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# **Coral Microcosms: Challenges and Opportunities for Global Change Biology**

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Patrick Schubert and Thomas Wilke

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<http://dx.doi.org/10.5772/intechopen.68770>

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## **Abstract**

Well-maintained coral-microcosm systems provide a good opportunity for performing global-change simulations under controlled conditions and allow long-term experiments while avoiding problems with natural fluctuations. However, despite rapid technical progress over the last few years in maintaining corals, microcosm experiments remain demanding and challenging. Therefore, this paper focuses on problems and opportunities associated with maintaining corals for global-change experiments, and the pitfalls associated with simulating natural and anthropogenic disturbances. We start in Section 1 with a brief assessment of the global situation of coral reefs and discuss problems and challenges associated with microcosm experiments. Section 2 covers the technical setup of coral-aquarium systems in respect to the necessary hardware and safety precautions. Section 3 provides information on coral-species selection, coral-propagation techniques, and the choice of associated fauna and flora. Problems with maintaining controlled conditions are deliberated in Section 4, including water chemistry as well as pest and disease control. The paper closes with conclusions for global-change studies in coral-microcosm systems (Section 5). As this review provides important insights into the rapidly developing field of coral-microcosm experiments, it might be of particular interest for graduate and post-graduate students in marine sciences, for global-change researchers, as well as for administrators and technicians interested in maintaining corals under fully-controlled conditions.

**Keywords:** aquaculture, coral propagation, environmental change, experimental design, reef aquarium, Scleractinia, simulation studies

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## 1. Introduction

Coral reefs belong to the most diverse and valuable ecosystems on Earth. They offer habitats for ca. 100,000 to >500,000 described species [1, 2] and the actual number might be higher by one magnitude [3]. Coral reefs also provide crucial ecosystem services as an important source of food for humans, as natural coastal defence, and as a recreational resource. Moreover, the biodiversity in coral reefs is seen as an important source for drug discovery [4].

Reef-building corals cover a total surface area of 260,000–600,000 km<sup>2</sup> [2]. They are typically restricted to latitudes between 25°N and 25°S. The optimal water temperature for most species is 23–29°C, and the optimal salinity is 32–42 parts per thousand (ppt). In addition, zooxanthellate corals require abundant light, restricting them to the euphotic zone of the oceans [5].

Reef-building corals are very sensitive to environmental change, both natural and anthropogenic, and it is estimated that around 50% of the world's reefs are threatened by human activities and that about 20% of the reefs have been destroyed already [6]. Major threats include destructive fishing practices and overfishing, habitat destruction, pollution, eutrophication, changes in food webs, unsustainable tourism, sedimentation, global warming and ocean acidification (e.g. [7, 8]).

There is ample empirical evidence for the adverse effects of these stressors on particular coral species or even entire reefs, and some of the causal links between environmental disturbances and biological responses of corals, such as increasing water temperature and coral bleaching or decreasing ocean-water pH and reduced calcification rates [9–11], are well understood.

However, as most adverse effects are multifactorial, a precise assessment of the individual contribution of stressors in natural systems, particularly those related to global change, remains challenging [12, 13]. Besides additive effects, multiple stressors could also act synergistically or antagonistically [14]. In fact, for developing effective management strategies, the individual contribution of stressors is of the utmost concern, enabling stakeholders to identify the most important parameters in a particular system. Therefore, scientists are interested in quantifying both the individual and combined effects of stressors acting on reef-building corals.

Although numerous field observations are being carried out to address these problems, studies in natural systems are typically confounded by the presence of variables other than those of interest. The relationship can thus be characterized, at best, as correlative, and a direct inference of cause and effect remains difficult [15]. In particular, environmental problems at the global scale can typically not be addressed using traditional scientific experiments [16]. The latter authors also argued that microcosms experiments (i.e. "experimental ecological systems at a small spatial scale") using model organisms could be a suitable methodology for addressing global problems, such as ecosystem responses to climate change.

Microcosms enable the manipulation of a single or few variables, and to compare the effects on organisms over time against control conditions. However, unlike natural systems, microcosm experiments are an abstraction from reality, and no single setup might explain the complex impact of global change on populations, species, and communities. Instead, each setup may help answer a specific question [15]. Besides generating such specific knowledge, microcosm studies can also help develop theories and meaningful policy implications [16].

Coral-microcosm experiments are a relatively new approach. Only some 30 years ago, several technical breakthroughs were achieved, enabling researchers to keep corals healthy in closed tanks [17]. However, controlled laboratory experiments add a level of complexity to keeping corals in aquarium systems, particularly in long-term microcosm studies. To ensure a stable growth of corals and to avoid a potential bias introduced by unintended variations of system parameters, a broad spectrum of environmental factors has to be regulated [18, 19]. Given the complex chemical nature of seawater and the dynamics of biological consumption processes, this remains a challenging task. Growth rates of corals, for example, are largely controlled by the  $\text{Ca}^{2+}$  content and alkalinity of the seawater. As growth processes deplete the water of  $\text{Ca}^{2+}$ , differential growth rates also have a differential feedback effect on the  $\text{Ca}^{2+}$  level and alkalinity of the water. These problems are of particular concern for global-change studies involving manipulations of  $\text{CO}_2$  and pH levels [15, 18]. Similar problems are of concern for the choice of associated animal (e.g. herbivorous fish) and plant species (e.g. coralline algae) to be maintained in the microcosm system for enabling a healthy growth of corals. Further challenges lie in the selection of the general experimental setup (e.g. size of tanks, natural vs. synthetic seawater, single vs. multiple water-circulation systems) and in the choice of the technical equipment (e.g. type of lighting, circulation and control systems).

All these considerations may have a profound impact on the quality of the data generated, on associated costs, on the maximum possible duration of the experiment, and on its susceptibility to failure. Moreover, microcosm experiments are increasingly being designed for long-term durations to enable an assessment of evolutionary adaptations of corals. Finally, a wealth of technical novelties has been introduced to the market in recent years. Therefore, complex decisions have to be made by the experimenter prior to the setup of coral-microcosm experiments.

For these reasons, this article aims at discussing the challenges and opportunities of utilizing coral microcosms for global-change studies. Based on literature reviews, expert interviews, and our own >15 years of experiences with maintaining stony corals, we inform about the technical setup of coral microcosms in Section 2, provide information on the study organisms in Section 3, discuss problems of maintaining controlled conditions in Section 4, and finish with conclusions concerning setup and operation of coral microcosms for global-change studies (Section 5).

The insights provided might be of particular interests for graduate and postgraduate students in marine sciences, for global-change researchers, for technicians and animal keepers, as well as for decision makers responsible for the administrative planning of coral-microcosm facilities.

## **2. Setup of coral-microcosm systems**

### **2.1. Experimental design**

Maintaining stony corals in tanks is a challenging task. Conducting controlled (long-term) microcosm experiments adds another level of complexity. Besides comprehensive technical, biological, and chemical knowledge, extensive experiences with experimental design and the manipulation of environmental variables are required in order to perform these experiments in a way that compelling conclusions can be drawn from the data generated [17, 18, 20, 21].

Many technical (e.g. size and number of tanks, choice of technical equipment) and biological decisions (e.g. study species, associated fauna and flora) have to be made prior to the setup of microcosm experiments. However, the first and most critical step is the selection of the general experimental design based on the study question, the study species chosen, and the intended duration of the experiment. Several key decisions have to be made. They include the choice of (i) natural versus synthetic seawater, (ii) open versus semi-closed versus closed systems and iii) number of water-circulation systems to be implemented.

Natural and synthetic seawater differ in various characteristics of significance for coral-microcosm experiments (see **Table 1**). Of particular interest are availability, overall quality, consistency, and toxicity. Particularly in coastal areas, natural seawater is readily available. The chemical composition of off-shore seawater is usually highly consistent. However, the overall quality strongly varies with source, mean of transportation (e.g. hygiene of ballast tanks, containers and delivery pumps), and subsequent treatment. A principal problem of natural seawater is chemical and biological contamination. In particular, the high abundance and diversity of bacteria, viruses, archaea, algae and fungi are of concern. Whereas for standard marine aquarium purposes, natural microbial communities could jumpstart the biological cycle in the system and can be an important source of food for vertebrates and invertebrates, the adverse impact on coral global-change experiments in microcosm setups could be considerable. Corals are holobionts that can adjust the composition of their microbial endosymbionts depending on environmental conditions. Therefore, natural seawater makes it more

	Synthetic seawater	Natural seawater
Availability	Always available	Depends on location and infrastructure available
Quality	Usually high, variation in consistency possible, low toxicity	Depends on the source of water and the transport process, consistency typically high, water often contaminated
Treatment	Only basic treatment required (dissolution in deionized or reverse-osmosis purified water, control/adjustment of pH, temperature and salinity)	Often complex treatment necessary (e.g. ultra-filtration, dark-treatment, ultraviolet sterilization, chlorination)
Costs	Medium	Low to high, depends on location, source and treatment
Pros for microcosm experiments	High quality, no contamination, readily available	Natural and consistent chemical composition, enables studies with natural microbial communities
Cons for microcosm experiments	Often variable chemical composition, deionized or reverse-osmosis water required for preparation	Biological and chemical contamination possible, often requires filtration and decontamination, cannot be stored over long periods of time

**Table 1.** Properties of synthetic and natural seawater.



difficult to maintain controlled conditions throughout the experiment. It requires ultrafiltration as well as extensive decontamination prior to use (e.g. chemical decontamination, dark or ultraviolet treatment). This, however, creates a new set of problems (e.g. the need for dechlorination after chlorine treatment). Moreover, due to its higher toxicity, natural seawater may perform inferior as a culture medium for juvenile invertebrates, compared to synthetic seawater [22, 23]. Besides a low toxicity, another advantage of synthetic seawater is its ready availability and overall high quality. However, chemical consistency may vary among brands, and sometimes even within lots and individual packages. Moreover, high-quality deionized or reverse-osmosis water is required for preparing synthetic seawater.

The second major decision to be made with regard to the planning of coral-microcosm setups is the question of closed versus semi-closed versus open systems (reviewed in [24]). According to the latter author, closed microcosm systems are barred from exchange of food energy, seawater, as well as associated fauna and flora. They only allow gas exchange, freshwater refill to replace evaporation and exchange of light and heat energy. By contrast, semi-closed systems also allow for partial seawater exchange to maintain water quality by replacing inorganic nutrients and trace elements. Finally, open systems permit gas and seawater exchange, but also moderate inputs of supplemental food for associated faunas as well as the replacement of dead plants and animals [17, 25, 26]. The major goal of coral-microcosm experiments is not self-sufficiency of the system but the manipulation of a single or few variables by maintaining all other variables as constant as possible. Therefore, closed systems are, in many cases, impractical as a constant water quality for the demanding stony corals cannot be assured (e.g. metabolized trace elements have to be replaced). Possible exceptions are short-term experiments. Whether semi-closed or open microcosm systems are more appropriate largely depends on the duration of the experiment, the study species, as well as the associated fauna and flora. Maintaining near-natural and relatively constant conditions in coral microcosms over a long time often requires the addition of fishes and other animals, which typically depend on external food supplies. Moreover, deceased animals and plants have to be replaced. Thus, long-term coral-microcosm experiments are mostly designed as open systems (*sensu* [24], see also **Figure 1**).

The third principal decision concerns the number of water-circulation systems to be implemented. This, in turn, depends on the statistical design of the experiment and the study question. In most cases, a design with a single water-circulation system is preferred in order to keep all but one or few target variables constant. This enables, for example, the exchange of planktonic organisms (including pathogens and parasites) throughout the system. Moreover, the associated larger water volume makes the system less susceptible to unintended fluctuations of water parameters. However, particularly for experiments studying the effects of biotic factors (e.g. the composition of microbial communities in seawater or the impact of pathogens) or chemical parameters (e.g. toxins), a single water-circulation system may be impractical. Another possibility for stabilizing water circulation is to integrate a larger 'buffer' tank containing fish for nutrient intake and a deep-sand bed for biological filtration (**Figure 1**). Moreover, an algae filter with an inverse lighting regime might compensate for diurnal fluctuations of pH values [27, 28].

*Recommendations:* The experimental setup is largely determined by the scientific question of interest, study species, associated fauna and flora (if applicable), and the intended duration of



**Figure 1.** 'Ocean 2100' global-change simulation experiment at Justus Liebig University Giessen. Nine experimental coral tanks (three hidden) are connected via a technical tank (not shown) to a 'buffer' reef tank with live rocks and a deep-sand bed (right). This long-term setup (intended duration 10 years) has been designed as an open microcosm system with synthetic seawater and a single water-circulation system. The replicate system (not shown here) has its own water-circulation system and 'buffer' tank.

the experiment. In fact, the longer the duration of the experiment, the more detailed planning is needed. Unless natural seawater of high quality is readily available or the composition of its natural microbial community is of interest, synthetic seawater is favoured in microcosm experiments. In that case, high-quality products should be preferred and whole packages must always be used for preparing the water [17, 25]. Moreover, important parameters (e.g. pH, alkalinity, salinity) have to be checked prior to use. Closed coral-microcosm systems are typically only applicable for short-term experiments (over few weeks) without the need for associated faunas (such as herbivorous fish). For medium-term experiments (several months) without associated faunas, semi-closed setups are preferable. Long-term experiments (several years) or setups that require supplementary food supply are typically designed as open microcosm systems (**Figure 1**). For semi-closed and open setups, a seawater exchange of at least 20% per month is recommended [28]. As for the water-circulation systems in coral microcosms, the least number with the largest effective volume should be chosen for each experimental system (for a review of the statistical needs in global-change experiments, see [18]).

## 2.2. Lighting

Light is fundamental to all photosynthetic processes and thus crucial for zooxanthellate corals [5]. Defined and controlled light conditions are also important for assuring reproducible

results obtained from coral-microcosm experiments. Among others, light (1) affects density and photosynthetic activity of hermatypic corals [29], (2) increases calcification rates in hermatypic corals [30], (3) influences the activity of associated faunas such as diurnal fishes [31, 32], (4) affects the metabolic efficiency of corals and thus survival [33, 34] and (5) influences the phenotype of scleractinian corals [35].

Although most stony corals require abundant light with a broad spectrum, conditions are often species-specific [36]. Under controlled microcosm conditions, it is therefore important to meet the requirements of the study species for achieving near-natural growth rates and physiological responses [37]. Too little light may, for example, decrease the metabolic efficiency and growth rates in stony corals, or may cause shifts in phenotype morphology [35]. Too much light could burn the zooxanthellae or cause coral bleaching [38].

For most coral-microcosm setups, no sufficient natural light is available. Therefore, artificial lighting has to be used with the appropriate intensity and colour spectrum. This is a challenging task as these characteristics, for example, change with water depth. Today, four popular artificial lighting systems are available, which differ in some of the main characteristics of relevance for global-change experiments in stony corals (see **Table 2** and **Figure 2**): T5 fluorescent lamps, metal-halide lamps, LED lamps and light-emitting plasma lamps.

The widespread introduction of metal-halide lamps into reef aquariums some 30 years ago made it possible to maintain stony corals with comparatively low effort, and for many years they have been the standard lighting equipment. They are well suited for high water columns, can be fitted to suit a wide range of tank sizes, are available with different colour temperatures and have, in general, a well-balanced spectrum. Disadvantages are their relatively low energy efficiency, a short lifetime and a high heat production.

The decline of metal-halide lamps over the past 10 years is mostly due to improvements in T5 fluorescent lighting, making the latter very popular for reef aquariums [39]. It is more energy efficient than metal-halide lamps, comes in a wide range of colour temperatures and its spectral characteristic is relatively good. Moreover, the spread of light is comparatively even, enabling relatively constant conditions across experimental tanks. However, similar to metal-halide lamps, T5 tubes have a comparably short lifetime. Moreover, light intensity and spectrum change over time, and spectral characteristics are affected by ambient temperature. T5 lighting is only suitable for shallow tanks.

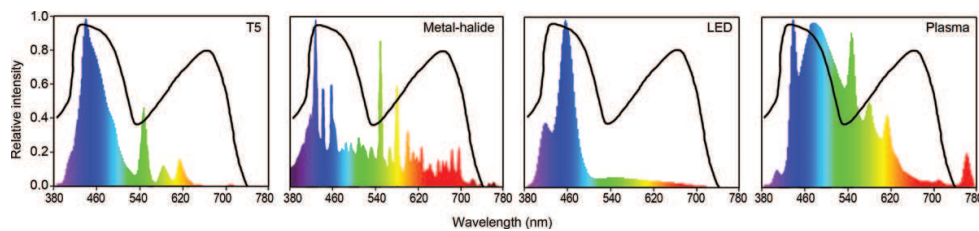
Very recently, LED lighting has advanced to the point where it can be used to maintain stony corals, as long as the quality of light meets the requirement of the study organism [40]. High-quality LED lighting combines excellent energy efficiency with long-term stability of spectrum and intensity. The spread of light can be controlled by lenses for individual LEDs. In sophisticated systems, intensity and colour temperature can be adjusted electronically, though achieving natural spectra remains a problem. As the respective colour spectrum is produced by an array of individual LEDs, partial failure of LEDs, which is often difficult to detect, changes the spectrum. Moreover, some LED lighting systems require active cooling, making them vulnerable to humidity and salt deposits. Finally, some coral species appear to be sensitive to LED light (Schubert, unpublished data).



	<b>T5 fluorescent lamp</b>	<b>Metal-halide lamp</b>	<b>LED lamp</b>	<b>Plasma lamp</b>
Technology	Gas-discharge lamp that uses internal electrodes	Metal-halide lamp that produces light by an electric arc	Light-emitting diode lamp	Gas-discharge lamp that uses an electric or magnetic field
Acquisition cost	Low	Medium	Medium to high	High
Maintenance requirements	Medium	Medium	Medium	Low
Energy efficiency	Medium	Low	High	High
Reliability	Medium	Medium	Low to medium	High
Lifetime	Ca. 10 months	Ca. 10 months	>48 months*	>48 months*
Spectral coverage	Good	Good	Good	Good
Spectral and brightness stability over lifetime	Change over time	Change over time	Relatively constant over time*	Relatively constant over time
Effective water column height	Up to ca. 60 cm	Up to several meters	Up to several meters	Up to several meters
Spread of light	Very even	Even	Depends on the lenses used, lamps may flicker	Even
Pros for microcosm experiments	Various types with different characteristics available	Common and well established	Some LED lamps allow an individual adjustment of spectral composition, little waste heat released to the water	Various types with different characteristics available, little waste heat released to the water, IP68 housings available
Cons for microcosm experiments	Spectrum affected by ambient temperature and age of bulb, air-cooled lamps often affected by humidity and salt	Much waste heat released to the water, decreasing number of manufacturers	High ambient temperature, humidity and mineral deposits decrease reliability and lifetime of the lamp, failure of individual LEDs often hard to detect	So far, no long-term experiences available
*High-quality lamps				

**Table 2.** Properties of popular artificial lighting systems used for coral-microcosm experiments.

The very latest editions to reef-aquarium lighting systems are modern plasma lamps. They are highly energy efficient, show a long-term stability of spectrum and intensity, and spectrum and colour-temperature can be custom-tailored by the manufacturer. Moreover, the spread of light is very even and typically no active cooling is necessary, allowing for the construction of housings according to the IP66 or IP68 standards. Though the equipment is still very expensive, energy and maintenance costs are very low. Thus, lifetime costs might be the lowest of



**Figure 2.** Irradiance spectra of common light sources. T5: ATI AquaBlueSpezial and BluePlus (ATI, Hameln, Germany); Metal-halide: BLV 12,000 K, 250 W (BLV, Steinhoring, Germany); LED: Orphek Atlantik V4 (Orphek, Sao Paulo, Brazil); Plasma: TGS029, 150 W (Aqua ECO Store, Kaltenkirchen, Germany). Spectra were generated using a Lighting Passport Spectrometer (Asensetek, New Taipei City, Taiwan). Black curves indicate PAR reference.

all lighting systems discussed. However, due to their recent introduction, so far no long-term experiences exist for the application of plasma lamps to coral-microcosm systems [36, 40].

*Recommendations:* The choice of lighting system for coral-microcosm experiments largely depends on the specific parameters investigated, the study species and the intended duration of the experiment. In general, high-quality T5, LED or plasma lamps should be considered. Some lighting systems are optimized to enhance coral growth and to ‘improve’ the colour intensity of the corals maintained. They are thus not suitable for most global-change experiments. All lamps/tubes used in a system should be at the same stage of lifetime. Open lighting systems have to be protected against heat, mineral deposits and water. All systems should be maintained regularly, which may also include the control of light intensity and spectrum. This is particularly important for LED lamps and respective hand-held LED testers and light metres are available on the market.

### 2.3. Water movement

Water movement in aquarium systems is crucial to the vitality of stony corals [41]. A controlled movement is also critical for obtaining reproducible results in global-change studies using microcosm setups. Among others, water movement increases the exchange rate of gases and thus photosynthetic efficiency [42], increases mass-transfer of materials across the tissue-water interface [43, 44], increases food capture and thus energy supply to the coral [43, 45], facilitates cleaning of corals and prevents build-up of detritus [46], and influences the phenotype of scleractinian corals [35].

Most stony corals are adapted to strong water movement and/or wave action [47, 48]. Insufficient water movement may, for example, enhance detritus and sediment build-ups, and could thus cause unintended and unpredictable local processes in nutrient balance (nitrification and denitrification). It may also foster the emergence of anaerobic zones in tanks, affects the biological filtration rate of the system and thus facilitates uneven growth rates of corals across experimental tanks. Excessive and/or strongly concentrated water movement, on the contrary, may increase the stress level of some corals, damage sensitive species and cause atypical growth forms.

Three popular systems for generating water movement in coral microcosms are available: (1) water-flow systems where pumps create a laminar or a turbulent water movement,

(2) water-oscillation systems (e.g. Wavebox®), which set the entire water body in motion and (3) water-spill systems where a water bucket equipped with a tilting mechanism creates a regular wave motion (**Table 3**).

Of these systems, the water-flow system is most commonly used. One or more pumps either create a laminar (i.e. streamlined) or turbulent (i.e. irregular or mixed) flow. Turbulent flows are typically found in oceans in water depths of less than 12–15 m, and laminar flows in depths more than that [49]. For generating the water flow, radial-flow and axial-flow velocity pumps are typically used [50]. The latter are preferred because the water flow is more uniform. Water-flow systems are relatively cost-efficient and can be installed in most tank systems. Disadvantages are that the direction and intensity of water movement vary across the tank. Moreover, flow velocity will be higher at the periphery of a bushy coral compared to its centre.

In recent years, another water-movement technology, the water-oscillation system (such as the Wavebox; Tunze, Penzberg, Germany), has made its way into coral-microcosm systems. A Wavebox consists of one or more axial-flow-pulsing pumps and a controller. Determined by tank resonance, the intermittent operation of the system sets the entire water body in motion, assuring water movement in all parts of the tank [51]. Maximal displacement at either end of the tank is several centimetres. Another advantage of the oscillating nature of water movement is the uniform growth morphometry of the corals seen in such systems. Disadvantages are the robustness of the construction required due to the resonance generated and the possible interferences with other tanks, the need to place the water overflow in the central part of the tank, and the need for additional pumps in larger systems to create a linear flow.

The third approach, water-spill systems, is less common and typically used for specific purposes [50]. It is a wave machine that usually consists of a bucket equipped with a tilting mechanism. The bucket is filled with water and once the water level reaches a certain level, it tips over and releases the water to create a spill. Water-spill systems are ideally suited to simulate

	Water-flow system	Water-oscillation system	Water-spill system
Technology	Radial- or axial-flow pumps	Pulsing pump with controller	Tilting water bucket
Acquisition cost	Low to medium	High	Low to high
Maintenance requirements	Low to medium	Low to medium	Low
Efficiency	Medium to high	High	High
Reliability	Medium to high	Medium to high	High
Pros for microcosm experiments	Applicable to tanks of various shapes and sizes of 20 to >20,000 L	High efficiency, optimal water movement, near-natural growth morphology of corals	Adaptable to all tank sizes
Cons for microcosm experiments	Low energy efficiency, high amount of waste heat	Restricted to rectangular, medium-sized tanks (0.6–3.5 m length)	Mostly individually manufactured

**Table 3.** Properties of popular water-movement systems for coral-microcosm experiments.

wave actions in littoral zones. They can be adapted to all tank sizes. As only few commercial products are available, custom-made solutions are typically required.

*Recommendations:* The choice of water-movement systems for coral-microcosm experiments depends on the specific parameters investigated, the study species, and the size and shape of the tanks used. The water-flow system, though widely distributed, might not be suitable for most questions related to global change due to difficulties in ensuring relatively constant conditions throughout the tank. A possible exception is the study of corals that live in water depths characterized by laminar water movements. The application of water-spill systems is also restricted to specific research questions. They are mainly suitable for studying processes in coral species that live in the littoral zone. By contrast, water-oscillation systems are applicable to a wide range of questions and species. They produce a relatively homogeneous water movement, thus preventing a potential bias of the study results. Though there are some size restrictions on the tanks (see **Table 3**), most coral-microcosm setups might fall within the suitable range. For larger systems, several Waveboxes can be combined and/or complemented with axial-flow pumps to increase water flow.

## 2.4. Miscellaneous supporting hardware

Coral reefs are characterized by relatively stable water conditions, and most inhabitants react very sensitively to sudden changes in environmental parameters [52]. Moreover, fluctuations in water chemistry may be caused by the reef organisms themselves due to metabolic processes. In natural systems, the physiological impact of organisms on water parameters is limited due to the comparatively low ratio of biomass and water volume [26]. However, compensating for these problems in microcosm systems remains challenging (see also Section 2.1.). This concerns the water chemistry but also other ‘tank effects’ such as temperature fluctuations, microbial contamination and the accumulation of pollutants.

With the growing popularity of seawater aquariums in general and reef aquariums in particular, the selection of devices and methods for controlling and maintaining healthy conditions for stony corals has significantly increased. However, some of the available commercial solutions and products are not suitable for a precise control of parameters; others may cause harmful side effects in coral-microcosm setups, and yet others have efficiency and reliability issues. Therefore, the appropriate equipment/method typically has to be chosen based on the design and specific goal of the microcosm experiment.

One of the most significant challenges in coral-microcosm experiments is to assure near-natural calcification conditions. In particular,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  levels have to be maintained, and alkalinity has to be stabilized [27, 53, 54]. Three approaches are commonly used: the calcium reactor, the Balling method and the Kalkwasser stirrer (**Table 4**). A calcium reactor is filled with  $\text{CaCO}_3$  material (such as coral rubble), which slowly dissolves when the pH value is lowered through the addition of  $\text{CO}_2$  [28, 55]. The efficiency of a calcium reactor largely depends on the type and size of the reactor, flow-through rate, type and grain size of the substrate used, as well as the pH value set. An alternative approach is the ‘Balling method’, that is, the individual addition of pre-mixed solutions of  $\text{CaCl}_2$ ,  $\text{MgCl}_2$  and  $\text{NaHCO}_3$  [17, 56]. The approach works well for short-term studies. However, for long-term experiments it requires considerable analytic



	Calcium reactor	Balling method	Kalkwasser stirrer
Principle	Dissolution of aragonite or lime through CO <sub>2</sub> enrichment and low pH	Individual addition of CaCl <sub>2</sub> , MgCl <sub>2</sub> and NaHCO <sub>3</sub>	Dissolves Ca(OH) <sub>2</sub>
Acquisition cost	Medium to high	Low	Medium
Operating cost	Medium	High	Low
Effecting pH	Decreases pH values	No effect	Increases pH values
Water volume	Applicable to tanks of various shapes and sizes of 200 to >20,000 L	Applicable to all tank sizes	Applicable to all tank sizes
Pros for microcosm experiments	Simultaneous increase of alkalinity and Ca <sup>2+</sup> content, easy to handle	Individual adjustment of parameters, applicable to small water volumes	Efficient approach for increasing pH values
Cons for microcosm experiments	Risk of nutrient pollution (e.g. PO <sub>4</sub> <sup>3-</sup> ), requires addition of CO <sub>2</sub>	Requires high analytical efforts, risk of nutrient pollution, might cause ionic shifts that need to be compensated with NaCl-free sea salt	No alkalinity control, only marginal Ca <sup>2+</sup> control

**Table 4.** Properties of popular systems/methods for controlling Ca<sup>2+</sup> supply and alkalinity in coral-microcosm experiments.

efforts to avoid miscalculations and to prevent ionic shifts. The Kalkwasser method is an older approach for increasing the Ca<sup>2+</sup> content by adding Ca(OH)<sub>2</sub> to the refill water [17, 28, 55]. Though its efficiency for Ca<sup>2+</sup> control is relatively low, it may well be used for balancing daily fluctuations of pH values caused by photosynthetic activities [28].

Organic wastes and nutrients are typically removed from coral-reef tanks by protein skimmers (foam fractionators) and phosphate-binding agents. Protein skimmers are important for coral microcosms because they enable removing suspended particles and organic wastes before they enter the nutrient cycle [17, 28]. They are also of importance for increasing gas exchange, and constitute a good location for the application of ozone (see subsequent text). Besides removal of organic wastes, phosphate control is very important in coral-microcosm systems because phosphate enhances unwanted growth of algae and may inhibit calcification processes [54]. Though protein skimmers also help reduce phosphate concentrations in the water (particularly organic phosphate before it is converted into inorganic orthophosphate), phosphate-binding agents are more effective. However, the latter only help remove inorganic orthophosphate and not inorganic polyphosphate or organic phosphate. Thus, they may not mitigate an algae problem in the system as this is typically caused by organic phosphate. Various commercial phosphate-binding agents are available that are either based on aluminium oxide or on iron oxides and hydroxides [57], though the latter are preferred by most experts. It should be noted that, besides phosphate, these agents may also remove other chemical compounds such as heavy metals and silicate, which may or may not be desired.

Pollutants in reef aquariums, such as toxins, heavy metals, chlorine, ozone and drugs, are usually removed via chemical filtration with activated carbon. This popular filtration method may also eliminate water discoloration and plays an important role in the prevention of pollutant

accumulation. Activated carbon is most efficiently used in a special flow-through filter arranged as bypass or equipped with an own pump. The direction of water flow is always upwards to reduce the risk of clogging. Filters with high water-flow-through (fluidized bed reactor) and low water-flow-through (slow flux filter) are in use. The former devices maximize reaction surface and respond rapidly. Disadvantages are high-carbon-abrasive rates and potentially abrupt changes in water parameters. The latter devices allow for an efficient usage of the substrate and enable a constant water quality but may increase sedimentation rates facilitated by detritus.

Reduction of parasite and pathogen loads may also help ensuring the health and vitality of the study organisms and the long-term stability of coral-microcosm systems. Moreover, depending on the goal of global-change experiments, it could be necessary to prevent the exchange of zooxanthellae across experimental tanks. This is most efficiently done through a combined ozone/ultraviolet (UV) radiation treatment—particularly in long-term experiments. UV irradiation is mainly effective in preventing infestations with pelagic microorganism. It does not introduce any harmful substances into the system, and high-quality products need relatively little maintenance. However, UV sterilizers will not be able to fully eradicate pathogens and have only little effect on benthic microbes. By contrast, an ozonizer uses the highly reactive ozone to efficiently kill pathogens in aquarium waters. Moreover, the gas helps transforming ammonia to nitrate, thus further increasing water quality. A disadvantage of ozone treatment is the high toxicity of the gas [58].

For global-change experiments in coral microcosms, a reliable temperature control is also crucial [52]. Depending on the tank size and goal of the experiment, several possibilities for temperature control exist, such as control via room-temperature regulation (heating/cooling), via a temperature-controlled water bath, or via heating rods. However, the internal temperature regulation of common heaters is often not reliable, typically requiring an independent sensor system.

Finally, for maintaining constant water conditions throughout the coral-microcosm system, an efficient water circulation is necessary. Pump selection depends on the capacity needed, the design of the delivery head, efficiency and the amount of excess heat produced [59].

*Recommendations:* Maintaining proper calcification conditions for long-term experiments is best achieved via a calcium reactor, whereas for smaller-water volumes (<200 L) and short-term experiments the 'Balling method' may be sufficient. For calculating the proper dosage, online calculators and apps are available (e.g. AquaCalculator; <http://www.aquacalculator.com>). Organic wastes and nutrients are efficiently removed from the system utilizing a combination of a protein skimmer and phosphate-binding agents. The latter could be used in a filter housing equipped with a slow-flux filter. To avoid unwanted side effects, phosphate-binding agents should be used cautiously. Moreover, phosphate levels in the system should be regularly monitored, and it is important to understand that these agents may also remove other chemical compounds.

Pollutants are typically eliminated from the system via chemical filtration with activated carbon. Good results can be obtained with a slow-flux filter and a daily-short time increase of the flow-through rate. However, since carbon loses its effectiveness when the surface pores close, its frequent replacement is important for optimal filtration. Efficient pathogen control is best done by combining an ozonizer and a UV sterilizer. As ozone is harmful to marine organisms

and humans, excess gas must not enter the experimental tanks or the air and its application has to be monitored carefully.

Water temperature fluctuations should not exceed 1°C in 24 h. Temperature control can be best attained by controlling the lowest target water temperature in the system via room-temperature or water-bath temperature control. Higher temperature in individual tanks can then be achieved via heating rods. In the latter case, it is important to adjust the performance of the respective heating rods to tank size. Moreover, they need to be calibrated prior to the start of the experiment. More reliable, however, is the control of the water temperature through external, computer-based sensors. The internal temperature regulation of the heaters could then be used as a ‘backup system’ by adjusting it to 1°C above the target temperature.

Water circulation throughout the system can be achieved by using high-quality adjustable radial-flow pumps.

2.5. Safety and control systems

Coral-microcosm systems are often highly complex in terms of electrical and mechanical devices integrated, water parameters to be monitored and (dangerous) organisms to be maintained. This places high demands on the equipment used and the safety procedures implemented. Seawater, for example, is a good electrical conductor and also promotes corrosion. Thus, electrical hazards are of particular concern [28]. Moreover, minor failures such as a short-term deviation from the target temperature may endanger the success of the experiment and/or the health of the study organisms [17, 60].

Discussing all safety and control equipment required for coral-microcosm experiments is beyond the scope of this paper. However, important devices are listed in **Table 5** together with some basic recommendations.

Device	Function
Ground fault circuit interrupter (GFCI)	Reduces the risk of electric shock to humans and animals
Grounding probe	Reduces the risk of electrical shock to humans and animals
Uninterruptible power supply (UPS)	Buffers short-time power failures for the most important electrical devices (e.g. delivery pumps; measuring, monitoring and control systems) and prevents sensitive electronical devices from harmful power fluctuations; note that the capacity of most UPSs is too low to buffer all electrical devices in the system over a long period of time
Emergency power supply	Buffers power failures over an extended period of time; note that powering on emergency power generators might generate harmful spikes
Sensor system	Controls and monitors a wide range of parameters (e.g. water level, pH, temperature, O <sub>2</sub> content); systems are typically computer-controlled and linked to an alarm system
Alarm device	Triggers an alarm (acoustically, visually or via messaging) in case of malfunctions of devices or unusual readings
Webcam	Remotely monitors the system

**Table 5.** Important safety and control devices for coral-microcosm systems.

Coral-microcosm experiments also require the implementation of a set of safety measures, including hazard assessments, safety-related labelling, emergency plans, staff training and regular security checks. A well-trained and experienced staff will not only help reduce the risk of accidents but also ensure a relatively problem-free operation of the experiment.

*Recommendations:* Depending on the goal, setup and duration of the experiment, several safety and control devices have to be installed. They include GFCIs as well as grounding probes for electrical safety purposes, a UPS (ideally in combination with an emergency power generator) for the continuous supply of electricity, sensor and webcam systems for monitoring proper operations and water parameters, as well as an alarm device to inform about malfunctions. It is highly recommended to spread electrical devices over several power circuits, each equipped with an own GFCI, to minimize the impact of failure of individual devices and to reduce electromagnetic interferences among electronical devices, respectively.

Prior to commissioning the installations, the responsible person should conduct a specific hazard assessment of the system together with the safety officer of the institution. This should include an evaluation of potential hazards through technical and electrical devices, irradiation (e.g. UV light), chemical substances (e.g. ozone or CO<sub>2</sub>), as well as poisonous or otherwise dangerous marine organisms. This hazard assessment should also be used as a basis for the mandatory hazard-related labelling of devices and tanks, as well as for all staff-training measures to be conducted. Moreover, an emergency plan has to be developed and prominently displayed in the microcosm facility. Essential information should include, among others, the telephone numbers of the emergency poison centre and the institution's first-aiders. Finally, regular safety checks by a certified electrician and/or the safety officer of the institution should be conducted.

### 3. Study organisms

#### 3.1. Selection of coral species

Selecting the proper study species for coral microcosms is a challenging task. Though the choice of species should largely be determined by the study question, other considerations such as availability, maintainability and legal aspects (e.g. CITES regulations [61]) also matter.

Scleractinian corals are a diverse and evolutionary old group that date back >250 million years ago [62]. However, many coral species are cryptic and (morphological) identification is not always straightforward (e.g. *Stylophora* spp.). Moreover, environmental parameters such as water temperature, water depth, water current, as well as light and nutrient availability not only effect the composition of species assemblages but also adaptations within species, leading to a variety of morpho- and ecotypes. Thus, different populations show different susceptibilities to changes in abiotic and biotic parameters [17].

Of relevance for microcosm experiments is also the fact that some species are more difficult to maintain than others. Moreover, there may be strong interspecific competition among species (e.g. *Galaxea* spp. have sweeper tentacles of up to 20 cm in length). In addition, branching



coral species such as *Acropora formosa* can have growth rates of up to 2–3 cm per month, thus increasing space constraints in the tanks over time.

Another important practical consideration is the question whether wild or farmed corals should be used for the experiment. Corals taken from the wild are of particular interest if the natural composition of their endosymbionts is of concern. Moreover, often detailed information on the geographical origin and ecological setting is available. However, they typically need a long time of acclimatization to microcosm setups, growth rates are often lower and the susceptibility to diseases can be higher [17, 28]. Furthermore, permitting laws to collect and export/import specimens are typically stricter. By contrast, farmed corals are often healthier and more resistant in experimental systems than colonies taken from the wild [17, 28]. They are often readily available and some ‘clonal lab strains’ are being used across laboratories, enabling comparative analyses. However, their associated endosymbiont diversity may be depleted and/or altered, affecting coral growth and survivorship in experiments [17, 63]. Moreover, often limited information about their geographic origin is available. Finally, the selection of farmed coral species is much lower compared to wild-caught taxa.

*Recommendations:* Many coral species are cryptic and/or difficult to determine. Therefore, in some cases a molecular characterization of the study individuals might be necessary. Robust species such as *Pocillopora damicornis*, *A. formosa* and *Montipora digitata* are more appropriate for long-term studies than very sensitive ones. High growth rates and strong defence mechanisms of some species need to be considered for species and tank-size selection. As different populations show different susceptibilities to changes in abiotic and biotic parameters, precise information about the ecological and geographical origin of the study specimens might be important. Finally, the choice of wild versus farmed corals may have implications for questions concerning growth rates, disease susceptibility and endosymbiont composition.

### 3.2. Coral propagation

One of the biggest advantages of using stony corals for global-change microcosm experiments is the possibility of fragmenting larger individuals. Though some colonies may show intercolonial variation [37], individual fragments are typically considered to be ‘clones’ of the mother colony. This has benefits for the statistical design of the experiment as the same individual can be simultaneously exposed to different environmental parameters. Therefore, fragment propagation of scleractinian corals often forms the basis for coral-microcosm experiments.

However, fragmenting corals is not always straightforward. Whereas some species are relatively easy to handle (e.g. *M. digitata*), others need more care during fragmentation (e.g. *Catalaphyllia jardinei*) [28]. Moreover, the size of the fragments as well as the quality of maintenance will determine survival rates within the first weeks after fragmentation [64–66].

The minimum size of the fragment has been discussed in detail elsewhere [28, 64–66] and mainly depends on species, experiences with fragmenting, condition of the mother colony and maintenance conditions. Similar aspects apply to the actual fragmentation technique [17, 28]. Of concern are, for example, the size of the polyps (large-polyp vs. small-polyp stony corals)

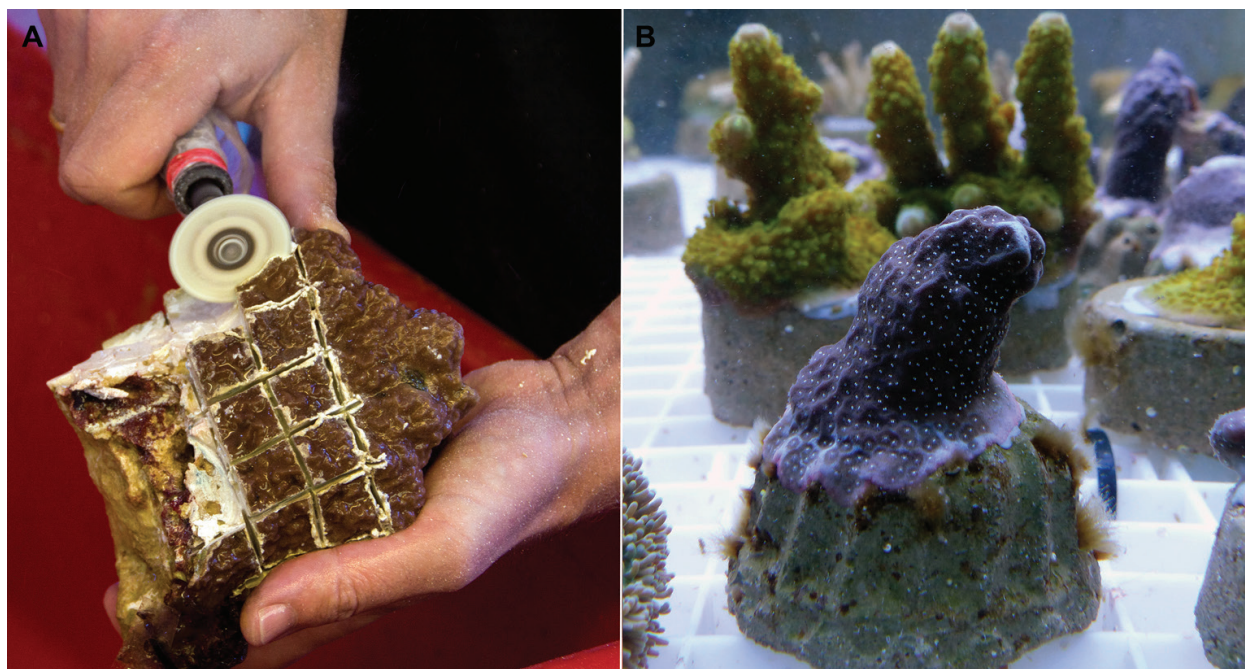
as well as shape (massive to fine-branched), hardness ('soft' to hard) and internal structure (dense to chambered) of the skeleton.

The most frequently used method involves a rotary tool equipped with a diamond-cutting disc (**Figure 3A**). It is applicable to most small-polyp stony coral species and works particularly well in species with a hard skeleton. For medium-hard and branched species, a coral clipper (bone cutter) is often used for the fragmentation of the mother colony. Finally, corals with a 'soft' skeleton such as *Alveopora* spp., *Goniopora* spp. and *Madracis* spp. are typically fragmented using a serrated knife.

All of the above methods may also cause injuries to humans through the tools used as well as through contact with toxic coral tissues or aerosols.

After fragmentation, different methods of treatment and rehabilitation can be realized. If the growth form of the corals is not of concern, the branched fragments are typically attached to a line hanging in the free water column. This approach reduces sedimentation and overgrowth by algae. If a more natural growth form is desired, the individual fragments are attached to a small pedestal using an adhesive [17, 28]. If the part of the coral that is to be connected to the pedestal is not covered by tissue, hot glue is used. Otherwise, cement, cyanoacrylate gel or epoxy adhesive provide good solutions. The latter two are particularly well suited for sensitive species and/or small fragments.

The choice of material for the pedestal depends on coral species and fragment size, as well as on the experimental design of the tanks. Common materials are unglazed tiles, specialized ceramic, plastic products ('reefplugs') or concrete. The latter can easily be used to produce custom-made structures with a range of labelling options (**Figure 3B**).



**Figure 3.** (A) Fragmentation of a *Porites rus* colony using a rotary tool. (B) Coral fragment attached to a concrete pedestal 4 weeks after propagation. Note: for the handling of some species or multiple colonies, the use of laboratory gloves is recommended to avoid human injuries or to minimize the risk of disease transmission among colonies, respectively.

*Recommendations:* The method to be used for fragmenting corals largely depends on the species, the size of the fragments as well as the treatment after propagation. All tools should be clean and sterile to avoid a potential transmission of coral diseases. The mother colony and fragments should be exposed as short as possible to the air, and high or low air temperatures must be avoided. Safety precautions have to be taken to prevent injuries to humans. Depending on method and species, this may include wearing laboratory gloves, safety glasses and a respirator. For most species, best fragmentation results are achieved by first superficially cutting the coral with a rotary tool and then carefully breaking off the fragment by hand, or using pliers or a small chisel. However, the heat generated by the cutting disc may harm the coral. If the fragments have to be mounted on individual pedestals, best results are obtained with cyanoacrylate gel and epoxy adhesive, though the former may dissolve in seawater after some time. A versatile and easy-to-handle material is low-pollutant Portland cement. To keep stress levels in the corals low, all fragments should remain within the origin water cycle for at least 1 week after fragmenting. Moreover, a slightly reduced light intensity and sufficient water movement might facilitate rehabilitation. To reduce sedimentation and to improve water circulation around the fragments, an elevated position within the tank might be helpful. This can be achieved, for example, via aquarium eggcrates ('lighting grids') (**Figure 1**).

3.3. Associated species

Semi-open and open coral microcosms often require the addition of associated species to ensure near-natural conditions, stabilize the system and facilitate the health of the corals [67, 68]. This applies in particular to long-term experiments. However, adding additional species also increases the complexity of the experiment and may affect the reproducibility of the data. A comprehensive discussion of individual species is beyond the scope of this paper. However, information for some of the most commonly associated organisms is listed in **Table 6**.

Taxon	Pros	Cons
Fishes		
<i>Acanthurus</i> spp.	Control macroalgae and periphyton	Large size, aggressive species
<i>Chaetodon</i> spp.	Control <i>Aiptasia</i> spp.	Feed on large-polyp stony corals
<i>Chelmon rostratus</i>	Controls <i>Aiptasia</i> spp.	Requires frozen food, may feed on <i>Tridacna</i> spp. and other invertebrates
<i>Chromis</i> spp.	Facilitate nutrient intake	–
<i>Ctenochaetus</i> spp.	Control periphyton	Potentially aggressive
<i>Halichoeres</i> spp.	Control some parasites (e.g. flatworms)	Require sand bed, feed on invertebrates
<i>Pseudochelinus</i> spp.	Control some parasites (e.g. flatworms)	Potentially aggressive, feed on invertebrates
<i>Salarias</i> spp.	Control periphyton	Need to be kept individually or in pairs
<i>Siganus</i> spp.	Control macroalgae	Large size, may be nervous, poisonous spines
<i>Synchiropus</i> spp.	Control some parasites (e.g. flatworms)	–
<i>Zebrasoma</i> spp.	Control macro algae and periphyton	Potentially aggressive

Taxon	Pros	Cons
<b>Mollusks</b>		
<i>Aeolidiella stephanieae</i>	Control <i>Aiptasia</i> spp.	Feed exclusively on <i>Aiptasia</i> spp.
<i>Euplicia</i> spp.	Control macroalgae and periphyton	–
<i>Nassarius</i> spp.	Control carrion and detritus	Require sand or detritus
<i>Stomatella</i> spp.	Control periphyton	–
<i>Tectus</i> spp.	Control periphyton	May relocate corals because of size
<i>Turbo</i> spp.	Control periphyton	May relocate corals because of size
<b>Crustaceans</b>		
Hermit crabs	Control periphyton and detritus	Larger species may damage corals
<i>Lysmata</i> spp. (peppermint shrimps)	Control <i>Aiptasia</i> spp.	May stress corals while removing food residues and mucus
<i>Mithrax</i> spp.	Control macroalgae and periphyton	–
<i>Percnon gibbesi</i>	Controls macroalgae and periphyton	Large size
<i>Stenopus</i> spp.	Control flatworms and polychaetes	–
<i>Trapezia</i> spp.	Control parasites and reduce sedimentation in bushy corals	–
<b>Echinoderms</b>		
Sea urchins	Control periphyton and encrusting algae	May relocate and/or feed on corals, some species are poisonous
<b>Macroalgae</b>		
<i>Halimeda</i> spp.	Easy to maintain and better to control than <i>Caulerpa</i> spp.	High calcification rate has to be compensated
<i>Chaetomorpha</i> spp.	Control nutrient levels and pH in algae filter	Floating, not attached to substrate

**Table 6.** Advantages and disadvantages of common associated species in coral-microcosm experiments.

Whereas larger organisms are often deliberately placed into the tanks, essential microorganisms are typically introduced with substrates such as sand, (live) rock and mud. They play an important role for stabilizing the water system, especially the nutrient cycle [69, 70]. Furthermore, the use of live rocks may significantly increase the risk of introducing diseases (see also Section 4.2.).

*Recommendations:* Associated species for coral-microcosm experiments have to be carefully selected, and species that feed on, stress and/or move corals should be generally avoided. Moreover, as associated species may influence the water parameters in the experimental tanks, each tank should contain the same species in the same quantities and with similar sizes. In some cases, it might be advisable to rotate associated species among tanks. As water parameters affected by substrate-bound microorganisms are difficult to control, it may be advantageous to refrain from using substrate within the individual experimental tanks. Instead, a



larger 'buffer tank' could be integrated in the water cycle, which contains a deep-sand bed as well as live rocks (**Figure 1**). The same may apply for some or all associated animal species discussed above.

#### 4. Quality control and maintenance of seawater

Slightest unintended variations in water parameters can cause significant effects to reef organisms [71–74]. Therefore, high-quality seawater is an important prerequisite for ensuring meaningful and comparable results in experimental systems [20, 26]. Some water parameters are relatively straightforward to measure and control, such as water temperature, pH and salinity. Others are more challenging to assess, including alkalinity,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  [14, 18, 75], and yet others, such as the concentration of many trace elements and some metabolic-degradation products, cannot be determined with standard water tests.

Therefore, water parameters in coral-microcosm systems are usually controlled and maintained through a set of common measures. This comprises the routine measurement of key water parameters, maintaining water levels and salinity in the system, regular exchange of parts of the seawater and active control of selected water parameters. Each coral-microcosm experiment requires a detailed plan for water testing. Whereas such a plan is also mandatory for regular reef aquariums, coral microcosms are even more demanding as fluctuations in water parameters have to be kept within narrow limits. A detailed description of all test procedures is beyond the scope of this paper and there is an extensive literature on this subject [75–78]. However, essential information can be found in **Table 7**.

Maintaining the water level and salinity in microcosm systems is a first step towards assuring a high quality of water parameters and to reduce unintended fluctuations. Strong water movements in combination with high air and water temperatures lead to high evaporation rates. To maintain salinity and other water parameters, the water volume in the system has to be kept constant. In coral microcosms, this is typically achieved through an automatic refill with deionized or reverse-osmosis water.

Fluctuations in chemical water parameters can also be mitigated through a regular and partial exchange of seawater in the system [17, 28]. This measure will help to replenish essential trace elements and reduce accumulation of harmful substances. Depending on the experimental design (e.g. filtration measures, biomass volume, feeding strategies), the exchange rate may vary between 20% per day and 20% per month [17, 26].

Though moderate seawater exchange helps stabilizing some water parameters, other factors such as alkalinity,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , pH,  $\text{PO}_4^{3-}$  and  $\text{NO}_3^-$  often require an active control (see also Section 2.4.). Adjusting nutrient levels in coral-microcosm systems (e.g.  $\text{PO}_4^{3-}$  and  $\text{NO}_3^-$ ) is even more difficult, particularly in long-term experiments. This is partly due to the fact that appropriate values for  $\text{PO}_4^{3-}$  and  $\text{NO}_3^-$  are close to the detection limit of most common water tests. Besides conducting partial water exchanges (see above), nutrient levels can also

Parameter	Target value*	Test frequency	Test method/equipment	Comments
Water temperature	24–28°C	Continuously or daily	Analogue or digital thermometer/sensors, data logging might be useful	Regular calibration required, at least two independent measurement systems required
Salinity	35 ppt	1 × per week	Analogue or digital refractometer	Regular calibration required
pH	8.1–8.3	Continuously or daily	Laboratory-grade pH meter, data logging might be useful	pH fluctuates during the day
Alkalinity	7–9 KH (2.5–3.2 mmol·L <sup>-1</sup> )	Daily to 2 × per week	Titration test	Use of standards is recommended, quality of commercial products differs considerably
Calcium (Ca <sup>2+</sup> )	380–440 mg·L <sup>-1</sup>	1–2 × per week	Titration test	Use of standards is recommended, quality of commercial products differs considerably
Magnesium (Mg <sup>2+</sup> )	1250–1350 mg·L <sup>-1</sup>	Biweekly	Titration test	Use of standards is recommended, quality of commercial products differs considerably
Phosphate (PO <sub>4</sub> <sup>3-</sup> )	<0.03 mg·L <sup>-1</sup>	1 × per week	Photometric test, colorimetric test	Available aquarium-grade kits often insufficient
Nitrate (NO <sub>3</sub> <sup>-</sup> )	<0.5 mg·L <sup>-1</sup>	1 × per week	Photometric test, colorimetric test	Available aquarium-grade kits often insufficient, some tests are not applicable to seawater
Nitrite (NO <sub>2</sub> <sup>-</sup> )	<0.1 mg·L <sup>-1</sup>	0.5–2 × per week**	Photometric test, colorimetric test	Available aquarium-grade kits often insufficient
Ammonia (NH <sub>3</sub> <sup>+</sup> /NH <sub>4</sub> <sup>+</sup> )	<0.1 mg·L <sup>-1</sup>	0.5–2 × per week**	Photometric test	Available aquarium-grade kits often insufficient
Redox potential	250–400 mV	Continuously	Laboratory-grade redox probe	Important for controlling ozone application

\*Value also depends on coral species and geographic origin.

\*\*Frequency of measurement depends on experimental design.

**Table 7.** Common water parameters to be monitored in coral-microcosm systems including typical target values, suggested test frequencies and test methods.

be reduced through a skimmer combined with an efficient water-flow-through system (see Section 2.4.). Other options include biological filters (see Section 2.4.) and chemical  $\text{PO}_4^{3-}$  adsorbers. Note that in some well-established coral tanks, nutrient values have to be increased and not decreased. This can be done through adding extra nutrients to the system [79] or by including associated animals, such as fish [80] (see Section 3.3.).

*Recommendations:* For maintaining salinity in larger microcosms, an automatic refill system for deionized or reverse-osmosis water equipped with double-protected sensors is recommended (see also Section 2.5.). The automatic refill system must be disabled during abstraction or exchange of saltwater.

Controlling alkalinity,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and pH requires a well-equipped laboratory (**Figure 4**; also see the technical recommendations provided in Section 2.4.). Particularly in systems with a high coral biomass, daily fluctuations of alkalinity need to be compensated. To buffer pH variations, either the addition of  $\text{Ca}(\text{OH})_2$  during the night or the use of an algae filter with an inverse lighting regime is suggested. All chemicals for controlling water parameters have to be administered individually and at places with a high water flow (e.g. outlet of pumps) to avoid precipitation. Moreover, if larger quantities of chemicals have to be added to the system, this should be done over a longer period of time. The use of special software tools (e.g. AquaCalculator; <http://www.aquacalculator.com>) is recommended.

If nutrients in the system are removed via a skimmer/water-flow-through system, skimmer size and water-circulation rates need to be adjusted carefully. As a rule of thumb, a 100-L tank requires a water-circulation rate of at least  $300 \text{ L} \cdot \text{h}^{-1}$ . If nutrient levels in the system need to be increased, particularly in long-term experiments, the use of carefully selected associated fish species might be less risky and achieves better results than the addition of extra nutrients. Special filter systems for nutrients, which are frequently used in fish aquacultures, are not recommended for coral-microcosm systems as accidentally released substances (e.g.  $\text{H}_2\text{S}$  or  $\text{NO}_2^-$ ) may jeopardize the entire system.



**Figure 4.** Laboratory workplace for seawater analyses. (1) Photometer, (2) test kits, (3) titration device with illuminated stirrer, (4) digital refractometer, (5) container for waste water and (6) lab shaker.

## 5. Pest and disease control

Maintaining stony corals and associated species in microcosms requires an effective pest and disease control. Particularly under experimental conditions, some usually inconspicuous organisms could become highly abundant [81], might compete with the study species for resources (e.g. light, nutrients or space) and may even prey on corals [28]. In addition, infectious diseases could be introduced into the system through animals, food and humans [17]. To minimize these risks, several procedures have to be implemented. They include a careful acclimation of study organisms, quarantine and prophylactic measures, a proper selection of associated species and the treatment of diseased corals.

When corals arrive at the facility, a slow acclimation to the new conditions is often suggested (but see the subsequent text). This is usually done via the drip method [17]. It eases acclimation stress both in the coral and its endosymbionts. After acclimation, the corals need to be inspected, unrelated organisms removed and the corals quarantined. Overlooked pest species or pest organisms introduced during the experiment should be eliminated through manual removal or chemical/biological treatment (see **Table 8**).

Moreover, a wide range of coral diseases is known [82–85] and the medication of diseased specimens is often problematic [81]. Typically, the infested tissue regions are removed, and the corals are treated with an iodine solution or other commercial products [17, 28]. Further spread of diseases may be reduced by covering infested parts with cyanoacrylate [17]. Often, the development of diseases is facilitated by inadequate water conditions. Therefore, an appropriate water exchange, filtration with activated carbon, or UV- and ozone treatments are typically used to reduce the risk of infection [86] (see also Section 2.4.). Note that UV- or ozone sterilization might unintentionally affect the exchange of symbionts among corals.

*Recommendations:* Experiences show that a slow-drip acclimation over ca. 30 min might be appropriate for most associated organisms. However, it might be better to directly transfer corals into fresh, temperature- and salinity-adjusted seawater (Schubert, unpublished data). Old transport water may contain high amounts of ammonium. If the pH rises during a slow acclimation process due to decreasing CO<sub>2</sub> levels, the proportion of toxic ammoniac will increase. This might harm the corals more than a sudden transfer into fresh seawater.

A common problem in newly arrived corals is flatworm infestations (e.g. *Amakusaplana* spp. [87]). Therefore, one treatment per week (10–20 min each) for a period of at least 2 weeks in an iodine bath is suggested. Particularly for *Acropora* spp., a prophylactic iodine treatment may be beneficial. Moreover, often it is useful to replace the complete base rock of the coral to reduce a potential parasite load. However, corals taken from the wild should be handled with particular care. At the beginning of quarantine, lighting should be dimmed to 50% and then gradually increased. After 3–4 weeks, the corals may be transferred to the experimental tanks.

Particularly in semi-closed and open microcosms, it is difficult to keep the coral tank free of pest species. They are best controlled through associated species such as fishes and invertebrates (**Table 8**). However, the former are often less suited for microcosm experiments due to their low abundances and more individual behaviour, potentially causing unintended differences among experimental tanks.



Pest species	Species/measures for pest control
<i>Aiptasia</i> spp. (Glass, rock or tube anemones)	<i>Aeolidiella stephanieae</i> (feeds exclusively on <i>Aiptasia</i> spp., high number of individuals needed for acute infestation or large tank, no preventive effect)  <i>Lysmata wurdemanni</i> , <i>L. rathbunae</i> , <i>L. seticaudata</i> ('peppermint shrimps') (preventive effect, well suited for smaller tanks, shy species with limited radius of action, might stress corals while removing feed from tentacles)  <i>Chelmon rostratus</i> (preventive effect, difficult to adapt to frozen food, may feed on other invertebrates)
<i>Amakusaplana</i> spp. (Acropora flatworms)	Treatment with iodine solution, fresh water or levamisole hydrochloride; removal of eggs (treatment might stress corals)  <i>Halichoeres cosmetus</i> , <i>H. marginatus</i> , <i>Thalassoma hardwicke</i> , <i>Pseudocheilinus hexataenia</i> (effectiveness uncertain, may also prey on other invertebrates)
<i>Convolutriloba</i> spp. (Acoelomorph flatworms)	<i>Halichoeres cosmetus</i> , <i>Synchiropus marmoratus</i> , <i>S. stellatus</i> (effectiveness uncertain)  <i>Chelidonura varians</i> (feeds exclusively on flatworms; specimens expensive—get easily sucked into pumps)  Manual removal; treatment with freshwater or levamisole hydrochloride (dying flatworms may secrete toxic substances)
<i>Embletonia</i> spp. (Montipora-eating nudibranchs)	<i>Halichoeres cosmetus</i> , <i>Pseudocheilinus hexataenia</i> (usually effective but may also prey on other invertebrates)
<i>Halofolliculina corallasia</i> (Ciliate that causes the Skeletal Eroding Band syndrome)	Improvement of water conditions, freshwater or iodine treatment (effectiveness uncertain)
<i>Heliocostoma</i> spp. (Ciliates that may cause Rapid Tissue Necrosis—'brown jelly')	Treatment with iodine solution or fresh water; removal of infested areas; improvement of water conditions (treatment might stress corals)

**Table 8.** Overview of common pest species and respective species/measures for pest control.

## 6. Conclusions

Coral-microcosm systems offer an excellent opportunity for performing global-change simulation studies under controlled conditions. They may thus help to identify the individual and combined effects of stressors acting on reef-building corals, to better understand stress response and resilience and to identify policy implications. However, coral-microcosm experiments are a relatively new approach. To avoid a potential bias caused by unintended variations of system parameters, a broad spectrum of environmental factors has to be regulated within narrow limits. In fact, maintaining healthy conditions for corals over a long period of time remains challenging. Therefore, several problems have to be addressed during planning, setup and operation of coral microcosms, and the following key recommendations should be considered:

- The experimental setup has to be determined by the scientific question of interest, study species, associated fauna and flora, and the intended duration of the experiment; the longer the duration of the experiment, the more detailed planning is required.
- Unless natural seawater of high quality is readily available or the composition of its natural microbial community is of interest, synthetic seawater should be preferred in microcosm experiments.
- Closed coral-microcosm systems are typically only applicable for short-term experiments without the need for associated faunas, semi-closed systems are preferred for medium-term experiments without associated faunas, and open microcosm systems for long-term experiments or experiments with associated animals.
- For lighting, high-quality T5, LED or plasma lamps should be considered, though the latter will likely become more popular in the future.
- For ensuring proper water movement, water-oscillation systems should be preferred in most global-change studies as they produce a relatively homogeneous water movement, thus preventing a potential bias of the study results.
- Maintaining good calcification conditions for long-term experiments is best achieved via a calcium reactor; for smaller water volumes or short-term experiments, the 'Balling method' may be sufficient.
- Pollutants are best removed from the system via chemical filtration with activated carbon; efficient pathogen control is best done by combining an ozonizer and a UV sterilizer.
- Water-temperature regulation can be best achieved by controlling the lowest target water temperature in the system via room temperature; higher temperature in individual tanks can then be attained with heating rods; temperature values should be controlled by two independent systems.
- Several safety and control devices should be installed, including GFCIs, grounding probes, a UPS ideally in combination with an emergency power generator, sensor systems and a malfunction alarm device.
- A specific hazard assessment has to be conducted, all devices and tanks necessitate a hazard-related labelling, all staff requires safety training, an emergency plan has to be developed and regular safety checks should be performed.
- All coral study species require proper species identification, and the choice of wild versus farmed corals should take the study question into account.
- As associated species in microcosms may influence water parameters, each tank should contain the same species in the same quantities and with similar sizes; potential adverse interactions with the study species have to be considered.

- Water parameters should be usually controlled and maintained through adjusting water levels and salinity in the system, regular exchange of parts of the seawater and active control of selected water parameters.
- All microcosms require an effective pest and disease control, including a careful acclimation of study organisms, quarantine and prophylactic measures, a proper selection of associated species, and the treatment of diseased corals.

In the years to come, we expect significant advances in coral-microcosm setups. They will likely involve improved lighting and water-circulation equipment, as well as sophisticated sensor systems for the continuous control of essential water parameters. Moreover, we might see important improvements in chemical water testing, aiming at quantifying essential trace elements and some metabolic-degradation products. This, in turn, may open new possibilities for closed microcosm setups and will likely further promote the use of synthetic seawater.

However, despite all technical improvements we may see in the future, the key factors for the success of global-change microcosm experiments are well-trained and dedicated people planning, setting up and operating the system. Therefore, the authors hope that this book chapter not only helps to better understand the advantages and pitfalls of coral-microcosm experiments, and the excellent opportunities such systems provide, but also encourages the reader to utilize this fascinating methodology for answering some of the key questions mankind faces relative to global-change processes in our 'rainforests of the oceans'.

## Acknowledgements

This work was supported by the CEMarin (Bogota, Colombia). We would like to thank Nina Paul (Leibniz-Zentrum für Marine Tropenforschung, Bremen, Germany), Kai Chepa (Vivarium, Staatliches Museum für Naturkunde, Karlsruhe, Germany), André Billion (pro marin, Giessen, Germany) and Thomas Tikatsch (Frankfurt Zoo, Frankfurt am Main, Germany) for the interesting discussions. Further thanks go to Ronny Schöpke (Bad Geiesbach, Germany) and Immo Gerber (Neuhausen ob Eck, Germany) for the information provided regarding LED and plasma lighting.

## Author details

Patrick Schubert\* and Thomas Wilke

\*Address all correspondence to: Patrick.Schubert@fg.bio.uni-giessen.de

Institute of Animal Ecology and Systematics, Justus Liebig University Giessen, Giessen, Germany

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