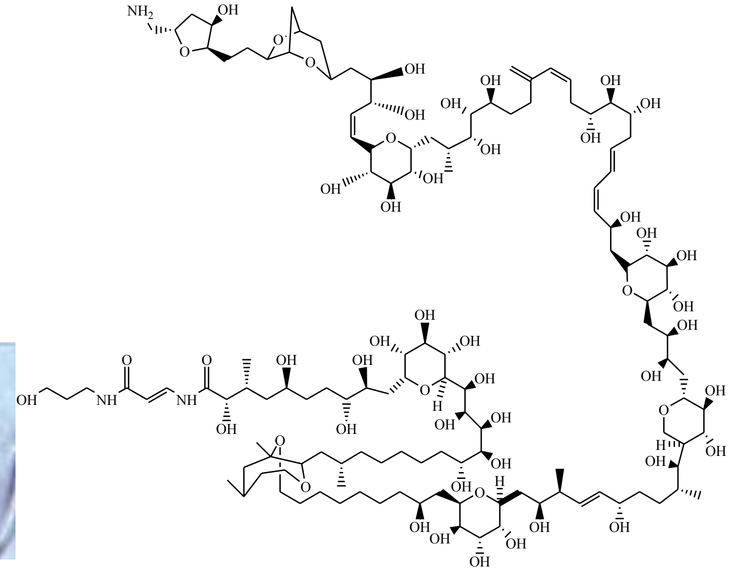
Ostreopsis spp. – palitoxin





Palytoxin and analogues

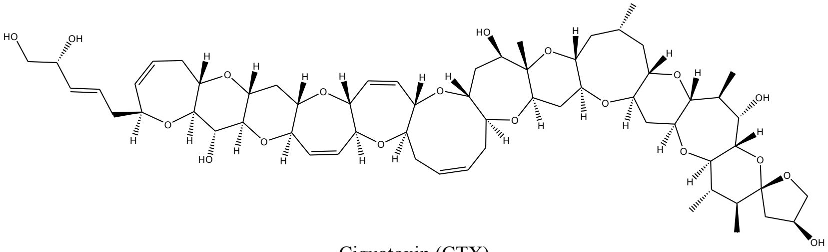




Organism	*Ostreopsis ovata*
Symptoms	Chest pain, respiratory distress, tachycardia, unstable blood pressure, hemolysis
Treatment	Life support system
Toxins	Palitoxin and ovatoxin-a
Mode of action	Open sodium channels

Ciguatera

Intoxication by ciguatoxins



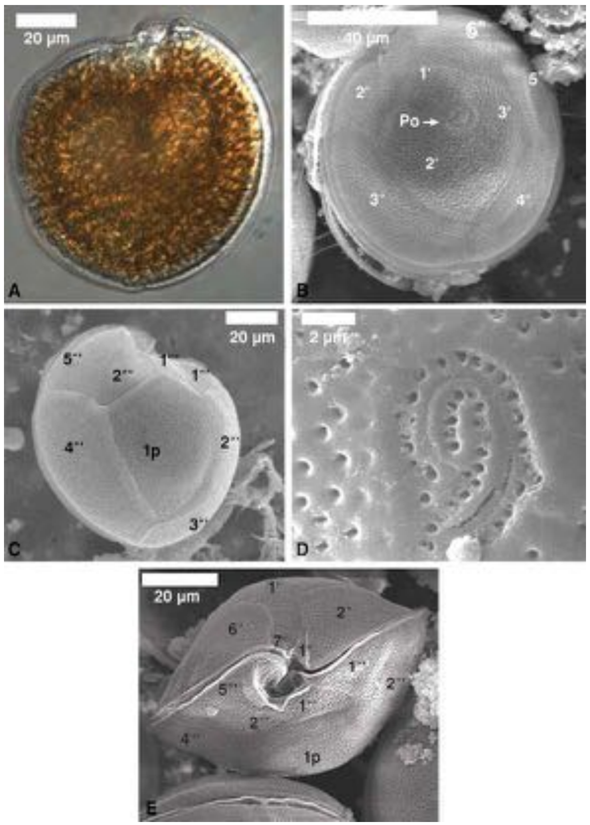
Ciguatoxin (CTX)
$C_{61}H_{88}O_{19}$
Mol. Wt.: 1125,34 g/mol

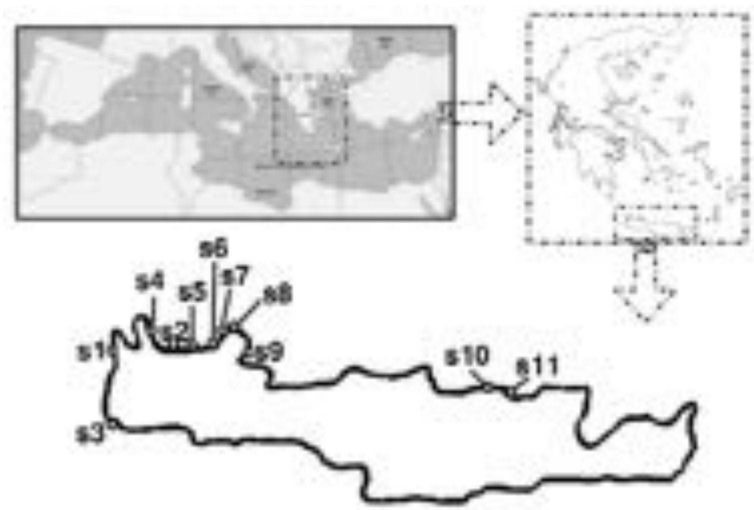
Organism	*Gambierdiscus toxicus* (vectors – carnivorous fish - barracuda)
Symptoms	Symptoms 6 hours after ingestion; onausea, diarrhoea, vomits, headache, vertigo, muscular weakness, prostation. Cardiovalscular symptoms, brachycardia or tachycardia, low blood pressure. Low mortality following respiratory or cardiac arrest .
Treatment	Life support system
Toxin	ciguatoxin
Mode of action	Block sodium channels

Vector fish of ciguatera toxins

WESTPAC/IOC/UNESCO

Gambierdiscus episodes in the Mediterranean





Samples collected from 2003-2007 showed the occurrence of *Gambierdiscus* in Greece.

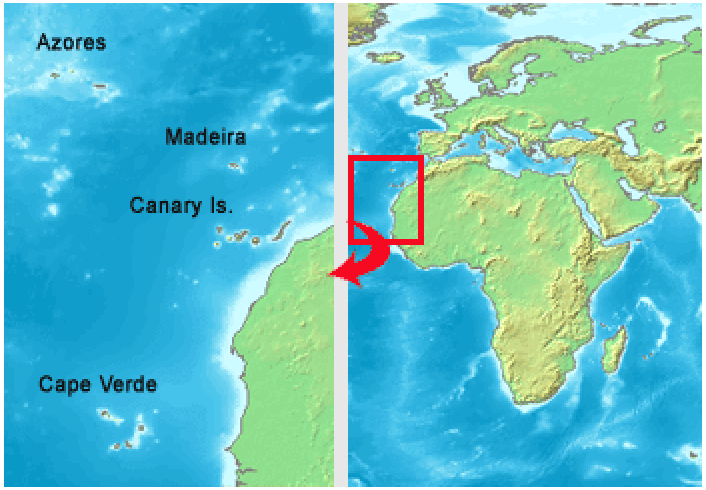
(Aligizaki and Nikolaidis, 2008)

Gambierdiscus episodes in the North Atlantic

Spain

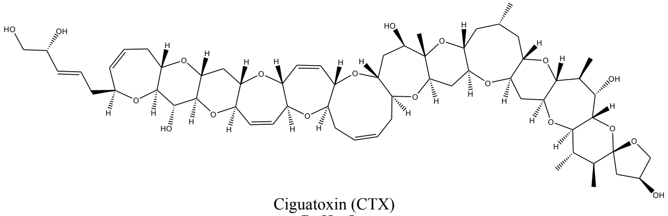
Perez-Arellano et al. [2005] reported for the first time the occurrence of CFP in the Canary Archipelago.

In January 2004, a 26-kg amberjack (*Seriola rivoliana*) was eaten and caused human intoxications.



Portugal

Six patients were intoxicated after consumption of various fish species *(Seriola sp., Sparisoma credence, Serranus atricauda, Bodianus scrofa, Balistes capriscus and Pagrus pagrus)* captured at the Selvagem Island (Madeira archipelago).



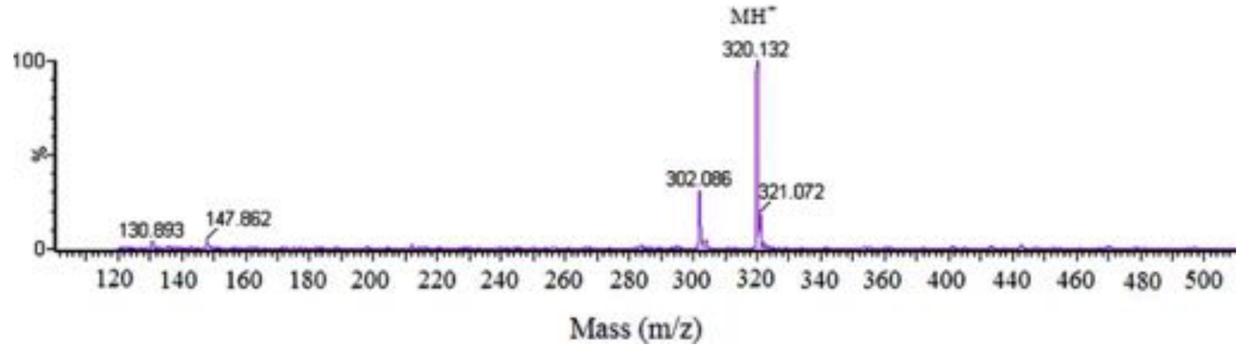
Ciguatoxin (CTX)
$C_{61}H_{88}O_{19}$
Mol. Wt.: 1125,34 g/mol

Otero et al (2010) established the profile of the ciguatoxins of the fish.

First Report of Tetrodotoxin in Europe (2007)

Trumpet Shell *Charonia lampas lampas*

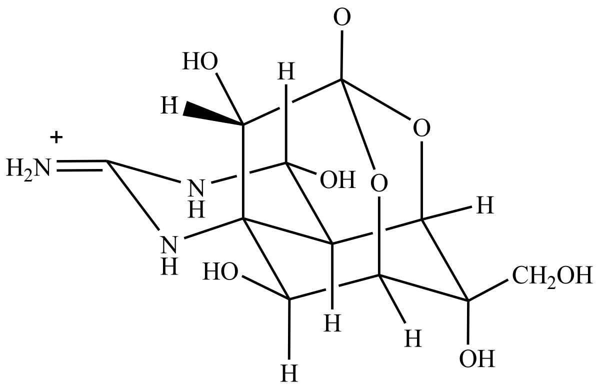
(Rodriguez et al., 2008)



C. lampas bought in Malaga market (molusks caught in Algarve, Portugal)

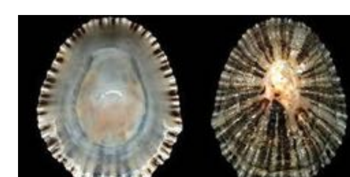
49 year old man with abdominal pain with nausea and vomiting, weakness, difficulty articulating words and keeping the eyelids open, and difficulty breathing. Fully recovered after 72 h

Intoxication by tetrodotoxin



Organism	*Vibrio* , *Serratia maecescens* and *Microbacterium arabinogalactanolyticum* (vectors – fish, gastropods, crustaceans, echinoderms)
Symptoms	20-30 minutes after ingestion, dry lips, paresthesia of face and limbs, dizziness, headache, nausea, vomits,. Muscle paralysis, cyanosis, low blood pressure,. Paralysis of the victim for 6-8 hours (zombie state) until death.
Treatment	Life support system
Toxin	tetrodotoxin
Mode of action	Block sodium channels

Seach of new marine toxin vectors



Mar. Drugs **2012**, *10*, 712-726; doi:10.3390/md10040712

OPEN ACCESS
Marine Drugs
ISSN 1660-3397
www.mdpi.com/journal/marinedrugs

Article

New Gastropod Vectors and Tetrodotoxin Potential Expansion in Temperate Waters of the Atlantic Ocean

Marisa Silva [1,2], Joana Azevedo [1,2], Paula Rodriguez [4], Amparo Alfonso [4], Luis M. Botana [4] and Vitor Vasconcelos [1,2,*]

Figure 1. Location of the sampling points in the North Atlantic Portuguese coast: 1 Viana do Castelo; 2 Esposende; 3 Póvoa do Varzim; 4 Angeiras; 5 Memória; 6 Valadares; 7 Aguda; 8 São Martinho do Porto; 9 São Torpes; 10 Porto Côvo; 11 Monte Clérigos; 12 Vila Nova de Milfontes; 13 Almograve.

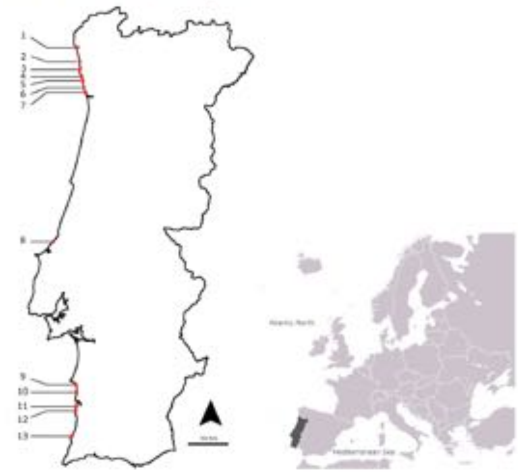


Table 1. TTX and analogues levels (µg/g) in marine gastropods from Portugal (pw-present work and [21]), China and Taiwan.

Species	Location	TTX	4-*epi*TTX	MonodeoxyTTX	5,6,11-trideoxyTTX	Ref.
G. umbilicalis	Memória			0.063		pw
M. lineata	Vila Nova de Milfontes	0.090	0.021			pw
C. lampas	Angeiras				0.006	pw
	Algarve	315.00 *			1004.00 *	[21]
N. nitidus	China	1350				[25]
N. semiplicatus		26.10	3.37			[12]
N. papillosus	Taiwan	42–60				[26]

* Data obtained from digestive gland only.

Impacts on human health

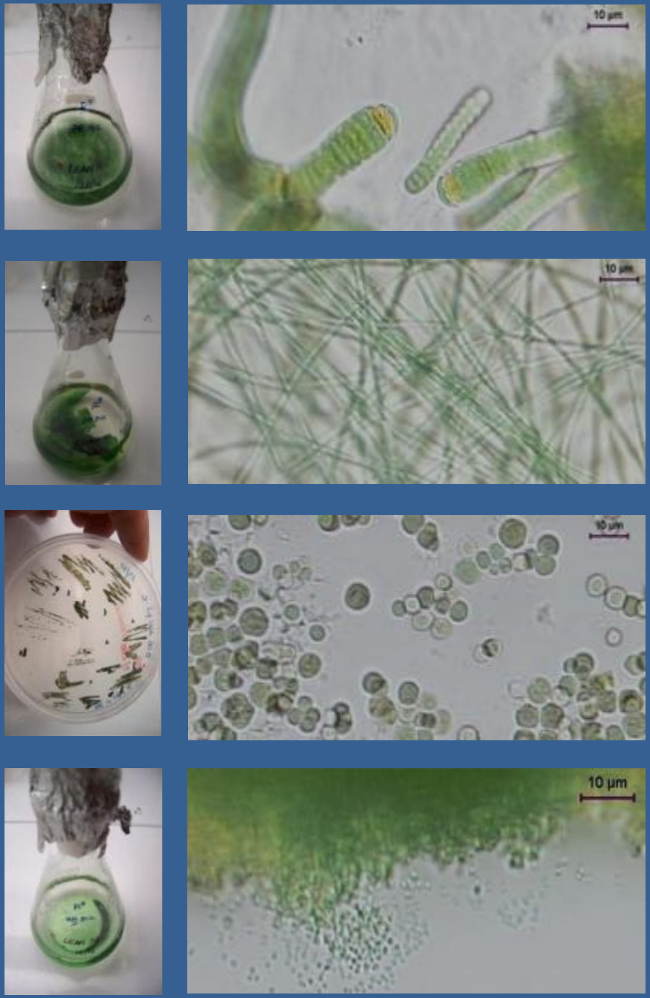
(-) Microbial contamination in recreational waters (bacteria, virus, HAB).

(-) Harmful Algal Blooms (fish and shellfish)

(+) New pharmaceuticals, nutraceuticals, cosmeceuticals and other industrial applications

Isolation and culture of microorganisms

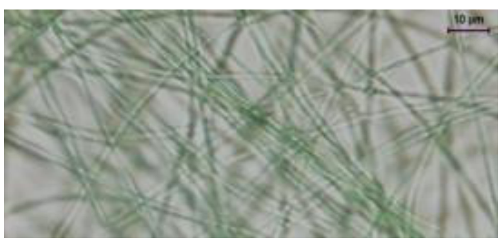
- discovery of new bioactive molecules isolated from bacteria, cyanobacteria and fungi



Cyanobacteria: Promise microbial group in the search of novel **bioactive compounds**

Cyanobactins:

- small ribosomal cyclic peptides
- antimalarial, antitumor, multidrug reversing activities
- potential as pharmaceutical leads



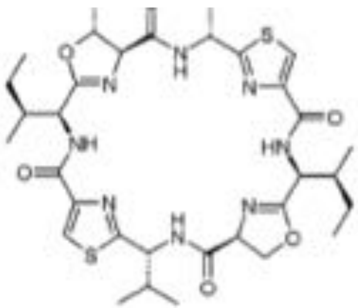
Free living cyanobacteria

Symbioses with sponges

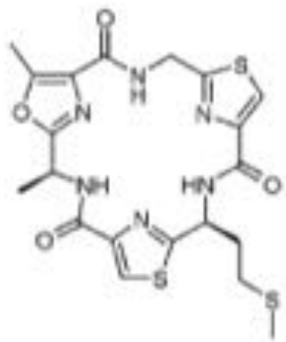
Symbioses with ascidians

Lissoclinum patella

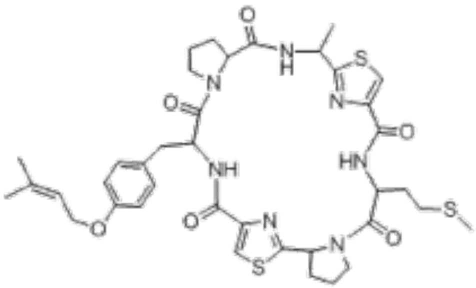
Cyanobactins chemical structures



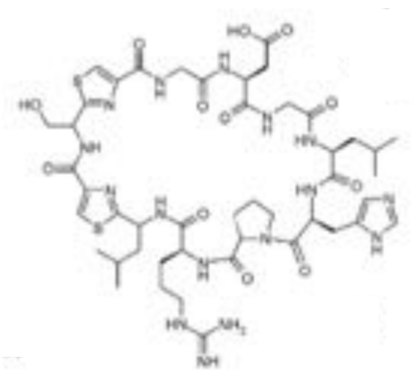
Patellamide A
Prochloron spp.



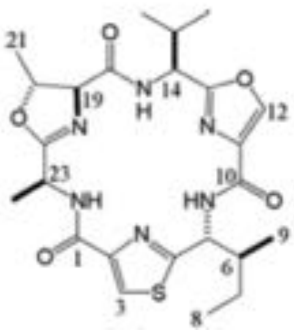
Tenuecyclamide C
Nostoc spongiaeforme



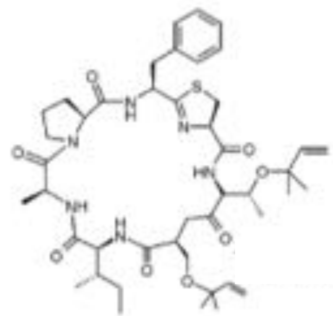
Lyngbyabactin A
Lyngbya aestuari



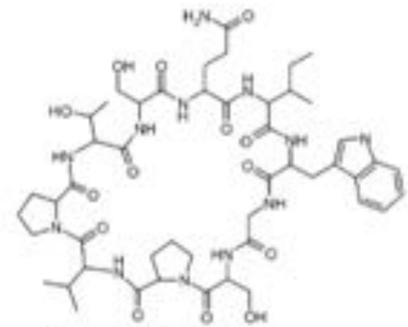
Trichamide
Trichodesmium erythraeum



Microcyclamide A
Microcystis aeruginosa



Trunkamide
Prochloron spp.



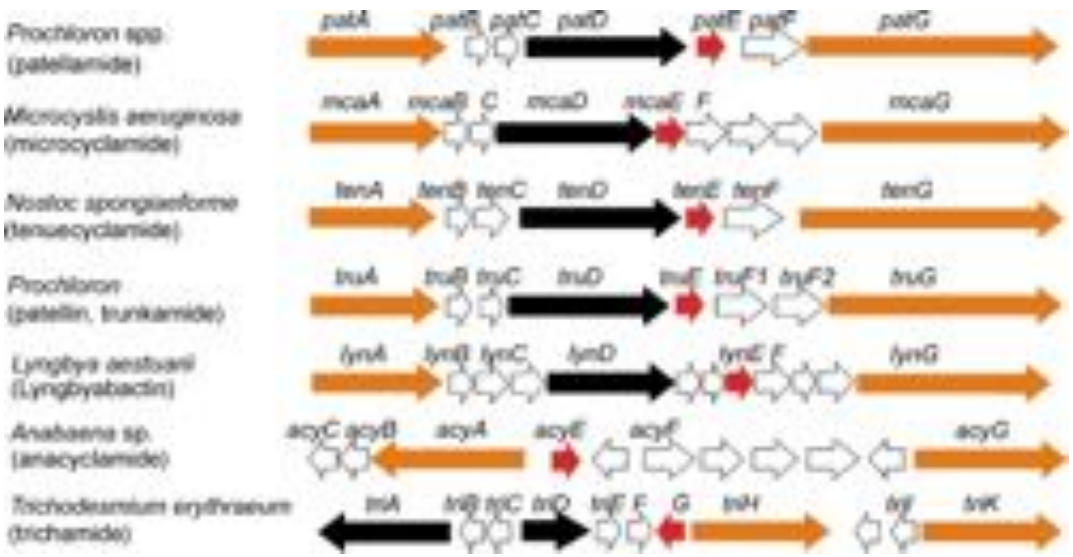
Anacyclamide A10
Anabaena sp.

Sivonen *et al.*, 2010; Donia *et al.*, 2008; Ziemert *et al.*, 2008;

Bioactivity of some selected cyanobactins produced by Cyanobacteria

Compound	Bioactivity	References
Patellamide A	Cytotoxic, antineoplastic	Ireland et al., 1982
Microcyclamide A	Moderate cytotoxicity against P388 murine leukemia cells	Ishida et al., 2000; Ziemert et al., 2008
Trunkamide	Cytotoxic, multidrug reversing activity	Caba et al., 2001; Salvatella et al., 2003; Donia et al., 2008
Trichamide	No effects found (tested for cytotoxic, antifungal, antibacterial and antiviral activities)	Sudek et al., 2006

Cyanobactin gene clusters published from seven distantly related cyanobacteria (Sivonen *et al.*, 2010)



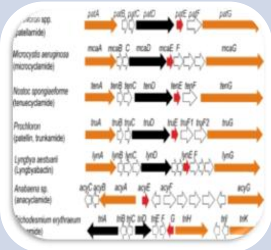
Genes Function:

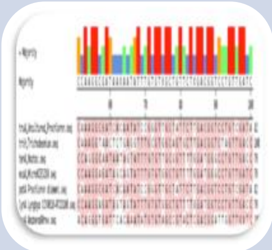
- Genes encoding proteases
- Genes encoding a short precursor peptide
- Genes encoding proteins involved in the maturation of the cyanobactin
- Conserved and hypothetical open reading frames

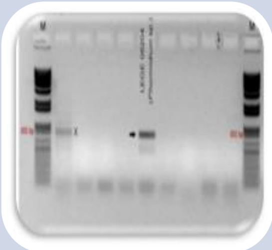
PHARMATLANTIC

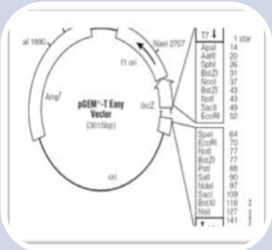
KNOWLEDGE TRANSFER NETWORK FOR PREVENTION OF MENTAL DISEASES AND CANCER IN THE ATLANTIC AREA

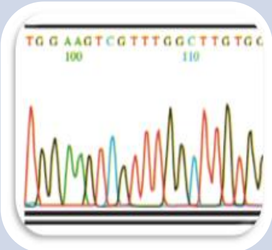
Molecular Screening: PCR analyses for cyanobactin (CBT) genes detection











Sequences from different cyanobacterial taxa

Primers design:

patA-like gene
patG-like gene

PCR: optimization and screening in LEGE CC

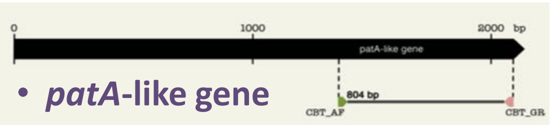
Cloning

- pGEM®-T Easy Vector Systems kit (Promega)
- transformation of chemically competent *Escherichia coli* ONE Shot TOP10 cells
- plasmid extraction with GenElute Plasmid Miniprep kit (Sigma-Aldrich)

Sequencing

Phylogenetic relationships

Primer set selection and optimization



- ***patA*-like gene**

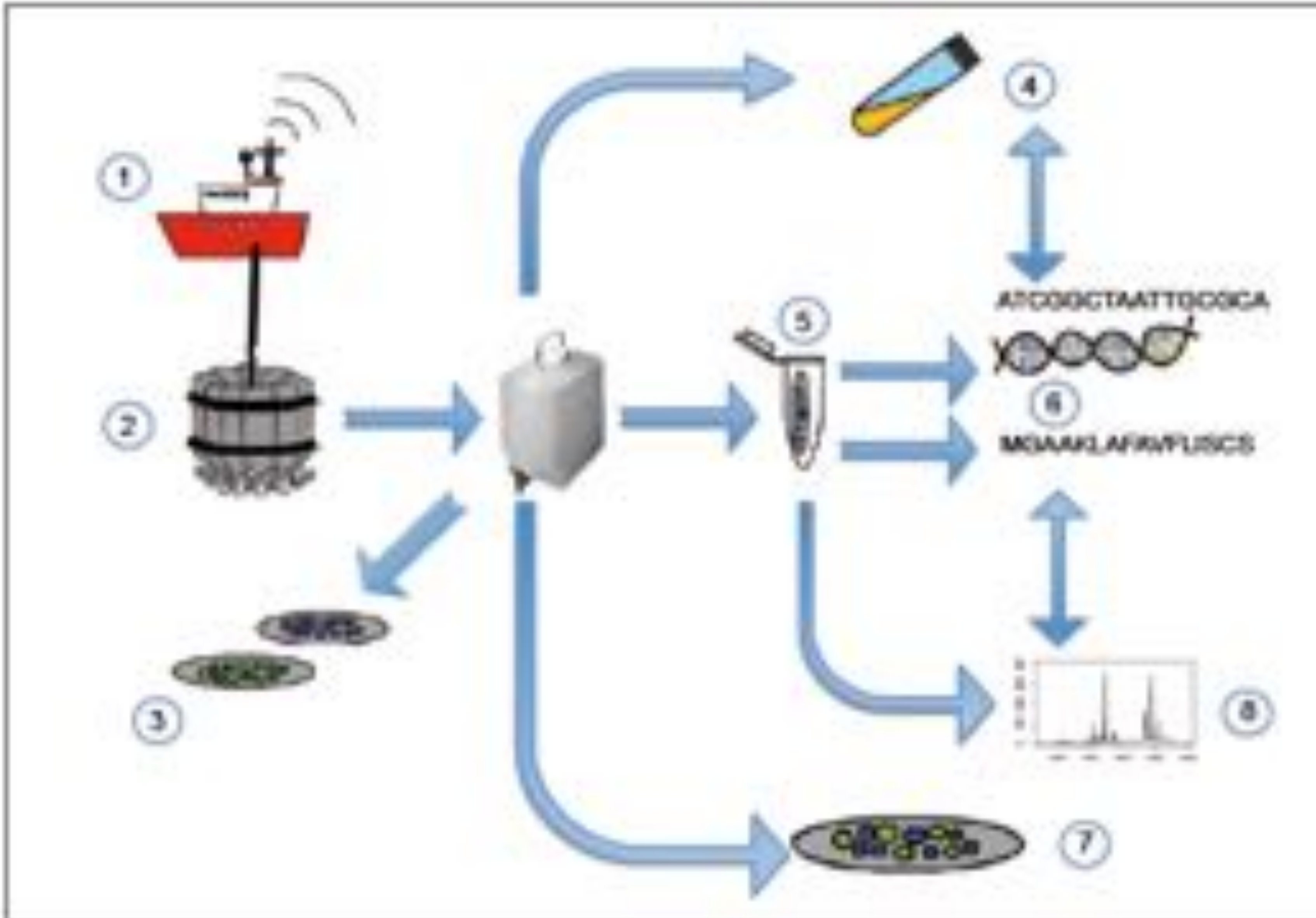


Figure 4.1. Types of data in studies of marine microbial diversity and function: (1) Geographic data; (2) Oceanographic data collected by the CTD sensors; (3) Chemical and Biological ancillary data; (4) Isolates from a given sample; (5) Microbial biomass or DNA stocks; (6) DNA, RNA and/or protein sequences; (7) Fluorescence In Situ Hybridization (FISH) samples; (8) Fingerprints of the distribution of different sequences of a specific gene. (Image by C. Ruiz-González and J.M. Gasol)

Needs – *ex situ* analysis

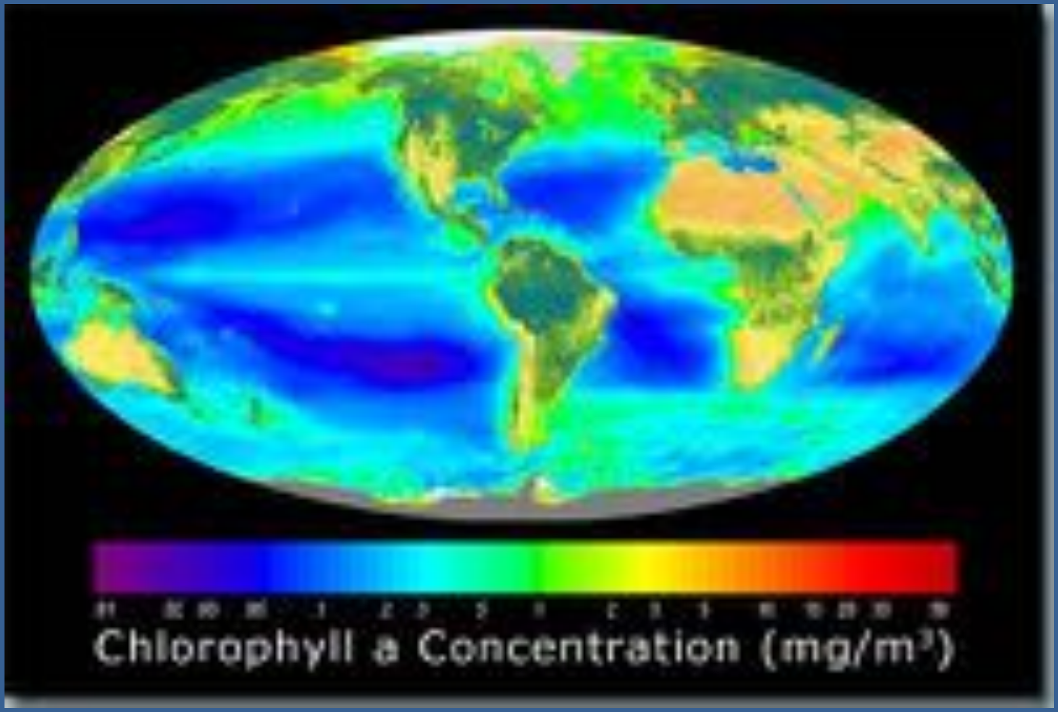
- Sampling devices for extreme environments (high depth, high temperature, high turbidity)
- Multiple sampling
- High volume or filtration/concentration of sample
- Possibility of simultaneous image capture in case of HAB (incorporated microscope?)

Needs – *in situ* analysis

- Chlorophyll mapping with alarm system

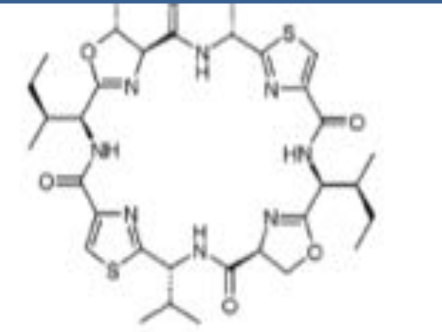
Ex: HAB in shellfish production areas and in recreational sites

http://www.gma.org

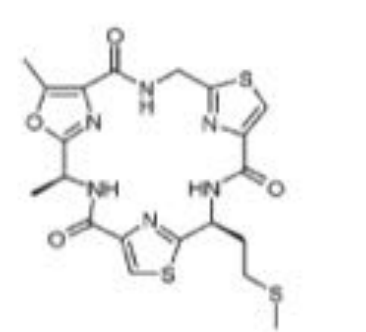


Needs – in situ analysis

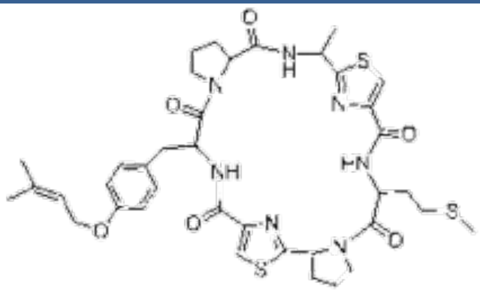
- RT-PCR (real time on line analysis of microbes) and possibility of sampling
- Ex genes PKS and NRPS responsible for interesting secondary metabolites.



Patellamide A



Tenuecyclamide C



Lyngbyabactin A

Needs – *in situ* analysis

Real time analysis of microbial contamination in recreational sites – sensors for microbes?

Ex. Blue flag standard

Sensors for HAB toxins (seafood safety)

Acknowledgements

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