

Stazione Zoologica "Anton Dohrn"

National Institute of Marine Biology, Ecology and Biotechnologies

Activity Report 2019



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1 EXECUTIVE SUMMARY

The Stazione Zoologica Anton Dohrn (*National Institute of Marine Biology, Ecology and Biotechnologies*) is a National Governmental Research Institute under the Ministry of University and Research (MUR), with the mission of improving our understanding of the structure and functioning of marine ecosystems, their biology and ecology and the evolution of marine life (*"To understand the marine biological systems and the evolution of marine life to improve the eco-sustainable use of marine resources"* Document of Vision 2015- 2025). The research conducted at the Stazione Zoologica contributes to the knowledge and provides new tools for the eco-sustainable management and exploitation of marine resources. The main features of the Stazione Zoologica can be summarized as follows:

- 1. SZN is the **top Italian institution among EPR in the field of Marine Biology and in the Area 05** (Biology, data from ANVUR).
- 2. SZN ranks **n. 27 among the research institutions worldwide** and number 5 in Europe (top 1 in Italy) in the field of Ocean and Seas (Ranking OceanExperts, 2019).
- 3. SZN includes top scientists conducting cutting-edge marine research and achieving **publications on top-ranked international journals**.
- 4. SZN is a truly international research institution, as evident also from the **large proportion of international scientists**.
- 5. SZN conducts a highly **multidisciplinary research** (including ecology, oceanography, genetics, cell and developmental biology, physiology, neuroscience, behavioural biology, microbiology, zoology, botany and biotechnology).
- 6. SZN is the Italian node of the **ESFRI European Research Infrastructure (EMBRC)**, with an important link to Industries, SMEs and other research institutions.
- 7. SZN is among the funders and is the headquarter of the Technological **Cluster Blue Italian Growth**.
- 8. The SZN is a leading institution in the **outreach and dissemination of marine research**, through the *Historical Aquarium*, the *Turtle Point*, the *Darwin- Dohrn Museum* and the largest marine scientific library in Europe.

The activities of the three-years Plan 2020-2022 of the SZN will include:

- 1) A continued investment in Human Resources with the recruitment of top scientists (researchers and technologists), able to ensure the turnover of retiring scientists in its top research fields (see attached *Piano di Fabbisogno Recruitment Plan*);
- 2) The growth of the new Department of Marine Biotechnologies and Third Mission Area (a Dept dedicated to dissemination, outreach and research on charismatic species);
- 3) The creation of the largest *Marine Farm and Factory* in Europe for promoting the production and use of marine models in research and industry (permission for the restoration of the dedicated building obtained in 2019);
- 4) The restoration of the Historical Aquarium and of the new Darwin Dohrn Museum and the completion of the new territorial seats (e.g., Sicily, Calabria, Marche) along with the implementation of the seats of Rome, Portici and Bagnoli.
- 5) The investments in research infrastructures for ca 16 million euros, including a *New Hightech Research Vessel* to implement the core facilities of the institution.

2 EXTENDED SUMMARY

Mission of the Institute - The Stazione Zoologica – National Institute of Marine Biology Ecology and Biotechnology (SZN) was founded in 1872 by Anton Dohrn. SZN is the oldest research institution in Italy and the oldest Marine Research Institution independent from a University in the world. SZN is a Public Research Institution (Ente Pubblico di Ricerca), belonging to the Ministry of University and Research (MUR), entirely devoted to marine research. The Headquarter is in Naples (Southern Italy), with Regional seats in Calabria, Sicilia, Rome and Marche. The core mission of the SZN is to "understand the marine biological systems and the evolution of marine life to improve the eco-sustainable use of marine resources" in a dual perspective: that of preserving a healthy ocean life and goods and services provided by marine biodiversity, and that of studying the biology and ecology of marine organisms so as to identify patterns and processes, at all levels of organization, that will improve the quality (e.g., biotech, Pharmaceuticals and of human life and the bio-economy of our country products for the industry). SZN conducts research in marine biology, ecology and biotechnology and develops research infrastructures able to support the Italian researchers and to support the sustainable development of the Country. SZN also collaborates with national and international research institutions, SMEs and industries.

Positioning of the Stazione Zoologica - The role of the Stazione Zoologica in the national and international context is recognized either in terms of research performance and for its ability to support, through the institutional research, the Ministries (MUR, MISE, MATTM) in solving some crucial issues (decontamination of sites of national interest, support to CITES to contrast illegal trading of protected/endangered marine species; contribution to the respect of the Nagoya Protocol). Current economic trends (see OECD, Ocean Economy) indicate that seas and oceans and their life offer a huge potential for both economic and occupational development. However, this potential can be exploited only if managed in an eco-sustainable way. To achieve this objective, the conservation of marine ecosystems (contribution to Aichi targets), the correct management of the marine biological resources (UN SDG 14), the sustainable development of the potential exploitation of the marine resources (EU Blue Growth and Italian Cluster Blue Growth) and the understanding of the impacts of global climate change (COP process), require the contribution of the knowledge and competences of the SZN.

Scientific performance – The ANVUR (*National Agency for the Evaluation of Universities and Research Institutes*) in 2017 reported that <u>SZN is the best performer among the Research Institutions of the Ministry for Education University and Research</u> in the Research Area Life sciences (Area 05). The scientific papers published by SZN display the highest number of citation per paper in the entire Area 05 (Biological Sciences) in Italy. SZN scientists show an increasing productivity (as number of ISI publications per researcher/technologist per year - source Web of Science, 'ISI journals' with Impact Factor): from 2.35 (in 2012-2014) to 2.60 (in 2015-2016) to 3,65 (in 2017). In the last 3 years (2015-2017), no scientists were inactive at SZN in terms of research publication outputs. The quality of the results produced by the Stazione Zoologica is also documented in top scientific journals.

SZN Organization - The 'Organization and Structure of the SZN' has been completely changed, from "Laboratories", in 2010 to "Research Sections in 2011-2014" to "Departments" in 2016. These changes were planned to optimize the use of equipment and infrastructure, allocation

of resources and overall functioning of the Institute. The 4 main Departments are: 1) Biology and Evolution of Marine Organisms; 2) Integrative Marine Ecology; 3) Services and Infrastructures for Marine Research, and 4) Department of Marine Biotechnologies, to which has been included The Third Mission Area in 2019, dedicated to dissemination, outreach and to the research on charismatic species.

Research Activities – The main strategic research areas are defined in the Document of Vision 2015-2025. The main research areas at SZN include: a) Marine Biodiversity; b) Marine model organisms; c) Biological Evolution; d) Marine Ecosystems; e) Global change; f) Conservation; g) Marine Biotechnology. SZN will continue the development of biological observatories, to understand the biodiversity and functioning of Mediterranean ecosystems as a model of the global ocean.

In particular, for the Three-year Plan 2020-2022, the SZN will focus its research on:

- 1) Marine organisms: adaptation and evolution: the SZN will launch novel models for multidisciplinary studies covering different aspects of basic and applied research. These "model organisms" will provide a new momentum in biological, ecological and biotechnological research. The specific topics for the 2020-2022 plan will be: 1) The evolution of biological complexity: molecular and adaptive aspects; 2) Neurons, sense organs and response to stimuli; 3) Immune response, natural variability, plasticity and symbiosis.
- 2) Multiscale marine biodiversity: SZN will continue the study of the biodiversity and ecological interactions and their contribution to the functioning of marine ecosystems. The topics for the 2020-2022 plan will be: 1) Innovative approaches to the description of biodiversity; 2) Biodiversity of extreme and degraded environments; 3) Large-scale, Spatial-temporal dynamics; 4) Biodiversity and halieutic resources.
- **3)** Conservation and management of marine biodiversity and ecosystems: The SZN aims at becoming the national reference for the solutions to the critical environmental problems as a part of its contribution to the National Research Plan (Blue growth) and Horizon 2020 and to contribute to the conservation of marine biodiversity. These ambitious objectives will be developed through the research in the following topics: 1) Response of marine assemblages to anthropogenic impacts and climate forcing; 2): Effects of multiple stressors on marine organisms; 3) Management and restoration of marine habitats; 4) Marine biodiversity conservation.
- 4) Marine biotechnologies: the SZN conducts eco-sustainable research on biotechnological potential of marine organisms: Biological products/molecules and processes, beneficial for humans and the environment, have a huge biotechnological potential, which will continue to grow in the near future. The topics for the 2020-2022 plan will be: 1) Enhance the role of marine biotechnology for human health and wellness; 2) Develop marine biotechnology for environmental health and biomaterials; 3) Innovative approach for production of marine bioactive biomass and bioactive compounds.

EU Research Infrastructures – The Department "*Research Infrastructure for Biological Marine Resources*" is devoted to the development and management of research infrastructures to provide: i) services for marine biological research; ii) technological development for the

research activities of SZN and iii) promote international collaborations within the European Research Infrastructures ESFRI (*European Strategy Forum on Research Infrastructures*) initiatives. In particular, during 2020-2022, the SZN will invest in the infrastructure of the PON PRIMA ca. 16 M€ for the implementation of the access to ecosystems, marine organisms and data, the enforcement of the territorial seats of SZN and the New High-tech Research Vessel.

Core facilities and research infrastructures – SZN is implementing the core facilities to promote scientific research. These include: a) production of marine biological resources; b) molecular biology, sequencing and bioinformatics; c) taxonomy of marine organisms; d) Microscopes and Bioimaging; e) Field sampling, scientific diving, and research vessels; f) research in extreme marine habitats; g) monitoring the quality of the marine environment and educational training. New infrastructures are being acquired, including a New Large Hightech Research Vessel (24 m), new robotics for the exploration at sea; new infrastructures for research in deep-sea and extreme environments; new technologies for animal tagging.

International collaborations and participation to consortia or any other organizations - SZN actively contributes to several prestigious international networks through research collaborations involving SZN and other organizations (i.e. OECD, UNESCO, G7 working groups). During the Three Year 2020-2022 SZN will participate to several events in collaboration with more than foreign institutions, including the Networks of Excellence on marine sciences (Euro-Marine Consortium).

Training and Education - The Stazione Zoologica contributes to the scientific and technical training of students from many countries through scholarships, PhD, MPhil, MS, research grants, job contracts, Marie Curie actions, ERC grants and internships. The Three Years Plan 2020-2022 will allow further implementing the offer of international summer schools, the launch of the new International Master Degree in Marine Biology, in collaboration with the University of Naples Federico II and the Open University (OU) PhDs along with doctoral grants in partnership with more than 10 universities.

Dissemination and Outreach - The new Third Mission Area will allow to significantly implement to dissemination and outreach activities at SZN, which in collaboration with the Dohrn Foundation, in the 2020-2022 will open to the following structures:

- i) **Research Centre for Sea turtles (Turtle Point)** in Portici (active since 2018).
- ii) **Historical Aquarium** (that will be expanded in the future by ca. 50% to the central part of the building, inauguration expected within 2020);
- iii) **Museum of Marine Biology and Evolution** (Darwin-Dohrn Museum in in Villa Comunale, inauguration expected within 2021);
- iv) **Marine Farm**, dedicated to the production of marine organisms for science and industry, and opened to the public, will be in Bagnoli (inauguration expected in 2022).
- v) Marine Library in Naples (in search for a new location for new opening in 2021).

The A3M, the Area of Third Mission, all the structures open to the public listed above, plus *Science and Society* initiative and all Dissemination & Outreach activities addressed to the public, the students and the schools, strengthening the environmental education and promoting a new scientific culture and awareness among the younger generations.

Financial resources and investments - The funding sources of the Stazione Zoologica for the period 2020-2022 are reported in section 'Financial resources' of the Three-Year Plan. The annual balance of SZN is robust and is progressively increasing thanks to the success in national and international projects. The SZN is making an important yet sustainable investment in scientific personnel with a plan to increase up to ca. 250 units (ca. 150 scientists) in 2022. This will require an effort in the structures needed to host the new researchers-technologists and infrastructures.

Financial resources for the restoration and building of new structures – A high priority will be given to finalize the restoration of the various historical seats. Besides the completion of the restoration of Villa Comunale in Naples, we started the restoration plan for the Ischia Marine Centre (Villa Dohrn). The seats of SZN Sicily (Milazzo- Palermo-Messina) are now fully active and Fano Marine Centre opened in early 2020. The Seat of SZN Calabria (Amendolara) will open by the end of 2020.



3 RESEARCH ORGANIZATION

3.1 THE RESEARCH AT SZN WITHIN THE NATIONAL RESEARCH PROGRAMME 2015-2020

For the three-year period 2020-2022 the Stazione Zoologica Anton Dohrn, pending the completion of the PNR, will continue to focus its research activities consistently with PNR 2015-2020, in the context of four research topics and in particular:

- 1. Marine Organisms: adaptation and evolution
- 2. Multiscale marine biodiversity
- 3. Integrated Approach to the study and management of the ecosystem
- 4. Marine Biotechnologies

The research activities included in these *Research Axes*, together with the *Core Activities* characterizing the research activities of the Departments, contribute fully to the advancement of knowledge and are believed to be perfectly integrated in the strategy outlined by the 2015-2020 National Research Program.

Studies on the biology, physiology, adaptation and evolution of marine organisms (Theme 1) respond to requests to facilitate research within the priorities of PNR 2015-2020: i) Health, ii) Quality of life, iii) Intelligent and sustainable industry, iv) Energy and environment. Research on fundamental biology and evolution contribute to research strategy at national and supra-national level.

The multi-scale approach to marine biodiversity of the SZN (**Theme 2**) is in line with EU guidelines that explicitly identify the need to protect biodiversity at its multiple levels: populations, species, habitats and ecosystems. The characterization of biodiversity and the definition of its conditions at spatial and genetic level are also considered fundamental aspects to define the good environmental status, as required by the Marine Strategy Framework Directive (GES, sensu Marine Strategy Framework Directive MSFD). In line with European guidelines, the 2015-2020 National Research Program identifies Blue Growth as a high-potential intervention sector, and together with Health and Agrifood are priority areas of intervention for the NRP. They are inseparable from a correct assessment of the biodiversity of marine resources.

The SZN will continue to invest in the development and attraction of highly qualified human capital in the field of research on the health of the marine environment (**Theme 3**), on the resilience and responsiveness of marine organisms and ecosystems to the impacts due to climate change and direct action of man. These activities represent a unique driving force for innovation in the field of management and sustainable exploitation of marine resources, considered strategic for the economic development of the country.

Finally, the exploration of marine biological resources (**Theme 4**) is clearly in line with the priorities of the 2015-2020 PNR ("Blue Growth") and supports the necessary networking activities within the National Technology Cluster «Blue Growth » further contributing to the development at national level of a network between business and research.

3.2 SZN IN THE NATIONAL AND INTERNATIONAL SCIENTIFIC CONTEXT

The Stazione Zoologica is one of the Public Research Bodies operating in Italy that represents one of the **excellences of marine biological and ecological research in the world**. In the panorama of national and international research institutions, the originality of the SZN derives from:

- co existence of biological and ecological research, essential elements for an understanding of the overall balance of the marine environment and the basic biological mechanisms that govern it;
- ability to make use of multi-faceted approaches such as genetics, cellular and developmental biology, ecology and oceanography;
- ability to explore and develop new experimental methodologies and protocols that have the potential to apply as a reference for studies transferred to other systems and / or organisms.
- competences on a wide spectrum of marine organisms that allow the Institution researchers to face the study integrating ecological aspects to the biological component in its complexity.



In addition to specific research activities, the Stazione Zoologica has a strong and consolidated ability to guarantee access to the use of marine organisms for the international scientific community, to provide <u>qualified advice to public bodies and Ministries</u>, and continues its tradition of contributing to <u>Higher Education</u>.

The ability to develop research activities and provide <u>specialized "scientific services"</u> is one of the main strengths of the Stazione Zoologica Anton Dohrn, a feature that makes it almost unique compared to other national research facilities.

Furthermore, in the context of research institutes, the SZN:

- has a <u>significant percentage of international researchers</u> (Canada, South Korea, Germany, France, Holland, Spain, New Zealand), which contributes to the internationalization of research in Italy;
- has the <u>highest concentration of researchers / technologists in the field of Marine biology</u>;
- is actively operating both through public competitions and through direct calls by excellent researchers for the "return of Italian brains" from abroad;

- develops new <u>methodologies of strategic interest in biological and biomedical research</u>, which constitute a <u>key component of the European EMBRC research infrastructure</u>, with a significant connection with Industries, SMEs and other research institutes;
- has the ability to provide <u>specialized scientific and technological services</u> rendered to third parties
- at local, national and international level;
- has a consolidated experience in disseminating to the general public, also thanks to the presence of the Aquarium of Naples and important outreach activities;
- takes care of and owns the <u>most important scientific library in marine sciences in Europe</u>, which is accompanied by an historical archive and a <u>biological collection of museum interest</u> (Darwin Dohrn) of great scientific value.

The **VQR** (evaluation of research quality) 2011-2014) conducted by the ANVUR notes that the Stazione Zoologica Anton Dohrn in Naples is positioned at levels of great value in the Reference Disciplinary Sector (Biological area). According to the evaluation of the ANVUR, the scientific production of the Stazione Zoologica is represented by a percentage of "excellent and high" products higher than the Area average, and that the Final Structure Indicator (IRFS) is higher than the share of expected products, which means that the SZN reaches a qualitative-quantitative weight higher than the quota of expected products.

The Stazione Zoologica is the MUR Organization with the best result in Area 05 (Life Sciences)

It is from the foundation in 1872 that the SZN has always been characterized by the predominant capacity for **international networking**. Still, the SZN is the center of an extensive network of international scientific collaborations.

Examples are the active role of the SZN in the following actions:

- a. The creation of the *EuroMarine Consortium* (support and coordination action derived from the FP7) and the consequent establishment of EuroMarinePlus. EUROMARINE is a European network for marine science that replaces the three ex-excellence networks (EUR-OCEANS, MarBEF and Marine Genomics Europe), as well as their follow-up structures (the EUR-OCEANS consortium and the MarBEF + Association), and aims to promote and implement the establishment of a non-profit organization that can promote networking actions in the field of marine sciences.
- b. The participation in the ASSEMBLEplus project, a program supported by the European Commission under the H2020, allowing researchers from member countries the access to infrastructures, guaranteeing the availability and support to conduct advanced experiments in the field of marine biology.
- c. The participation in different European "clusters", such as: EMBRIC, ENVRI, CORBEL, aimed at networking Research Infrastructures, as well as "COST actions", aimed to foster international collaborations in science and technology.
- d. Active participation in the <u>European Marine Biological Resource Center (EMBRC)</u> Research Infrastructure part of the European Strategy Forum on Research Infrastructures (ESFRI) of the

European Union recognized on 20 February 2018 as ERIC (European Research Infrastructure Consortium) by the European Commission.

- e. Coordination of the Italian node of EMBRC (EMBRC IT), which gathers the major National Institutions and Consortia operating on the sea through a JRU recognized by the Ministry of Research
- f. Participation in EMSO ERIC, with an active role in the network of oceanographic observation points of the national network of the European research infrastructure.
- g. Participation in <u>LifeWatch ERIC</u>, a European infrastructure of the ESFRI roadmap, in which the SZN plays an active role in the analysis of the plankton and benthos biodiversity, areas in which the SZN has a consolidated experience recognized at the highest national and international levels.
- h. <u>Collaboration and support to the OECD</u> as part of the "Fostering innovation in Ocean economy" project, which intends to emphasize the role of Research and Technological Innovation in the sustainable development of the 'Seas and Ocean Economy', in the optics of the new perspective of observation and analysis of global sustainable economic development.
- i. Participation in the <u>working groups on Ocean Literacy</u> (promoted by UNESCO)
- j. Participation in the <u>working group on "Ocean and</u> <u>seas" of the G7.</u>
- Participation in bilateral forums and meetings / initiatives: Italy-China (promoted by MIUR), Italy-Iran and Italy-Korea, Italy-Japan.
- Subscription of a series of bilateral agreements (e.g. SZN - South Korea / MABIK, promoted thanks to initiatives of the Italian Embassy in South Korea and the SZN).



3.3 SUMMARY OF THE 10 YEAR VISION DOCUMENT OF RESEARCH OF THE INSTITUTE

The Stazione Zoologica Anton Dohrn aims to <u>consolidate its role as a national and international</u> reference point for competences in the biology of marine organisms and ecosystems.



Marine <u>biodiversity represents an important source of still unexplored knowledge of the planet</u> and the research that the SZN will conduct in the coming years will provide an important contribution to the advancement of knowledge and new tools for sustainable development.

The <u>development of new technologies</u> that allow us to study the biology of organisms - from molecules to the global scale - offers unprecedented research opportunities. These opportunities can be seized by acquiring the best tools and infrastructures for research, taking up the challenge of complexity that characterizes the living and strengthening human resources capable of transforming research into data and data into knowledge. Although considerable progress has been made in the past three decades in the process of integrating knowledge and multidisciplinarity, much remains to be done to fully exploit the potential offered by the integration of different scientific approaches. The SZN intends to seize these opportunities, on the one hand increasingly opening up to intersectoral collaboration with areas that are not within its competence (e.g., geology, chemistry, physics, economics, engineering, robotics) and on the other to further enhance its own multidisciplinary competence within the Biology Area.

Priority of marine biological research of the SZN in the decade 2015-2025

Marine biodiversity: towards innovative and integrated approaches aimed at enhancing and protecting it

Marine model organisms: new opportunities for fundamental and applied research Marine genomics: understanding the structure and function of genes in Mediterranean organisms Biological evolution: integrating genetics, epigenetics, ecology and behavior

Adaptations to extreme marine environments: new opportunities to know the limits of life Marine biotechnologies: sustainable use of marine resources for nutraceuticals, pharmacology, environmental remediation and biomaterials

Global changes: *understanding the response of marine ecosystems with a focus on the Mediterranean* **Conservation:** *protecting biological resources and the marine environment for sustainable blue growth*



3.4 SCIENTIFIC AND TECHNOLOGICAL RESEARCH STRUCTURES

Since 2018, the Stazione Zoologica has been organized into four Departments, which have the primary task of promoting and carrying out scientific and technological research. The departments seek to enhance human, financial and instrumental resources with the aim of allowing all Researchers and Technologists to be able to enhance their business research consistent with the 3-yr PLAN OF ACTIVITIES 2020 - 2022, the DVS of the Stazione Zoologica, and the recommendations of the PNR, in compliance with the European Researchers Charter. The main objective of the Departments is to:

- *i.* conduct basic and applied research of excellence at an international level;
- *ii.* improve the SZN performance compared to ANVUR criteria;
- iii. obtain more research funding, and participate in national and international competitive tenders;
- *iv.* promote the training and recruitment of young researchers, also in accordance with the European Researcher Charter and the new SZN statute;
- v. contribute to the visibility of Italy in international research:
- vi. contribute to the growth and well-being of the country.

The **Scientific Research Departments** are divided into functional areas, i.e. laboratories and spaces for using the instruments and available infrastructures. The **Dept. Research Infrastructures for marine biological resources** is divided into Core Facility Units, operational structures with the primary task of facilitating and improving the research carried out by researchers and technologists of the SZN through "state-of-art" services and core facilities. To this end, the Department promotes and implements technological research. The **Department Research Infrastructures for marine biological resources** primarily offers technical and technological support for the research of the other Departments through a portfolio of activities. The portfolio of services is also implemented with a view to participation and the contribution that SZN offers to the EMBRC infrastructure.

Biologia ed Evoluzione Organismi Marini

Biology and Evolution of Marine Organisms Ecologia Marina Integrata (EMI)

Integrative Marine Ecology Infrastrutture Ricerca Risorse Biologiche Marine Research Infrastructures Marine Biological Resources

Biotecnologie Marine Sostenibili

Marine Biotechnologies

Area III Missione (A3M) Disseminazione Divulgazione Strutture Aperte al Pubblico

	RIMAR	EMBRC-IT Management	
		RIMAR Administration	
		Access to Marine Ecosystems and Environmental Analysis	Infrastructure for Marine Research (IRM)
			Infrastructure for Rearing of Marine Organisms (IMOM)
			Technological Marine Implementation & Robotics (ITR)
			Environmental Monitoring & Analysis (MAA)
		Technologial Platforms	Classical and Molecular Taxonomy (Motax)
			Advanced Microscopy Center (CeMA)
			Molecular sequencing & Analysis Center (CSAM)
			Bioinformatics, Computational Analysis & Data Manag. (BAC)
Research	BluBio	Administration BluBio	
Departments			Functional Area - Nutraceutical
Deparemento		-	Functional Area - Pharmaceuticals and Cosmeceuticals
			Functional Area - Environment and Biomaterials
	EMI	EMI Administration	
			Functional Area - Molecular Ecology
			Functional Area - Plankton Ecology
			Functional Area - Benthos and Necton Ecology
			Functional Area - Microbial Ecology
	BEOM	BEOM Administration	
			Funtctional Area - Molecular Developmental Biology
0			Funtctional Area - Physiology & Behavior
			Funtctional Area - Functional Genomics
			Funtctional Area - Biochemistry & Cellular Biology



3.4.1 Department of Biology and Evolution of Marine Organisms

The Department of *Biology and Evolution of Marine Organisms* (BEOM) studies biological mechanisms as the basis of Life on Earth, and of organismal diversity. BEOM applies multidisciplinary approaches ranging from molecular and cellular biology to physiology, biochemistry, ecotoxicology, population biology and genetics, phylogeny, immunology, epigenetics, including functional genomics and neuroscience. The scientific activities focus on:

- regulatory processes and gene networks underlying development, from fertilisation to larval stages;
- comparative genomics and molecular evolution of Metazoa;
- responses to stressors and adaptive capabilities of marine organisms to changes in the environment;
- neural mechanisms underlying behavioural plasticity including learning and the responses to stimuli.

Labs and staff - BEOM is organised into four Functional Areas located in the East Wing of the main building of the Stazione (Villa Comunale in Naples), a section of the Institute dated back to the beginning of the XX century, but renewed about 20 years ago thanks to the contribution of the European Commission. The Department is equipped with infrastructures including microscopes (imaging *in vitro* and *in vivo*), microinjection, nucleic acids and proteins analysis, behavioural analysis, histology, electrophysiology. BEOM is implementing open-space labs characterised by infrastructures and equipment that allow the identification of *Core Facilities* open to staff scientists and students and intraand extra-mural collaborators and coworkers. The functional areas are designed to maximise and facilitate access through ERIC-EMBRC. BEOM Functional Areas, including the main equipment characterising them and the related activities are shown in the following scheme:



At present, 22 researchers, two technologists and four technicians constitute the staff members of BEOM Department. A total of 5 new researchers and one technician have been hired in BEOM during 2019. The staff of the Biology and Evolution of Marine Organisms Department includes various collaborators, young research associates, scholarship holders, PhD students and external associates from national and international organizations. Many international collaborations are active, and about 15% of people working in the Department (including students and



collaborators) originate from other countries, thus facilitating BEOM visibility and networking capacity. BEOM has also a long and strong tradition of hosting and training PhD students originating from Italian and EU-Universities, as well as numerous students working on their Master's degree thesis.



The Department intends to strengthen its research capacity by integrating new fields and expertise e.g., epigenetics, functional genomics, structure and function of proteins, system biology, neuroscience. This expertise will join those currently in place, thus further enhancing the research capacity.

BEOM Research Objectives - P3-yr PLAN OF ACTIVITIES 2020 - 2022

BEOM staff scientists actively contribute to the Three-year Activity Plan (2019-2021) with a large part of the foreseen activities in the four proposed research themes, with particular attention to the Theme: a) Physiology, adaptation and Evolution in marine organisms; b) Multi-scale marine biodiversity; c) Exploration of the marine biological resources.

Some scientists collaborate also to the activities of the Department of Marine Biotechnology by



exploring biologically active molecules of marine origin, primarily produced by diatoms, dinoflagellates and sponges, or by evaluating the effects of environmental contaminants and stressors on the 'functioning' of the cells and organisms. Furthermore, in line with the principles instilled in the European Charter for Researchers and thanks to resources arising from some research projects funded by competitive national and international grants, BEOM continues to significantly contribute to the scientific research of the Stazione Zoologica Anton Dohrn through *Core Research Activities* as summarised below. **Study of the development mechanisms of the marine organisms.** One of the core research activities of BEOM is focused on the study of the mechanisms governing animals' development from fertilisation to adulthood. This research is facilitated by the study of emergent animal models as molluscs, echinoderms, tunicates, chordates and fishes. By using live imaging and gene and proteins expression and genome analysis a complete analysis of the intracellular signals (e.g., calcium signalling) necessary for the activation of the egg and the first phases of the embryonic development is carried out. A focus is also given to the possible impact of environmental stressors, contaminants and secondary metabolites, and to the way these factors can interfere with organisms' physiological functions, inducing reproductive and development "disorders".

Furthermore, the characterization of organs and tissues determination is key of BEOM core activities. This is achieved through the analysis of the *Gene Regulatory Networks* (GRN) in different species at the basis of the diversification of Chordates, thus allowing comparative analysis (evo-devo approach) and the study of the GRN evolution.

Finally, studies on the regenerative abilities and homeostasis of tissues and organs in key species integrate approaches of developmental and functional biology. These approaches allow to expand the contribution and research on *systems biology*.

Neurobiology and behavioral biology of marine organisms. Another key Core activity characterizing BEOM research is the analysis of the behavioural and physiological 'status' of marine organisms. By utilizing different approaches (imaging and morphological analysis, anatomy and morpho-functional analysis of the nervous system) scientists study interindividual variability and biological and neural plasticity. Strong emphasis is given to organismal plasticity and their capacity to respond to stimuli. Research focuses on the study of various behaviours such as predatory response, learning, memory, social behaviour in crustaceans, cephalopods and fishes. Studies are based on a multidisciplinary approach, including behavioural biology, neurophysiology, neuroanatomy, immunohistochemistry, *in situ* hybridisation, live-imaging and gene expression analysis. The study of the evolution of cognitive capabilities of organisms and their neural complexity are at the basis of this pillar of the BEOM research.

Evolutionary patterns in marine organisms. The study of evolutionary patterns is another well established research activity of BEOM Department. It is achieved considering different evolutionary scales including genetics, genomics, protein and organisms. A comparative analysis of the genic *pathways*, together with the changes in the organisation of the genomes and with the study of the structure/function relationship of new protein "folding", contribute also to the comprehension of the mechanisms at the basis of the evolutionary innovations and of the adaptive strategies. This BEOM Core is based on a functional description of the evolutionary process occurring within complex ecological scenarios. Genome architecture, for example, is also studied with particular reference to the functional and evolutionary implications of the structure, composition in bases and the retrotransposon activity, with the purpose of understanding how these contribute to the expression, regulation and function of genes as organisms evolve.

3.4.2 Department of Integrative Marine Ecology

Research lines - The Department of Integrative Marine Ecology Research (EMI) carries out

interdisciplinary research on biodiversity, structure and functioning of marine ecosystems. Understanding the functioning of marine ecosystems requires aN holistic approach that integrates the study of the diversity and biological traits of organisms with the knowledge of modes of interaction between individual organisms and between organisms and the abiotic environment. On this basis, EMI also investigates the sustainable use of marine resources and the conservation of biodiversity and habitats, with particular regard to

marine protected areas. The marine environment



undergoes a considerable anthropic pressure, coming by the exploitation of different types of resources, the increasing impact of climate change and of pollutants conveyed in the trophic network, in the spread of alien species and/or toxic substances that alter the structure and functioning of the ecosystems. The diversity of marine organisms is widely unexplored and the knowledge about the functioning of the "sea ecosystem" and the related goods and services is still limited as well as its buffering response capability to the growing anthropic pressure.

Functional areas and personnel - EMI Functional Areas are equipped with multiple facilities for research. The four EMI Functional Areas with the main equipment that characterize them and related activities are shown in the following diagram:



They are equipped with microscopy systems, chambers and mesocosms at controlled temperature and light for experimentation on planktonic and benthic organisms, optical system for video filming of planktonic organisms, turbulence production system (TurboGen), photobioreactors, instrumentation for molecular biology and ecology.

The research lines of the Department also include field worK, which includes collection, monitoring and in situ experimentation on marine organisms and ecosystems. Currently the EMI department is composed of 40 researchers and 5 technicians. EMI researchers and technicians are spread among the main central headquarters of Naples and the territorial laboratories and offices of Ischia, Fano and Sicily. EMI researchers cover skills that include the classical and molecular taxonomy of marine organisms, various aspects of their biology (from physiology, to life cycles, to behaviour, to adaptive response to environmental perturbations), ecology, the study of molecular mechanisms that regulate biological responses, physical and ecological modeling, experimental design and the meta-analysis of ecological data. The Department also includes 24 associate researchers from both national and international external institutions. The research makes use of a wide national and international network of collaborations. The EMI Department has a consolidated tradition in higher education, where PhD students are trained through international and national doctoral programs. The doctoral projects have attracted numerous Italian, European, and non-European students. The Department also hosts numerous masters-level students for their thesis work.

EMI objectives for the 3-yr PLAN OF ACTIVITIES 2020 - 2022

For the Three Year Activity Plan 2020-2022, researchers from the EMI Department contribute to the activities foreseen in the four proposed Research Themes, with particular attention to the three Themes Function, adaptation, and evolution in marine organisms, Multi-scale marine biodiversity and Integrated approach to the study and management of the ecosystem. The EMI Department contributes to the scientific research of the Stazione Zoologica Anton Dohrn also through Core Research Activities

aimed at the integrated study of planktonic and benthic ecosystems and of the management of halieutic resources. The strength of the research conducted at EMI is the high level of integration of approaches ranging from the study of genes to that of organisms to that of ecosystem and socio-ecological systems. Also in this direction, efforts are aimed at interpreting the role of the microbiota in the functioning of organisms (seen as holobionts) and their ecosystem. In line with the tradition and experience gained since its foundation, the Stazione Zoologica has supported long-term ecological research that combines the environmental observations with the study of biological components in terms of biodiversity and of functional characteristics. Such research represents a fundamental contribution to environmental management, to the achievement of good environmental status (Good Environmental Status - GES, sensu Marine Strategy Framework Directive MSFD Marine Strategy Framework Directive), which are essential for human well-being and for the sustainable use of marine resources. The acquisition of this knowledge is part of the themes of the National Plan of Research (Blue growth) and



Horizon2020 "Protection of the marine environment as a source of livelihood, and biotechnology and for the choice of the necessary tools for the decisions of the policy makers ".

Ecology of the benthic system. Long-term studies on benthic ecosystems are conducted on seagrass beds of *Posidonia oceanica* and on other marine vegetated systems (LTER site of Lacco Ameno, Ischia Island). Over the last few years, the Stazione Zoologica has made available to the scientific community the logistic infrastructures for the study of natural CO₂-vents sites present along the coast of the Ischia Island, characterized by high CO₂ concentration and reduced pH with condition very closed to those predicted world-wide by modelists for the end of this century and during the next one. These sites represent a natural laboratory and a unique opportunity for the study of the impact of sea acidification on coastal ecosystems and to study the adaptational potential of benthic species to future environmental conditions. Examples are studies based on functional traits applied to distribution of benthic organisms collected along a pH gradient (from extremely low to normal) that allowed identifying a decrease in the number of species and the number of functional entities, suggesting that acidification is a key factor in determining the simplification of populations with the decline of top consumers and the dominance of lower-order consumers in the trophic network.

The effects of OA in decline is species particularly evident on calcifying taxa, however the non calcifers may show diverse responses. Porifera, which is а dominant phylum on the marine benthos, reveals certain tolerance to OA, yet species specific adaptability traits to acidified conditions in relation to the associated microbial composition, and metabolite profiles. Studies on Posidonia



oceanica, a key species for the Mediterranean coastal environment, also included manipulative experiments conducted both in natural conditions and in indoor mesocosms, with the aim of examining changes in the gene expression and in the physiological response in individuals subjected to single (CO₂, light or temperature) or multiple stressors (CO₂ and nutrients, temperature and nutrients). A new approach is being implemented for looking at the role of epigenetic changes in modulating gene expression and enhancing response potential to environmental changes in highly clonal species such as seagrasses. These studies made it possible to identify the thresholds of acclimatization of the species to climatic variations and to begin to clarify the molecular and physiological mechanisms underlying these responses. The variation of the composition of communities from vegetated systems and hard bottom, has also been studied along direct impact gradients, as in the Bay of Bagnoli, subjected to a long history of industrial pollution. Several studies highlighted the effects of such chronic industrial pollution and its interaction with global changes on a hierarchy of biological scales, from genes to habitats. Research on intra and interspecific forms of interaction, such as kleptopredation and protocooperation, casts new light on our understanding of the flows of matter and energy within the benthic systems and between the benthos and the plankton.

Ecology of the pelagic system - Observations on the pelagic ecosystem also make use of the studies of the Long-Term Ecological Research station MareChiara (LTER - MC) in the Gulf of Naples , whose results are made available online within a few days of sampling (<u>http://szn.macisteweb.com/campionamenti/ultimo-campionamento</u>). MareChiara has been "augmented" by the program called NEREA - Naples Ecological Research for Augmented observatories, a multidisciplinary observatory of the water column established in February 2019 and integrating traditional and disruptive technologies (see Highlights). Recently, thanks to the acquisition of new equipment (e.g., thermo-salinograph and a continuous fluorimeter for the definition of hydrographic patterns on a spatial scale, on-going flow cytometers for the real time assessment of phytoplankton and bacteria concentrations, and of a vertical

microturbulence profiler) data have been further enriched. Furthermore, two elastic beacon buoys flanked by flow cytometers were activated for the analysis of the picoplankton component (prokaryotic and eukaryotic). The study of organisms of the planktonic trophic network is also conducted with High Throughput Sequencing approaches used for characterization the of taxonomic and bacterial functional diversity of the component, of bacteria, protists (diatoms, dinoflagellates, ciliates) and metazoans. The more than 30-yrs time-series of data allows to trace seasonal patterns and trends of the plankton communities and of the environmental variables that drive them, shedding light not only on the functioning of the system but also on the general ecological characteristics of plankton organisms. The multi-year results of the long term investigations also offer background а knowledge of the optimal ecosystem in order to be able to face specific questions with in situ or laboratory experiments (e.g. prokaryoticphytoplankton interactions, role of life cycles in population dynamics, effect of secondary metabolites produced by diatoms on other community components, identification of



trophic interactions etc.). Functional genomics studies allow to investigate the mechanisms at the molecular level following specific environmental forcing (e.g. diatom growth under different temperature conditions, copepod responses to phycotoxins) or the mechanisms that regulate the life cycles of diatoms. These studies will provide information necessary for metatranscriptomic approaches of the planktonic community in key periods of the annual cycle. Finally, the use of a modeling approach, including ecological-network approaches to reproduce food-web and other interaction processes and predict the systemic outcome of biotic interactions, allows the integration of the different scales of observation, from the cell to the ecosystem, and across components (e.g. from plankton to benthos and nekton) to obtain useful information for management as well as for the conservation of marine resources.

Fish and Fisheries management and conservation - This research activity aims at investigating how to ensure socio-ecologically sustainable exploitation of fisheries resources and conserve marine biodiversity and its ecosystem functioning. Various studies carried out by the SZN aim to describe the dynamics and state of exploitation of fishery resources, from single stocks to complex communities of species. These studies are based on a multidisciplinary approach where the functional traits of the exploited stocks (natural mortality, sexual maturity, growth curves), the species interactions (competition, prey-predator, consumption) and both the human (e.g. fishing pressure) and environmental drivers (climatic variations, habitats, acidification, system productivity) are included in different modeling frameworks. Such modelling effort is aimed at delivering advice for the sustainable exploitation of marine resources in line with the EU and FAO-GFCM policy goals, also through the minimization of fishing impacts on marine ecosystems. Fisheries oceanography and biophysical interactions during fish early life history stages is one of the key study subjects at SZN to understand the spatio-temporal dynamic of recruitment. Additionally, a growing number of national and regional funded research activities (e.g. FEAMP Campania) is focusing on integrating the existing knowledge on the status of deeper benthic communities, spanning across the upper continental shelf deep to the deeper slope.



New integrated ecosystem-based investigations have been developed to allow detecting hotspots of vulnerable marine ecosystems (species and habitat), threatened by human activities and deserving of conservation to ensure a correct ecosystem functioning and the maintenance of the related ecosystem services (e.g. associated marine resources inhabiting spawning and nursery areas, crucial food source for the national economy). In this context researchers at Stazione Zoologica are already creating synergies among plankton, benthos and nekton specialists by integrating ecosystem components monitoring to disentangle the effects of both fisheries and aquaculture activities on benthic communities. Patterns of disturbance on communities and ecosystem responses (e.g. biodiversity and functioning measures) are analyzed and modelled to investigate recovery times over which tailor future sustainable management measures to be translated to social capital (e.g. stakeholder engagement from

policy-makers to end-users).

Resources management now is usually framed in the context of Blue Growth, that implies environmentally sustainable and socially equitable development. Therefore in the context of sustainable exploitation of marine resources, a key aspect is played by the human dimension that should be considered in a wider context of socio-ecological systems (including both the natural and the human components). SZN has investigated the human dimension of small scale fisheries, that represents a key extractive use of coastal areas, and the role of institutional frameworks and governance in affecting socio-ecological effectiveness of management measures. In this perspective marine protected areas (MPAs) represent relevant case studies being one of the most widely promoted spatially explicit conservation tools and policy solutions to address many of the well-documented problems of marine habitat degradation and overfishing





3.4.3 Marine Biotechnology Department

Research lines - The Marine Biotechnology Department carries out interdisciplinary research, which aims to study marine organisms for the development of products or molecules to protect the environment and to improve human well-being. The "target" organisms for the production of these products or molecules are bacteria, fungi, micro/macroalgae and marine invertebrates (crustaceans, sponges, molluscs and cnidarians), with the aim of exploring their potential biotechnological applications. Currently the research conducted in the department focuses on applications in the nutraceutical, cosmeceutical, pharmaceutical, biomaterials and environmental fields.

A common tool for all these applications is "culturomics", that is the technological development of a cultivation platform for marine organisms of biotechnological interest. The most promising organisms for this culturing approach are bacteria, fungi and microalgae which are isolated from aquatic environments, grown in culture media, and subsequently subjected to screenings aimed at identifying strains possessing the most promising features for biotechnological applications. Microalgae, that are the test-organisms of many studies of the department, need innovative validation cultivation strategies (e.g. new generation of photo-bioreactors) in order to implement and maximize the production of



bioactive molecules for biotechnological applications.

Image of photobioreactors @ SZN (capacity 100 L).

Research on products from microalgae with nutraceutical and cosmeceutical applications focuses on primary and secondary metabolites. Primary metabolites (e.g. lipids, sugars and proteins) are also considered a good alternative to animal-derived products for aquaculture. Secondary metabolites include antioxidants such as polyphenols, carotenoids, sterols, vitamins. These compounds are studied, separately and/or in a mixture, for their properties in human health defense against diseases or to stimulate the immune system.

Research in the pharmaceutical field aims at exploring marine biodiversity to identify new bioactive molecules with anti-proliferative, anti-inflammatory, anti-microbial, antidiabetes and anti-neurodegenerative activities. Bioactive compounds for the nutraceutical, cosmeceutical and pharmaceutical sectors are

isolated by various chemical extraction protocols, purified by HPLC and identified/characterized by GC/MS, LC/MS and NMR.

Research in environmental biotechnology aims at identifying bacteria, fungi and microalgae able to contribute to environmental decontamination of aquatic environments through biodegradation or

bioaccumulation of pollutants. To date, 39 National Reclamation Sites and 18 others at the Regional level have been identified. National Reclamation Sites need to be decontaminated from industrial wastes and are often located in marine coastal and transitional areas. Contamination of marine sediments does not only concern sites of interest, but also harbour areas. Two major contaminants are present in these coastal marine areas: heavy metals and hydrocarbons, but new emerging contaminants have recently attracted scientific interest as well. We aim at investigating microorganisms inhabiting such polluted environments in order to clarify their detoxification mechanisms. Within this context, environmental bioremediation methods relying on biodegradation, bio-leaching, biotransformation, bioaccumulation or bio-immobilization of organic and/or inorganic contaminants exploit the capacity of marine microorganisms to enhance natural removal processes. The production of biomolecules that enhance the contaminant uptake and their removal from environmental matrices is also explored, as a possible alternative to synthetic compounds commonly used in the bioremediation field.

Research in the field of biomaterials has been recently introduced at SZN and is mainly focused on exploiting some microalgae for their ability to produce biopolymers with a protective function. For example, species of the genus *Nannochloropsis* have the outer layer of their cell wall made of algaenan, a highly refractory biopolymer that seems to be attractive for the development of bioplastics. Furthermore, the major algaenan monomer, i.e. long chain diols and their precursors, long chain hydroxy fatty acids, possess a chemical structure similar to that of some plant lipids (ricinoleic and lesquerolic acids) that are used for the development of fuel lubricants. In addition, marine collagen has been recently studied by SZN researchers as a promising biomaterial with great potential in tissue engineering and drug delivery applications, due to its unique properties. A new research line aims at the exploitation of marine discards and underused species of the Mediterranean Sea, such as by-catch species and waste from fish processing industries and aquaculture facilities, for the development of a sustainable process of collagen extraction.

Laboratories and staff - The laboratories are currently located in the main building in the Villa Comunale in Naples and a new one is in the Messina premises. Currently, various platforms are available:

- "Culturomics" platform, which aims to optimize the growth of marine organisms, maximizing the production of bulk biomass and/or highly valuable molecules of biotechnological interest;
- "Screening" platform for the identification of antitumor, anti-inflammatory, antioxidant, antidiabetes and anti-neurodegenerative activities. This platform, in addition to the identification of compounds of interest, also provides preliminary information on their mechanism of action, thereby predicting possible applications of the compounds of interest in the biomedical field;

• "OMICS" platform, which is dedicated to the study of genomic, transcriptomic, proteomic and metabolomic data for marine organisms of interest, searching for enzymatic pathways involved in the synthesis of bioactive metabolites, the degradation of specific organic pollutants, and the bioaccumulation of toxic substances. A gene approach is also implemented mining for genes of interest in marine organisms that are difficult to massive cultivation.

- "Metabolites analysis platform" aimed at the study of bioactive compounds.
- "Bioremediation" platform, dedicated to the study of bacteria, fungi and microalgae useful for bioremediation environmental biotechnologies.

The department was created in 2018 and is composed of researchers, technologists and technicians with skills in the biomedical, environmental and biomaterial fields. The department also includes research associates from national research institutes and universities and includes a wide network of collaborators at the national and international levels.

Goals of the Marine Biotechnology Department for the 3-yr Plan of Activities 2020 - 2022

Research conducted by the Marine Biotechnology Department contributes to the long-term challenges (2020-2030) launched by the European Community to improve human and ecosystem health and wellbeing. The goal is to create and enhance research infrastructures and activities, aiming to become a point of reference in the BluBiotec sector in Italy. Particular interest is dedicated to research applied to the development of patents and product marketing innovative deriving from micro and macro organisms. The research conducted by the Department are included in the framework of the themes of the National Research Plan (Blue growth) and Horizon2020.

For the Three-Year Activity Plan 2020-2022, the SZN staff of the Marine Biotechnology Department will focus their research on the above-mentioned Research Topics, with particular attention to the Themes: "Sustainable Exploration of Marine Biological Resources" and "Integrated Approach to the Study and Management of Marine Ecosystems".

Recently, the department has grown in terms of research staff increasing the multidisciplinary of research and interactions within and outside the Department.

The objectives for the three-year period are:

• Implementing studies on the sustainable exploitation of marine biodiversity for the discovery of new bioactive molecules. This objective involves the isolation of new bacteria, diatoms and fungi from marine compartments, as well as the isolation of other marine organisms, the development of new strategies to cultivate new microorganisms and development of microbial biotechnologies for the mass production of bioactive compounds to meet industrial demands;

• Developing bioremediation methods to decontaminate marine sites of national interest through the use of marine microbes and their products using biostimulation (stimulation of autochthonous microbes) and/or bioaugmentation approaches (use of non-native microbial biomass to develop *ex situ techniques* of depollution), and by employing the natural skills of some marine microorganisms to adsorb/incorporate persistent pollutants (heavy metals) and degrade hydrocarbons;

• Developing new biomaterials from marine organisms;

• Implementing the platform for the analysis of "metabolites" to include polyphenols, sterols and vitamins;

• Implementing the screening platform for the characterization of the biological activity of compounds of marine origin, taking advantage of chemical ecology issues to identify possible relationships among organisms and info chemicals.

• Implementing the OMICS platform for the discovery of metabolic pathways involved in the synthesis of products of biotechnological interest.

• Identifying new techniques for aquaculture production of model organisms, to be used for the test and the discovery of new bioactive molecules.



Figure. Schematic representation of drug discovery from microalgae with *in silico approaches* (i.e., genomics, transcriptomics, proteomics and metabolomics) in order to identify marine natural products (From Lauritano et al. 2019, *Marine Drugs*). Once the compound of interest is identified, bioactivity screening, heterologous expression, genetic engineering and/or chemical synthesis can follow. This will allow us to characterize the activity and produce the desired bioproducts with pharmaceutical, nutraceutical, cosmeceutical and biofuel production applications.

3.4.4 Research Infrastructures for marine biological resources Department

Research lines- Research Infrastructures is one of the pillars of the Stazione Zoologica activities. The Department of Research Infrastructures for marine biological resources (RIMAR) provides high technology services and access to infrastructure and research platforms to the national and international scientific community, as well as support third mission activities such as third-party contracts. At the same time, RIMAR conducts applied research and technological development in the field of marine biology and in the specific fields of interest of the units of which it is composed. To achieve this goal RIMAR staff have set themselves the following objectives: to provide high quality scientific services in the SZN research fields, managing equipment, carrying out analysis, provide support, advice and training to users, and promote research for the purpose of protection of marine biodiversity. The RIMAR Department also has an important role to play in to ensure the contribution of the SZN within the ESFRI European Research Infrastructure European Marine Biological Resource Centre (EMBRC), for which the Stazione Zoologica Anton Dohrn coordinates the Italian node (EMBRC-IT). In the field of international and national infrastructures RIMAR also guarantees the participation to the Italian nodes of the European Research Infrastructures ESFRI-EMSO and Lifewatch, and participates in GLOMICON (Global Omics Observatory Network) the coordination network for "omics" observatories with the coordination of GEOBON. Finally, the Department of Research Infrastructures for marine biological resources manages at the European level the transnational access program of RIs Cluster Project EMBRIC and the INFRAIA-01-2016-2017 ASSEMBLE Plus (H2020) both associated to the EMBRC.



Facilities and personnel – The Dept. RIMAR

Department is currently organized into two sections each divided into four Service and Technological Research Units as reported in the following scheme:

	EMBRC-IT Node Management RIMAR Administration		
	Access to marine ecosystems and environmental analysis	Environmental Monitoring & Analysis (MAA)	
		Infrastructures for rearing of Marine Organisms (IMOM)	
		Infrastructure for Marine Research (IRM)	
RIMAR		Technological Implementation & Robotics (ITR)	
		Advanced Microscopy Center (CeMA)	
	Tasky alogical Diatforms	Bioinformatics, Computational Analysis & Data Management (B AC)	
	Technological Platforms	Classic and Molecular Taxonomy (MoTax)	
		Sequencing and Molecular Analysis Center (CSAM)	

The Department is organized in two sections: 1) Access to marine ecosystems and environmental analysis and 2) Technology platforms. The first section consists of 4 core facilities: (a) infrastructures for marine research; (b) infrastructures for rearing of marine organisms; (c) environmental monitoring and analysis; (d) technological implementation and robotics. The second consists of 4 core facilities: a) advanced microscopy center; (b) sequencing and molecular analysis; (c) classical and molecular taxonomy; (d)bioinformatics, computational analysis and data management. The RIMAR department also includes the management structure for the EMBRC-ERIC Italian node.

Access to marine ecosystems and environmental analysis Section

The section "Access to marine ecosystems and environmental analysis" consists of the Units IRM, IMOM, MAA and ITR, in a multidisciplinarity context created by the interaction between the different units. Thanks to the contribution of the research and infrastructure projects PON PRIMA, PON INSEA, PON PLaCE, and others forthcoming, the Section will be equipped with high technological value instrumentation and multidisciplinary fixed observatories both coastal and deep that are fully part of the European and international context for ecosystem monitoring services, for the preservation of the state of environmental quality, needed for preserving and regenerate natural capital (see e.g. EMSO-ERIC, EMBRC-ERIC, CLUSTER-BIG). This is in consideration of the fact that marine resources will be in the near future the object of greater use by human activities.

The expansion of marine research must interact strongly with the many economic activities that take place at sea and eco-sustainable exploitation of its natural resources. In this scenario the role of the Stazione Zoologica and in particular of the Section for Access to Marine Ecosystems and Environmental Analysis of the RIMAR Department, is preeminent and it will therefore be necessary to focus on technological development thanks to the strong interaction of the connection between Core facilities and Infrastructure for research at sea (IRM), Implementation, Technology & Robotics (ITR) and Environmental Monitoring and Analysis (MAA), as well as with the help of of the Rearing of Marine Organism Infrastructure Unit (IMOM).

Core Facility – Infrastructure for marine Research (IRM) is in charge of the logistics of the operations at sea, the sampling and the environmental analysis. It manages two coastal vessels (M/N Vettoria and Hippocampus)



equipped with instrumentation and oceanographic instrumentation for measurement of environmental variables and for the sampling. It provides management and maintenance of fixed research infrastructures (elastic MEDA-type buoys, seabed multiparameter platforms, mooring) and other advanced oceanographic instrumentation. It collects material used for research (samples, data, etc.). It carries out activities of material collection, visual census and instrumentation maintenance in diving, with the help of Underwater Technical Operators (OTS). Technological development and research is aimed at the implementation of new observation techniques and procedures and the management and analysis of the data produced by advanced oceanographic instrumentation

Infrastructures for rearing of Marine Organisms (IMOM) (IMOM) deals with animal housing and breeding of marine organisms used in scientific research. The Unit develops methodologies and procedures aimed at optimizing the use of marine resources by avoiding, when possible, field sampling.

Its operation is divided into two main lines: research and cultivation/breeding. The technological development is aimed at optimizing the techniques for the production/housing of organisms in a confined environment. The culture allows breeding and housing the species commonly used for embryology, ecotoxicology, genomics, plant and animal physiology. Research is in progress to define the procedures for the rearing in continuous of key model species, such as *Ciona intestinalis* and *Paracentrotus lividus*, starfish, using systems on land and at sea. At present the facilities are undergoing restructuring and the Housing activities are continuing in temporary locations, in the western building area.



Core facilities for Monitoring & Environmental Analysis (MAA) - The core facility previously included in the MEDA unit, offers support for laboratory activities and sampling of principal environmental variables. The facility can provide instrumentation and provide know-how to users, or directly conduct chemical analysis. The facility carries out service activities for internal users, activities for third parties projects and institutional projects.

Core facility for Technological & Robotic Implementation (ITR) develops prototypes of interest for research, developed on the basis of ideas and requests from the technical and scientific staff of the Stazione Zoologica, within the framework of the activities of the Departments and the Functional Areas, subject to feasibility analysis. The unit deals with design through 3D modelling software, rapid prototyping using a 3D FDM printer, creation of cards, etc. and programming of microcontrollers to

manage sensors and actuators, creation of graphic interfaces for the management of the prototype, mechanical processing (drilling, turning, cutting and milling), power lines and lighting technology, as well as selection and research of materials suitable for the intended purpose. It also takes care of the management, maintenance and implementation of oceanographic instrumentation in synergy with the Technology Platforms Section.



Technology Platforms Section

The Technology Platforms Section, in combination with the internal laboratories and the Access to Marine Ecosystems and Environmental Analysis, taking advantage of the different expertise developed, will try to combine multidisciplinary expertise to develop new approaches and strengthen the institutional portfolio of activities. The section consists of 4 cores facilities.

Core facility - Taxonomy of Marine Organisms (MOTax)

provides specialised services for taxonomy of marine organisms using integrated morphological and molecular approaches. The primary objectives are: (i) the creation of an Italian network of taxonomists with expertise in marine organisms (ii) the dissemination



of this science through the advanced training of marine organisms with an integrated approach to the study of taxonomy. In order to facilitate the communication within the network a newsletter "MOTax" was created to receiving and disseminate information on marine taxonomy. The newsletter is periodically published on the SZN website and the numbers published to date are available in the MOTax newsletters archive.

(<u>http://www.szn.it/index.php/it/servizi/identificazione-e-tassonomia-organismimarini/-e-taxonomy-organismimarini/motax-newsletters</u>). As part of SZN's higher education activities, the service MOTax promotes specialised courses on the taxonomy of marine organisms. In November 2018 an international advanced course on taxonomy of zooplankton was organized for the first time.



Centre for Advanced Microscopy (CeMA) provides the necessary skills to the application of optical microscopy and conventional fluorescence techniques (epifluorescence or confocal). The Unit provides both internal and external users with advice on scientific, technical assistance and training for the application of live-cell technologies imaging. It provides techniques and preparation equipment and observation of samples for the optical microscopy, fluorescence microscopy and electronic transmission and scanning (TEM and SEM). Recent installation of the environmental SEM will make possible to promote the development of new methodologies for improvement the provision of services. In addition, the Unit provides assistance to analyse the results of the acquired images.



Molecular Sequencing & Analysis Center (CSAM) offers services, training and consulting in the field of Molecular Biology, from the execution of complex procedures to specific consultancy and development services and on-demand protocols. The Unit has developed a sequencing service which includes both the classic Sanger method that the Ion deep sequencing technology GeneStudio S5, a service for the Real Time PCR and a service for the droplet digital PCR that will be improved by the purchase of the sonicator "Covaris" for Chromatin immunoprecipitation experiments". The unit is also equipped with a automated liquid handler for low to mid throughput processes the unit also integrates the cytometry service with the setting up of a new laboratory equipped with BD equipment, the sorter "Influx" and




the "FACS Verse" cytometer extending the portfolio of offered technologies to users. In addition, at this platform studies and tests are underway for the development of new applications, such as the isolation of new microorganisms, nuclei from diatom cells, etc.

Bioinformatics, Computational Analysis & Data Management. (BAC) – From 2017 the activities of the new structure have begun with the support and coordination of the Prof. Maria Luisa Chiusano, Professor of Bioinformatics at Federico II. The core facility was strengthened by the recruitment of a permanent researcher with expertise in marine metagenomics, two technicians and three research assignees, dedicated to the setting up of advanced computing systems for the analysis of "omics" data and to the implementation of methodologies, software, IT procedures and databases, maintenance and release of data produced by the institute, and also training activities supporting all activities of interest to the Institute, with the aim of supporting the research based on massive data and computational modeling also.



RIMAR OBJECTIVES FOR 3-yr PLAN OF ACTIVITIES 2020 - 2022

Technological upgrading - The RIMAR Department will see a substantial technological upgrading through the financing of PON Infrastructures approved by decree MIUR 461 (dated 14.03.2019) for an eligible grant of more than 15 Million €. This financing, which refers to infrastructure, instrumentation and support systems, will realize the strengthening of the entire Department as well as of the institution as a whole, not only in the its Institutional headquarters but also for the development of other outstation by increasing the research capabilities not only of the Stazione Zoologica but of the entire EMBRC-IT community and finally of the entire scientific community, at large.

Core Facility	Expected evolution			
Infrastructure for marine research (IRM)	Expansion of the SZN naval units and consequent augmented access to the environment capacity, study and experimentation. Enhancement of the instrumental park and fixed infrastructures for the detection of marine weather parameters			
Infrastructures for rearing of Marine Organism (IMOM)	Enhancement of life supporting systems infrastructure and technologies			
Environmental Monitoring & Analysis (MAA)	Enhancement of the instrumental park with the acquisition of new analytical instrumentation			
Technological Implementation & Robotics (ITR)	Acquisition of a modern ROV and enhancement of management and ancillary technologies			
Classical and Molecular Taxonomy (Motax)	Introduction of IFCB technology as automatic <i>in situ</i> detection system			
Advanced Microscopy Centre (CeMA)	Enhancement of the instrumental park both related to the observation/preparation of the samples and the image analysis			
Sequencing & Molecular Analysis Center (CSAM)	Enhanced instrumentation to support the sequencing and gene expression analysis platforms			
Bioinformatics, Computational Analysis & Data Management. (BAC)	Enhanced computing, storage and networking capacity			

Within the EMSO Joint Research Unit, the Infrastructures for Sea Research (IRM) and Technological & Robotic Implementation (ITR) will participate in the technological development of a new generation of deep multidisciplinary observatory to monitor the carbonation processes induced on electrified structures (within the framework of the Blue Growth, Industrial Research project to evaluate the possible conversion of off-shore platforms for multiple eco-sustainable uses). Capacity building and the acquisition of new skills, new technicians and technologists will include specific training in order to manage and make fully usable the acquired technological potential.

3.4.5 Third Mission Area



The structure of the Third Mission Area is new and can be summarized as follows:

The organization of the III Mission Area (A3M) formally started in October 2019 with the definition and reorganization of the functions based on priorities and following objectives: 2020

- Organization of the Area
- Organization of communication and outreach activities: revision of Institutional imaging and logo
- Re-opening and inauguration of the Aquarium in Naples, design and renovation of the Aquarium of Fano
- Local renovation and content design, furnishings and DaDoM flow management
- Logistical assessment of Incoming activities for all the sites opened to public view, starting from Turtle point (Portici) within the agreement between SZN and the Dohrn Foundation
- Implementation of Grant and Innovation Office activities and training
- Marine farm and aquarium renovation concept in Bagnoli
- Implementation of rescue and research projects, complementary to those carried out in the Departments, aimed at large mega- and charismatic species, which are particularly suitable for carrying out communication and dissemination activities (including citizen science) aiming at active conservation of marine environment.

2021:

- DaDoM Opening event and inauguration
- Marine Library opening

To carry out all these activities, A3M general meetings and target calls by theme have been carried out, reviewing the entire organization and setting the identification of expertises and gradual implementation of resources. This plan should be completed in 2022, provided full operation of the different sites.

The Historical Aquarium - the works for the renovation of the historical aquarium are in progress and will be completed in the autumn of 2020.



During the three-year period 2020-2022 a further extension to the central body is planned below the former library.



Interior rendering of the former library that will house the expansion of the Historical Aquarium

Darwin-Dohrn Museum aims to explain to the general public the reason for the foundation of the Stazione Zoologica and the importance of the study of biodiversity and evolution of life at sea. The central part of the museum will be a space for special exhibitions and conferences, with a weekly calendar of initiatives, in order to produce a communication program, open to the public, in which the research conducted at the Stazione Zoologica Anton Dohrn will be explained. Visiting researchers will contribute to the program, with special presentations. This formula will be a world first, which will combine history, science and art. The restoration works of the building "Casina del Boschetto", is ongoing. In the meanwhile the databases of museum collections are being implemented: http://www.anms.it/collmap/

The Marine Library (Biblioteca del Mare)

contains the historical heritage of ancient texts, photographs, scientific instruments, correspondence, miscellaneous and collections. This structure enhances the assets of the museum and the archive exhibitions through setting up for conferences or other events, internal and external to the SZN. It provides useful information to implement the scientific databases that store up the collections and collaborates with Italian and foreign researchers for the study and the enhancement of the assets held.

Turtle Point is located in Portici and is a unique place where research and education are carried out to coexist and to

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achieve the common goal of preservation of Mediterranean marine ecosystems. This will be attained through the following objectives: i) Improving well-being of animals and success of rehabilitation; ii) Expanding research in the field of sea turtle veterinary and study of their ecology; iii) Increasing the involvement of public with environmental education and awareness-raising and citizen science activities; iv) active protection of turtle nesting sites in the Western Mediterranean. Turtle Point is equipped with advanced laboratories for environmental and biological analysis, a clinic with surgical and radiological rooms, an educational display and two multimedia rooms. Obviously, there are in addition the spaces for the care and rehabilitation of sea turtles injured by human activities.



Images of the laboratories at the Turtle Point

Visitors are welcomed in the hall of thematic aquariums dedicated to environmental quality descriptors of marine ecosystems defined by the Marine Strategy Framework Directive of the European Community. Real-size models of the great Mediterranean marine vertebrates inspire visitors to discover these fascinating animals and the problems linked to their conservation. The visit continues with the meeting of sea turtles in the rehabilitation tanks. A wide multimedia space provides the opportunity to explain, more in-depth, the research conducted at the Turtle Point through, for example, migrations of sea turtles released after rehabilitation.

3.5 THE SZN SEATS

3.5.1 Seat in Villa Comunale

The Seat is under renovation and refurbishment, the external part has been completed, and further work is underway for the structural consolidation of the historical Aquarium, both for the expansion of the Aquarium in the central body and the new ex-library course. The restructuring of the West Wing and of the - 1 area of the Institute is also underway. An executive project of complete renovation has been developed for the former library and asbestos is being removed from the center of the former library.



3.5.2 Villa Dohrn – Ischia

The facility of Ischia, called Villa Dohrn being built in 1906 as a private residence of the Dohrn family, was established in 1969 as a research center on the sea to study the ecology of benthic organisms and communities that live in close contact with the seabed. Its mission is to study the functioning of benthic systems at the organism, community and ecosystem level. Particular emphasis is given to organism biology, taxonomy, physiology, biomolecular analysis, chemistry, and evolutionary processes, paying attention to the organism-organism and organism-environment interactions and their implications for biodiversity conservation. The objectives of the facility are to host research and training activities carried out in the field of benthic ecology, plankton of organism biology and marine biotechnology according to the new guidelines established by the SZN organs.

3.5.3 Headquarter of the Marine Biotechnology Department (Naples)

The Marine Biotechnology Department was established in 2018. The consequent increase in staff and the need for ad hoc infrastructures will require the identification of a suitable site near the Villa Comunale. This process was started with the Municipality of Naples and the Campania Region.







3.5.4 The Portici Centre

The Turtle Point, inaugurated in 2017, is located in the former Bourbon slaughterhouse, and is on free loan for twenty years (since 2016) from the Municipality of Portici. It extends over 600 m² roofed and 7000 m² uncovered area and is an exceptional example of urban redevelopment. The Centre is equipped with advanced laboratories for environmental and biological analysis, a clinic with surgery room and X-ray, a rich didactic exhibition, a multimedia room and, of course, the part dedicated to marine turtles.

The Turtle Point is a leading center in the Mediterranean for research, rehabilitation and education on the conservation of marine vertebrates with the following objectives:

• Expand research in the field of veterinary medicine, animal welfare and ecological niche modeling,

• Involve the public through environmental education programs and awareness raising activities

• Conservation actions applied on nesting sites in the western Mediterranean







4 SCIENTIFIC ACTIVITIES

4.1 RESEARCH RESULTS OF THE SZN IN 2019



The pillars driving SZN activities are illustrated here below.

Reported are from left to right: 1) new challenges in marine research; 2) contribute to the national research; 3) Improve international competitivity; 4) increase the research personnel; 5) enhance and support scientific research; 6) contribute to the socio-economic development; 7) contribute to high education.

The Ten-year Vision document of the SZN predicts the development of the following activities as priorities:

Marine Biotechnologies	Identify new marine organisms and their molecules and products of pharmaceutic, nutraceutical, environmental and industrial interest
Global Climate Change	Develop a global network of observatories to integrate biological and environmental variables to understand the effects of global climate change on seas and oceans
Conservation Biology	Develop a network of protection for marine species and habitats able to sustain the targets of the marine strategy framework directive and to reach a good environmental
Marine Biological Resources	Develop new approaches and solutions for the sustainable use of marine biotic and abiotic resources

The scientific issues developed in the Departments of the SZN during 2019 and their interdepartmental implementation are summarized below:

ΤΟΡΙϹ	BEOM	EMI	RIMAR	BLUEBIO	A3M
1: Function, adaptation and evolution in marine organisms	\checkmark	\checkmark	\checkmark		
2: Multiscale marine biodiversity	\checkmark	\checkmark	\checkmark	\checkmark	
3: Integrative approach to environmental research and ecosystem management		\checkmark	√		\checkmark
4: Sustainable exploration of marine biotic resources	~	\checkmark	\checkmark	\checkmark	

The BEOM Department has contributed to research on the biology, evolution and adaptation of marine organisms included in Theme 1 (Physiology, adaptation and evolution) and Theme 2 (Multiscale marine biodiversity). The EMI Department has contributed to research developed within all four themes, an aspect favored by the fixed-term recruitment process in the last three years and the recent permanent one, which has allowed EMI's scientific staff to explore new areas of research. The RIMAR Department participated in the research activities of all four themes with an essentially supporting role. The activities envisaged under Theme 4 are the basis for the establishment of the new Department of Marine Biotechnology.

Theme 1: Function, adaptation and evolution in marine organisms

The research conducted in the context of Theme 1 has the objective to characterize biological complexity identifying the molecular, metabolic, and physiological mechanisms that determine it, including the mechanisms of adaptation of the organisms to environmental conditions altered by human impact.

The development of 'omics' approaches has provided an enormous boost to the studies in the field of functional and evolutionary biology (Theme 1). The sequencing of messenger RNA organisms of marine aimed at the identification of genes differentially expressed in physiological conditions compared to the organism exposed to perturbations - represent nowadays the basis of biological experimentation.

The information collected is fundamental for studies aimed to reconstruct the mechanisms



regulating specific responses or functions and their evolutionary paths. The data produced, made accessible to the entire scientific community - as indicated by the European Dissemination Plans - amplify the dissemination process and its effects and contribute to the enhancement of the scientific reputation, recognized both nationally and internationally, of the SZN. The scientific staff of SZN has significantly contributed in recent years to the knowledge of numerous marine organisms.

The results obtained were favoured by the development of optimized methods for the study of gene expression as well as differential analysis in specific biological processes or in relation to events that induce plasticity and adaptation.

An important focus within Theme 1 is the study of molecular mechanisms that regulate the life cycles of planktonic organisms, their capability to perceive and respond to environmental and biological stimuli. The integration of this 'functional' information into an ecological context promotes an integrative approach among molecular biology, genomics and marine ecology. A solid understanding of these mechanisms is also essential for the interpretation of metagenomics and metatranscriptomic data, which represent the new frontier of biological oceanography.

The dynamics of the diatom life cycle is a target process. Several papers have been produced in the past years on this topic, most of them on the planktonic diatom *Pseudo-nitzschia multistriata*, due to the possibility to finely control its genetics. The project DIsCO (see highlights) is allowing further boosting of the studies on this marine diatom with a focus on the sexual phase and on the implication for genome evolution. Information on the gene expressed during the sexual phase has been gained also for *Skeletonema marinoi*, another ecologically relevant species whose genome sequencing is in progress. We detected an additional set of genes expressed during the sexual phase and shared with *P. multistriata* that expands the 'sex toolbox'. These studies open two future avenues: comparative studies with other diatoms with a different lifestyle, such as benthic species, and the exploration of the distribution of diatom sex genes in the environment using metaG/T datasets (produced in TARA but also in the ongoing project NEREA, see highlights). The vast background knowledge gained over the past decade on the genus *Pseudo-nitzschia* is now allowing more comprehensive analyses of metaG/T data to explore the ability of this genus to colonize specific ecological niches in the oceans.

Species of the genus Chaetoceros, another extremely abundant diatom genus in the oceans, present another intriguing feature which is the capability to produce resting stages. C. socialis was selected as a model to investigate the mechanisms that induce this life cycle transition. A detailed mechanistic description of spore formation has been obtained, this will be followed by a study of the gene expression changes associated with the transformation of vegetative cells into spores, with the aim to identify the pathways involved in the perception of cues inducing it and the master genes orchestrating the profound cellular rearrangements required, currently completely unknown for phytoplankton.



Life cycles are only one of the endogenous processes shaping populations and communities. Many other factors affect diatom populations dynamics, including exogenous factors such as light and nutrients, but also temperature, especially at our latitudes. Part of the efforts are therefore devoted to understanding diatom adaptation to changes in these parameters, with a focus on the enzymes and proteins that are most likely providing plasticity, such as nutrient transporters. Among the most recent and interesting results is the identification of the role of transposon genes in the cold-adaptation of a key-species of the Gulf of Naples, *Leptocylindrus aporus*, and the recognition of a high intraspecific variability and of in-culture adaptation processes, which hint at a great complexity in the mechanisms underlying functional diversity in diatoms.

To enable functional studies in planktonic organisms, EMI researchers work steadily on the development of genomic resources and tools, to optimize them in model species as well as to set them up on a selection of novel species which are representative of groups with distinctive features and adaptation potentials. These activities include novel genome sequencing projects (a project funded on the genus Chaetoceros, collaboration for the benthic genus Seminavis), transcriptome generation, metabolomics explorations, and application of genome editing methodologies, including the CRISPR/Cas9 technology to obtain knockout strains. This method will be useful for the dissection of many processes, allowing to switch off candidate key genes for the regulation of life cycle transitions, for nutrient transport, for the production of chemical mediators or for the response to selected environmental changes.



Marine phanerogams have long been the subject of study and research by SZN researchers. The omic approach based on the analysis of gene expression patterns in Posidonia oceanica and in Cymodocea nodosa, in different plant organs, has allowed us to enrich the information on the molecular mechanisms activated when environmental conditions

change, in particular temperature and luminosity, and the combined effect of multiple stress factors. The genomic regions of the genes related to the dynamic response to atmospheric agents are those with lower methylation rates. *P. oceanica* flowering caused by an increase in temperature, a stress response strategy that was previously only hypothesized, was observed for the first time under controlled conditions. Again, genes involved in the regulation and induction of epigenetic mutations were highly expressed.

Studies carried out under controlled conditions on Posidonia have allowed us to establish that different plant tissues and leaves of different ages have different methylation rates. Expression patterns, specific to the thermal ranges to which plants live, are the basis of the plant's ability to acclimatize to simulated conditions of heat waves. Finally, the role of adaptation to the local environmental conditions of the mother plant in conferring a different ability to respond to changes in environmental conditions in seedlings kept in common garden conditions, has been studied in *C. nodosa*.

The increase in CO2 emissions in the atmosphere is one of the most important factors contributing to global climate change. This rising CO2 concentration generates, not only global warming, but also the ocean acidification phenomenon (OA). Several studies described detrimental effects due to OA under laboratory conditions and across many taxa such as reduced calcification, metabolic stress and energetic constraints, altered growth and reproduction, highlighting that predicted OA conditions will have negative consequences for marine organisms. Besides, the natural CO2 vents of Ischia represent a pivotal in situ laboratory to study



the future ocean conditions, helping to predict changes in biodiversity and ecophysiology adjustments of marine species associated with OA. Several studies have been performed by SZN researchers in such natural laboratories showing that divergent and compensatory biological responses to OA can occur, driving some species to counteract the effects of OA through a range of adaptive processes including acclimatisation (phenotypic plastic adjustment) and genetic adaptation. A recent study highlighted a high phenotypic plasticity that might lead to adaptation in a sea urchin species, Paracentrotus lividus, which has both economical (being harvested for human consumption) and ecological value (having a key role in shaping benthic marine communities). In particular, in this study no differences in several physiological parameters (e.g. including acid-base balance, respiration and nitrogen excretion) were found when sea urchins from the vents were compared with those collected in the control sites. Another benthic species was found to be plastic in response to OA. Individuals of two species of polychaetes, Platynereis dumerilii and P. massiliensis (Nereididae), sampled from a natural CO2 vent system and from a control site in three different periods (spring, fall and winter), were compared highlighting signals which suggested the ability of both species to acclimatize to high pCO2-low pH with slight seasonal variations of their antioxidant efficiency and the absence of disturbances of the oxidative status of *Platynereis spp.* tissues.

In another study, it has also been found that the ascidian *Ciona robusta* can be particularly resilient to OA. In this study, two experimental approaches were used, by conducting in situ transplant experiments at the CO2 vents of the Castello Aragonese (Ischia) and as well as microcosm experiments, to evaluate the short-term effects of the predicted near-future levels of OA on sperm quality after parental





exposure. After the first days of exposure to acidified conditions in both field and laboratory conditions, alterations on sperm motility, morphology and physiology were found, followed by a rapid recovery of physiological conditions that provide a new evidence of resilience of ascidian spermatozoa in response

to ocean acidification.

A new research line within Theme 1 concerns the study of immunity in Tunicata, with a main interest in the inflammatory response and the development of the immune memory, by means of multidisciplinary approaches and functional assays. The exposure of the thaliacean salp Thalia democratica to probiotics, and of the ascidian *C. robusta* to industrial pollution and to stimuli of bacterial origin, has allowed to i) characterize the immunocytes responsible for the inflammatory response in salps, important components of the gelatinous zooplankton, ii) evaluate the impact of resuspended heavy metals and hydrocarbons on host-microbiote interactions, innate immunity and gastrointestinal epithelial morphology in *C. robusta*, and iii) study the development and the expression (potentiation and tolerance) of immune memory in the pharynx of *C. robusta*.

Theme 1 research has also dealt with the mechanisms that regulate embryonic development. The approach of reconstructing Gene Regulatory Networks for the study of the development and evolution of body parts has been integrated with several "omics" technologies, including chromatin accessibility assays and single cell RNA-Seq, and applied to the digestive system and nervous system in sea urchins and sea stars.



The SZN has a strong tradition in the study of some marine organisms, such as the amphioxus *Branchiostoma lanceolatum*, a species described to belong to the chordate phylum for the first time in Naples in the early nineteenth century, with the contribution of the SZN. In amphioxus, three NOS (Nitric Oxide Synthase) genes were isolated by SZN researchers and characterized. The inhibition of their activity during embryonic development has highlighted a novel role of nitric oxide as a signaling molecule indispensable for the formation of embryonic head territories, such as the primary mouth. Ongoing research aims to discover if this is a feature typical of all chordates.

The current collaborations will work on two main areas:

- 1) Evolution of the chordate jaw and mouthparts. The development of the mouth in animals has fascinated researchers for decades, and recently researchers from the Stazione Zoologica have expanded their knowledge on the preserved features of the formation of the mouth in the chordates, testing the hypothesis that nitric oxide (NO) is a potential regulator of this development process in the European amphioxus *Branchiostoma lanceolatum*. In Japan we will work with embryos and larvae of the sea lamprey *Lethenteron japonicum*, a very important animal model because it belongs to the Agnatha (jawless fishes) that represent a very early evolutionary divergence from the line that produced the Gnathostomata (jawed vertebrates).
- 2) The evolution of circadian rhythms in chordates. The genetic mechanism that controls the circadian clock was studied in an initial version of a project to sequence the genome of the

hagfish *Eptatretus burgeri*. The hagfish is one of the most primitive extant vertebrates, and the study of the circadian clock and non-optical photoreception in this taxon is useful for understanding the evolution of the circadian timekeeping system and of photic entrainment.



The SZN also has a consolidated experience in ascidian studies. The SZN continues to contribute significantly to the study of the basic mechanisms that determine the onset of compartments in the nervous system of ascidia, which favours the understanding of the complex regionalization of the central nervous system in vertebrates. During 2019, mechanisms involved in the transcriptional regulation of genes essential for the development of the ascidian nervous system were studied. In addition, the function of regulatory elements, phylogenetically conserved among evolutionary distant species were studied. Finally, the molecular evolution of the Rab family of genes, implicated in vesicular traffic among organelles within cells, have been studied phylogenetically.

During 2019, another mollusc has been introduced as a laboratory model at Stazione Zoologica, the Mytilus *galloprovincialis*. Efficient protocols to obtain spawning from this mollusc were established with the aim of studying the role of DNA methylation during the development.



Theme 1 is also characterized by an important study of the nervous system from an evolutionary point of view. Research on the cephalopod mollusc Octopus vulgaris - along the long-standing tradition of the SZN - provided important contribution to the understanding neural regeneration phenomena such and the involvement cellular proliferation and reprogramming, also utilizing innovative imaging approaches. addition, studies have In contributed to: i) the analysis of



protocadherins distribution and contribution neural complexity in cephalopods; ii) studies of the

evolution of cognitive capacities of cephalopods, including asymmetry in behavioural responses and inter-individual differences; iii) the application of neural tracing to decipher neural connectome. The study of the mitochondrial genome (mitogenome) of the species belonging to the fish family Sparidae, commonly called sea breams and porgies, represents a new research line that embraces both basic (genome evolution, taxonomy) and applied aspects (mtDNA extraction, new barcode markers). The complete sequences of the mitogenomes of Dentex dentex, Dentex gibbosus, Dentex angolensis, Pagellus acarne e Pagellus erythrinus have been published; moreover, interspecific analysis of sparid mitogenomes allowed to identify a region within the gene NAD5 showing a higher discriminatory potential compared to classical barcodes like COI e CytB.

Through comparative transcriptomic approaches it has been possible to reconstruct the evolutionary history of genes coding for cytolytic and antihemostatic proteins in the Mediterranean hematophagous gastropod *Cumia reticulata*, highlighting the adaptive role of such proteins in maintaining such a peculiar trophic strategy.



Theme 2: Multiscale marine biodiversity

The diversity of the marine biota, or marine biodiversity, is one of the main topics on which research activities were focused in the last twenty years mainly due to its gradual loss linked to the direct or indirect anthropogenic impact. For its characterization, protection and analysis, many initiatives are underway and one of the recent European directives, the Marine Strategy Framework Directive (MSFD), considered it one of the most important descriptors for the determination of Good Environmental Status (GES) and placed its conservation as one of the main objectives to be achieved by 2020. The contribution of the SZN in this area is of considerable importance thanks to its traditional taxonomic expertise enriched by the introduction and use of the most modern approaches, including imaging and molecular approaches, and constant interaction with the research conducted within the other themes, focusing on the characterization of biodiversity of the single organism at the functional level (Theme 1) or of its role in an ecosystem (Theme3). The results should be considered as an integrated study of complementary research developed within the various Themes, focusing mainly on the distribution of organisms and their spatial and temporal variability, aiming at the integrated characterization of species, morphotypes or genotypes.



The quantification of the diversity of planktonic organisms, which represent one of the most important components of marine ecosystems, derives from the sequence analysis of specific target regions, located both in coding regions and in apparently non-coding regions of the DNA. These analyses are conducted on individual species or on DNA or RNA extracted from natural populations. In the second case we deal with metagenomics or metatranscriptomics. This approach makes it possible to discriminate the diversity of entire natural populations, and is linked to the availability of reference sequences, which allow us to assign the individual sequences to a specific genotype and/or morphotype. The activity in this field is continuous and only through the accumulation of material and sequences it is possible to bridge the gap in the existing knowledge. Important results have been obtained at specific sites, such as the Venice Lagoon and Gulf of Venice (Adriatic Sea), as well at the global scale in the framework of the Tara Oceans project, in which the SZN has a prominent role: among the recent achievements is the assessment of global diversity patterns across taxa inhabiting the epipelagic zone of the global ocean and the prediction of their possible trends under climatic variations. The OceanObs meeting that took place in autumn 2019 was the chance for several efforts of the international Scientific Community towards the definition of the elements of the Global Ocean

Observing System. In this context the experience gained through LTER observations in the pelagic system of the Gulf of Naples allowed to contribute to the international discussion on the integrated approach to coastal and biological observations, recognizing the fundamental role of the LTER research in the coastal ocean and moving forwards towards the development of the next generation of Marine Observatories for science and society, in strict connection with "augmented observatories, such as the NEREA one (see highlights).

To enhance the value and increase the comparability of the precious biogeochemical dataset collected at the LTER-MC site over more than 30 ys, a step-wise quality control procedure has been designed. The procedure consists of 9 analytical steps merging statistical tests and expert judgement, and has allowed to characterize the quality of ~ 84,000 data-points, assigning quality flags to individual data. These results contribute to bridge the gap between the need of objective QC criteria and the intrinsic noise of coastal datasets, promoting the discussion on this topic, and improving proper management and sharing of coastal data.

Biodiversity studies in benthic systems have been conducted in Mediterranean and other many geographical areas (e.g., strait of Gibraltar, Hawai'i, Vietnam, Panamá, Arctic and extreme environments including hydrothermal vents and seamounts). The results obtained include:

studies on molluscs, with the review of two groups of species and the description of four new species for science, one of which from the Gulf of Naples; the analysis on the species-specificity of the mollusc fauna associated with different algae species of *Cystoseira simpatriche* among them in Ischia; the review of the species of molluscs described by the malacologist Raffaello Bellini; of the alien species living in the Italian seas and finally of the protocols for the assessment of the impact of alien species in the European context. Knowledge on distribution areal of numerous alien species has been increased through specific research projects and approaches based on citizen science;



- studies data on Mediterranean gorgonians, Mediterranean mesopelagic and large pelagic fish, organisms particularly important because they structure the environment, on the Antarctic continental shelf, on the diversity of crustaceans of rocky bottoms of the Egyptian coast, on meiofauna populations in Nisida, in the Gulf of Naples, on the variations of the infralittoral communities in Liguria, on benthic communities associated to hydrothermal vents (e.g Panarea), and seamounts (Palinuro Seamount).
- studies, on the diversity and distribution of sponges and gastropods of tropical environments, hotspots of diversity, on the description of new species of zoanthids and fish associated with cold water corals in the deep Atlantic Ocean, as well as of sponges from shallow tropical habitats and brackish waters.

Another aspect in the field of marine biodiversity has been explored through studies on marine parasitology. Parasitic species are extremely diverse, and often display complex life cycles that involve several hosts, serving as proxies to diagnose host populations' health status. Such cycles are enormously unknown, but are highly relevant for trophic webs and ecosystem functioning. Parasites are key in controlling local host populations, but the introduction of alien parasitic species, instead, may cause devastating effects on the community structure. International market exchange, degeneration of natural habitats and climate change are major drivers of such events. Indeed, a number of our studies increasingly report cases of allochthonous parasites in our coasts, in particular in species from commercial fisheries.

Overall, research has confirmed that a large proportion of biodiversity is still unknown with respect to ecologically important and relatively well studied groups. The strategy for a real advancement of knowledge includes the integration of classic methods with more modern methods, and expanding searches to different habitats, with a particular focus on those of less explored deep waters. These research activities will certainly produce long-term results and will allow in the short term to lay the necessary foundations to answer fundamental questions about the evolution and logic of living beings.

Theme 3: Integrative approach to environmental research

This is one of the most complex research themes, since it is the framework in which biology, diversity, evolution and interaction of organisms take place. Moreover, this theme should provide the knowledge to address the management strategies for the marine environment as well as the basis for the exploitation of the biotechnological potential of marine organisms (fully explained in Theme 4).

Studies deal with various ecosystems and levels of their biological organization, spanning from the habitat distribution along the Italian coast to the distribution of organisms among habitats to the impact of environmental changes on species distributions to the interactions favouring their persistence, to the structure of communities and trophic webs and their changes when exposed to drivers of change.

The SZN continued the research on the ecology of habitats of priority interest, including studies aimed at understanding the feasibility of restoration actions, as in the case of *Posidonia oceanica* meadows, as well as studies aiming at assessing the effects of anthropogenic stress on valuable habitats, such as the endemic Mediterranean reefs of the coral *Astroides calycularis*, also aiming at better planning management actions for these habitats.



Environmental shifts induce modifications in species behaviours that can propagate at community and ecosystem levels. As species are interconnected by several kinds of biological relationships, most of which are antagonistic (e.g. predator-prey relationships and allelopathic interference), the amplitude of community-level responses depends from the way interaction/ecological networks modify in response to those shifts. In addition, due to the intricacy of these networks, systemic changes emerge in consequence of both direct (proximate, species-species) and indirect (remote, species-distant neighbours) effects. A number of studies have been carried out at SZN to characterize community-level responses using network approaches, at both time and spatial scales.

A common property of antagonistic networks is the subdivision of the community in different subcommunities, or modules, including smaller groups of species interacting more frequently. This depends from the fractal nature of ecological communities, which are "communities of communities" - e.g. marine communities are made of different compartments, such as plankton, nekton, and benthos, and these are in turn subdivided in many lower compartments, down to species level. In the course of

SZN investigations, modularity changes have been tracked in marine communities at different hierarchical levels.

For instance, modularity reshuffling in planktonic communities in response to intermittent nutrient inputs in the coastal Gulf of Naples and iron-fertilization in the open Ocean were related with the rewiring of food webs and gene-regulatory networks, respectively; furthermore, strong modifications of the overall modular structure of the phytoplankton communities across time and space scales were related to the differential release of oxylipins by the planktonic diatom genus Pseudo-nitzschia, which appears to interfere with other species by means of allelopathy. Network approaches allow us to integrate biodiversity, community structure and ecosystem functioning, and are



also useful to characterize the ecological status of communities in impacted vs- pristine environments. To this latter purpose, these approaches were more recently applied by SZN scientists also to assess the stability of benthic communities, from bacteria in the sediments to demersal fish, in the Bay of Bagnoli (AbBaCo project), across a gradient of pollution. Finally, network approaches offer useful tools to integrate the information generated by research in the other Themes and will be employed in the frame of integrative analysis targeting data generated by the augmented observatory NEREA (see highlights).



Research on symbiotic interactions, parasitism or antagonism is at the forefront of marine research aiming at a better understanding of ecosystem functioning, and a field in which SZN has played a pioneering role. The most recent studies at SZN have focused on the role of symbiotic associations in nutrient cycles, and in particular in the nitrogen cycle. Research has shown how a chemosynthetic symbiosis between lucinid bivalves and sulphur-oxidizing bacteria, which use chemical energy to fix carbon, also actively carry out the process of Nitrogen fixation in Posidonia oceanica sediments. In particular, experiments with isotopic tracers have highlighted how these symbioses can contribute substantial amounts of ammonium to the ecosystem. Given the plant's preference for this form of nitrogen, the contribution of lucinid bivalves can be important to increase the productivity of these important marine ecosystems. In another study of this kind, combining molecular techniques and biogeochemical assays, it has been demonstrated that some insect larvae (Chironomus plumosus) that burrow into aquatic sediments, besides creating oxidized and ammonium-rich micro-niches through their bioturbation activity, play the role of hosts for complex microbial communities involved in the Nitrogen cycle. Worth mentioning are also the results obtained about the mechanisms of interaction between pathogens of the digestive system and metazoans as well as those related to the chemical ecology, i.e. chemical defences, of e.g. Antarctic bryozoans. Finally, other important research has been carried out on the toxic effects produced by substances synthesized by marine organisms. This latter category includes the study of oxylipins and derived products synthetized by diatoms. New results showed that these toxins exert effects on the sea urchin as well as on bacteria, and maps of molecular response for these organisms have been depicted.

Manipulative experiments in naturally stressed environments, such as geothermal vents, allowed the prediction of response of ecosystems to the interplay of several drivers of change. This is particularly

important, since global change other than causing continuous temperature increase, causes variation of atmospheric events and increase in their power. Changes in abundance and structures of marine communities exposed to acid vents and alien invasions were assessed. Opportunities offered by the numerical modelling of marine ecosystems should be also considered. This research field can now profit from information deriving from "omics". Notwithstanding, this appears a promising field, it is not easy to fully profit from such an opportunity since models are not properly implemented yet and the functional information provided by metagenomics and metatranscriptomics is not fine enough. This approach is however part of the exploration of ecological modelling of SZN.



Theme 4: Sustainable exploration of marine biotic resources

In recent years there has been a growing interest in the potential of the oceans to contribute to sustainable economic growth, which goes by the name of Blue Growth. Among the various sectors that are considered relevant, one is undoubtedly that of blue biotechnology, which aims to explore marine organisms in order to discover new compounds with properties that allow them to be used in the nutraceuticals, pharmaceuticals and cosmeceutical industries or inspiring the synthesis of new molecules. The development of this sector is favoured by the high biodiversity of marine ecosystems that offers multiple opportunities for the search for new products, as demonstrated by the growing number of therapeutic agents of marine origin currently in clinical use for cancer treatment and pain management.

The use of marine organisms and communities to address problems of environmental pollution arising from anthropic impact falls within another field of marine biotechnology, known as environmental biotechnology. Several biotechnological approaches have been tested and developed to study environmental damage and test interventions of bioremediation in contaminated sites of national interest identified as priorities for reclamation interventions, such as the Sites of National Interest (SIN) Bagnoli-Coroglio (due to the dismission of a steel factory) on the Tyrrhenian coast and the SIN ex Montedison (former chemical industry) of Falconara Marittima on the Adriatic coast. These sites are ideal models for testing and validating new environmental intervention technologies based on bioremediation strategies through use of marine microbes and their products with specific removal actions towards organic and inorganic pollutants, biostimulation processes (stimulation of autochthonous microbes) and bioaugmentation (use of non-native microbial biomass). These biotechnologies have particular relevance for their eco-compatibility, efficiency in reducing the degree of contamination and versatility of use for different types of contaminants and in different environmental contexts.

Many of the studies for sustainable exploration of marine biological resources have been carried out on microalgae which, like other microorganisms, have received little attention despite representing a potential goldmine for the discovery of new molecules with biological activity. Microalgae are a potentially continuous source of compounds useful for human health, having the advantage of being suitable for cultivation even on a large industrial scale. To fully exploit this resource, it is necessary to optimize production costs and cultivation methods. For example, recent studies at the SZN have shown how the growth rate and the production of polyphenols, carotenoids and vitamins C, and therefore the antioxidant activity of the diatom *Skeletonema marinoi* are modulated by the light spectrum, the intensity of light, the distribution of light throughout the day, and the concentration of some nutrients, combined with the method used for mixing and oxygenation of microalgal cultures. These results have important implications for maximization of algal production and the design of large-scale algal growth bioreactors.

Recent results have identified antitumor activity in an extract of the dinoflagellate *Alexandrium minutum*, which induces death by mitophagy in lung cancer cell lines without inducing toxicity towards somatic lines of the same tissue derivation. Previous research also identified the anti-cancer activity of benthic diatoms against various solid tumours and the search for a very selective apoptogenic compound produced by diatoms of the genus Cocconeis is still active in cooperation with various international partners, also within funded projects.



Amphidinol 22 planar structure (From Martinez et al., 2019 Marine Drugs)

Recent studies on microalgae led to the identification of a new compound from the dinoflagellate *Amphidinium carterae*. The compound named Amphidinol 22 showed antibacterial and antiproliferative activity on human cells.

Monoacylglycerides produced by the diatom *Skeletonema marinoi* showed selective cytotoxic activity against haematological cancer cell line and colon cancer cell compared to normal cells. These compounds also have anti-inflammatory activity and represent a promising class of bioactive molecules for the development of new therapeutic agents.

The study of microalgae and their products was also carried out using transcriptomic approaches to identify enzymes with biotechnological applications. Recent results have shown the presence, in the flagellated *Tetraselmis suecica*, of enzymes involved in the synthesis of secondary metabolites with high biological activity (polyketide synthase and lipoxygenase) and the enzyme nitrilase, which may be involved in the degradation of toxic nitriles.

With the same approach, we showed the occurrence in the transcriptome of the diatom *Thalassiosira rotula* of the biosynthetic pathway involved in the synthesis of Secologanin, a molecule involved in a complex metabolic pathway leading to the synthesis of Monoterpenoid Indole Alkaloids (MIAs), a large class of chemically different bioactive molecules considered very attractive for the pharmaceutical industry, due to their activity against cancer and other severe diseases in humans.

Polyketide synthase enzymes are also likely to be involved in the biosynthesis of long chain diols, that are the building blocks of biopolymers, known as algaenans, making up the outer layer of Nannochloropsis cell wall. Culturing manipulations combined with stable isotope labelling experiments and transcriptome sequencing predicted indeed that a polyketide synthase and a fatty acid elongase enzymes are involved in the biosynthesis of long chain hydroxy fatty acids. Long chain diols would then be formed after the reduction of the carboxylic group in long chain fatty acids.



Putative pathways for the biosynthesis of long chain hydroxy fatty acids, long chain alkenols and long chain diols in Nannochloropsis oceanicaCCMP1779. Lipids known to be present in Nannochloropsis Spp. are in bold, whereas those that have been predicted are in red (from Balzano et al. 2019, doi:10.1093/pcp/pcz078).

The growth of the Department in terms of research staff has introduced new lines of research and expertise in the biotechnology field. The planned goals for the three-year period will be achieved by extending research on specialized bacterial communities, and their exploitation for bioprospecting purposes. In details, new expertise will be spent in the discovery of novel bacteria as producers of molecules with antibacterial, antibiofilm, antiviral and bioremediative activity. Great insights are envisaged in the search for new biosurfactant producers, from temperate and cold environments. Biosurfactant and exopolysaccharides are gaining increasing interest because of their high specificity of action and environmental-friendly character. Nevertheless, stronger efforts are necessary for the optimization of production processes, with low costs and high yield. For this reason, we aim at extending the research activities to underexplored sources for the isolation of new producers. Particular interest is focused in the association of bacteria with marine invertebrates, and on hydrocarbon-oxidizing and heavy-metal chelating microorganisms. Indeed, the bacterial fraction associated with higher organisms in marine environments has been recently evidenced as a really promising hotspot for bioprospecting researchers. The search in extreme polar environments is also particularly targeted, as cold-adapted bacteria have evolved special metabolic and physiological adaptation strategies to

cope with the harsh conditions, thus potentially corresponding to a wide chemo-diversity in terms of structurally and functionally novel biologically active molecules. Several researchers and technologists of the Department of Marine Biotechnology claim specific competences for carrying out such types of investigation, ranging from culture-dependent and -independent procedures, to the introduction of appropriate screening procedures, the application of OSMAC ("one strain many compounds") approach, biosurfactant extraction techniques and chemical characterization.

SZN expertise has also been applied to improving the efficiency of aquaculture of the sea urchin *Paracentrotus lividus*. The effect on *P. lividus* of four benthic diatoms, *Nanofrustulum shiloi*, *Cylindrotheca closterium*, *Diploneis sp.*, and *Cocconeis scutellum*, isolated from the leaves of *Posidonia oceanica*, was studied for the first time. Combining morphological and metabolomic approaches and transcriptomics, a noxious effect was demonstrated on the embryos generated by female sea urchins fed with these benthic diatoms, with the only exception of C. scutellum, which did not induce any morphological effect. Chemical analysis revealed that *C. closterium*, *N. shiloi*, and *C. scutellum* produced several polyunsaturated aldehydes and linear oxygenated fatty acids (LOFAs) related to the products of LOX pathways in planktonic species.

This work also included some preliminary experiments to evaluate the effects and the daily quantity consumed by *P. lividus* of two foods, the seagrass *Posidonia oceanica* and the green algae *Ulva rigida*. Feeding for three months with *U. rigida* and *P. oceanica* had no effect on growth or reproduction of sea urchin adults. In contrast, the diet based on commercial pellets caused a significant increase in the gonadal index, with a lack of gamete production, due to a follicular hypertrophy. The relationships between benthic diatoms and invertebrates have been studied using *P. lividus* and other species.

Chemical ecology can explain plant-animal relationships.

Acidification has also physiological effects on aquatic organisms, including marine phanerogams and coralligenous organisms. Such effects can be more deleterious than direct effects that can be easily demonstrated on the physiology of calcifying organisms. Equally, the presence of some cyanobacteria on *Posidonia oceanica* leaves, ascribable to a species that had been considered only as a symbiont of various invertebrates, shows how organisms can colonize very different environments due to the production of numerous chemical "weapons".

In addition, direct and indirect effects of acidification (O.A.) may represent means to identify newer biotechnological applications and, contemporaneously, they represent environmental issues requiring biotechnological approaches for restoration. Thus, O.A. should be considered both as a target for new biotechnologies and a source of new applications.



4.2 HOT TOPICS AND RESEARCH HIGHLIGHTS 2019

The Stazione Zoologica contributes to dissemination of scientific knowledge also through its webpage. In the Homepage highlights provide an insight of some significant results described in scientific publications considered to potentially have a high scientific impact. These are described in the "Hot Topics" section as a vehicle for this dissemination activity. Hot topics also provide visibility to the tangible outputs and activities carried out by students, doctoral candidates and postdocs.

AUGMENTED OBSERVATORIES - Integrative approaches to the study of marine ecosystems

The Sea hosts priceless biodiversity providing humans with a multitude of goods and services. Assessing the ecological status of the sea is fundamental to face actual social-economic issues, as marine ecosystems are threatened by multiple human activities. In this context, addressing pressing threats requires "augmenting" the observation of the Sea using integrative approaches and, possibly, link these to well-established monitoring structures, such as Long-Term Ecological Researches (LTER).

Building on these needs, the Naples Ecological REsearch for Augmented observatories (NEREA), a multidisciplinary and integrative observatory of the water column, was established in the Gulf of Naples in February 2019, (coordinated by Casotti, Conversano, D'Alelio, and Iudicone). Inspired by the Tara Oceans project, an oceanographic expedition applying cutting-edge molecular techniques, morphological and environmental data for studying the huge variety of organisms present in marine microbiome, NEREA expanded the technical experience of the LTER MareChiara, a monitoring program established in 1984. NEREA's augmented observation is characterized by monthly sampling of plankton in the Gulf of Naples using both traditional (i.e. physical, chemical, and biological measurements) and innovative approaches (i.e. meta-omics, trace metals analysis, environmental DNA, etc.). NEREA aims to operate as an interdisciplinary structure, which involves the coordinated efforts of different operational units (oceanographers, biologists and chemists), from the experimental design phase and sampling activities that promote the data interoperability, to data analysis, to open access datasets.



This new conceptual, methodological and technological infrastructure is contributing to consolidate the relative weight of SZN at international level as pertaining to ocean observation and, therefore, it is attracting funds. The newly-funded project Atlantic ECOsystems Assessment, Forecasting & Sustainability (AtlantECO, 2020-2023, coordinated by SZN) will develop and apply a novel, unifying framework for providing knowledge-based resources to design policies, support decisions making and engage with citizens to encourage responsible behaviour to manage the Atlantic system and protect its Ecosystem Services (ES) provision. AtlantECO will determine the structure and function of Atlantic

microbiome in the context of ocean circulation and presence of pollutants, e.g., plastics, to assess its role in driving the dynamics of Atlantic ecosystems at basin and regional scales; its potential of being used as a sensor of ecosystem state and the mechanisms by which it drives the provision of ecosystem services. This is key to improve our predictions on future provision of ES in the basin and to favour the establishment of a sustainable Blue Growth strategy for an All-Atlantic community.



Conceptualisation of modelling workflow

Interdisciplinarity and ambitious challenges in the project DIsCO 'Diatom life cycles, molecular controls and contribution to ecosystem dynamics'

The single-celled organisms of plankton are the basis of marine trophic networks. Like multicellular plants and animals, these microscopic organisms have life cycles characterized by the possibility of growing rapidly, giving rise to blooms, of going through quiescent phases, through sexual reproduction or through sudden massive death events. Life cycles therefore play a fundamental role in the dynamics of the populations of these organisms. Thanks to a very generous contribution from the Gordon and Betty Moore Foundation, which funds cutting-edge research in marine biology, SZN researchers are studying the mechanisms that regulate the life cycle of a planktonic diatom, Pseudo-nitzschia multistriata, common in our seas and object of various studies in recent years. The DIsCO project (http://www.szn.it/index.php/it/ricerca/ecologia-marina-integrata/progetti-di-ricerca-emi/disco) is characterized by a marked interdisciplinarity and includes laboratory studies with advanced methods of functional genetics, genetics, bioinformatics and "single cell" approaches, studies at sea in the context of LTER-MC and NEREA, which include population genetics and genomics analyses, and a conceptual part to model genome evolution dynamics and species succession. Among the first results obtained, the indication that the process of sexual reproduction is linked to a control of the growth of the entire population represents an entry point into the molecular control of cell proliferation, and provides an additional element to be included in conceptual models on plankton dynamics.

These studies touch on basic aspects of diatom biology, from the regulation of gene expression, to transcriptional control, to signal transduction, and hopefully will provide valuable information for interpreting the 'invisible life' of diatoms in their natural environment.

Microplastics: a habitat in itself

Plastics are widespread in the marine environment where they accumulate at the surface, along the water column and at the seafloor. Once entering the ocean, plastic debris is fragmented by physical, chemical and biological factors into pieces milli-, micro- and nano- meters in size. At sea, microplastics (<5 mm) are fast colonized by a specialized microbial biofilm, the so-called "microbial plastisphere", that can affect their properties, behaviour and ultimately ecotoxicity. Due to their small size, comparable to those of sediments and some planktonic organisms, microplastics are, in fact, ingested by a wide range of marine organisms, thus negatively affecting several biological processes, including feeding, energy reserves, growth rates and reproduction. We investigate the abundance of microplastics at the surface of the ocean and along the water column with a focus on the microbial communities attached to the pieces, with the hypothesis that these communities represent vectors for diffusion and spreading of organisms (also potentially pathogens) and that their effect on consumers amplifies the effect of plastic alone, triggering stress response that strongly influence their health and value as food for higher trophic levels, including humans. To do so, we use the sea urchin Paracentrotus lividus as a model species, also for its commercial value as food (the gonads). In addition, the sea urchin is a model for the immunological responses as its sentinel cells, the coelomocytes, have proven to be key elements in the response to microplastics, as shown by morphological analysis and oxidative status monitoring (Murano et al. 2020 Environ Pollut). The SZN Flagship Project MicroMARE (PI R. Casotti and A. Palumbo), the JPI Oceans project MicroPlastiX (PI R. Casotti, with MG. Mazzocchi and D. Iudicone) and several other initiatives, including 2 PhD projects (C. Murano and V. Donnarumma) have supported these studies, also in collaboration with I. Corsi of the University of Siena, M. Sprovieri and F. D'Agostino of CNR-IA, L. Amaral Zettler and E.R. Zettler of NIOZ (NL).

Not venom acquisition but conquest of the depths drove terebrid diversification.

Terebridae are a large family of marine gastropods, collected by amateurs for their slender, elegant shells. Not many people are aware that these animals are able to inject a potent mixture of neurotoxins into their preys (polychaete worms). Could the onset of a venom apparatus have induced Terebridae diversification, as it happened in other groups of venomous organisms? To answer this question, an international research team reconstructed a large time-calibrated phylogeny of Terebridae. In parallel, data were collected on a number of morphological, functional and ecological traits, to evaluate their potential impact on the diversification dynamics of the family through a phylogenetic comparative approach (Modica et al., 2019, 10.1093/sysbio/syz059). The obtained results indicate that Terebridae have originated about 50 mya and are presently represented by ca 600 species, organized into at least 11 lineages. The diversification rate began to increase in the whole family about 25 mya. However, no differences were detected between the clades equipped with a venom apparatus and the numerous clades that lack it. Instead, a concurrent increase of the bathymetric range occupied by the Terebridae was observed. Additionally, the higher diversification rate was due to the decrease of the extinction rate beginning in the Eocene. It was thus possible hypothesizing that terebridae, after their origin in shallow water, progressively adapted to deeper environments following the rise of sea level in the early Eocene that made available a high number of new potential habitats. The consequent release of competitive pressure would have lowered the extinction rate. This is the first study where a multifactorial macroevolutionary analysis was applied to venomous marine invertebrates.



The molecular mechanisms that allow the sea urchin to live in the future ocean acidification scenarios

The sea urchin, a key organism for the functioning of coastal vegetated ecosystems, is present in the CO₂ volcanic vents, naturally acidified systems occurring off the island of Ischia (Castello Aragonese). In particular, they can thrive in the areas where the emissions cause a lowering the pH to around 7.8, a value comparable to the future acidification conditions of the oceans expected for the end of the century, compared to the normal value of the control areas (8.0). Through the study of both physiological parameters, such as respiration, nitrogen excretion and mineralogy of the skeleton, and of the immune response at the level of the coelomocytes of animals residing in the acidified and in the control areas, the molecular mechanisms allowing sea urchins to live in conditions with reduced pH, have been outlined (Migliaccio et al., 2019. Science of the Total Environment 672, 938–950). The coelomocytes, the sentinel cells of the sea urchin, have proven to be key elements in the response to acidification stress. These peculiar cells have been examined using different approaches, such as morphology, biochemistry and proteomics. The identification of the proteins expressed differentially in the coelomocytes of the animals of the acidified area have allowed, together with biochemical assays, to reveal that the sea urchins adapt to live at lower pH values by modulating the metabolism of the



immune system with an increase in defense mechanisms and in particular antioxidant processes. This study reveals a high plasticity of the sea urchin' immune system that will allow this species to overcome the climate changes related to future ocean acidification scenarios.

First evidence of ascidia sperm resilience to ocean acidification

By the end of this century, it is predicted a drop of oceans pH value to 7.8. To determine whether the

ocean acidification affects the gamete quality and the reproductive fitness of marine animals, adults of the ascidian Ciona robusta were exposed for a shortterm period (7 days) to acidified conditions in microcosm and in the naturally acidified area of the Ischia island. A set of sperm quality parameters underlying fertilization competence and offspring quality evaluated were as toxicity endpoint. It has been highlighted that acidification induces а significant alteration of motility, morphology and sperm function in the first days of exposure followed by a rapid recovery of physiological conditions. This first evidence of the resilience of ascidian sperm to acidification stress opens a new scenario on the ability of marine species to adapt and reproduce in adverse environmental conditions. (Gallo et al., 2019, Science of the Total Environment, 697,134100).



THE PROJECT LIFE ELIFE - Elasmobranch Low Impact Fishing Experience - LIFE18 NAT/IT/000846

Since the mid-1980s, chondrichthyans (cartilaginous fish) have been under growing pressure from fishing in the Mediterranean Sea mainly as a result of incidental by-catch. Despite some recent efforts to reduce shark by-catch and mortality (i.e. adoption of the European Commission's Action plan for the Conservation and Management of Sharks), the EU goals are far from being achieved.

The 5-years project LIFE ELIFE (https://www.elifeproject.eu/), leaded by the SZN and funded by the LIFE Programme of the European Commission (https://ec.europa.eu/easme/en/life) aims at improving the conservation of elasmobranch species (sharks and rays) by promoting best conservation practices in EU professional fishing in the Mediterranean Sea, including both bottom trawl and longline fishing. The project will allow pilot and demonstrative actions for turning commercial fishers towards low-impact fishing devices that decrease elasmobranch by-catch and it will be carried out by-catch in the some of the most important Italian harbours (like Chioggia, Gallipoli, Lampedusa, Cirò Marina, Porto Cesareo, Marsala, Mazara del Vallo, Lampedusa), some Italian MPAs, and also in some relevant areas of Cyprus and Greece. Additionally, the project will support marine management authorities in Cyprus, Greece and Italy by providing the latest data for assessing the status of shark species and will support fishers in fund raising activities (e.g. grant applications) to adopt sustainable practices. Mediterranean fishers will therefore enhance their role in marine biodiversity conservation.



The project also aims to transfer good practices for shark by-catch mitigation and lowering mortality to other Mediterranean countries and to increase the awareness of people and stakeholders about the value vulnerability and of the elasmobranchs; thus promoting a more sustainable fishing and responsible fish consumption. In the picture: Specimen of blue shark, Prionace glauca, common bycatch during the pelagic professional fishery (©Alberto Luca Recchi).

LIFE ELIFE will contribute to achieving the European Commission goals through conservation actions on shark species listed in the European Red List of marine fishes (IUCN, 2015), in accordance with the EU Action Plan for the Conservation and Management of Sharks; the aims of the EU Marine Strategy Framework Directive (in particular, Descriptor 1 biodiversity and Descriptor 3 fishery); the European Biodiversity Strategy to 2020; and of the Regulations 1380/2013 and 72/2016. The project is also consistent with the 7th EU Environment Action Programme, the Birds Directive and the Habitats Directive, and several international conventions on marine conservation, biodiversity and migratory species. The ELIFE project has started in October 2019 and it will continue until October 2024: it is coordinated by the SZN, with the collaboration of Italian National Research Council, University of Padua, Marine Protected Area of Pelagie Islands, Marine Protected Area of Tavolara-Punta Coda Cavallo, Mer Research Ltd., Mediterraneum Consortium, Legambiente, Costa Edutainment and Softeco Sismat, and with the endorsement of the Italian Ministry of Environment, the Italian Fishery Direction, the Greek Department of Fisheries-Aquaculture Technology, the EU-MEDAC and many others international public bodies and stakeholders.

The role of epigenetic mechanisms during marine organisms development and adaptation/acclimation to environmental changes

The ocean global change biology is a multidisciplinary field whose major research goal is to better understand the processes that might be involved in supporting organismal resistance to environmental changes. Phenotypic plasticity has emerged as an important area of study as it can occur on ecological rather than evolutionary time scales. Among various mechanisms responsible for supporting phenotypic plasticity in marine organisms, epigenetic modifications affecting gene expression often enable rapid (and eventually heritable) reactions to environmental challenges.

Accumulating evidence clearly established that epigenetic alterations consist of complex regulatory pathways (including DNA methylation, histone modifications and micro-RNA mediating gene silencing) that modulate gene expression changes independently of DNA sequence alterations. Importantly, current evidence suggests these modifications also include heritable changes, which, besides having a key role in many developmental and ph Environmental/ecological epigenetics can be applied to many critical issues in marine research such as aquaculture, biomonitoring, biological invasions, and conservation, therefore providing new insights into how marine taxa react to global climate change and local disturbances. Very recently, marine epigenetic studies started to be a hot topic for the Stazione Zoologica, focusing their attention mostly on the role of DNA methylation in marine plants (i.e. *Posidonia oceanica* and *Cymodocea nodosa*) and the mollusc *Mytilus galloprovincialis*. The ultimate goal of these two research lines is to determine the mechanistic basis of DNA methylation in the species of interest during their development and to investigate epigenetic changes following environmental changes, which can support long-term adaptation of marine organisms.

Posidonia oceanica

Recent works in seagrasses have proven global DNA methylation level changes depending on leaf age. Yet, whole genome hyper-methylation was generally observed following stress events, as for instance after heat stress and low-light exposure, possibly as a way to down-regulate the expression of the transcriptome slowing down the overall metabolism, which would allow to save energy needed to overcome the temporary challenge. Differences in DNA methylation level and in the activation of genes



involved in epigenetic regulative pathways were also detected among seagrass populations extending along depth and latitudinal gradients. The role of epigenetic mechanisms in regulating the expression of key genes related to flowering and stress Ρ. tolerance in oceanica was also highlighted as well as the first evidence of the relationships between intragenic methylation and flexible expression of specific genes across latitudinal gradients and under warming conditions obtained from in silico transcriptome analysis of normalized CpG content (CpGO/E ratio). Current works are exploring the role played by epigenetic mechanisms in seagrass stress memory and how epi-transcriptomic mechanisms based on RNA methylation could influence/regulate seagrass circadian rhythms.



Mytilus galloprovincialis

The pattern of DNA methylation changes during development and embryogenesis. As a consequence, differentiated cells develop a stable and unique DNA methylation pattern that regulates tissue-specific gene transcription: a critical mechanism for development. DNA methylation however remains poorly investigated in molluscs. To shed light on this process, we screened the genome of the mollusc *M. galloprovincialis*, and we first employed database search, detecting three major groups of DNMT enzymes, three MBD members and one TET protein, which were subject to phylogenetic studies and protein domain characterization. We then investigated the expression of these genes both during development and in different adult tissues together with the dynamics of global DNA methylation levels during embryogenesis. Finally, we found that embryos treated with a specific DNMT1 inhibitor, 5-aza-cytidine, display delayed or arrested development: a likely consequence of altered DNA methylation. Our findings, therefore, provide a first insight into epigenetic mechanisms responsible for proper development of *M. galloprovincialis*. Since epigenetic mechanisms are important in promoting phenotypic plasticity, we think that our data may help improving the efficiency of *M. galloprovincialis* husbandry, one of the most important aquaculture resources worldwide, and more generally of other Lophotrochozoans.



The evolutionary landscape of the Rab family in chordates

Intracellular traffic amongst organelles represents a key feature for eukaryotes evolution and animal diversification, and is orchestrated principally by members of Rab family, the largest within Ras superfamily. Rabs are involved in a myriad of basic biological processes, being key regulators of membrane transport and vesicular trafficking in the cellular endomembrane system. The work of
Coppola et al published in Cellular and Molecular Life Sciences in 2019 (76: 4117–4130) provided the most exhaustive survey regarding the Rab toolkit of chordates, revealing the existence of 42 metazoan conserved subfamilies from cnidarians to vertebrates and we also proposed a new Rab classification based on phylogenetic analysis. The Rab complement of chordates showed a dramatic diversification due to genome duplications (known as R), as well as lineage-specific gene duplications and losses with sharp differences among cephalochordates, tunicates and gnathostomes (vertebrates). Therefore, our data depicted a scenario in which chordates represent a hotspot of Rab variability, with many events of gene gains and losses impacting intracellular traffic capabilities.

The octopus, researching the origins of animal intelligence

The study of the evolution of intelligence among large-brained vertebrates –corvids among birds; cetaceans and primates among mammals – has suggested that complex cognitive abilities emerged multiple times and independently over the course of evolution. At the basis of intelligence – as we see it today – are the capabilities to: i) engage in stable and enduring social bonds ii) solve complex foraging problems. In addition to this, there is a slow life history, i.e. long average life and prolonged care of offspring. Recent studies on some species of cephalopod molluscs challenge the view that these three factors are essential for intelligence to evolve. Cephalopods have, in fact, evolved a large brain supporting extremely sophisticated behavioural repertoires despite they have fast life histories (e.g. most species live 1-2 years) and do not establish long-lasting social bonds. SZN researchers have developed a comparative analysis of the cephalopod (octopus) brain, nervous system plasticity and cognitive abilities. In some publications in Trends in Ecology and Evolution, Frontiers in Physiology and JEB, the authors suggested that a. it is possible to identify clear functional analogies between mammalian brain areas (primates and cetaceans) and that of the octopus; b. cephalopod cognitive

abilities have been favoured by evolutionary pressures related to the loss of the shell, the emergence of flexible mating strategies, fast life histories, but sophisticated communication skills between individuals; c. the animals are able to compensate for losses due to accidental damage, not only through the nerve regeneration ability, but also thanks to the marked plasticity of the central nervous system that allows the reactivation of the function through alternation of the nervous circuit and its compensation (sprouting).



Uncovering the mechanisms of decentralized vision in sea urchins

In 2019, a small consortium led by the molecular marine biologist Arnone from the Stazione Zoologica Anton Dohrn was able to secure funding from the prestigious "Human Frontier Science Program Organization (HFSPO)" for a three year project to study the architecture of the sea urchin nervous system to understand its information processing underlying the visually guided behaviour of the animal.

Sea urchins have no eyes, yet they can respond to light and accurately react to visual stimuli through photoreceptor cells distributed across numerous "tube feet," which are small flexible appendages that allow them to move. The discovery that seaurchins photoreceptor may use cells distributed around their whole body for vision was made in 2011 when a team led by Maria Ina Arnone, working at Stazione Zoologica Anton Dohrn in Naples, Italy, characterized for the first-time photoreceptor cells in a sea urchin. The results of the 2011 paper, published in P.N.A.S., led to the speculation



that sea urchins must have a sort of decentralized visual system, which may work like a giant compound eye, that allows them to detect light stimuli. The question arose: how can the sea urchin, which possesses only a decentralized nervous system distributed along 5 principal nerves connected by a nerve ring around their mouth, integrate external light stimuli and respond to them?

To answer this question, an integral approach is required that combines molecular, morphological, electrophysiological, behavioral and computational studies. This is exactly what the international team of scientists led by Dr. Arnone plans to do. The team includes Prof. Dan-Eric Nilsson of Lund University in Sweden, an expert in animal vision; ecologist and morphologist Carsten Lüter of the Museum für Naturkunde in Berlin, Germany and Giancarlo La Camera, a computational neuroscientist of the State University of New York at Stony Brook, USA.

The ultimate goal of the project is to obtain a computational model of the decentralized "spherical" vision of the sea urchin from its molecular, morphological and physiological makeup.

Decentralized vision itself, using the sea urchin as a model organism, can prove useful beyond the biological realm, as it may lead to applications in the field of biomimetics. Potential biomimetic applications include robotic miniaturization, smart probes, and intelligent materials where dispersed light detectors control the properties of the material. It's too early to say how much we will learn from this venture, but the road is full of exciting possibilities.

Using chemical language to shape future marine health

Infochemicals are information-conveying chemicals and they dominate much of the underwater communication in biological systems. They influence the movement and behavior of organisms, the ecological interactions between and across populations, and the structure of marine food webs. However, relative to their terrestrial equivalents, the wider ecological and economic importance of marine infochemicals remains understudied and a concerted, cross-disciplinary effort is needed to reveal the full biotechnological potential of marine chemical ecology. In a recent paper (1) published in cooperation with ecologists from other SZN departments, current challenges have been highlighted with specific examples and suggest how research on chemical ecology of marine organisms could

provide opportunities for implementing new biotechnologies for "blue growth" and maintaining healthy marine ecosystems. In marine environments, species interactions that form the basis of food webs and shape ecosystem functioning are dependent on chemical communications, as demonstrated by the case of the shrimp *Hippolyte inermis* and its diatom foods. In particular, two distinct challenges to sustainable growth in marine aquaculture and maritime operations, and propose solutions that require major interdisciplinary efforts, the development of a strengthened knowledge base, improved innovation and predictive capacity, and adaptive management plans for sustainable use of marine resources and their biotechnological exploitation. The enormous diversity of very small organisms (sub-millimetre sizes) that form the basis of the marine food web strongly influences carbon and nutrient cycling, and provides the scaffold for stable, healthy ecosystems and fisheries. Various challenges, as sustainable aquaculture, marine biofilms, biotechnological exploitation of the "chemical language", are sketched as possible approaches to the sustainable use of provisional services provided by the sea.



The role of infochemical gradients (indicated by the diffused red shading emanating from the point sources) in guiding marine interactions among living organisms and with non-living substrates. (a) Parasites on farmed salmon: salmon release infochemicals that enable host finding in parasitic sea lice. (b) Cell-to-cell communication: quorum sensing using, for example, N-acyl homo- serine lactone (AHL) in bacteria, results in settlement and biofilm formation. (c) Biofouling: ship hulls, oil rigs, and wind farm turbine foundations are prone to fouling that can cause detrimental biocorrosion. (d) Epibiosis on farmed seaweeds: micro- and macrofouling by bacteria and filamentous green algae is triggered by infochemicals. (e) Larval settlement: larvae of oysters, mussels, and corals are attracted to infochemicals from conspecifics, as well as specific flora and fauna living on the seafloor surface, leading to gregarious larval settlement. Persistent chemical gradients act as a directional cue to these colonizing organisms. (Da Saha et al., 2019. Frontiers in Ecology and the environment. IF= 10.94).

Effects of a Simulated Acute Oil Spillage on Polar Bacterial Communities

The cold-adapted microbial communities arouse great scientific interest for the peculiar adaptive strategies which have evolved to face extreme environmental conditions. The study has explored the effects of hydrocarbon addition on bacterial communities of Arctic and Antarctic sediment by simulating an acute oil spill in microcosms. The results evidenced differences in the biodegradation patterns between the two polar communities, with a biodegradation potential up to 100% for longer chain hydrocarbons. The bacterial communities' evolution in terms of diversity and abundance profile was similar. Indeed, both communities showed an initial limited microbial growth, in the times immediately following the contaminant addition, and a growth-stimulating effect afterwards. This finding is presumably due to the occurrence of first biodegradative processes with appearance of chemical intermediates which contributed to the development of more active oil degrading consortia. The study revealed the appearance of specialized phylotypes in dependence on the carbon source (crude oil or diesel oil) and the sediment origin (Arctic or Antarctic). Interestingly, some bacterial species, e.g. Microbulbifer, Thiobacillus, and Novosphingobium were never reported in a cold environment for hydrocarbon degradation. The study underlined the higher potential of cold-adapted bacteria, thus providing a possible way for novel biotechnological applications in bioremediation processes (Microorganisms 2019, 7, 632; doi:10.3390/microorganisms 7120632).



Venn diagrams showing phylotypes distribution in Arctic and Antarctic microcosms.

Microalgae as a sustainable source of bioactive natural products

Microalgae, due to their huge taxonomic and metabolic diversity, have been shown to be a valuable and eco-friendly source of bioactive natural products with pharmaceutical, nutraceutical, and cosmeceutical interests. Microalgae can be cultivated in huge quantities and this advantage overcomes the bottleneck of drug discovery from marine macro-organisms and destructive collection practices. In addition, many studies have focused on optimizing the culturing conditions in order to obtain the metabolites of interest or produce them in large amounts. SZN results allowed us to identify different classes of microalgal-derived compounds with specific biological activities, such as anti-cancer, antioxidant, anti-inflammatory, anti-tuberculosis, anti-epilepsy, anti-microbial and immune-regulatory.

Diatom oxylipin effect on sea urchin reproduction

Microalgae represent a potential goldmine for the discovery of new molecules with biological activity. Diatoms have been regarded as beneficial to the growth and survival of primary consumers such as planktonic and benthic filter feeders. However, many planktonic diatoms produce a wide range of oxygenated fatty acid derivatives (called oxylipins), which affect diatom growth or have negative effects on the reproduction and development of marine invertebrates. Moreover, some oxylipins (the diatom-derived polyunsaturated aldehydes) activate a process of cell death in human cancer cell lines. In

contrast to chemical ecology of planktonic diatoms that is better documented, a few studies investigated the chemistry of benthic diatoms, also due to difficulties in their isolation, quantification and cultivation, with respect to planktonic species. Combining morphological, metabolomic and de novo transcriptome approaches, for the first time, the effect of four benthic diatoms, Nanofrustulum shiloi, Cylindrotheca closterium, Diploneis sp. and Cocconeis scutellum, isolated from leaves of the seagrass Posidonia oceanica, was investigated on the reproductive success of the sea urchin Paracentrotus lividus. A



noxious effect was demonstrated on the embryos generated by female sea urchins fed on these benthic diatoms, with the only exception of C. scutellum, which did not induce any deleterious effect. Chemical analysis revealed that *C. closterium, N. shiloiand, C. scutellum* produce several polyunsaturated aldehydes and linear oxygenated fatty acids (LOFAs) related to the products of LOX pathways in planktonic species. *Diploneis sp.* also produces other unidentified fatty acid derivatives that are not related to LOX metabolism. The levels and composition of oxylipins in benthic diatoms match their negative effects on the reproductive success of the sea urchin *P. lividus*. In agreement with this correlation, the most toxic species *N. shiloh* revealed the same LOX pathways of *Skeletonema marinoi and Thalassiosira rotula*, two bloom-forming planktonic diatoms that affect copepod reproduction. These results open new perspectives on the identification of new bioactive compounds from these benthic diatoms, as candidates for biotechnological applications.

4.3 STRATEGIC GEOGRAPHICAL AREAS

Global ocean - The Stazione Zoologica has been involved for years in projects of a global scale, such as the Tara Ocean which has made it possible to carry out innovative research on the genomics and function of plankton on a megascale. Other Tara spin-off initiatives are underway for the project on benthic communities and tropical environments and may have important repercussions on the results of the institution's research.



Shinichi Sunagawa et al. Science 2015; 348:1261359

Mediterranean Sea - A basin of particular interest because, due to its geomorphology and circulation, it functions as a small ocean though influenced by connections to the Atlantic Ocean and Red Sea. Furthermore, it is located in a transition zone between two different climatic cells which expose it to different large-scale forcers and make it particularly sensitive to climate changes. It is considered to be an important centre of biodiversity for marine flora and fauna. The eastern and western reaches of the Mediterranean have very different characteristics, the former being very oligotrophic and also susceptible to invasion by species of Indo-Pacific origin. With a very high population density along its coasts, the Mediterranean is also strongly influenced by various anthropogenic impacts and it has been identified it as one of the regions most impacted by contamination by microplastics (project TARA-Mediterranean: Assessing the impact of plastic debris on Mediterranean ecosystem health and function). The Mediterranean population of the common marine turtle *Caretta caretta* uses this basin for its entire life cycle and is demographically independent of other populations. Genetic exchange with the Atlantic populations is in fact almost nil, and any threat or change in this closed marine system has a direct impact on the local population. It thus requires targeted interventions and strategic research, monitoring and management plans.

Tyrrrhenian Sea - The island of Panarea, the smallest of the Aeolian archipelago, is volcanically active and possesses different gas emission systems (especially CO₂) and hot water vents distributed around the coast and the numerous surrounding rocks and islets. It is the most extensive hydrothermal area in

the Mediterranean, and provides a rich natural laboratory for the study of oceanic acidification and its effects on marine organisms and habitats. The island hosts the ECCSEL - NatLat Italy laboratory, an facility financed by MiUR and managed by OGS, and which is one of the Italian components of the European Carbon Dioxide Capture and Storage Laboratory Infrastructure (ECCSEL) for the study of the CCS (Carbon Dioxide Capture and Storage), and is accessible to the national and international research community. Eleswhere in the Aeolian Islands, the island of Salina has extensive *Posidonia oceanica* meadows, where the SZN has established, in collaboration with the Blue Marine Foundation and the Eolian Preservation Fund, some fixed monitoring points to establish the state of the seagrass habitat over time. This work is also relevant to the possible establishment of the Marine Protected Area of the Aeolian Islands.

Decommissioned industrial sites can leave behind accumulated contaminants and environmental degradation that threatens human health, biodiversity and the functioning of ecosystems. It is thus frequently necessary to institute active restoration programmes to eliminate contamination and return industrial sites to a state that provides ecosystem goods and services. The science of the restoration of marine ecosystems is in its infancy, and undertaking research of this type in Italy appears strategic to provide scientific leadership (especially in the Mediterranean area) in the field of sustainable management of the coastal environment, in accordance with the EU blue growth philosophy.

The Gulf of Naples has been studied intensively since the founding of the Stazione Zoologica. Its geographical position, the geomorphology of the seabed and the variety of environmental conditions make it an ideal area for the study of both plankton and benthos. In an area of approximately 570 km², relatively uncontaminated and highly biodiverse areas coexist with heavily impacted areas, including Sites of National Interest (SIN) that require restoration. The presence of naturally acidified areas due to volcanic emissions also allows research on the possible impacts of climate change on the marine ecosystem. With the two study sites of *Long-Term Ecosystem Research* (L-TER_MC plankton, since 1984, szn.macisteweb.com) and the *Posidonia oceanica* L-TER LA grasslands, the Gulf of Naples is part of the LTER international network. The Napoli area has one of the highest concentrations of research bodies and university departments that deal with the sea, and the skills present at SZN makes it a key area internationally for marine biodiversity studies.

The island of Ischia, where the Stazione Zoologica has operated the Centre of Villa Dohrn since the early 1970s, has a complex volcanic and tectonic history. Some coastal areas located in areas of fault/fracture in the north-east part of Ischia, are characterized by phenomena of gas emission from the seabed (vents), 95% of which consists of CO₂ that comes out at the same temperature as the surrounding waters. This results in natural acidification of marine waters, albeit with high levels of variability, and true pCO₂ gradients, such as along the north and south coasts of the islet of the Aragonese Castle. These areas represent natural laboratories for studying the long-term effects of acidification on marine organisms. There have been five vent systems identified along the coast of Ischia to date, at depths between one and 48 m, representing a range of typical Mediterranean habitats. These systems provide the opportunity to determine acidification effects upon shallow rocky habitats, caves and grottos, sandy bottoms, and *Posidonia* meadows.

Adriatic Sea - The Po Delta area is affected by intense and repeated blooms of *Skeletonema marinoi*, a model species for the production of secondary metabolites belonging to the oxilipin group. In addition, the marine area is affected by significant and highly polluting inputs from the Po valley. The balance of this ecosystem is therefore extremely interesting for impact studies and for the applications of the Marine Strategy Framework Directive.

Straits of Sicily - Since 1989 the Stazione Zoologica has carried out studies concerning threatened biogenic habitats, including the distribution, ecology and conservation of *Sabellaria* reefs in the Mediterranean.

Strait of Messina. The Strait of Messina is an important upwelling area in the middle of the Mediterranean Sea and its hydrodynamic processes are interesting on a world-wide scale. This peculiar ecosystem is characterized by several intense and complex hydrodynamic conditions, having a remarkable effect on the abundance and structure of plankton and nekton: different communities can coexist in this area, as the result of passive horizontal and vertical transport and mixing. The upwelling currents, whose intensity is regulated by moon phases, concentrate mesopelagic food resources in this area and also cause the regular stranding of mesopelagic and bathypelagic organisms. Therefore, this upwelling system attracts predators of different trophic levels, probably regulating their dietary rhythm. The concentration of pelagic resources allowed the development of ancient and important fishery traditions (e.g. swordfish harpoon fishery). These features make also possible the presence of very different bottom communities in a relatively small area, with the presence of species unknown in other Mediterranean areas, such as kelp forests, Errina aspera and Pachylasma giganteum populations, considered belonging to Mediterranean relict fauna of the Tethys Sea. For these reasons, the Strait of Messina is considered a hotspot of biodiversity and was the favoured destination of several marine biologists of the past, aiming to collect fresh samples for their research. Among these scientists, August David Krohn (1803–1891) underlined the importance of this area, describing the Strait as 'the paradise of zoologists' and Anton Dohrn tried to establish here its Stazione Zoologica, before he decided to fulfil its objective in Naples.

Red Sea (Israel) - The Red Sea is home to 12 species of seagrasses belonging to two families: Cymodoceaceae and Hydrocharitaceae. Within the latter, the species *Halophila stipulacea* has recently caught the attention of researchers as it entered the Mediterranean Sea via the Suez Canal becoming invasive. SZN established collaboration with Dr. Gidon Winters from the Dead Sea & Arava Science Centre (Israel) to study the physiological mechanisms and the "early-warning" molecular signals of stress in *H. stipulacea* exposed to eutrophication and elevated temperatures. Studies are performed in mesocosms and in the field in the Gulf of Aqaba and analysed in comparison to similar studies in *Posidonia oceanica*, the keystone species from the Mediterranean, which is threatened due to anthropogenic impacts and global warming. These studies will help to shape the future of seagrass conservation in both the Red Sea and the Mediterranean basin.

Southern Ocean and Antarctica –The Stazione Zoologica has been carrying out scientific studies of the ecology and physiology of phytoplankton and on the trophic dynamics of the Southern Ocean since 1989. The global mean temperature has increased by more than 1°C in recent years, and more than 70% of this excess heat has been absorbed by the Southern Ocean. The Ross Sea is an important deepwater formation site and has much influence on ocean circulation and global climate. The Ross Sea is one of the most productive areas of the Southern Ocean and includes a mosaic of functionally different marine ecosystems. It is therefore an ideal area for studying the potential effects of climate change on biological and ecological characteristics. Recent data collected as part of the ROME project by our group have highlighted a change in the trophic structure in the pelagic zone of the Ross Sea. A large amount of data has been generated by a series of projects (including ROSSMIZE, CLIMA, ROME) in both pelagic and neritic areas (including PIPEX, SICAF and CEFA). These constitute important resources for the evaluation of changes in pelagic food webs over the last 20 years.

Pacific Ocean (Australia) - Australia's seas are home to a high diversity of marine seed plants. Consider that the genus Posidonia is present in the Mediterranean with a single species and in Australia with 8 different species. SZN has signed a collaborative agreement with the Climate Change Cluster of the University of Technology in Sydney, directed by Prof. Peter Ralph. The collaboration focuses on the acclimatation of marine plants to variation in environmental factors. Mesocosm studies are performed on congeneric species living in the Mediterranean and Australia, and the ability to adapt to temperature increases is analysed at a



morphological, physiological, transcriptomic and epigenetic level.

Pacific Ocean (Japan) – The SZN has set up a further collaborative agreement with the Laboratory of Evolutionary Morphology at RIKEN, in Kobe, directed by Prof. Shigeru Kuratani. Professor Kuratani is known around the world for his studies of marine cyclostomes: lampreys and hagfishes.



4.4 SZN STRATEGIC VISION FOR THE THREE YEAR PERIOD 2020-2022

The strategy pursued by the SZN aims to integrate research, innovation, technology transfer, training, and dissemination of results. The SZN promotes participation in national projects and in community and international programs. This in order to promote an integrated and 'multidisciplinary' approach, and efficient use of the institution's extensive infrastructure, also in the context of its participation and role as an active partner of the ESFRI Research Infrastructures.

Research and innovation represent important tools to encourage the creation of skilled career opportunities for future generations, and accelerate the development of a knowledge-based economy that is rooted in environmental awareness, sustainability and inclusivity.

The SZN has the ambition to contribute to increasing the competitiveness of the country, especially in the south, strengthening the capacity to produce research and infrastructure of excellence. It will do this by assisting young research talent, and by raising awareness of the social and economic value of research, science and culture in contemporary society.

SZN's 3-yr plan of activities 2020 – 2022 includes the following priorities:

- 1) The development of the new Department of Marine Biotechnology, and the reorganization of the research structures in accordance with the new Statute and the new body of organization and operation rules,
- 2) The development of territorial offices of the SZN in southern regions with high potential for research (Sicily, Calabria)
- 3) The creation of a marine center in Bagnoli, with a joint research and training structure with Federico II University,
- 4) The creation of the largest marine farm in Europe to promote the use of marine models in research and industry,
- 5) To invest in new research areas and in the development of young researchers,
- 6) To invest in the implementation of research infrastructures, technologies and robotics,
- 7) The inauguration of the renovated aquarium and renovation of the current library,
- 8) investment in targeted dissemination, communication & outreach actions.

4.5 SCIENTIFIC PROGRAMMING OF SZN FOR THE THREE YEARS 2020-2022

The Stazione Zoologica Anton Dohrn will consolidate scientific research and activities as previously described (*Core Activities* of the Departments) over the three-year period and, in order to promote a strong integration between research, innovation, technology transfer, training and dissemination of results will promote the development of scientific activities organized into four research themes as described below.

4.5.1 Function, adaptation and evolution in marine organisms

The SZN contributes to biological research and the study of evolution through projects aimed at understanding the biological and physiological mechanisms that regulate the reproduction, development, survival of organisms and species, with attention to adaptation processes and biological and neural plasticity, fundamental for evolutionary history of organisms. Theme 1 is designed to contribute to this aim. The study of molecules and cells, embryos and adults, single species of marine organisms - animals and plants - involves members from different Departments and with different expertise. Understanding the function and adaptability of marine organisms considering the diversity of marine life - in its complexity and evolutionary context -, provides a fundamental contribution to knowledge, and is instrumental for the increased sensitivity towards the well-being of the planet and of man and sustainability of resource use. Theme 1 contributes to the understanding of mechanisms that regulate the reproduction, development and survival of species, with attention to adaptation processes and biological plasticity. In this context, the SZN has the intention to increase the spectrum of organisms, functions and mechanisms to study in the three-year period. The study of physiological, metabolic and behavioral processes using a large diversity of marine organisms will help to decipher their functional diversity and complexity and provide important information about their evolution. Experimental activities require use of different approaches including anatomy, taxonomy, biochemistry, molecular biology, pharmacology, genetics, cell and developmental biology, phylogeny, physiology, immunology, neuroscience, and epigenetics, as well as the use of technologies and platforms for sequencing, bioinformatics and bioimaging.

Context and consistency with Horizon 2020 and with the Italian National Research Program

The activities of Theme 1 are closely related to Horizon 2020 framework program and in particular are part of the "European Bioeconomy Challenges" and "Health & Wellbeing". Questions relating to the processes underlying well-being and health, envisaged in this area, will find answers thanks to the studies of the mechanisms underlying gene and protein expression, and the regulation of plasticity phenomena at the molecular and physiological level. Furthermore, a detailed understanding of the relationships between cells, systems, organisms and environmental factors will allow us to better appreciate the effects of anthropogenic perturbations and possible environmental changes, a contribution in line with the "Climate Actions" axis. The theme "Function, Adaptation and Evolution of Marine Organisms" is strongly oriented to promote Knowledge Driven Research.

The SZN has a consolidated tradition of study of marine organisms and benefits from the ideal condition for the ability to access different Mediterranean environments. The studies conducted at SZN contribute substantially to the expansion of knowledge of multiple aspects of biology, physiology and the functioning of both plant and animal species, ranging from deciphering the functioning of nucleic acids to the molecular mechanisms that regulate complex phenomena. The scientific activities are also consistent with the needs identified as priorities in the 2015-2020 NRP: i) Health, ii) Quality of life, iii) Smart and sustainable industry, iv) Energy and environment.

Research objectives and expected results for the three-year period

The strategic research theme "Function, Adaptation and Evolution of Marine Organisms" part of the three-year plan 2020-2022 aims to achieve results that as a whole will allow to identify distinctive and characteristic mechanisms of marine organisms and mechanisms shared with other "systems". In particular, the research will focus on the following three priority objectives:

1. The evolution of biological complexity: molecular and adaptive aspects

The molecular and genetic mechanisms underlying the development and functioning of marine organisms (unicellular or multicellular), are still largely unexplored.

The diversity of organisms forms and functions specific to marine life represents a unique opportunity that is central to the research activities planned by the SZN for the three-year time course. Research activities will be focused on genomes of marine organisms in order to highlight genomic innovations in relation to their complexity and adaptation to the environment. The increased research capacity acquired over the past two years and the one expected in the three-year period will allow us to explore environments, organisms and functions, providing an opportunity to significantly contribute to the knowledge of biological complexity and its evolution.

The focus of the activities is the analysis of genomic innovations (e.g., retrotransposition events, acquisition of *de novo* genes or enhancers sequences) typical of the various marine taxa and so far largely unexplored. This analysis will be supported by the study of biochemical, enzymatic, morpho-functional adaptations and 'innovations'. The studies will also focus on the analysis of adaptive strategies to environments (including extreme ones) on macro-evolutionary dynamics. The research conducted in this area will also help to deepen knowledge about the role of cell reprogramming and epigenetic modifications to adaptive response and plasticity to stimuli or to variation in environmental conditions.

These studies will be carried out on various marine organisms, including algae, marine phanerogams, molluscs, echinoderms, tunicates and fish. The availability of reference genomes for various organisms (i.e., diatoms, echinoderms, molluscs) to which SZN-Theme 1 scientists are contributing, and availability of increasingly rapid, efficient and economic HTS sequencing methods will allow to intraspecific explore diversity by re-sequencing whole genomes of multiple strains of same



species to be analysed with phylogenomics approaches. The in-depth study of specific features and their phylogenomics mapping will be a necessary tool to identify evolutionary schemes and to formulate working hypotheses to be tested within a functional context.

The study of the evolution of the function of genomes also requires attention to the molecular mechanisms that make the information encoded in the genome of marine organisms functional. In this sense, SZN researchers apply multidisciplinary approaches that integrate functional genomics, transcriptomics, epigenetics, as well as molecular and physiological analyzes, for the study of the regulation of the determination of cell fate and the mechanisms of response to stimuli and adaptation in marine systems.

Disentangling phenotypic plasticity in the three main components of adaptation, acclimation and migration will also be approached looking at the relative role of epigenetic mutations, and genetic diversity and connectivity in conferring species resistance and resilience to environmental challenges. The use of these approaches will allow to explore specific selective pressures on genes coding on adaptive traits, and to explore the influence of key traits on evolutionary rates and the phyletic diversification of evolutionary lines, confirming its role as evolutionary drivers in the context of synthetic theory of Darwinian evolution. The SZN will continue to explore different biogeographic regions further expanding its operational borders, continuing to study the polar and temperate regions, but starting a systematic exploration of organisms of the deep sea, and the superficial and coastal regions.

Another substantial part of the activities will be aimed at clarifying the mechanisms underlying fundamental processes in the life of a marine organism, such as reproduction, embryonic development (with particular attention to organogenesis), and processes related to the control of the different phases of life cycles. These studies will complement the "Core" scientific activities using systems biology approaches. Research will allow identification of embryonic development and cellular differentiation programs, gene regulation networks, signal transduction pathways, intracellular signals needed to begin the early stages of sexual reproduction or regeneration (muscle and nerve), and will allow us to identify conserved phenomena and explore 'novel' ones.

During the next three years, marine organisms will be investigated using biochemistry, genetics, transcriptomics, structural and functional genomics, proteomics, phylogeny, real-time and live cell imaging and epigenomics. The state-of-the-art methods for both genomic and transcriptomic sequencing (High Throughput Sequencing), the analysis of gene expression (RNA-seq, qPCR, genome skimming), and protein (e.g., MALDI-TOF mass spectrometry), of protein and small molecule interaction



(advanced binding assays), the study of structure-function relationships (proteins and carbohydrates), the interference of the protein function, the interaction protein-DNA (ChIPseq), DNA accessibility (ATACseq) and mutagenesis (genome editing). We will integrate "single cell sequencing" (MARIE SKŁODOWSKA - CURIE Innovative Training Networks project EvoCELL) to explore fundamental mechanisms in development and evolution. This will allow to explore questions such as: how many types of cells an animal has, how it generates a new cell in the course of evolution, how many cell types are shared between different animals and how many types unique cell lines have emerged in different lineages (or evolutionary lines). We will also use Spatial Transcriptomics approaches to significantly increase knowledge on the biological and neural complexity of marine organisms (e.g., molluscs).

The use of different approaches and the development of cutting-edge methodologies will allow us to reconstruct the processes that take place within a cell or an organism, even in the case of systems in which the traditional tools and genomic resources for functional studies are still not available, very common in the study of marine organisms. In this context, we will also implement methods and

reagents (such as gene transduction, live imaging, cell-free assays and clickchemistry) that, to date, have found limited application in marine model organisms. These approaches will allow the study of biological processes at the cellular and subcellular level, reducing the existing technical gap with vertebrate and mammalian models. The possibility of tackling some of the above questions in diatoms has been boosted by substantial funding from the Gordon Foundation and Betty Moore for a three-year project called DIsCO (Diatom life cycles, molecular controls and contribution to ecosystems dynamics).



2. Neurons, sense organs and response to stimuli

In the course of evolution marine organisms have developed extraordinary sensory, neural and behavioural strategies to cope with life and adapt to marine environments. Theme 1 at the SZN will continue in the search and identification of sensory and neural 'markers' that - from the most simplified to the most complex forms - characterize the capacities of the marine organisms to control, perceive, and respond to chemical (e.g., odours), light (i.e., light and bioluminescence), and mechanical stimuli (vibrations and sounds). The study of the nervous system at different levels of biological and phylogenetic complexity will allow the identification of fundamental neural mechanisms and describe biological, physiological and behavioural plasticity that greatly differentiate marine taxa. Particular attention will be given to chordates and lophotrochozoa in order to extend the evolutionary and comparative analysis.

Research activities will range from genes to behaviour. Classical approaches will be integrated by highresolution imaging and behavioural analysis to describe locomotor strategies (e.g., copepods, molluscs, chordates), variations into patterns (i.e. body pattern-semantic, cephalopods), inter- and intraindividual variations of behavioural performance with the aim of evaluate the effects of "environmental" cues (e.g., food choice, environmental complexity, light spectrum) on biological and behavioural plasticity.

The molecular mechanisms underlying the differentiation of sensory and neuronal cells will be investigated in different marine species to identify the function of genes, regulatory elements, retrotransposition events, and genomic characteristics. Furthermore, proteins involved in chemoreception and odorant-binding from marine invertebrates will be researched and characterized by bioinformatic and biochemical approaches. These proteins, well known in insects and vertebrates, are responsible for the solubilization, transport, presentation to receptors and perception of lipophilic molecules, including pheromones. However, their presence and function in aquatic systems have never been explored.

We will continue to characterize sensory and neuronal cell types (endocannabinoids, neurotrophins, protocadherins), we will characterise neuromodulatory territories and functions (e.g., octopaminergic and dopaminergic receptors) that are relevant to the control of reward. Focus will also be given to the understanding of how the nervous system controls biological plasticity including learning and memory recall. Studies aimed at evaluating the somatic mosaicism of the 'brain' in some species of marine organisms will be undertaken in order to correlate learning and cognitive skills with neural organization. These studies will be accompanied by research on transcription factors and molecular pathways involved in the development of receptors including mechanoreceptors, photoreceptors and nociceptors. The characterization of the nervous system and its complexity will also be focused on the study of the neural connectome in key species of marine invertebrates. In this context, the light behavior of the adult Mediterranean sea urchin (Paracentrotus lividus) will be studied, in the context of the HFSPO funded international project, through an integrative (at the molecular, morphological, physiological and neural modelling level) with the aim to unravel the mystery of spherical vision in echinoderms. The mechanisms by which the brain keeps itself in constant register with the external environment are of primary importance to understanding the evolution and behavior of animal species. A paradigmatic example of the adaptive capacity of the nervous system is represented by adult neurogenesis, a pervasive phenomenon in the brain and retina of Teleostei. In this sense, the research will focus on the evolutionary study of adult neurogenesis in marine vertebrates through the use of immunohistochemistry and in situ hybridization techniques, in representative species of the basal branches of the chordates (agnates, chondrichthyes, chondrostei, osteoitti). In the same context, the neurotrophins of agnates and chondrichthyes in terms of distribution and biological activities will be investigated.

3. Immune response, natural variability, plasticity and symbiosis

The research planned for the next three-year period will extend ongoing studies on the immune response and memory in marine invertebrates. The immune system will be challenged at different life-cycle stages by bacterial, fungal and viral stimuli in order to examine the inflammatory response at transcriptomics, proteomics and metabolomics levels. Also, the mechanisms of action of immunoadjuvant compounds of marine origin will be analyzed through the implementation of a high-throughput pipeline based on differential gene transcription in juvenile ascidians (project ADVISE). Such research will allow to acquire important information to understand the strategy adopted by plants and marine animals to resist re-infections. Studies on innate immune system will also extend to eco-immunology, a sector recently expanded interdisciplinary, focusing on ecological factors, be they biotic or abiotic, which determine the functioning and evolution of the immune system. Moreover studies will be carried out on the relationships between symbiotic microorganisms and host organism (for example in corals, phanerogams, sponges and echinoderms), taken together as a single biological entity called

"olobionte". The focus will be on the diversity of microbiome, on microbiome dynamics in variable environments, and on interactions functional in relation to metabolic responses in ad hoc studies of holobionts. Studies on the immune system also have important conservation implications: the infectious diseases are considered a major threat to marine organisms, especially in the context of the global biodiversity crisis, which can only be countered by understanding the mechanisms underlying the interaction between the body's defenses and pathogens.

In the next three years, SZN will identify viruses found in starfish that are adapted to the Antarctic continent, with the objective of identifying the viral agents responsible for pathological forms observed in the last years in marine invertebrates of the polar regions.



4.5.2 Multiscale approach to marine biodiversity

The Stazione Zoologica contributes to the study of the biodiversity of marine environments through a multiscale approach so as to understand the different levels of organization of life, from molecules to ecosystems. The SZN staff uses morphological, genetic, genomics and modeling methods that are applied to areas of the Mediterranean Sea to native and invasive species. The Mediterranean has a peculiar value as a hotspot due to the presence of invasive species, partly due to the ancient and rooted human presence on its coastal areas and partly due to the massive perturbations originating from the opening of the Suez Canal (lessepsian migrations). Moreover, despite being the most studied basin, its cryptic biodiversity is still far from being entirely discovered. Particular attention is dedicated to extreme environments, such as those with low pH, to deep environments and to the polar areas, the latter still little characterized in terms of biodiversity. Finally, especially for some taxa (e.g., gastropod molluscs, scleractinian corals and porifers), the research activity is also focused in areas of high biodiversity, such as tropical ecosystems. A correct assessment of biodiversity constitutes a fundamental prerequisite both for the development of comparative and applied research areas and for the effective implementation of environmental management strategies through the use of quantitative biodiversity as a tool for analyzing the quality of the environment. In such perspective, the study of marine biodiversity not only integrates perfectly with other research activities carried out at SZN, but it is also an essential prerequisite. The central role of Stazione Zoologica in the characterization of marine biodiversity is reflected in participation to the European ESFRI infrastructure LifeWatch and in the recent creation of the MOTax network (Marine Organisms Taxonomy).

Coherence with Horizon2020, PNR, PNRA and PNIR:

The multiscale approach to marine biodiversity developed by the SZN is in line with the EU guidelines, which explicitly identify the need to protect biodiversity at multiple levels: populations, species, habitats and ecosystems. The anthropization of the coasts in synergy with the effects of climate change contributes substantially to the reduction of biodiversity both on a local and a global scale. This impoverishment compromises ecosystem function, negatively affecting resilience (see also Theme -Integrated approach to the study and management of the ecosystem). The characterization of existing biodiversity at the species level and the definition of its conditions at the spatial (distribution pattern) and genetic (including populational patterns) levels are considered fundamental aspects to define the Good Environmental Status, as also requested by the Marine Strategy Framework Directive (GES, sensu Marine Strategy Framework Directive MSFD). Protecting the marine environment is one of the challenges identified in Horizon2020, in line with the idea of developing an economy based on a sustainable relationship man - nature (Societal Challenge 5), for which it is essential to gain detailed knowledge on biodiversity. This knowledge is also a fundamental prerequisite for the sustainable development of marine biotechnology as indicated in H2020 Societal Challenge 2 (Blue Growth; see also Theme - Exploration of marine biological resources). In line with European guidelines, the 2015-2020 National Research Program identifies Blue Growth as a high-potential sector, while Salute and Agrifood are considered priority areas of intervention. These three sectors explicitly identified in the PNRs as strategic are fundamental for a correct assessment of marine resources biodiversity.

Research objectives and expected results for the three-year period

In the next three years, the SZN will continue to carry out research on marine biodiversity using an approach of integrated taxonomy, also applied to alien and invasive species. Distribution patterns will

be addressed at the community level by including benthic, planktonic and nektonic organisms (algae, seagrasses, invertebrates, fish) in relation to the characteristics of the habitats using the time series available to the SZN. Particular attention will be given to the following four priority objectives sustained through funding from competitive national and EU international projects.

Innovative approaches to the description of biodiversity - HTS metabarcoding approaches, currently limited to microbial communities (planktonic diatoms, sediment protists), will be extended to a large number of marine organisms and will also be integrated into the study of the variability of various morpho-physiological, behavioral and ecological traits, including trophic associations between organisms. For example, a metabarcoding characterization (from samples of environmental DNA) will be performed of planktonic and necton communities of metazoans, as the stomach contents of gastropods and fish, and the symbiosis with microorganisms in corals, sponges and starfish (see also Theme 1 - Biology, evolution and adaptation). The SZN also aims at contributing to the development of an implementation of genomic technologies to be integrated into classic monitoring observatories classic (e.g. LTER). Genetic diversity will be explored in diatoms with approaches of population genomics, with the aim of continuing an ongoing study of populations since 2008 and to identify the genomic features that underlie strain and population variabilities (DIsCO project). The use of phylogenetic approaches in the evaluation of biodiversity will make it possible to integrate the simple measure of species richness in the communities with evolutionary distances among species. It will thus be possible to obtain a more accurate evaluation of biodiversity gradients and their temporal dynamics. Studies on a wide range of marine organisms will enable the development of new hypotheses to be experimentally tested to investigate the underlying patterns and processes that affect the distribution of marine biodiversity, in the context of recent and future changes on local and global scale.

Biodiversity in extreme and degraded environments

It is planned to extend the study of biodiversity in deep environments, which host communities that are still poorly known and characterized in terms of species composition, both in the Mediterranean Sea (see DEEPTYR Project) and the Atlantic and the Pacific Oceans, to obtain a valuable biogeographic and comparative framework. The SZN will continue to address the study of biodiversity in extreme environments such as the polar areas, in particular Antarctica (PIED project of PNRA). Biodiversity studies will also be carried out in acidified and/or environments with strong anthropogenic impact and in conditions of environmental degradation. For example, in acidified environments (Ischia and Panarea; NEMO Award Project; Marine Hazard Project; IPANEMA Project)) in-depth studies will be conducted on species composition, functional traits, and patterns of abundance and distribution in the benthic communities (projects Future4Oceans and High-CO2 Seas), with a particular focus on phenology, epiphytes and fungi associated with Posidonia and Cystoseira, and on the settlement pattern of benthos associated with Posidonia and Cystoseira, in comparison with control environments. Furthermore, it is planned to evaluate the biodiversity at a site of the SIN Bagnoli Coroglio, characterized by the presence of polluting substances (e.g. heavy metals and hydrocarbons) related to past industrial activity, comparing the current situation with data obtained from reconstructions of historical distribution in species and habitats for the Gulf of Naples (FISR ABBaCo Project). This research activity integrates with the research that seeks to decipher the biological mechanisms that have allowed the organisms to adapt to particular environments and will allow them to build maps indicative of species and habitat change over time.

Large-scale spatial-temporal dynamics

During the three-year period the SZN will address research activities aimed at identifying diversity

patterns in the plankton compartment, focusing on the space-time variations of phytoplankton. The analysis of the multidecadal plankton dataset will be aimed at the detection of long-term trends related to interannual climatic variations. Within a UNESCO-IOC working group on phytoplankton long term trends, the temporal patterns of cosmopolitan species will be compared among long-term datasets from distant locations, which is expected to provide important knowledge on biological and ecological characteristics of key phytoplankton components. Literature and data-based overviews of Harmful Algal Blooms (HABs) trends will finally provide a sound answer to the questions whether HABs are actually increasing at regional and global scales. As for the metagenetic approaches, further studies are conducted on samples collected in the long station term LTER - MC on a larger time scale and the development of new molecular targets for a more accurate identification of evolutionarily very similar species (e.g., diatoms of the genus Chaetoceros, Pseudo-nitzschia and Leptocylindrus; various groups of Dinoflagellates). Morphological analysis will be combined with metabarcoding approaches in samples collected during Tara Ocean surveys and within the Ocean Sampling Day project. The NEREA project (Naples Ecological REsearch for Augmented observatories), putting together the multifunctional approach of TARA and the skills and information acquired over the years at the LTER station in Marechiara, aims to achieve the development of a multi and trans-disciplinary approach to the study of spatial and temporal dynamics of the Gulf of Naples marine ecosystem, under an end-to-end perspective, from viruses to necton. A fixed station will also be set in deep waters (under the auspices of EMSO International), to carry out sampling aimed at characterize the biodiversity and physics of the Dohrn Canyon, which crosses the Gulf of Naples transversal to the coast.

Biodiversity of fishery resources

The exploitation of fish resources is one of the main factors inducing the impoverishment of marine biodiversity, especially in the Mediterranean and in its deep environments that are particularly sensitive to overfishing as characterised by long-living vulnerable habitat forming benthic species (i.e. Vulnerable Marine Ecosystems) generally regulating the ecosystem functioning by providing high valuable ecosystem services (e.g. food and refuge for fish - Essential Fish Habitat). In addition to studying the taxonomic biodiversity of fishing resources, SZN researchers focus on describing the functional biodiversity of exploited communities highlighting how fishing causes a selection of specific traits, which could lead to the loss or alteration of the functionality of the entire ecosystem. The research at SZN will apply genetic and genomic approaches for a correct evaluation of biodiversity of species of commercial interest (e.g. mussels, cephalopods, pagro, fragolino, anchovies, sardines), and their responses to fishing pressure and environmental changes, so as to being able to contribute to the sustainable management of native fish resources. In particular, the genetic variability of the stocks of these species will be evaluated on an inter-annual scale, and genetic tagging techniques will be developed to identify regional catches. Moreover, new molecular tools for taxonomic and biogeographic identification will be developed through the study of the entire mitochondrial genome of fish species in the family Sparidae. The management of human activities and related threats at sea (e.g. fisheries and aquaculture) is a cornerstone for researchers at SZN, that by following an Ecosystem Based Approach through the implementation of Integrated Ecosystem Assessment, or rather an integrated - multidisciplinary - approach (Theme 4) inform the monitoring and evaluation of marine resources strategies. Global change drivers have been recently integrated into this approach to produce dynamic / adaptive climate resilient management strategies, tailored to species and ecosystem responses (SZN priorities 2015-2025).

4.5.3 Integrated approach to the study and management of the ecosystem

The marine environment is a precious legacy that must be protected and, where possible, restored with the ultimate aim of maintaining biodiversity and preserving its vitality so that seas and oceans are clean, healthy and productive. Anthropogenic impacts have reached critical levels, causing changes in the physical, chemical and biological structure of marine ecosystems, with loss of biodiversity, erosion of natural capital and of the goods and services they represent. It is only through a holistic and multidisciplinary approach that it is possible to correctly evaluate and understand the mechanisms underlying the changes in the structure and functioning of ecosystems, thus informing appropriate management interventions. The activities carried out at the SZN, through both experimental and observational approaches, contribute to the development of methods of proper environmental management, which are essential for the sustainable exploitation of marine resources. At SZN, research on this topic focuses on the study of the effect that environmental variations have on marine organisms and on how these can influence the interactions among the organisms that shape communities. The aim is to evaluate the responsiveness of the species and to identify key ecosystem structures and functions to be preserved through appropriate forms of management (dynamic/adaptive management) that include conservation and restoration approaches.

Context and consistency with Horizon 2020 and with the National Research Plan

This topic is in line with the Horizon 2020 program, in particular the social challenge is to achieve: a) a society and economy resilient to climate change; b) the protection and sustainable management of natural resources and ecosystems and the sustainable supply and use of raw materials for world population growth within the limits of the planet's natural resources and ecosystems (call SC5-7-2015, More effective ecosystem restoration in the EU). Objective 2 of the EU biodiversity strategy calls for the restoration of at least 15% of degraded ecosystems. The EU policy on regional development has always supported restoration actions, particularly in the framework of INTER-REG programs. In the Italian context, the scientific activities of this theme are consistent with the areas identified as priorities by the National Research Plan (PNR) 2015-2020: i) Health, ii) Quality of life, iii) Intelligent and sustainable industry, iv) Energy and the environment. The theme therefore fits within the framework of actions envisaged by the PNR, as it intends to invest in the development and attraction of highly qualified human capital in the area of research on environmental and human health. Thanks to its pioneering objectives, the PNR represents a unique driving force of innovation in the field of sustainable exploitation of marine resources, considered strategic for the economic development of the country. In the context of integrated management of the various activities that take place in the maritime environment, it is fundamental to understand how the concepts of Marine Spatial Planning (MSP) and Integrated Marine Policy (IMP) and other European policies that regulate the exploitation of natural resources can be applied in reality, given that the characteristics of the different zones and their idiosyncrasies drastically determine the success of the implementation of these policies. In this sense, continuity will be given to the work begun in recent years on conservation, fishing and tourism management in the context of the Mediterranean MSP.

Research objectives and expected results for the three-year period

We intend to fill important knowledge gaps in the responses of marine communities to anthropogenic and climatic forcing as well as defining new and more appropriate conceptual models of biotic interactions. This knowledge will also be an indispensable prerequisite for the development of appropriate strategies for managing and restoring coastal environments. Over the next three years, ecosystem research will be dedicated to the following three main objectives.

Response of marine communities to anthropogenic and climatic forcing - Biological monitoring will be strengthened utilizing the Observatory Systems of the Gulf of Naples and the Central Tyrrhenian (see NEREA observatory). Implementation of a multidisciplinary approach will link the activities in the laboratory with those at sea. We will use spatial-temporal dynamic analysis of planktonic food webs in relation to the main environmental variables. Research will range from the study of metabolic processes at the cellular and molecular level with experiments and field investigations, to the analysis of biological data detected with technologically advanced instruments, up to a system modelling that integrates the different types of information. Such an approach is made possible thanks to recent advances in the field of functional biology and to the development of a new generation of sensors designed for the observation of biological traits and processes.

The automatic observation systems installed in the Gulf of Naples and Pozzuoli will be brought into operation and one will be installed in the Gulf of Trieste, in collaboration with the OGS in Trieste. In order to characterize microbial communities in relation to biotic and abiotic environmental factors, we will perform а bioinformatic analysis of HTS obtained sequences during sampling. The bacterial communities associated with planktonic diatoms will be identified during spring and autumn blooms through high-



resolution metagenomic and morphological analyzes. Innovative technologies based on automated and programmable scanning flow cytometry will be developed for the continuous analysis at close spatial and temporal scales of microbial communities aimed at the development of systems of early warning of environmental risk. We will analyze temporal patterns at the seasonal and decadal scale at the LTER-MC Station, in order to identify long-term variability in plankton abundance and / or phenology. The samples collected at the surface and at different depth within the NEREA observatory will be analyzed with various -omic techniques. At a larger scale, we will study the distribution of diatoms in European seas based on the HTS sequences collected within the EU-Biomarks and MIUR-BioDiversitalia projects, while the distribution of phytoplankton in the Arctic Ocean, will be analyzed comparing morphological and HTS data collected during the recent Tara Arctic expedition. The dynamics of zooplankton in relation to environmental factors will be analyzed on a large scale within the integrated observation systems of the Mediterranean (BG - 12-2016 proposal MedOS). Finally, on a local scale in the Gulf of Naples, the seasonal trend of reproductive and recruitment rates of key zooplankton species will be evaluated at the LTER-MC Station, in relation to the temporal dynamics and the metabolic profile of phytoplankton. This study will allow us to evaluate the ecological role of secondary metabolites produced by diatoms (oxyilipins), in situ phytoplankton-zooplankton interactions.

The effects of climate change on benthic communities will be studied in depth in the projects "High-CO₂ Seas" and "Future4Oceans", through composition analysis, abundance patterns, distribution and functional traits. Moreover, within the project NEMO, we will address the fungal and bacterial component and the benthos settlement pattern (with artificial collectors) associated with both *Posidonia oceanica* and *Cystoseira* in systems subject to natural acidification (Ischia and Panarea) and in control systems. The benthic communities and the adaptations of fish species in the coastal hydrothermal systems of Panarea and near the "Smoking Land" will also be analysed, as part of the IPANEMA Project which will allow to acquire and systemise instrumentation aimed at monitoring the effects of acidification. around the island of Panarea. Data will also be analysed on the *P. oceanica* of Lacco Ameno (Ischia) (LTER-LA site), along a depth gradient, in order to detect temporal changes in the spatial anisotropy of the rhizome density caused by anthropogenic pressures.

The results of these activities will allow to improve the understanding of the responses of marine ecosystems to human pressure and climate change and will be useful for the eco-sustainable management of the "sea resource", as well as training a new generation of researchers predisposed to multidisciplinary work. The large amount of information generated may also have important applications in biotechnology and the development of sensors for the robotized monitoring of the state of ecosystems.

Multiple stress effects on marine organisms - We will examine the effects of environmental factors and their combinations that are capable of influencing marine biodiversity and the functioning of food webs, thus exerting an impact on the structuring species and on the whole systems. In particular, the effects of temperature, acidification, sedimentation, chemical pollution and microplastics, and toxic algal blooms on various levels of biological organization, will be analyzed from the molecular level to the ecosystem level. Descriptive and manipulative laboratory and field experiments will be carried out focusing on ecologically and/or economically important species (for example, madrepores, sea urchins, tunicates, mussels, seaweeds and seagrasses forming habitats, phytoplankton primary producers, primary consumers, zooplankton, fishes). The response of marine flowering plants to multiple effects of eutrophication and temperature increase will be characterized, with particular emphasis on the transcriptomic and epigenetic response. Early molecular stress indicators will be sought to be used for monitoring coastal environments. The role of natural pre-adaptation (plants living in different environments) and induced pre-adaptation (hardening or priming) in conditioning the level of adaptive response will also be evaluated in marine plants. The impact of microplastics on the immune system of sea urchins will be investigated. Particular attention will be paid to the response of organisms to gradients of environmental pressure, or to single perturbations, or to experimental combinations thereof, to understand the biota's responses to present and future environmental changes in superficial coastal, pelagic and deep systems. In this regard, we will characterize the sea urchin population that lives in the low pH area of the Ischia acidification site through a wide-ranging study of immune cells, the coelomites. Cell and biochemical investigations are planned, including differences in gene expression, by de-novo sequencing techniques, compared to celomocytes from control animals. These results, together with the examination of the physiological parameters of animals, will allow us to understand the molecular mechanisms allowing sea urchins to live at low pH values. Particular emphasis will be placed on the study of the effects of climatic factors, introduction of non-native species, classical, emerging and potential contaminants, analyzing them separately or in their complex interactions. Thanks to a multidisciplinary and integrated approach, these researches will provide essential information for understanding the ecological dynamics and the management and

conservation of natural resources. In particular, over the next three years we will work to:

- identify biological indicators of environmental status, with particular attention to the definition
 of model organisms and biological responses allowing to assess the ecological and economic
 impact of aquaculture activities;
- develop predictive systems for changes in distribution, abundance and diversity of key species in relation to various forms of anthropogenic disturbance;
- identify the effects of stressors, in particular temperature and other environmental factors linked to climate change, on the response capacity of structuring species such as seagrasses, in order to predict possible regression and mortality scenarios;
- identify early molecular stress indicators that can be used in monitoring coastal environments;
- test the level of adaptive response in marine plants, with repercussions on the material of choice in transplants;
- investigate assisted evolution through hardening of adults and juveniles in marine plants, and assessing the role of epigenetic mutations conferring stress memory;
- identify the effects of environmental contamination (including that of nanoparticles, micro- and nano-plastics), but also toxic substances and signal molecules of biological origin (chemical ecology) on species and ecosystems and on the embryonic development of tunicates; particular attention will be devoted to the study of the quality of gametes, the mechanisms of fertilization and embryonic / larval development that are the basis of reproductive success, a key factor for the survival and continuity of marine species;
- study the adaptability, and therefore the potential epigenetic changes, in the mollusc *Mytilus galloprovincialis* following exposure to different emerging contaminants.
- understand the role played by zooplankton and benthic organisms in the transfer of contaminants (e.g., microplastics) from water to food webs;
- understand the mutual interactions between native and non-indigenous species and the related modulating factors, and evaluate the ability of habitat-forming species to mitigate the effects of climate change on the populations associated with them;
- investigate the effects of environmental variability on the distribution and behavior of sea turtles thanks to the use of cutting-edge telemetric technologies that allow to monitor the animal and the environment it occupies at the same time. Furthermore, the study of the effects of climate change on the distribution of sea turtles in the Western Mediterranean will continue. The data obtained will be integrated into ecological niche models to define the habitat needs of sea turtles and identify critical areas for the long-term management of the species;
- identify the crossed effect of environmental drivers of change (e.g. temperature increase scenarios) and human activities at sea (e.g. fisheries and aquaculture) to predict the effects on species-specific functional traits and the rebounds on population (e.g. fish stocks), an informative layer to be integrated into processes of marine spatial planning and to use when evaluating dynamic/adaptive management strategies.

Management and recovery of coastal habitats - In the next two years, thanks to the ABBaCo project (see project sheet), research will continue to assess the environmental status of model study areas, such as the SIN Bagnoli-Coroglio, for which a targeted management action is requested. This allows us to characterize the current conditions and evaluate their evolution in response to environmental changes, including environmental restoration actions, potentially applicable also in other similar areas. Therefore, following the philosophy of the MSFD, and in particular the definition of the GES descriptors, studies are being conducted to evaluate:

• the biodiversity of the area from bacteria to large vertebrates, with particular attention to the presence, abundance and invasiveness of non-native species, and to the abundance and distribution of marine organisms exploited for commercial purposes;

• the physical, chemical and biological state of the water column, the hydrography of the area (also through the use of oceanographic models), the assemblage structure and the state of integrity of the seabed;

• the presence and concentration of pollutants in the environment and in fishing products intended for human consumption, the concentration of marine litter on the seabed and in the water column, and the types and extent of underwater acoustic pollution. Furthermore, the search for benchmarks for the management and restoration of coastal habitats, such as seagrass meadows, bioconstructions, will be



conducted through the analysis of historical data and collections designed to construct maps capable of representing the diversity and distribution in the Gulf of Naples. This will allow the identification of the forms of pressure potentially responsible for their current regression and will provide fundamental information for environmental restoration actions. Taking advantage of this information and in collaboration with other Italian research institutes, pilot actions are underway to replant Posidonia oceanica meadows and madreporarian bio-constructions (including ecosystem engineers and habitat formers). The know-how acquired through the

ABBaCo project will be used to carry out pilot studies and research aimed at environmental restoration of other contaminated sites in the Mediterranean, such as the SIN of Falconara Marittima. This site is the subject of study by the BioBluTech project (Fondazione Cariverona), and activities are currently underway aimed at the recovery / restoration of the 'sabellarieto', a typical habitat of the Adriatic coasts and a priority according to EU regulations. Furthermore, the Algal Forest Restoration In the Mediterranean Sea (AFRIMED, EU EASME) project, of which SZN is a partner, will develop and promote restoration protocols for Mediterranean macroalgae (Cystoseira) forests and will provide the knowledge framework for the management of these important coastal habitats. In the context of the integrated management of the different activities taking place in the maritime space, it is fundamental to understand how concepts such as those of the Marine Spatial Planning and Integrated Marine Policy (IMP) and other European policies that regulate the exploitation of natural resources can be applied in reality, given that the characteristics of the different zones and their idiosyncrasies drastically determine the success of the implementation of these policies. In this sense, continuity will be given to the work begun in recent years on conservation, fishing and tourism management in the context of the Mediterranean MSP. This objective proposes the application of these policies in the research strategy. The activities of the next few years will also aim to increase the knowledge of deep marine systems, focusing attention on the Mediterranean and in particular on the Tyrrhenian Sea (e.g., Canyon Dohrn). In particular, also thanks to the EARTHCRUISERS project, and in collaboration with other EPRs, targeted studies are underway to characterize biodiversity in the deep sea, a virtually unknown ecosystem, and to understand the role of deep environments in the ecological functioning of the marine system-

4.5.4 Sustainable Exploitation of Marine Biological Resources

Marine biotechnology has seen strong growth both at the scientific and industrial level over the past five years. The most recent data indicate that "Blue biotechnology" has an estimated market value of over 3 billion euros (in Europe alone) and with a growth potential to reach 4.3 billion euros by 2020 and up to 5.7 billion by 2025, considering that industry and scientific research can move in synergy.

The great biodiversity that characterizes the biological communities that inhabit the sea, resulting from the adaptation to the properties of the ecosystem in which they live, offers a unique opportunity for research aiming, through biological or chemical investigations, at the optimization of the sustainable exploitation of marine organisms. The great potential represented by marine biodiversity is already demonstrated by the increasing number of therapeutic agents of marine origin currently in clinical use for cancer therapies, analgesics and painkillers, as well as new products developed in the nutraceutical and cosmeceutical fields.

For years the SZN has focused on the biotechnological potential of microalgae, such as witnessed by the numerous national (PON01_02782; PON01_00117; PON01_02093) and international projects (PharmaSea; PharmaDeep) SZN scientists have participated in. Furthermore, the SZN also studies the biotechnological potential of some marine invertebrates such as sponges, tunicates and echinoderms. Sponges, in particular, in the last 50 years have been considered an inexhaustible source of natural marine products and currently four products, derived from sponges, are commercially available as anticarcinogens and antivirals. Finally, fungi, bacteria and marine microalgae are objects of research at the SZN in different fields of applications, including the pharmaceutical, nutraceutical, cosmeceutical and environmental biotechnology fields.

field In the of bioremediation of contaminated marine environments, in recent years researchers at the SZN have tested and developed biotechnological approaches to study environmental impacts and possible interventions of bioremediation in sites of national interest identified as priorities for interventions of reclamation, such as the SIN on the Tyrrhenian coast and the SIN former Bagnoli-Coroglio Montedison on the Adriatic coast of Falconara Marittima. These sites are ideal models for testing and validating new environmental intervention technologies based on bioremediation strategies through the use of marine microbes and their



products with biostimulation processes (stimulation of autochthonous microbes) and bioaugmentation (use of non-native microbial biomass). The development of these biotechnologies is particularly relevant as an eco-friendly and efficient way to reduce the degree of contamination, with versatility of use for different types of contaminants and in different environmental contexts.

Context and consistency with EU projects and with the PNR

This research is in line with the objectives of the "Blue Growth" and "Health and Welfare". They are also in line with one of the priorities of the PNR 2015-2020, "Blue Growth", identified as an area with the highest potential for innovation among the twelve areas. It is worth noting that such research will help compose the strategic framework adopted by the PNR which also provides support for the necessary networking activities also within the National Technological Cluster «Blue Growth» in order to promote development at the national level of a network between business and research. The challenge of using marine resources in different fields of application is also perfectly in line with the SZN Vision for the decade 2015-2025 for the "study of marine organisms for the identification of new molecules for biomedical and biotechnological applications". The proposed topic may also benefit from the advantages provided by infrastructures operating at the national and European levels, such as EMBRC to which the SZN belongs - an infrastructure that aims to provide research support services on marine biological resources in Europe by providing the right research environment for users of academia, industry, technology and other sectors.

Research objectives and expected results for the next three-year period

Marine biotechnologies for nutraceuticals and cosmeceuticals - Among the objectives for the next three years, there is the aim to analyse highly antioxidant compounds, such as polyphenols, flavonoids, sterols and vitamins (B, D, E), which will be added to the already optimized analyses conducted in the department (carotenoids and phycobiliproteins). This will allow us to run one screening of these compounds in different microalgal classes of biotechnological interest (diatoms, green algae and cyanophytes). The screening platform will be implemented with the aim of specifically characterizing the type of activity of biological products with potential applications in the nutraceutical and / or cosmeceutical fields.

Furthermore, the modulation of the production of these compounds in microalgae will be studied in order to maximize their production and increase their bioactive properties as antioxidants. The molecular study of the synthetic pathways of these compounds will be undertaken in targeted microalgal species. Another objective concerns the maximization of the production of microalgal biomass with high added value in terms of activity antioxidants. This goal will be achieved by two means: the construction of a new generation photobioreactors that allows easy parameter manipulation of environmental growth and therefore the physiological and biochemical modulation of compounds of interest (expected also within the INTENSE project); the second way is to investigate and optimize cultivation between two microalgal species and between microalgae and bacteria.

Marine biotechnology for pharmacology – Studies will be deepened and expanded on marine organisms with particular attention to microalgae, for which the SZN has for years been the first institute in Italy for its ability to exploit the biotechnological potential in the pharmacological field. It is known that varying the culture conditions (e.g. in lack of nutrients) greatly influences the bioactivity of most species of microalgae. Therefore, in the next three years we aim to maximize the synthesis levels of compounds of interest. The conditions for the synthesis of primary and secondary metabolites (such as ovothiol oviotol) will be optimized. Ongoing studies have revealed that the key enzyme in ovothiol biosynthesis, OvoA, is present in many centric and pennate diatoms and that the diatom *S. marinoi* produces ovothiol B. Moreover, the biosynthesis of ovothiol in *S. marinoi* is affected by different light conditions, opening new perspectives for the biotechnological production of ovotiols which are receiving a great interest for their anti-proliferative and anti-inflammatory properties. An eco-friendly

production system will be also developed through diatom engineering for the enzyme involved in the biosynthesis of ovothiol. Some studies will also continue on a marine dinoflagellate, capable of inducing the death of cancer cell lines, for which a patent for the compound is under consideration. The candidate compounds to be transferred for pre-clinical and clinical trials will be accompanied by specific information concerning the type of activity induced at the molecular level, the receptors involved and graphic representation of the predicted signalling pathways activated at the cellular level. This information will enrich the activity data by providing an overall framework on drug kinetics action potential of the compounds. It will also continue study of diatoms with particular regard to enzymes involved in lipid metabolism.

This metabolic pathway determines, in part, the nature and quantity of the lipids available in the cells and is therefore relevant for increasing the exploitation potential of microalgae in the production of polyunsaturated fatty acids beneficial to humans (omega - 3) of great interest for the food industry and for nutraceuticals. Diatoms will also be engineered for the enzyme involved in the biosynthesis of the oviotol for the development of a production system that is eco-friendly. Even benthic diatoms, that have been poorly studied due to difficulties in their cultivation in the laboratory as opposed to planktonic species, will be further explored, since previous results have identified bioactive fractions with potential in biomedical applications.

further objective will А concern the identification and characterization of an active compound produced by a cyanobacterium, which has previously been shown to have powerful antimicrobial activity. This study could lay the foundation for a possible development of the active compound as an anti-parasite in aquaculture facilities, to manage planktonic toxic algal blooms and for possible applications in the biomedical field, since the activity of extracts of this cyanobacterium against flagellated parasites is superior to that of medical devices already approved for human use (Flagyl). Moreover, we will continue to explore the biotechnological



potential of marine macroorganisms such as sponges, tunicates and echinoids, in order to identify bioactive compounds and explore the potential application of already identified molecules, such as ovothiol from sea urchins. To discover other possible applications related to human health, ongoing studies on the role of ovothiol on sea urchin will be completed. Studies on the temporal and spatial expression of the enzyme involved in its biosynthesis, together with data on gene perturbation, will allow us to get further insights into the functional role of ovotiol in sea urchin.

Previous studies have indicated that crude extracts derived from the marine sponge *Geodia cydonium* and the tunicate *Ciona intestinalis*, possess anti-inflammatory activity and inhibit cell proliferation. We also intend to continue studies on the bio-functional potential of the sea urchin *Arbacia lixula* and other species of echinoderms, such as sea cucumbers (*Holothuria spp.*), by screening crude extracts deriving from different body compartments of these organisms. At the same time, attempts will be made to develop cultivation methods to overcome problems for their use related to the seasonality of the reproductive period, to provide sufficient biomass for the isolation of bioactive metabolites.

In parallel, approaches that integrate transcriptomic and proteomic sequencing will be developed to identify bioactive peptides in specific tissues involved in feeding in poisonous venom-producing marine invertebrates. This methodology is complementary to the approaches of large-scale screening, based on the knowledge available on the natural history, the phylogenetic relationships, and ecological adaptations of these organisms to select the most promising targets in a biodiscovery perspective.

Attention will also be paid to the identification of other organisms belonging to poorly explored species in the macrobenthos, in order to identify new biological resources for biotechnological applications. Using SZN's taxonomy competences, within the project ExPO, species belonging to groups such as annelids, sipunculids, nemertids, gastropods, and others will be collected according to different ecological criteria, such as the capacity to live in extreme and / or polluted environments, invasiveness, breeding skills, etc. The screening activity which will take place mainly at the SZN, will cover biological tests carried out on animal and cellular models selected ad hoc for the in vitro assessment of different bioactivity potentials: anticancer, anti-inflammatory, antibiotic and antioxidant. In particular, the research activity will make use of innovative methods for the study of neuroactive substances based on automated screening of the locomotor phenotype of embryos and larvae of ascidians and fish to determine the presence of potential anti-neuroinflammatory, anti-convulsive and neuroprotective activity of marine compounds. We also intend to initiate the study of microorganisms that live in association with marine invertebrates, as it is widely acknowledged that these microorganisms may be the true producers of most secondary metabolites present in marine organisms. Several microbiomes will be characterized associated with macroorganisms, through the use of metagenomic approaches to assess their potential as a source of biologically active natural marine products.



Biomonitoring of Pollutants and Bioremediation - In the past few decades, marine environmental pollution has been on the rise due to increased human activities, such as the marine traffic development and the rapid industrialization. Amongst the pollutants, polycyclic aromatic hydrocarbons (PAHs), heavy metals (HM) and emerging pollutants represent a serious threat to marine organisms and, therefore, to human health. This research line is aimed at monitoring pollutants in seawater, sediments and endemic marine organisms, that represent excellent bioindicators, and the assessment of the impact that pollutants have on marine fauna. Moreover, the remediation of polluted sites through the use of microorganisms (bioremediation) has proven to be an effective and reliable approach thanks to its ecofriendly features. One of the objectives of the SZN Marine Biotechnology Department is to identify the autochthonous bacterial marine community, ecologically important in polluted environments, and to select species tolerant to traditional and emerging chemical pollutants, with particular attention to the identification of hydrocarbonoclastic bacteria and new bioactive compounds involved in bioremediation (e.g. siderophores, biosurfactants). Another objective of the SZN Marine Biotechnology Department is to isolate and culture microalgae from HM polluted environments in order to evaluate their potential use for HM bioremediation. Understanding and characterising interactions between HMs and microalgae may pave the way to improve our knowledge on the bioremediation of marine environments. HMs can be immobilised by passive adsorption onto cell walls, and microalgae can also actively transport HMs through their cytosol and accumulate them within chloroplasts, mitochondria, and vacuoles. This research line is aimed at evaluating the effectiveness of microalgae as biosensors of HM pollution, and their ability to remove metals from aquatic environments.

Symbiosis as underexplored sources of biologically active molecules - Symbiosis naturally occurs in marine environments as survival strategies, by involving different kinds of interactions and several taxa of marine organisms, often including marine invertebrates and microorganisms. The establishment of complex interactions networks between cells, which often is accompanied by the microbial production of secondary metabolites, relies on symbiotic relationships. This aspect is still poorly explored in extreme environments. The research line is focused on the study of microbial symbionts of temperate and polar origin as producers of molecules (i.e. biosurfactants and exopolysaccharides) with potential application in several fields, e.g. pharmaceutical, medical and bioremediation. The final aim is the discovery of new producers/compounds with higher efficiency and sustainable application.



Biotechnology for biomaterials - Microalgae offer extraordinary opportunities for research and development of eco-sustainable materials due to their ability to grow and accumulate biomass at a higher speed than terrestrial plants. Microalgae also contain an extraordinary diversity of secondary metabolites, some of which constitute biopolymers with a protective function. Numerous compounds deriving from terrestrial plants and currently undergoing biotechnological research, such as cellulose, could be replaced by compounds of microalgal origin. Microalgae are able to produce biopolymers with a protective function known as algaenans.

Algaenan is a highly refractory biopolymer that seems to be attractive for the development of bioplastics and is present in the cell wall of Eustigmatophyceae, as well as green algae from the genera *Scenedesmus, Tetraedron, Chlorella, Haematococcus,* and *Botryococcus.* Furthermore, the major monomers of algaenans from Eustigmatophyceae, that are long chain diols and long chain hydroxy fatty acids, possess a chemical structure similar to that of some plant lipids (ricinoleic and lesquerolic acids) that are used for the development of fuel lubricants.

Recently, marine organisms have been considered as promising sources of collagen. Due to its unique properties, collagen is well-known as a structural support for biomedical devices, dermal implants and emerging health applications, as well as being used in the fields of nutraceuticals, cosmeceuticals, food and beverages. This research line aims to exploitation of underused marine raw materials of the Mediterranean Sea (fishery by-catch marine species, and waste from fish processing industries and aquaculture facilities) to the development of a sustainable and efficient process for collagen extraction, with a significantly reduced impact on the environment and economy. The use of discarded and underused biomass allows to achieve the European Zero-Waste Strategy, which supports all three generally accepted goals of sustainability: sustainable economic well-being, environmental protection, and social well-being.



4.6 METRICS OF SCIENTIFIC PRODUCTIVITY

The main scientific findings obtained during 2019 were reported 309 scientific publication in peer reviewed international journals with impact factor, with an increase compared to the previous year and a significant increase on the average of the three-year period 2016-2018 (average: 220 papers / year, time interval: 2016-2018). This corresponds also to an increase in the potential scientific impact of the publications of the SZN, especially given that in the course of the previous three years (2016-2018), the average of the ISI papers published in journals classified in the first quartile of ISI impact factors stood at around 104 publications, which was increased to 137 publications in Q1 journals in 2019 (source Web of Science).

The growth in capacity for scientific dissemination seen in 2019 is the result of:

- i. increases in the research capability of SZN
- ii. growth in the understanding of SZN researchers of the need for scientific dissemination of results
- iii. the strategy of scientific publication incentives adopted by the institution

The incentive mechanisms are unique among Italian research institutions (EPR). These have formed the basis of funding distribution systems that are linked to the scientific performance of researchers and technologists, which allows for a fairer performance-based distribution of financial resources. This in turn increases research capacity and encourages innovation and the exploration of alternative resources of funding, while rewarding productivity and initiative. Many initiatives have been aimed at strengthening the participation of SZN researchers in the European Research and Innovation Programs, first and foremost those falling within the scope of Horizon 2020. Ample space has been given to the enhancement of the 'training' tools available, through innovative PhDs thus encouraging the contribution of doctoral students to scientific production.

The recent VQR assessment (2011-2014) conducted by ANVUR found that the Stazione Zoologica of Naples Anton Dohrn is positioned at levels of great value in the Disciplinary Sector (Biological Sciences). In particular, the percentage of products delivered relative to that expected is on average 98.2%, higher than the average of research bodies supervised by MIUR (95.4%). According to the ANVUR assessment, the scientific production of the Stazione Zoologica Anton Dohrn of Naples is characterized by a percentage of products rated as "excellent" or "high" that is above the Area average. The value of IRFS is higher than the share of expected products, which means that the institution has a qualitative-quantitative weight higher than the share of expected products.

We anticipate that the ANVUR evaluation for the period 2015-2019 will see the presentation of 100% of the expected research products.

Istituzione	Somma punteggi (v)	# Prodotti attesi (n)	Voto medio (I=v/n)	Voto medio normalizzato (R)	Num comp	Num. istituzioni compl.	% prodotti A+B	X
SZN	77,1	107	0,72	1,24	3	6	74,77	1,29
CNR	1221,5	2165	0,56	0,97	4	6	55,94	0,96
CREA	51,4	100	0,51	0,88	6	6	48	0,83
OGS	22,1	47	0,47	0,81	7	6	42,55	0,73

List of surveyed research institutes classified in Area 05. The table contains the sum of the scores obtained, the number of expected products, the average mark, the R indicator, the percentage of excellent products. The column that reports the ranking refers to the position of the institution in the absolute ranking. The table also contains information on the total number of entities that have presented at least 7 products in the area. The rankings are built on the basis of the R indicator.



Scientific publication per year (1929-2019)

Trend of publications in the last 91 years @SZN (source Web of Science) Search for: AD = (Napoli OR Naples OR NA OR Ischia OR Messina OR Palermo OR Milazzo) AND AD = (dohrn OR dorhn OR dhorn OR st* zool* OR stazione). Data exclude: DATA PAPER OR MEETING ABSTRACT OR CORRECTION. Temporal framework: 1929-2019



Sum of Times Cited per Year



Citation trend for SZN publications in the 1990 – 2019

Number of articles and citations in the last two decades (1999-2019)

Number of articles published	2775	
Sum of citations	84352	
Average citations per article	30,62	
H-index	118	

In the last three years the SZN rate of publication (data from the Web of Science Core Collection, only journals with Impact Factor) has grown significantly compared to the average of the previous decade.

Number articles published in 2019	309
Number articles published in 2013	95
Number art. published 2015-2018	713

534 MARINE FRESHWATER BIOLOGY	226 Multidisciplinary science	114 2001.05Y	68 BIODIVERSIT CONSERVATI	66 FISHERIES	65 Chemistry Medicinal	65 MICROBIOL
244 BIOCHEMISTRY MOLECULAR BIOLO	193 environmental sciences	102 Developmental Biology	62 NEUROSCIEN	ICES 54 REF		50 BIOPHYSICS
242 oceanography	132 PLANT SCIENCES	81 GEOSCIENCES MULTIDISCIPLINARY	59 EVOLUTIONA BIOLOGY	IRY 44 PHY	SIOLOGY	37 ENDOCRING
226 ECOLOGY	124 CELL BIOLOGY	74 BIOLOGY	58 TOXICOLOGY	43 BIO APP	Technology Lied Robiology	24 ANATOMY MORPHOLD

Distribution of SZN publications in Web of Science categories for the period 1999-2019

70 MARINE FRESHWATER BIOLOGY	30 ECOLOGY	18 Chemistry Medicinal	13 zoology	11 BIOTE APPLI MICR	CHNO IED DBIOLO	11 MICROBIC	11 TOXICOLOC
48	23	18 FISHERIES					
ENVIRONMENTAL SCIENCES	MULTIDISCIPLINARY SCIEN		10 GEOSCIENCES MULTIDISCIPLINARY		9 9 FOOD SCIENCE P		9 PLANT SCIENC
32	22	17 EVOLUTIONARY BIOLOG	Part the other bard		20000		
OCEANOGRAPHY	BIODIVERSITY CONSERVAT		9 BIOLOGY		7 BIOCHEMICAL RESEARCH		6 NEUROSCIEI
30 BIOCHEMISTRY MOLECULAR BIOL	18 CELL BIOLOGY	17 GENETICS HEREDITY	9 DEVELOPME BIOLOGY	NTAL	6 MATH COMP BIOLO	EMATICAL PUTATIONA	4 ENVIRONME

Distribution of SZN publications among WoS categories during 2019.

373 MARINE FRESHWATER BIOLOGY	153 Oceanography	73 PLANT SCIENCES	63 CELL BIOLOG	56 CHEMISTRY MEDICINAL	55 BIOTECHNO APPLIED MICROBIOLO	54 BIODIVERSI CONSERVA
197	128 BIOCHEMISTRY MOLECIII A	73 2001.06Y				
MULTIDISCIPLINARY SCIENCES	BIOLOGY		53 DEVELOPMENTAL BIOL		18 MCROBIOLOGY	28
170 ENVIRONMENTAL SCIENCES	104 GENETICS HEREDITY	69 FISHERIES 65 GEOSCIENCES MULTIDISCIPLINARY				REOROSA
			53 TOXICOLOGY		26	20
					20 REPRODUCTIVE SIGLOGY	20 PHYSIOLOG
154 ECOLOGY	77		48			
			BIOLOGY		21 BIOCHEMICAL RESEARCH METHODS	16 Immunolo

Distribution of SZN publications within WoS subject categories, ten-year period 2010-2019

The relevance of the international collaborations in SZN research is apparent also from the co-authored list of ISI-listed publications, years 2018-19.

Countries/Regions	records	Countries/Regions	records
FRANCE	95	ARGENTINA	5
USA	95	CHILE	5
SPAIN	84	ESTONIA	5
GERMANY	77	ALBANIA	4
ENGLAND	57	CZECH REPUBLIC	4
GREECE	43	HUNGARY	4
CANADA	29	LEBANON	4
SCOTLAND	28	PANAMA	4
PORTUGAL	27	THAILAND	4
AUSTRALIA	25	WALES	4
CROATIA	25	JORDAN	3
NORWAY	24	MALAYSIA	3
BELGIUM	20	MEXICO	3
JAPAN	20	TAIWAN	3
SWEDEN	20	BARBADOS	2
CYPRUS	18	CAPE VERDE	2
NETHERLANDS	16	EGYPT	2
SWITZERLAND	16	IRAN	2
TURKEY	15	KENYA	2
DENMARK	14	NORTH IRELAND	2
IRELAND	14	SERBIA	2
ISRAEL	14	SOUTH KOREA	2
MONTENEGRO	11	UKRAINE	2
MALTA	10	BANGLADESH	1
AUSTRIA	9	BOTSWANA	1
SAUDI ARABIA	9	COSTA RICA	1
SLOVENIA	9	CURACAO	1
BRAZIL	8	ECUADOR	1
NEW ZEALAND	8	GIBRALTAR	1
RUSSIA	8	ICELAND	1
INDIA	7	LATVIA	1
LITHUANIA	7	LUXEMBOURG	1
MOROCCO	7	NEW CALEDONIA	1
BULGARIA	6	PAKISTAN	1
FINLAND	6	PERU	1
LIBYA	6	PHILIPPINES	1
MONACO	6	POLAND	1
PEOPLES R CHINA	6	ROMANIA	1
SOUTH AFRICA	6	SINGAPORE	1
TUNISIA	6	SYRIA	1
ALGERIA	5	URUGUAY	1

4.7 INCENTIVISING RESEARCH

The SZN is the first research institution to launch a program of scientometric evaluation of research. These procedures are not alternatives to those established by ANVUR. They represent a way to incentivise and improve the performance of individual researchers, thus contributing to improving the scientific and managerial performance of the Body.

The ANVUR evaluation provides reward mechanisms for funding of Public Research Bodies, based on the excellence of scientific research and on the ability to attract funding with competitive evaluation. To encourage this process, the SZN, in recent years has established a research incentive fund to be distributed to the Researchers and Technologists of the Stazione Zoologica within the following areas:

SCIENTIFIC PUBLICATIONS: scientific papers in ISI journals are rewarded with research funds on the basis of:

IF (Impact Factor) \geq 10 as first or last author;

IF \ge 10 as co-author; IF \ge 7 as first or last author, IF \ge 7 as co-author.

If there are multiple SZN co-authors, the rewards will be shared among the eligible peers.

APPLICATIONS FOR RESEARCH FUNDING: applications for external research funding are also rewarded, depending on the specific role (coordinator or partner).


5 INSTITUTIONAL RESEARCH ACTIVITIES

5.1 STRATEGIES FOR THE INSTITUTIONAL RESEARCH OF THE SZN

The SZN believes that the mission and the institutional mandate are divided into three strategic areas (see also the SZN Performance Plan): 1) scientific (curiosity driven) research, 2) institutional research 3) third mission.

The three strategic objectives of the SZN research are (1) Curiosity driven research, (2) Institutional Research, (3) Third Mission). These strategic areas correspond to strategic objectives that identify activities related to the development and management of large infrastructures, recognizing the key national and European role of the SZN and characterizes the institutional research of the SZN.

The management, promotion and development of Research Infrastructures has been one of the main aims of SZN since its foundation in 1872. SZN recognizes important institutional research activities, conducted in close connection with scientific research proper. This type of research activity, firmly founded on the availability within the Body of specific scientific and technological skills, allows the creation of a fruitful integration between fundamental research, applied research and institutional activities.

The institutional research pursued by SZN is carried out through the creation and management of research infrastructures designed to promote international collaborations. The SZN plays a key role in the creation of the Italian nodes of the ESFRI European Research Infrastructures, in particular EMBRC.

EMBRC is a distributed European research infrastructure working to facilitate the exploration and exploitation of marine organisms, a centre open to all researchers, European and non-European, built on the strong synergy developed between all partner "stations/institutions".

The recognition of the legal form of ERIC for the EMBRC European Infrastructure has been achieved through a two-step path, defined as step-one and step-two, to which all partners have been called to contribute. Each of the two steps is carried out through a verification by the European Commission. In particular, the SZN has coordinated the preparatory phase (step-one) of EMBRC, which was successfully completed on 31 January 2014, and which saw in 2018 the recognition of EMBRC as ERIC (European Research Infrastructure).

The SZN is the institutional headquarters of EMBRC Italia (Italian Joint Research Unit), the national scale component of the European Marine Biological Resource Center. EMBRC: The European Marine Biological Resource Centre is a European research infrastructure that aims to facilitate the investigation of marine organisms, a center open to all European researchers built on the strong synergy developed between all partner "stations / institutions".

SZN also carries out institutional research on behalf of MUR in relation to the FISR-CIPE activities for the characterization and remediation of the SIN in Bagnoli Coroglio.

5.2 MANAGEMENT OF THE EUROPEAN RESEARCH INFRASTRUCTURE - EMBRC

The management, promotion and development of Research Infrastructures is one of the founding reasons for the SZN since its foundation in 1872. As part of its institutional mission and in accordance with the provisions of the applicable PNR, the SZN shall recognize in its institutional mandate and carries out institutional research activities by implementing the design, implementation, management and maintenance of research infrastructures of an international and particular nature complexity.

The SZN manages, internally, a constellation of "Infrastructures". These infrastructures are the In-kind contribution of SZN to ESFRI Infrastructures to which SZN is committed to participate. SZN's research facilities have the following objectives:

- to provide services for research;
- to promote research and technological development for the research activities of SZN and third parties;
- to foster international collaboration, to contribute to the implementation of the Italian nodes of the European Research Infrastructures ESFRI: EMBRC, EMSO and Lifewatch;
- to provide internal and external users with a range of scientific and technological research services contributing to the realization of the third mission of the Institute.

The strengthening and maintenance of complex research infrastructures, including the implementation of the EMBRC-IT project, is the key aspect of Institutional Research conducted by SZN, as described above.

As exemplified in the Performance Plan, SZN's contribution to the project is implemented at European level and at the level of the Italian Joint Research Unit. At the national level, also as a contribution to the implementation of the European IR, the services offered by the Italian node to the ERIC have been defined. Some of these services have been tested in the context of pilot actions funded by European projects of the H2020 programme, such as EMBRIC and ASSEMBLE Plus. The integration of these services into the European ERIC is currently being defined.

The mission of the European Marine Biological Resource Centre (EMBRC) is to facilitate access to ecosystems and marine organisms and related data (e.g. long-time historical series, LTER) thus facilitating their use by the scientific community at large. The establishment of this research infrastructure will have positive medium- and long-term effects on regional, national and international socio-economic systems, allowing the acquisition of knowledge of the complexity of the marine ecosystems, forcing mechanisms at different spatial scales, and approaches for their conservation and sustainable use.

EMBRC is therefore based on the continuous dialogue between research and "service", encouraging studies that enable knowledge to be acquired, for example, on new molecules, genes and/or functions, and metabolic activities that can be considered as models for the development of new processes. EMBRC also aims to provide theoretical and practical training to the new generations of researchers, to prepare the logistics for *ex-situ* experiments including access to modern equipment for the study of biological phenomena, the development of new prototypes (with significant technological spin-offs and possible patents), assistance for the transfer of knowledge and technology and logistics to foster the hospitality of researchers.

On a national scale, the SZN coordinates the Italian JRU (Joint Research Unit) which involves the National Research Council (CNR, after a reorganization of the institutes now with IAS, IRBIM and ISMAR), the Inter-University Consortium for Marine Sciences (CoNISMa) and the National Institute of Oceanography and Experimental Geophysics (OGS). This node is known as EMBRC-IT. Among them, the headquarters of ISMAR in Venice and the headquarters of IRBIM in Messina together with the SZN Ischia and Naples sites are included in the European project ASSEMBLE Plus. The General Assembly of the Joint Research Unit in SZN was held on 2 and 3 April 2019. The General Assembly approved the action plans proposed by the Director of the EMBRC-IT, deciding to define the services to be proposed in the EMBRC-IT and agreed to set up a working group with the objective of analyzing the available services within the JRU, as well as identifying the main services to characterize the node and the "core" business of EMBRC- IT. The JRU manager presented the data of the working group to the General Assembly on 13 November 2019, which carefully evaluated them and finally produced a list of services that are currently available in the EMBRC offer. This action has in fact inaugurated the full operation of the Italian node. In order to enhance and complete the offer, the JRU is committed in the short term to address other points, such as the implementation of the Nagoya protocol, the optimization of Knowledge and Technology Transfer processes, and the sharing of good working practices through the exchange of personnel.



5.3 NETWORKING AND MANAGEMENT ACTIVITIES IN RESEARCH INFRASTRUCTURES

As specified in section 1.3 (National and International Scientific Context) the SZN continues to actively operate in a series of networking activities that stem from the Participation in the Network of Excellence (NoE) on Marine Biology launched by the European Union during the Sixth Framework Programme (FP6) and subsequent programmes Framework, such as: MARBEF, Marine Genomics, and Euroceans. These projects and NoE were the basis for the subsequent development of ESFRI Research Infrastructures, such as the EMBRC, which saw the coordination of Italy, through the Stazione Zoologica, of the first preparatory phase. EMBRC, which has now become a European Research Infrastructure Consortium (EMBRC-ERIC) has facilitated the creation of EMBRIC as a large cluster of IR at European level, with the general objective of creating interconnectivity along three dimensions: the science, industry and regional policies for research, development and innovation (RDI). The final result is the formation of a stable group of research Institution federated in research Infrastructures (RIs) to foster innovation in the field of marine biotechnology. CORBEL, another European cluster with the participation of the SZN, favours at European level an alignment between RIs, including at the national level, by encouraging the development of a plan for synergistic coordination for research infrastructures as represented in the National Plan of Research Infrastructures (PNRI) with the aim of strengthening the research system with (i) a strategy guided by competitiveness and integration of resources (ii) a coherent action plan, avoiding unnecessary redundancies. The type of actions described above will allow the development of different approaches for access to research sectors and national and international markets. With regard to the sector of protection and management of the marine ecosystem, rather than that of the farming of marine organisms of aquaculture and industry, there is ample scope for satisfaction of domestic demand and, since there is no dominant position by the industries concerned. In some cases, it will be possible to access the international market. With regard to the area of medicines and new approaches to human health, the current industrial structure sector present in the country can, only in some cases, go as far as supporting the launch of the product at international level, but it will be able to benefit to a large extent from agreements on exploitation of international licences and patents.



5.4 CHARACTERIZATION AND RESTORATION OF CONTAMINATED SITES OF NATIONAL INTEREST

Defunct industrial activities are responsible for persistent and ongoing environmental degradation, mainly due to long-term accumulation of toxic contaminants in the environment. Such a chronic form of pollution represents a major threat for human health, biodiversity and ecosystem functioning. Necessary environmental remediation practices should however be coupled to restoration plans aiming at revert the degradation trend and give back healthy areas able to provide valuable ecosystem goods and services. Albeit fully integrated into the EU Restoration Agenda, marine environmental restoration is a new challenging issue in ecology, with Italy coordinating MERCES, the first European project in this field. The environmental restoration of Bagnoli-Coroglio Bay is a unique challenge at European level. ABBACO will develop new approaches for the removal and remediation of contaminated sediments and restoration of marine habitats in collaboration with the Government Chief for the area and INVITALIA. Actions include: i) identifying the environmental benchmark of the area; ii) assessing its present health status, iii) studying the effects of contaminated sediments on biodiversity and ecosystem functioning (MSFD), iv) assessing the combined effects of multiple stress at a hierarchical level; (v) experimenting innovative methods of transplantation and restoration of key species and habitats, and new biotechnological instruments for the remediation of sediments (bioremediation, bioaugmentation) in degraded habitats. ABBACO will provide novel expertise and stimulate new initiatives within the Blue Economy Agenda.



5.5 THE NAGOYA PROTOCOL ON THE ACCESS TO MARINE GENETIC RESOURCES

The Nagoya Protocol which came into force on 2014, regulates access to genetic resources and the fair and equitable sharing of benefits arising from their utilization. The Nagoya Protocol has been implemented in the EU by Regulation (EU) No 511/2014 on compliance measures for users deriving from the protocol. The Regulation aims at uniformly implement in the European Union the Nagoya Protocol on access to genetic resources and on the fair and equitable sharing of the benefits deriving from their use, in particular the so called "user compliance pillar". This expression identifies the set of Protocol's rules that establishes clear due diligence duties upon Member States with the goal of guaranteeing that the users operating within their respective jurisdiction comply with the domestic legislation or regulatory requirements of the contracting party providing genetic resources. The Regulation applies to any user, natural or legal person, that utilises genetic resources or traditional knowledge associated with genetic resources" (Art. 3, par. 4 Regolamento ABS), regardless of its size or use for which the resources are intended (commercial or non-commercial). In the ABS Regulation 'utilisation of genetic resources' means to conduct research and development on the genetic and/or biochemical composition of genetic resources, including through the application of biotechnology as defined in Article 2, let c, of the Nagoya Protocol. In particular, the access and benefit-sharing (ABS) of marine genetic resources (MGR) within the national jurisdiction received little interest among scientists and in the international debate. This may seem surprising, given that most of the MGRs with the potential for scientific discovery and industrial applications, including deep-water organisms, are currently (predominantly) of national competence. Many countries could undoubtedly benefit from better guidance on how to implement effective national regulatory frameworks on ABS with respect to MRG and marine bioprospecting activities in maritime areas under their jurisdiction. Inform the scientific community on the evolution of the international framework governing the access and use of these resources would be just as relevant. Italy has not yet adopted any legislative measures to implement the ABS Regulation, placing itself lagging behind the majority of European countries that have largely already fulfilled these obligations (including, for example, Denmark, Finland, France, Spain, Germany, Great Britain, Hungary).

In the next three-years 2020-2022, the SZN will support the Italian Ministry of the Environment (MATTM) in the drafting of the Regulation, which requires the adoption of some specific measures, namely:

- a) the designation of the Authority, responsible for the application of this Regulation;
- b) the designation of Checkpoint (the authority involved in the monitoring phase);
- c) the adoption of a control system for registered collections and users;
- d) the adoption of a framework of penalties for the violation of regulatory obligations.

A draft of the bill for transposition in the Italian legislation of the EU ABS Regulation n. 511/2014, in accordance with the provisions of the Nagoya Protocol, is currently under discussion. While awaiting Italy's full implementation of the ABS Regulation, it would be appropriate if all potential recipients, who work in our country, are informed of the new obligations deriving from European and International legislations, to help them to evaluate the risk of "non-compliance" under the ABS Regulation and to adopt all the necessary precautions to reduce such risks. The absence of a specific penalties framework for the violation of the Regulation cannot exempt from the obligations regarding ABS.

The Regulation requires the establishment and maintenance of a European register of reliable collections of genetic resources that meet the Regulation criteria for their acquisition, preservation and

management. Users that obtain a genetic resource from a collection included in the register should be considered to have exercised due diligence as regards the seeking of all necessary information.

Article 8 of the Body of Rules ABS establishes a framework of best practises (procedures, tools or mechanisms) developed by associations of users or other interested parties that must be applied in the different sectors in accordance with the requirements of the ABS Regulation.

- Competence in the identification of organisms with different techniques;
- Identification of potential MGR holders (museums, MPAs, aquariums, research institutes and universities, foundations;
- Certified collections of living (research institutes, universities, aquariums) and preserved (research institutes, universities, museums, herbaria) organisms;
- Type of collections and origin of the MGR present;
- Documents preparation to access marine genetic resources;
- Didactics on the use of genetic resources in the scientific research.



5.6 SUPPORT TO CITES FOR MARINE PROTECTED SPECIES

CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) is an international agreement between governments since 1973. Its aim is to ensure that international trade in specimens of wild animals and plants does not threaten their survival. CITES is part of the UN activities for the environment (UNEP) and its implementation is legally binding for all signing States (Parties). Currently, all parties that have joined the convention are UN members. Since January 1st 1984, the Community has been implementing the provisions of CITES through common Regulations that, with regard to certain species, impose stricter conditions than those required by CITES itself.

The Regulations currently in force are as follows:

- Council Regulation (EC) No 338/97 on the protection of species of wild fauna and flora by regulating trade therein;
- Commission Regulation (EU) No 865/2006 (layng down detailed rules concerning the implementation of Council Regulation No 338/97);
- Commission Regulation (EC) No 100/2008 amending Regulation (EC) No 865/2006;
- Commission Regulation (EU) No 791/2012 amending Regulation (EC) No 865/2006 laying down detailed rules for the implementation of Council Regulation (EC) No 338/97;
- Commission Regulation (EU) No 870/2015 amending Regulation (EC) No 865/2006;
- Commission Regulation (EU) No 865/2006 (consolidated text);
- Commission Implementing Regulation (EU) No 792/2012 laying down rules for the design of permits, certificates and other documents provided for in Council Regulation (EC) No 338/97 and amending Commission Regulation (EC) No 865/2006;
- Commission Regulation (EU) No 57/2015 amending Regulation (EC) No 792/2012;
- Commission Regulation (EU) No 792/2015 (consolidated text);
- Commission Implementing Regulation (EU) No. 2019/1587 prohibiting the introduction into the Union of specimens of certain species of wild fauna and flora;
- Commission Regulation (EU) No 2017/160 (amending the Annexes of the Regulation No 338/97) Relevant Italian legislation
- Law No 150 of 7 February 1992 sanctioning the offences related to the implementation of the Convention on International Trade in Endangered Species of Wild Fauna and Flora in Italy, amended by Law No 59 of 1993, by Law No 426 of 1998 and by the Legislative Decree No 275/2001; (Consolidated text Law 150/1992)
- Decrees of the Ministry for the Environment of 19 April 1996 and 26 April 2001 listing all the species (dangerous live animals) whose introduction into the national territory is forbidden.

The SZN carries out institutional support activities for the harbour authorities and the Finance Guard, in particular for the identification of marine species, especially, for example, reptiles. In the three-year period 2020-2022, the SZN intends to develop a Memorandum of Understanding with the Ministry for Economic Development (MIISE) for strengthening the identification capacity of species and products originating from protected and endangered species.

5.7 RECOVERY AND REHABILITATION OF MARINE TURTLES

The SZN represents the largest center in Italy for research, recovery, care and rehabilitation of marine turtles. The Turtle Point (where is conducted the research on marine turtles) is equipped with a surgery room and a radiological unit, and with spaces dedicated to the care and rehabilitation of the marine turtles impacted by human activities. The SZN carries out education and training activities for the staff of the Regions (e.g., Lazio, Campania) and receives specimens that found in difficulties on Campanian coasts, but also, upon request, from Lazio, Puglia, Calabria, Sicily, Basilicata. The center collaborates actively with sanitary service ASL in Campania and with the Coast Guards.



A research program on animal welfare criteria for temporary recovery and maintenance of sea turtles authorized by the Ministry of the Environment, has been implemented in 2019. This evaluation will take into consideration all the parameters involved in the management of single individuals at the center and after release in the natural environment.

To raise public awareness on the protection of marine ecosystems, this program with such charismatic species, is also carried out in presence of students and public visitors for educational purposes.

5.8 DATABASE MANAGEMENT

In the course of normal research operations, the SZN has created and maintains a series of databases, of which some briefly described examples are:

Website on Mediterranean Phytoplankton (WEMP) - WEMP provides a general taxonomic description for ten algal groups and for certain genera and species, along with biology and distribution information. The species listed in the WEMP were selected mainly on the basis of a list of the most abundant or important species in the Mediterranean area. The illustrations are largely taken from the collection of images from the Optical Microscopy, Scanning Electron Microscopy and Transmission Electron Microscopy of SZN's Electron Microscopy Service, confocal and bioimaging facilities, and the Marine Organisms Taxonomy Service of the Stazione Zoologica. A considerable number of images have also been produced using new technologies (digital photography, both optical and electronic).



Zoological Collections Database (http://szn.i.hosei.ac.jp/HTML/index.php) - This database is composed of 4 373 videos, 18 212 static images, and 7 771 collection labels relating to specimens preserved and reared at the SZN since 1910. In addition, the database contains the digitized version of 38 documents (for a total of 8 406 pages) containing important taxonomy, ecology and phylogenetic information.

The LTER-MareChiara Gulf of Naples website (<u>http://szn.macisteweb.com/</u>) - The MareChiara research program, started in 1984, is aimed at studying the structure and functioning of the plankton of the coastal pelagic system of the Gulf of Naples, in relation to environmental characteristics and climate change. The LTER-MC sampling site is located at a depth of around 75m, two miles off the city of Naples, in a dynamic area situated as it is between coastal habitats that are affected by terrestrial activities, and offshore habitats that characterise open waters of the Southern Tyrrhenian Sea. For the management and analysis of environmental and planktonic data collected at the LTER-MC station we use the MACISTE system. MArine Coastal Information SysTEm is an integrated information system that manages interdisciplinary environmental data (physical, chemical and biological) and specialized cartography by a flexible means of interrogating the database to address specific queries (reporting, maps, WMS WFS services, etc.).

6 THIRD MISSION ACTIVITIES

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6.1 PURPOSE AND OBJECTIVES

The third mission activities of the SZN fall within the statutory objectives of the institution (articles 2 and 3). In particular, the purposes and activities of the third mission are:

- contribute to the protection of the environment and the improvement of quality of life by promoting partnerships with companies and other entities for the purpose of developing knowledge and applying research in the marine sciences and biomedical fields;
- contribute to the promotion and development of social, cultural, and economic progress of the country by promoting industrial collaborations, spin-offs, patents, scientific dissemination, and training;
- conduct industrial research and product development in collaboration with, or at the request of companies;
- promote the training and career growth of researchers through involvement with both private and public entities, including support for degree courses and research doctorates organised through agreements with both Italian and foreign universities;
- promote and disseminate scientific culture and knowledge of the marine environment, the latter through the aquarium, scientific collections and the library;
- collaborate with the regional and local administrations in order to promote, through joint research initiatives, the development of local natural resources;
- conduct client-driven technical or scientific consultancy services for public or private entities.
- implementation rescue and research projects complementary to those developed out in the Reserach Departments, on mega- and charismatic marine species, which are particularly suitable for carrying out communication and dissemination activities for conservation of the marine environment.

The SZN has identified a series of objectives for the next three years:

- increase the number of doctoral students carrying out thesis research at SZN by 10% per annum;
- development of a new course for the International Master Degree in collaboration with the University Federico II of Naples;
- increase the number of university courses offered;
- organise at least three events (summer school or workshops) per year;
- increase third party activities by at least 20% compared to the previous three years;
- participate in at least three television programmes per year;
- publication of at least three scientific publications per researcher per year;
- interact with at least 1000 primary or secondary school pupils per year;
- conduct seminars or other events for at least 10 000 people per year;
- renegotiate the activities with Fondazione Dohrn
- opening of DaDoM (Darwin Dohrn Museum) at the Casina del Boschetto
- restoration and opening of Aquarium and Fano aquarium
- become fully operational with the public incoming at Turtle point
- full operation of the Grant and Innovation Office (BoD Resolution No. 3 of 29 June 2016)
- filing of at least three patent applications;
- creation of at least 1 Spin off.

In addition, SZN is working on the creation of a regional hub for biotechnology of marine origin, and the creation of a sea farm at Bagnoli that will, over the next few years, constitute two important initiatives to increase the capacity for innovation in marine biotechnology and aquaculture.

To this end, an important step forward was the signing of the Inter-institutional Agreement between Invitalia SpA and SZN which took place on 18/04/2018 for the concession of the above-mentioned Bagnoli areas. This agreement also saw the authorization of the Municipality and Region for the new developments.



The III Mission Area formally started in October 2019 with the definition of priorities and the reorganization of the functions and related activities. The organization of the area provides work by objectives which, in addition to those mentioned above and, can be detailed as follows:

2020 Goals:

A. **reopening and inauguration of the Aquarium by carrying out the following activities:** a series of design restoration, and reviews led to substantial changes to be made to allow the re-activation of the aquarium

- preparation of curatorial spaces, quarantine rooms, laboratories, food preparation rooms, veterinary and necropsy room: essential to function and support animal management and husbandry

- applications for ministerial permits to be activated to allow the opening (Wildlife exhibition, fishing authorization for structures open to the public, CITES and institution registration as a scientific institute, loading and unloading of sea water, wake for service rooms and curators etc.)

- reorganization and start-up of Animal Welfare Committee and, possibly, restoration of an Ethics Committee to carry out ethical assessments related to animal welfare and other ethical clearances such as environmental, social, scientific correctness, protection of any human beings involved in the various research steps, etc. etc.

- restructuring and authorization of Animal Facility for the Animal welfare regulation

- preparation of authorized animal transport vehicle and procedure

-preparation of educational panels to be included in the exhibition context, for the public and for the development of laboratories and workshops (the latter is expected to involve Casina Pompeiana thanks to an agreement being drafted with the Municipality)

- conception of the SZN communication materials: an agreement was made with the Accademia delle Belle Arti to create an institutional identity profile (including logo and gadgets declination, panels, signs etc) taking into account the synergy necessary between the different facilities; for the latter, the name and acronym was defined in order to respect unicity in communication and image identity. In the same area of collaboration there is also support for the revision of the signs inside the Villa to standardize it to all the structures and support for the preparation of the exhibition teaching materials.

- logistical organization of the incoming reception and access facilitation to the Villa for events and for the daily management of the public: this work involves the Municipality and the bodies responsible for urban planning, safety, culture and tourism and the Dohrn Foundation.

B. **DaDoM** renovation and content design, assessing pathways and flow management, aiming at opening to the public in spring 2021. A working group made up of AIIIM staff, architects and the President of Dohrn Foundation is working on defining the Museum scientific content and storyboard. This context is extremely complex to harmonize the ancient with modern with a clear and linear display of the items within the museum exhibits. This will allow dissemination about marine sciences and increase awareness on human impact on the protection of ecosystems

C. **Turtle Point** reorganization of Portici for guided visits and for public display of animals in compliance with current legislation. Carry out research projects on mega- and charismatic species that facilitate outreach and engagement of the public in active conservation of marine environment.

D. Fano aquarium design and renovation

E. **Marine farm and Aquarium in Bagnoli**: contribute to marine animal farming and algal cultures and will provide model organisms for the scientific and industrial research at contribute to restoration actions and re-stocking.

F. **Grant and Innovation Office:** implementation of the activities and training in compliance with the above-mentioned programme, providing for up to 4 permanent figures at the GIO and an additional technician in the training sector, within the general objectives of the Third mission Area

G. **Communication/dissemination and outreach**: organization of the entire sector which primarily includes the activity of the SZN press office, together with the preparation of activities on the web and social networks and revision of the SZN website. This work started in the first month of 2020 and will take at least 6 months to be implemented in an organic and operational way also through the criteria defined by SZN. In general, the communication activity among different facilities will be harmonized, the signage will allow identification of the individual structures for tourists and citizens within the Villa Comunale, and identifiable as SZN.

H. **Dohrn Foundation**: implementation of the agreement to carry out the activities related to the management of the various categories of visitors in facilities open to the public: reception management, ticketing systems, gadgets and gift shops, transfer of content and educational materials for guided tours and workshop

Goals 2021:

A. DaDoM inauguration: this important event will also represent an opportunity for the launch of an educational multimedia App with professional underwater stories and images about the sea
B. Marine library opening is expected to take place at the turn of the following year.

Human resources analysis:

To carry out all these activities, numerous specific topic meetings have been scheduled. The implementation of staff dedicated to individual sectors was necessary and the review of the structure of the Area provides the identification of specific activities and expertise (often inerdisciplinary) to harmonize the work with all the others, in order to achieve common objectives. The forecast of implementation of resources will be gradually completed in 2022 when most structures should be able to be considered fully operational (apart from Bagnoli which will take a few more years).



6.2 SERVICES FOR RESEARCH AND THIRD PARTIES

SZN collaborates with national and international institutions to carry out research services and thirdparty activites. Research services offered by SZN are:

- 1) Analysis of environmental and biological variables,
- 2) Bioinformatics, building and maintenance of databases with emphasis on marine organisms,
- 3) Molecular biology, gene expression and sequencing,
- 4) Cell sorting and flow cytometry of marine samples,
- 5) Management of research vessels for institutional and client-driven at-sea sampling,
- 6) Taxonomic and molecular identification of marine organisms for impact assessment and broader scientific study,
- 7) Facilities for mesocosm studies,
- 8) Advanced microscopy, including SEM, TEM, confocal and bioimaging,
- 9) Environmental monitoring for public and private bodies,
- 10) Underwater sampling (Professional Technician for underwater operations, OTS, and Scientific Scuba Divers, OSS),
- 11) Experimental research in marine habitats,
- 12) Hydrographic surveys,
- 13) Census of marine biological resources.

The main projects are:

- Environmental monitoring of marine platforms for ENI-SYNDIAL in the Adriatic Sea,
- Port Authority of Salerno, environmental monitoring of port dredging and sediment dumping operations
- Environmental monitoring and sediment characterization of the Fusaro lake (three years project duration)
- Conversion of off-shore platforms for multiple sustainable uses;
- TERNA: Implementation of the environmental monitoring plan of the connection of the Campanian islands to the national transmission network, an alternating current connection of 150 kw Nuova SE of Sorrento and SE of Capri (acronym TERNA2) of 02/08/2018.

The creation of the Grant and Innovation Office provides support for the supply, promotion and negotiation procedures of contracts, making the SZN more administratively streamlined and provides a greater quantity of services for third parties. These improvements have already been demonstrated with previous clients based upon the quality and quantity of results produced.

In the 3-yr plan of activities 2020-2022, third-party revenue is expected to increase, as the following actions will be implemented:

- growth of the Grant and Innovation Office to reach a critical mass of at least four full time staff, with at least one dedicated the private contracts,
- management of research services provided within the European EMBRC infrastructure;
- implementation of a marine organism taxonomy service,
- implementation of a bioinformatics service,
- creation updating and promotion of a catalogue of SZN research services,
- enhancement of SZN research infrastructure,

- additions to the scientific and industrial network to promote the research skills available at SZN,
- promotion of public-private partnerships,
- organization of events, workshops and demonstrations aimed at creating useful contacts for the promotion of SZN research services,
- establishment of consistent contract models and administration for management of third-party contracts,
- implementation of a system of incentives for researchers and technical staff to be active in undertaking contract work for third parties.

In addition, the creation of the regional hub for marine biotechnology and the Marine Farm in Bagnoli will contribute to increasing the potential of the SZN for marine biotechnology and marine organism breeding.



6.3 MANAGEMENT OF INTELLECTUAL PROPERTY

Proper intellectual property management of SZN research results is a fundamental activity to protect both the good work of researchers and the SZN and to contribute effectively to the economic and social development of our country. The SZN, with the establishment of the Patent Commission and the Grant and Innovation Office, pursues the strengthening of technology transfer activities and, in particular, the enhancement of research results. In terms of patents, the following table shows the SZN patents, patent applications and applications in preparation, and demonstrates the growing SZN activity in terms of attention to the protection of intellectual property

Title	Inventors	Patent (or Patent Application) Number	Filing Year	Status
Light system for aquatic photosynthetic organisms	Brunet C. <i>,</i> Corato F	EP 13196793.7 EP2883950	2013 2020	EP Patent Application EP Patent (8.04.2020). Validation pending (ITA, DE, FR, UK, IS, Netherlands)
Procedimenti e composizioni per la produzione di astaxantina da organismi marini	Cirino P., Toscano A., Sansone C., Brunet C.	ITA 102017000053831	2017	Patent ITA
Automated apparatus and process for cultures of aquatic Organisms	Zupo V., Mutalipassi M.	PCT/IB2016/052128 EPO 16726161.9 EP 3282834	2016 2018 2019	PCT Application. EPO Patent Application Patent Validation (ITA, DE, FR, UK)
Ovotioli per il trattamento dell'Infiammazione Sistemica Cronica di Basso Grado (ISC) e delle patologie ad essa correlate	Palumbo A., Pandolfi A., Castellano I., Di tomo P.	ITA 102017000104529	2017 2020	ITA Patent Application (Al 31.12.19) ITA Patent (Al 28/01/2020)
Ovotiols for the treatment of chronic low-grade systemic inflammation (CLGSI) and related diseases	Palumbo A., Pandolfi A., Castellano I., Di tomo P.	PCT/IB2018/057098 PCT/IB2018/057098 USA 16/646716 EP 18782202.8 CN 2018800606770	2018 2020	PCT Application (il 17/09/2018). Patent Application Validation Pending national phases in 2020: USA EPO CN

5-tioistidine e derivati metilati (ovotioli) come inibitori di attività gamma- glutamil-transpeptidasi (GGT)	Castellano I., Palumbo A., D'Argenio G.	ITA 102018000010907	2018	ITA Patent Application (10.12.2018)
5-thioistidine compounds and methylated derivatives (ovothiols) as inhibitors of gamma- glutamyl-transpeptidase (GGT) activity	Castellano I., Palumbo A., D'Argenio G.	EP N. 19210282.0	2019	EP Patent Application (20/11/2019)
Dispositivo per la rilevazione di profili di	Passarelli A., Formisano F.,	ITA 102019000003391	2019	ITA Patent Application (08.03.2019)
temperatura, e relativo sistema di monitoraggio (SOPHIA)	De Vito S., Esposito E.	PCT/EP2020/056091	2020	PCT Application (06.03.2020)
Processo per la produzione	Fioretti S.,	ITA 02019000001577	2019	ITA Patent Application (04/02/2019)
di perle da bivalvi e gasteropodi commestibili	Patti F. P.	PCT/EP2020/052135	2020	PCT Application (29/01/2020)
Metodo e kit per predire la morte cellulare in risposta a stimoli biotici e/o abiotici	lanora A., Sansone C., Chiusano M., Romano G., Galasso G., Tangherlini M., Brunet C.	ITA 102019000012624	2019	ITA Patent Application (26/08/2019)
Dispositivo per il supporto,	Alagna A.	N/A	2019	Application under
di piante acquatiche da semi	F. Musco L.	ITA 102020000009046	2020	ITA Patent Application (27/04/2020)
GRow Alga Better - Sviluppo e costruzione di un fotobioreattore di nuova generazione	Brunet C., Sansone C., Galasso C., Balzano S.	N/A	2019	Application under Preparation
Genetic engineering system in diatoms for the eco-sustainable production of ovothiols	Russo M.T. Castellano I. Ferrante M.I. Palumbo I.	N/A	2019	Application under Preparation

The continuous scouting of new patentable results and internal awareness raising will allow a further increase in the SZN patent portfolio.

The legal protection of intellectual property also controls permission for the use of the results or prototypes, secrecy agreements and the definition of criteria for the protection of joint results from research projects or third-party contracts. The SZN has equipped itself with models to be used in the case of a Material Transfer Agreement and a Non - Disclosure Agreement which are used with the expert support of the staff of the Grant and Innovation Office. In addition, the Office assists SZN researchers in the negotiation phases with clients or scientific partners to better protect the previous and future intellectual property rights of the SZN.

The discoveries are patentable only if they are original inventions with industrial applications. The SZN has strategically focused on the enhancement of research products, giving a mandate to the Grant and Innovation Office to assist inventors in all phases of:

- searching for potential licensees of SZN patents and the possible negotiation of concession contracts;
- creation of spin-offs for the commercial development of inventions by the inventors themselves;

Training events have been organised for research staff on the basic concept of patenting, on the use of patent databases, and the concept of *open innovation*.

In the years 2020-2022, we have the following aims:

- submit further patent applications,
- raise awareness among SZN researchers on the importance of adding value to research,
- gather ideas for new patents and assist researchers to file applications,
- organise training events for inventors and those wishing to establish start-ups,
- participate in exhibitions, workshops and conferences to promote SZN patents.



6.4 PRIVATE COMPANIES PARTICIPATED BY SZN

The SZN supports the enhancement of research outputs developed at its structures also by supporting the establishment and development of Spin-off companies. With the support and direct or indirect participation in the Spin-off initiatives, the SZN intends to encourage:

- the transfer of scientific and technological knowledge to the economic and entrepreneurial systems, to provide opportunities for innovation and commercial development;
- the creation of new professional outlets for staff trained in the SZN;
- promoting relations with the production system;
- economic growth at local and national scales.

The SZN has approved the dismission of all private companies to which it participated (i.e., AMRA scarl, in 2015, BIOGEM scarl in 2018) and now participates only to the Dohrn Foundation and the spin-off Biosearch.

The active spin-off was set up in 2016: BIOSEARCH SRL is a joint stock company owned by SZN (10% of share capital) and CNR (10% of share capital), by researchers and research fellows of SZN and the CNR, and by other external individuals. BIOSEARCH operates in the marine biotechnology sector and aims at the industrial exploitation of molecules discovered in the SZN and CNR.

In 2017, the FARD Foundation (Antonio and Rinaldo Dohrn Foundation) was reactivated and renamed the Dohrn Foundation. It is a body governed by public law controlled by the SZN and aimed at managing the activities open to the public (e.g., aquarium, Turtle Point and SZN museum system). The turtle center was established in Portici and inaugurated on 20 January 2017. The historic aquarium is located at SZN headquarters and is undergoing renovation, and the Darwin-Dohrn Museum is being built inside the Villa Comunale of Naples at the Casina del Boschetto.

The Dohrn Foundation is a strategic resource for the viability of third mission activities of the SZN, rectifying past problems with overly costly outsourcing of ticketing to private companies. The Foundation will assist SZN in the management of public visits, ticketing, merchandising, donation collection, public engagement, and research in the field of scientific dissemination to the public.



Denomination	Tipology	Year of constitution	Notes
Dohrn Fundation	Foundation	1955 Reactivated on 2018	Called "Fondazione Antonio e Rinaldo Dohrn" until 17.02.2017 when renamed <i>Fondazione Dohrn</i> . Manages in-house facilities aimed at the public by promoting scientific dissemination and outreach and financing the Stazione Zoologica for its research The exclusive purpose is to manage in house the structures aimed at the public by promoting scientific dissemination and outreach and funding the Stazione Zoologica for its research
BioSEArch	SRL	2016	Spin-off formed from public research institutes, researchers, and ItalBiotec

In the three-year period 2020-22, the following objectives will be pursued:

- organize courses on entrepreneurship for young researchers and SZN students;
- stimulate and assist participation in national and international competitions to present business ideas;
- assist potential new entrepreneurs
- helping to draft business plans and in the creation of new companies;
- support the start-up phase of spin-offs and monitor their progress;
- contribute to the creation of at least one new spin-off.

SZN also intends to contribute to the creation of spin-off companies capable of using and marketing patents and inventions arising from the operation of the new marine biotechnology department of the Marine Farm & Factory in Bagnoli.





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6.5 PARTNERSHIPS WITH SCIENTIFIC INSTITUTIONS AND RESEARCH INFRASTRUCTURES

Organism	Activity	Start
ЕМВ	European Marine Board	2019-2020
OECD	Organization for Economic Co-operation and Development	2017
EMBRC	THE EUROPEAN MARINE BIOLOGICAL RESOURCE CENTRE European Research Infrastructure - ERIC	2011
EMBRC-IT	Italian Node of EMBRC coordinated by SZN Joint Research Unit (including OGS, CNR, CoNISMa)	2016
EMSO	European Multidisciplinary Seafloor and water column Observatory (EMSO) European Research Infrastructure - ERIC	2013
EMSO-IT	Italian Node of EMSO coordinated by INGV Joint Research Unit (including SZN)	2017
Euromarine	International Association of Marine research institutions	2014
LifeWatch Lifewatch - Italia	Pan-European E-infrastructure for censusing biodiversity and ecosystems coordinated by CNR, and including SZN and BTC Italian structure	2008

SZN is a member of the following international scientific partnerships:

Since 2018 SZN is member of APRE



6.6 PUBLIC-PRIVATE PARTNERSHIPS

SZN collaborates with the private sector at various levels: 1) Research; 2) Promotion and disclosure; 3) Enhancement of research.

1. Research

- SZN collaborates with the private sector in several research and development projects funded by the European Commission, MUR, MISE, the Region and other public and private funding bodies;
- SZN is a co-founding partner and institutional headquarters of the Blue Growth National Technology Cluster, which involves the Italian entrepreneurial fabric active in the economy of the sea and is aimed at creating joint research initiatives in the Blue Growth sector;
- SZN participates in international networks and research infrastructures aimed at facilitating public-private collaboration for innovation;
- SZN provides research services for companies and in partnership, with consequent
- shared ownership of the results, or as a third-party research contractor with complete transfer of ownership of the results;

2. Promotion and disclosure

- SZN participates in national and international dissemination and promotion events organized also by private bodies and associations;
- SZN controls and collaborates with the Dohrn Foundation, aimed at managing facilities open to the public;
- SZN has reactivated the Dohrn Foundation an in-house body for the dissemination and opening to the public of the body's structures.

3. Enhancement of research

- SZN participates in the Spin-off BioSEArch srl;
- SZN is active in the search for companies potentially interested in the industrial exploitation of SZN patents;
- SZN is active in the organization of thematic events or workshops to present the results of SZN research to companies and other stakeholders.



6.7 HIGHER EDUCATION

SZN hosts research activities for Master's thesis and for post-graduate internships. These activities are supervised by the SZN research staff, whose work is recognized as thesis supervisor or co-supervisor. The main outcome of this strategic area is the increase of the training and research capacity at a national level, through the enhancement of human resources, and strengthening and diversification of postgraduate training in the South of Italy.

Nel 2019 have been carried out the following activities:

- 47 students from several Universities have conducted the thesis at SZN under the supervision of SZN scientists;
- 19 students of different Italian Universities have conducted a stage at the SZN labs
- 14 students from EU Universities have carried out a stage at the SZN Labs (UBO France, Erasmus Vilnius University, University of Corse, Erasmus University of Porto (Instituto de Ciências Biomédicas Abel Salazar), EBI (*Ecole de Biologie Industrielle*), France, Agrocampus ouest. Kiel University CAU, Erasmus Univ. Klobenz, Landau Germany, Intechmer France, Université de Lorraine, Erasmus Universidad de Alcala, Spain, Agrocampus ouest, France)
- 11 students from the International Master of Science in Marine Biological Resources (IMBRSea), of which SZN is a partner have conducted the professional practice at SZN.

Higher education (Master and Post-degree)

SZN is part of Master Degree in Biology and Ecology pf the marine environment and sustainable use of the resources - MaRE in collaboration with the University of Naples Federico II (<u>http://www.mare.unina.it/).</u>

In addition, in 2019 SZN contributed to the following activities:

- Marine Genomics Course (Caterina Missero), Laurea MaRe Università di Napoli Federico II;
- Marine Ecology Course (Prof. Corsolini), Università degli Studi di Siena;
- Zoology course, Università degli Studi di Trieste;
- Bioscience coruse (Prof. Della Torre e Prof. Binetti), Università degli Studi di Milano;
- Water Resources and Waste Management: World Oceans as socio-ecosystem, MaGER -Master in Green Management, Energy and Corporate Social Responsibility, Università Bocconi di Milano;
- Animal Production, Università di Scienze Gastronomiche di Pollenzo.

SZN scientists are teachers at the PhD courses of the Stazione Zoologica and in a number of Doctoral courses for Italian Universities.

The following PhD courses are organized by the Office Higher Education of the SZN:

- Data Carpentry 06-07/02/2019
- Evo Devo Arnone, 1-4,9,11 e 12/04/2019
- Basic Course in Bioinformatics, 15-17/07/2019
- Grant Proposal Writing , 17/10/2019
- Data wrangling & visualization in R: a tour of the "tidyverse", 05/10/2019
- Protein Function and Evolution, 5,.9,10/12/2019

PhD – Research Doctorates

- Since 1998 SZN is *Affiliated Research Center* of the Open University (OU) for a programme of higher studies (PhD e MPhil).
- In 2019 the following PhD / Doctorates have been activated in Agreement with Universities:
- 1. Università degli Studi di Napoli Federico II Biologia 31° ciclo (1 student)
- 2. Università Politecnica delle Marche Ingegneria Industriale curriculum Ingegneria Meccanica 31° ciclo (1 student)
- 3. Università degli Studi di Napoli Federico II Scienze Veterinarie 31° ciclo (2 students)
- 4. Università Politecnica delle Marche Scienze della Vita Curriculum "Biologia ed Ecologia Marina" 32° ciclo (1 student)
- 5. Università degli Studi di Napoli Federico II Biologia 32° ciclo (1 student)
- 6. Università Politecnica delle Marche Scienze della Vita Curriculum "Biologia ed Ecologia Marina" 33° ciclo (1 student)
- Università degli studi di Siena Scienze e Tecnologie Ambientali, Geologiche e Polari 33° ciclo (1 student)
- 8. Università degli Studi di Trieste, Dottorato di Ricerca in Ambiente e Vita 33° ciclo (2 students)
- 9. Scuola Superiore Sant'Anna BioRobotica, 33° ciclo (2 studenti)
- 10. KU Leuven (Belgium) (1 student)
- 11. Università degli studi di Siena Scienze e Tecnologie Ambientali, Geologiche e Polari 34° ciclo (2 students)
- 12. Università degli Studi di Trieste, Dottorato di Ricerca in Ambiente e Vita 34° ciclo (2 students)
- 13. Università degli Studi di Napoli Federico II Biologia 34° ciclo (1 student)
- 14. Università Politecnica delle Marche Scienze della Vita Curriculum "Biologia ed Ecologia Marina" 34° ciclo (1 student)
- 15. Università della Calabria Dottorato in Scienze della Vita 34° ciclo (1 student)
- 16. Universität Bremen, Germany Doctoral Programme in Natural Sciences
- 17. Scuola Superiore Sant'Anna BioRobotica, 35° ciclo (1 student)
- 18. Università degli Studi di Napoli Federico II Biologia 35° ciclo (3 students)
- 19. Università Politecnica delle Marche Scienze della Vita Curriculum "Biologia ed Ecologia Marina" 35° ciclo (1 student)
- 20. PhD Program in Natural Sciences in the field of Life Sciences, University of Vienna, Austria (1 student).

In 2019 higher education activities SZN involved 62 Phd students and support 40 PhD grants.

At present SZN does not carry out Life-Long Training, but undertake and aim at improving the advanced training, organizing Summer Schools and International Workshops. In the next three years, SZN plans to increase its level of summer school activities, organizing of at least one course per year at the Ischia seat or at the other seconded seats.

Organized by the Grant and Innovation Office:

- "Managing a Horizon 2020 project, 10/12/2019, Valentina Fioroni, APRE, Agenzia per la Promozione della Ricerca Europea.
- "How to write a successful proposal in Horizon 2020", 10/12/2019, Bruno Mourenza, APRE, Agenzia per la Promozione della Ricerca Europea

Organized by the Office Higher Education

- PhD students supervision Training Session, 12/03/2019, I. D'Ambra, G. Procaccini (SZN)
- Corso sulla politica e buona pratica di supervisione di studenti di dottorato

Organized by the Servizio Prevenzione e Protezione

• Corso dirigente sicurezza, 21/02/2019

Organized by the Department RIMAR

- First Aid & CPR (cardiopolmunar resuscitation, B. lacono (SZN)
- Oxygen in diving emergencies B. lacono (SZN)
- OSS / OTS course.

SUMMER SCHOOLS

The SZN carries out and intends to strengthen the higher education activities managed through summer schools and international workshops. In 2019, the SZN organized:

1st International Summer School on Marine Ecotoxicology (SSME1) - July 1-5, 2019 - Stazione Zoologica Anton Dohrn, Naples, Italy

The first International Summer School on Marine ecotoxicology, SSME1, is organized by the Stazione Zoologica Anton Dohrn under the patronage of *Ordine Nazionale dei Biologi* and will take place in July 2019 from the 1st to the 5th, 2020. SSME1 provided an advanced training on different classes of traditional and emerging marine pollutants and how biological systems respond to and are affected by these contaminants at all biological levels. The participants focused on:

Marine chemical stressors: metals, antifoulants, nanomaterials, plastics, Climate changes: global warming, ocean acidification, Marine natural toxins, Marine model organisms in ecotoxicology, Biomarkers and bioindicators in marine environmental monitoring, reproduction, toxicology, Case studies, Ecotoxicity tests. The program consisted of lectures and practical sessions held by internationally recognized experts and researchers in the field. Practical session included innovative and standardized toxicity tests for the assessment of ecotoxicological effects in different marine organisms. This course allowed the participants to acquire methods and approaches for the detection and assessment of the impact of environmental stressors on marine biota. SSME1 was open to 20 people with basic knowledge in ecotoxicology.

Summer School on: "PROTEIN EVOLUTION - from Environmental Adaptations to Biotechnological applications" July 24-26, 2019 Stazione Zoologica Anton Dohrn Naples, Italy

The Summer School on "Protein Evolution" was held at the Stazione Zoologica Anton Dohrn in Naples on July 24 – 26, 2019. The Program included lectures on theoretical aspects of molecular evolution,

sequence data manipulation and alignment, protein structure, molecular modelling, and docking analysis. Lectures have been integrated with practical tutorials; and participants are encouraged to work on their own laptop. The number of participants was limited to 22, and admission is on a first come, first served basis. Protein evolution is determined by different factors: mutations of the encoding genes, selection on protein structure and function, regulation of gene expression patterns, position in biological networks. As protein sequence evolves, enzymes can change their reaction profile and accommodate different substrates. Indeed, many enzymes perform multiple reactions and are called promiscuous. Many studies have revealed a possible role of promiscuous enzymes as intermediates in the evolution of moonlighting functions. Ocean offers a unique opportunity to study protein evolution as life began in the ocean and tells the story of how the most complex organisms evolved from primordial bacteria. The ability of organisms to adapt to the changing conditions of their habitat is crucial to guarantee their survival and reproduction. Adaptation to the living environment drives the innovation, exchange, and demise of enzyme function. At the metabolic level, this process of adaptation is related to the ability of enzymes to evolve beneficial functions and improve the fitness in an environment of changing conditions. On the other hand, mankind can get ideas from nature and exploit the unique properties displayed by specialized proteins and molecules for biotechnological and pharmaceutical applications.

International Summer School of Natural Products (ISSNP) July 1- 5, 2019 University Centre - CESTEV in Naples and Hotel Pianeta Mare in Maratea

A combination of lectures, seminars and interactive sessions provided an immersive and challenging experience and team-based problem-solving activities helped the participants to be prepared for future research studies in these fields.

In addition, interactive sessions on transferable skills, including teamwork, leadership, personal effectiveness, entrepreneurship and innovation, gave the participants training in order to combine research excellence with novel approaches in communication and dissemination strategies.

As an international summer school, ISSNP provided the opportunity to enjoy an exciting experience in a highly stimulating environment and in one of the most inspiring countries in the world, Italy.

Previous participants have highlighted that ISSNP is an enriching event both professionally and personally. All students are expected to submit an abstract and may apply for a short oral communication and/or a poster presentation.

The poster communications are briefly presented by the authors during lively open sessions, in line with the main aim of the school to create a friendly environment and provide the opportunity to young scientists, post-docs and PhD students to meet leading scientists of high international repute in a very relaxed and informal atmosphere.

During the next 3 years, SZN will continue the proposal of new Summer schools at the Ischia Marine Centre or in other territorial seats.

Summers schools and courses organized in 2019:

International Summer School of Natural Products (ISSNP) 2019, July

International Summer School "Protein Evolution: from Environmental Adaptations to Biotechnological Applications" 2019, July

1st International Summer School on Marine Ecotoxicology (SSME1) 2019, July

Summer school proposed for 2020*

SUMMER SCHOOL SCHMID TRAINING COURSE 2020: Establishing and Emerging Model Organisms for Marine Science - Stazione Zoologica Anton Dohrn, Napoli 18-29 maggio 2020*

PLAnkton NetworKs and long-Term trend analysis: Ecological Research and System approaches Stazione Zoologica Anton Dohrn, Napoli 4-12 giugno 2020* Ecologia e funzionamento degli ecosistemi a fanerogame marine dell'Isola di Salina (Isole Eolie) Isola di Salina (Isole Eolie) 03-09 luglio 2020* The Evolution of Enzymes and Metabolic Pathways in marine organisms: analysis, understanding and implications for biotechnology Stazione Zoologica Anton Dohrn, Napoli 14-16 luglio 2020* Disegno sperimentale, tecniche di campionamento ed analisi negli studi ecologici ed etologici di organismi marini degli ambienti rocciosi nelle AMP- 2020 Favignana TP settembre 2020* BENTACS - BENthos TAxonomy CourseS - TAXONOMY OF POLYCHAETES Stazione Zoologica Anton Dohrn, Napoli 26-30 October 2020*

* all summer schools planned for 2020 have been postponed due to the emergency COVID-19



7 RESEARCH INFRASTRUCTURES

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7.1 DEVELOPMENT OF INNOVATIVE TECHNOLOGIES FOR ENVIRONMENTAL MONITORING

In addition to the ESFRI Research Infrastructures referred in the previous paragraphs, SZN operates a set of internal infrastructures working for the development of the scientific resource and technological development.

<u>Objective 1</u>: oceanographic technological development and management of platforms and oceanographic instrumentation through the dayby-day maintenance of the infrastructures, to ensure correct and continuous use, also preparing quality protocols, to assess the standard performance, and ad hoc training of staff for each individual instrument or platform. Thanks to the experience gained and the expansion of the working group, it is planned to work on the technological implementation and on the interconnection of different instruments construction of multidisciplinary for the infrastructures (seabed platform, junction box, mooring), within the activities of the PON InSEA and PON Place. The Technological and Robotic Implementation activities will ensure the supply of a product on request for specific research needs, through the various stages of design, preparation and assembly of measuring instruments. More emphasis will also be placed on the production, processing and analysis of oceanographic acquired data, from any instrument or platform, as meteorological data,



oceanographic, geophysical and chemical-biological. The data will be validated and organized in a special database, to which SZN researchers will have free access and specific rules will be defined for external researchers.

<u>Objective 2</u>: consolidation/development of QA/QC procedures. In this context, it ongoing the participation in the intercalibration activities that officially attest to the quality of the data produced. With this in mind, in 2018 the unit participated in the Inter-laboratory Comparison Study for Nutrients Certified Reference Material in a seawater matrix organized the International Ocean Carbon Coordination Project (IOCCP) and the Japan Agency for Marine-Earth Science and Technology (JAMATEC) and the intercalibration results were published. A paper has been published on the definition of the procedures for the quality control of biogeochemical data (Sabia et al., 2019). This task, started in the framework of the of the RITMARE project, will be continued and completed with the publication of datasets. Finally, it will be placed particular attention to the procedures, management and archiving of data. Considering the value of the data collected and the possibility of their use for multiple purposes (re-analysis, meta-analysis, models etc.) it is necessary to organize the datasets according to the international standard, including data policy, both for both indoor and outdoor use.

<u>Objective 3</u>: meta-dating of biological material collected at the request of users according to established standards. This activity, which is complementary to the sampling of biological material for research, significantly contributes to the creation of a database of resources of the Gulf of Naples, enriching the heritage of information that the SZN can offer available to its researchers and the scientific community, as well as to other users. It will be also realized the morphological and geological characterization of the seabed of areas of interest with construction of a database in GIS integrated system with underwater images and videos.

<u>Objective 4</u>: Study of the interactions between nutrient dynamics, physical drivers and microbial communities at different spatial and temporal scales. The temporal and vertical dynamics of the main functional groups of phytoplankton (chemo-taxonomic approach) is analyzed at the LTER-MareChiara station, in relation to the dynamics of macro-nutrients and particulate.

Climate changes and possible implications on the trophic structure of the Ross Sea are analysed using data collected during different mission to Antarctica realized in the austral summer. Some papers have been published in recent years (Mangoni et al. al., 2017; Escalera et al., 2019) and other are under preparation. Finally, recent studies have highlighted the great dominance of the small phytoplanktonic fraction for which the development of innovative techniques is required for the identification of some organisms, very abundant and never reported in the study area, through metagenomic analysis and high-resolution morphology.



7.2 MAINTENANCE/BREEDING OF MODEL ORGANISMS

<u>Objective 1:</u> The facility for breeding and maintaining model organisms (Unit IMOM) is undergoing a reorganization and structural adjustment. The intervention will be carried out with funds from the PRIMA project financed under the PON Research and Innovation 2014-2020 and provides for the expansion and technological enhancement of the research infrastructure for maintenance, breeding, experimental handling and preservation of marine organisms. The new research infrastructure, designed in accordance with the requirements of the Legislative Decree 26/2014, will allow to improve the activities of maintenance and breeding of marine organisms for research, as well as to carry out in an optimal manner and according to excellent quality standards all activities related to handling on marine organisms.

<u>Objective 2:</u> To develop and improve methodologies and procedures to optimize the use of marine resources in order to reduce naturally occurring harvesting. This principle of "optimization of the resource" is applied to each species used as an animal model, and is dedicated to particular interest in model species that are found to be under high fishing pressure



such as, for example, the common sea urchin, *Paracentrotus lividus*, renowned animal model for embryology and eco-toxicology studies, as well as much sought after edible species for the consumption of the gonads. Intensive harvesting, sometimes using destructive methods, to respond to market demand, has caused a dramatic impoverishment of natural populations, leading in some areas to the complete disappearance. It is therefore essential to optimize the procedures already established such as, for example, for sea urchin, and to develop new ones to apply to the other animal models used in order to improve both their performance for experimental purposes (quantity of organisms and/or their products) and their efficiency (quality of the organisms and/or their products) as a physiological response).

<u>Objective 3:</u> Identification and breeding of new marine organisms as a model for research. The layout and facilities serving the facility of the IMOM Unit, which will be built with the planned empowerment, were aimed to maximize the versatility of the entire structure to respond to the different experimental needs for research. Therefore, it will be possible the rearing of new marine model organisms, to be selected according to the needs of the scientific community. In other words, in addition to the traditional models, new species of model organisms to be used for research, according to the demand of the scientific community. Objective 3 is expected to setup the protocols and to optimize the rearing/breeding techniques for selected species and to produce standard operational procedures.

7.3 NEW TECHNOLOGIES FOR MARINE RESEARCH

Objective 1: New technologies for molecular analysis. The "Omics - Sciences" are of fundamental importance in the classification, structural and functional characterization of new organisms. The RIMAR Section is working hard on the implementation of the technologies to address these approaches. In particular, the Centre for Sequencing and Molecular Analysis (CSAM) has been dedicated to the development of the Thermofisher's ION Gene Studio S5 parallel massive sequencer, which allows to produce data for metabarcoding, de novo sequencing of bacterial genome, resequencing of reference genomes, transcriptomes and microRNA, gene pression profiles (mRNA, small RNA and other). Metabarcoding experiments were carried out on both environmental samples and from laboratory cultures, both prokaryotic and eukaryotes. Protocols for the sequencing of bacterial genomes transcriptomes and microRNAs are being developed and will be added to what is already offered to the scientific community. The collaboration with the bioinformatics team to perform data analysis products, will allow users to fully exploit the result and its final interpretation. The Real Time PCR and Sanger sequencing, techniques widely used by the CSAM staff, and the PCR digital droplet, currently under implementation, complete the of the Unit's overall offering. We will consider to the possibility of integrating these techniques to provide the scientific community with a comprehensive gene expression platform. The management of the high number of samples was considered as cornerstone for these technologies, for which further automation protocols are being developed which allow procedures to be speeded up and the possibility of errors to be significantly reduced during the preparation phases. Following the recent integration of the service of flow cytometry in the CSAM is considering the possibility of extending technology capacities of the Unit for genomic and/or transcriptomic analysis of single, isolated cells through FACS. In addition, studies and tests for the development of new applications, such as the isolation of new microorganisms, nuclei from diatoms, etc. Finally, methods are being developed for the exogenous production of proteins from marine organisms with potential biotechnological applications. This technological development will be a benefit for all departments of the Stazione Zoologoca, as well as participants in EMBRC and the scientific community at large.



7.4 NEW BIO-IMAGING TECHNOLOGIES

Activities for the three-year period 2020 - 2022 include the expansion and improvement of services offered by the Unit. The actions that will be taken to this end are:

- 1. application of scanning electron microscopy (E-SEM) which will provide technology for imaging hydrated samples;
- 2. development of advanced live imaging technologies (confocal and epifluorescence) for the study of dynamic and rapid intracellular events using fluorescent probes specific to cellular components;
- 3. organization of a computerized catalogue for protocols for electron microscopy samples preparation;
- 4. development of experimental approaches for single-cell analysis;
- 5. application of spectroscopic/morphological analysis in confocal microscopy for the characterization of natural fluorescent emissions of organisms by the identification of molecular markers for the analysis of biological phenomena in connection with Omics methodologies;
- 6. application of digital microscopy image processing and analysis techniques to electron, optical and fluorescence microscopy.


7.5 DATA INFRASTRUCTURE

The enormous amount of information available makes it increasingly necessary to recruit researchers able to turn this information into knowledge. A recent international trend of the sector also involves the creation of centers for the analysis and synthesis of ecological data.

SZN concluded this prospect in 2014, in collaboration with Stanford University, a convention for the creation of the Marine Ecological Data Analysis and Synthesis Centre (MEDAS). The aim of this center is to analyze existing information and to provide answers to the relevant questions and economic demands in the context of Blue Growth.

The SZN intends, with this center, to promote the formation of working groups, summer schools, research activities, visiting scientists and PhDs. MEDAS will focus on the marine systems and the definition of new research and management policies of marine resources. The SZN intends to improve its experience in the field of ecological data metanalysis and in knowledge and technology transfer, thus also providing new opportunities for development to the regional territory and at national level.



7.6 ADVANCED MARINE RESEARCH AND INFRASTRUCTURE CENTRE

The Centre for Advanced Marine Research and Infrastructures (CRIMA) has the objective of

creating a centre of excellence at European level for marine infrastructure and research and for the teaching of marine biology. The creation of the marine research center of Bagnoli aims to revitalize the area by placing it at the centre of a long-term project that sees research and jointly promoted training by SZN and UNINA, bodies of excellence to international level in its areas of activity. The CRIMA project in the area of Bagnoli sees a close collaboration with



INVITALIA, owner of the structures concerned and in charge of the SIN Bagnoli-Coroglio transfer. This project will allow to create a joint and integrated structure between the Research Institution (Stazione Zoologica) and the Academy (University Federico II) through:

- The recovery and re-functionalization of the former Turtle Point Exhibition Centre at Bagnoli to create the largest European infrastructure in the field of production of organisms for marine research, marine biotechnology and innovative aquaculture, also for the restocking of natural environments (ecological restoration).

- The expansion of the infrastructure and the expansion of the activities of CISMAR-DEB in order to create, in collaboration with the Stazione Zoologica, a research center carrying out activities aimed to study, monitor and preserve the environment and their biodiversity.

- The creation of a structure open to the public and aimed at teaching and training, knowledge and public awareness on the themes of ecology, biology and marine biotechnology.

The strengthening of the infrastructures for breeding and maintenance of marine organisms will be realized with the financial support of the PON PRIMA.



8 RESEARCH PROJECTS AND INNOVATION

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8.1 INTERNATIONAL RESEARCH PROJECTS

PONDIV: PseudO-Nitzschia: DIVersity behind an image

Duration: Jan 2018 – Jun 2020 - Research field: population genetics

Funding: SZN funded project (Cooperation with non-EU countries) - SZN role: Coordinator - PI M.

Montresor

Marine phytoplankton include toxic or harmful species that represent a threat for human health, aquaculture activities and economy. Monitoring programs are required to assess the presence of these microalgae and the toxins they produce. To this end, various automatic systems are implemented and Imaging Flow Cytobot (IFCB) is the most advanced equipment for automatic identification of phytoplankton. This instrument has operated since 2007 in the Gulf of Mexico at Port Aransas on the coast of Texas (Texas A&M University).

The genus *Pseudo-nitzschia* includes diatom species that produce the neurotoxin domoic acid; their identification at the species level is particularly difficult and in most of the cases requires combined ultrastructural and molecular analyses.

We propose to: i) identify the main *Pseudo-nitzschia* species present along the coast of Texas through molecular and ultrastructural approaches. ii) Focus on the morphotypes that look like *P. multistriata* and test the genetic relatedness (level of gene flow) between Mediterranean and US populations with already available microsatellite markers. iii) Test the capability of IFCB to resolve at least some morphotypes of *Pseudo-nitzschia*.

LIFE ELIFE (*Elasmobranch Low Impact Fishing Experience***)** - LIFE18 NAT/IT/000846 Duration: Oct 2019 - Sep 2024. Research field: biodiversity, conservation biology, ecology, environmental and marine biology. Funds: UE LIFE Program. SZN role: Coordinator - <u>PI M. Bottaro</u>

Since the mid-1980s, chondrichthyans (cartilaginous fish) have been under growing pressure from fishing in the Mediterranean Sea mainly as a result of incidental by-catch. Despite some recent efforts to reduce shark by-catch and mortality (i.e. adoption of the European Commission's Action plan for the Conservation and Management of Sharks), the EU goals are far from being achieved.

The 5-years project LIFE ELIFE (https://www.elifeproject.eu/), leaded by the SZN and funded by the LIFE Programme of the European Commission (https://ec.europa.eu/easme/en/life) aims at improving the conservation of elasmobranch species (sharks and rays) by promoting best conservation practices in EU professional fishing in the Mediterranean Sea, including both bottom trawl and longline fishing. The project will allow pilot and demonstrative actions for turning commercial fishers towards low-impact fishing devices that decrease elasmobranch by-catch and it will be carried out by-catch in the some of the most important Italian harbours (like Chioggia, Gallipoli, Lampedusa, Cirò Marina, Porto Cesareo, Marsala, Mazara del Vallo, Lampedusa), some Italian MPAs, and also in some relevant areas of Cyprus and Greece. Additionally, the project will support marine management authorities in Cyprus, Greece and Italy by providing the latest data for assessing the status of shark species and will support fishers in fund raising activities (e.g. grant applications) to adopt sustainable practices. Mediterranean fishers will therefore enhance their role in marine biodiversity conservation. The project also aims to transfer good practices for shark by-catch mitigation and lowering mortality to other Mediterranean countries and to increase the awareness of people and stakeholders about the value and vulnerability of the elasmobranchs; thus promoting a more sustainable fishing and responsible fish consumption. LIFE ELIFE will contribute to achieving the European Commission goals through conservation actions on shark species listed in the European Red List of marine fishes (IUCN, 2015), in accordance with the EU Action

Plan for the Conservation and Management of Sharks; the aims of the EU Marine Strategy Framework Directive (in particular, Descriptor 1 biodiversity and Descriptor 3 fishery); the European Biodiversity Strategy to 2020; and of the Regulations 1380/2013 and 72/2016. The project is also consistent with the 7th EU Environment Action Programme, the Birds Directive and the Habitats Directive, and several international conventions on marine conservation, biodiversity and migratory species. The ELIFE project has started in October 2019 and it will continue until October 2024: it is coordinated by the SZN, with the collaboration of Italian National Research Council, University of Padua, Marine Protected Area of Pelagie Islands, Marine Protected Area of Tavolara-Punta Coda Cavallo, Mer Research Ltd., Mediterraneum Consortium, Legambiente, Costa Edutainment and Softeco Sismat, and with the endorsement of the Italian Ministry of Environment, the Italian Fishery Direction, the Greek Department of Fisheries-Aquaculture Technology, the EU-MEDAC and many others international public bodies and stakeholders. Specimens of blue shark, *Prionace glauca*, common by-catch during the pelagic professional fishery (©Alberto Luca Recchi)

Epic Sea: Role of EPIgenetic mutations in plastic response. Comparison between southern and north hemisphere SEAgrass species

Duration: Jan-Dec 2019 – extension 2020 - STAZIONE ZOOLOGICA ANTON DOHRN - EXTRA-EU SCIENTIFIC RESEARCH & COOPERATION - CALL FOR PROJECT PROPOSAL - 2017 - PI G. Procaccini

The projects aims to assess the role of epigenetic mutations in affecting plasticity response to environmental disturbance in seagrasses. Species with different lifestyle (i.e. pioneer or late succession species) are compared. Congeneric species, representing comparable ecological stages in the Mediterranean climatic regions, both within the Mediterranean Sea and along the temperate coast of Australia, are analyzed. Plants are cultivated in high temperature conditions, to assess their response



to warming, and the interaction existing with the epiphytic cover. Response is assessed at morphological, physiological and molecular level, and correlated with the amount of epigenetic mutations. Expected results include the assessment of plant response to warming, with particular reference to the assessment of DNA methylation level, in order to assess the importance of epigenetic mutations for the evolutionary success of species. The comparison of such response between species with different life styles and the comparison between congeneric species

at the extremes of their distribution, aims to assess if species-specific features have been acquired early in the evolutionary history of the species or if similar ecological role and life style are driven by or influence at the same way the species response to stress.

Genomics for a Sustainable Economy

January 2018- December 2020 - Area Genomics and Biotechnologies Source of funding: Protocol of Scientific Cooperation Italy-South-Africa Role SZN: Coord - PI G. Romano

Marine organisms produce a wide variety of biologically active molecules which are often new and/or have new mechanisms of action. Such compounds may find applications for the treatment of diseases

and to improve the quality of human life. Although there is a growing interest in natural marine products (MNP) as potential therapeutic, few have reached the market. To make full use of the promising resources marine biology, overcome existing obstacles and ensure the production of marine bio-molecules of high value, new research strategies are needed, as well as a new generation of scientists trained in interdisciplinary approaches. For these reasons, the search for new MNPs increasingly makes use of innovative study approaches such as genomics, transcriptomics and proteomics, which already have successfully guided the discovery of new molecules. The main objective of this project is to identify, through approaches interdisciplinary and innovative, new bioactive compounds and bring them to preclinical testing. The objective will be achieved through the identification of new genes and "clusters" responsible for the production of metabolites with antimicrobial and anticancer activity in marine microorganisms isolated from partner laboratories (two Italian, one South African). The compounds derived from these microorganisms will be characterized and developed through the collaboration with international partners with whom the proposers already have active collaborations in the context of European projects. We are confident that the establishment of this research network international will contribute significantly with its synergies to advancing the visibility of the international laboratories involved in the discovery of new marine pharmaceuticals.

INBALANCE - INvertebrate-BActeriaL Associations as hotspots of benthic Nitrogen Cycling in Estuarine ecosystems Jan 2018-Dec 2021 Biogeochimica/Simbiosi Fonte Finanziamento: Research Council of Lithuania (LMT) under the European Social Funds (ESF) programme - SZN: Partner - <u>PI U. Cardini</u>

While traditionally, bacteria have been considered driving much of the Earth's nitrogen (N) cycle, recent

research shows that ecological interactions between meio-, macrofauna and bacteria are important in regulating N cycling in soft sediments. Thus, the INBALANCE Project aims to unveil the quantitative importance of ecological interactions between microorganisms and their benthic invertebrate hosts in regulating N cycling in shallow estuarine sediments. In particular, this project will investigate the identity and activity of bacteria associated with benthic invertebrate hosts, the most abundant functional group of meio- and macrofauna in estuarine systems, across the full range of possible interactions, from strict symbioses to casual associations.



DIsCO -Diatom life cycles, molecular controls and contribution to ecosystem dynamics Dec 2018-Nov 2021 Research area: Genomica funzionale/genetica di popolazione Source: Gordon and Betty Moore Foundation. SZN: Coord. - **PI M. Ferrante**

Diatoms are eukaryotic microalgae responsible for 20% of the global CO₂ fixation ubiquitous in aquatic habitats. Sex in diatoms, a rare but great event impact on ecosystems, is governed by endogenous processes and external signals, but the mechanisms involved are unknown. The project focuses on the marine species *Pseudo-nitzschia multistriata*, model species for the study of life cycles and population genetics. The objectives include the definition of transcriptional and epigenetic controls orchestrating the transitions of the vital cycle, and the effects of sexual reproduction on genome evolution. The project plans to integrate laboratory, *in-situ* and modelling approaches to respond to different questions, and it is divided in three parts:

- *In the lab*- what are the endogenous mechanisms that control the diatom life cycle? Which signal transduction pathways and which genetic networks are involved? Is there a epigenetic control in key life cycle transitions?

- In the environment- how are natural populations made up? Which gene/regions or genomic traits are affected by variations during adaptation and microevolution in natural populations? What is the balance between cloning and sexual reproduction? In which way is population dynamics related to genomic variation?

- Conceptual Integrative and modelling studiesin which measures the processes of genetic diversification are driven by environmental factors and/or of "neutral" interaction between cell division, genetic mutation and recombining? Which one is the expected effect of environmental disturbances on the division, on the mutation and on recombining?

The project includes a component for the development of new tools, including the application of the ATAC-seq and single cell transcriptomics.



High-CO₂ Seas

Dec 2016-Dec 2019 (Extended to Dec 2020) Source: Total Foundation, BIO 2016 081 4 . SZN Role: Coord. - PI N. Teixido - MC Gambi

The aim of the High-CO₂ Seas project is to assess the effects of ocean acidification (OA) on marine biodiversity and to estimate species adaptation. OA is expected to profoundly alter the diversity and function of whole marine ecosystems, impairing their capacity to deliver goods and services to society. Thus, understanding how future oceans will function in the face of OA represents one of the major challenges and needs for marine science and management. To date, however, much of what is known concerning the impacts of OA to benthic marine organisms has been derived from laboratorycontrolled experimental studies. Naturally acidified sites capture the emergent effects of OA on the structure, function and long-term interactions of entire ecosystems, not just single species; thereby providing novel and crucial information. The shallow volcanic CO₂ vents near Castello Aragonese in Ischia (Italy) cause local acidification of seawater by as much as 1.5 pH (pH on total scale) units below the average ocean pH of 8.1-8.2. Corresponding to this pH drop, the diversity and biomass of benthic organism decrease. Recently, various new vent systems have been discovered along the coast of Ischia across depths of 3-48 m. These new CO₂ vent sites span a variety of different habitats such as *Posidonia* oceanica seagrass meadows, gravel and sandy bottoms, semi-dark caves, and coralligenous outcrops, the latter dominated by calcifying organisms that are particularly vulnerable to OA. The specific objectives of High-CO₂ Seas are: 1) to characterize water chemistry and pH variability; 2) to assess the vulnerability of marine benthic biodiversity to OA based on trait-functional approaches; 3) to assess the role of acclimation/adaptation in determining responses to OA of the coral Astroides calycularis. In addition, we will use virtual reality to communicate OA impacts to the public and policy makers. The High-CO₂ Seas project is highly collaborative, international, and multidisciplinary with the collaboration of top scientists from different fields from France, Italy, and the USA. Overall, we expect that this project will advance the field and will have a significant scientific and broader social impact. The scientific results, virtual reality set up, still images and videos that will be produced through this project will provide ample material for outreach to communicate the anticipated impacts of OA.

"EXCITES" bilateral Italy/Israel "Induction of marine sex shift processes towards applied environmental and mariculture biotechnologies - Duration: 20 Jul 2018 - 20 Jul 2020 (extended until 17 Dec 2020) - Research field: Marine Aquaculture – Marine Biotechnology

Funding: Ministero degli Affari Esteri e della Cooperazione Internazionale. SZN Role: Coord. - PI V. Zupo

Sexual changes occur naturally in marine organisms as a strategy for ensure maximum reproductive success. However, cases of changes have been reported as a result of exposure to pollutants, environmental stressors, and acidification of the oceans, which can produce ecological imbalances and major losses. In addition, sex changes can contribute to the creation of biotechnology for single sex populations very useful in the aquaculture sector. So, it is important to understand the molecular basis of sex changes to deal with various scientific and environmental issues and application challenges. Decapod crustaceans, among others



invertebrates, have a wide range of reproductive strategies and their differentiation is controlled by a hormone produced by the androgenic gland. An ideal model for such a study is the shrimp *Hippolyte inermis* that undergoes a sex change from male to female with the complete disappearance of the male gonad without an apparent intersex stage. In this species, the sexual transition from male to female is a natural process and can be induced both in nature that in the laboratory, from the ingestion of certain algae. This model species, distributed in the prairies of phanerogams in several European coastal waters, has been intensively studied in Ischia (Italy), where it produces stable populations on *Posidonia oceanica* leaves. The aim of the project is to study the genes keys related to the control of sex changes, gonad degeneration through the latest generation of sequencing approaches (i.e. sequencing of the RNA). The selected genes will also be tested for their function in a shrimp important in aquaculture.

VIPurchin - Studying sea urchin dermal photoreception to unravel principles of decentralized spherical vision. Duration: Sep 2019- Aug 2022 - Research field: evolutionary molecular biology; neurobiology, physiological; neuroscience, computational - Funding: Human Frontiers Science program Organization (HFSPO). SZN Role: Coord. - <u>PI MI Arnone</u>

The core of the proposed project is to investigate and model the neural mechanisms of information processing, which enables sea urchins, animals lacking eyes and proper brain, to perform spherical vision by deploying an obviously very different mechanism from today's technology, and also very different from visually guided behaviour in most other animals. Our study includes molecular and morphological identification of cell types, measurements of behavioural responses and electrophysiological photoreceptor responses, mapping the connectome of sea urchin photoreceptors and nervous system (NS), and theoretical modelling of the information processing underlying visually guided behaviour. We will map the connectomics of the NS and record the activity from key positions in the processing of visual information and generation of locomotory responses. The data will be used

for computational modelling of the entire process from visual input to motor control. Special focus will be given to behavioural decisions where small changes in stimuli cause behavioural switches. We will also use genetic approaches to test the agreement between theoretical models and actual behaviour. The team is coordinated by the molecular and development biologist Maria Ina Arnone at Stazione Zoologica Anton Dohrn of Napoli, Italy and includes Prof. Dan-Eric Nilsson of Lund University in Sweden, an expert in animal vision; ecologist and morphologist Carsten Lüter of the Museum für Naturkunde in Berlin, Germany and Giancarlo La Camera, а computational neuroscientist of the State University of New York at Stony Brook, USA.



INTENSE "INtegrated operating devices for inTElligent eNvironmental Services" Duration: 2018-2021 Area di ricerca: Marine Aquaculture – Marine Biotechnology Funding: Ministero degli Affari Esteri e della Cooperazione Internazionale SZN: Coord. - PI V. Zupo

This project of "Industrial Research and Experimental Development" aims at the implementation of activities for the development of advanced integrated systems for management, monitoring, control and automation of specific operations, including: i) Industrial research, for "Intelligent Environments" and support for process innovation related to structures production and monitoring and control infrastructure; ii) Experimental development of intelligent integrated systems, advanced information technologies (hardware (HW) and software (SW) components) and microenvironment demonstrators; iii) Surveys, studies and analyses - through networking of medium and large scale monitoring - and aquaculture, and implementation systems suitable for process automation standard. Innovative technologies, products and services capable to implement the "IoT" (Internet of Things) and "IoE" (Internet of Everythings) paradigm will be produced. We want also demonstrate how the IOTes (internet of things evoluted systems) paradigm is able to to implement "IT nodes (IOTes Things) able to interact and cooperate in a network such as Operators able to perform assigned tasks, even dynamic variables, based on an "Operating Contract or Service Contract" in relation to "Competences held or Published Services" within Production Processes or "integrated and distributed" Services.

SEA- Stress Durata: biennale 01/08/2018 - 31/07/2020 Extended until 20 Jan 2021 - Area di ricerca: Ecologia marina. Funding: MAECI - MINISTRY OF SCIENCE AND TECHNOLOGY OF THE STATE OF ISRAEL. SZN Role: Coord. - **PI G. Procaccini**

Application of molecular tools for the detection of early signs of stress in Israeli and Italian marine phanerogams (seagrasses). Seagrass meadows provide key ecosystem services with an estimated annual value of 28,106 US \$/km⁻². The loss of such ecosystems will lead to serious ecological and socio-economic consequences. In Israel (in the north of the Gulf of Aqaba, GoA) and along the western coasts of the Italian peninsula, the areas of interest for this bi-national proposal, seagrasses meadows are

dominated by *Halophila stipulacea* and *Posidonia oceanica*, respectively. The objectives of this project are: 1) to understand tolerance levels and resilience potential of *H. stipulacea* and *P. oceanica* exposed to high temperature levels, in presence and absence of high nutrient levels and 2) to develop early indicators of physiological and molecular stress to detect changes when still undetectable at the morphological and population level. Experimentation is conducted in the mesocosm systems available both in Israel (ADSSC) and in Italy (SZN). The project will contribute to: 1) identify nutrient and temperature tolerance levels in the two target species; 2) identify the key genes and molecular and physiological mechanisms associated with each individual stress factor and their own combination, 3) identify and validate early warning indicators of stress to be used as a molecular tool for monitoring and managing Italian and Israeli seagrasses, with potential applications also in other species and in other geographical areas



8.2 EUROPEAN RESEARCH PROJECTS

Eurosea

Duration: 11/2019-10/2023 - Research areas: Oceanography Funding: EU H2020 SZN role: Task leader - <u>PI D. Iudicone</u>

The overall aim of EuroSea is to significantly improve the European ocean observing system as an integrated entity within a global context, delivering ocean observations and forecasts to advance scientific knowledge about ocean climate, marine ecosystems, and their vulnerability to human impacts and to demonstrate the importance of the ocean for an economically viable and healthy society. EuroSea will improve: 1) the design of the observing system adapted to European needs; 2) *in-situ* observing networks and data integration; 3) integration of remote and in-situ data; 4) the forecasting systems. This flagship initiative will enable a quantum leap in making the European ocean observing a better public utility and a key enabler for a sustainable blue economy in the long term.

MPA-Engage: Engaging Mediterranean key actors in Ecosystem Approach to manage Marine Protected Areas to face Climate Change

Duration: 32 months from 11/2019 to 06/2022 - Research area: Climate change Funding Interreg MED Role SZN: partner - PI E. Azzurro

Climate change is dramatically affecting the Mediterranean Sea, which is warming at a rate 20% faster than the world's average. Marine Protected Areas (MPAs), despite the nature-based solution they offer to support efforts towards climate change adaptation and mitigation, are the first ones to feel the climate change effects. Several Mediterranean MPAs are already facing major biodiversity and functional alterations due to climate change, whereas others will likely face them in the next few decades. There is, therefore, an urgency to mitigate these risks and consider adaptation options in partnership with local communities; decision-makers at local, national and regional level; civil society, civil society organizations; research bodies and other socio-economic actors. MPA-ENGAGE, led by the

Institute of Marine Sciences of the National Spanish Research Council, is funded by the Interreg MED program with a budget of about 3 million euros. MPA-ENGAGE aims primarily to support Mediterranean MPAs adapt to to and mitigate the ongoing climate change effects in the Mediterranean Sea. Through а participatory approach, MPA-Engage will monitor in



a harmonized way the climate change impacts, will elaborate vulnerability assessments and will develop climate change adaptation action plans in 7 Marine Protected Areas located in 6 Mediterranean countries, namely Albania, Croatia, France, Greece, Italy, Malta, Spain.

The main goal of the project is to put Mediterranean Marine Protected Areas in the frontline for adaptation to Climate Change. Main lines of action are: 1. Engaging local communities, small-scale fishermen and citizen scientists in monitoring climate change impacts; 2. Promoting MPAs as nature-based solutions for climate change adaptation; 3. Facilitating a Mediterranean policy dialogue with scientists, MPA managers, decision-makers, environmental NGOs and socio-economic stakeholders on climate change adaptation strategies.

MarPipe - Improving the flow in the pipeline of the next generation of marine biodiscovery scientists Nov 2016 – Oct 2020 - Research Area: Marine Biotechnology Source: European Commission, H2020-H2020-MSCA-ITN-2016-Marie Curie ITN Human Capital –SZN: Partner - PI A. Ianora

MarPipe is a consortium of 11 partners (IBP - CNR, SZN, UiT, UNIABDN, GEOMAR, KULeuven, UCC, eCOAST, MEDINA, MicroDish, Italbiotec) based in 8 countries (I, N, UK, D, B, IRL, E, NL), 3 of which come from the non-academic sector. The goal is to train 11



young marine biotechnology researchers dedicated to the discovery of new drugs, providing them unique skills to become world leaders in this field of research and to advance their careers in academia or industry. The "MarPipe" doctorates will be trained in a program that includes joint courses to acquire technical skills, scientific and knowledge transfer, including active participation in public scientific events and an intense exchange plan between the various laboratories related to the Partnership. Marine organisms have the ability to produce a variety of biologically active natural products, including antibiotic and anticancer compounds. MarPipe aims at the further development of molecules with antimicrobial and antitumor activity coming from a previous European project (PharmaSea). Deep water samples will also be explored (5000 m) collected during the recent Eurofleet-2 project in the sub-Antarctic area. The PhD students will therefore be involved in all phases of the "pipeline" of the discovery of new pharmaceuticals, from the isolation of new microbial strains to the preclinical development of lead compounds. The PhD students will also be trained to deal with the obstacles still present in this field, eg. low yields, isolation of known compounds, toxicity of the compounds. The aim is therefore to improve the detection rates of new antimicrobial and antitumor molecules. As a final result of the project, we envisage the creation of a marine biodiscovery start-up company, which will include most of MarPipe partners. PhD students will in fact be trained to become aware of the socioeconomic and political context of their work, since some MarPipe doctoral research projects focus on legal, political, innovation and entrepreneurship issues.

EvoCell - Animal evolution from a cell type perspective Jan 2018-Dec 2021. Research field: evolutionary developmental biology. Funding: EU-H2020 - H2020-MSCA-ITN-2017 - Marie Curie ITN - Role SZN: Partner - **PI MI Arnone**

The aim of EvoCELL is to lay the foundation for a new branch of evo-devo focussing on cell types. We will study fundamental questions in animal evolution and development - eg. how new cell types arise in evolution, how many are in common between different animal groups and how many unique cell types have evolved in different animal lineages- using a new technology, single cell sequencing, which we will for the first time employ outside of lab models to sample the great diversity of animal phyla. EvoCELL will train a new generation of multidisciplinary scientists skilled in exploring the vast breadth of animal differentiation. We will jointly sample data from all major animal lineages, richly represented in the biodiversity of European waters, and develop new tools for comparative analyses, through which we will together pioneer three branches of cell evo-devo: evolution of stem cells; emergence of animal life cycles, and the stunning diversity of neural cell types. Through their excellent interdisciplinary and intersectoral training, from single-cell biology and palaeontology to bioinformatics and public outreach, our graduates will be in prime positions to assume leadership roles in academia, industry, and science outreach.

What we do: We are one of ten partners and are contributing to the diversity and evolution of neuronal cell types.



AFRIMED

Duration: 01/01/2019 - 31/12/2021. Funding: EASME/EMFF/2017/1.2.1.12- Sustainable Blue Economy SZN Role: Partner - **PI - S. Fraschetti**

As the deployment of blue economy sectors across Europe accelerates, it is vital that the resilience of marine and coastal ecosystems is maintained and restored in order to allow economic growth to be achieved in a sustainable manner. However, despite best efforts to conserve and sustainably manage marine ecosystems, attempts to mitigate human threats are generally proving inadequate to halt biodiversity loss and habitat degradation. Consequently, there is a need for the European Union to turn policy objectives relating to secure, safe, clean and sustainably managed oceans into actions and impacts. This is particularly true in coastal habitats, where the effects of multiple stressors are causing widespread loss of critical coastal habitats, a pattern which is projected to increase with climatic change. Macroalgal forests, one of the most productive and valuable, yet undervalued, habitats in the

Mediterranean Sea, are being rapidly lost. Due to their role in supporting biodiversity and food webs, their loss is leading to a decrease in critical ecosystem services, such as fisheries, and a reduction in the capacity of oceans to sequestrate carbon dioxide and help mitigate climate change. Consequently, prompt and novel actions are urgently required to stimulate their recovery. Active restoration (e.g. the planting of seeds or seedlings) is one of the most profitable strategies to return ecosystems to their original state in a reasonable time frame. Appreciating the potential of restoration as a method to bring

about much needed change, the EU recently funded the "MERCES" (http://www.mercesproject project.eu/), which seeks to revise current concepts and understanding of restoration in the marine environment. Here we seek to improve existing, and develop new innovative restoration actions in order to restore damaged or degraded macroalgal forests (Cystoseira) for increasing the good and services and promote the industrial development, providing a framework from which to up-scale the approach.



DEMERSTEM - DEMERsal ecosySTEMs

Duration: March 2019-February 2022. Research Area: Improved Regional Fisheries Governance in Western Africa. Funding: European Development Fund, EuropeAid/158370/DD/ACT/ SZN: Partner - **PI P. Licandro**

DEMERTSEM is part of the PESCAO program, the EU funded programme for improved regional fisheries governance in Western Africa, which aims at enhancing the contribution of fisheries resources to sustainable development, food security and poverty alleviation in West Africa.

The main objective of DEMERSTEM is to produce scientific advice on the state of selected demersal stocks and reinforce regional cooperation from ecosystem monitoring to advice for management. This in order to contribute to the implementation of a sustainable exploitation of target demersal fisheries in the countries involved in the project, as well as in the CECAF area.

Specific actions of DEMERSTEM include: 1. an assessment of the identity of demersal stocks, which are shared across NW African countries; 2. the identification of sensitive habitats (e.g. nurseries and/or spawning grounds) that should be protected and taken into consideration when developing marine spatial planning directives aiming to improve the sustainability of demersal fisheries; 3. An assessment of 'real' fishing pressures from artisanal and industrial fisheries on demersal stocks at local and cross-regional level; 4. the identification of key environmental indicators and clarification of the effects of environmental pressures on demersal stocks and ecosystems. The Stazione Zoologica (SZN) is mainly involved in WP4 ("Ecosystem approach to the fisheries"), co-leading this Workpackage and contributing to the case studies that will investigate low trophic levels (phyto. and zooplankton) and the impact of jellyfish on other Ecosystem groups.

8.3 NATIONAL RESEARCH PROJECTS

IPANEMA – Implementation of the Natural laboratory ECCSEL Panarea and the marine observatory Durata: 06/2019-02/2023 - Aree di ricerca: Marine Ecology and Innovation. Fonte Finanziamento: PON "Ricerca e Innovazione" 2014-2020, Asse 2 - Azione II.1. soggetto finanziatore e schema di finanziamento MIUR Ruolo SZN: PARTNER, OR1 - <u>PI T. Romeo</u>

The project coordinated by OGS has as final objective the enhancement of a limited part of the ECCSEL-ERIC infrastructure with particular reference to the Panarea natural laboratory with new scientific laboratory and field instrumentation for sampling, *in situ* observation and the acquisition of data in the Panarea hydrothermal system, which is highly innovative and allows multidisciplinary studies in the field of physical, chemical, biological and geological sciences. As part of this objective, the SZN (OR1) will acquire new scientific instrumentation for the study of biodiversity in hydrothermal environments, in order to evaluate the effects of changes in CO2, CH4 and other environmental parameters on benthic communities, habitats and species fish associated with these environments. The instrumentation consists of a STEREO BRUV system equipped with cameras with LED headlights for capturing high resolution videos and images even at depths of 1000 m which will be positioned in areas with different levels of hydrothermal emissions. CO₂ and CH₄ sensors, multiparameter probe including dissolved oxygen sensor, weather station and Benthos MODEM systems on which to install the probes that will be connected to the probe and weather station and will allow to continuously acquire data on the variables related to hydrothermal emissions.

TECHNOLOGICAL INNOVATION FOR THE PROTECTION AND ENHANCEMENT OF MARINE ECOSYSTEMS (ITEM) - Duration: 11 June 2019-10 June 2022, Research areas: Economics of the sea, environmental technologies and biotechnologies: Funding: MIUR. SZN role: Participant - <u>PI A. Zingone</u>

The blue economy is a resource with enormous potential for Italy. At the same time, the protection of the quality of the marine environment is a precise responsibility of our country not only for the conservation of biotic and abiotic resources, but also for the development possibilities of blue tourism. The ITEM project stems from the awareness of serious deficiencies in technological innovation which risk limiting sustainable development of the economy of the sea. ITEM brings together a community made up of 50 research entities, universities, small and medium enterprises and large industries who will work in an integrated way to: 1) identify the main technological gaps and opportunities for competitive industrial development; 2) develop industrial research by testing new technologies patented by project partners; 3) design new observational technologies to assess and guarantee the quality of the marine environment; 4) design new technologies for production systems for the sustainable use of marine biotic and abiotic resources; 5) design biotechnologies for environmental remediation and for the identification of new products, food, materials and drugs of marine origin. The innovation of marine research in Italy promoted by ITEM will help create tools for economic and social growth based on a sustainable use of resources and opportunities offered by the blue economy. With the ITEM project, Italy aims to become one of the leading European countries in the research and development of a new generation of marine technologies and biotechnologies capable of ensuring environmental quality, entrepreneurial development and new employment opportunities.

Global Change Impact in Deep Sea Economy (GLIDE) Duration: April 2020-April 2023 - Research areas: Benthic marine ecology, global changes; source: Progetti di Ricerca Nazionali - MIUR – PRIN SZN role: partner - <u>PI M. Tangherlini</u>

Global climate change is progressively altering also deep-sea ecosystems, which represent the largest ecosystem on Earth and provide good and vital services for humans. Changes involve both physical-chemical conditions of the environment and the supply of food to the ocean bottom. Although global change is of increasing concern for its potential consequences at global scale, its impact on deep-sea ecosystems remains largely unknown. The project GLIDE will use a multi- and interdisciplinary approach, including remotely operating vehicles, along with state-of-art methodologies to provide an unprecedented information on the impacts of global change in the deep ocean. In particular, GLIDE aims at investigating the responses of the biodiversity and ecosystem functioning to changes in temperature and primary productivity, comparing historical data (collected by the GLIDE team 15-30 years ago in deep-sea regions the NE Atlantic Ocean and in the Mediterranean Sea) with new data and developing innovative strategies for the monitoring of the impacts of global changes on the deep-sea.

Marine Hazard: Development of innovative technologies for the identification, monitoring, remediation of sources of natural and anthropogenic contamination Started 12/2019 – end 06/2022 - Aree di ricerca: Ricerca e Innovazione Tecnologica Fonte Finanziamento: PON "Ricerca e Competitività 2007-2013" -soggetto finanziatore e schema di finanziamento MIUR Ruolo SZN: PARTNER, OR1, OR4 - PI F. Andaloro e G. Procaccini

The project aims to enhance the marine research infrastructures located in the Convergence Regions of Sicily, Campania and Puglia, and to create synergy between scientific research and the industrial sector, for an advanced and appropriate management of the coastal marine environment and to develop an effective prevention of effects that may be consequent to mining activities in deep environments. The project coordinated by the CNR is organized into 4 research objectives: OR1 / Prevention of effects related to finding marine resources; OR2 / Remediation of sediments and waters polluted by priority pollutants; OR3 / Development of new sensors and integrated approaches to coastal marine Remote / sensing; OR4 / Development of management technologies and strategies for environmental protection and restoration of *Posidonia oceanica* meadows. Within OR1, the activity of the SZN is aimed at the acquisition and processing of previous data on hydrothermal emissions in the area of the Southern Tyrrhenian Sea and the Sicilian Strait, planning and carrying out of exploratory surveys to be carried out in the southern Tyrrhenian area, Palinuro Seamount and the Sicilian Straits for the study of the distribution, intensity and characteristics of submarine hydrothermal systems, as well as assessment of possible anthropogenic impacts related to extraction / withdrawal from hydrothermal systems, including the development of models. OR4 The activities of the SZN within OR4 are focused on two main directives. (i) The development of early warning indicators through massive sequencing techniques of the Posidonia oceanica transcriptome, in order to select target genes that respond to particular types of stress and (ii) The definition of genetic discontinuities existing between the putative areas of sampling and replanting, through a fine genetic mapping of the Posidonia oceanica meadows along the Italian coasts, with the development of standard laboratory protocols for the individual / specific genotyping of *Posidonia oceanica* plants.

Cosmeceuticals And Nutraceuticals From Antarctic Biological REsources (CAN FARE) Durata: Novembre 2017-Novembre 2020 - Aree di ricerca: Biotecnologie Fonte Finanziamento: Programma Nazionale di Ricerca in Antartide - Ruolo SZN: Partner - <u>PI A. Ianora</u>

Antarctica is a still an unexplored area for many aspects, including biological diversity and, even more, chemical diversity. Thanks to its evolutionary history and ecological isolation, Antarctic marine environments are expected to harbour unique biological communities that could biosynthesise novel bioactive compounds, potentially valuable for many applications such as pharmaceuticals, nutritional supplements and cosmeceuticals. The main objective of this two-year project is to identify novel marine bioactive molecules that can function as model compounds for the design of new products for nutraceutic and cosmetic applications. This is in view of the recent expansion in the diagnosis of genetic and life-style related diseases, and the increased interest in finding new products aimed at improving human well-being by treating these diseases. Our aims are: 1. Collection of marine micro- and macroorganisms, putative producers of bioactive secondary metabolites and functional lipids having an untapped biotechnological potential; 2. Screening of Antarctic samples by biological assays for nutraceutic and cosmetic properties using in vitro selected human cell lines; 3. Isolation and characterisation of bioactive molecules by chromatographic, spectroscopic and spectrometric methods, mainly mass spectrometry and Nuclear Magnetic Resonance spectroscopy. CAN FARE will have societal impacts by (i) providing novel substances for human health care, (ii) stimulating the acquisition of novel expertise and promoting innovative business models and entrepreneurship, (iii) increasing our knowledge of Antarctic biological resources. Furthermore, the project will have an economic impact derivied from patenting and commercial exploitation of the novel compounds.

Place - Off-shore Platform Conversion for Eco-sustainable Multiple Uses November 2018-April 2021 - Research Area: Blue Growth – Source: MIUR Role SZN: partner - Pl A. Toscano

The offshore oil and gas infrastructures are ending their operational life in most areas of the world, and the impact of decommissioning activities is still largely unknown. Policies of complete removal are based on the assumption that 'leaving the seabed as you found iť' represents the most environmentallysound decommissioning option. However, we



know that oil structures are capable of supporting abundant and diverse marine communities during

their production lives. Removal of such structures is unlikely to represent best environmental practice and this has prompted some countries leaving obsolete structures in place as artificial reefs or to find alternative solutions for their sustainable re-use. PLaCE aims at investigating for the first time at national level cutting-edge technologies and solutions for the re-use of offshore platforms located in the Adriatic Sea in front of the Abruzzo region coastline at the end of their productive phase. In particular, a life-extension strategy for reutilization of offshore platforms into new and eco-sustainable economy, based on mineral accretion technology under low voltage electrolysis of seawater to protect offshore structures from corrosion will be tested. Cutting-edge activities of the project will include the application of innovative eco-sustainable strategies of aquaculture based on integrated shellfish and holothurians farming, design and development of innovative systems for renewable energy generation needed for multipurpose platform activities, the development of innovative integrated systems for the assessment of ecological sustainability of multipurpose platform activities and their maintenance, costbenefit analyses and business scenarios taking into account also recreational aspects. To achieve its goals this project relies on a consortium composed by a big industrial partner of the oil & gas sector, a SME and 6 top ranked research institutes/universities. This project will represent a major opportunity to modify the business as usual approach to the sea and the use of its resource and will contribute to the blue growth of the sectors involved promoting diversification and synergies and therefore improving attractiveness, competitiveness and innovation at regional and national level.

Peptides and Enzymes from cold-adapted microorganisms foR melanoma and leukemia TREATment (PERTREAT)

2020-2022 - Research Area: Marine Biotechnology, Drug discovery, Anticancer activity Source: PNRA 2018 Role SZN: SZN: Coordinator - PIC. Lauritano

The Antarctic ecosystem is characterized by a high level of biological and genetic diversity and is largely unexplored from the bioactivity point of view. This project aims to discover bioactive peptides and proteins with anticancer activity from bacteria and microalgae collected during a previous Antarctic expedition (XXXIII Expedition, Mario Zucchelli Station, 2017-2018). There are more than 200 different types of cancers, but the project will focus only on two: 1) human melanoma cancer cells, because Antarctic marine organisms may have developed physiological and biochemical mechanisms for reducing UV-induced damage; 2) leukaemia (acute monocytic and lymphoblastic leukaemia), because several peptides from bacteria are known to be active against leukemia and because we recently identified for the first time in the transcriptome of the dinoflagellate Amphidinium carterae the sequence encoding for the enzyme L-Asparaginase used for the treatment of leukaemia. The aims of the project are: i) to screen for anticancer activities in Antarctic microorganisms, ii) identify and characterize active peptides and proteins, iii) identify genes/transcripts encoding for proteins/enzymes useful for cancer treatment by genomic, metatranscriptomic and/or transcriptomic analyses of the bioactive samples. The main reasons why we expect a successful outcome of the project are a strong focus on well-defined activities (selected organisms, specific bioactivity and a defined class of bioactive compounds) and the exploration for anticancer compounds in a relatively unexplored Antarctic system. The project is highly interdisciplinary, requiring expertise ranging from cultivation of marine microorganisms to evaluation of gene expression, protein and peptide profiling and their structure elucidation. The activities will be carried out by two operative units, experts in these activities and well equipped for the required analyses: OU1-Stazione Zoologica Anton Dohrn, leaded by Chiara Lauritano, coordinator of the project and researcher at SZN in the Marine Biotechnology Department, and OU2-CNR leaded by Daniela Giordano.

ABBACO - Sperimentazioni pilota finalizzate al Restauro ambientale e balneabilità del SIN Bagnoli-Coroglio. Duration: triennale 08/03/2017 - 07/03/2020 Research Area: Marine Ecology Funding: MIUR FISR –CIPE SZN Role: Coordinator - <u>PI L. Musco</u>

Dismissed industrial activities are responsible for persistent environmental degradation, mainly due to long-term accumulation of xenobitic contaminants in the environment. Such a chronic form of pollution represents a major threat for human health, biodiversity and ecosystem functioning. Environmental restoration practices should however be coupled to restoration plans aiming at revert the degradation trend and give back healthy areas able to provide valuable ecosystem goods and services. Albeit fully integrated into the EU Restoration Agenda, marine environmental restoration is a new challenging issue in ecology, with Italy coordinating MERCES, the first European project in this field. The environmental restoration of Bagnoli-Coroglio Bay is a unique challenge at European level. ABBACO will develop new approaches for the removal and remediation of contaminated sediments and restoration of marine habitats in collaboration



with the Government Chief for the area and INVITALIA. Actions include: i) identifying the environmental benchmark of the area; ii) assessing its present health status, iii) studying the effects of contaminated sediments on biodiversity and ecosystem functioning (MSFD), iv) assessing the combined effects of multiple stress at a hierarchical level; (v) experimenting innovative methods of transplantation and restoration of key species and habitats, and new biotechnological instruments for the remediation of sediments (bioremediation, bioaugmentation) in degraded habitats. ABBACO will provide novel expertise and stimulate new initiatives within the Blue Economy Agenda.

DEMBAI

Duration: Three Years triennale 2017- 13 Nov. 2019 (Extended until 13 May 2020) Funding: PNRA 2016 SZN Role: Partner - PI S. Stefanni

DEMBAI Diversity and Evolution of Marine Microbial Communities Associated with Invertebrates benthic antarctics. The associations between microbes and marine metazoa play a fundamental role in influencing host functions, nutrition and health, but study concerning such associations in Antarctic ecosystems are still very limited. Ecosystems antarctics can be an ideal laboratory for exploring biodiversity and the microbial community functions associated with benthic metazoa, and their adaptation to extreme conditions, also to understand conservation strategies and potential biotechnology of polar ecosystems. The main objectives of the project are: 1) to study the diversity and functions of microbial communities (bacteria, archaea and eukaryotes, such as fungi) associated with the most representative taxa of the macro and megafauna of Antarctic sediments through metagenetic and metatrascrittomic analyses; 2) evaluate the influence of the environmental factors (depth, trophic conditions, latitude) on taxonomic composition and gene expression of the microbiota of Antarctic benthic invertebrates, also by comparing the microbiota of Antarctic species with conspecifics from temperate ecosystems (already comparing the taxonomic composition and functions of the microbiota and communities microbes in the water column and surrounding sediments; 4) identify potential interactions between microbiota and Antarctic invertebrates and their co-evolutionary processes through the analysis of the microbiota of phylogenetically related invertebrates. Sediment samples for the analysis of the microbiota associated with metazoa will be collected using the motor vessel Malippo (or Skua) along two bathymetric transects characterized by different organic loads in Newfoundland Bay. At the same time, samples of sediment and water to explore the origin of the associated microbes

will be collected. Benthic invertebrates will be identified by means of a combined microscopy approach and on Sanger sequencing and their DNA and RNA will be extracted, amplified and sequenced on high throughput platforms to assess the diversity of bacteria, archaea and fungi and their functions. The main scientific results expected for this project, in addition to the new information about the ecological interactions between microbes and hosts, their evolutionary processes and the adaptations in Antarctica, also include the expansion of the databases with new gene sequences of microbes and identification of sequences/functions of potential biotechnological interest.



8.4 FINANCING FOR INFRASTRUCTURES

Research and marine infrastructure strengthening (EMBRC-IT) PRIMA

20/06/2019 – 19/02/2022 - Research area: strengthening research infrastructures. Source: PON Research and Innovation 2014-2020. SZN: Coord - M. Borra e F. Vecchi

Italy has an extraordinary potential in the field of marine research thanks to the heritage of about 2000 high-level researchers. However, this potential remains largely unexpressed due to the limit of the infrastructures. The project PRIMA aims to fill this gap by increasing the Italian competitiveness on the basic and applied marine research in the European context by tightly linking this effort to the EMBRC-ERIC infrastructure and then contextually increasing the importance and impact of Italy in the marine research at European level. The project aims to strengthen the research infrastructures through: i) the acquisition of new instruments not available in this research field at national level and instruments of relevant technological complexity that allow investigations that are currently not feasible in the marine environment; ii) the completion and strengthening of existing infrastructures to make them updated and competitive in the light of new research developments; iii) the creation of hubs able to allow researchers of the Stazione Zoologica Anton Dohrn, and overall of the country, to access and investigate marine habitats, thus increasing the potential development of some key regions in Italy. In this perspective, particular interest has been dedicated to:

- the development of infrastructures in the Calabria region, the only Italian southern region which currently has no marine research centers despite the extraordinary coastal development and a totally unexplored potential in marine ecosystems;
- the strengthening of infrastructures in the Sicily region;
- the creation of a new inter-institutional research center in collaboration with CNR and three academia located on the Adriatic Sea (Marche region), where the Stazione Zoologica Anton

Dohrn actively works in the monitoring of marine platforms for the hydrocarbons extraction. lt is therefore evident that also the investments developed in the central-northern regions, more specifically Fano and Trieste, will receive a direct logistical and operational facilitation for the work with the headquarters in Naples.



Iniziative in Supporto al consolidamento e potenziamento dell'infrastruttura EMSO e delle sue attività (InSEA)

Duration: June 2019-February 2022 - Research areas: Oceanography, Biology and Marine Ecology, Technological development Funding Source: PON RICERCA E INNOVAZIONE 2014-2020 (CCI: 2014IT16M2OP005) INFRASTRUTTURE DI RICERCA Role of the SZN: non-coord part - <u>M. Borra</u>

The InSEA Project is the Italian contribution to the consolidation of the European research infrastructure EMSO ERIC in synergy with other ERIC research infrastructures or otherwise included in the latest ESFRI Roadmap. Contributes to the promotion of sustainable use of the seas and the conservation of marine ecosystems in line with the indications of the European Commission (Marine Strategy Framework Directive, the Maritime Spatial Planning Directive, the Blue Growth Strategy and the G7 Future of the Sea and Ocean Initiative), implemented by ESFRI for research infrastructures. As part of the Project, the construction of an integrated fixed observatory (SZN-fix) is planned which will integrate an existing mobile system (SZN-mob) conceived as monthly sampling in a coastal station in the Gulf of Naples (LTER-MC), where the entire water column is sampled for the determination of biological parameters with an end-to-end approach (from microbes to fish). SZN-fix will be built in 2020 and positioned in the Dohrn canyon (from 600 to 1000 m) and will consist of a seabed platform and a mooring with a series of sensors. The ecological information of the two modules will be complemented by further ad hoc sampling at key moments and by experimental activities aimed at contextualising the sites on a large scale. The observatory therefore aims to represent a first step towards the establishment of a network of augmented observers, with an end-to-end approach in the multidisciplinary framework necessary for the assessment of the health of the oceans. The knowledge generated will be useful for directing management policy and decisions on ecological footprint mitigation in the current Marine Strategy Framework Directive and the definition of good environmental status (GES-Good Environmental Status).

Marine Farm and Factory (MAFF) - Research center and advanced marine infrastructure Duration: March 2018 - March 2022 Research area: Infrastructures for marine research. Funding Agency: ISR MUR PON research and Innovation SZN Role: Partner - PI F. Maffucci

The Marine Farm will offer new opportunities to the biotechnological sector and to researchers and it will allow to expand experimental capabilities promoting innovation in marine, biological, biomedical and productive research. This new research infrastructure will offer the unique opportunity to discover and produce new products derived from marine organisms and develop innovative concepts in marine biological science. It will make possible the scaling –up of tools and technological innovations for the production of marine organisms. The aspects of scientific dissemination will also be fundamental and to this respect the large aquariums present in the former Tematic Aquarium will be reorganized to create an exhibition on marine biological resources that will be an important and innovative tourist attraction for the Bagnoli area.

Summarising, the Marine Farm will play a strategic role for the development of the South Italy since: 1) it will create in Campania an excellence in the field of marine organism production for basic and applied research; 2) This new Blue biotech advanced infrastructure built in the former industrial site of Bagnoli-Coroglio, will have strong environmental implications, 3) It will create an innovative Exhibition Center to promote the importance of marine research that will also be a unique attraction which will strengthen the tourist vocations of the Bagnoli-Coroglio area. **EMBRIC - European Marine Biological Research Infrastructure Cluster to promote Blue Economy** Duration: Jun. 2015-May 2019 (Extended until 31 Dec 2019). Funding Agency: EU-H2020 SZN Role: Partner - <u>PI W. Kooistra</u>

The general objective of EMBRIC (European Marine Biological Resource Infrastructure Cluster) was to build connections on three axes: science, industry and regional CSR policies. The results are lasting collaborations, a memorandum of understanding among the partners and a series of bilateral agreements between the RIs (EMBRC-ERIC, EU-OpenScreen-ERIC, MIRRI-ERIC and ELIXIR, which promote innovation in the marine biotechnology sector. This project focuses on two specific sectors of marine biotechnology, in particular (i) the isolation and development of new molecules and natural marine products, and (ii) the identification of markers in aquaculture. EMBRIC has been designed to promote scientific research and innovation policies related to marine bio-resources. This objective was achieved through the development of multidisciplinary research lines in the technological field (WP 2-4), joint development activities (WP 6-8), training and knowledge transfer (WP9) and access to cluster and service pilot structures (WP10). 24 projects have enjoyed TA services offered by EMBRIC. EMBRIC has also developed technology transfer (TT) and innovation policies at the European level (WP5) promoting greater cohesion in TT practices. The EMBRIC project ended on 05/31/19.

Corbel - Coordinated Research Infrastructures Building Enduring Life-science Services. Duration September 2015-August 2019 (Extended May 2020) Funding: European Commission, H2020-INFRADEV SZN Role: Partner - PI MI Arnone

The CORBEL project involved 35 European partners in order to establish a network of common service collaborations between the ESFRI Biological and Medical Research Infrastructures. Its objective was to translate, within the European scientific community, discovery of the fundamental biological mechanisms all the way into medical applications. It did so through the creation of a unified interface, at the level of typology and coordination of services, in order to obtain a wide access to all the technological platforms involved. The SZN, as an EMBRC partner, participated in the CORBEL project for work-package 4 (WP4), Use case 4, coordinated by the CNR partner - Villefranche - sur - Mer (coordinator Evelyn Houliston), which aimed to create databases based on the NISEED platform for the use of marine organisms in biomedical research. The marine reference organisms in this project were the jellyfish *Clytia hemisphaerica*, the sea urchin *Paracentrotus lividus* and the amphioxus *Branchiostoma lanceolatum*. SZN participated mainly for the creation of a database of sea urchin

Paracentrotus lividus and, in part, for that of the amphioxus Branchiostoma lanceolatum. To carry out these activities, the SZN received the contribution corresponding to 18 months of contract for a postdoc with experience in bioinformatics. Furthermore, in 2018 the SZN also saw the access of Dr. Matteo Rauzi, University of Nice, France, winner of Corbel's first "access call". Dr. Rauzi received access to the microinjection station for sea urchin at the BEOM department, "Functional Genomics Area" and received training for the collection of gametes and microinjection in *Paracentrotus lividus*. This first access was also a test bench for the future activities of the SZN in the context of the EMBRC-ERIC.

C R B E L

Assemble Plus

Duration: Oct. 2017 - 30 Sep.2021 Funding Agency: EU-H2020-INFRAIA-2016-2017 SZN Role: Partner - <u>W.</u> Kooistra

The main objective of the Association of European Marine Biological Laboratories Expanded "ASSEMBLE Plus" is to organize, operate and improve the provision of Transnational Access (TA) to European marine stations, ie to marine ecosystems, local marine biological resources, modern infrastructure and the scientific and technological knowledge necessary to perform innovative research with these resources.

The TA is supplied to a total of 33 marine stations (some of which consist of connected Third Parties) in 16 countries. The stations that supply the TA have modern research laboratories and a broad spectrum of



ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES EXPANDED

specialized research facilities to support users. Virtual Access (VA) will include internet access to biological datasets generated over decades in various marine stations, for example, in long-term ecological research and monitoring programs. Networking Activities (NAs) and Joint Research Activities (JRAs) are focused on supporting and improving TA. The TA in ASSEMBLE Plus operates through national EMBRC nodes, where present, in order to attract a diverse community in the field of life sciences, pharmaceutical and biomedical sciences and the private sector. These activities increase the importance of marine stations in European research. The objectives of the NAs are to develop the TA program, to improve it, to attract new categories of users including those from the private sector, and to ensure the long-term sustainability of EMBRC-ERIC. The objectives of the JRAs are to solve the critical issues in the supply of the TA and improve the quality and quantity of TA.

SZN coordinates WP NA1 (WP3: Improving access provision), participates in WP NA2 (WP4: Improving virtual access- Design Data Management Plan), WP NA3 (WP5: Engaging with User communities), WP NA4 (WP6: Long-Term Sustainability), JRA1 (WP7: Genomics observatories), WP JRA3 (WP9: Functional Genomics) and WP JRA4 (WP10: Development of instrumentation), and coordinates WP TA12 (WP23: Transnational Access to EMBRC-Italy) which includes access to the SZN and to the Italian Third Parties: CNR - ISMAR Venice and CNR - IRBIM Messina.

EMSO MedIT

Duration: completed but undergoing further implementation with PON 2018. Financing source: PON R&C 2007-2013 - PAC Enhancement of public research infrastructure for environmental monitoring. Role SZN: Partner - <u>PI R. Casotti</u>

EMSO -MedIT is a project to upgrade marine research infrastructures in Sicily, Campania and Puglia and represents the Italian contribution to the ESFRI EMSO infrastructure (www.emso-eu.org).

The project is funded under the NOP R&C 2007-2013 - PAC Enhancement public research infrastructures and pursues the objective of strengthening the infrastructures for the research in the field of marine sciences in the Convergence Regions of Sicily, Campania and Puglia.

EMSO - MedIT is the Italian contribution to the consolidation of the European research infrastructure EMSO coordinated by Italy, which, as part of EMSO - MedIT, goes in synergy with the other ESFRI with Italian coordination and with the Italian initiative for marine research: the RITMARE flag project. The actions foreseen by the EMSO - MedIT Project aim at:

• enhance marine infrastructures and scientific and technological facilities in order to consolidate and expand the network for multidisciplinary monitoring of the marine environment coastal and deep and the water column;

• network all the existing infrastructures and enhance the real transmission time / near - real time

integrating the measurements of fixed and relocatable observing systems;

• set up a mobile intervention system to be used for monitoring campaigns in sites of strategic interest or in the event of environmental emergencies.

The network of monitoring infrastructures is further enhanced through the creation of an exchange information system that allows the sharing of large data mass of data produced, with access by a vast community of Italian users and foreigners of various origins. We are project partners and WP2 managers "Enhancement of Campania", together with INGV for the Gulf of Pozzuoli. The upgrade provides for the acquisition of different oceanographic instrumentation, including a WaveGlider, a ROV, and several probes, but above all two MEDA-type elastic beacon to be located in the Gulf of Naples and the Gulf of Pozzuoli for real-time monitoring and environmental data transmission and physical conditions to the SZN operation center.

Partners - National Institute of Geophysics and Volcanology (INGV), Stazione Zoologica Anton Dohrn (SZN), National Institute of Nuclear Physics (INFN), National Research Center (CNR), Higher Institute for Environmental Protection and Research (ISPRA).



8.6 MINISTRY UNIVERSITY AND RESEARCH FUNDED PROJECTS

TERABIO (INFN Coordination) - PI S. Stefanni

In the framework of the 2014 TERABIO Award Project financed by the MIUR, our unit in the Stazione Zoologica Anton Dohrn in Naples will have the task of identifying new fluorophores genetically coded. Sampling of organisms will be carried out first zooplankton and marine phytoplankton from coastal and offshore waters in the Gulf of Naples, spectral analyses of the collected organisms will be carried out, RNA extraction and analysis to define transcriptomic profiles of the selected organisms. Bioinformatics and molecular evolution studies will allow for identification by sequence homology, of potential new fluorescent proteins. At the same time, computational analyses will be carried out on genomes and transcripts already sequenced and made public for *in silico* identification of possible fluorescent proteins to exploit the enormous potential of the data -not yet public of the TARA expedition (http://marinemicroeukaryotes.org/) and the amphioxus *Branchiostoma lanceolatum*. The coding sequences for the new biofluorescent proteins will be used as reporter genes for further in vivo testing. Final and long-term objectives will be to identify fluorescent proteins with new spectral characteristics and study their optics properties optics to allow their modelling.

EARTH CRUISERS

EARTH's CRUst Imagery for investigating SEismicity, volcanism and marine natural Resources in the Sicilian offshore - Duration: 1 Jun 2018 - 31 Dec. 2020 Research Area: Financing Source: Altri ministeriali SZN Role: Partner - PI <u>S. Stefanni</u>

EARTH CRUISERS is a project coordinated by OGS (National Institute of Oceanography and Experimental Geophysics) in collaboration with the Stazione Zoologica A Dohrn and INGV (Vesuvian Observatory) and proposes to identify and characterize the main crustal tectonics structures present in the Sicilian offshore, in order to: i) reconstruct the kinematics of the microplates that make up this sector of the Central Mediterranean; (ii) understand and modelling the geodynamic processes that control seismicity and volcanism in the offshore environment of Sicily and its smaller islands; (iii) to provide a useful tool for the estimation of dangerousness seismic, volcanic and tsunami-like coastal areas of the region. In particular, the deep geological depth knowledge of this region of the Central Mediterranean, so densely populated and notoriously affected by destructive seismic events, it is indispensable to understand the seismic hazard and therefore to provide an indispensable element in the to future risk reduction. The project also aims to develop some themes relating to the offshore marine extractive industry, and to the regulation and environmental protection to (i) characterize the hydrothermal systems identified in the Tyrrhenian Sea (ii) assess the effects of hydrocarbon extraction activities on the seabed (iii) estimate the hazardousness of an offshore area subject to sudden gas releases (Channel of Sicily). The refinement of knowledge on the evolution of the Central Mediterranean in the last millions of years represents the starting point to understand the current geodynamic processes and develop a seismic hazard model in the coastal areas of Sicily (connected to the presence of structures seismogenic and potentially tsunamigenic tectonics), characterized by the highest values of seismic energy released on the entire national territory, also through the realization of of hazard maps for two pilot areas: the Etna coast and the Gulf of Patti; (ii) of the hazard volcanic of the largest underwater volcano in the Mediterranean Sea, Marsili, and the Banco Graham who produced the last historical Italian underwater eruption (the Ferdinandea island). Two areas of the Sicilian Channel have been chosen as test sites for the application of marine geophysics. The first site is the seabed on which the Vega-A platform rests exploits the main

hydrocarbon field in the Italian offshore, which is the subject of an geophysical monitoring experiment, able to study possible deformations of the seabed and seismicity traceable to mining activity. The second one is the area between the Graham, Nerita and Terrible, and the Sicilian coast of Sciacca, where extensive pockmark fields have been recognized, which will be the subject of an integrated study for the estimation of the hazard in an area of high environmental value, repeatedly requested for mining exploration activities. In the south-eastern Tyrrhenian Sea, at the underwater volcanoes Marsili and Palinuro, hydrothermal systems and massive sulfide deposits have been identified, which could be exploited for the cultivation of minerals rich in economically valuable metals. The project It is proposed to characterize the geology of the areas affected by these hydrothermal systems, to assess future economic exploitation that protects the marine environment. (This research objective is also identified and promoted in the H2020 and EU Marine calls Strategy Framework Directive). SZN researchers contribute in the different work packages: Dissemination Component (WP0), Innovative Geophysical Applications to the Geophysics Industry. marine extractions in Sicily (WP2), and Hazard Assessment in offshore areas for the regulation of the exploitation of marine resources and protection of the marine environment (WP3). The activities of WP2 fall within the two areas of the "BLUE" area of specialization GROWTH", "Marine Extraction Industry" and "Research, Regulation and Protection environmental', the object of which is, respectively, the exploitation of the deposits present in the marine environment and the assessment of the dangerousness of natural phenomena, aimed at regulating mining activities. Task 2.3 concerns the study on diversity of the benthic community associated with the hydrothermal activity of volcanic submarines structures and is of particular interest to SZN. WP3 will instead develop the theme of the 'research, regulation and environmental protection' sector of the 'BLUE GROWTH' specialization area, which has as its object the evaluation of the dangerousness of phenomena natural, preparatory to the regulation of the exploitation of natural resources offshore. Task 3.2 sees SZN's involvement in identifying possible key species that are associated with an exploitation of this type, and at the same time evaluate an recovery timeframe in terms of biodiversity for those deposits already decommissioned and focuses on the Biological environmental impact indicators.ù

PHOTOSYNTHESIS 2.0 Duration: 2017-2019 Research Area: AgriFood – Marine Biotechnology Financing Source: MIUR - SZN Role: Partner - **M. Costantini**

The objective of the project is to use the new scientific and technological tools to overcome the limitations of photosynthesis (the milestone of life on earth for millions of years allows you to produce clean food and energy) and make the process more efficient and stable, especially in adverse environmental conditions. The project consists in workpackages and activities that examine photosynthetic limitations according to the classical hierarchical scheme, which starts from the photosynthetic organelle (the chloroplast) and reaches the plant community (culture agricultural or forestry or natural ecosystem). New attention is given to the possibility of use symbioses with microorganisms to improve the photosynthetic process, in particular in unfavorable conditions, and the possibility of using physical methods and genetic engineering for increase the efficiency of the various parts of the process, from the capture of photons of light into photosystems to the distribution of photosynthesis products in the various organs of the plant. Particular attention is given to the production of algal biomass, as the algae constitute a particularly efficient photosynthesis model and promising applications in various fields of green chemistry, producing for biorefining of the photosynthesis products a broad spectrum of industrial commodities. At the end of the project, information able to optimize photosynthesis will be achieved and therefore to produce better and with fewer resources, and the Italian scientific community will have consolidated a position of excellence, contributing significantly to international efforts for the improving the planet's productivity.

CIBO E SALUTE - Duration: 2016-2019 (Extended 31/12/2021)Research Area: AgriFood – MarineBiotechnology - Financing Source: MIURSZN Role: Partner - M. Costantini

The project Cibo & Salute (Ci&Sa) intends to contribute to strengthening and innovating the system Italian agri-food by identifying appropriate and effective solutions to growing and new food needs of the population, and problems of food security and sustainability agribusiness. Ci&Sa will address food quality, food safety and food security in an interdisciplinary and innovative manner, with the aim that food, at any scale (local, global), both healthy, safe and of quality, and produced in a sustainable and accessible manner, in particular to those segments of the population with eating disorders and/or pathologies connected to incorrect feeding. In the proposed project, the SZN will contribute with the analysis of the primary processes of contaminant transfer in the marine trophic network and with the exploration of substances of nutraceutical interest derived from marine organisms.



8.7 FLAGSHIP RESEARCH PROJECTS FUNDED BY SZN

BIOINforMA "core facilities" in BIOINformatica per la biologia MArina. Department: RIMAR - **M.L.Chiusano**

This is a Flagship Project of the Stazione Zoologica Anton Dohrn. It aims to set up bioinformatics resources for the genomics of model species for marine biology, favouring the already widespread functional analysis activities in the institute and genomics comparative approaches. In addition, the project aims at the organization of a reference infrastructure for bioinformatics, preparing basic and advanced methodologies, with the aim to contribute to innovate research to discover, enhance and protect marine life (Vision 2015-2025). The initiative aims to channel multidisciplinary skills, to be trained with one integration effort that fosters synergy effects, versatility and interoperability for address the growing demand for bioinformatics in support of "omics" activities in the institute. This will also allow for the development of an organized infrastructure, with centralized hardware and software resources, helping to optimize management costs, of training and outsourcing, and to preserve and increase know-how in the sector. The creation of a reference structure for bioinformatics, inserted in a context as the Stazione Zoologica, ready to experiment the continuous evolution of omics technologies to meet ambitious scientific challenges running and in perspective, will foster a mutual exchange, establishing a language, common sensitivities and objectives, to address frontier approaches, technological advances, and promote cutting-edge research. With the main objective of developing genomics resources accessible via the web, and to implement local data analysis methodologies in order to define value-added collections and disseminate the associated results, favouring the development of procedures that will be shared and offered as service to the SZN scientific community. This preliminary scientific effort will favour the organization of a basic infrastructure paving the way for the definition of strategies, resources, databases, computational scientific platforms for the entire community concerned, inside and outside the institute. The establishment of a coordinated bioinformatics service in the field of marine genomics will be essential to allow the Stazione Zoologica to expand its key role in marine genomics and ecology.



8.8 **REGIONAL PROJECTS**

FEAMP Campania

Duration: 08/08/2019-30/10/2022 - Research areas: AGR/20, LS, SH3_1, Source: Fondo Europeo per gli Affari Marittimi e la Pesca 2014-2020 SZN: Coordinator - <u>F. Colloca e S. Fraschetti</u>

Fishery and aquaculture represent for the Campania Region a critical productive, economical, occupational and social opportunity that can be developed through the innovation and sustainability of the productive processes. The region is featured by a widespread activity of artisanal fishery, very rich in anthropological, gastronomical and cultural traditions together with industrial and semi industrial activities within a complex natural system. In the area, Marine Protected Areas (MPAs) and Biological Protection Zones, coexist with polluted sites, relevant touristic fluxes linked to the use of the sea, IUU fishing, maritime and port activities, marine pleasure craft market and other productive activities. Aquaculture, and especially shellfish farming, is also an important activity in the coastal area. The project is developed through the collaboration of 9 research partners and has the aim to integrate the ecosystem approach in the current "Campania fishery and aquaculture management model" to achieve an economic and environmental sustainable development of maritime activities in the global context of Blue Growth. The project takes into account of the Good Environmental Status targets of the Marine Strategy Framework Directive (MSFD) and the objectives of Common Fisheries Policy (PCP). The ultimate goal is to produce the spatial planning of aquaculture as key component of a broader maritime spatial planning of anthropogenic uses of the sea at a regional scale and in accordance with the MSP directive 2014/89 / EU.

NETTUNO (Contabilità ambientale)

Start: 1 2018 - End 4 2020 - Aree di ricerca: Area 5-BIO/07 - Soruce: Area Marina Protetta Regno di Nettuno - Ruolo SZN: coinvolta nella fase 0 - M.C. Buia

The project "Environmental accounting in the Italian marine protected areas", financed by the Italian Ministry of the Environment and Protection of Land and Sea (MATTM), is aimed at implementing an environmental accounting system for the Italian Marine Protected Areas (MPAs). The main goal of this project is the biophysical and economic assessment of natural capital stocks and ecosystem services flows generated by the MPAs. The methodological approach for the environmental accounting of the MPAs is articulated in six Phases. In particular, the Phase 0 (object of the present project) dealt with the collection and analysis of data specific for the MPA "Regno di Nettuno", among which bathymetry and bionomic maps, the characterization and the area of the habitats, and the quantitative biomass assessment of macro-benthic organisms and sedentary fish fauna. Ad hoc sampling and visual census activities were performed on hard bottom (Sciaphilic and Photophilic), soft bottom, and *Posidonia oceanica* beds habitats. The collected samples were analyzed in laboratory and the biomass values calculated for macro-benthic organisms and fish fauna were entered into a database that represents the prerequisite for the implementation of the other Phases of the project.

"MARINE HAZARD OF SEAFOOD" CONVENZIONE ISTITUTO ZOOPROFILATTICO DELLA SICILIA PIANO REGIONALE PREVENZIONE-, LINEA PROGETTUALE 4, ACCORDO STATO- REGIONE NR. 64 DEL 14 APRILE 2016. end: 12/2020. - PI F. Andaloro

The project aims to create an expert system that through a dedicated portal and utilising the the social media can develop an effective prevention of the negative effects on human health both produced by marine hazards, related to the consumption of fish products, and related to the bathing, the diving and the other direct uses of the sea, such as the risks represented by jellyfish, the harmful algae and the toxic or the poisonous animals. The portal will contain a general part relating to basic information on food risks from fish products such as the counterfeiting, the alteration, the microbiological contamination, the zoonoses, the traditional and emerging contaminants, the histamines and the toxic or the poisonous species. The risks from species toxic and poisonous animals on contact will also be treated. The portal will report in an updated form, also in spatial terms, the dangers that may arise, even occasionally, at particular times. Finally, an early-warning system will also be created to deal with particular emergencies that require timely information. The system will have a specific data policy also involving Coast Guard and other Public Administrations and will also be based on an interactive system, making the most of the potential offered by the Citizen Science in acquiring information's. The goal of the project is on the one hand to improve the health and well-being of citizens and sea workers and on the other to reduce health public expenditure.

CRIMAC - Research Center and Advanced Marine Infrastructure in Calabria Start: 01/2020 end 01/2025 - Research Area: Marine Biology and Ecology, Innovation, Biotechnology, Sustainable Use of the Land. Source: CIPE soggetto finanziatore e schema di finanziamento MIUR Role SZN: Coordinator - PI S. Greco

The project was prepared with the aim of promoting National research in the Southern Region and starting new scientific collaborations with National scientific associations, SZN Scientific Community of reference, University and Public Research Institutes. In this context, this project was launched as an internal SZN Call with the aim of financing 7 project proposals (3 years) and 5 grants (2-years) to enhance research at the SZN Research Center in Amendolara. The project proposals must be presented by researchers / technologists of the SZN in partnership with other institutions (with at least one partner from Institutions, University of Calabria) and must be developed according to a multidisciplinary approach, in order to enhance the biodiversity of territories and marine habitats and for environmental protection. Curiosity Driven can be included, as long as it is linked to biological models, organisms or habitats of the Calabrian seas. Research activities will be carried out aimed at the protection of marine environments and fishing areas (Secca Amendolara), coastal environmental quality for the promotion of activities and blue tourism in the Calabria region.

Mediterranean spearfish – Assessment of the distribution and status of the Mediterranean spearfish Tetrapturus belone. Start: 01/2019 end 12/2021 - Research area: Fishery Science and Marine Ecology Fonte Finanziamento: REGIONE SICILIA EMFF 2014-2020 Role SZN: Coordinator - <u>PI T. Romeo</u>

The UE Reg. n. 1380/2013 on Common Fisheries Policy (CFP) has the long-term aim of guarantee that the fishing activities are carried out and managed, in sustainable manner. Based on these assumptions, the art. 40 del Reg. (UE) n. 508/2014 (European Maritime and Fisheries Fund - EMFF), to protect and restore biodiversity and ecosystems by sustainable fishing activities, also promotes the involvement of fishing operators. In this context, has been funded the project to fill the knowledge gaps on

Mediterranean spearfish (*Tetrapturus belone*), Until now this resource is underexploited by local professional fisheries. To date, the information on the stock status, migratory movements and reproductive areas of this species in the Mediterranean is almost completely lacking or otherwise fragmentary. For this reason, in order to acquire essential information necessary for the stock management in agreement with ICCAT recommendations, the following activities will be carried out: 1) Acquisition of basic information (ecological and biological data as well as catch and effort) and creation of the first database on the presence and distribution of the Mediterranean spearfish in the Sicilian and Italian waters. 2) Use of satellite archival pop-up tag on at least 10 specimens of Mediterranean spearfish in order to define the migratory patterns and the seasonal movements of the species to identify aggregation spots, reproductive areas and main feeding grounds; 3) Analysis of the genetic structure of the population, study of the trophic ecology, analysis of age structure of catches. 4) Evaluation of socio-economic aspects.



REsPoNSo (RiduzionE Pesca faNtasma in Sicilia)

Durata: Giugno 2020/Dicembre 2020 - Aree di ricerca: Ecologia marina Fonte Finanziamento: MIPAAF, (FEAMP) 2014-2020, misura 1.40 Ruolo SZN: Coordinatore - <u>PI P. Consoli</u>

The aim of the project is to improve the management of litter generated by fishing activities and to retrieve abandoned fishing gear. The project will take place in three areas of Sicily, "Portorosa", "Licata -Gela" and "Lampedusa Linosa" where local management plans of small-scale fishery have already been established. In the above-mentioned areas units. the most affected by the seafloor



litter will be identified through preliminary investigations (interviews, ROV surveys). The presence of litter will be verified in areas of conservation concern such as the nursery areas (already designated in the management plans) and Natura 2000 sites (SPA "Torre Manfria, Biviere and Piana di Gela" ITA 050012; SPA "Pelagie Archipelago - marine and terrestrial area" ITA040013; SIC "Oliveri-Tindari Lagoon" ITA 030012; SIC "Pelagie Islands seabed" ITA040014 and SIC - SACS "Lampedusa Island and Lampione" ITA040002; SIC - SAC "Linosa Island" ITA040001). The recovery of marine litter will be arranged and, in the case of abandoned fishing gear, the recycling even partial of the equipment will be promoted in order to promote the sustainability of fishing sector. The project will also sensitize fishermen in order to prevent the future release or loss of marine litter, and will adopt best practices for the recovery and storage of litter produced and collected during fishing activities. The investment, therefore, focuses on the development and dissemination of a removal plan for the seafloor litter in order to mitigate its impact and to restore biodiversity and marine resources.

ADVISE - Antitumor Drugs and Vaccines from the SEa Duration: JAN 2019- DEC2020 (to be extended to 2021) Aree di ricerca: Marine Biotechnology, Marine Biodiversity, Human Health Fonte Finanziamento: Regione Campania, IT Ruolo SZN: Non-Coord (WP LEADER) - PI A. Ianora

The ambitious goal of the ADViSE proposal is the creation of a platform to discover and validate small molecules of marine origin to be used as immunogenic chemotherapy, chemo-preventive agents and therapeutic vaccines against cancer. Specific natural substances can act at different levels including i) chemoprevention, ii) direct efficacy on cancer cells, iii) ability to modify tumor / host interactions, iv) possibility of enhancing a specific immune-mediated antitumor response as adjuvants in vaccines. We shall characterize the effect of the candidate natural compounds for the direct activities on the neoplastic cells as well as through the evaluation and monitoring of the immune system response in animal models. Therefore, the proposal concerns the realization of the first drug discovery platform for the identification and development of natural products with chemotherapy and immunomodulating activities, chemo-preventive agents and active components of cancer vaccines. As a proof of concept, the drug discovery platform will initially target chronic forms of neoplasms, without validated biological markers of Lung Cancer (LC), Melanoma (Mel) and Multiple Myeloma (MMi). The proposal also includes the testing of preventive antineoplastic treatments using vaccines capable of activating the immune system specifically towards cancer cells. At a minimum, three distinct models of therapeutic anticancer vaccines will be tested, through the preclinical evaluation of three distinct classes of vaccine adjuvants, based on the patented molecule of SULFAVANT (PCT / IB2014 / 062098 (10/06/2014), a sulfolipid of marine origin, whose efficacy has already been proven on a mouse melanoma model (Sci. Rep., 2017, 7: 6286).

8.9 PROJECTS FROM OTHER FINANCIAL SOURCES

INPUT - Start: January 2018 – July 2021 Aree di ricerca: Marine ecosystems, marine microbiology, marine biotechnology Source: SZN, Role SZN: Ruolo SZN: Coord - **PI C. Brunet**

Plastic pollution of the seas is becoming a main environmental threat along with the continuous increase of plastic pieces concentration in worldwide ocean systems. Plastics, micro-plastics and nanoplastics affect the organisms inhabiting aquatic systems, both at the top and at the base of the marine food web, as described by recent in-laboratory studies. While the knowledge on the effects of plastics on some biological compartments is increasing, together with the reports of plastics and microplastics concentrations at sea, the impact of micro- and nano-plastics on ecosystem functioning remains unknown. The INPUT project ("Integrative Pelagic Ecosystem Responses to nUtrients fertilization and microplasTics addition") aims to investigate the effects of microplastics on the pelagic ecosystem functioning, with a particular attention on the microbial community and their interactions in the food web and with the water chemistry (carbohydrates, doc, metals). A 14-days lasting experiment deploying six large mesocosms was carried out in the gulf of Naples, with a daily sampling plan to follow the temporal distribution of a plethora of parameters regarding water chemistry, viruses, bacteria, microalgae, micro- e meso-zooplankton. Results showed that microplastics affect coastal ecosystem functioning, altering the dissolved organic matter dynamics together with the microbial community with cascading effects on the first links of the pelagic food web. Ecosystem based-statistical analysis are now on-going to decipher the effects and the cascading events induced by microplastics on the trophic food web and on the overall ecosystem functioning.

Modres

Durata: 1/1/2018-31/12/2020 - Research area: LS1-8, LS8-1, LS8-8. Source: Ricerca Fondi Interni Ruolo SZN: Role: SZN Coordinator - <u>PI V. Zupo</u>

MODRES - Several model organisms have been selected and traditionally tested using widely distributed species and various allochtonous species for the Mediterranean. The Mediterranean Sea, and in particular the Gulf of Naples, contains an extraordinary biodiversity, especially in some specific hotspots that were widely investigated in the last centuries. Several invertebrate species contain physiological, morphological or genetic peculiarities useful for the scientific research, although specific investigation is needed to test the properties of new candidate models. The main objective of this project was to exploit the enormous biodiversity of forms and functions that characterizes the Bay of Naples, to produce new models for the scientific research, according to the SZN 3-yr plan of activities 2020 - 2022. The selection of candidate models, according to the Vision document, was based on some key elements: I. position in the tree of life, II. commercial and pharmaceutical interests, III. genome size, IV. ecological niche, V. importance for biology and marine ecology, VI. knowledge about the life cycle, VII. geographical distribution. In fact, we investigated all these aspects with the involvement of the whole Stazione Zoologica community, that is historically concerned in the culture and use of marine animal and plant models for scientific purposes. We have studied the model Paracentrotus lividus (Echinodermata) and clarified its relationship with algal feeds, the possibility for culturing in industrial plants, and obtained deep information of molecular ecology and physiology. We investigated the model Hippolyte inermis (Crustacea, Decapoda) and obtained deep insights on its physiology and genomic expression in response to environmental variables. We investigated the models Botryllus spp. and

Botrylloides spp. (Tunicata) and tested their ability to react to various allochemicals and bioactive molecules. We investigated the model *Ciona robusta* (Tunicata) and defined the main feature of its industrial culture for scientific purposes. Research is still running to complete the above tasks and reach mature conclusions and comparisons.

Blue Biotechnologies for restoring marine ecosystems of the contaminated Site of National Interest (SIN) ex Montedison (Falconara M.ma) - BIOBLUTECH - Duration: 36 months Research Area: Environmental Biotechnology - Funding Agency: Fondazione Cariverona SZN Role: Partner - PI A. Ianora

The BIOBLUTECH project aims to develop innovative biotechnological and eco-technological approaches and applications aimed at the reclamation and redevelopment of the marine area adjacent to the disused industrial plant of the "Montedison", located in the municipality of Falconara Marittima (Ancona). This area (ca. 12 km²) is part of the contaminated sites of national interest and is therefore considered one of the areas with high environmental risk by the Marche region and the European Environment Agency. The industrial activities of the Montedison plant, over a span of nearly 7 decades (1919-1990), have affected coastal marine ecosystems in various ways, causing deleterious long-term effects. A redevelopment project of the terrestrial area is in progress but the recovery of the marine area adjacent to the plant has never Been taken into consideration. This is also due to the difficulty of implementing effective solutions in the marine environment. The solution proposed by BIOBLUTECH is based on: I) new environmental biotechnologies that use bacteria, fungi and their metabolites, to remove and / or render the contaminants inert; II) innovative ecological technologies for the restoration of degraded habitats able to restore healthy and productive marine habitats. BIOBLUTECH offers an unprecedented opportunity for the development of sustainable solutions aimed at restoration / recovery of marine habitats, providing important benefits for the blue growth of the region and country, within the EU strategic framework.



CTN Blue Growth –Cluster Project "BIG" TECHNOLOGICAL INNOVATION FOR THE PROTECTION AND VALORIZATION OF MARINE ECOSYSTEMS (ITEM) - PI <u>A. Zingone</u>

The ITEM project see the participation of 4 national research bodies, the National inter-University Consortium for marine sciences (which includes 33 universities) and 3 companies with activities and skills that cover the main areas of interest of the project. The project covers 4 of the 6 research and development trajectories of the cluster (Environmental protection and coastal zone, blue Biotechnology, abiotic marine resources, biotic marine resources). The ITEM project has gathered a large and diversified



interest in the Italian scientific and entrepreneurial community with many companies that have signed letters of intent and contributed to their drafting and implementation of the project. ITEM is divided into seven workpackages, one of which is dedicated to the gap analysis in the ITEM areas of interest of the cluster, three WPs dedicated to research, one WP to experimental development. The last two WPs are dedicated to cross-cutting activities between partners of the project and between national clusters and to the coordination of the project as a whole. The the main objectives of the project are the preparation of measures and necessary design for the development of new technologies to fill the Italian technological gap and the experimental validation of available technologies. The three main research sub-projects and technological development include: technologies for the implementation of observational or predictive systems at sea; technologies for the eco-sustainable production / use of marine resources; marine biotechnology. The ITEM project will enable the identification and design of new technologies of interest, to test and develop technologies of the very latest patenting and develop new collaborations between research and companies to make the system competitive to develop national competitiveness on technological innovation and the sustainable use of marine resources.

The area of specialization of the project is "Marine Economy". In particular, the project develops topics included in the technological trajectories:

- 1. Marine environment and coastal zone;
- 2. Abiotic marine resources;
- 3. Marine biotic resources;
- 4. Blue biotechnology.

Summary of the project - Italy with more than 8000 km of coastline and 40% of the marine territory underwater sees the Blue Economy as a resource with enormous potential for expansion. However, any current development policy cannot be done without preserving the environment and Italy has precise responsibilities in achieving quality objectives. A sustainable use of marine resources is also essential for the development of blue tourism and of biotic and abiotic resources. The present ITEM project has identified a serious shortcoming in development and technological innovation that risks limiting any possible development of the marine economy.

For this reason, ITEM has formed a community of 50 research reality, universities, small, medium and
large enterprises that will be working in integrated and inclusive way to: 1) identify the main technological gaps and opportunities for the national competitive industrial development; 2) to develop industrial research by testing new technologies patented by project partners; 3) to design new technologies useful to improving the observation systems necessary to ensure the quality of the marine environment; 4.) designing new technologies for production systems for the sustainable use of biotic resources 5) to design new marine biotechnologies useful for the recovery of the identification of new products, foods, materials and pharmaceuticals of marine origin.

The ITEM project aims to innovate marine research in Italy and to create a instrument for sustainable economic and social growth based on the sustainable use of resources and opportunities offered by the blue economy. With the ITEM project, Italy aims to become one of the leading European countries in research and development of technologies, and new generation marine biotechnology capable of ensuring quality of the environment, Entrepreneurial development and new employment opportunities based on the "marine resource".

The Cluster will work on:

- Environmental protection, control and monitoring systems, including the potential for blue tourism
- Abiotic resources (marine extraction industry and mineral resources)
- Biotic resources (fisheries and aquaculture)
- Blue biotechnology

Type of activity.

ITEM includes:

(A) NEW TECHNOLOGIES TO BRIDGE THE ITALIAN TECHNOLOGY GAP

(B) EXPERIMENTAL VALIDATION OF AVAILABLE TECHNOLOGIES

With regard to the development of new technologies and approaches, the specific objectives of ITEM are aimed at 3 major sub-projects (WPs) of research and technological development:

- A1. TECHNOLOGIES FOR THE IMPLEMENTATION OF MARINE OBSERVATION SYSTEMS (WP3)
- A2. TECHNOLOGIES FOR THE ENVIRONMENTALLY SUSTAINABLE PRODUCTION / USE OF MARINE RESOURCES (WP4)
- A3. MARINE BIOTECHNOLOGY (WP5)

SZN participates in all sectors and coordinates the A3 Marine Biotechnology sector.

8.11 PROJECT FINANCING FOR INFRASTRUCTURES

Assemble Plus

Durata: Ottobre 2017-Settembre 2021 Fonte Finanziamento: EU-H2020 - H2020-INFRAIA-2016-2017 Ruolo SZN: Partner - **PI W. Kooistra**

The main objective of the Association of European Marine Biological Laboratories Expanded "ASSEMBLE Plus" is to organize, operate and improve the provision of Transnational Access (TA) to European marine stations, i.e., to marine ecosystems, local marine biological resources, modern infrastructure and the scientific and technological knowledge necessary to perform innovative research with these resources. The TA is supplied to a total of 33 marine stations (some of which consist of connected Third Parties) in 16 countries. The stations that supply the TA have modern research laboratories and a broad spectrum of specialized research facilities to support users. Virtual Access (VA) will include internet access to biological datasets generated over decades in various marine stations, for example, in long-term ecological research and monitoring programs. Networking Activities (NAs) and Joint Research Activities (JRAs) are focused on supporting and improving TA. The TA in ASSEMBLE Plus operates through national EMBRC nodes, where present, in order to attract a diverse community in the field of life sciences, pharmaceutical and biomedical sciences and the private sector. These activities increase the importance of marine stations in European research. The objectives of the NAs are to develop the TA program, to improve it, to attract new categories of users including those from the private sector, and to ensure the long-term sustainability of EMBRC-ERIC. The objectives of the JRAs are to solve the critical issues in the supply of the TA and improve the quality and quantity of TA. SZN coordinates WP NA1 (WP3: Improving access provision), participates in WP NA2 (WP4: Improving virtual access- Design Data Management Plan), WP NA3 (WP5: Engaging with User communities), WP NA4 (WP6: Long-Term Sustainability), JRA1 (WP7: Genomics observatories), WP JRA3 (WP9: Functional Genomics) and WP JRA4 (WP10: Development of instrumentation), and coordinates WP TA12 (WP23: Transnational Access to EMBRC-Italy) which includes access to the SZN and to the Italian Third Parties: CNR - ISMAR Venice and CNR - IRBIM Messina.



EMSO MedIT

Duration: completed but undergoing further implementation with PON 2018. Source Financing: PON R&C 2007-2013 - PAC Enhancement of public research infrastructures Line of intervention B. Interventions for the adaptation and consolidation of infrastructures for environmental monitoring. SZN role: Partner - **PI R. Casotti**

EMSO - **MedIT** is a project to upgrade marine research infrastructures in Sicily, Campania and Puglia and represents the Italian contribution to the ESFRI EMSO infrastructure (www.emso-eu.org).

The project is funded under the NOP R&C 2007-2013 - PAC Enhancement public research infrastructures and pursues the objective of strengthening the infrastructures for the research in the field of marine sciences in the Convergence Regions of Sicily, Campania and Puglia.

EMSO - MedIT is the Italian contribution to the consolidation of the European research infrastructure EMSO coordinated by Italy, which, as part of EMSO - MedIT, goes in synergy with the other ESFRI with Italian coordination and with the Italian initiative for marine research: the RITMARE flag project.



The actions foreseen by the EMSO - MedIT Project aim at: i) enhance marine infrastructures and scientific and technological facilities in order to consolidate and expand the network for multidisciplinary monitoring of the marine environment coastal and deep and the water column; ii) network all the existing infrastructures and enhance the real transmission time / near - real time integrating the measurements of fixed and relocatable observing systems; iii) set up a mobile intervention system to be used for monitoring campaigns in sites of strategic interest or in the event of environmental emergencies.

The network of monitoring infrastructures is further enhanced through the creation of an exchange information system that allows the sharing of large data mass of data produced, with access by a vast community of Italian users and foreigners of various origins. We are project partners and WP2 managers "Enhancement of Campania", together with INGV for the Gulf of Pozzuoli. The upgrade provides for the acquisition of different oceanographic instrumentation, including a WaveGlider, a ROV, and several probes, but above all two MEDA-type elastic beacon to be located in the Gulf of Naples and the Gulf of Pozzuoli for real-time monitoring and environmental data transmission and physical conditions to the SZN operation center.

Partners - National Institute of Geophysics and Volcanology (INGV), Stazione Zoologica Anton Dohrn (SZN), National Institute of Nuclear Physics (INFN), National Research Center (CNR), Higher Institute for Environmental Protection and Research (ISPRA).

8.12 PARTICIPATION IN INTERNATIONAL ORGANIZATION

OECD - The SZN following its participation in the OECD project "Fostering innovation in Ocean economy" and "The ocean economy: Preparing the innovation of tomorrow" contributes to the OECD project

"The Ocean Economy: preparing the Innovation of Tomorrow 2019-2020". This Project aimed at understanding and improving innovation policies for science and technology for seas and oceans; providing guidance in the use of economic instruments to enrich



innovative and integrated management of seas and oceans; developing and refining an OECD industry database for the sea and ocean; analysing the economic value of marine and oceanographic research and observation. To develop these topics investigations are also planned in close cooperation with other OECD Directorates, in addition to the DSTI, including: Direction Environment; Management; Trade and Agriculture; Department of Economics; Centre for Entrepreneurial Development and Territorial and SME; International Forum for Transportat; Shipbuilding Section and International Agency of Energy. One of the major output is represented by the OECD publication "Rethinking innovation for a sustainable ocean economy".

UNESCO - SZN participates in the working group on Ocean Literacy promoted by UNESCO. The oceans (Atlantic, Pacific, Indian; Mediterranean, Baltic, Northern) are actually a unique system, globally connected and fundamental to life on earth: the ocean provides oxygen, absorbs anhydride is a source of food, regulates temperatures and weather. The first forms of life on our planet arise from the sea. Dealing with Ocean Literacy means to spread the understanding of the influence that the ocean have on our life and the influence that our choices and our actions have on the ocean (and therefore, again, on our lives). The need for the spread of Ocean Literacy, as a fundamental educational theme, was emerged about twenty years ago in the United States, when scientists, scholars and educators noted that in the guidelines for teaching there was no trace of ocean and science. Since then the topic has been studied, debated, planned, regulated and the Ocean Literacy has become a basic element of educational programmes in many countries, including Europe, from seven very simple and clear principles. But it is not yet in Italy. The construction of an Italian network for Ocean Literacy has the objective of spreading also in our country, surrounded by the sea and historically, culturally, economically linked to the sea, the culture of the ocean, sharing these principles starting from the schools and the civil associations. It will thus be simpler and more natural to protect what is the fundamental element for life on Earth.

SZN also participates with UNESCO, MAECI, MIT to the MSPglobal Initiative (Marittime Spatial Planning). The overall objective of the project is to support international marine/maritime spatial planning (MSP) for the sustainable development of the blue economy, by enhancing cross-border and transboundary cooperation where it already exists as well as through the promotion of MSP processes in areas where it is yet to be put in place. The specific objective aims at improving planning of sustainable economic activities at sea by promoting the establishment of MSP plans and by creating an environment conducive to transnational cooperation through the development of international guidance for cross-border and transboundary MSP. The project is designed to support the implementation of the Joint Roadmap to accelerate marine/maritime spatial planning processes worldwide, adopted by the Directorate General for Maritime Affairs and Fisheries of the European Commission (EC-MARE) and the Intergovernmental Oceanographic Commission of UNESCO (IOC-UNESCO) as part of the conclusions of the 2nd International Conference on MSP jointly organised in Paris, March 2017

G7 - Of particular importance, moreover, is the contribution made, in representation of Italy, in the context of the G7 work group Ocean and seas and G7 *sherpas*. This commitment is reflected in the active participation to the working group and to the produced documents, become part of the G7 science official communication as Ii was also under the Canadian G7 presidency (2018) and the USA G7 Presidency (2019)

Italy-China - The SZN, participates in the Italy-China working table (promoted by the MIUR), was part of the Italian delegation to China and to Italy-China Science, Technology & Innovation Città della Scienza, Naples.

Italy-Korea - The SZN, finally, as part of the Memorandum of Understanding, signed the 12 July 2016 in Naples with the National Marine Biodiversity Institute of Korea (MABIK) participates in the bilateral table Italy/Korea under the coordination of the Italian Embassy in Seoul and the collaboration of the MAECI.



8.13 S.W.O.T. ANALYSIS

The researchers and the council of Departments discussed and proposed a SWOT ANALYSIS that was initiated in 2017 and is reported here to be updated in 2020. This analysis can be useful for identifying the strengths, weaknesses, potentials and opportunities of SZN and its research activities, and to identify progresses made into the solutions to face the weaknesses and threats that can affect the research activities at SZN.

STRENGTHS

- 1. Highly qualified and multidisciplinary researchers in the field of life sciences.
- 2. Participation to infrastructure, in house funding and advanced research equipment.
- 3. International reputation and networking skills.
- 4. High quality approach to research.
- 5. Recruitment of international scientists.
- 6. Large critical mass in one single location (Villa Comunale).
- 7. Great professionalism in the preparation and management of events.
- 8. Large participation to SZN seminars and training events.
- 9. Highly qualified and international level training activities.

WEAKNESSES

- 1. SZN has been perceived as an expression of the local territory, rather than a National institution.
- 2. Lack of experience and ability in fundraising and international planning.
- 3. Low scientific leadership in international scientific collaboration activities.
- 4. Difficulty in planning the workflow.
- 5. Infrastructure management not sufficiently efficient for a full exploitation of the potential.
- 6. Insufficient qualification of part of the technical-administrative staff.
- 7. Insufficient organization and repartition of responsibilities.
- 8. Poor self-criticism with respect to the real problems of the Institution and difficulty in exploiting the best practices
- 9. Structures and laboratories with architectural constraints and not always functional to research.

OPPORTUNITIES

- 1. Recruitment of several new scientists offers new opportunities for strengthening of the international reputation, lowering of average age and return of Italian scientists abroad.
- 2. International credit allows inclusion in the most prestigious global projects.
- 3. The link with the territory offer opportunities for funding and collaborations (Regione Campania, Municipality of Naples, University of Naples).
- 4. New BlueBio department and new core facilities (eg, Bioinformatics) can enhance innovative research sectors.
- 5. National Cluster Blue Growth and EMBRC offer opportunities to enhance marine research.
- 6. The strengthening of structures open to the public (Aquarium, Museum, Library, Turtle Point) can enhance the Third Mission of SZN.
- 7. Strategic role at national level and participation in OECD, G7, UNESCO, Bilateral agreements, etc.
- 8. Network of new territorial offices on a national scale can give impetus to new sectors of research, access to new marine habitats improve research funding.
- 9. The enhancement of the Grant Innovation Office and the BlueBio Dept. can increase the potential for patenting and collaborations with the business sector.

RISKS / THREATS

- 1. Recruitment unable to intercept the new skills necessary for competitive marine research.
- 2. Slowness in adapting to the new structure and organization can leave the SZN in an indeterminate condition with repercussions on the quality of internal life and research.
- 3. Dissemination of internally-generated and unfounded news generates confusion and uncertainty compared to the real strategies of the organization.
- 4. Insufficient sharing of the SZN vision may limit the achievement of all expected goals.
- 5. Non-completion of the infrastructure may limit the future activity of the Institution.
- 6. Insufficient administrative / managerial ability can limit the potential to grasp the research opportunities.
- 7. Staff's energies risk of being distracted from research.

9 PUBLICATIONS

9.1 ISI PUBLICATIONS - 2019

List of publication ISI for the year 2019

1. Adelfi MG, Vitale RM, d'Ippolito G, Nuzzo G, Gallo C, Amodeo P, Manzo E, Pagano D, Landi S, Picariello G, Ferrante MI, & Fontana A (2019) Patatin-like lipolytic acyl hydrolases and galactolipid metabolism in marine diatoms of the genus Pseudo-nitzschia. Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids 1864(2):181-190.

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3. Agnetta D, Badalamenti F, D'Anna G, Sinopoli M, Andaloro F, Vizzini S, & Pipitone C (2019) Sizing up up the role of predators on Mullus barbatus populations in Mediterranean trawl and no-trawl areas. Fisheries Research 213:196-203.

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11. Ambrosino L, Tangherlini M, Colantuono C, Esposito A, Sangiovanni M, Miralto M, Sansone C, & Chiusano ML (2019) Bioinformatics for Marine Products: An Overview of Resources, Bottlenecks,

and Perspectives. Marine Drugs 17(10).

12. Ambrosino L, Vassalli QA, D'Agostino Y, Esposito R, Cetrangolo V, Caputi L, Amoroso A, Aniello F, D'Aniello S, Chatzigeorgiou M, Chiusano ML, & Locascio A (2019) Functional conserved non-coding elements among tunicates and chordates. Developmental Biology 448(2):101-110.

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Meglio N, Roul M, Gregorietti M, Mazzucato V, Pellegrino G, Giacoletti A, Paraboschi M, Zampollo A, de Lucia GA, & Hochscheid S (2019) Turtles on the trash track: loggerhead turtles exposed to floating plastic in the Mediterranean Sea. Endangered Species Research 40:107-121.

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