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Position Paper 15

Marine Biotechnology: A New Vision and Strategy for Europe

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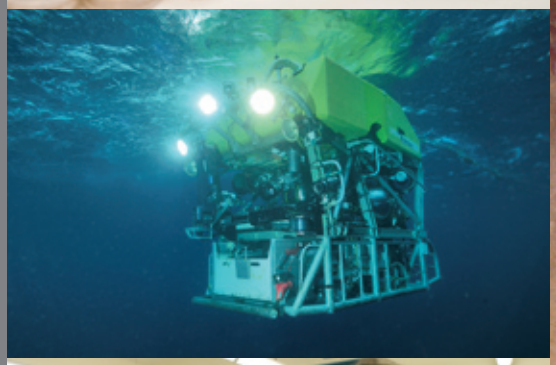

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Marine Board-ESF

The Marine Board provides a pan-European platform for its member organisations to develop common priorities, to advance marine research, and to bridge the gap between science and policy in order to meet future marine science challenges and opportunities.

The Marine Board was established in 1995 to facilitate enhanced cooperation between European marine science organisations (both research institutes and research funding agencies) towards the development of a common vision on the research priorities and strategies for marine science in Europe. In 2010, the Marine Board represents 30 Member Organisations from 19 countries.

The Marine Board provides the essential components for transferring knowledge for leadership in marine research in Europe. Adopting a strategic role, the Marine Board serves its Member Organisations by providing a forum within which marine research policy advice to national agencies and to the European Commission is developed, with the objective of promoting the establishment of the European *Marine* Research Area.

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Marine Biotechnology: A New Vision and Strategy for Europe

Marine Board-ESF Position Paper 15

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Foreword

In 2001, the Marine Board-ESF published its Position Paper 4, *'A European Strategy for Marine Biotechnology'*, to highlight the many benefits that Marine Biotechnology could offer for Europe if its development was sufficiently supported. This first Position Paper called for a European initiative in Marine Biotechnology to mobilise the scattered human capital and strategically refocus the extensive but dispersed infrastructure into concerted action. Four key objectives were highlighted: (i) the development of Marine Biotechnology industries; (ii) the identification of R&D requirements to establish Europe as a world leader in marine bio-screening and derived bio-products; (iii) the promotion of networking between European actors in Marine Biotechnology; and (iv) recommendations to directly impact on future European Union Framework Programmes. In 2002 the US National Academy of Sciences published a report entitled *Marine Biotechnology in the Twenty-first Century: Problems, Promise, and Products*. This report made broadly similar recommendations to the Marine Board Position Paper and stressed the need to develop new advanced techniques for detection and screening of potentially valuable marine natural products and biomaterials.

Today, European countries are facing complex and difficult challenges that will shape our common future. Issues that top the agenda include a sustainable supply of food and energy, climate change and environmental degradation, human health and aging populations. The current global economic downturn has made these issues even more pressing. Marine Biotechnology can and should make an important contribution towards meeting these impending challenges and contribute to economic recovery and growth in Europe. Not only can it create jobs and wealth, but it can contribute to the development of greener, smarter economies, central components of the new Europe 2020 Strategy[1]. The potential contribution of Marine Biotechnology is, therefore, even more relevant now than it was ten years ago and a sound strategy for its development in Europe is urgently needed to allow for this potential to be realised.

Surrounded by four seas and two oceans, Europe benefits from access to an enormous and diverse set of marine ecosystems and to the corresponding biodiversity. These marine ecosystems are largely unexplored, understudied and underexploited in comparison with terrestrial ecosystems and organisms. They provide a unique environment with an enormous potential to contribute to the sustainable supply of food, energy, biomaterials and to environmental and human health. Marine Biotechnology is, and will become even more, central to delivering these benefits from the sea. Therefore, it is appropriate that this Position Paper uses these 'Grand Challenges' to structure the logical analysis of the current and possible future development of Marine Biotechnology set against its capacity to deliver products and processes to address these high-level societal needs and opportunities.

Marine Biotechnology developments in each of these areas cannot be seen in isolation from the wider European and global scientific and political landscape which has changed considerably since 2001. If the most significant developments in Marine Biotechnology during the 1990s were the result of the molecular biology revolution, it is clear that the primary driving force during the last decade was the genomic revolution. The overwhelming role of marine biodiversity for the future of marine resources, ecosystem management, bioprospecting and Marine Biotechnology was also recognised. The EU research policy was responsive to some extent, notably through support for the Marine Genomics and Marine Biodiversity (MarBEF) FP6 Networks of Excellence and other on-going collaborative projects. Recent efforts to support and coordinate European coastal and marine research infrastructures to improve, for example, access to research vessels, stations and laboratories indicate some level of recognition that action is needed to fully exploit the vast but fragmented research infrastructure available for marine sciences in Europe, including for Marine Biotechnology research. However, it is clear that objective number 2 of the 2001 Marine Board Position Paper on Marine Biotechnology, i.e. establishing Europe as a world leader in marine bio-screening and derived bio-products, has not been achieved.

The present report was initiated by the Marine Board to provide an updated view of Marine Biotechnology to policy makers at EU and national levels and to EU and national scientific and administrative officers involved in research in marine sciences and their interacting fields in health, food, environment and energy. The report has been produced by the members of the Marine Board Working Group on Marine Biotechnology (WG BIOTECH), established by the Marine Board in order to:

(i) provide a strategic assessment of the current scientific understanding of Marine Biotechnology relevant to European Union and Member State policies;
(ii) identify the priorities for further research in this field;
(iii) analyse the socio-economic context in which Marine Biotechnology is evolving; and
(iv) formulate recommendations for future policies and critical support mechanisms.

1 http://ec.europa.eu/eu2020/index_en.htm

Foreword

The resulting product of this joint effort is this new Marine Board Position Paper on Marine Biotechnology which calls for a collaborative industry-academia approach by presenting a common Vision and Strategy for European Marine Biotechnology research which sketch the contours of the research and policy agenda in the coming 10-15 years.

On behalf of the Marine Board, we would like to sincerely thank the Working Group Chair, Dr Joel Querellou, and its expert participants, whose efforts resulted in a comprehensive overview of Marine Biotechnology research achievements and future challenges. Their work has been crucial to highlight the diverse and exciting opportunities in this field of research and in providing a decisive contribution to further develop the Marine Biotechnology sector in Europe to its full potential. We are also very grateful for the many constructive suggestions and critical comments provided by various industry representatives and experts. In particular we would like to thank Dermot Hurst, Bill Fenical, Yonathan Zohar and Meredith Lloyd-Evans for their valuable comments and inputs. Finally, we take this opportunity to acknowledge the hard work of Jan-Bart Calewaert from the Marine Board Secretariat, who provided unstinting support to the Working Group.

Lars Horn and Niall McDonough
Chairman and Executive Scientific Secretary,
Marine Board-ESF

Executive Summary

Biotechnology, the application of biological knowledge and cutting-edge techniques to develop products and other benefits for humans, is of growing importance for Europe and will increasingly contribute to shape the future of our societies. Marine Biotechnology, which involves marine bioresources, either as the source or the target of biotechnology applications, is fast becoming an important component of the global biotechnology sector. The global market for Marine Biotechnology products and processes is currently estimated at € 2.8 billion (2010) with a cumulative annual growth rate of 4-5%. Less conservative estimates predict an annual growth in the sector of up to 10-12% in the coming years, revealing the huge potential and high expectations for further development of the Marine Biotechnology sector at a global scale.

This Position Paper, developed by the Marine Board Working Group on Marine Biotechnology, presents a shared vision for European Marine Biotechnology whereby:

By 2020, an organised, integrated and globally competitive European Marine Biotechnology sector will apply, in a sustainable and ethical manner, advanced tools to provide a significant contribution towards addressing key societal challenges in the areas of food and energy security, development of novel drugs and treatments for human and animal health, industrial materials and processes and the sustainable use and management of the seas and oceans.

This 2020 Vision for European Marine Biotechnology will only be achieved through a coordinated implementation in a joint effort with active support and involvement from all relevant stakeholders, of the following high level recommendations:

- RECOMMENDATION 1: Create a strong identity and communication strategy to raise the profile and awareness of European Marine Biotechnology research.
- RECOMMENDATION 2: Stimulate the development of research strategies and programmes for Marine Biotechnology research and align these at the national, regional and pan-European level.
- RECOMMENDATION 3: Significantly improve technology transfer pathways, strengthen the basis for proactive, mutually beneficial interaction and collaboration between academic research and industry and secure access and fair and equitable benefit sharing of marine genetic resources.
- RECOMMENDATION 4: Improve training and education to support Marine Biotechnology in Europe.

Marine Biotechnology contribution to key societal challenges

In the context of a global economic downturn, European countries are now facing complex and difficult challenges such as the sustainable supply of food and energy, climate change and environmental degradation, human health and aging populations. Marine Biotechnology can make an increasingly important contribution towards meeting these societal challenges and in supporting economic recovery and growth in Europe by delivering new knowledge, products and services.

Sustainable supply of high quality and healthy food

Marine Biotechnology is essential to satisfy the growing demand for healthy products from fisheries and aquaculture in a sustainable way. The growing demand for marine food will need to be increasingly delivered through intensive aquaculture. Since 2001, rapid biological and biotechnological progress has resulted in a more efficient and environmentally responsible aquaculture and a greater diversity of marine food products. Marine Biotechnology has contributed significantly to increasing production efficiency and product quality, to the introduction of new species for intensive cultivation and the to the development of sustainable practices through a better understanding of the molecular and physiological basis for reproduction, development and growth, and a better control of these processes. However, commercial aquaculture continues to face challenges in understanding and controlling reproduction, early life-stage development, growth, nutrition, disease and animal health management and environmental interactions and sustainability.

Sustainable alternative sources of energy

The ocean is an untapped, sustainable source of bio-energy. There are many examples of the production of bio-energy from marine organisms, but the production of biofuel from microalgae presents perhaps the most promising option to harvest this huge energy potential. The theoretical production of oil from microalgae is considerably higher than that of terrestrial crops but, to achieve viability, the cost of production will need to be significantly reduced and the scale of production increased, while maintaining environmental sustainability. To cultivate microalgae for the generation of bio-energy is an important challenge for Marine Biotechnology in the 21st century.

Securing environmental health

Marine Biotechnology is playing an increasingly important role in the protection and management of the marine environment. Achievements in this field have been less substantial than expected during the last decade and most of the applications routinely used nowadays still rely on traditional methods based on chemistry and microbiology. This is mainly the result of the complexity of marine ecosystems on one hand, and the gap between results in marine genomic approaches and the development of derived commercial assays and products on the other hand. However, the potential contribution of Marine Biotechnology for environmental applications is enormous and requires urgent attention.

Securing human health and well-being

In recent years, the chemistry of natural products derived from marine organisms has become the focus of a much greater research effort. Currently there are around 15 marine natural products in various phases of clinical development, mainly in the oncology area, with more on the way and several products already on the market. Nevertheless, the seas and oceans represent a huge potential source of new drugs, innovative treatments and diagnostic tools for human health. The main challenges facing pharmaceutical discovery from marine bioresources are linked to: legal aspects (secure access to marine resources, property rights and intellectual property); quality of marine resources (identification and variability); technology (screening of active compounds and dereplication, preventing repeated rediscovery); and structural costs of drug discovery from natural products and especially marine products.

Industrial products and processes

Proteins and enzymes from marine organisms already contribute significantly to industrial biotechnology but can also support novel process development in the food and pharmaceutical industries or in molecular biology and diagnostic kits. For example, the luminescent properties of the jellyfish *Aequorea victoria* led to the characterisation of the green fluorescent protein (GFP). GFP and the luciferase enzyme from *Vibrio fischeri* have widespread applications in molecular biology as a reporter protein.

In the past decade, biopolymers of marine origin have received increasing attention from the medical, pharmaceutical and biotechnology industries for numerous applications ranging from biodegradable plastics to food additives, pharmaceutical and medical polymers, wound dressings, bio-adhesives, dental biomaterials, tissue regeneration and 3D tissue culture scaffolds.

However, marine-derived biomaterials science is still relatively new and the marine environment is, as yet, a relatively untapped resource for the discovery of new enzymes, biopolymers and biomaterials for industrial applications.

This Position Paper analyses the contributions Marine Biotechnology can make to address key societal challenges and identifies the associated future research priorities which are summarised in Executive Summary Box A.

Executive Summary Box A Marine Biotechnology research priorities to address key societal challenges	
Target research area for development	**Research priorities and objectives**
Food: Development of food products and ingredients of marine origin (algae, invertebrates, fish) with optimal nutritional properties for human health	- Develop innovative methods based on -omics and systems biology for selective breeding of aquaculture species; - Develop biotechnological applications and methods to increase sustainability of aquaculture production, including alternative preventive and therapeutic measures to enhance environmental welfare, sustainable production technologies for feed supply, and zero-waste recirculation systems; - Integration of new, low environmental impact feed ingredients to improve quality of products and human health benefits.
Energy: Development and demonstration of viable renewable energy products and processes, notably through the use of marine algae	- Produce an inventory of microalgae resources for biofuel production to support optimisation of the most appropriate strains; - Improve knowledge of basic biological functions, tools for steering the metabolism and cultivation methods of marine microalgae to improve the photosynthetic efficiency, enhance lipid productivity and obtain microalgae with optimum characteristics for mass cultivation (mixed & mono cultures), biofuel production and biorefinery; - Develop efficient harvest, separation and purification processes for micro- and macroalgae.
Health: Development of novel drugs, treatments and health and personal care products	- Increase the focus on the basic research (taxonomy, systematics, physiology, molecular genetics and chemical ecology) of marine species and organisms from unusual and extreme environments to increase chances of success in finding novel bioactives; - Improve the technical aspects of the biodiscovery pipeline, including the separation of bioactives, bio-assays that can accommodate diverse material from marine sources, dereplication strategies and structure determination methods and software; - Overcome the supply problem to provide a sustainable source of novel pharmaceutical and healthcare products through scientific advances in the fields of aquaculture, microbial and tissue culture, chemical synthesis and biosynthetic engineering.
Environment: Development of biotechnological approaches, mechanisms and applications to address key environmental issues	- Develop automated high-resolution biosensing technologies allowing *in situ* marine environmental monitoring to address coastal water quality, including prediction and detection of Harmful Algal Blooms and human health hazards; - Develop cost-effective and non-toxic antifouling technologies combining novel antifouling compounds and surface engineering; - Consolidate knowledge on DNA-based technologies for organism and population identification and support the development of commercial tools and platforms for routine analysis.
Industrial Products and Processes: Development of marine-derived molecules exploitable by industry including enzymes, biopolymers and biomaterials	- Develop enabling technologies for high throughput enzyme screening and for the expression of marine proteins and enzymes through dedicated hosts; - Produce marine biopolymers as novel competitive commercial products in food, cosmetics and health.

Drivers, barriers and enablers of Marine Biotechnology in Europe

While it is difficult to predict major innovations in life science and their future impact on society, it is clear that **developments in life science technologies have been, and will continue to be in the future, one of the key drivers of Marine Biotechnology research**. In the 1990s Marine Biotechnology developments were largely the result of the molecular biology revolution. During the last decade, the genomic revolution was clearly the primary driving force. Aside from advances in -omics, the development and optimisation of appropriate bio-engineering tools and the cultivation of microorganisms and the use of marine model organisms need to be stimulated as they are expected to have a large impact on future progress in Marine Biotechnology. Research and Development priorities associated with key marine biotechnological toolkits are presented in Executive Summary Box B.

Since the year 2000, the European Commission has been working with Member and Associated States towards development of the European Research Area (ERA), one of the goals of which is to **better integrate scientific communities and the research infrastructures they need**. Through support for marine research Networks of Excellence and other collaborative projects, EU research policy has been responsive to the growing awareness of the important role of marine biodiversity for the future of marine resources, ecosystem management, bioprospecting and Marine Biotechnology. Recent efforts to support and coordinate European coastal and marine research infrastructures to improve, amongst others, the access to research vessels, stations and laboratories also indicate some level of recognition that action is needed to fully exploit the vast but fragmented research infrastructure available for marine sciences and hence Marine Biotechnology in Europe.

Executive Summary Box B
Marine Biotechnology toolkit research priorities

Target research area for development	Research priorities and objectives
Genomics and meta-genomics, molecular biology in life sciences	- Implement genomic analyses of marine organisms, including the systematic sampling of different microorganisms (viruses, bacteria, archaea, pico and micro-plankton), algae and invertebrate taxa; - Implement metagenomic studies of aquatic microbiomes and macrobiomes.
Cultivation of marine organisms	- Develop enabling technologies for culture and isolation of uncultivated microorganisms; - Develop innovative culture methods adapted to vertebrate or invertebrate cell lines for production of active compounds.
Bio-engineering of marine micro-organims	- Optimise microalgal cultivation systems with respect to energy supply, productivity and cost; - Develop innovative photobioreactors adapted to different species of interest and production sites; - Promote research on the biorefinery approach based on microalgae production to develop a long-term alternative to petrochemistry.
Marine Model Organisms	- Identify and prioritise new marine model organisms that are still not investigated in the tree of life and which are needed to fill critical knowledge gaps; - Investigate identified marine model organism cultivation and perform genomic and chemical analysis.

We are now in a much better position to collectively address key challenges for the successful development of Marine Biotechnology. However, a strategic approach at EU level is critical to build on the progress that has already been made. The **EU currently lacks a coherent Marine Biotechnology RTD policy and needs to prepare one without delay**. As it stands, individual European countries support, to varying degrees, national Marine Biotechnology initiatives, programmes, and RTD policies and/or strategies. As a result, the European Marine Biotechnology effort is fragmented and based on national rather than common European needs and priorities. A coordinated effort is also needed at pan-European level to mobilise and optimise human resources and available infrastructures. Such efforts should address both fundamental research and advanced application-oriented research and take an approach which supports industry-academia collaboration.

A multi-disciplinary industry-academia collaborative approach will be critical for the success of European Marine Biotechnology. With a few notable exceptions, most industrial contributions to Marine Biotechnology in Europe are generated through specialised Small and Medium-sized Enterprises (SMEs). These small companies assume most of the risks inherent in RTD in a highly unstable economic environment and are characterised by a rapid turn-over. There is a danger that the current global financial crisis, coupled with reductions in available venture capital and public research funding, may reduce the capacity of Marine Biotechnology SMEs to continue to play a key role in developing new technologies products and processes. Nevertheless, efforts to involve larger, established companies should also be intensified as the technology transfer is often incomplete if they are not involved.

At the same time, **specific education and training initiatives and pathways are necessary to provide both research and industry with skilled graduates**. The future of life sciences in the 21st century is closely linked to the ability of scientists to develop and participate in interdisciplinary projects, embracing skills and concepts from other disciplines. Hence, training the next generation of marine biotechnologists must focus on the use of interdisciplinary and holistic approaches to solve technological problems specific to dealing with marine organisms and the marine environment.

An important barrier to the further development of Marine Biotechnology in Europe is linked to the **lack of identity and profile of Marine Biotechnology as a research field in its own right**. This is partly due to the broad range of disciplines and activities which contribute to Marine Biotechnology. This lack of a coherent identity in Europe is also a result of inadequate efforts to coherently communicate the needs, benefits and opportunities to the wider scientific community, to policy makers and to the public in general. There is an urgent need to communicate how marine biotechnological knowledge and applications can provide advances in, for example, industrial biotechnology, health and agriculture. In particular, there is insufficient awareness within the pharmaceutical industry of the potential for novel drug discovery based on bioactive molecules and compounds derived from marine organisms.

There is also an **urgent need to improve information exchange among those who are actively involved in European Marine Biotechnology**. Mechanisms need to be developed to mobilise and facilitate the efficient pooling of knowledge, data and research capacities distributed throughout Europe. Mobility of researchers should be encouraged at all levels. The effective dissemination of novel Marine Biotechnology research discoveries can improve greatly Europe's capacity to generate new commercial opportunities. Creating a common identity and information exchange platform will also reduce the apparent gap which currently exists between researchers and high-tech companies (notably companies from the healthcare sector).

Vision, Strategy and recommended actions

This Paper, which is the result of a collaborative effort of the members of the Marine Board Working Group on Marine Biotechnology, presents a Vision and a Strategy with a set of concrete and achievable recommendations and actions designed to support and develop European Marine Biotechnology research, enhance the European biotechnology and bioscience industries and provide a considerable contribution to the Knowledge Based Bio-Economy (KBBE). Central to the Strategy is the shared vision for European Marine Biotechnology whereby:

By 2020, an organised, integrated and globally competitive European Marine Biotechnology sector will apply, in a sustainable and ethical manner, advanced tools to provide a significant contribution towards addressing key societal challenges in the areas of food and energy security, development of novel drugs and treatments for human and animal health, and the sustainable use and management of the seas and oceans.

This 2020 Vision will only be achieved through the coordinated implementation of all of the recommendations and actions presented in this new Strategy for the future development of Marine Biotechnology in Europe. The Strategy aims to enable

the sector to much better contribute to the resolution of some of the most important social, economic, environmental and health challenges which we will encounter in the coming decade and beyond. In the context of a weakened global economy, the strategy will focus on optimising the use of marine biological resources, better coordination of research programmes at EU and national levels, and maximising the benefits for European citizens from products and services derived from Marine Biotechnology.

The strategy is designed such that its full implementation should contribute to wealth and job creation in EU Member and Associated States. It also aims to position Europe as a globally competitive leader in Marine Biotechnology research, in the advancement of associated technologies and in the development of marine derived products and services through biotechnological applications. At the same time, the strategy must provide the means to assist countries with limited access to marine resources and/or the means to valorise them. An underlying tenet of the strategy is that its recommendations must be implemented according to the principles of sustainability, ensuring the protection and preservation of coastal and marine ecosystems and their resources for future generations. In fact, Marine Biotechnology can itself better contribute to the appropriate protection, remediation and management of the marine environment.

Four recommendations with a set of specific implementation actions constitute the core of the strategy to achieve the joint vision for Marine Biotechnology in Europe. These are presented in Executive Summary Box C.

Successful implementation of the strategy will require a **joint effort with active support and involvement from all relevant stakeholders**. Europe needs to mobilise the necessary support in terms of funding, human resources and research infrastructures, and to secure the engagement of all of the relevant actors. These actors include the science community, the private sector (e.g. individual companies, associations and technology platforms) policy makers and advisors at national and European level, national strategy and programme developers and managers, and ultimately the public at large. As each actor has an important responsibility to bring forward key elements of the strategy, mobilising, in a coordinated way, this diverse range of actors will be critical.

Executive Summary Box C
Overview of recommendations and associated actions for implementation as a central component of the Strategy for European Marine Biotechnology

RECOMMENDATION 1: Create a strong identity and communication strategy to raise the profile and awareness of European Marine Biotechnology research.

Recommended Actions:

1a) Create a central European information portal which provides a one-stop-shop for state-of-the-art reports on novel discoveries and success stories, challenges and opportunities.*

1b) Conduct an audit of Marine Biotechnology effort in Europe to allow an economic evaluation of the benefits of Marine Biotechnology in Europe and facilitate the development of strong support policies.*

1c) Initiate a series of Marine Biotechnology demonstration projects that target the utilisation of marine materials in defined sectors.

1d) Develop promotional and education support materials that highlight the potential and the successes of European Marine Biotechnology research.

RECOMMENDATION 2: Stimulate the development of research strategies and programmes for Marine Biotechnology research and align these at the national, regional and pan-European level.

Recommended Actions:

2a) Create a European Marine Biotechnology Institute or Centre, at least virtual, charged with developing Europe's Marine Biotechnology research capabilities through a range of collaborative actions including establishing and operating the European Marine Biotechnology Portal (see recommendation 1a).*

2b) Develop a coherent European Marine Biotechnology RTD policy to strengthen the integration at EU level of Marine Biotechnology research and corresponding infrastructures, among others through a future Framework Programme support action or a dedicated ERA-NET.*

Executive Summary Box C

2c) Strengthen common European platforms in the field of omics research which include corresponding bioinformatics and e-infrastructures and the development of centres for systems biology and synthetic genomics, recognising that Marine Biotechnology draws from a wide range of multi-disciplinary research outputs and tools.

2d) Develop high level European Marine Biotechnology research programmes taking an industry-academia collaborative and multidisciplinary scientific approach in the thematic areas of Food, Energy, Health, Environment and Industrial Products and Processes.

RECOMMENDATION 3: Significantly improve technology transfer pathways, strengthen the basis for proactive, mutually beneficial interaction and collaboration between academic research and industry and secure access and fair and equitable benefit sharing of marine genetic resources.

Recommended Actions:

3a) Better adapt future FP financial rules and Grant Agreements to ensure SMEs are attracted to participate in a way that maximises the reward and minimises economic risks.

3b) Establish completely new mechanisms and policies to circumvent the high risk of investments in critical novel drugs developed from marine bioresources, in particular for the development of new antibiotics of marine origin.

3c) Harmonise the property rights and procedures for the protection of intellectual property for marine-derived products at European level but with a global relevance. Develop new European protocols to facilitate the publication of academic research results whilst protecting, through innovative procedures, the intellectual property on new discoveries.

3d) Develop a common European position on the simplification and harmonisation of regulations on access and fair and equitable benefit sharing from the exploitation of marine genetic resources taking into account three 'territories': (i) inside Europe; (ii) outside Europe; and (iii) international waters.

3e) Conduct a survey of industry stakeholders to guide research towards applications and processes to address current industry needs.

RECOMMENDATION 4: Improve training and education to support Marine Biotechnology in Europe.

Recommended Actions:

4a) Assure that appropriate biotechnology modules are included in all bio-science undergraduate educational programmes.

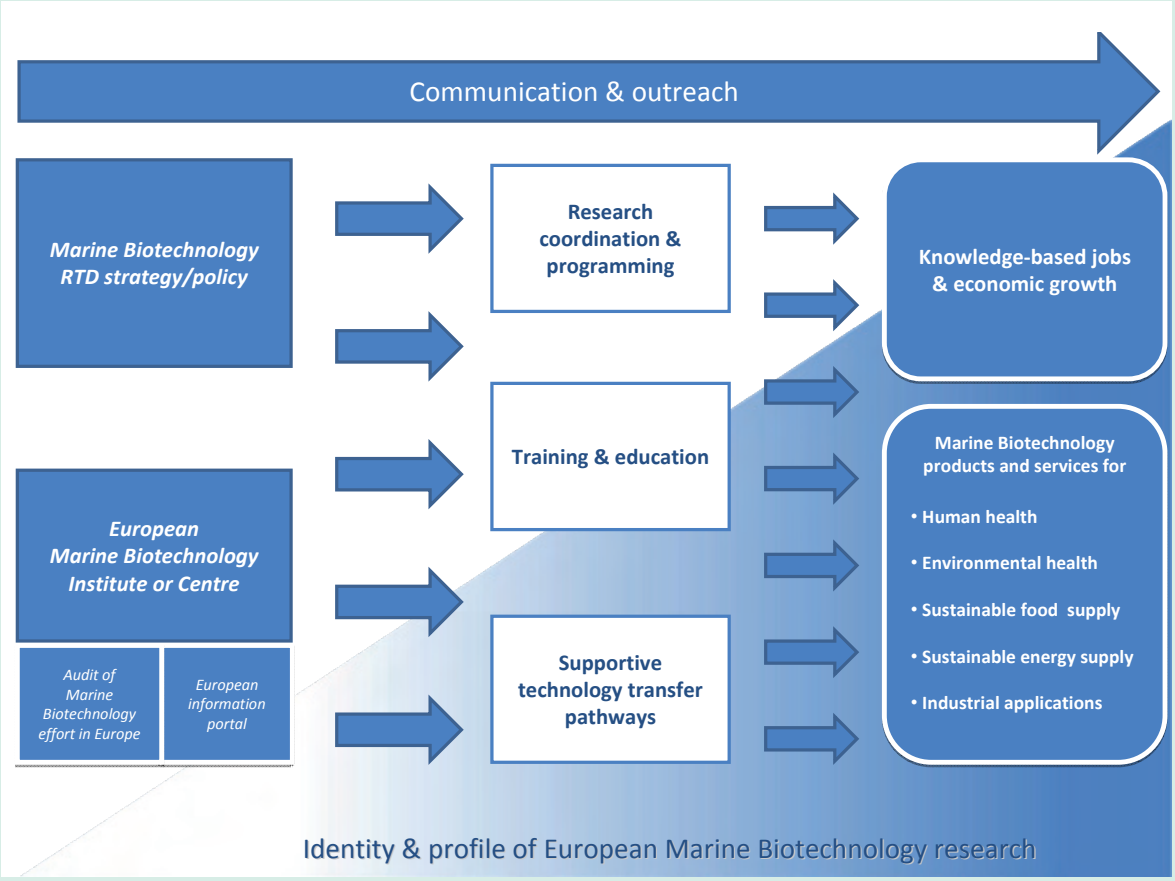
4b) Initiate actions that will ensure the participation of researchers from non-marine backgrounds in Marine Biotechnology, thus ensuring that a growing pool of exceptional research talent is available to the Marine Biotechnology sector.

4c) Organise regular trainings or summer schools on Marine Biotechnology subjects supported, for example, by the EU Framework Programme.

4d) Create a European School or Course on Marine Biotechnology (virtual and distributed) and a European PhD programme on Marine Biotechnology both of which need to include business and entrepreneurship training as standard.

*Actions which should be implemented without delay

Executive Summary Box D
Flow-chart of recommended priority actions for immediate implementation and their expected impact



Some of the recommended actions provide a structural basis for realisation of the strategy and should be prioritised for early implementation. These are highlighted (with *) in Executive Summary Box C and presented in a flow-chart in Executive Summary Box D. Once up and running, these activities will act as a catalyst to drive implementation of the other recommended actions that make up the strategy. For example, a European Marine Biotechnology Institute or Centre could develop a roadmap for implementation of the strategy, coordinate its implementation and mobilise the relevant actors. A Framework Programme support action or ERA-NET, bringing together national funding organisations which support Marine Biotechnology research, will play a key role in aligning existing programmes, coordinating investments and informing the development of new research programmes and initiatives.

There is now a strong momentum to drive progress in European Marine Biotechnology in the coming decade. If Europe does not act now through a concerted effort by all of the identified actors and stakeholders and through increasing its support with targeted funding and coordinated research, it will begin to lose ground on other global leaders in this field such as the USA, Japan and China. The successful implementation of the integrated strategy presented in this Marine Board Position Paper has the potential, not only to significantly advance European research in Marine Biotechnology, but, in turn, to contribute significantly towards the development of knowledge-based jobs and smart economic growth, and to create innovative solutions to meet critical societal challenges in the areas of food, environment, energy and health in the coming decade and beyond.

1. Introduction

Biotechnology is of growing importance for the European Union and will increasingly contribute to shape the future of our societies. The rapid rate of progress in the life sciences makes it difficult to predict our future capabilities and their potential impacts on our knowledge and in some cases our economies. Nonetheless, it remains crucial to analyse the limits of previous RTD policies both at European and national level, and to formulate recommendations for future research priorities and supporting policies in order to enhance the competitiveness of European countries and to improve the social benefits of their inhabitants. This Position Paper attempts to address these questions specifically focusing on Marine Biotechnology (see Information Box 1 and Figure 1).

Information Box 1.
What is Marine Biotechnology?

Biotechnology, and in turn, 'Marine Biotechnology', mean different things to different people. A very broad and simple definition of biotechnology is 'the application of biological knowledge and techniques to develop products and other benefits for humans'. As such, the definition covers all modern biotechnology but also many more production related and traditional borderline activities used in agriculture, food and beverage production (e.g. cheese and beer). Nowadays, biotechnology is more often considered in terms of cutting-edge molecular or genomic biological applications where molecular or genetic material is manipulated to generate desirable products or other benefits.

What we consider as biotechnology, therefore, largely depends on what techniques we include and this is linked, in turn, to what we wish to address. This is illustrated by the varying definitions for biotechnology used by different organisations. For example, in a single provisional and deliberately broad definition, the Organisation for Economic Co-operation and Development (OECD) defines biotechnology as 'The application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services'. This broad definition includes both modern and more traditional techniques and, for that reason, the definition comes with a non-exhaustive list of biotechnology techniques which functions as an interpretative guideline to the overarching definition and which is considered to evolve over time.

Marine Biotechnology encompasses those efforts that involve marine bioresources, either as the source or the target of biotechnology applications. In many cases this means that the living organisms which are used to develop products or services are derived from marine sources. At the same time, if terrestrial organisms are used to develop a biosensor which is used in the marine environment to assess the ecosystem health then it also falls within the sphere of Marine Biotechnology.

A useful website which provides general information on Marine Biotechnology and a wide range of examples is www.marinebiotech.org.

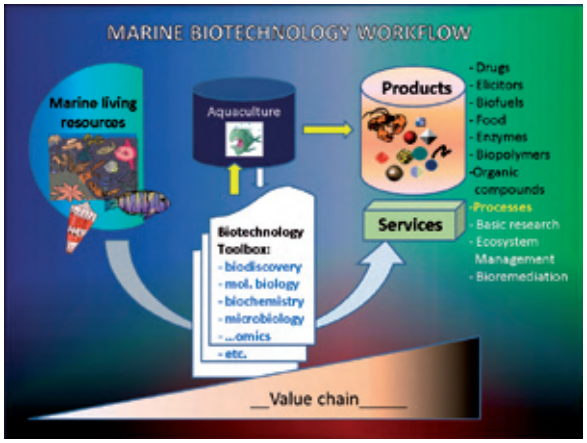


Figure 1. Marine Biotechnology Workflow. Marine Biotechnology is part of global biotechnology and its specificity lies in the uniqueness of marine living resources and their derived products and services through the use of a set of tools ranging from biodiversity assessment to systems biology, from cultures to engineering.

In recent years there has been a rapid increase in the inventory of marine natural products and genes of commercial interest derived from bioprospecting efforts. The rapid growth in the human appropriation of marine genetic resources (MGRs) with over 18,000 natural products and 4,900 patents associated with genes of marine organisms, the latter growing at 12% per year, illustrates that the use of marine bioresources for biotechnological applications is no longer a vision but a growing source of business opportunities[2].

While it is difficult to predict major innovations in life science and their future impact on society, a crystal

2. From Arrieta J., Arnaud-Haond S. and Duarte C. Marine Reserves Special Feature: What lies underneath: Conserving the oceans' genetic resources. PNAS 2010

ball is not required to foresee the importance of the ongoing omics revolution for biotechnology, and by extension, for Marine Biotechnology. For that reason, **Chapter 2** of this Position Paper opens with one of the key drivers of Marine Biotechnology research: life science technologies, including developments in the field of omics, cultivation of marine living resources and bio-engineering. We expect that this chapter will contribute to highlight possible developments, evolutions and changes for each of the Marine Biotechnology domains.

The seas and oceans represent a unique environment with the potential to contribute enormously to the sustainable supply of food, energy, biomaterials and to environmental and human health. Marine Biotechnology is now, and will become even more, central to delivering these benefits from the sea. It is appropriate then that **Chapter 3** provides a logical analysis of the achievements and the current and possible future development of Marine Biotechnology set against its capacity to deliver products and processes to address these high-level societal needs and opportunities.

The **sustainable supply of high quality and healthy food** is a fundamental and recurrent issue and was considered so strategically important by the EU founders that it led to the early introduction of dedicated Common Policies in the fields of Agricultural and Fisheries. Marine Biotechnology can contribute to the maintenance and improvement of food quality, can support sustainable production of aquaculture products or other marine biomass feedstocks and help to provide viable sources of food in developing countries. The role of Marine Biotechnology in addressing food safety and supply, including its past and potential future applications, is considered in **Section 3.1** of this Position Paper.

While there might be controversy over the current rates and impacts of climate change and the respective contributions of greenhouse gases and other factors, it is beyond doubt that the use of fossil fuels will have to be reconsidered within the next decades owing to limited reserves and increasing costs. Already the race is on to find viable and **sustainable alternative sources of energy**. It is becoming increasingly recognised that Marine Biotechnology could provide a potentially major contribution to the production of bioenergy, either by providing novel biocatalysts for second generation biofuels, or directly by producing algae to build up a third generation of biofuels. The development of marine bio-energy as a viable and renewable energy source is clearly in its infancy, but given the impending energy crisis, there is an urgent need to ensure that all necessary building blocks and support mechanisms are in place to fast-track marine bio-energy research (**Section 3.2**).

It is hardly surprising that **human health** has traditionally been one of the best supported fields of research. With our rapidly changing societies and environments, there are always new challenges to add to the list of issues which endanger the health and well-being of our growing populations. Among many acute problems, the increasing development of antibiotic resistance combined with a lack of novel antibiotic families raises major concerns. Terrestrial ecosystems have long provided most of the natural products used to generate drugs and to serve as templates for combinatorial chemistry to design novel drugs. In the meantime, marine environments and marine living resources have largely been ignored. With appropriate supporting policies and research investment, marine resources and Marine Biotechnology can and should contribute significantly to address human health concerns in the future (**See Section 3.3**).

One other major trend is the ongoing global migration of populations to coastal regions. This is generating significant pressures on fragile marine ecosystems located close to major coastal population centres which receive the by-products of increasing human activities. Again, marine biotechnological solutions might help to deal with and mitigate against human-induced environmental degradation through the development of novel products and services. The potential contribution of Marine Biotechnology to monitor and protect the **environmental health** of our oceans and seas is discussed in **Section 3.4** of this paper.

Finally, marine living resources provide a huge and almost untapped reservoir of genes, organisms, and various products which may present unique **solutions for industrial and biotechnological applications**. Preliminary research has provided evidence that products derived from some marine living resources can be used to generate innovative biomaterials as discussed in **Section 3.5** of this report.

Then, in **Chapter 4**, we discuss important additional support mechanisms and needs for the development of Marine Biotechnology and, more specifically, the issue of access to marine resources and common infrastructures.

From chapters two to four it will become clear that Europe urgently needs to implement a sound strategy for development of Marine Biotechnology research in Europe to allow for its full potential to be realised. The Position Paper therefore concludes in **Chapter 5** by presenting a common vision for the future development and impact of Marine Biotechnology in Europe and a strategy, with concrete recommendations, to deliver this vision by 2020. To guide further Marine Biotechnology research in Europe, the chapter also provides a

Figure 2. Sirens Reef Natural Park of Cabo de Gata Nijar in Almería (Spain). The marine environment presents a vast and largely unexplored source of bioresources for biotechnology applications.

summary of research priorities for each of the strategic areas discussed in the Position Paper.

This Position Paper is based on the activities of the Marine Board Working Group on Marine Biotechnology which convened in Brussels on 22 September 2009 and on 18-19 March 2010. The preliminary conclusions were presented and discussed during the Marine Board-ESF-COST High Level Research Conference on Marine Biotechnology[3] (20-24 June 2010, Acquafredda di Maratea, Italy) which provided additional insight on the future challenges and research priorities for European Marine Biotechnology research which are taken into account in this document.

3. Information and outputs of the Marine Board-ESF-COST High Level Research Conference on Marine Biotechnology (20-24 June 2010, Acquafredda di Maratea, Italy) are available on the Marine Board website http://www.esf.org/marineboard/.

2. Developments and perspectives of key tools and technologies

The life sciences, and specifically synthetic biology, promise to engineer organisms for the benefit of humanity with potential applications in medicine, agriculture, industry and environmental management. However, these promises cannot obscure the fact that synthetic biology may change the human relationship with nature. Public debate and dedicated ethics committees should establish clear limits to its use, which must be anticipated now whilst synthetic biology is still in its formative stages. This Chapter provides a brief presentation of those technologies which are expected to have the largest impact on future progress.

Courtesy Mike Thorndyke

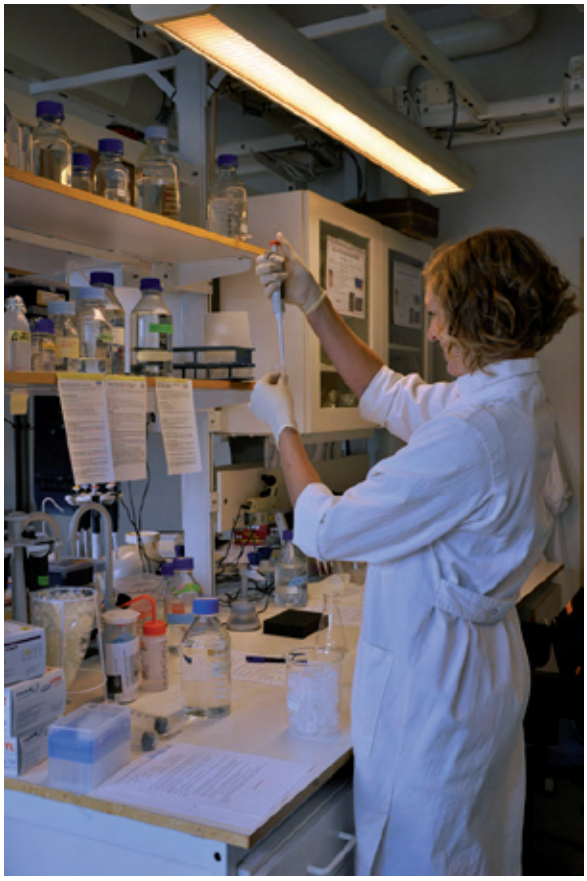


Figure 3. Marine scientist preparing samples in a molecular biology laboratory

2.1 'Omics' driven technologies

In the mid 1990s, the 'omics' revolution started to change biology and its application in biotechnology. Omics focus on a large-scale, holistic approach to understand life in encapsulated omes such as the genome, transcriptome, proteome, metabolome, etc. ('ome' stems from Greek for 'all', 'whole' or 'complete'). This view, supported by informatics and the internet, had a strong influence on all life sciences and provided an efficient means to integrate and understand complex biological knowledge and systems.

2.1.1 Genomics of marine organisms

Central to the understanding of the biotechnological potential of marine organisms is the assessment of their genetic capabilities, i.e. sequencing of their genome and annotation of the genes. This understanding is the focus of genomics. Currently, about 1000 prokaryotic genomes have been sequenced and annotated. More than half of these genomes are of medical or industrial relevance and no phylogenetically systematic genome sequencing has been carried out until recently. Sequencing of phylogenetically diverse microbial genomes still results in the discovery of many novel proteins per genome and the trend is linear, demonstrating the existence of a huge reservoir of undiscovered proteins. Given that about 7500 bacterial species have been validly described, it follows that still hundreds of thousands of new proteins will be discovered by sequencing, in a systematic manner, all cultured bacterial species. Another level of diversity has to be expected from the uncultured prokaryotes which make up about 70% of the more than 100 bacterial phyla. This uncultured diversity became apparent when the first whole genome analysis of marine microbial communities revealed as many new clusters of ortholog groups (COGs) as were already known at the time (2004). On the other end of the phylogenetic diversity, i.e. comparing different strains of a bacterial species, it is becoming clear that each new strain can add hundreds of new genes. This means that, the pan-genome of a microbial species, comprising all genes of all strains of that species, is several times larger again than the core genome.

In addition to bacteria, aquatic ecosystems contain viruses which are the most common biological entities in the marine environment. The abundance of viruses exceeds that of prokaryotes at least by factor of ten and they have an enormous impact on the other microbiota, lysing about 20% of its biomass each day. Recent metagenomic surveys of marine viruses demonstrated their unique gene pool and molecular architecture. Their host range covers all major groups of marine organisms from archaea to mammals. Metagenome-

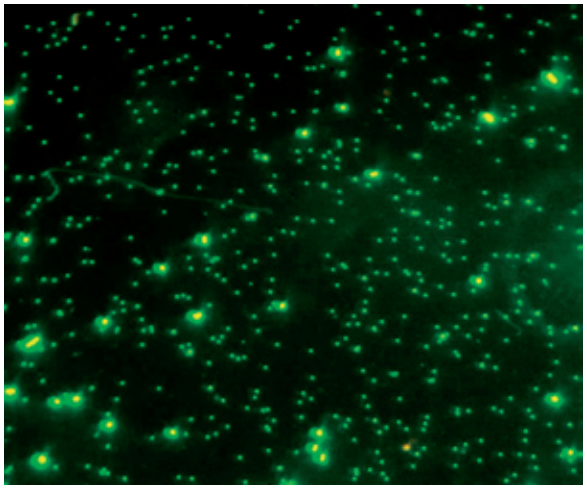


Figure 4. Epifluorescence micrograph of prokaryotes and viruses in a seawater sample stained with a fluorescent dye, SYBR Green I. The dye specifically stains doubled-stranded DNA (dsDNA). Smallest dots are viruses and larger ones are prokaryotes (bacteria or archaea). With about 1 billion bacterial cells and 10 billion viral particles per liter of seawater, viruses are by far the most common biological entities in the marine environment.

based estimates of the marine viral diversity indicate that hundreds of thousands of different species exist with genes completely different from any other form of life. Therefore, marine viruses are an untapped genetic resource of truly marine character and could provide novel proteins, genetic tools and unexpected functions.

In contrast to prokaryotes, the era of the genomics of marine eukaryotes, comprising microalgae, macroalgae (seaweeds) and protozoa, has just begun. The slower progress is a result of their large genome size and high cellular complexity. This group of mainly aquatic organisms is very old, highly diverse and taxonomically still vaguely defined. Currently not much more than 30 microalgal genomes have been completed, ranging from 12 to 165 Mb in size. Algal genome sizes can even vary about 20 fold within a genus, as illustrated with *Thalassiosira* species. The overall size range for microalgal genomes is 10 Mb to 20 Gb, with an average size of around 450 Mb, except for *Chlorophyta*, that are on the average four times larger. Many marine microalgae are highly complex single celled organisms containing chromosomal DNA as well as mitochondrial and chloroplast DNA. They have a complex nucleus that has been subjected to extensive exchange of genes between the organelles and the nucleus (endosymbiotic gene transfer) as well as horizontal gene transfer during their hundred millions years of evolution. In addition, the first genome of a macroalgae (*Ectocarpus*) has been sequenced and several others are being completed. The challenge here will be to analyse this novel 'terra incognita' through post-genomics, biochemical approaches and genetic developments. The reward for taking on this challenge is an improved understanding of the biochemical functioning of key players in aquatic ecosystems with new insights into the regulatory genetic network of eukaryotes and their early evolution, and moreover, with great potential for the production of a huge variety of bioproducts.

For protozoan genomics the situation is even more difficult because of their extremely diverse phylogeny, their complex life cycles and their even larger range of genome sizes than for microalgae. Protozoan genomes range from 8 Mb to 1400 Gb for *Chaos chaos* which is a free-living amoeba with the largest genome reported to date. The accuracy of the measurements of these very large genomes is questionable and complicated by the highly polyploid nature of many protozoan genomes that can also contain hundreds of small chromosomes. Overall, this complexity and diversity illustrate the basic research problems of protozoan genomics and explain the low number of completed protozoan genomes (25 genomes, most of them of medical relevance).

The study of metazoan genomes is highly biased towards vertebrates, especially mammals, due to their medical and economic relevance. Marine invertebrates, ranging from sponges to crustaceans, comprise only 11% of the currently planned sequence analyses of metazoan genomes, despite their substantially larger phylogenetic diversity. Only a few commercially relevant marine invertebrates such as mussels and oysters have

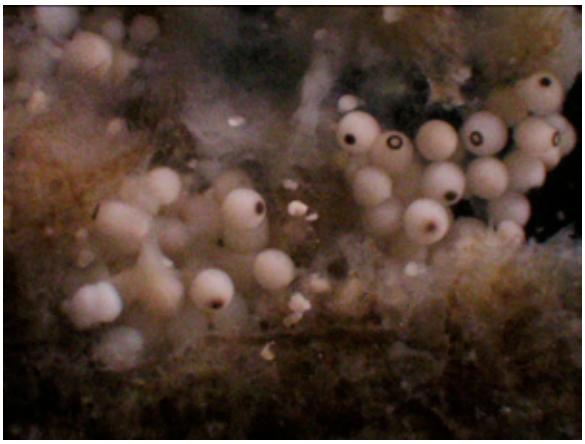


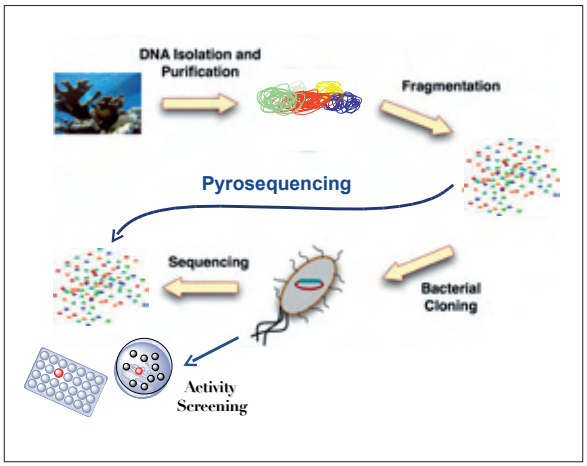
Figure 5. *Amphimedon queenslandica* is a demosponge native to the Great Barrier Reef which has been the subject of various studies on the evolution of metazoan development. In landmark effort its genome has recently been sequenced.

been sequenced, largely because of their importance as aquaculture species. Teleost fishes have, on average, a genome size of around 1 Gb. Interestingly, lungfish have a much larger genome, ranging from 50 Gb to 130 Gb with the marbled lungfish (*Protopterus aethiopicus*) having the largest genome of all animals. Only a very few teleost fish genomes have been completed, such as *Takifugu rubripes* and the zebrafish (*Danio rerio*), which are of interest to fisheries and developmental biology, respectively.

For prokaryotes, the size of the genome is a very good indicator of its gene content and thereby its biotechnological potential. This correlation vanishes for eukaryotes for several reasons: (i) basic molecular genetics are very different and substantially more complex (exons, introns, splicing), (ii) highly complex RNA infrastructure (small and long non-coding RNAs, RNA interference, RNA editing, etc.), (iii) large amounts of non-coding DNA (can be more than a hundred fold of the coding DNA), (iv) polyploidy, and (v) epigenetics. How this complexity has evolved and how it is changing for the major taxa is far from understood. This knowledge gap has major implications for the use of higher organisms for biotechnological purposes. Some of these important consequences are: (i) eukaryotic genome projects will take longer and demand more resources to complete annotation, (ii) genetic engineering opportunities are very different according to the species, and (iii) transcriptomics and proteomics are very complex and cannot be used easily to understand the relationship between phenotype and genotype. Overall, these major differences present a difficult challenge for using 'omics' approaches on a large scale for higher marine organism for the benefit of biotechnology.

2.1.2 Metagenomics of marine communities

Metagenomics, comprising the analysis of all genes of a given community of organisms, is even younger than the 'omics' revolution, with the first successful study published in 2001. Metagenomics only became technically possible through the availability of Bacterial Artificial Chromosomes (BACs) and the possibility to clone and sequence long stretches of environmental DNA. Metagenomics works like a shotgun by taking all the genes of a community apart by complete DNA extraction and putting these genes in large clone libraries to make them available for later use in biotechnological applications. The first metagenomic studies concentrated on bacterioplankton which can easily be separated from higher organisms by filtration. Current metagenome studies target all domains of life and a broad range of environments. Meta-transcriptomics and meta-proteomics have been successfully applied



Courtesy Oded Beja

Figure 6. Schematic overview of the metagenomics process

to bacterioplankton providing exciting insights into the functioning of microbial communities. However, these approaches lack broader application owing to their complexity and are of limited value for biotechnological exploitation.

A biological bottleneck for exploitation of newly discovered genes from marine genome and metagenome projects is the heterologous expression of recombinant proteins in well characterised biotechnological workhorses like *Escherichia coli* or *Bacillus subtilis*. Innovative molecular approaches are needed whereby enzymes or secondary metabolites, useful for biotechnology, can be obtained directly from targeted marine systems. In addition, it has become apparent that two technical bottlenecks can impede metagenomic studies: (i) massive sequencing is needed; and (ii) massive computing capacity is essential. The first bottleneck has been overcome with the development of deep and ultra deep sequencing technology (see below). The second bottleneck, however, is becoming even more problematic because of the enormous amount of sequence data generated and the need for massive parallel data processing capability.

2.1.3 Deep sequencing

About five years ago, a set of new sequencing technologies reached the market (referred to as second-generation sequencing) enabling 10 to 100 times faster — and thereby substantially cheaper — automated sequencing of nucleic acids. These technologies, allowing so-called 'deep' sequencing, were based on sequencing by synthesis, also called pyrosequencing, and advanced opto-electronics. Currently, depending on the specific technology used, these new sequencing

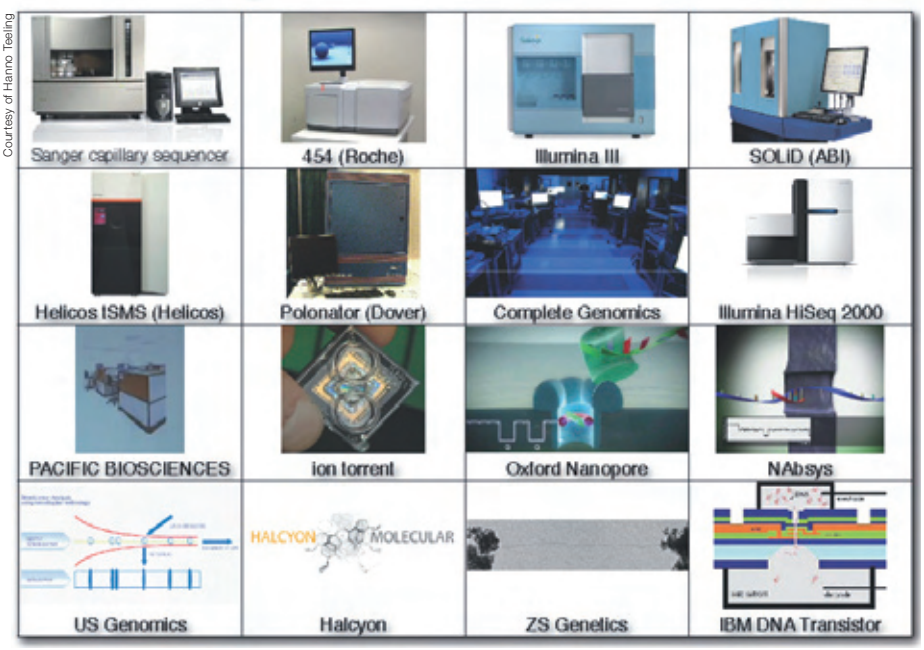


Figure 7. (R)Evolution in Sequencing Technology

technologies provide read length of 50 to 450 nucleotides and generate 20 to 200 Mb of raw sequence data per run. They enable *de novo* sequencing of genomes as well as re-sequencing of individual genomes of the same species at a price that is about 100 times cheaper than the classical Sanger-based, automated sequencer. It is expected that the next (third) generation of sequencing technology (nanopore) will add, probably during the next five years, another order of magnitude in terms of speed and reduction of price. It is expected that these ultra-deep sequencing technologies will enable single DNA molecule sequencing with read length in the kilo base pair (kbp) range, thereby eliminating gene amplification bias and providing improved data for metagenome assembly. However, the rate at which new tools and instruments become available is not always in line with the ability of laboratories and researchers to learn and use them and the outputs produced are not always comparable.

The application of more and more genomic and metagenomic analyses and deep sequencing will generate large datasets from marine environments. Bioinformatics resources and tools have been developed in an attempt to maximise the capacity to analyse these vast datasets. This so-called e-infrastructure (equivalent to 'cyber-infrastructure' which is the term used in the United States) has to support advanced data acquisition, data storage, data management, data integration, data mining, data visualisation and other computing and information processing services over the Internet.

The provision of dedicated web-based resources and e-infrastructures is essential for advanced research in marine ecology and biotechnology. At the same time, there is a growing need to interpret the sequence data via laboratory biochemical studies.

Summary Box 1. Recommendations for marine genomics research

The screening of marine genomes with molecular tools must be intensified to fully capitalise on the novel genes, proteins, enzymes and small molecules found in marine macro and microorganisms. This requires:

- Genomic analyses of marine organisms, including the systematic sampling of different microorganisms (viruses, bacteria, archaea, pico and micro-plankton), algae and invertebrate taxa;
- Metagenomic studies of aquatic microbiomes and macrobiomes;
- Establishment of integrated databases for marine organisms and communities;
- The development of bioinformatics resources and e-infrastructures;
- Relevant annotations for marine specific genes through the use of biochemical techniques.

2.2 Metabolic engineering and systems biology

Knowledge of metabolic pathways and their link with genomics and other omics aspects of marine organisms are an important basis for the production of unique compounds. However, the productivity (the amount of product produced per volume of culture over time) of the original organisms is often much too low to make commercial production possible. In many cases it is necessary to increase productivity in the marine organism or to introduce the metabolic pathways into a new host organism that can be grown much more easily.

Metabolic engineering is defined as the optimisation of genetic and regulatory pathways to increase the production of certain compounds by cells. Many techniques for this purpose have been developed for prokaryotic systems and need to be developed further for eukaryotic systems.

Better processes can be developed if the right targets for metabolic engineering are properly chosen. The target of metabolic engineering will always be determined by the biochemical bottlenecks in the process and the economic limitations of the individual steps in the production chain. Various modelling approaches can be used to identify these bottlenecks, including mathematical models, metabolic flux models and process design models (see also Section 2.4.3). For example, there is currently a strong focus on lipid production by microalgae for biofuel applications. It is generally assumed that the process will be improved if the lipid productivity is increased. However, in most microalgae the cell wall is so thick that extraction of the lipids is actually the bottleneck in the process. In this case, the goal of metabolic engineering should be to reduce the thickness of the cell wall instead of increasing the productivity of lipids. Thus models help identifying interesting targets to be addressed by metabolic engineering.

The application of engineered cells produced in contained systems could certainly improve the prospects for commercial production of certain bioactive compounds for medicines, reduce the cost price for production of food ingredients or make the production of energy ingredients more sustainable. Engineered organisms are expected to become more commonly used in the future but the biosafety and consumer acceptance aspects will need to be taken into account.

Systems biology is an emergent field that aims at system-level understanding of biological systems. In systems biology organisms are studied as an integrated and interacting network of genes, where these interactions determine the functions of an organism. Systems biology studies this network largely on mathematical tools to understand gene function relationships.

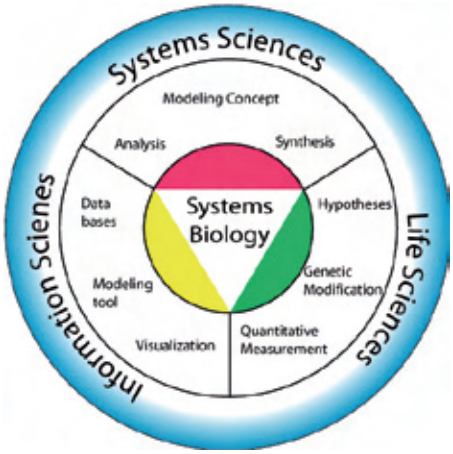


Figure 8. Systems biology is the study of an organism, viewed as an integrated and interacting network of genes, proteins and biochemical reactions which give rise to life. Instead of analysing individual components or aspects of the organism, such as sugar metabolism or a cell nucleus, systems biologists focus on all the components and the interactions among them, all as part of one system.

System-level understanding has been a long standing goal in the biological sciences. In the early days of molecular biology, only phenomenological analysis was possible and it is only recently that system-level analysis can be grounded on discoveries at molecular-level. With the progress of genome sequencing and a range of other molecular biology projects that accumulate in-depth knowledge of the molecular nature of biological systems, we are now at the stage where a system-level understanding based on a sound molecular-level understanding, is possible.

2.3 Cultivating the uncultured

During the last decade it became more and more evident that many bioactive molecules are produced by unknown and uncultivated microorganisms (the so-called dark matter), or microorganisms associated with invertebrates, often through symbiosis. Metagenomic approaches can sometimes give a direct access to the gene(s) of interest, but in many cases, it is still necessary to culture the organisms to produce enough bioactive compounds for further detailed characterisation. In some cases, culture techniques for marine organisms are similar to the general culture techniques used in

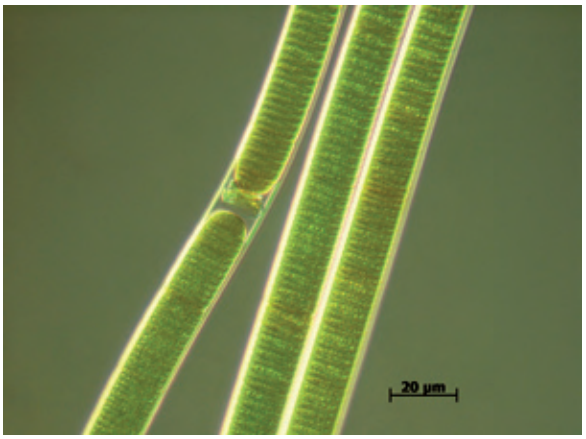


Figure 9. Micrograph of *Lyngbya*, a benthic marine filamentous cyanobacterium forming microbial mats in coastal areas which is known for producing many bioactive compounds

biotechnology. However, marine environments induce specific culture requirements for most marine organisms. This section will address the technical challenges associated with cultures: how can we (i) access the marine microbial dark matter through cultures; and (ii) improve the cultivation of microbial marine invertebrate symbionts and cell lines of marine invertebrates?

2.3.1 Access to the uncultured marine microbial majority

To date, it has been practically impossible to grow on a synthetic medium more than a minute fraction of the global diversity present in any crude sample. This phenomenon, one of the oldest problems of microbiology, is known as 'the great plate count anomaly' has erroneously been perceived as being of minor importance since the emergence of molecular

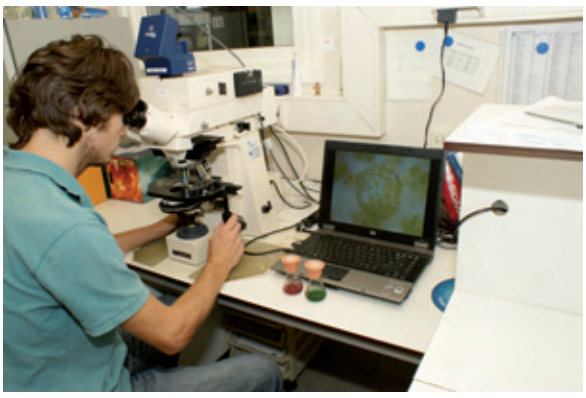


Figure 10. Analysing cultures of marine microorganisms in the laboratory

environmental microbiology and more recently the advent of metagenomics. The result is an exponentially growing amount of microbial sequences, most of them unrelated to cultivated microorganisms. The gap for prokaryotes (bacteria and archaea) is increasing fast. While in 1987 much of our knowledge derived from pure culture techniques with cultured representatives of all the known phyla, twenty years later only 30 of the 100 bacterial phyla identified possess a cultivated representative. With a doubling of sequencing efficiency every 12 months versus a linear trend in isolation of novel prokaryotic species there is no sign of improvement. Molecular biology and metagenomics opened the lid of the microbial diversity box and provided an efficient access to the corresponding genetic diversity. They contributed to shape our evaluation of the importance of the 'dark matter' or the uncultivated majority of prokaryotes, not just from marine environments but from all parts of the biosphere. Access to the gene resources is a first step. A second one is to gain access to the uncultured majority through innovative culture methods.

Why is it so important to improve the number and diversity of cultivated microbes?

Firstly, while the output of meta-omics are of high interest for data mining, they currently have their own limits: sequence errors, length of reads and subsequent assembly limitations, gene fragmentation, high frequency of hypothetical genes, and the difficulty of relating gene resources to complex products other than proteins and enzymes. In the case of drug research it is also difficult to identify and isolate the 'host' organisms to demonstrate their absence of pathogenicity. Secondly, metagenomics and other meta-omics approaches are as yet of little help to unveil and to characterise the interactions between organisms and the complex networks that control population dynamics, especially when threshold phenomena are involved or when viruses play key roles in ecosystem regulation. The discovery of novel signalling compounds still relies on the ability to control cultivation. Finally, prokaryotic and picoeukaryotic strain collections either in private collections or in public BRCs (Biological Resource Centres) are the cornerstone of marine cellular biodiversity research and conservation. DNA and genomes cannot replace culturable cells, at least not yet. And if synthetic genomics fulfils its promise, it will likely remain cheaper for some time to isolate, to culture and then to curate a new strain than to produce it through synthetic genomics.

Why does it remain so difficult to improve microbial cultivation efficiency?

At present, there seems to be no solution to solve the problem of microbial cultivation other than tedious and time consuming work at the bench in the microbiology laboratory. The slow progress can be mainly attributed to the low priority given to research in this supposedly old-fashioned field. More specific interdependent reasons that could explain the failure to grow many prokaryotes by classical approaches include:

- Fundamental lack of knowledge. Most microbes are not amenable to culture using classical approaches probably because of our insufficient knowledge of (i) the organisms themselves; (ii) the chemistry of their natural habitats; (iii) the natural biotic and abiotic interactions; and (iv) the global functioning of their ecosystems at microbial level;
- Lack of patience (partly because of the pressure to publish results) and a lack of sensitive detection methods for low cell yields;
- Most *in vitro* cultivation techniques aim paradoxically at isolating strains in pure culture, while most organisms in nature live in community and establish complex relationships including communication and cooperation. Thus the very first stage of isolation results in a break in intraspecies communication, and the disruption of all interspecific interactions. In practice, the social life of microbes has largely been underestimated and could be the key to developing techniques to cultivate many of them. It could also be an invaluable source of novel signalling compounds potentially interesting for biotechnology;
- During the enrichment-isolation process the abiotic interactions are most of the time broken off. This suggests again that a better understanding of marine chemical ecology must be developed.

The same factors explain the difficulties associated with the cultivation of prokaryote and eukaryote microorganisms. To improve the cultivation efficiency of unknown microbes, the following conditions need to be satisfied:

- A radical change in isolation rates and a substantial increase in the use of medium or high throughput based approaches in cultures and isolation procedures;
- An unprecedented effort towards gaining a better understanding of the various types of cell-to-cell communication in the microbial world and, more generally, of the social life of microbes; and
- The development of innovations enabling the combination of optimised methods, specific devices and robotics.

Summary Box 2. Research priorities to improve the cultivation efficiency of unknown microbes

To improve the cultivation efficiency of unknown microbes, future research priorities should include:

- Extraction of relevant metabolic information from genomic data and the use of molecular data to trace the cells of interest among community cultures;
- Improvement in the detection of cultures at low and very low densities;
- Refinement of culture media with additional information from metagenomics and knowledge of chemical ecology;
- Mimicking nature through *in situ* cultivation systems;
- Design of devices enhancing cell-to-cell communication;
- Development of automated procedures through robotics in combination with different approaches;
- Development of rapid identification methods for efficient dereplication and selection of novel strains and species.

Figure 11. Preparing, maintaining and analysing cultures in the marine microbiology laboratory is tedious and time consuming.

Cultivation of microbial marine invertebrate symbionts

Marine invertebrates are the richest source of newly discovered bioactive metabolites. In addition, many marine invertebrates host a large variety of symbiotic bacteria, archaea and other microorganisms. Therefore, it was not surprising that many bioactive compounds that were previously ascribed to the host are actually produced by microbial symbionts. For example, halichondrin B and discodermolide are among the most promising anti-tumour molecules that have (to date) been discovered in sponges. Other potent marine invertebrate-derived compounds with anti-tumour or potentially anti Alzheimer's disease activity are the tunicate-derived ecteinascidin 743 and bryozoan-derived bryostatin-1. Halichondrin B, discodermolide and bryostatin-1 are type I polyketides, metabolites that are mostly associated with bacterial metabolism. For bryostatin-1 it has been confirmed that it is produced by an uncultured gammaproteobacterial endosymbiont of the bryozoan. None of these compounds could be obtained by cultivation of marine invertebrate-associated bacteria.

The unculturability of the producers of bioactive compounds confirms the general unculturability of marine invertebrate-associated bacteria. New cultivation approaches are necessary to overcome this hurdle as cultivation will remain an important technique in the era of genomic analysis. Cultivation will give access to 'clean' genomes from environmental samples and, in addition, allow initial production of complex secondary metabolites that are found in marine invertebrates (and cannot easily be expressed in a heterologous host). New approaches that have to date only scarcely been employed are co-cultivation of host and symbionts. They could be cultivated for example in 'together but apart' systems, such as diffusion chambers. Co-cultivations can be seen as an intermediate step between the natural environment and pure culture.

2.3.2 Cell cultures of sponges and sponge cells

Marine sponges are a rich source of bioactive compounds. In some cases, sponge symbionts are responsible for production of these compounds and in other cases it is the sponge itself which produces the compound.

A number of avenues for the supply of bioactive compounds can be explored. Harvesting the producing species and extracting the active compound is seldom sustainable owing to variability in yield with location, season and biological conditions, and such an approach is also deemed ecologically unsound. Moreover, using

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Figure 12. Marine sponge *Amphilectus fucorum*

aquaculture to produce raw material is, in most cases, uneconomical. For these reasons synthesis or semi-synthesis could be a better approach. However, given the complexity of the molecules involved, most chemical synthesis approaches, if viable, would require a large number of synthetic steps. The consequence is that, in most cases, chemical synthesis is impractical and unviable in terms of chemical yield. Hence, more efforts are needed to understand the metabolism that is involved in the biosynthesis of the required compound.

Ideally we would like to produce the bioactive compounds in immortalised continuous cell lines. Immortalised continuous sponge cell lines are not yet available. Animal cell lines from insects and mammals usually are transformed cells that have an unlimited capacity to proliferate (immortal). For mammals, transformed cells can be obtained from tumour tissue or induced artificially by, for example, hybridisation of normal cells with other transformed cells (e.g. hybridomas), by subjecting the cells to mutagenic agents such as carcinogenic compounds, viruses or radioactivity, or by transfecting the cells with oncogenes. Sometimes, immortal cells evolve spontaneously by mutation of normal cells growing in rich media. So far, no reports on successful immortalisation of sponge cells have been published.

It would appear that sponges are very dynamic organisms with a very slow net growth that is the result of fast division of cells and a high rate of apoptosis. For the development of continuous growing cell lines it will be necessary to exploit the strong capability of sponge cells to divide and to prevent cells from apoptosis. More information is now becoming available on this subject from amongst others research on the demosponge *Amphimedon queenslandica* (see Figure 5).

Summary Box 3. Recommendations to address microbial cultivation challenges

- Recognise that the microbial cultivation challenge is critical for the future of marine microbiology, microbial ecology and microbial biotechnology and actively support the development of innovations in this field;
- Promote basic research in the field of marine microbial ecology in order to understand and access compounds and mechanisms which regulate intraspecies and interspecies cellular communication which might, in turn, lead to new discoveries and possibly to novel antibiotics;
- Develop innovative culture methods for symbionts producing active compounds and cell line cultures of invertebrates of biotechnological interest.

2.4 Technological advances in bio-engineering beneficial to the development of Marine Biotechnology

2.4.1 Culture of microalgae and use of photobioreactors

A huge variety of cultivation systems have been developed for microalgae but the most important are based on the use of open raceway ponds and photobioreactors. The only one which has been used on a large scale and a commercial basis is the shallow open raceway pond. These ponds are usually no more than 30 cm deep and the water containing nutrients and microalgae is circulated by a paddle wheel. CO_2 or CO_2-containing exhaust or flue gases can be sparged through the culture. Major drawbacks of these open systems are that there is almost no possibility for temperature control (unless a source of cheap surplus heat is available) and that they are very susceptible to invasion of algal predators, parasitic algae or other algal strains that grow better at the applied conditions and therefore out-compete the desired species. Only a few species can be grown in these open systems through a selective environment. For example, *Dunaliella salina* requires a high salinity while *Spirulina platensis* requires a highly alkaline environment. Moreover biomass concentration and thus volumetric productivity is very low due to the long light path and poor mixing. Despite these major drawbacks these ponds can allow a simple use of largely unexploited shallow coastal regions.

A photobioreactor can be described as an enclosed, illuminated culture vessel designed for controlled biomass production of phototrophic liquid cell suspension cultures. While an open pond could be seen as photobioreactor, the term photobioreactor mostly refers to closed systems having no direct exchange of gases and contaminants with the outside environment. Photobioreactors are considered to have several major advantages over open ponds. In short they can (i) prevent or minimise contamination, allowing the cultivation of algal species that can not be grown in open ponds; (ii) offer better control over cultivation conditions (pH, pCO_2, pO_2, Temperature, etc.); (iii) prevent evaporation and reduce water use; (iv) lower CO_2 losses due to outgassing; and (v) attain higher cell concentrations and, therefore, higher volumetric productivity. Certain requirements of photobioreactors (e.g. cooling, mixing, control of oxygen accumulation and biofouling) make these systems more expensive to build and operate than open ponds. In spite of their numerous advantages, the viability of photobioreactor technology on very large scales remains to be demonstrated. Nonetheless, many microalgae which are promising for the production of an enormous variety of compounds and their products require maintenance of monocultures and for that, enclosed photobioreactors have to be used. Photobioreactors, as completely closed systems, could also be of high interest for Genetically Modified Organism (GMO) production of targeted compounds for pharmaceutical industry. However, certain requirements of photobioreactors such as cooling, mixing, control of oxygen accumulation and biofouling, make these systems more expensive to build and operate than ponds. New cheaper innovative photobioreactor systems are being designed and waste streams are used to make the production of microalgae commercially attractive.

Information Box 2. Photobioreactor optimisation

The fundamental design elements of photobioreactors are targeted at the control of light gradient and light/dark cycles, surface to volume ratio, mixing and degassing. The Surface-to-Volume (S/V) ratio of the bioreactor (i.e. the ratio between the illuminated surface of the reactor and its volume) determines the amount of light that enters the system per unit volume and the light regimen to which the cell population is exposed, and is consequently one of the most important factors in photobioreactor design. The hydrodynamic behaviour of the culture is also affected by this as higher S/V ratios can lead to shorter light/dark cycles. For these reasons, in recent years a general trend towards the reduction of the diameter of tubular reactors and the thickness of

flat panels can be seen. The type of device used to mix and circulate the culture suspension is essential in the design of a successful photobioreactor. Both the productivity of a photobioreactor and the cost of its construction and operation are influenced to a great extent by the type of mixing mechanism used. Mixing is necessary for a number of reasons: to prevent cells from settling, to avoid pH and temperature gradients, to distribute nutrients, to supply CO_2 and remove O_2. Yet, excessive mixing can lead to cell damage and eventually cell death. For this reason the choice of mixing intensity and mixing system must be dictated by the characteristics of the organism to be cultivated. Finally, scaling up from a prototype design to full-scale commercial size system is still a very challenging issue.

Summary Box 4. Recommendations to improve the use of photobioreactors for the culture of microalgae

- Optimise microalgal cultivation systems with respect to energy supply, productivity and cost;
- Develop innovative photobioreactors adapted to the different species of interest and define optimal scaling-up approaches taking into account local space constraints and availability of inputs: CO_2, light, downstream processing;
- Develop design criteria for culture systems and advise professionals in the construction of industrial scale systems in the near future. Achieve cost reduction to be fully compatible with market needs.

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Figure 13. Vertical photobioreactor

2.4.2 Culture of macroalgae

Currently, the European seaweed industry relies on macroalgae collected from the wild with the exception of some Asian and African seaweeds such as *Kappaphycus* and *Eucheuma* which are cultivated for carrageenan extraction. The growing demand for raw material for food, cosmetics and bioactives, raises questions surrounding the sustainability of the European industry. There is an urgent need to upscale or develop methods for mass production of native seaweeds.

The development of culture methods, particularly for rare and slow-growing plants, is expected to have a significant environmental benefit in the conservation of genetic resources and of algal-associated biodiversity. There are several approaches used to cultivate seaweeds: fragments of plants, sporelings or spores can be seeded onto ropes or other substrates and grown to maturity in the wild. An alternative to open sea culture is the cultivation of seaweed in artificial enclosures, such as tanks or ponds, where seaweeds can be grown in high densities on otherwise low value land. The nursery phase of open-ocean cultures is also operated in controlled conditions with techniques for intensive land-based seaweed aquaculture with air rotation of the seaweed biomass in tanks (tumble culture). Bioreactors designed for tiny species allow even higher productivity values. There are clear advantages to land-based seaweed aquaculture over cultivation in the sea including: better control of both epiphytes and photoinhibition by maintaining high algal density within the tank and the possibility of high levels of productivity all year round. This is made possible through the provision of (i) year-round supply of nutrients, and (ii)

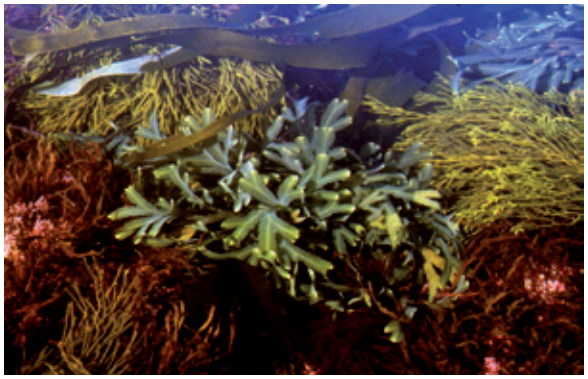

Figure 14. Marine macroalgae in a tidal pool

artificial light in the winter and the manipulation of day length (photoperiod) and thus of plant seasonality to control reproductive and nutritional physiology. Heat-sensitive seaweed species may be cultivated on land throughout the summer by use of cold water pumped from 20-30m ground depth and cooling the seawater tanks by heat exchangers.

At a first glance, the costs of land-based seaweed aquaculture may appear higher than for seaweed cultivation performed in the sea, but integration of seaweed biomass as a nutrient scrubber into existing land-based marine animal farms may reduce the cost to such an extent that in future land-based seaweed cultivation may compete with seaweed cultivation in the sea.

Both open sea-based aquaculture and alternative growing methods are likely to be important for Europe. Regardless of the specific technique, these activities require detailed information about the biology and life cycle of the algal crops and the different production options. Marine genomics research is generating new tools, such as functional molecular markers and bioinformatics, as well as new knowledge about statistics and inheritance phenomena that could increase the efficiency and precision of algal crop improvement. Marker-assisted breeding and selection will be largely accelerated by these novel approaches. In addition, it is expected that population genomics will help in the exploitation of algal genetic resources as well as in the development of association genetics.

Among the traits which are of interest for the selection of algal crops are their defenses against stress and especially biotic stress. Populations of algae (Phaeophyceae) can be affected by various pathogens, including fungi, oomycetes, bacteria, viruses and pathogenic algal endophytes. Intensive algal mariculture however, may facilitate disease outbreaks. As aquaculture continues to rise worldwide, pathogens of algal crops are becoming a significant economic burden. Algal chemical defenses which are known to exist include secondary metabolites such as terpenoids and polyphenolics, as well as fatty acid-derived compounds, which are either antimicrobial, anti-herbivore or act as signalling compounds.

2.4.3 Optimisation of production systems for Marine Biotechnology

The production of microbes, microalgae, macroalgae or invertebrates for bioactive compounds, biorefinery or energy applications is a complex process that needs considerable optimisation. Very different but complementary approaches can be employed, including metabolic flux modelling, biorefinery, bioprocess and chain design, and up-scaling.

Metabolic flux modelling

Establishing industrial production and maximising productivity requires in-depth knowledge of basic biological functions and tools for steering the metabolism. This can be achieved through generating optimal conditions inside a reactor or through metabolic engineering.

A key technology for optimal metabolic design is the metabolic flux model. A metabolic network model can be constructed with the known stoichiometry of the biochemical reactions. Next, by assuming steady state and constructing mass balances over the intracellular metabolites, the rates with which these biochemical reactions take place (the fluxes) can be connected to the consumption of substrates and production of biomass and other compounds, including bioactive compounds. This is currently difficult to achieve because of our fundamental lack of knowledge of biochemical processes in marine organisms and notably of the equilibrium and rate constants for the reactions. For development of metabolic flux models and metabolic engineering, the availability of well annotated genomes and quantitative tools for genome-scale metabolic models that permit understanding and manipulation of the genome are important. An integrated approach using state-of-the-art omics technologies is therefore needed in order to gain the best possible insight into metabolic pathways leading to the product of interest.

Biorefinery

Research is often only focused on production of biomass or specific biomass ingredients at high efficiencies and high volumetric productivities. The biorefinery concept is, however, about more than just downstream processing. The focus in downstream processing is usually to

isolate one specific compound while in a biorefinery the biomass is fractionated resulting in several isolated products from the biomass. The biorefinery approach is, therefore, analogous to today's petroleum refinery, which produces multiple fuels and chemical products from petroleum. In order to maintain the functionality of the isolated products in a biorefinery, isolation should be performed under mild conditions. In this respect, production in the biomass should be optimised whilst allowing mild extraction and fractionation of the different products. For an efficient biorefinery, new extraction and fractionation processes will need to be developed.

Bioprocess and chain design

For the manufacturing of new marine products the process design should be done early on. In the early stages of development much of the basic information for an optimal design is still unknown. Even if many aspects are unknown it makes sense (and often assumptions need to be made) to make a general design of predicted processes. The result of these designs is that bottlenecks in the process are identified which will determine the agenda of the research programmes. As know-how increases, more accurate designs can be made and research objectives can be narrowed down such that processes are developed more rapidly.

The whole production process, upstream and downstream, should be developed and tested at pilot and demonstration scale. Production at large scale will be complex with respect to logistics and space requirements, especially for bulk applications such as food, feed and fuels. Resources for production, such as sunlight, land, water, CO_2 and nutrients should be available. Availability and cost of transport (both in terms of economy and energy) will determine the scale at which production is efficient. Transport of the different feedstocks over long distances is most probably not a feasible option. A design of the whole system, including the logistics and analysis of the life cycle is a good basis to analyse the sustainability and viability of the technology.

Scale-up

Developments in technologies aimed at commercial production are mainly driven by end users. For new processes, the end users usually have a limit in supply of biomass to develop further processes. For this reason, some production capacity needs to be realised straight away. With such a production capacity, end products can be manufactured and tested, and research in biorefinery can be further developed. In addition, for new technology there is little or no experience with production at larger scales for longer periods of time. It is very important, therefore, not only to do research at a laboratory scale but also to develop pilot scale production experiments to evaluate and compare their performance as a basis for the design of demonstration scale facilities.

In order to facilitate rapid development of the technology, research at laboratory scale, pilot scale and demonstration scale should run parallel with a good exchange of information such that technology developed in the laboratory can be tested under realistic conditions and research at laboratory scale can be targeted at addressing the problems encountered at large scale.

Summary Box 5.
Recommendations for the optimisation of production systems for Marine Biotechnology

- Develop the use of metabolic flux modelling as early as possible in Marine Biotechnology pilot scale production projects;
- Promote research on the development of biorefinery technologies and approaches based on microalgae production to develop a long-term alternative to petrochemistry (see also Summary Box 10 in Section 3.2.2);
- Increase the support to Marine Biotechnology research and development initiatives at European levels which integrate bioprocess and chain design through cooperation between academic research teams and industry;
- In parallel to laboratory research, support the development of demonstration-scale facilities based on projects integrating the knowledge of academic research groups and the know-how of industry.

2.4.4 Fish culture in recirculating aquaculture systems

In Recirculating Aquaculture Systems (RAS) seafood production is combined with water purification to maintain a healthy culture environment. RAS refers to the process of re-using some (or all) of the water in a fish culture facility, for example by circulating it through filters to remove fish waste and food and then recirculating it back into the tanks. The technology reduces rates of water consumption, improves opportunities for waste management and nutrient recycling, allows for disease and hygiene management, reduces potential wildlife interactions (no escapees), and minimises the visual impact of farms. In addition, the application of RAS technology enables the production of a diverse range of (also exotic) seafood products in close proximity to (urban) markets, thereby reducing CO_2 emissions

Courtesy Yonathan Zohar, CCMB

Figure 15. Recirculated fish tank with biofilter (drum on right), which uses beneficial microorganisms to remove chemical wastes from the water

associated with food transport. Other benefits of the RAS approach include:

- Being generic, RAS allow for diversification of species. As such the selection of species is dictated by the economic opportunity, as opposed to the geographical location;
- Being fully biosecure, they are the only aquaculture practice that might be considered safe to farm non-native and transgenic fish;
- Being able to fully tailor the environmental conditions in these systems, the fish will perform better and grow significantly faster compared to open sea cage culture;
- Solid waste produced by RAS is converted to methane, bioenergy that is captured to offset the energy cost of the operation;
- RAS systems do not have to be in the proximity of a source of seawater thus can be developed anywhere, close to the markets or to transportation venues, reducing the carbon footprint of the operation;

RAS are advanced and complex aquaculture systems with technology that relies on both physical and biological processes. The biological processes are primarily microbial and therefore can benefit from advances in marine microbial ecology. There is also potential to integrate microalgal systems into recirculating aquaculture systems and further downstream in the management of fish processing outflows.

Although small, the European RAS industry has a 25 year commercial history placing it ahead of the US and Japan both in terms of size and scope. To maintain this competitive advantage, the industry should focus on:

- Minimising the ecological impact of fish farming by closing the system, where possible, in terms of water and nutrient use;
- Maintaining top quality organisms in quality systems;
- Producing healthy and safe seafood products.

RAS outcompetes any other mode of animal food production in terms of consumption of water and discharge of nutrients to the environment. Important research challenges include fine tuning the quality of the wastes produced and maximising waste removal efficiency in biofilters while minimising discharge to the outside environment. This calls for the development of specific RAS feeds and feeding strategies paving the way to both reliable and efficient biofiltration and profitable production.

The welfare of culture animals in RAS can be closely monitored and controlled. Important welfare related research topics in RAS include: (i) fish resilience to changes in water quality; (ii) the effect of accumulation of substances resistant to microbial breakdown (e.g. humic acids) that might bind toxins, metals, steroids, etc.; (iii) poor flavour caused by stress; and (iv) welfare impacts in relation to the accumulation of bio-active compounds in combination with high culture densities. Our understanding of the ecology of microbial communities in RAS and its interaction with the microbiota in the food and gut of culture organisms is still poorly understood. In addition, microbiota present during larval development are highly variable, and are believed to influence larval viability and health.

Summary Box 6.
Recommendation for the improvement of Recirculating Aquaculture Systems (RAS)

Improve the knowledge of microbial communities within RAS and consequently optimise its management in order to support larval development and the establishment of gut microbiota in cultured seafood organisms that will contribute to both their health and vitality over their entire life cycle.

2.5 Model species for Marine Biotechnology

Conventionally, model organisms are organisms that have been selected for in-depth study by scientists according to various criteria such as generation time, ability to be cultivated in the lab, facility to be genetically transformed, genome size, facility to work with, evolutionary position, etc. Due to time and human and financial capacity limitations it is not possible to perform in depth studies of all (marine) organisms of interest. For this reason, scientists strive to select a limited number of model organisms to focus their attention on, assuming that knowledge gained from these model organisms could be, to a certain extent, transferred to related organisms (e.g. from mouse to human). For reasons of space limitations, in this section the discussion focuses on marine species only, although it is clear that non-marine species whether of terrestrial or freshwater (e.g. zebrafish) origin are often used as model organisms for Marine Biotechnology purposes as well.

Only a few model organisms that are currently investigated in biological institutes around the world are of marine origin. Among eukaryotes, most of them are animals (e.g. sea urchin, sea squirt, lamprey, polychaete, platyneris) with a few macro and microalgae and a few blue-green algae and archaea. Although they have been selected for their interest in fundamental biology, model organisms can be very useful for biotechnological applications (an overview of marine model organisms with their applications can be found in Annex 4).

For example, sea urchin embryos are good models for cancer research or neurodegenerative disorders. This echinoderm represents a powerful research model that has brought almost everything we know about the chromosomal basis of development, maternal determinants, fertilisation and maternal messenger RNA. The genome sequence of the California Purple Sea Urchin *Strongylocentrotus purpuratus* (see Figure 17), obtained recently, provides a unique opportunity to address crucial questions in developmental biology and cell cycle regulation. Using sea urchins as a model is important since these organisms occupy a key evolutionary position with respect to vertebrates. Indeed, the echinoderms and their sister group, the hemichordates are the only other deuterostome animals besides the chordates. The sea urchin is thus more closely related to humans than other major invertebrate models in use. Therefore, knowledge obtained from sea urchin studies gives the opportunity to discover potential new targets for therapy in humans.

Extremophiles found in coastal and deep-sea hydro-thermal vents harbour a huge diversity of microorganims

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Figure 17. California Purple Sea Urchin *Strongylocentrotus purpuratus*. Sea urchins are important as research models in developmental biology, cell biology, gene regulation molecular biology, evolutionary biology, metabolic biochemistry and marine biology. The genome of *Strongylocentrotus purpuratus* was sequenced and published in 2006 providing a unique opportunity to address crucial questions in developmental biology and cell cycle regulation.

belonging to Bacteria, Archaea and their related viruses. The nucleic acid processing machinery (DNA synthesis, replication, repair and recombination) is similar in eukaryotes and archaea, with the latter displaying a simplified version. The DNA replication machinery -or replisome- of *Pyrococcus* (hyperthermophilic archaea) does not only offer thermostable DNA polymerases commonly used in high fidelity PCR, but also provides a set of proteins and enzymes that might contribute to solving unanswered questions about the human replisome like the resolution of its 3D structure which is of much interest in the design of new anti-cancer therapies.

It is difficult to estimate the importance of models for Marine Biotechnology innovations and there is no ideal model that should be developed specifically for biotechnological purposes, but there is a wealth of information and data that could be of interest for developing new products and services, that are either not produced, made available or that are underutilised by the wider scientific community. Moreover, marine models could lead to entirely new insights, particularly at the larval stages where different kinds of host-defence mechanisms are operating.

Information Box 3. Exploration of marine life

The oceans are the cradle of life and the three domains of the tree of life, namely Bacteria, Archaea, and Eukaryotes have evolved in the marine environment from a common ancestor. Prokaryotic life originated in the oceans about 3.6 billion years (Gyr) ago. Eukaryotic life originated between 0.6 and 1 Gyr later and the most ancient fossils currently known of multicellular organisms dates back to 2.1 Gyr. Land became colonised by fungi about 1 Gyr ago and by green plants only 0.7 Gyr ago. Thus the very long evolution period of marine life compared to terrestrial life has generated a massive biodiversity at the gene, the genome, the species, the lineage and the ecosystem level. For example, the animal eukaryotic lineage that includes sponges, molluscs, invertebrates and mammals, is simply one single independent lineage in the tree of life that contains tens of lineages, all of which comprise marine organisms. This diversity includes lineages that have evolved multi-cellularity such as animals, green plants, red and brown algae and fungi but most of them are unicellular (microbial) eukaryotes. The same evolutionary diversity can be found in the two other prokaryotic domains, Archaea and Bacteria. Even more importantly, bacterial and archaeal diversity is surpassed ten-fold by the diversity of viruses.

This evolutionary richness combined with an adaptation to a wide range of environmental conditions (temperature, salinity, tides, pressure, radiation, light, etc.) and to a specific aquatic habitat, makes marine organisms a huge reservoir for new developments in both basic knowledge and biotechnological innovations and both aspects are related. At present only a few marine lineages have been investigated with modern biological approaches, and many remain as yet totally unexplored or even undiscovered.

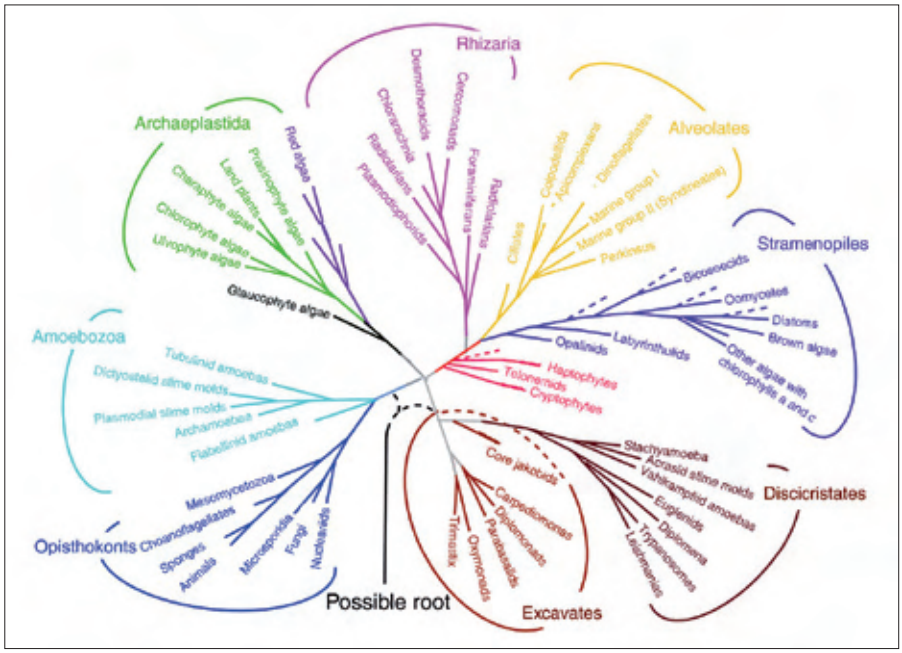

Figure 16. Eukaryotic tree of life (modified from Baldauf S., 2008)

There are many different marine models being investigated in European laboratories that may be of interest for biotechnological purposes for various applications or molecules such as:

- New enzymes (bio-catalysts);
- Proteins and peptides;
- Secondary metabolites;
- Polysaccharides (bacteria, archaea, algae, marine plants);
- Fatty acids and lipids (microalgae).

Nevertheless from a phylogenetic and evolutionary point of view, only a limited number of marine models even within the animal lineage, are established as biological models and this deficit is detrimental to the advancement of Marine Biotechnology. While the recent revolution in sequencing techniques now makes it possible to sequence many more genomes at much lower cost, the establishment of a model organism is much more than genome sequencing. The knowledge gained from the development of new marine models could provide the basis for more targeted studies in closely related marine organisms with specific interests for biotechnological applications. We can now access whole genomes of marine organisms and metagenomes more easily. The major challenge facing us is to mine the genes of interest, to identify novel functions and to store and utilise all these metadata. This requires novel bioinformatics developments as well as the establishment of collective e-infrastructures.

Besides the typical biological models concept, one needs to emphasise the value of defining ecological models that are not relevant biological models because they do not satisfy the criteria detailed before, but which play a significant role in the marine ecosystem. This is the case for instance for the diatom *Thalassiosira pseudonana*, or the haptophyte *Emiliania huxleyi*. This is the case also with seagrass populations in coastal areas where they play a critical role in marine ecosystems and have a huge impact on aquaculture grounds and fisheries as well as a significant role in carbon sequestration. In a similar manner, fish such as seabream, seabass and salmon or shellfish such as oysters, mussels, clams that are supplied through fisheries and aquaculture can be considered as economic models.

Interaction between marine organisms is critical in our choice of model organisms. Communication in the marine environment is different than in the terrestrial environment and marine organisms have developed a whole set of molecules (aldehydes, halogenated compounds…) that are used for communicating among communities of the same species, in defence responses against pathogens, as signals for larval development or in host-symbiont interactions. Very little is known about these signal molecules which most of the time, are produced at very low concentrations and that could offer novel options for biotechnological applications. Marine chemical ecology, the discipline that addresses these questions, is still in its infancy worldwide with a very restricted scientific community in Europe.

Once a molecule/compound/enzyme from a marine organism has been identified as being valuable for biotechnological application, the immediate subsequent question is one of access to the biomass. When the molecule is an enzyme, then expression in heterologous systems is often possible provided that the gene is identified and available. If the molecule is not easily synthesised and belongs to an organism that is rare and not amenable to cultivation then biotechnological development may take different routes according to the cost/benefit outcome. Access to the natural resources is also an important issue and exploitation or sampling should be performed according to the international policy on biodiversity protection and using sustainable management practices.

Summary Box 7
Recommendations to improve the use of marine model organisms for Marine Biotechnology

- Identify and encourage the development of new priority marine models that have not yet been investigated in the tree of life, to fill critical gaps;
- Improve access to the knowledge generated from model organisms for biotechnological purposes. Identify the mechanisms that should be implemented for facilitating the transfer of knowledge from scientists studying marine models for biological reasons to more applied research;
- Foster and support the development of the newly emerging field of marine chemical ecology;
- Ensure that marine organisms of biotechnological interest are exploited in a sustainable way. Always consider cultivation issues and access to the biomass in parallel to the screening and research activities for biotechnological development.

2.6 High throughput tools for proteins, enzymes and biopolymers

Enzymes have for many years been the driving force of biotechnology. There is an ever increasing demand for novel enzymes for a variety of applications ranging from the degradation of natural polymers such as cellulose, starch and proteins, or for use in the pharmaceutical and chemical industries, involving numerous chemically and structurally diverse molecules. It is clear that Moore's Law[4] that applies to sequencing technology does not fit to enzyme screening, expression of novel recombinant proteins and structural genomics despite all the recent innovations in proteomics. Filling these gaps is a challenge that is not specifically limited to Marine Biotechnology. However, it is even more important in this case due to the size of the untapped protein reservoir provided by marine life.

Every genome or metagenome project increases the number of putative genes whose functions are often unknown and at best deduced from sequence analysis. But even the best annotation provides little information about detailed substrate specificity and functionality.

Sensitive high throughput screening methods to identify genes encoding novel enzymes for specific applications need to be established. These methods need to be based on easily identifiable phenotypes such as colourimetric assays, that can ideally be automated, combine many substrates in one assay system and be compatible with liquid high-throughput screening facilities. The challenge is to use existing enzymatic activity detection methods based on changes in spectroscopic properties for the design of high throughput chips that can identify the product formed. Thus, the development of high throughput technologies based on robotic systems to directly screen samples or colonies for specific substrates should be a priority.

Future advances should focus on designing cell-free systems with the aim of increasing substrate bioavailability and reducing the inhibitory and cross reactivity of cellular components. Small molecule microarrays (SMMs), involving the use of synthetic molecules as capture agents, will contribute to an expansion in the capabilities in high-throughput screening for novel enzymes. Interesting contributions to direct mapping of metabolic pathways have recently been made with the design of tools capable of generating non-destructive, real time functional and dynamic knowledge with microbial reactomes.

While a number of expression systems are already available for expressing genes encoding enzymes, additional expression systems are urgently required for marine invertebrates and plants. The situation is even worse for exopolysaccharides where the production of the targeted products is often strictly dependent upon the ability to induce production, e.g. through stress, and to recover end-products which implies time consuming and labour intensive control of cultures of the strain or species of interest. This illustrates the urgent need for basic and applied research to develop and improve high throughput tools for proteins, enzymes and biopolymers from marine bioresources which will be beneficial for a wide range of biotechnological applications.

4. Moore's law describes a long-term trend in the history of computing hardware where the number of transistors that can be placed inexpensively on an integrated circuit has doubled approximately every two years. The trend has continued for more than half a century and is not expected to stop until 2015 or later and will continue to profoundly impact all applications and technologies that rely on transistor power for their processing.

3. Marine Biotechnology: achievements, challenges and opportunities for the future

3.1 Marine Food: Marine Biotechnology for sustainable production of healthy products through fisheries and aquaculture

3.1.1 Science driving aquaculture development

The world's oceans harbour a wide range of environmental niches, host an as yet largely untapped and underutilised source of biodiversity, and remain a significant source of food. Marine fisheries have leveled off and an increasing number of fish stocks are now overexploited or even in danger of extinction. There is a general consensus, therefore, that the oceans have reached their maximal sustainable yield. Most fisheries scientists agree that, if current trends continue, many fisheries could collapse by 2050.

In securing healthy food from the seas and oceans, Marine Biotechnology can contribute by selection and captive breeding of stock for return to their natural environment, in order to replenish wild stocks and mitigate the effects of overfishing to some extent. However, to satisfy the growing demand for seafood, marine food will need to be increasingly delivered through intensive aquaculture. In fact, according to FAO statistics, close to 50% of the seafood produced globally today originates from farming operations. While salmonids are probably the most well known farmed finfish species in the western world, other species like seabass, seabream, catfish, tilapia, turbot and pangasius, are among a growing number of species being farmed today, demonstrating that aquaculture is only at the early stage of conquering the marketplace. Aside from fish and shellfish aquaculture, macroalgae are also harvested and cultivated for a range of components, including food additives. The cultivation issues related to macroalgae are discussed in Section 2.4.2.

To meet the challenge of supplying growing seafood markets, aquaculture will need to become more efficient and cost-effective, whilst simultaneously reducing its environmental impact. Thus both the aquaculture research community and the industry itself have focused on increasing production efficiency, increasing product quality, introducing new species for intensive cultivation and on developing sustainable practices. In order to achieve these goals, there was a need to better understand the molecular and physiological aspects of reproduction, development and growth, and to better control these processes. Science has contributed significantly to achieving these goals.

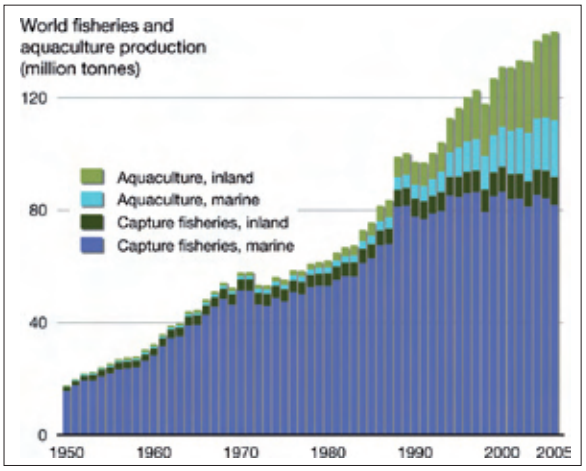

Figure 18. Evolution of world capture fisheries and aquaculture production from 1950 to 2005. Current FAO Statistics indicate that in 2010 about 50% of the aquatic food produced globally originates from aquaculture activities. (Source: FAO. 2009, FishSTAT Fishery Statistical Collections Global Aquaculture Production. Produced by Hugo Ahlenius, Nordpil)

Some examples of progress include:

- Molecular diagnostics and novel immunisation strategies which have decreased the impact of diseases and their transmission;
- Traditional selection has led to growth improvements of up to 25% per generation in some aquaculture species, a value in which has never been achieved in farm animals;
- Marine genomics projects at EU and national levels have also had a significant impact on selective breeding, particularly through the integration of quantitative genetics and molecular screening, whole genome wide association studies and marker assisted selection;
- Ecological and genetic approaches have largely contributed to a better assessment of chemical and biological interactions between aquaculture and the environment and to develop strategies to reduce the harmful environmental impacts from intensive production systems;
- Microbial bioremediation, particularly in land-based mariculture, and improved microbial control of intensive production systems have improved containment and environmental compatibility;
- A better understanding of the life cycle of cultured organisms has improved the ability to support sustainable aquaculture through improved nutrition, intensified selection and disease management, resulting in improved food quality.

As such, through rapid biological and biotechnological progress, a more efficient and environmentally

responsible aquaculture has been achieved and food products from the marine sector are diversifying. Moreover, the feed which we use in the culture of fish is fast becoming the key to deliver a healthier fish and, in turn, a healthier consumer and environment.

Fish oil and fish meal, derived from the wild catch sector, are critical components of the artificial diets used in carnivorous finfish aquaculture. Aquaculture enterprises represent the major consumer of fish meal and oil using almost half of the global production. Pessimistic forecasts concerning fisheries catches have prompted a major research focus on delivering alternative oil and meal sources in the diets used for these aquaculture species. In this respect, molecular approaches are of use to investigate the effectiveness of marine product substitution by e.g. plant-derived materials and more recently algal products. There has been a particular surge in research investigating the effects of dietary oils on the fatty acid profile of fish and it has been demonstrated that the fatty acid profile in the end product can be specifically modified by the design of the feed used and thus be adjusted based on consumer preferences. Dietary shifts may also induce an impact on other aspects of fish physiology including, for instance, metabolism, health and immunity which can be monitored by molecular tools studying metabolic profiling, liver enzymes-biomarkers and immune parameters.

In another example, carotenoid substances are commonly included in the diets of farmed species such as salmon and trout to produce a natural colouration in the final product. Research also indicates additional benefits from dietary carotenoids other than colouration of the flesh. Other biological functions of these substances related to growth, reproduction and tissue health have been evident in salmonids and shrimp. However, the full function of such compounds is not yet completely understood, and considerable research is still required to 'tailor' artificial aquaculture diets with ingredients designed to increase the functional and health properties of the end-product. It follows that a healthier diet which benefits the farmed species, will also ultimately benefit the consumer. This is a valid reason for delivering improvements in aquaculture diets, as many new options open up for using aquatic animals as carriers of essential nutrients in human nutrition.

Figure 19. Finfish aquaculture

3.1.2 Development of new methods for the optimisation of marine aquaculture

There is a strong rationale for the move towards using the aquatic environment to grow food. It opens a production volume representing more than 90% of the culturable biosphere of the planet and two-thirds of the surface, and in contrast to terrestrial farming, production can be achieved utilising a three dimensional space. However, commercial aquaculture is currently faced with several important bottlenecks at the level of overall performance, reproduction (no or unpredictable spawning), early (larval) development (low survival, cost), growth, nutrition, disease/health management and interactions with the environment.

The significant challenges to farming the oceans need to be addressed in a wider context. Challenges include the physical constraints of temperature and weather conditions at the surface, and light and pressure deeper in the water. There are also many factors to consider when choosing organisms to target, including adaptation or efficiency at prevailing environmental conditions and nutrient availability, the capacity of the organism to deliver food of optimal quality and health for the consumer and securing a sustainable environment. While our understanding of the nutritional loops in the oceans is good, the technological potential of typical complex marine biological systems is still widely under-investigated. Intensifying production of a target organism by supplying feed presents challenges of balancing the local biosphere.

It is thus necessary to select physical conditions optimal for sustainable production and minimal risk. Development of key technologies for overcoming the natural constraints is necessary to release the vast potential of aquaculture. The application of molecular and biotechnological tools will be particularly important to support the development of sustainable aquaculture. Better understanding of reproduction, development and growth will result in better control of those processes, and continually improving methods for diagnostics and immunisation will decrease the impact of diseases and their transmission. Novel ecological and genetic

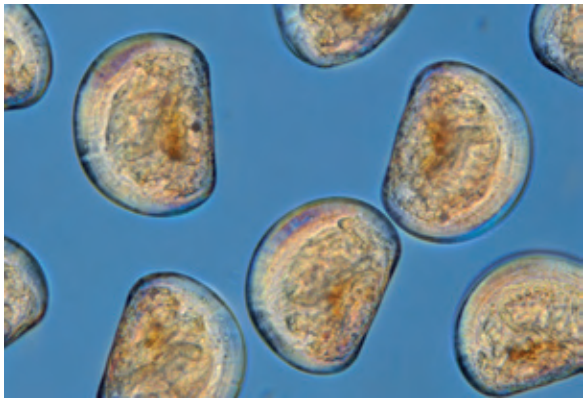

Figure 20. Scallop larvae (approximate size about 1/10 mm) grown in Argenton, France. Global scallop production amounted up to 1,265,000 tonnes in 2005 with aquaculture producing more than fisheries as early as 1992.

approaches are required to assess chemical and biological interactions between aquaculture and the environment, and improved understanding of microbial control and bioremediation in mariculture will improve environmental compatibility.

Which organisms to culture, and how?

The common perception of aquaculture today is the farming of finfish, crustaceans or molluscs in sea cages or other confined structures suitable for supporting growth. Some attempts have been made at 'ocean ranching', but with limited success. In some areas macroalgae are successfully grown (see also Section 4.2.4).

The number of aquaculture species has been increasing over the years, but is still limited relative to the number of species with potential for farming. Further, most aquaculture is intensive monoculture. This concept has several disadvantages both from an environmental point of view, and from the perspective of disease development.

Polyculture production systems could represent ways of increasing production volume and utilising the biodiversity of marine ecosystems. The most advanced systems are termed Integrated Multi-Trophic Aquaculture (IMTA) and combine the cultivation of fed species (e.g. finfish fed with sustainable commercial diets) with extractive species, which utilise the inorganic (e.g. seaweeds) and organic (e.g. suspension and deposit feeders) excess nutrients from the fed aquaculture. Seaweeds and invertebrates produced in IMTA systems could be considered as candidates for nutrient/carbon trading credits within the broader context of the ecosystem, and IMTA could become an integral part of coastal regulatory and management frameworks. The challenge is to establish safe and stable systems with an economically feasible output. Hosts (particularly invertebrates) may harbour bacteria that may be detrimental or pathogenic to other organisms within the same culture or ecosystem. This is a particular challenge in the marine environment, where the principles of host-microbe interactions, symbiosis, commensalism and epidemiology are not yet well understood, while the medium is open to pathogen transmission. Consequently, a fundamental understanding of aquatic ecology, including competition and symbiotic principles, is necessary for the sustainable farming of marine organisms in complex systems such as IMTA. Indeed, a better mastery of host-microbe interactions within such confined marine ecosystems may provide us with completely new tools to understand disease development in general and how to fight new diseases in a more ecological way.

The concept of a disease-free animal production system is unrealistic and may lead to strive towards the development of systems that are ecologically unstable and unreliable. The challenge will be to understand complex ecosystem processes in order to further develop production of existing species and to introduce new species, while maintaining the basic biological foundations of health, competition and interactions in such systems.

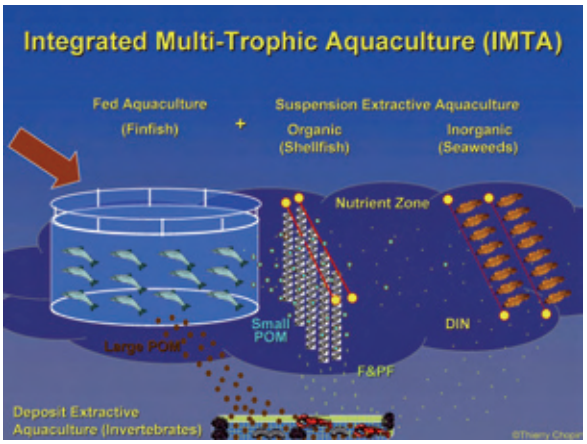

Figure 21. Conceptual diagram of an Integrated Multi-Trophic Aquaculture (IMTA) operation including the combination of fed aquaculture (e.g. finfish) with suspension organic extractive aquaculture (e.g. shellfish), taking advantage of the enrichment in small particulate organic matter (POM), inorganic extractive aquaculture (e.g. seaweeds) taking advantage of the enrichment in dissolved inorganic nutrients (DIN), and deposit organic extractive aquaculture (e.g. echinoids, holothuroids and polychaetes), taking advantage of the enrichment in large particulate organic matter (POM) and faeces and pseudo-faeces (F&PF) from suspension-feeding organisms. The bioturbation on the bottom also regenerates some DIN, which becomes available to the seaweeds. (Source: Thierry Chopin)

Marine Biotechnology contribution to aquaculture

Intensive production of genome and transcriptome sequence information from a variety of organisms, including some farmed fish species, has been made possible by major technological developments in the 21st century. There is a growing interest in applying genomics approaches to aquaculture, creating the opportunity of making new aquatic products through altering their genetic make up. Moreover, progress in transgenic technologies has provided considerable opportunities, including improved growth rates and improved health and quality of broodstocks.

However, there is increasing consumer concern regarding genetically modified organisms (GMOs) and transgenic products. Manipulation of the genes controlling growth hormone production has resulted in a 30-fold increase in growth rate in some fish species (e.g. in salmonids). Interestingly, both the enhanced growth phenotype and the genotype can be inherited to the progeny thus producing second and third generation transgenic offspring. Therefore, the introduction of transgenic technology necessitates the need for production of sterile progeny and the development of better engineered aquaculture systems such as RAS (see Section 2.4.4) in order to minimise the risk of released transgenic stocks mixing with wild populations, and this may, in turn, question the long-term benefits of such manipulations. For these reasons biotechnological applications should focus on disease control and production of healthy fish instead of boosting growth rates.

To develop a new species for aquaculture requires the ability to control its reproduction. Common methods of spawning induction have largely relied upon external stimulation of reproduction through control of environmental parameters or direct administration of gonadotropin releasing hormones. For example, spawning can be induced in tuna with the help of hormones (see Figure 22) administered through implants. Sex manipulation can be optional for particular species displaying sex-specific growth differences where monosex culture is preferred. This can be accomplished through a number of methods with the most common being the treatment of fish with methyl testosterone to produce the male phenotype, although other methods such as specific crossing of closely related species with varying genetic make-up can also result in single sex hybrids. For the production of sterile fish, with a high market value, triploidy can be induced through heat or pressure-shock to inactivate the sperm. Sex inversion using steroids can also be used to produce fertile males with the female genotype, allowing the production of single-sex offspring. However, the question is whether

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Figure 22. Spear guns are used to implant Tuna with hormone releasing implants to induce spawning in attempts to domesticate this fish with a high market value which is under growing pressure from over-consumption.

such methods would be acceptable to the consumer in the near future.

Combating disease in cultured fish and shellfish stocks is a critical issue for the commercial aquaculture sector and has thus been a key area for applied research. Firstly, bacterial pathogens are usually treated with antibacterial agents (some of the most commonly used globally include oxytetracycline, oxolinic acid, potentiated sulfa), a practice which can eventually lead to antibacterial resistance with a subsequent and direct impact on human health. The control of ectoparasites, notably Atlantic salmon lice, has commonly been achieved with emamectin benzoate (SLICE®) and teflubenzuron (Ektobann). As with antibacterials, the repeated use of the same products to fight parasites over a number of years increases the probability of acquired resistance by the parasite as demonstrated with emamectin on lice. As a consequence, there is clearly the need for alternative strategies.

Secondly, marine organisms with increased resistance to pathogens can be produced with the use of transgenic technology, incorporating genes encoding antimicrobial activity. Derived from specific organisms, these genes can deliver increased resistance to pathogenic infection when incorporated in the genotype of other fish species. Even though some improved resistance from genetic manipulation has been claimed for *Vibrio anguillarum* in salmon, there are reasons to question the long-term efficiency of transgenic technology for disease control

of intensively cultured species. This is particularly true in the marine environment, where many organisms may harbour commensal microorganisms that do not cause disease, but are potentially pathogenic. Moreover, there are occasions where gene alteration may compromise fish immunity. A much more promising approach that has been neglected in the past is the development of advanced vaccines. DNA vaccines are now ready to be recruited to the battle against fish diseases. This novel method is based on the administration of the gene encoding the vaccine antigen, rather than the antigen itself, providing a valuable tool for more sustainable aquaculture production.

A third approach is based on the preventive use of probiotics and immunostimulants such as yeasts. In the marine environment a remarkably high percentage of the potentially pathogenic microflora may be resident in a host without causing disease, or could be harmless to one host and cause disease in others. Aquatic systems lend themselves perhaps better than any other, to disease management through the use of probiotics, which has mainly concentrated on pathogen exclusion, competition or microflora manipulation. Improved microbial control could result in improved disease management, as well as having positive effects on the environment. Up to now, such studies have been largely empirical, but some promising results are beginning to emerge, particularly at critical stages such as larval production, or stress situations. During recent years, data has emerged on the genetic basis of host colonisation in the zebrafish, and such studies could provide a better basis for microflora manipulation or pathogen exclusion, either by competition, exclusion or interference with the microflora signalling or sensing systems.

Seafood and health – Opportunities in aquaculture

Scientific and technological developments in food science have led to a marked shift in how consumers deal with food and health. The beneficial health effects of seafood consumption have primarily been attributed to the ω-3 fatty acids EPA and DHA. Emerging research suggests that other components in seafood are also beneficial as they may have synergistic or additive effects with ω-3 fatty acids. Taurine is a component that is relatively abundant in seafood, and synergistic effects with ω-3 polyunsaturated fatty acids (PUFAs) have been found on markers for cardiovascular disease. There is also evidence to support the contention that eating seafood is more beneficial than taking ω-3 fatty acid supplements, suggesting that the isolation of single components is too simplistic.

Much of the research on the beneficial health effects of fish has been focused on ω-3 fatty acids. However, recent and emerging research on seafood proteins suggests that the contributing health effects from the proteinaceous part of fish have to date been largely ignored. Eating fish proteins has, in some dietary studies, been found to protect against the development of type II diabetes and obesity. Also, it is worth noting that a meal of seafood generally replaces another protein source, for instance red meat, and thus the health effects of seafood consumption may arise from the low content of saturated fat.

Aquaculture offers opportunities to modify the nutritional contents of farmed species. Dietary modulation enables sea farmers to produce seafood with added health benefits by incorporating functional components into the feed, and thus increasing the beneficial components in the fish. However, the optimal method to manipulate and optimise the content and profile of fatty acids in cultured fish is still a matter of research and debate. It is a matter of research since innovative methods based on genomics and molecular biology could be established in the future to directly allow for the production of genetically modified fish with enhanced fatty acid synthesis capabilities. It is a matter for debate since another route to a similar end would be to rely on the production of genetically modified plants with the capability of synthesising the right fatty acids to be used as food for fish. Obviously it is also a matter of debate since the culture of those GMOs would be controversial. While not cost effective yet, a viable and promising approach might therefore be to use cultured algae in the fish feed for obtaining the desired fatty acid profile in the fish for consumers.

Summary Box 8. Research priorities for Marine Biotechnology applications in aquaculture

- Ensure a better control of reproductive processes in aquaculture species to improve the quality of juveniles;
- Develop innovative methods, based on omics and systems biology, for selective breeding and integration of feed ingredients to improve the quality and health benefits of the end products;
- Develop new methods for environmental quality preservation, animal disease and welfare control (including vaccines and probiotics) and for feed ingredient optimisation to ensure the economics and sustainability of aquaculture production;
- Develop and employ alternative therapeutic measures including plant extracts to limit the use of antibiotics and chemicals in intensive aquaculture systems.

3.2 Marine Energy: Marine Biotechnology for energy supply

3.2.1 New opportunities and challenges

There is a growing need for supply of energy on a global scale and Marine Biotechnology can provide an important contribution to satisfy these energy needs in very different ways. For example, Microbial Enhanced Oil Recovery (MEOR), a bio-based approach to improve the efficiency of recovery of fossil oil reserves, could help to increase the life of mature oil reservoirs. At the same time, the ocean itself is an untapped, sustainable source of bio-energy. The production of biofuel with microalgae poses one option to harvest this huge potential but there are many more examples related to the production of bio-energy from marine organisms.

The efficiency of oil recovery from petroleum reservoirs is about 50%. The efficiency of collection can be enhanced by decreasing the viscosity of oil and/or the permeability of rock material in oil reservoirs. Two main strategies are employed in MEOR: (i) injection of nutrients to stimulate growth and/or metabolic activity of indigenous bacteria already thriving in the oil field; or (ii) injection into the reservoir of specific bacteria and a nutrient solution. The results of MEOR trials are controversial and their conclusions and benefits are still a matter of debate. The future challenges for MEOR will be to make it technically and economically feasible and environmentally friendly.

A lot of research has already been done demonstrating that both microalgae and seaweeds are possible resources for the production of renewable fuels. In some European regions, like Brittany (France), indigenous seaweeds constitute a valuable resource for food and cosmetics industries but thousands of tonnes of undesirable seaweeds are polluting the coasts and regularly need to be collected. Despite various trials, it appears that bioconversion of this huge quantity of seaweeds is technically feasible but not yet profitable. Producing seaweeds specifically for biofuel production has also been suggested but its viability remains questionable.

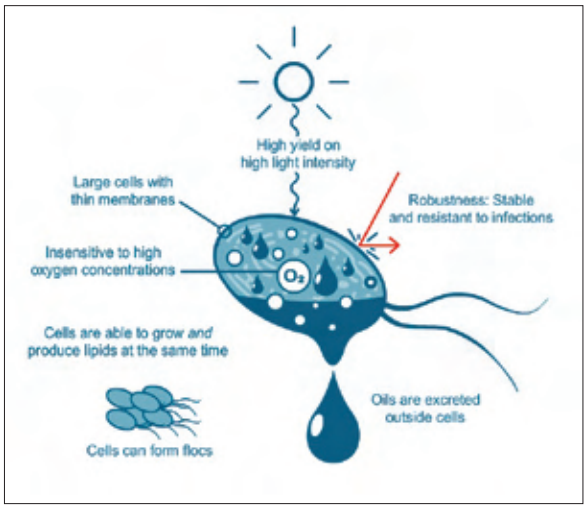

Figure 23. The ideal photosynthetic cell factory for production of biofuels (from Wijffels and Barbosa, 2010)

Microalgae can accumulate large quantities of hydrophobic compounds which can be converted into biodiesel and the production of biodiesel from microalgal tri-acylglycerides is the focus of much interest. The concentration of lipids in microalgae varies between 10 and 60%. Accumulation to these high concentrations in lipid globules generally takes place as a result of stress such as that induced by Nitrogen limitation. However, any benefit gained through an increase in oil content is usually offset by lower production rate induced by the same stress. Therefore, using the whole algal biomass is essential for achieving a good energy balance.

Based on previous work on a limited scale it is estimated that a theoretical production of 20,000-80,000 litres of oil per hectare, per year can be achieved from microalgal culture although on the basis of the present technology the maximum productivity will not be higher than 20,000 litres per hectare per year. This is considerably higher than production from terrestrial crops; for example, palm and rapeseed oils are produced at 6,000 and 1,500 litres per hectare, per year, respectively. In contrast to terrestrial crops, there is no debate about algae as an energy crop competing with food crops. This is because the productivity of algal biomass is higher than for terrestrial crops and, even more importantly, because microalgae can be grown in closed systems with close to complete recycling of nutrients and water. Also, for cultivation of microalgae, areas can be used that are not suitable for agriculture. To cultivate microalgae for the generation of bio-energy is a great challenge and one of the key objectives for Marine Biotechnology in the 21st century. The detailed challenges are manifold because of the enormous up-scaling which will be needed for commercial production (from a few hectares to thousands of square kilometres). Therefore, a highly multidisciplinary approach is needed and success will depend upon the overall energy efficiency and environmental sustainability at all levels of the production chain. The following main challenges are foreseen:

- Exploration and understanding of the biodiversity of microalgae at the molecular level and on a global scale;

- Exploitation of the physiological potential of microalgae to produce biofuels with the bioengineering tools of the 21st century;
- Harvesting and processing of large amounts of microalgae for the production of the optimal mix of bio-energy and bioproducts;
- Achievement of a net energy gain along the whole production chain necessary to convert microalgal biomass into biofuels;
- Achievement of full sustainability of the whole production chain in terms of regional and global impact.

3.2.2 Perspectives for the future of microbial enhanced oil recovery

The principles of Microbial Enhanced Oil Recovery (MEOR) have long been established. The basic mechanisms behind the technique are, however, poorly understood. The technique has enormous prospects if it can be applied in a controlled way with little or no negative impact on the environment.

Summary Box 9. Research priorities to improve Microbial Enhanced Oil Recovery (MEOR)

- Identification and isolation of new strains or communities of marine bacteria suitable for MEOR;
- Monitoring of bacterial populations using *in vitro* and *in situ* tests (with molecular and genomic tools) and tracing of metabolic activities in experimental models and in pilot experiments;
- Understanding and optimisation of the MEOR process on a pilot and field scale;
- Reservoir engineering for *in situ* trials of MEOR and assessment of its environmental impact and sustainability.

Biofuels from microalgae

Worldwide research programmes have been initiated to develop technologies for the production of biodiesel from microalgae giving rise to the generation of many new companies in this field with a major emphasis in the USA. In contrast to terrestrial crops, only a very low production capacity for microalgae exists at this stage. Production is done in niche markets for high-value products such as carotenoids and ω-3-fatty acids. The world production of microalgae is about 5 million kg of dry biomass with a total market volume of € 1.25 billion. Today, the market price of microalgae is on average 250 €/kg dry biomass. For microalgae to be used for the production of bio-energy, the cost

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Figure 24. Conservation room for microalgal strains. Laboratory of algal physiology and biotechnology, Nantes, France (2008).

of production will need to be significantly reduced and the scale of production significantly increased. There are many challenges to be faced in reaching this goal but with a major multi-disciplinary effort it should be possible within ten to fifteen years.

Economic and environmental sustainability are the major issues for the production of biofuels from microalgae. The overall process will need to be optimised in terms of the input or use of energy, water, land and materials, relative to energy output and a close to complete recycling will be needed. Indeed, achieving environmental sustainability will complement the achievement of economic sustainability. Microalgal production will help bio-remediate our environment, through the use of nutrients in waste-water and CO_2 in flue gases in large scale algal production. As less production resources are needed, and more are recycled, energy output per volumetric production unit will be improved. For example, the use of industrial waste products for an efficient and low cost supply of water, nutrients and CO_2, will be crucial for achieving a good energy budget. Based on these 'waste' resources, the right algal cultures (mixed cultures, mono cultures) will need to be adapted to the prevailing climate and seasonal conditions (though there are limitations for higher latitudes and cold climate regions), and an optimal cultivation strategy determined based on an increased knowledge of their physiology. Therefore, the major goals for biofuel production from microalgae are: (i) a high rate of algal production per volume/area; (ii) a high oil content of the biomass; (iii) an efficient low-cost and low-energy harvest procedure; (iv) a highly positive energy balance; and (v) an overall high sustainability.

Microalgae for biorefineries

Microalgae produced for energy could reach profitability more rapidly if the uses of their organic compounds were optimised. The high number and the diversity of organic compounds are a strong incentive not to limit the use of microalgae to energy production alone but to develop a new algal-chemistry that would, on long-term basis, deliver multiple and profitable uses of all components of the algal biomass. This is a key step towards viability of microalgae for biofuels.

Summary Box 10. Recommendations for the development of sustainable production systems for biofuel from microalgae

- Initiate a major programme of bio-prospecting of aquatic environments and strain collections to provide a global inventory of algal species and their habitats to understand their natural growth conditions and requirements;
- Develop an in-depth knowledge of basic biological functions and tools for steering the metabolism of microalgae, with the objective of improving photosynthetic efficiency and enhancing lipid productivity; develop metabolic flux modelling as a tool to steer the cellular metabolism;
- Develop cultivation methods with the objective to obtain microalgae with excellent characteristics for mass cultivation (mixed and mono cultures), for biofuel production and biorefinery;
- Design pond systems/photobioreactors in which high volumetric productivities can be obtained and which can be scaled up for large-scale biofuel production;
- Develop efficient harvest, separation and purification processes to biorefine crude biomass not used for biofuel production into different compounds such as ω-3-fatty acids, carbohydrates, vitamins and proteins (e.g. study of autoflocculation processes of algae and enzymatic driven use of algal biomass);
- Develop a complete production chain from algal cultivation to biofuel and by-products with detailed life cycle analysis, optimising both the individual stage processes and the process chain as a whole;
- Assess the sustainability and socio-economy of the biofuel production from microalgae at all levels of the production chain.

3.3 Human Health: biodiscovery of novel marine-derived biomolecules and methodologies

Because of the physical and chemical conditions in the marine environment, almost every class of marine organism possesses the capacity to produce a variety of molecules with unique structural features. These molecules offer an unmatched chemical diversity and structural complexity, together with a biological potency and selectivity. In recent years, the chemistry of natural products derived from marine organisms has become the focus of a much greater research effort. This is due in large part to the increased recognition of marine organisms as a source for bioactive compounds with pharmaceutical applications or other economically useful properties. The fact that marine resources are still largely unexplored has inspired many scientists to intensify their efforts by using novel technologies to overcome the inherent problems in discovering compounds which may have potential for further development as pharmaceuticals or as functional products such as cosmetics, nutritional supplements and functional foods.

3.3.1 Achievements: marine natural products in various phases of clinical development

Currently there are around 15 marine natural products in various phases of clinical development, mainly in the oncology area, with more on the way. It is now almost five decades since spongothymidine and spongouridine were isolated from the marine sponge *Tethya crypta* which eventually led to the development of Ara-C used against leukemia and Ara-A for treating viral infections (FDA approval 1969/1976). However, it was not until 2004 that the next marine natural product ziconotide (Prialt®) was approved followed by trabectedin (Yondelis®) in 2007, both of which were commercialised by European companies (Prialt by Elan, Ireland and Yondelis by Pharmamar, Spain).

Table 1. Selected marine natural products in development as anticancer drugs (based on Sashidhara K et al., 2009)

clinical trial	name	source	target	developed by
In Clinical Use	ectenaiscidin 743 (Yondelis)	tunicate	tubulin	PharmaMar, Rinehart
phase III	E7389 (halichondrin B inspired)*	synthetic	tubulin	Eisai
phase II	dehydrodidemnin B (Aplidine)	tunicate	ornithine decarboxylase	PharmaMar, Rinehart
phase II	soblidotin (aka TZT1027, dola-10 insp.)	synthetic	tubulin	Teikoku, Pettit
phase II	synthadotin (aka ILX651, dola-15 insp.)	synthetic	tubulin	ILEX
phase II	bryostatin 1	bryozoan	PKC	GPC Biotech, Pettit
phase II	squalamine	shark	angiogenesis	Zasloff
phase II	kahalalide F	mollusk	multiple	PharmaMar, Scheuer
phase I	PM02734 (kahalalide insp.)	synthetic	solid tumor	PharmaMar
phase I	Zalypsis (jorumycin insp.)*	synthetic	DNA	PharmaMar
phase I	E7974 (hemiasterlin insp.)*	synthetic	tubulin	Eisai
phase I	taltobulin (aka HTI286, hemiasterlin insp.)*	synthetic	tubulin	Wyeth, Andersen
phase I	salinosporamide A (aka NPI0052)	bacteria	proteasome	Nereus, Fenical
phase I	spisulosine (aka ES285)	clam	Rho	PharmaMar
phase I	KRN-7000 (agelasphin insp.)*	synthetic	NKT	Koezuka-Kirin
phase I	NPI 2358 (halimide insp.)	synthetic	tubulin	Nereus, Fenical
phase I	LBH 589 (psammaplin insp.)*	synthetic	HDAC	Novartis
Discontinued				
phase II (<2004)	dolastatin 10	sea hare	tubulin	Pettit
phase II (<1999	didemnin B	tunicate	antineoplastic	Rinehart
phase II (<2004)	cemadotin (dola-15 insp.)	synthetic	tubulin	BASF, Pettit
phase II (<2002)	cryptophycin 52 (≈ arenastatin)*	synthetic	tubulin	Lilly, Valeriote
phase I (2004)	discodermolide*	sponge	tubulin	Novartis, HBOI
phase I (2002)	LAF 389 (bengamide insp.)*	synthetic	MetAP	Novartis, Crews
phase I (<2006)	LAQ 824 (psammaplin insp.)*	synthetic	HDAC	Novartis, Crews
phase I (<2000)	girolline (aka girodazole)*	sponge	protein synthesis	Potier

* Substances from marine sponges

Figure 25. Left: Schematic representation of the three-dimensional structure of ziconotide ($C_{102}H_{172}N_{36}O_{32}S_7$; coloured from N-terminal end (red) to C-terminal end (blue) with disulfide bonds shown in gold), a synthetic version of a peptide first isolated from the venom of the cone snail *Conus magus*. Right: Cone shell (*Conus* sp.) ingesting a small fish after incapacitating its prey by injecting a neurotoxin which can be dangerous to humans. Ziconotide was developed into a synthetic drug for the treatment of patients suffering from neuropathic pain. It was approved for sale under the name Prialt® by the FDA in December 2004 and by the EU in February 2005.

Information Box 4. The case of Trabectedin, a unique marine compound with anti-cancer properties

Yondelis® (Trabectedin, ET-743), is a unique anti-cancer agent that binds the minor groove of DNA. Extracts of *E. turbinata* were shown to have anti-tumour effects in 1969, but isolation of the active compound was not achieved until 1990. Phase I and II clinical trials were carried out using material derived from aquaculture, but this was not deemed feasible for the larger phase III trials. For this reason, an economically viable semisynthetic process was developed starting with the natural product safracin B isolated from the microorganism *Pseudomonas fluorescens*. Pharmamar received approval to market Yondelis® in Europe in September 2007 for the treatment of soft tissue sarcoma and this was the first drug to be approved for this condition for more than 25 years. Centocor Ortho Biotech Products, L.P. has obtained worldwide marketing rights for Yondelis® except in Europe. In September 2009, they received a Complete Response letter from the FDA regarding the NDA for Yondelis® when administered in combination with DOXIL® for the treatment of women with relapsed ovarian cancer. In November 2009, Pharmamar won final European approval for the use of Yondelis® in the treatment of ovarian cancer. This drug has now been approved for use in 57 countries worldwide, of which 26 are outside Europe. Subsequent to approval for ovarian cancer, sales have increased to € 17 million in the first quarter of 2010, delivering a net profit for Pharmamar.

Figure 26. A colony of the marine tunicate *Ecteinascidia turbinata* which is at the basis of a commercially available anti-cancer agent (Yondelis®) with sales at € 30 million in 2008, rising to € 45 million in 2009

3.3.2 Development of novel products and methodologies for Human Health and well-being

While a number of marine-derived products have found, and continue to find, industrial applications in areas such as functional foods, nutraceuticals and cosmeceuticals, the recent approvals of two important marine-derived drugs has renewed interest in compounds produced by marine macro and microorganisms, not only from within the scientific community but also from large pharmaceutical companies and SMEs. However, in the quest to discover structurally interesting new products, it is clear that it will be necessary to go beyond the current frontiers in terms of both the source of the materials that can be potentially exploited and the technologies currently being employed. In order to fully exploit the enormous opportunities and potential from Marine Biotechnology, the challenges will keep getting bigger.

Challenges in pharmaceutical discovery

The lack of interest by industry in natural products from all sources can be attributed to a number of common problems, some of which are perceived to present insurmountable obstacles. Some of these problems (taxonomy, variability, supply) are particularly acute for marine-derived compounds. These reasons, coupled with an industry-wide preference for technology-intensive discovery methods has led to the pharmaceutical industry largely turning away from natural products as a source of chemical diversity. Industry also has a lack of familiarity with the marine environment and prefers the traditional source of terrestrial resources for biodiscovery. The increasing awareness that nature covers a greater chemical diversity than synthetic chemicals and that many of these perceived obstacles can be addressed using modern technologies has resulted in a renewed interest from industry in natural products as part of their portfolio of approaches to find new chemical

entities. However, without adequate and relevant high-content and high-throughput screens and associated data capture and analysis systems, all the novelty in the marine environment will simply overwhelm any potential end-user. Where to place the screening power in the chain from biodiversity explorer to end-user is also challenging, as industry is often reluctant to take on novel molecules without adequate evidence of likely efficacy and safety, but it is unlikely that researchers discovering novel molecules have the capacity or resources to carry out such high-content broad-target screening.

The main challenges facing pharmaceutical discovery from marine bioresources are:

- Legal aspects: secure access to marine resources, property rights and intellectual property[5];
- Quality of marine resources: identification and variability;
- Technology: screening of active compounds and dereplication (preventing repeated rediscovery);
- Structural costs of drug discovery from natural products and especially marine products.

Addressing the challenges

(i) Biodiversity issues

The lack of taxonomic expertise for marine species can lead to either the unnecessary recollection of a particular species or, conversely, difficulty in targeting the recollection of a species when this is required for further study. A similar situation exists for marine microorganisms where there are very few experts in taxonomy and systematics. An increase in marine invertebrate and microorganism taxonomy expertise and effort, employing both classical and DNA methods, is required to increase the speed and efficiency of marine biodiscovery. Another problem for biodiscovery to address is that the same marine invertebrate from separate locations often produces different metabolites, making it difficult to recollect specimens to isolate material for follow-up studies. This may result from the fact that symbiotic microorganisms are often believed to be the true source of the metabolites, causing this observed variability with different microorganisms present in the same invertebrate species collected in different locations.

(ii) Supply issue

The lack of a sustainable supply has completely stopped further development of several promising marine-derived compounds. In some case, to overcome this synthesis or semi-synthesis has yielded kilogram quantities of compounds or their analogues for clinical trials and applications. In other cases material for clinical evaluation has been supplied through aquaculture using adaptations of existing methods for the seafood industry. The increased focus on culturable marine microorganisms is in part due to the need to overcome this supply problem and has led to the discovery of a number of highly active compounds, one of which, Salinosporamide (NPI0052), is in phase II clinical trials sponsored by Nereus Pharmaceuticals of San Diego, USA. The search for methods to cultivate microorganism symbionts from marine invertebrates to produce the bioactives found in the invertebrate needs to be intensified. Finally, methods to find, identify, clone and genetically manipulate complex biosynthetic pathways will need to be greatly improved to have an impact in resolving the supply issue.

(iii) Technical issues

A recent statistical survey analysing chemical space indicated that marine-derived compounds showed the broadest coverage of this space, including many drug-relevant areas. In addition, fungal and actinobacterial products populate the boundaries between natural product and drug-like space, making them worthy of investigation. This study (Grabowski, Baringhaus and Schneider, 2008) also highlighted that few plant-derived natural products populated drug-like space. Therefore marine natural products, particularly those from actinobacteria and fungi should be regarded as favoured sources of chemical diversity for drug discovery, and must be made compatible with current industrial paradigms.

New chromatographic instruments and media together with simple protocols are available to prepare extract libraries with drug-like properties. In addition, these methods prepare extracts much more suited to modern high-throughput or high-content screening platforms. The use of such methods would also increase the speed of follow-up isolation and structure determination once an active compound has been identified. Bioactive compounds found at this stage have to be screened for their novelty. This is done through the use of dereplication ('de-discovery') at multiple levels. The use of organisms from unexplored and extreme environments is an excellent starting point together with the use of reliable taxonomy to source genetically distinct organisms will increase the chances of finding novel chemistry. Extracts and fractions can be screened using biological methods to pinpoint compounds with known activity profiles. Coupled liquid chromatographic methods can identify known compounds, and more recently, statistical methods have been applied to spectroscopic data of extracts as a dereplication tool.

5 This challenge is not limited to the pharmaceutical industry but applies to discoveries from marine bioresources for all other industries as well

Figure 27. Life near an active smoker at the Rainbow hydrothermal vent field located southwest of the Azores on the Mid-Atlantic Ridge (MAR) at 2270- 2320 m depth in international waters. The rainbow hydrothermal system is associated with vent fluids at temperatures as high as 364°C. The use of organisms from unexplored and extreme environments is an excellent starting point to source genetically distinct organisms which increases the chances of finding novel chemistry. (Picture taken by the ROV Victor 6000)

At the stage of pure compound, dereplication can easily be achieved through interfacing spectroscopic information with databases. Further development of novel rapid dereplication tools is required.

The use of modern spectroscopic methods utilising very sensitive high field NMR spectrometers and extremely accurate MS systems has reduced the difficulty in determining complex structures from limited amounts of material. The advent of computer aided structure elucidation packages using a combination of expert systems and constraint satisfaction paradigms offers the possibility of reducing the time taken to deliver accurate and reliable solutions. These tools need to become more widely available, faster and easier to use as well as being able to deal adequately with the relative and absolute stereochemistry of complex compounds.

(iv) Lack of a coherent rationale

It is now becoming clear that there are evolutionary reasons, elucidated at the genetic and protein fold level, why natural products fit so well into target active sites. More studies to understand and validate the coupling between genetics, protein folding, targets and natural products are necessary. The rational use of such information will lead to a greater acceptance of natural products in the drug discovery pipeline. In addition, the use of chemical ecology in the selection of organisms will improve the 'hit rate' in assays where the target and ecology of the organism are carefully matched. For instance, proven antifeedants have been used to derive anti-cancer, anti-viral and anti-aging products; toxins have been used to develop pain-killers; and functional products have been obtained from the materials used by sessile organisms to colonise free surfaces. Knowledge of eco-physiological interactions may serve as a platform to facilitate the search for new biotechnological candidates, as well as to optimise culture conditions and achieve production of biomass for industrial applications.

Information Box 5. The search for novel antibiotics: an urgent challenge

Hundreds of publications and public reports have drawn attention to the problem of antimicrobial resistance resulting from the intensive use of antibiotics worldwide for human health and veterinary purposes. Multi-resistance dramatically reduces the possibility of treating infections effectively and increases the risk of a fatal outcome. A better use of existing antibiotics is certainly needed but new antibiotics to tackle resistant bacteria are also urgently needed. However, the antibiotic development pipeline is dry and for several reasons the industry has been, and is still, reluctant to invest in research and development of antibiotics. The lack of investment, despite various incentives to encourage drug companies to invest in this field, is mainly due to the fact that a novel antibiotic may be less profitable than drugs in other therapeutic areas (low frequency prescriptions for severe infections, limited duration of use compared to drugs for chronic diseases, possible rapid growth of resistance shortening clinical lifespan, etc.). There may need to be a policy shift to support increased research and focus from the industry in this area.

The World Health Organisation has identified antimicrobial resistance as one of the three greatest threats to human health. The European Centre for Disease Prevention (ECDP) and the Infectious Diseases Society of America (IDSA) have both, in recent reports, highlighted that there are few candidate drugs in the pipeline offering benefits over existing drugs (for more information see: http://www.ecdc.europa.eu/en/healthtopics/Pages/Antimicrobial_Resistance.aspx). The ECDP and IDSA analysed changes in policy needed to tackle this problem. Meanwhile the European Union has committed to the development of innovative solutions to make sure that the boon of antibiotics is never lost and that we do not have to face a post-antibiotic era (http://www.consilium.europa.eu/uedocs/cms_data/docs/pressdata/en/lsa/111608.pdf).

For the same reasons the IDSA has committed to the 10x'20 initiative which entails the development of ten new antibiotics by 2020.

In 2009, the EU and USA agreed, through the Washington Declaration,

"To establish a transatlantic task force on urgent antimicrobial resistance issues focused on appropriate therapeutic use of antimicrobial drugs in the medical and veterinary communities, prevention of both healthcare- and community-associated drug-resistant infections, and strategies for improving the pipeline of new antimicrobial drugs, which could be better addressed by intensified cooperation between us."

The fact that most antibiotics in current use are from terrestrial origin, and that screening projects mainly focus on samples from terrestrial environments, suggests that the probability of finding novel antibiotic families from the same type of environments is rather low. In order to increase the probability of finding novel bioactives, it seems reasonable to target untapped reservoirs of genes. Obviously the greatest such reservoir on the planet is the marine environment with its enormous range of ecological niches.

For the above reasons, we recommend that special attention is given to the search for novel antibiotics from marine environments. Referring to the IDSA 10x'20 initiative, appropriate measures should be taken to strengthen the focus on marine environments, e.g. developing a 5m x'20 initiative (5 new antibiotics of marine origin by 2020).

Summary Box 11. Recommendations to improve biodiscovery of novel marine-derived biomolecules and the development of new tools and approaches for human health

- Simplification of access and benefit sharing agreements throughout Europe and its territories through the development of a common template agreement. However, simplification and harmonisation of regulations on access and 'fair and equitable' benefit sharing (commonly called ABS) from the exploitation of genetic resources should not be limited to the European level but also needs to be addressed at the international level;
- Resolution of the clash between the UN Convention on Laws of the Sea and intellectual property rights with the assistance of legal experts and practitioners/industry;
- A workable legal environment must be developed to bring functional products to the market safely, quickly and at low cost;
- Increased focus on the taxonomy, systematics, physiology, molecular genetics and (chemical) ecology of marine species via additional targeted basic research funding, including training programmes in taxonomy of marine species. Greater emphasis is needed on organisms from unusual and extreme environments to increase the chances of success in finding novel bioactives;
- Improvements in technical aspects of the biodiscovery pipeline are necessary to make marine derived compounds acceptable to the pharmaceutical industry. This includes separation of bioactives, dereplication strategies and structure determination methods and software;
- For sustainable modes of supply there will need to be a focus on integrated development of aquaculture, tissue culture, microbial isolation/culture, chemical synthesis/semi-synthesis, molecular genetics and the availability of appropriate central resources for scale-up by these methods;
- Develop appropriate measures to strengthen the focus on marine environments to secure a 5m x'20 initiative (5 new antibiotics of marine origin by 2020). These measures should be included in the comprehensive action plan on 'innovative incentives for effective antibiotics' that the European Commission has to publish by December 2011.

3.3.3 Nutraceuticals and cosmetics

Currently there is also great interest in marine derived products as nutraceuticals and cosmeceuticals because of their beneficial effects on human health. These often have drug-like properties (hence the term –ceutical) and contain active ingredients such as vitamins, phytochemicals, enzymes, antioxidants and essential oils which are finding uses as natural additives in foods, as nutritional supplements including colour additives and antioxidants, and as vitamins, oils, and cofactors which enhance general well-being. These products form the middle ground between bulk products such as biofuels and the high value pharmaceuticals in that they are medium-volume/medium-value products which have a rapid route to market. In fact many companies have chosen to go along the functional product route as it offers lower risk and a quicker potential return on investment than the high-risk high-reward pharmaceutical market.

Information Box 6. Astaxanthin as an example of a multi-functional high value compound derived from marine biotic resources

The carotenoid astaxanthin is a pigmented anti-oxidant produced by many microalgae which is responsible for the red colour often associated with crustaceans such as shrimps, crabs, and lobsters. Astaxanthin has been used extensively in the feed of farmed fish as a nutritional supplement and is partly responsible for the strong colouration often observed in farmed salmon, a fish which naturally accumulates astaxanthin in the wild resulting in the pink hue of its flesh. Recent studies have shown that the carotenoid astaxanthin is effective in reducing inflammation and in stimulating the immune system in humans. *In vitro* and *in vivo* studies have also shown anti-tumour effects, as well as effectiveness in preventing and treating retinal oxidative damage and macular degeneration. A demand for natural astaxanthin is now emerging in the fast-growing, multi-billion dollar nutraceutical market. In fact, increasing evidence suggests that astaxanthin is a stronger antioxidant than vitamins C and E, or than other carotenoids such as β-carotene, lycopene, lutein and zeaxanthin. Algatech based in the Arava desert in Israel is a worldwide leader in the production and supply of natural astaxanthin for human applications but there are many other examples of biotechnology companies which produce astaxanthin as dietary supplements.

Current high value chemical markets from marine organisms are mostly focused on a limited number of high value chemicals such as carotenoids due to their high market value, projected to reach € 77 billion per year by 2010. Carotenoids have much potential as food colourants, feed supplements, nutraceuticals, and for cosmetic and pharmaceutical purposes.

More revealing is the discovery of the role that another carotenoid, fucoxanthin, may play in reducing obesity. So far, only animal studies have been conducted but these show that fucoxanthin, found in edible brown seaweed and other marine sources, promotes the loss of abdominal fat in obese mice and rats. Although it is not fully understood how fucoxanthin works, it appears to target protein UCP1 that increases the rate at which abdominal fat is burned. Fucoxanthin has also been found in animal studies to decrease insulin and blood glucose levels. Researchers hypothesise that fucoxanthin may have anti-diabetes effects because it stimulates the formation of ω-3 fatty acids which are thought to increase insulin sensitivity, improve triglycerides and reduce LDL ('bad') cholesterol.

The other major market is for polyunsaturated ω-3 fatty acids such as EPA, DHA and ARA which are purported to have a range of beneficial effects including improved heart health and reduced inflammation. Several population studies report that dietary ω-3 fatty acids or fish oil may also reduce the risk of developing breast, colon, or prostate cancer. A recent report from the Global Organisation for EPA and DHA (GOED) indicates that the ω-3 market had reached US$ 13 billion in 2008. In addition to the traditional sources of the ω-3-desirable fish oils, there are other sources either just entering the marketplace or in the pipeline. These other sources are much more expensive than fish oil but are now of interest as the price of fish oil has increased hugely since 2006. One of the new products on the market is krill oil. This oil is different from the traditional fish oils because it contains three active components: ω-3 fatty acids, phospholipids, and astaxanthin. For example, Norway's Aker BioMarine markets a range of vitamins and nutraceuticals derived from krill and krill by-products which are very rich in ω-3 fatty acids. There are also many companies and institutions evaluating different marine algae for ω-3 oils. The effort in this case is directed toward finding organisms that will produce high levels of oil, which will increase yields and improve the economics. A potential spin-off might come from the biofuels development work going on around the world with the production of algal oils in addition to biofuels.

The European market for cosmetics is booming and is the biggest in the world, at over € 27.6 billion per year. The results published by COLIPA (The European Cosmetics Association), related to cosmetic market in the year 2006, revealed that Germany had the highest consumption rate of cosmetic products (€ 11.7 billion), which covered 18.7% of the whole European market. France was in second position (€ 11.4 billion), followed by Great Britain (€ 10 billion). The first five countries, including Italy at forth position, covered more than 77% of the total world market of cosmetics. The most consumed articles were skin care products with 25.7% of the cosmetic market, followed by hair products (23.7%) and 'toiletries' (23.4%). In 2008 the innovative products (and those with high technological index) registered the most significant growth in production and sale: facial serviettes (+4.9%), whitening agents (+6.0%), and anti-age/anti-wrinkle creams (+0.5%) produced a modest but very significant income of € 447 million. Undoubtedly, the most sold products were the anti-ageing creams, covering 38% of skin care cosmetic market. In this context, safety, innovation and efficacy are always the most requested parameters by consumers, and therefore represent an important priority for cosmetics industries. Consumers are always more concerned about the mechanisms of action of the products they use, and are therefore generally interested in understanding the scientific base of the product efficacy. The trend is moving forward cosmetic natural products, in particular those derived from plant and marine organisms, as long as they are certified as free of any biological or chemical pollutant.

Various metabolites such as terpenoids, nitrogenous compounds, tocopherol, polysaccharides, carotenes, phenolic compounds, mycosporine-like amino acids, parabens, chitin, chitosan and unsaturated fatty acids from different types of marine organisms such as bacteria, micro and macroalgae, crustaceans and fish have been investigated for their potential applications as cosmeceuticals. Some specific examples include the cosmetics giant Estée Lauder which uses pseudopterosin, an anti-inflammatory extracted from a sea fan in skin lotions (Resilience™), and the French company Phytomer, a specialist in marine cosmetics which uses a blue-green algal extract in some of its products. The French cosmetics company, Clarins, uses the algae *Durvillea antarctica* in its Extra Firming Day Cream, which it markets for the treatment of mature skin. Sederma (France) sells Venuceane™, a skin protection product that includes a radical-scavenging enzyme originally discovered in the extremophile bacterium *Thermus thermophilus*.

One of the main concern for any functional products is safety and efficacy, and it is likely that legislation covering pharmaceuticals will change to include nutraceuticals and cosmeceuticals if they wish to make efficacy claims. This legislation needs to be adapted so that it does not damage a nascent industry creating unique and highly marketable functional products.

Summary Box 12. Recommendations for the development of functional products with health benefits from marine living resources

- Develop a workable legislation to bring functional products to the market safely, quickly and at low cost;
- Implement further investigations to verify the actual health benefits of functional products of marine origin;
- Develop sustainable and economically viable production methods for functional product ingredients;
- Increase industry awareness of opportunities for drug discovery based on marine resources.

Towards a common platform for biobanks

Many of the concerns outlined above, particularly those expressed by industry, can be addressed by coordinated activity between academic scientists and industry. To streamline the biodiscovery pipeline, a number of overarching changes must be made so that the content, format, and speed of academic research outputs match that required by the pharmaceutical and functional product industry. Together with these changes, a re-education is necessary to convince industry that marine bioresources are an important source of chemical diversity for drug discovery. A major focus of this campaign must be on the unique characteristics and high activity found in chemical diversity from marine species which makes the perceived risk and cost acceptable for the development of new pharmaceuticals and functional products.

Throughout Europe, researchers in the field of biodiscovery utilise a vast array of methods and protocols to obtain, extract and fractionate bioresources and interact in a variety of ways with screening facilities. If this resource could be harnessed and utilised efficiently through the use of common protocols and procedures, it would be a powerful adjunct to Europe's pharmaceutical and functional product industry. A common online 'portal' could give access to all the biobanks, compound and extract libraries and bioscreening facilities. Such a portal, which could for example be maintained by the proposed European Marine Biotechnology Institute or Centre (see Section 5.2), will only work if ownership of

resources and libraries is maintained by the institutions and the portal works only as a zero cost 'dating agency' between academic partners, institutes and industry. Once a contact has been formalised, the legal aspects can be addressed. The portal should contain different types of information for different facilities, for example:

- Biobanks: type, organism focus, taxonomy of organisms, amount available, format (preserved, freeze dried, chemical extract, DNA, strain) and any residual ownership/IP rights associated;
- Extract libraries: origin of extracts, preparation, format, complexity, known activities and any residual ownership/IP rights associated;
- Compound libraries: origin of compound, structure, format, known activities and any residual ownership/IP rights associated;
- Screening facilities: targets & validation, types of screen, format, presentation of extract/compound libraries required, volume of testing possible at facility, how results should be interpreted and facility for validation and follow-up testing of actives.

Greater simplification could be attained if these facilities could agree on a common set of standards for the preparation of extracts and libraries that can be used in the majority of screening formats, as well as a common format for data exchange. This could include: the use of common extraction procedures giving drug-like purified extracts; accepted concentrations of pure compounds and assay-plate format; common presentation of data for the screening facility; common reporting format of screening data; and procedures for prioritising hits. Capability to carry out validation of hits and conduct follow-up studies to translate a hit into a lead or products is also a necessity.

In effect this would generate a number of European academic Small to Medium-sized Enterprises (SMEs) in a number of different disease areas, coordinated via a central portal. These could provide compound and extract libraries and screening services to larger pharmaceutical companies, as well as executing drug discovery projects on neglected diseases. The model of accessing a natural compound library in a non-exclusive fashion is becoming a more common mode of operation, with SMEs providing extract and compound libraries and with the large pharmaceutical companies providing expertise to effectively manage and complete the expensive large scale clinical trials.

Many of these goals are compatible with, and complementary to, the aims of the EU-OPENSCREEN project (http://www.eu-openscreen.eu/) promoted by ESFRI (European Strategy Forum on Research Infrastructures). The recommendations above will require the broadening of the OPENSCREEN project to include biobanks and extract libraries rather than just pure compound collections. On the screening side the inclusion of applications outside of chemical biology and pharmaceuticals will be necessary to evaluate potential use of materials in the personal care and nutrition market. There is also a need to integrate this activity with other elements of the biotechnology chain, including Marine Biotechnology SMEs with dedicated screens in-house, SMEs set up to provide innovative screening activities, and end-user companies, some of which may be large multinationals, since there is still a question of the minimum data-sets required to support further industrial development of novel molecules from marine sources. Industry associations should therefore be part of strategic thinking on how best to make use of marine biodiversity.

Care also needs to be taken that setting up a system of standard procedures does not lead to a limitation or restriction on innovative thinking, and an over-codification of the types of screens that might be used, which could artificially limit the scope for commercialisation of novel marine-derived molecules into truly innovative therapeutic areas and uses. Innovative predictive screens are an essential element of progress in marine bioactives, in addition to better and faster characterisation of molecules.

Summary Box 13. Recommendations to improve the use of biobanks, compound and extract libraries and bioscreening facilities for Marine Biotechnology applications

- Develop a common online 'portal', operated as a zero cost 'dating agency' between academic partners, institutes and industry, giving access to all relevant biobanks, compound and extract libraries and bioscreening facilities, where ownership of resources and libraries is maintained by the institutions;
- Collaborate with the OPENSCREEN project to incorporate biobanks and extract collections and include screens for functional products;
- Create new opportunities for interactions between marine-derived molecules and innovative high-content screens;
- Increase industry awareness of opportunities for drug discovery based on marine resources.

3.4 Marine Environmental Health: Marine Biotechnology for protection and management of marine ecosystems

Marine Biotechnology is playing an increasingly important role in optimising the sustainable use of marine environments by the wide range of human activities which require marine space and resources. Environmental applications of Marine Biotechnology are numerous and diverse, ranging from biofouling control, environmental monitoring for ecosystem and human health to marine habitat restoration, bioremediation and more generally natural resource and environment management. At the same time, it is becoming increasingly clear that the oceans represent a huge reservoir of biomolecules, genetic resources and organisms of which we still know very little. A rational exploitation of these resources for economical purposes, together with adequate preservation and management of marine environments, will be beneficial for the future use of these resources through existing Marine Biotechnology knowledge and methods or from further developments in this field.

3.4.1 Promises and achievements

Achievements in this field have been less substantial than expected during the last decade and most of the methods and techniques routinely used nowadays still rely on the use of traditional methods based on chemistry and microbiology. This is mainly the result of the complexity of marine ecosystems on one hand, and the gap between results in marine genomic approaches and the development of derived commercial assays and products on the other hand.

Biofouling can be defined as the settlement and accumulation of organisms on surfaces immersed in an aquatic environment. It is a worldwide problem with significant economical and safety implications for sectors such as shipping and aquaculture, and for the maintenance of any man-made structures deployed in the marine environment including platforms, pipes and coastal defence and monitoring equipment (see Figure 29) as a result of (amongst other):

- Increased frictional resistance (drag) of fouled ship hulls;
- Structural deterioration (corrosion) of engineered materials;
- Restricted flow through fouled aquaculture cage netting;
- Mechanical blockage of intake and outfall pipes;
- Losses in heat-transfer efficiency of marine cooling systems; and

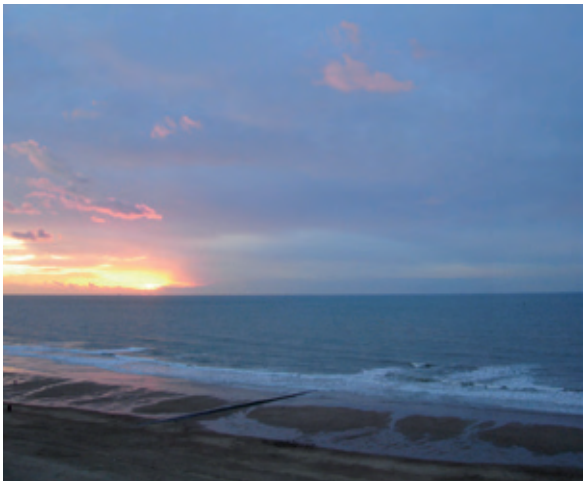

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Figure 28. North Sea sunset at Ostend (Belgium)

- Increased costs for maintaining/replacing all of the above.

Marine biofouling is the result of a succession of events occurring over time on immersed materials. Initial steps involve adsorption of dissolved organic and inorganic molecules and early microbial colonisers followed by macroorganisms like barnacles and mussels. Traditional antifouling strategies involved the use of biocidal tin-oxide marine paints (e.g. on the hulls of vessels). These paints successfully inhibited fouling for several years but resulted in contamination of surrounding waters with tributyltin (TBT) which has been shown to have a high toxicity for many invertebrates and coastal ecosystems. Following the worldwide ban on use of TBT in anti-fouling paints, there is an urgent need to develop effective and more environmentally acceptable alternatives. However, up to now it has been impossible to substitute the use of chemical paints with an efficient and lasting biobased process. While a better understanding of the biofouling process will be critical, Marine Biotechnology has already delivered some interesting results in the search for anti-fouling strategies. They notably include:

(i) The investigation of methods for immobilising bacteria including the marine bacterium *Pseudoalteromonas tunicata* which produces antifouling compounds in gels, and the use of enzymes to inhibit bio-fouling;

(ii) The evaluation of novel classes of chemical compounds isolated from marine organisms such as bacteria, microalgae, cnidaria, bryozoa, chordata other sessile benthic invertebrates and chordata with more than 160 compounds displaying biofouling inhibition properties. However, in most cases the issue of supply is a major obstacle;

(iii) The evaluation of antimicrobial peptides from crustaceans to prevent biofilm formation;
(iv) The discovery of compounds like furanone, synthesised by macroalgae that impair bacterial cell-to-cell communication systems and prevent microbial biofilm formation on engineered surfaces, of a preliminary stage of substrate exploration by barnacle larvae characterised by a weak adhesive bond that might be targeted by enzymes in order to disrupt the microbial community; and
(v) The development of biomimetic analogues of mussel adhesive proteins (MAPs) which could ultimately lead to MAP-inspired antifouling coatings which inhibit biofouling.

In spite of these and other promising research results, we are still a long way off the development of cost-effective commercial paint products containing functional biological ingredients which efficiently inhibit biofouling. The main problem is that many of these actives (e.g. antifoulants) have 'fine chemical' structures and cannot be produced in bulk, as required for commercial production. The agrochemical industry experience could serve as a model of how this can be achieved.

Ex situ **detection of marine pathogenic bacteria, viruses and toxic compounds** produced by harmful algal blooms (HABs) is still the rule and despite significant advances in research, commercial applications of Marine Biotechnology for *in situ* monitoring of marine ecosystems are not yet available. The current situation is that for most groups of toxins, even if biosensor technologies are sufficiently sensitive to comply with the regulatory limits, few of these methods have been validated and/or accepted as an alternative to the mouse bioassay[6]. In most cases, these techniques would be good tools to be used at least as screening methods in order to reduce the number of animal bioassays. Biosensor technologies offer several advantages over analytical methods and animal bioassays including low cost, speed and ease-of-use, and avoidance of legal and/or ethical issues related to the use of laboratory animals. The gene probe-based monitoring technologies currently under development for detection and quantification of bacterial and viral disease agents are still expensive. Some molecular probes able to detect the presence of toxic species have already been developed but they do not provide information on the level of expression of genes controlling toxin production.

The monitoring of man-made chemicals in the marine environment is still mainly based on chemical analytical techniques (chemical concentrations in water, sediment and biota). However, to evaluate the true impact of these substances on the different compartments of coastal and open sea ecosystems, there is a clear need to fully implement state-of-the-art environmental risk assessment procedures which combine both exposure and effect assessments. Future European monitoring programmes should, therefore, include both chemical analyses and biological effects measurements. Marine Biotechnology can play an important role in the further development, evaluation and validation of efficient biological effects assessment tools operating at different levels of biological organisation. To achieve this, more research is needed on both conventional and new techniques such as genomics and proteomics. The latter are, due to the current lack of knowledge on the ecological relevance of associated endpoints, not yet ready for inclusion in long-term monitoring efforts.

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Figure 29. A tripod used for the deployment of oceanographic measuring instruments heavily fouled by hydroids (genus Tubularia) after two months in the sea off the harbour at Zeebrugge (Belgium) in spring 2010

Bioremediation of marine ecosystems has a long history and progress is often determined by our reaction to cleaning up major oil spills. In many cases contaminants have been removed over time from the marine ecosystems by natural processes based on the activity of endogenous hydrocarbon compound degrading microbes. In case of major oil spills, residual oil can be further broken down through a process of 'biostimulation', whereby fertilisers (Nitrogen and Phosphorous) are added to enhance the activity of microbial communities. Protocols employed during the cleanup of the 1989 Exxon Valdez oil spill in Prince William Sound, Alaska, demonstrated that the application of fertilisers led to a significant increase in the rate of biodegradation, with oil-contaminated shorelines appearing significantly cleaner after a few

6 While still *ex situ*, the Jellet tests for a range of biotoxins are accepted in the USA in place of the mouse bioassay.

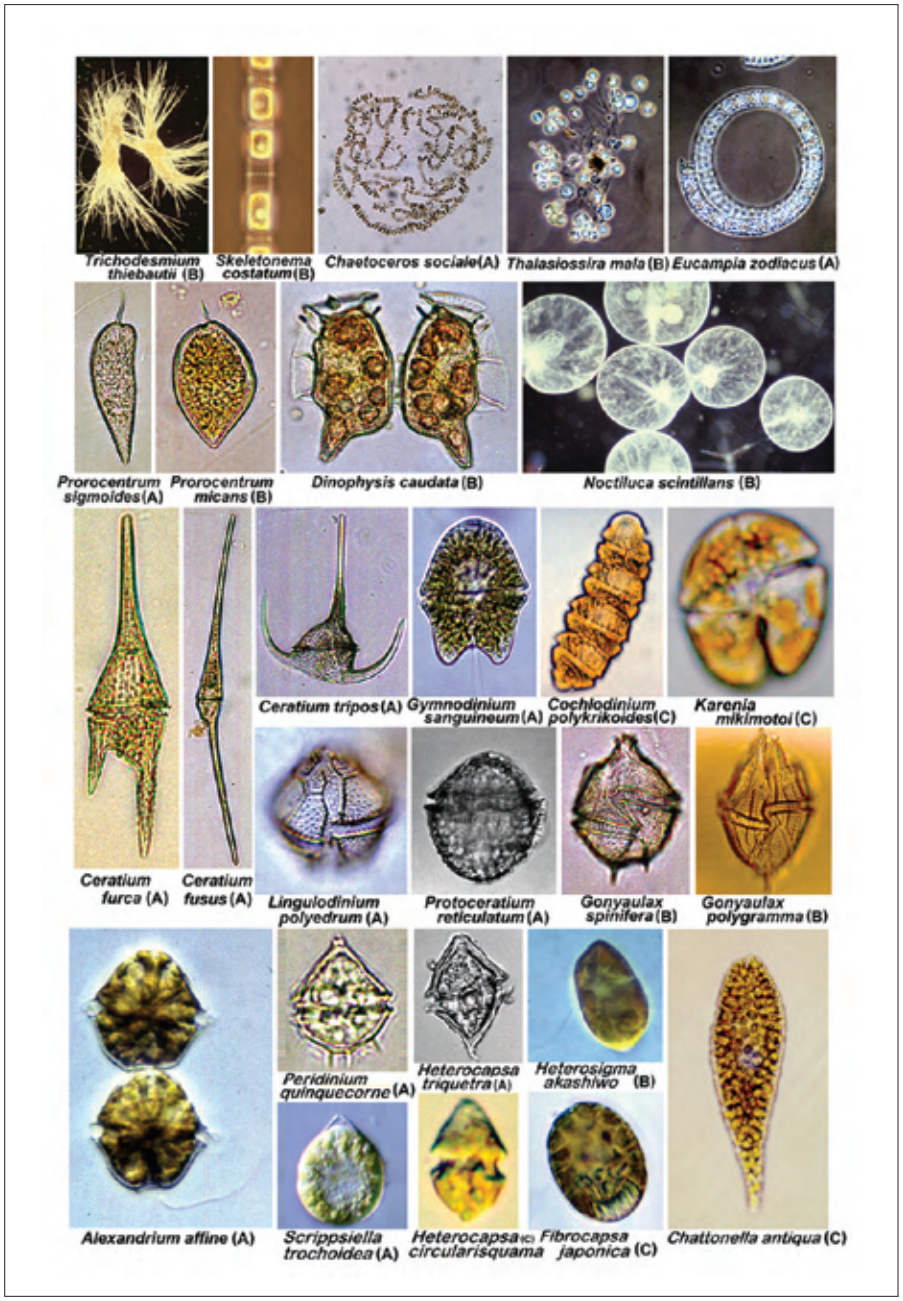

Figure 30. Red Tide Microalgae (A: Useful, mostly harmless; B: Potentially harmful by oxygen depletion; C: Harmful, responsible for fish mass mortality)

weeks of application. The time it takes for an oil spill to breakdown and dissipate naturally depends on several factors including the volume of the spill, the physical characteristics of the crude oil, the weather and sea state conditions, and whether the spill remains at sea or is carried onshore. The key benefit of biostimulation in the case of the *Valdez* oil spill was that it sped up the natural recovery process and reduced the possibility of exposure of wildlife to dangerous oil contamination. The current view is that biostimulation can shorten the recovery time for severely oil-impacted shorelines to as little as 2-5 years, compared with 5-10 years if sites are left untreated. With the ability to cut the site recovery time by half, there is a strong argument in favour of biostimulated remediation as a cost effective component of a marine oil spill response strategy.

Figure 31. National guard dressed in protective gear during the cleanup after one of the worst oil spills in Norwegian history (August 2009)

Biostimulation is based on the fact that rare taxa displaying oil degrading properties are present in most marine ecosystems. Less used and more controversial is the process called bioaugmentation: the introduction of additional oil-degrading microbial populations to the marine environment. Despite very active research in this field, the evidence remains mostly empirical and this is again demonstrated by the BP oil spill in the Gulf Mexico (April-July 2010). This is not surprising given the complexity of microbial communities and, more generally, food chains in marine environments. Detailed knowledge about the physiological aspects and bioremediation capacity of specific microbial strains may begin to emerge as whole-genome sequencing studies are initiated for key biodegrading taxa. Once entire genomes are elucidated, whole-genome DNA microarray analyses would allow detailed examination of the expression of all the genes in the genome under a variety of environmental conditions.

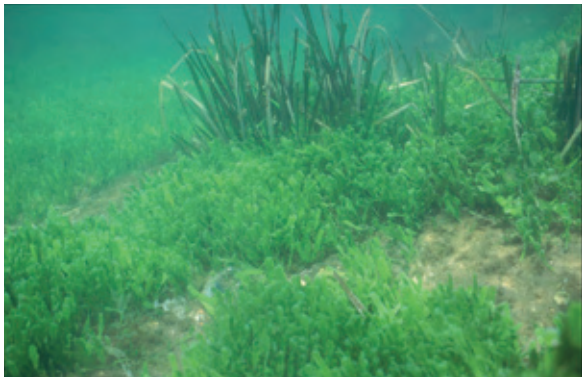

Figure 32. *Posidonia oceanica* (commonly known as Neptune Grass or Mediterranean tapeweed) surrounded by *Caulerpa taxifolia* (Théoule-sur-Mer, France). *Posidonia oceanica* is a seagrass species that is endemic to the Mediterranean Sea where it forms large underwater meadows that are an important part of the ecosystem. *Caulerpa taxifolia* is a species of seaweed native to the Indian Ocean which produces a large amount of a single chemical that is toxic to fish and other would-be predators. *Caulerpa taxifolia* is one of two algae on the list of the world's 100 worst invasive species compiled by the IUCN Invasive Species Specialist Group. Marine Biotechnology could efficiently contribute to the restoration of specific marine habitats such as seagrass beds.

Obviously, to minimise the impacts of future oil spills in Europe on both marine environments and on human activities which rely on their use, a specific action plan should be developed and corresponding conclusions enforced. The isolation and maintenance of stocks of microbial strains suitable for biostimulation and bioaugmentation should be evaluated.

Marine Biotechnology can also contribute to better management of marine resources as illustrated by several examples where DNA-based methods were used for this purpose. This is the case, for example, for whales and more generally for marine mammals and sharks. The technology used is rather simple and requires characterisation of appropriate and specific molecular markers and routine PCR or multiplex PCR. DNA-based methods have been developed to identify species or populations within species for salmon, rockfish, abalone, seals, tuna, halibut, crab, and many other marine fish, shellfish and macroalgae. Highly variable DNA markers can be used to determine if a sample comes, for example, from a threatened or a stable population. In addition, preliminary work has demonstrated that Marine Biotechnology could efficiently contribute to the restoration of specific marine habitats such as coral reefs, coastal wetlands and seagrass beds. However, much more basic research on the functioning of these complex ecosystems is still required in order to understand the mechanisms controlling processes such as reproduction, inter-organism communication and stress responses and interactions.

Another management issue relates to the reduction of aquaculture impact on the environment and the containment of aquaculture risks for human health. One of the increasingly acute problems for marine aquaculture management is the control of fish diseases in more or less open environments where the use of antibiotics contributes to the increase in antibioresistance of bacterial pathogens, generating multidrug resistant bacteria that might ultimately impair both fish and human health. Depending on the diseases, diverse strategies have been developed in the past to meet profitability criteria but with little consideration of environmental issues and impacts such as the effects of high nutrient loads on aquatic primary producers and effects on altered foodweb dynamics in general. Reconsidering these strategies and, more specifically, the development of alternative approaches based on environmentally-friendly techniques is urgently required.

3.4.2 Fast forward: Marine Biotechnology innovations for environmental applications

Antifouling

To control fouling on immersed structures and notably ship hulls, requires a coating or system that will provide a clean smooth hull, resistance to slime, resistance to roughness, resistance to fouling, be long lasting and have a negligible impact on the marine environment. Despite much research and some progress in this field, there is still no viable non-toxic antifouling agent (green AF) which can meet these requirements. Up to now, the promises of laboratory assays are rarely if ever confirmed by field trials and considerable improvements should be made to stabilise green AF in commercial paints. In addition, the supply of the most promising green AF molecules is often limiting and efforts are needed to solve this problem. The development of more efficient nanostructured coating mimicking (or not) physical defences of marine macroorganisms is also needed (see AMBIO project[7]). Therefore, establishing cost-effective optimal combinations of non-toxic AF compounds for paints and designing novel surfaces remain very important objectives for the future. Multidisciplinary approaches including materials science and engineering, chemistry, toxicology, biology, omics, ecology, and modelling will be critical in solving this problem, not only for limiting the dramatic impacts of current antifouling measures on the environment, but to limit energy consumption in maritime transport and to provide a competitive advantage to Europe in this potentially lucrative field.

7. http://www.ambio.bham.ac.uk/

Tools to monitor environmental or microbial-related variables

The development of tools based on automated sensing technologies to monitor environmental or microbial-related variables is of great interest for adaptive sampling and management. Several approaches should be investigated in parallel. The first one does not belong to Marine Biotechnology *sensu stricto* but is an adaptation of innovations from medical, food control and terrestrial environmental sectors such as gene probe-based tools and Surface Plasmon Resonance (SPR)-based technology which uses chips with probes targeting toxic compounds. Specific equipment for *in situ* analyses, with technical specifications adapted to the various types of environments (e.g. coastal waters, seabed and deep-sea) monitoring stations should be developed. Some other approaches belonging strictly to Marine Biotechnology should also be further investigated, including bioluminescence-based microbial biosensors, whole-cell biosensors and the development of biomarkers. Until recently, it seemed impossible to predict the interactions of thousands of toxic compounds released in the oceans with thousands of marine organisms. Marine Biotechnology might well contribute to address this in the future using relevant biomarkers such as enzymes and hormones produced by living organisms in response to toxic compounds. The main advantage of this strategy is that it is integrative. The use of marine models coupled with omics technologies opens new perspectives in this field and deserves an increased research effort. These tools should be cost-effective, reliable (notwithstanding biofouling), and as efficient as traditional laboratory methods. Despite recent advances in gene probe-based technology, the use of derived assays on a routine basis is still not practical. In short, there is an urgent need for simple, cheap and reliable methods and techniques to circumvent these problems.

The field of marine bioremediation and habitat restoration is characterised by the fact that the intervention takes place in the open environment, resulting in interactions between physico-chemical parameters, dissolved or particulate matter and marine biota. Therefore, Marine Biotechnology will have intrinsic limits owing to our lack of knowledge and to the limits in our ability to reliably predict the effects on the environment of chemicals (fertilisers, dispersants) or microbial inoculates. Despite these limits, enzymes from marine microbes may also have a role in increasing the efficiency of oil recovery and in the bioremediation of Arctic soils resulting from increased oil operations and in human activity in the Arctic. For example hydrocarbon cold seeps and oil spills have led to the adaptation of certain marine microbiota which utilise alkanes as carbon sources.

Such bacteria and their enzymes have a potential role in bioremediation and oil processing. Indeed alkane hydroxylase genes, encoding enzymes with specificity for alkanes of varying lengths, have been isolated from deep sea and cold seep metagenomic libraries (See Annex 3). Environmental bioremediation using microorganisms is characterised by relatively low-tech empirical practices and the limited ability to use advances in microbial ecology for management. A long term approach should be followed, combining microbial ecology, including the development of mathematical models, whole genome studies for key biodegrading taxa, and field assays in mesocosms in order to improve our future capability to predict how diverse assemblages will respond in defined conditions.

Marine resource management

How to best manage marine resources on a global basis is critically important for the current and future sustainability of those resources and the environments they occupy. Marine resource management and sustainable development require an holistic approach, integrating biological, environmental, economic and social components. In many cases, the status of marine resources, notably fish and shellfish resources, is determined by the will to enforce optimal management options based on already available management tools. Paper-based traceability can be enforced by developments in molecular barcoding. DNA probe technology has reached the maturity to identify taxa routinely and will soon do so at the origin level. DNA-based analysis of marine shellfish, fish and mammals is one of the most promising approaches for detailed assessment and control of fisheries and the traceability of fresh and processed fish products. Similar tools will also be needed:

(i) To improve the monitoring and the management of the most dangerous invasive species, especially during the very first stages of colonisation;
(ii) For the control of fish and shellfish populations escaping from farms or culture grounds; and
(iii) For the control of aquacultured GMOs in case of introduction in Europe.

These capabilities should be fully developed in order to evaluate the efficacy of trade agreements and treaties on specific resources either threatened or endangered. In addition, the restoration of marine ecosystems through either habitat restoration or protection measures could benefit from molecular evaluation tools besides traditional population dynamic approaches.

A common feature of all these aspects of environmental monitoring, including bioremediation, restoration and management, is that they will benefit very much from advances in Marine Biotechnology. The most obvious step changes will be realised through DNA-based technologies and improvements in omics approaches. Developing Marine Biotechnology tools to that end could contribute to improve marine ecosystem management and reconcile the biotech development and the sustainable management of the marine ecosystems. This is both a challenge and an opportunity.

A more basic aspect impacting environmental issues is linked to the knowledge of the interactions among the various components of marine ecosystems. To a large extent these interactions are linked to issues such as the control of biofilm formation and maturation, the regulation of the microbial world and changes in ecosystems and their resilience. In order to understand, to describe and to predict these interactions, it will be very important to enhance the understanding of the social life of microbes and to obtain a more complete picture of the factors which control interactions between microbes and invertebrates.

Summary Box 14. Recommendations for the development of marine biotechnological applications for the protection and management of marine ecosystems

- Develop cost-effective and non-toxic antifouling technologies combining novel antifouling compounds and surface engineering;
- Develop automated high-resolution biosensing technologies allowing *in situ* marine environmental monitoring of coastal water quality, including prediction and detection of HABs and human and environmental health risks;
- Consolidate knowledge on DNA-based technologies for organism and population identification and support the development of commercial tools and platforms for routine analysis;
- Support basic research in all aspects of marine sciences contributing to a better knowledge of biotic interactions, including the social life of microbes, microbe/invertebrate interactions, chemical ecology, and connectivity;
- Incorporate novel Marine Biotechnology approaches in existing and new action plans for combating marine oil spills based on marine biotechnological products or processes.

3.5 Enzymes, biopolymers, biomaterials for industry and the development of other life science products

Many Marine Biotechnology advances including the isolation, synthesis or use of enzymes, biopolymers and biomaterials can have applications in several domains. Proteins and enzymes from marine organisms contribute significantly to industrial biotechnology but can also support novel process development in the food industry or in molecular biology and diagnostic kits. In the past decade, the medical, pharmaceutical and biotechnology industries have directed increasing attention towards biopolymers of marine origin for numerous applications ranging from biodegradable plastics, food additives to pharmaceutical and medical polymers, wound dressings, bio-adhesives, dental biomaterials, tissue regeneration and 3D tissue culture scaffolds. However, marine-derived biomaterials science is still relatively new and the marine environment is, as yet, a relatively untapped resource for the discovery of new biopolymers and biomaterials. All of these products with very diverse applications are of high interest for the future and will, in many cases, contribute to answer to the grand challenges discussed in Chapter 1.

3.5.1 Proteins and enzymes

To date, over 3,500 microbial enzymes have been isolated, with the majority being derived from either mesophilic bacteria or fungi, predominantly sourced from terrestrial environments. In contrast, marine environments have received little attention, with the possible exception of extreme environments. Because they live in a unique environment, marine organisms can provide some potentially useful characteristics such as an increased salt tolerance, hyperthermostability, cold adaptivity and/or barophilicity, together with other potentially novel chemical and stereochemical properties. While some enzymes derived from marine organisms have been isolated (See Annex 3), the potential of isolating enzymes from the diversity of available unique marine ecosystems has until now been largely underexploited.

Enzymes have, however, been isolated from marine extremophiles such as psychrophiles, acidophiles, thermophiles and hyperthermophiles. A limited number of biocatalysts such as amidases, lipases, proteases and carbohydrases, have been isolated, biochemically characterised, and in some cases, optimised through protein engineering. For example, enzymes from marine hyperthermophilic archaea are used in molecular biology research, diagnostics, food safety and environmental monitoring. They include DNA-dependent DNA polymerases, DNA ligases from marine *Thermococcales* (*Thermococcus* and *Pyrococcus*) that are the enzymes of choice for high-fidelity *in vitro* gene amplification.

Figure 33. *Pandalus borealis* is a species of shrimp found in cold parts of the Atlantic and Pacific Oceans. *P. borealis* is an important food resource, and has been widely fished since the early 1900s in Norway followed by other countries. At the same time this shrimp species is also the source of Shrimp Alkaline Phosphatase (SAP), a hydrolase enzyme used in molecular biology for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. Shrimp Alkaline Phosphatase (SAP) has become one of today's highest-selling DNA modifying enzymes beacause in contrast with other alkaline phosphatases (from *E. coli* or Calf intestine) SAP can be completely inactivated by heating it for 15 minutes at 65 °C.

In addition, Shrimp Alkaline Phosphatase (SAP) from Marine Biochemicals has become a popular DNA-modifying enzyme due to its heat inactivation properties. The luminescent properties of the jellyfish

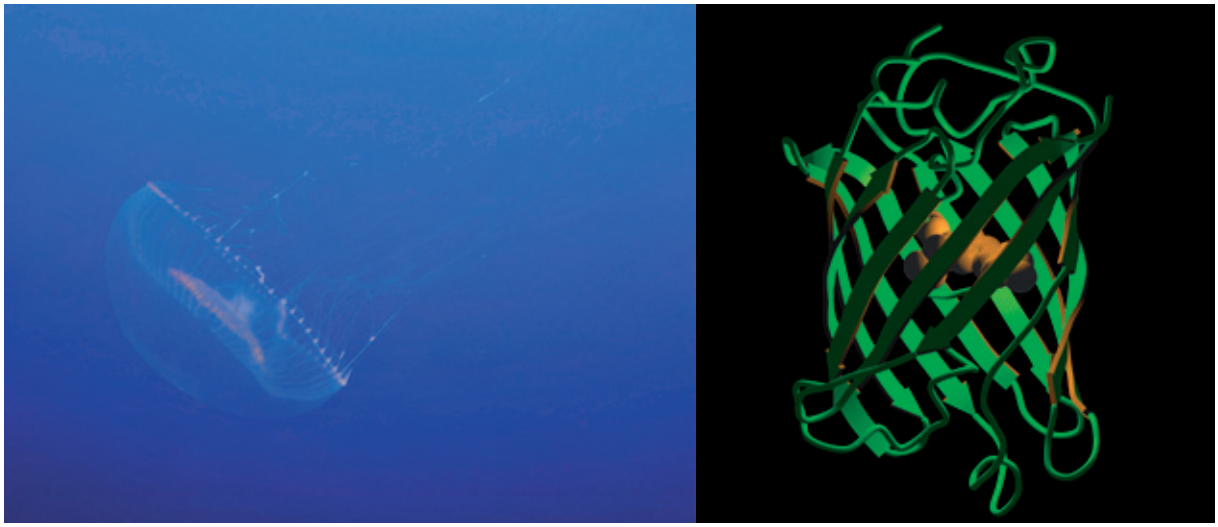

Figure 34. Jellyfish *Aequorea victoria* (left – picture taken at the Monterey Bay Aquarium, USA) and Green Fluorescent Protein (right – Courtesy Roger Tsien, UCSD). The luminescent properties of the jellyfish *Aequorea victoria* led to the characterisation of the green fluorescent protein (GFP) which has widespread uses in molecular biology as a reporter protein. Martin Chalfie, Osamu Shimomura, and Roger Y. Tsien were awarded the 2008 Nobel Prize in chemistry on 10 October 2008 for their discovery and development of the green fluorescent protein.

Aequorea victoria led to the characterisation of the green fluorescent protein (GFP). The GFP and the luciferase enzyme from *Vibrio fischeri* have widespread uses in molecular biology as a reporter protein. By using DNA technology, researchers can now connect GFP to other interesting, but otherwise invisible, proteins. This glowing marker allows them to watch the movements, positions and interactions of the tagged proteins. The aequorin protein from the *Aequoria* jellyfish has also found use as a biosensor for Ca^{2+} signalling in the research laboratory.

Enzymes such as silicateins involved in biosilicia production in marine sponges and enzymes involved in dissolving or etching silica such as silicases, have enormous potential in nanobiotechnology and biomedicine. For example, biosilica can be used as a coating for metal implants used in surgery, for drug delivery via encapsulation of bioactive compounds, and in microelectronic fabrication. In addition, silicateins and silicases may also find uses in organosilicon chemistry, specifically in drug design through the synthesis of novel drug analogues by the replacement of specific carbon atoms with silicon, or the regeneration of tooth and bone defects. Future applications may be in structuring nanoengineered fibre-optics and etching nanoscale silica structures.

The Arctic Ocean, where sea ice temperatures range from -1.9°C to 3°C, provides one of the coldest habitats on earth for marine life, and has been targeted by a number of biotechnology companies for novel enzymes. Focusing in particular on highly cold-adapted and salt-tolerant enzymes, these companies now have a number of products either in development or already on the market.

3.5.2 Marine-derived biopolymers and biomaterials

Biopolymers of marine origin are currently being examined for a wide variety of applications. There is a particularly strong interest in the biomedical sphere, with developments such as pharmaceutical and medical polymers, bio-adhesives, wound dressings, dental biomaterials, tissue regeneration and 3D tissue culture scaffolds.

Polysaccharides (also called glycans) are an emerging class of marine-derived biopolymer with numerous applications. In addition to their potential direct use as biomaterials, marine derived polysaccharides are readily amenable to chemical modification, permitting a greater flexibility in the design of, for example, novel alginate co-polymers which have significant promise as drug delivery systems. Marine macroalgae synthesise a great diversity of polysaccharides, which constitute their cell wall and energy storage. They are characterised by their high levels of sulphated polysaccharides which have no equivalent in land plants and which are currently being investigated as potential immune boosters in cattle. The ban on using antibiotics in cattle feed adds extra

impetus to the development of these novel approaches using compounds derived from marine organisms.

Red algae produce agars and carrageenans and brown algae produce alginates, fucans and laminarins. These hydrocollids are well-known for their gelling properties and are used in a variety of laboratory and industrial applications. Laminarin for example is used for the stimulation of natural defences in terrestrial crop cultures (Iodus 2®), thus allowing the partial replacement of pesticides used in conventional agriculture. In this application, the priority is given to the crop protection rather than to the pathogen destruction.

Chitin (and its derivative chitosan), derived principally from shellfish waste (prawn, crab, crayfish), can be used in combination with natural or synthetic polymers and is widely used in biomedical applications due to its lack of toxicity, biodegradability, anti-bacterial and gel-forming properties. Chemical deacetylation of chitin improves the reactivity and solubility of the resulting product, chitosan. Chitosan is also widely employed in biomedicine. In addition to the characteristics detailed above, chitin and chitosan are capable of forming films and chelating metal ions.

Figure 35. Experimental bioreactor for marine bacterial exopolysaccharide production

Figure 36. Young *Chondrus Crispus,* a species of red algae which grows abundantly along the rocky parts of the Atlantic coast of Europe and North America. *Chondrus crispus* is an industrial source of the polysaccharide carrageenan, which is commonly used as a thickener and stabiliser in milk products such as ice cream and processed foods including meat.

Collagen-based marine sponges have been utilised as a potential collagen biomaterial for bone repair. Once again, native sponge materials and chemically modified derivatives have been investigated.

Hydrothermal conversion of calcium carbonate to hydroxyapatite, a calcium phosphate compound found at high levels in mineralised tissue/bone of vertebrates, has received much attention in the past 10-15 years. Hydroxyapatite formed in this manner permits synthesis of a compound with a similar microstructure to that of bone. In recent years, bioceramics based on calcium phosphates, have been examined extensively as bone substitutes, since these materials may be bioactive (hydroxyapatite, bioactive glasses), resorbable (tricalcium phosphate), porous for tissue in-growth (hydroxyapaptite coated metals) or composites. Recently, the hydrothermal conversion of coralline algae to hydroxyapatite has also been investigated.

Bioplastics such as polyhydroxyalkanoate (PHA) have also received considerable attention owing to their high molecular weight, thermoplastic/elastomeric properties, biodegradability, biocompatibility, non-toxicity and potential for production from renewable carbon sources. PHAs are synthesised by a wide variety of Gram-positive and Gram-negative bacteria, by members of the family Halobacteriaceae of the archaea and recently by marine bacteria including *Paracoccus seriniphilus* strain E71, *Bacillus* sp. NQ-11/A2, *Pseudomonas* sp. CMG607w, a *Pseudomonas guezennei* sp, *Halomonas profundus* sp, *Pseudomonas raguenesii* and the cyanobacterium *Spirulina subsalsa*.

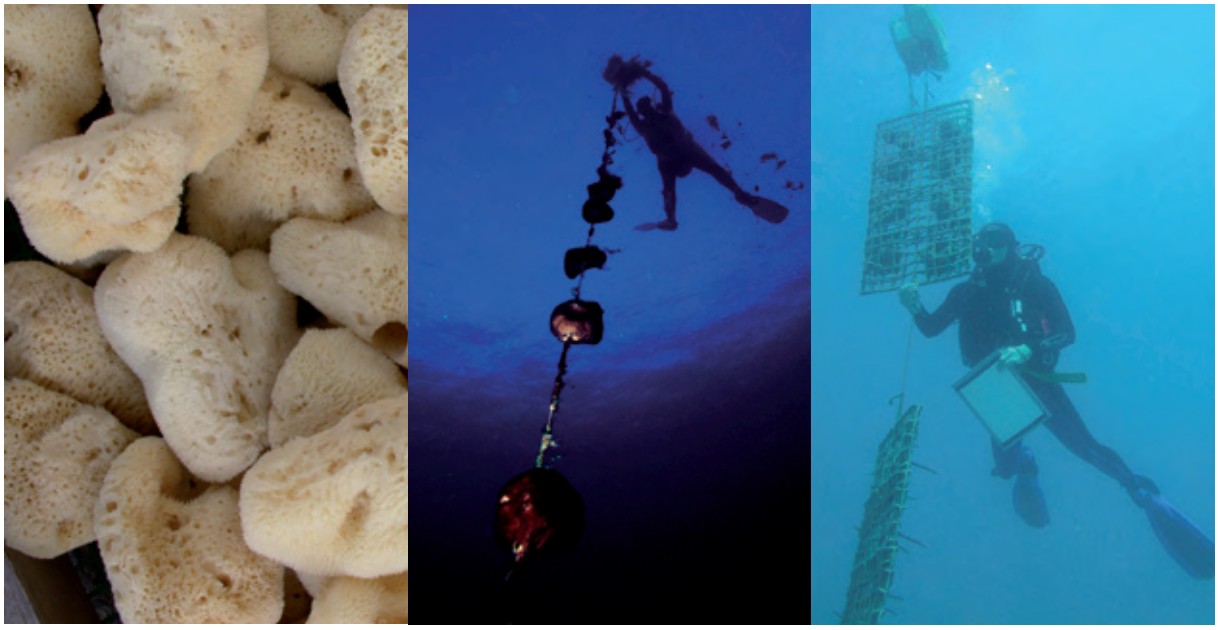

Figure 37. Research to underpin the technology and environmental management of marine sponge aquaculture to obtain high value collagen

Finally, spicules in marine sponges, which contain silicia are excellent light transmitters and exhibit some advantages over technical optical fibres such as enhanced fracture toughness and fibres with higher refractive indexes.

3.5.3 Future perspectives and avenues

There is currently an upsurge in interest by biotechnology companies in isolating and characterising novel enzymes, biopolymers and biomaterials with properties which meet identified needs and circumvent existing barriers. Biomolecules and biomaterials from marine sources are of particular interest as they likely to have novel characteristics such as increased salt tolerance, pressure tolerance, cold adaptivity, heat tolerance and may have novel physical, chemical/stereochemical as well as original biochemical properties.

Mining for enzymes

If we consider (i) the vast reservoir of enzymes identified through large-scale sequencing projects of genomes and metagenomes (mostly marine for the latter); and (ii) the current and near-future advances in screening and expression technologies (see Section 2.6), the potential to unveil novel interesting enzymes from marine sources is very high. But this does not automatically guarantee novel commercial products. Of course, the issues of supply of raw material, property rights and intellectual property, combined with the current limitations in screening and expression technologies are important (as stated in Section 2) and deserve attention. However, the main obstacle to progress in this case is the inability of academic and industry partners at EU level to work in a coordinated fashion in order to develop common projects. This cooperation is, to some extent, a prerequisite for enzyme development and commercialisation. Indeed, hundreds of novel enzymes without industrial applications have been identified through past projects and a better focus on detailed specifications of enzymes required for new processes or for the improvement of existing ones is required. Since the detailed specifications of a product, notably the precise substrate specificity and conversion efficiency can be of high value, they are not easily shared. This implies that huge screening projects of enzymes should preferentially be operated by the industry possessing the precise technical specifications of the enzymes of interest.

Marine Biomaterials

Chemical and enzymatic modification of marine-derived polymers can improve their mechanical and functional properties, biocompatibility, solubility and biodegradability. In addition, chemical modifications permit a wider application of such biopolymers and can result in significant improvements to their reactivity and the ease with which they are processed. Chemically modified, marine-derived biopolymers are

now being used in the areas of wound healing, drug delivery systems and tissue engineering. Alginates have also been investigated for their ability to act as gel entrapment systems and for their potential use in bone repair.

In recent years, there has been increasing industry interest in the isolation of novel marine microorganisms which produce extensive exopolysaccharides (EPS). Many such microorganisms produce EPS of novel chemical composition which may have applications in adhesives, textiles, pharmaceuticals and food additives. Strong sampling infrastructure/capabilities will be required for this type of approach, since most gains are likely to be made from microorganisms derived from extreme marine environmental niches.

Perhaps the area of greatest potential importance will be the development of marine-derived biomaterials which are suitable for tissue repair and regeneration. This will require a multidisciplinary approach to translate advances in the characterisation and chemical modification of lead materials and culture scaffolds to clinical applications. Identification of scaffolds onto which regenerative cells (stem cells, neuronal cells, osteoblasts or chondrocytes) can be seeded and retained to generate functional three dimensional tissue scaffolds represents a major future direction of biomaterial-based regenerative medicine.

Summary Box 15. Recommendations for the discovery and application of novel enzymes, biopolymers and biomaterials from marine bioresources

- Actively support the development of enabling technologies for high-throughput enzyme screening and for the expression of marine proteins and enzymes through dedicated hosts;
- Actively support projects aiming at the emergence of marine biopolymers as novel competitive commercial products in food, cosmetics and health.

4. Supporting the development of Marine Biotechnology

4.1 Facilitating access to marine resources, biodiscovery and marine bioresource information

The coasts, seas and oceans around Europe host an incredibly diverse set of ecosystems and habitats ranging from coastal marshes to lagoons, bays, deep-sea, polar and tropical marine environments, tropical and cold coral reefs, deep-sea anoxic sediments and brine lakes, continental margin cold seeps and mud-volcanoes, and coastal and deep-sea hydrothermal vents. These ecosystems, in turn, host an extraordinary biodiversity.

The European Union has a complex mode of regulation and exploitation of the marine resources in its EEZ (Exclusive Economic Zone), ranging from common policies (for aquaculture and fisheries) to national and in some cases regional policies for other types of living resources, including resources for biotechnology. Sampling of marine resources for biodiscovery ('bioprospecting') is controlled by each Member State and special permits are normally required to sample habitats of interest. In many cases, this means that bioprospecting in the waters of a third country is allowed only for the purposes of academic research, even if the material taken is of negligible intrinsic value. In fact, the 'gold mine' syndrome (according to which each crude sample contains a hidden treasure) hampers the ability of partners to agree on an *a priori* chain value. An alternative approach would be to uncouple access from benefit sharing as in the case of biodiversity legislation used in Queensland, Australia[8].

Another obstacle has regularly been identified in EU research projects which involve collaboration between academia and industry. Often academic scientists and commercial enterprises will differ on how to utilise and/or protect promising research results. Complex intellectual property (IP) and consortium agreements can act as a disincentive for SMEs to participate in research partnerships with academic institutions. Where appropriate, SMEs should own the IP derived from SME/academic collaborations since SMEs need it to raise investment. On the other hand, the interests of universities and public research organisations must also be preserved in the frame of partnership with the industry to avoid negative effects.

Beyond the EU-EEZ, European countries have access to the 'zone' under international jurisdiction thanks to research fleets, deep-sea submarines and ROVs from several countries. The United Nations Convention on the Law of the Sea (UNCLOS) governs access to the seabed, and rights to conduct scientific research in international waters (see also Section 4.2 below). The sea and seabed are regarded as common human heritage, and the convention states that research in these waters must address '*peaceful uses of the seas and oceans, the equitable and efficient utilisation of their resources, the conservation of their living resources, and the study, protection and preservation of the marine environment*'. The convention was developed before the biotechnological potential of the oceans had been anticipated, and many of its statements refer to large scale mining of the seabed for resources, and not for the small-scale sampling necessary for biotechnological applications. The main issue to be resolved is the clash between the need for ownership to claim intellectual property protection over an invention derived from marine genetic resources, and the convention's statement that '*no state shall claim sovereignty over any part of the area or its resources*' (although there is no specific reference to marine genetic resources). Additionally, the freedom to conduct scientific research in the oceans requires the sharing of results, again incompatible with the need to keep inventions secret until a patent application has been filed. UNCLOS states that the International Seabed Authority '*shall provide for the equitable sharing of financial and other economic benefits derived from activities in the area*', although it provides no clear mechanism for doing so. It is also worth noting that a number of countries bordering the Arctic (Denmark, Norway, Canada, Russia and the US) are currently claiming territorial rights to Arctic water, with a view to future exploitation. All of these issues need to be resolved before we can develop a genuinely equitable and transparent process to open access for the sampling of marine resources for biotechnological applications.

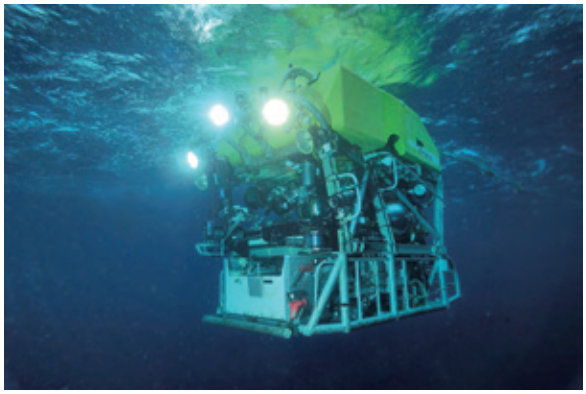

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Figure 38. Remotely Operated Vehicle (ROV) Victor (Ifremer, France). ROVs are indispensible tools to explore the deep-sea.

8 See Queensland Nature Conservation Act 1992 and Biodiscovery Act 2004 available at http://www.environment.gov.au/biodiversity/science/access/contacts/qld/index.html

The absence of a common policy to provide equitable access to marine resources for biodiscovey is further compounded by a complete lack of coordination between Member States on this issue and concrete coordination bodies such as a 'Marine Biotechnology Centre or Institute' operated at the European level or within the frame of an intergovernmental agreement. It is, therefore, difficult to get an accurate up-to-date figure on the status of biodiscovery based on marine resources in Europe, at least for the primary steps in the process which include sampling, and possibly culture, fractionation, and screening for active compounds. An analysis of the scientific literature will shed light on some of the marine biodiscovey effort that is ongoing but will provide little if any information on screening activities being undertaken by the private sector which is missing.

For marine drug discovery, the last decade was characterised by a very low level of investment from major pharmaceutical companies. Moreover, the pharmaceutical industry has tended to privilege R&D on chronic diseases with less attention paid to infectious ones, driven by the need to minimise risk and maximise profitability. In fact, marine drug biodiscovery has been mainly conducted by academic teams funded by governments and a few small and medium sized pharmaceutical companies willing to assume the risks of this preliminary step in drug R&D.

However, EU policy during the last decade has already paved the way for further coordination in marine biodiscovery at European level. Multiple initiatives have been developed and there is already a very active network of marine biological stations with a specific policy dedicated to the management and conservation of various marine resources (see Section 4.2 below).

4.2 Marine bioresource and biotechnology research infrastructures

Since the year 2000, the European Commission has been working with Member and Associated States towards the development of the European Research Area (ERA), one of the goals of which is to better integrate the scientific communities and the infrastructures they need to conduct interdisciplinary and collaborative research. The Networks of excellence (NoEs) funded under FP6 have contributed to this integration, including some marine-focused projects. The overall objective of the **MarBEF** NoE, was to investigate the relationships between marine biodiversity and ecosystem functioning and to understand the economic, social and cultural value of marine biodiversity. The major goal of the **Marine Genomics Europe** NoE was to integrate genomics with marine biology in order to implement high-throughput approaches in genomics and post-genomics in the biology and ecology of marine organisms and ecosystems. **EUR-OCEANS** NoE focused on the development of models for assessing and forecasting the impacts of climate and anthropogenic forcing on food web dynamics of pelagic ecosystems. Even though the communities covered by these three large projects were only partially overlapping, their members came from the main marine research institutes in Europe as well as a number of other partners from transdisciplinary fields of expertise (e.g. genomics, data bases, outreach). In total, over one hundred marine institutes and close to two thousand scientists have contributed to the activities of the three marine Networks of Excellence and this large community represents an impressive potential both in academic and applied research (see Annex 2 for some of the major achievements of these marine Networks of Excellence). While the project funding has ended, all of the marine NoEs are pursuing, through different mechanisms, lasting integration of their networks.

This momentum has deeply shaped the area of marine biology and although there is still progress to be made, we are now in a much better position to collectively address key challenges for the successful development of Marine Biotechnology. The community is much less fragmented than before and several infrastructures such as Marine Institutes and Marine Biological Resource Centres (MBRC) have gained visibility and are more accessible to scientists from throughout Europe. MBRC, however, do not cover all academic groups in Europe involved in Marine Biotechnology and further interaction with, and involvement of, other relevant research groups and infrastructures is needed.

Other activities and networks supported by the EU, which can contribute to the integrated view of Marine Biotechnology, include ASSEMBLE, TARA Oceans, EUROFLEETS, MARS and, above all, projects under ESFRI, the European Strategy Forum on Research Infrastructures.

ASSEMBLE is an EU FP7 research infrastructure initiative comprising a network of marine research stations which provide transnational access to a comprehensive set of coastal marine ecosystems, research vessels, state-of-the-art experimental facilities and to a wide variety of marine organisms. A major objective of ASSEMBLE is to enhance complementarity and interoperability of the various Marine Biological Resource Centres and to collectively provide a representative set of experimental ecological and biological systems to feed, for example, developments in genomics and post genomics. The **TARA Oceans** project is a three-year

Figure 39. ROV deployment from RRS James Clark Ross operated by the National Oceanography Centre, Southampton, UK, on behalf of NERC.

international scientific expedition aiming to study the composition and dynamics of planktonic ecosystems and certain unexplored coral reefs around the globe. TARA Oceans is developing a new integrated marine biobank to integrate all the data acquired in the course of the expedition. The goal is to provide a tool to extract functional correlations between genes, the diversity of organisms present in a given region and its physical environment. While the main objective of TARA Oceans expedition is to study the effects of climate change on marine biodiversity, the integrated biobank could be screened for biotechnological applications.

There are also a number of initiatives for improving the coordination of European Marine Research fleets. **EUROFLEETS** is another EU FP research infrastructures project which aims to bring together the European marine research fleets to enhance their coordination and promote a more efficient, collaborative and cost-effective use of the vessels and their associated heavy equipment. **OFEG**, the 'Ocean Facilities Exchange Group' represents Europe's leading oceanographic research organisations and provides a forum to consider exchange and co-operation opportunities for the Global and Ocean Class research fleet. OFEG aims to maximise the overall scientific output of its partners, using its state-of-the-art facilities in support of the worldwide oceanographic community. Finally, **ERVO**, a network of European Research Vessel Operators, aims to promote the co-ordination of small to medium sized research vessel operators in Europe. The harmonisation of the European fleet is essential for facilitating international access to marine biodiversity and to remote/extreme ecosystems such as deep sea habitats, polar environments or the open ocean. These areas will surely provide numerous new species, novel molecules and biocatalysts for biotechnological applications.

Another level of integration is realised with the **MARS** foundation, a network of European marine research institutes and stations which pursue common goals such as generating a critical mass and focus for European and global marine research activities, engaging stakeholders and facilitating 'durable integration' of marine research stations in the long-term.

ESFRI, the European Strategy Forum on Research Infrastructures will also have a major impact on marine research infrastructures and will play a role in Marine Biotechnology development in Europe since the forum aims to identify the scientific needs for research infrastructures for the next 10-20 years in the context of the Lisbon agenda. ESFRI projects are generally characterised by large investments and long project lead-times, with associated needs for long-term commitment. In the ESFRI policy, research infrastructures are at the core of the knowledge triangle comprising Research, Education and Innovation. So far, four marine projects have been adopted by ESFRI, some of which are in the negotiation phase while others are still in the preparatory phase:

- **EMSO** 'European Multidisciplinary Seafloor Observatory' is a distributed infrastructure for long term monitoring of environmental processes;
- **EURO-ARGO** is a global ocean observing infrastructure delivered through an array of Argo autonomous monitoring floats throughout the ice-free areas of the deep ocean;
- **EMBRC** 'European Marine Biological Resource Centre' is a distributed pan-European infrastructure including the main existing coastal marine laboratories for providing access to model marine organisms and related genomic resources;
- **LIFE WATCH** 'Science and Technology infrastructure for Biodiversity data and observatories' is not strictly marine but is a distributed e-infrastructure for providing access to interoperable biodiversity databases and biological collections.

These diverse initiatives truly reflect an unprecedented momentum that is fundamentally changing the European landscape for marine sciences and marine research infrastructures. This momentum can be characterised by the following keywords: critical mass, interoperability, integration, complementarity, and the sharing of access to resources, infrastructures and data. At the European level, the challenges are now to build on the achievements of the projects mentioned above, keep the momentum to improve collaboration and to streamline the access to 'marine knowledge' through the shared access to marine research infrastructures and

to samples that are deposited in marine biobanks. The new context described here could provide a beneficial background and novel opportunities for fostering Marine Biotechnology provided that the topic is clearly acknowledged as a priority.

Summary Box 16. Recommendations to improve access to marine bioresource and biotechnology research infrastructures

- Identify recognised Marine Research Centres, promote synergies and facilitate interactions between these centres where the scientific expertise, the resources and the technological platforms are established, and where there are new emerging Marine Biotechnology start-ups or companies. One model could be to develop 'incubators' or marine biotechnological institutes co-located with marine laboratories as demonstrated at the European Centre of Marine Biotechnology, based at the Scottish Association for Marine Sciences (SAMS) in Oban, Scotland;
- Biobanks and Biological Resource Centres (BRCs) must meet their obligations with respect to the convention on biodiversity. If the bioresources held at these biobanks are to be shared throughout Europe, then very clear access and benefit sharing agreements must be put in place;
- Encourage and establish linkages between current and planned European scientific research infrastructures networks and Marine Biotechnology. This could be part of the activities of the proposed European Marine Biotechnology Institute or Centre (see below);
- Establish a European Marine Biotechnology Institute or Centre, at least virtual, through a permanent Secretariat and network with key nodes, to capitalise the knowledge and experience gained in different Member States for further optimisation of nationally based projects, cooperative initiatives and funding.

  The proposed European Marine Biotechnology Institute or Centre should be set up at the EU level or as a major inter-governmental research organisation, with equivalent standing to the European Molecular Biology Laboratory (EMBL). It should be charged with developing Europe's Marine Biotechnology research capabilities through a range of collaborative actions including: the provision of research leadership in Marine Biotechnology; establishing and operating a European Marine Biotechnology Portal; facilitating access to essential infrastructure to ensure that European Marine Biotechnology research remains competitive; training early stage scientists via a European Marine Biotechnology graduate development programme; operating a Marine Biotechnology resources centre and strengthening the awareness of Marine Biotechnology across European industry. In doing so, the European Marine Biotechnology Institute or Centre would capitalise the knowledge and experience gained in the different member states for further optimization of nationally based projects, cooperative initiatives, biobanks and funding. Such a Centre would also contribute to securing adequate supplies of biological material and provide a basis for much stronger industry-academic collaborations and partnerships. The establishment of the European Marine Biotechnology Institute or Centre could play a catalysing role in strengthening European Marine Biotechnology research capability and would enable critical recommendations put forward throughout this Position Paper to be addressed.

4.3 Education, outreach, integration and interdisciplinarity

Education: training the next generation of marine biotechnologists

To promote Marine Biotechnological innovation, training of the next generation of scientists is critical. They must have more interdisciplinary expertise and use tools from various disciplines to address questions related to marine organisms and to solve problems posed by the marine environment. This statement is not specific to Marine Biotechnology; in fact the future of life sciences in the 21st century will depend upon the ability of scientists to develop interdisciplinary projects embracing skills and concepts from, for example, phylogeny, mathematics, chemistry, and the physical, engineering, computational and social sciences. The challenge for the development of the Marine Biotechnology sector is to ensure that undergraduate and graduate training programmes related to marine sciences include adequate training in biotechnology.

Figure 40. Marine science fairs provide excellent opportunities to present the opportunities and societal benefits offered by biotechnological applications based on marine organisms to children and the public at large.

Outreach: training the European citizen and other relevant stakeholders

The proficient dissemination of novel research discoveries will unquestionably provide new opportunities in marine biotechnologies for the industrial sector. Nevertheless, the great potential of marine biology for biotechnology innovation is still not realised. Marine Research centres in academic institutions are largely focused on fundamental research and there is insufficient interaction between scientists from these centres and potential end-users in the private sector or in governmental decision-making bodies. There are many obstacles which can block the effective transfer of knowledge from the science community to the business world such as insufficient support and motivation, legal issues and cultural disparity. Therefore, improving the dissemination channels that will facilitate this knowledge transfer is an important challenge for the successful development of Marine Biotechnology in Europe. The Biosciences Knowledge Transfer Network (BKTN) in the UK, which provides neutral information on the value and applications in various biotechnology fields, provides a good example that could serve as a model for such initiatives.

Summary Box 17. Recommendations to improve education, training and outreach activities related to Marine Biotechnology research

Training the next generation of marine biotechnologists

- Include biotechnology in all marine biology training programmes;
- Create a European School or Course on Marine Biotechnology, which could be virtual and distributed. A distributed virtual institute would also be useful as a central point of interaction with the commercial sector;
- Create European MSc and PhD programmes in Marine Biotechnology;
- Create schools information packs based on an inventory of existing information and adaptation/extension of this information, in relevant languages;

Training the European citizen and other relevant stakeholders

- Create a series of journalist and media briefings on Marine Biotechnology;
- Identify the needs for modern biotechnology resulting from the development of basic and applied research in life sciences;
- Understand and take into account the diversity of needs of different stakeholders, from specialised marine biology researchers to SMEs;
- Remove or minimise the barriers to cooperation between researchers and high-tech companies (notably companies from the healthcare sector);
- Develop improved transfer pathways for data and results between marine researchers based in academic institutions and the private sector.

The proposed European Marine Biotechnology Institute or Centre could play an important role in all of the above activities.

5. A European Strategy for Marine Biotechnology

5.1 A vision for the future development of Marine Biotechnology Research in Europe

Considering:

- the uniqueness of marine ecosystems and the physical and chemical variability in marine environments which support adapted life forms from intertidal mud-flats to deep sea hydro-thermal vents;
- the extreme originality and diversity of marine organisms, the fact that they represent a unique source of genetic information and biochemical complexity;
- that the marine environment is a critical source of products and services of societal value including food, bio-active compounds and biomaterials with both medical and industrial applications;
- that marine ecosystems, and in particular marine organisms (excluding fisheries products), are largely unexplored, understudied and underexploited in comparison with terrestrial ecosystems and organisms;
- that Europe possesses a wide variety of marine habitats and has a long-standing tradition of marine bio-science research, a strong biotechnology expertise and significant relevant research infrastructures; and
- that Europe now needs to be highly strategic in its approach at national and European level, to ensure development of a strong Marine Biotechnology sector which can contribute to meeting the significant environmental and societal challenges ahead;

This Position Paper presents a shared vision for European Marine Biotechnology whereby:

By 2020, an organised, integrated and globally competitive European Marine Biotechnology sector will apply, in a sustainable and ethical manner, advanced tools to provide a significant contribution towards addressing key societal challenges in the areas of food and energy security, development of novel drugs and treatments for human and animal health, industrial materials and processes and the sustainable use and management of the seas and oceans.

5.2 Strategic recommendations and actions

The strategy for the future development of Marine Biotechnology in Europe presented in this paper aims to enable the sector to much better contribute to the resolution of some of the most important social, economic, environmental and health challenges which we will encounter in the coming decade. In the context of a weakened global economy, the strategy will focus on optimising the use of marine biological resources, better coordination of research programmes at EU and national levels, and maximising the benefits for European citizens from products and services derived from Marine Biotechnology.

The Strategy is designed such that its full implementation should contribute to wealth and job creation in EU Member and Associated States. It also aims to position Europe as a globally competitive leader in Marine Biotechnology research, in the advancement of associated technologies and in the development of marine derived products and services through biotechnological applications. At the same time, the strategy must provide the means to assist countries with limited access to marine resources and/or the means to valorise them. An underlying tenet of the strategy is that its recommendations must be implemented according to the principles of sustainability, ensuring the protection and preservation of coastal and marine ecosystems and their resources for future generations. More than that, there is a need for Marine Biotechnology to better contribute to the appropriate protection, remediation and management of the marine environment. Given that knowledge of marine ecology is critical in the understanding of the impacts of human activities on the oceans and for the design of conservation measures, recognition and better support of marine ecology research will be crucial in this respect.

At the background of these strategic recommendations and actions, it must be recognised that most of the knowledge, tools and progress to date in Marine Biotechnology arose from advances in basic disciplines such as marine microbiology, biology, biochemistry, biophysics and bioengineering. Hence, the strengthening of the European research potential in marine biology *sensu lato* will be one of the major enabling factors in the future development of Marine Biotechnology. As such, the integration of marine biology research and related policies at the European level is an important condition for a successful implementation of this strategy.

Four recommendations for specific actions constitute the core of the strategy to achieve the vision for Marine Biotechnology in Europe. These are:

RECOMMENDATION 1: Create a strong identity and communication strategy to raise the profile and awareness of European Marine Biotechnology research.

The broad range of disciplines and activities which contribute to Marine Biotechnology mean that it often suffers from a lack of identity and profile as a research field in its own right. This lack of a coherent identity in Europe is also a result of inadequate efforts to coherently communicate the needs, benefits and opportunities to the wider scientific community, to policy makers and to the public in general. There is an urgent need to communicate how marine biotechnological knowledge and applications can provide advances in, for example, industrial biotechnology, health and agriculture. In particular, there is insufficient awareness within the pharmaceutical industry of the potential for novel drug discovery based on bioactive molecules and compounds derived from marine organisms.

At the same time there is an urgent need to improve information exchange among those who are actively involved in European Marine Biotechnology. Mechanisms need to be developed to mobilise and facilitate the efficient pooling of knowledge, data and research capacities distributed throughout Europe. Mobility of researchers should be encouraged at all levels. The effective dissemination of novel Marine Biotechnology research discoveries can improve greatly Europe's capacity to generate new commercial opportunities. Creating a common identity and information exchange platform will also reduce the apparent gap which currently exists between researchers and high-tech companies (notably companies from the healthcare sector).

Recommended Actions:

1a) *Create a central European information portal (e.g. www.marinebiotechnology.eu), based on a European Marine Biotechnology open network, which provides an one-stop-shop for reports on novel discoveries and success stories, challenges and opportunities.*

This portal should also provide access to documentation on marine resources, marine biotechnological research, bioactive molecules, cultures of marine microorganisms and non-registered strains in international collections. In providing a central portal with access to all biobanks, compound and extract libraries and bioscreening facilities, it should be easier to avoid duplication of scientific effort or rediscovery of molecules. Ideally, this should be under the responsibility of a European Marine Biotechnology Institute or Centre (see recommendation 2a).

1b) *Conduct, as soon as possible, an audit of Marine Biotechnology effort in Europe, providing accurate and up-to-date information on active research centres and laboratories, training programmes and outputs, companies, discoveries, patents and market information. This will allow an economic evaluation of the benefits of Marine Biotechnology in Europe and facilitate the development of strong support policies.*

1c) *Initiate a series of Marine Biotechnology demonstration projects that target the utilisation of marine materials in defined sectors – e.g. food, biomaterial and environmental remediation.*

1d) *Develop promotional and education support materials, based on, amongst others, the recommended audit and demonstration projects (see recommendation 1b and 1c), that highlight the potential and the successes of European Marine Biotechnology research.*

RECOMMENDATION 2: Stimulate the development of research strategies and programmes for Marine Biotechnology research and align these at the national, regional and pan-European level.

The EU currently lacks a coherent Marine Biotechnology RTD policy and needs to prepare one without delay. Instead, individual European countries support, to varying degrees, national Marine Biotechnology initiatives, programmes, and RTD policies and/or strategies. As a result, the European Marine Biotechnology effort is fragmented and based on national rather than EU needs and priorities. There is a need, therefore, to better co-ordinate and plan existing Marine Biotechnology activities and to identify and coordinate future R&D needs at multiple geographical scales, taking into account the variable levels of access to marine resources. A coordinated effort is also needed at pan-European level to mobilise and optimise human resources and available infrastructures. Such efforts should address both fundamental research and advanced application-oriented research and take an industry-academia collaborative approach.

Recommended Actions:

2a) *Create a European Marine Biotechnology Institute or Centre at the European level or as an intergovernmental research organisation (at least virtual, through e.g. a permanent Secretariat, coordinated network and key nodes) charged with developing*

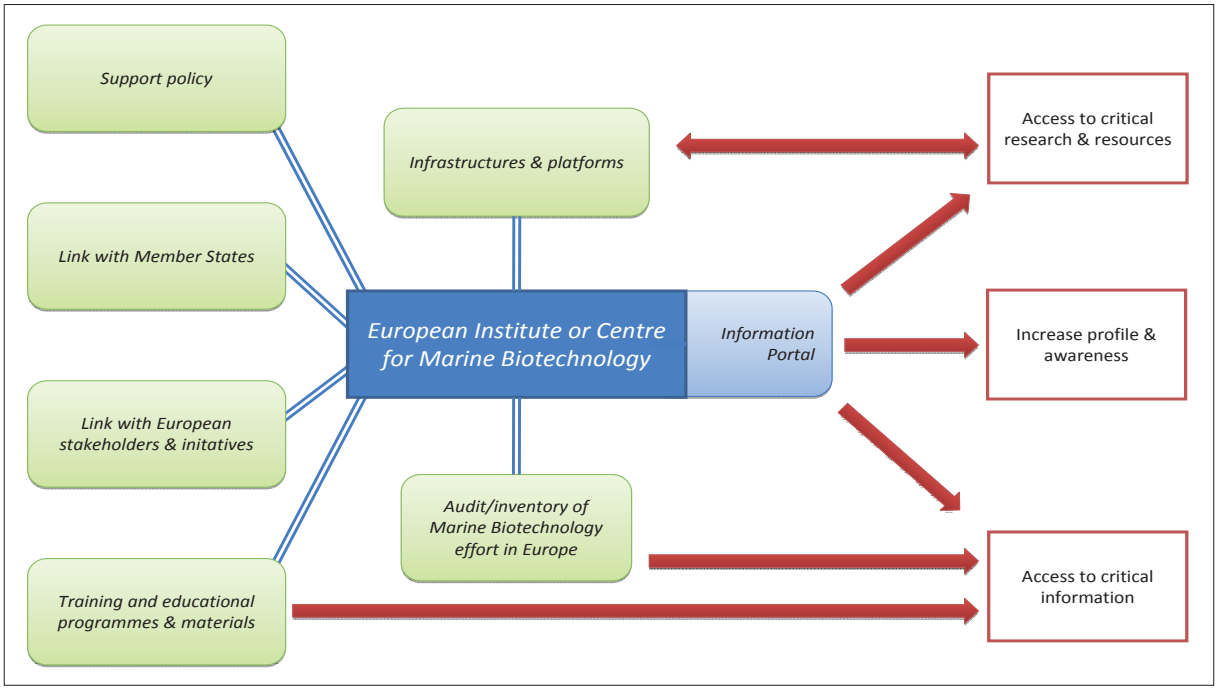

Figure 41. Overview of core functions and outputs of the proposed European Marine Biotechnology Institute or Centre

Europe's Marine Biotechnology research capabilities through a range of collaborative actions including:

- *the provision of research leadership in Marine Biotechnology;*
- *establishing and operating the European Marine Biotechnology Portal (see recommendation 1a);*
- *providing essential infrastructure to ensure European Marine Biotechnology research remains competitive (see also recommendation 2c below);*
- *training early stage scientists via a European Marine Biotechnology graduate development programme (see recommendation 4d below);*
- *operating a Marine Biotechnology resources centre and strengthening the awareness of Marine Biotechnology across European industry.*

In doing so, the European Marine Biotechnology Institute or Centre would capitalise the knowledge and experience gained in the different Member States for further optimisation of nationally based projects, cooperative initiatives, biobanks and funding. Such a Centre would also contribute to securing adequate supplies of biological material and provide a basis for much stronger industry-academic collaborations and partnerships.

2b) *Develop a coherent European Marine Biotechnology RTD policy to strengthen the integration at EU level of Marine Biotechnology research and corresponding infrastructures, among others through a future Framework Programme support action or a dedicated ERA-NET.*

2c) *Strengthen common European platforms in the field of omics research which include corresponding bioinformatics and e-infrastructures and the development of centres for systems biology and synthetic genomics, recognising that Marine Biotechnology draws from a wide range of multi-disciplinary research outputs and tools;*

2d) *Develop high level European Marine Biotechnology research programmes taking an industry-academia collaborative and multidisciplinary scientific approach in the following thematic areas:*

- **Food:** *Development of food products and ingredients of marine origin (algae, invertebrates, fish) with optimal nutritional and functional properties for human health.*
- **Energy:** *Development and demonstration of viable renewable energy products and processes, notably through the use of marine algae for biodiesel, biogas and by-products for biorefineries.*
- **Health:** *Development of novel drugs and healthcare products involving all levels of industry and research in an integrated manner*

with targeted outcomes (e.g. five new antibiotics of marine origin by 2020).

- **Environment:** *Development of biotechnological approaches, mechanisms and applications to address key environmental issues such as biofouling, harmful algal blooms, oil spills and bioremediation in the marine environment.*
- **Industrial Products and Processes:** *Development of marine derived biomaterials and molecules including enzymes and biopolymers that have applications in human and animal health, in industrial and environmental areas.*

RECOMMENDATION 3: Significantly improve technology transfer pathways, strengthen the basis for proactive, mutually beneficial interaction and collaboration between academic research and industry and secure access to, and fair and equitable benefit sharing of, marine genetic resources.

With a few notable exceptions, most industrial contributions to Marine Biotechnology in Europe are generated through specialised SMEs. These small companies assume most of the risks inherent in RTD in a highly unstable economic environment and are characterised by a rapid turn-over. There is a danger that the current global financial crisis, coupled with reductions in available venture capital and public research funding, may reduce the capacity of Marine Biotechnology SMEs to continue to play a key role in developing new technologies, products and processes. Nevertheless, efforts to involve larger, established industries should also be intensified as the technology transfer is often incomplete if they are not involved.

The participation of SMEs in EU research projects can place significant pressure on their cash flow which is a disincentive for them to get involved. It is crucial that future research and technology development funding mechanisms open to industry are designed to minimise the risks for participating SMEs and to maximise their potential to gain commercial advantage. Research contracts could allow, for example, for up to 100% of the additional costs to be reimbursed to participating SMEs, with a partial reimbursement clause to be defined in case of takeover by a large company.

Another reason why academic scientists do not always cooperate effectively with industry is related to different objectives and interests in terms of publications and intellectual property protection and this also needs to be addressed.

Recommended Actions:

3a) *Better adapt future EU Framework Programme financial rules and Grant Agreements to ensure that SMEs are attracted to participate in a way that maximises the reward and minimises economic risks.*

3b) *Establish completely new mechanisms and policies to circumvent the high risk of investments in critical novel drugs developed from marine bioresources, in particular for the development of new antibiotics of marine origin.*

3c) *Harmonise the property rights and procedures for the protection of intellectual property for marine-derived products at European level but with a global relevance. Develop new European protocols to facilitate the publication of academic research results whilst protecting, through innovative procedures, the intellectual property on new discoveries, thereby addressing the divergent goals of the academic and industrial spheres and enabling them to cooperate more effectively.*

3d) *Develop a common European position on the simplification and harmonisation of regulations on access and fair and equitable benefit sharing from the exploitation of marine genetic resources to strengthen Europe's voice at the International level. In its analysis, Europe must take into account three 'territories': (i) inside Europe; (ii) outside Europe; and (iii) international waters.*

3e) *Conduct a survey of industry stakeholders to guide research towards applications and processes to address current industry needs. Such a survey should consist of a wide consultation on the kind of marine products that industry would like to see developed, followed by specific requests for concrete applications.*

RECOMMENDATION 4: Improve training and education to support Marine Biotechnology in Europe.

While the strengthening of fundamental science is essential, specific education and training pathways are required to provide both research and industry with skilled graduates. The future of life sciences in the 21st century is closely linked to the ability of scientists to develop and participate in interdisciplinary projects embracing skills and concepts from other disciplines. Hence, training the next generation of marine biotechnologists must focus on the use of interdisciplinary and holistic approaches to solve technological problems specific to dealing with marine organisms and the marine environment.

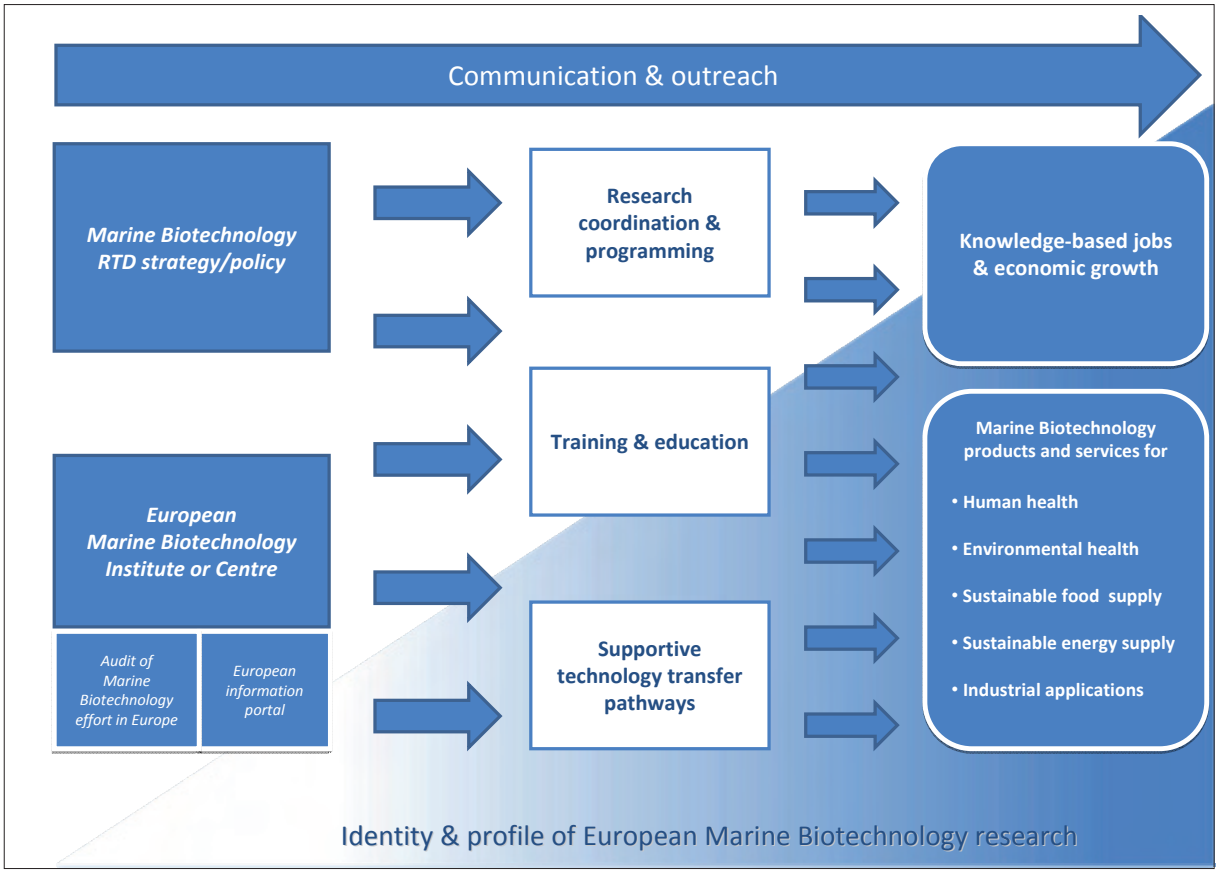

Figure 42. Flow-chart of recommended priority actions for immediate implementation and their expected impact

Recommended Actions:

4a) *Assure that appropriate biotechnology modules are included in all bio-science undergraduate educational programmes.*

4b) *Initiate actions that will ensure the participation of researchers from non-marine backgrounds in Marine Biotechnology, thus ensuring a growing pool of exceptional research talent is available to the Marine Biotechnology sector.*

4c) *Organise regular trainings or summer schools on Marine Biotechnology subjects supported, for example, by the EU Framework Programme.*

4d) *Create a European School or Course on Marine Biotechnology (virtual and distributed) and a European PhD programme on Marine Biotechnology both of which include business and entrepreneurship training.*

5.3 Strategic research priorities

Throughout this Position Paper, strategic areas for further development of Marine Biotechnology in Europe have been discussed, highlighting past achievements, current gaps and challenges and future research priorities. The implementation of the presented strategy should enable these research priorities to be addressed through advanced, collaborative and interdisciplinary research projects and programmes. For this reason, a summary overview of the research priorities is given in Summary Box 18.

Summary Box 18. Overview of strategic areas for Marine Biotechnology development in Europe and associated research priorities	
Target research area for development	**Research priorities and objectives**
A. Marine Biotechnology contribution to key societal challenges	
Food: Development of food products and ingredients of marine origin (algae, invertebrates, fish) with optimal nutritional properties for human health	- Develop innovative methods based on -omics and systems biology for selective breeding of aquaculture species; - Develop biotechnological applications and methods to increase sustainability of aquaculture production, including alternative preventive and therapeutic measures to enhance environmental welfare, sustainable production technologies for feed supply, and zero-waste recirculation systems; - Integration of new, low environmental impact feed ingredients to improve quality of products and human health benefits.
Energy: Development and demonstration of viable renewable energy products and processes, notably through the use of marine algae	- Produce an inventory of microalgae resources for biofuel production to support optimisation of the most appropriate strains; - Improve knowledge of basic biological functions, tools for steering the metabolism, and cultivation methods of marine microalgae to improve the photosynthetic efficiency, enhance lipid productivity and obtain microalgae with optimum characteristics for mass cultivation (mixed & mono cultures), biofuel production and biorefinery; - Develop efficient harvest, separation and purification processes for micro- and macroalgae.
Health: Development of novel drugs, treatments and health and personal care products	- Increase the focus on the basic research (taxonomy, systematics, physiology, molecular genetics and (chemical) ecology) on marine species and organisms from unusual and extreme environments to increase the potential for success in finding novel bioactives; - Improve the technical aspects of the biodiscovery pipeline, including the separation of bioactives, bio-assays that can accommodate diverse material from marine sources, dereplication strategies and structure determination methods and software; - Overcome the supply problem to provide a sustainable source of novel pharmaceutical and healthcare products through scientific advances in the fields of aquaculture, microbial and tissue culture, chemical synthesis and biosynthetic engineering.
Environment: Development of biotechnological approaches, mechanisms and applications to address key environmental issues	- Develop automated high-resolution biosensing technologies allowing *in situ* marine environmental monitoring to address coastal water quality, including prediction and detection of HABs and human health hazards; - Develop cost-effective and non-toxic antifouling technologies combining novel antifouling compounds and surface engineering; - Consolidate knowledge on DNA-based technologies for organism and population identification and support the development of commercial tools and platforms for routine analysis.

Industrial Products and Processes: Development of marine derived molecules exploitable by industry including enzymes, biopolymers and biomaterials	- Develop enabling technologies for high throughput enzyme screening and for the expression of marine proteins and enzymes through dedicated hosts; - Produce marine biopolymers as novel competitive commercial products in food, cosmetics and health.
B. Marine Biotechnology toolkit research priorities	
Genomics and meta-genomics, molecular biology in life sciences	- Implement genomic analyses of marine organisms, including the systematic sampling of different microorganisms (viruses, bacteria, archaea, pico- and micro-plankton), algae and invertebrate taxa; - Implement metagenomic studies of aquatic microbiomes and macrobiomes.
Cultivation of marine organisms	- Develop enabling technologies for culture and isolation of uncultivated microorganisms; - Develop innovative culture methods adapted to vertebrate or invertebrate cell lines for production of active compounds.
Bio-engineering of marine micro-organisms	- Optimise microalgal cultivation systems with respect to energy supply, productivity and cost; - Develop innovative photobioreactors adapted to different species of interest and production sites; - Promote research on the biorefinery approach based on microalgae production to develop a long-term alternative to petrochemistry.
Marine Model Organisms	- Identify and prioritise new marine model organisms that are still not investigated in the tree of life and which are needed to fill critical knowledge gaps; - Investigate identified marine model organism cultivation and perform genomic and chemical analyses.

5.4 Implementing the strategy

The strategy presented in this Position Paper contains a set of concrete and achievable recommendations and actions designed to support and develop European Marine Biotechnology research, enhance the European biotechnology and bioscience industries, and provide a considerable contribution to the Knowledge Based Bio-Economy (KBBE).

It is not the first time that Marine Biotechnology has been championed as a sector where progress will be considerable if properly supported. Previous position papers (e.g. Marine Board Position Paper 4[9]), science-policy meetings (e.g. Bremen Meeting[10]) and working groups (e.g. EC Collaborative Working Group on Marine Biotechnology CWG-MB, the EU-US Task Force on Biotechnology[11]) have highlighted the potential of the sector and provided clear recommendations to support the development of Marine Biotechnology in Europe. Unfortunately, the recommendations arising from previous strategic initiatives have only been partially implemented and, as a result, the final objectives have never been fully met. Meanwhile the scientific and policy landscape has changed significantly and awareness has grown to a point where decisive support through an up-to-date and coordinated strategy can provide a renewed impetus for European Marine Biotechnology research. The strategy proposed in this Position Paper, therefore, builds on previous initiatives whilst bringing new insights and highlighting current needs and opportunities. At the same time, implementation of the strategy will require openness, flexibility and integration within a wider policy and strategic framework.

9. Marine Board Position Paper 4 on Marine Biotechnology was published in 2001 and is available for download on www.esf.org/marineboard/publications

10. http://ec.europa.eu/research/press/2007/maritime-briefing/pdf/37-bremen-marine-biotechnology-research_en.pdf

11. http://ec.europa.eu/research/biotechnology/ec-us/tf_en.html

Successful implementation of the strategy will require a joint effort with active support and involvement from a range of stakeholders. Europe needs to mobilise the necessary support in terms of funding, human resources and research infrastructures, and to secure the engagement of all of the relevant actors. These actors include the science community, the private sector (e.g. individual companies, associations and technology platforms), policy makers and advisors at national and European level, national strategy and programme developers and managers. As each actor has an important responsibility to bring forward key elements of the strategy, mobilising, in a coordinated way, this diverse range of actors will be critical.

The 2020 vision presented at the beginning of the strategy will only be achieved through the coordinated implementation of all the recommendations and actions presented. However, some of the recommended actions provide a structural basis for realisation of the strategy and should be prioritised for early implementation. These include: (i) the preparation of a European Marine Biotechnology RTD Policy; (ii) the creation of a European Marine Biotechnology Institute or Centre and Information Portal (recommended actions 1a and 2a); (iii) an audit of Marine Biotechnology effort in Europe (including an economic evaluation of the benefits of Marine Biotechnology) (recommended action 1b); and (iv) development of a dedicated support action or ERA-NET to coordinate the programming and investments of national research funding organisations (recommendation 2a). Once up and running, these activities will act as a catalyst to drive implementation of the other recommended actions that make up the strategy. For example, a European Marine Biotechnology Institute or Centre could develop a roadmap for implementation of the strategy, coordinate its implementation and mobilise the relevant actors. A Framework Programme support action or ERA-NET, bringing together national funding organisations which support Marine Biotechnology research, can also play a key role in aligning existing programmes, coordinating investments and informing the development of new research programmes and initiatives.

Summary Box 19. Priority actions for immediate implementation

Key recommended actions which provide a structural basis for realisation of the strategy should be prioritised for early implementation. These include:

- Preparation of a European Marine Biotechnology RTD Policy;
- Creation of a European Marine Biotechnology Institute or Centre and Information Portal;
- An audit of Marine Biotechnology effort in Europe (including an economic evaluation of the benefits of Marine Biotechnology); and
- Development of a dedicated support action or ERA-NET to coordinate the programming and investments of national research funding organisations.

There is now a strong momentum to drive progress in European Marine Biotechnology in the coming decade. If Europe does not act now through a concerted effort by all the identified actors and stakeholders and through increasing its support with targeted funding and coordinated research, it will begin to lose ground on other global leaders in this field such as the USA, Japan and China. The successful implementation of the integrated strategy presented in this Position Paper has the potential, not only to significantly advance European research in Marine Biotechnology, but, in turn, to contribute significantly towards the development of knowledge-based jobs and economic growth and to meet critical societal challenges in the areas of food, environment, energy and health in the coming decade and beyond.

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List of abbreviations and acronyms

AF: Antifouling
AMBIO: Advanced Nanostructured Surfaces for the Control of Biofouling (sixth Framework Programme project)
ARA: Arachidonic acid (a polyunsaturated omega-6 fatty acid)

BAC: Bacterial Artificial Chromosome
BRC: Biological Resource Centre

COGs: Clusters of Ortholog Groups
COLIPA: The European Cosmetics Association
COMB: Center of Marine Biotechnology of the University of Maryland Biotechnology Institute

DHA: Docosahexaenoic acid (an omega-3 fatty acid)
DIN: Dissolved Inorganic Nutrients
DNA: Deoxyribonucleic acid
dsDNA: doubled-stranded Deoxyribonucleic acid

EC: European Commission
EC CWG-MB: European Commission Collaborative Working Group on Marine Biotechnology
ECDP: European Centre for Disease Prevention
EEZ: Exclusive Economic Zone
EMBL: European Molecular Biology Laboratory
EMBRC: European Marine Biological Resource Centre
EMSO: European Multidisciplinary Seafloor Observatory
EPA: Eicosapentaenoic acid (an omega-3 fatty acid)
EPS: Exopolysaccharides
ERA: European Research Area
ERA-NET: European Research Area Network
ERVO: European Research Vessel Operators
ESF: European Science Foundation
ESFRI: European Strategy Forum on Research Infrastructures
EU: European Union
EU-OPENSCREEN: European Infrastructure of Open Screening Platforms for Chemical Biology
EURO-ARGO: European component of a world wide *in situ* global ocean observing system, based on autonomous profiling floats (Argo floats)
EUR-OCEANS: Ocean Ecosystems Analysis (EU Network of Excellence)
F&PF: Faeces and Pseudo-Faeces
FAO: Food and Agriculture Organization of the United Nations
FDA: United States Food and Drug Administration
FP: European Commission Framework Programme

GFP: Green Fluorescent Protein
GMO: Genetically Modified Organisms
GOED: Global Organization for EPA and DHA Omega-3s
Gyr: Gigayear, i.e. 1 billion years

HAB: Harmful Algal Bloom

IDSA: Infectious Diseases Society of America
IMTA: Integrated Multi-Trophic Aquaculture
IP: Intellectual Property

KBBE: Knowledge Based Bio-Economy
KBBE-NET: Experts Group of officials from Member States on the Knowledge Based Bio-Economy
Kbp: Kilo base pair

LDL: Low-density lipoprotein
LIFE WATCH: Science and Technology infrastructure for Biodiversity data and observatories

MAP: Mussel Adhesive Proteins
MAR: Mid-Atlantic Ridge
MarBEF: Marine Biodiversity and Ecosystem Functioning (EU Network of Excellence)
MARS: European network of Marine Research Institutes and Stations
MEOR: Microbial Enhanced Oil Recovery
MGE: Marine Genomics Europe (EU Network of Excellence)
MGR: Marine Genetic Resource
MS: Mass spectrometry

NIOO-KNAW: Netherlands Institute of Ecology
NMR: Nuclear Magnetic Resonance
NoE: Networks of Excellence

OECD: Organisation for Economic Co-operation and Development
OFEG: Ocean Facilities Exchange Group

PCR: Polymerase Chain Reaction
PHA: Polyhydroxyalkanoate
POM: Particulate Organic Matter
PUFA: Polyunsaturated fatty acid

R&D: Research and Development
RAS: Recirculating Aquaculture Systems
RNA: Ribonucleic acid
ROV: Remotely Operated Vehicle
RTD: Research and Technical Development

SAMS: Scottish Association for Marine Sciences
SAP: Shrimp Alkaline Phosphatase
SMEs: Small to Medium sized Enterprises
SMM: Small molecule microarray
SPR: Surface Plasmon Resonance

TBT: Tributyltin

UK: United Kingdom
UNCLOS: United Nations Convention on the Law on the Sea
UCSD: The University of California, San Diego, USA

VLIZ: Flanders Marine Institute

WG BIOTECH: Marine Board Working Group on Marine Biotechnology

Annex 1. Members of the Marine Board Working Group on Marine Biotechnology (WG BIOTECH)

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Annex 2. Overview of major achievements of the marine Networks of Excellence Marbef, MGE and EUR-OCEANS

A. The Network of Excellence MGE 'Marine Genomics Europe'

Bringing together about 450 scientists from 45 institutions in 16 countries, MGE has considerably improved integration and strongly promoted interaction and collaboration in the field of marine genomic research. Some examples of MGE outputs and achievements include:

- MGE promoted, developed and spread a better understanding of the functioning of marine ecosystems and the biology of marine organisms throughout the European Union;
- Contributed significantly to the acquisition of important genomics data on marine model organisms and to the development of new genomics tools;
- Established agreement to focus on a few model organisms on which genomics and post-genomics resources could be collectively developed in order to structure the community around specific research priorities and to gain access to large scale sequencing centres. Results included a significant contribution to the sea urchin genome project that was headed by BaylorCollege (Houston, Texas) Human Genome Centre;
- Contributed to education and outreach with 16 short training courses (mainly in Bioinformatics, Transcriptomics and Proteomics) and 10 summer courses (Marine Evolutionary & Ecological Genomics, Marine diversity, Life History Strategies, and plankton bloom dynamics);
- MGE members have presented their data or the activities of the consortium in about 350 conferences; more than 230 articles acknowledging MGE were published in International peer reviewed journals with an average impact factor of 4.557.

http://www.marine-genomics-europe.org

B. The Network of Excellence MarBEF 'Marine Biodiversity and Ecosystem Functioning'

Bringing together over 700 scientists from around Europe to integrate their research, Marbef addressed the scientific challenges of the most topical marine biodiversity questions and provided new insights and answers at a scale of research never before attempted in this field in Europe. With 95 member institutes, MarBEF developed the critical mass to promote, unite and represent European marine biodiversity research at a global scale. Examples of major outputs and achievements include:

- MarBEF has established a baseline from which trends in marine biodiversity change can be detected at the relevant spatial and temporal scales;
- Recent advances in molecular technologies allowed MarBEF scientists to identify the key microbes that participate in biogeochemical cycling in different areas in Europe;
- Marine biological valuations in the form of maps developed by MarBEF could be used as baselines for future spatial planning in the marine environment;
- MarBEF scientists applied the most advanced genetic technologies to study marine biodiversity and phylogeographic structures which is of use to help improve the way fisheries are managed;
- MarBEF scientists gave us a better understanding of the role of secondary metabolites in maintaining marine biodiversity and driving ecosystem functioning;
- MarBEF scientists have shown that alterations of key species abundances affect ecosystem functioning more than changes in species diversity;
- Capture of 5.2 million distribution records of 17,000 species and a total of 137 species new to science have been added to the European Register of Marine Species (ERMS) by MarBEF. Publication of 415 scientific articles, 82% of which are 'open access'.

http://www marbef.org/

C. Network of Excellence EUR-OCEANS 'EURopean network of excellence for OCean Ecosystems Analysis

From 2005 to 2008, the EUR-OCEANS Network of Excellence brought together more than 160 Principal Investigators and 350 Associated Scientists, from 61 member research institutes and universities in 25 countries in Europe and beyond. As such, EUR-OCEANS has significantly contributed to the integration European research organisations working on global change and pelagic marine ecosystems. Selected achievements:

- Fostered integration through education and training with funded PhD projects for 19 students, a postdoctoral programme with eleven scientists, organisation or co-organisation of 40 summer schools and workshops and creation of mobility opportunities;
- Design and implementation of a database for sharing more than 110 major facilities (mesocosms, mass spectrometers, equipment at sea, etc.);
- Development of models for assessing and forecasting the impacts of climate and anthropogenic forcing on food-web dynamics (structure, functioning, diversity and stability) of pelagic ecosystems in the open ocean.

http://www.eur-oceans.eu/

Annex 3. Selected examples of enzymes discovered from marine biotic sources

Activity	Source	Habitat
Esterase	Metagenome	Deep-sea sediment
	Metagenome	Deep-sea basin
	Metagenome	Surface seawater
	Metagenome	Arctic sediment
	Vibrio sp.	Sea Hare Eggs
	Pseudoalteromonas haloplanktis	Antarctic Seawater
Feruloyl esterase	*Pseudoaltermonas haloplanktis*	Antarctic Seawater
Lipase	Metagenome	Tidal Flat
	Metagenome	Deep-sea sediment
	Metagenome	Baltic Sea sediment
	Pseudoalteromonas haloplanktis TAC125	Antarctic Seawater
	Aureobasidium pullulans HN2.3	Sea saltern
Cellulase	*Pseudoalteromonas* sp. DY3	Deep-sea sediment
	Pseudoalteromonas haloplanktis	Antarctic Seawater
	Teredinibacter turnerae T7902T	Shipworm
	Marinobacter sp. MSI032.	Marine sponge
Chitinase	Metagenome	Estuary
	Arthrobacter sp. TAD20	Antarctic ice
	Rhodothermus marinus	Marine hot spring
Amidase	Metagenome	Marine sediments / sludges
Amylase	*Aureobasidium pullulans* N13d	Deep-sea sediment
	Metagenome	Deep-sea hydrothermal vent
	Nocardiopsis sp.	Deep-sea sediment
Phytase	*Kodomaea ohmeri* BG3	Fish gut
Protease	*Pseudomonas* strain DYA,	Deep-sea sediment
	Aerpyrum pernix K1	Antarctic Seawater
	Pseudoalteromonas, Shewanella, Colwellia,	Sub-Antarctic sediment
	Planococcus species	Coastal solfataric vent
Alkane hydroxylase	Metagenome	Hydrocarbon seep
	Metagenome	Deep-sea sediment
Xylanase	*Pseudoalteromonas haloplanktis*	Antarctic Seawater
Alanine ehydrogenase	Psychrophilic bacterium strain PA-43	Sea Urchin
Chitinase	*Arthrobacter sp.* TAD20	Antarctic ice
	Rhodothermus marinus	Marine hot springs

Activity	Source	Habitat
β-Galactosidase	*Arthrobacter* sp. SB	Antarctic seawater
	Guehomyces pullulans	
Alcohol ehydrogenase	*Flavobacterium frigidimaris* KUC-1	Antarctic seawater
Malate dehydrogenase	*Flavobacterium frigidimaris* KUC-1	Antarctic seawater
Isocitrate ehydrogenase	*Colwellia psychrerythraea*	Arctic marine sediment
Isocitrate lyase	*Colwellia psychrerythraea*	Arctic marine sediment
Catalase	*Vibrio salmonicida*	Fish Microbiota
Uracil Dna Glycosylase	Marine bacterium strain BMTU3346	Marine sample
Epoxide hydrolases	*Erythrobacter litoralis* HTCC2594	Seawater
Aminopeptidase	*Colwellia psychrorythraea* strain 34H	Marine sediment
Subtilisin	*Bacillus* TA41	Antarctic seawater
Trehalase	*Rhodothermus marinus*	Marine Hotsprings
Pectate lyase	*Pseudoalteromonas haloplanktis* strain ANT/505	Antarctic sea ice
β-D-glucosidase	*Shewanella* sp. G5	*Munida subrrugosa*
Homoserine ranssuccinylase	*Thermotoga maritima*	Marine sediment
Quinol oxidase	*Shewanella* sp. strain DB-172F	Deep-sea sediment
Agarase	*Pseudoalteromonas gracilis B9*	Marine macroalgae
	Microbulbifer sp.	Deep-sea sediment
	Microscilla sp.	Marine sediment
	Pseudoalteromonas carrageenovora	Marine macroalgae
	Zobellia galactinovorans	Marine macroalgae
	Pseudomonas atlantica T6C	Marine macroalgae
	Alteromonas agarylytica	Marine macroalgae
	Agarivorans sp. JAMB-A11	Marine macroalgae
	Vibrio sp. JT0107	Seawater
Carrageenase	*Pseudoalteromonas carrageenovora*	Marine macroalgae
	Zobellia galactinovorans	Marine macroalgae
	Alteromonas fortis	Marine macroalgae
Porphyranase	*Zobellia galactinovorans*	Marine macroalgae
Fucanase	*Marineflexile fucanivorans*	Alginate plants waste waters
Fucoidan-degrading enzyme	*Fucophilus fucoidanolyticus*	Marine echinoderme
Sulfatase	*Zobellia galactinovorans*	Marine macroalgae
	Rhodopirellula baltica	Water column Batic sea
Mannuronan C5-epimerase	*Laminaria digitata*	Sea shore
Galactose sulfurylase	*Chondrus crispus*	Sea shore

Annex 3. Selected examples of enzymes discovered from marine biotic sources

Activity	Source	Habitat
Alginate-lyase	*Haliotis discus discus*	Sea shore
	Pseudoalteromonas atlantica AR06	Coastal water
	Pseudoalteromonas sp. IAM14594	Marine macroalgae
	Pseudoalteromonas sp. CY24	Seawater
	Vibrio sp. A9m	Deep-sea sediment
	Cobetia marina	Marine environment
	Agarivorans sp. JAM-A1m	Marine macroalgae
	Porphyra yezoensis	Sea shore
	Streptomyces sp.	Marine macroalgae
	Pseudomonas alginovora XO 17	Marine macroalgae
Halo-peroxidase	*Laminaria digitata*	Sea shore

Annex 4. Overview of marine model organisms

Below table presents an overview of marine organisms currently used as model organisms for various purposes. To improve contributions of marine model organism studies for biotechnological purposes, there is a need for an in-depth evaluation to identify, prioritise and select a limited number of appropriate marine model organisms which could provide critical knowledge to stimulate the development of biotechnological applications.

Taxonomy (phylum or class)	Applications	Species	Genomic resources	Other resources (enabling technologies, post-genomics)
Eukaryotes				
Animals				
Cnidaria	Evo-Devo Phylogenomics	*Clytia hemisphaerica* Jellyfish	Genome sequencing in progress ESTs	
Ctenaires	Evo-Devo Ecology Phylogenomics Regeneration	*Pleurobrachia pileus* comb jellies	Genome sequencing in progress ESTs	
Acoela	Evo-Devo Ecology Phylogenomics Regeneration Photosymbiosis	*Symsagittifera roscoffensis*	ESTs, BACs	*In situ* hybridization Immuno-localization
Echinodermata	Evo-Devo Ecology Ecotoxicology Immunologie Human disease (cancer)	*Strongylocentrotus purpuratus* Sea urchin	Genome sequence available ESTs, BACs	Knock down using morpholinos Transient transformation
Echinodermata	Evo-Devo Human disease (cancer)	*Paracentrotus lividus* Sea urchin	ESTs	*In situ* Hybridization
Echinodermata	Evo-Devo Regeneration			
Echinodermata	Evo-Devo Ecology Immunology Regeneration	*Asterias rubens* starfish	Genome sequencing on going	
Ascidiacea	Genetics Evo-Devo System biology Study of the nervous system	*Ciona intestinalis* *Ciona savignyi* tunicate	Genome sequence available ESTs, BACs	Electroporation injection technics, Knocking out using morpholinos and transformation

* Evo-Devo = **Evolutionnary and Developmental Biology**

Annex 4. Overview of marine model organisms

Taxonomy (phylum or class)	Applications	Species	Genomic resources	Other resources (enabling technologies, post-genomics)
Cephalochordata	Evo-devo Phylogenomics	*Branchiostoma lanceolatum* *Branchiostoma floridae* Amphioxus, Lancelet	Genome sequence available for B. floridae	Knock down using morpholinos Transient transformation
Chordata, Actinopterygii	Fisheries/ Aquaculture	*Salmo salar* Atlantic Salmon	ESTs, BACs Genome sequence available	Physical and genetic maps, QTL Transcriptomics
Chordata, Actinopterygii	Fisheries/ Aquaculture	*Sparus Auratus* Seabream	ESTs, BACs, BAC end sequences	Molecular markers, Genetic map, Radiation hybrid map, Transcriptomics
Chordata, Actinopterygii	Fisheries/ Aquaculture	*Dicentrarchus labrax* Seabass	ESTs, BACs, Draft genome sequence	Molecular markers Genetic map, Radiation hybrid map Transcriptomics
Chordata, Actinopterygii	Fisheries/ Aquaculture	*Gadus mohrua* Cod	ESTs	Molecular markers
Chordata, Actinopterygii	Evo-Devo	*Tetraodon nigroviridis* Green spotted Puffer fish	ESTs, Genome sequence	Genetic map
Chordata, Actinopterygii	Evo-Devo	*Takifugu rubripes* Japanese puffer fish	ESTs, cDNA, Genome sequence	
Chordata, Actinopterygii	Evolution Ecotoxicology	*Fundulus heteroclitus mummichog*		linkage map; transcriptomics
Chordata, Actinopterygii	Fisheries/ Aquaculture	*Gadus morhua* cod	Genome fully sequenced	linkage map, SSR/ SNP, SNP-array,
Chordata, Actinopterygii	evolution/ ecology	*Gasterosteus aculeatus* threespined stickleback	Genome fully sequenced and well assembled EST, BAC	linkage map, oligo expression array, SNP chip
Chordata, Actinopterygii	Fisheries/ Aquaculture	*Paralichthys olivaceus* bastard halibut	ESTs BAC	linkage map, SSR markers
Chordata, Actinopterygii	Fisheries/ Aquaculture	*Scophthalmus maximus* turbot	ESTs	linkage map; SSR and SNP markers
Chordata, Actinopterygii	Fisheries/ Aquaculture	*Solea senegalensis* Senegal sole		transcriptomics, SSR markers
Chordata, Actinopterygii	Fisheries/ Aquaculture	*Solea sole* sole		transcriptomics, SNP-chip

Taxonomy (phylum or class)	Applications	Species	Genomic resources	Other resources (enabling technologies, post-genomics)
Chondrichthyes	Ecotoxicology Evo-Devo	*Scyliorhinus canicula* dogfish	ESTs	*In situ* Hybridization
Cephalaspido-morphs	Ecotoxicology Evo-Devo	*Petromyzon marinus* Sea lamprey	Genome sequencing on going	*In situ* Hybridization Knocking out using morpholinos Transient transformation
Polychaeta	Evo-Devo	*Platynereis dumerilii* Marine annelid worm	ESTs BACs	*In situ* hybridization Knock down using morpholinos Transient transformation
Platyhelminthes	Evo-Devo Stem cells biology Apoptose regeneration	*Schmidtea mediterranea* flatworm	Genome sequencing done ESTs	*In situ* hybridization RNAi gene knock out transcriptomics
Bivalvia	Aquaculture	*Crassostrea gigas* Oyster	ESTs, BACs, Genome sequencing in progress	RNAi gene knock out *In situ* hybridization Transcriptomics
Bivalvia	Aquaculture	*Mytilus galloprovincalis* Mussel	ESTs	Molecular markers Genetic map Transcriptomics
Gastropoda	Neurobiology Human disease (cancer)			
Green plants				
Monocotyledons Alistmatidae	Ecology	*Zostera Marina*	ESTs Genome sequencing in progress	
Green algae				
Prasinophytes (unicellular)	Cellular Biology Ecology Cellular cycle Photosynthesis Circadian rythms	*Ostreococcus taurii*	Genome sequence available, BACs, EST	Transformation Transcriptomics Ecotypes collection

Annex 4. Overview of marine model organisms

Taxonomy (phylum or class)	Applications	Species	Genomic resources	Other resources (enabling technologies, post-genomics)
Heterokonta				
Phaeophyta	Evo-Devo Ecophysiology, Ecology, stress Cell-wall Photosynthesis	*Ectocarpus siliculosus*	Genome sequence available EST	Transcriptomics, Mutants, proteomics, Tiling, genetic map Protoplasts, ecotypes collection, molecular markers
Phaeophyta	Ecology, Polysaccharides Aquaculture	*Laminaria digitata*	EST	protoplasts
Diatoms	Ecology Development Cell division Environmental genomics Photosynthesis	*Phaeodactylum tricornutum*	Genome sequence available EST	Genetic transformation Transcriptomics
Diatoms	Ecology Development Cell division Environmental genomics Photosynthesis	*Thalassiosira Pseudonana*	Genome sequence available EST	Genetic transformation Transcripomics
Rhodophyta (red algae)				
Rhodophyceae	Biology, ecology, ecophysiology, polysaccharides Photosynthesis aquaculture	*Chondrus crispus* Irish moss	Genome sequencing in progress, ESTs	Transcriptomics, protoplasts
Bangiophyceae	Biology, ecology, ecophysiology, Photosynthesis aquaculture	*Porphyra umbilicalis* nori	Genome sequencing in progress ESTs	Protoplast fusion Molecular markers Mutants
Haptophytes				
Prymnesio-phyceae	Environmental genomics, Ecology, Paleoclimatology	*Emiliania huxleyi* Coccolithophores	Genome sequencing in progress, ESTs	Transcriptomics

Taxonomy (phylum or class)	Applications	Species	Genomic resources	Other resources (enabling technologies, post-genomics)
Prokaryotes				
Archaea				
Euryarchaeota	Environmental Biotechnology Biology	*Pyrococcus sp*	Genome sequence available	Transcriptomics
Eubacteria				
Cyanobacteria	Environmental genomics	*Prochlorococcus marinus Strain* PCC9511	Genome sequence available	Transcriptomics
Cyanobacteria	Environmental genomics	*Synechococcus* WH7803, WH8102, RS9916	Genome sequence available	Transcriptomics Mutants
Bacteroidetes	Environmental genomics, metabolism, carbohydrate modifying enzymes	*Zobellia galactinovorans*	Genome sequence available	Transcriptomics Genetic transformation Mutants
Gamma proteobacteria, Vibrionales	Environmental genomics, aquaculture	*Vibrio tapetis CECT*	Genome fully sequenced	Genetic transformation GFP
Gamma proteobacteria, Vibrionales	Environmental genomics, aquaculture	*Vibrio tapetis LP2*	Genome fully sequenced	GFP
Gamma proteobacteria, Vibrionales	Environmental genomics	*Vibrio harveyi, ORM4*	Genome fully sequenced	Genetic transformation, GFP
Gamma proteobacteria, Vibrionales	Environmental genomics	*Vibrio harveyi, 7890*	Genome fully sequenced	Genetic transformation, GFP
Gamma proteobacteria, Vibrionales	Environmental genomics	*Vibrio nigripulchritudo*	Genome fully sequenced	Genetic transformation, GFP
Gamma proteobacteria, Vibrionales	Environmental genomics	*Vibrio aesturianus*	Genome fully sequenced	Proteomics, Genetic transformation, GFP
Gamma proteobacteria, Vibrionales	Environmental genomics	*Vibrio splendidus*	Genome fully sequenced	Proteomics, Genetic transformation, GFP

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