doi:10.20944/preprints201801.0220.v1

Peer-reviewed version available at *Sustainability* **2018**, *10*, 526; doi:10.3390/su10020526

A European Community COST ACTION grant proposal

MARISTEM - STEM CELLS OF MARINE/AQUATIC INVERTEBRATES: FROM BASIC RESEARCH TO INNOVATIVE APPLICATIONS

Loriano Ballarin[1]*, Baruch Rinkevich[2], Kerstin Bartscherer[3], Artur Burzynski[4], Sebastien Cambier[5], Matteo Cammarata[6], Isabelle Domart-Coulon[7], Damjana Drobne[8], Juanma Encinas[9], Uri Frank[10], Anne-Marie Geneviere[11], Bert Hobmayer[12], Helike Löhelaid[13], Daniel Lyons[14], Pedro Martinez[15], Paola Oliveri[16], Lorena Peric[17], Stefano Piraino[18], Andreja Ramšak[19], Sebastian Rakers[20], Fabian Rentzsch[21], Amalia Rosner[22], Tiago Henriques da Silva[23], Ildiko Somorjai[24], Sherif Suleiman[25] and Ana Varela Coelho[26]

[1] Dipartimento di Biologia, University of Padova, Via U. Bassi 58/B 35100 Padova - Italy; loriano.ballarin@unipd.it

[2] Israel Oceanography and Limnological Research, National Institute of Oceanography, Tel Shikmona, PO Box 8030, Haifa 31080, Israel; buki@ocean.org.il

[3] Max Planck Institute for Molecular Biomedicine, Röntgenstraße 20, and Medical Faculty, University of Münster, Albert-Schweitzer-Campus 1, 48149 Münster, Germany; kerstin.bartscherer@mpi-muenster.mpg.de

[4] Institute of Oceanology, Polish Academy of Sciences, Powstańców Warszawy 55, 81-712, Sopot, Poland; aburzynski@iopan.gda.pl

[5] Luxembourg Institute of Science and Technology, 5, avenue des Hauts-Fourneaux, L-4362 Esch-sur-Alzette, Luxembourg; sebastien.cambier@list.lu

[6] Department of Earth and Marine Sciences, University of Palermo, Via Archirafi 22, 90123 Palermo, Italy; matteo.cammarata@unipa.it

[7] Muséum National d'Histoire Naturelle, MCAM Laboratory (UMR 7245CNRS-MNHN) 75005 Paris, France; icoulon@mnhn.fr

[8] Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia; damjana.drobne@bf.uni-lj.si

[9] Achucarro Basque Center for Neuroscience, Science Park of the UPV/EHU, Sede Building, Barrio Sarriena, E-48940 Leioa, and Ikerbasque, The Basque Foundation for Science. María Díaz Haroko Kalea, 3, 48013 Bilbo, Spain; jm.encinas@ikerbasque.org

[10] School of Natural Sciences, Biomedical Science Building, Dangan, National University of Ireland, Galway, Ireland; uri.frank@nuigalway.ie

[11] Sorbonne Université, UPMC, Univ Paris 06, CNRS, Biologie Intégrative des Organismes Marins (BIOM), Observatoire Océanologique, F-66650 Banyuls/Mer, France; anne-marie.geneviere@obs-banyuls.fr

[12] Institut für Zoologie, Universität Innsbruck, Technikerstraβe 50, A-6020 Innsbruck, Austria; Bert.Hobmayer@uibk.ac.at

[13] Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia; helike.lohelaid@ttu.ee

[14] Center for Marine Research, Ruđer Bošković Institute, G. Paliaga 5, HR-52210 Rovinj, Croatia; lyons@irb.hr

[15] Departament de Genetica, Universitat de Barcelona, Av. Diagonal 643, 08028-Barcelona, Spain and Institut Català de Recerca i Estudis Avancats (ICREA), Barcelona, Spain; pedro.martinez@ub.edu

[16] GEE & CDB Departments, University College London, Darwin Building, Gower Street, London, WC1E 6BT, UK; p.oliveri@ucl.ac.uk

[17] Rudjer Boskovic Institute, Laboratory for Aquaculture and Pathology of Aquaculture Organisms, Bijenicka cesta 54, HR-10000 Zagreb, Croatia; lorena.peric@cim.irb.hr

[18] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, Strada Provinciale Lecce-Monteroni, 73100 Lecce, Italy; stefano.piraino@unisalento.it

[19] National Institute of Biology, Marine Biology Station, Fornače 41, SI-6330 Piran, Slovenia; andreja.ramsak@nib.si

[20] Fraunhofer Research Institution for Marine Biotechnology and Cell Technology, Mönkhofer Weg 239a, 23562 Lübeck, Germany; sebastian.rakers@emb.fraunhofer.de

[21] Sars International Centre for Marine Molecular Biology, University of Bergen, Thormøhlensgt. 55, N-5006 Bergen, Norway; Fabian.Rentzsch@uib.no

[22] Israel Oceanography and Limnological Research, National Institute of Oceanography, Tel Shikmona, PO Box 8030, Haifa 31080, Israel; amalia@ocean.org.il
[23] Biomaterials, Biodegradables and Biomimetics, University of Minho, AvePark - Parque de Ciência e Tecnologia, 4805-017 Barco, Guimarães, Portugal; tiago.quinteiros@gmail.com
[24] Scottish Oceans Insitute, East Sands, University of St Andrews, KY16 8LB and School of Biology, Biomedical Sciences Research Complex, North Haugh, University of St Andrews, St Andrews, KY16 9ST, UK; imls@st-andrews.ac.uk
[25] Anatomy Department, Faculty of Medicine & Surgery, University of Malta, Msida, MSD2080, Malta; sherif.s.suleiman@um.edu.mt
[26] Universitade Nova de Lisboa, ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Av. da República, 2780-157 Oeiras, Portugal; Varela@itqb.unl.pt

* Correspondence: loriano.ballarin@unipd.it; Tel.: +39 049 827 6197

Abstract: The “stem cells” discipline represents one of the most dynamic areas in biomedicine. While adult marine/aquatic invertebrate stem cell (MISC) biology is of prime research and medical interest, studies on stem cells from organisms outside the classical vertebrate (e.g., human, mouse, zebrafish) and invertebrate (e.g., *Drosophila*, *Caenorhabditis*) models have not been pursued vigorously. Marine/aquatic invertebrates constitute the largest biodiversity and the widest phylogenetic radiation on Earth, from morphologically simple organisms (e.g. sponges, cnidarians), to the more complex mollusks, crustaceans, echinoderms and protochordates. These organisms illustrate a kaleidoscope of MISC-types that participate in the production of a large number of novel bioactive-molecules, many of which are of significant potential interest for human health. MISCs further participate in aging and regeneration phenomena, including whole-body regeneration.

For years, the European MISC-community has been highly fragmented and scarce ties were established with biomedical industries in attempts to harness MISCs for human welfare. Thus, it is important to: i) consolidate the fragmented European community working on MISCs; ii) promote and coordinate European research on MISC biology; iii) stimulate young researchers to embark on research in MISC-biology; iv) develop, validate, and network novel MISC tools and methodologies; v) establish the MISC discipline as a forefront interest of biomedical disciplines, including nanobiomedicine; vi) establish collaborations with industries to exploit MISCs as sources of bioactive molecules. In order to fill the recognised gaps, the EC-COST Action 16203 “MARISTEM”, has recently been launched. At its initial stage the consortium unites 26 scientists from EC countries, Cooperating countries and Near Neighbor Countries.

Keywords: aging; bioactive molecules; blue biotechnology; cancer; cell culture; COST Action; Europe; marine/aquatic invertebrates; regeneration; stem cells

---

Peer-reviewed version available at Sustainability 2018, 10, 526; doi:10.3390/su10020526

1. Science and Technology Excellence of the Project

1.1 Challenge

1.1.1 Description of the Challenge (Main Aim)

The primary aim of this COST Action is to foster, at the European level, knowledge of the biology of marine/aquatic invertebrate stem cells (MISCs) to develop innovative ideas relevant to various environmental and biomedical disciplines. In line with the Ostend declaration [1] and the European Marine Board strategic recommendations [2], specific aims of this Action are: (a) to overcome the scientific boundaries and obstacles facing the European MISC community; (b) to consolidate the fragmented community; (c) to integrate the MISC field with environmental (e.g. ecotoxicity and impacts of environmental stressors) and biomedical disciplines (such as aging, cancer, immunology, regeneration, stem cell biology, etc.); (d) to strengthen the European research community on MISCs. This will be accomplished by promoting: i) joint research on stem cell biology; ii) the sharing innovative ideas and discussion on compared models; iii) coordination of research in MISC biology; iv) establishing ties with biomedical and biotechnology industries to harness MISCs for animal and human welfare, as well as sources of molecules of interest for biotechnological purposes; v) training the next generation of biologists in MISC research.

1.1.2 Relevance and Timeliness

Marine/aquatic invertebrates represent a large biodiversity assemblage of multicellular organisms and the widest phylogenetic radiation on Earth, with more than 2,000,000 species formally described (95% of the overall animal biodiversity). They have been employed as laboratory models for more than 150 years and have contributed to the elucidation of various biological problems. Of particular significance were the first experiments on phagocytosis in sea star larvae [3], the first studies on biological chimeras in corals [4], the importance of the sea urchin to understand the molecular basis of development [5-7], including the "gene regulatory networks", the molecular control of proliferation (cyclins identified there) [8], the studies on primitive immunity in colonial organisms (sponges, hydrozoans, corals, urochordates, bryozoans), the plasticity of development, the use of aquatic flatworms for regeneration studies [9] and the discovery of green fluorescent protein in cnidarians [10], among many others.

While up until a few years ago, the use of invertebrates as model organisms was limited by the paucity of "–omics" data, the situation has rapidly changed and is still changing. Today, the genomes and various transcriptomes of many marine/aquatic invertebrate species (Non-Bilateria, Ecdysozoa, Spiralia and Deuterostomata), as well as many recombinant proteins of invertebrate origin, are available. New technologies such as RADseq, RNAseq, ChIP-sequencing and other next-generation sequencing methods), epigenome characterization, mass spectrometry and protein profiling, reverse-phased protein microarrays, combined with novel bioinformatic tools, have revolutionized the available tool-box of research methodologies. These can be used to further develop marine/aquatic invertebrates as reliable model organisms in a variety of biological research, including their stem cells. There are three major routes of involvement where MISCs are highly relevant and timely:

a) The understanding of fundamental biological processes. MISCs are key players in the biology of aquatic invertebrates, with a central role in many biological processes [11-12]. While much is known about adult stem cells and their properties in vertebrates (primarily mammals) and some model terrestrial invertebrates (i.e., *Drosophila*), very little has been learnt on the nature and properties of MISCs. Aquatic invertebrates exhibit multiple cell types with stem cell attributes. Studies revealed that, in contrast to the prevalence of diverse oligopotent and unipotent stem cells in vertebrates, marine invertebrates appear to display the communal spread of multipotency and pluripotency [10], with adult stem cells that give rise to cell lineages characteristic of more than a single germ layer, sometimes with somatic and germ line potential. In addition, unlike vertebrates, in many aquatic/marine invertebrates, stem cells are disseminated and widespread inside the animal body, i.e., not associated with a regulatory microenvironment (niche) [12-13]. It is also of note that transdifferentiation (today, a topic of great interest when trying to understand how to "reprogramme" a cell) is prevalent in both anatomically simple and "morphologically complex" invertebrates [14-15]. These observations delineate common and unique properties of MISCs, possibly tailored to suit the varied life history traits, ecology and developmental modes characteristic of aquatic invertebrates [10].

b) Alternatives to the use of vertebrates. The recent directive of the EU (2010/63/EU) [16] on the protection of animals used for scientific purposes is highly restrictive for the use of complex, classical vertebrate models in biological research. Following the general scientific trend and to bypass restrictions imposed by the directive on behalf of the protection of animals, researchers turned to the use vertebrate cell lines and primary cultures to provide answers to a wide array of biological questions. This approach greatly limits the comprehension of biological phenomena at the organismal level as well as from an evolutionary perspective. Unlike vertebrates, invertebrates (cephalopods excluded) are not included in the EU directive and offer the possibility of *in vivo* analyses. In many cases, they not only successfully replace mammals or other vertebrates as laboratory animals in biological research but also provide added value due to their simpler body organization or reduced genetic complexity, and by presenting biological phenomena that do not occur in vertebrates (like whole body regeneration, concealed vs. programmed aging, etc.).

c) The importance of MISCs for the biomedical and biotechnology industries. Marine/aquatic biotechnology is an emerging discipline aiming at the use of marine bio-resources for biotechnological applications (Blue Biotechnology) [2, 17-20]. However, industries traditionally lack familiarity with the marine environment. The latter, with its vast genetic richness, is a potential source of new products of socio-economic value, as marine organisms produce molecules (enzymes, biopolymers, bioactive compounds, secondary metabolites) with applications in various fields, such as nutraceutics, cosmetics, antibiotics, disease-fighting drugs, antifouling products, biomaterials and more [21-25]. In addition, marine invertebrates exhibit a kaleidoscope of MISC types that participate in the production of this plethora of novel bioactive molecules (antitumor, antimicrobial, and anti-inflammatory), with significant potential interest for human health and well being. MISCs also participate in unique aging and regeneration phenomena, including whole-body regeneration, implying lack of senescence/aging [9, 26], and are responsible of the presence of unique stemness systems, without the formation of tumors, the knowledge of which could be of great help in medicine. The increasing availability of genomes from marine animals will allow the rapid detection of the gene networks involved in these biological phenomena, as well as biosynthetic pathways of new useful bioactive molecules and metabolites through the functional analysis of cloned gene libraries. In addition, marine organisms and their cells are of great use to study the impacts of environmental stressors, global warming and ocean acidification on biota.

The European MISC community, however, confronts these scientific boundaries and obstacles:

a) Ties with biomedical and biotechnology industries. Current European marine/aquatic biotechnology initiatives are mainly focused on identifying novel molecules and biosynthetic pathways, but there is a critical lack of cellular models able to express biomolecules and test their effect on environmentally significant organisms. At present, most of the research on MISCs is primarily fundamental, leading to papers published in scientific journals; but precisely this may stand in the way of commercialization and economic criteria. All this knowledge needs to be translated into innovation. The future of marine biotechnology (including MISC research) for industrial and medical applications is very promising. The global market for marine biotechnology is estimated at 4.1 billion USD in 2015, and has the potential to reach 4.8 billion USD by 2020 and 6.4 billion USD by 2025 [27]. The marine environment accounts for the great majority of the ecosystems and living species on Earth. Marine biotechnology follows the diverse opportunities that have emerged in a wide variety of biotechnology areas and medical fields, from basic research to industrial applications. In the European Community, interest in marine biotechnology by the scientific community, and to some extent by the industry, has grown rapidly in the past decade. The

Peer-reviewed version available at Sustainability 2018, 10, 526; doi:10.3390/su10020526

main drivers are the recognition of the sheer scale of opportunity presented by the largely unexplored and immense biodiversity of European seas and oceans and the increasing availability of molecular tools to explore this biodiversity. However, cellular tools are critically lacking to validate these molecular studies. Such important barriers and challenges need to be tackled at various levels for Europe to remain a key player in marine biotechnology research [2]. Part of the problem is the relatively low spending of gross domestic product on research and development in the marine arena. Another obstacle is that the EU, it takes too long to transform research and innovation results into marketable products.

b) Social implications. Boosting marine innovation through biotechnology-related activities is specifically highlighted in Horizon 2020: under the priority 'Better Society' [28]. This is widely supported by the notion that the successful development of the marine biotechnology sector in Europe would be performed within an industry-academic collaborative environment. However, recent developments in green biotechnology, nanotechnology and synthetic biology have shown how critical the social acceptability of new technological knowledge has become. Social acceptance of the MISC technology, and marine biotechnology as a whole, faces an important challenge in convincing a large array of stakeholders that, on one hand, it does not build on irresponsible knowledge, and, on the other hand, it can fulfill the numerous promises made [29]. This COST Action will help to identify the main bottlenecks that could impede the development of the MISC discipline and, therefore, provide standards and guidance in dealing with the corresponding policy issues, with input into the recent European move towards Responsible Research and Innovation initiative.

c) Need for coordinated European research on marine/aquatic stem cells. The community engaged in MISC in COST countries is highly fragmented, each laboratory working on its organism or cell of choice using different methodologies. A preliminary census in COST countries led to the identification of >200 MISC scientists in different institutions, located in coastal and in inland laboratories, employed by the government, universities and research institutions. Additionally, numerous scientists are employed by biotechnology entities. In other marine sciences fields (e.g., oceanography), the need for sharing costly research instruments (e.g., vessels) has led to higher research integration. Since this is not the case for MISC research in COST countries, far fewer scientific innovations have been produced, negatively affecting this discipline.

d) Training of next generation researchers. Unlike the ongoing European Blue Biotech projects, focused on molecules and genes of biological interest, this COST Action is mainly biodiversity oriented, with particular attention to stem cells and their potential for applied research. In general, there is a lack of European students and young scientists with a thorough knowledge of the marine/aquatic biodiversity who also receive the practical training in cutting-edge techniques and cellular approaches that underpin ongoing developments in marine biotechnology, including MISC. There is also a clear need to support high quality new students and young researchers with a broad education in marine science, medicine and biotechnology that are ready to become leaders in this rapidly expanding field. Additionally, there is a shortage of university programmes which focus on the sea in integrated courses, research and practical experience, as well as few partnerships of the universities with the local biotechnology industry. It is therefore a prime mission to prepare a new generation of talented students for careers in the MISC discipline (research and industry). This field will not be developed without a constant flow of young scientists actively involved in the various aspects of MISC research. Up to now, only a small number of graduate students have engaged with MISCs, primarily due to the lack of established and standardized methodologies. Addressing specific needs in education and training in the field of marine biotechnology (including MISCs), will bring together crosscutting disciplines such as marine biology, cell biology, bio-informatics, systems biology, synthetic biology, etc.

1.2 Objectives

1.2.1 Research Coordination Objectives

MARISTEM has a pan- European focus on stem cells from marine (as well as aquatic) invertebrates (MISCs) and connects to the EC- International Partner Countries (IPCs), EC-Associated Countries (AC) and Near Neighbor Countries (NNCs) to achieve its Research Coordination Objectives. The following objectives aim to overcome the fragmentation within the European MISC community, strengthen the collaboration among the European research community on MISCs, foster the MISC discipline at the academic level, and link the MISC research area with biomedical disciplines (such as aging, cancer, immunology, regeneration, stem cell biology, etc.). Research coordination activities include:

1. Consolidation of the European community of scientists involved in MISC research. This will be accomplished through the: i) organization of annual meetings for data sharing; ii) promotion of Short-Term Scientific Missions for technical training; iii) creation of an Action website and a newsletter acting as a discussion forum for MISC issues central to the MISC community; iv) setting up new collaborations among participants; v) organization of workshops on MISC and specific methodologies related to MISCs, in particular addressing the bottlenecks in MISC identification and *in vitro* culture; vi) publication of an updated review on MISCs in a qualified scientific journal; vii) organization of participants in Working Groups to address the scientific tasks described below.
2. Presentation of MISC research, sharing of methodologies/databases used in MISC research in various European countries, and updating scientific and technical guidelines for standardization of methods, techniques and protocols, to maximize the extent and the quality of the results. The objectives should be achieved through an integrated effort to develop technologies that allow the isolation, phenotyping and culture of stem cells, and those state-of-the art methodologies that allow us to test the effectiveness of MISC-derived bioactive molecules in biomedicine and biotechnology.
3. Establish ties with biomedical and biotechnology industries for the exploitation of MISCs and the derived results (for instance, in the fields of immunity, regeneration and aging, bioactive molecules).
4. Coordinate funding applications, at European level, emphasizing doctoral and post-doctoral research opportunities.
5. Coordinate collaborative and scientific ties, at the international level, with scientists working on MISCs, primarily from the USA and Japan.

1.2.2. Capacity- Building Objectives

MARISTEM seeks to drive both scientific progress and technological innovation in the MISC field of research through the following four major Capacity Building Objectives:

1. Strengthening the European Community on MISC through the setting up of new collaborations among participants and the promotion of Short-Term Scientific Missions for technical training.
2. Promoting interactions of Action members to establish a defined identity and profile in the European field of MISC; establishing ties with European networks, scientific societies/institutions and/or large-scale funded projects in related fields (e.g., European Society for Marine Biotechnology, Assemble Plus, EuroMarine, EuroStemCell, EMBRC-ERIC, Corbel, EuroSyStem, Neurostemcell, Neurostemcellrepair, OptiStem, ESTOOLS).
3. Stimulating contacts and the development of a joint research agenda in order to strengthen future research on MISCs.
4. Establishing working groups, as listed below, to collaborate on specific topics with defined tasks.

Peer-reviewed version available at *Sustainability* **2018**, *10*, 526; doi:10.3390/su10020526

1.3 Progress beyond the State-of-the Art and Innovation Potential

1.3.1 Description of the State-of-the Art

Stem cells in multicellular organisms possess the unique ability to remain in undifferentiated state and, upon demand, originate cells that differentiate. This has been well established in vertebrates and terrestrial invertebrates, where much of the research activities have been linked to applied aspects of stem cell biology. However, the excessive focus on the applied outcomes of stem cell biology poses the risk of missing the wide range of stem cell properties that are represented in various multicellular taxa, primarily in taxa belonging to the aquatic invertebrates. In addition, working on vertebrate systems is extremely expensive and raise several ethical concerns. In many activities, invertebrates can replace vertebrates and, in addition, they can offer new perspectives on MISC function, properties and evolution.

Not only do MISCs share a whole range of novel biological properties but, unlike vertebrates, they are also key participants in aging and regeneration phenomena. The comparison of well-known biological processes in model systems with novel phenomena, associated with the biology of MISCs, can help to better understand events in mammalian stem cells biology - including natural chimerism and cancer, aging and senescence, immunity and autoimmune responses - all representing phenomena difficult to directly address within the human context.

A careful literature search attests to the fact that studies on stem cells from organisms that not defined as the classical model systems (such as human, mouse, zebrafish, *Drosophila*, *Caenorhabditis*, etc.), have not been pursued vigorously. This is because these cells are generally few in number, sometimes not specifically characterized, are studied in biological systems not amenable for *in vitro* work and, probably, are less plastic.

Recent studies have shown that the best organisms for stem cell research are the marine/aquatic invertebrates. These organisms possess numerous types and lineages of stem cells that can offer, once studied in the lab, important clues to the understanding of stem cell biology. Stem cells are present in marine/aquatic organisms, in either (morphologically) simple organisms, such as cnidarians, sponges and flatworms, or in anatomically more complex taxa, such as crustaceans, echinoderms and protochordates (urochordates and cephalochordates) (Fig. 1). Unique to many of the aforementioned marine invertebrates, is the property that some adult MISCs are pluripotent, capable of developing both the germ line and somatic tissues, and are involved in asexual reproduction and regeneration. Marine organisms challenge the prevailing dogmas on stem cell structures, niches and cell lineages biology. They also challenge the existing concepts on the genetic and epigenetic control of stem cell differentiation. Due to their simpler morphological and tissue organization and the accessibility of some of them to genetic manipulation, marine/aquatic invertebrates are reliable and efficient model systems to investigate the molecular basis of stemness and stem cell regulation. This problematics can be accomplished by analyzing the molecular interactions between stem cells and their niches, under controlled *in vivo* conditions.

Peer-reviewed version available at *Sustainability* **2018**, *10*, 526; doi:10.3390/su10020526

Figure 1. Colony of the ascidian *Botryllus schlosseri*. In this species, zooids are grouped in star-shaped systems (two systems are visible here) and stem cells assure the rejuvenation of the colony through the cyclical production of palleal buds, as well as the whole body regeneration (also known as vascular budding), when all the zooids and buds are ablated and new zooids originated from the hemocytes in the tunic vasculature. **A**: ampulla (blind, contractile ending of the tunic circulation); **B**: bud; **CS**: cloacal siphon of the system; **OS**: oral siphon of the zooids; **T**: tunic; **V**: tunic vessel; **Z**: zooid. Scale bar: 1 mm.

1.3.2 Progress beyond the State-of-the Art

Many of the features revealed in MISCs have not been recorded in stem cells from vertebrates, including the almost absence of association with regulatory microenvironments (niches), their widespread distribution (and high percentage) in all marine animals, their pluripotency, and the feeble distinction between somatic and germ stem cells lineages, among others [30]. However, while the literature on stem cells from vertebrates is rich and expanding at an exponential rate, investigations on MISCs are scarce and limited in scope, despite the results underscoring the importance of MISCs in the understanding of various biological processes, such as the mechanisms promoting cell growth and differentiation, regeneration and budding typical of marine invertebrates, tissue homeostasis of marine organisms (including those that may live for decades), aging and senescence.

The concepts of stem cell-based organogenesis, aging, cancer and regeneration are interrelated and shared among evolutionary lineages. Therefore, stem cells are not only entities of biological organization, accountable for the formation and regeneration of specific tissue and organ systems, but are also units in the complex evolutionary selection process [31]. Thus, a clear understanding of the relationships between stem cells and the aforementioned processes, and the possibility of their conservation across systems will push the MISC community in Europe far ahead of the current state-of-the-art.

This COST Action, which proposes to strengthen the European research community on MISCs and promotes joint research among Action members on comparative stem cell biology, developing innovative ideas and technology, is the most appropriate tool for significant European progression, breaking through the current state-of-the-art. Exponential growth in the number of investigations on MISCs, as well as a more integrated European scientific community engaged with MISC research,

Peer-reviewed version available at Sustainability 2018, 10, 526; doi:10.3390/su10020526

will offer exciting new avenues for advancing knowledge of MISC biology as well as providing novel systems for medical and economical applications.

1.3.3 Innovation in Tackling the Challenge

Interdisciplinary approaches are crucial to tackle the challenges posed by MISC research and are central to the proposed holistic, integrated approach to managing the MISC community. Pluripotent MISCs provide powerful and physiologically-relevant systems to characterize the regulatory mechanisms that control cellular differentiation at all organizational levels of biology, also impacting on mammalian systems. We identify the following three key strategies to target current and future scientific and economic awareness:

a) development of standardized and optimized protocols. Innovative technologies and strategies that are currently and will continue to be employed and will be employed to decipher the mechanisms that control the directed differentiation of pluripotent stem cells are the base rationale for the scientific approach in tackling the challenge. There is the need to set up and share good protocols for rearing marine invertebrates and for MISC isolation, culture and phenotyping. This should be done on a wide spectrum of marine invertebrate species in order to support subsequent comparative analyses. This approach requires the integration of different laboratories, in EC countries and within universities/research institutions, and small biotech companies in each specific EC state. The COST umbrella is probably the best and most innovative approach to tackle all the challenges that have been presented here. Innovation will be achieved by addressing scientific problems with clear objectives, such as those indicated in section 3 of this manuscript, and through the frequent interactions among the Action members. This will also enhance competitiveness of the EC research.

b) consolidation of the fragmented scientific arena. The scientific community in the European countries currently engaged in MISC biology is fragmented and composed by too few established scientists. The recruitment of a new generation of young scientists interested in MISCs, the consolidation of the fragmented MISC community in Europe, the frequent meeting among members, through participation in workshops, Action meetings and short term visits to other laboratories, will favor the generation of new ideas and joint research projects in the field of MISC research.

c) Fostering contacts with industries. The MISC field needs to develop connections with relevant biotechnology industries. One of the challenges faced is to convince the multiple types of stakeholders (i.e., those reported in para 2.2.1) that, through its know-how and expertise, it can achieve what is being promised. Contacts with industries interested in exploiting MISCs for their regenerative potential and/or the production of bioactive molecules or metabolites useful in animal/human welfare and/or biotechnological applications will be established. Marine/aquatic organisms have revealed (and still can reveal) unexpected gene regulatory pathways of interest to regenerative biology. In addition, they are known sources of valuable biologically active substances for the pharmaceutical, biotechnology and food industries. Many marine secondary metabolites are known for their highly effective antioxidant, antibacterial, antifungal, antifouling and antitumor activities. Ideally, the possibility to produce such bioactive compounds using MISC-derived immortalized cell lines would be of great importance in developing societal improvements and advances. An academic-industrial collaborative approach can add new value to the applied MISC research.

1.4. Added Value of Networking

1.4.1. In relation to the Challenge

Stem cell science is an emerging global industry in which European countries fiercely compete for economic advantage in an arena where, currently, the USA and Japan dominate. Most European groups (academics, as well as companies and SMEs [Small and Medium size Enterprises]) are dispersing their scientific efforts using different biological models and methodologies, in an area where no stream of knowledge and intellectual property are still available. There is thus a pressing need for developing expertise, approaches and tools. The only way forward is by consolidating a network of MISC groups sharing expertise. We cannot be competitive in the international arena if we do not promote our own research groups/projects through collaborative, intra-EU actions. Networking is one of the most efficient approaches for consolidation of the European MISC community and for the raising of European competitiveness and leadership. Such networking should focus on the biological diversity of MISCs and their structures, pluripotency properties, development of understudied, unique or culturally valuable (e.g., aging, cancer) biological/applied aspects. Thus, the Network will develop European leadership and expertise in the MISC discipline.

The fragmented research community currently limits European competitiveness also in technology transfer to potential end-users. For example, there are currently few SMEs in COST countries that consider MISCs as part of their R&D plan. Thus, our Action will not only offer a unique nucleus of commercial importance, but also the pooling of individually acquired knowledge within a single community, offering the participants better access to international grant agencies or products under development in each country/group. Thus this COST Action will have a competitive ability to develop innovative, effective, and flexible adaptation strategies that will address multiple national, regional, and global priorities in key economic and social sectors.

Networking will promote the discussion of novelties and research results, will attract students to the MISC discipline, will provide opportunities to mix newcomers with important players in the MISC arena and stem cells biology discipline and learn about the potential offered by the markets. In addition, amalgamating the EC community working on MISC (both academic and industries) may pose significant challenges to the future of EC stem cell science. Particular attention will be paid to the training of early-stage researchers by organizing summer schools, by favoring their mobility through short-term scientific missions and by promoting their participation in scientific meetings and workshops. We firmly believe that the coming generations of young researchers (trained in the MISC discipline) will be the future leaders of marine science.

1.4.2. In Relation to Existing Efforts at European and/or International Level

Despite the great efforts and the variety of funding schemes and actions within Horizon 2020, no former or existing scientific networks or projects in Europe dealt/are dealing with MISCs and, up to now, no individual entity (either private or national institute) in the European MISC discipline has gathered a critical mass of researchers and knowledge to become a globally leading contributor. There is an urgent need to increase the cohesion between scientific institutions and industry in this field via the creation of a COST network. This multidisciplinary collaboration would eventually bypass methodological bottlenecks, gaps and barriers. Indeed, because of the fragmentation of the scientific community, we do not know whether methodologies developed for other animal systems are being utilized in developing MISC or have failed; likewise, failed experiments and approaches are not being presented or published in peer-reviewed journals. This makes exchange of information utterly crucial in the research community, sharing promising methods and avoiding replication of failed or redundant procedures. De-fragmentation of the MISC scientific community is therefore expected to revolutionize this field, resulting in novel and generic technologies and products.

This COST Action will be tightly connected with several European associations that deal with cell cultures. One such important organization is the European Collection of Authenticated Cell Cultures (ECACC), a supplier of authenticated and quality controlled cell lines [32]. ECACC was established in 1985 as a cell culture collection to service the research community and to provide an International Depository Authority for patent deposition for Europe. The same implies for the Horizon 2020 research and innovation program called EuroStemCell [33] that tries, among its major goals, 'to help European citizens make sense of stem cells, by providing independent, expert-reviewed information and road-tested educational resources on stem cells and their impact on society'. The MISC partners will also team with the ECVAM [34], which is the EC reference Centre for the development and validation of alternative testing methods to replace, reduce or refine the use of laboratory animals in biomedical sciences, with an emphasis on toxicology assessment.

Peer-reviewed version available at Sustainability 2018, 10, 526; doi:10.3390/su10020526

The proposed COST Action will be integrated within the Blue growth initiatives included in other EU science consortia/networks/platforms (Assemble PLus, EuroMarine, EuroStemCells, EMBRC-ERIC, Corbel, ERA-MBT, EuroSyStem, Neurostemcell, Neurostemcellrepair), and will contact participants in current and former EU projects on marine and stem cell science, such as MarBEF, EurOceans, MarineGenomics, OptiStem, ESTOOLS.

2. Impacts

2.1. Expected Impact

2.1.1. Short-term and Long-term Scientific, Technological, and/or Socioeconomic Impacts

The aim of this proposal is to mobilize the European scientific expertise in MISC research and associated technological resources to provide professionals with relevant state-of-the-art information, methods and tools for the amelioration and treatment of diseases (gene regulatory networks, antimicrobials, antimitotic compounds), and for pharmaceutical and biotechnological purposes (enzymes, polymers, secondary metabolites). The heavy investment in developing and sharing technologies, from genomic/proteomic tools to those that use high throughput analysis of chemical compounds, necessitates the support of a solid network of scientists with complementary expertise. The MISC team will provide such a wide range of expertise. The Network will use the following resources to determine how and when to involve end users in knowledge dissemination plans. Knowledge translation (e.g. synthesis, dissemination, exchange, and ethically sound application of knowledge) will be used to improve MISC activities within a system of interactions between researchers and knowledge users. Interactions may vary in intensity, complexity, and level of engagement depending on the research and findings, as well as the needs of particular knowledge users.

Scientific-technological short-term impacts

- creation of a Europe-wide research network to answer key questions on MISCs;
- promotion of interactions among scientific institutions interested in MISCs;
- release of updated standardized scientific protocols/technical guidelines for rearing of marine/aquatic invertebrates and MISC isolation and culture;
- creation of a registry/repository for sharing data on MISC research;
- creation of a website and a newsletter as a forum to link the MISC community;
- common publications in peer-reviewed and open access scientific journals describing optimized protocols for MISC isolation and rearing.

Socio-economic short-term impacts:

- dissemination of COST Action results and public awareness on importance and potential of MISCs;
- starting of collaboration with industries for the exploitation of MISCs;
- training of talented students/young researchers;
- stimulating the creation of new networks for fund-raising opportunities.

Scientific-technological long-term impacts:

- new insights on the biology of MISCs and on mechanisms controlling their *in vitro* growth and evaluation of the possibility of the production of bioactive compounds from *in-vitro* culture of MISCs. Results will represent a commonly distributed know-how;
- contribution to a wide range of biomedical disciplines, including regenerative medicine, aging and cancer. The study of MISCs will increase commonly shared and evolutionary perspectives in these disciplines, towards a more comprehensive understanding of these cells;
- positive impact on regeneration biology as marine/aquatic invertebrates have unique regeneration potential and can contribute to the comprehension of the constraints preventing large scale regeneration in vertebrates. MISCs can also be used to assay the impact of different chemicals in their ability to regenerate tissues;

Peer-reviewed version available at Sustainability 2018, 10, 526; doi:10.3390/su10020526

- new strategies for sustainable exploitation of marine/aquatic bioproducts, and for development of alternative ecotoxicological tests, meeting international regulations, that can be used by biomedical and biotechnological industries. The management of intellectual and industrial property rights arising from this Action will ensure that eventual benefits of MISC project results are shared fairly and reasonably among the institutions of the COST Action participants;
- better understanding the impact of environmental stressors (temperature, acidification, etc.) in regeneration processes and the resilience of challenged aquatic ecosystems;
- MISCs can differentiate in a variety of cell lines, including hemocytes and, among them, immunocytes. Therefore, the study of MISC differentiation to immunocytes can provide a better elucidation of the behavior of the immune system in reared, edible marine/aquatic invertebrates and help in the control of diseases and viral infections in aquaculture.

Socio-economic long-term impacts:

- change of public perception concerning marine/aquatic invertebrates leading towards a full awareness of the socio-economic importance of marine/aquatic biodiversity. As a matter of fact, degraded marine ecosystems provide fewer goods and services than healthy habitats via decreased abundance of living species. As regards invertebrates, as reported below, they can be the source of cellular systems which could be used for sustainable biotechnological production of new bioactive molecules useful for human and animal health, and other applications (e.g., antifouling, enzymes for biocatalysis, biopolymers, products of interest to pharmaceutics, nutraceutics and cosmetics) of interest to biomedical and biotech industries. We cannot imagine a more powerful impact for a project like this one;
- change of the social acceptability for the MISC importance in day to day life, not differently from other bio- technologies;
- efficiently delivery of MISC results to potential stakeholders through specific meetings with SMEs representatives;
- while still at early stages, MISC research is opening up a competitive niche of potentially lucrative avenues for the development of protocols and technologies to isolate, cultivate and exploit MISCs. Similarly, the MISC market is backed by biomedical research and bioprocessing;
- the availability of MISCs will also increase potential monetary benefits to society by adding novel tools for scientific research, including mammalian stem cells biology. This reflects the objectives of the European Strategy for Marine & Maritime Research and the last European Science Foundation positional paper on marine biotechnology [2]. MISCs also represent one of the targeted topics in the EC consortia ASSEMBLE [35] and EMBRC [36], aiming to promote marine laboratory infrastructures;
- preparing young European researchers to launch careers in the MISC discipline and become the new generation of MISC researchers in Europe.

2.2. Measures to Maximize Impact

2.2.1. Plan for Involving the most Relevant Stakeholders

At the start of the project, a dissemination plan will evaluate maximizing impacts using a: -who (relevant end-users) -how (dissemination plan) -when approach.

The most relevant stakeholders that we identified are the following:

- SMEs, in particular: i) the antifouling paint sector that can take advantage from new natural antifouling products preventing the growth of the bacterial film that triggers the adhesion of encrusting organisms, without any concern for the environment and alternative to those currently in use, which have profound effects on the biocoenoses once released in the environment [37]; ii) the fine chemical sector, for a wide range of materials; iii) the nutraceutics and cosmetic sector, interested in new useful bioactive molecules; iv) the pharmaceutical and medical device sector, where new antimicrobials are required to face the increasing number of bacterial strains resistant to penicillin-based antibiotics; v) the human health sector, as new antimitotic compounds can be of

Peer-reviewed version available at Sustainability 2018, 10, 526; doi:10.3390/su10020526

great help in the treatment of some kinds of cancer [23] and MISCs can provide new diagnostic and treatment devices based on nanobioengineering [38, 39];

- the medical community, which can gain new knowledge on alternative molecular mechanisms of aging, differentiation, tumor formation and regeneration operating in marine/aquatic invertebrates;
- the toxicologist, who can get new methods, tests and standards for safety evaluation of existing and new substances;
- the broader scientific community studying stem cells, their role and differentiation pathways, which can gain additional knowledge from the behavior of MISCs;
- the general public, who can gain benefits from the results of MISC research;
- the European networks interested in stem cells and MISCs, such as: i) EuroStemCell [33]; ii) the Horizon 2020 research and innovation program aiming to help European citizens make sense of stem cells; iii) ECVAM [33], European Commission reference Centre for the development and validation of alternative testing methods to replace, reduce or refine the use of laboratory animals in biomedical sciences; iv) ECACC, European Collection of Authenticated Cell Cultures, a supplier of authenticated and quality controlled cell lines [32].

2.2.2. Dissemination and/or Exploitation Plan

Scientific community

The diffusion of the results of the project, via publication of joint research articles and presentation at some of the most important international conferences in the field, together with organization of courses and seminars will help us to improve the visibility and impact of our network on an international scale. The detailed list of these dissemination activities includes:

- writing collaborative review articles on MISC research in peer-reviewed, high impact, open access scientific journals;
- editing a scientific book focused on MISCs and/or regenerative biology;
- exploiting courses/workshops/meetings to disseminate the main outcomes of the Action among scientists;
- promoting courses/teaching activities on MISCs in the European universities through the initiative of the participants of this COST Action;
- promoting inter-university agreements aiming at an International PhD program on MISCs;
- introducing students, in a mentoring capacity, to research on MISCs for their degree thesis;
- creating new networks, within the MISC community, supporting applications for research funding at national/international levels;
- participating in international conferences on stem cells. This will provide good opportunities to share the results obtained within the proposed COST Action with a wider scientist network;
- proposing technical documents for standardization.

SMEs

- promoting transfer of knowledge, expertise and technical skills from the proposed COST Action to the stakeholders as possible end-users through specific meetings/workshops;
- organizing specific workshops/meeting with industries to help the interaction with the biotechnology world. Contact with some SMEs interested in areas reported above has already been initiated and they will be invited to specific workshops, as indicated in section 3.1.2. Furthermore, the aforementioned European networks will be contacted by the management committee of this COST Action, and representatives will be invited to the meetings/workshops.

General public

It is clear to all of us that a pressing problem faced by EU countries in recent times is the communication between scientists and the general public. The striking features of simple aquatic organisms to which the general public is exposed during leisure activities at the seaside are good ambassadors to communicate about the potential of European research to improve their daily life. We want to emphasize that these planned activities are of special concern to us by:

Peer-reviewed version available at *Sustainability* **2018**, *10*, 526; doi:10.3390/su10020526

• activating and maintaining active, even after the closure of this COST Action, an open website as a preferential platform to share protocols, methods, etc. and to offer accessible knowledge to the general public;

• working together with Innovation and Press offices at our Institutions to organize outreach and public engagement activities for the general public, and to introduce the lay public to the research performed by our network.

2.3. Potential for Innovation versus Risk Level

2.3.1. Potential for scientific, technological and/or socioeconomic innovation breakthroughs

Stem cell biology in vertebrates has a great deal to offer to society and industry. The ability to culture vertebrate cells in the lab has supported tremendous breakthroughs in science over the years, from the very foundations of cell biology to the cell therapy and tumor stem cell biology. It is evident from the mammalian stem cell biology literature, that stem cells have been invaluable for treating a number of intractable diseases, and that boundaries are continuously pushed and frequent discoveries made. It is beyond dispute that innovative technologies in the mammalian stem cells arena continue to proliferate, striving to advance the research. There are two main critical issues in the clinical/commercial translation of stem cell intellectual property and products: (i) entrepreneurial exploitation of breakthrough ideas and innovations, and (ii) regulatory market approval. Thus, the commercial development of stem cells products and innovations reflects potential high risks due to technological challenges, changing policies and markets, as well as management changes, in this highly dynamic field. However, on the other hand, the benefits incurred from a successful approach are tremendous. What is surprising about the recent stem cell breakthrough in mammalian systems is that researchers make new discoveries that would otherwise go uncharted if the research was not specifically focused on the stem cells biology. The same applies for the MISC discipline, which answers the strategic breakthrough needs of various applied biotechnology and healthcare issues, and provides additional innovative facets that are not found in the mammalian stem cell discipline:

a) Comparative aspects. Comparative approaches on MISCs essentially consist of examining whole genome structures, gene arrangement and rearrangement, stem cells lineages, stem cells properties (such as stemness capabilities, structures, etc.) with the aim of delineating the evolution of gene families and cell lineages, the cellular and molecular basis of adaptation (including the identification of cells potentially involved in niche adaptation) as well as evolutionary relationships at various taxonomic levels in the Tree of Life. The high *in vivo* plasticity of MISC shapes, structures, cell replacements, proliferation processes and cell lineages encountered in different invertebrate taxa, make a comparative approach highly valuable. In the mammalian stem cell arena, relatively few comparative studies are available, and this lacuna severely constrains the potential value of many predictions on stem cells origins, activities and fates.

b) Environmental approaches. They deal with the understanding of the functional significance of cellular variation in MISCs - the basic unit of selection [31] - in natural biological entities. This includes the use of various genotyping approaches to delineate the structure of inter- and intraspecific biodiversity of MISCs, as well as the metagenomics approach of MISC, which treats entire organisms (sometimes even populations) as carrying a single living entity. This has never been addressed in the vertebrates in spite of various theoretical approaches [40].

c) Evolutionary perspectives. MISCs may also provide some understanding of evolutionary relationships among different phyla and within-phylum groups. This is particularly valid for organisms that possess MISC types with major evolutionary importance (such as stem cells in colonial urochordates), either with respect to phyletic novelty or to structural cell lineages that can only be investigated using an evolutionary approach. Looking to stem cell theories, we still have only a poor understanding of stem cell origins and their importance in governing the dynamics of stem cell populations over evolutionary time.

<u>d) Changing of current dogma(s)</u> such as disposable soma [41], irreversibility of aging [42], germ/somatic cell barriers [43], as demonstrated by the capacity of whole body regeneration from small fragments [26] or the ability to rejuvenate [44], easily found in marine/aquatic invertebrates.

3. Implementation

3.1. Description of the Work Plan

3.1.1. Description of Working Groups

We identified 4 working groups (WG), each involved in the analysis and the development of different specific topics, as described below.

<u>WG 1- Developing protocols for raising marine/aquatic invertebrate stem cells under *in vitro* conditions</u>

WG1 coordinates the activities of a series of tasks devoted to the development of common protocols, problem solutions and tools (such as the development of resource services) in order to foster integration of research institutions. It will focus on the tasks listed below. This will guide the development of shared services and solutions not only within the research institutions but also into the working environments of stakeholders and users to lay a solid foundation for long-term cooperation.

Task 1.1 - new marine invertebrate models and access to marine resources

Task 1.2 - the problem of endosymbionts in establishment of pure or mixed cell cultures of MISC

Task 1.3 - methods for stem cell enrichment in culture

Task 1.4 - immortalization of marine/aquatic invertebrate stem cells

Task 1.5 - cryopreservation of marine/aquatic invertebrate stem cells

Deliverables:

- List of reference laboratories/institutions/marine stations for the supply of marine/aquatic invertebrates
- Common protocols for MISC identification, isolation, rearing and storage
- Strategies to solve the problem of endosymbiont contamination that, up to now, made fruitless the efforts of *in vitro* rearing of MISCs

<u>WG 2- “omics” to characterize the MISC phenotypes</u>

Technical and scientific capabilities to support the cooperation are coordinated by WG2: molecular/biochemical profiling of novel model organisms needs data services for annotation, analysis and archiving.

Task 2.1 - comparative functional genomics and transcriptomics of marine/aquatic invertebrate tissues or derived MISCs

Task 2.2 - comparative proteomics of MISCs

Task 2.3 - differentiation molecular pathways of MISCs

Task 2.4 - development of strategies for “manipulating” stem cells (knockdown, CRISPR, transgenesis, etc.)

Deliverables:

- Stem cell markers for aquatic invertebrate organisms
- Shared, trans-European open access database with molecular data of the organisms of interest, with the possibility of continuous implementation by COST Action participants

<u>WG 3- Blue technology: MISCs as model systems for the study of (see tasks):</u>

Task 3.1 - evolutionary aspects of stem cell differentiation and development

Task 3.2 - cancer, aging and senescence phenomena

Task 3.3 - regeneration

Deliverables:

- Genes, signal transduction pathways, proteins involved in development, senescence, regeneration, and suppression/induction of cancer
- Conserved detoxification pathways

Peer-reviewed version available at *Sustainability* **2018**, *10*, 526; doi:10.3390/su10020526

- Evolutionary steps/passages in the evolution of development, senescence, regeneration

WG 4- Networking with stakeholders

The evaluation of the potential of MISCs to provide useful biomolecules is the focus of WG4.

Task 4.1- bioactive molecules. The technology developed in culture may be instrumental in solving some practical tasks in marine biotechnology, including the generation of cell cultures producing complex bioactive compounds with therapeutic potential. Stakeholders, some of which are already part of the proposing network, will be contacted from the beginning of the COST project and invited to participate in specific workshops in which the potential applications of MISCs will be discussed.

Deliverables:

- Bioactive molecules (antimicrobials, anticancer, opsonins, enzymes) of potential use in human health, pharmaceuticals, nutraceutics, cosmetics, antifouling paint formulation

The proposed timeline of the activities in the *MARISTEM* Action is shown in Table. 1.

Activity	Year 1		Year 2		Year 3		Year 4	
Initial Action meeting	+							
Management Committee (MC) meeting		+		+		+		+
Supervision Board (SB) meetings	+	+	+	+	+	+	+	+
Launch of Action website	+							
Workshops with stakeholders		+		+		+		
Courses on MISC			+		+		+	
Final meeting/conference								+
Summer/winter training school				+		+		+
WG1								
Task 1.1	+	+	+	+	+	+	+	+
Task 1.2		+	+	+	+			
Task 1.3			+	+	+	+	+	+
Task 1.4			+	+	+	+	+	+
Task 1.5		+	+	+	+	+	+	+
WG2								
Task 2.1	+	+	+	+	+	+	+	+
Task 2.2	+	+	+	+	+	+	+	+
Task 2.3		+	+	+	+	+	+	+
Task 2.4				+	+	+	+	+
WG3								
Task 3.1	+	+	+	+				
Task 3.2					+	+	+	+
Task 3.3			+	+	+	+	+	+
WG4								
Task 4.1		+	+	+	+	+	+	+

Table 1. Gantt diagram showing the planned COST activities to be applied in the MARISTEM consortium.

3.1.2. Risk and Contingency Plans

Several risks to the success of the *MARISTEM* Action have been identified that could impact the outcomes. We also defined remediation activities that minimize or eliminate the risks.
The main risks to the project are related to scientific tasks of WG1, as, up to now, none of the scientific approaches employed succeeded in getting cultures of immortalized MISCs. We contend that the failures in cultivating MISC were directly related to the major obstacles that are listed above, including the fragmentation of the scientific community working on MISC and the unavailable networking opportunities, the lack of European students and early career scientists with a thorough knowledge and expertise in the MISC discipline, the lack of connections with industries (technology transfer to potential end-users), the general lack of knowledge of MISC biology and the importance of knowing the intraspecific and interspecific communication of MISC cellular components for their survival. This is the reason why this project targets the weak points mentioned above. The envisaged positive aspects of the network - in terms of developing protocols, advancing the use of marine systems, understanding of the stem cell biology or the regeneration processes, the study of the effects of toxic components - far outweigh the current lack of a cultured stem cell system and the main risks of the project. The need for networking and coordination in this field are so evident that, even in the presence of negative results in establishing immortalized MISCs, the Action will undoubtely generate new ideas and tools for research, and exert a positive influence on Action members, next-generation researchers and European researchers in the field of invertebrate stem cells.

3.2. Management Structures and Procedures

The Action will be carried out according to "Rules and Procedures for Implementing COST Actions". This COST Action proposes a wide geographical network involving (at the initiating stage) 26 institutions from 15 countries (14 COST countries and 1 NNC represented by 2 institutions). Among the COST countries, 4 are ITC (Inclusiveness Target Countries, represented by 5 institutions) and 1 is the cooperating state of Israel (Table 2). Contacts and collaboration with additional COST countries, as well as biotech industries, are underway. The overview of the structure of the *MARISTEM* Action, and the interplay among the 4 working groups (WGs) and the management committee (MC) are depicted in Fig. 2.

Country	ITC/non ITC/other	Number of Institutions from that country	Number of researchers from that country	Percentage of proposing network
Austria	non ITC	1	1	3.85%
Croatia	ITC	1	1	3.85%
France	non ITC	4	4	15.38%
Germany	non ITC	1	1	3.85%
Greece	non ITC	1	1	3.85%
Ireland	non ITC	1	1	3.85%
Israel	non ITC	2	2	7.69%
Italy	non ITC	5	5	19.23%
Norway	non ITC	1	1	3.85%
Poland	ITC	1	1	3.85%
Portugal	ITC	1	1	3.85%
Russian Federation	other	2	2	7.69%

Slovenia	non ITC	2	2	7.69%
Spain	non ITC	1	1	3.85%
United Kingdom	non ITC	2	2	7.69%

Table 2. Geographic distributions of proposers in the *MARISTEM* Action. ITC= Inclusiveness Target Countries.

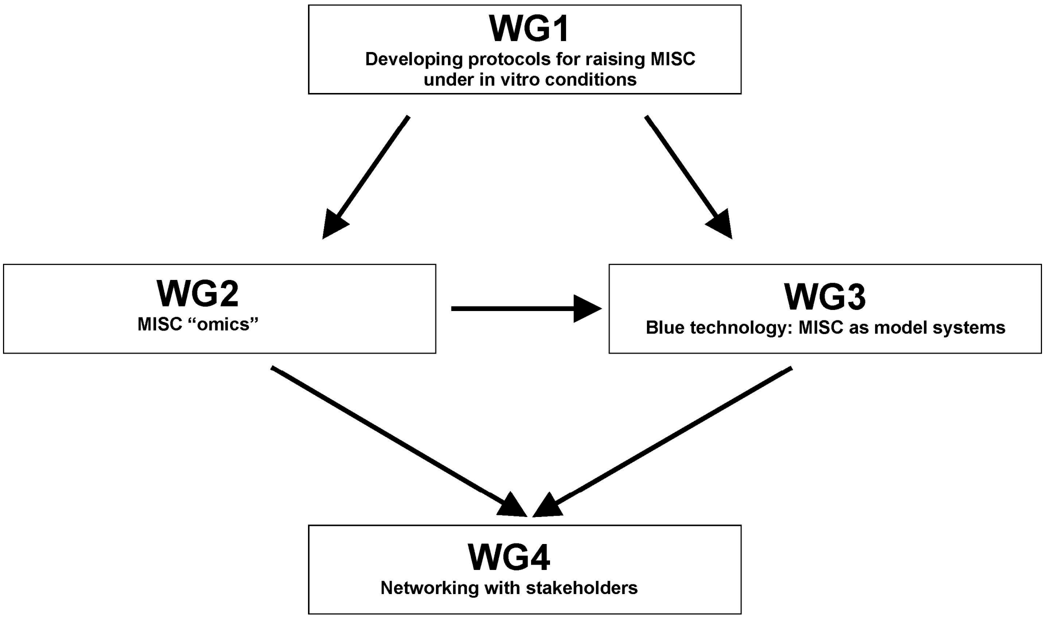

Figure 2. Pert Chart showing an overview of the structure of the *MARISTEM* Action and the interplay relationships between the major components of the Action.

The management structure of the *MARISTEM* Action is as follows:
A Core Group (CG), composed of Action Chair, Vice chair, WG leaders and vice leaders, STSM coordinator, meeting in person twice a year, will be the Consortium's highest decision-making body. The Management Committee (MC) will coordinate the COST Action and will include senior scientists from participating laboratories in each of the participating member states; it will be elected at the outset of the Action. It will meet at least once a year. An international advisory board of 3-4 international scientists, experts in different areas of our program, will be invited to attend the annual meetings in order to give critical feedback. Within the MC, a chair and a vice-chair will be elected. The CG and the MC members will be elected at the kick-off meeting. The kick-off meeting will be organized by the main proposer with the help of some secondary proposers.

<u>The MC will:</u>

- elect the Action Chair, Vice-Chair and task coordinators;
- nominate a coordinator for Short term scientific mission;
- nominate a coordinator for training schools;
- organize scientific workshops, training schools and the final meeting/conference;
- evaluate the progress of the scientific tasks from WG reports (every six months);
- evaluate the progress of other deliverable (e.g., workshops, schools);
- prepare the annual report;
- take care of the Action website;

Peer-reviewed version available at *Sustainability* **2018**, *10*, 526; doi:10.3390/su10020526

- promote the contacts with other relevant EU networks;
- ensure that COST policies are followed, and specifically encourage active involvement of Early Career researchers;

The CG will:

- overview the rationale for activities;
- ensure smooth operation of activities of the MC;
- decide conflicts between WG leaders and between participants;
- back the coordinator activities;

MC Chair: The MC chair will be the reference point for the Action, chair the annual conference/meetings (together with the MC), be responsible for the preparation of all scientific reports and the final report. The MC chair will be elected during the first meeting of the MC.

MC Vice-Chair: The MC Vice-Chair is also elected through the MC and should represent a different research field than the MC Chair. The MC Vice Chair will primarily focus on practical issues (organization of the Action) and represent the MC in relation to the "external world".

WG leaders: Each WG will have two leaders from different countries/research backgrounds. Junior researchers shall be actively promoted to take a lead in the WG. The WG leaders will coordinate the WG networking and capacity building activities, stimulate Short Term Scientific meetings (STSMs) and contacts with other WGs. The WG leaders are in charge of further subdividing the working groups into sub-groups, coordinate the progress of these and prepare the WG output for the MC reports.

3.3. Network as a Whole

Cellular (stem cells), genomic, proteomic and bioinformatic technologies are advancing rapidly and it is often difficult for those involved in fundamental biological research aspects to keep up-to-date and have access to the latest tools. These tools are often being developed by laboratories that are restricted in their access to suitable and tractable models with which to fully exploit the full potential of their powerful tools and their application to important healthcare problems. We believe that our project will be unique in combining a network of EC laboratories that are at the forefront of using organismal, cellular and genomic technologies with biologists studying the fundamental aspects (such as stem cell biology, aging, regeneration and tumor formation), end users that are associated with industries (five different sectors were outlined in section 2.2.1) and human well-being in the EU, also tackling with the pressing environmental challenges our seas and oceans face right now.

This COST Action MARISTEM aims to create a novel research collaboration platform within a scientific community that has, until now, been highly fragmented. It will lead to the consolidation of research on MISCs at the European level in order to strengthen this emerging field in the academy (e.g., by promoting the institution of university courses devoted to MISCs) and create synergy with R&D institutions. Thus far, research on MISCs in Europe has been very limited, with scattered expertise, and hampered by low funds and scarce attention by the scientific community. Bringing together candidates from more than 20 European research institutions to work on common objectives related to MISCs has the potential of producing a strong scientific impact in the field. In this context, our project will be a comprehensive, integrated, multidisciplinary genomic and proteomic approach to understand the basic biology of stem cells and regeneration. One of the main aims will be, in the long term, to improve and enhance treatment of disease by utilizing homologous gene networks and gene products to mobilize natural adult stem cell populations and to create pluripotent cells. Others aims are to provide the scientific community with new biomolecules for applied research.

The network will be the connecting avenue for all the people and institutions carrying out work on aquatic organisms in Europe, with a critical mass of more than a hundred researchers, using various model organisms, such as: Sponges, Cnidarians, Platyhelminthes, Mollusks, Echinoderms, Crustaceans, Cephalochordates and Tunicates.

Peer-reviewed version available at *Sustainability* **2018**, *10*, 526; doi:10.3390/su10020526

Acknowledgments: This study is supported by the European Cooperation in Science & Technology program (EU COST). Grant title: "Stem cells of marine/aquatic invertebrates: from basic research to innovative applications" (MARISTEM). The project idea developed as a direct outcome of a EuroMarine (European Marine Research Network) working group meeting, held in Padua on March 9-10, 2016.

Author Contributions: L.B. and B.R. conceived, initiated and designed the Action; L.B., B.R., P.M., I. D.-C., A.-M.G. contributed to the writing of the text. L.B., B.R., K.B., A.B., S.C., M.C., I.D-C., D.D., J.E., U.F., J-M. G., B.H., H.L., D.L., P.M., P.O., L.P., S.P., A.R., S.R., F.R., A.R., T.H.S., I.S., S.S., A.V.C. (the Management Committee) manage all activities of the *MARISTEM* project.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Ostend declaration. Available online: www.vliz.be/events/eurocean2010/attachments/Ostend%20Declaration.pdf (accessed on 16 January 2018).
2. Marine Board Position Paper 15, September 2010. Available online: www.marineboard.eu/science-strategy-publications (accessed on 16 January 2018).
3. Gordon, S. Phagocytosis: the legacy of Metchnikoff. Cell **2016**, *166*, 1065-1068, DOI: 10.1016/j.cell.2016.08.017.
4. Duerden J.E. Aggregated colonies in madreporarian corals. *Am. Nat*. **1902**, *36*, 461–471.
5. Vacquier, V.D., Tegner, M.J., Epel, D. Protease released from sea urchin eggs at fertilization alters the vitelline layer and aids in preventing polyspermy. Exp Cell Res **1973**, *80*, 111-119. DOI: 10.1016/0014-4827(73)90281-4.
6. Moy, G.W., Vacquier, V.D. Immunoperoxidase localization of bindin during the adhesion of sperm to sea urchin eggs. *Curr. Top. Dev. Biol.* **1979**, *13*, 31-44. DOI: 10.1016/S0070-2153(08)60688-2.
7 Terasaki, M., Sardet, C. Demonstration of calcium uptake and release by sea urchin egg cortical endoplasmic reticulum. *J. Cell Biol.* **1991**, *115*, 1031-1037.
8. Evans, T., Rosenthal, E.T., Youngblom, J., Distel, D., Hunt, T. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* **1983**, *33*, 389–396. DOI: 10.1016/0092-8674(83)90420-8.
9. Gehrke, A.R., Srivastavam M. Neoblasts and the evolution of whole-body regeneration. *Curr. Opin. Genet. Dev.* **2016**, *40*, 131-137. DOI: 10.1016/j.gde.2016.07.009.
10. Shimomura, O., Johnson, F.H., Saiga, Y. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, *Aequorea*. *J. Cell. Comp. Physiol*. **1962**, *59*, 223–239. DOI: 10.1002/jcp.1030590302.
11. Rinkevich, Y., Matranga, V., Rinkevich, B.. Stem cells in aquatic invertebrates: common premises and emerging unique themes. In: *Stem cells in marine organisms*, Rinkevich, B., Matranga, V., Eds.; Springer: London, UK, **2009**; pp. 61-103, ISBN: 9789048127665.
12. Sköld, HN., Obst, M., Sköld, M., Åkesson, B. Stem cells in asexual reproduction of marine invertebrates. 2009. In: *Stem cells in marine organisms*, Rinkevich, B., Matranga, V., Eds.; Springer: London, UK, 2009; pp. 105-137, ISBN: 9789048127665.
13. Gold, D.A., Jacobs, D.K. Stem cell dynamics in Cnidaria: are there unifying principles? *Dev. Genes Evol.* **2013**, *223*, 53-66. DOI: 10.1007/s00427-012-0429-1.
14. Knapp, D., Tanaka, E.M. Regeneration and reprogramming. *Curr. Opin. Genet. Dev.* **2012**, *22*, 485-493. DOI: 10.1016/j.gde.2012.09.006.
15 Arenas-Mena, C. Indirect development, transdifferentiation and the macroregulatory evolution of metazoans. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2010**, *365*, 653-669. DOI: 10.1098/rstb.2009.0253.
16. Directive 2010/63/eu of the European Parliament and of the Council. Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0063&from=EN (accessed on 16 January 2018).
17. Blue Growth opportunities for marine and maritime sustainable growth. Available online: https://ec.europa.eu/maritimeaffairs/sites/maritimeaffairs/files/docs/body/com_2012_494_en.pdf (accessed on 16 January 2018).

18. Innovation in the blue economy. Available online: http://www.eubusiness.com/topics/fisheries/blue-economy (accessed on 16 January 2018).
19. Innovation in the Blue Economy: realising the potential of our seas and oceans for jobs and growth. Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=COM:2014:254:REV1&from=EN (accessed on 16 January 2018).
20. MARINE KNOWLEDGE 2020: marine data and observation for smart and sustainable growth. Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52010DC0461&from=EN (accessed on 16 January 2018).
21. Adrian, T.E. Novel marine-derived anti-cancer agents. *Curr. Pharm. Des*. **2007**, *13*, 3417-3426. DOI: 10.2174/138161207782360500.
22. Gordaliza, M. Cytotoxic terpene quinones from marine sponges. *Mar. Drugs* **2010**, *8*, 2849-2870. DOI: 10.3390/md8122849.
23. D'Incalci, M., Galmarini, C.M. A review of trabectedin (ET-743): a unique mechanism of action. *Mol. Cancer Ther*. **2010**, *9*, 2157-2163. DOI: 10.1158/1535-7163.MCT-10-0263.
24. Sunassee, S.N., Davies-Coleman, M.T. Cytotoxic and antioxidant marine prenylated quinones and hydroquinones. *Nat. Prod. Rep*. **2012**, *29*, 513-535. DOI: 10.1039/c2np00086e.
25. Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H., Prinsep, M.R. Marine natural products. *Nat. Prod. Rep*. **2016**, *33*, 382-431. DOI: 10.1039/c5np00156k.
26. Rinkevich, B., Shlemberg, Z., Fishelson, L. Whole-body protochordate regeneration from totipotent blood cells. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7695–7699. DOI: 10.1073/pnas.92.17.7695
27. Smithers Rapra's Market Reports. Available online: https://www.smithersrapra.com/market-reports (accessed on 16 January 2018).
28. HORIZON 2020. Available on line: https://ec.europa.eu/programmes/horizon2020/sites/horizon2020/files/InfoKit_UK_240214_Final.pdf (accessed on 16 January 2018).
29. Joint OECD/NNI International symposium on assessing the economic impact of Nanotechnology. Available online: https://www.oecd.org/sti/nano/Washington%20Symposium%20Report_final.pdf.
30. Rinkevich, B., Matranga, V., Eds. Stem Cells in Marine Organisms. Springer: London, UK, **2009**, 369 pp, ISBN: 9789048127665.
31. Weissman, I.L. Stem cells: units of development, units of regeneration, and units in evolution. *Cell* **2000**, 100, 157-168. DOI: 10.1016/S0092-8674(00)81692-X.
32. Public Health England, culture collections. Available online: https://www.phe-culturecollections.org.uk (accessed on 16 January 2018).
33. Eurostemcell. Available online: https://www.eurostemcell.org (accessed on 16 January 2018).
34. EURL-ECVAM, European Union Reference Laboratory for alternatives to animal testing. Available online: https://eurl-ecvam.jrc.ec.europa.eu (accessed on 16 January 2018).
35. ASSEMBLE, Association of European Marine Biological Laboratories. Available on line: http://www.assemblemarine.org (accessed on 16 January 2018).
36. EMBRC, European Marine Biological Resource Centre. Available online: http://www.embrc.eu (accessed on 16 January 2018).
37. Van Wezel, A., Van Vlaardingen, P. Environmental risk limits for antifouling substances.. *Aquat. Toxicol*. **2004**, *66*: 427-444. DOI: 10.1016/j.aquatox.2003.11.003. DOI: 10.1016/j.aquatox.2003.11.003.
38. Kaur, S., Singhal, B. When nano meets stem: the impact of nanotechnology in stem cell biology *J. Biosci. Bioeng*. **2012**, *113*, 1-4. DOI: 10.1016/j.jbiosc.2011.08.024.
39. Mooney, E., Dockery, P., Greiser, U., Murphy, M., Barron, V. Carbon nanotubes and mesenchymal stem cells: biocompatibility, proliferation and differentiation. *Nano Lett*. **2008**, *8*, 2137-2143. DOI: 10.1021/nl073300o.
40. Rinkevich, B. A critical approach to the definition of Darwinian units of selection. *Biol. Bull*. **2000**, *199*, 231-240. DOI: 10.2307/1543179.
41. Kirkwood, T.B.L., Holliday, F.R.S. The evolution of ageing and longevity. *Proc. R. Soc. Lond. B* **1979**, *205*, 531-546. DOI: 10.1098/rspb.1979.0083.
42. Kirkwood, T., Melov, S. On the programmed/non-programmed nature of ageing within the life history. *Curr. Biol*. **2011**, *21*, R701-707. DOI: 10.1016/j.cub.2011.07.020.

43. Ridley, M. *Evolution*, 3rd edition; Blackwell Publishing: Oxford, UK, 2004. ISBN: 1405103450.
44. Schmich, J., Kraus, Y., De Vito, D., Graziussi, D., Boero, F., Piraino, S. Induction of reverse development in two marine Hydrozoans. *Int. J. Dev. Biol.* **2007**, *51*, 45-56. DOI: 10.1387/ijdb.062152js.