

Experimental techniques to assess coral physiology in situ: current approaches and novel insights

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Abstract: Coral reefs are declining worldwide due to global changes in the marine environment. The increasing frequency and severity of massive bleaching events in the tropics are highlighting the need to better understand the stages of coral physiological responses to extreme conditions. Moreover, like many other coastal regions, coral reef ecosystems are facing additional localized anthropogenic issues such as nutrient loading, increased turbidity, and coastal development. The changes in coral metabolism under local or global stress conditions is studied largely through laboratory manipulation and field observations. Different strategies have been developed to measure the health status of a damaged reef, ranging from the resolution of individual polyps to an entire coral community, but techniques for measuring coral physiology in situ are not yet widely implemented. For instance, while there are many studies of the coral holobiont response in single or limited-number multiple stressor experiments, they provide only partial insights to metabolic performance under more complex temporally and spatially variable natural conditions. Here, we discuss the current status of coral reefs and their global and local stressors in the context of current experimental techniques that measure core processes in coral metabolism (respiration, photosynthesis, and biocalcification) and their role in indicating the health status of colonies and communities. The state of the art of in situ techniques for experimental and monitoring purposes is explored. We highlight the need to improve the capability of in situ studies in order to better understand the resilience and stress response of corals under multiple global and local scale stressors.

Keywords: environmental monitoring; underwater respirometry, fluorometry, coral metabolism

1. Introduction

Coral reef ecosystems are hotspots of biodiversity and productivity in the ocean [1] that exceed that of tropical rainforests [2]. They provide crucial ecosystem functions and services such as providing goods for subsistence and economic fisheries, coastline protection from storms, and centers of the growing field of ecotourism [3–5]. As a key habitat-forming taxa, corals are critical to both reef systems and the coastal human populations that rely on them, and it is imperative to accelerate advances to ensure the longevity and survival of corals and coral reefs.

Coral reefs have drastically declined worldwide in the last thirty years because of recruitment failures, reduced growth rates, and acute and chronic mortalities [6,7], with only a fraction expected to survive in the next three decades in the Indo-Pacific region [8,9]. One of the most significant and widespread anthropogenic causes of this degradation is the change in climate drivers associated with the rise in atmospheric carbon dioxide

(CO₂) and other greenhouse gases [10,11]. Local stressors also go hand in hand with global stressors, such as coastline erosion or development, which threaten the resilience of corals through pollution and sedimentation.

Increased energy consumption since the Industrial Revolution has led to the highest CO₂ levels recorded in the atmosphere since human evolution (> 410 ppm) [12]. Of the multiple greenhouse gases (such as N₂O, CH₄ or CFCs) affecting global biogeochemical cycles, biodiversity, and human health [13,14], CO₂ has been the most relevant to marine ecology because of its dual role in marine heatwaves [15] and ocean acidification [16]. Overall average seawater temperatures in tropical regions have increased by almost 1°C over the past 100 years and are projected to continue increasing at 1° to 2°C per century [17]. Increased seawater temperatures are a major contributor to coral bleaching and are considered as the limiting factor for coral survival [7,18]. Roughly half of the CO₂ emitted into the atmosphere dissolves into the surface ocean, reacting with water to form several dissolved inorganic components of the carbonate system [19] and lowering seawater pH [20]. In comparison with pre-Industrial Revolution levels, seawater pH has decreased by approximately 0.1 [21], which equates to roughly 30% increase in acidity and may decrease further by 0.06 to 0.32 based on emission scenarios [16]. This process of ocean acidification is particularly disruptive to marine organisms like reef-building hard corals that create calcium carbonate skeletons, increasing the energy requirements for growth and survival [20,22,23]. Thus, corals and coral reefs may be significantly more vulnerable than previously thought when ocean acidification and warming combine [24,25].

Although climate drivers are widely recognized as dominating factors in coral loss and reef ecosystem shifts [18], localized stressors also are impacting coral health, where a survival-resilience pattern is observable in urban subtropical reefs subjected to several anthropogenic stressors [26]. Here, there are indications that stress-tolerant hard coral species have been selected to foster more resistant though less diverse reefs [27]. Impacts from eutrophication, increased turbidity, and lowered dissolved oxygen significantly affect coral metabolism, changing energy pathways and reef ecology (Figure 1). A coral as a holobiont, including all its functional, genomic and epigenetic associated symbionts, is capable of ecological plasticity in order to survive environmental changes, but not all coral species showed the same response patterns under anthropogenic threats.

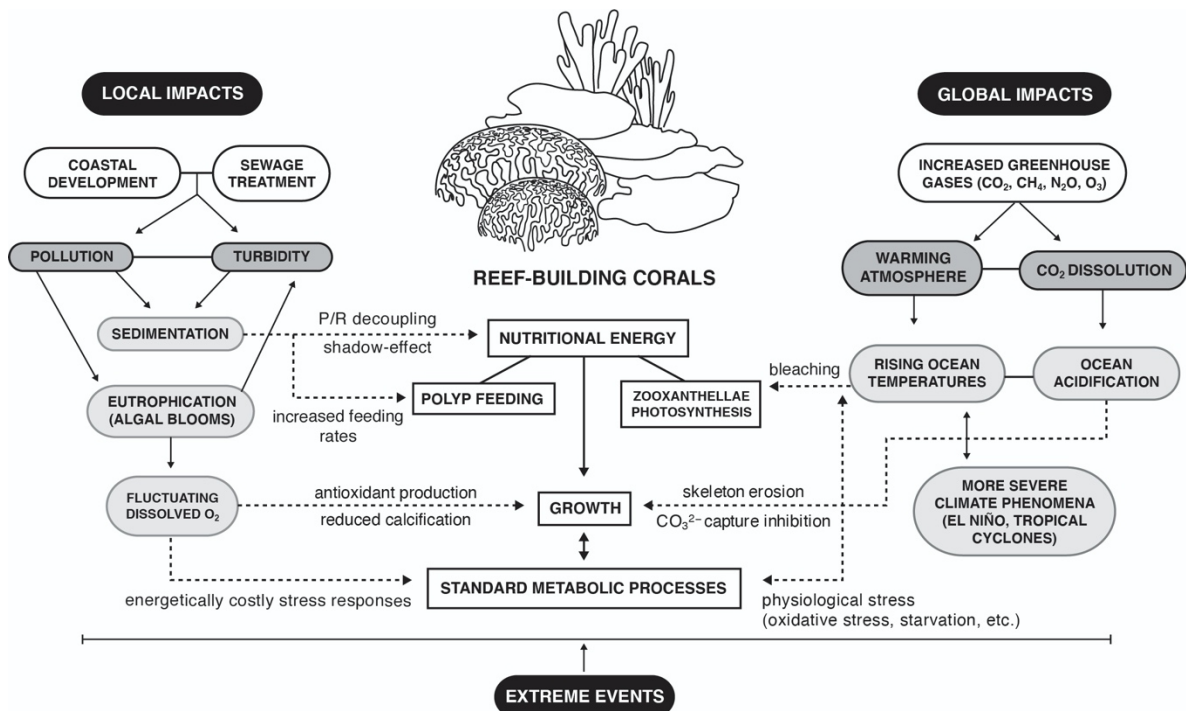


Figure 1. Local and global impacts affecting the nutritional energy of reef-building corals. Full lines indicate direct interactions, dotted lines indicate indirect interactions.

Turbid water conditions commonly occur within inshore shallow coastal waters [28], owing to the collective interactions of river runoff and the natural re-suspension of sediments (e.g. tides or storms) as well as anthropogenic activities (e.g. ship wakes, coastline modification, storm and other sewage discharge). The adaptive responses required to survive these conditions are both stressful and energetically costly [29], utilizing energy that otherwise would be put towards growth and reproduction. One of the consequences of increased turbidity is the reduction of the in situ irradiance supporting photosynthesis, and settling sediments can further smother coral polyps [30]. The resulting decreases in photosynthetic efficiencies and increases in respiration reduce the daily productivity of corals (measured as the ratio of photosynthesis to respiration, P/R) as well as the relative nutritional energy requirements, such as reduced lipids content and change in lipid class composition [31]. Lower P/R also can increase coral susceptibility to infection and bleaching [32], generating community- and ecosystem-level impacts. Corals adjacent to urban developments are frequently exposed to acute sedimentation but do not suffer tissue mortality, because they appear to have acclimated to low light conditions [33] with increased feeding rates to offset energy deficits from photosynthesis [34]. However, the increased frequency and severity of acute sedimentation in coastal waters contributes additional stress to corals that potentially are functioning near the limits of their physiological tolerances [31,35,36].

High oxygen concentrations from over-primary production (e.g. the first stage of an algal bloom) can bring oxygen intoxication, resulting in coral damage and photorespiration [37], due to a shift of the use of energy into the production of antioxidants rather than calcification [38]. On the other hand, natural fluctuations of dissolved oxygen concentrations in coastal seawater and shallow coral reef can stimulate physiological rates and promote adaptation to the adjusted environment. However, low oxygen concentrations may have a significant effect on the survival behavior of corals. Although hypoxic events and dead zones in the tropical coral reef are poorly investigated and rarely reported, episodic or seasonal hypoxia has been recently associated with bleaching and mortality in deeper corals rather than shallow colonies [39].

Eutrophication has degraded many coral reefs in coastal regions, and high-nutrient waters exacerbate the effect of global warming on coral survival [40]. The nutrient gradient affects the nutritional status of corals, in particular when the calcification rate is reduced but heterotrophy enhanced under high nutrient loading [41]. At the community level, a shift from net community calcification to dissolution can occur under high nutrient conditions [42]. However, several factors influencing the susceptibility to eutrophication have to be included, such as hydrodynamic, connectivity and location [43].

Gaining knowledge of the stress responses of corals and their effects on reef ecology, along with the pathways to best minimize these impacts, depends on two related tasks; understanding coral health from polyp-endosymbiont symbiosis to the community level, and achieving early detection of the onset of the stress responses. The first provides the foundation for studying and developing potential mitigation and managements strategies, and the second is crucial for implementing these strategies soon enough to help minimize impacts.

While most early studies of environmental change on coral health focused on the effects of single drivers (e.g., temperature, ocean acidification, turbidity, etc.), their interactive effects require addressing multiple stressor effects on physiological processes at both the holobiont and colony level if we are to comprehensively understand their impacts on coral communities. Some of these effects are major (e.g., temperature and light), but there undoubtedly are other interactions subtle enough to challenge recognition but significant enough that they can affect the resistance and recovery response of coral communities; factors critical for reef management in proactive preventative planning. But, our current understanding of coral stress responses is largely based on experimental manipulation studies in laboratory systems that are poor replicants of their natural habitats. In situ studies, by either SCUBA or automated sensors, could provide a better understanding of local and global impacts, but few studies have undertaken the logistical complexities of studying fine-scale physiological processes of corals in situ recently [44–46]. The primary objective of this review is to summarize the current strategies used to measure the metabolism of corals to highlight the benefits of non-destructive methodologies. A list of recommendations is provided that would expand the efficacy of underwater studies for improving local knowledge and better understanding of how corals respond to stressors.

2. Overview of the coral metabolism

2. 1. Metabolic responses at coral polyp level

The concept of the coral as a “holobiont” was introduced in the 2000s [47,48] which comprises the coral polyps and the associated symbiotic organisms, including photosynthetic endosymbionts, bacteria, viruses, fungi and protists. These have a fundamental role in nutrient and energy acquisition processes of coral polyps [49], genome evolution in the coral host and microbial partners, maintaining holobiont homeostasis, and the overall health of the holobiont [50]. The functional diversity of these microbes contributes to the stress response and acclimation to abnormal conditions, e.g. high seawater temperature and bleaching events [51,52].

The family Symbiodiniaceae includes several genera of dinoflagellates which reside in the tissue of corals and other marine organisms [53]. The coral-Symbiodiniaceae association is an obligate symbiosis for the coral, where the photosynthetic dinoflagellates provide the coral oxygen and energy in the form of glucose for aerobic respiration [54,55]. While most of the oxygen arising from photosynthesis is immediately utilized in coral respiration [56–58], the excess oxygen is released to the surrounding seawater throughout the day [59–62], supporting the oxygen availability on the reef. Therefore, at night, corals must acquire oxygen from the surrounding environment to fuel the respiration process [59]. As a consequence, the energy acquisition follows a circadian pattern where endosymbiont-guided photosynthesis is dominant during the day while polyp-guided respiration is dominant at night.

This continuous loop is stable in normal conditions, but the symbiotic dynamics within the coral holobiont is changing rapidly in the Anthropocene [63]. Temperature-induced stress damages a key protein (D1) in the photosystem II within the dinoflagellate (PSII; [64]), inactivating the Rubisco center [65], affecting the production of ATP [66], and leading to the overproduction of reactive oxygen species, inducing damaging conditions for both the endosymbiont and coral host. The increased frequency and severity of temperature anomalies will lead to negative consequences for coral survival [20,67]. Under warming and eutrophic conditions, this symbiosis may shift from a mutualistic to parasitic strategy for nutritional resources allocation [68], and the positive feedback loop for nutrient acquisition can be disrupted when conditions are not favorable to support the benefits of the O₂ / CO₂ fluxes [69,70].

The coral symbioses also can be influenced by other types of stress related to environmental changes. The impact of high seawater pCO₂ / low pH on corals is highly variable, where it can stimulate growth and photosynthetic efficiency in certain Symbiodiniaceae species [71], which suggests that the presence of carbon-concentrating mechanisms in the endosymbiont can lead to strain in the symbiotic relationship [72]. Bleaching, where the endosymbionts may decrease their chlorophyll content or be ejected from the coral host, signals severe physiological consequences arising from oxidative stress and starvation, leading to reduced respiration rates and energy acquisition [73]. Although bleaching can be a reversible condition, it often leads to coral tissue death if sustained too long. However, endosymbionts have developed adaptive mechanisms to limit thermal stress by increasing electron flow in photosystem I as photoprotection [74], thereby limiting the extent of bleaching. Moreover, corals polyps may partially compensate for the lost photosynthetically-derived energy during bleaching by enhanced feeding if sufficient prey are available [32].

2. 2. Metabolic responses of the coral community

When magnified across coral tissues, the polyp-level metabolic responses can affect reefs at community level. Growth of coral colony, or net community calcification, is measured in terms of the deposition of its calcium carbonate (CaCO₃) foundation, which is determined largely by coral energy reserves and the need to maintain optimal chemical conditions at the tissue/calcium carbonate interface. Hard corals build their skeleton of CaCO₃ through the uptake of calcium and carbonate ions from seawater. The reaction occurs in the calciblastic cells lining the surface where primary crystal secretion occurs [22] and is facilitated at pH levels above that of seawater. However, ocean acidification reduces the seawater pH and the aragonite saturation state (Ω_a) [23], which can have a strong effect on net community calcification (NCC), along with the balance of organic matter production and respiration, or net community production (NCP) [75]. Moreover, the projected decreases

in pH in the future ocean eventually will affect the stability of existing reef ecosystems, because the CaCO_3 formed by corals (aragonite) is more susceptible to dissolution than that formed by other bio-calcifying organisms [76]. These effects overlay natural seasonal variations in the carbonate chemistry in reef systems, and the question remains of what extent does globally and locally derived ocean acidification have on the seasonal balance of NCP and NCC [77]. However, these estimates remain imprecise, owing to the lack of information about seawater residence time and volume, which would modulate reef chemistry [78]. The relationship between corals and their symbiotic dinoflagellates is exacerbated under the combined effects of ocean acidification and warming, where warming can decrease photosynthetic production and increase respiration, leading to lower energy reserves for growth [6,20,79,80].

Even with these impacts though, it is possible for coral communities to recover after destructive events. For example, the coral reef in Kane'ohe Bay showed positive NCC and NCP about one year after the last drastic bleaching event in 2015 [81], indicating the capacity of local rapid post-bleaching recovery. However, these estimates considered only the abiotic factors, such as carbonate chemistry and oceanographic patterns, without considering potential effects from the benthic community which would contribute to the depletion or repletion of alkalinity in the coral reef [82].

Defining the health of corals and coral communities is complex because of array of elements that contribute the status of reef ecosystems. The coral micro-environment comprises ecologically-dependent niches that contribute to the general homeostasis and plasticity—i.e., resilience—to the coral holobiont. But understanding the responses to variable multistressor conditions in their in situ environments is not adequately informed by only laboratory-based manipulation studies, so the adaptive resilience of coral species and their influence the reef systems remains only partially understood. It is difficult then to chart ways forward for pragmatic and effective steps towards protection and restoration. Determining how to overcome these limitations will depend on applying new, in situ observational techniques that can bridge from single organisms to ecosystems and then to the global scale.

3. Methods

We conducted a literature review based on case studies of in situ observation of coral metabolism from published peer-reviewed scientific literature. We included cases involved in original research on direct (e.g. using SCUBA divers, benthic chambers, optical sensors) underwater measurements of metabolic rates on both coral individuals and coral communities. We excluded sample collections (e.g. coral fragmentation), laboratory experiments, and indirect measurements of metabolic fluxes (e.g. ex situ from sample incubations). The literature screening was categorized in eight separate categories based on the methodology used (Table 1), including: 1) SCUBA FRR fluorometry; 2) SCUBA PAM fluorometry; 3) Clark-type O_2 sensors; 4) boundary layer; 5) SCUBA imaging; 6) benthic chambers; 7) submersible chambers; and 8) automated sensors. The database was further classified based on the system type (open, semi-closed, enclosed); measured parameters (O_2 , Fv/Fm, ETR, CA); sampling frequency (minutes, hours, days); sampling scale (symbionts, polyp, colony, community); and aim of the study (monitoring, experiment).

We used the advanced search on Google Scholar to identify studies with the keywords “coral in situ metabolism” and “underwater”, excluding the keyword “collection”, in articles published between 1990 and 2020. The search yield ($n = 2090$) was scrutinized and the literature was manually reviewed to fulfil our selection criteria described above. A final list of 52 studies were included for the analysis in this review.

Through the analysis of the literature screened, we ranked the database by publication year (Table 2) and identified the most commonly used techniques for studying in situ coral metabolism. Finally, we analyzed the objectives of the studies included in this review (Fig. 2) in order to give an overview on the topics mostly studied between 1990 and 2020.

4. Methodologies for coral in situ metabolism

Several tools and instruments have been developed for measuring coral physiology in both field and laboratory studies. The ability to non-invasively measure metabolic and physiological processes makes these methods well-suited for studying both healthy and stressed organisms. The use of diver-portable technologies, designed to non-destructively estimate energy production and expenditure, such as respirometers and fluorimeters, is discussed in this review. However, there is little consensus about the extent to which apparently healthy corals are adapting to the changing conditions [83]. Measurements of metabolic proxies such as variations in dissolved oxygen and pH have been used at both individual coral and coral community scales to infer the metabolic state of benthic ecosystems [84], and link these indicators to the wider contribution the ecosystem’s nutrient cycle and biogeochemistry.

Previous convention for laboratory-based manipulation studies for coral metabolism have involved destructive sampling of individual or fragments of a coral colony. Although this framework provides crucial knowledge on separate isolated physiological mechanisms and pathways, these controlled conditions do not comprehensively reflect the stochasticity and cyclical fluctuations in environmental conditions on a reef and the effects of multiple chronic or acute stressors. In the last decades, experimental techniques to detect the metabolic status of coral directly in situ have been developed, using specific tools engineered for automated analysis on coral surface and reef communities. Among these, microsensors and benthic chambers for respirometry and fluorometry techniques became the most popular for coral surface studies even with different applications (Table 1).

Table 1. Experimental techniques available for in situ monitoring of coral physiological processes.

Method	System	Parameters	Sampling frequency	Sampling scale	Aim
SCUBA FRR fluorometry	Open	ETR, Fv/Fm	Minutes	Symbionts	Monitoring
SCUBA PAM fluorometry	Semi-closed	ETR, Fv/Fm	Minutes	Symbionts	Monitoring
Clark-Type O ₂ microsensor	Open	O ₂	Hours	Symbionts, Polyp	Experiment, Monitoring
Diffusive Boundary Layer	Semi-closed	O ₂ , CA	Minutes	Symbionts, Polyp	Monitoring
SCUBA imaging	Open	O ₂	Hours	Symbionts, Polyp, Colony	Monitoring
Benthic chamber	Enclosed	O ₂ , CA	Days	Colony	Experiment, Monitoring
Submersible chamber	Enclosed	O ₂ , CA	Hours	Colony, Community	Experiment, Monitoring
Automated sensors	Open	O ₂ , CA	Hours	Community	Monitoring

SCUBA FRR: SCUBA-based fast respiration rate fluorometer; SCUBA PAM fluorometry: SCUBA-based pulse amplitude modulation; O₂: Dissolved oxygen; CA: Calcification; ETR: Electron transport rate; Fv / Fm: maximum quantum yield of photosystem II.

4. 1. Underwater fluorometry

The photochemical performance of coral endosymbionts is a key aspect influencing the health of scleractinian corals, so measures of holobiont photophysiology provide insight to coral metabolic status. Fluorometry can provide insight in two broad ways: a measure of the abundance, character, and distribution of light-harvesting pigments and fluorescent proteins in corals (i.e., the potential energy source), and the photo-physiological status of the endosymbiont i.e. how well these pigments are being used to generate energy for the coral.

A fundamental measure of coral status is quantification of the light harvesting pigments of the endosymbionts over time, whereby decreasing trends can provide early warning of the onset of bleaching events. The underwater fluorescence imaging has been adapted to conduct large-scale in situ assessments of coral reefs, through the detection of green fluorescent proteins in corals [85,86]. Because the concentration of these pigments and fluorescent proteins are directly involved in photoprotection and photo-acclimation, quantifying the concentrations present in a coral population or community can provide crucial information on bleaching susceptibility or resistance [87–89]. In addition, this imaging technique can also make early signs of disease and coral juveniles easier to detect for accurate enumeration [90], providing a facet of coral reef health [86].

Early in situ studies on coral physiology estimated the rates of photosynthesis and respiration within recirculating chambers by measuring changes in oxygen concentrations [91]. While still a highly useful approach (see below), the introduction of variable fluorescence techniques has greatly increased the ability to assess the photophysiology of the endosymbionts, and thus provide a sensitive indicator of their metabolic status. With variable fluorescence, very rapid pulses of light are used to probe the photosystems and inform on a number of photosynthetic parameters including the quantum yield of photosystem II (PSII), which is used as a metric for photosynthetic rates [92] and is a key indicator of the physiological status of the endosymbiont. Changes in photosynthetic efficiency provides an early signal of stress, some of which is a natural response to changes in irradiance but become more prolonged and severe when conditions of imminent bleaching occur.

The estimation of the potential quantum yield of PSII as a metric for photosynthetic rates was first applied to underwater organisms by Beer et al. [93]. Pulse amplitude modulated (PAM) fluorometer uses light pulses of relatively long duration (300 - 1200 ms, 3000-10000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) to modify the fluorescence yields, generating multiple turnover for the reduction of PSII, allowing non-invasive chlorophyll fluorescence analysis, quantum yield and electron transport rate of coral endosymbionts. The introduction of an underwater version of this instrument (Diving-PAM, Walz) facilitated the direct observation of changes in coral health in situ conditions [94]. The introduction of in situ PAM fluorometry enabled the construction of photosynthesis-irradiance models for corals based on varying sun exposures in reefs [93,94]. For example, observations of decreased quantum yield during the day reflected photoinhibition through loss of photosynthetic capacity; a response seen in shallow but not deeper water corals [95]. Similar reduced photosynthetic efficiency has been observed in corals exposed to seasonal turbid conditions [96]. The use of Diving-PAM continuous monitoring during bleaching events also showed that combined thermal and light stress generated greater reductions in photosynthetic efficiency, and was a sensitive indicator of metabolic stress in impacted areas [97]. Even so, variable responses in the coral community to this stress suggested coral species-specific physiological responses [98–101]. Further development on PAM technology is the high-resolution imaging fluorometry used for analysis of heterogeneity within colonies, from coenosarc tissue to polyp tentacles, but so far this methodology is not available for underwater studies [102].

A second approach for measuring variable fluorescence, the FRR fluorometer uses a different approach to saturate the photochemistry during measurement, whereby shorter (150 – 400 μs , >20000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) but more intense light is used to generate a single turnover and reduction of the primary electron acceptor [103,104]. The methodology is more sensitive than PAM, which can be beneficial when endosymbiont abundance (or chlorophyll concentrations) are very low in corals. The SCUBA-based FRR fluorometry was developed by Gorbunov et al. [105] for measuring chlorophyll fluorescence from PSII reaction centers in corals, sea grasses, macroalgae and algal turfs. FRR fluorometry has been also used to monitor coral physiology during bleaching events, and their later recovery [106,107]. Although nutrient availability (as nitrogen or iron) and sunlight irradiance were already recognized as the main factors directly affecting photosynthesis in the aquatic ecosystem [108,109], the use of FRR fluorometry enabled more direct measurements to the effects of nutrient limitation on photosynthetic efficiency of corals. Indeed, the FRR fluorometry predicted which corals might be more susceptible to bleaching under stress conditions even when no signs of stress are visible, but photosynthetic quantum yield was reduced [110].

The use of submersible chambers combined respirometry and fluorometric techniques (see below), and have been used to study the evolution of diurnal hysteresis in coral photosynthesis in situ [111,112]. However, some discrepancy in the findings were found, and were interpreted as being derived from the different scales of measurements, where FRR and PAM fluorometry measured the fluorescence signal at polyp-symbiont scale [105] while oxygen measurements integrate the whole colony [113]. Moreover, differences are in the system design, where FRR and PAM are fluorometry probes in open systems, while benthic chambers encloses the studied organisms in a confined system. This caveat aside, fluorescence instruments (Diving-PAM, FRR and imaging systems) have become popular for studying photosynthetic performance of coral symbionts and monitoring the diel change of coral productivity, favored by accessible costs and portability of these instruments for underwater studies.

4.2 Benthic chambers, diffusive flux methods, and flow analyses

Early in situ studies on coral biology used the strategy of encapsulating single or multiple individuals within a closed benthic systems [114]. These chambers enabled the non-destructive measurement of coral physiological rates through changes in water chemistry to better understand community level responses to perturbation [114,115]. For example, incubation chambers have been used to study in situ coral calcification and photosynthesis rates, where the effect of ocean acidification was shown to lower the calcification rates [116]. Large incubation systems have enabled measurements of benthic community metabolism as well as conducting in situ experiments, providing better insight to ecological responses in reefs than can more focused studies of host-symbiont relationships.

The common metrics to assess the health of corals are the levels of photosynthesis by the endosymbionts, respiration of the holobiont, and ideally the rates of biocalcification (or growth). Benthic chambers are well-suited to measure these essential parameters in situ [117–120]. For example, differences in coral metabolism were measured between shallow (reef flat) and deeper area (reef slope) at Ishigaki Island, Japan, with negative net calcification rates being attributed to nighttime decreases in pH due to benthic respiration [121]. In another study, Sawall et al. [41] used benthic chamber to study the coral nutritional status at elevated anthropogenic nutrient loading. The design of a transparent 3-L urine bag as the "Flexi-Chamber" made it easily usable at low-cost by any underwater scientists, where short incubations detected changes in biological processes with no visual signs of coral stress or endosymbionts concentration [120]. A similar design was used as benthic chambers to show that abiotic conditions including light intensity were drivers of spatial-temporal patterns of reef metabolism [122], although some limitations on the use of these incubation chambers emerged when applied on large boulder colonies. For instance, the amount of light available for photosynthesis, and relative energetic budget, can have different role based on colony shape (micro niches) and surround environment (backscattering light) [123]. However, non-invasive diver-portable in situ incubation chambers (~70 L, 0.2 m² area) have been developed for reproducible measurements of biogeochemical processes in both simple and structurally complex reef communities, allowing the in situ study of various benthic habitats, such as corals, sediments and seagrass meadows [124]. The introduction of benthic and submersible chambers has made it possible to link individual metabolic host-symbionts focuses to community-wide responses, improving the generality and robustness of previous metabolic and physiology studies.

An ambitious program to use benthic chambers as flow respiratory systems to study the in situ response of a coral community was begun in the early 2000's. The "Submersible Habitat for Analyzing Reef Quality" was designed to maintain and measure a turbulent flow of water over benthic substrates for extended periods while measuring temperature, oxygen, salinity, pH and irradiance (photosynthetically active radiation) and to monitor the daily variations in photosynthesis, respiration, and calcification [125]. This system enabled some of the first studies of the direct influence of high CO₂ levels on coral communities in short term experiments [125], although the strength of this approach is the ability to study of the ecology and biogeochemistry of coral communities over multiple days. A similar system was used as automated closed-chamber for application over a variety of substrate types [126].

These flow systems have launched new investigations into the effects of environmental stressors on reef metabolism, ecology, and biogeochemistry. The combination of in situ flow chambers and fluorometry techniques in experimental studies, like that conducted at the underwater laboratory NOAA Aquarius [127], demonstrated the effect of increased water flow and temperature on coral physiology. These applications were used to demonstrate asymmetric patterns of photosynthetic yield across coral colonies [128], where the upper side has a reduced quantum yield [129]. Other experimental studies have been conducted using flow chambers to study coral physiology under chemical enrichment in a benthic chamber experiment [130] adding new findings on coral responses to high $p\text{CO}_2$ and the combined effect of chemical inputs and global warming. A new diver-portable tool has been developed that enables short-term (minutes) experimentation with flow and chemical manipulations, as well as in situ measurements of coral metabolic health and rates of calcification [131]. Although its use has been limited to date, it offers the ability for the assessment of individual corals across and among reef environments.

Although benthic chambers offer the chance to assess the variability of responses within and among reefs, by design these systems alter water flow dynamics around the study corals and restrict water exchange sufficiently to enable changes to accumulate in water chemistry (i.e., the measurement parameters at a minimum). It is unclear at what stage these changes in water flow and bulk chemical conditions may begin influencing coral responses. Unenclosed, diffusive boundary layer (DBL) approaches were developed to avoid these potential issues, whereby sensors placed well above the coral interface make unobstructed measurements of vertical gradients in velocity, temperature, and chemical constituents on the open reef to calculate the flux of momentum, heat, and O_2 in the boundary layer [132].

4.3. Micro- and automated sensors

The use of microsensors at coral tissue levels [123,133–136] and automated sensors at community level [137–139] reveals new insights into coral metabolism by showing the fluctuations in chemical conditions under different natural regime of dissolved oxygen, marine pH and light intensity. For example, the oxygen saturation at coral-water interface fluctuates from supersaturated during daytime due to production by endosymbionts, to hypoxic at night due to respiration by both polyps and symbionts [55,58,140]. However, despite these broad chemical changes, oxygen microsensors used in the microenvironment of coral-turf and coral-corallinae algae showed that low oxygen concentrations were not generally found at the interface of *Porites* spp., turf algae, and crustose coralline algae [134]. More microenvironment studies are needed to better understand the metabolic regulation between corals and the surrounding environment [123]. Technological development of microsensors in a modified diver-operated system [141] allowed the quantification of irradiance at the coral surface and the measurement of the efficiency of photosynthetic system at polyp and coenosarc microscale [142], confirming the ability of corals to adapt to environmental changes, such as temperature or irradiance [143]. On a broader scale, micro- and automated sensors increasingly have been used in both closed (benthic chamber) and open (reef-scale) approaches to studying coral and reef ecologies.

4.4. Reef scale experiments

The study of corals in open or unconfined natural systems using DBL techniques was made possible by the advent of sensors for measuring both water flow and relevant aspects of water chemistry. A common approach is to use eddy correlation techniques [144] where non-invasive acoustic doppler velocimeters measuring water flow are coupled with O_2 sensors to provide three-dimensional fields of oxygen distribution over benthic environments [145]. This application can examine in situ dynamics of O_2 production and consumption (i.e., respiration) in different habitats, including highly productive reef crests relative to reef slopes [145] as well as cold-water (deep) coral reefs [146,147]. These ecosystem-scale measurements show how high oxygen fluxes are possible, even in nutrient replete environments such as reef slopes and deep waters. The advantage of such techniques has been discussed by Takeshita et al. [148] who introduced a new autonomous system (the benthic ecosystem and acidification measurement system) for simultaneous measurement of NCP and NCC from a coral reef through the autonomous use of O_2 and pH sensing technology. Moreover, direct observation of the effect

of increased $p\text{CO}_2$ / lowered pH environments on corals showed temporal and spatial metabolic response of reef diversity and water chemistry to high CO_2 / low Ω_a , at temporal and spatial patterns [137].

Comparison between the enclosed and open system approaches showed good general agreement with respect to discrete measurements of oxygen from the gradient flux (GF) method at the DBL and a multiparametric probe in a benthic chamber [125], although other findings indicate that GF method can offer more accurate measurements of these fluxes [132]. The low physical disruption with DBL methods means both less direct impacts on corals and more spatially integrative observation and interpretation of coral reef responses to changes in the natural environment at colony or community levels. Their smaller infrastructure requirements also make them an attractive strategy for investigations. Even so, these approaches can be complementary. For example, DBL studies show that water flow can be a factor affecting the light-limited photosynthesis in coral colonies [60], and more spatially focused studies have used benthic chambers to show that increased water flow leads to increased calcification rates, carbonate deposition, protein concentration and endosymbionts density [149].

Experiments at reef scale represent a key step forward in the in situ studies of coral responses in the natural environment [44,150–152], but they also have provided the opportunity to quantify reef-scale responses to environmental perturbations. For example, manipulations to lower Ω_a by ~20% led to a 34% decrease in NCC consistent with the effect of lower pH on biocalcification [153], although this finding may have been influenced by a high abundance of crustose coralline algae living in the reef community [152]. Another free ocean CO_2 enrichment (FOCE) system was used to incubate coral reef communities at ambient $p\text{CO}_2$ (393 μatm) and high $p\text{CO}_2$ (949 μatm), and a decrease in daily NCC by 49% under high $p\text{CO}_2$ was observed over a 21 days experiment, corresponding to 26% reduction in NCC per unit of Ω_a [150]. These results were in line with previous studies on ecosystem-level responses of coral reef communities to ocean acidification level projected in the next century. Indeed, a modification of such an experimental system, the coral-*proto* free ocean carbon enrichment system, allowed the short-term in situ study of the induced ocean acidification on coral reef organisms and diel changes of the seawater carbonate system [151]. Srednick et al. [44] introduced another novel FOCE for spatial and temporal studies on shallow reefs, with the aim of studying a coral reef community in situ under controlled conditions of current and projected levels of $p\text{CO}_2$. Such a system allowed a direct estimation of the hysteresis of seawater carbonate chemistry along a reef transect, using high accuracy and precision measurements of seawater pH, $p\text{CO}_2$ and the biological responses of the reef community over high temporal resolution [44]. Other high frequency sampling of coral reef carbonate dynamics and metabolic rates have used automated systems for measurements of alkalinity, pH and $p\text{CO}_2$ at high frequency sampling [45,138]. Through automated measurements with the slack water and flow respirometry approaches, it is now possible to characterize the net calcification and productivity of a reef system (either reef flats or crests), as well as reveal long-term changes driven by global changes (ocean acidification and global warming) or hysteresis under local changes [138,154]. However, dissimilarities in the methods used in community metabolism studies [155], create potential uncertainties when applied to coral reefs, in terms of community calcification and seawater carbonate conditions. Limitations of metabolic studies at community level are related to lack of suitable controls, spatial heterogeneities and thus replicability among sites, as well as confounding factors in open systems, such as the introduction of organisms and differing oceanographic settings in the surrounding reef environments.

5. Discussion and conclusions

Underwater methodologies from 52 studies on coral metabolism and physiology conducted since 1990 have been collated (Table 1). The current knowledge of combined effects of local and global stressors comes from a wide breadth of manipulation studies on coral responses under predicted future scenarios but limited in representing complex abiotic factors in ecosystems. The interaction among environmental variables, such as temporal changes in inorganic carbon chemistry, physical parameters, or nutrient loads, is an important factor affecting the biogeochemistry of coral health [150] and can play a key role in population dynamics. Though invaluable as a study tool, manipulation studies are limited in the ability to mimic important spatiotemporal patterns and interactions. On the other hand, comprehensive long-term in situ metabolic measurements still are

lacking but nevertheless necessary to understand the energetics and trophodynamic of reef ecosystems. The focus of instrumentation development for in situ studies of coral metabolism has shifted over the last three decades from the host-symbiosis relationship using fluorometric techniques to measure the photosynthetic efficiency of endosymbionts to a more comprehensive investigation of individuals and communities (Table 2). Indeed, through the use of benthic chambers it is possible to run experimental manipulations of carbon fluxes or nutrients and monitor long-term ecosystem responses via automated sensors. With this shift, the biogeochemical processes involved in corals and coral reefs have received advanced understanding of ecosystems functioning with the possibility to predict benthic conditions using modelling of future scenarios [124].

Table 2. Time ranking of published peer-reviewed studies on coral in situ metabolism according to this study.

Year	Methodology	Objective	Sampling scale
1991 - 2005	fluorometry, benthic and submersible chamber	productivity, diel change	Polyp, colony
2006 - 2010	fluorometry, benthic chamber, automated sensors	diel change, productivity, physiology under stress	Colony, community
2011 - 2015	Benthic and submersible chamber, diffusive boundary layer	physiology under stress, productivity	Colony, polyp, community
2016 - 2020	benthic/submersible chamber, automated sensors	seasonal change, productivity, physiology under stress	Colony, community

Among the objectives of coral physiology studies, investigating the diel or seasonal changes in primary productivity comprised 57% of the published works, highlighting that quantification of natural variations in energetic fluxes are a central concern for the assessment of coral conditions (Fig. 2). Moreover, coral productivity was the only objective of study being covered by all methodologies at different sampling scales, from polyp to community. Conversely, 26% of studies addressed coral physiology under stress, including bleaching responses, environmental changes and bathymetric change conditions (transplantation). The assessment of recovery of health status was investigated by 10% of the studies. The remaining papers centered on issues of zonation (4%) and photo-inhibition (3%).

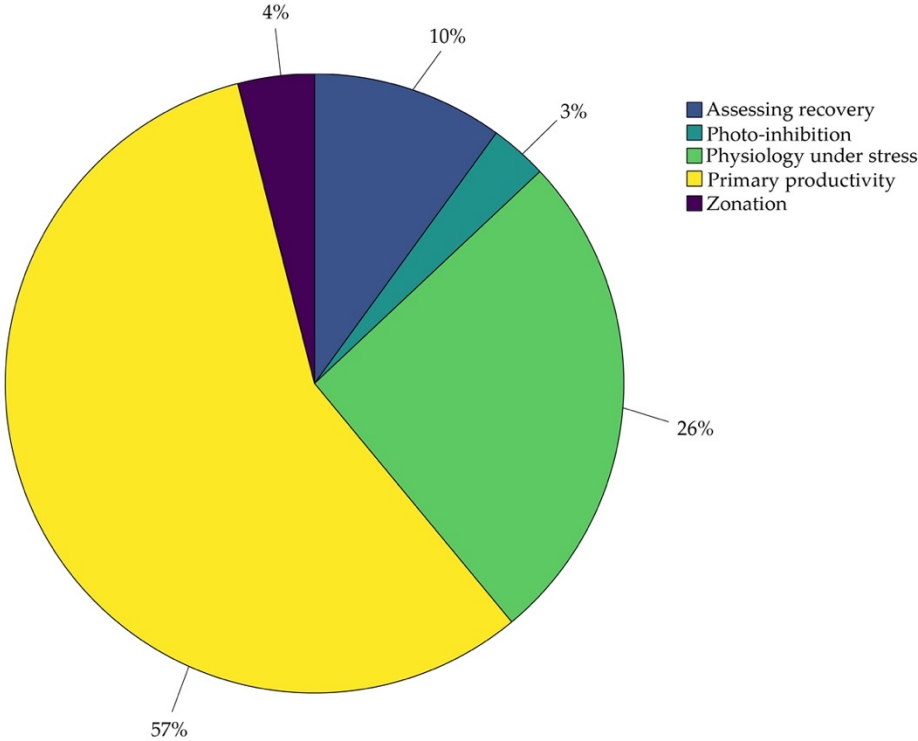


Figure 2. Study objectives of in situ methodologies for the measurement of coral physiology

Laboratory-based studies have provided a strong foundation for understanding coral metabolism and the responses to stress, and they will continue to serve as a primary means of research under controlled conditions. However, the expanding role of in situ-based studies of coral systems is essential for extrapolating and modulating these laboratory-based findings to the temporal and spatial complexity of natural reef and environmental conditions. The advantages of in situ experimental techniques described relate to the ability for measuring metabolic and biogeochemical properties of different benthic habitats having both simple and complex structures. The replicability of experimental studies, for example using same methodology or sensors, allows the simultaneous monitoring of coral health in different locations with the ability to compare the ecosystem functions, e.g. standardization of the methods. However, there are limitations to in situ methodologies. In the case of benthic chambers, there are restrictions to which substrate surfaces are suitable for study, and they are not well suited, even with enhanced flow capabilities, for longer-term monitoring of coral health. In the case of DBL approaches, limitations include the needed maintenance and fragility of microsensors, which can limit deployments to shorter duration, or under more quiescent weather conditions. Co-deployment of multiple instrumentation approaches will be needed to ensure both accuracy and logistic practicality in monitoring fluxes of O₂ and pH in coral reefs. Even with these accounted for, most studies on coral metabolic rates rely on observation of changes in DIC and alkalinity in a specific (small) area to calculate the carbon fluxes for productivity and calcification estimates [156]; the precise movement of seawater flow and aspects of reef heterogeneity cannot be fully taken into account, leading to potentially biased findings that may not adequately reflect natural conditions. In reality then, a combination of in situ techniques likely will be most useful, such as the application of flow respirometry approaches to estimate carbon fluxes over a small spatial scales and different substrate type within a reef, coupled with DBL methods that provide an integrative assessment of the temporal variability in coral productivity and growth [138].

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