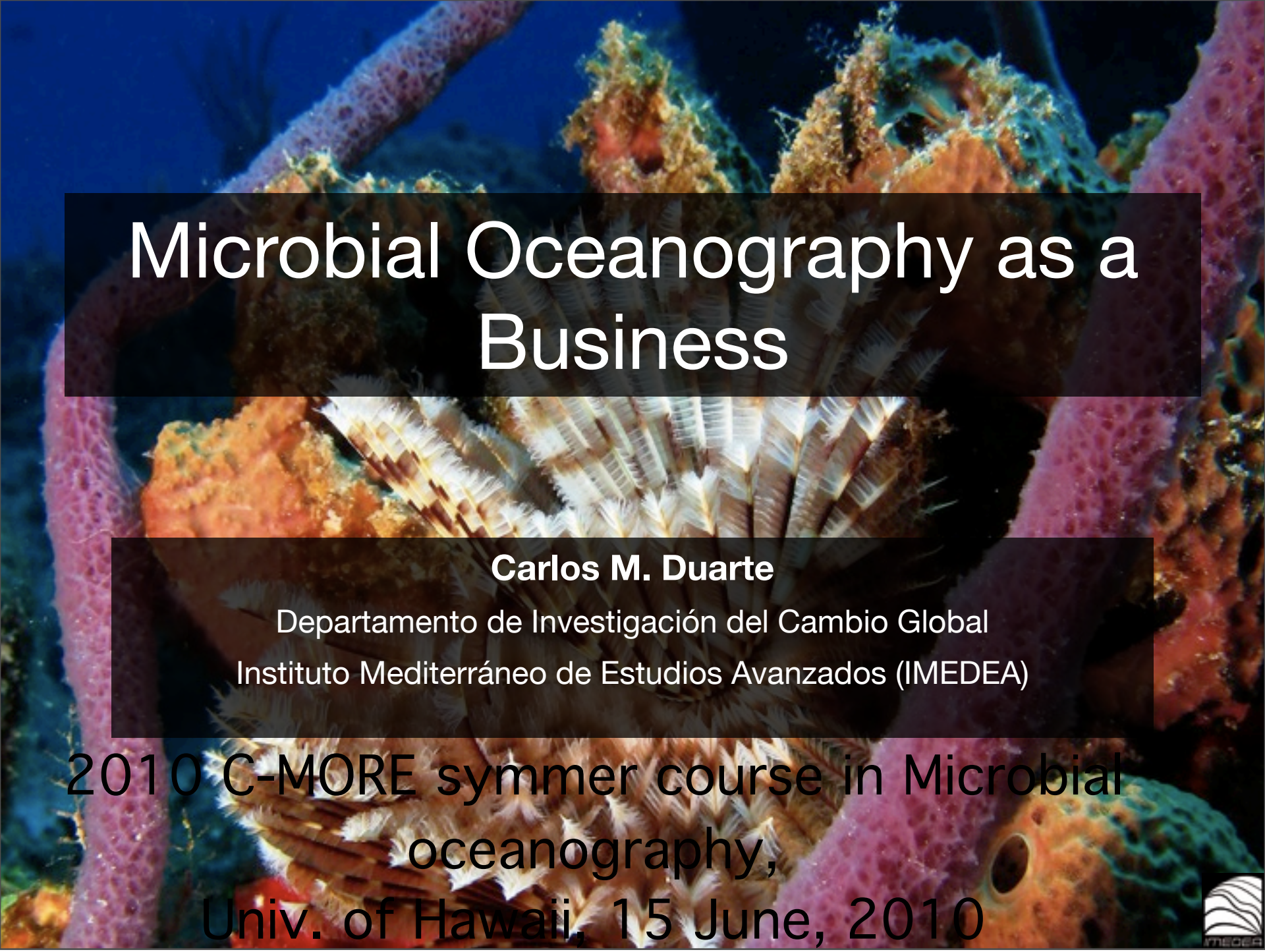


Microbial Oceanography as a Business

Carlos M. Duarte

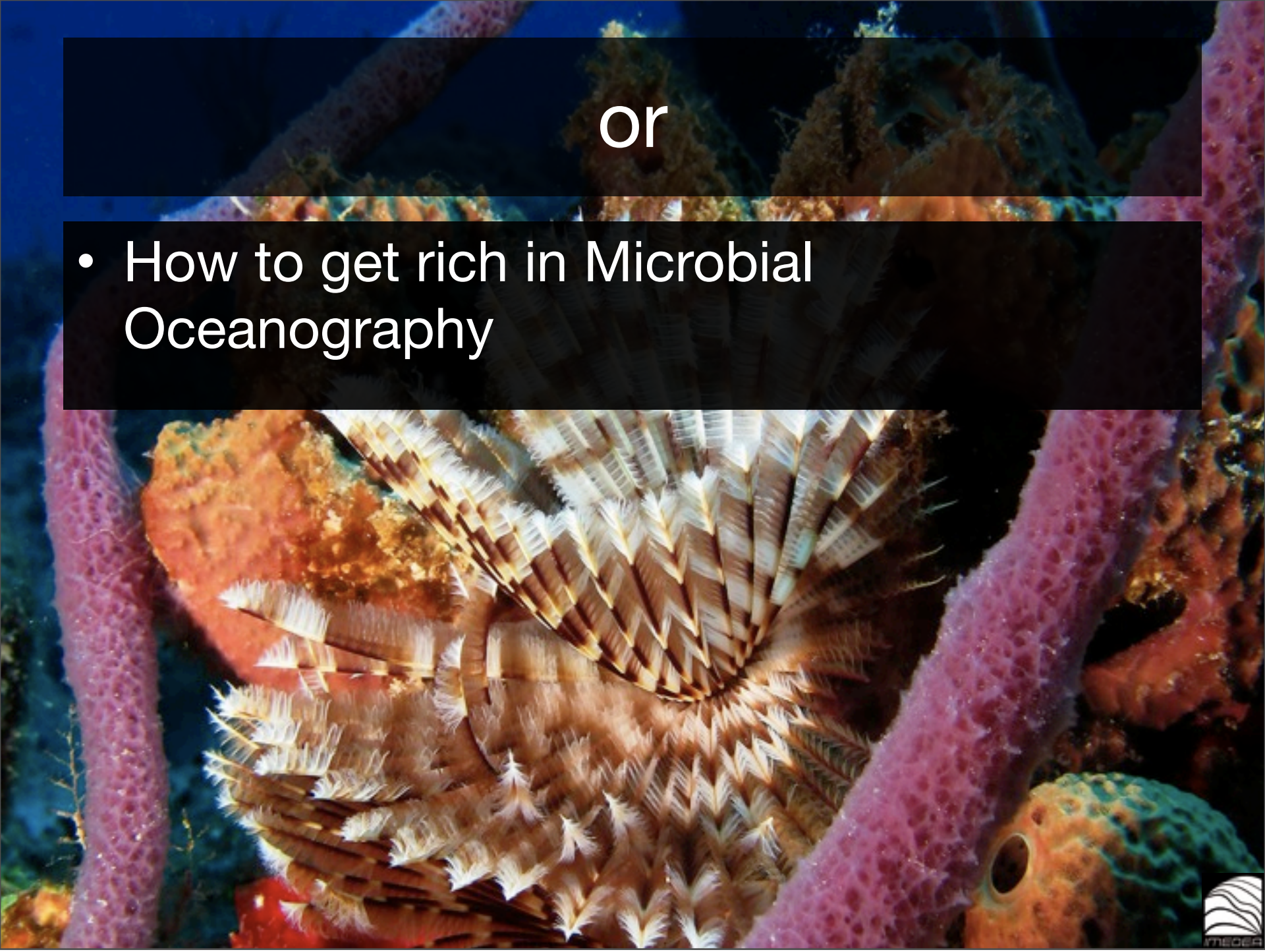
Departamento de Investigación del Cambio Global

Instituto Mediterráneo de Estudios Avanzados (IMEDEA)

2010 C-MORE symmer course in Microbial oceanography,
Univ. of Hawaii, 15 June, 2010

or

- How to get rich in Microbial Oceanography

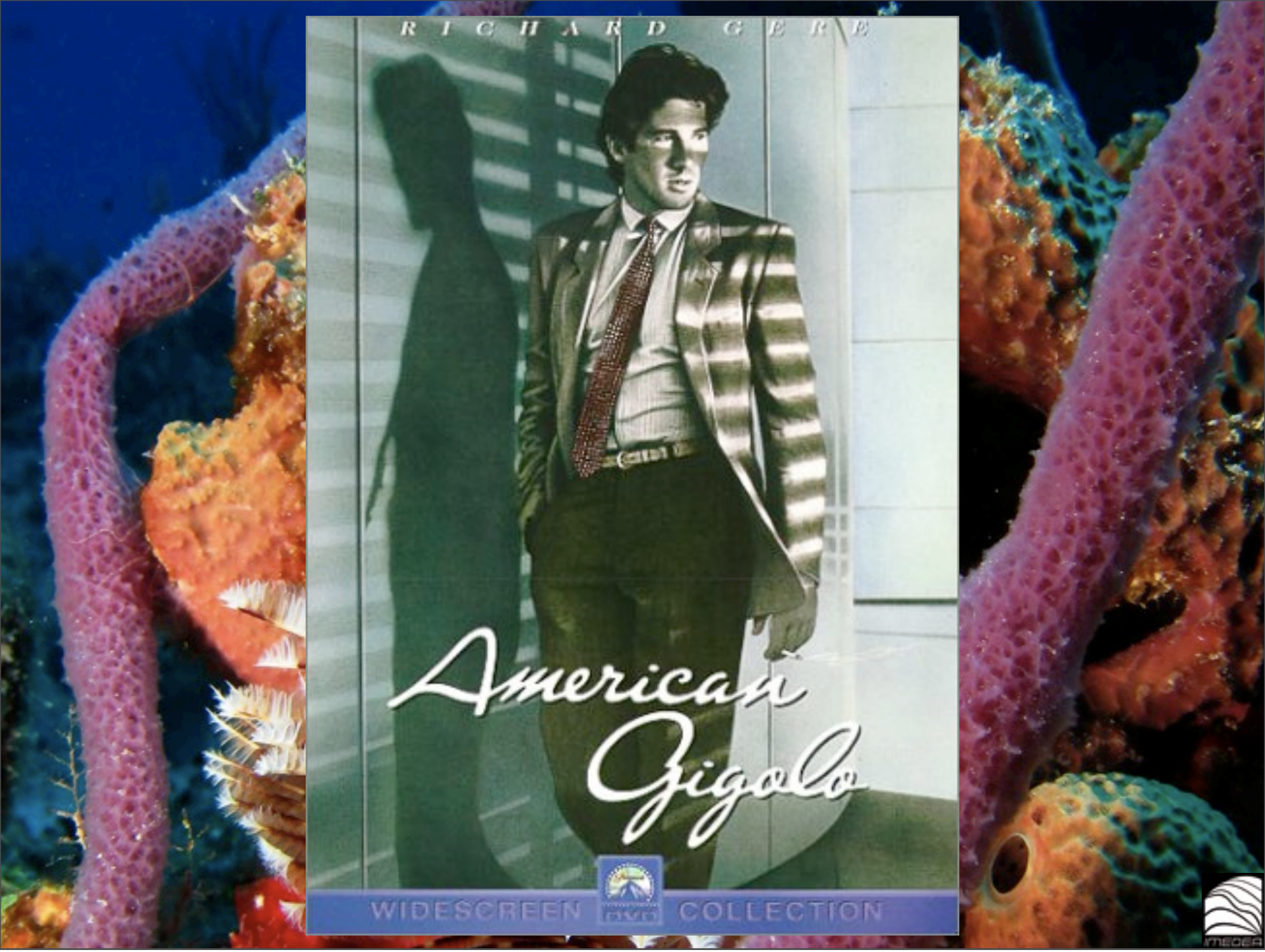


or

- How to get rich in Microbial Oceanography

My bank statements shows a 0 balance


THE LOVE BOAT
abc


RICHARD GERE
American Gigolo
WIDESCREEN DVD COLLECTION

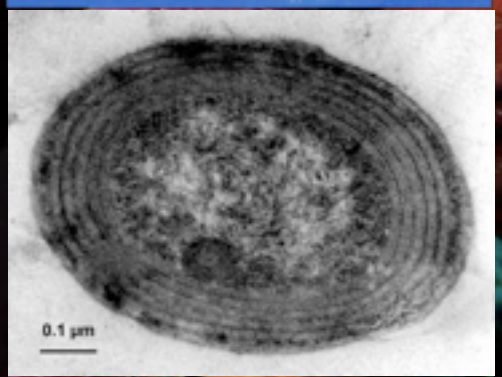

NSF

Marine Biodiversity

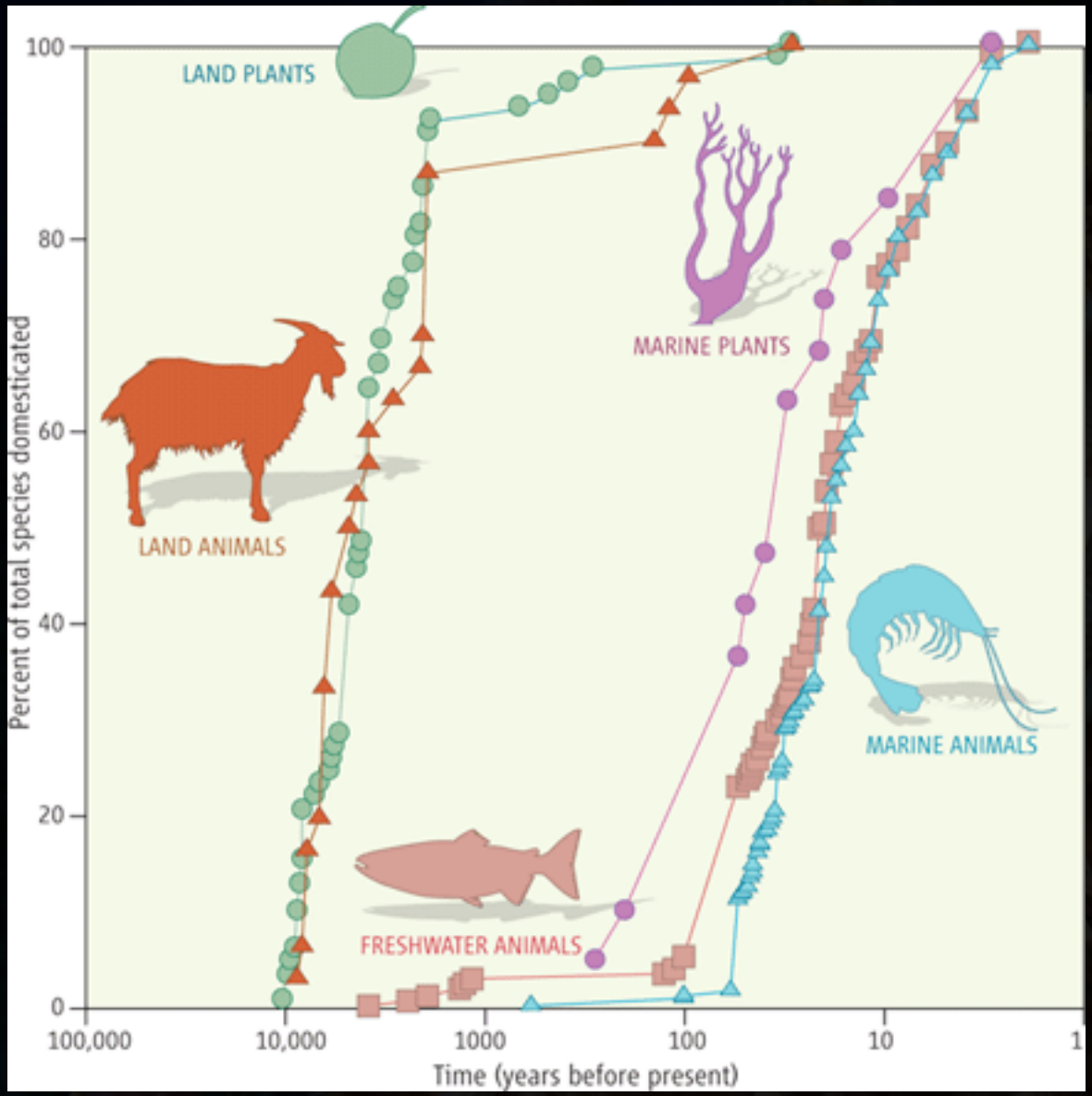
- Oceans encompasses >70% of the sea surface.
- Research on marine biodiversity ~10% of global biodiversity research.
- Approximately 10% of all named species are marine.
- Growth rate of marine species 0.9% per year.
- Large surprises despite limited respurces alocated to marine research.

Recent milestones in the exploration of the “inner space”

- 1977. Chemosynthetic ecosysems discovered (hydrothermal vents).
- 1983. A new marine phyllum Loricifera, discovered.
- 1988. *Prochlorococcus, possibly the most abundant phototroph in the plnet discovered.*



Use of living resources: Domestication



Duarte et al 2007

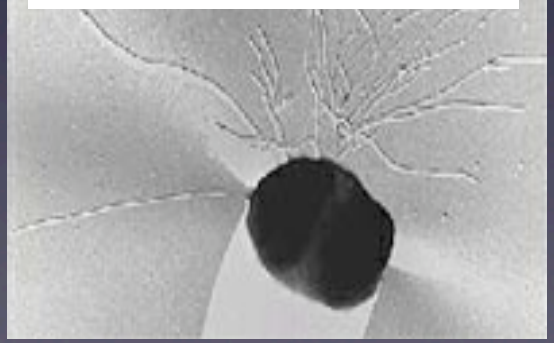
On land, > 90% of species domestiated > 2000 yrs ago.

At sea, 90% of species, domesticated within last 100 yeas

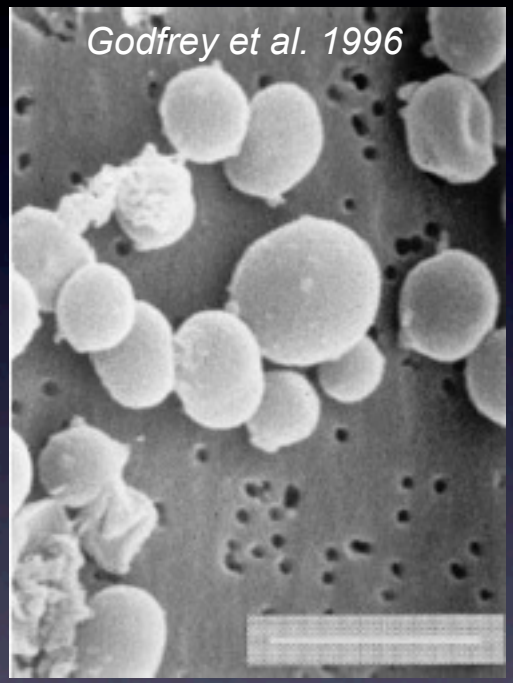
Marine Genetic Resources

- Linked with discovery (exploration of) marine biodiversity
- Highest potential in extreme habitats, where the highest potential for biodiversity discoveries are:

Pyrococcus furiosus

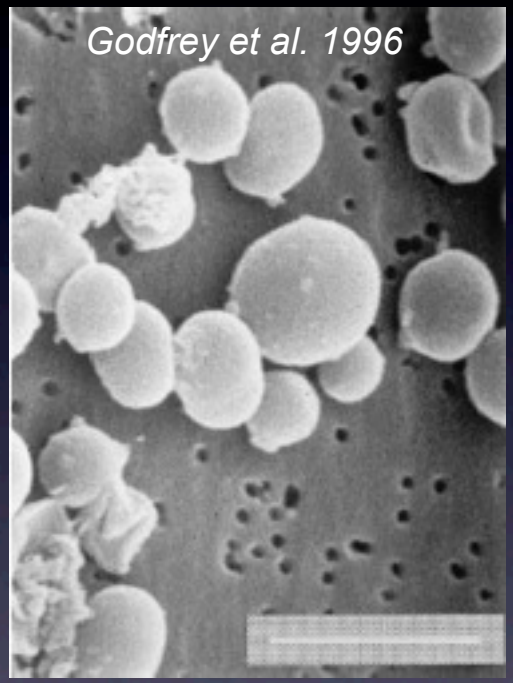


The bacteria *Pyrococcus furiosus*, recently discovered by NASA grows at enormous speed (1 duplication every 37 minutes) at 100 º C in submarine volcanoes , and is the only known living organism able to Tungsten



- Proteins from bacteria isolated from submarine volcanoes are stable at high temperature and can be used for development of more efficient and sure methods for genome sequencing.

- e.g. polymerase *Tfu* used in PCR, isolated from bacteria *Thermococcus fumicolans* out hydrothermal vents by



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Genetic Resources

- Natural Products
  - Molecules produced by organisms that can be used as medicines, cosmetics, nutritional supplements...
- DNA Sequences
  - Genes that code for a protein or enzyme of interest, or sequences of DNA useful for diagnostics or detection.

Business opportunities in marine biodiversity

- Observational technologies
- Biotechnology
- Food Industry
    - Aquaculture

  (production of new species)
- Cosmetics industry
- Pharmaceutical Industry

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Patented Genes

- The PAT division of Genbank (release 165) lists more than 5 million records of DNA sequences deposited in different patent offices worldwide.
- Most sequences from humans, model organisms and domestic/food animals and plants.
- The requirements to patent a gene is that both the sequence and the function of the protein/enzyme that the gene codes for be described.
- No required information on organismal identity (e.g. species name) or source (location where sample

The New York Times

Business

Judge Invalidates Human Gene Patent

By JOHN SCHWARTZ and ANDREW POLLACK
Published: March 29, 2010

A federal judge on Monday struck down patents on two genes linked to breast and ovarian cancer. The decision, if upheld, could throw into doubt the patents covering thousands of human genes and reshape the law of intellectual property

Enlarge This Image

United States District Court Judge Robert W. Sweet issued the 152-page decision, which invalidated seven patents related to the genes BRCA1 and BRCA2, whose mutations have been associated with cancer.

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Examples of marine natural products

Product	Use	Source
Pharmaceuticals		
Ara-A (acyclovir)	Antiviral drug (herpes infections)	Marine sponge, *Cryptotethya cryta*
Ara-C (cytosar-U, cytarabine)	Anticancer drug (leukemia and non-Hodgkin's lymphoma)	Marine sponge, *Cryptotethya cryta*
Molecular Probes		
Okadaic acid	Phosphatase inhibitor	Dinoflagellate
Manoalide	Phospholipase A_2 inhibitor	Marine sponge, *Luffariella variabilis*
Cosmetic additives		
Resilience (Estée Lauder)	"Marine extract" additive	Caribbean gorgonian, *Pseudopterogorgia elisabethae*
Nutritional Supplements		
Formulaid (Martek Biosciences)	Fatty acids used as additive in infant formula nutritional supplement	Marine microalga
Pigment		
Phycoerythrin	Conjugated antibodies used in ELISAs and flow cytometry	Red algae

MARINE NATURAL PRODUCTS

Marine Natural Products under clinical trials (January 2004)

Compound name	Origin	Supply	Discovered by	Developped by	Biological Activity	Clinical Phase
APLIDINE Depsipeptide	*Aplidium albicans* **Tunicate**	Chemical synthesis	**Univ. of Illinois** (USA) **PharmaMar** (Spain)	PHARMAMAR (Spain)	**Cytotoxic**	**Phase II**
BRYOSTATIN Macrocyclic lactone	*Bugula neritina* **Bryozoan** (Pos. bacterial origin)	Recollection / Aquaculture	**Arizona State Univ.** (USA)	GPC Biotech (Germany)	**Antitumor** (modulator of protein kinase C- PKC activity)	**Phase II** Esophageal cancer and others cancers.
CONOTOXINS Polypeptides (more than 100 reported)	*Conus spp.* **Mollusk**	Chemical synthesis	**Univ. of Utah** (USA)	**PRIALT** (Ziconotide) : ELAN PHARMACEUTICALS (Ireland) **AM336:** AMRAD (Australia)	Analgesic (neuropharmacologic activity)	**PRIALT** (Ziconotide): **Phase III. AM336: Phase II** Severe pain.
DISCODERMOLIDE Polyketide	*Discodermia sp.* **Sponge**	Chemical synthesis	**Harbor Branch** (USA)	NOVARTIS (USA)	**Antitumor**	**Phase I** Pancrealic cancer
DOLASTATINS (Cemadotin, ILX-651 and TZT-1027 are synthetic analogues of Dolastatins) Polypeptides	*Dolabella auricularia* **Mollusk** (Possible origin cyanobacterium species: *Lyngbya sp., Symploca sp., Schizothrix sp.*)	Chemical synthesis Polypeptide	**Arizona State Univ.** (USA)	**Cemadotin** (LU103793): Dol-15 Synt. anal. ABBOTT GmbH & Co. KG (Germany) (formally Knoll Pharma). **ILX-651:** Dol- 15 anal. from BASF Pharma, licensed to ILEX ONCOLOGY (USA). **TZT-1027:** Dol-10 derivative: TEIKOKU HORMONE (Japan) **DOL-10:** NATIONAL CANCER INSTITUTE (USA)	**Cytotoxic** (inhibition of microtubule assembly)	**Cemadotin: Phase II** Melanoma **ILX-651: Phase I.** Advanced solid tumors **TZT-1027: Phase I. DOL-10: Phase I-II**
ES-285 Anti-Beta-aminoalcohol	*Mactromeris polynyma* **Mollusk**	Synthesis	**Univ. of Illinois** (USA) **PharmaMar** (Spain)	PHARMAMAR (Spain)	**Antitumor**	**Phase I** Solid Tumors
ET-389	Axinella sp. **Sponge**		**Arizona State U.** (USA)	**NCI-Approved California Cancer Consortium** (USA). EISAI Inc. (Japan)	**Antitumor**	**Phase I**
GTS-21, DMXBA (Anabaseine analogue) Alkaloid	*Amphiporus lactifloreus* **Nemertine**	Chemical synthesis	**Univ. of Florida** (USA)	TAIHO PHARMACEUTICALS CO (Japan)	**Inhibition of neuronal nicotinic receptors**	**Phase I-II** Alzheimer disease.

http://www.bioaqua.net/mnp/MNP/clinical.asp

Examples of Genes

Product	Use	Source
Molecular Probes		
Aequorin	Bioluminescent calcium indicator Reporter gene	Bioluminescent jellyfish, *Aequora victoria*
Green fluorescent protein (GFP)	Reporter gene	Bioluminescent jellyfish, *Aequora victoria*
Enzymes		
Vent and Deep Vent DNA polymerase (New England BioLabs)	Polymerase chain reaction enzyme	Deep-sea hydrothermal vent bacterium
Fish proteases	Fish scaling Ripening of salted fish Tenderization of squid	Many fish species.
Cold adapted enzymes	Proteases, lipases for detergents Extraction of carotenoproteins for food coloring Caviar production Meat tenderizing	Psichrophylic microorganisms

Food Industry

Business potential

- US$2.1 billion in 2002, increasing at a rapid 9.4% from the previous year.
- 1 amylase, from a hydrothermal vent organism, used to liquify corn biofuel deliver 150M $ every year on patent use rights.

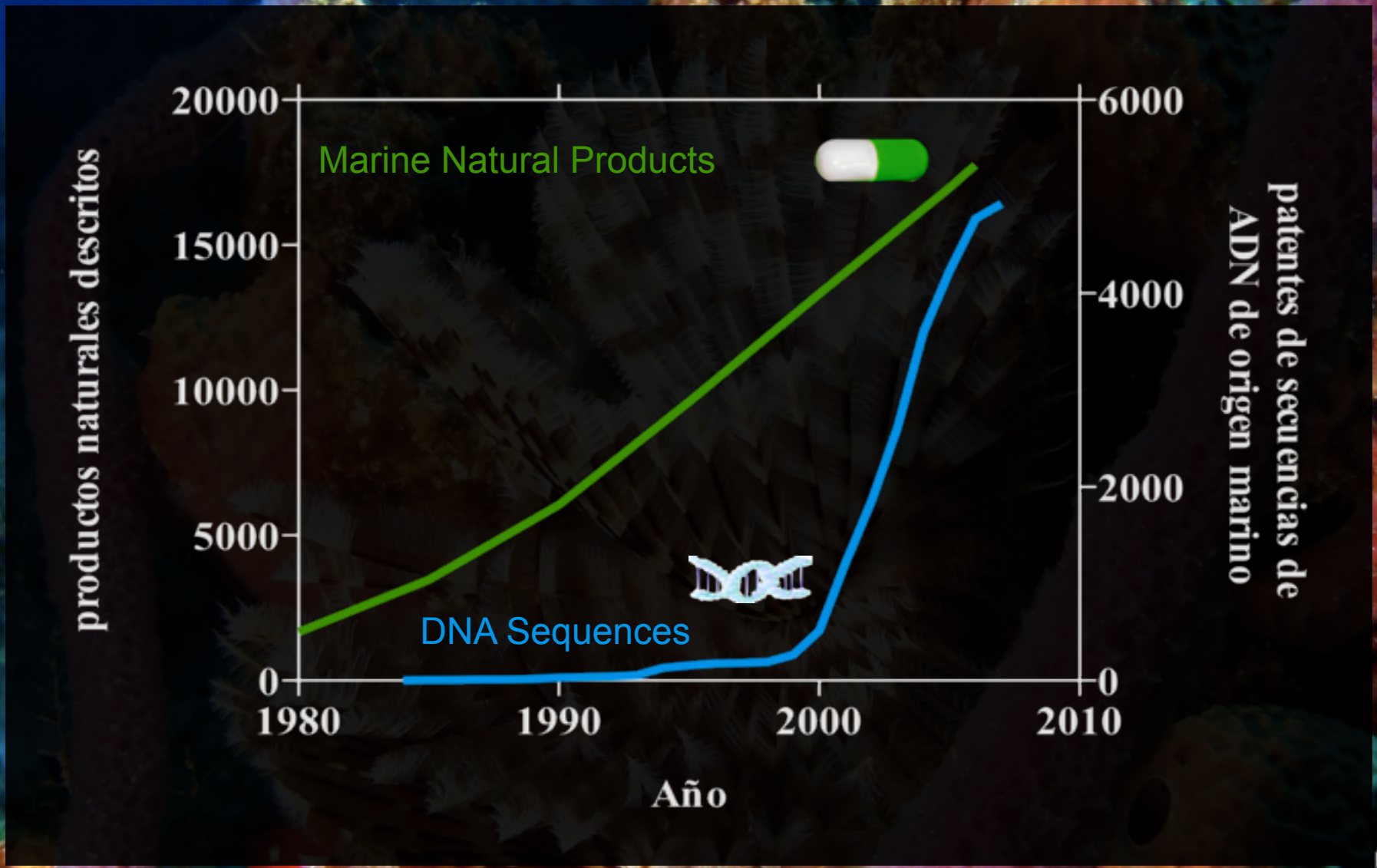
Deep Ocean MGR

Blue Gold

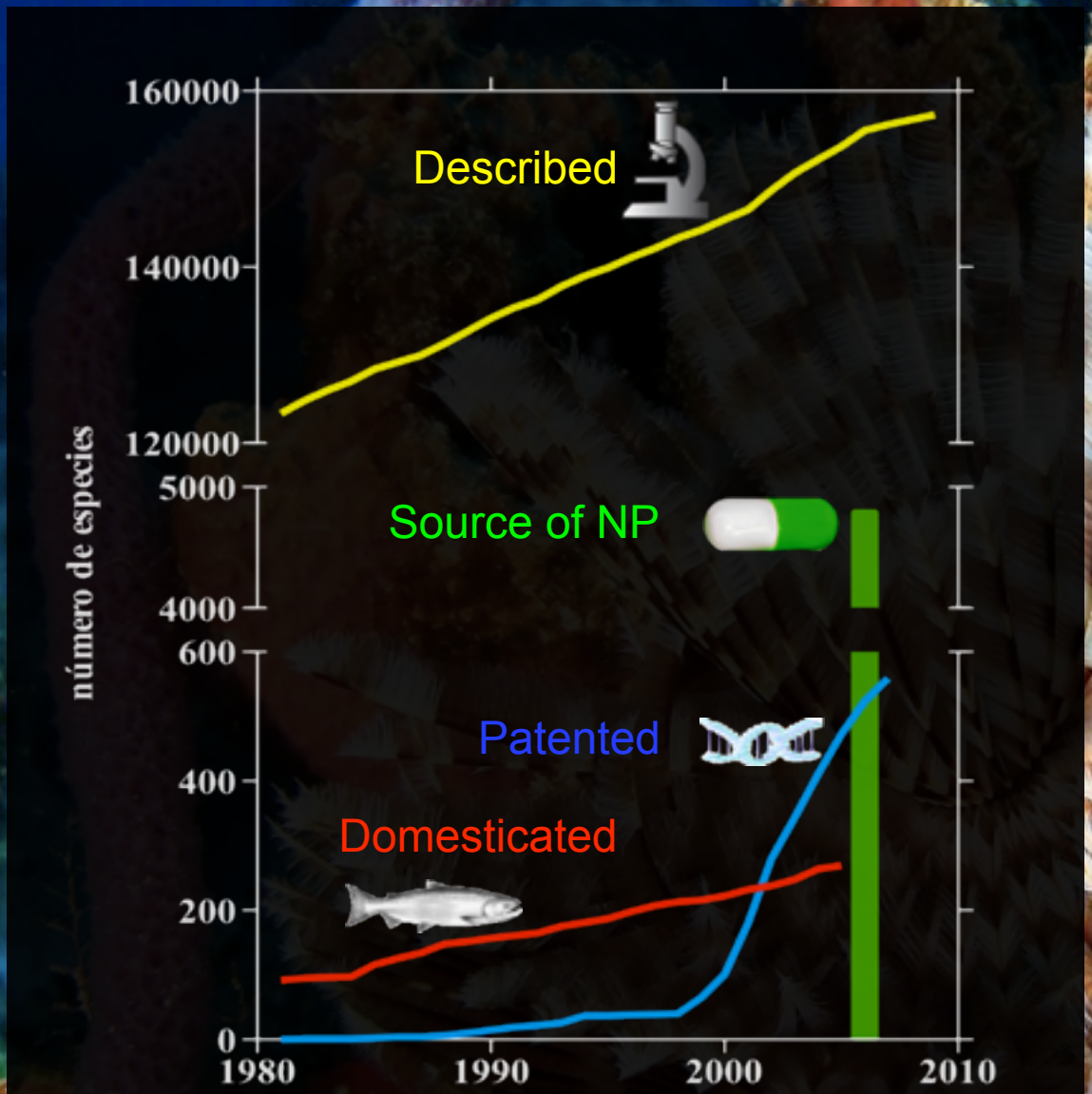
Table 1 Selected marine biotech patents 2005

Patent	Assignee	Publication date	Patent number
Novel polysaccharide obtained from marine bacterium *Altermonas macleodii*	Ifremer, Issy-les-Moulineaus, France, and Cooperative Laitiere Ploudaniel, Pancoet, France	January 19, 2005	EP1171625B1
Fluorescent and colored reporter proteins isolated from marine organisms	University of Florida Research Foundation, Gainesville, Florida	March 3, 2005	WO2005019252A2
Novel Agrobacterium with improved production of carotenoids derived from marine strain	Tosoh, Tokyo	March 10, 2005	JP2005058216A
Novel compound derived from marine actinomycete for treating mammalian cell proliferative disorder or bacterial infection	University of California, Oakland, California	March 24, 2005	WO2005025490A2
Equipment for sampling microorganisms from the Earth's crust	Japan Marine Science and Technology Center, Yokosuka, Japan	May 19, 2005	US2005106751A1
Marine yeast component for cancer drug screening	Orient Cancer Therapy, Tokyo	June 16, 2005	WO2005054497A1
Substrate for detection of marine microorganisms	Mitsubishi Jukogyo, Tokyo	July 7, 2005	JP2005176791A
Novel marine Actinomycete	University of California Oakland, California	July 14, 2006	US2005153389A1

Nature Biotechnology (2005)


20000
15000
10000
5000
0
6000
4000
2000
0
1980
1990
2000
2010
Año
productos naturales descritos
patentes de secuencias de ADN de origen marino
Marine Natural Products
DNA Sequences

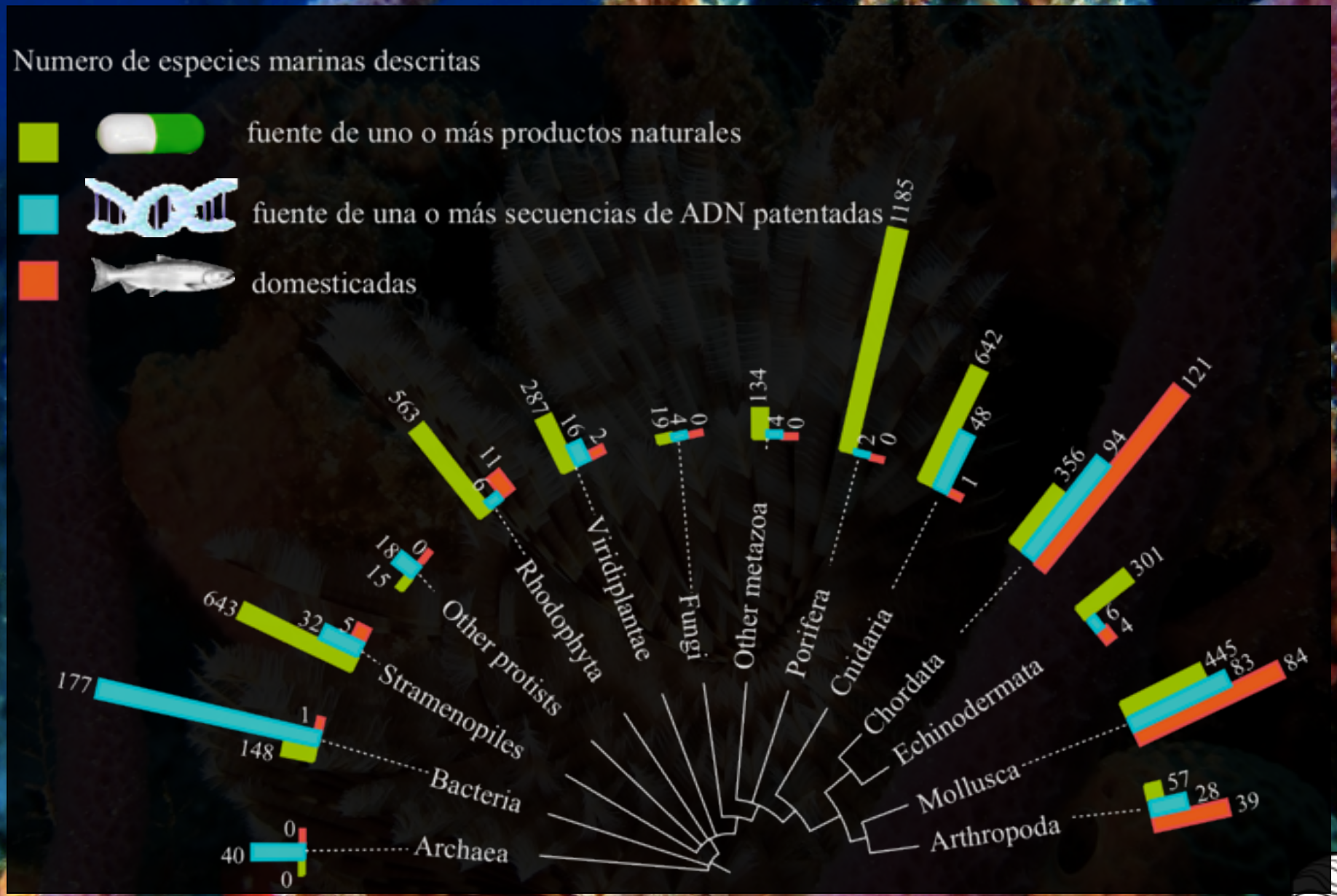
Number of marine species used



Annual Growth Rate

Described
0.91%
Domesticated
2.75%
Patented
12%

Number of marine species



Comparison with terrestrial species

	Especies descritas			Especies en patentes			Porcentaje de las descritas en patentes		
	Total	terrestres	marinas	Total	terrestres	marinas	Total	terrestres	marinas
procariotas	7928	7173 90.48%	755 9.52%	1619	1401 86.53%	218 13.47%	20.42%	19.53%	28.87%
eucariotas	1800000	1653045 91.84%	146955 8.16%	2019	1679 83.16%	340 16.84%	0.11%	0.10%	0.23%
Total	1807928	1660218	147710	3638	3080	558	0.20%	0.19%	0.38%

- Prokaryots (Bacteria, Archaea) are the best candidates to derive useful genes.
- About twice as many marine species have yielded patentable genes as terrestrial ones.
- The probability to derive a natural product from a marine species in 500 times than that for a randomly selected terrestrial species (Venugopal, 2008)

Marine biodiversity

- About 300000 named marine species.
- Likely about 1.4 to 1.6 million marine species in total.
- At present rate of description, 250 to 1000 years for a full inventory of marine species.

Microbial diversity

Some recent examples:

- Rowher et al (2002) → 430 species most new from 14 coral fragments.
- Venter et al. (2004) → 1800 OTUsen in a m^3 of Sargasso Sea waters, 148 of them unknown.
- Rusch et al (2007) → 811 OTUs in 45 coastal stations, of which 52% were thus unknown.
- Sogin et al (2006) → >6000 OTUs in a L of water, statistical extrapolation: ~25000 species.

Globally, there may be several million species of marine microbes

Sustainable use of marine genetic resources

- Most yet to be discovered → huge potential for future discoveries.

- Bioprospección of genes and natural products does not require large biomass harvest in most cases → a sustainable process.

Are MGR's threatened?

- A third of marine coral reefs are endangered (Carpenter, 2008)
- Los of Arctic ice (ACIA, 2004).
- Out of the 340 especies of marine eukaryotic species described as sources of useful genes:
  - Only 36 appear as in the Red list of IUCN
    - 2 as "Endangered"
    - 6 as "Vulnerable"
    - 7 as "Near Threatened"
    - 10 as "Data deficient"

  More will appear…

Biopiracy

- Bioprospecting for genes and natural products require sophisticated methods and is dominated by developed countries.

- Marine genetic resources are often in the waters of developing countries or in international waters.

- Biopiracy: Apropiation of natural biological matterials by a technological advanced country without a just compensation to the nations in which territory the matterials were found

Prospection of natural resources

- UN Convention for the Law of the Sea (UNCLOS)
  - Stablish the boundaries of national waters
  - Regulates the use of mineral resources in international waters.
  - Does not regulate the use of biological resources in international waters.
- Convention for Biological Diversity (CBD)
  - Stablishes the rights of the nations to biological resources and obligates signatory countries to share the benefits.

Not all nations have ratified these conventions

- The use of marine genetic resources meet a legal void in international waters.

Circunnavegation Cruises
The Pioneer

Opening the Global Marque, 16th Century

Magallanes / Elcano, 1519-1522 on board ships Trinidad (capitana), Victoria, San Antonio, Concepción y Santiago.

Goal: Open a route from Occident to the land of the spices (Molucas)

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Magallanes / Elcano, 1519-1522 on board ships Trinidad (capitana), Victoria, San Antonio, Concepción y Santiago.
Goal: Open a route from Occident to the land of the spices (Molucas)

Francis Drake, 1577–1580, on board Pelican / Golden Hind.
Goal: Dispute commercial interests to Spain

Cinrcunnavigation cruises

The Illustration

Exploring the Planet, Siglo XVIII

James Cook, 1ª 1768-1771 on board HMS *Endeavour*;
2ª 1772–1775 on board HMS *Resolution*.

Goal: Funded by the Royal Society astronomy, botany and zoology

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Incomplete

Cinrcunnavigation cruises

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Incomplete

Alejandro Malaspina, 1789–1794, on board *Atrevida y la Descubierta*. Scientific research, oceanography and politics
Incomplete

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Welcome to SORCERER II Expedition

A Global Voyage of Discovery

ENTER SITE

J. Craig Venter Institute's Sorcerer II Expedition

Funding provided by: Office of Science U.S. DEPARTMENT OF ENERGY · MOORE FOUNDATION · Discovery Channel · JCVI

Results

Sampling and the Metagenomic Dataset

Microbial samples were collected as part of the *Sorcerer II* expedition between August 8, 2003, and May 22, 2004, by the S/V *Sorcerer II*, a 32-m sailing sloop modified for marine research. Most specimens were collected from surface water marine environments at approximately 320-km (200-mile) intervals. In all, 44 samples were obtained from 41

Sample collection.

A YSI (model 6600) multiparameter instrument (http://www.ysi.com) was deployed to determine physical characteristics of the water column, including salinity, temperature, pH, dissolved oxygen, and depth. Using sterilized equipment [91], 40–200 l of seawater, depending on the turbidity of the water, was pumped through a 20-μm nytex prefilter into a 250-l carboy. From this sample, two 20-ml subsamples were collected in acid-washed polyethylene

Deep Malaspinomics:

Microbial genetic and functional diversity in the world's deep ocean

A few facts about the Dark Ocean

- Enormous genetic variability contained in the DO
- Not only phylogenetic but also functional
- And, very dynamic (much more than previously thought)
- Microbial life-style adapted to large MW DOM
- High production of exoenzymes
- Microbial life-style adapted to survival on particles
- Very little known of viruses and protists
- Unclear, yet, effect of pressure on interaction with DOM
- Dark Ocean is not homogeneous: water mass interfaces appear to be hotspots of microbial activity and diversity

Prokaryote diversity studies in the deep Ocean

- FISH
  - Crenarcheaota most abudant in the deep ocean
- Fingerprinting studies
  - richness decreases 25% with depth
  - diff. communities in diff. water layers
  - according to water-mass characteristics
- Clone libraries
  - Lots of piezophiles closely related to psychrophiles (same ecol. constraints?)
  - enrichment of gamma- and deltaproteobacteria, Actinos, Acidobacter... lack of Bacteroidetes

Backgroup on Marine Deep "Omics"

- Metagenomics (i.e. no PCR) of the Deep Sea:

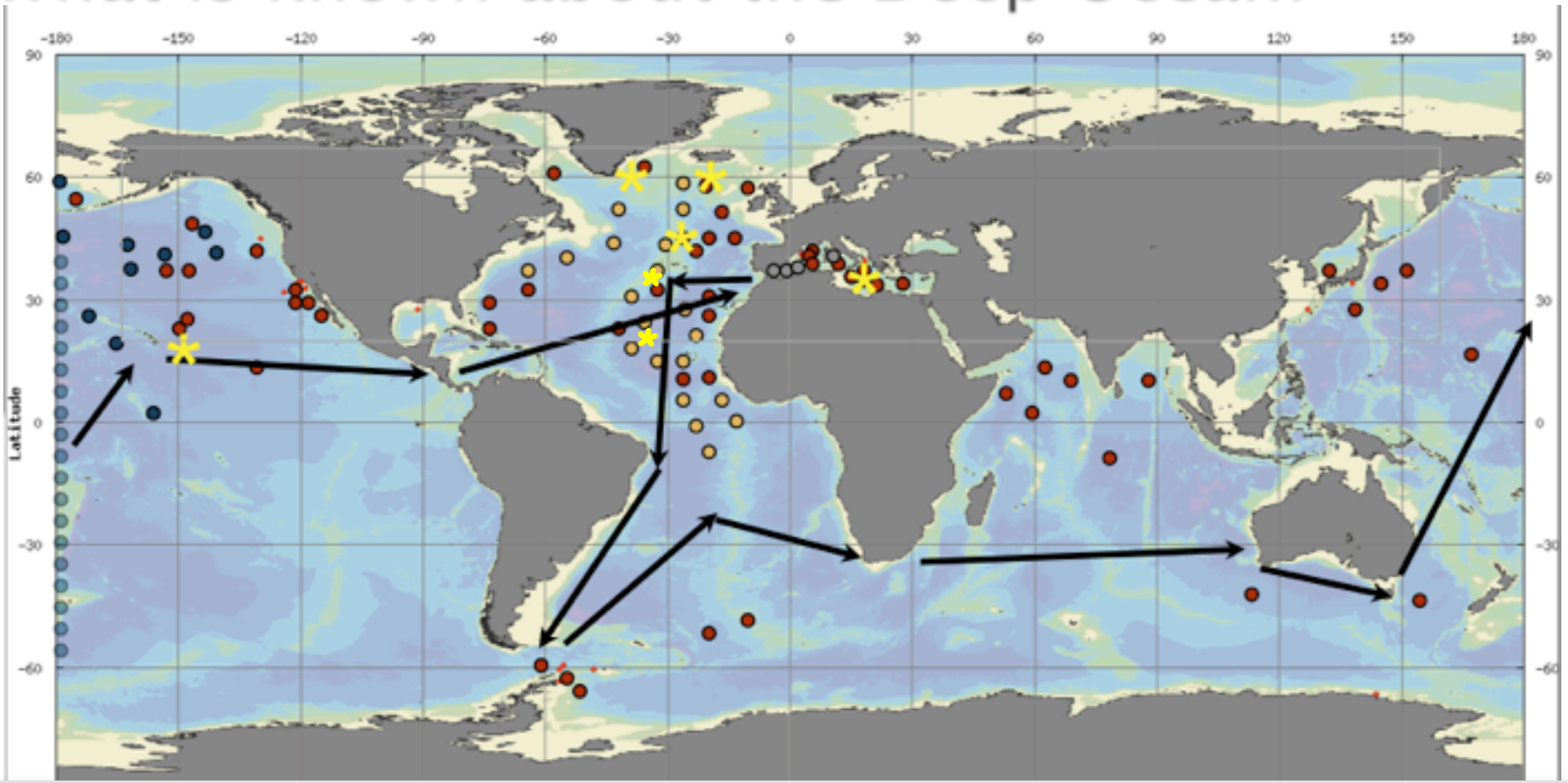
DeLong et al. 2006 (Science)

North-Pacific Subtropical Gyre ALOHA station, ranging from 10 to 4000 m (Temp: 2°C)

Martín-Cuadrado et al. 2007 (PLoSOne)

Comparison btween DeLong dataset and a deep Mediterranean metagenome from 3000 m depth (Km3)

What is known about the Deep Ocean?



The points (in different colors) belong to different studies where microbial deep ocean data exists based on a recent review (Arístegui et al. 2009). The yellow asterisks indicate the sites of 454 tag-sequencing and/or metagenomic studies of the deep ocean, all in the Northern Hemisphere. The Arrows illustrate the Malaspina 2010 path mostly along uncharted deep ocean waters.

The Malaspina cruise (2010-2011)

- ca. 180 stations with profiles to <4000 m
- Bathypelagic DNA samples of 4 z per station
- High quality DNA and RNA samples of
  - 60 stations – > 150 L – one depth (at ca. 4000 m)
- Ancillary data for all stations:
  - Prokaryote abundance and viability
  - HNF and viruses
  - Prokaryote heterotrophic activity and chemolitoautotrophic CO_2 fixation
  - Exoenzymatic activity
  - Biolog Ecoplates
  - Respiration rates
  - FISH and $^{14}CO_2$ and ^{3}HLeu MARFISH samples
  - Physical profiles

What is Deep Malaspinomics?

First sequencing effort:

Proposal for CSP2011 Program by DOE JGI under review in their second stage.

PI: Silvia G. Acinas (ICM, CSIC, Spain)
Co-PIs: José M. González (ULL, Spain), Josep M. Gasol (ICM, CSIC, Spain), Carlos Pedrós-Alió (ICM, CSIC, Spain), Jesús M. Arrieta (IMEDEA, CSIC, Spain), Dolors Vaqué (ICM, CSIC, Spain) and Matthew Sullivan (Univ. Arizona, US)
Collaborators: Ramunas Stepanauskas and Michael Sieracki, Bigelow Laboratory for Ocean Sciences (US)

Technological State of the Art
454 pyrosequencing and Illumina

60 selected samples
From Malaspina deep ocean

30 samples

30 samples

Pyrotagging
(Bacteria & Archaea)
Who is there?

16S rDNA (present)
versus
16S rRNA (active)

Metagenomics
Bacteria and
Viruses

What genes are present?

Transcriptomics
Bacteria

What are they doing?

Main Goals of Deep Malaspinomics:

- Explore the biogeography of deep ocean prokaryotes and associated viruses as it correlates to water masses and to the ecological and oceanographic settings
- obtain insights on relevant prokaryotic metabolic pathways and their interaction with viruses.
- usage Single Amplification Genomes (SAGs) as reference genomes for assemblage of the prokaryotic metagenome of the selected deep ocean samples.

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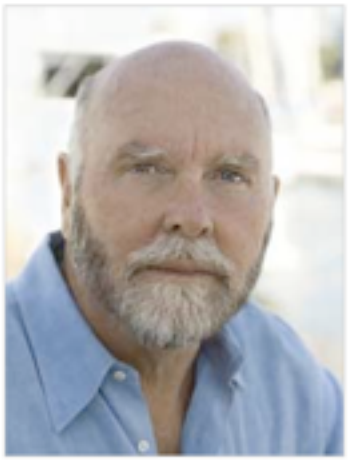
What's next for synthetic life?

By Katherine Harmon



COLD SPRING, N.Y.— J. Craig Venter and his colleagues recently announced that they had created the first cell to run on a fully artificial genome. So what's next for this synthetic strain of microscopic *Mycoplasma mycoides* and the new technology?

The "synthetic cell" achievement has been lauded, condemned and undercut, but it has yet to fully demystify life's underlying code, the genome. "It's amazing how little we know about genomics," Venter said June 1 at the Cold Spring Harbor Symposia on Quantitative Biology in Cold Spring, N.Y.

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From *Canadian Business* magazine, July 19, 2010

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Genetics

Microbiology: The business side of synthetic life

J. Craig Venter is more focused on practical applications than playing God.

By Laura Cameron

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Jun 3, 2010 01:45 PM in Basic Science | 7 comments

What's next for synthetic life?

By Katherine

Synthetic Genomics is investigating a naturally occurring organism capable of generating biofuels. ExxonMobil has invested $600 million in the company to research the possibility of synthesizing algae that converts carbon dioxide into fuel. The partnership is looking at blue-green algae, a one-celled organism found in the ocean that turns carbon into fuel. Venter claims an industrial cell with this function could be worth over $1 trillion.

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50 million candidate genes to play with...

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OPINIONS, ARGUMENTS & ANALYSES FROM THE EDITORS OF SCIENTIFIC AMERICAN

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Jun 3, 2010 01:45 PM in Basic Science | 7 comments

What's next for synthetic life?

By Katherine

Synthetic Genomics is investigating a naturally occurring organism capable of generating biofuels. ExxonMobil has invested $600 million in the company to research the possibility of synthesizing algae that converts carbon dioxide into fuel. The partnership is looking at blue-green algae, a one-celled organism found in the ocean that turns carbon into fuel. Venter claims an industrial cell with this function could be worth over $1 trillion.

The "synthetic cell" achievement has been lauded, condemned and undercut, but it has yet to fully demystify life's underlying code, the genome. "It's amazing how little we know about genomics," Venter said June 1 at the Cold Spring Harbor Symposia on Quantitative Biology in Cold Spring, N.Y.

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From *Canadian Business* magazine, July 19, 2010

Genetics

Microbiology: The business side of synthetic life

J. Craig Venter is more focused on practical applications than playing God.

By Laura Cameron

Resumen

1. Marine ecosystems hold great potential to deliver MGRs of commercial interest.
2. The large unexplored biodiversity together with the limited impact of bioprospecting makes this a potentially sustainable process.
3. MGR's must be incorporated into schemes to conserve marine biota.
4. Legal and ethical issues must be addressed and resolved.
5. 21st Century will be the century of Biology.
6. This is very exciting... Open your Eyes!