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Novel Tools for the Evaluation of the Health Status of Coral Reefs Ecosystems and for the Prediction of Their Biodiversity in the Face of Climatic Changes

Stéphane La Barre

Additional information is available at the end of the chapter

1. Introduction

Coral reefs concentrate between one quarter and one third of the total marine biodiversity, according to different estimates, though they only cover about 0.1% of the global oceanic surface and are confined to tropical and sub-tropical latitudes. The extraordinary diversity in invertebrates and fish species is often compared to that of arthropods and vertebrates of tropical primary rainforests. Half a billion humans depend partly or totally on the goods and services provided by coral reef ecosystems.

However, coral reefs are now recognized as being among the most fragile of all environments in the face of localized anthropic pressures (overfishing and various atmospheric and water pollutions) and of their climatic consequences of planetary dimensions. Within the last 30 years, about 20% of all coral reefs have being totally destroyed while another estimated 60% are damaged to some extent – a few beyond recovery - and only 20% can still be regarded as unharmed.

With a human population growing at faster rates in developing nations, biodiversity concerns are conflicting with the pressures to exploit local mineral resources and to develop agricultural and seafood production. While residents often deplore the gradual changes in the natural habitats in which they were brought up, socio-economic pressures originally due to post-colonial emancipation and now to global trade tend to resist any policy that may be considered restrictive or receding. Almost every aspect of this economic growth relies on fossil fuel energy consumption and to a lesser extent on vegetal biomass burning, which links development with climatic changes, and also with the generation of polluting wastes.

Educational programs and non-government associations propose alternatives to established practices in habitat and resource management and waste disposal by end users. Research scientists continue to explore natural biodiversity in remote pristine environments (especially *biodiversity hotspots*, a very useful conservation-promoting concept) and to observe its losses in degrading habitats. Programs on bioremediation of impacted sites are attracting funds, and classification of natural habitats as protected sites is gaining public support. Yet the scientific community has very little leverage on the decision-making of potentially impacting industrial, commercial and urban development projects, and on the say-so broadcast by their promoters in the media. In-house consultancy is often hampered by the lack of appropriate analytical tools, and reports are often biased in favor of employment and cash return prospects.

In a previous chapter of this series on biodiversity [1] the various ecological consequences of climatic changes, of chemical and microbial pollutions and of overexploitation of natural resources have been reviewed for coral reef ecosystems. Suggestions have been made on the basis of recent publications by experts on various subjects, as to how modern techniques and innovative approaches could be used appropriately to complement the above initiatives.

In this chapter, a holistic concept is proposed that (i) integrates cutting-edge molecular research and standard technologies with field sampling and laboratory simulations of natural habitats (ii) using holobiont-based *sentinel* systems, (iii) into a single tool that “shows evidence” of ongoing degradation rather than aftermath “score loss”. Corrective action can then be taken in specific directions before no-return limits have been reached and total ecosystem collapse is on the way.

2. Biodiversity is our responsibility: The future of mankind depends on it

2.1. If the origins of life remain controversial, biodiversity is a miracle of sorts

Basic forms of life like bacteria are reputedly capable of withstanding extreme conditions, to the point that scientists of repute such as Sir Francis Crick, Carl Sagan or Stephen Hawking have speculated on an alien origin to terrestrial life, which is now held as a tenet of the modern panspermia theory by some exobiologists. Dormant bacterial spores or alike would have been seeded on our planet, possibly from different sources and at different times. Those that encountered favorable “starting” conditions, supposedly in the chemolithotrophic environments around oceanic ridges, would have initiated the evolutionary scenario we know. Posed under such terms, the true *origin of life* escapes our observation, but the *origin of biodiversity* does not, as it remains intrinsically terrestrial.

Satellite views of the ionized portion of our atmosphere show it as a barely perceptible glow that outlines the shape of our planet against the black outer space background. Just under it, blue expanses of oceanic waters spread as a delicate film less than one-thousand times thinner than the supporting “blue marble”, but over two thirds of its surface (Figure1). Life forms occur at the sea-air and soil-air interfaces, just where geoclimatic fluxes and exchanges are the most rapid and subjected to biotic influences. Thus marine biodiversity thrives under specific

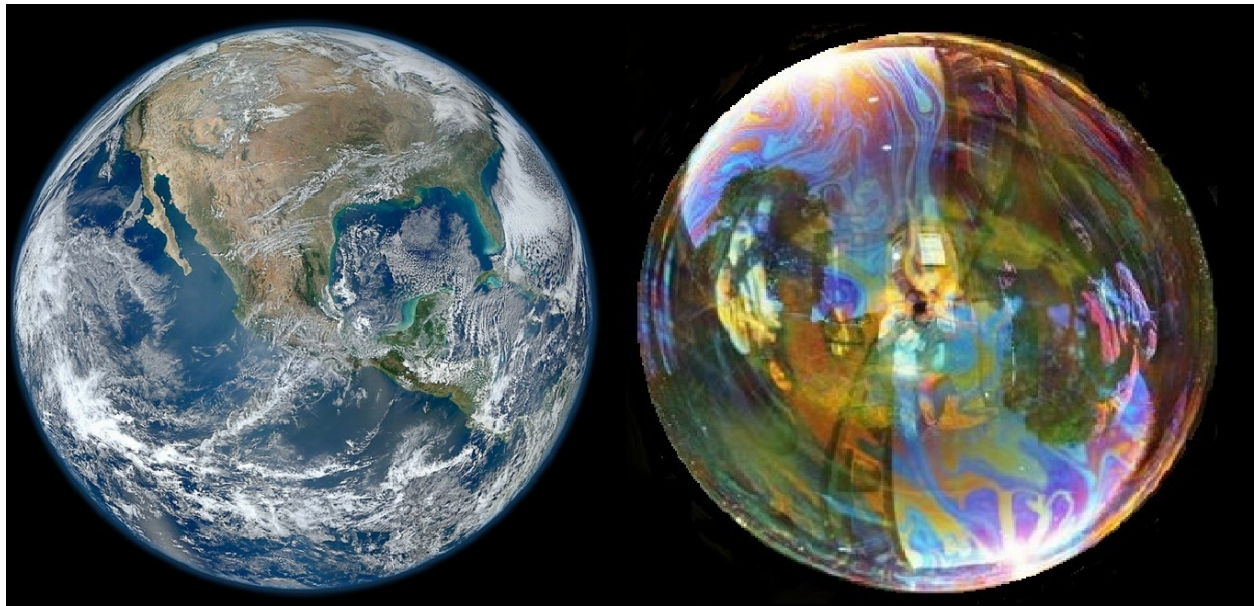


Figure 1. (left) - Composite image of Planet Earth viewed from orbiting satellite Suomi NPP on January 4, 2012 (<http://npp.gsfc.nasa.gov/>), scaled here at approximately 5×10^{-9} ; (right) -The biosphere is about one-thousandth the diameter of the planet – just as thin as the surface of a soap bubble that size, a metaphor illustrating its instability and fragility.

conditions that are only found between the oxygen-rich lower atmosphere (at cloud canopy level on Figure 1), and the sunlit oceanic surfaces.

One-third of all known marine species is concentrated on confettis occupying one-thousandth of the oceanic surfaces, also thanks to favorable conditions afforded by land influence, i.e. marine volcanoes and continental shelves under tropical-subtropical latitudes. This circumstantial miracle is called coral reefs.

2.2. Extinction events are natural over long periods after which biodiversity has to reinvent itself

Any lasting change in the biogeochemistry of any of the three components (atmosphere, seawater and land) will disrupt the interfacial equilibrium that supports the many thousands of life forms that interact constantly within an ecosystem. Mass species extinctions occurred several times (6 or 7 described) in the history of our planet since it became life-supporting, every time followed with new and better adapted life forms and a biodiversity climax attained after long periods of environmental stability. Changes in soil mineral strata indicate the occurrence of biodiversity-modifying events such as occupation by seas or the occurrence of an ice-age. Discrete organic layers may indicate the presence of a tropical rainforest or of a dry land savannah. Datable fossil evidence within these strata, together with paleontological reconstructions, point out the floristic and faunistic peculiarities of the times. Core drills in ice provide datable evidence of biogeoclimatic episodes within the last few millennia, while core drills in massive scleractinian corals give accurate calendar-like records of recurrent or of accidental climatic events affecting their biotope.

Speciation usually goes along with occupation of new territories and new habitats, the first colonizers having acquired the necessary adaptations to cope with evolving external demands – the Cambrian explosion (545 million years ago) being the most dramatic example of such adaptive diversification at all scales.

Along with this, evidences of accidental episodes of massive species extinctions are witnessed by the sudden “disappearance” of terrestrial and of marine life, that are attributable to tectonic, telluric or meteoritic impacts and to their profound and lasting climatologic and geochemical consequences. The most significant mass extinction is undoubtedly the Permian-Triassic Great Dying [2] where a 96% loss of all non-microbial marine life occurred within ten million years. The precise causes of mass extinction events may be in connection with continuous tectonic movements with their telluric and volcanic outbreaks and their climatic consequences, to collisions with meteoritic bodies, and to a lesser extent to the appearance of dominant predators, parasites or microbial diseases, or to combinations thereof. Common to many extinction events, however, is the massive release of greenhouse and of toxic gases (carbon dioxide, methane, hydrogen sulfide etc.). The water solubility of CO₂ being nearly 30 times that of oxygen, water acidification occurs that impacts preferentially all calcifying organisms with low metabolic rates and weak respiratory systems: most coral genera died out during the Great Dying, along with calcareous sponges, calcifying algae, echinoderms, bryozoans etc. [3].

Interestingly, profound taxonomic changes in all major phyla seem to follow extinction events, resulting in a better adapted biodiversity. Nothing is known, however, on the consequence of such changes in microbial life or on the putative role microbial associates had on the *reinvention* (understand: adaptive evolution) of new species. As Falkowski [4] puts it, animals and plants are merely new incarnations of ancient metabolic processes, but the ultimate key to biodiversity may be held by bacteria ferrying the set of core genes that are necessary for life to express itself.

2.3. Brutal human influence may lead to hazardous extinction events

Man is of very recent occurrence in the history of terrestrial life, and until the advent of the industrial age (especially since the middle of the 19th century) his planetary influence was minimal, and the destructive potential of its inventions was purely local. Since then, his population has increased seven fold while consuming or exploiting 42% of the terrestrial net primary production [5]. The single most environment-impacting activity today is the production of energy from fossil fuel, and the single most mechanically destructive invention is that of nuclear weapons. More insidious are the thousands of new chemical species (mostly organic) that are produced and improperly disposed of once used, and the new strains of mutant microorganisms that pop up unpredictably and threaten to create worldwide pandemics affecting both wildlife and humans.

Without reviewing the subject of negative traces of human doings (see [1]), a reflection on the accelerating pace of carbon volatilization in the atmosphere is necessary to appreciate its effect on marine biota and on coral reef ecosystems in particular. In one year, we extract the equivalent of one million years' worth of fossil fuel natural production [4]. In other words, using primitive technology e.g. internal combustion engines, we enrich our atmosphere in carbon

dioxide at a pace at which terrestrial and marine autotrophs will not be able to keep up with in their efforts to create new biomass and quench free carbon excess and reduce seawater acidification rates.

3. Coral reef management: between the economy's hammer and the climate's anvil

Ecosystems at immediate risk are biodiversity hotspots (primary rainforests and land-connected reefs systems), but most ecosystems with endemic species at all latitudes will be profoundly affected by acclimation of competing aliens. Being able to analyze, quantify and predict changes is the first step to avoid losing control... indeed environmental scientists should endorse the role of a general practice physician making an overall check-up on an ailing patient and prescribing a course of medications and exercises for recovery, or that of an investigator using state-of-the-art forensics such as DNA amplification to evidence causes of a crime and provide evidence to lawyers. This will become a necessity, beyond economic pressures worldwide (q.v. [6]).

3.1. Climate changes have profound biogeoclimatic consequences

Climatic changes act globally, and the effects of naturally occurring destructive episodes are now superseded by those due to ever-growing man-induced emissions of various sorts. The latter include carbon, nitrogen or sulfur transfers that are generated essentially through the combustion of fossil organic matter or of agricultural or livestock breeding activities, and manifesting themselves differently according to their biogeochemical state.

- i. First, their volatilization as simple molecular species which are responsible for temperature rises through the glasshouse shielding effect, while halocarbon emissions tend to destroy the protective ozone layer of the upper atmosphere. Elevated surface water temperatures and genotoxic radiations are responsible for the bleaching effect and cellular stresses observed on subtidal corals, and death may follow if the exposures are lasting. Also, strong seasonal evaporation of large volumes of surface waters leads to atmospheric pressure instabilities, resulting in the occurrence of more frequent and more severe hurricanes. The mechanical damage due to wave action and to the sudden input of large volumes of freshwater and alien material (silt and debris) in coastal zones may entirely destroy some reef portions in single episodes;
- ii. Second, the restitution of most of the above molecules at the air-water interface and their incidence on biomineralization of many marine invertebrates and of their larvae, and of coralline algae; In the 22nd century, seawater acidification may be as large as 0.5 pH units below the pH levels recorded in early 20th century. The extent of seawater acidification due to a shift in bioavailability of HCO_3^- concentration, if still debated in 2012, may shortly become dramatic for the survival of calcifying invertebrates (corals, mollusks, echinoderms, some sponges etc. and their larvae) and of coralline algae.

3.2. Societal changes: think globally, act locally

Socio-economic determinants are linked to the development of industries, commerce and urbanism, their primary effects being direct, localized and traceable.

- industrial activities typically generate important volumes of polluting agents that dissipate during the processing stages. Furthermore, residues are managed according to criteria of productivity and cost-effectiveness i.e. with little concern about recycling alternatives, and about speciation into toxicants that make their way from the discharge areas into waterways and eventually into the sea. Ore excavation or grading of metal-rich top soil cause faunistic and floristic deteriorations, i.e. loss of endemic wildlife, facilitate bioerosion, cause the constant remodeling of river mouths with the finer particles and destroy almost life forms with the accumulation of heavier deposits in basins. In the sea, bioaccumulation of organic pollutants by primary consumers can expectedly reach very high concentrations in top predators, e.g. lipid-bound chlorinated biphenyls. Heavy metals that leach out from anti fouling formulations are accumulated by filter-feeding invertebrates, and take a heavy toll on the reproductive success of many life forms found in intertidal and subtidal life forms around harbor waters;
- intensive agriculture and farming generate various type of pollution, (i) enrichment by fertilizers and farm animals disjecta, (ii) pesticides and systemic herbicides, (iii) veterinary products, hormones that alter the quality of underground water reserves, while excess runoff benefits undesirable primary production in coastal zones, e.g. “green tides” and microalgal blooms;
- global commerce favors dissemination of alien species by shifting large amounts of ballast water by container ships and cleaning of hulls;
- urban development in tropical zones eliminates mangrove forests and seagrass beds that are important coastal component of fringing reefs. Shifting into the sea of very large volumes of untreated sewerage generated on land causes eutrophication in coastal reef systems with the introduction of novel microbial diseases.

3.3. Scientists should play a central role in biodiversity issues

At present, there are no reliable estimates of the rate at which coral reef biodiversity is going to be affected by climate and anthropic forcings by year 2100. The various proxies used by climatologists and reef ecologists predict two types of scenarios: (i) short term collapse of tropical reef biodiversity as early as 2050 if current trends are not curbed, (ii) a good level of resilience if present-day conditions are maintained as an absolute maximum, allowing photosymbiotic systems to adapt gradually. By limiting direct interference (q.v. *overfishing*, *algal proliferation*, *alien species*) that introduce phase-shifts in the complex and otherwise self-regulated food chains, acclimatization of most common coral species can be made possible in the face of climatic changes. The second alternative represents the only and narrow line of action, knowing that it will not be realistic to completely stabilize CO₂ emissions to safe levels, i.e. natural quenching capacity of excess by phytoplankton.

In 2012, scientific expertise is mostly directed to the discovery and documentation of new species in remote or in hitherto neglected regions, or to report the loss of biodiversity in areas impacted by human activities. Consulting is sought for the delineation of protected areas and establishing quotas for fishing. Science-based management is necessary, in particular in regions suffering from direct and rapidly growing human influence [7]. Particularly at risk are young developing nations and small islands that are placed under tremendous pressure from major industrial countries to exploit and export their natural resources – a socio-political scenario that tends to divide local populations and sometimes accelerate environmental issues. Thus, care must be taken of the “growth crises” of these culturally fragile ethnic groups undergoing these economic pressures, and a premium should be placed on the management of their natural patrimony, in which their culture is firmly rooted.

What comes out most often in recent discussion groups is: (i) that new monitoring tools, new environmental technologies and new models must be developed within a consensus mode between interested parties (ii) scientific expertise and field experience must be federated into autonomous consortia, (iii) implementation stages must follow a rigorously coordinated and step-wise approach. The present feeling is that scientific expertise is only marginally called upon and often misquoted in the decision-making of matters of economic development and urbanization in developing countries. By following the above recommendations, habilitation of the scientist as an advisor or as a mediator playing a crucial role in decision-making will be greatly facilitating the social dialogue on ecological matters.

4. State-of-the-art: New concepts, novel approaches, cutting-edge technology etc...

How to find a suitable management compromise between climate and human forcings without altering the economy and the social development of developing nations? This is everybody's concern: entrepreneurs, merchants, economists, businessmen, politicians, educators, end-users and consumers and of course, scientists.

From the scientist's point of view, each identifiable environmental issue has its specific set of solutions, and should be treated with the same care as an outpatient's condition is in a hospital. The physician makes a global assessment and recommends that a series of analyses be made by specialists before he confirms his diagnosis and prescribes an adapted treatment. In most cases, the patient will recover successfully – sometimes he has to be hospitalized for some time, and on rare occasions he may not leave the hospital alive.

After an overall check-up in which the probable stressor(s) is (are) identified, the environmental investigator will hand the case over to specialists who will each carry out a set of biochemical, molecular, microbiological and imaging tests on specific model organisms, on their tissues, cells, body fluids and associated microbes. Once the diagnosis is confirmed, a preliminary report is made that may contain special recommendations, followed by regular visits to evaluate the resilience of the system and the potential for the recovery of its lost biodiversity.

To be able to adapt this medical approach to an ailing ecosystem, the environmentalist needs to find representative biological study models (i.e. sentinel species that are sensitive to the stressors, but not to the point of immediate eradication at the onset of a mild exposure). He also needs to study characteristic and observable symptoms, their evolution and their succession. Early markers of an organism exposed to a stressor can be detected using functional genomics with suitable molecular tools, and the evolution of the responses can be followed thenceforth. For instance, an organism undergoing abiotic stress is usually more prone to microbial infection than a conspecific control organism, calling for physiological and bacteriological / fungal / viral / parasitic analyses. Unfortunately, very few field investigators are in a position to use these tools routinely, let alone in association - especially molecular tools that have been adapted from the medical world to specific biological models, and only for research.

4.1. Shifting from a consumer-minded to a conservation-minded attitude

Coral reef organisms tend to live in close association in order to gain optimal access to essential resources such as hard substratum, access to light, appropriate food etc., the co-occurrence of which is limited in shallow tropical waters [8], which are naturally efficient at nutrient cycling (oligotrophic regime). These constraints are reflected in the highly sophisticated communal assemblages formed by coralline and fleshy algae, invertebrates, vertebrates, protists etc. which communicate essentially via surface-to-surface or distant interactions of immunological and/or chemical nature. All forms of associations are encountered, ranging from obligate parasitism to symbiosis via commensalism, from prey hunting to filter feeding via surface browsing, etc.

The scientific literature on coral reef biology has traditionally emphasized on competitive aspects between members sharing a same habitat, especially in connection with secondary metabolites emitted by sessile or sedentary invertebrates and having allelopathic or growth inhibiting activities [8,9]. Only recently have the cooperative and functional aspects of interspecies and cross - phyletic communication been explored - stepping from a more "medical" attitude (i.e. pharmacologically-oriented) to a more "ecological" one (i.e. conservation-oriented). Discoveries such as bacterial inter - communication via quorum-sensing signals, biofilm studies, and of course the progresses made in genomics have greatly contributed to this attitude change (e.g. the coral probiotic hypothesis of Reshef et al. [10]).

In the eighties, the term *biodiversity* was publicized by [11] to account for the need to evaluate and to manage biological resources in endangered habitats. It gained immediate recognition as a key concept in life sciences, and public adoption followed relayed by media channels, e.g. "citizen science" in which data collection by non-scientists was encouraged to feed statistics on endangered species, as well as public initiation to scientific issues [12]. Ecosystem services and human well-being oriented approaches in general, while often assimilated to biodiversity-conservation strategies, may not ultimately pursue the same goals, calling for trade-offs in difficult issues [13].

The biodiversity concept certainly helped in the funding of sampling expeditions in diversity *hotspots*, another term useful to conservationists and to taxonomists. The Tree of Life (<http://>

tolweb.org/tree/) is a collaborative web project in which over 10,000 web pages provide information about extant groups of organisms and their evolutionary history.

Rapid progress in molecular science, and genomics in particular, has made it theoretically possible to retrieve useful information from crude environmental samples, i.e. doing away with cultivation restrictions and going beyond conventional taxonomy based on morphological traits. However, metagenomic characterization of marine microorganisms from the plankton, from biofilms or associated with macrobiota and their exsudates, generates phenomenal volumes of data from which crucial information extraction is a difficult and costly task for biocomputing specialists. Advocated by some as a new paradigm for biodiversity studies [14], “data-intensive science” taking a naïve (data-driven, i.e. non-theory-based) approach looks for truly novel and surprising patterns that are “born from the data”. But because of meta-data sorting problems and costs, and since environmental problems cannot wait for new hypotheses to emerge, others think that “knowledge-based science” can at least confront existing hypotheses against the meta-data background and guide investigators into detecting novel information [15].

4.2. The holobiont as an evolutionary concept, the *extended holobiont* as a conservation concept

Closer to hands-on science, genomists have coined a very useful concept, originally to account for the functional dynamics associated with bioconstructing organisms such as coral, sponges, or coralline algae in tropical marine ecosystems: that of the *holobiont*, an entity that includes the host (basibiont) and its microbial associates (microbionts) – the *hologenome* being the sum total of the associated genomes [16]. Photosymbiotic systems may function of both autotrophic and heterotrophic modes with the assistance of, respectively, symbiotic microalgae or cyanobacteria during the day, and element-recycling bacteria inside bacteriocytes or associated with the mucus, tissues or skeletal cavities followed by directed uptake at night. This dual mode of operation is conferring an adaptive advantage to short-term disturbances [17].

The hologenome theory was later generalized to terrestrial eukaryotic-prokaryotic systems, including man and its microflora [18], in order to account for the coevolutionary and cross-kingdom aspects of symbiosis [19].

Finally, the holobiont is also a practical concept insofar as it can be conveniently transposed from its natural sites to a microcosm or mesocosm aquarium setup, in order to evaluate stress impacts on both the host and its associated microbiota.

The concept of *extended holobiont* is proposed here, as the urge is now felt to develop practical tools to directly address biodiversity issues. In their definition of the coral holobiont, [20] include cryptic associates that are structurally associated with the host (fungi, encrusting sponges and algae, protists). In order to maintain some evolutionary coherence between the holobiont *sensu stricto* and the hologenome concepts of Rosenberg, we may include the dozens of specialized and mobile life forms that live in trophic or parasitic or commensal relationship with the host (e.g. little crabs, shrimps, echinoderms, ascidians, polychaete worms, shelled or naked mollusks, planarians, fish, crustose algae, fungi, foraminifera, etc.) into a wider concept

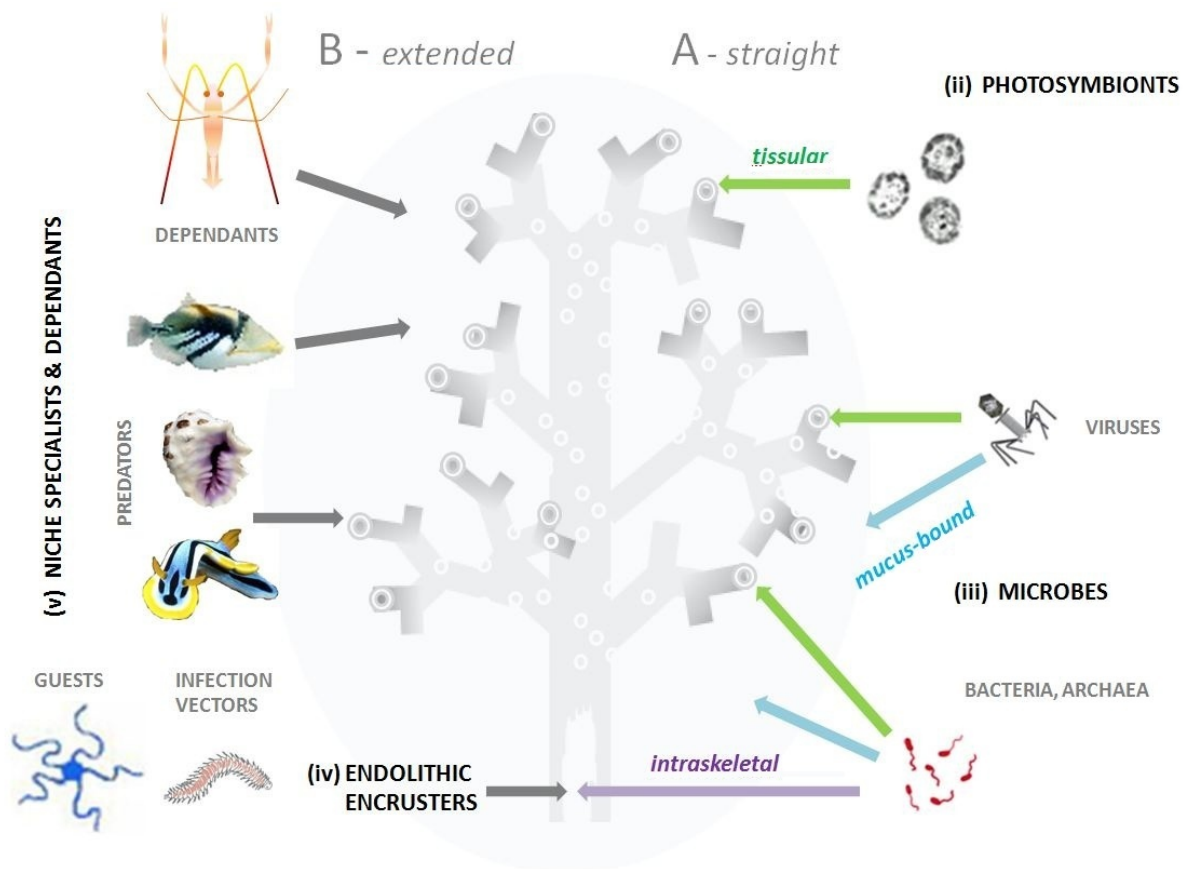


Figure 2. (right) A - Representation of the coral holobiont *sensu stricto* i.e. basically a functional 3-way photosymbiotic system with (i) the sedentary or sessile basibiont (center) and its mucosphere (pale grey halo) with (ii) its photosymbiotic associates and (iii) prokaryotic microbiome; (left) B - The *extended holobiont*, which may include (iv) endolithic associates (fungi, encrusting sponges and algae, protists) and (v) biodiversity that lives in association with the coral and may be critically affected by the loss of the host

which may account for the host- dependant biodiversity. We propose to refer to it as the *extended holobiont*.

4.3. The “omics” or high-dimensional biology revolution

“Omics” technologies are primarily aimed at the universal detection of genes (*genomics*), mRNA (*transcriptomics*), proteins (*proteomics*) and metabolites (*metabolomics*) in a specific biological sample [21]. Omics can be used in a wide variety of applications, ranging from biomedical to environmental, from biotechnological to ethical, from single-cell to ecosystem-wide, from systems biology to phylogeny. In short, “omics” technologies adopt a holistic view of the molecules that make up a cell, tissue or organism. This integrative approach, together with the “extended holobiont” as a functionally comprehensive biological model, provides a useful conceptual framework when dealing with complex environmental issues.

Transcriptomics - Perhaps the single most informative approach to studying the fitness of a test organism being subjected to an environmental stress is transcriptomics. In essence, the aim is

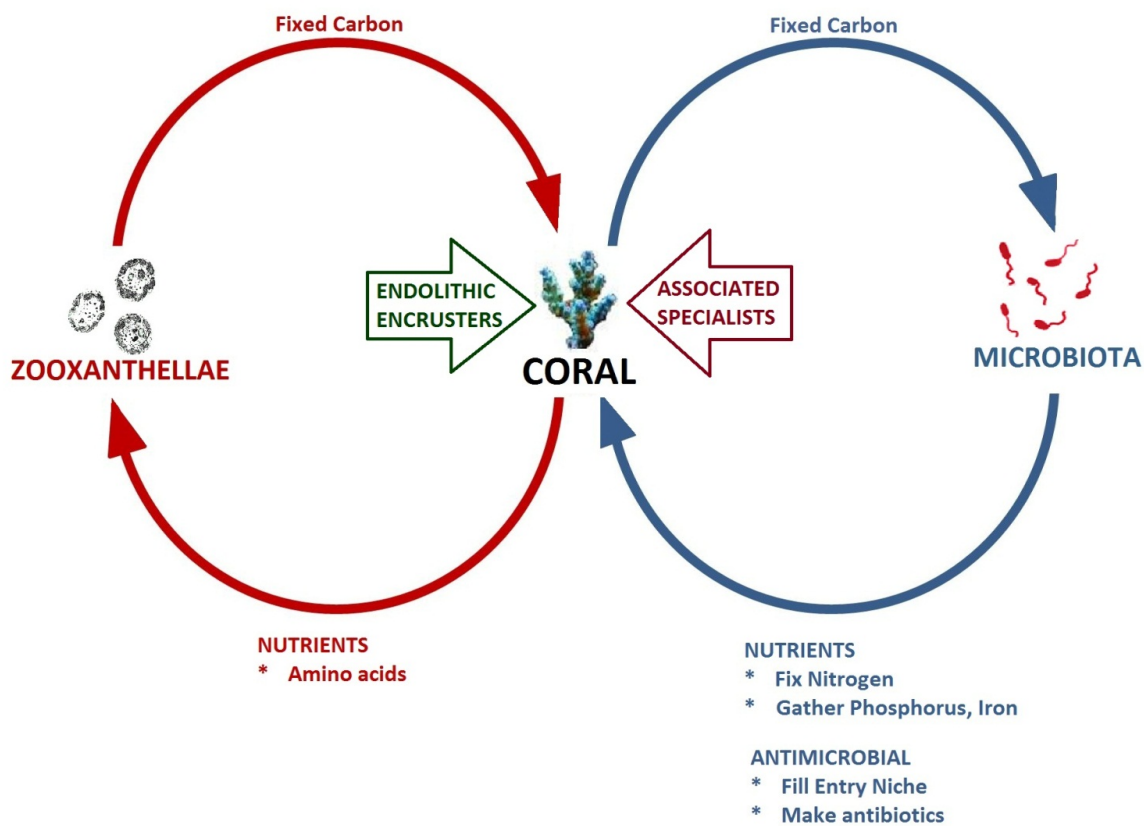


Figure 3. Relationships between the components of the coral holobiont system (developed from [20], with the addition of cryptic organisms and of associates (the *extended holobiont*)

(i) to catalogue all species of gene transcripts, including mRNAs, non-coding RNAs and small RNAs (non-coding bacterial RNAs), (ii) to determine the transcriptional structure of genes in terms of their start sites, 5' or 3' ends, splicing patterns and other post-transcriptional modifications, and (iii) to quantify the changing expression levels of each transcript during development and under different conditions [22]. The set of coding transcripts from activated genes will encode proteins as products of the ribosomal assembly line. Initially, complementary DNA (cDNA) copies are created from the mRNA templates, from which two analytical techniques have been developed: (i) hybridization-based and (ii) sequence-based. In hybridization transcriptomics, DNA microarrays composed of collections of a collection of microscopic DNA probes are used to hybridize cDNA targets - here a gene is activated when a spot is highlighted with a visual label, allowing quantification as well as characterization. Yet, the method relies on existing knowledge about the genome sequence. Modern sequence-based methods (RNA-seq) determine cDNA sequences directly, the latter (30-400 bp.) being attached terminally to adaptors. Each short sequence is aligned with the reference genome or transcriptome and classifies as exon read, junction reads and poly(A) end reads, from which a base-resolution expression profile is generated. A critical comparison between the different transcriptomic techniques is proposed in [22], with an emphasis on RNA-seq.

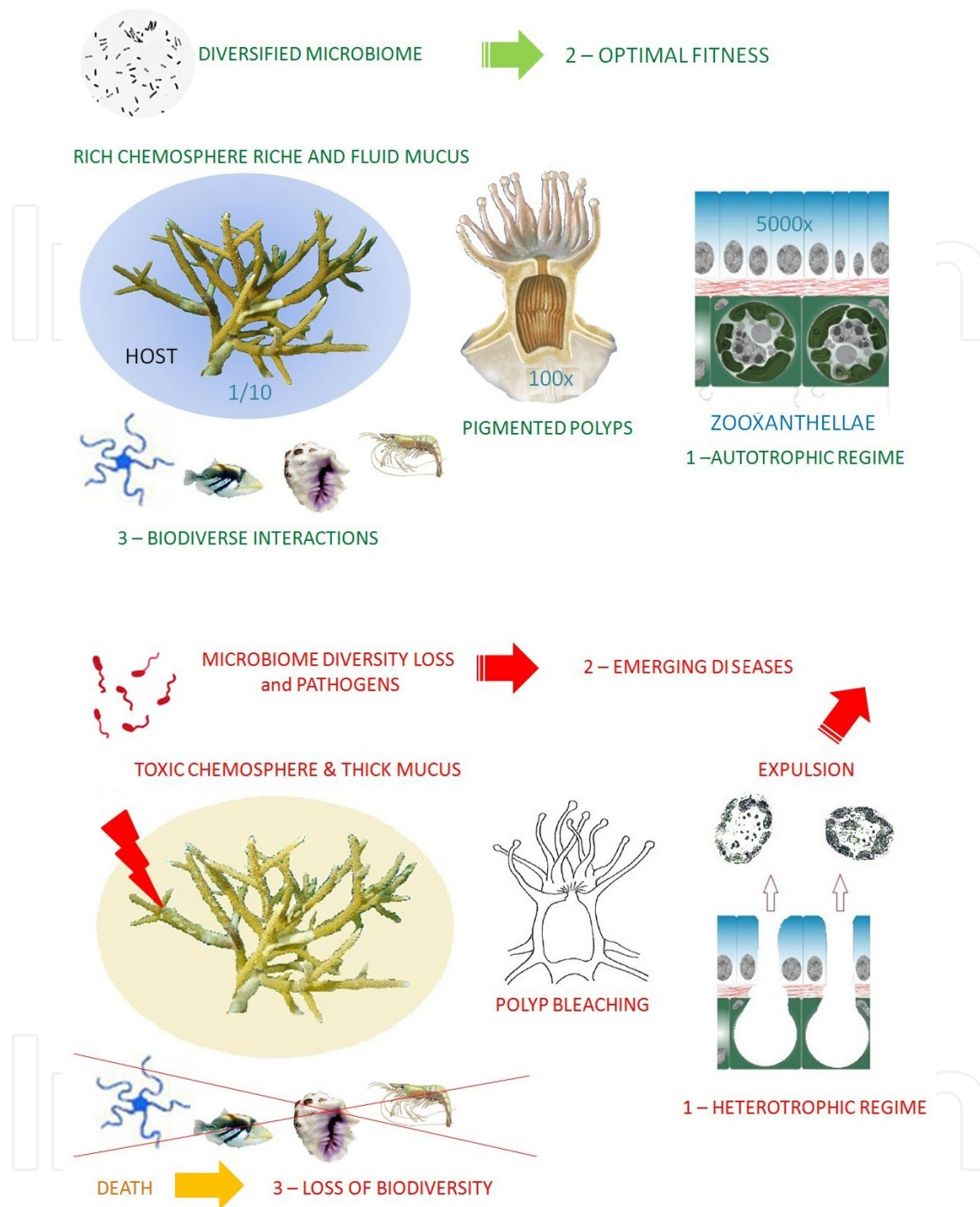


Figure 4. The extended holobiont of a scleractinian sentinel system under contrasting conditions: (1) top: under optimal conditions, the fit colony produces a fluid mucus which hosts a well-diversified and functional microbial flora; the polyps are colourful thanks to a dense population of symbiotic zooxanthellae that colonize the endodermal layer and provide the substantial autotrophic part of the energy budget of the holobiont. Eukaryotic associates are numerous, engaging into a network of interactions centered on the host. (2) Bottom: under lasting stress, e.g. sea water temperature above 32°C, the colony may produce a thicker mucus, in which a growing population of pathogenic bacteria tends to displace less competitive strains. Zooxanthellae leave the polyp tissues as a result of loss of immune recognition, and no longer provide photosynthates to the holobiont system which survives on heterotrophic/direct diffusion regime. Rate of associate biodiversity loss depends on the type of relationship with the host, from obligate to occasional

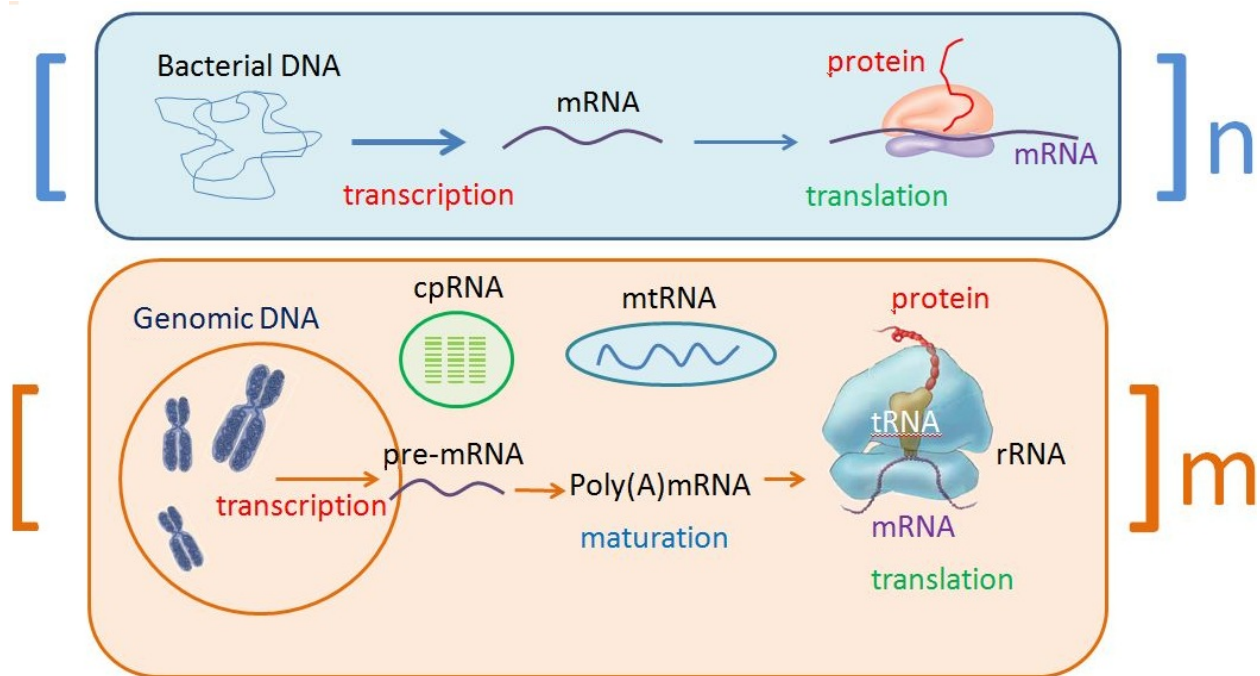


Figure 5. The total transcriptome of a holobiont system (1) top: the total set of bacterial transcripts from a population of n cells (2) bottom: the total set of eukaryotic transcripts from a population of m cells. Separating the coding RNAs from the non coding ones is made on the basis of the presence or absence of a poly-adenosine monophosphate tail, but only the final mRNAs are usually selected in transcriptome analyses

Stress reactions have been studied on of components of the coral holobiont. In [23] De Salvo and collaborators performed a medium-scale micro transcriptomics experiment on the Caribbean coral *Montastrea flaveolata* undergoing thermal stress and bleaching vs. unexposed conspecifics. Their complementary microarray experiment containing 1310 genes revealed that thermal stress and bleaching in this species affected the following processes: oxidative stress, Ca^{2+} homeostasis, cytoskeletal organization, cell death, calcification, metabolisms, protein synthesis, heat-shock protein activity and transposon activity. A dedicated transcriptome database was recently produced on *Pocillopora damicornis*, a ubiquitous and environmentally sensitive scleractinian [24], [<http://cnidarians.bu.edu/PocilloporaBase>]

Proteomics - Physical or chemical stress (i) inactivates or down-regulates many genes, including many housekeeping genes, (ii) while up-regulating stress genes that perform orchestrated induction of key proteins necessary for cellular protein repair. Stress expressions of organisms, or comparative analysis of ecotypes of a species naturally found in contrasting environments, can highlight the production of stress-related enzymes, such as heat-shock proteins. Heat stress does denature proteins, causing weakening of polar bonds, unfolding and exposure of core hydrophobic groups. Beyond the cell's tolerance, heat stress will cause its death. Thus the cellular stress response (heat-shock response) protects organisms from damage resulting from critical fluctuations of e.g. heat, UV light, trace metals, and xenobiotics. Stress genes are activated to rapidly synthesize stress proteins, a universal and highly conserved response (from bacteria to humans in which similar roles are played).

Indeed, candidate genes that are directly implicated (e.g. corals' response to stress) or functionally interconnected (e.g. to genes related to immunity) with cnidarians-dinoflagellate symbiosis point out the major role of key proteins [25], and their fast-evolving adaptation in the face of environmental challenges. Oxidative stress in zooxanthellae produces reactive oxygen species (ROS) with hydrogen peroxide diffusion into the host cell which activates a cellular cascade resulting into the photosymbiont expulsion and polyp bleaching. It may be that recognition of a suitable zooxanthellae clade by the coral host is a selective process during which other strains are actively expelled through immunity and apoptosis [26], the photosymbiont being more susceptible than the host to e.g. elevated temperatures and possibly UV levels [27]. Massive and laminar species are supposedly more resistant to environmental fluctuations than shorter-lived branched or encrusting species. Investigators in [28] showed that laminar corals (using *Turbinaria reniformis*) undergo transient Hsp60 heat-shock protein induction under either light or thermal overexposure, and prolonged induction of Hsp60 if the two treatments were combined – whereas branched *Stylophora pistillata* were relatively unresponsive, highlighting differences in potential for resilience between different growth forms and nutritional regime.

Metabolomics - Notwithstanding their dedicated specialist predators and parasites, scleractinian corals in general are poor producers of secondary (i.e. defence) metabolites since they enjoy protection from a biomineralized skeleton into which polyps can retract – a situation quite different to that of soft corals (alcyonarians) which produce a whole range of cyclized terpenoids against predators, for space occupation and larval protection. However, the presence of several classes of compounds, naturally produced or stress-elicited, has been described for massive coral species (e.g. *Porites* and *Montipora*), and in a number of branched forms. In the former, thermal stress followed by pathogen-associated molecular patterns (PAMPS) triggered defensive responses against alien threat as well as protective responses, e.g. phenoloxidase activity (production of melanin pigment [29], antibacterial activity, peroxidase and ROS scavenging (oxidative stress) and fluorescent protein [30]. Branched forms are regarded as more sensitive to thermal stress than massive forms, and produce natural antibiotics. Ectodermal cell of *Pocillopora damicornis* release damicornin, a 40-(residue antimicrobial peptide) in response to non-pathogenic challenges, but its expression is repressed by pathogen *V. corallilyticus* [31]. Various *Pseudoalteromonas* strains are known to produce antibiotics, e.g. diketopiperazines, that help them control the bacterial profile sharing the same host-associated resources [32], especially during changes in bacteriome profiling [33]. Mucus-associated *Pseudoalteromonas* spp. are generally considered efficient at protecting the coral holobiont's defense against potential Gram-positive pathogens [34], and are already known to participate in the antibiotic defense of green algae, against surface fouling organisms [35] and fungal epiphytes [36]. Each of these molecules is a potential chemomarker of stress.

Genomics - Marine eukaryotes span 35 phyla, 14 of which are exclusively marine. Genomics is useful in complementing taxonomy beyond classical morphological keys, in exploring evolutionary traits and similarities of small and large taxa, and in investigating the therapeutic potential of target taxa. Furthermore, molecular barcoding allows accurate assessments of environmental samples (especially protists and prokaryotes) – a very useful tool in estimating

biodiversity. Corals genomes include 23677 genes (fewer than human genome with 36036 but also than paramecium genome with 39581 genes). The genomes of several common species of scleractinian corals have been characterized at least partially. *Acropora millepora* [37], *Acropora digitifera* [38, 39], genomes have revealed a more complex than hitherto suspected complexity, especially regarding calcification and innate immune repertoire, though scleractinians seems to have lost the ability to carry out *de novo* synthesis of photoprotective mycosporin-like amino acids (MAA) family of compounds. Innate immune responses provide valuable information as predictors of thermal stress susceptibility and disease. A recent paper by [40] usefully reviews key features in coral immunology (recognition, signalling pathways and effector responses) in the general context of invertebrate immunology. Gene expression markers have been recently developed to monitor early responses to acute heat-light stress in *Porites* species, on the basis of differential expression of Hsp16 and actin genes [41]. Genes involved in the immune response against bacterial pathogens present clear differences in their expression patterns between *Vibrio corallilyticus* – exposed *Pocillopora damicornis* vs. unexposed control conspecifics [42]. Such predictors of stress responses can prove very useful if integrated in monitoring tools.

A comprehensive expressed sequence tags (EST) transcriptomics dataset on the symbiotic zooxanthellae has recently indicated some unique regulatory characteristics not found in free dinoflagellates – once completed and annotated, the complete *Symbiodinium* genome will represent a considerable asset in interpreting the coordinated responses of the coral holobiont under stress [43], beyond completely elucidating the molecular bases of the host-photosymbiont association. A recent worldwide survey spanning 20 years of data on coral-*Symbiodinium* photosystems revealed that the transmission mode correlates positively with photosymbiont specificity, not with coral specificity [44]. The authors found fewer generalist species than specialist species. The former group include the common Pocilloporid scleractinians and several Acroporids that can accommodate more than one zooxanthellae clade, and are more environment-sensitive than e.g. common massive corals which may be more resilient to contemporary stressors, especially those genera that are vertical transmitters such as *Porites* and *Montipora*. These findings point out the necessity to select one sentinel species of each group (branched vs. massive) in order to obtain a less biased assessment of the health status and of the resilience potential of a given reef locality under stress.

The third major functional component of the coral holobiont is the bacterial and archaeal microbiome, also susceptible to large composition shifts during e.g. heat stress [45], as revealed by metagenomic studies. Bacterial consortia are described as host-specific, each profile having its specific and its generalist strains, the relative composition of which being affected by environmental conditions.

Ideally, holobiont-wide analysis will benefit from the combined knowledge of the molecular biology of coral, zooxanthellae and prokaryotes that are necessary to define a fully functional system used as a control. The fitness descriptors will need to take into account (i) geographic variations, e.g. chemotypes for widely dispersed taxa), as well as (ii) short natural environmental fluctuations that fall into the natural physiological tolerance of a given population.

The introduction of a stressor (significant in intensity and/or duration) will allow investigators to model the precise interactions of molecular events that affect the three components of this holobiont, and place the physiological responses of the different parties into a system-wide sequence, from early responses to total collapse. Using a holistic and clinical-like approach [46], issue - specific network models can be created by confronting data collected on “stressed” holobionts against homeostatically regulated “no-stress” conspecific controls.

4.4. Merging “omics” with imaging and physiological / ecotoxicological approaches

Creating a multi-approach and comprehensive tool to evaluate the health status of corals under climatic or direct anthropic threat provides a more robust assessment than when using a single analytical method. The omics revolution is coming of age, and large scale data collection (metadata) are more easily tractable using modern bioinformatics algorithms (next generation sequencing) than before. The number of research papers dealing with molecular aspects of photosymbiosis in corals, with stress responses leading to the host-symbiont rupture, with the detection of early markers of stress (before actual symptoms are physiologically or visually expressed), with the resilience potential of massive vs. branched growth forms, with innate immunity, with the onset and development of bacterial pathogenicity following stress, has increased tremendously within the last few years. Each paper brings its unique and useful light into one of the most fascinating biological phenomenon. As of today, however, scientists are little more than spectators of a dramatic acceleration of the destructive impacts of civilization on the most fragile of all marine ecosystems. Politicians and the media are only implementing or relaying preventive anti-pollution policies, and what high-tech research has to offer to monitor what is actually occurring goes well beyond the understanding of the layman.

What is proposed here is to select the most informative analytical strategy as the core component, molecular biology (omics) being the choice alternative to detect early stress responses, and to complement it with physiological measures that are privileged for evidencing (i) adaptability and (ii) loss of function. Physiological monitoring is important in comparing the tolerance range of corals various growth forms, to short lived or limited stress exposure that do not cause changes in the composition of zooxanthellae [47], and allow for gradual acclimation. This laboratory experiment on *Acropora millepora*, a favourite model, may explain why in some localized coral populations, holobionts were capable of resisting temperatures well above the known tolerance limit for the species. Whatever the analytical method used for physiological measurements on the coral host or on its photosymbionts, the instrumentation must be adapted and the measurements repeatable in order to have strong and scalable metrics both in measuring the level and duration of the stressor, and the responses on behalf of the holobiont component under investigation. Respiration and photosynthesis, oxygen production, calcification rates, mucus rheology, pigmentation etc. can be very useful as complementary analyses along with omics.

Imaging tools have a greater impact to non-scientists, and their can offer excellent visual “proof” of an ongoing stress response, in the field (e.g. time-lapse photography of entire holobiont), or inside the component under examination. For example high-resolution imaging

mass spectrometry or NanoSIMS can make isotope tracing at single cell level. When linked to molecular visualization methods, such as *in situ* hybridization and antibody labeling, these techniques enable *in situ* function to be linked to microbial identity and gene expression [48]. Fluorescent pigments, e.g. multi-use pocilloporins, that have a photoprotective and antioxidant roles as well as helping dissipate excess light energy [49] can be detected by classical UV-VIS spectrometry. In fact green fluorescent protein-like (GFP-like) coral fluorescent pigments are routinely used as biomarkers in medicine and can be used to detect coral growth and tissue proliferation, as well as monitoring reactive oxygen species scavenging during stress. Hyperspectral pigment imaging in combination with oxygen profiling provide useful information on competitive interactions between benthic reef organisms, and demonstrate that some turf and fleshy macroalgae can be a constant source of stress for corals, while crustose coralline algae are not [50].

In combination, -omics, physiological/ecotoxicological and imaging tools provide a potentially formidable combination for measuring stress responses in coral holobionts and their separate components.

5. What modern technologies can do for the environment

Ideally, we need a multi approach diagnosis tool focused on the sentinel species undergoing stress, on the profile of its microbial associates, and to be able to estimate the loss of the epibiotic and encrusting macrofauna and flora which lives in association with the living host, i.e. the evolution of the overall biodiversity from the earliest stress symptoms detectable on the host, to its death.

The basic requirements for a diagnostic tool are:

- to consider different components of the holobiont model (i.e. host, photosymbionts and microbiota) and then integrate the results of the different analyses into a single comprehensive “holistic” diagnosis;
- for each analysis and each approach (molecular, microscopic, ecotoxicologic), to be able to define dose/exposure limits of the stressor that correspond to threshold responses along a continuum such as: normal tolerance/acclimation/resilience/no-return/rapid death;
- to propose corrective measures wherever some critical point is reached in one of the above limits.

The basic requirements for a biodiversity-assessment tool are:

- to list the algal and animal species that are usually associated with the sentinel holobiont;
- to categorize each species with respect to its location within the holobiont system (encrusting, epibiotic, mucus-bound, free-living) and to its degree of dependence to the host for each type (predator, commensal, parasite, symbiont).

5.1. Measuring stress responses, diagnosing overall fitness and proposing corrective measures

On the basis of the above requirements for a diagnostic tool, we propose an 8-step procedure to achieve a compromise between experimental robustness and implementation simplicity. The sequence is shown in Fig. 6, and each step is detailed in the text.

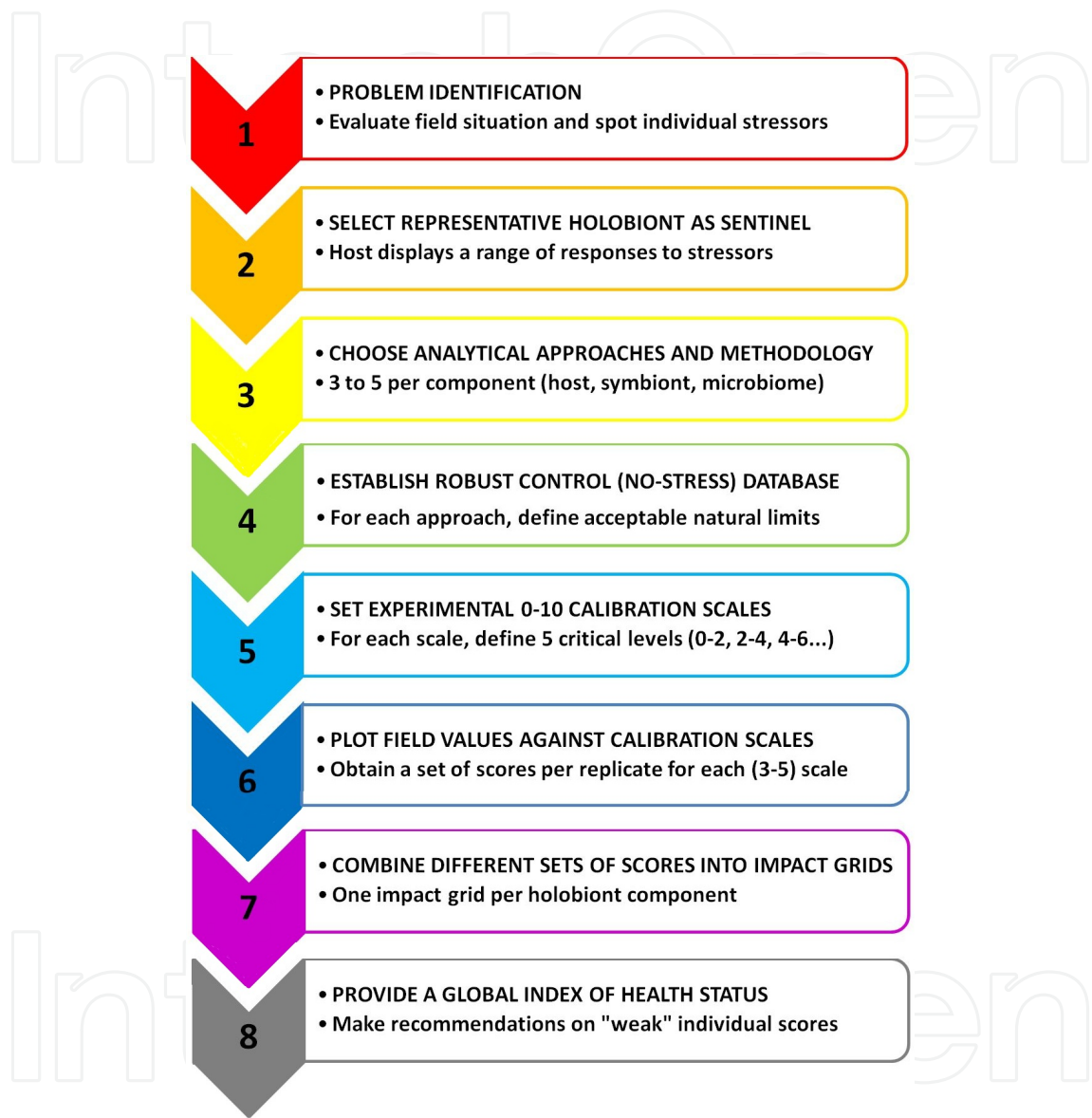


Figure 6. The 8-step logical sequence to create a customized tool (the INDICORAL procedure), each step being described in the text

STEP ONE (ISSUE) - Identification of the problem (in the field)

Each biodiversity issue is different, and the first task is to identify the source of the problem. Abiotic stresses should not include first-degree biological interference due to competition, predation or outbreaks of invasive or of alien opportunists, including benthic and pelagic macro and microbiota. On the other hand, we shall consider the departures from “normal” or

standard macro and microbiodiversity components resulting from the host species being under abiotic stress, e.g. expulsion or “bail out” of photosymbionts and loss of useful bacterial strains, or disappearance of vegetal and animal associates, which are at the very heart of the biodiversity loss issue. Most common abiotic stressors include: thermal stress, dessication, irradiation, hypo-hypersalinities, silting, heavy metal accumulation, organic compounds, mechanical damage due to wave or wind action and to anchoring. Thus, abiotic stressors can be climatic or pollution bound, chronic or accidental and have their effects combined. It may be necessary to combine field and laboratory (aquarium) studies in which stressors can be analyzed and modulated individually.

STEP TWO (METHODS) - The choice of the adequate holobiont (field)

The choice of a biological taxon may depend on the type of stressor. Corals and coralline algae might be more suitable to evaluate thermal, ultra-violet and acidity stresses, whereas bioaccumulators such as sponges or bivalve mollusks might be more suitable for silting and heavy metal stresses. Furthermore, a good model is one which is sensitive enough to the stressor, at the same time displaying a range of responses that can be calibrated usefully against different concentrations / exposure times. Finally, the model host-species must be (i) representative of the area under investigation, (ii) common enough for sampling at statistically significant scale and (iii) amenable to aquarium studies.

STEP THREE (METHODS) - How to measure stress in a model holobiont system

Abiotic stress will affect all components of the holobiont diversely and in a network connection manner, with gradual loss of function and accompanying morbidity symptoms. Stress studies will therefore be dealing with the host organism, its photosymbionts, and also with its associated microbiome. This will be achieved through a combination of cutting-edge and classical approaches, e.g. (i) *in aquario*: transcriptomics on the basibiont, microscopy/cytometry and molecular biology on the photosymbiont, (ii) *in the field*: bacterial and viral metagenomic sampling on the associated microbiomes, and ecotoxicology/physiology on the holobiont.

In aquario studies will use a significant number of the selected sentinel species from undisturbed environments, or cloned fragments of same where applicable.

STEP FOUR (DATABASE) - Making a robust set of control data for each analysis

The sentinel holobiont must be sampled in reputedly undisturbed areas, whatever the type of analysis: physiological, metagenomic, taxonomic (associate biodiversity) etc. and natural variations from a statistically significant number of replicates must be recorded.

When dealing with a photosystem holobiont, aquarium studies should consider the three components separately: (i) the holobiont e.g. coral, sponge..., (ii) the photosymbionts, e.g. zooxanthellae, cyanobacteria, (iii) the associated microbes e.g. mucus and tissue-bound bacteria. In each case, a minimum of three (and up to five) independent analyses using different analytical approaches must be undertaken, e.g. one or two –omics, one or two physiological or ecotoxicological, one using imaging etc.

For each analysis, the average values or estimates will set the 0 mark or control score of a future 0-10 scale. This step is crucial and the natural variability must not be too large with scores never exceeding those of responses to mild-severe stresses.

STEP FIVE (DATABASE) – Set experimental calibration scales (Fig. 7)

A useful way to calibrate a response scale *in aquario* is to expose the holobiont or its separate components to different stress regimes (concentration and duration) and establish a lethal value (10 on the 0-10 scale) and in between define threshold values (first symptoms, loss of photosymbionts, loss of microbial biodiversity, irreversible loss of function, death < 12h). Each analytical method will highlight a sequence of events along this “control to immediate death” gradient.

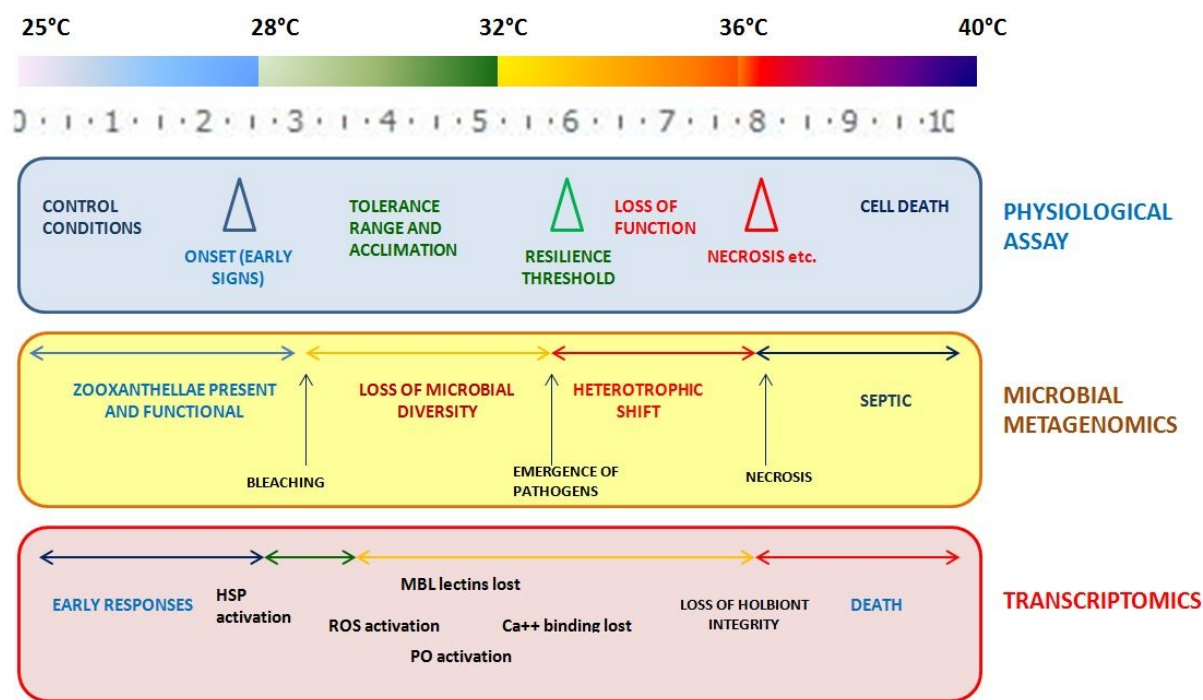


Figure 7. Studying responses of a coral holobiont during experiment heat/light stress using different analytical methods, and establishing a 0-10 scale of responses with critical values. This diagram is indicative only (values and sequences are fictional)

STEP SIX (ANALYSIS) - Compare stress vs. standard profiles

Once each experimental scale is established per analysis, environmental samples can be rated by attributing an average performance score based on *n* replicates.

STEP SEVEN (TREATMENT) - The single grid of impact or radar chart (Fig. 8 top and middle)

Then scores for each of the 3-5 analyses on each of the biological components will be reported to a single grid of impact, e.g. on a coral (5), its photosymbionts (4) and its microbiome (3).

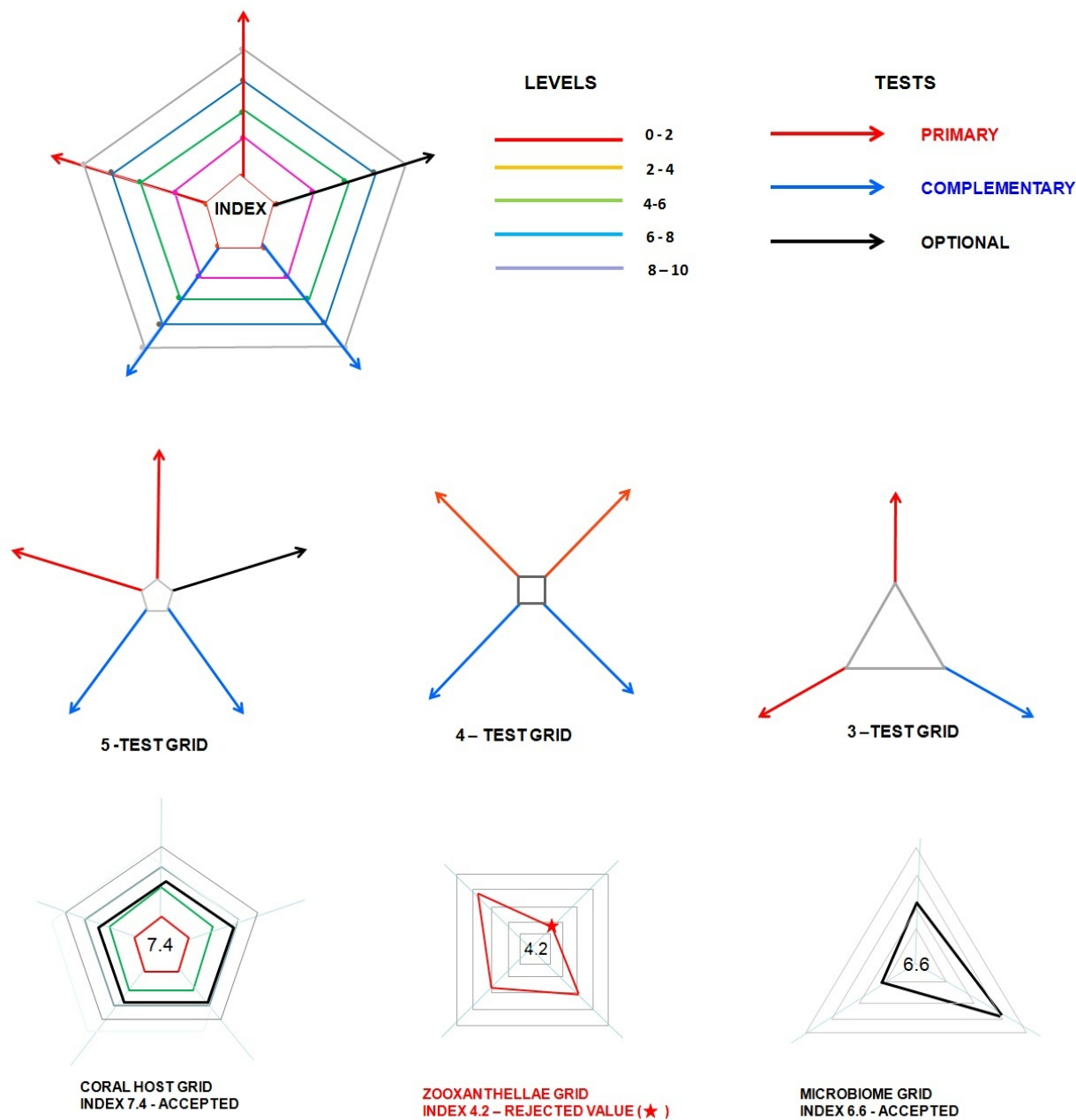


Figure 8. Radar charts – *Top*: typical radar chart - each apex of this polygon is a calibrated scale. *Middle*: types of radar charts with 3, 4 or 5 scales (red: important tests, blue: complementary tests, black: optional test). *Bottom*: Example of a sentinel coral host under heat/UV stress, using a 5-test grid on coral host, a 4-test grid on zooxanthellae photosymbionts and a 3-test grid on bacterial microbiome. Here, the coral holobiont undergoes preliminary signs of stress on photosymbiotic component (red star = oxidative stress on zooxanthellae).

STEP EIGHT (REPORT) - Health status of sentinel species and recommendations for amendment (Fig. 8 bottom)

Let us say we have a 7.4 average score on the coral host, 6.6 on the microbiome and 4.2 on the photosymbiont.. This indicates (i) what biological component is most affected by the environ-

mental stress (ii) where appropriate action is to be taken. Here, holobiont looks normal but early signs of stress are detected on the zooxanthellae (ROS and oxidative stress products), without signs of microbial dysfunction.

On the basis of these visual diagrams and numerical scores that are explicit enough to be understood and followed by non-scientists. Useful recommendations can be made to customers - if the principle behind this nicknamed "INDICORAL" environmental tool can be validated as a standard, legal enforcement can follow.

5.2. Strengths, limitations and future of environmental diagnosis tools

What is described here is a custom-designed diagnostic tool that integrates the critical snapshot information from different analytical approaches into a single easy-to-read layout, with a consensus fitness index. Using the multivariate "radar chart" model in conjunction with the fitness index then allows us to point out the weaknesses of the holobiont health at a given time, after which corrective measure can be proposed. Such a chart is made up of radiating spokes each representing a performance scale, for example rated from 0 to 10 in a given test. The spokes or performance scales correspond to a single test type, either molecular or visual or physiological. A robust radar chart typically integrates at least one test of each sort, in order to miss as little useful information as possible when establishing the final diagnosis. For example, the holobiont may "look normal" (as compared to control conspecifics) using a visual scale, perform close to optimal using a physiological assay, yet present strong signs of stress using an omics approach that detects early molecular responses. Or else, the holobiont may be suffering from a pollutant that acutely undermines its respiration, with no apparent effect on its microbiome nor on its appearance, and so on.

The objection that immediately comes to mind is that such an endeavour is time and money consuming, knowing how difficult it is to perform discriminating omics tests alone, especially when dealing with meta-data and the mathematical treatment that follows. The answer has to be optimistic: giant strides are being made in analyzing environmental meta-data more efficiently and cost-effectively, a good thing since some environmental issues are becoming rapidly critical. The other objection is that you need a whole team of specialists to devise and run a single "radar experiment".

The answer is two-fold: (i) once a suitable sentinel holobiont is chosen, the tedious part is to establish a reliable control database for each experiment type, mostly in the laboratory and using precise instrumentation. This database may initially require a panel of experts to set it up, but it does not have to be repeated for subsequent investigations. The other aspect is that we are creating a tool, not a thorough investigation into fundamentals, i.e. the experts must make sure that only essential information is retained, e.g. use identified molecular or microbial markers instead of profiles, set critical or threshold values within the 0-10 scale of responses (e.g. onset of symptoms appearance, loss of pigmentation, resilience limits, and so on), 0-2 representing the natural variability observed in controls, and 8-10 representing loss (no-return point to immediate death). This simplified representation relies on strong metrics, not on checking hypotheses which is the job of researchers. The custom association of 3 to 5 different

tests types on the coral host and/or the photosymbionts and/or the mucus-bound bacterial flora spans a whole range of dysfunction possibilities.

5.3. Predicting biodiversity loss using the extended holobiont concept

The health status of the coral holobiont directly affects all associated life forms according to the degree of dependence on the host. Ultimately, the loss of the host determines the biodiversity loss of all dependant flora fauna and microbes. Changes in fish biodiversity and pressures on feeding niches have been reported in relation to coral bleaching and loss [51].

Pocillopora damicornis has been used as a choice model in biodiversity issues, not only because of its pan-tropical distribution in the Indo-Pacific region and because of its sensitivity to climatic stress, but also because it provides shelter to a number of commensals that are dependant to varying degrees on the fitness of the coral host [52] and on the state of its bacterial flora. Metagenomic barcoding of the whole extended holobiont system, coupled with appropriate bioinformatics, will be a powerful asset in determining biodiversity loss associated with environmental stressors, and provide a useful link with the analysis of detritic biota after the host's death.

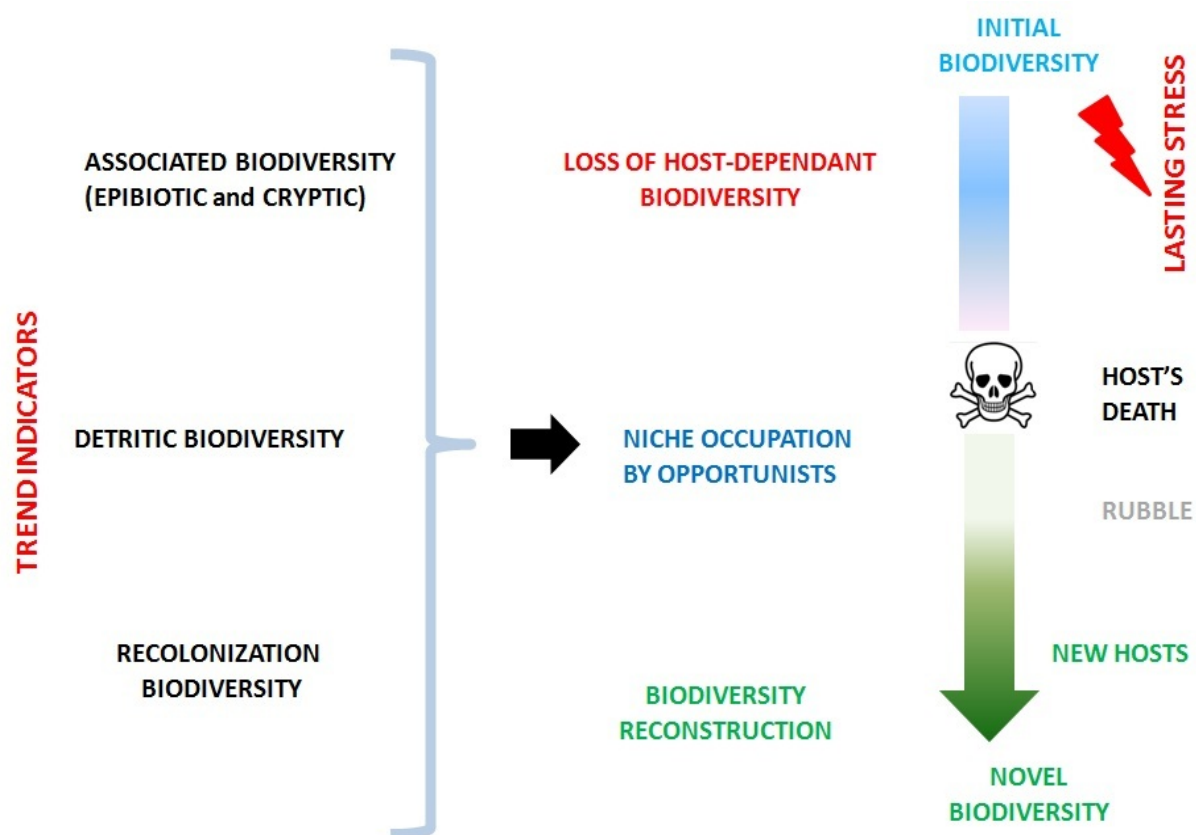


Figure 9. Loss of host-associated biodiversity (i) of original holobiont during lasting stress episode until host's death, (ii) detritic biodiversity finding refuge in coral rubble (iii) replacement biodiversity as substrate is recolonized with new hosts.

6. Measuring, predicting and hopefully mending

As pointed out earlier, over half a billion human live off goods and services of coral reef ecosystems at large, mostly in Asia. But not only: research on some 20,000 chemicals extracted from reef invertebrates has inspired the design of novel anticancer agents, antibiotics, anti-inflammatory, painkillers etc. and the exploration of the complex biosynthetic pathways leading to the production of these “miracle” molecules is only starting. Knowing the paramount importance of host-microbial symbioses in the making of these molecules, biodiversity loss will inevitably lead to chemodiversity loss, and opportunities will no longer exist to investigate the full bio-inspiration potential of what our holobiont systems can produce better than we can.

Marine ecology is a young science, and its developments are accelerating in response (i) to the urge of measuring the reactions of organisms facing the fluctuations of their environment, (ii) to the understanding of how they form complex interaction networks around key molecules acting as mediators of antibioses and symbioses, feeding hierarchies and occupational strategies for essential resources. Holobiont-wide systems biology is coming of age and will allow us to understand how various components of a holobiont system respond to stress in a coordinated manner, in the face of sudden and brutal environmental disasters, or of steadily increasing climatic or anthropogenic forcings, against the background of naturally fluctuating levels of stress. Ecosystem-wide resilience to environmental challenges can no longer be hoped for, and total biodiversity wipe-out of coral reefs is highly unlikely. The most probable scenario is that some reef systems might face near-total biodiversity collapse (e.g. in tropical zones that are within direct influence of urban expansion), while coral species from subtropical and remote localities will be constantly trying to acclimatize, each ecosystem striving to reach an overall “resilience equilibrium” at the cost of some of its biological diversity.

In 2013, most biological phenomena can be measured at all scales, from single cells to whole ecosystems, directly or using proxies and appropriate metrics. In combination, -omics, physiological/ecotoxicological and imaging tools provide a potentially formidable combination for measuring stress responses in coral holobionts and their separate components. Designing environmental tools such as proposed here might soon or later become a necessity, not only for coral reefs, but for all endangered ecosystems, marine and terrestrial.

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I wish to dedicate this environmental tool concept to those who guided and encouraged my early endeavours as an enthusiastic observer of nature: to the great naturalist René Catala (1901 - 1987), and to my Australian and New Zealander academic mentors, from Sydney to Townsville via Auckland.

Author details

Stéphane La Barre^{1,2}

1 Université Pierre et Marie Curie-Paris 6, UMR 7139 Végétaux marins et Biomolécules, Station Biologique F-29680, Roscoff, France

2 CNRS, UMR 7139 Végétaux marins et Biomolécules, Station Biologique F-29680, Roscoff, France

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