The Deep-Sea Frontier

Biotechnology Opportunities and Conservation Challenges

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Workshop 1: Mobilisation and Engagement of Funding Agencies and Stakeholders

Hotel Real Marina, Olhão, Portugal

26th – 27th April 2012

(Marine) Biotechnology

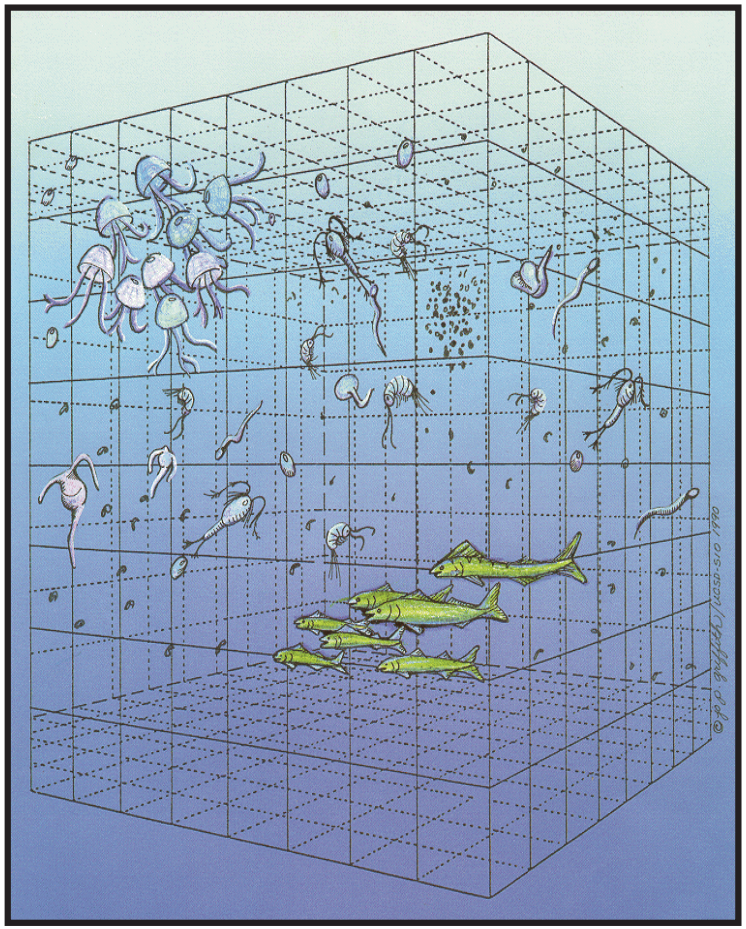
Importance of (Marine) Biotechnology for Mankind

- Aim to contribute to:
  - Sustainable supply of high quality and healthy food
  - Sustainable alternative sources of energy
  - Securing environmental health
  - Securing health and well being
  - Industrial products and processes
- Our last hope in a overcrowded, unstained, unhealthy and changing world?

Presentation Outline

- Biodiversity as Resource for Biotechnology
- Beyond the Blue: the deep-sea frontier
- Hot-spots: ex. Hydrothermal vents
- Research examples in extreme environments
- Conservation needs and challenges
- Sharing of benefits and access regulations

The Oceans
Huge & Diverse



- ***Huge*** 70% of the planet's surface and more than 90% of the available volume: 170 times more space available to life than all other combined ecosystems.
- ***Unknown*** In deep sea only an area corresponding to a few football fields has actually been sampled from a scientific point of view.
- ***Diverse*** A predicted number of marine species is 2.2 million, of which 91% still waiting description.
- ***Miscellaneous*** deep ocean is a miscellany with several levels of productivity and biomass.
- ***Rich*** In the seafloor and sub-seafloor there are abundant minerals, biominerals and energy deposits.

Serrão Santos R et al. 2012. Natural Resources, Sustainability and Humanity, Ch5. Springer

How Many Species in the Ocean?

Table 2. Currently catalogued and predicted total number of species on Earth and in the ocean.

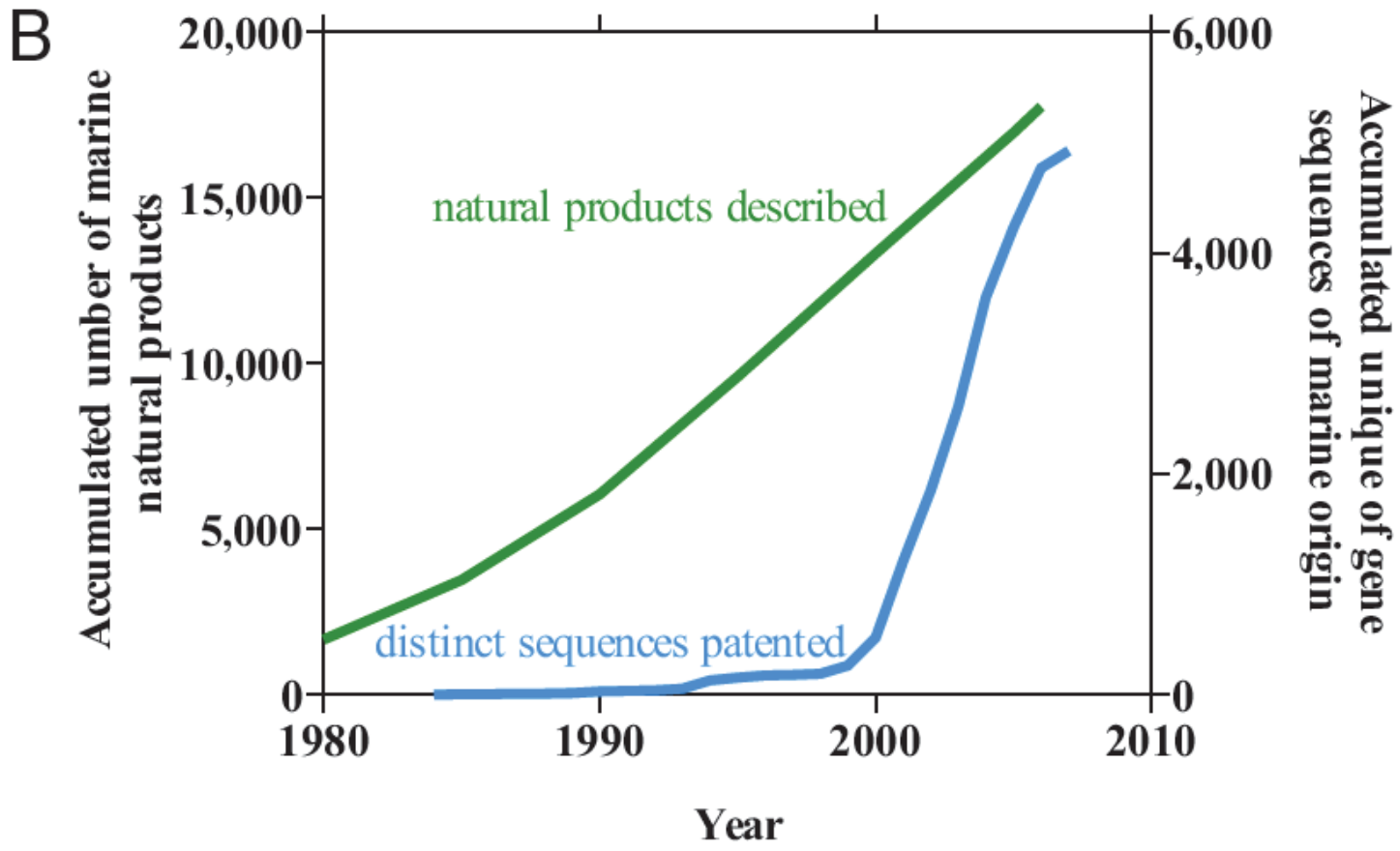
Species	Earth			Ocean		
	Catalogued	**Predicted**	**±SE**	**Catalogued**	**Predicted**	**±SE**
Eukaryotes						
Animalia	953,434	7,770,000	958,000	171,082	2,150,000	145,000
Chromista	13,033	27,500	30,500	4,859	7,400	9,640
Fungi	43,271	611,000	297,000	1,097	5,320	11,100
Plantae	215,644	298,000	8,200	8,600	16,600	9,130
Protozoa	8,118	36,400	6,690	8,118	36,400	6,690
Total	1,233,500	8,740,000	1,300,000	193,756	2,210,000	182,000
Prokaryotes						
Archaea	502	455	160	1	1	0
Bacteria	10,358	9,680	3,470	652	1,320	436
Total	10,860	10,100	3,630	653	1,320	436
Grand Total	**1,244,360**	**8,750,000**	**1,300,000**	**194,409**	**2,210,000**	**182,000**

Predictions for prokaryotes represent a lower bound because they do not consider undescribed higher taxa. For protozoa, the ocean database was substantially more complete than the database for the entire Earth so we only used the former to estimate the total number of species in this taxon. All predictions were rounded to three significant digits.

doi:10.1371/journal.pbio.1001127.t002

Bioprospecting Efforts Increases

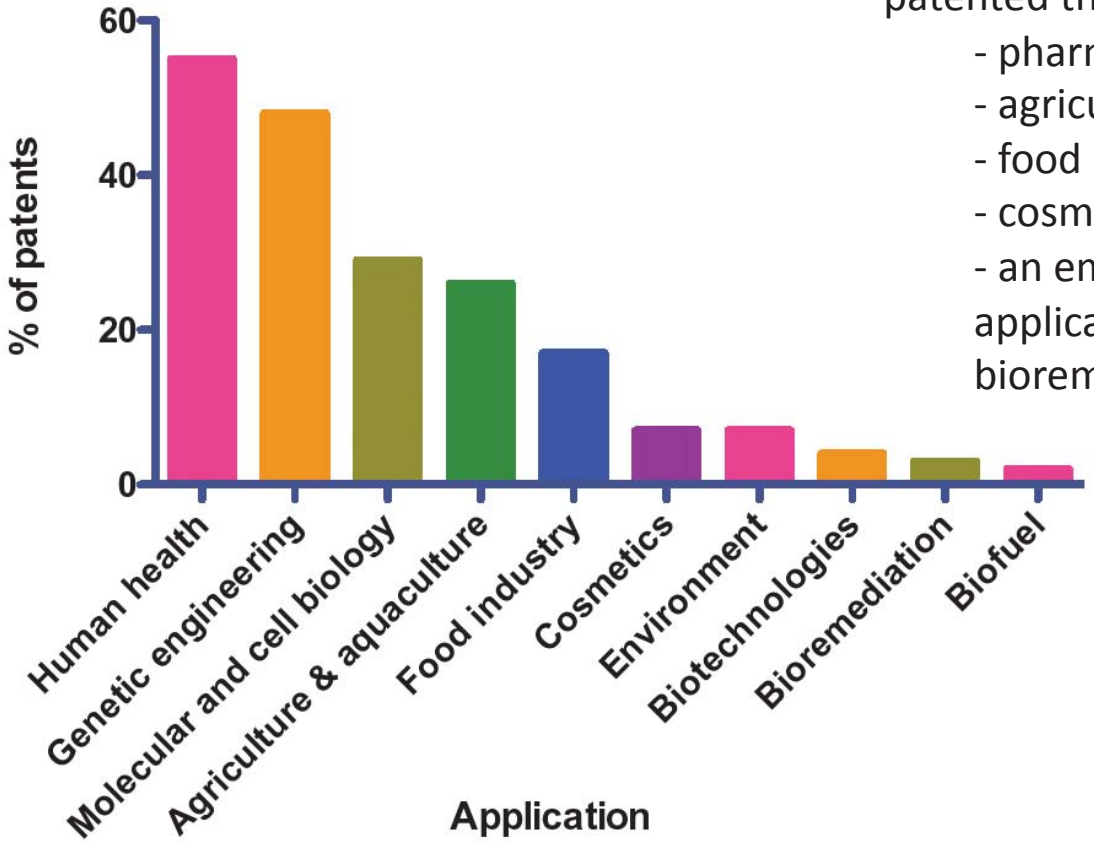
Marine natural products & genes of commercial interest



Arrieta et al. 2010 PNAS | October 26, 2010 | vol. 107 | no. 43

Marine Biotechnology

Broad human use of biological resources



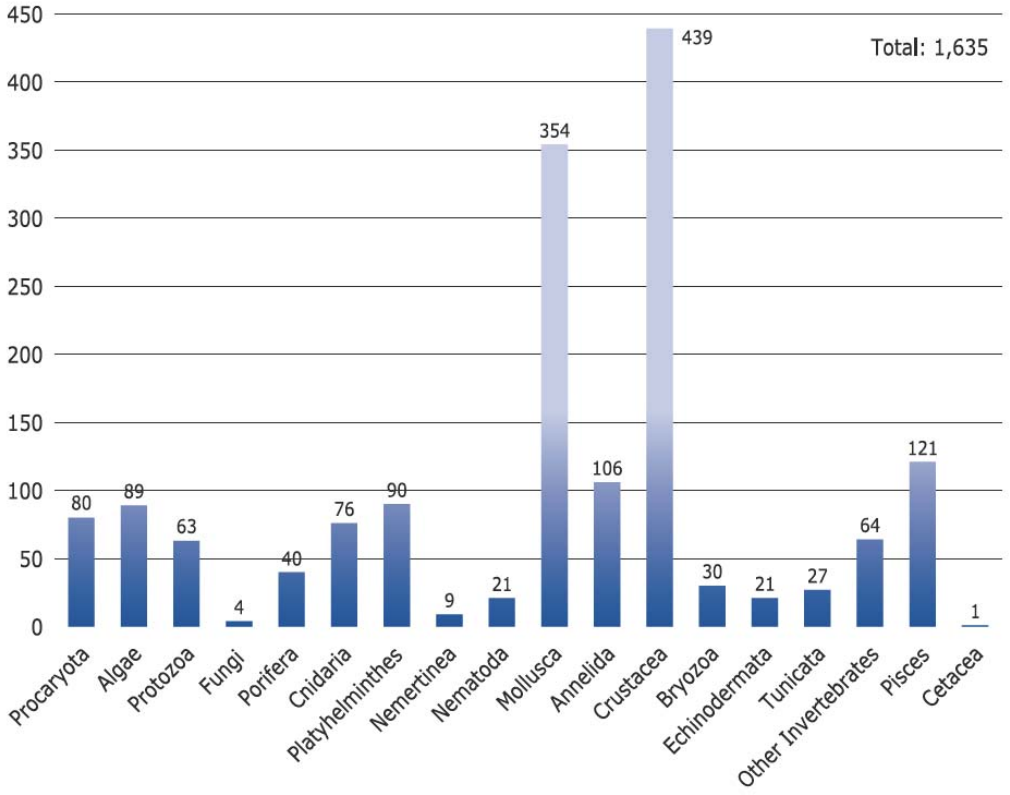
The applications of genes of marine organisms patented thus far range widely:

- pharmacology and human health (55%),
- agriculture or aquaculture (26%),
- food (17%),
- cosmetics (7%) industry
- an emerging and growing number of applications in the fields of ecotoxicology, bioremediation, and biofuel production

Arrieta et al. 2010 PNAS | October 26, 2010 | vol. 107 | no. 43

Average New Species / year

Figure 2.1: Yearly average number of marine species described in 2002-2003 by taxonomic group



Bouchet P 2006. The Magnitude of Marine Biodiversity

Hidden in Hotspots

The majority of species that remain to be discovered are likely to be small-ranged occurring in hotspots and less explored areas such as the deep sea.

Yeti crab & Michel Segonzac IFREMER

Bouchet P 2006. The Magnitude of Marine Biodiversity

Beyond the Blue

Finding Nemo © Walt Disney Pictures & PIXAR Studios

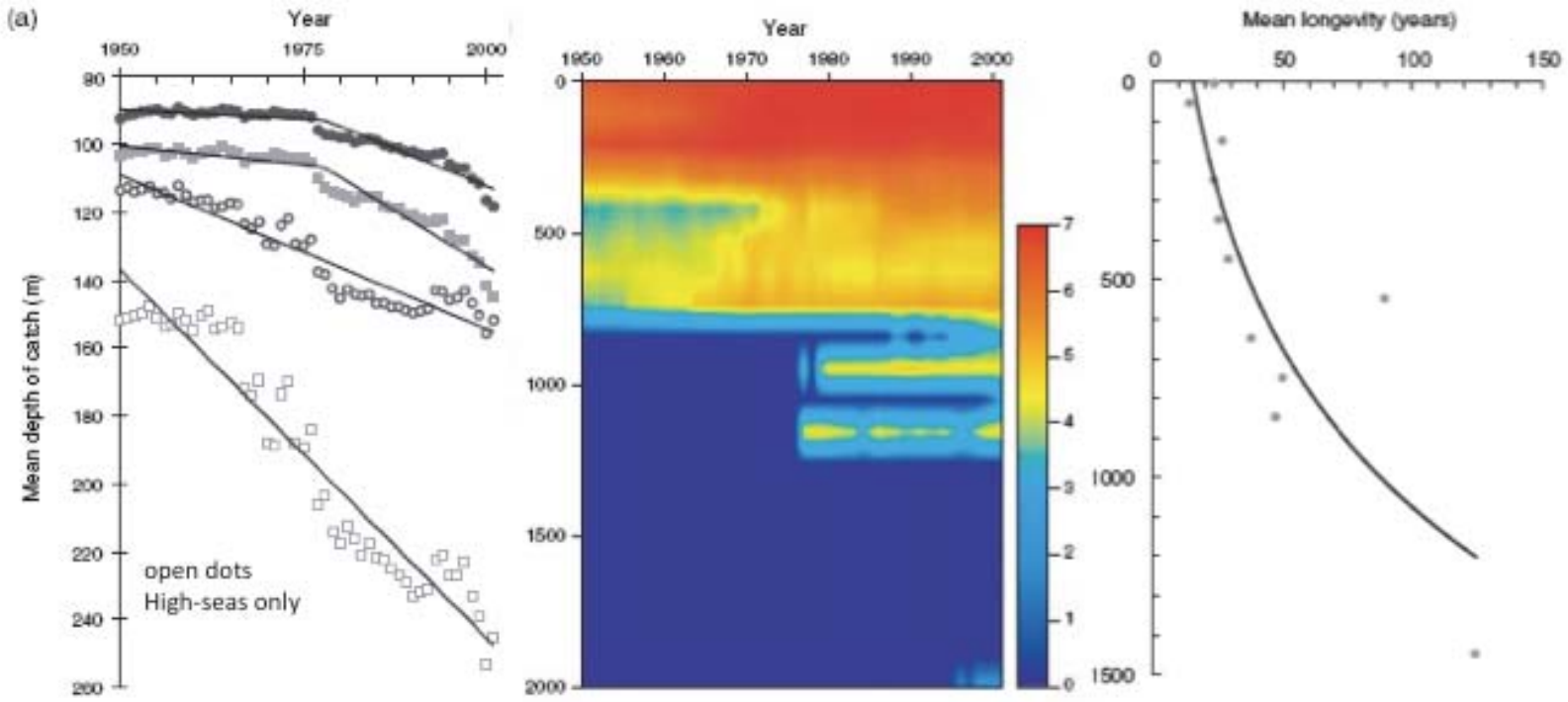
The dimension of our ignorance

The global area covered by many of these habitats is still largely unknown and the proportion studied is minimal.

Habitat	Area (km^2)	% ocean floor	% investigated
Deep sea floor	326 000 000 km^2	100%	0,0001%
Abyssal plains	244 360 000 km^2	75%	< 1%
Ocean ridges	30 000 000 km^2 (ca. 50 000 km)	9,20%	10%
Seamounts	8 500 000 km^2	2,6%	0,25-0,28%
Coral reefs	280 000 km^2	0,08%	mínimo
Hydrothermal fields	Approx. 2000 (Unknown area)	Unknown	10% of the 200 hundred know fields
Cold seeps	10 000 km^2	0,003%	2%

Ramirez-Llodra E et al. 2010. *Biogeosciences*, 7: 2851-2899

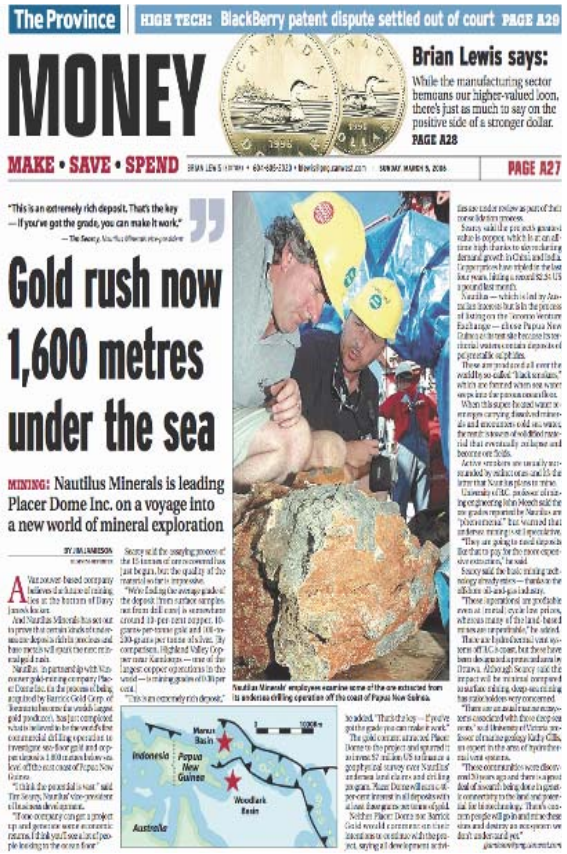
Growing Interest on the Deep-sea Fisheries



Global tendencies of mean depth in world fisheries from 1950 -2001

Morato T et al. 2006, Fish and Fisheries: Fishing down the deep
Serrão Santos R et al. 2012. Natural Resources, Sustainability and Humanity, Ch5. Springer

Growing Interest on the Deep-sea Mining of Minerals



The Province | HIGH TECH: BlackBerry patent dispute settled out of court PAGE A29

MONEY

Brian Lewis says:
While the manufacturing sector bemoans our higher-valued loon, there's just as much to say on the positive side of a stronger dollar.
PAGE A28

MAKE • SAVE • SPEND PAGE A27

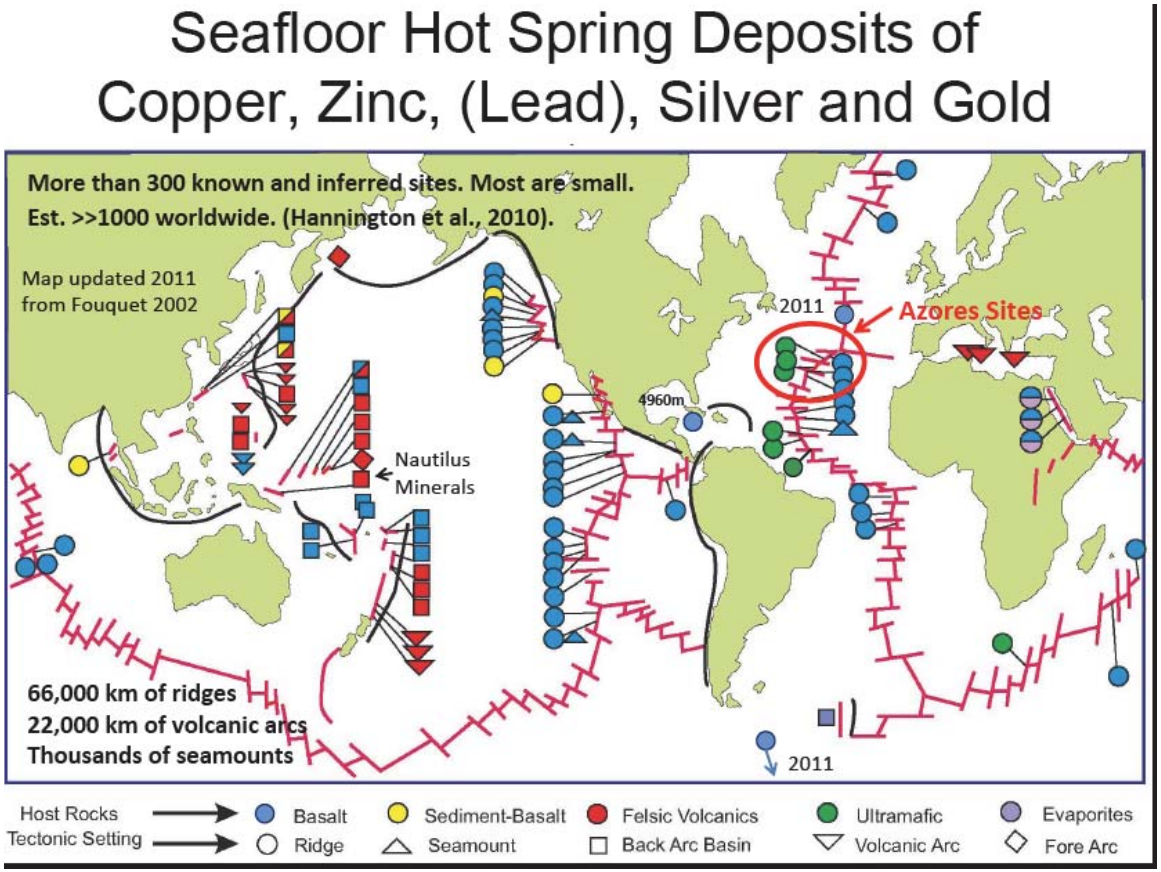
"This is an extremely rich deposit. That's the key — if you've got the grade, you can make it work."

Gold rush now 1,600 metres under the sea

MINING: Nautilus Minerals is leading Placer Dome Inc. on a voyage into a new world of mineral exploration

Nautilus Minerals' employees examine some of the ore extracted from its undersea drilling operation off the coast of Papua New Guinea.

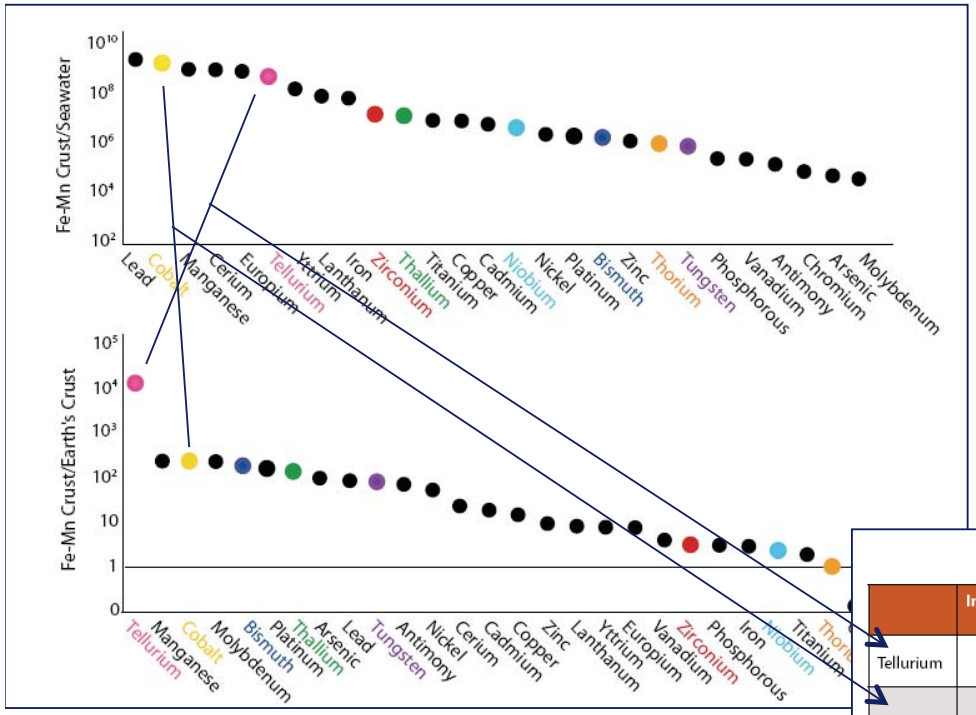
Manus Basin
Indonesia
Papua New Guinea
Woodlark Basin
Australia



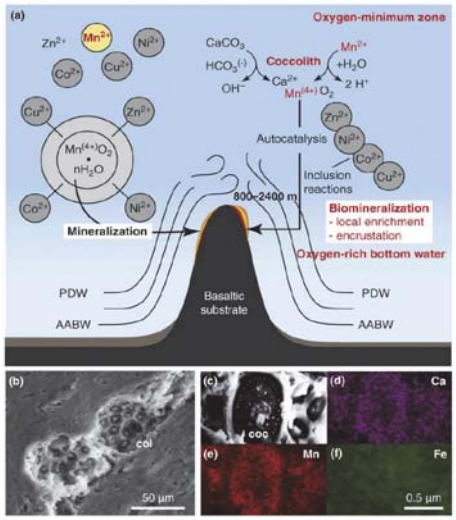
Map – Steve Scott courtesy

Serrão Santos R et al. 2012. Natural Resources, Sustainability and Humanity, Ch5. Springer

Growing Interest on the Deep-sea Rare Earth/ High-tech Metals



Wang & Muller 2009. Trends in Biotechnology



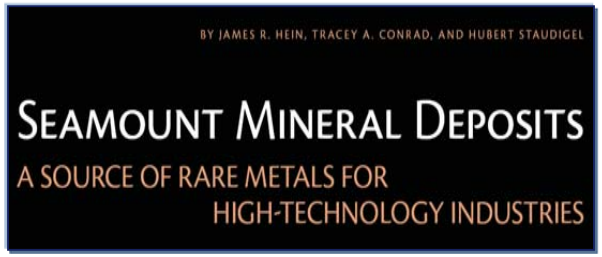


Table 1. United States 2008 imports, exports, and uses for selected high-tech metals

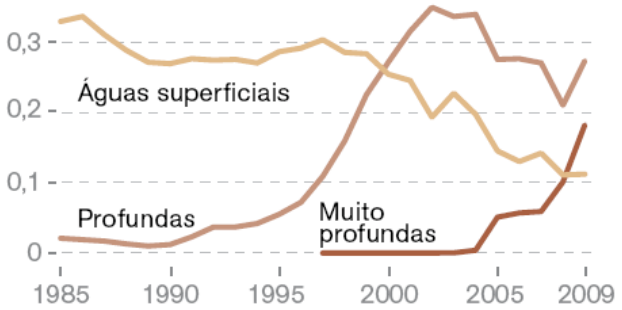
	Imported (Tons)	Exported (Tons)	Main Uses	Emerging and Next-Generation Technologies
Tellurium	90[1]	50[1]	Steel, Cu, and Pb alloys, pigment	Photovoltaic solar cells, computer chips, thermal cooling devices
Cobalt	11,000	2,900	Steel superalloys (e.g., jet engines), batteries, chemical applications	Hybrid and electric car batteries, storage of solar energy, magnetic recording media, high-T superalloys, supermagnets, cell phones
Bismuth	3,480	566	Metallurgical additives, fusible alloys, pharmaceuticals, chemicals	Liquid Pb-Bi coolant for nuclear reactors, Bi-metal polymer bullets, high-T superconductors, computer chips
Tungsten	12,700	5,675	Wear-resistant materials, superalloys, electrical products, chemicals	Negative thermal expansion devices, high-temperature superalloys, X-ray photo imaging
Niobium	10,500	600	Steel and superalloys	High-temperature superalloys, new-generation capacitors, superconducting resonators
Platinum	195	27	Catalytic converters, liquid-crystal and flat-panel displays, jewelry, petroleum refining, electronics	Hydrogen fuel cells, chemical sensors, cancer drugs, electronics

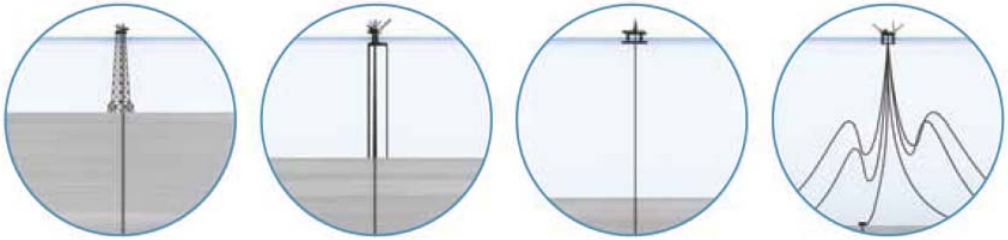
Hein JR 2010. Oceanography, 23 (1)

Growing Interest on the Deep-sea Offshore Petroleum Industry

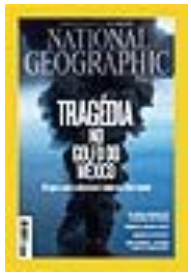
Petróleo do golfo nos EUA a partir de licenças federais, 1985 a 2009

Mil milhões de barris por profundidade



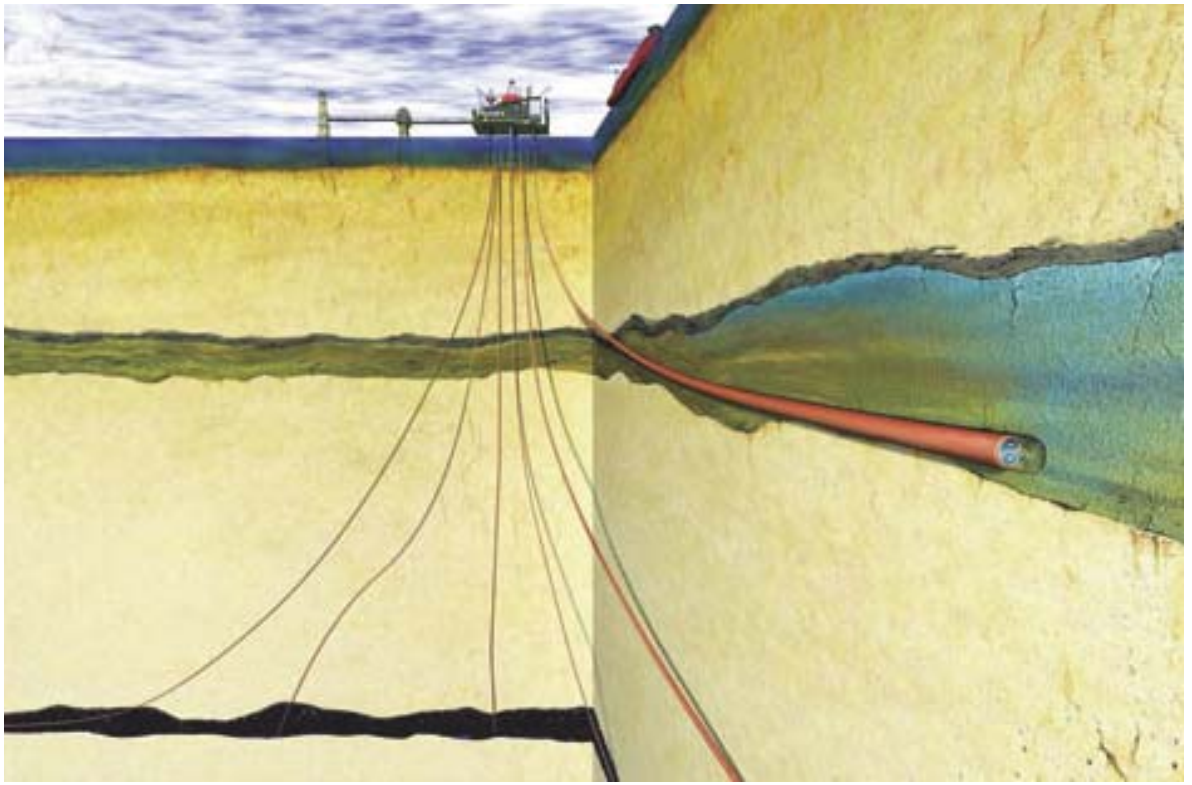


Tipo	FIXO	PERNAS EM TENSÃO	SEMI-SUBMERSÍVEL	SFP*
Implementado	1938	1989	1963	2000
Profundidade	Até 535m	Até 1.500m	Sem limite	Sem limite
Exemplo	1 VK821	2 Ram/Powell	3 *Deepwater Horizon*	4 *Na Kika*



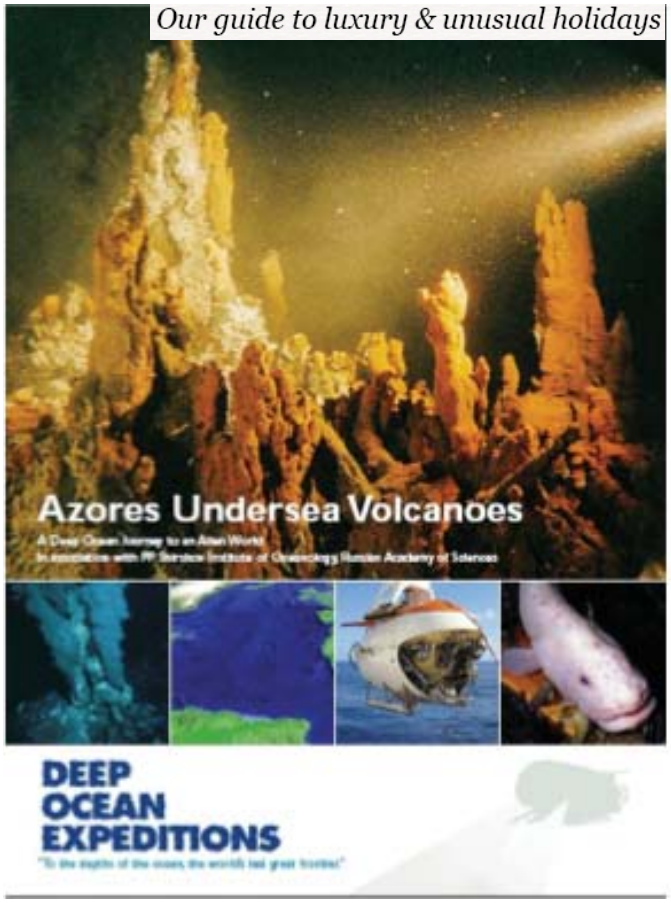
NATIONAL GEOGRAPHIC PORTUGAL

Growing Interest on the Deep-sea CO^2 Sequestration and Deposition

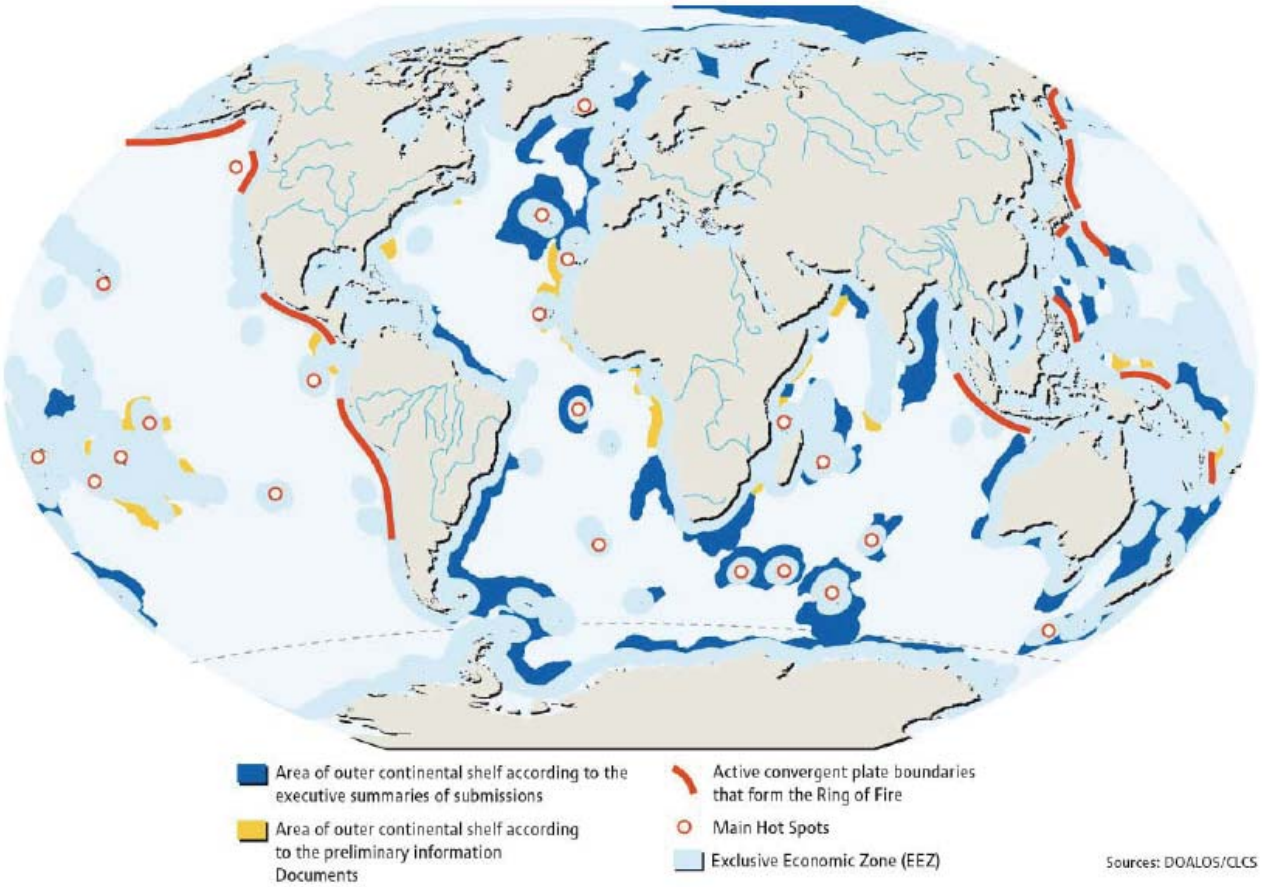


At Statoil's Sleipner West rig in the North Sea, carbon dioxide produced along with natural gas is stored in an aquifer more than 800 meters below the sea floor. (Courtesy of Statoil Corp.)

Growing Interest on the Deep-sea Tourism and Filming



Growing Interest on the Deep-sea Jurisdiction



UNEP GRID ARENDAL

Environmental Knowledge for Change

Growing Interest on the Deep-sea Scientific Research

Growing Interest on the Deep-sea Biotechnology

Examples of commercial products derived from deep-sea species and materials

Company name	*Product and related properties*
Sederma	Enzymes isolated from deep-sea bacteria used in skin protection products (UV-resistant)
California Tan	*T. thermophilus enzymes* (same type of products as above)
Roche	*T. thermophilus, Thermotoga maritime* and other deep-seabed species which thrive at high temperatures Several DNA polymerases (a polymerase is an enzyme that builds new strands of DNA)
Diversa Corporation	Pyrolase™ 160 enzyme, used in industry to reduce viscosity; ThermalAceTM DNA Polymerase
New England BioLabs Inc.	Deep Vent® DNA Polymerase, Therminator[a] DNA Polymerase
Aquaartis	BactoScreen™, a library of extracts of some 1000 marine bacteria isolated from marine organisms and sediments with several potential applications
HyTest Ltd	*Thermus aquaticus* DNA polymerase Taq Red
Promega	Thermostable Tth DNA Polymerase[a]

Life at Extreme Conditions

Structurally Unique Molecules

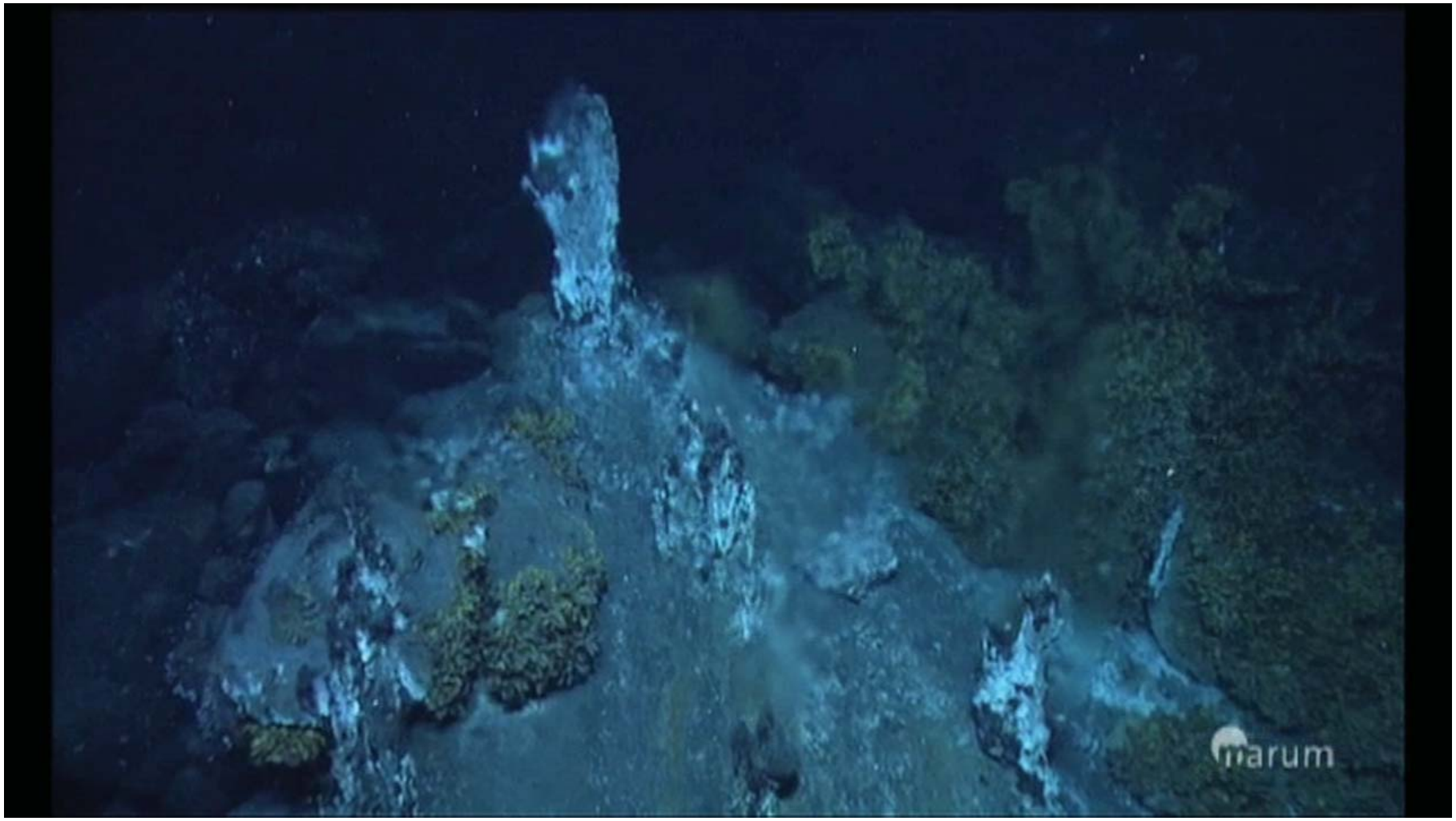
- Deep-sea organisms survive under:
  - absence of light, low levels of oxygen, intensely high pressures, increasingly low temperatures
- At volcanic active areas, where hydrothermal vents exist generating chemosynthetic communities life proliferate under
  - low pH, toxic metals, high temperature, low oxygen, seismic activity, radio-active elements.
- Deep-sea fauna are expected to have a greater genetic diversity, than their shallow-water counterparts, and a higher probability of containing structurally unique molecules with potential application in biotechnology.

Skropeta D 2008. Natural Product Reports, 25 (6): 989–1216
Pettit R K 2011. Marine Biotechnology,13:1–11

Extreme Enzymes

- Patents are associated with genes of marine organisms inhabiting extreme environments, such as hydrothermal vents.
- Discovered in a hydrothermal vent bacteria, FUELZYME® alpha-amylase is thermostable enzyme that is effective over an exceptionally wide temperature and pH range allowing ethanol producers greater operational flexibility.

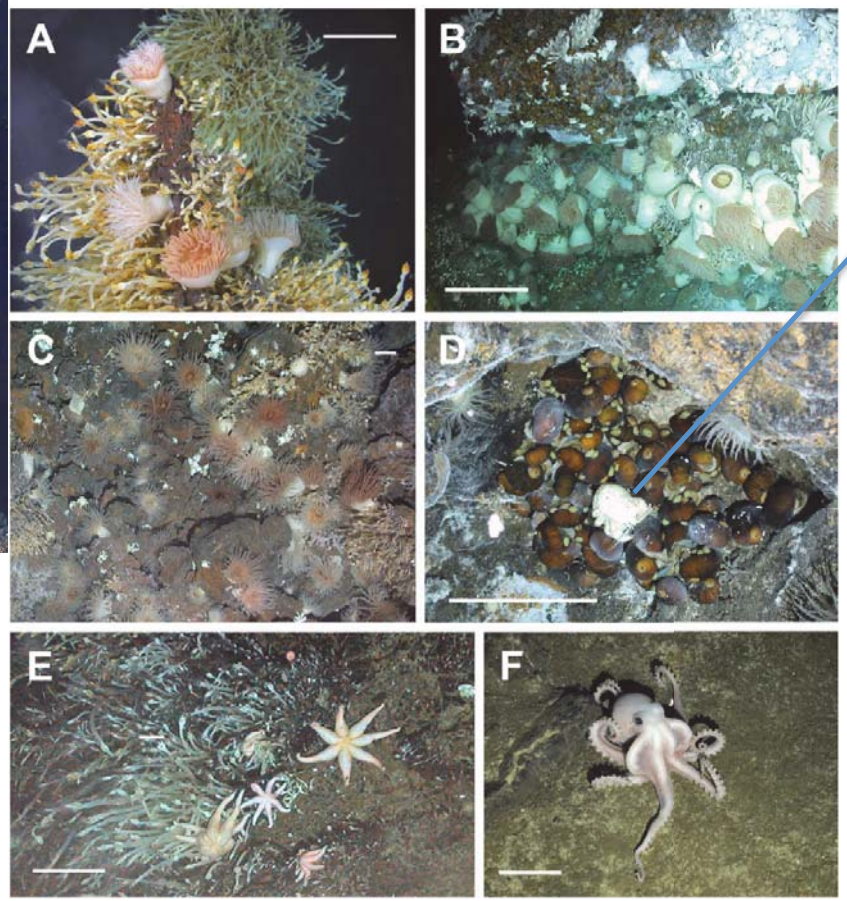
Deep-sea Vents

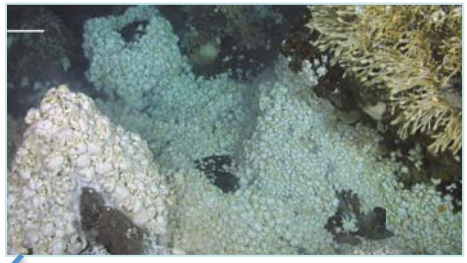


Antarctic Hydrothermal Vent

ca. - 2400 m deep

2009 -2010





Undescribed peltospiroid gastropod surrounding **undescribed** single Kiwa n. sp. and partially covered by Lepetodrilus **n. sp.**

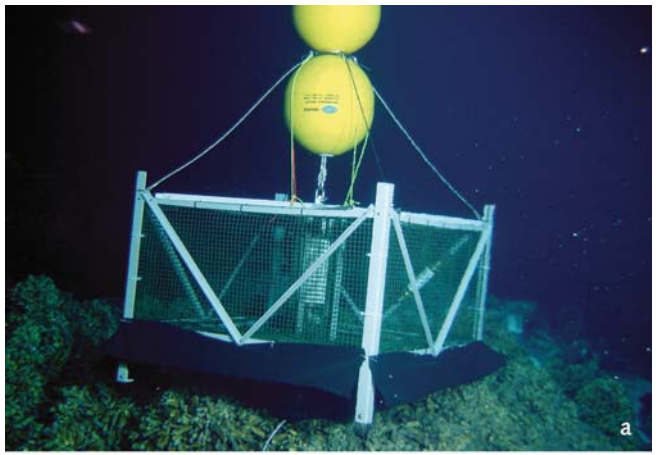
An **undescribed** seven-arm sea star predatory on the stalked barnacles cf. Vulcanolepas

Unidentified octopus

Rogers et al. 2012 PLoS Biology | Januay, 2012 | vol. 107 | Issue 1

Vulnerable Habitats and Harvesting for Natural Products

- Huge collections of marine organism from natural populations may pose considerable impacts in habitat conservation and sustainability.
- Impacts may be avoid with alternative laboratory controlled production schemes.



LabHorta – "Large Scale Facility" For Experimental Studies with deep-sea Vent Organisms

a) Acoustic Retrievable Cage with Vent Mussels at the Hydrothermal Vent Field Menez Gwen: - 870 metres deep. ©Ifremer- ATOS2001

b) Recovery of Cage with RV Arquipélago ©*Imag*DOP;

c) Inside Acclimatised LabHorta with Pressured Chambers and Sulphide / Methane Chemically Controlled Seawater Aquaria. ©*Imag*DOP

ELSEVIER

Journal of Experimental Marine Biology and Ecology 333 (2006) 166–171

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Ana Colaço et al. 2006

Annual spawning of the hydrothermal vent mussel, *Bathymodiolus azoricus*, under controlled aquarium, conditions at atmospheric pressure

ELSEVIER

Journal of Experimental Marine Biology and Ecology 318 (2005) 99–110

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Eniko Kádár et al. 2005

Experimentally induced endosymbiont loss and re-acquirement in the hydrothermal vent bivalve *Bathymodiolus azoricus*

Raul Bettencourt et al. 2011

ICES Journal of Marine Science (2011), 68(2), 357–364. doi:10.1093/icesjms/fsq119

Out of the deep sea into a land-based aquarium environment: investigating physiological adaptations in the hydrothermal vent mussel *Bathymodiolus azoricus*

Ana Colaço et al. 2011

ICES Journal of Marine Science (2011), 68(2), 349–356. doi:10.1093/icesjms/fsq120

LabHorta: a controlled aquarium system for monitoring physiological characteristics of the hydrothermal vent mussel *Bathymodiolus azoricus*

Bettencourt *et al. BMC Genomics* 2010, **11**:559
http://www.biomedcentral.com/1471-2164/11/559

BMC Genomics

RESEARCH ARTICLE Open Access

High-throughput sequencing and analysis of the gill tissue transcriptome from the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus*

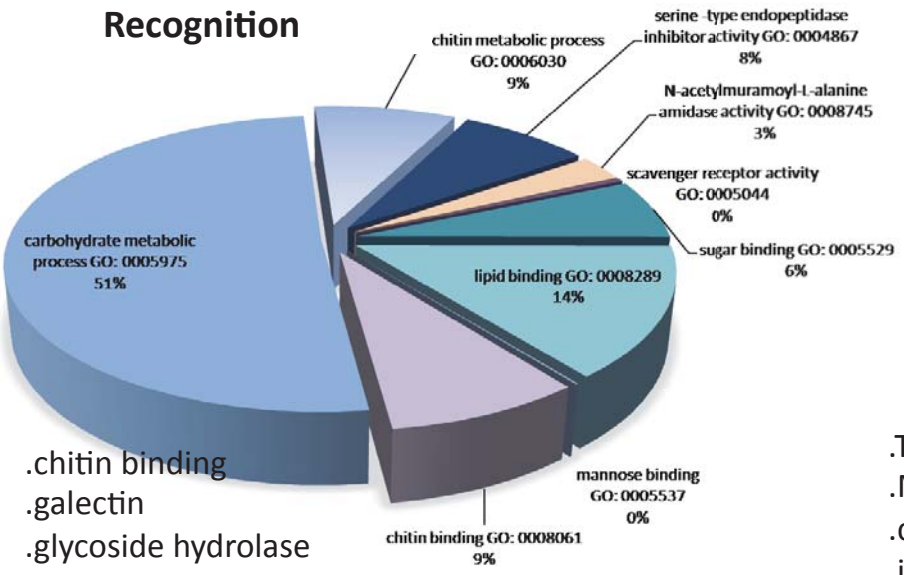
Raul Bettencourt[1,2*], Miguel Pinheiro[3], Conceição Egas[4,5], Paula Gomes[4], Mafalda Afonso[2], Timothy Shank[6], Ricardo Serrão Santos[1,2]

86065 potential genes were sequenced from which 44000 proteins were identified

New genes were identified, like the genes involved on immunological and inflammatory reactions of the mussels.

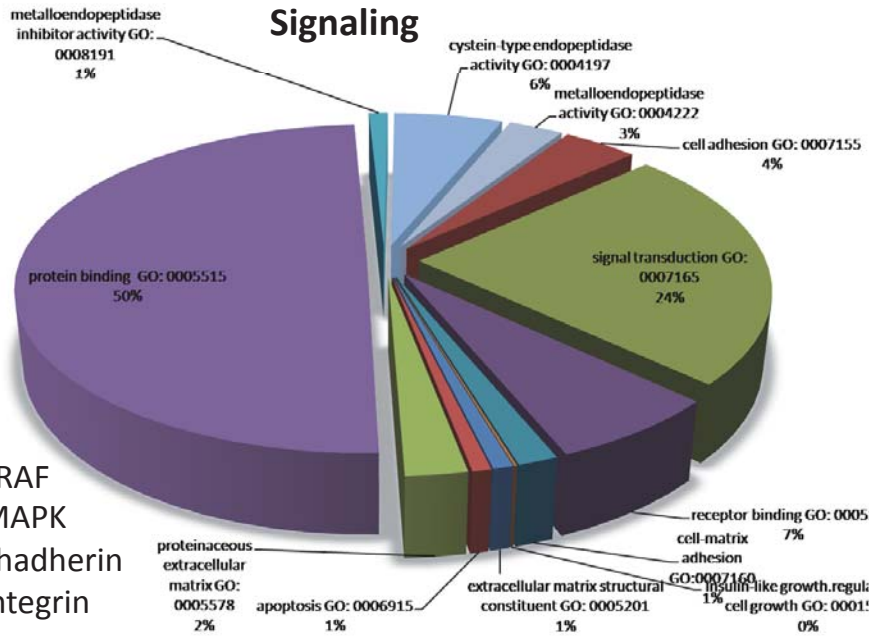
Bettencourt R et al. 2010. BMC Genomics, 11

Recognition



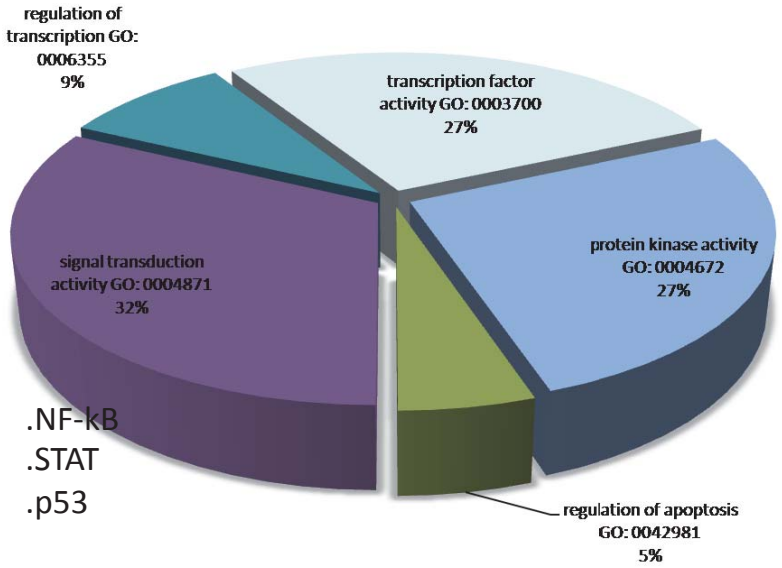
.chitin binding
.galectin
.glycoside hydrolase
.scavenger receptor

Signaling



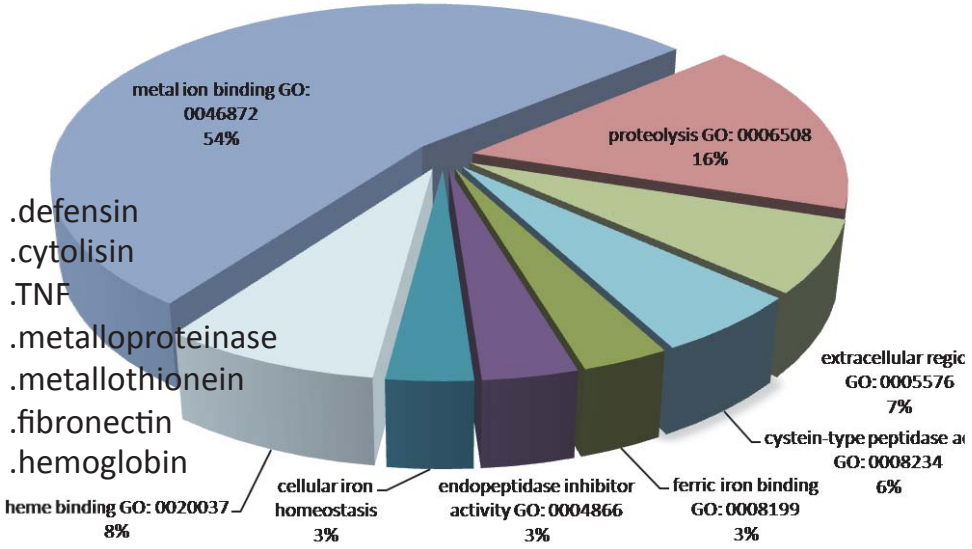
.TRAF
.MAPK
.chadherin
.integrin

Transcription



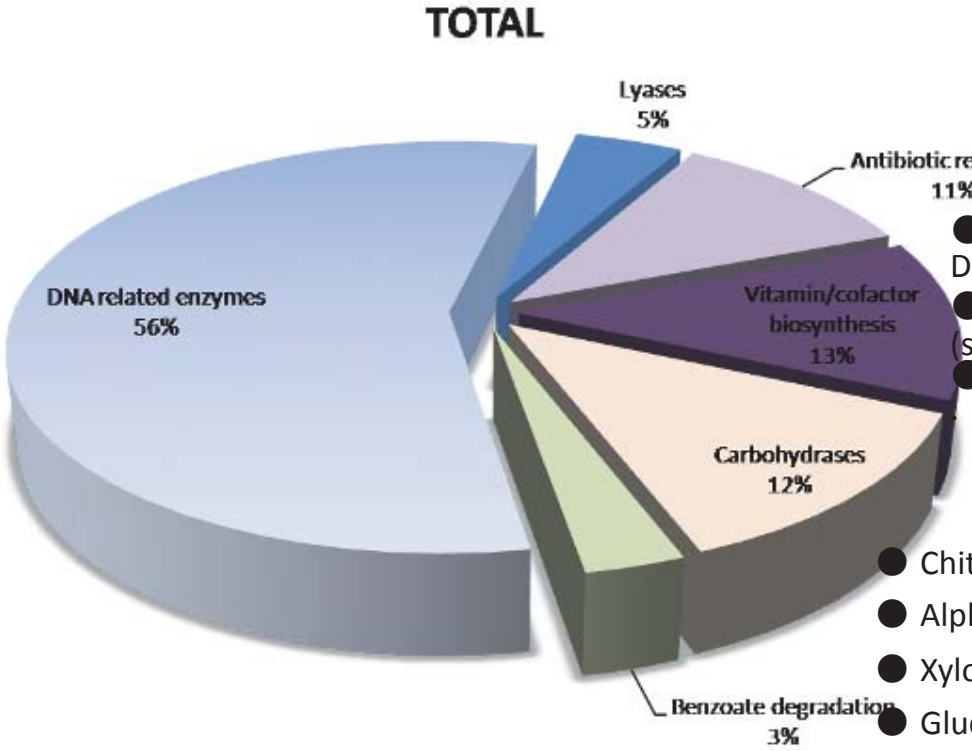
.NF-kB
.STAT
.p53

Effector



.defensin
.cytolisin
.TNF
.metalloproteinase
.metallothionein
.fibronectin
.hemoglobin

Marine enzymes from symbiotic bacteria with potential biotechnological use

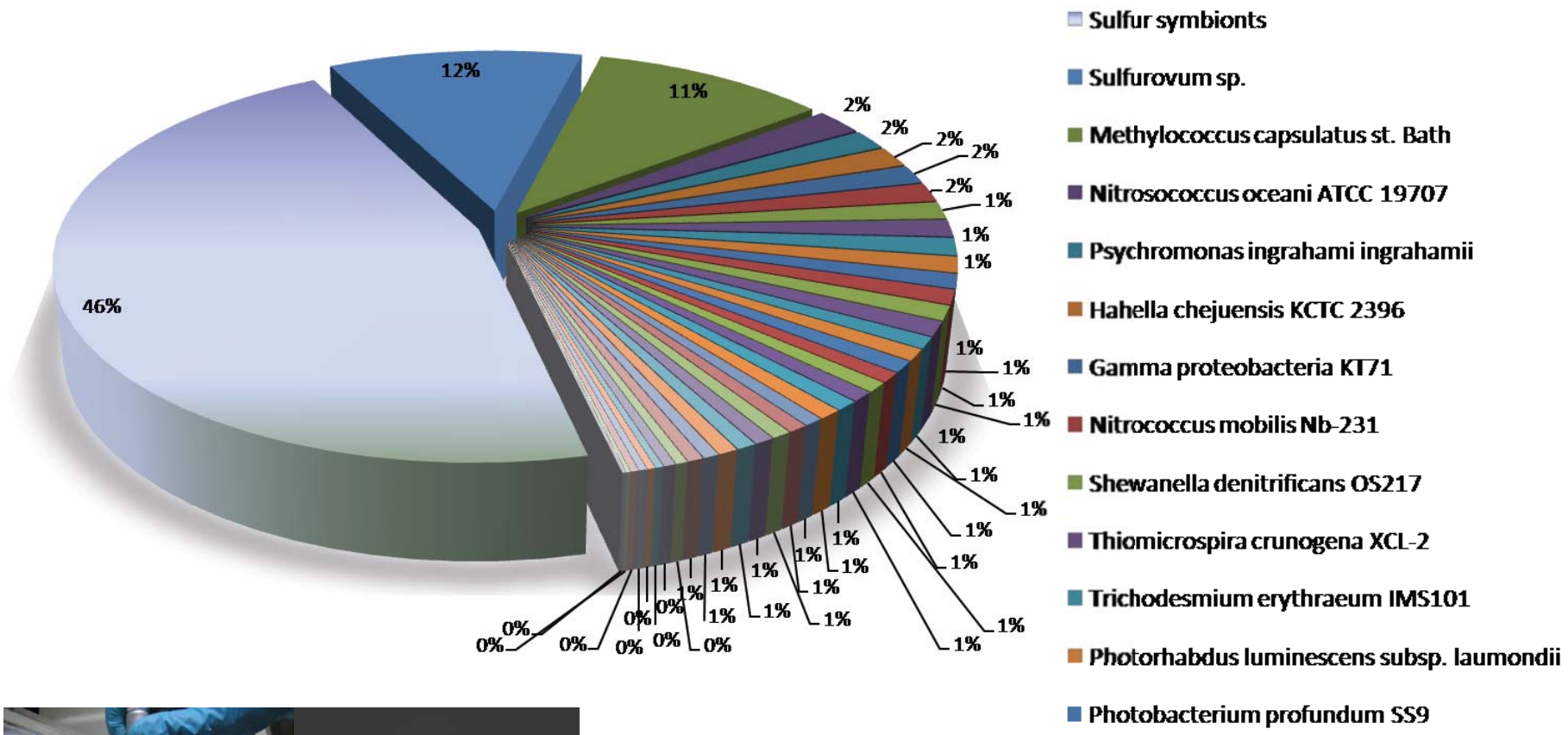


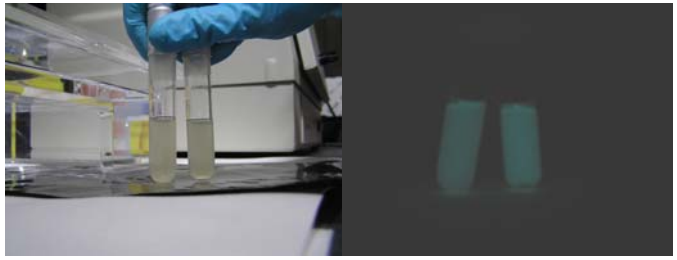
- 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (terpenoids)
- 1-deoxy-D-xylulose 5-phosphate synthase (steroid)
- polyketide synthase (metabolites,bryostatin?

- Chitinase (anti-fungal)
- Alpha-amylase (starch)
- Xylose isomerase (biofuel)
- Glucose dehydrogenase (sugar monitoring)
- Beta-glucuronidase (bacterial detection)

Bettencourt R et al. 2010. BMC Genomics, 11

Microbes are not likely to be endangered.
But symbiotic microbes, are likely to disappear along with their hosts.





Bioluminescent bacteria

Farming Bacteria for Food

This species of yeti crab "farms" colonies of bacteria on its claws.

To help them grow, it waves its pincers over methane and sulfide vents, fertilizing the bacteria and making them good enough to eat.

Mark Brown, Wired UK

Thurber AR et al. 2011 PLoS one | November 2011 |Volume 6 | Issue 11 | e262431

Threaten Species

- About 36 of the 340 marine eukaryotic species reported as a source of genes included in patents, of which:
  - 10 appear as “data deficient,”
  - 2 as “endangered,”
  - 6 as “vulnerable,”
  - 7 as “near threatened.”
- The need to identify threats and determine conservation priorities for MGRs.

Arrieta et al. 2010 PNAS | October 26, 2010 | vol. 107 | no. 43

Hot Spots for MGR Threaten Habitats

- Marine Genetic Resources of economic interest are estimated to be particularly abundant in biodiversity hot spots, such as cold water reefs and gardens, seamounts, sponge aggregations and in extreme environments, such as hydrothermal vent ecosystems.
- Deep-sea coral reefs/gardens, seamounts, sponge aggregations and hydrothermal vent ecosystems are classified as threatened.

Arrieta et al. 2010 PNAS | October 26, 2010 | vol. 107 | no. 43

Threaten Habitats

OSPAR CONVENTION FOR THE PROTECTION OF THE MARINE ENVIRONMENT OF THE NORTH-EAST ATLANTIC

MEETING OF THE OSPAR COMMISSION (OSPAR)

REYKJAVIK: 28 JUNE – 1 JULY 2004

OSPAR List of Threatened and/or Declining Species and Habitats

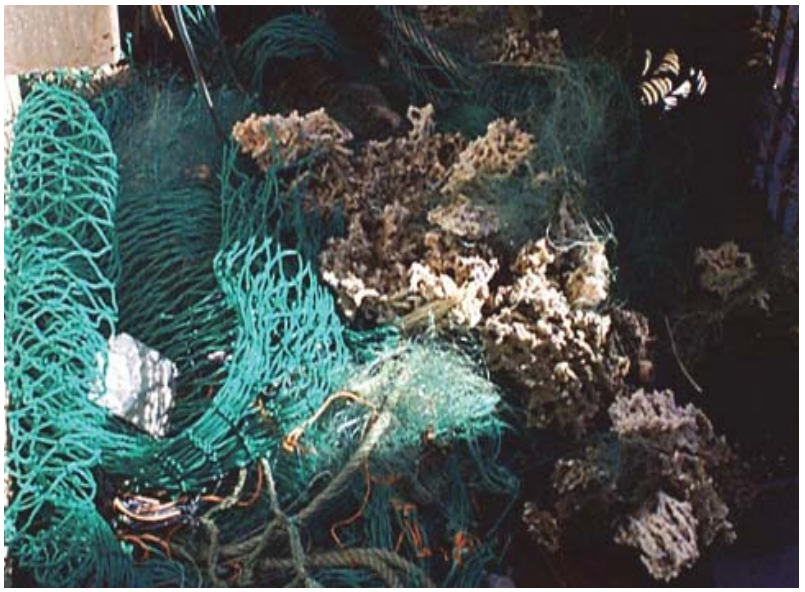
(Reference Number: 2004-06)

DESCRIPTION	OSPAR Regions where the habitat occurs	OSPAR Regions where such habitats are under threat and/or in decline
HABITATS		
Carbonate mounds	I, V	V[7]
Deep-sea sponge aggregations	I, III, IV, V	All where they occur
Oceanic ridges with hydrothermal vents/fields	I, V	V
Intertidal mudflats	I, II, III, IV	All where they occur
Littoral chalk communities	II	All where they occur
Lophelia pertusa reefs	All	All where they occur
Maerl beds	All	III
Modiolus modiolus beds	All	All where they occur
Intertidal *Mytilus edulis* beds on mixed and sandy sediments	II, III	All where they occur
Ostrea edulis beds	II, III, IV	All where they occur
Sabellaria spinulosa reefs	All	II, III
Seamounts	I, IV, V	All where they occur[8]
Sea-pen and burrowing megafauna communities	I, II, III, IV	II, III
Zostera beds	I, II, III, IV	All where they occur

Competing Activities in the Deep-sea

Seamounts face increasing pressure by deep-sea fisheries, which results in high environmental impacts and potential extinctions.

Stripped boulders;



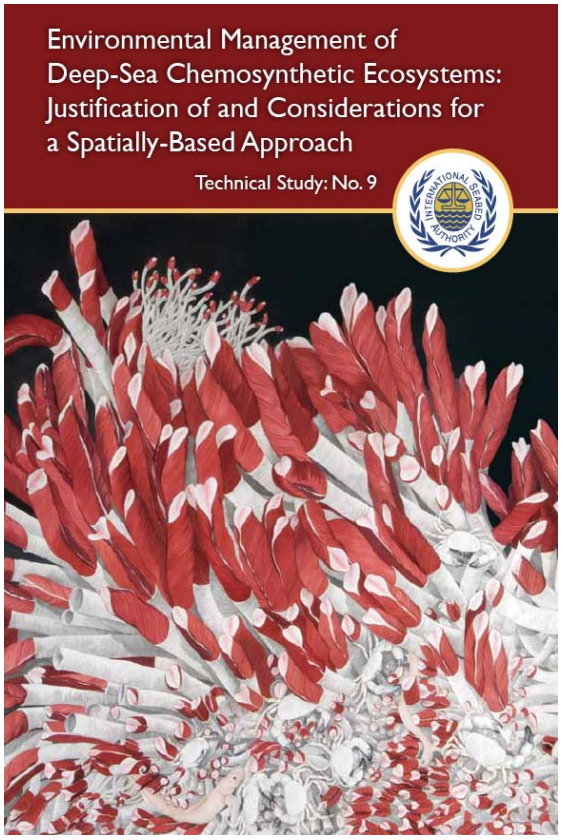
smashed corals >4500 years old

[photos: courtesy of Jason Hall-Spencer]

Serrão Santos R et al. 2012. Natural Resources, Sustainability and Humanity. Springer

Competing Activities in the Deep-sea

Potential impacts of mining for sulfide deposits rich in gold and other metals located near hydrothermal vent ecosystems, likely to be severe because these sites support the highest biomass concentrations in the deep sea.

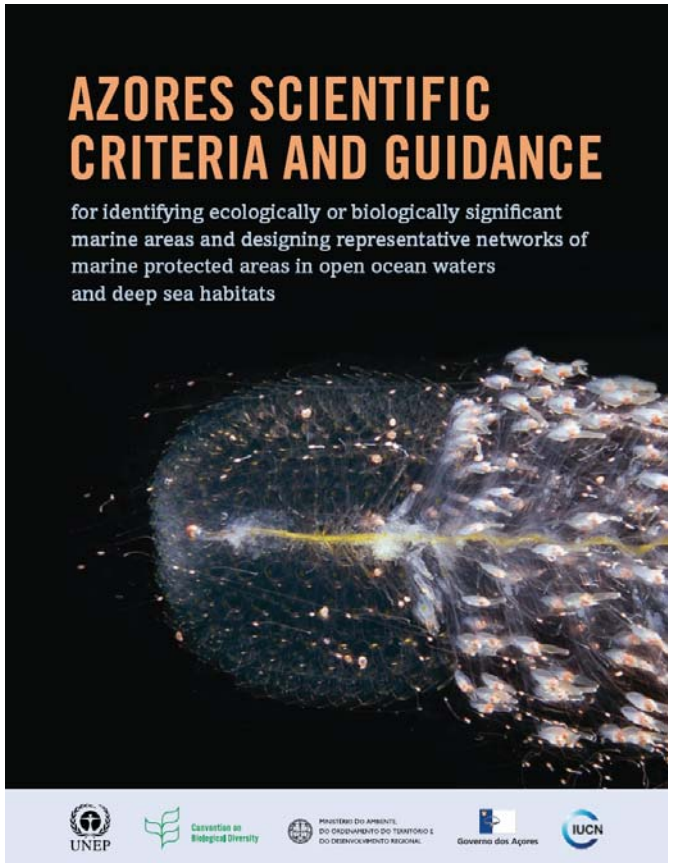


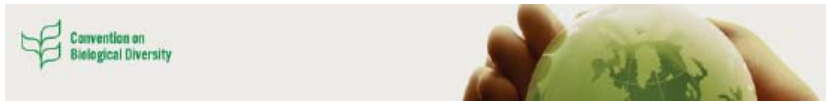
Marine Protected Areas for Species and Habitats

- MPAs with general conservation goals are suitable for the preservation of MGRs because they target both known and yet to be discovered species.
- Carefully designed MPA networks are probably the best tool to meet both fishery, mineral exploration and exploitation, bio-prospection and conservation goals.

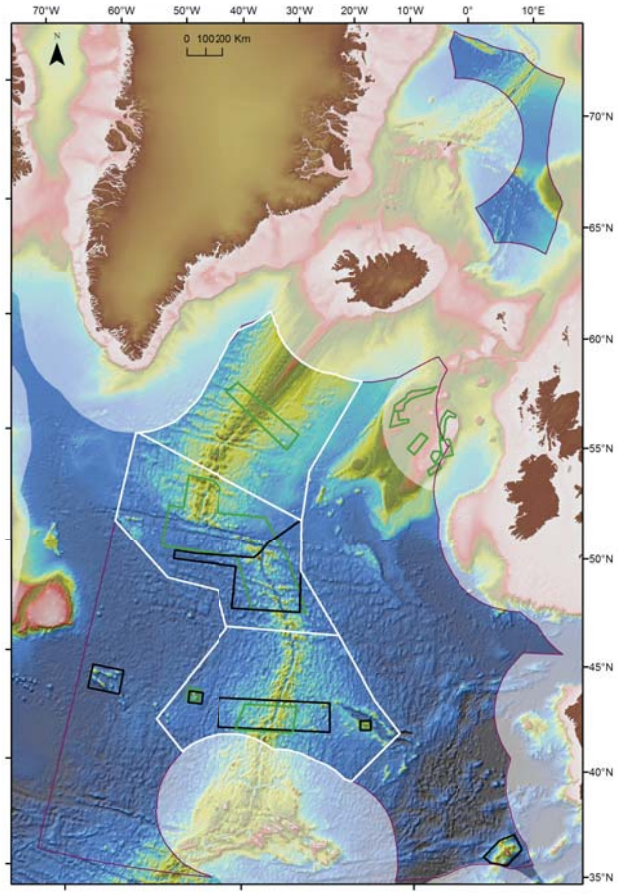
Arrieta et al. 2010 PNAS | October 26, 2010 | vol. 107 | no. 43
Serrão Santos R et al. 2012. *Natural Resources, Sustainability and Humanity*, Ch 5, Springer

Identifying
Ecologically or Biologically Significant Areas

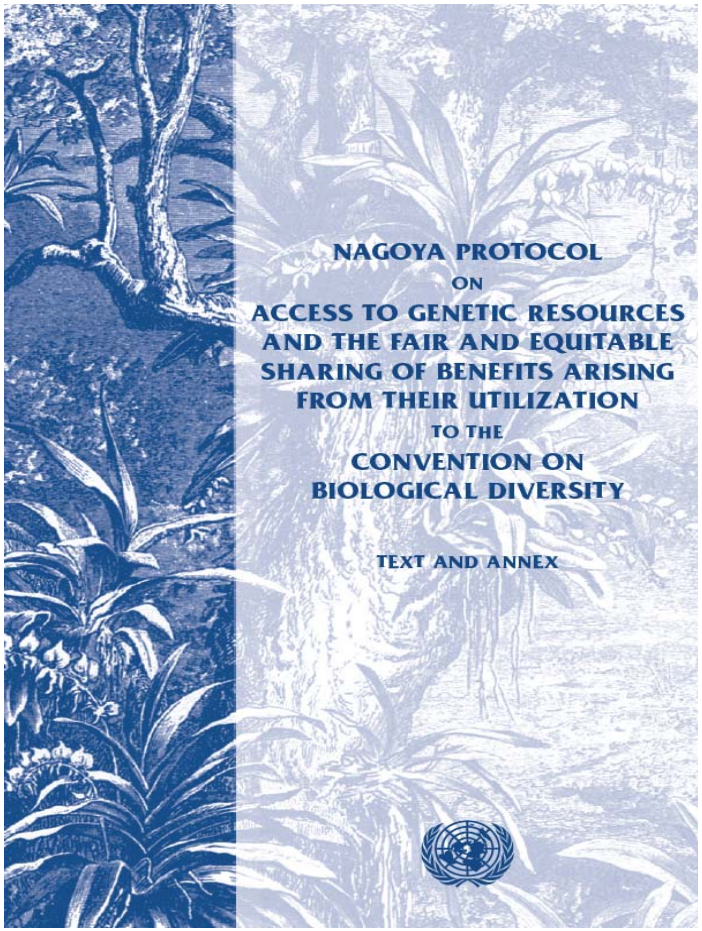




OSPAR Network of MPAs and Proposed Large EBSA



Addressing access to MGR



SCIENCE VOL 331 25 MARCH 2011

GLOBAL GENETIC RESOURCES

Marine Biodiversity and Gene Patents

Sophie Arnaud-Haond,[1]* Jesús M. Arrieta,[2] Carlos M. Duarte[23]

PATENT CLAIMS FOR A GENE OF MARINE ORIGIN WITH SOURCE

Country	Marine organism patent claims
USA	199
Germany	149
Japan	128
France	34
United Kingdom	33
Denmark	24
Belgium	17
Netherland	13
Switzerland	11
Norway	9

Of the genes associated with WIPO patents, 17% are of **unknown taxonomic origin**, and almost none of the patent claims examined **disclosed** the **geographic origin** of material.

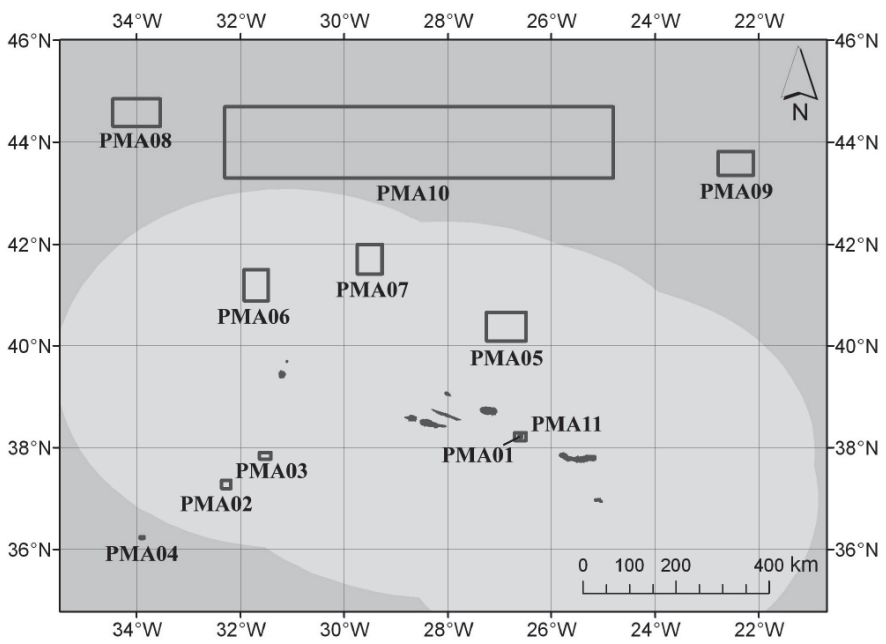
Although states compromised in promoting establishment of sharing agreements under CBD, this is not a legally binding agreement and so does not imply that companies will necessarily comply.

Decreto Legislativo Regional n.º 28/2011/A

Estrutura o Parque Marinho dos Açores

Nos termos do disposto no Decreto Legislativo Regional n.º 15/2007/A, de 25 de Junho, que procede à revisão da Rede Regional de Áreas Protegidas da Região Autónoma dos Açores e determina a reclassificação das áreas protegidas existentes, pelo presente diploma procede-se à estruturação do Parque Marinho dos Açores. Este parque

Diário da República, 1.ª série — N.º 217 — 11 de Novembro de 2011



Artigo 29.º

Transposição do Protocolo de Nagoya

Artigo 30.º

Convenção sobre a Diversidade Biológica

A utilização dos recursos biológicos e genéticos submetidos a amostragem e ou acedidos de acordo com as normas constantes do presente diploma e das normas regulamentares que o desenvolvem não pode contrariar o disposto na Convenção sobre a Diversidade Biológica, ratificada pelo Decreto n.º 21/93, de 21 de junho.

REGIÃO AUTÓNOMA DOS AÇORES

Assembleia Legislativa

Decreto Legislativo Regional n.º 9/2012/A

Regime jurídico do acesso e utilização de recursos naturais da Região Autónoma dos Açores para fins científicos

A Região Autónoma dos Açores não dispõe no seu ordenamento jurídico de normas que regulamentem, de forma específica, o acesso a amostras de recursos naturais, sobretudo quando em causa estão os fins científicos.

Diário da República, 1.ª série — N.º 57 — 20 de março de 2012

CTT - 2006

Hydrothermal Vents at the Post Office