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Experimental Techniques to Assess Coral Physiology In Situ Current Approaches and Novel Insights

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1 Experimental techniques to assess coral physiology in situ: 2 current approaches and novel insights

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14

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

32 **Keywords:** environmental monitoring; underwater respirometry, fluorometry, coral metabolism
33

34 1. Introduction

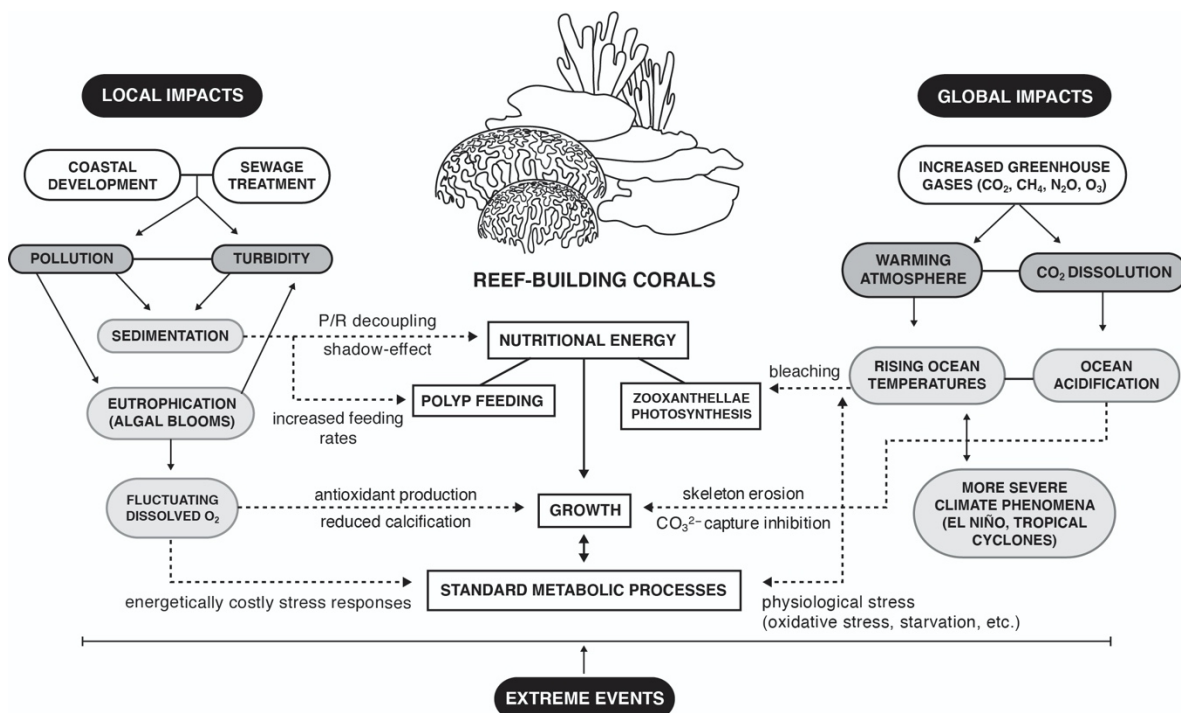
35 Coral reef ecosystems are hotspots of biodiversity and productivity in the ocean [1] that exceed that of
36 tropical rainforests [2]. They provide crucial ecosystem functions and services such as providing goods for
37 subsistence and economic fisheries, coastline protection from storms, and centers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

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

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

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

62 Although climate drivers are widely recognized as dominating factors in coral loss and reef ecosystem
 63 shifts [18], localized stressors also are impacting coral health, where a survival-resilience pattern is observable
 64 in urban subtropical reefs subjected to several anthropogenic stressors [26]. Here, there are indications that
 65 stress-tolerant hard coral species have been selected to foster more resistant though less diverse reefs [27].
 66 Impacts from eutrophication, increased turbidity, and lowered dissolved oxygen significantly affect coral
 67 metabolism, changing energy pathways and reef ecology (Figure 1). A coral as a holobiont, including all its
 68 functional, genomic and epigenetic associated symbionts, is capable of ecological plasticity in order to survive
 69 environmental changes, but not all coral species showed the same response patterns under anthropogenic
 70 threats.
 71



72
 73 **Figure 1.** Local and global impacts affecting the nutritional energy of reef-building corals. Full lines indicate
 74 direct interactions, dotted lines indicate indirect interactions.
 75

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

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

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

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

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

125 2. Overview of the coral metabolism

126 2.1. Metabolic responses at coral polyp level

127 The concept of the coral as a “holobiont” was introduced in the 2000s [47,48] which comprises the coral
128 polyps and the associated symbiotic organisms, including photosynthetic endosymbionts, bacteria, viruses,
129 fungi and protists. These have a fundamental role in nutrient and energy acquisition processes of coral polyps
130 [49], genome evolution in the coral host and microbial partners, maintaining holobiont homeostasis, and the
131 overall health of the holobiont [50]. The functional diversity of these microbes contributes to the stress response
132 and acclimation to abnormal conditions, e.g. high seawater temperature and bleaching events [51,52].

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

142 This continuous loop is stable in normal conditions, but the symbiotic dynamics within the coral
143 holobiont is changing rapidly in the Anthropocene [63]. Temperature-induced stress damages a key protein
144 (D1) in the photosystem II within the dinoflagellate (PSII; [64]), inactivating the Rubisco center [65], affecting
145 the production of ATP [66], and leading to the overproduction of reactive oxygen species, inducing damaging
146 conditions for both the endosymbiont and coral host. The increased frequency and severity of temperature
147 anomalies will lead to negative consequences for coral survival [20,67]. Under warming and eutrophic
148 conditions, this symbiosis may shift from a mutualistic to parasitic strategy for nutritional resources allocation
149 [68], and the positive feedback loop for nutrient acquisition can be disrupted when conditions are not favorable
150 to support the benefits of the O₂ / CO₂ fluxes [69,70].

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

162 2.2. Metabolic responses of the coral community

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

172 in pH in the future ocean eventually will affect the stability of existing reef ecosystems, because the CaCO₃
173 formed by corals (aragonite) is more susceptible to dissolution than that formed by other bio-calcifying
174 organisms [76]. These effects overlay natural seasonal variations in the carbonate chemistry in reef systems, and
175 the question remains of what extent does globally and locally derived ocean acidification have on the seasonal
176 balance of NCP and NCC [77]. However, these estimates remain imprecise, owing to the lack of information
177 about seawater residence time and volume, which would modulate reef chemistry [78]. The relationship
178 between corals and their symbiotic dinoflagellates is exacerbated under the combined effects of ocean
179 acidification and warming, where warming can decrease photosynthetic production and increase respiration,
180 leading to lower energy reserves for growth [6,20,79,80].

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

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

197 3. Methods

198 We conducted a literature review based on case studies of in situ observation of coral metabolism from
199 published peer-reviewed scientific literature. We included cases involved in original research on direct (e.g.
200 using SCUBA divers, benthic chambers, optical sensors) underwater measurements of metabolic rates on both
201 coral individuals and coral communities. We excluded sample collections (e.g. coral fragmentation), laboratory
202 experiments, and indirect measurements of metabolic fluxes (e.g. ex situ from sample incubations). The
203 literature screening was categorized in eight separate categories based on the methodology used (Table 1),
204 including: 1) SCUBA FRR fluorometry; 2) SCUBA PAM fluorometry; 3) Clark-type O₂ sensors; 4) boundary
205 layer; 5) SCUBA imaging; 6) benthic chambers; 7) submersible chambers; and 8) automated sensors. The
206 database was further classified based on the system type (open, semi-closed, enclosed); measured parameters
207 (O₂, Fv/Fm, ETR, CA); sampling frequency (minutes, hours, days); sampling scale (symbionts, polyp, colony,
208 community); and aim of the study (monitoring, experiment).

209 We used the advanced search on Google Scholar to identify studies with the keywords “coral in situ
210 metabolism” and “underwater”, excluding the keyword “collection”, in articles published between 1990 and
211 2020. The search yield (n = 2090) was scrutinized and the literature was manually reviewed to fulfil our selection
212 criteria described above. A final list of 52 studies were included for the analysis in this review.

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

218 4. Methodologies for coral in situ metabolism

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

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

237
 238

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

239 SCUBA FRR: SCUBA-based fast respiration rate fluorometer; SCUBA PAM fluorometry: SCUBA-based
 240 pulse amplitude modulation; O₂: Dissolved oxygen; CA: Calcification; ETR: Electron transport rate; Fv / Fm:
 241 maximum quantum yield of photosystem II.
 242

243 4. 1. Underwater fluorometry

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

250 A fundamental measure of coral status is quantification of the light harvesting pigments of the
251 endosymbionts over time, whereby decreasing trends can provide early warning of the onset of bleaching
252 events. The underwater fluorescence imaging has been adapted to conduct large-scale in situ assessments of
253 coral reefs, through the detection of green fluorescent proteins in corals [85,86]. Because the concentration of
254 these pigments and fluorescent proteins are directly involved in photoprotection and photo-acclimation,
255 quantifying the concentrations present in a coral population or community can provide crucial information on
256 bleaching susceptibility or resistance [87–89]. In addition, this imaging technique can also make early signs of
257 disease and coral juveniles easier to detect for accurate enumeration [90], providing a facet of coral reef health
258 [86].

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

268 The estimation of the potential quantum yield of PSII as a metric for photosynthetic rates was first applied
269 to underwater organisms by Beer et al. [93]. Pulse amplitude modulated (PAM) fluorometer uses light pulses
270 of relatively long duration (300 - 1200 ms, 3000-10000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) to modify the fluorescence yields,
271 generating multiple turnover for the reduction of PSII, allowing non-invasive chlorophyll fluorescence analysis,
272 quantum yield and electron transport rate of coral endosymbionts. The introduction of an underwater version
273 of this instrument (Diving-PAM, Walz) facilitated the direct observation of changes in coral health in situ
274 conditions [94]. The introduction of in situ PAM fluorometry enabled the construction of photosynthesis-
275 irradiance models for corals based on varying sun exposures in reefs [93,94]. For example, observations of
276 decreased quantum yield during the day reflected photoinhibition through loss of photosynthetic capacity; a
277 response seen in shallow but not deeper water corals [95]. Similar reduced photosynthetic efficiency has been
278 observed in corals exposed to seasonal turbid conditions [96]. The use of Diving-PAM continuous monitoring
279 during bleaching events also showed that combined thermal and light stress generated greater reductions in
280 photosynthetic efficiency, and was a sensitive indicator of metabolic stress in impacted areas [97]. Even so,
281 variable responses in the coral community to this stress suggested coral species-specific physiological responses
282 [98–101]. Further development on PAM technology is the high-resolution imaging fluorometry used for analysis
283 of heterogeneity within colonies, from coenosarc tissue to polyp tentacles, but so far this methodology is not
284 available for underwater studies [102].

285 A second approach for measuring variable fluorescence, the FRR fluorometer uses a different approach to
286 saturate the photochemistry during measurement, whereby shorter (150 – 400 μs , $>20000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$)
287 but more intense light is used to generate a single turnover and reduction of the primary electron acceptor
288 [103,104]. The methodology is more sensitive than PAM, which can be beneficial when endosymbiont
289 abundance (or chlorophyll concentrations) are very low in corals. The SCUBA-based FRR fluorometry was
290 developed by Gorbunov et al. [105] for measuring chlorophyll fluorescence from PSII reaction centers in corals,
291 sea grasses, macroalgae and algal turfs. FRR fluorometry has been also used to monitor coral physiology during
292 bleaching events, and their later recovery [106,107]. Although nutrient availability (as nitrogen or iron) and
293 sunlight irradiance were already recognized as the main factors directly affecting photosynthesis in the aquatic
294 ecosystem [108,109], the use of FRR fluorometry enabled more direct measurements to the effects of nutrient
295 limitation on photosynthetic efficiency of corals. Indeed, the FRR fluorometry predicted which corals might be
296 more susceptible to bleaching under stress conditions even when no signs of stress are visible, but
297 photosynthetic quantum yield was reduced [110].

298 The use of submersible chambers combined respirometry and fluorometric techniques (see below), and
299 have been used to study the evolution of diurnal hysteresis in coral photosynthesis in situ [111,112]. However,
300 some discrepancy in the findings were found, and were interpreted as being derived from the different scales
301 of measurements, where FRR and PAM fluorometry measured the fluorescence signal at polyp-symbiont scale
302 [105] while oxygen measurements integrate the whole colony [113]. Moreover, differences are in the system
303 design, where FRR and PAM are fluorometry probes in open systems, while benthic chambers encloses the
304 studied organisms in a confined system. This caveat aside, fluorescence instruments (Diving-PAM, FRR and
305 imaging systems) have become popular for studying photosynthetic performance of coral symbionts and
306 monitoring the diel change of coral productivity, favored by accessible costs and portability of these instruments
307 for underwater studies.

308

309 **4.2 Benthic chambers, diffusive flux methods, and flow analyses**

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

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

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

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

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

366

367 4.3. Micro- and automated sensors

368 The use of microsensors at coral tissue levels [123,133–136] and automated sensors at community level
369 [137–139] reveals new insights into coral metabolism by showing the fluctuations in chemical conditions under
370 different natural regime of dissolved oxygen, marine pH and light intensity. For example, the oxygen saturation
371 at coral-water interface fluctuates from supersaturated during daytime due to production by endosymbionts,
372 to hypoxic at night due to respiration by both polyps and symbionts [55,58,140]. However, despite these broad
373 chemical changes, oxygen microsensors used in the microenvironment of coral-turf and coral-corallinae algae
374 showed that low oxygen concentrations were not generally found at the interface of *Porites* spp., turf algae, and
375 crustose coralline algae [134]. More microenvironment studies are needed to better understand the metabolic
376 regulation between corals and the surrounding environment [123]. Technological development of microsensors
377 in a modified diver-operated system [141] allowed the quantification of irradiance at the coral surface and the
378 measurement of the efficiency of photosynthetic system at polyp and coenosarc microscale [142], confirming
379 the ability of corals to adapt to environmental changes, such as temperature or irradiance [143]. On a broader
380 scale, micro- and automated sensors increasingly have been used in both closed (benthic chamber) and open
381 (reef-scale) approaches to studying coral and reef ecologies.

382

383 4.4. Reef scale experiments

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

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

397 Comparison between the enclosed and open system approaches showed good general agreement with
398 respect to discrete measurements of oxygen from the gradient flux (GF) method at the DBL and a
399 multiparametric probe in a benthic chamber [125], although other findings indicate that GF method can offer
400 more accurate measurements of these fluxes [132]. The low physical disruption with DBL methods means both
401 less direct impacts on corals and more spatially integrative observation and interpretation of coral reef responses
402 to changes in the natural environment at colony or community levels. Their smaller infrastructure requirements
403 also make them an attractive strategy for investigations. Even so, these approaches can be complementary. For
404 example, DBL studies show that water flow can be a factor affecting the light-limited photosynthesis in coral
405 colonies [60], and more spatially focused studies have used benthic chambers to show that increased water flow
406 leads to increased calcification rates, carbonate deposition, protein concentration and endosymbionts density
407 [149].

408 Experiments at reef scale represent a key step forward in the in situ studies of coral responses in the natural
409 environment [44,150–152], but they also have provided the opportunity to quantify reef-scale responses to
410 environmental perturbations. For example, manipulations to lower Ω_a by ~20% led to a 34% decrease in NCC
411 consistent with the effect of lower pH on biocalcification [153], although this finding may have been influenced
412 by a high abundance of crustose coralline algae living in the reef community [152]. Another free ocean CO_2
413 enrichment (FOCE) system was used to incubate coral reef communities at ambient $p\text{CO}_2$ (393 μatm) and high
414 $p\text{CO}_2$ (949 μatm), and a decrease in daily NCC by 49% under high $p\text{CO}_2$ was observed over a 21 days experiment,
415 corresponding to 26% reduction in NCC per unit of Ω_a [150]. These results were in line with previous studies
416 on ecosystem-level responses of coral reef communities to ocean acidification level projected in the next century.
417 Indeed, a modification of such an experimental system, the coral-*proto* free ocean carbon enrichment system,
418 allowed the short-term in situ study of the induced ocean acidification on coral reef organisms and diel changes
419 of the seawater carbonate system [151]. Srednick et al. [44] introduced another novel FOCE for spatial and
420 temporal studies on shallow reefs, with the aim of studying a coral reef community in situ under controlled
421 conditions of current and projected levels of $p\text{CO}_2$. Such a system allowed a direct estimation of the hysteresis
422 of seawater carbonate chemistry along a reef transect, using high accuracy and precision measurements of
423 seawater pH, $p\text{CO}_2$ and the biological responses of the reef community over high temporal resolution [44]. Other
424 high frequency sampling of coral reef carbonate dynamics and metabolic rates have used automated systems
425 for measurements of alkalinity, pH and $p\text{CO}_2$ at high frequency sampling [45,138]. Through automated
426 measurements with the slack water and flow respirometry approaches, it is now possible to characterize the net
427 calcification and productivity of a reef system (either reef flats or crests), as well as reveal long-term changes
428 driven by global changes (ocean acidification and global warming) or hysteresis under local changes [138,154].
429 However, dissimilarities in the methods used in community metabolism studies [155], create potential
430 uncertainties when applied to coral reefs, in terms of community calcification and seawater carbonate
431 conditions. Limitations of metabolic studies at community level are related to lack of suitable controls, spatial
432 heterogeneities and thus replicability among sites, as well as confounding factors in open systems, such as the
433 introduction of organisms and differing oceanographic settings in the surrounding reef environments.

434 5. Discussion and conclusions

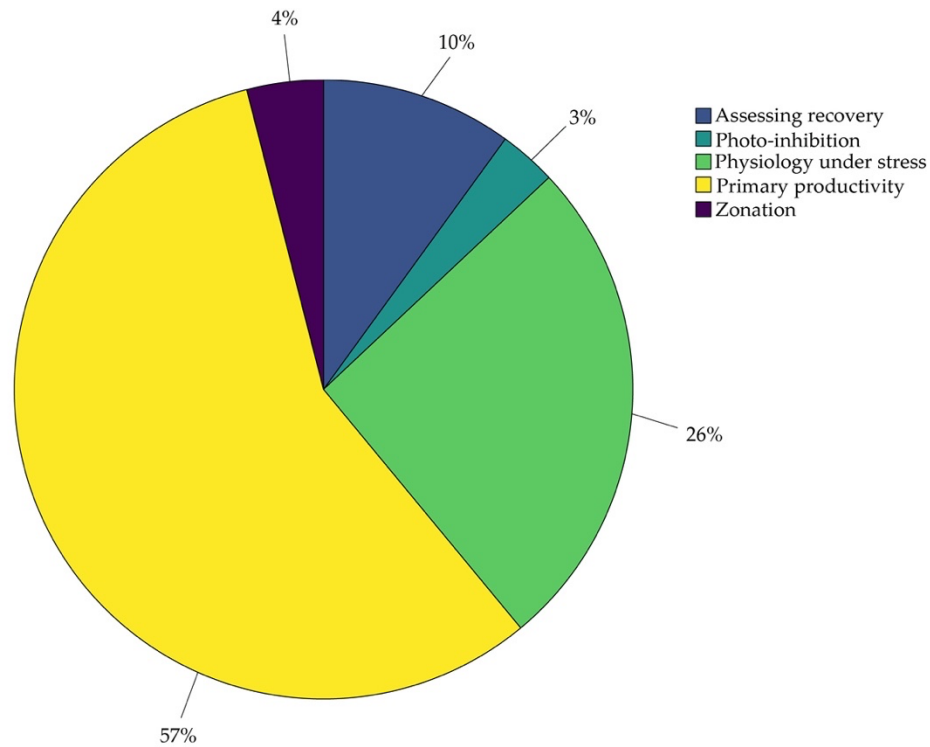
435 Underwater methodologies from 52 studies on coral metabolism and physiology conducted since 1990
436 have been collated (Table 1). The current knowledge of combined effects of local and global stressors comes
437 from a wide breadth of manipulation studies on coral responses under predicted future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

443 lacking but nevertheless necessary to understand the energetics and trophodynamic of reef ecosystems. The
 444 focus of instrumentation development for in situ studies of coral metabolism has shifted over the last three
 445 decades from the host-symbiosis relationship using fluorometric techniques to measure the photosynthetic
 446 efficiency of endosymbionts to a more comprehensive investigation of individuals and communities (Table 2).
 447 Indeed, through the use of benthic chambers it is possible to run experimental manipulations of carbon fluxes
 448 or nutrients and monitor long-term ecosystem responses via automated sensors. With this shift, the
 449 biogeochemical processes involved in corals and coral reefs have received advanced understanding of
 450 ecosystems functioning with the possibility to predict benthic conditions using modelling of future scenarios
 451 [124].

452 **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

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



461
462 **Figure 2.** Study objectives of in situ methodologies for the measurement of coral physiology

463
464 Laboratory-based studies have provided a strong foundation for understanding coral metabolism and the
465 responses to stress, and they will continue to serve as a primary means of research under controlled conditions.
466 However, the expanding role of in situ-based studies of coral systems is essential for extrapolating and
467 modulating these laboratory-based findings to the temporal and spatial complexity of natural reef and
468 environmental conditions. The advantages of in situ experimental techniques described relate to the ability for
469 measuring metabolic and biogeochemical properties of different benthic habitats having both simple and
470 complex structures. The replicability of experimental studies, for example using same methodology or sensors,
471 allows the simultaneous monitoring of coral health in different locations with the ability to compare the
472 ecosystem functions, e.g. standardization of the methods. However, there are limitations to in situ
473 methodologies. In the case of benthic chambers, there are restrictions to which substrate surfaces are suitable
474 for study, and they are not well suited, even with enhanced flow capabilities, for longer-term monitoring of
475 coral health. In the case of DBL approaches, limitations include the needed maintenance and fragility of
476 microsensors, which can limit deployments to shorter duration, or under more quiescent weather conditions.
477 Co-deployment of multiple instrumentation approaches will be needed to ensure both accuracy and logistic
478 practicality in monitoring fluxes of O₂ and pH in coral reefs. Even with these accounted for, most studies on
479 coral metabolic rates rely on observation of changes in DIC and alkalinity in a specific (small) area to calculate
480 the carbon fluxes for productivity and calcification estimates [156]; the precise movement of seawater flow and
481 aspects of reef heterogeneity cannot be fully taken into account, leading to potentially biased findings that may
482 not adequately reflect natural conditions. In reality then, a combination of in situ techniques likely will be most
483 useful, such as the application of flow respirometry approaches to estimate carbon fluxes over a small spatial
484 scales and different substrate type within a reef, coupled with DBL methods that provide an integrative
485 assessment of the temporal variability in coral productivity and growth [138].

486

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489 review and editing, W.D., J.C., C.C., J.W., M.W., L.C.; visualization, W.D. and C.C.; supervision, L.C.; project administration,
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499

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