The Marine Biodiscovery Pipeline: From Cruise to Commercialisation

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Abstract Marine biodiscovery is the search for marine-derived natural products which may have marketable applications. It is an area of science generating much interest in discussions regarding the need for new governance mechanisms to ensure the conservation and sustainable use of marine biodiversity in areas beyond national jurisdiction. Pivotal to these discussions is the question of if and how benefits arising from research on marine genetic resources sourced from areas beyond national jurisdictions should be shared. This document provides an overview of the marine biodiscovery process to scientifically inform these discussions. It clarifies common misconceptions and provides a non-technical summary of key steps in relation to the sampling, study and commercial exploitation of marine genetic resources.

Introduction The urgent need to protect marine biodiversity in areas beyond national jurisdiction (ABNJ) and to ensure its sustainable and equitable use has been the subject of ongoing discussions in the international arena in recent years (Druel & Gjerde, 2013). In 2018 these discussions reached the level of an Intergovernmental Conference to draft an International Legally Binding Instrument (ILBI) under the United Nations Convention on the Law of the Sea (UNCLOS). The envisaged scope of this ILBI is a package of issues to be addressed as a whole, namely: marine genetic resources (MGR), including questions on the sharing of benefits; measures such as area-based management tools including marine protected areas and environmental impact assessments; capacity-building and the transfer of marine technology.\textsuperscript{1}

Currently, access to MGR from ABNJ is limited to those countries with the financial resources and necessary infrastructure to sample in these challenging marine environments. An access- and benefit-sharing (ABS) mechanism for MGR from ABNJ could theoretically widen this bottleneck by facilitating greater access to ex-situ MGR and their associated derivatives and data, and ensure that benefits arising from research and development on these resources are equitably shared. The entry into force of the Nagoya Protocol\textsuperscript{2} to the Convention on Biological Diversity in October 2014, which provides for an ABS framework for genetic resources (including marine) within national jurisdiction, further highlights the question of whether a regulatory gap now exists in relation to marine biodiversity beyond the limits of national jurisdiction. However, the freedom to carry out marine scientific research is a central tenet of UNCLOS and any new regulation must ensure that this freedom is not constrained. The creation of benefits (monetary or non-monetary) arising from research and development on MGR is intrinsically linked to marine scientific research. One area of marine scientific research receiving considerable attention as a potential benefit-generation engine is marine biodiscovery, a complex, science-driven process with multiple stages, actors and potential outcomes. This article provides a non-technical summary of the process from seabed to application dividing it into discrete steps.


\textsuperscript{2} The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (ABS) to the Convention on Biological Diversity is a supplementary agreement to the Convention on Biological Diversity. It provides a transparent legal framework for the effective implementation of one of the three objectives of the CBD: the fair and equitable sharing of benefits arising out of the utilization of genetic resources.
Current best practice is described, and technical obstacles are highlighted to assist those involved in policy making in this field. Rapid scientific advances mean that some processes that are currently rate-limiting may be accelerated in the near future, and thus any policy decisions need to be able to accommodate such changes.

**A note on terminology** The search for biological material (marine or terrestrial) with interesting properties which may be developed into marketable applications is often termed ‘bioprospection.’ This traditionally referred to the process of targeted ‘in situ’ sampling of terrestrial, and less commonly, coastal biodiversity with potential biological activity (bioactivity), where the associated traditional knowledge of local or indigenous communities may have facilitated the identification of the target organisms’ potential application. It is this activity that the Nagoya Protocol aims to regulate by ensuring that the providers of the genetic resources and/or associated traditional knowledge are fairly compensated for facilitating access to these valuable resources and knowledge. It became clear during the negotiations for the Nagoya Protocol, however, that the transfer of resources from providers to users is seldom a ‘one step process,’ but more often a convoluted chain involving many steps and actors, each with varying intents. Biodiscovery can and does occur without intentional, targeted in situ sampling for commercial purposes in the first instance, for example where the biological material used is sourced from ex-situ samples e.g. from a biorepository where it may have been deposited in the first instance by a non-commercial actor. This would be particularly applicable to areas beyond national jurisdiction where much of the research activity which results in the collection of the samples (MGR) would be for basic research purposes and little in the way of traditional ‘bioprospection’ would take place for reasons which will be explained below. For these reasons the term biodiscovery is preferable and more accurately describes the activity than bioprospecting. The term biodiscovery reflects the reality of the process, in that in most cases the idea or concept discovered from bioresources is the essential part. Most current products derived from marine bioresources are not made from the original resource, but are manufactured using chemical or biotechnological processes.

From a scientific perspective considering ‘MGR from ABNJ’ is nonsensical. The term ABNJ, encompassing ‘the Area’ and “the high seas” refer to marine areas defined not by environmental characteristics, but according to UNCLOS maritime zones. Also the term ‘marine genetic resources’ is not commonly used in any scientific discipline. Yet an ABS regime for MGR specifically from ABNJ could be within the scope of any new ILBI and so for consistency this document will refer to biological organisms found in, but not necessarily restricted to, marine areas beyond the limits of national jurisdiction as ‘MGR from ABNJ.’

**What is biodiscovery?** Biodiscovery is the discovery of compounds and ideas from natural sources to treat disease, increasingly the broader definition includes the use of these compounds and ideas as biomedical research tools, antifoulants, enzymes/catalysts, nutritional supplements (nutraceuticals) and personal care products (cosmeceuticals) (Querellou et al. 2010). Whilst a natural product can be any compound or substance produced by a living organism, in the field of organic chemistry natural products refers to small molecules, often called secondary metabolites, which confer an evolutionary advantage on the producing organism. They often play a role in ecological relationships e.g. predator-prey interactions, competition for space and food and communication strategies. Research has shown that these natural products can have extremely interesting bioactivity with potential therapeutic value. The use of natural products in drug discovery has been particularly successful. In the period 1981-2010, 71% of the 1355 new chemical entities introduced into the clinic originated from, or were

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3 As determined by the State having sovereign rights over these natural resources.
4 The term ‘marine genetic resources’ derives from the term ‘genetic resources,’ the latter of which is defined by the Convention of Biological Diversity (CBD) as genetic material of actual or potential value.
http://www.cbd.int/convention/text/default.shtml
inspired by, natural products (Newman & Cragg, 2012). Despite this, with the advent of high throughput testing and combinatorial chemistry, the pharmaceutical industry had largely turned away from natural products as a source of lead compounds for therapeutics. Natural products were perceived as difficult to obtain both initially and for repeat or follow-up studies. They were thought to be structurally complex, not sufficiently drug-like, with the possibility of associated ownership issues. Additionally it was believed that the throughput for natural products was lower and structural characterisation slow. While such misconceptions have been shown categorically to be ill-founded (Bull and Stach 2007), the result is that the pipelines of many large pharmaceutical companies are no longer viable. The recent resurgence of interest in natural products has been led by small to medium-sized enterprises (SMEs), often focusing on one particular class of natural products, or source of natural products. However, if screening of natural products is to compete as a lead-generation engine, the time and resources needed to produce and assay organism extract/fraction and pure natural product libraries must be reduced. Many of the improved economies can be attained by improving extract/fraction and natural product libraries themselves, and the means by which they are screened. This can be achieved by a range of means, including, but not limited to, accessing unique biodiversity, using genetics to identify chemically ‘talented’ organisms and using innovative high-content screening. A general paradigm in natural product research is that interesting environments give rise to organisms with unique cellular chemistry which in turn implies interesting biological activity. With this in mind attention has turned to the marine environment as a promising and as yet largely untapped source of natural products.

The marine environment: promises and challenges

The marine environment constitutes a vast biome characterised by an extensive range of ecological niches, including cold seep, mid-ocean ridge, trench and hot vent ecosystems. These diverse and hostile environments harbour a huge biodiversity uniquely adapted to these conditions.

About 95% of the volume of the global ocean is more than 1,000 m deep and the average depth is 3,790 m. Just over half of the ocean can be defined as abyssal (3,000-6,000 m deep) whereas only a tiny proportion (ca 1%) is defined as hadal (6,000-11,000 m deep), and mainly consists of trench ecosystems. At depths greater than 2,000 m, oceans have high hydrostatic pressures (>200 Atm), a constant temperature of 2.0 - 5.0°C, pH 8 and 3.5% salinity (Skropeta 2008). The deep sea is a dark place; light penetration decreases exponentially with depths such that photosynthetically useful light stops at ca. 250m. The cold biosphere ranges from -2°C to 4°C, including the polar zones, and has high primary productivity (Lebar et al. 2007). Hot vents are one of the most extreme environments on Earth, with a combination of high temperature, toxic metals, extreme pH and reducing power often combined with high hydrostatic pressure.

Whilst these extreme environments give rise to organisms with potentially interesting bioactivity (Jorgensen and Boetius 2007, Kennedy et al., 2010), they also present significant challenges in terms of physical access. Despite more than a decade of technological advances which have resulted in a significant increase in deep ocean exploration leading to the discovery of many new species, still only 0.0001% of the deep-sea has been sampled biologically (European Marine Board, 2013). This is reflected in the fact that while more than 30,000 marine natural products have been reported to date, only a very small number of compounds have been reported from abyssal and hadal environments, with less than 10 reported up to the end of 2007 (Skropeta 2008) with a further 7 identified in 2010 (Abdel-Mageed et al. 2010). It is important to note that simply because most of the deeper part of the global oceans are also beyond the limits of national jurisdiction, such extreme environments and the resultant ecosystems are mostly (although not exclusively) to be found beyond the limits of national jurisdiction.
Early marine biodiscovery efforts focused on larger organisms which could be collected relatively simply by scuba diving or conventional fishing methods. In recent years, for various reasons including sustainability of supply, potential bioactivity and relative ease of collection, marine biodiscovery has increasingly focused on microorganisms. These will now be discussed in more detail.

**Why microbes?** Planktonic and benthic microbes such as *Bacteria*, *Archaea*, viruses, *Fungi* and protists, including microalgae, comprise up to 90% of the living biomass in the ocean (Heip and McDonough, 2012). In contrast to terrestrial environments, marine environments have a very high bacterial diversity at the higher taxonomic levels (Lozupone and Knight 2007). A detailed assessment of marine pelagic and benthic bacterial ecosystems showed that surface and deep water, coastal and open ocean, oxic and anoxic habitats host very different communities at all taxonomic levels and confirmed that there is minimal taxon overlap between habitats (Nemergut et al. 2011). The unique metabolic pathways acquired by these communities of microbes in order to survive in these harsh environments have been shown to produce some of the most interesting bioactive compounds known today and offer a particularly exciting prospect for the discovery of novel bioactivity for use in the pharmaceutical, nutrition and personal care areas (Lebar et al., 2007, Thornburg et al., 2010, Martins et al., 2014, Rocha-Martin et al., 2014). Recent figures show that of the approximately 1,000 new marine natural products that are discovered each year, 27% are from microbial sources, rising year-on-year (Blunt, Copp et al. 2013 & 2014). One marine biodiscovery success story is the drug Yondelis®, marketed for cancer treatment, which is produced by modifying a microbial product. Other marine microbial products currently in clinical trial include Salinosporamide 1 and Plinabulin. Many more microbially derived compounds are in pre-clinical evaluation for a number of diseases and novel chemistry from marine bacteria and fungi is now to be found in biological screening for the personal care and nutrition areas. Table 1 lists some products in development or on the market, their uses, sources and potential markets.

The postgenomic era in marine natural products biodiscovery has been, and will continue to be, facilitated by the genome sequencing of numerous microorganisms. Large scale sequencing projects such as those supported by the Gordon and Betty Moore Foundation have resulted in the generation of 1000s of marine microbial genome and transcriptome sequences, all of which have been made accessible via public databases (Keeling et al., 2014). Bioinformatic-based interrogation of the marine bacterium, *Salinispora tropica*, shows that 10% of its genome is dedicated to biosynthesis, greater than for similar terrestrial species (Udwary et al. 2007). Adopting a metagenomic approach which involves extraction and sequencing of the total DNA of an environmental sample enables simultaneous and direct access to the genomes of whole communities of microorganisms without prior knowledge of which microorganisms are present or the need for independent cultivation (Handelsman 2004). These and other emerging strategies for the discovery of novel marine bioactive compounds from microorganisms (Rocha-Martin et al., 2014) coupled with the challenges of physical access to deep-sea ecosystems, means that biotechnological research on organisms from extreme ecosystems, such as those found in areas beyond national jurisdiction, focuses increasingly on *microorganisms* (Querellou et al. 2010).

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5 [http://camera.calit2.net/microgenome/](http://camera.calit2.net/microgenome/)
**Table 1.** Examples of marine natural products in the pharmaceutical, nutritional and personal care markets.

<table>
<thead>
<tr>
<th>Category</th>
<th>Product</th>
<th>Organism</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapeutic</td>
<td>Yondelis® (Cancer)</td>
<td><em>Ecteinascidia turbinata</em> (Ascidian)</td>
<td>c. €60M in 2012</td>
</tr>
<tr>
<td>Therapeutic</td>
<td>Prialt® (Neuropathic Pain)</td>
<td><em>Conus magus</em> (Mollusc)</td>
<td>est. $20M in 2012</td>
</tr>
<tr>
<td>Therapeutic</td>
<td>Halaven® (Cancer)</td>
<td><em>Halichondria okadai</em> (Sponge)</td>
<td>est. &gt;$200M 2011</td>
</tr>
<tr>
<td>Therapeutic</td>
<td>Salinisporamide (Cancer)</td>
<td><em>Salinispora tropica</em> (Bacterium)</td>
<td>Phase I</td>
</tr>
<tr>
<td>Biofilm inhibitor</td>
<td>Brominated furanones (Quorum sensing inhibitor)</td>
<td><em>Delisea pulchra</em> (Red alga)</td>
<td>In trials</td>
</tr>
<tr>
<td>Sunscreen</td>
<td>Mycosporine like amino acids (UV absorbing)</td>
<td>Coral <em>Zooxanthellae</em></td>
<td>In trials</td>
</tr>
<tr>
<td>Cosmetic</td>
<td>Pseudopterosins (anti-inflammatory)</td>
<td><em>Pseudopterogorgia elisabethae</em> (Soft coral)</td>
<td>Commercial</td>
</tr>
<tr>
<td>Cosmetic</td>
<td>Venuceane (anti-free radicals)</td>
<td><em>Thermus thermophilus</em> (Bacterium)</td>
<td>Commercial</td>
</tr>
<tr>
<td>Nutrition</td>
<td>ω-3 fatty acids</td>
<td><em>Crypthecodinium cohnii</em> (Microalga)</td>
<td>Commercial</td>
</tr>
<tr>
<td>Nutrition</td>
<td>Carotenoids (anti-oxidant)</td>
<td><em>Dunaliella salina</em> (Microalga)</td>
<td>Commercial</td>
</tr>
</tbody>
</table>

**The Marine Biodiscovery Process: A linear overview** The marine biodiscovery process is linear with a number of feedback loops which inform sample selection at a number of decision points (Figure 1). The first part of the process involves obtaining the biological material (MGR), either via targeted *in situ* sampling or through access to curated *ex-situ* samples held by colleagues, institutes or public collections. These can be whole macroorganisms such as sponges or sea squirts, or can be sediments / cores of sediment from which microorganisms can subsequently be isolated. In addition, microorganisms can be isolated from water samples, surfaces and interiors of marine organisms and from the surfaces of submerged material. Having obtained the organism(s) of interest, the next steps are to obtain sufficient biomass from which the small and large biomolecules are extracted followed by purification. Extracts and pure molecules can then be tested for biological activity or alternative functions which could potentially lead to applications following additional and extensive development work and may sometimes, but not always, result in a marketable product. There follows a description of the marine biodiscovery process in detail highlighting, where possible, aspects relevant to MGR sourced from ABNJ. From a scientific perspective, no physical sampling or R&D experimental procedure makes any distinction between MGR sourced from within or beyond national jurisdictions.
Figure 1. A flow scheme of the biodiscovery and development process

Discovery Phase

- Sampling
- Curation
- Biomass
- Extraction
- Assay
- Purification
- Active NCE (Lead)

Development Phase

- Pre-clinical studies
- Clinical trials
- Market

Pre- and post-clinical studies can be iterated.
**In situ Sampling** Sampling bioresources in ecosystems found in ABNJ requires access to ocean-going vessels. These can belong to national marine research organisations / institutions (e.g. National Oceanographic Centre in Southampton), not for profit organisations (The Schmidt Foundation) or commercial operations (oil, salvage). The vast majority of deep sea biological sampling is carried out by scientists onboard publicly funded research vessels. The daily rate for these vessels can be very high, circa US$ 20,000-50,000 per day, especially if it involves the use of a submersible Remotely Operated Vehicle (ROV) to retrieve samples. The number of suitable research vessels is limited globally and their capability varies enormously. Access is, therefore, very competitive, via bids to national agencies or transnational projects such as Eurofleets. Between bid and cruise, a period of 1-2 years can elapse in many cases. Ship time is intensively used and must serve the needs of the many multidisciplinary scientists on board which can often lead to compromises. Added to this, transit to sampling sites and weather conditions may limit useful time available for sampling. Globally there are very few manned submersibles or ROVs that can reach hadal depths. Most manned submersibles and ROVs have a maximum depth of 4,000m, with few going as deep as 7,000m. Exceptions exist and include James Cameron’s Deepsea Challenger which reached 11,000m and the Wood’s Hole Oceanographic Institution hybrid ROV, Nereus, which has reached similar depths. The latter was irretrievably damaged in operation in 2014.

To reduce costs and provide access to expensive infrastructure, public/private partnerships have been developed for marine researchers to utilise downtime on remotely operated vehicles used in the offshore oil industry (http://www.serpentproject.com/). In another transnational initiative the Ocean Facilities Exchange Group (OFEG) “provides a forum to consider barter exchange and co-operation opportunities for the Global and Ocean Class research fleet” and currently lists 6 European nations as members (http://www.ofeg.org/). Because of the limited cruise time available globally and the relatively small number of ships, cruise paths are recorded and sampling logged carefully. Many national agencies and international organisations have extensive websites that record all this information and allow access to existing repositories once an agreement has been signed. Examples of such websites are shown in Table 2.

Cruises are primarily led by scientists engaged in fundamental marine research, and may sometimes host biodiscovery scientists. The former will often decide the cruise path to enable optimal study of particular organisms or ecosystems found in different components of the marine system (coastal to deep sea, pelagic, benthic, sub-seabed, etc.) in different geographical areas (e.g. Porcupine Abyssal Plain, Atacama Trench). A cruise plan will attempt to combine scientific goals with pragmatism (ease of access to particular environments, needs of other scientists, etc.). Legal or administrative requirements will be relevant in the context of obtaining permits or providing notifications to coastal states and are usually dealt with by research vessel services.

The sampling methods employed on any one cruise will vary depending on end usage and often different forms of sampling cannot be carried out concurrently. A range of sampling methods is listed in Table 3. Considering the time available during a research cruise, the quantity of opportunities for sampling are limited and must be carefully planned to satisfy the varying requirements of the multidisciplinary teams on board. Apart from sampling with a ROV, most sampling methods do not allow for specific species or taxa to be targeted. Exact locations of sample collections are based on surface GPS coordinates, and when collecting a sample close to the limits of the exclusive economic zone of a coastal state, there may be a lack of legal certainty as to where on the seafloor the sample was actually collected. The vertical transboundary situation that may exist where an extended continental shelf underlies the High Seas (ABNJ) can be more easily dealt with because sample data will always record whether the sample was taken from the water column or substrate.
<table>
<thead>
<tr>
<th>Platform</th>
<th>Aim</th>
<th>Website</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan Agency for Marine-Earth Science and Technology (JAMSTEC)</td>
<td>A data search portal which records a large dataset on every sample collected.</td>
<td><a href="http://www.godac.jamstec.go.jp/dataportal/viewer.htm">http://www.godac.jamstec.go.jp/dataportal/viewer.htm</a></td>
</tr>
<tr>
<td>British Oceanographic Data Centre (BODC)</td>
<td>Housed at the National Oceanographic Centre in Southampton.</td>
<td><a href="http://www.bodc.ac.uk/">http://www.bodc.ac.uk/</a></td>
</tr>
<tr>
<td>The Schmidt Ocean Institute</td>
<td>Records its cruises on Google Earth</td>
<td><a href="http://www.schmidtsonian.org/maps">http://www.schmidtsonian.org/maps</a></td>
</tr>
<tr>
<td>Ocean Biogeographic Information System (OBIS)</td>
<td>An international effort to provide data about marine species globally. It allows users to identify “biodiversity hotspots and large-scale ecological patterns, analyse dispersions of species over time and space, and plot species’ locations with temperature, salinity, and depth.”</td>
<td><a href="http://www.iobis.org/">http://www.iobis.org/</a></td>
</tr>
<tr>
<td>The International Ocean Discovery Program (IODP)</td>
<td>IODP is “an international marine research collaboration that explores Earth’s history and dynamics using ocean-going research platforms to recover data recorded in seafloor sediments and rocks and to monitor subsea floor environments.” Sample materials collected during IODP drilling operations are made available to researchers via repositories.</td>
<td><a href="http://www.iodp.org/">http://www.iodp.org/</a></td>
</tr>
<tr>
<td>InterRidge</td>
<td>An international organization that pools the resources of its member countries to drive oceanic ridge research forward in a cost-effective and cooperative manner.</td>
<td><a href="http://www.interridge.org/">http://www.interridge.org/</a></td>
</tr>
<tr>
<td>EMBRC</td>
<td>The European Marine Biological Resource Centre (EMBRC) is a distributed research infrastructure that aims to provide a strategic delivery mechanism for excellent and large-scale marine science in Europe.</td>
<td><a href="http://www.embrc.eu/">http://www.embrc.eu/</a></td>
</tr>
</tbody>
</table>
Table 3. Different sampling methods to access marine genetic resources

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water sampling</td>
<td>Plankton netting throughout the water column.</td>
</tr>
<tr>
<td></td>
<td>Water sampling at different depths (e.g. Niskin bottle)</td>
</tr>
<tr>
<td></td>
<td>Water filtering at different depths (e.g. stand-alone pump systems; SAPS)</td>
</tr>
<tr>
<td>Trawling</td>
<td>Bottom (benthic) trawling using small trawl nets (e.g. the Agassiz trawl).</td>
</tr>
<tr>
<td></td>
<td>Mid-water (pelagic) trawling using closable nets (e.g. RMT)</td>
</tr>
<tr>
<td>Trapping</td>
<td>Baited traps (for small invertebrates or larger fish)</td>
</tr>
<tr>
<td>Grabs/coring</td>
<td>Grab sampling (e.g. Day, Eckman, Peterson grabs)</td>
</tr>
<tr>
<td></td>
<td>Sediment coring – typically using multiple core design (e.g. Megacore).</td>
</tr>
<tr>
<td></td>
<td>Maximum lengths of cores are ca 1 m.</td>
</tr>
<tr>
<td></td>
<td>Box coring, where large volume of sediment are retrieved</td>
</tr>
<tr>
<td></td>
<td>Targeted coring using ROVs or manned submersibles.</td>
</tr>
<tr>
<td></td>
<td>Piston coring – this can reach sub surface depths of over 10 m.</td>
</tr>
<tr>
<td>Drilling</td>
<td>There are a limited number of ocean going drilling vessels that are dedicated to geological/oceanographic research – the international JOIDES Resolution and JAMSTEC’s Chikyu.</td>
</tr>
</tbody>
</table>

Owing to the high costs of ship time and the lack of suitable vessels, commercial expeditions to collect marine bioresources are rare. On the whole, sampling activity in ABNJ is relatively limited and already transparent and well recorded as the majority takes place on logged research vessel cruises. There is a considerable degree of coordinated capacity already in place through the initiatives mentioned above, and with limited oceanographic research funds available, the pressure to share facilities to an even greater extent will increase. Whilst there is a degree of coordination at national and regional levels, global level coordination could lead to optimised usage of available resources.

Sample Curation Samples obtained from ABNJ during research cruises (macroorganisms, sediments/cores, microorganisms) must be correctly curated to maintain optimal condition of the sample for the intended research. Once samples have been collected, the norm on research cruises is for georeferenced samples to be stored and linked with associated environmental data including, for example, depth, temperature, pH, oxygen content, seafloor conditions. The conditions under which samples are stored will vary depending on the planned research and can vary from liquid nitrogen storage to the use of preservation fluids and more commonly -20°C and 4°C coolers. These conditions must be maintained during sample transfer to long term storage. Excess sample may be available to other users (e.g. cores for geological research may be used for microbiology) as long as the appropriate storage conditions have been maintained at all stages.

Generating Biomass The next step in the biodiscovery pipeline is the generation of biomass from the original sample. The purpose of obtaining biomass is to provide sufficient material for downstream uses. In the case of macroorganisms, the collected sample is a form of biomass, and ca. 1 kg wet weight of organism is typically sufficient for further work. Microorganisms can be isolated from the sediment / cores and also the macroorganisms themselves, which often harbour a large number of unique microorganism strains. For these purposes a few grams of material is sufficient, whereas for metagenomic studies hundreds of grams of material (invertebrate or sediment) may be needed. Microorganisms can be isolated using standard microbiological cultivation techniques, resulting in pure strains representing single species (or operational taxonomic units). These can be grown in larger scale batch cultures to generate enough biomass for downstream processing. Once the microorganism is isolated and preserved correctly, if it is culturable it is in principle possible to generate biomass when desired, although there are some important caveats to this. “Culturable” is a relative term in a quantitative (less than qualitative) sense and the amounts of biomass accessible through culturing
within a reasonable framework of time, labour and cost can vary widely between a few mg to kg or tons.

**Using Synthetic Biology Approaches to Access Useful Products and Processes** Many microorganisms, particularly those from extreme marine environments cannot be cultured. Metagenomics enables access to the genetic information contained within the genomes of all microorganisms present in an environmental sample without individual isolation and culturing. This data can be searched for useful genes which can then be synthesised with or without modifications and placed in an easy to grow microorganism for expression of useful products (Schmidt, Nelson et al. 2005). Batch culture of these engineered strains can then be carried out to give biomass which can be used for downstream processing. Current developments allow the incorporation of genes from different organisms into a single host organism which will then produce novel substances. Although there are not many examples of this currently, this may be a major route for this type of work in the future and should be considered carefully from a legal perspective because a company could develop a commercial product based on genetic information derived from one or more MGR without ever having had physical access to the MGR.

**Product Extraction** Once the biomass has been generated, the next step is to extract the useful products. The process varies depending on end use, but typically involves disruption of the cells (grinding, solvents/reagents, ultrasound) followed by extraction with a solvent (water, buffer, chemical solvents). At this point the desired materials are present in the solvent, at which point the solvent is partially or fully removed (dialysis, freeze drying, removal of solvent under reduced pressure), leaving a concentrate or extract. Libraries of extracts are created and can be stored for long-term use. These can also be distributed, but as materials are often limited in amount and hard to re-obtain this approach has a limited lifetime. Examples of such repositories exist at the US National Cancer Institute’s Natural Product Branch under the Open Repository scheme (http://www.dtp.nci.nih.gov/branches/npb/repository.html) which allows access to materials after signing a material transfer agreement.

**Screening for Bioactivity – Assay** The next step is to obtain biological activity (bioactivity) data on the materials obtained. Extracts can be subject to preliminary fractionation, or be subjected to assays directly, depending on how the assays deal with crude samples containing, amongst other things; salts, fats, sugars and proteins making up ca 80% of sample mass. These major components can often be removed leaving only materials with the desired characteristics. Sample preparation is carried out before assay screening to prevent false negatives and false positives occurring. Assays fall into a variety of types, from whole organism (e.g. zebrafish), whole cell (e.g. cancer, antibacterial) and mechanistic assays (e.g. enzyme inhibition) (Crawford, Esguerra et al. 2008). For extracts derived from bioresources, most commonly whole organism or whole cell assays are carried out. Enzyme assays are often carried out on purified compounds to confirm suspected mechanisms of action.

**Purification of active compound** Once initial activity has been pinpointed in a fractionated organism extract, the next step is to isolate and identify the active principle from that extract. This can be achieved via a series of chromatographic steps using a variety of techniques. A number of chromatographic steps may be necessary to obtain a pure compound, and in each case fractions are collected. In many cases, the purification process is guided by the assay data, in which the chromatographic fractions are tested and the most active fraction is carried forward to the next step. Once a pure compound is obtained, the structure of the compound can be clarified using x-ray crystallography or a combination of spectroscopic methods such as nuclear magnetic resonance spectroscopy and mass spectrometry.
Active New Chemical Entity (NCE) At this point you may have a ‘hit’ compound, that is, a compound with a defined structure and associated bioactivity. The structure may need chemical optimisation to increase potency and reduce side effects; this is termed improving its therapeutic index. Compound optimisation can be done using a range of methods. It is important to remember that this ‘hit’ is not likely to be used unchanged as a pharmaceutical. Many manufacturing options exist ranging from total chemical synthesis, semi-synthesis, production by a microorganism and biotechnological methods relying on genetically modified microorganisms. Once the therapeutic index and physical properties (e.g. bioavailability) have been tuned, the compound may be regarded as a ‘lead’ compound which may enter development as a new chemical entity. This marks the end of the ‘discovery’ phase (typically several years) and the beginning of the ‘development’ phase. The development of a lead compound can take many years, with many apparently promising candidates falling by the wayside. There are multiple hurdles to be cleared, including production of the active pharmaceutical ingredient, preclinical evaluation followed by clinical trial phases, approval by regulatory agencies and finally marketing. For the eight FDA or EMEA approved marine derived drugs currently on the market, the process from lead discovery to entry into the market took between 20 and 30 years (Martin et al 2014). It is possible that more than one lead may be obtained within these time-frames if different selective screens were employed at the assay stage. Therefore, increased access by multiple actors to an initial resource could increase the number of potential leads developed.

A study by the Tufts Centre for the Study of Drug Development has estimated the cost of developing a new prescription medicine that gains market approval to be US$ 2,558 million. This compares to their 2003 study which estimated the cost per approved new drug to be US$802 million (at 2000 prices). According to the study author, rising drug development costs have been driven mainly by increases in out-of-pocket costs for individual drugs and higher failure rates for drugs tested in human subjects. Lengthening development and approval times were not considered to be responsible for driving up development costs. Up to 70% of these costs, and much of the time taken to bring a drug to market, are associated with clinical trials. For this reason mechanisms are being discussed to reduce the timelines and complexity of bringing a drug to market safely. This is particularly salient for neglected diseases prevalent in developing countries and could reduce the cost too far below the figure estimated by the Tufts study.

Discussion There are a number of challenges inherent in the marine biodiscovery process and these are discussed in this section. MGR from extreme environments, found within or beyond the limits of national jurisdiction, present unique challenges for collection and curation. A deep sea core sample or a marine macroorganism collected during one sampling expedition represents a finite sample which could be sub-sampled but which cannot be easily duplicated or propagated. Re-sampling at the same GPS coordinates and depth cannot guarantee isolation of a duplicate sample. It would be entirely unfeasible to consider a captive breeding option to generate ex situ breeding lines of deep sea organisms as can be maintained for many terrestrial taxa and which could be deemed as conserving biodiversity ex situ. The unique and extreme environmental conditions to which deep sea organisms are adapted cannot be easily simulated. Whilst some marine microbial strains are maintained in

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6 On November 18, 2014 Tufts Centre for the Study of Drug Development held a press conference to present results of its latest study, updating the total average cost of developing and winning market approval for a new prescription drug. For full press release together with background information on the methodology used to develop the cost estimate and slides presented at the briefing can be found at the following link.
http://csdd.tufts.edu/news/complete_story/cost_study_press_event_webcast

http://www.cgdev.org/files/1425588_file_Bollyky_Clinical_Trials_FINAL.pdf
culture collections, more than 90% cannot currently be cultured under standard laboratory conditions (Glockner et al., 2012). Moreover, the natural products of interest for biodiscovery are often produced by the metabolic processes developed by organisms in response to in situ environmental stimuli. These conditions and also the unique microbial community interactions found in situ would also need to be replicated ex situ in order for certain microbes to synthesise particular natural products of interest. The challenges of collection in the marine environment are reflected in the fact that there are no examples of a MGR-derived product in the pharma sector that requires large-scale harvesting of the resource. In all cases, alternative and sustainable methods of supply have been found; examples include Prialt®, Halaven® and Yondelis® (Table 1). This scenario will likely continue given the increasing focus within marine biodiscovery on microorganisms together with current advances in synthetic biology.

Facilitating greater access to ex situ samples and related data would promote and encourage marine scientific research. It would multiply the potential for exploitation of these valuable resources and mitigate the environmental impact of sampling in unique and fragile marine environments by preventing unnecessary resampling at the same location. In practice, such a scenario could require those sampling MGR from ABNJ to deposit a duplicate sample together with all associated environmental data in a centralised or distributed (virtual) repository. There are significant questions regarding the potential feasibility of a single, centralised multinational biorepository holding duplicates of all ABNJ-derived MGR. Some relevant bodies that might facilitate this are given in Table 2. Where would such a repository be based and how would samples be sub-sampled, maintained and transferred to it? Furthermore, sample collection methods and storage conditions would need to take account of all potential future applications. If, for example, a sample was taken and stored in a preservative medium with the intention of future taxonomic study, then use of that sample for biotechnology research may not be feasible, as more stringent storage conditions would be required. What requirements should be imposed concerning the quantity of sample to be deposited, given that the amount of biological material needed can vary broadly depending on the nature of the intended research?

Alternatively, a virtual repository, akin to a ‘dating agency,’ could be envisaged to allow collectors/providers of material to share excess materials collected in ABNJ with other researchers. Any such virtual repository would require a coordinated fully-searchable database with all information on sample quantity, storage conditions, method of sampling, taxonomic information (if available) and associated environmental metadata. The question then arises as to how to prioritise users requesting material from finite samples. Additionally, who should maintain the database but also who should be responsible for the financial costs of sample storage given that the host institution could not be expected to derive any benefit from maintaining and distributing these samples? Current good scientific practice is that samples of MGR collected by scientists are usually logged at laboratory, institutional and / or sometimes national level as part of a coordinated national system. Samples are also routinely shared amongst the research community through requests from colleagues, collaborating institutes or project partners. So whilst a bottleneck does exist in terms of which countries have capacity to undertake oceanographic research, once samples are returned to laboratories, there exists within the global research community an extensive network for the sharing of samples, data, and knowledge. Improved transparency in terms of what samples are held where and by whom would of benefit to the research community.

Another means of exploring the potential of MGR from ABNJ is via bioinformatics databases which create the potential for sharing enormous amounts of data and information. However, many of the functions attributed to genes identified are putative, based on predictions. For many marine-derived

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8 For the reasons mentioned previously this would be unlikely to represent a true duplicate but would be a sub-sample.
genes, no clear function can be attributed and further laboratory analysis is required to define the function of the gene products. While the accessibility of bioinformatics databases is a major benefit, it is often not clear how the person who deposited the gene sequences obtained the MGR from which the sequences are derived. This may become critical when an attempt is made to protect IP based on this particular MGR and its genetic information (Bagley and Rai 2013). Since these databases are open access, what must also be considered is the impact of cheap gene synthesis, allowing anyone to synthesise a gene deposited in an open access bioinformatic database. This allows possible access to gene products (proteins, small molecules), and eventually organisms, all without access to the original source genetic material and raises the obvious issue of traceability.

This aim of this paper was to provide a non-technical synopsis of both the biodiscovery process and some of the most important associated issues. In doing so, it is hoped to inform the discussions that are currently taking place, for example, in the context of the Intergovernmental Conference to draft an ILBI on BBNJ for UNCLOS. We do not propose to provide here the answers to these complex problems, but rather to provide some clarity around the scientific aspects of sampling, research and development which the ILBI may seek to address.

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