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

**Walter Dellisanti [1, 2], Jeffery T. H. Chung [1], Cher F. Y. Chow [1, 4], Jiajun Wu [1], Mark L. Wells [5, 6], Leo L. Chan [1, 2, 3] ***

[1] State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong S.A.R., China;

[2] Department of Biomedical Sciences, City University of Hong Kong, Hong Kong S.A.R., China;

[3] Hong Kong Branch of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Hong Kong, China;

[4] Centre for Biological Diversity, Scottish Oceans Institute, School of Biology, University of St Andrews, Fife, UK;

[5] School of Marine Sciences, University of Maine, Orono, USA;

[6] State Key Laboratory of Satellite Ocean Environment Dynamics, Second Institute of Oceanography, Ministry of Natural Resources, 36 Baochubei Road, Hangzhou, China 310012

**\*** Correspondence: leochan@cityu.edu.hk; Tel.: +852 34424125

**Abstract:** Coral reefs are declining worldwide due to global changes in the marine environment. The increasing frequency of massive bleaching events in the tropics is 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. 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 experimental techniques that measure core processes in coral metabolism (respiration, photosynthesis, and biocalcification) in situ, and their role in indicating the health status of colonies and communities. 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; photobiology; 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 ($CO_2$) 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.

45    Increased energy consumption since the Industrial Revolution has led to the highest $CO_2$ levels recorded
46  in the atmosphere since human evolution (> 410 ppm) [12]. Of the multiple greenhouse gases (such as $N_2O$, $CH_4$
47  or CFCs) affecting global biogeochemical cycles, biodiversity, and human health [13,14], $CO_2$ has been the most
48  relevant to marine ecology because of its dual role in marine heatwaves [15] and ocean acidification [16] (Figure
49  1). Overall average seawater temperatures in tropical regions have increased by almost 1°C over the past 100
50  years and are projected to continue increasing at 1º to 2°C per century [17]. Increased seawater temperatures are
51  a major contributor to coral bleaching and are considered as the limiting factor for coral survival [7,18]. Roughly
52  half of the $CO_2$ emitted into the atmosphere dissolves into the surface ocean, reacting with water to form several
53  dissolved inorganic components of the carbonate system [19] and lowering seawater pH [20]. In comparison
54  with pre-Industrial Revolution levels, seawater pH has decreased by approximately 0.1 [21], which equates to
55  roughly 30% increase in acidity and may decrease further by 0.06 to 0.32 based on emission scenarios [16]. This
56  process of ocean acidification is particularly disruptive to marine organisms like reef-building hard corals that
57  create calcium carbonate skeletons, increasing the energy requirements for growth and survival [20,22,23]. Thus,
58  corals and coral reefs may be significantly more vulnerable than previously thought when ocean acidification
59  and warming combine [24,25].
60    Although climate drivers are widely recognized as dominating factors in coral loss and reef ecosystem
61  shifts [18], localized stressors also are impacting coral health, where a survival-resilience pattern is observable
62  in urban subtropical reefs subjected to several anthropogenic stressors [26]. Here, there are indications that
63  stress-tolerant hard coral species have been selected to foster more resistant though less diverse reefs [27].
64  Impacts from eutrophication, increased turbidity, and lowered dissolved oxygen significantly affect coral
65  metabolism, changing energy pathways and reef ecology (Figure 1). A coral as a holobiont, including all its
66  functional, genomic and epigenetic associated symbionts, is capable of ecological plasticity in order to survive
67  environmental changes, but not all coral species showed the same response patterns under anthropogenic
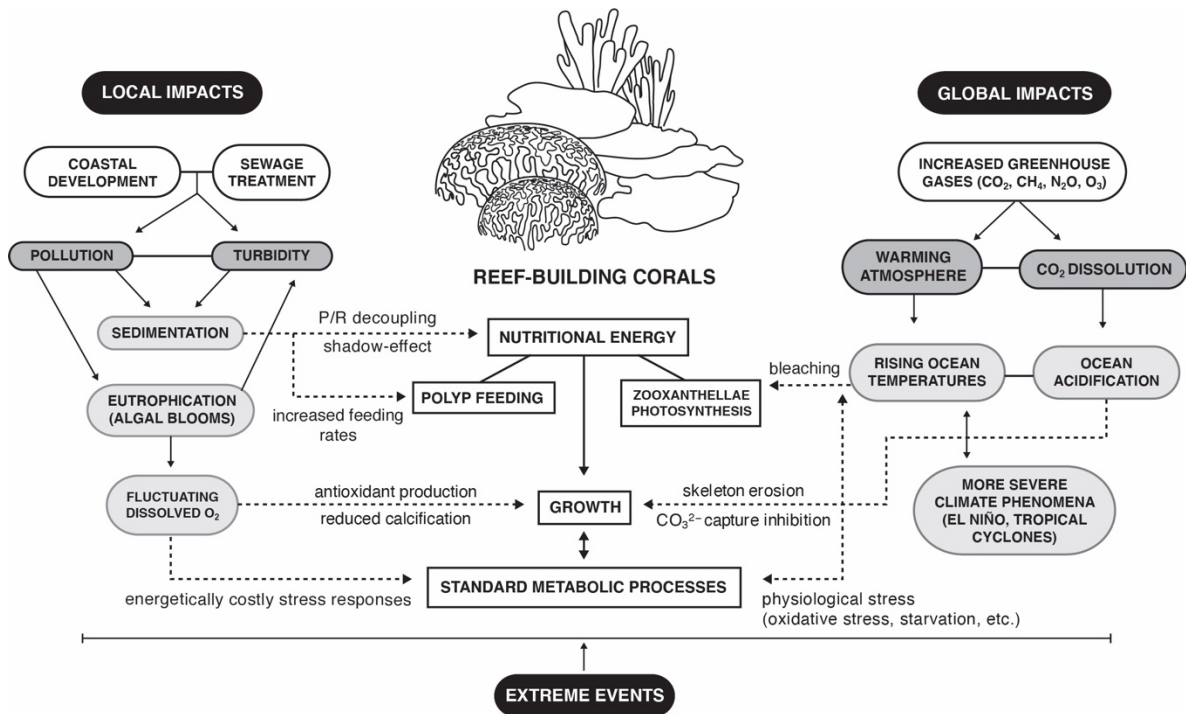68  threats.
69



70
71  **Figure 1.** Local and global impacts affecting the nutritional energy of reef-building corals. Full lines indicate
72  direct interactions, dotted lines indicate indirect interactions.
73
74    Turbid water conditions commonly occur within inshore shallow coastal waters [28], owing to the
75  collective interactions of river runoff and the natural re-suspension of sediments (e.g. tides or storms) as well as

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

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

97    Eutrophication has degraded many coral reefs in coastal regions, and high-nutrient waters exacerbate the
98    effect of global warming on coral survival [40]. The nutrient gradient affects the nutritional status of corals, in
99    particular when the calcification rate is reduced but heterotrophy enhanced under high nutrient loading [41].
100   Indeed, traces of nitrate pollution can be found in both hard and soft corals through the analysis of radioisotopes
101   $\delta^{15}N$ in the tissue [42,43] and $\delta^{13}C$ as an estimate of particulate organic matter ingestion [44,45]. At the
102   community level, a shift from net community calcification to dissolution can occur under high nutrient
103   conditions [46], due to combination of direct (physiological status) and indirect (biogeochemical status)
104   responses of corals [46,47]. Eutrophic conditions can increase the productivity of reef waters by increasing food
105   availability (e.g., particulate matter) [30], although macroalgae, turf and bioeroders can inhibit competitively
106   the coral recruitment [47]. However, several factors influencing the susceptibility to eutrophication have to be
107   included, such as hydrodynamic, connectivity and location [48].

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

114   While most early studies of environmental change on coral health focused on the effects of single drivers
115   (e.g., temperature, ocean acidification, turbidity, etc.), their interactive effects require addressing multiple
116   stressor effects on physiological processes at the holobiont level if we are to comprehensively understand their
117   impacts on coral communities. Some of these effects are major (e.g., temperature and light), but there
118   undoubtably are other interactions subtle enough to challenge recognition but significant enough that they can
119   affect the resistance and recovery response of coral communities, factors critical for reef management in
120   proactive preventative planning. But our current understanding of coral stress responses is largely based on
121   experimental manipulation studies in laboratory systems that are poor replicants of their natural habitats. In
122   situ studies, by either SCUBA or automated sensors, could provide a better understanding of local and global
123   impacts, but few studies have undertaken the logistical complexities of studying fine-scale physiological
124   processes of corals in situ recently [49–51]. The primary objective of this review is to summarize the current
125   strategies used to measure the metabolism of corals to highlight the benefits of non-destructive methodologies.

126 A list of recommendations is provided that would expand the efficacy of underwater studies for improving
127 local knowledge and better understanding of how corals respond to stressors.

128 **2. Overview of the coral metabolism**

129 **2. 1. Metabolic responses at coral polyp level**

130 The concept of the coral as a "holobiont" was introduced in the 2000s [52,53] which comprises the coral
131 polyps and the associated symbiotic organisms, including photosynthetic endosymbionts, bacteria, viruses,
132 fungi and protists. These have a fundamental role in nutrient and energy acquisition processes of coral polyps
133 [54], genome evolution in the coral host and microbial partners, maintaining holobiont homeostasis, and the
134 overall health of the holobiont [55]. The functional diversity of these microbes contributes to the stress response
135 and acclimation to abnormal conditions, e.g. high seawater temperature and bleaching events [56,57].
136 The family Symbiodiniaceae includes several genera of dinoflagellates which reside in the tissue of
137 corals and other marine organisms [58]. The coral-Symbiodiniaceae association is an obligate symbiosis for the
138 coral, where the photosynthetic dinoflagellates provide the coral oxygen and energy in the form of glucose for
139 aerobic respiration [59,60]. While most of the oxygen arising from photosynthesis is immediately utilized in
140 coral respiration [61–63], the excess oxygen is released to the surrounding seawater throughout the day [64–67],
141 supporting the oxygen availability on the reef. Therefore, at night, corals must acquire oxygen from the
142 surrounding environment to fuel the respiration process [64]. As a consequence, the energy acquisition follows
143 a circadian pattern where endosymbiont-guided photosynthesis is dominant during the day while polyp-
144 guided respiration is dominant at night.
145 This continuous loop is stable in normal conditions, but the symbiotic dynamics within the coral holobiont
146 is changing rapidly in the Anthropocene [68]. Temperature-induced stress damages a key protein (D1) in the
147 photosystem II within the dinoflagellate (PSII; [69]), inactivating the Rubisco center [70], affecting the
148 production of ATP [71], and leading to the overproduction of reactive oxygen species, inducing damaging
149 conditions for both the endosymbiont and coral host. The increased frequency and severity of temperature
150 anomalies will lead to negative consequences for coral survival [20,72]. Under warming and eutrophic
151 conditions, this symbiosis may shift from a mutualistic to parasitic strategy for nutritional resources allocation
152 [73], and the positive feedback loop for nutrient acquisition can be disrupted when conditions are not favorable
153 to support the benefits of the $O_2$ / $CO_2$ fluxes [74,75].
154 The coral symbioses also can be influenced by other types of stress related to environmental changes. The
155 impact of high seawater $p$$CO_2$ / low pH on corals is highly variable, where it can stimulate growth and
156 photosynthetic efficiency in certain Symbiodiniaceae species [76], which suggests that the presence of carbon-
157 concentrating mechanisms in the endosymbiont can lead to strain in the symbiotic relationship [77]. Eutrophic
158 conditions of seawater, such as elevated concentration of nitrates ($NO_3^-$) or phosphate ($PO_4^{3-}$), can stimulate both
159 photosynthesis and respiration rates in corals, but not concomitant increase of net calcification [46] bringing to
160 unbalanced efficiency of symbiosis in the coral health. However, ammonia ($NH_4^+$) enrichment can support the
161 resistance to thermal stress, but not $NO_3^-$ which can bring to increased levels of reactive species and oxidative
162 damage [78].
163 Bleaching, where the endosymbionts may decrease their chlorophyll content or be ejected from the coral
164 host, signals severe physiological consequences arising from oxidative stress and starvation, leading to reduced
165 respiration rates and energy acquisition [79]. Although bleaching can be a reversible condition, it often leads to
166 coral tissue death if sustained too long. However, endosymbionts have developed adaptive mechanisms to limit
167 thermal stress by increasing electron flow in photosystem I as photoprotection [80], thereby limiting the extent
168 of bleaching. Moreover, corals polyps may partially compensate for the lost photosynthetically-derived energy
169 during bleaching by enhanced feeding if sufficient prey are available [32].

170 **2. 2. Responses of the coral community**

171   When magnified across coral tissues, the polyp-level metabolic responses can affect reefs at community
172   level. Growth of coral colony, or net community calcification, is measured in terms of the deposition of its
173   calcium carbonate ($CaCO_3$) foundation, which is determined largely by coral energy reserves and the need to
174   maintain optimal chemical conditions at the tissue/calcium carbonate interface. Hard corals build their skeleton
175   of $CaCO_3$ through the uptake of calcium and carbonate ions from seawater. The reaction occurs in the
176   calicoblastic cells lining the surface where primary crystal secretion occurs [22] and is facilitated at pH levels
177   above that of seawater. However, ocean acidification reduces the seawater pH and the aragonite saturation state
178   ($\Omega a$) [23], which can have a strong effect on net community calcification (NCC), along with the balance of organic
179   matter production and respiration, or net community production (NCP) [81]. Moreover, the projected decreases
180   in pH in the future ocean eventually will affect the stability of existing reef ecosystems, because the $CaCO_3$
181   formed by corals (aragonite) is more susceptible to dissolution than that formed by other bio-calcifying
182   organisms [82]. These effects overlay natural seasonal variations in the carbonate chemistry in reef systems, and
183   the question remains of what extent does globally and locally derived ocean acidification have on the seasonal
184   balance of NCP and NCC [83]. However, these estimates remain imprecise, owing to the lack of information
185   about seawater residence time and volume, which would modulate reef chemistry [84]. The relationship
186   between corals and their symbiotic dinoflagellates is exacerbated under the combined effects global drivers of
187   stress, leading to lower energy reserves for growth [6,20,85,86], and local impacts of eutrophic conditions of
188   seawater surrounding the coral reefs, causing reduced calcification, oxidative stress, and eventually leading to
189   bleaching events [46,78].

190   Even with these impacts though, it is possible for coral communities to recover after destructive events. For
191   example, the coral reef in Kane'ohe Bay showed positive NCC and NCP about one year after the last drastic
192   bleaching event in 2015 [87], indicating the capacity of local rapid post-bleaching recovery. However, these
193   estimates considered only the abiotic factors, such as carbonate chemistry and oceanographic patterns, without
194   considering potential effects from the benthic community which would contribute to the depletion or repletion
195   of alkalinity in the coral reef [88].

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

205
206   **3. Methods**
207   We conducted a literature review based on case studies of in situ observations of coral metabolism from
208   published peer-reviewed scientific literature. We included cases involved in original research on direct (e.g.,
209   using SCUBA divers, benthic chambers, optical sensors) underwater measurements of metabolic rates on both
210   coral individuals and coral communities. We excluded sample collections (e.g., coral fragmentation), laboratory
211   experiments, and indirect measurements of metabolic fluxes (e.g., ex situ from sample incubations).

212   We used the advanced search on Google Scholar to identify studies with the keywords "coral in situ
213   metabolism" and "underwater", excluding the keyword "collection", in articles published between 1991 and
214   2020. The search yield (n = 2090) was scrutinized, and the literature was manually reviewed to fulfil our selection
215   criteria described above. A final list of 54 studies was included for the analysis in this review.

216   The literature screening was categorized in eight separate categories based on the methodology used (Table
217   1), including: 1) SCUBA FRR fluorometry; 2) SCUBA PAM fluorometry; 3) Clark-type $O_2$ sensors; 4) boundary
218   layer; 5) SCUBA imaging; 6) benthic chambers; 7) submersible chambers; and 8) automated sensors. The
219   database was further classified based on the system type (open, semi-closed, enclosed); measured parameters

220 (O$_2$, Fv/Fm, ETR, CA); sampling frequency (minutes, hours, days); sampling scale (symbionts, polyp, colony,
221 community); and aim of the study (monitoring, experiment).
222      Through the analysis of the literature screened, we ranked the database by publication year (Table 2) and
223 identified the most commonly used techniques for studying in situ coral metabolism. Finally, we analyzed the
224 objectives of the studies included in this review (Fig. 2) in order to give an overview on the topics mostly studied
225 between 1991 and 2020.
226

227 **4. Results**

228 **4.1. Methodologies and objectives for coral in situ metabolism**

229      Several tools and instruments have been developed for measuring coral physiology in both field and
230 laboratory studies. The ability to non-invasively measure metabolic and physiological processes makes these
231 methods well-suited for studying both healthy and stressed organisms. The use of diver-portable technologies,
232 designed to non-destructively estimate energy production and expenditure, such as respirometers and
233 fluorometers, is discussed in this review. However, there is little consensus about the extent to which apparently
234 healthy corals are adapting to the changing conditions [89]. Measurements of metabolic proxies such as
235 variations in dissolved oxygen and pH have been used at both individual coral and coral community scales to
236 infer the metabolic state of benthic ecosystems [90], and link these indicators to the wider contribution the
237 ecosystem's nutrient cycle and biogeochemistry.
238      Previous convention for laboratory-based manipulation studies for coral metabolism have involved
239 destructive sampling of individual or fragments of a coral colony. Although this framework provides crucial
240 knowledge on separate isolated physiological mechanisms and pathways, these controlled conditions do not
241 comprehensively reflect the stochasticity and cyclical fluctuations in environmental conditions on a reef and the
242 effects of multiple chronic or acute stressors. In the last decades, experimental techniques to detect the metabolic
243 status of corals directly in situ have been developed, using specific tools engineered for automated analysis on
244 coral surface and reef communities. Among these, microsensors and benthic chambers for respirometry and
245 fluorometry techniques became the most popular for coral surface studies even with different applications
246 (Table 1).
247

248      **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 |

249  SCUBA FRR: SCUBA-based fast respiration rate fluorometer; SCUBA PAM fluorometry: SCUBA-based
250  pulse amplitude modulation; $O_2$: Dissolved oxygen; CA: Calcification; ETR: Electron transport rate; Fv / Fm:
251  maximum quantum yield of photosystem II.
252

253  The focus of instrumentation development for in situ studies of coral metabolism has shifted over the last
254  three decades from the host-symbiosis relationship using fluorometric techniques to measure the
255  photosynthetic efficiency of endosymbionts to a more comprehensive investigation of individuals and
256  communities (Table 2). Indeed, through the use of benthic chambers it is possible to run experimental
257  manipulations of carbon fluxes or nutrients and monitor long-term ecosystem responses via automated sensors.
258  With this shift, the biogeochemical processes involved in corals and coral reefs have received advanced
259  understanding of ecosystems functioning with the possibility to predict benthic conditions using modelling of
260  future scenarios [51].

261  **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 |

262

263  Among the objectives of coral physiology studies, investigating the diel or seasonal changes in primary
264  productivity comprised 59% of the published works, highlighting that quantification of natural variations in
265  energetic fluxes are a central concern for the assessment of coral conditions (Fig. 2). Moreover, coral productivity
266  was the only objective of study being covered by all methodologies at different sampling scales, from polyp to
267  community. Conversely, 25% of studies addressed coral physiology under stress, including bleaching
268  responses, environmental changes and bathymetric change conditions (transplantation). The assessment of
269  recovery of health status was investigated by 9% of the studies. The remaining papers centered on issues of
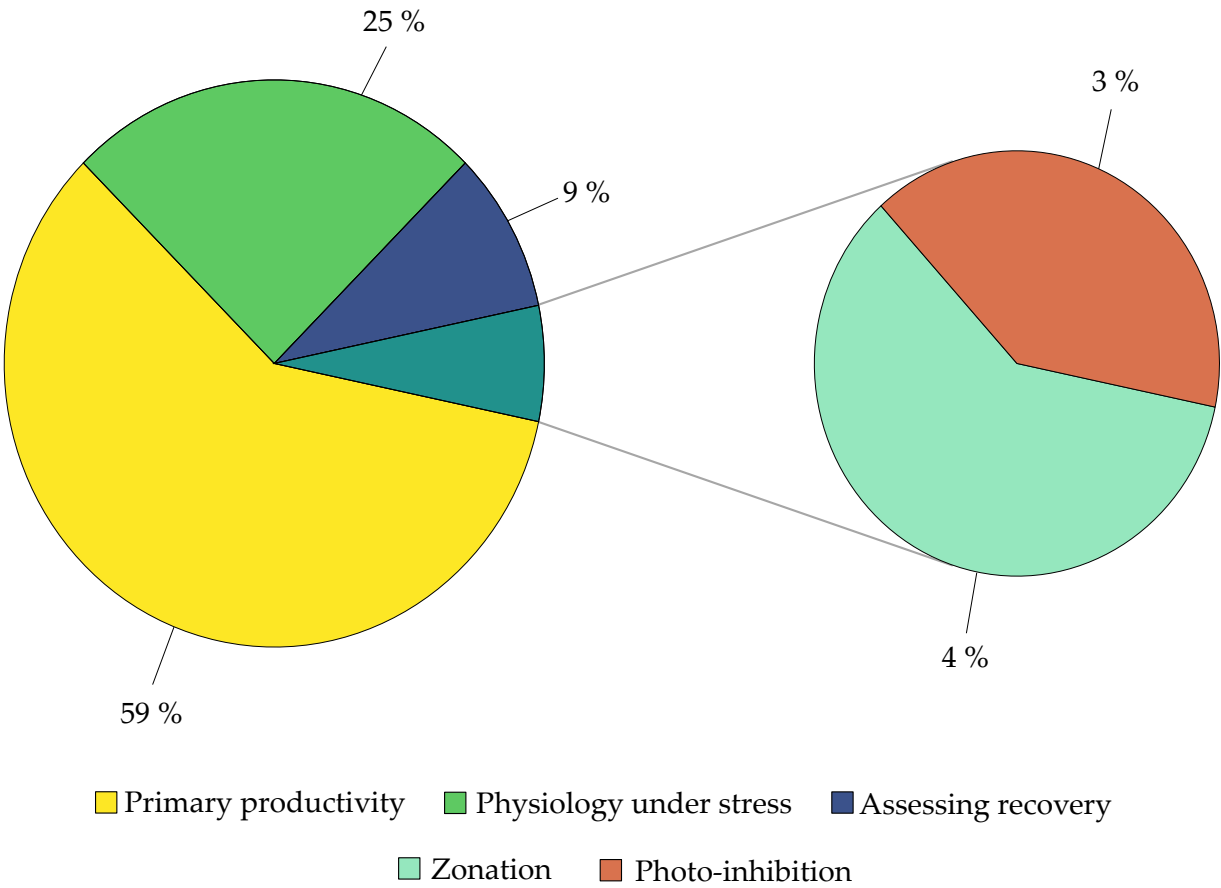270  zonation (4%) and photo-inhibition (3%).

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

### 4. 2. 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 [91,92]. 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 [93–95]. In addition, this imaging technique can also make early signs of disease and coral juveniles easier to detect for accurate enumeration [96], providing a facet of coral reef health [92].

Early in situ studies on coral physiology estimated the rates of photosynthesis and respiration within recirculating chambers by measuring changes in oxygen concentrations [97]. 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 [98] 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. [99]. Pulse amplitude modulated (PAM) fluorometer uses light pulses of relatively long duration (300 - 1200 ms, 3000-10000 µmol photons $m^{-2}$ $s^{-1}$) 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 [100]. The introduction of in situ PAM fluorometry enabled the construction of photosynthesis-irradiance models for corals based on varying sun exposures in reefs [99,100]. 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 [101]. Similar reduced photosynthetic efficiency has been observed in corals exposed to seasonal turbid conditions [102]. 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 [103]. Even so, variable responses in the coral community to this stress suggested coral species-specific physiological responses [104–107]. 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 [108].

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 µmol photons $m^{-2}$ $s^{-1}$) but more intense light is used to generate a single turnover and reduction of the primary electron acceptor [109,110]. The methodology is more sensitive than PAM, which can be beneficial when endosymbiont abundance (or chlorophyll concentrations) is very low in corals. The SCUBA-based FRR fluorometry was developed by Gorbunov et al. [111] 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 [112,113]. Although nutrient availability (as nitrogen or iron) and sunlight irradiance were already recognized as the main factors directly affecting photosynthesis in the aquatic ecosystem [114,115], 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 [116].

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 [117,118]. 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 [111] while oxygen measurements integrate the whole colony [119]. 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.

## 4.3. 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 [120]. These chambers enabled the non-destructive measurement of coral physiological rates through changes in water chemistry to better understand community level responses to perturbation [120,121]. 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 [122]. 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 [123–126]. 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 [127]. 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 [126]. 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 [128], 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) [129]. However, non-invasive diver-portable in situ incubation chambers (~70 L, 0.2 m$^2$ 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 [51]. 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 [130]. This system enabled some of the first studies of the direct influence of high $CO_2$ levels on coral communities in short term experiments [130], 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 [131].

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 [132], 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 [133], where the upper side has a reduced quantum yield [134]. Other experimental studies have been conducted using flow chambers to study coral physiology under chemical enrichment in a benthic chamber experiment [135] adding new findings on coral responses to high $p$$CO_2$ and the combined effect of chemical inputs and global warming. Another system, such as the collapsible benthic isolation tents (cBITs) [136], was used to assess the combined effects of benthic communities, from corals to algae and microbes, over diurnal cycles, highlighting the importance of contribution to reef metabolism and DOC by different compartments.

A new diver-portable tool has been developed for community in situ metabolism (CISME) that enables short-term (minutes) experimentation with flow and chemical manipulations, as well as in situ measurements

390   of coral metabolic health and rates of calcification [137]. Although its use has been limited to date, it offers the
391   ability for the assessment of individual corals across and among reef environments with high accuracy and
392   resolution. Similarly, small benthic chambers enclosing ~70 L [51] were used to study complex benthic structures
393   and to measure coral reef community budgets of primary production over coral-dominated or alge-dominated
394   reef communities [138] adding new findings on the biogeochemical cycles of coral reef ecosystems.

395         Benthic chambers offer the chance to assess the variability of responses within and among reefs, but by
396   design these systems alter water flow dynamics around the study corals and restrict water exchange sufficiently
397   to enable changes to accumulate in water chemistry (i.e., the measurement parameters at a minimum). It is
398   unclear at what stage these changes in water flow and bulk chemical conditions may begin influencing coral
399   responses. Unenclosed, diffusive boundary layer (DBL) approaches were developed to avoid these potential
400   issues, whereby sensors placed well above the coral interface make unobstructed measurements of vertical
401   gradients in velocity, temperature, and chemical constituents on the open reef to calculate the flux of
402   momentum, heat, and $O_2$ in the boundary layer [139].
403

### 4. 4. Micro- and automated sensors

405         The use of microsensors at coral tissue levels [129,140–143] and automated sensors at community level
406   [144–146] reveals new insights into coral metabolism by showing the fluctuations in chemical conditions under
407   different natural regime of dissolved oxygen, marine pH and light intensity. For example, the oxygen saturation
408   at coral-water interface fluctuates from supersaturated during daytime due to production by endosymbionts,
409   to hypoxic at night due to respiration by both polyps and symbionts [60,63,147]. However, despite these broad
410   chemical changes, oxygen microsensors used in the microenvironment of coral-turf and coral-corallinae algae
411   showed that low oxygen concentrations were not generally found at the interface of *Porites* spp., turf algae, and
412   crustose coralline algae [141]. More microenvironment studies are needed to better understand the metabolic
413   regulation between corals and the surrounding environment [129]. Technological development of microsensors
414   in a modified diver-operated system [148] allowed the quantification of irradiance at the coral surface and the
415   measurement of the efficiency of photosynthetic system at polyp and coenosarc microscale [149], confirming
416   the ability of corals to adapt to environmental changes, such as temperature or irradiance [150]. On a broader
417   scale, micro- and automated sensors increasingly have been used in both closed (benthic chamber) and open
418   (reef-scale) approaches to studying coral and reef ecologies.
419

### 4. 5. Reef scale experiments

421         The study of corals in open or unconfined natural systems using DBL techniques was made possible by the
422   advent of sensors for measuring both water flow and relevant aspects of water chemistry. A common approach
423   is to use eddy correlation techniques [151] where non-invasive acoustic doppler velocimeters measuring water
424   flow are coupled with $O_2$ sensors to provide three-dimensional fields of oxygen distribution over benthic
425   environments [152]. This application can examine in situ dynamics of $O_2$ production and consumption (i.e.,
426   respiration) in different habitats, including highly productive reef crests relative to reef slopes [152] as well as
427   cold-water (deep) coral reefs [153,154]. These ecosystem-scale measurements show how high oxygen fluxes are
428   possible, even in nutrient replete environments such as reef slopes and deep waters. The advantage of such
429   techniques has been discussed by Takeshita et al. [155] who introduced a new autonomous system (the benthic
430   ecosystem and acidification measurement system) for simultaneous measurement of NCP and NCC from a coral
431   reef through the autonomous use of $O_2$ and pH sensing technology. Moreover, direct observation of the effect
432   of increased $p$CO$_2$ / lowered pH environments on corals showed temporal and spatial metabolic response of reef
433   diversity and water chemistry to high $CO_2$ / low $\Omega$a, at temporal and spatial patterns [144].

434         Comparison between the enclosed and open system approaches showed good general agreement with
435   respect to discrete measurements of oxygen from the gradient flux (GF) method at the DBL and a
436   multiparametric probe in a benthic chamber [130], although other findings indicate that GF method can offer
437   more accurate measurements of these fluxes [139]. The low physical disruption with DBL methods means both
438   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 [65], 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 [156].

Experiments at reef scale represent a key step forward in the in situ studies of coral responses in the natural environment [49,157–159], but they also have provided the opportunity to quantify reef-scale responses to environmental perturbations. For example, manipulations to lower $\Omega$a by ~20% led to a 34% decrease in NCC consistent with the effect of lower pH on biocalcification [160], although this finding may have been influenced by a high abundance of crustose corallinae algae living in the reef community [159]. Another free ocean $CO_2$ enrichment (FOCE) system was used to incubate coral reef communities at ambient $p$CO$_2$ (393 µatm) and high $p$CO$_2$ (949 µatm), and a decrease in daily NCC by 49% under high $p$CO$_2$ was observed over a 21 days experiment, corresponding to 26% reduction in NCC per unit of $\Omega$a [157]. 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 [158]. Srednick et al. [49] 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 $p$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, $p$CO$_2$ and the biological responses of the reef community over high temporal resolution [49]. Other high frequency sampling of coral reef carbonate dynamics and metabolic rates have used automated systems for measurements of alkalinity, pH and $p$CO$_2$ at high frequency sampling [50,145]. 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 [145,161]. However, dissimilarities in the methods used in community metabolism studies [162], 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

Underwater methodologies from 54 studies on coral metabolism and physiology conducted since 1991 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 [157], 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.

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

486    measuring metabolic and biogeochemical properties of different benthic habitats having both simple and
487    complex structures. The replicability of experimental studies, for example using same methodology or sensors,
488    allows the simultaneous monitoring of coral health in different locations with the ability to compare the
489    ecosystem functions, e.g., standardization of the methods. However, there are limitations to in situ
490    methodologies. In the case of benthic chambers, there are restrictions to which substrate surfaces are suitable
491    for study, and they are not well suited, even with enhanced flow capabilities, for longer-term monitoring of
492    coral health. Yet, benthic chambers are restricted to limited benthic area of the reef, with no accounting of the
493    energy processes from the entire ecosystem (macroalgae, fish, etc.) In the case of DBL approaches, limitations
494    include the needed maintenance and fragility of microsensors, which can limit deployments to shorter duration,
495    or under more quiescent weather conditions. Co-deployment of multiple instrumentation approaches will be
496    needed to ensure both accuracy and logistic practicality in monitoring fluxes of $O_2$ and pH in coral reefs. Even
497    with these accounted for, most studies on coral metabolic rates rely on observation of changes in DIC and
498    alkalinity in a specific (small) area to calculate the carbon fluxes for productivity and calcification estimates
499    [163]; the precise movement of seawater flow and aspects of reef heterogeneity cannot be fully taken into
500    account, leading to potentially biased findings that may not adequately reflect natural conditions. In reality
501    then, a combination of in situ techniques likely will be most useful, such as the application of flow respirometry
502    approaches to estimate carbon fluxes over a small spatial scales and different substrate type within a reef,
503    coupled with DBL methods that provide an integrative assessment of the temporal variability in coral
504    productivity and growth [145].

519    **References**

520    1.    Roberts, C. M.; McClean, C. J.; Veron, J. E. N.; et al. Marine biodiversity hotspots and conservation priorities for
521          tropical reefs. *Science (80-. )*. **2002**, 295(5558):1280-1284. doi:10.1126/science.1067728.

522    2.    Ray, C. G. Ecological Diversity in Coastal Zones and Oceans. In Wilson, E. O., Peter, F. M., eds. *Biodiversity*; National
523          Academies Press (US), **1988**. https://www.ncbi.nlm.nih.gov/books/NBK219271/.

524    3.    Cesar, H.; Burke, L.; Pet-Soede, L. *The Economics of Worldwide Coral Reef Degradation*. International Coral Reef Action
525          Network; **2003**. https://agris.fao.org/agris-search/search.do?recordID=GB2013202743.

526    4.    Knowlton, N.; Brainard, R. E.; Fisher, R.; Moews, M.; Plaisance, L.; Caley, M. J. Coral Reef Biodiversity. In *Life in the
527          World's Oceans*; Wiley-Blackwell, **2010**; 65-78. doi:10.1002/9781444325508.ch4.

528    5.    Van Zanten, B. T.; Van Beukering, P. J. H.; Wagtendonk, A. J. Coastal protection by coral reefs: A framework for
529          spatial     assessment     and     economic     valuation.     *Ocean     Coast.     Manag.*     **2014**,     96:94-103.

530        doi:10.1016/j.ocecoaman.2014.05.001.

531    6.    Hughes, T. P.; Bellwood, D. R.; Baird, A. H.; Brodie, J.; Bruno, J. F.; Pandolfi, J. M. Shifting base-lines, declining coral
532          cover, and the erosion of reef resilience: Comment on Sweatman et al. (2011). *Coral Reefs*. **2011**, 30(3):653-660.
533          doi:10.1007/s00338-011-0787-6.

534    7.    Hughes, T. P.; Kerry, J. T.; Baird, A. H.; et al. Global warming impairs stock–recruitment dynamics of corals. *Nature*.
535          **2019**, 568(7752):387-390. doi:10.1038/s41586-019-1081-y.

536    8.    Descombes, P.; Wisz, M. S.; Leprieur, F.; et al. Forecasted coral reef decline in marine biodiversity hotspots under
537          climate change. *Glob. Chang. Biol.* **2015**, 21(7):2479-2487. doi:10.1111/gcb.12868.

538    9.    Bruno, J. F.; Selig, E. R. Regional decline of coral cover in the Indo-Pacific: Timing, extent, and subregional
539          comparisons. *PLoS One*. **2007**, 2(8). doi:10.1371/journal.pone.0000711.

540    10.   Goulletquer, P.; Gros, P.; Boeuf, G.; et al. Status and Trends. In *Biodiversity in the Marine Environment*; Springer
541          Netherlands, **2014**;21-84. doi:10.1007/978-94-017-8566-2_3.

542    11.   Hannah, L. Impacts of Anthropogenic $CO_2$ and Climate Change on the Biology of Terrestrial and Marine Systems.
543          In Kendall, R. J., Lacher, T. E., Cobb, G. C., Cox, S. B., Lacher, T. E., eds. *Wildlife Toxicology: Emerging Contaminant*
544          *and Biodiversity Issues*; CRC Press, **2016**;165-190. doi:10.1201/9781439817957-11.

545    12.   Dlugokencky, E.; Tans, P. Global Monitoring Laboratory - Carbon Cycle Greenhouse Gases. US Department of
546          Commerce, NOAA, Global Monitoring Laboratory. Published 2020. Accessed July 6, 2020.
547          www.esrl.noaa.gov/gmd/ccgg/trends.

548    13.   Bustamante, M. M. C.; Nardoto, G. B.; Pinto, A. S.; Resende, J. C. F.; Takahashi, F. S. C.; Vieira, L. C. G. Potential
549          impacts of climate change on biogeochemical functioning of Cerrado ecosystems. *Brazilian J. Biol.* **2012**, 72(3
550          SUPPL.):655-671. doi:10.1590/S1519-69842012000400005.

551    14.   Galloway, J. N.; Dentener, F. J.; Capone, D. G.; et al. Nitrogen cycles: Past, present, and future. *Biogeochemistry*. **2004**,
552          70(2):153-226. doi:10.1007/s10533-004-0370-0.

553    15.   Gruber, N. Warming up, turning sour, losing breath: ocean biogeochemistry under global change. *Philos. Trans. R.*
554          *Soc. A Math. Phys. Eng. Sci.* **2011**, 369(1943):1980-1996. doi:10.1098/rsta.2011.0003.

555    16.   Ciais, P.; Sabine, C.; Bala, G.; Peters, W. Carbon and other biogeochemical cycles. In *Climate Change 2013 the Physical*
556          *Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate*
557          *Change*; Vol 9781107057. Cambridge University Press, 2013;465-570. doi:10.1017/CBO9781107415324.015.

558    17.   Kuffner, I. B.; Lidz, B. H.; Hudson, J. H.; Anderson, J. S. A Century of Ocean Warming on Florida Keys Coral Reefs:
559          Historic In Situ Observations. *Estuaries and Coasts*. **2015**, 38(3):1085-1096. doi:10.1007/s12237-014-9875-5.

560    18.   Hughes, T. P.; Kerry, J. T.; Álvarez-Noriega, M.; et al. Global warming and recurrent mass bleaching of corals. *Nature*.
561          **2017**, 543(7645):373-377. doi:10.1038/nature21707.

562    19.   Zeebe, R. E.; Wolf-Gladrow, D. CO2 in Seawater: Equilibrium, Kinetics, Isotopes. Elsevier Oceanography Series 65.
563          Published October 15, **2001**. https://www.elsevier.com/books/co2-in-seawater-equilibrium-kinetics-
564          isotopes/zeebe/978-0-444-50946-8.

565    20.   Anthony, K. R. N.; Kline, D. I.; Diaz-Pulido, G.; Dove, S.; Hoegh-Guldberg, O. Ocean acidification causes bleaching
566          and productivity loss in coral reef builders. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, 105(45):17442-17446.
567          doi:10.1073/pnas.0804478105.

568    21.   Caldeira, K.; Wickett, M. E. Anthropogenic carbon and ocean pH. *Nature*. **2003**, 425(6956):365. doi:10.1038/425365a.

569　22.　Cohen, A.; Holcomb, M. Why Corals Care About Ocean Acidification: Uncovering the Mechanism. *Oceanography*.
570　　　**2009**, 22(4):118-127. doi:10.5670/oceanog.2009.102.

571　23.　Eyre, B. D.; Andersson, A. J.; Cyronak, T. Benthic coral reef calcium carbonate dissolution in an acidifying ocean.
572　　　*Nat. Clim. Chang.* **2014**, 4(11):969-976. doi:10.1038/nclimate2380.

573　24.　Hoegh-Guldberg, O.; Mumby, P. J.; Hooten, A. J.; et al. Coral reefs under rapid climate change and ocean
574　　　acidification. *Science*. **2007**, 318(5857):1737-1742. doi:10.1126/science.1152509.

575　25.　Pandolfi, J. M.; Connolly, S. R.; Marshall, D. J.; Cohen, A. L. Projecting coral reef futures under global warming and
576　　　ocean acidification. *Science (80-. ).* **2011**, 333(6041):418-422. doi:10.1126/science.1204794.

577　26.　Heery, E. C.; Hoeksema, B. W.; Browne, N. K.; et al. Urban coral reefs: Degradation and resilience of hard coral
578　　　assemblages in coastal cities of East and Southeast Asia. *Mar. Pollut. Bull.* **2018**, 135(July):654-681.
579　　　doi:10.1016/j.marpolbul.2018.07.041.

580　27.　Darling, E. S.; Alvarez-Filip, L.; Oliver, T. A.; Mcclanahan, T. R.; Côté, I. M. Evaluating life-history strategies of reef
581　　　corals from species traits. *Ecol. Lett.* **2012**, 15(12):1378-1386. doi:10.1111/j.1461-0248.2012.01861.x.

582　28.　Browne, N.; Precht, E.; Last, K.; Todd, P. Photo-physiological costs associated with acute sediment stress events in
583　　　three near-shore turbid water corals. *Mar. Ecol. Prog. Ser.* **2014**, 502:129-143. doi:10.3354/meps10714.

584　29.　Brown, B.; Bythell, J. Perspectives on mucus secretion in reef corals. *Mar. Ecol. Prog. Ser.* **2005**, 296:291-309.
585　　　doi:10.3354/meps296291.

586　30.　Fabricius, K. E. Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. *Mar. Pollut.*
587　　　*Bull.* **2005**, 50(2):125-146. doi:10.1016/j.marpolbul.2004.11.028.

588　31.　Jones, R.; Giofre, N.; Luter, H. M.; Neoh, T. L.; Fisher, R.; Duckworth, A. Responses of corals to chronic turbidity.
589　　　*Sci. Rep.* **2020**, 10(1):1-13. doi:10.1038/s41598-020-61712-w.

590　32.　Anthony, K. R. N.; Connolly, S. R.; Hoegh-Guldberg, O. Bleaching, energetics, and coral mortality risk: Effects of
591　　　temperature, light, and sediment regime. *Limnol. Oceanogr.* **2007**, 52(2):716-726. doi:10.4319/lo.2007.52.2.0716.

592　33.　Dubinsky, Z.; Falkowski, P. G.; Porter, J. W.; Muscatine, L. Absorption and utilization of radiant energy by light-
593　　　and shade-adapted colonies of the hermatypic coral *Stylophora pistillata*. *Proc. R. Soc. London. Ser. B. Biol. Sci.* **1984**,
594　　　222(1227):203-214. doi:10.1098/rspb.1984.0059.

595　34.　Anthony, K. R. N.; Fabricius, K. E. Shifting roles of heterotrophy and autotrophy in coral energetics under varying
596　　　turbidity. *J. Exp. Mar. Bio. Ecol.* **2000**, 252(2):221-253. doi:10.1016/S0022-0981(00)00237-9.

597　35.　Bessell-Browne, P.; Negri, A. P.; Fisher, R.; Clode, P. L.; Duckworth, A.; Jones, R. Impacts of turbidity on corals: The
598　　　relative importance of light limitation and suspended sediments. *Mar. Pollut. Bull.* **2017**, 117(1-2):161-170.
599　　　doi:10.1016/j.marpolbul.2017.01.050.

600　36.　Loiola, M.; Cruz, I. C. S.; Lisboa, D. S.; et al. Structure of marginal coral reef assemblages under different turbidity
601　　　regime. *Mar. Environ. Res.* **2019**, 147:138-148. doi:10.1016/j.marenvres.2019.03.013.

602　37.　Mass, T.; Genin, A.; Shavit, U.; Grinstein, M.; Tchernov, D. Flow enhances photosynthesis in marine benthic
603　　　autotrophs by increasing the efflux of oxygen from the organism to the water. *Proc. Natl. Acad. Sci. U. S. A.* **2010**,
604　　　107(6):2527-2531. doi:10.1073/pnas.0912348107.

605　38.　Wijgerde, T.; Silva, C. I. F.; Scherders, V.; Van Bleijswijk, J.; Osinga, R. Coral calcification under daily oxygen
606　　　saturation and pH dynamics reveals the important role of oxygen. *Biol. Open.* **2014**, 3(6):489-493.
607　　　doi:10.1242/bio.20147922.

608  39.  Altieri, A. H.; Harrison, S. B.; Seemann, J.; Collin, R.; Diaz, R. J.; Knowlton, N. Tropical dead zones and mass
609      mortalities on coral reefs. *Proc. Natl. Acad. Sci.* **2017**, 114(14):3660-3665. doi:10.1073/pnas.1621517114.

610  40.  DeCarlo, T. M.; Gajdzik, L.; Ellis, J.; et al. Nutrient-supplying ocean currents modulate coral bleaching susceptibility.
611      *Sci. Adv.* **2020**, 6(34):eabc5493. doi:10.1126/sciadv.abc5493.

612  41.  Sawall, Y.; Teichberg, M. C.; Seemann, J.; Litaay, M.; Jompa, J.; Richter, C. Nutritional status and metabolism of the
613      coral Stylophora subseriata along a eutrophication gradient in Spermonde Archipelago (Indonesia). *Coral Reefs.* **2011**,
614      30(3):841-853. doi:10.1007/s00338-011-0764-0.

615  42.  Duprey, N. N.; Wang, X. T.; Thompson, P. D.; et al. Life and death of a sewage treatment plant recorded in a coral
616      skeleton Δ15N record. *Mar. Pollut. Bull.* **2017**, 120(1-2):109-116. doi:10.1016/j.marpolbul.2017.04.023.

617  43.  Baker, D. M.; Kim, K.; Andras, J. P.; Sparks, J. P. Light-mediated 15N fractionation in Caribbean gorgonian
618      octocorals: Implications for pollution monitoring. *Coral Reefs.* **2011**, 30(3):709-717. doi:10.1007/s00338-011-0759-x.

619  44.  Baker, D. M.; Jordán-Dahlgren, E.; Maldonado, M. A.; Harvell, C. D. Sea fan corals provide a stable isotope baseline
620      for assessing sewage pollution in the Mexican Caribbean. *Limnol. Oceanogr.* **2010**, 55(5):2139-2149.
621      doi:10.4319/lo.2010.55.5.2139.

622  45.  Conti-Jerpe, I. E.; Thompson, P. D.; Wong, C. W. M.; et al. Trophic strategy and bleaching resistance in reef-building
623      corals. *Sci. Adv.* **2020**, 6(15):5443-5453. doi:10.1126/sciadv.aaz5443.

624  46.  Silbiger, N. J.; Nelson, C. E.; Remple, K.; et al. Nutrient pollution disrupts key ecosystem functions on coral reefs.
625      *Proc. R. Soc. B Biol. Sci.* **2018**, 285(1880):2-10. doi:10.1098/rspb.2017.2718.

626  47.  D'Angelo, C.; Wiedenmann, J. Impacts of nutrient enrichment on coral reefs: New perspectives and implications for
627      coastal management and reef survival. *Curr. Opin. Environ. Sustain.* **2014**, 7:82-93. doi:10.1016/j.cosust.2013.11.029.

628  48.  Fabricius, K. E. Factors determining the resilience of coral reefs to eutrophication: A review and conceptual model.
629      In *Coral Reefs: An Ecosystem in Transition*; Springer Netherlands, 2011;493-505. doi:10.1007/978-94-007-0114-4_28.

630  49.  Srednick, G.; Bergman, J. L.; Doo, S. S.; et al. Shallow coral reef free ocean carbon enrichment: Novel in situ flumes
631      to manipulate pCO $_2$ on shallow tropical coral reef communities. *Limnol. Oceanogr. Methods.* **2020**, 18(3):116-128.
632      doi:10.1002/lom3.10349.

633  50.  Cyronak, T.; Takeshita, Y.; Courtney, T. A.; et al. Diel temperature and pH variability scale with depth across diverse
634      coral reef habitats. *Limnol. Oceanogr. Lett.* **2020**, 5(2):193-203. doi:10.1002/lol2.10129.

635  51.  Roth, F.; Wild, C.; Carvalho, S.; et al. An in situ approach for measuring biogeochemical fluxes in structurally
636      complex benthic communities. Trueman, C., ed. *Methods Ecol. Evol.* **2019**, 10(5):712-725. doi:10.1111/2041-210X.13151.

637  52.  Rohwer, F.; Seguritan, V.; Azam, F.; Knowlton, N. Diversity and distribution of coral-associated bacteria. *Mar. Ecol.*
638      *Prog. Ser.* **2002**, 243:1-10. doi:10.3354/meps243001.

639  53.  Rosenberg, E.; Koren, O.; Reshef, L.; Efrony, R.; Zilber-Rosenberg, I. The role of microorganisms in coral health,
640      disease and evolution. *Nat. Rev. Microbiol.* **2007**, 5(5):355-362. doi:10.1038/nrmicro1635.

641  54.  Peixoto, R. S.; Rosado, P. M.; Leite, D. C. de A.; Rosado, A. S.; Bourne, D. G. Beneficial microorganisms for corals
642      (BMC): Proposed mechanisms for coral health and resilience. *Front. Microbiol.* **2017**, 8(MAR):1-16.
643      doi:10.3389/fmicb.2017.00341.

644  55.  Thompson, J. R.; Rivera, H. E.; Closek, C. J.; Medina, M. Microbes in the coral holobiont: partners through evolution,
645      development, and ecological interactions. *Front. Cell. Infect. Microbiol.* **2015**, 4. doi:10.3389/fcimb.2014.00176.

646  56.  Hume, B. C. C.; Voolstra, C. R.; Arif, C.; et al. Ancestral genetic diversity associated with the rapid spread of stress-
647       tolerant coral symbionts in response to holocene climate change. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, 113(16):4416-
648       4421. doi:10.1073/pnas.1601910113.

649  57.  Jones, A. .; Berkelmans, R.; van Oppen, M. J. .; Mieog, J. .; Sinclair, W. A community change in the algal
650       endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proc.*
651       *R. Soc. B Biol. Sci.* **2008**, 275(1641):1359-1365. doi:10.1098/rspb.2008.0069.

652  58.  LaJeunesse, T. C.; Parkinson, J. E.; Gabrielson, P. W.; et al. Systematic Revision of Symbiodiniaceae Highlights the
653       Antiquity and Diversity of Coral Endosymbionts. *Curr. Biol.* **2018**, 28(16):2570-2580.e6. doi:10.1016/j.cub.2018.07.008.

654  59.  Muscatine, L. The role of symbiotic algae in carbon and energy flux in reef corals . In Dubinsky, Z., ed. *Ecosystems of*
655       *the World*; Vol 25. Elsevier, **1998**;75-87.  https://www.scienceopen.com/document?vid=c3e2e66a-41da-4a83-8fb9-
656       88316db544bf.

657  60.  Gardella, D. J.; Edmunds, P. J. The oxygen microenvironment adjacent to the tissue of the scleractinian Dichocoenia
658       stokesii and its effects on symbiont metabolism. *Mar. Biol.* **1999**, 135(2):289-295. doi:10.1007/s002270050626.

659  61.  Al-Horani, F. A.; Al-Moghrabi, S. M.; De Beer, D. The mechanism of calcification and its relation to photosynthesis
660       and respiration in the scleractinian coral Galaxea fascicularis. *Mar. Biol.* **2003**, 142(3):419-426. doi:10.1007/s00227-002-
661       0981-8.

662  62.  Al-Horani, F. A.; Al-Moghrabi, S. M.; De Beer, D. Microsensor study of photosynthesis and calcification in the
663       scleractinian coral, Galaxea fascicularis: Active internal carbon cycle. *J. Exp. Mar. Bio. Ecol.* **2003**, 288(1):1-15.
664       doi:10.1016/S0022-0981(02)00578-6.

665  63.  Kuhl, M.; Cohen, Y.; Dalsgaard, T.; Jorgensen, B. B.; Revsbech, N. P. Microenvironment and photosynthesis of
666       zooxanthellae in scleractinian corals studied with microsensors for 02, pH and light. *Mar. Ecol. Prog. Ser*. **1995**,
667       117:159-172.

668  64.  Al-Horani, F. A.; Tambutté, É.; Allemand, D. Dark calcification and the daily rhythm of calcification in the
669       scleractinian coral, Galaxea fascicularis. *Coral Reefs*. **2007**, 26(3):531-538. doi:10.1007/s00338-007-0250-x.

670  65.  Finelli, C. M.; Helmuth, B. S. T.; Pentcheff, N. D.; Wethey, D. S. Water flow influences oxygen transport and
671       photosynthetic efficiency in corals. In *Coral Reefs*; Vol 25. Springer, 2006;47-57. doi:10.1007/s00338-005-0055-8.

672  66.  Mass, T.; Genin, A.; Shavit, U.; Grinstein, M.; Tchernov, D. Flow enhances photosynthesis in marine benthic
673       autotrophs by increasing the efflux of oxygen from the organism to the water. *Proc. Natl. Acad. Sci. U. S. A.* **2010**,
674       107(6):2527-2531. doi:10.1073/pnas.0912348107.

675  67.  Yonge, S. C. M.; Nicholls, A. G.; Yonge, M. J. The relationship between respiration in corals and the production of
676       oxygen by their zooxanthellae. *Sci. Reports / Gt. Barrier Reef Exped. 1928-29*. **1932**, 1:213-251. Accessed July 7, 2020.
677       https://www.biodiversitylibrary.org/part/183504.

678  68.  Hughes, T. P.; Barnes, M. L.; Bellwood, D. R.; et al. Coral reefs in the Anthropocene. *Nature*. **2017**, 546(7656):82-90.
679       doi:10.1038/nature22901.

680  69.  Hill, R.; Brown, C. M.; DeZeeuw, K.; Campbell, D. A.; Ralph, P. J. Increased rate of D1 repair in coral symbionts
681       during bleaching is insufficient to counter accelerated photo-inactivation. *Limnol. Oceanogr.* **2011**, 56(1):139-146.
682       doi:10.4319/lo.2011.56.1.0139.

683  70.  Lilley, R. M.; Ralph, P. J.; Larkum, A. W. D. The determination of activity of the enzyme Rubisco in cell extracts of
684       the dinoflagellate alga Symbiodinium sp. by manganese chemiluminescence and its response to short-term thermal
685       stress of the alga. *Plant, Cell Environ.* **2010**, 33(6):995-1004. doi:10.1111/j.1365-3040.2010.02121.x.

686   71.   Franklin, D.; Hoegh-Guldberg, O.; Jones, R.; Berges, J. Cell death and degeneration in the symbiotic dinoflagellates
687         of the coral Stylophora pistillata during bleaching. *Mar. Ecol. Prog. Ser.* **2004**, 272:117-130. doi:10.3354/meps272117.

688   72.   Randall, C. J.; Szmant, A. M. Elevated temperature affects development, survivorship, and settlement of the elkhorn
689         coral, Acropora palmata (Lamarck 1816). *Biol. Bull.* **2009**, 217(3):269-282. doi:10.1086/BBLv217n3p269.

690   73.   Lesser, M. P.; Stat, M.; Gates, R. D. The endosymbiotic dinoflagellates (Symbiodinium sp.) of corals are parasites and
691         mutualists. *Coral Reefs.* **2013**, 32(3):603-611. doi:10.1007/s00338-013-1051-z.

692   74.   Douglas, A. E. Conflict, cheats and the persistence of symbioses. *New Phytol.* **2008**, 177(4):849-858. doi:10.1111/j.1469-
693         8137.2007.02326.x.

694   75.   Baker, D. M.; Freeman, C. J.; Wong, J. C. Y. Y.; Fogel, M. L.; Knowlton, N. Climate change promotes parasitism in a
695         coral symbiosis. *ISME J.* **2018**, 12(3):921-930. doi:10.1038/s41396-018-0046-8.

696   76.   Brading, P.; Warner, M. E.; Davey, P.; Smith, D. J.; Achterberg, E. P.; Suggett, D. J. Differential effects of ocean
697         acidification on growth and photosynthesis among phylotypes of *Symbiodinium* (Dinophyceae). *Limnol. Oceanogr.*
698         **2011**, 56(3):927-938. doi:10.4319/lo.2011.56.3.0927.

699   77.   Comeau, S.; Edmunds, P. J.; Lantz, C. A.; Carpenter, R. C. Daily variation in net primary production and net
700         calcification in coral reef communities exposed to elevated $pCO_2$; *Biogeosciences.* **2017**, 14(14):3549-3560.
701         doi:10.5194/bg-14-3549-2017.

702   78.   Fernandes de Barros Marangoni, L.; Ferrier-Pagès, C.; Rottier, C.; Bianchini, A.; Grover, R. Unravelling the different
703         causes of nitrate and ammonium effects on coral bleaching. *Sci. Rep.* **2020**, 10(1):11975. doi:10.1038/s41598-020-68916-
704         0.

705   79.   Williams, D. E.; Miller, M. W.; Bright, A. J.; Pausch, R. E.; Valdivia, A. Thermal stress exposure, bleaching response,
706         and mortality in the threatened coral Acropora palmata. *Mar. Pollut. Bull.* **2017**, 124(1):189-197.
707         doi:10.1016/j.marpolbul.2017.07.001.

708   80.   Hoogenboom, M. O.; Campbell, D. A.; Beraud, E.; DeZeeuw, K.; Ferrier-Pagès, C. Effects of light, food availability
709         and temperature stress on the function of photosystem II and photosystem I of coral symbionts. *PLoS One.* **2012**, 7(1).
710         doi:10.1371/journal.pone.0030167.

711   81.   Albright, R.; Benthuysen, J.; Cantin, N.; Caldeira, K.; Anthony, K. Coral reef metabolism and carbon chemistry
712         dynamics of a coral reef flat. *Geophys. Res. Lett.* **2015**, 42(10):3980-3988. doi:10.1002/2015GL063488.

713   82.   Shaw, E. C.; Mcneil, B. I.; Tilbrook, B.; Matear, R.; Bates, M. L. Anthropogenic changes to seawater buffer capacity
714         combined with natural reef metabolism induce extreme future coral reef CO2 conditions. *Glob. Chang. Biol.* **2013**,
715         19(5):1632-1641. doi:10.1111/gcb.12154.

716   83.   Cyronak, T.; Andersson, A. J.; Langdon, C.; et al. Taking the metabolic pulse of the world's coral reefs. Voolstra, C.
717         R., ed. *PLoS One.* **2018**, 13(1):e0190872. doi:10.1371/journal.pone.0190872.

718   84.   Courtney, T. A.; Lebrato, M.; Bates, N. R.; et al. Environmental controls on modern scleractinian coral and reef-scale
719         calcification. *Sci. Adv.* **2017**, 3(11):e1701356. doi:10.1126/sciadv.1701356.

720   85.   Hoegh-Guldberg, O. Coral reefs in the anthropocene: Persistence or the end of the line? *Geol. Soc. Spec. Publ.* **2014**,
721         395(1):167-183. doi:10.1144/SP395.17.

722   86.   Hughes, T. P.; Anderson, K. D.; Connolly, S. R.; et al. Spatial and temporal patterns of mass bleaching of corals in
723         the Anthropocene. *Science (80-. ).* **2018**, 359(6371):80-83. doi:10.1126/science.aan8048.

724   87.   Courtney, T. A.; De Carlo, E. H.; Page, H. N.; et al. Recovery of reef-scale calcification following a bleaching event

725          in Kāne'ohe Bay, Hawai'i. *Limnol. Oceanogr. Lett.* **2018**, 3(1):1-9. doi:10.1002/lol2.10056.

726  88.   Page, H. N.; Courtney, T. A.; Collins, A.; De Carlo, E. H.; Andersson, A. J. Net community metabolism and seawater
727          carbonate chemistry scale non-intuitively with coral cover. *Front. Mar. Sci.* **2017**, 4(MAY):161.
728          doi:10.3389/fmars.2017.00161.

729  89.   Baker, A. C.; Glynn, P. W.; Riegl, B. Climate change and coral reef bleaching: An ecological assessment of long-term
730          impacts, recovery trends and future outlook. *Estuar. Coast. Shelf Sci.* **2008**, 80(4):435-471.
731          doi:10.1016/j.ecss.2008.09.003.

732  90.   Bates, N. R.; Amat, A.; Andersson, A. J. Feedbacks and responses of coral calcification on the Bermuda reef system
733          to seasonal changes in biological processes and ocean acidification. *Biogeosciences*. **2010**, 7(8):2509-2530.
734          doi:10.5194/bg-7-2509-2010.

735  91.   Oh, T.; Sermsripong, J.; Hicks, B. W. Using scuba for in situ determination of chlorophyll distributions in corals by
736          underwater near infrared fluorescence imaging. *J. Mar. Sci. Eng.* **2020**, 8(1):53. doi:10.3390/JMSE8010053.

737  92.   Treibitz, T.; Neal, B. P.; Kline, D. I.; et al. Wide Field-of-View Fluorescence Imaging of Coral Reefs. *Sci. Rep.* **2015**, 5.
738          doi:10.1038/srep07694.

739  93.   Kenkel, C. D.; Traylor, M. R.; Wiedenmann, J.; Salih, A.; Matz, M. V. Fluorescence of coral larvae predicts their
740          settlement response to crustose coralline algae and reflects stress. *Proc. R. Soc. B Biol. Sci.* **2011**, 278(1718):2691-2697.
741          doi:10.1098/rspb.2010.2344.

742  94.   Roth, M. S.; Deheyn, D. D. Effects of cold stress and heat stress on coral fluorescence in reef-building corals. *Sci. Rep.*
743          **2013**, 3(1):1-8. doi:10.1038/srep01421.

744  95.   Salih, A.; Larkum, A.; Cox, G.; Kühl, M.; Hoegh-Guldberg, O. Fluorescent pigments in corals are photoprotective.
745          *Nature*. **2000**, 408(6814):850-853. doi:10.1038/35048564.

746  96.   Ramesh, C. H.; Koushik, S.; Shunmugaraj, T.; Ramana Murthy, M. V. A rapid in situ fluorescence census for coral
747          reef monitoring. *Reg. Stud. Mar. Sci.* **2019**, 28:100575. doi:10.1016/j.rsma.2019.100575.

748  97.   Patterson, M. R.; Sebens, K. P.; Olson, R. R.; et al. *In Situ Measurements of Flow Effects on Primary Production and Dark
749          Respiration in Reef Corals*. Vol 36.,; 1991.

750  98.   Schreiber, U.; Schliwa, U.; Bilger, W. Continuous recording of photochemical and non-photochemical chlorophyll
751          fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* **1986**, 10(1-2):51-62.
752          doi:10.1007/BF00024185.

753  99.   Beer, S.; Ilan, M.; Eshel, A.; Weil, A.; Brickner, I. Use of pulse amplitude modulated (PAM) fluorometry for in situ
754          measurements of photosynthesis in two Red Sea faviid corals. *Mar. Biol.* **1998**, 131(4):607-612.
755          doi:10.1007/s002270050352.

756  100.  Ralph, P.; Gademann, R.; Larkum, A.; Schreiber, U. In situ underwater measurements of photosynthetic activity of
757          coral zooxanthellae and other reef-dwelling dinoflagellate endosymbionts. *Mar. Ecol. Prog. Ser.* **1999**, 180:139-147.
758          doi:10.3354/meps180139.

759  101.  Winters, G.; Loya, Y.; Röttgers, R.; Beer, S. Photoinhibition in shallow-water colonies of the coral *Stylophora pistillata*
760          as measured in situ. *Limnol. Oceanogr.* **2003**, 48(4):1388-1393. doi:10.4319/lo.2003.48.4.1388.

761  102.  Piniak, G. A.; Storlazzi, C. D. Diurnal variability in turbidity and coral fluorescence on a fringing reef flat: Southern
762          Molokai, Hawaii. *Estuar. Coast. Shelf Sci.* **2008**, 77(1):56-64. doi:10.1016/j.ecss.2007.08.023.

763  103.  Roder, C.; Wu, Z.; Richter, C.; Zhang, J. Coral reef degradation and metabolic performance of the scleractinian coral

764  Porites lutea under anthropogenic impact along the NE coast of Hainan Island, South China Sea. *Cont. Shelf Res.*
765  **2013**, 57:123-131. doi:10.1016/j.csr.2012.11.017.

766  104.  Banaszak, A. T.; Ayala-Schiaffino, N.; Rodriguez-Romàn, A.; Enriquez, S.; Iglesisas-Prieto, R. Response of Millepora
767        Alcicornis (Milleporina: Milleporidae) to Two Bleaching Events at Puerto Morelos Reef, Mexican Caribbean -
768        PubMed. *Rev. Biol. Trop.* **2003**, 51(4):57-66. Accessed June 29, 2020. https://pubmed.ncbi.nlm.nih.gov/15264554/.

769  105.  Frade, P. R.; Englebert, N.; Faria, J.; Visser, P. M.; Bak, R. P. M. Distribution and photobiology of Symbiodinium
770        types in different light environments for three colour morphs of the coral Madracis pharensis: Is there more to it
771        than total irradiance? *Coral Reefs*. **2008**, 27(4):913-925. doi:10.1007/s00338-008-0406-3.

772  106.  Okamoto, M.; Nojima, S.; Furushima, Y.; Nojima, H. Evaluation of coral bleaching condition in situ using an
773        underwater pulse amplitude modulated fluorometer. *Fish. Sci.* **2005**, 71(4):847-854. doi:10.1111/j.1444-
774        2906.2005.01036.x.

775  107.  Suggett, D. J.; Kikuchi, R. K. P.; Oliveira, M. D. M.; Spanó, S.; Carvalho, R.; Smith, D. J. Photobiology of corals from
776        Brazil's near-shore marginal reefs of Abrolhos. *Mar. Biol.* **2012**, 159(7):1461-1473. doi:10.1007/s00227-012-1925-6.

777  108.  Ralph, P. J.; Schreiber, U.; Gademann, R.; Kühl, M.; Larkum, A. W. D. Coral photobiology studied with a new
778        imaging pulse amplitude modulated fluorometer. *J. Phycol.* **2005**, 41(2):335-342. doi:10.1111/j.1529-8817.2005.04034.x.

779  109.  Kromkamp, J. C.; Forster, R. M. The use of variable fluorescence measurements in aquatic ecosystems: Differences
780        between multiple and single turnover measuring protocols and suggested terminology. *Eur. J. Phycol.* **2003**,
781        38(2):103-112. doi:10.1080/0967026031000094094.

782  110.  Suggett, D. J.; Oxborough, K.; Baker, N. R.; MacIntyre, H. L.; Kana, T. M.; Geider, R. J. Fast repetition rate and pulse
783        amplitude modulation chlorophyll *a* fluorescence measurements for assessment of photosynthetic electron transport
784        in marine phytoplankton. *Eur. J. Phycol.* **2003**, 38(4):371-384. doi:10.1080/09670260310001612655.

785  111.  Gorbunov, M. Y.; Falkowski, P. G.; Kolber, Z. S. Measurement of photosynthetic parameters in benthic organisms
786        in situ using a SCUBA-based fast repetition rate fluorometer. *Limnol. Oceanogr.* **2000**, 45(1):242-245.
787        doi:10.4319/lo.2000.45.1.0242.

788  112.  Lombardi, M. R.; Lesser, M. P.; Gorbunov, M. Y. Fast repetition rate (FRR) fluorometry: Variability of chlorophyll a
789        fluorescence yields in colonies of the corals, Montastraea faveolata (w.) and Diploria labyrinthiformes (h.) recovering
790        from bleaching. *J. Exp. Mar. Bio. Ecol.* **2000**, 252(1):75-84. doi:10.1016/S0022-0981(00)00238-0.

791  113.  Lesser, M.; Gorbunov, M. Diurnal and bathymetric changes in chlorophyll fluorescence yields of reef corals
792        measured in situ with a fast repetition rate fluorometer. *Mar. Ecol. Prog. Ser.* **2001**, 212:69-77. doi:10.3354/meps212069.

793  114.  Behrenfeld, M. J.; Kolber, Z. S. Widespread iron limitation of phytoplankton in the south pacific ocean. *Science (80-.
794        ).* **1999**, 283(5403):840-843. doi:10.1126/science.283.5403.840.

795  115.  Falkowski, P. G.; Kolber, Z. Variations in chlorophyll fluorescence yields in phytoplankton in the world oceans.
796        *Aust. J. Plant Physiol.* **1995**, 22(2):341-355. doi:10.1071/PP9950341.

797  116.  Lesser, M.; Gorbunov, M. Diurnal and bathymetric changes in chlorophyll fluorescence yields of reef corals
798        measured in situ with a fast repetition rate fluorometer. *Mar. Ecol. Prog. Ser.* **2001**, 212:69-77. doi:10.3354/meps212069.

799  117.  Levy, O.; Achituv, Y.; Yacobi, Y. Z.; Dubinsky, Z.; Stambler, N. Diel "tuning" of coral metabolism: Physiological
800        responses to light cues. *J. Exp. Biol.* **2006**, 209(2):273-283. doi:10.1242/jeb.01983.

801  118.  Levy, O.; Dubinsky, Z.; Achituv, Y. Photobehavior of stony corals: Responses to light spectra and intensity. *J. Exp.
802        Biol.* **2003**, 206(22):4041-4049. doi:10.1242/jeb.00622.

803    119.    Schneider, K.; Levy, O.; Dubinsky, Z.; Erez, J. In situ diel cycles of photosynthesis and calcification in hermatypic
804             corals. *Limnol. Oceanogr.* **2009**, 54(6):1995-2002. doi:10.4319/lo.2009.54.6.1995.

805    120.    Patterson, M. R.; Sebens, K. P.; Olson, R. R. In situ measurements of flow effects on primary production and dark
806             respiration in reef corals. *Limnol. Oceanogr.* **1991**, 36(5):936-948. doi:10.4319/lo.1991.36.5.0936.

807    121.    Gattuso, J.-P.; Yellowlees, D.; Lesser, M. Depth-and light-dependent variation of carbon partitioning and utilization
808             in the zooxanthellate scleractinian coral Stylophora pistillata. *Mar. Ecol. Prog. Ser.* **1993**, 92:267-276.

809    122.    Okazaki, R. R.; Swart, P. K.; Langdon, C. Stress-tolerant corals of Florida Bay are vulnerable to ocean acidification.
810             *Coral Reefs.* **2013**, 32(3):671-683. doi:10.1007/s00338-013-1015-3.

811    123.    Nakamura, T.; Nakamori, T. A geochemical model for coral reef formation. *Coral Reefs.* **2007**, 26(4):741-755.
812             doi:10.1007/s00338-007-0262-6.

813    124.    Wild, C.; Rasheed, M.; Jantzen, C.; et al. Benthic metabolism and degradation of natural particulate organic matter
814             in carbonate and silicate reef sands of the northern Red Sea. *Mar. Ecol. Prog. Ser.* **2005**, 298:69-78.
815             doi:10.3354/meps298069.

816    125.    Ferrier-Pagès, C.; Gevaert, F.; Reynaud, S.; et al. In situ assessment of the daily primary production of the temperate
817             symbiotic coral *Cladocora caespitosa. Limnol. Oceanogr.* **2013**, 58(4):1409-1418. doi:10.4319/lo.2013.58.4.1409.

818    126.    Camp, E. F.; Krause, S. L.; Santos, L. M. F.; et al. The "Flexi-Chamber": A novel cost-effective in situ respirometry
819             chamber for coral physiological measurements. *PLoS One.* **2015**, 10(10):1-21. doi:10.1371/journal.pone.0138800.

820    127.    Nakamura, T.; Nakamori, T. Estimation of photosynthesis and calcification rates at a fringing reef by accounting for
821             diurnal variations and the zonation of coral reef communities on reef flat and slope: A case study for the Shiraho
822             reef, Ishigaki Island, southwest Japan. *Coral Reefs.* **2009**, 28(1):229-250. doi:10.1007/s00338-008-0454-8.

823    128.    Pramneechote, P.; Sinutok, S.; Wongkamhaeng, K.; Chotikarn, P. An assessment of the net ecosystem metabolism
824             and respiration of a tropical coral reef. *Appl. Ecol. Environ. Res.* **2020**, 18(1). doi:10.15666/aeer/1801_18631881.

825    129.    Wangpraseurt, D.; Polerecky, L.; Larkum, A. W. D.; et al. The in situ light microenvironment of corals. *Limnol.
826             Oceanogr.* **2014**, 59(3):917-926. doi:10.4319/lo.2014.59.3.0917.

827    130.    Yates, K. K.; Halley, R. B. Measuring coral reef community metabolism using new benthic chamber technology. *Coral
828             Reefs.* **2003**, 22(3):247-255. doi:10.1007/s00338-003-0314-5.

829    131.    Gevaert, F.; Delebecq, G.; Menu, D.; Brutier, L. A fully automated system for measurements of photosynthetic
830             oxygen exchange under immersed conditions: an example of its use in *Laminaria digitata* (Heterokontophyta:
831             Phaeophyceae). *Limnol. Oceanogr. Methods.* **2011**, 9(9):361-379. doi:10.4319/lom.2011.9.361.

832    132.    Shepard, A.; Dinsmore, D.; Miller, S.; Cooper, C.; Wicklund, R. Aquarius undersea laboratory: the next generation.
833             Published online 1996. Accessed July 14, 2020. archive.rubicon-foundation.org/xmlui/handle/123456789/4703.

834    133.    Carpenter, L. W.; Patterson, M. R.; Bromage, E. S. Water flow influences the spatiotemporal distribution of heat
835             shock protein 70 within colonies of the scleractinian coral Montastrea annularis (Ellis and Solander, 1786) following
836             heat stress: Implications for coral bleaching. *J. Exp. Mar. Bio. Ecol.* **2010**, 387(1-2):52-59.
837             doi:10.1016/j.jembe.2010.02.019.

838    134.    Carpenter, L. W.; Patterson, M. R. Water flow influences the distribution of photosynthetic efficiency within colonies
839             of the scleractinian coral Montastrea annularis (Ellis and Solander, 1786); implications for coral bleaching. *J. Exp.
840             Mar. Bio. Ecol.* **2007**, 351(1-2):10-26. doi:10.1016/j.jembe.2007.05.022.

841    135.    Biscéré, T.; Lorrain, A.; Rodolfo-Metalpa, R.; et al. Nickel and ocean warming affect scleractinian coral growth. *Mar.*

842            *Pollut. Bull.* **2017**, 120(1-2):250-258. doi:10.1016/j.marpolbul.2017.05.025.

843    136.    Haas, A. F.; Nelson, C. E.; Rohwer, F.; et al. Influence of coral and algal exudates on microbially mediated reef
844            metabolism. *PeerJ*. **2013**, 2013(1):e108. doi:10.7717/peerj.108.

845    137.    Dellisanti, W.; Tsang, R. H. L.; Ang Jr., P.; Wu, J.; Wells, M. L.; Chan, L. L. A diver-portable respirometry system for
846            in-situ short-term measurements of coral metabolic health and rates of calcification. *Front. Mar. Sci.* **2020**, 7:932.
847            doi:10.3389/FMARS.2020.571451.

848    138.    Roth, F.; Rädecker, N.; Carvalho, S.; et al. High summer temperatures amplify functional differences between coral-
849            and algae-dominated reef communities. *Ecology*. Published online October 17, 2020. doi:10.1002/ecy.3226.

850    139.    Mcgillis, W. R.; Langdon, C.; Loose, B.; Yates, K. K.; Corredor, J. Productivity of a coral reef using boundary layer
851            and enclosure methods Coastal Ocean Observing: Platforms, Sensors and Systems View project BIOWATT View
852            project Productivity of a coral reef using boundary layer and enclosure methods. *Artic. Geophys. Res. Lett.* Published
853            online 2011. doi:10.1029/2010GL046179.

854    140.    Long, M. H.; Berg, P.; de Beer, D.; Zieman, J. C. In Situ Coral Reef Oxygen Metabolism: An Eddy Correlation Study.
855            Davies, A., ed. *PLoS One*. **2013**, 8(3):e58581. doi:10.1371/journal.pone.0058581.

856    141.    Wangpraseurt, D.; Larkum, A. W. D.; Ralph, P. J.; Kühl, M. Light gradients and optical microniches in coral tissues.
857            *Front. Microbiol.* **2012**, 3(AUG). doi:10.3389/fmicb.2012.00316.

858    142.    Takeshita, Y.; McGillis, W.; Briggs, E. M.; et al. Assessment of net community production and calcification of a coral
859            reef using a boundary layer approach. *J. Geophys. Res. Ocean.* **2016**, 121(8):5655-5671. doi:10.1002/2016JC011886.

860    143.    McGillis, W. R.; Langdon, C.; Loose, B.; Yates, K. K.; Corredor, J. Productivity of a coral reef using boundary layer
861            and enclosure methods. *Geophys. Res. Lett.* **2011**, 38(3):n/a-n/a. doi:10.1029/2010GL046179.

862    144.    Takeshita, Y.; Cyronak, T.; Martz, T. R.; Kindeberg, T.; Andersson, A. J. Coral Reef Carbonate Chemistry Variability
863            at Different Functional Scales. *Front. Mar. Sci.* **2018**, 5(MAY):175. doi:10.3389/fmars.2018.00175.

864    145.    McMahon, A.; Santos, I. R.; Schulz, K. G.; Cyronak, T.; Maher, D. T. Determining coral reef calcification and primary
865            production using automated alkalinity, pH and pCO2 measurements at high temporal resolution. *Estuar. Coast. Shelf*
866            *Sci.* **2018**, 209:80-88. doi:10.1016/j.ecss.2018.04.041.

867    146.    Cyronak, T.; Andersson, A. J.; Langdon, C.; et al. Taking the metabolic pulse of the world's coral reefs. *PLoS One*.
868            **2018**, 13(1):1-17. doi:10.1371/journal.pone.0190872.

869    147.    Shashar, N.; Cohen, Y.; Loya, Y. Extreme diel fluctuations of oxygen in diffusive boundary layers surrounding stony
870            corals. *Biol. Bull.* **1993**, 185(3):455-461. doi:10.2307/1542485.

871    148.    Weber, M.; Faerber, P.; Meyer, V.; et al. In situ applications of a new diver-operated motorized microsensor profiler.
872            *Environ. Sci. Technol.* **2007**, 41(17):6210-6215. doi:10.1021/es070200b.

873    149.    Wangpraseurt, D.; Weber, M.; Røy, H.; et al. In situ oxygen dynamics in coral-algal interactions. *PLoS One*. **2012**,
874            7(2). doi:10.1371/journal.pone.0031192.

875    150.    Brown, B. E.; Dunne, R. P.; Goodson, M. S.; Douglas, A. E. Experience shapes the susceptibility of a reef coral to
876            bleaching. *Coral Reefs*. **2002**, 21(2):119-126. doi:10.1007/s00338-002-0215-z.

877    151.    Berg, P.; Røy, H.; Janssen, F.; et al. Oxygen uptake by aquatic sediments measured with a novel non-invasive eddy-
878            correlation technique. *Mar. Ecol. Prog. Ser.* **2003**, 261:75-83. doi:10.3354/meps261075.

879    152.    Long, M. H.; Berg, P.; de Beer, D.; Zieman, J. C. In Situ Coral Reef Oxygen Metabolism: An Eddy Correlation Study.

880          Davies, A., ed. *PLoS One*. **2013**, 8(3):e58581. doi:10.1371/journal.pone.0058581.

881     153.    Rovelli, L.; Attard, K. M.; Bryant, L. D.; et al. Benthic $O_2$ uptake of two cold-water coral communities
882             estimated with the non-invasive eddy correlation technique. *Mar. Ecol. Prog. Ser.* **2015**, 525:97-104.
883             doi:10.3354/meps11211.

884     154.    de Froe, E.; Rovelli, L.; Glud, R. N.; et al. Benthic Oxygen and Nitrogen Exchange on a Cold-Water Coral Reef in the
885             North-East Atlantic Ocean. *Front. Mar. Sci.* **2019**, 6:665. doi:10.3389/fmars.2019.00665.

886     155.    Takeshita, Y.; McGillis, W.; Briggs, E. M.; et al. Assessment of net community production and calcification of a coral
887             reef using a boundary layer approach. *J. Geophys. Res. Ocean.* **2016**, 121(8):5655-5671. doi:10.1002/2016JC011886.

888     156.    Mass, T.; Brickner, I.; Hendy, E.; Genin, A. Enduring physiological and reproductive benefits of enhanced flow for
889             a stony coral. *Limnol. Oceanogr.* **2011**, 56(6):2176-2188. doi:10.4319/lo.2011.56.6.2176.

890     157.    Doo, S. S.; Edmunds, P. J.; Carpenter, R. C. Ocean acidification effects on in situ coral reef metabolism. *Sci. Rep.* **2019**,
891             9(1):1-8. doi:10.1038/s41598-019-48407-7.

892     158.    Kline, D. I.; Teneva, L.; Schneider, K.; et al. A short-term in situ $CO_2$ enrichment experiment on Heron Island (GBR).
893             *Sci. Rep.* **2012**, 2(1):1-9. doi:10.1038/srep00413.

894     159.    Albright, R.; Takeshita, Y.; Koweek, D. A.; et al. Carbon dioxide addition to coral reef waters suppresses net
895             community calcification. *Nature*. **2018**, 555(7697):516-519. doi:10.1038/nature25968.

896     160.    Albright, R.; Caldeira, L.; Hosfelt, J.; et al. Reversal of ocean acidification enhances net coral reef calcification. *Nature*.
897             **2016**, 531(7594):362-365. doi:10.1038/nature17155.

898     161.    Albright, R.; Takeshita, Y.; Koweek, D. A.; et al. Carbon dioxide addition to coral reef waters suppresses net
899             community calcification. *Nature*. **2018**, 555(7697):516-519. doi:10.1038/nature25968.

900     162.    Shaw, E. C.; Phinn, S. R.; Tilbrook, B.; Steven, A. Natural in situ relationships suggest coral reef calcium carbonate
901             production will decline with ocean acidification. *Limnol. Oceanogr.* **2015**, 60(3):777-788. doi:10.1002/lno.10048.

902     163.    Anthony, K. R. N.; A. Kleypas, J.; Gattuso, J. P. Coral reefs modify their seawater carbon chemistry - implications for
903             impacts of ocean acidification. *Glob. Chang. Biol.* **2011**, 17(12):3655-3666. doi:10.1111/j.1365-2486.2011.02510.x.

904