RESEARCH PROGRAM

EFFECTS OF CUMULATIVE HUMAN IMPACT ON MEDITERRANEAN CORALS AS BIO-INDICATORS OF MARINE ECOSYSTEM HEALTH

BACKGROUND

The integrity of marine ecosystems has long been threatened by the combined effects of a series of stressors that jeopardize the survival of individual species and the goods and services they provide\(^1\). Organic and inorganic pollution (from agricultural, urban and industrial discharges), overfishing, increased shipping, and coastal tourism are among the main anthropogenic drivers of change in marine ecosystems\(^2\). In addition, the effects of ongoing climate change, with ocean acidification, temperature increase and fluctuations, and extreme atmospheric events, may impair the survival and the distribution of organisms and, ultimately affect ecosystem functioning\(^3,4,5,6\).

The semi-enclosed Mediterranean Sea can be considered a “miniature ocean” responding faster than the global ocean to environmental change\(^7\). For centuries, the Mediterranean basin has been one of the areas most subject to anthropogenic pressures in the world\(^8\). Threats to its marine life derive from impressive coastal urbanization, shipping activities, and overexploitation of resources that cause pollution, eutrophication, habitat degradation, collapse of fish stocks, algal blooms, spread of pathogen organisms, and invasion by non-indigenous species, among others\(^7,9,10,11\). Thus, the Mediterranean Sea is significantly affected by the combined effect of global climate change and an array of pressures whose synergy is expected to heavily impact marine biodiversity and ecosystem functioning. For instance, during the last few decades, prolonged periods of high temperatures during summer have been linked to the mass mortality events that have periodically impacted Mediterranean benthic communities\(^12,13,14,15,16\).

In recent years, several attempts to weigh the impact of anthropogenic drivers in spatially explicit ways have been proposed both at the global scale\(^2\) and at the Mediterranean level\(^17,18\). Data on anthropogenic pressures are abundant in the Mediterranean context, albeit the weight of some of them (e.g., shipping, fishing) is sometimes underestimated and the resolution level is rough. Additionally, attempts to investigate the impacts of those pressures on biodiversity and ecosystem functioning assume a linear relationship between the intensity of a given pressure and its impact, but different response curves are also plausible. Moreover, estimates of anthropogenic impact on the ocean hardly take into account the possible synergy among multiple pressures, since their synergistic effect is almost impossible to test in the field beyond the local scale. These are among the main reasons why the combined potential impact of multiple pressures and drivers is often measured as the sum of the impacts in a cumulative way\(^2,17,18\), although synergy or antagonism among impacts may occur\(^19\). Micheli et al.\(^18\) identified and quantified human impacts for the Mediterranean and Black Sea, producing a map of cumulative human impact by combining a diverse set of anthropogenic pressures and climatic drivers, of both land-based and sea-based origin, along with their potential impact on marine habitats.

Shallow water organisms are among the first to experience anthropogenic impacts of both land and sea-based origin\(^20\). The single or combined effect of these pressures can have negative consequences on vital functions of marine organisms such as reproduction, feeding and growth, ultimately leading to population declines\(^3,21,22\). When the aim of a study is to estimate the health status of a species in the wild and little is known on its biology and ecology, it is almost impossible (and of limited interest) to disentangle the effects of each single stressor from the others. In such cases, a holistic approach, such as the one proposed by Halpern et al.\(^2\) and Micheli et al.\(^18\), taking into account the array of potential threats to wildlife and their weight, would better depict the actual risk to the conservation status of a species.

The aim of this study is to investigate the effects of cumulative human impacts, including the vicinity to fish mariculture, on Mediterranean corals as biological indicators of the health status of the marine ecosystem. In
particular, it aims at i) investigating ecological and physiological parameters in corals at locations along a gradient of cumulative human impact and in proximity to open-cage aquaculture installations and nearby sites, and ii) identifying the main origin (land based or sea based) of the threats to the investigated species.

**PROJECT IMPLEMENTATION AND TIMING**

The project comprises four interconnected work packages (WPs: Fig. 1). WP timing is illustrated in Figure 2.

**WP1 Fieldwork**

T1.1 Preliminary survey: Selection of sites along a cumulative human impact gradient and in proximity to open-cage aquaculture installations and nearby sites. Locations will be selected in advance based on the results obtained by Micheli et al.\(^23\). In particular, the method is based on the assessment of cumulative impacts produced by 22 different drivers (representative stressors of the most important forms of human impact on the marine system) of 17 Mediterranean ecosystems.

T1.2 Coral sampling: Characterization of transcript profiles and metagenomes of native populations along a cumulative human impact gradient and in proximity to open-cage aquaculture installations will identify the most important host genes and microbiome functions to be studied. Corals will be snap frozen in liquid nitrogen and stored on dry ice for transcriptomics and metagenomics (WP4) and their skeletons will be cleaned for biomineralization and skeletal characterization (WP3).

T1.3 Environmental parameters: Temperature, pH, and salinity will be measured with multi-parametric probes. Other environmental descriptors (e.g., CHL.a, intensity of ambient light, suspended sediment, wave parameters) will be gathered preferably from the literature or other available sources (e.g., maps, satellite data).

**WP2 Percent cover**

T2.1 Benthic surveys: The percentage cover of selected species will be quantified in 3 sites hundreds of meters apart within each location. At each site, percentage of cover will be determined by taking 6 photos, acting as replicates. The camera is equipped with two laser pointers set at a fixed 20 cm distance, which produces a reference scale for each picture.

T2.2 Image analysis: The digitizing software ImageJ will be used to calibrate each photo setting the reference distance given by the two pointers, and to measure the area occupied by the investigated species inside 50 x 50 cm randomly place quadrats by tracing their outline with a hand-controlled mouse on the digital image.

**WP3 Physiology and skeletal features**

T3.1 Photosynthesis and respiration: Photosynthesis and respiration will be measured in the field, thanks to the collaboration with Prof. Dubinsky at Bar-Ilan University, using the unique computerized metabolic chambers by WALZ Instruments, funded by the EU FP7 ERC CoralWarm grant of which the tutor is co-holder. Upon completion of physiology measurements, the corals will be bleached, rinsed and dried at 50°C before further analysis.

T3.2 Skeletal parameters (porosity, bulk density and micro-density): Micro-density (density of the carbonate skeleton, excluding pores), bulk density (total density, including pores) and porosity (percentage of pores connected with the external surface) will be measured by buoyant weight at the laboratory of coral ecology and biology [Prof. Stefano Goffredo; Department of Biological, Geological and Environmental Sciences (BiGeA)]. Pore-size distribution of collected corals will be determined through the innovative application of
time-domain nuclear magnetic resonance at the laboratory of magnetic resonance in porous media [Prof. Paola Fantazzini; Department of Physics and Astronomy (DIFA)].

T3.3 Biochemical and mineralogical properties: Skeletal microstructures, mineral phases and the associated organic matrix will be investigated by last generation diffractometry, spectroscopy, thermogravimetric and SEM analyses at the laboratory of biomineralization and biocrystallography [Prof. Giuseppe Falini; Department of Chemistry (CHIM)].

T3.4 Mechanical properties: Mechanical properties of the mineralized structures will be measured through innovative nanoindentation techniques at the laboratory of mechanical properties of calcium carbonate-based composites of Prof. Luca Pasquini (DIFA), from the elastic to plastic regime, including hardness, ductility and fracture toughness.

**WP4 Transcriptomics and metagenomics**

T4.1 Temporal profiles of targeted gene expression: Analysis of quantitative time-dependent expression of selected genes involved in biomineralization, metabolism, growth and reproduction will be performed at the animal and environmental physiology lab (Prof. Silvia Franzellitti; BiGeA). Zooxanthellae clade harbored by test coral samples will be determined based on nuclear ribosomal DNA.

T4.2 Microbial metagenomics: Microbial metagenomes and metavirome analyses from tissue, skeleton and mucus of samples from WP1 will be obtained by shot gun sequencing at the unit of microbial ecology of health [Prof. Marco Candela; Department of Pharmacy and Biotechnology (FaBiT)]. By means of multidimensional data analysis, the metagenome determinants conferring phenotypic plasticity will be defined.

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**Fig. 1** Pert chart showing project activities (divided in work packages) and how they are interconnected
Fig. 2 Activities and time schedule

REFERENCES


TRAINING PROGRAM (OR ACTIVITY PLAN)

The postdoctoral researcher will learn and apply the following research methodologies:

• **Fieldwork** through scientific SCUBA diving for collection of coral samples and environmental data along a cumulative human impact gradient and in proximity to open-cage aquaculture installations and nearby sites. *Infrastructures involved*: UniBo, Department of Biological, Geological and Environmental Sciences (BiGeA), Prof. Stefano Goffredo;

• **Percent cover and image analysis** – to investigate the effect of environmental parameters on coral abundance. *Infrastructures involved*: UniBo, BiGeA, Prof. Stefano Goffredo;

• **Buoyant weight** – to measure coral skeletal parameters (porosity, bulk density and micro-density). *Infrastructures involved*: UniBo, BiGeA, Prof. Stefano Goffredo;

• **X-ray diffraction (XRD) and Fourier Transform Infra Red (FTIR) spectroscopy** – to determine variations in skeletal mineralogy. *Infrastructures involved*: UniBo, Department of Chemistry (CHIM), Prof. Giuseppe Falini;

• **Scanning Electron Microscopy (SEM)** – to observe shape and architectural assembly of crystalline units. *Infrastructures involved*: UniBo, CHIM, Prof. Giuseppe Falini;

• **Time-Domain Nuclear Magnetic Resonance (TD-NMR)** – to determine pore-size distribution of coral skeletons. TD-NMR, and in particular magnetic resonance relaxometry of water 1H nuclei for analyzing internally connected skeletal porosity, has several advantages compared to other methods because it is a non-destructive and non-invasive procedure, allowing intact specimens to be further analyzed with other techniques. Here, the transverse magnetization component, with transverse relaxation time T2, will be studied. In porous media saturated by water, under the assumptions that the relaxation rate of the unconfined water is negligible and the molecular diffusion is fast enough to maintain the magnetization uniform within the pores, the distribution of T2 corresponds to specific “pore-size” distributions. The area below each distribution is proportional to the total NMR signal and therefore is proportional to the volume of water saturating the skeletal pore volume. *Infrastructures involved*: UniBo, Department of Physics and Astronomy (DIFA), Prof. Paola Fantazzini;

• **Nanoindentation** – to measure skeletal mechanical properties. The nanoindentation technique involves the application of a controlled force by an indenter, to produce a local deformation of the surface, with a typical penetration depth of 100-1,000 nm. To perform nanoindentations on coral skeletons, preliminary treatments will be performed, which involve three processing phases: embedding, cutting, and polishing. The hardness and the stiffness will be obtained from the analysis of the load–displacement curves according to standard methods (Oliver & Pharr 2004 J. Mater. Res. 19:3-20). The hardness is a measure of the resistance of the material to plastic deformation and the stiffness quantifies the resistance to reversible (elastic) deformations (Goffredo et al. 2015 Coral Reefs 34:121-132). *Infrastructures involved*: UniBo, DIFA, Prof. Luca Pasquini;

• **Metabolic chambers** – to investigate effects of decreasing pH on metabolism (photosynthesis and respiration). The metabolic chambers use high intensity LEDs and a dedicated software to generate photosynthesis versus energy curves and simulate diurnal light cycles. Oxygen uptake/evolution rates are measured by optodes, while variable fluorescence is also measured simultaneously. Calcification rates are determined from changes in the alkalinity and calcium content in water samples withdrawn from the cuvette. The chambers are designed for simultaneous measurements of 3-6 cuvettes of phytoplankton or any aquatic organism under experimentally changing pH and temperature combinations. They allow examining the effects of pH and temperature on the interrelation among photosynthesis, respiration and calcification rates. *Infrastructures involved*: Bar-Ilan University (Israel), Prof. Zvy Dubinsky;