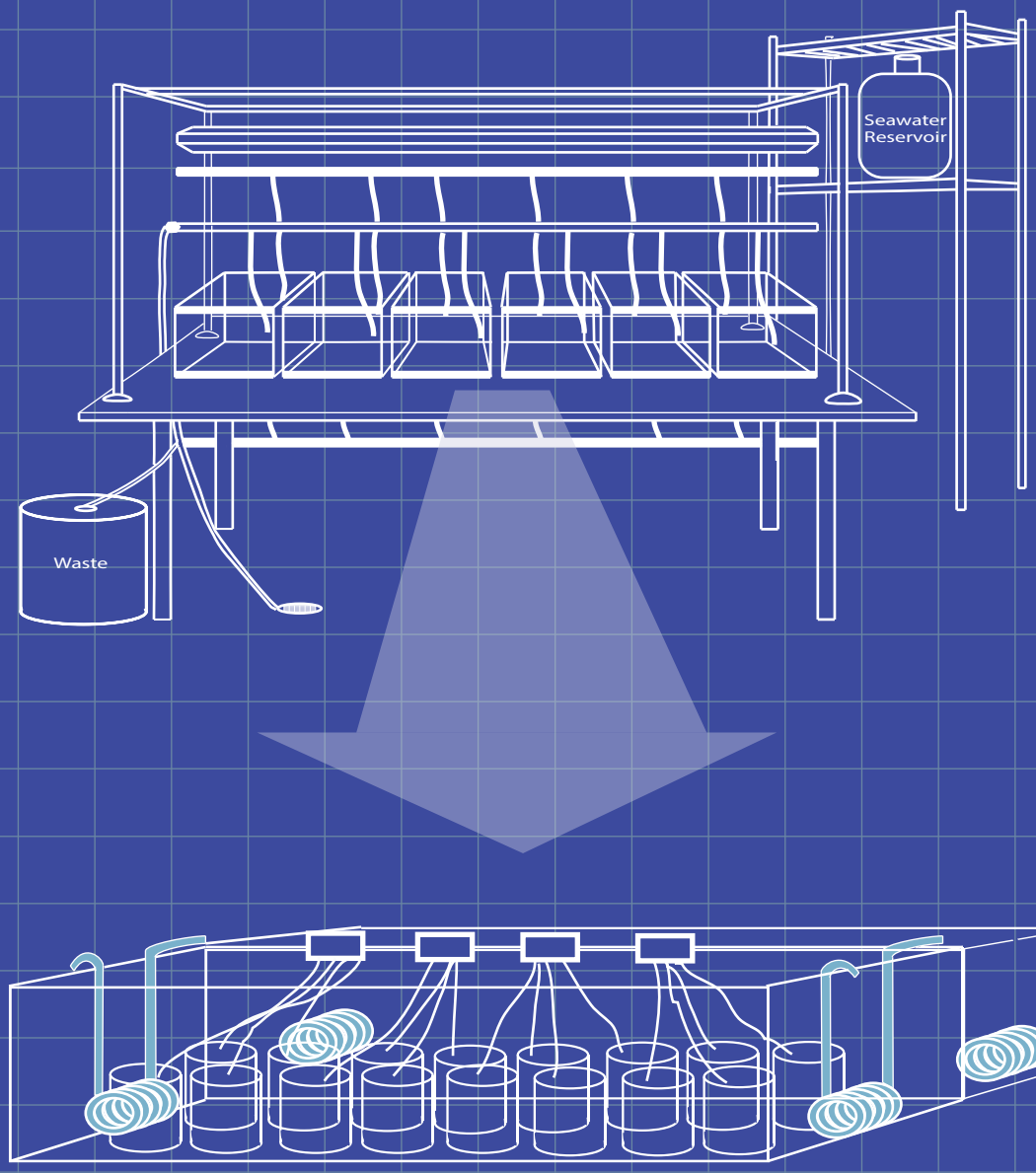
Coral Disease & Health Consortium
solutions today for reefs tomorrow.

NOAA Coral Reef Conservation Program

SMALL SCALE EXPERIMENTAL SYSTEMS FOR CORAL RESEARCH: CONSIDERATIONS, PLANNING, AND RECOMMENDATIONS



National Oceanic and Atmospheric Administration
U.S. Department of Commerce

March 2013

NOAA Technical Memorandum NOS NCCOS 165

NOAA Technical Memorandum CRCP 18

This report has been reviewed by the National Ocean Service of the National Oceanic and Atmospheric Administration (NOAA) and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for their use by the United States government.

Acknowledgements

The author would like to thank the following people for their guidance and review of this manuscript: Sylvia Galloway, Lisa May, Athena Avadanei, and Carl Miller. Cover illustration courtesy of Athena Avadanei. This project was funded in part by NOAA's Coral Reef Conservation Program and NOAA/NOS/NCCOS/CCEHBR.

About the CDHC

The Coral Disease and Health Consortium (CDHC) is a Working Group of the U.S. Coral Reef Task Force charged with organizing and coordinating the scientific resources of the U.S. and its territories to meet the challenge presented by globally declining coral reefs. Its mission is to preserve and protect the health of coral reef ecosystems through an understanding of the effects of natural and anthropogenic stressors on reef-building communities. The CDHC serves to unify the coral health and disease research community, identify research priorities, and encourage a new generation of coral researchers through education and outreach. The biomedical perspective and innovative technologies developed from Consortium efforts is envisioned to give scientists, resource managers, and industry new tools to identify and alleviate hidden stresses before they become environmental health crises. Currently over *125 partners,* including federal agencies, NOAA, DOI, EPA, along with academia, non-profit and industry, contribute their time and expertise to the CDHC, while organizational infrastructure is supported by the congressionally funded NOAA Coral Reef Conservation Program.

Citation for this Report

Bartlett, T.C. 2013. Small Scale Experimental Systems for Coral Research: Considerations, Planning, and Recommendations. NOAA Technical Memorandum NOS NCCOS 165 and CRCP 18. 68pp.

Small Scale Experimental Systems for Coral Research: Considerations, Planning, and Recommendations

Thomas C. Bartlett

Jardon & Howard Technologies, Inc.
NOAA, National Ocean Service
National Centers for Coastal Ocean Science
Center for Coastal Environmental Health and Biomolecular Research
Hollings Marine Laboratory
331 Fort Johnson Rd.
Charleston, South Carolina 29412-9110

NOAA Technical Memorandum NOS NCCOS 165 and CRCP 18

March 2013

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Only a few decades ago, keeping coral alive and healthy in closed, captive systems proved to be challenging. However, by the mid-1980s several breakthroughs were made and success with captive systems became more commonplace. In the short time since, there has been a surge of interest, knowledge, and technological advances with significant contributions from marine science, the aquarium industry, and aquarium hobbyists. Currently, there are several strategies for maintaining live coral outlined in many books, articles, and online resources. The majority of information now available focuses on success in either home and public aquaria (for aesthetic purposes) or commercial production.

Likewise, the difficulty in keeping coral in captivity has limited their use in well controlled laboratory experimentation. Much of the research on coral health and disease has been observational (vs. manipulative), and until recently, experimentation has been performed in a field setting which is subject to many uncontrollable variables, some measurable and some not. Furthermore, experimentation with infectious agents of coral diseases and toxicant effectors (i.e., chemical or biological agents eliciting a response) in the wild carries the risk of harming natural populations and raises ethical dilemmas. As a result of these issues, we and others are working to create laboratory life support systems for corals that can be used for experimentation under well controlled and monitored parameters. This is in contrast to the hobbyist or public aquaria approaches that focus on enhancing aesthetic properties (i.e., color, feeding behavior etc.) rather than approximating optimal physiological condition of the coral. Laboratory life-support systems strive to control as much variability as possible thus providing greater statistical power (i.e., the ability to detect significant differences from collected data).

In the following technical memorandum, techniques and procedures are presented to assist researchers in developing small experimental systems for coral and attempts to identify possible confounding factors to consider when setting up laboratory experiments with coral. The system features presented here are intended for relatively simple experiments when funding, space, and time (i.e., experimental duration from days to one or two months) are limiting. While focused on scleractinian coral, often referred to as stony or hard coral, the following information can be applicable to studies involving other cnidarian model organisms, such as anemones (Order Actiniaria) or soft coral (Order Alcyonacea).

Extensive and thorough planning is required before any scientific experimentation can begin. Cost and space constraints will be major factors when determining what experiments are feasible. The following is an overview of the experimental elements to be considered as part of the planning process.

Test species: relevance to study; source and ease/cost of acquisition; size and quantity; tolerance to effectors; growth rates, ability to propagate, and survivability in captivity (i.e., hardiness).

Experimental parameters: the stressor/effector to be studied; compatibility of construction material (e.g., plastic, glass, Teflon®) with testing parameters; design agreement with specific statistical analyses; duration of experiment.

Tank or vessel sizes and numbers: Based on coral species size and quantity, experimental parameters, duration of the experiment, and suitability to statistical analyses.

Lighting: artificial vs. natural; quantity (i.e., intensity and duration); quality (i.e., spectrum).

Water chemistry/quality control: maintaining critical parameters; reducing harmful metabolites; interval and quantity of water changes; impact on exposure material and waste generation.

Closed system vs. flow-thru: Dependent on effector to be studied and availability of natural seawater.

Other equipment/filtration: maintaining proper water quality and hydrodynamics; dependent on all the aforementioned considerations.

SECTION 1 – TEST SPECIES SELECTION

1.1 Relevance

The coral species chosen for any particular research should be based on its relevance to that particular study. Relevance must first be dictated by the experimental question being posed. These may involve considering a species' biogeography, importance to the reef community structure, current population status, and susceptibility to a specific disease.

While many stressors that affect coral health are ubiquitous, coral reefs have been impacted to various degrees in different geographic regions around the world (Table 1) with many localized even to specific embayments or watersheds. For example, if the question is whether a particular insecticide is toxic to coral, it is important to know where that particular chemical is being used and the species that are possibly at risk of encountering the pollutant as part of the species selection criteria.

TABLE 1. Coral reef regions that have been impacted the most by a particular environmental threat. Cumulative threats include all integrated local threats and thermal stress. Values are percentage of reef area at medium or greater risk. Adapted from Burke *et al.* 2011.

Threat	**Geographic Regions at Greatest Risk**	
Coastal Development	SE Asia (36%)	Indian (28%)
Watershed Pollution	SE Asia (45%)	Indian (32%)
Marine-based Pollution	Atlantic/Caribbean (23%)	Middle East (20%)
Thermal Stress	Atlantic/Caribbean (56%)	Indian (50%)
Cumulative	SE Asia (95%)	Atlantic/Caribbean (92%)

Similar to environmental threats, disease has affected coral reefs around the world to varying degrees. Specifically, disease refers to any impairment that interferes or modifies normal functions, including responses to environmental factors, infectious agents, and inherent or congenital defects (Wobeser 1981). More appropriately, it is the combination of many factors and co-factors that ultimately contribute to disease (Wobeser 1994). In many cases, the impact of disease is thought to be limited to certain species or genera (Table 2). Choosing the appropriate test species for disease research should take into consideration the corals' susceptibility to a specific disease. Again, biogeography will be an important factor since a coral species from one geographic location may be more or less resistant to a particular disease than one from a different location (e.g., *Porites lobata* from the Eastern Pacific vs. SE Asia).

Coral bleaching is an interesting example as it is often considered a form of disease and a symptom of environmental threats and climate change. While there will be reef communities that may never recover from a bleaching event, some "resistant" reef communities are able to tolerate or avoid bleaching (Grimsditch and Salm 2006; Obura and Grimsditch 2009); other

"resilient" communities may bleach but recover to their previous state more quickly (Sheppard *et al.* 2008; Lasagna *et al.* 2009; Obura and Grimsditch 2009). In addition, past exposure to stress may increase the tolerance of a coral species to future incidences of the same stress (Baird and Maynard 2008; Maynard *et al*. 2008). Thus for bleaching, it is important that the species selected for study is based on the research question being asked as this condition is a normal physiological response in some contexts and pathological in others.

TABLE 2. Some common diseases/syndromes affecting coral and the proposed causative agent. Species and causative agents have been separated by region when possible. Adapted primarily from Rosenberg *et al.* 2007.

Disease	Commonly Affected Species	Proposed Causative Agent
Bleaching†	*Oculina patagonica* (EM) *Pocillopora damicornis* (RS, IO)	*Vibrio shiloi* (EM) *Vibrio coralliilyticus* (RS, IO)
Black-band	*Montastraea annularis*, *Montastraea cavernosa*, *Diploria strigosa*, *Colpophyllia natans*, *Siderastrea siderea* (WAC)[1] *Favia* spp., *Acropora* spp. (RS, IP)	cyanobacteria/bacterial consortium
Brown-band	Acroporidae, Pocilloporidae, Faviidae (IP, RS)	*Porpostoma guamensis* (philasterine scuticociliate)[2]
White plague*	*Dichocoenia stokesii, Montastraea* spp., *C. natans*, *S. siderea* (WAC)[1] *Favia favus* (RS)	*Aurantimonas coralicida* (WAC) *Thalassomonas loyana* (RS)
White pox	*Acropora palmata* (WAC)	*Serratia marcescens*
White-band*	*Acropora cervicornis, A. palmata* (WAC) *Goniastrea* spp., *Favia* spp., *Acropora* spp. (RS, IP)[1]	*Vibrio harveyi/carchariae*
Yellow blotch/band	*Montastraea* spp. (WAC) *Diploastraea heliopora*, *Herpolitha* spp., *Fungia* spp (IP)[3] *Acropora* spp., *Porites* spp. (ME)[4]	*Vibrio* spp.

Regions: Indian Ocean (IO), Middle East (ME), Red Sea (RS), Eastern Mediterranean (EM), Indo-Pacific (IP), West Atlantic/Caribbean (WAC).
†Bleaching can be caused by a number of different factors. The examples listed here are only incidences believed to be caused by bacterial pathogens.
*Did not distinguish between different types of specified disease.
[1]Green and Bruckner 2000; [2]Lobban *et al*. 2011; [3]Cervino *et al.* 2008; [4]Korrubel and Riegl 1998.

In addition to the impact of environmental stressors or pathogenic agents, the abundance and ecological importance of a particular species in respect to the reef community can also be an important factor when choosing an experimental species. Such coral species can often be distinguished by their predominance in a given geographic region (Table 3). Test species can also be selected based on critical management needs, two good examples of relevant coral species for research are *Acropora palmata* and *Acropora cervicornis*. These two major reef building corals in the Atlantic have been decimated by white-band disease and are currently listed as "threatened" under the Endangered Species Act (ESA) (Aronson and Precht 2002;

Federal Register 2006). Very recently, however, NOAA proposed the reclassification of *A. palmata* and *A. cervicornis* from "threatened" to "endangered" (Federal Register 2012). These species are also subjected to the many harmful stressors that are correlated with urbanization and other human activity in the region. Globally, 200 reef-building corals are vulnerable as a result of localized threats, bleaching, disease, and climate change (Carpenter *et al.* 2008). The research needs for these species focus on the causes of their decline and mitigation actions to allow their recovery. In cases dealing with vulnerable species such as *Acropora*, the acquisition of specimens is often difficult due to their protective status. This may be true for other species from marine sanctuaries, marine protected areas (MPAs), and other protected regions like in the Northwest Hawaiian Islands. Thus, it is important to identify regulatory authorities governing a particular region which may include federal, state, regional and/or local authorities. For instance, coral collected in all U.S. jurisdictions requires permitting by the local governing agency(s). Internationally, it is most important to not only determine the local permitting laws but if specimens are to be transported back into the U.S. then CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) export and import permits will likely be required (see Woodley *et al.* 2008 for more information).

TABLE 3. The predominant coral species within regions of the U.S., Pacific Freely Associated States, Malaysia, Australia, and the Red Sea.

Geographic Region	Predominant Corals	References
South Florida	*Montastraea cavernosa*, *Siderastrea siderea*, *Porites astreoides*, *Meandrina meandrites*	Gilliam 2011
Bahamas/Caribbean†	*Montastraea*, *Porites*, *Diploria*, *Agaricia*	Bruckner 2012; Rothenberger *et al.* 2008
Hawaiian Islands	*Porites lobata*, *Pocillopora meandrina*, *Porites compressa*, *Montipora capitata*	Friedlander *et al.* 2008a; Friedlander *et al.* 2008b
Guam	*Porites*, *Favia*, *Montastraea*, *Pocillopora*	Burdick *et al.* 2008
Micronesia	*Porites*, *Montipora*, *Acropora*	Houk and Starmer 2007
Pacific Remote Islands	*Montipora, Porites*, *Pocillopora*, *Acropora*	Miller *et al.* 2008
American Samoa	*Porites rus, Pavona varians, Montipora, Pocillopora*	Mundy 1996; Fenner *et al.* 2008
Malaysia	*Acropora, Porites, Montipora, Goniastrea*	Toda *et al.* 2007
Australia (GBR)	Acroporidae, Favidae, Pocilloporidae, Poritidae	Sweatman *et al.* 2008
Red Sea	*Stylophora pistallata, Porites lutea, Porites, Acropora*	Riegl *et al.* 2012

Much of the information regarding the Indo-Pacific coral reefs has been omitted due to the vastness of the area, the wide dispersal of literature, and the difficulty finding/accessing relevant data.
The "spp." abbreviation has been intentionally left out for the sake of brevity.
†*A. cervicornis* and *A. palmata*, the two major reef building corals in these areas, are not included presumably due to their steady decline within the last several decades (Aronson and Precht 2001; Gardner *et al.* 2003).

In 2009 the Coral Disease and Health Consortium (CDHC) recognized that coral reef research could greatly benefit from having a model species to focus mechanistic studies on gaps in our understanding of the basic biology, physiology, and genetics of corals. The working group recommended six Indo-Pacific species as models for coral physiological research (Galloway *et*

al. 2009) instead of one species due to their diverse habitats and life histories. In addition to the selection criteria listed within this document (i.e., disease susceptibility, prevalence and distribution, hardiness), the CDHC included biogeography (habitat), sexual reproduction, growth rate, and morphology (e.g., branching vs. boulder) as other important considerations. The six species that fulfilled these criteria were *Pocillopora damicornis*, *Stylophora pistillata*, *Porites rus*, *Galaxea fascicularis*, *Fungia scutaria*, *Acropora formosa*, and *Acropora millipora*. Other previously recommended coral model species include *Plesiastrea versipora* (Ritchie *et al.* 1997) and *Acropora* spp. (Miller and Ball 2000).

1.2 Captive vs. Collected

Once identified, acquiring the desired experimental coral species can pose a challenge. For the purposes of this document, coral origins are categorized into those that have been held in captivity (e.g., maintained and/or propagated in an aquaculture/mariculture facility) or freshly collected from its natural environment (wild coral) and added directly to the end-users system. However, there is some gray area between these two categories wherein recently harvested coral are held in a secondary captive system for a short period, typically a wholesaler or retailer, and then transferred to the end-user.

In many cases, unless the researcher lives within driving distance of the experimental coral's natural reef community and has access to snorkeling/SCUBA equipment and a boat, collecting experimental coral directly from the wild can be logistically and monetarily infeasible. Obtaining the necessary permits to import coral internationally (i.e., a CITES permit), collect coral from protected areas, or to maintain non-indigenous species in a facility could take months. In addition, acquiring coral directly from the wild can introduce significant physiological and genetic variability in an experimental setting. Optimally, coral stocks should be established from a recognized reference area, and genetic clones (see Section 6) propagated in a captive setting in which as many potential stressors as possible have been removed. However, the capacity of wild coral to acclimate to a new artificial environment can be highly variable and may require weeks, months, even years to determine the optimal parameters to maintain healthy coral stocks in a closed system.

Fortunately, there are numerous resources for acquiring coral livestock whether it is retail, wholesale, organizational, academic, or governmental. It may even be possible to purchase the desired coral from the local aquarium supply store; however, these animals are typically from unknown origins, have been maintained under various conditions, and have interacted with a number of other organisms which they would not naturally encounter. An important key to acquiring captive coral, especially from a retailer, is to have an open line of communication. When there is a reliable point of contact (POC) within the organization/business, it is sometimes possible to coordinate the acquisition of desired coral species at a certain time and from a specific geographic region, reducing the amount of time held in captivity and having a better knowledge of the corals' origins. The POC can better describe the conditions in which the corals are held once they arrive at the facility. This information could include quarantine procedures, lighting, temperature, water chemistry, and other coral species/animals held in the same system.

1.3 Hardiness

Some species have been found to be more amenable to captivity than others (Table 4).

TABLE 4. The physical parameter requirements of some common coral and their capacity to be kept in captivity (hardiness). Hardiness is based on how many aquarists out of ten would be likely to succeed in growing the coral. Light and flow is ranked from 0 (lowest) to 10 (highest strengths). Adapted from Sprung 1999.

Genus	Common Species	Hardiness	Lighting	Flow
Madracis	*decactis, mirabilis*	9	2-8	2-8
Stylophora	*pistillata*	8	4-10	4-10
Pocillopora	*damicornis, verrucosa, meandrina*	7	4-10	4-10
Acropora	*palmata, cervicornis, formosa*	3, 4, 7*	4(5)*-10	4-10
Montipora	*capitata, digitata*	6	3-8	1-6
Porites	*astreoides, lobata, divaricata, porites*	7	5-10	2-10
Siderastrea	*siderea, radians*	6	3-10	3-10
Pavona	*cactus, decussata, varians, venosa*	9	3-9	3-9
Agaricia	*agaricites, lamarcki*	7	3-9	2-9
Fungia	*fungites, danai*	8	3-10	1-10
Montastraea	*annularis, faveolata, cavernosa, curta*	9	3-9	3-9
Favia	*fragum, favus, speciosa*	8	4-9	3-10
Favites	*adbita, flexuosa*	8	4-9	3-10
Diploria	*strigosa, labyrinthiformis*	7	4-10	4-9
Colpophyllia	*natans*	8	4-9	3-8
Oculina	*diffusa, varicosa, patagonica*	7	4-8	3-9
Goniastrea	*aspera, australensis, retiformis*	6	4-10	4-10

(*) *A. palmata* is considered the least hardy (3) and requires lighting levels of 5-10. *A. cervicornis* and *A. formosa* have hardiness ratings of 4 and 7, respectively, and both require light levels of 4-10.

The values listed above should only be used as a guideline and can be somewhat deceptive. Even the hardiest of corals will not survive if improperly handled, infected with pathogens or parasites, or if any number of parameters are not optimized. Some species that are considered hardy in the wild (e.g., *Goniastrea,* Veron 2000) may not fare as well in captivity (Sprung 1999). Furthermore, the success of a coral species in captivity is not only determined by the coral's inherent hardiness, but also the suitability of the captive conditions. For example, relatively hardy species that thrive in deeper reef ecosystems may prove unsuccessful in holding systems designed to simulate outer fringe reef conditions (i.e., high flow rates, more intense lighting). In our experience there can be significant differences between species within a genus and sometimes between different colonies of the same species. For example, we have had far more success with the branching/finger-like *Porites* species*, P. divaricata* and *P. porites*, than the mounding species, *P. astreoides* and *P. lobata*. In addition, of two *M. capitata* colonies, obtained from the same source at the same time and held under identical conditions, one has grown extensively while the other bleached and eventually lost tissue. We have also had good success maintaining *A. cervicornis*, considered a less hardy species. It should be noted that this *A. cervicornis* was donated from stock that had been growing in captivity for more than 20 years.

SECTION 2 – EXPERIMENTAL DESIGN

The scientific method forms the basis of experimental design and consists of four basic steps: (1) observation and description of a scientific phenomenon, (2) formulation of a problem statement and hypothesis, (3) use of the hypothesis to predict the results of new observations or the existence of other phenomena, and (4) the performance of experimental methods or procedures to test the hypothesis.

2.1 Identifying Experimental Variables

In the laboratory, "scientific phenomena" refers to the experimental organism's response to a specific stimulus, treatment, or challenge and these can be divided into three groups: (1) chemical, (2) physical, and (3) biological. Chemical challenges can include exposure to toxicants found in agrochemicals (herbicides, pesticides, fertilizers), petroleum products, industrial waste, personal care products, and toxins derived from other biological sources. Physical effectors can include temperature differences, light intensity, light spectrum, water quality, pH, salinity and sedimentation. Biological challenges can include either bacterial or viral infectivity or the introduction of other invasive organisms (e.g., fungi, worms, copepods). In some cases, the experimental parameters can be a combination of, or an interaction between these groups. For example, the effect of increased temperature on bacterial virulence in coral or the differences in coral growth rates under variable water quality parameters such as increased ammonia or phosphate levels. In the latter case, water quality could be considered both a physical and chemical variable.

A major challenge for coral experimentation is the necessity to control many critical parameters while manipulating experimental variables. Some more important parameters include, but are not limited to, lighting (duration, intensity, and spectrum), temperature, water quality and chemistry, and water circulation. These parameters can vary temporally and spatially (e.g., where a coral is positioned under a light source). Fortunately, through constant vigilance and a well-designed experimental plan, many variables can be eliminated or, at the least, their variation reduced. Controls also need to be implemented within the experimental design in order to distinguish between a valid treatment effect and one caused by unknown, random variables. Negative controls can be untreated animals or those treated with a placebo; the latter is useful for determining the effects of the act of being treated or the treatment vehicle. For example, a negative (vehicle) control consisting of dilute acetone should be included when acetone is used to solubilize the chemical compound under investigation. Positive controls are often used to ensure that the experimental procedure is capable of detecting a treatment effect. Failure of the positive control to demonstrate the expected response could indicate a problem with the experimental plan or apparatus.

2.2 Experimental Plan

The experimental plan is dependent on a number of factors which are predetermined in order to comply with the rules and assumptions of a particular statistical analysis. Textbooks are written that describe these statistical tests in detail; most of which are beyond the scope of this document. However, there are a few key components that can be discussed that will help when planning an effective experimental layout.

2.2.1 Treatments

The number of different variables, or effectors, to which the experimental organism will be exposed. Controls are often included to elucidate the effects of unknown or unwanted variables (see Johnson and Besselsen 2002). The complexity and size of the experiment is directly proportional to the number of treatments. More treatments require more replicates and, thus, more test organisms.

2.2.2 Experimental Unit

The entity, either an individual or group, which is being studied. In this case, the experimental unit will almost always be a coral fragment or group of fragments although coral or zooxanthellae cell cultures are being used more routinely and the cell population would be the experimental unit. Multiple measures can be obtained from each experimental unit and each will also be a unit of statistical analysis. Each experimental unit, or replicate, must be independent of the others; that is, the treatment response of one replicate shall have no effect on the response of another. For example, three coral fragments held in one treatment tank maintained at 30^{o}C can only be considered one true replicate, not three separate replicates, since they all share the same water and can possibly affect the behavior of the others. The measurable qualities of each fragment can all still be combined or averaged, but the tank remains one experimental unit. Misidentifying experimental units can result in pseudoreplication (Section 2.2.4) and invalidate statistical results.

2.2.3 Experimental Group/Sample Size

The number of units, or replicates, assigned to each treatment. Commonly determined by a power analysis, the sample size is inversely related to the effect size which is the minimal measurable difference between two means divided by the standard deviation among experimental units. For example, a low effect size results in an experiment when a relatively small difference between two treatments is anticipated but the replicates have a large variability; therefore, more replicates will be needed to detect such small differences with confidence. Alternately, in order to detect significant differences between treatments in which variability is low, fewer replicates will be required. The anticipated differences between experimental groups and their inherent variability are often not known. In this case, a pilot study may be necessary to test the logistics of a proposed experiment and estimate the experimental means and standard deviations in order to determine sample size. For more detailed information on sample size refer to Dell *et al.* (2002) and Festing and Altman (2002). There are also numerous online calculators dedicated to determining sample size.

2.2.4 Pseudoreplication

Hurlbert (1984) defines **pseudoreplication** as "*the use of inferential statistics to test for treatment effects with data from experiments where either treatments are not replicated (though samples may be) or experimental units are not statistically independent*".

With **simple pseudoreplication** (Fig. 1A), the investigator fails to recognize that multiple measurements are being taken in a single replicate of a treatment and considers each measure a separate experimental unit. In other words, animals intended to serve as replicates for a given treatment are all placed together in a single tank. Probably the most common form of pseudoreplication, this misrepresentation artificially inflates the effective sample size of the experiment. An example of simple pseudoreplication compared the effect of three different phosphate levels on coral growth rates. The different phosphate levels were administered in three separate tanks which held multiple coral fragments. The growth of each fragment was measured over time and statistical analysis was implemented using each fragment as a replicate to compare the means between treatments (phosphate levels). Since the fragments for each treatment were kept in a single tank, they are not independent and should not be considered true replicates. Each treatment tank is the true experimental unit in which multiple samples are taken. Thus, rather than representing the analysis as possessing nine replicates, there was actually only one experimental unit (the tank) per treatment, and as a result, no true replication. In this design, there is no way to test whether growth rates were affected by phosphate levels or by some unknown or uncontrolled variable in each tank.

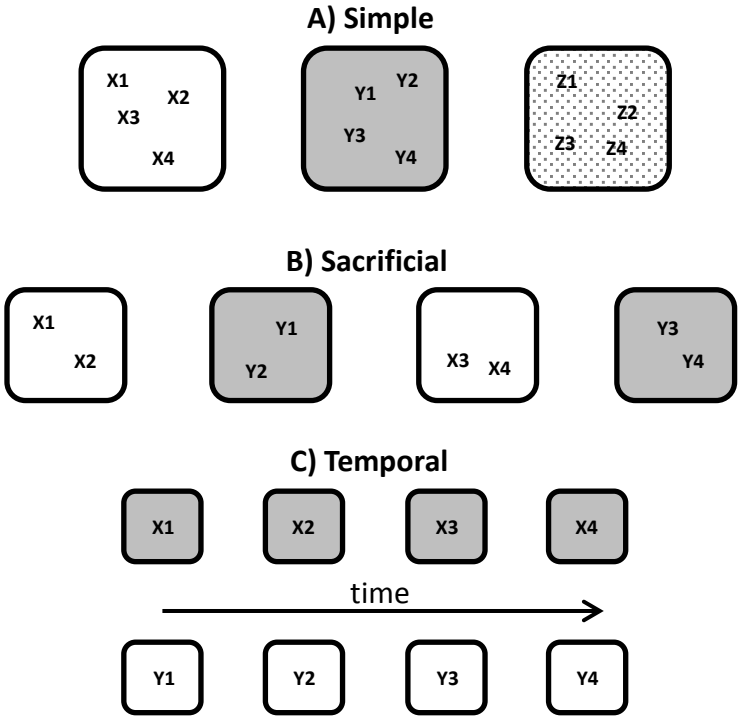


Figure 1. Three common types of pseudoreplication. Different colored boxes represent experimental units receiving a unique treatment. Each numbered letter represents a sample or measurement. Adapted from Hurlbert (1984).

Sacrificial pseudoreplication (Fig. 1B) occurs when true treatment replication exists, but the variance among treatment replicates is lost when multiple samples within a replicate are pooled with those of another or when the samples from each experimental unit are treated as individual replicates. For example, in an experiment designed to evaluate the effect of two different light intensities on coral tissue regeneration, an investigator placed three fragments in each of two replicate tanks per light intensity thereby having four separate tanks each containing three coral fragments. Upon completion of the experiment, measurements were

taken on each fragment and the data for each treatment was combined prior to statistical analysis. With this design, true replication of treatments exists (each light intensity had two separate tanks); however, when the researcher combined all the measurements from each replicate, the sample size was artificially inflated because each fragment was then inappropriately considered an experimental unit (replicate). Rather than two true replicates per treatment there are now six false replicates. Furthermore, any information regarding variance among treatment replicates was lost. For instance, suppose the bulb over one of the replicates was somehow defective and emitted a slightly shifted light spectrum which affected tissue growth within that one tank. By pooling all the samples, any differences between the two replicate tanks cannot be statistically determined and can result in a spurious treatment effect.

Temporal pseudoreplication (Fig. 1C) is similar to simple pseudoreplication, however, rather than simultaneous measurements within a single experimental unit, measurements are taken sequentially over time and each measurement is represented as a treatment replicate. Since multiple measurements of a single unit are obviously not independent of each other, this approach is statistically invalid. A simple example of pseudoreplication studied the effect of elevated alkalinity on coral growth rate. Two tanks were setup each containing one coral fragment; one tank was maintained at an alkalinity level of 7 KH (average reef concentrations) and the other at 10 KH (well above average). Over a period of eight weeks, the growth rate of each fragment was determined weekly. After eight weeks the eight measurements from each treatment were statistically compared. Although there was only one true replicate per treatment in this experimental design, the researcher has treated each temporal measurement as an independent data point, artificially inflating the number of replicates to eight per treatment. Since the growth rate of an individual over time is obviously not an independent measure, this particular experimental design is incorrect.

2.2.5 Replicate Interspersion

The spacing of treatments and replicates in order to prevent spurious spatial effects and segregation. Figure 2 illustrates some acceptable methods (A-C) and some unacceptable methods (D-H) for interspersing replicates in a two-treatment experimental design. In these examples, each unit can be considered as being a tank holding coral that has been treated independent of other units within the same treatment. Figure 2(A-C) shows the best methods to prevent unwanted spatial effects between treatments. However, sometimes randomization can lead to unintentional segregation, especially in smaller experiments. In this event, a systematic design may be more appropriate or the layout can be “re-randomized” until an acceptable level of interspersion has been obtained. In addition, each design may have a corresponding statistical test which is more appropriately used with that particular layout.

Design types illustrated in Fig. 2(D-H) allow segregation and increase the chances of spatial and spurious treatment effects. Simple and clumped segregation increase the risk of gradient effects which could induce artificial treatment effects. Practical examples in coral experimentation would be a temperature or light intensity gradient from one side of a room to another, for example having one end of an experimental system closer to an air conditioner unit or having a light bulb fail at one end of the setup. Isolative segregation can occur when

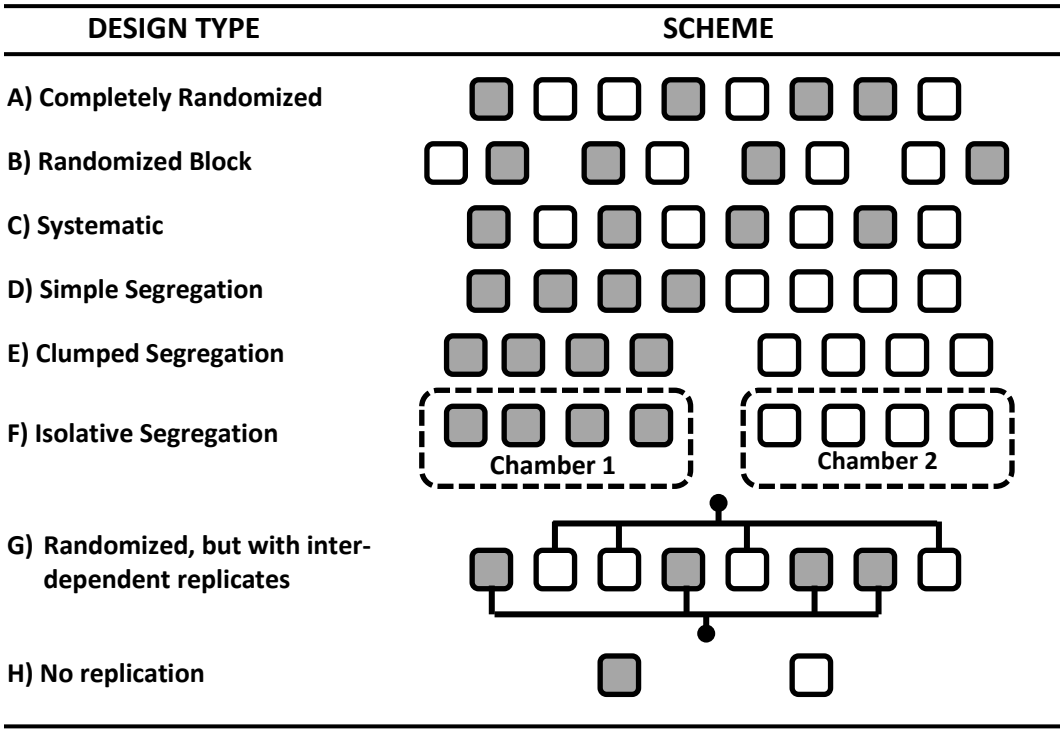


Figure 2. Randomization and interspersion of treatment replicates. Each different colored box represents a unique treatment. Adapted from Hurlbert (1984).

treatments are kept in separate incubators or some similar situation, and the chances of spurious treatment effects are much more likely. In design Fig. 2G, the treatments are suitably randomized; however, the replicates share a common systemic component such as heating, aeration, or filtration. This design is at risk of artificial treatment effects (e.g., faulty heater or contamination) similar to isolated segregation and should be avoided.

2.3 Experimental Design and Statistical Test Compatibilities

An in-depth discussion on the different statistical applications for various experimental designs is beyond the scope of this manuscript. Briefly, depending on experimental design, researchers will often use parametric or nonparametric statistical tests as well as correlation and regression analyses. In order to use parametric tests a few assumptions must be fulfilled: 1) the data have a normal distribution, 2) the variances of each group are approximately equal, and 3) the observations are independent of each other. Common parametric methods include the *t*-test and the ANOVA. If the previous assumptions are not met, non-parametric methods of analysis include the Wilcoxon rank sum test and the Mann-Whitney test (non-parametric equivalents of

the *t*-test) as well as the Kruskal-Wallis test (the non-parametric equivalent of the one-way ANOVA). Correlation analysis is used to determine the linear relation between two variables but does not assume that the variation of one causes the variation of the other. On the other hand, regression analysis is used to quantify the relation between two continuous variables where variation of one is assumed to cause variation in the other.

Fortunately there are numerous resources to help understand statistical analysis and its implementation with various experimental designs. For a good overview of design and statistical analysis, a series of papers in the ILAR Journal are particularly helpful (see Dell *et al.* 2002; Festing and Altman 2002; Johnson and Besselsen 2002). For discussions on the inappropriate use of statistical analysis and experimental design ("what not to do") refer to Hurlbert (1984), Heffner *et al.* (1996), and Kilkenny *et al.* (2009). Detailed explanations of statistical tests and their applications can be found in the books by Seltman (2012) and Sokal and Rohlf (2012). In addition, statistical software is available which greatly facilitates and in many cases is required for the analysis of data (e.g., JMP®, SAS® Institute Inc., Cary, NC).

SECTION 3 – IMPORTANT COMPONENTS

3.1 Water

In an experimental setting, the use of a defined media (i.e., artificial seawater) is preferred over natural seawater. However, if artificial seawater is not available, natural seawater may be suitable for experimental use if it is conveniently supplied (i.e., already being pumped into the facility) and if it is actually "natural" to the coral species being used. Coral from a "pristine" reef environment may not respond well to water pumped in from a harbor, river mouth, or estuary. Natural seawater composition can vary throughout the day as a result of tides and freshwater influx, so critical parameters need to be monitored and adjusted if necessary. Furthermore, depending on the source (i.e., distance from shore and depth), there is a risk of introducing harmful chemical or biological contaminants. A comprehensive analysis of natural source water is required, especially if experimentation involves chemical or biological exposure. Regardless of the source, natural seawater should be mechanically filtered at least down to 50 µm (preferably lower) before being used for experimentation. Further filtration with activated carbon and UV sterilization may remove any harmful contaminants.

Some coral research has been conducted using flow-thru designs in which new seawater constantly flows through the experimental tanks at a predetermined rate and then exits without being recirculated. Often used only when natural seawater is available, flow-thru systems can be designed for use with a continuous artificial seawater supply. While these provide the benefits of supplying new seawater and removing waste, as well as eliminating the need for artificial circulation and biological filtration, flow-thru systems are not practical for most experimental applications. The constant flow of new seawater makes it extremely difficult to maintain consistent chemical or biological dosages as well as any altered water chemistry parameter that is being studied. Such designs require technically sophisticated and often very expensive equipment. In addition, the continuous flow of water generates copious amounts of waste which would need to be collected, decontaminated, and properly disposed. Given the large volumes, treatment of any contaminated wastewater may be inadequate and, without proper monitoring, could potentially harm the environment.

Many commercially available artificial seawater formulations are now designed to closely mimic the water chemistry parameters typically found on coral reefs (see Section 4); however, these formulations can be highly variable. Therefore it may be advantageous to either consult with colleagues having success culturing coral or test several brands of artificial sea water in your system and then purchase a sufficient amount of the lot performing the best. Extensive testing of each production lot of commercial sea salts is not commonly done or practical for small scales experiments. However knowing the typical values of important components (e.g., pH, alkalinity, calcium) is important. More thorough analysis using complex instrumentation like inductively coupled plasma mass spectrometry (ICP-MS) for metals or HPLC or GCMS for other compounds, unless performed in-house, is costly. Sea salt mixes are also available from reputable scientific suppliers (e.g., sea salts from Sigma-Aldrich®) that may conform to higher manufacturing standards and have specific lot analysis. While the composition may be more consistent and less ambiguous, the unit prices for these mixes are high and may only be

practical for very small scale experiments. The relative cost of high quality, lot analyzed sea salts may be 30 times more expensive than commercial aquarium-grade sea salts. A third alternative is to prepare one's own sea salt formulations from analytical grade components.

To reduce variability between batches of artificial salt mixes, prepare the largest supplied package of salts applicable to meet the needs of the experiment. For example, salt mixes can come in 10, 25, or 50 gallon boxes/bags and even 160 gallon buckets. If 40 gallons will be required for an experiment, prepare an entire 50 gallon bag or multiple smaller bags at one time (if possible) rather than removing only 40 gallons worth from a 50 or 160 gallon unit. Since they are mixes of many different salts which may settle or become heterogeneously distributed, homogeneity can be improved by using the entire portion. Artificial seawater should be prepared in deionized (DI), reverse osmosis (RO), or combination RO/DI freshwater (Fig. 3) that is vigorously agitated and/or aerated. A combination of agitation using an aquarium pump and aeration (Fig. 4) dramatically improves the dissolution efficiency of the salt mix and reduces precipitation and insoluble debris accumulation. It is strongly recommended that the salt solution mix overnight so that it can equilibrate with atmospheric carbon dioxide; freshly prepared artificial seawater

Figure 3. Mixed bed deionizing system for preparing artifical seawater. Tap water passes through a 1 µm pre-filter (**white cylinder**), an activated carbon tank (**black band**), and then through the mixed bed working and polishing deionizer tanks (**yellow bands**). A light indicates when the working tank is spent.

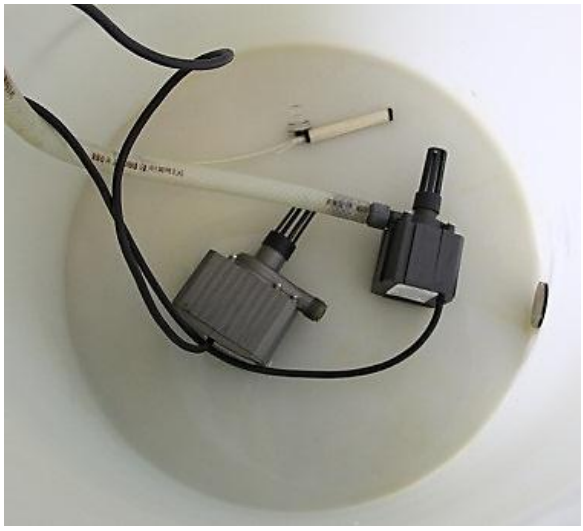


Figure 4. A combination of submersible utility pumps and aeration is used to dissolve and equilibrate freshly mixed artificial seawater.

Figure 5. Artificial seawater mixing (**left**) and holding (**right**) tanks. After mixing overnight fresh artificial seawater is pumped through 5 µm and 0.35 µm pleated filters (**arrows**) into the holding tank where it is aerated until use.

can be somewhat caustic (i.e., elevated pH) if not allowed to equilibrate. Once mixed any important parameter can be checked (at least salinity), adjusted, and then pumped into a separate holding tank with aeration before use (Fig. 5). Mechanical filtration of freshly mixed seawater is recommended (e.g., filter sequentially through 5 µm and 0.35 µm pleated cartridge filters when the seawater is pumped from the mixing tank to the holding tank). Carbon filtration and UV sterilization can also be added depending on the experimental application.

3.2 Experimental Tanks

The choice of tank size will depend on available space, practicality, bioload (i.e., biomass per volume) and to some extent, the duration of the experiment. Common tank sizes are listed in Table 5 and can be purchased from any local pet or aquarium store. Smaller jars or bowls, such as those in Fig. 6, may have to be specially ordered by the fish store or obtained from an online vendor. Tanks or dosing chambers made from special materials can be constructed from Teflon®-lined carboys available from specialty plastic distributors or scientific supply companies (e.g., U.S. Plastics Corp.; Welch Fluorocarbon; Savillex).

TABLE 5. Common tank volumes, dimensions, and fill weights.

Tank Volume gal (L)	Dimensions (L x W x H) in (cm)	Weight Filled lbs (kg)
2.5 (9.5)	12x6x8 (30x15x20)	27 (12)
5 (19)	16x8x10 (40x20x25)	62 (28)
10 (38)	20x10x12 (50x25x30)	111 (50)
15 (57)	24x12x12 (60x30x30)	170 (77)
20 high (76)	24x12x16 (60x30x40)	225 (102)
20 long (76)	30x12x12 (75x30x30)	225 (102)
25 (95)	24x12x20 (60x30x50)	282 (128)
29 (110)	30x12x18 (75x30x45)	330 (150)
30 (113)	36x12x16 (90x30x40)	348 (158)

Unfortunately, there is no defined standard ratio for tank size relative to biomass. Larger tanks with more seawater capacity will be more stable under higher bioloads and longer experimental durations, but this deficiency of smaller tanks can be compensated for by more frequent water changes. Larger tanks will obviously take up more space, thus reducing the number of possible replicates and potentially sacrificing statistical power. In addition, when experiments involve specific chemical or biological dosages, the excessive volume of larger tanks may require impractical quantities of a specified treatment and more hazardous waste to handle and dispose. We find that for fragments 2.5-5 cm in length that two

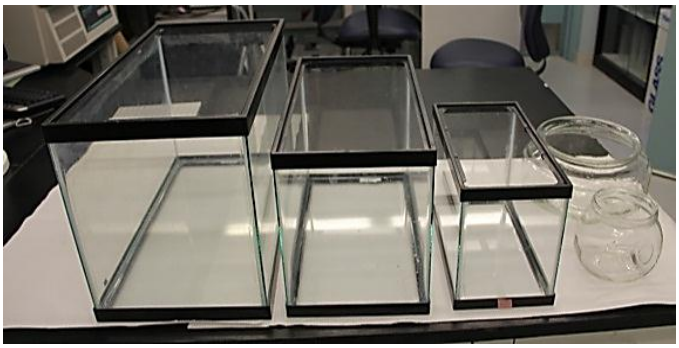


Figure 6. Some examples of tanks and bowls which can be used for coral experiments. These range from 10 gallons down to 0.5 gallons.

to three per one gallon of seawater with adequate circulation and frequent water changes is adequate. This will not suffice, however, for larger single polyp species (ranging from 5 to 10 cm in diameter) such as *Fungia* spp. which require *at least* one gallon per individual, preferably two, depending on the size of the animal.

3.3 Lighting

Unlike many other experimental marine invertebrates, most coral require light of a particular intensity, duration and quality (i.e., spectrum) in order to thrive and remain healthy. Lighting is an important and complex issue that can and has been discussed at length in many references (Delbeek and Sprung 2005; Calfo 2009; Joshi 2005, 2010; Borneman 2001; Riddle 2007). Deciding on the proper lighting regime should be based on the coral species, region of origin, biogeography (i.e., location and depth on the reef) or, if acquired from captive stock, its previous lighting regime. If most of this information is not known, there are some general guidelines (Table 4). Intensities between 100-200 µmol/m^2/s measured at the depth of the coral appear to be sufficient for most coral species. A light cycle between 10-12 hours per day controlled with a timer (Fig. 7) will also mimic natural photoperiods. Note: regardless of origin, when first acquired, coral need to be acclimated to any new lighting regime slowly.

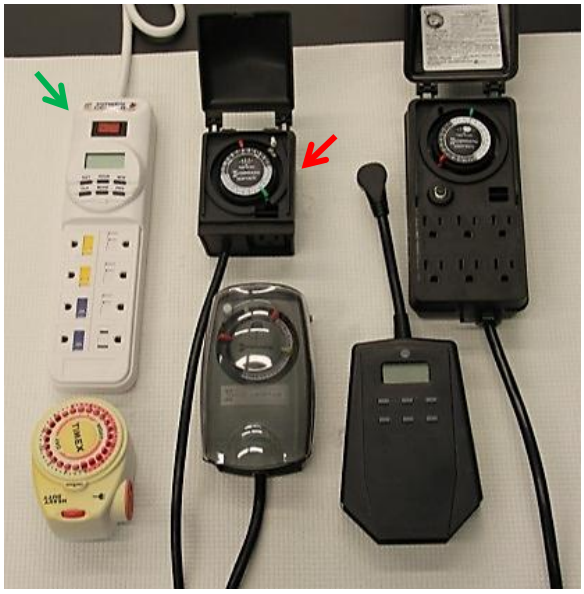


Figure 7. Various models of timers can be used to control light duration. These range from single-outlet mechanical timers (**red arrow**) to multi-outlet digital timers (**green arrow**).

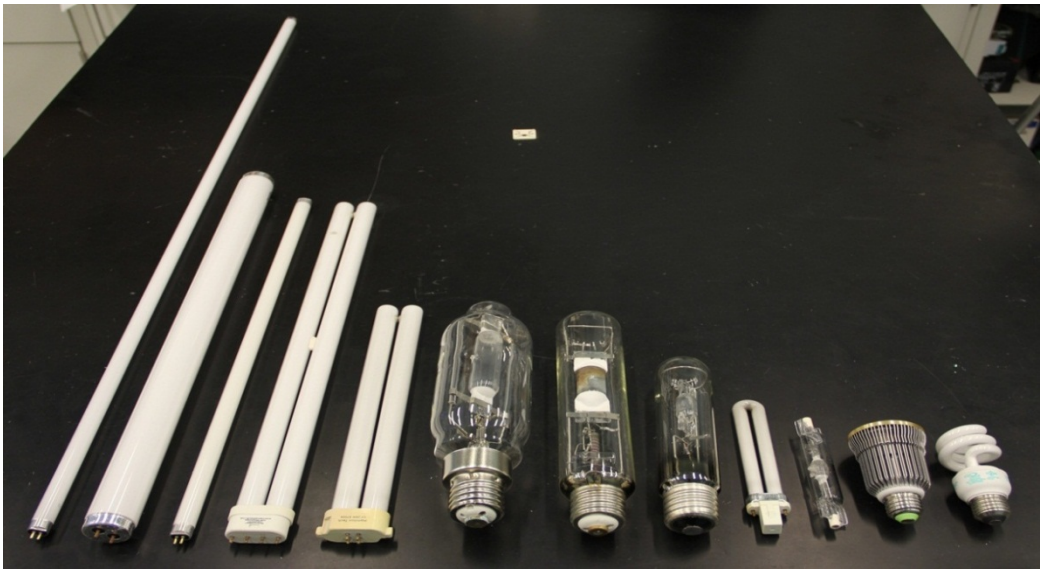


Figure 8. Examples of various light bulbs used for coral aquaculture. Left to right: 54 W T5 fluorescent, 20 W T12 fluorescent, 24 W T5 fluorescent, 55 W power compact (PC) fluorescent, 26 W PC fluorescent, 1000 W metal halide, 400 W metal halide, 250 W metal halide, 9 W PC fluorescent, 150 W metal halide, 12 W LED, 5 W compact fluorescent (CFL).

In the case of experimental applications, the key to proper lighting is consistency, unless the purpose is to study the effects of different types, intensities, or qualities of light. A plethora of different light bulbs and fixtures are available (Fig. 8), all of which provide varying intensities and unique spectral characteristics (Fig. 9). While being held in experimental tanks which are relatively shallow, high intensity bulbs such as metal halides probably will not be necessary. A good alternative would be individual LEDs, compact fluorescence, or longer high-output T5 fluorescent fixtures which can span over multiple tanks (see Section 7.1 for example). These light fixtures have added benefits of being more energy efficient and producing less heat, which could confound an experiment, if left unchecked.

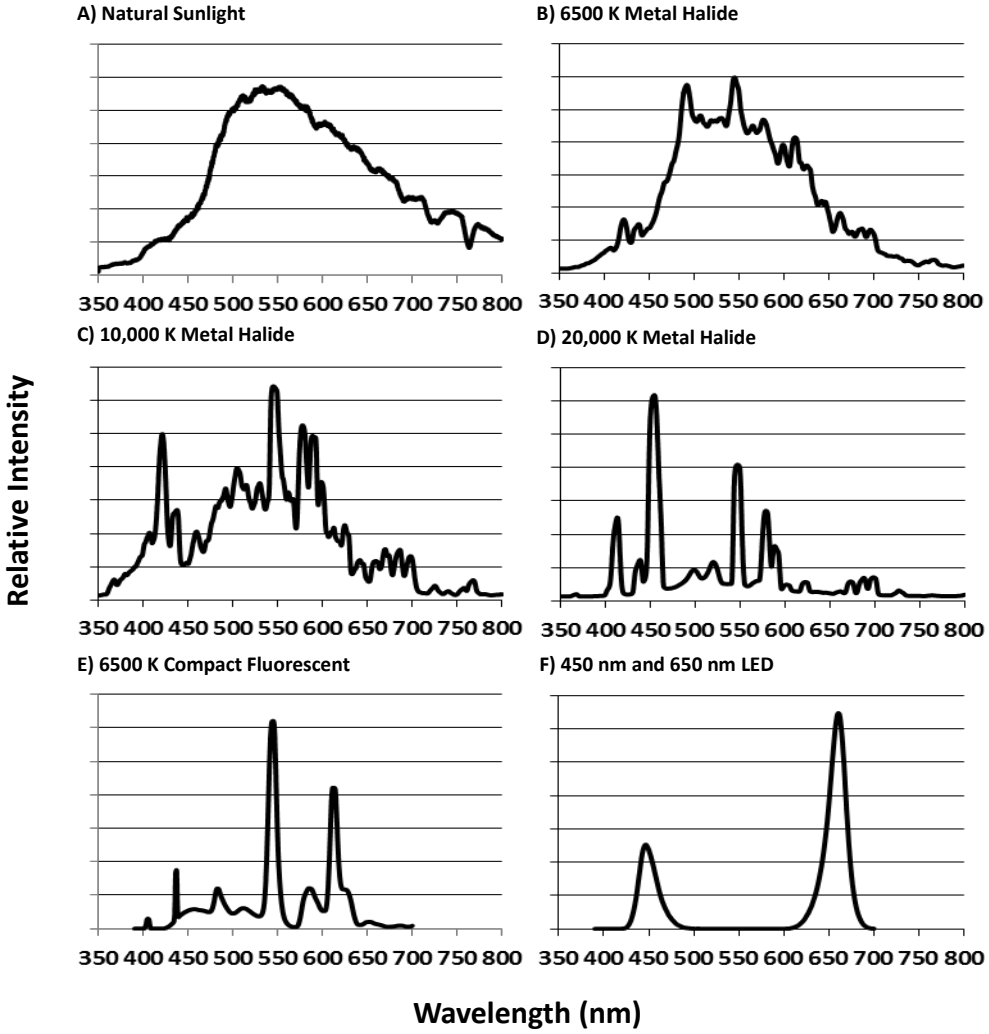


Figure 9. Irradiance spectra of different light sources. Spectra A-D were measured using a USB2000 spectrometer with SpectraSuite software (Ocean Optics, Inc., Dunedin, FL), and spectra E-F were measured with an Olympus BX-51 microscope equipped with a PARRIS® hyperspectral system (LightForm, Inc., Asheville, NC). Spectral data acquired by David Allen and Jim Nicholson, respectively. Figure is intended to show differences between spectral profiles, not for the comparison of intensities between light sources.

Light intensities can be adjusted by either adjusting the height of the fixture above the tank or coral fragment in the tank or by using neutral density filters or shade cloth (e.g., screen material

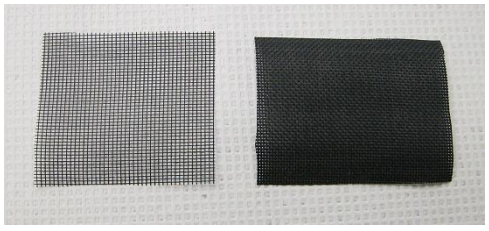


Figure 10. Shade cloth used to attenuate light intensity.

from a hardware store) to attenuate the light (Fig. 10). All the light intensities should be uniform between all the replicates within the experiment and should be measured with a light meter. A number of different light meters are available through the aquarium trade and specialized manufacturers which can measure Lux, lumens, or photosynthetically active radiation (PAR). Although more expensive, PAR meters (Fig. 11) measure the amount of photons between the 400 nm to 700 nm wavelength region. Also referred to as photosynthetic photon flux density (PPFD), the integrated quantity is then expressed in terms of irradiance as either micromoles/m^2/s, µmol/m^2/s, or sometimes µE/m^2/s where E equals Einsteins. Measuring PAR is more relevant to coral research as it is more biologically significant and is recommended for coral experimentation. The sensor used with the meter should be submersible in order to get an accurate intensity measurement at the depth of the coral in the tank and not just at the water surface. Keep in mind, the PAR sensor measures light differently when submersed than when exposed to air, and involves using a different immersion effect correction, or correlation coefficient, with the meter. In addition, planar, or 2π, sensors provide measurements from light perpendicular to the sensor (i.e., directly above), whereas spherical 4π sensors can provide total light measurements not only from above but also from the sides and below.

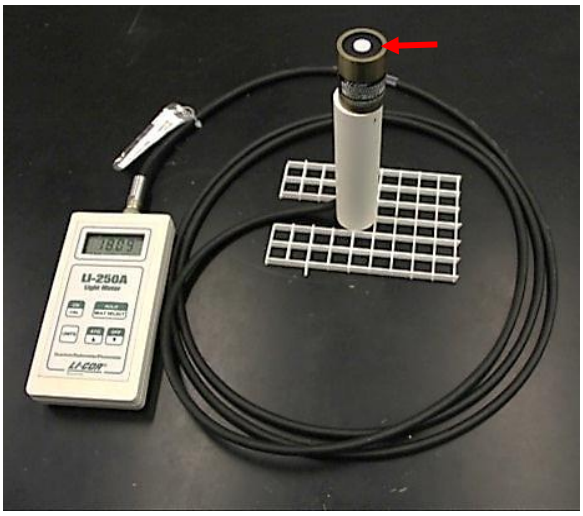


Figure 11. Light meter and sensor used to measure photosynthetically active radiation directly above the sensor (**arrow**). The sensor has been positioned on a stand constructed from PVC pipe and plastic egg crate material to keep the sensor stable and at a specific height in the water column.

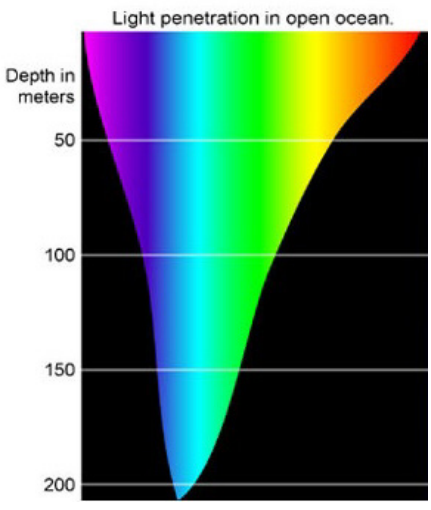


Figure 12. Attenuation of solar radiation in the open ocean. *Image credit: Kyle Carothers, NOAA-OE.*

The proper light quality, or spectrum, is another important consideration when working with coral. In nature, direct sunlight at the ocean's surface demonstrates a very broad irradiance spectrum (Fig. 9A); however, as depth increases longer wavelengths are "filtered" out until only blue light remains (Fig. 12). Light bulbs used in coral aquaculture are designed to reproduce the different spectral qualities found at depth on the reef. The specific spectral emissions of a particular bulb are typically indicated by their color temperature in kelvins (K), as the color temperature increases there is a shift from longer, red wavelengths to shorter, blue wavelengths. Usually referred to as daylight bulbs, 5500 K or 6500 K bulbs most closely resemble the

spectral characteristics of natural surface sunlight (Fig. 9B), and bulbs with lower temperatures (i.e., more long, red wavelengths) are generally never used. However, dependent on the typical biogeography of the coral species, it is usually more desirable to use or include bulbs with higher color temperatures and thus shorter, blue wavelengths like those seen with 10,000 K to 20,000 K bulbs (Fig. 9C&D). This is supported by research that indicates photosynthesis in corals is enhanced by providing light primarily in the blue spectrum and less so in the longer wavelengths (Kinzie *et al.* 1984; Kinzie and Hunter 1987). High-output fluorescent light fixtures are useful for providing various light qualities as they possess multiple locations where bulbs of different color temperatures can be combined. Light emitting diode (LED) fixtures are a fairly recent and unique technology to coral aquaculture that allows the researcher to use very specific, narrow wavelengths during experimentation (Fig. 9F). These specific wavelengths can also be combined with broad-spectrum (white) diodes that resemble natural sunlight. Unfortunately, there is no easy, inexpensive means of measuring light quality in the experimental setting. Spectrometers with submersible probes can cost thousands of dollars. Fortunately, most light bulb manufacturers supply the spectral output of each bulb or the information can be found online (e.g., www.manhattanreefs.com/lighting).

3.4 Hardware

Almost all the equipment and hardware necessary can be found at the local hardware store or online aquatic vendors (e.g., www.aquaticeco.com) (Fig. 13). Some hard-to-find fittings may be ordered from specialty plastic vendors or online hardware retailers (e.g., U.S. Plastics Corp. or McMaster-Carr®, Atlanta, GA).

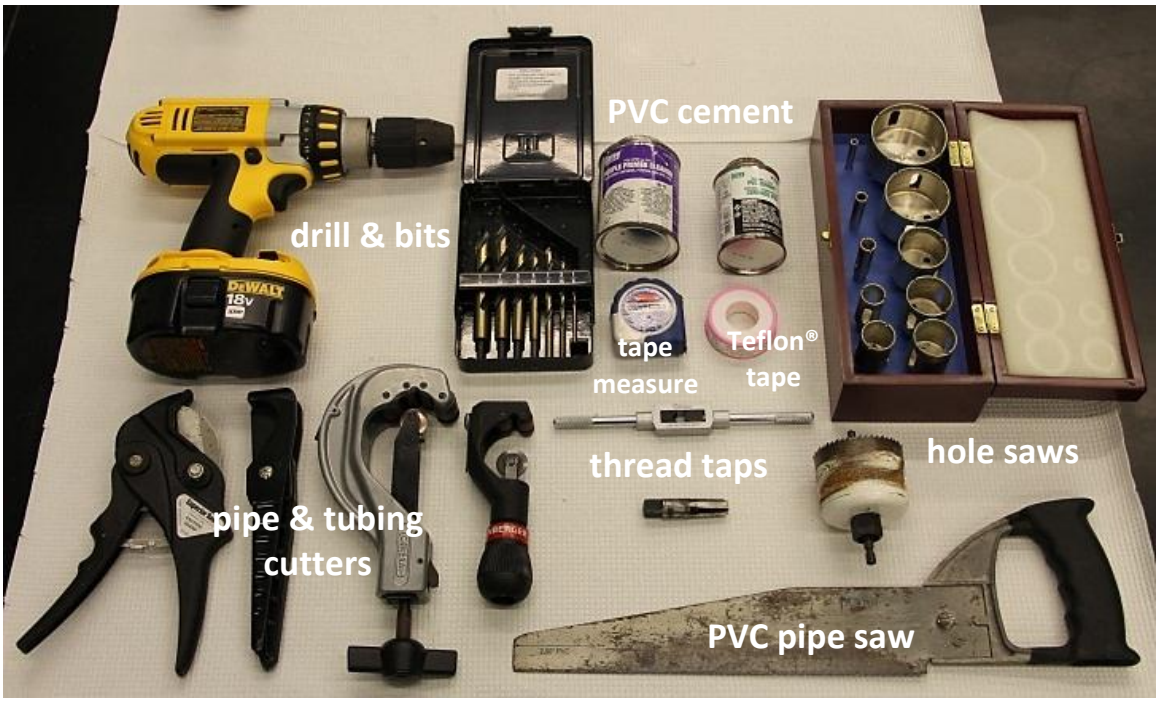


Figure 13. Tools typically needed to construct experimental setups. Most can be found at the local hardware store. Some like diamond-coated hole saws and plastic-specific drill bits may need to be ordered (e.g., www.mcmaster.com). Other tools sometimes used but not shown here are a circular saw (for cutting acrylic sheets or wood) and a Dremel® rotary tool (for cutting, grinding, and sanding).

Circulation within each tank is important for the overall health of the experimental coral. Similar to lighting, there is no defined standard for how much flow is required, but there are some guidelines based on the typical biogeography of individual coral species (Table 4). Generally most small polyp stony (SPS) corals benefit from strong water motion; however, some species that are adapted to low-flow environments such as *Cynarina lacrymalis*, *Nemenzophyllia turbida*, and *Plerogyra sinuosa* will respond negatively to excessive current (Delbeek and Sprung 2005). A general "rule of thumb" is that the combined total flow per hour should be at least ten times the water volume (Pawlowsky 2008). For instance, a ten gallon tank holding eight gallons of water will do well with two small powerheads rated at 50 gallons per hour (GPH), a combined total of 100 GPH. Experiments which can be conducted in small bowls, such as those shown in Fig. 6, will probably suffice with aggressive aeration; this will work to agitate and aerate the water. However larger tanks (2.5 gallons or more) will require small powerheads (Fig. 14). It is important to note that any devices producing water circulation should be positioned such that they create random, turbulent eddies rather than unidirectional laminar flow directed at the coral. Some devices are available that are designed to randomize or produce surge flow; however, most of these are very expensive and designed for larger tanks, thus impractical in an experimental setting. Reasonable alternatives would include using an inexpensive rotating deflector (Fig. 15) or connecting pumps to timers (Fig. 7) so that their output is variable.

Figure 14. Various sizes and models of powerheads.

Figure 15. Inexpensive rotating deflector.

Certain experimental designs may require implementing some sort of filtration, be it biological, chemical, or physical. A simple and inexpensive solution for all three is to use a "hang-on-the-back" (HOB) powerfilter (e.g., Marineland® Penguin® Filters). These filters come in a variety of models suitable for many different tank sizes (5 gallons or more), create beneficial water flow, and provide three-stage filtration (Fig. 16). The BIO-Wheel® acts as a biological filter and filter pads can be placed internally that contain activated carbon. To improve the biological filtration efficiency of a BIO-Wheel® filter or sponge filter, they can be seeded, or inoculated, by keeping them in an already

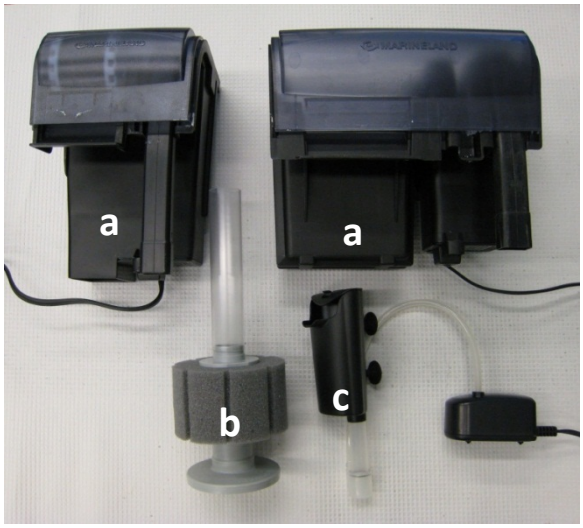


Figure 16. BIO-Wheel® powerfilters (**a**) hang on the back of tanks while sponge (**b**) and small air-driven filters (**c**) are placed inside tanks.

established system days/weeks prior to using them in an experimental tank.

Protein skimmers can also provide beneficial filtration by removing suspended particles and dissolved organic compounds while also improving aeration, gas exchange, and water flow (see example in Section 5.3). Unfortunately, most skimmers are costly and designed for larger tanks; although there are a few models (e.g., "Nano" skimmers by AquaC, Hydor, or AquaEuroUSA) that are relatively inexpensive and can either hang on the back of a tank or be placed directly in the tank. Even with these models there may be a minimum five gallon capacity limitation.

Figure 17. Aquarium heaters come in many different sizes and wattages. The smaller 25W heater (**top**) is useful in smaller tanks up to 10 gallons. The larger 200W heater (**bottom**) is suitable for tanks between 50-75 gallons.

Temperature control is an obvious necessity when working with experimental coral. If the temperature is uniform between treatments, a properly functioning environmentally controlled room should suffice. However, any temporal or spatial temperature variations should be determined beforehand. Often experiments will require temperature differentials between treatments which can be accomplished by simply placing an aquarium heater in each replicate tank (Fig. 17). Compact heaters down to 7.5 watts will fit 2.5 gallon tanks and should maintain water five to ten degrees above ambient room temperature. For larger tanks, 2.5-5 watts per gallon is recommended to maintain temperature. One should err on the higher side if temperatures are needed well above ambient room temperature. **Please note:** *Aquarium heaters are notoriously inaccurate and the actual water temperature usually does not reflect the set temperature; therefore, each heater should be checked and standardized before use.*

For experiments using small containers (one liter or less), an external, temperature-regulated water bath can house each treatment (see example in Section 7.3). For proper experimental design and statistical purposes, placing experimental tanks in separate incubators, environmental chambers, or temperature controlled rooms is not recommended as it creates isolative segregation and increases the risk of artificial, or spurious, treatment effects as described in Section 2.2.5.

The temperature of each tank should be monitored daily to ensure consistency and proper temperature control. Fluid-filled or small digital thermometers can be added to each tank, reducing the possibility of cross-contamination. A more accurate digital probe-type thermometer can be used when cross-contamination is not a concern, when measurements are taken in a sequential order as to minimalize treatment effects (i.e., measuring from lowest to highest dosage), or when the probe can be adequately decontaminated between measurements (Fig. 18a). Fluid-filled thermometers are designed to be either partially or completely immersed in water and using them inappropriately will affect their accuracy (Fig. 18b).

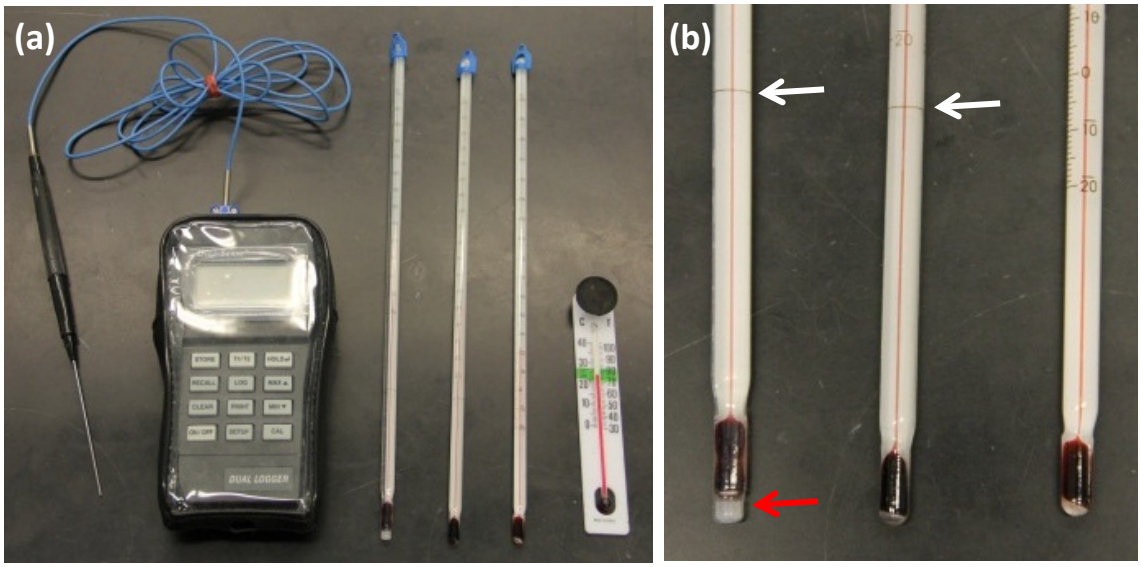


Figure 18. Various thermometers that can be used to monitor temperature during coral experiments. They can range from very accurate thermocouples connected to dataloggers (**a; left**), fairly accurate fluid-filled models from scientific supply vendors (**a; middle three**), or questionably accurate hobbyist brands (**a; right**). Some fluid-filled models are designed for either partial or complete immersion (**b**). The white arrows indicate the depth marking on partial immersion thermometers. Teflon®-coated (**red arrow**) thermometers are also available that are resistant to chemical exposure.

SECTION 4 – CRITICAL PARAMETERS AND MAINTENANCE

NOTE: The following pertains to coral in experimental systems as well as animals being held in quarantine.

Monitoring important water chemistry and water quality parameters and performing routine maintenance (i.e., water changes, salinity and chemical adjustments) will help ensure consistency, replication among treatments, and that the experimental coral are not being adversely affected by anything other than what is being investigated. Critical parameters (Table 6) may vary based on the origin of the experimental coral. For example, a coral species acquired from the Red Sea (e.g., *Stylophora pistillata* or *Favia favus*), where average summer surface water temperatures can range from 26 ^{o}C to 30 ^{o}C and average salinity is approximately 40 ppt, may benefit from maintaining slightly higher temperatures and salinities.

Table 6. Critical parameters to control in coral aquaria. Adapted primarily from Holmes-Farley 2004.

Parameter	Aquaria Recommendation	Typical Values on Reef[1]
Temperature	24.5-28 ^{o}C†	25-30 ^{o}C[2]
Salinity	35 ppt†	34-36 ppt
pH	8.1-8.3	8.0-8.5
Alkalinity	8-10 KH	7 KH
Calcium	380-450 ppm	420 ppm
Magnesium	1250-1350 ppm	1280 ppm
Ammonia (total)‡	*< 0.1 ppm*	*< 0.1 ppm*
Nitrite	*< 0.2 ppm*	*< 0.0001 ppm*
Nitrate	*< 0.2 ppm*	*< 0.04 ppm*[2]
Phosphate	*< 0.03 ppm*	*<0.02 ppm*[2]

[1]Millero 1996; [2]Kleypas *et al.* 1999.
†If possible, these parameters should be based mostly on the origin of the coral species used.
‡Total ammonia includes the concentrations of both free ammonium ion and free un-ionized ammonia. See Section 7.1.6 for more detail.

4.1 Water Changes

Water changes are simply removing a known portion of used, waste seawater and replacing it with the same volume of new ASW or natural seawater. Performing routine water changes is an excellent way to maintain proper levels of important water chemistry parameters as well as reducing harmful waste products (i.e., nitrogenous compounds and phosphates). The volume of seawater replaced and the frequency in which it is replaced is dependent on a number of factors including bioload, biofiltration capacity, and duration of the experiment. For example, an experimental design using smaller tanks containing a relatively high number of coral fragments (e.g., 10-20 fragments per 5 gallon tank), with very little or no biofiltration (e.g., powerheads only), and held for over three to four weeks, could require very frequent (i.e., daily or every other day) or at least larger volume water changes. In most cases, given the need for less complicated experimental designs, biofiltration will be a limiting factor and some sort of water change will need to be scheduled.

In terms of maintaining water quality in established aquaria, it is recommended to perform the equivalent of a 1% daily water change which would translate to 15% every two weeks or 30% every month. Usually established aquaria have higher biofiltration capacities, so this figure would need to be modified to compensate for the relatively poor biofiltration capacities inherent in experimental systems. We would recommend no less than the equivalent of a 20% water change per week. Again, the frequency and volume of water changes is strongly dependent on bioload and duration and should be adjusted accordingly. However, we do not recommend performing more than a 50% water change at any given time, as this may induce stress on the coral. In cases where experimental dosage is important, whether chemical or bacterial, water changes may need to be much more frequent (i.e., daily) to ensure that the challenge agent is at the same concentration throughout the duration of the exposure.

Experimental systems should be designed to facilitate the water changing process, reducing the labor involved, the potential stress on the experimental animals, and the likelihood of cross-contamination. Siphon tubes connected to each tank with shut-off valves provides for the rapid removal of seawater from experimental tanks without disturbing the coral inside (see examples in Sections 7.1 and 7.2). For a more permanent fixture, a bulkhead affixed to a side of the tank attached to flexible tubing with a valve will also do (Fig. 19). The waste seawater can then be collected in a centralized container which can be treated prior to disposal. Manifolds connected to the common seawater source with individual valves and fill tubes to each tank will allow for quick replacement of seawater. If the particular experiment demands, the source water can also be treated prior to exchange by connecting a small in-line UV sterilizer or carbon filter between the seawater tank and manifold (see example in Section 7.1).

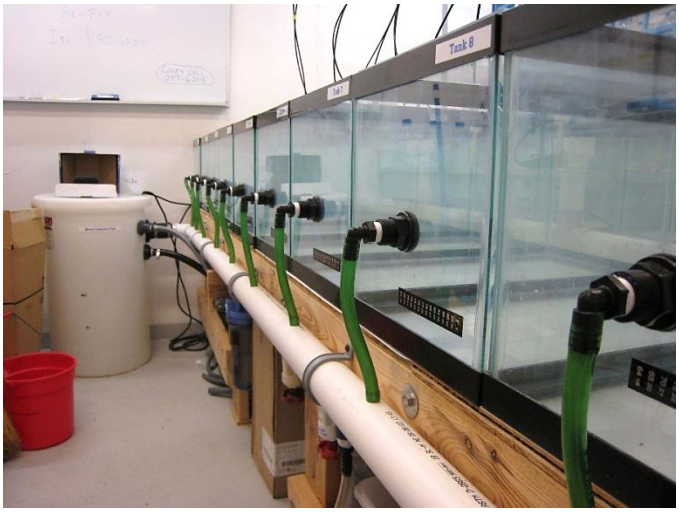


Figure 19. Waste seawater can drain through bulkheads and be collected in a central waste container where it can be decontaminated prior to disposal. Photo of a toxicology dosing system at the Hollings Marine Laboratory, Charleston, SC.

4.2 Water Quality Testing and Adjustment

Depending on the experimental tank volume, small aliquots of seawater can be removed from each tank and multiple tests can be performed daily to help streamline the process and reduce the chance of cross-contamination (Fig. 20). While some water quality measurements require only small volumes of test material such as a few drops on a refractometer, others require more, such as 5 ml of tank water for calcium tests. Therefore, water quality testing should be considered when planning for experimental tank size and seawater volume; testing may have to be limited when using smaller experimental containers (<1 L). Alternatively, water quality testing can coincide with routine water changes where the water being removed is measured,

eliminating the need to remove additional experimental tank water. Routine water changes with new ASW (10-20% twice weekly) will help maintain beneficial parameters while reducing levels of harmful waste products, especially in experiments with higher bioloads and longer durations.

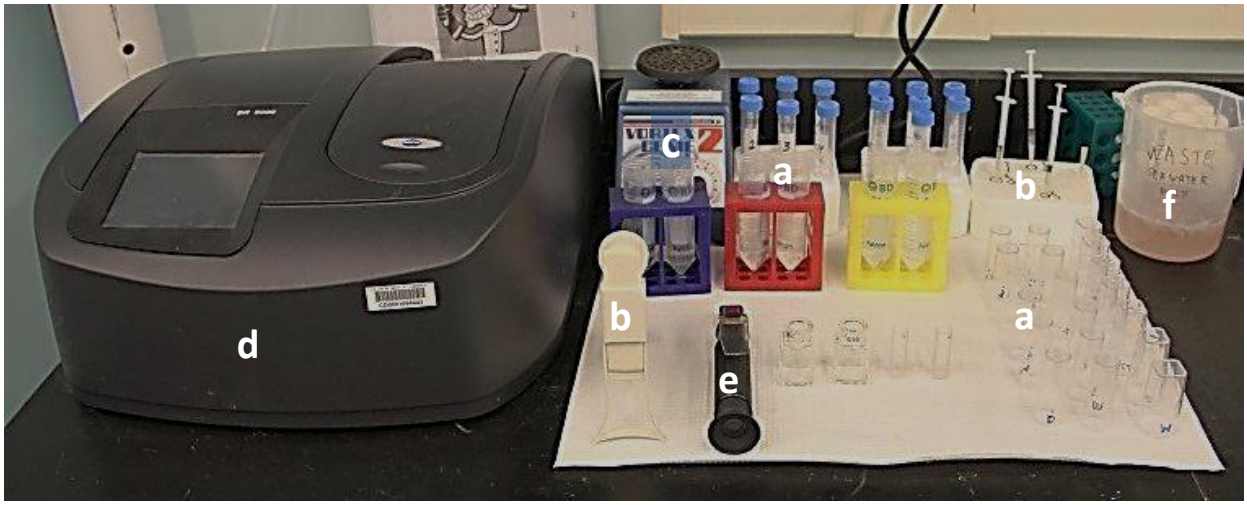


Figure 20. A well organized, clean work station will help streamline water quality measurements, reducing time spent and increasing consistency. Within easy reach, the area should include reagents for each test (not shown), testing vials or tubes for each experimental unit (**a**), volumetric measuring devices (**b**), a vortex for mixing solutions (**c**), any special measuring instruments such as a spectrophotometer (**d**) or refractometer (**e**), and a waste container (**f**).

4.2.1 Salinity

Water will evaporate and salinity will increase daily; tank lids will obviously reduce the rate of evaporation. Salinity is best measured with a refractometer; however, some refractometers are designed specifically for seawater (e.g., SR-6 (Vital Sine™) or STX-3 (Vee Gee®)) others for sodium chloride, saline or brine solutions (e.g., MR100ATC (Milwaukee Instruments, Inc)), and they are not always obviously indicated. Seawater refractometers can be calibrated to zero with DI water. Sodium chloride refractometers, often marketed as "salinity" refractometers, require calibration with a 3.65% (w/w) sodium chloride solution then adjusting the calibration to 35 ppt. If the type of refractometer is not known, set the calibration to zero with DI water and read a sample of 3.65% sodium chloride solution. If the refractometer reads 36.5 ppt, it is not a seawater specific refractometer, and the salinity reading for the 3.65% sodium chloride should be calibrated to 35 ppt. The discrepancy between the two types of refractometers is due to the different refractive indices of seawater and saline solutions. Regardless of refractometer type, calibration to 35 ppt with a 3.65% sodium chloride standard will increase the refractometer's accuracy within the working measurement range (~34-36 ppt). For a more detailed explanation of refractometers and salinity measurement refer to Holmes-Farley (2006).

Deionized water should be slowly added directly to the tank in an area of high water flow to adjust salinity. **A fill line or water level mark indicating the initial water level will facilitate the process**. The approximate volume of DI needed can be calculated if the new salinity and water volume are known.

Avoid measuring seawater that contains organic solvents such as diesel fuel as they can damage the refractometer optics. Digital refractometers (e.g., Milwaukee MA887 Seawater Digital Refractometer) are available which may be more resistant to chemicals since they do not have any plastic optical parts. However, according to the specifications of these instruments, they are not as accurate (±2 ppt) as hand-held refractometers (±1 ppt). Conductivity probes are an alternative for measuring salinity, but they can be costly and their resistance to solvents and other potentially harmful chemicals is uncertain.

4.2.2 pH

The pH of water samples can be measured with a laboratory grade pH probe and meter. Portable meters also can be used if contamination is not a concern. Some probes are compatible with organic solvents and other potentially damaging substances. Aquarium-grade colorimetric test kits and litmus paper can be used, but these should be considered semi-quantitative. They do not usually possess the resolution of most pH probes (~±0.2 pH units compared to ±0.01 pH unit) and are prone to user error and subjectivity since the sample color is compared to a color chart. In addition, they could be more time consuming; for example, colorpHast® pH strips (EMD Millipore, Billerica, MA) could take 1-10 minutes for the completed color change in the weakly buffered seawater.

The pH will fluctuate throughout the day, usually decreasing at night and increasing during the day as a result of photosynthesis. In addition, pH is strongly correlated with alkalinity (Section 4.2.3). If alkalinity is maintained, pH should be stable. However, tanks with higher bioloads may see a gradual decline in pH (i.e., become more acidic) as metabolites are produced.

Routine water changes will help maintain pH levels. Although not recommended, the careful and slow addition of a dilute anhydrous sodium carbonate (<10 g/L) or calcium hydroxide (<1.5 g/L) solution in DI water will increase pH but also will affect other important tank parameters (i.e., alkalinity and calcium). Inappropriate use of these additives can cause drastic pH spikes and calcium carbonate precipitation events, or “snowstorms.”

4.2.3 Alkalinity

Alkalinity refers to the buffering capacity of seawater and can be reported using different units: milliequivalents per liter (mEq/L), carbonate hardness (KH), or parts per million calcium carbonate (ppm $CaCO_3$), where 1 mEq/L = 2.8 KH = 50 ppm $CaCO_3$. The German unit of carbonate hardness (KH), or degrees carbonate hardness (dKH), is very common and our preferred unit of measure. Predominately a function of bicarbonate and carbonate ion concentrations, proper alkalinity levels are required for the formation of coral skeleton and can be measured somewhat reliably with aquarium test kits (see Dickson (1981) for a more comprehensive explanation of alkalinity). Most kits are based on the same chemical principles for alkalinity measurement (see APHA 2012; Holmes-Farley 2002); however some kits appear to be more accurate than others (Riddle 2007). Accuracy and consistency can be improved by making in-house titration reagents. Using the pH indicator dye included in the Salifert® KH/Alk Profi Test Kit, we perform the test as described but replace the titration reagent supplied in the kit with 0.023 N hydrochloric acid in 0.7 M sodium chloride. This reagent is stoichiometrically

correct and is verified using a carbonate standard solution. In addition to improved consistency, in-house reagents also extend the capacity of test kits and reduce overall costs.

In addition to water changes, alkalinity levels can be adjusted using known concentrations of a sodium bicarbonate (i.e., baking soda) solution (60 g/L), or a combination of sodium bicarbonate and anhydrous sodium carbonate (4:1 w/w). The latter mixture will also increase pH levels. The addition of a calcium hydroxide solution can be used to adjust alkalinity, but the precise proportions are difficult to determine and the risk of misuse is high; therefore, it is not recommended for experimental purposes. An online calculator is very helpful in determining the correct dosage of different alkalinity products needed to achieve a target concentration (http://reef.diesyst.com/flashcalc/flashcalc.html).

4.2.4 Calcium

Proper calcium levels are as important as alkalinity in the formation of coral skeleton and can be measured using common aquarium test kits, most of which are based on a complexometric titration with ethylenediaminetetraacetic acid (EDTA). But like alkalinity test kits, some seem to be more accurate than others. Kits should be verified with in-house produced calcium standards in artificial seawater or commercially available standards (e.g., PINPOINT® Calcium Standards, American Marine, Inc.). Again, stoichiometrically correct and verified titrants can be made to improve accuracy and consistency. For example, we use a 0.069 M disodium EDTA, pH 8.0 solution prepared in DI water as a replacement for the titrating reagent supplied in the kit.

Commercially available calcium probes can be used to monitor calcium levels in experimental systems. In our experience, however, they seem to have a number of disadvantages: (1) they are expensive, (2) their response time is slow (5-10 minutes), (3) their accuracy is affected by a number of interfering factors such as pH, temperature, and ionic activity, and (4) they are delicate and require a fairly high degree of user proficiency.

Calcium adjustments can be made with known concentrations of either anhydrous calcium chloride or calcium chloride dihydrate solutions (~60 g/L of either). As with alkalinity, a calcium hydroxide solution can be used, but the risks remain the same and it is not recommended. The same online calculator mentioned in Section 4.2.3 is also useful for calcium dosing calculations.

4.2.5 Magnesium

Magnesium is a physiologically important element which aids in maintaining a correct balance between alkalinity and calcium levels in seawater. The measurement of magnesium (a complexometric titration with ethyleneglycoltetraacetic acid (EGTA)) is based on a similar principle as the measurement for calcium, and commercial aquarium-grade test kits are probably the most affordable and practical means of magnesium measurement. As with calcium tests, the accuracy of each kit (even each batch) should be determined using a magnesium standard. We have yet to identify any suitable replacement reagents for magnesium tests.

Magnesium concentrations tend not to fluctuate as much as pH, calcium, or alkalinity; thus, its measurement is not as critical. However, if adjustment is required, solutions containing known concentrations of either magnesium chloride, magnesium sulfate, or a combination of the two can be used. The hydrated forms of these salts are also acceptable. It is recommended to combine the chloride and sulfate salts in proportions similar to seawater (i.e., chloride:sulfate ≈ 19.5:1 by molarity or 7.2:1 by mass) so as to not shift the overall ionic balance. The same online calculator mentioned above (Section 4.2.3) is also useful for magnesium dosing calculations.

4.2.6 Ammonia, Nitrite, Nitrate, and Phosphate

Ammonia, nitrite, nitrate, and phosphate are all waste or breakdown products that can accumulate over time and negatively impact coral health or affect experimental parameters. The risk of accumulation and impact increases with greater bioloads and insufficient biological and chemical filtration. In an experimental setting the best and perhaps only way to reduce the levels of these unwanted compounds is through increased frequency or volume of water changes. The addition of commercially available chemicals that neutralize ammonia, nitrite, nitrate, or phosphate is not recommended due to their unknown effects on coral health and other water quality parameters.

Elevated ammonia poses the greatest threat to coral health. Although the toxic effects of ammonia on fish are well studied (the 96 h LC_{50} for marine fish ranges from 1.3 to 50 ppm total ammonia; see Eddy 2005), far less is known of its direct effects on coral. For some perspective, levels up to approximately 20 µM (~0.34 ppm) total ammonia increase zooxanthellae growth, while 50 µM (~0.85 ppm) tend to show toxicity (Hoegh-Guldberg 1994; Muller-Parker *et al.* 1994). In addition, Bassim and Sammarco (2003) observed significant mortality in *Diploria strigosa* larvae at concentrations as low as 20 µM (~0.34 ppm) total ammonia.

A number of ammonia tests are available either from reputable commercial water quality specialists (e.g., HACH®) or aquarium-grade test kits. These tests often report total free ammonia which includes toxic ammonia (NH_3) and the far less toxic ionic ammonium (NH_4^-) that exist in an equilibrium based largely on pH and temperature. In typical aquarium parameters (i.e., 26^oC, pH 8.2), only about 9% of total ammonia is the toxic un-ionized form (NH_3). In our experience, however, we have found that neither source supplies a truly accurate, reliable measure of ammonia.

The HACH® protocol for ammonia-nitrogen (Method 8155) combines two reagents to produce a colorimetric change in the presence of ammonia and is measured using a spectrophotometer. It appears to be significantly affected when measuring seawater samples, usually under-reporting actual ammonia concentrations as determined using spiked samples. Presumably this is caused by the many interfering substances found in seawater, some of which are even listed in the protocol (e.g., sulfate above 300 mg/L). Aquarium test kits do not fare any better, since they lack sensitivity (i.e., the detectable limits and the measureable increments are usually too high) and are based on a highly subjective colorimetric change that is compared to a color card.

To compensate for the deficiencies inherent in these tests, we have combined the principles of both into a single, high resolution, accurate test. Using the reagents and protocol of a typical

aquarium-grade ammonia test kit (e.g., Red Sea® Ammonia Test Kit), we can measure the resulting color change with a spectrophotometer (Fig. 20) and calculate ammonia concentrations using a predetermined standard curve. Spectrophotometers are common in most research facilities and smaller, more affordable models are also available. A standard curve should be performed for each test kit reagent batch and requires spiking freshly made ASW with known concentrations of an ammonia standard (HACH®, CO).

There is little evidence demonstrating nitrite toxicity in marine invertebrates especially within corals, although it appears considerably more toxic in freshwater systems (see Holmes-Farley (2005) for review). One of the most relevant studies was by Siikavuopio *et al.* (2004) in which gonadal development in the green sea urchin *Strongylocentrotus droebachiensis* was reduced at concentrations as low as 1.6 ppm; however, death did not occur until concentrations over 33 ppm.

Nitrate and phosphate can stimulate unwanted algal growth (Millero 1996; Pedersen and Borum 1996) in experimental tanks without any noticeable detriment to coral. However, elevated levels of phosphate may affect coral growth and possibly cause mortality. Long-term exposure to elevated phosphate (2 µM; ~0.19 ppm) on a patch reef reduced calcification rates by more than 50% (Kinsey and Davies 1979). In addition, significant mortality in *Pocillopora damicornis* was observed after long-term enrichment of approximately 5 µM (0.5 ppm) phosphate (Koop *et al.* 2001). The effects of nitrate on coral growth are not as clear and there is no scientific evidence of acute or chronic toxicity. Experiments using *Porites porites* and *Montastaea annularis* demonstrated decreased calcification rates after exposure to 1 µM (0.06 ppm) nitrate for 40 and 30 days, respectively (Marubini and Davies 1996). However, in a later study increased nitrate levels (0.5-5.0 µM; 0.03-0.3 ppm) did not significantly affect calcification rates in *Porites compressa* after five weeks (Marubini and Atkinson 1999).

Nitrite, nitrate, and phosphate should also be measured using spectrophotometric methods (e.g., HACH® Method 8507, 10049, and 8048, respectively) even though more affordable aquarium-grade test kits are available. We have found that the HACH® reagents and protocols for these analyses to be suitably accurate with low detection limits and high sensitivity. To note, there appears to be some background nitrate levels when measuring ASW with the HACH® Nitrate UV Screening Method. We compensate for this by replacing the DI blank with one made from freshly made ASW.

Depending on bioload and filtration capacities, it may be necessary to test for ammonia and phosphates weekly or less frequently. Since nitrite and nitrate are nitrification products of ammonia and pose less threat to coral health, these may be a concern only if ammonia is detected or if increased algal growth is observed. If feeding is required during the duration of the experiment, testing frequencies should be increased, especially for phosphates and nitrates, due to the possible introduction of these compounds from food sources.

NOTE: Ammonia, nitrite, and nitrate can be reported either as their ionic form or as their nitrogenous form (e.g., nitrate (NO_3^-) vs. nitrate-nitrogen or nitrate as nitrogen (NO_3^--N)). Using the wrong unit will misrepresent the actual concentration of the compound. For example, 1.0

ppm nitrate-nitrogen equals 4.4 ppm nitrate ion. Similarly, phosphate can be reported as either orthophosphate (PO_4^{-3}) or phosphate-phosphorous (PO_4^{-3}-P) where 1.0 ppm phosphate-phosphorous equals 3.1 ppm orthophosphate ion. These conversions are critical when determining acceptable water quality parameter limits. Different water quality tests may report the results in any one of the specific compounds forms; HACH® spectrophotometric protocols will even allow the user the select the desired form. The reported form is not as important as being consistent with the measurements and comparing values in the proper context.

4.3 Feeding

For short term experiments (<2 weeks) feeding is often omitted; however, for longer durations, feeding may be required to prevent nutrient deprivation and attenuated coral growth. This will need to be experimentally determined based on the experimental design and species under investigation. Unfortunately, the act of feeding, especially overfeeding, can introduce and elevate levels of harmful ammonia, nitrite, nitrate, and phosphate. If a small number of coral fragments are being used, one option is to feed them in individual separate containers and then return them to the experimental tank. However, this may not be practical with larger numbers of animals that cannot be removed from their tanks (e.g., high risk of contamination). In these cases, the quantity of food should be measured and used sparingly, and it would be beneficial to determine the appropriate amount of a particular food source prior to experimentation. If this practice is integrated into the experimental procedures, care must be taken to assure that the added food does not interfere with or create artifacts.

Many different coral foods are available online and at local fish stores. They range from liquid cultures to freeze dried powders to frozen zooplankton. For experimental purposes liquid foods and powders that contain numerous ingredients may introduce unexpected variables into the experiment. Liquid nutrients are more likely to cause negative water quality issues and powdered foods tend to settle and decay in tanks without sufficient circulation and filtration. Frozen foods are usually a single ingredient (e.g., copepods, roe, *Mysis* shrimp) and come in a variety of sizes that can be tailored to suit the particular coral species. For example, small-polyped coral like *Acropora* or *Porites* would benefit more from foods composed of roe or copepods, whereas larger polyped coral (e.g., *Fungia* or *Goniastrea*) can consume meatier foods such as *Mysis* shrimp.

Frozen foods need to be thoroughly rinsed with DI or seawater before feeding since the liquid "juice" will negatively affect water quality. The food is then resuspended in new seawater, preferably at a predetermined concentration, and a known volume is applied over each individual fragment using a transfer pipet, small baster, or by similar means. Meatier foods may need to be chopped into smaller pieces or applied using a large bore pipet. Turning off pumps and filters will help keep the food directed at the coral rather than circulating around the whole tank. If at all possible, any uneaten food should be siphoned out of the tank during the next water change. The overall goal is to give each fragment a well-controlled, known amount of food to limit variability between replicates and to maintain desirable water quality parameters.

4.4 Suggested Testing and Maintenance Guidelines

4.4.1 Short Term Experiments

For shorter experiments (less than two weeks), usually the most important parameters to check are temperature and salinity. If maintained in a well-controlled chamber, room, or water bath, temperature should vary slightly, well within acceptable limits. However, experiments using individual heaters should be monitored daily as heaters can malfunction and either stop heating or heat continuously. Furthermore, the researcher should be aware of the effects of certain closures on the temperature of experimental units. For instance, if experimental vessels are enclosed inside a glass tank or a larger chamber with a lid, this can act to elevate the temperature of the experimental unit above that of the surrounding environment, particularly during periods of illumination. So, even though the chamber and experimental units are being kept in a well-controlled environmental chamber at 26 ^{o}C, the actual temperature inside that chamber and thus the experimental units can be 27-28 ^{o}C for example.

Salinity will consistently increase especially in instances of higher temperatures, increased air circulation, and greater water/air interface surface areas. Using clear lids over experimental units or humidifying the ambient air such as in a humidified environmental chamber can help reduce evaporation. If it determined prior to the experiment that salinity will only fall within an acceptable range (e.g., 34.5 to 35.5 ppt), then no extra monitoring or adjustment is probably required. Otherwise, salinity should be monitored daily and adjusted with DI when necessary. Predetermining a fill line can reduce the time and effort needed to check and adjust numerous experimental units.

In cases where temperature and salinity cannot be monitored in each experimental unit (e.g., too many replicates, possible contamination, small volumes), separate vessels with replicates can be added to the experiment dedicated for this sole purpose. Although adjustments to individual experimental units should not be made based on measurements from these vessels, they can be useful for recording and reporting trends observed throughout the duration of the experiment.

Other important parameters such as pH, calcium, alkalinity, and ammonia can be measured at the end of the experiment to ensure that these values remained within acceptable limits and did not cause a spurious treatment effect. Over such a short duration, water exchanges may not be required; however, if the parameters mentioned above fall outside their optimal values (e.g., pH, calcium, and alkalinity levels drop or ammonia levels increase), partial or full water exchanges may be needed to maintain proper water chemistry/quality levels.

4.4.2 Long Term Experiments

For experiments running longer than two weeks, there is a greater risk that important water chemistry and water quality parameters will not remain within acceptable limits. In addition to regularly monitoring temperature and salinity, if it is feasible, pH, alkalinity, calcium, and ammonia should be measured at least weekly. The measurement of each parameter can be staggered throughout the week to reduce the workload on any one day. Over time pH,

alkalinity, and calcium levels will decrease while ammonia can possibly start to accumulate especially during experiments with greater bioloads. Performing partial or full water exchanges one to three times per week will help maintain constant water chemistry values and deter harmful ammonia levels. Elevated ammonia can also result in the buildup of nitrites and nitrates; if increased ammonia levels are observed, including tests for nitrites and nitrates may be warranted.

Incorporating a feeding regime will help prevent nutritional deficiencies over the course of longer experimental durations. One feeding per week may probably be adequate assuming proper irradiance intensity and quality (i.e., spectrum). If lighting is inadequate, the symbiotic zooxanthellae photosynthesis can be reduced and the host coral health compromised. In such a case more frequent feedings may be required. Feeding performed within the experimental vessel can also contribute to undesirable levels of nitrates and phosphates which should be measured at some point during the experiment, at least at the end, and especially if there is excessive algae/cyanobacteria growth observed.

When it is not practical to perform measurements on all the critical water chemistry/quality parameters, regular water exchanges is probably the best means of maintaining beneficial parameters while reducing levels of harmful waste products.

NOTES: This maintenance schedule will need to be adjusted depending on the experimental parameters. As mentioned in Section 4.1, if a specific chemical or biological dosage is required, daily and/or complete water changes may be necessary to ensure a consistent exposure through the duration of the experiment.

It is recommended to make any water chemistry adjustments prior to measuring salinity and topping off with DI, since the addition of salt solutions can affect the salinity. In addition, if water changes are performed after adjusting salinities, the amount added will be equal to the amount removed without worrying about salinity or water level changes. Salinity can occasionally be adjusted by topping off with DI to a predetermined fill line without direct measurement with a refractometer assuming there is no excessive addition of other salt solutions.

4.5 Handling Chemical and Biological Agents

The use of chemical and biological agents in coral experimentation generates a number of challenges (i.e., special considerations concerning material compatibility, handling and disposal, and biosecurity). Common materials used in marine experimental setups are glass (predominantly holding tanks), a variety of common plastics (e.g., PVC, nylon, acrylic), silicone, and other chemically inert fluoropolymers such as polytetrafluoroethylene (PTFE) and perfluoroalkoxy (PFA) (e.g., Teflon®). Some chemicals that may be used in experiments are not compatible with many plastics. For example, PVC, a major component of aquarium plumbing, is incompatible with many organic solvents and petroleum. In such cases, a compatible plastic polymer, glass, or Teflon® alternative would need to be used. In addition, most plastics leach chemical byproducts that mimic or interfere with chemicals under evaluation. Acrylonitrile and

phthalates, components of some plastics, are known to have chronic aquatic toxicity (Lithner *et al.* 2011). This is of particular concern because low level exposures to chronic toxicity can result in erroneous responses of cellular physiological or biochemical parameters which are the basis for many diagnostic tests. Furthermore, chemically inert materials like glass and Teflon® are used in toxicological studies to minimize the risk of absorption of hydrophobic substances.

When performing experiments with potentially hazardous or pathogenic substances, biosecurity and biocontainment of the materials are of utmost importance. This may require increased ventilation if the agent can aerosolize increased personal protective gear (i.e., footbaths, disposable lab coats, showers etc.) and secondary containment. **Biosecurity** practices prevent agents from entering clean areas like coral stock holding tanks. **Biocontainment** practices prevent the spread of infection or contamination between animals as well as prevent the agent from leaving the designated areas. Some measures that can be implemented to provide biocontainment and biosecurity include the following:

- Designate specific area where contaminants are allowed ("dirty") and where they are prohibited ("clean") using physical barriers and/or brightly, well labeled signs and boundaries. A separate, environmentally controlled room with its own air supply is ideal for this purpose.

- Designate equipment only to be used for contaminant work and that is kept only in dirty areas.

- Provide plenty of consumables to be used in dirty areas (e.g., gloves, pipets, paper towels) and adequate hazardous waste containers for disposal.

- Provide sufficient disinfectant that can be easily administered (e.g., spray or squirt bottle) to disinfect contaminated equipment, surfaces, or hands. For biological contaminants, 10% bleach and 70% ethanol are commonly used, but chemical contaminants may require acetone or other similar solvents.

- Reduce the risk of contaminated clothing from leaving designated areas either by supplying clothing that remains in these areas (e.g., lab coat, scrubs, booties) or by providing a means to decontaminate clothing (e.g., Virkon® Aquatic (Western Chemical, Inc., Ferndale, WA) applied to a disinfecting foot mat).

- Design experimental system to reduce cross-contamination between animals (e.g., reduce splashing, use lids, keep tubing separated) and never enter/manipulate a tank with contaminated equipment or hands.

- Decontaminate all sample tubes, surfaces, equipment, and waste water before leaving designated areas.

Proper waste handling and disposal is also an important consideration during the planning these experiments. For example, sea water from experimental tanks are subject to local public wastewater plant's daily input of seawater, seawater containing chemical toxicants or

pathogenic microbes cannot be drained directly into the sewer system; they must either be treated prior to discharge or collected and disposed properly according to institutional policy, federal, state and local laws.

Most organizations, institutions, and facilities have developed standard protocols for handling hazardous chemical and biological waste, typically in the form of a chemical hygiene or waste management plan. However, these protocols usually need to be tailored to fit the needs of the experiment (i.e., concentration and volume of hazardous discharge) and the design layout (i.e., how and where hazardous material will be collected). Depending on discharge volumes, biological waste can be drained into a separate container (e.g., bucket or carboy) and decontaminated with the appropriate agent prior to disposal (see Appendix 1). A 10% final concentration of bleach (~5000 ppm free chlorine) is commonly used for immediate (~10 minutes) disinfection; however, the concentration of free chlorine can be reduced by increasing the contact time with the biological waste before disposal. If excessive volumes of biological waste are generated, the chlorinated water may need to be neutralized with sodium thiosulfate before disposal or special permission may be required from local sewage treatment facilities or natural resource authorities. Alternatively, plumbing a UV sterilizer in-line with waste water discharge can also effectively decontaminate biologically hazardous material prior to disposal (Table 7). Similarly, chemical waste can be collected, neutralized, or treated depending on concentration and volume generated. Small volumes are easily collected in bottles or larger carboys, but large volumes of contaminated water may need to be treated by filtering through materials such as activated carbon (e.g., MatrixCarbon™, Seachem Laboratories, Inc., Madison, GA) that physically removes the hazardous chemical from the waste water.

Table 7. Recommended UV dose (fluence) to inactivate some representative microorganisms at a given log reduction.

Microorganism	**micro-watt seconds per square cm (μWsec/cm^2) at 254 nm***	
Bacteria	**1-log (90%)**	**3-log (99.9%)†**
Aeromonas salmonicida	1,500	3,100
Bacillus subtilis	5,800	11,000
Escherichia coli	3,000	6,600
Pseudomonas aeruginosa	5,500	10,500
Pseudomonas fluorescens	3,500	6,600
Serratia marcescens	2,420	6,160
Staphylococcus aureus	2,600	6,600
Staphylococcus epidermidis	NA	5,800
Staphylococcus hemolyticus	2,160	5,500
Streptococcus viridans	2,000	3,800
Vibrio cholera	3,375	6,500
Fungi		
Aspergillius flavus	60,000	99,000
Penicillium digitatum	44,000	88,000
Saccharomyces carevisiae	6,000	13,200
Saccharomyces spores	8,000	17,600
Protozoa		
Chlorella vulgaris (algae)	13,000	22,000
Entaboeba hystolytica	NA	84,000
Nematode eggs	45,000	92,000
Paramecium	11,000	20,000
Viruses		
Bacteriophage - *E. Coli*	2,600	6,600
Infectious Hepatitis	5,800	8,000
Influenza	3,400	6,600
Poliovirus - Poliomyelitis	3,150	6,600

*milli-Joules per square cm (mJ/cm^2) is another unit commonly used to measure UV fluence (1 mJ/cm^2 = 1,000 μWsec/cm^2).
†individual UV efficiency is determined by many variables including UV length, power output, water clarity, and flow rate.
(NA) indicates that the information was not available.
Adapted from http://www.americanultraviolet.com. For more information refer to USEPA 2006, Hijnen *et al.* 2006, and Chevrefils *et al.* 2006.

SECTION 5 – ACCLIMATING, TREATING, AND QUARANTINING NEW CORAL

5.1 Acclimation

The acclimation process is critical for reducing stress imposed on coral during shipment and transport and to prepare it for the new and, most likely, different conditions of its new habitat. Acclimation time should first be based on an initial assessment of the coral condition. Especially applicable with transhipped animals, if the water conditions are too poor, a complete and quick exchange with new seawater may be the best option. A slower acclimation may actually prove more harmful than good. Either way, basic measurements of the shipment water (i.e., salinity, temperature, pH, and possibly ammonia) will help determine the acclimation duration.

A generally accepted method of acclimation is by slowly dripping system water (water from the holding or quarantine tank in which the new coral will be placed) into a separate container, usually a 5-10 gallon tank, containing the coral and enough shipment water to cover them (Fig. 21). A rate-adjustable siphon can be made using a short length of air-line tubing and a small ball valve. A small powerhead placed in the tank helps mix the new water. In addition, light should be attenuated (no more than approximately 50 µmol/m^2/s so as to not further shock the light-starved new arrivals. The acclimation process should take between 30 minutes to an hour or more, although some believe 15 to 30 minutes is sufficient (Calfo 2009). During that time, occasionally removing some water from the acclimation tank will increase the ratio of new system water to old shipment water, thereby further diluting the transport water of presumed poor quality with water of desired quality. After acclimation, the new coral are further treated (see Section 5.2) and placed in quarantine.

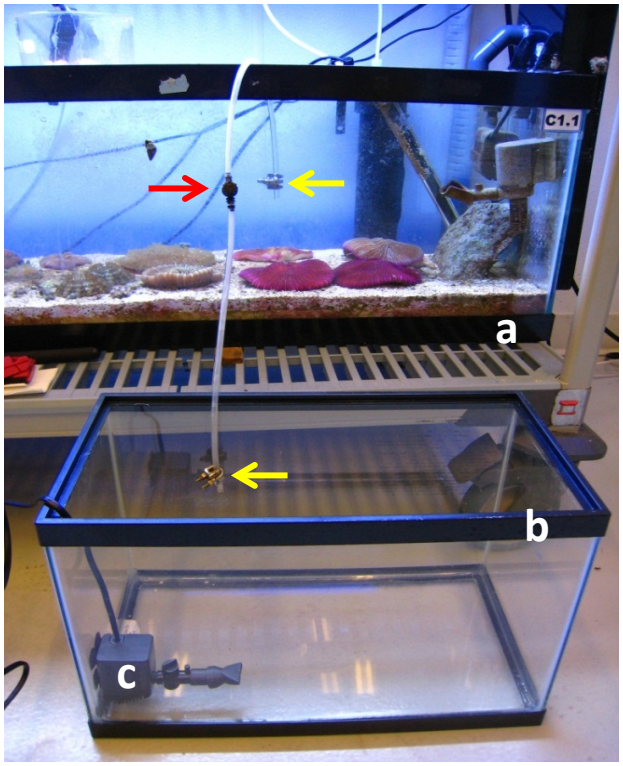


Figure 21. Simple drip acclimation setup. Seawater is slowly siphoned from the holding tank (**a**) to the temporary acclimation tank (**b**) through air-line tubing. A small ball valve (**red arrow**) regulates flow rate and stainless steel clamps (**yellow arrows**) keep the tubing in place. Water is circulated by a small powerhead (**c**).

Another acclimation technique is to float or prop the shipment bags containing the new coral in a separate container (never the main holding system) with system water. Every 10-15 minutes a portion (20-25%) of the shipment water is replaced with system water, occurring over the same amount of time as the drip method. Again, after acclimation, the new coral are further treated and placed in quarantine and all shipment water is discarded.

5.2 Prophylactic Treatment

Treating coral before they enter the quarantine system is a good precaution to reduce the risk of bacterial infection and help eliminate parasites and unwanted hitchhikers that could possibly devastate the entire stock. Often referred to as "coral dips", these treatments are usually iodine-based (e.g., Lugol's solution, Tropic Marin® Pro-Coral Cure) or contain natural extracts (e.g., ReVive Coral Cleaner™, Coral Rx). Whichever product is used, the specific manufacturer's instructions should always be followed and the treated coral should be monitored closely in case of any adverse reactions as certain species may tolerate treatment less than others. Testing prophylactic dips on a few small fragments or a less important coral specimen can help prevent any major losses due to coral species/dip incompatibility.

Lugol's solution is a popular dip that is used as a proactive precaution against disease and parasites as well as a treatment for corals displaying symptoms of infection. There are a number of retail products which contain unspecified concentrations of iodine; however, a concentrated 5% stock solution can be made easily by mixing 5 g elemental iodine in 100 ml of 10% (wt/v) potassium iodide solution in DI water. Recommended dosages vary. Most commercial manufacturers recommend 30-40 drops per gallon of seawater which is similar to Borneman (2001) who recommends 5-10 drops (~0.5ml) of a 5% solution per liter. Carl (2008) suggests using a dip containing 1.32 ml/L (assuming a 5% Lugol's concentrated stock). However, a dosage of as little as one drop per five gallons of heavily aerated seawater has also been recommended (Calfo 2009). It is generally agreed that treatment with Lugol's (protocol below) should only last about 10-15 minutes.

Recommended treatment prior to quarantine: After acclimation, closely inspect corals and any accompanying rock or base. Physically remove any noticeable parasites, eggs, dead or dying organisms, or other undesirable hitchhikers (see Section 5.4) with suction, tweezers, or forceps. It may be necessary to remove the live rock or base entirely and reaffix the coral on a new base (see Section 6.1 for affixing corals to new bases). In a separate tank or container with adequate aeration or circulation using a small powerhead add 0.5 ml of 5% Lugol's solution per liter of new system seawater. Add coral to diluted Lugol's and let sit for 10-15 minutes. After this time, blow a stream of water over the coral using a turkey baster, pipet, or powerhead to dislodge anything that has not detached. Rinse the coral in new system seawater thoroughly and place coral in quarantine tank. The length of the dip and concentration may be adjusted depending on the tolerance of the particular coral species which should be determined empirically. It is recommended to place the coral in Lugol's that has already been completely mixed rather than adding Lugol's directly to corals in a separate tank so as to avoid exposure to excessively high localized iodine concentrations. If Lugol's is to be added directly, it should be done very slowly and in areas of high circulation.

5.3 Quarantine

A quarantine system is simply a separate tank isolated from the main holding system in which new corals can be stabilized and observed for the presence of disease, parasites, or undesirable biota. The characteristics of a quarantine system are inherently uncomplicated, inexpensive,

and transient in nature. The minimum requirements for any quarantine system is an aquarium tank of the appropriate size (depending in size and number of coral) (Table 5), a heater (2.5-5 watts per gallon), small powerheads (Fig. 14) for gas exchange and circulation, and adequate lighting (compact or high-output (HO) fluorescent lights should suffice) (Fig. 8). Proper water quality and chemistry can be maintained through regular, small water changes with new natural or artificial seawater (e.g., 10% twice a week or 20% weekly). The quarantine period should last between two weeks to 30 days; anything pathogenic or problematic should have manifested by this time (Calfo 2009). Using a tank without substrate and elevating the coral using PVC and egg crate (plastic light-diffusing material) will help identify any pests that have dislodged from the coral. These pests can then be easily siphoned out of the system. During this time, important water quality and chemistry parameters should be routinely monitored and adjusted accordingly (see Section 4). After the quarantine period, the tank and all its components should be disinfected (see Appendix 1) thoroughly to eliminate the risk of contaminating any other new arrivals.

Lighting is not critical during the quarantine period. Initially, it should remain attenuated (~50 µmol/m^2/s) to reduce light stress on newly acquired corals. During the quarantine process, light levels could be increased slowly to those of the experimental or main holding system. Alternatively, the lighting in the quarantine system could remain attenuated throughout the entire process and then the coral can acclimate slowly to the more intense light regime in the new system using either neutral density filters, shade cloth, or varying light heights. However, during periods of attenuated light, corals should be fed regularly since the nutritive output of the endosymbiotic zooxanthellae will be reduced.

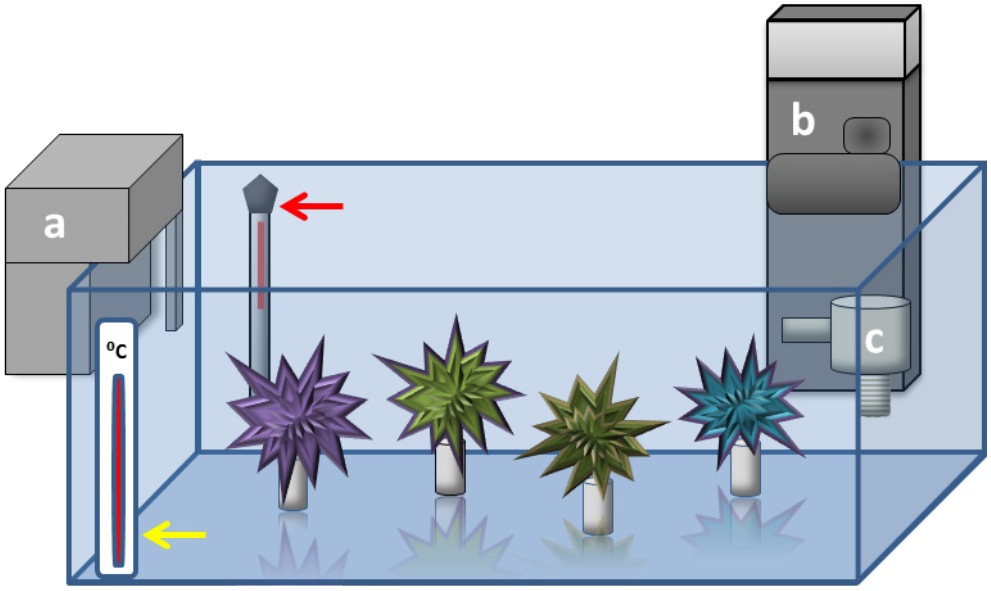


Figure 22. Possible setup for a quarantine tank. HOB powerfilters and skimmers (**a** and **b**, respectively) provide biological and chemical filtration while a powerhead (**c**) increases water circulation and gas exchange. A small heater (**red arrow**) and thermometer (**yellow arrow**) are necessary to regulate temperature. Sediment on the bottom of the tank is not recommended in quarantine tanks (see main text), thus coral colonies are supported above the tank bottom by a natural or artificial base (see Section 6.1.1 for examples).

Other components can be added to the quarantine system which may improve stability and water quality (Fig. 22). These include, but are not limited to, skimmers, media filtration, biological filters (e.g., BIO-Wheel® or sponge filter), and UV sterilization. A small "hang-on-the-back" (HOB) skimmer is very useful to eliminate dissolved organics, suspended solids, and provides circulation and aeration. A BIO-Wheel® power filter, while not commonly used in coral systems, is useful on small tanks to provide some biological filtration as well as circulation and some chemical filtration (Fig. 16). The biological filtration efficiency can be increased by seeding the BIO-Wheel® prior to use with seawater from an already established system (as described in Section 3.4). Media filtration such activated carbon in a canister filter (e.g., Marineland® H.O.T. Magnum®) will help remove harmful metabolites and other chemicals while also providing circulation (Fig. 23a). A small UV sterilizer to deter the proliferation of pathogenic suspended bacteria can be plumbed to the tank using tubing and a small powerhead (Fig. 23b).

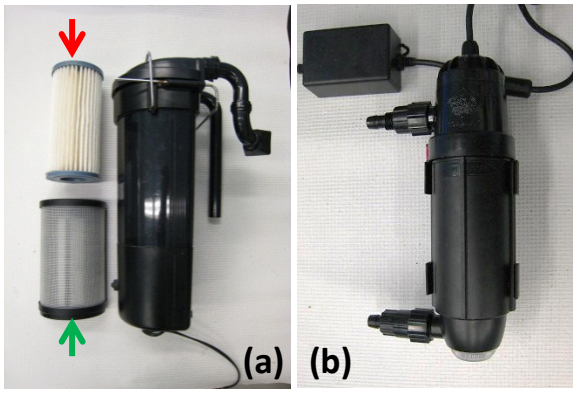


Figure 23. a) The HOT Magnum® canister filter can provide either mechanical (**red arrow**) or chemical (**green arrow**) filtration. **b**) Potentially harmful bacterial populations can be reduced with a small UV sterilizer like the Coralife® 9W Turbo-Twist™ (Franklin, WI).

5.4 Common Pests, Parasites, and Disease Seen With New Coral

Most reputable sources of live coral perform their own treatment and quarantine procedures so as to not contaminate their own stock. However, there is always a possibility that some undesirable organism has survived and can become established in the new system. The quarantine process is to separate new organisms from other stocks to reveal any health issues that arrive with the new coral and allow the researcher to identify the problem and treat it accordingly. Below are some examples of common problems seen in new coral and recommended treatments. It should be stressed, however, that the following treatments are only suggestions and not guaranteed to be successful. In some cases, especially if performed other than as recommended, they can do more harm than good.

5.4.1 Acoel Flatworms

Synonyms: Rust Brown Flatworms, *Convolutriloba* or *Waminoa* spp., planaria.

Description (Fig. 24): Tan, brown, red, or rust colored often with a red dot. Oval and slightly elongated with two tail-like appendages at their posterior, usually 3-6 mm in length.

Treatment: Commercial products (e.g., Flatworm eXit, Coral Rx), manual removal, Lugol's or other iodine-based

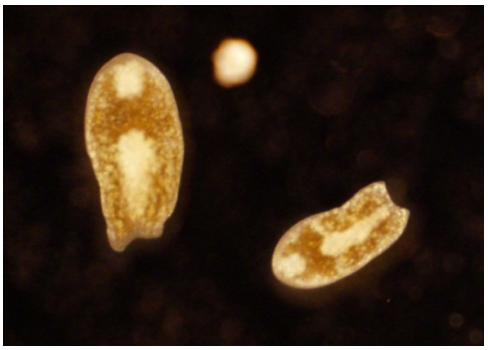


Figure 24. Common acoel flatworm. While those pictured are tan, flatworms are very often seen as red or rust colored.

dip (see Section 5.2), levamisole hydrochloride (refer to Delbeek and Sprung (2005) for additional information).

Notes: The acoel flatworms are considered more of a nuisance and a pest rather than a threat to coral, but they may ingest zooxanthellae from dying coral tissue (Calfo 2009). They can quickly overrun tanks, especially with high detritus and nutrient loads. Strong water movement and aggressive skimming can help control invasive populations.

5.4.2 *Acropora*-Eating Flatworms

Synonyms: AEFW, *Amakusaplana acroporae* (Rawlinson *et al.* 2011), may include other predatory flatworms.

Description (Fig. 25): White, tan to opaque in color, mostly oval in shape, 1-5 mm in length. Often very difficult to detect on coral except for eggs which are golden brown masses on the base or underside of white, dead coral tissue (inset).

Treatment: Commercial products (e.g., Flatworm eXit, Coral Rx), manual removal, Lugol's or other iodine-based dip, levamisole hydrochloride, freshwater dip (see Section 5.4.9). Treatment does not usually affect eggs which should be removed manually by scraping or water jet. Multiple treatments may also be necessary.

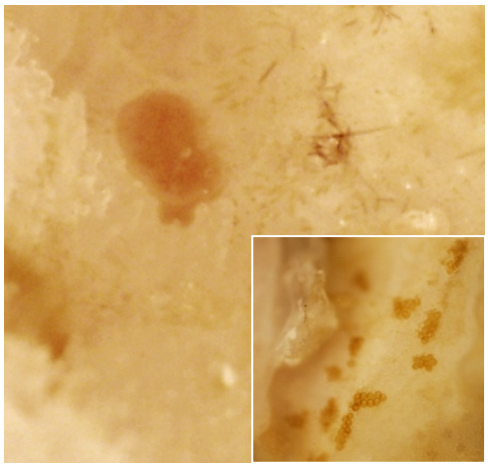


Figure 25. *Acropora* eating flatworm (AEFW) on bare skeleton of an *Acropora* fragment. Golden egg masses (**inset**) are usually the first sign of AEFW invasion.

Notes: AEFW are specific to *Acropora* spp., but not all species appear to be susceptible (Delbeek and Sprung 2005). Other predatory flatworms exist as well. These flatworms are extremely detrimental to coral health.

5.4.3 Red Bugs

Synonyms: Red Acro Bugs, *Tegastes acroporanus.*

Description (Fig. 26): A harpacticoid copepod crustacean, yellow or golden with a prominent red spot, approximately 0.5 mm in size and affecting only *Acropora* spp.

Treatment: Lugol's or other iodine-based dip, manual removal, Interceptor® (milbemycin oxime) (refer to Delbeek and Sprung (2005) for additional information).

Figure 26. Red bugs infesting an *Acropora* branch. The body of the copepod is actually yellow with a bright red head (**inset**). *Photos courtesy of Bruce Wilfong.*

5.4.4 Nudibranchs

Synonyms: *Montipora*-eating nudibranch, may include other predatory nudibranchs.

Description (Fig. 27): *Montipora*-eating nudibranchs are pale white to tan in color, up to 0.5 cm in length, and have many branch-like appendages along their back. Other predatory nudibranchs vary in size and color.

Treatment: Commercial products (e.g., Coral Rx), manual removal, Lugol's or other iodine-based dip, levamisole hydrochloride. Treatment does not usually affect eggs which should be removed manually by scraping or water jet. Multiple treatments may also be necessary.

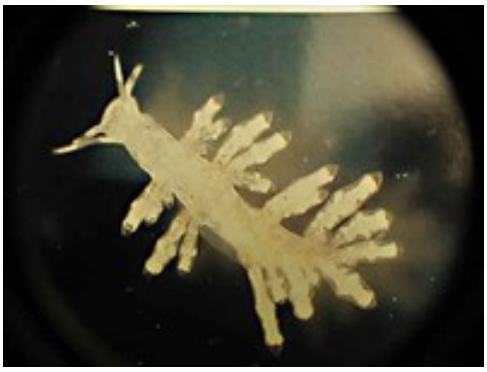


Figure 27. *Montipora*-eating nudibranch. *Photo courtesy of Advanced Aquarist.*

5.4.5 *Aiptasia* Anemone

Synonyms: *Aiptasia pallida*, *Aiptasia pulchella*, glass anemone

Description (Fig. 28): Light tan to brown, semi-translucent with sweeping tentacles and can range from millimeters to centimeters in length.

Treatments: Commercial products (e.g., Aiptasia X). Injecting single animals directly with boiling water, saturated calcium hydroxide, dilute sodium hydroxide, dilute hydrochloric acid, hydrogen peroxide, or vinegar have had moderate rates of success (Borneman 2001). Mechanical removal is not recommended since remnant pedal lacerations will propagate into new individuals (inset). New technology has recently emerged that uses electricity to electrolyze the anemone tissue (e.g., the AipTaser by Tropical Reef Corals, Orlando, FL).

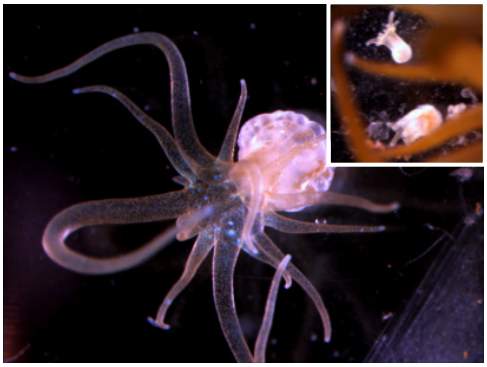


Figure 28. A relatively young *Aiptasia* anemone. Over time or as a result of attempts to physically remove the anemone, pedal lacerations will mature into new, whole animals (**inset**).

Notes: Like acoel flatworms, *Aiptasia* are considered more of a nuisance than harmful; although, their stinging tentacles could harm neighboring coral tissue, and they will compete for resources. Also similar to the flatworms, they thrive in nutrient rich systems and will reproduce quickly. So, aggressive skimming, limited feeding, and proper water quality will deter anemone populations.

5.4.6 Skeletal Eroding Band

Synonyms: SEB, Caribbean ciliate infection, *Halofolliculina corallasia* (Antonius 1999; Page and Willis 2008).

Description (Fig. 29): Sessile, folliculinid ciliate protozoan, unicellular, approximately 100-150 µm in length. Individuals form a distinct black band between healthy coral tissue and bare skeleton. Under magnification, a single specimen possesses a flask-shape body (lorica) that is

lying on or burrowed into the coral skeleton. From the lorica protrude two conspicuous peristomial "wings" which will retract quickly into the lorica if disturbed.

Treatment: Little is reported about this pest. Our observations indicate that Lugol's dip has little effect on the ciliate; however, freshwater dips may have some limited success. Removal may also be facilitated by mechanical scrubbing of the infected area.

Notes: *H. corallasia* does not directly feed on coral tissue, but the physical and chemical process of reproduction and borrowing into the coral causes tissue death. These ciliates appear to be more opportunistic, taking advantage of slow-growing, unhealthy coral tissue and high nutrient loads. Proper nutrient control and water quality may deter ciliate populations.

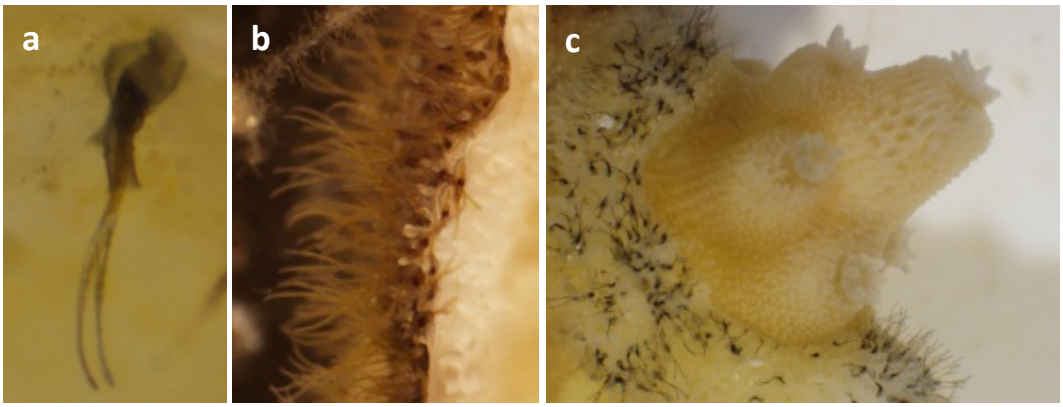


Figure 29. *Halofolliculina corallasia* infestation on an *Acropora* fragment. The unicellular, protozoan (**a**) can form dense thickets on coral skeleton (**b**). The infestation is often seen as healthy tissue surrounded by black spots or a black band of ciliates (**c**).

5.4.7 Rapid Tissue Necrosis

Synonyms: RTN, rapid tissue degeneration, shut-down reaction.

Description (Fig. 30): Sloughing of tissue, usually starting at the base. Complete tissue loss can occur within hours to days. It occurs more commonly in *Acropora* and *Pocillopora* spp.

Treatment: Commercial products (e.g., Coral Rx), Lugol's or other iodine-based dips, chloramphenicol (refer to Borneman (2001) for additional information). Regular water changes and/or chemical filtration may alleviate or prevent tissue loss.

Figure 30. Rapid tissue necrosis (RTN) occurring on a fragment of *Pocillopora damicornis*. The reaction was likely a stress response to poor water quality. *Photo courtesy of Athena Avadanei.*

Notes: It is not known what specifically causes RTN, but it has been correlated with coral stress levels like those associated with shipping. Possible causes include an autolysis reaction to stress (Borneman 2001), an "allergic reaction" to allelopathic chemical toxins (Calfo 2009), or a bacterial pathogen (Luna *et al.* 2007). Although the treatments suggested above have been

described by some to successfully treat RTN, due to the unknown etiology of RTN, these treatments should not be considered fail-safe cures for the disease.

5.4.8 Brown Jelly

Synonyms: protozoan infection, *Helicostoma* sp. (Borneman 2001).

Description (Fig. 31): Brown, gelatinous mass usually brought on by tissue trauma and subsequent infection by opportunistic protozoans. Disease progression is rapid and contagious. It is more commonly seen in large polyp corals such as *Galaxea*, *Euphyllia*, and *Xenia* spp.

Treatment: Lugol's or other iodine-based dips, freshwater dip, antibiotics.

Notes: Because of the highly contagious nature of brown jelly infection, affected tissue should be removed by siphoning before treatment.

Figure 31. Brown jelly infection. *Photo courtesy of Anthony Calfo.*

5.4.9 Additional Notes

Freshwater dips can be an effective means of eliminating some parasites and disease; however, it should be used with extreme caution. Dechlorinated tap or mineralized water (not distilled, DI, or reverse osmosis (RO) fresh water) should be used that has been temperature and pH adjusted to that of the holding or quarantine tank. The pH adjustment can be made with sodium bicarbonate (i.e., baking soda). Dips should be no longer than one to three minutes and it is not recommended for thin tissues, small polyped coral such as *Acropora* (Borneman 2001). A safer alternative may be to use a hypo-osmotic (15 ppt) seawater solution for no more than three minutes as recommended by Sweet *et al.* (2012). This solution should also be adjusted for pH.

Concentrated Lugol's iodine can also be applied topically to an affected region of coral. With the coral out of the tank, the Lugol's is diluted 1:2 or 1:4 with seawater and is applied to the coral using a cotton swab at the boundary between healthy and affected tissue. After a brief rinse in seawater, the coral is returned to the holding or quarantine tank. This technique should be used carefully and sparingly as the concentrated Lugol's will cause tissue damage.

Cyanoacrylate glue (e.g., Loctite® Super Glue Gel Professional, Henkel Corporation, OH) can also be applied at the healthy/affected tissue boundary to prevent further progression of a disease or infection with little or no damage to coral tissue.

Many parasites and pests have natural predators that will control or eliminate the issue (e.g., *Berghia* nudibranchs to *Aiptasia* anemones), but are not discussed here due to their extra care, possible harmful effects to coral, and impractical usage in a quarantine setting.

Antibiotics have been used effectively to treat some coral diseases (refer to Borneman (2001) for additional information). Because little information is known about adverse effects on the coral or their symbiont, the specific drug selected and the treatment regime will need to be determined experimentally. As an added caution, any antibiotics remaining in treatment water or surplus stock solutions should be inactivated with bleach before disposal.

Many other diseases and infections can be present on new coral arrivals, such as those listed in Table 2. These too can benefit from the treatments listed above.

The progression of many diseases and infections can be completely halted on most occasions by excising, or fragmenting, the coral at the healthy tissue region just above the affected area. Fragmenting is described in more detail in Section 6.

SECTION 6 – FRAGMENTING CORAL

In most instances, experimental setups will not require single large colonies of coral, but rather multiple small pieces. Therefore, the larger parent colonies must be physically fragmented to produce the smaller colonies. Fragmenting allows the researcher to control the physical dimensions (e.g., width, height, biomass, tissue area) of the experimental animal and, if obtained from the same parent colony, provides identical ecological and genetic clones which reduces the experimental variability due to differences between animals. In addition to the benefits of having multiple (or many) physically and genetically identical clones, fragmenting is useful for removing unwanted areas (e.g., bases covered in undesirable hitchhikers or algae covered skeleton) and preventing disease progression. Fragmenting also is used to provide a self-sustaining supply of experimental animals.

Figure 32. Examples of tools used for fragmenting coral. They include a hammer, a hand drill fitted with a hole saw, a Dremel® rotary tool, various sizes of chisels and corers or leather punches, "coral clippers" similar to pruning shears or bone cutters, and adhesives.

There are a variety of techniques and tools used to fragment corals, depending mostly on the size and shape of the parent colony (Fig. 32). For example, fragmenting a branching coral such as *Acropora cervicornis* or *Pocillopora damicornis* can be accomplished using clippers, bone-cutters, or even pruning shears of various sizes. However, mounding or boulder-like coral such as *Porites astreoides* or *Porites lobata* may require a chisel, electric cutting wheels, or even a tile saw to get suitable fragments. An acceptable procedure for fragmenting branching coral is described below; this procedure may be modified to suit other coral species.

6.1 Recommended Procedure for Fragmenting Branching Coral

6.1.1 Equipment

- Nitrile gloves
- Protective eyewear
- Coral clippers or shears
- Bases (Fig. 33)
- Cyanoacrylate glue (gel)
- At least two stands made from plastic egg crate (light-diffusing material), PVC, or Teflon®.
- Multiple holding tanks of adequate size (at least two)
- Paper towels
- Clean surface or cutting board
- Optional: heater, small powerhead or air stone for circulation

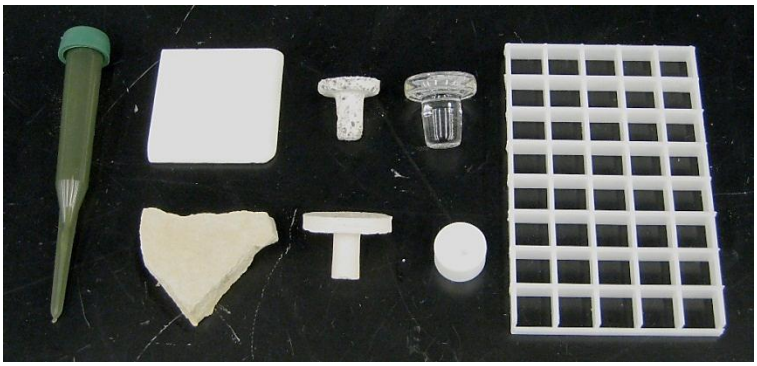


Figure 33. Bases to mount coral fragments vary in material, shape, and size. They can be composed of plastic, ceramic, concrete, glass, and Teflon®. The bases are supported on Teflon® or plastic stands.

6.1.2 Method

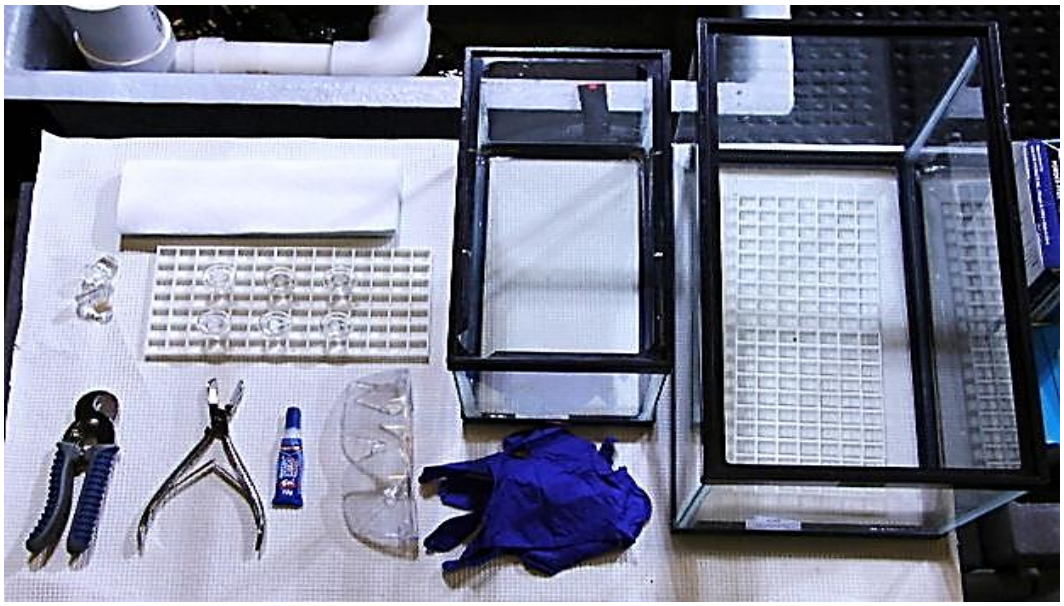


Figure 34. The fragmenting, or propagation, station should have all the necessary equipment at hand (see text) and be well organized to avoid unnecessary delays when handling live coral. This will help reduce the stress imposed on the organism as it is being manipulated.

- All tools and equipment should be thoroughly cleaned and disinfected before use to prevent possible disease transfer or contamination (see Appendix 1 for disinfection guidelines). After disinfection rinse equipment with DI and clean seawater.

- Set up coral fragmenting area with all the necessary equipment (Fig. 34) and fill holding tanks with enough system water to completely cover the anticipated sizes of coral fragments, or nubbins, on bases.
- With gloved hands, remove parent colony from holding or quarantine tank.
- Using the appropriate size clipper and avoiding excessive handling, cut branches to the desired size (see additional notes below). Place clipped nubbins into first holding tank until ready for adhesion to bases. If possible, clip parent colony directly over the water in the holding tank and let fragments drop into the tank (Fig. 35).
- When fragmenting is complete or when the parent colony has been exposed to the air for an extended period (5-10 minutes), return it to its original tank and position.
- Support bases in egg crate stand (or similar base holder) on a level surface and apply an appropriate amount of cyanoacrylate gel to the base surface about the size, or slightly less, of the exposed fragmented skeleton. Allowing gel to cure for five to ten minutes will provide a tacky area that will better support the new fragments.
- Remove new fragments from holding tank, pat exposed skeleton dry with paper towel, and place in the center of tacky gel, supporting the fragments for 30-60 seconds to insure adhesion.
- When the cyanoacrylate gel has cured enough so that the attached fragment can be moved without risk of detachment (approximately five minutes), move nubbins to the second holding tank, supporting the base on an egg crate stand (Fig. 36).
- When all the coral clippings have been attached or the second holding tank is at capacity, move fragments to holding or quarantine tank (see additional notes below).

Figure 35. Newly clipped fragments will gently fall into the first holding tank containing seawater if the parent colony is held directly over the water surface.

Figure 36. Once the fragments are attached to the bases, they can be placed in the second holding tank until it is time to place them in the system or quarantine tank.

6.1.3 Additional Notes on Fragmenting

Parent colonies to be fragmented should be healthy and stabilized for at least the duration of the quarantine process. Colonies that have recently moved or have destabilized for any other reason (e.g., infection) should be given ample time to recover. In extreme cases, unhealthy or infected areas of a colony may need to be removed by fragmentation so as to prevent disease progression into healthy regions. After excision the exposed skeleton can be sealed with cyanoacrylate glue or artists clay (e.g., Roma Plastalina #2, Rex Art, Miami, FL) to deter the growth of algae, fungi, and cyanobacteria or the settlement of other organisms such as ciliates or boring worms. A prophylactic dip as described above (Section 5.2) may also assuage disease progression. A good indication of recovery is new tissue growth enveloping the exposed or sealed skeleton which can be observed within a week.

There are numerous types of cyanoacrylate and epoxy adhesives. The gel form of cyanoacrylate (e.g., Loctite® Super Glue Gel Professional), as opposed to the more liquid type, is preferred since it provides additional stability, it is easier to apply, and is considered nontoxic when cured. Epoxies, often supplied as a two-part stick that is kneaded together before application, require more effort, are not as efficient, and can irritate living tissue. Furthermore, cyanoacrylate-based gels actually adhere to living tissue, whereas other "glues" merely form around the fragment, holding it in place once hardened (Calfo 2009).

If the time required to fragment is excessive, placing a heater and small pump or air stone in the holding tanks is recommended. The circulation will help remove excess mucus from the newly fragmented corals.

Calfo (2009) suggests that in order to increase the likelihood of success, the new fragments should be no less than about 2.5 cm (length or diameter), the rationale being that larger segments are hardier and demonstrate greater survivability. This guideline is somewhat arbitrary and relative to the polyp sizes of different coral species. On the other hand, Shafir *et al.* (2006) describe a means for successfully producing coral nubbins of approximately 0.25 cm^2, incorporating as few as a single polyp, to be used for experimental purposes.

Prior to returning new coral nubbins to the holding or quarantine tank, a prophylactic dip in diluted Lugol's (see Section 5.2 for recommended treatment procedure) or other commercial products (e.g., ReVive™, Coral Rx) may improve recovery and survivability of freshly clipped coral.

If at all possible, avoid placing new fragments in a confined space with other coral species. The stress of fragmenting will cause the production of mucus and other noxious allelopathic compounds which could harm other corals, a possible cause of RTN. The increased mucus production may also increase the risk of infection among the stressed coral by promoting the growth of potentially harmful bacteria (Calfo 2009).

SECTION 7 – THREE EXPERIMENTAL DESIGNS AT WORK

7.1 Small-Scale Biological Challenge Experiment Using *Fungia*

Although only six experimental units are illustrated here, this particular setup can accommodate up to 12 to 16 replicates depending on experimental design and available space. Animals and treatments are randomized to prevent segregation. New ASW is supplied using a common manifold that is plumbed to a central aerated ASW reservoir (Fig. 37; see Appendix 2 for equipment list). A small UV sterilizer is plumbed between the ASW reservoir and the seawater manifold to sterilize water immediately prior to use. Air is supplied using a common manifold connected to a central air pump. Waste seawater is siphoned out of each tank and directed through a collection manifold to a common waste bucket which is then decontaminated with bleach and drained into the sewer.

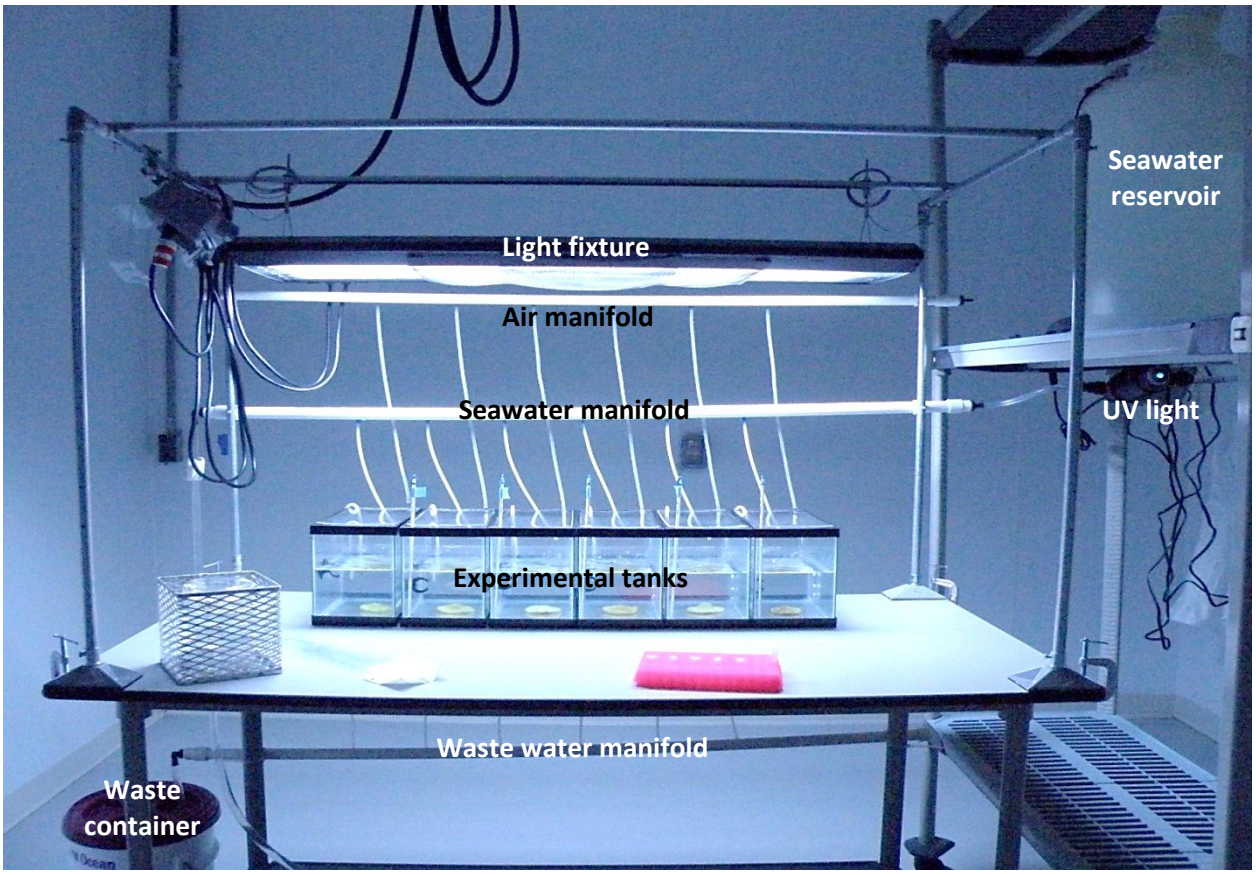


Figure 37. Overview of biological dosing setup and its major components.

Each 2.5 gallon tank is fitted with an air supply (for aeration and circulation), a thermometer, heater, new ASW supply tube, and a waste seawater siphon tube (Fig. 38). A clear lid covers each tank to prevent cross-contamination and reduce evaporation. Air flow and new ASW flow are regulated using needle valves which are threaded into their respective manifold using a thread tap. Pinch valves are used to control waste seawater siphoned from each tank during twice weekly water changes.

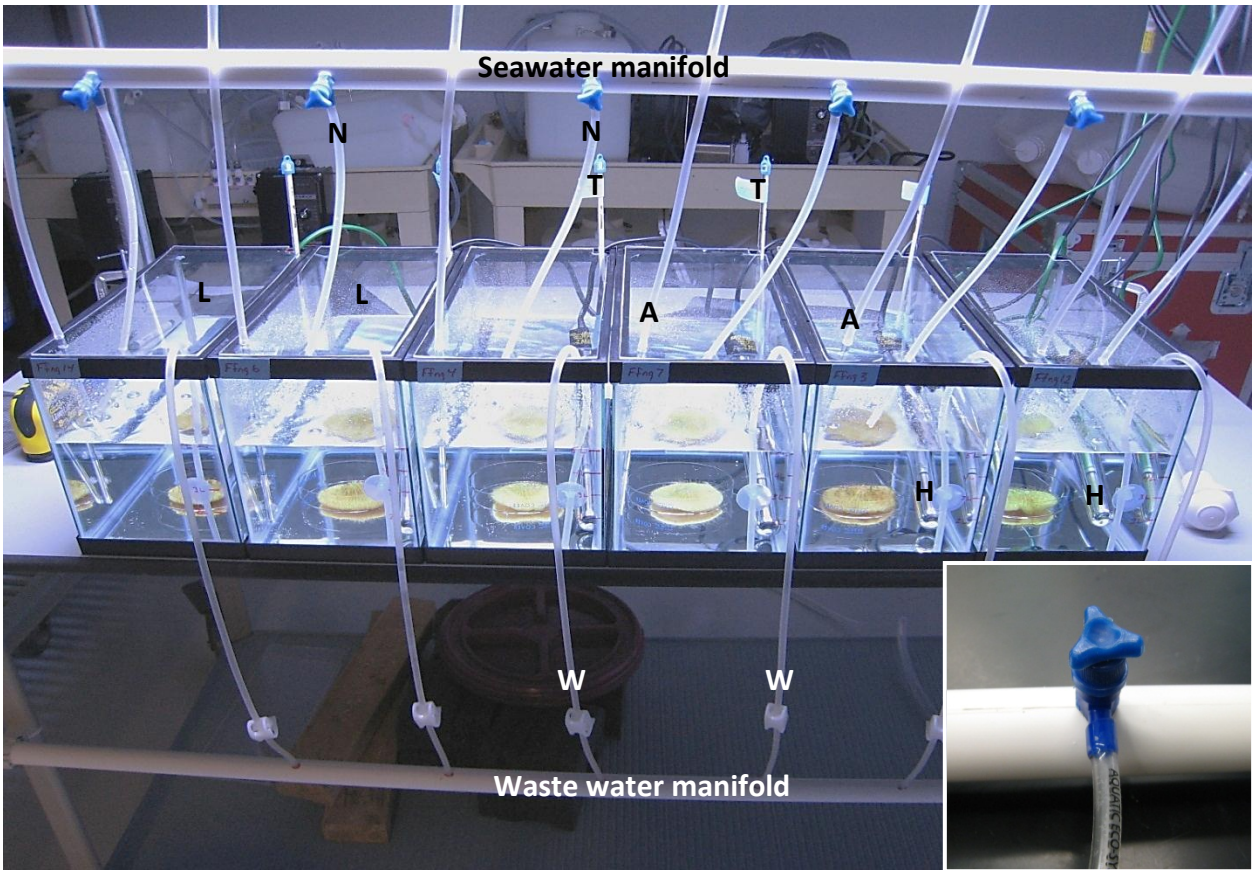


Figure 38. Front view of biological dosing setup. Each tank is equipped with a thermometer (**T**), heater (**H**), air line (**A**), new ASW line (**N**), waste water siphon tube (**W**), and a clear lid (**L**). (**inset**) Closeup of plastic needle valve that regulates air and new ASW.

The intensity of the T5 HO fluorescent light source was standardized over each tank by measuring with a LI-192 Underwater Quantum Sensor (LI-COR®) and adjusted with shade cloth material (Fig. 39a). Volume levels were marked on each tank to ensure accurate and consistent water changes (Fig. 39b). Air lines, new ASW lines, and waste water siphon tubes are inserted into the experimental tank through holes drilled in the lids. The air supply is kept rigid and in place by inserting a 1 ml glass serological pipet (cotton filter removed) into the end of the flexible air-line tubing. In addition, the siphon tube is affixed to the side of the glass experimental tank with a plastic suction cup.

Biocontainment is managed by using an isolated room that can be washed down, designated lab coats, plenty of consumables and waste storage, and disinfectants including a disinfectant foot mat containing Virkon® Aquatic (see Appendix 1) before exiting the room (not shown).

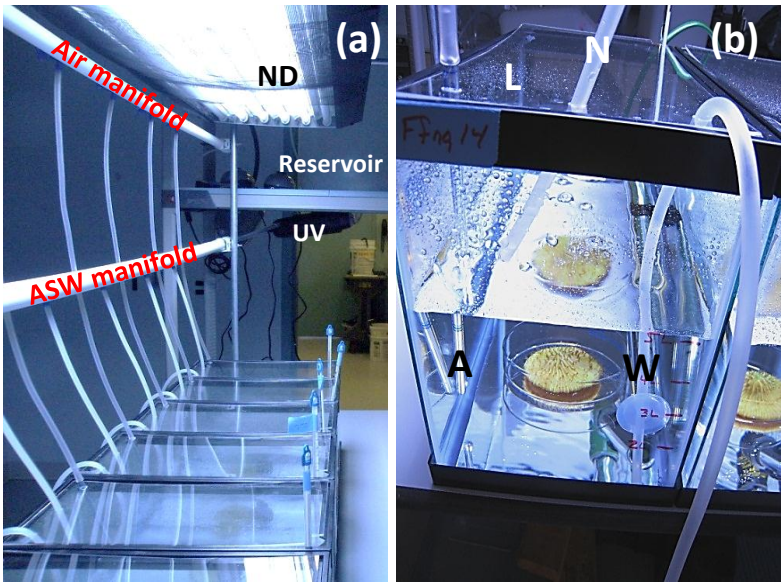


Figure 39. a) Side view of biological dosing setup. Neutral density shade cloth (**ND**) standardizes the light intensity across all the experimental tanks. **b**) Close-up of individual experimental tank. The air line (**A**), new ASW line (**N**), and waste siphon tube (**W**) enter the tank through holes drilled into the lid (**L**).

7.2 Effects of Different Light Sources on *Acropora cervicornis*

This experiment was designed to investigate the effect of two different light sources, compact fluorescence (CF) and light-emitting diode (LED), on the rate of coral tissue regeneration. The actual spectral output of each light source can be seen in Fig. 9 (E&F). The six experimental units (two treatments with three replicates each) are systematically interspersed between two different shelf units to reduce segregation. For non-laminar circulation, gas exchange, and limited biological filtration, each 5 gallon tank is fitted with a small BIO-Wheel® powerfilter and powerhead (Fig. 40; see Appendix 2 for equipment list). Both are positioned in the same place on each tank to reduce variability and prevent spurious treatment effects as a result of circulation differences. Due to the relatively long duration of this experiment (30-40 days), the limited biological filtration offered by the BIO-Wheel® powerfilter can help maintain acceptable water quality parameters. Opaque barriers and black plastic sheeting are used to prevent ambient and stray neighboring light from interfering with treatments. Consistent temperatures between treatments are maintained in a temperature controlled room and daily monitoring with a digital thermometer. *Caveat: With this particular design there is a possibility of some segregation effects such as slight temperature differences between tanks located on different shelving units. With proper replication and interspersion, these effects can be detected by statistical analysis.*

Figure 40. Overview of light experimental setup. Each replicate has its own light source (**CF** or **LED**), BIO-Wheel® powerfilter (**F**) and small powerhead (**P**) and are separated by cardboard and black plastic sheeting (not shown) to reduce stray light. Only three of the six replicates are shown here.

Light intensity over each tank is standardized at 100 µmol/m^2/s by measuring the PAR in the center of the tank at a fixed depth (Fig. 41a), and the height of each lamp is adjusted accordingly. A 10:14 hour light:dark cycle is controlled by a single mechanical timer. Individual fragments are secured to Teflon® bases and are then centered under their respective light source at a fixed height atop a stand (Fig. 41b). Coral fragments are placed randomly in each tank to prevent any experimental bias.

Given the relatively low bioload in each tank (one 2.5 cm fragment per ~19 L of seawater), more fragments (up to about ten) could be added to each. However, additional fragments **CAN NOT** be considered additional replicates since the independent experimental unit is the individual tank, not the coral within the tank. Measurements of multiple fragments within a single tank would then need to be averaged and considered one replicate or used as repeated measures, removing a fragment at a predetermined timepoint. Also keep in mind that increasing the bioload in each tank could also negatively affect water quality and require more frequent or larger volume water changes to maintain acceptable water quality parameters (see Section 4).

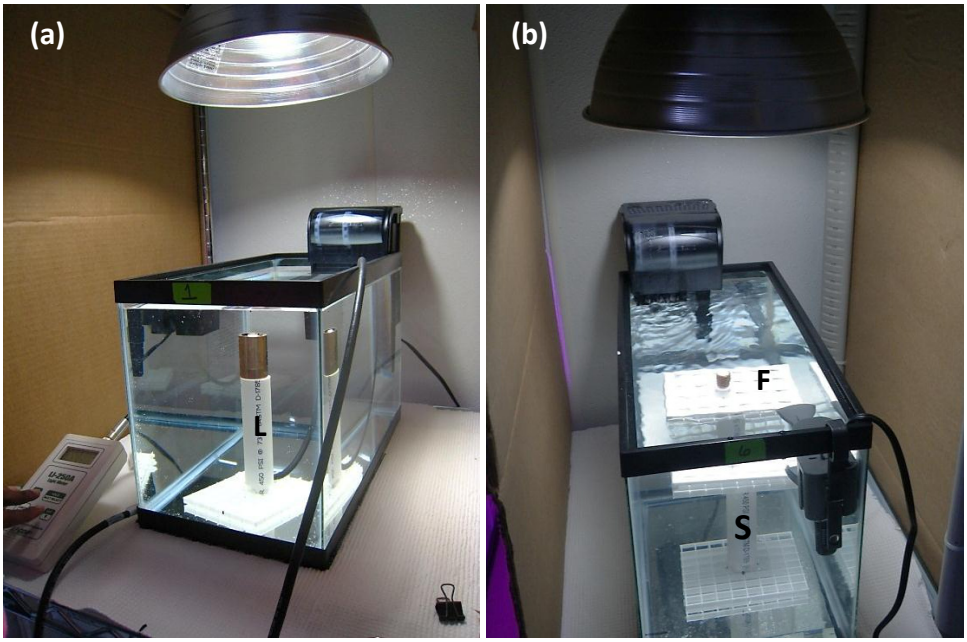


Figure 41. a) Each light is standardized to 100 µmol/m^2/s using an LI-192 underwater light sensor (LI-COR®) mounted on a PVC stand (**L**). **b**) The single coral fragment (**F**) is centered in the middle of the tank directly under the light source at a specific height on a PVC stand (**S**).

Siphon tubes are placed in each tank to allow for convenient removal of tank seawater during water changes (Fig. 42). New ASW is then added directly to each tank until the predetermined fill-line is reached. By maintaining a constant water level, light intensity will not vary as a result of the fragment being in different depths of water.

Because of the longer duration of this experiment (over five weeks), each coral fragment is fed once a week in a separate container with new ASW and 0.01 g Golden Pearls 100-200 µm Larval Diet (Brine Shrimp Direct, Ogden, UT). The food slurry is pipetted directly over the coral nubbin using a transfer pipet and allowed to sit for ~10 minutes before being returned to its respective experimental tank. Feeding in a separate container improves consistency between each replicate and reduces the potential harmful effects of remnant food material on water quality.

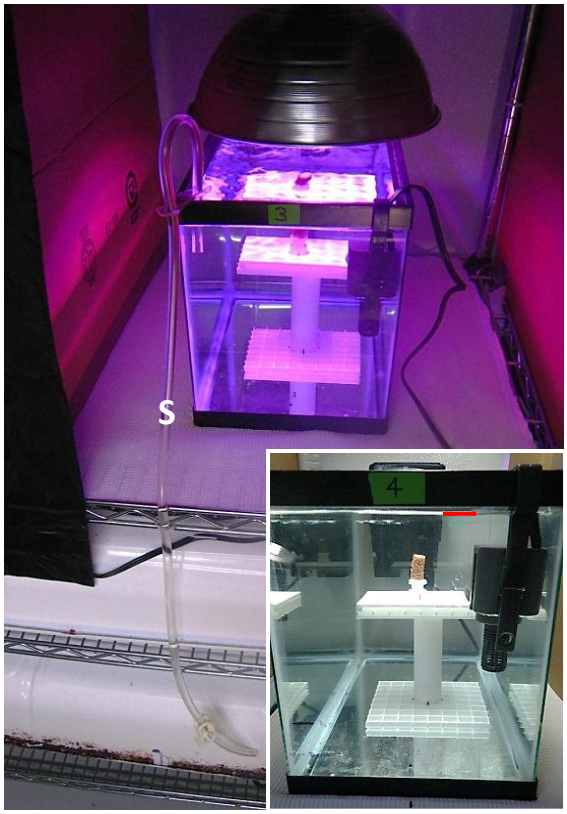


Figure 42. A siphon tube (**S**) allows for quick and accurate water changes along with a marked fill-line (**inset; red line**).

7.3 Chemical Dosing Experiment on *Pocillopora damicornis*

This chemical dosing system is designed to contain up to 36 one liter isolated experimental units which are divided between two separate holding tanks. A diagram of a single holding tank is shown below (Fig. 43; see Appendix 2 for equipment list). Isolative segregation is prevented by dividing all the treatments evenly between the two holding tanks rather than placing some treatments in one and some in the other. However, replicates are distributed randomly within each holding tank.

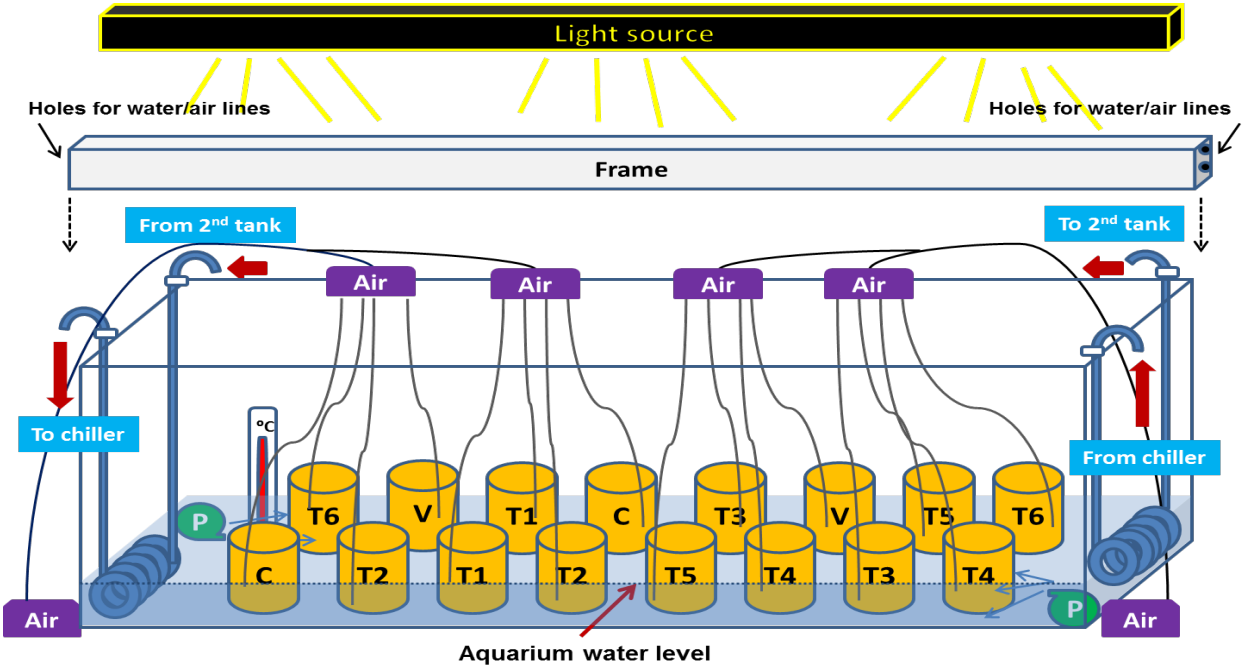


Figure 43. Schematic overview of chemical dosing system. *Schematic designed by Lisa May.*

Four air pumps supply two gang valves each; each gang valve then controls the air flow of four separate air tubes. The gang valves are adjusted such that each unit maintains a consistent bubble rate that produces adequate water movement within the beaker without excessive splashing. Air lines and recirculating water lines are channeled through holes drilled into the PVC frame (Fig. 44). A 10:14 hour light:dark cycle is controlled by a digital timer/powerstrip which also serves as a continuous power supply for the numerous water and air pumps.

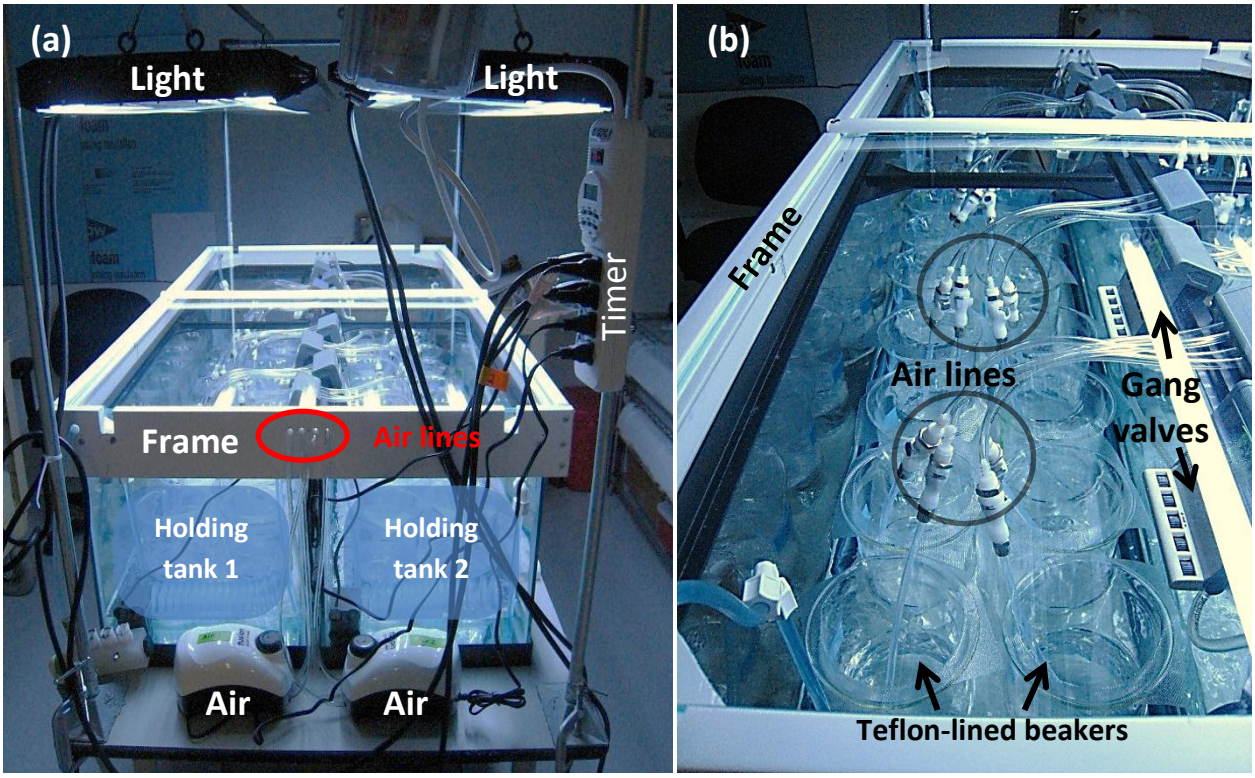


Figure 44. **a**) Side overview of chemical dosing system. Treatments are divided evenly between two holding tanks. **b**) Closeup side view of dosing system. Gang valves regulate the air flow in each experimental unit.

Each experimental unit consists of a 1 L beaker fitted with a Teflon® liner and a single Teflon® air tube (Fig. 45). Teflon® is used exclusively to eliminate sequestering of the compound of interest by glass or plastic materials.

Given the large number of replicates, it is not practical to use individual heaters; and due to unacceptable temperature fluctuations in such small volumes, the temperature controlled room is also unreliable. As an alternative, a water bath is used to maintain consistent temperatures in each experimental unit (Fig. 46). An appropriate amount of fresh water is added to each holding tank, at or around the same level as the seawater in each beaker. A temperature controlled water circulator pumps conditioned fresh water through manifolds that are connected to coils placed in each holding tank. The conditioned water

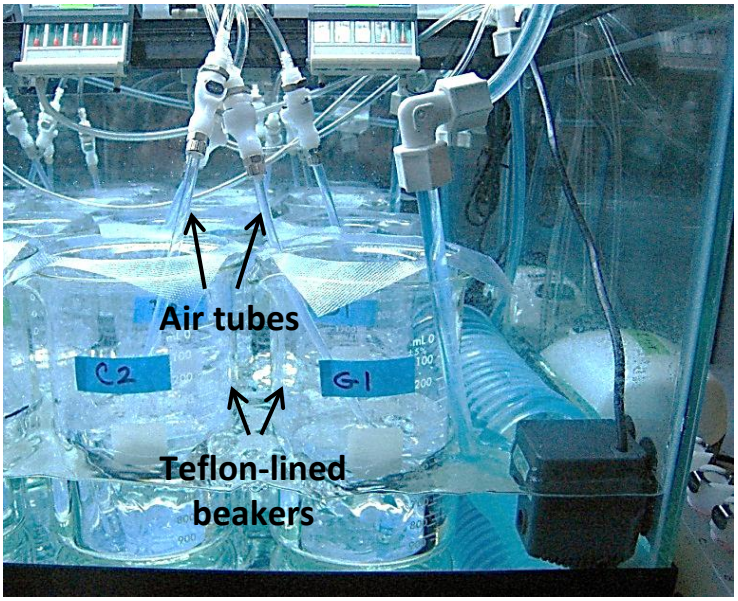


Figure 45. Each replicate consists of a Teflon®-lined beaker and a single Teflon® air tube.

passes through each holding tank twice and then returns to the circulator. Two small powerheads are placed on either side of each holding tank next to the coils to effectively mix the water bath and generate a homogenous temperature distribution.

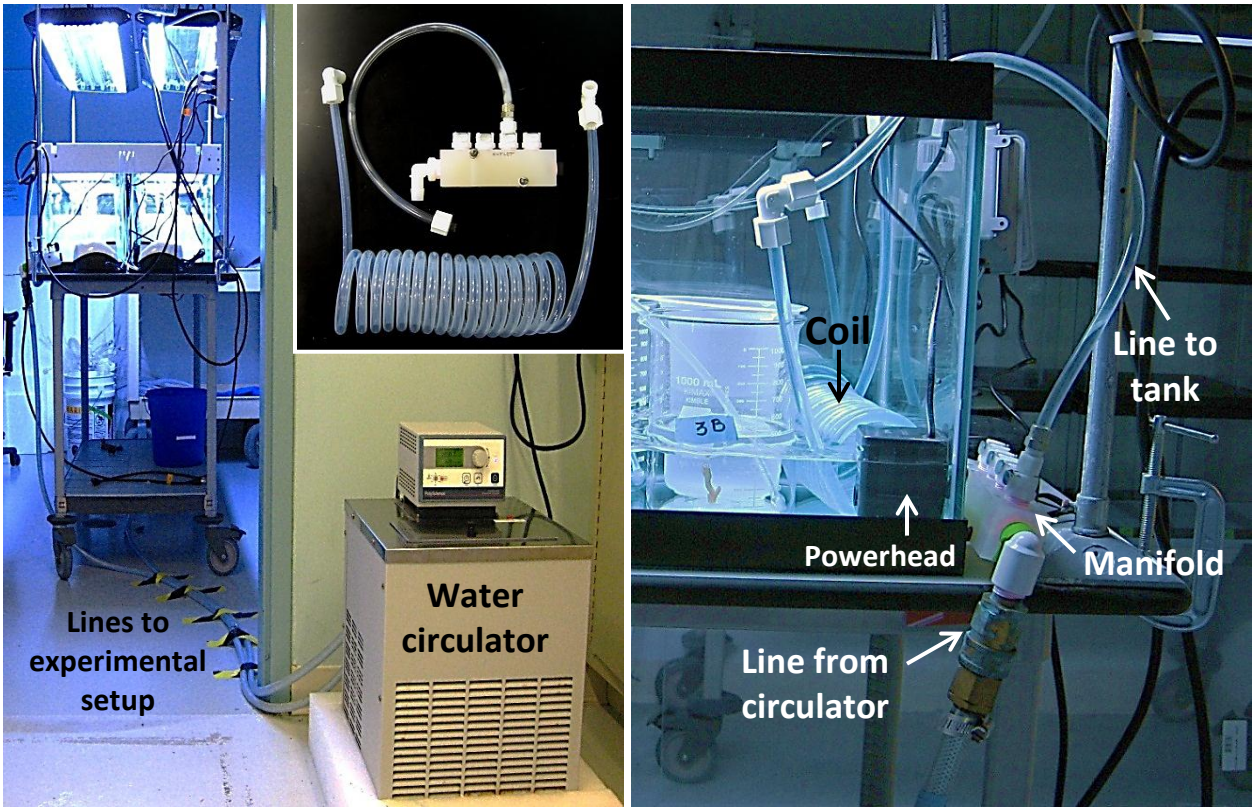


Figure 46. Water recirculation system for regulating temperature in the chemical dosing system. A temperature controlled water circulator pumps fresh water through a manifold and coils (**inset**) placed in each water bath. Small powerheads ensure evenly distributed water temperatures.

Preliminary work with this system showed that water quality did not significantly decrease over the short duration of the experiment (4-5 days). As a result the only maintenance necessary is daily salinity checks and the occasional adjustment with DI water. Note that although water quality seemed stable this does not address the chemical agent being tested and maintaining constant concentrations. Thus water changes are recommended as frequently as every 12 hr to maintain the desired concentration of the chemical. The simplest method of conducting a water change is to remove the coral fragment quickly and place it in a beaker of freshly made exposure media that has been properly equilibrated to the dosing conditions (i.e., temperature, pH). **Caution:** All contaminated waste water should be disposed of in the proper waste container.

Adding any chemical substance to seawater at relatively high concentrations, as is usually the case in chemical dosing experiments, can have significant negative impacts on water chemistry and consequently coral health. For example, some chemicals may act as chelators (e.g., EDTA) and remove necessary ions such as calcium and magnesium from the system. Others may produce extreme pH fluctuations which would induce stress in the exposed animal. Any

unknown effects of an experimental substance on water chemistry should be elucidated before use. If possible, steps to counteract or neutralize any negative effects on water chemistry should be employed (e.g., adjust pH with appropriate acid or base) which would not otherwise affect the mode of action of a particular compound.

7.4 Additional Notes on Experimental Designs

The experimental designs described above are meant to illustrate only a few of the ways in which the researcher can address the many variables inherent in coral experimental research. Many factors are involved in shaping the design of an experiment including, but not limited to, available facilities and equipment, the model coral species, required water parameters, and the specific experimental question. It is imperative that the investigator first identify any available resources, limitations, and variables in order to determine the optimal experimental system design that will effectively address these issues and reduce as much experimental variability as possible.

SUMMARY

The design and construction of small scale experimental systems for the study of scleractinian corals under a variety of stressor conditions demands considerable attention due to the many facets one should consider before undertaking the process. There are four main components of the experimental system and each should be thoroughly vetted in light of the others: the animal, the physical containment system, the media/water, and lighting. The system should further be designed to meet stringent experimental design factors such as numbers of treatments, replication of treatments, and sample size that will result in statistically sound data. This treatise provides guidance which fosters sound design, provides insight into possible materials and approaches to develop a functional system, and a means of handling corals for use in the test system.

GLOSSARY

- **Acclimation:** The process by which a coral is slowly conditioned to a change in environment. Example – Using the seawater drip method to acclimate a coral colony to a new tank.
- ***Aiptasia*:** A genus of hardy anemones which may be considered an aquarium pest due to its rapid rate of growth.
- **Air stone:** A device usually made of porous glass, sand, or ceramic material that is attached to an air-line to produce fine bubbles. Also termed an "air diffuser."
- **Alkalinity:** The acid-buffering capacity of seawater equal to the stoichiometric sum of the bases in solution and is often *incorrectly* used interchangeably with basicity. Alkalinity can be expressed in milliequivalent per liter (mEq/L), parts per million calcium carbonate (ppm $CaCO_3$), or carbonate hardness (KH or dKH).
- **Allelopathy:** The method by which a living organism produces one or more chemicals that can positively or negatively influence the growth, reproduction or survival of another organism.
- **Aragonite:** One of two naturally occurring carbonate minerals. It is formed in the shells of mollusks and in the calcareous exoskeleton of scleractinian corals. Commercially it is used in the saltwater aquarium trade to provide a substrate for animals, buffer seawater, and as a supplemental carbonate source for stony corals.
- **Biocontainment:** The type of physical isolation used to prevent the release of an organism into the environment. This usually pertains to highly pathogenic or recombinant organisms, but can also serve as a method for preventing the release of non-native species into an environmentally sensitive area.
- **Bioload:** A term commonly used in the aquaculture and aquarium trade, the relative proportion of biomass per a given volume in a tank or system. As bioload increases, so too do the negative effects on water quality (e.g., elevated nitrogenous waste). Systems with higher bioloads may require additional mechanical, chemical, or biological filtration or more frequent/greater volume water changes.
- **Biomass:** The total living biological organisms in an ecosystem at a given time.
- **Biosecurity:** The practices and procedures put into place to reduce the risk of release of undesirable organisms into the environment.
- **Brown Jelly:** A coral disease almost exclusively found in aquaria. It presents as a brown-colored slime which may float above the coral colony or appear on the surface of the coral. The etiology is unknown, however it usually appears after a colony has undergone physical injury.
- **Bulkhead:** A mechanical fitting with a flange, rubber gasket and a retaining nut, which attaches to a drilled hole in an aquarium or sump. It serves as a port for water circulation and drainage in the system.
- **CITES:** (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) is an international agreement between governments. Its aim is to ensure that international trade in specimens of wild animals and plants does not threaten their survival. Corals are included in this agreement.

- **Closed loop (recirculating) aquarium system:** An aquaculture design in which seawater from the tank is refreshed within a sump using various physical, biological and chemical filtration devices before being returned to the tank.
- **Cnidarian:** A phylum of radially symmetrical aquatic (mostly marine) animals having cnidocytes, or stinging cells, for defense and prey capture.
- **Deionized:** A type of purified water in which ions and other compounds have been removed using ion exchange resins.
- **Flow-through aquarium system:** An aquaculture system design in which natural or artificial seawater is passed through the tank only once prior to being treated and released back into the aquatic environment or down the sewer system.
- **Fragment:** A piece of coral that has been excised from a larger coral colony, usually as a method of coral propagation in aquaculture.
- **Live rock:** Aragonite skeletons from long-dead corals which are inhabited by many kinds of marine organisms, including coralline algae, sponges and other invertebrates. It is often harvested for use as a natural biological filter.
- **Live sand:** A natural reef coral sand that either is collected live from the ocean, or is non-living coral sand that is cultured to make it live. As in the case of live rock, it can serve as the main base for biological filtration in a saltwater aquarium.
- **Lorica:** The thick, shell-like cuticle enclosing the animal body of some members of the Phyla Protozoa and Rotifera.
- **Lugol's solution:** A solution of elemental iodine and potassium iodide in water, used as an antiseptic and disinfectant. It is used to treat newly acquired coral colonies, or corals exhibiting signs of disease.
- **Manifold:** A mechanical device used to regulate fluid or air flow in an aquaculture system.
- **Neutral density filter:** Used in photography and optics, a filter typically composed of glass or plastic which can be used to diffuse light without altering the light spectrum.
- **Nubbin:** A small coral fragment.
- **Nudibranch:** A clade of carnivorous marine gastropods which shed their shell after the larval stage. Often termed "sea slugs," nudibranchs feed on some species of coral.
- **Photosynthetically active radiation (PAR):** The spectral range of light potentially useful for photosynthesis (400-700 nanometers).
- **PAR meter:** An instrument used to measure the amount of available PAR usually expressed as $\mu mol/m^2/s$ or $\mu E/m^2/s$.
- **Pathogen:** A microorganism which causes disease in a host.
- **pH:** The measure of the molar concentration of hydrogen ions in a solution (i.e., whether a solution is acidic or basic).
- **Powerhead:** A small, submersible water pump used to circulate water in the aquarium. Strategic placement of these pumps can create various types of water flow in the tank.
- **Protein skimmer:** A device used in saltwater aquaria to remove dissolved and particulate organic material from the water by creating a large water/air interface.
- **Protozoan:** A diverse group of eukaryotic, unicellular organisms such as amoebas, ciliates, and foraminiferans that live in the water or as parasites.

- **Pseudoreplication:** An experimental design in which the treatments are not replicated or the experimental units are not statistically independent.
- **Quarantine:** The physical isolation of an organism, usually to prevent the spread of disease.
- **Randomization/Replicate interspersion:** An experimental design in which treatment replicates are not ordered. It is important to ensure uncontrollable parameters do not have a significant impact upon experimental results.
- **Rapid tissue necrosis (RTN):** A fast-acting coral disease in which the coral tissue sloughs from the skeleton. Possibly related to stress-related necrosis and shut-down reaction, it usually affects *Acropora* or *Pocillopora* species.
- **Red bugs:** A common parasitic crustacean (copepod) that infects only Acroporid corals. They are less that 0.5 mm and appear yellow with a prominent red spot.
- **Refractometer:** An instrument used to measure the refractive index of a substance. In aquaculture it is used to determine the salinity and specific gravity of seawater.
- **Refugium:** Integrated within a sump or separate, a protected area, or refuge, that shares the same water supply as the main holding tank and usually contains macroalgae and harbors smaller, more delicate invertebrates such as amphipods, copepods, and isopods. A refugium can be a means of denitrification, nutrient export, and plankton production.
- **Reverse osmosis:** A method of water purification using membrane filtration to remove many types of large molecules and ions by applying pressure to the solution when it is on one side of a selective membrane.
- **Salinity:** The dissolved salt content of a body of water.
- **Scleractinian:** A group of corals (Order Scleractinia) having hard calcium carbonate skeletons.
- **Shade cloth:** Similar in function to neutral density filters, material (e.g., window screen mesh) which can be used to diffuse light without altering the light spectrum.
- **Skeletal eroding band:** A disease of corals that presents as a black or dark gray band that slowly advances over the colony, leaving a progressive region of dead coral. The causative agent is a protozoan, *Halofolliculina corallasia*.
- **Sump:** In aquaculture, a holding tank, usually underneath the aquarium which serves as a reservoir for biological, chemical and physical treatment of used tank water.
- **Transhipped:** Animals transported from the collection source to the point of entry in another country (e.g., Indonesia to Los Angeles) usually by a broker or wholesaler. Transhipped animals undergo severe stress and survival is usually low, especially if distributed immediately to a secondary destination.
- **UV dosage:** Also referred to as UV fluence, the amount of UV irradiance, expressed as milli-Joules per square cm (mJ/cm^2) or micro-watt seconds per square cm ($\mu Wsec/cm^2$), needed to inactivate a specific microorganism by a given amount. This amount is usually measured as a logarithmic (base 10) reduction such that a 1-log reduction corresponds to 90% inactivation efficiency; a 2-log reduction corresponds to a 99% inactivation efficiency, etc.

REFERENCES

Antonius A (1999) *Halofolliculina corallasia*, a new coral killing ciliate on Indo-Pacific reefs. Coral Reefs 18: 300.

APHA (2012) Standard Methods for the Examination of Water and Wastewater. 22nd Edition. APHA/AWWA/WEF, Washington, DC. 1496p.

Aronson RB and Precht WF (2001) Applied paleoecology and the crisis on Caribbean coral reefs. Palaios 16: 195-196.

Aronson RB and Precht WF (2002) White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia 460: 25-38.

Baird A and Maynard JA (2008) Coral adaptation in the face of climate change. Science 320: 315.

Bassim K and Sammarco P (2003) Effects of temperature and ammonium on larval development and survivorship in a scleractinian coral (*Diploria strigosa*). Marine Biology 142: 241-252.

Borneman EH (2001) Aquarium Corals: Selection, Husbandry, and Natural History. T.F.H. Publications, Inc. Neptune City, NJ. 432p.

Bruckner AW (2012) Factors contributing to the regional decline of *Montastraea annularis* (complex). In: D. Yellowlees and T.P. Hughes (eds.), Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia, July 9-13.

Burdick D, Brown V, Asher J, *et al.* (2008) The State of Coral Reef Ecosystems of Guam. pp. 465-509. In: J.E. Waddell and A.M. Clarke (eds.), The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008. NOAA Technical Memorandum NOS NCCOS 73. NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team. Silver Spring, MD. 569p.

Burke L, Reytar K, Spalding M, *et al.* (2011) Reefs at Risk Revisited. World Resources Institute. Washington, DC. 130p.

Calfo A (2009) Book of Coral Propagation: Reef Gardening for Aquarists. Volume 1. 2nd Edition. Reading Trees Publications. Monroeville, PA. 416p.

Carl M (2008) Predators and pests of captive corals. pp. 31-36. In: R.J. Leewis and M. Janse (eds.), Advances in Coral Husbandry in Public Aquariums. Public Aquarium Husbandry Series, vol 2. Arnhem, Netherlands.

Carpenter KE, Abrar M, Aeby G, *et al.* (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science 321: 560-563.

Cervino JM, Thompson FL, Gomez-Gil B, *et al.* (2008) The Vibrio core group induces yellow band disease in Caribbean and Indo-Pacific reef-building corals. Journal of Applied Microbiology 105: 1658-1671.

Chevrefils G, Ing B, and Caron E (2006) UV dose required to achieve incremental log inactivation of bacteria, protozoa and viruses. IUVA News 8(1): 38-45.

Delbeek JC and Sprung J (2005) The Reef Aquarium Volume Three: Science, Art, and Technology. Ricordea Publishing. Miami Gardens, FL. 680p.

Dell RB, Holleran S, and Ramakrishnan R (2002) Sample size determination. ILAR Journal 43(4): 207-213.

Dickson AG (1981) An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. Deep-Sea Research 28A: 609-623.

Eddy FB (2005) Ammonia in estuaries and effects on fish. Journal of Fish Biology 67(6): 1495-1513.

Federal Register (2006) Endangered and Threatened Species: Final Listing Determinations for Elkhorn Coral and Staghorn Coral. Federal Register 71(89): 26852-26872.

Federal Register (2012) Endangered and Threatened Wildlife and Plants: Proposed Listing Determinations for 82 Reef-Building Coral Species; Proposed Reclassification of *Acropora palmata* and *Acropora cervicornis* from Threatened to Endangered. Federal Register 77(236): 73220-73262.

Fenner D, Speicher M, Gulick S, *et al.* (2008) The State of Coral Reef Ecosystems of American Samoa. pp. 307-351. In: JE Waddell and AM Clarke (eds.), The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008. NOAA Technical Memorandum NOS NCCOS 73. NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team. Silver Spring, MD. 569p.

Festing MFW and Altman DG (2002) Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR Journal 43(4): 244-258.

Friedlander A, Aeby G, Balwani S, *et al.* (2008a) The State of Coral Reef Ecosystems of the Northwestern Hawaiian Islands. pp. 263-306. In: JE Waddell and AM Clarke (eds.), The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008. NOAA Technical Memorandum NOS NCCOS 73. NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team. Silver Spring, MD. 569p.

Friedlander A, Aeby G, Brainard R, *et al.* (2008b) The State of Coral Reef Ecosystems of the Main Hawaiian Islands. pp. 219-261. In: JE Waddell and AM Clarke (eds.), The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008. NOAA Technical Memorandum NOS NCCOS 73. NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team. Silver Spring, MD. 569p.

Galloway SB, Bruckner AW, and Woodley CM (eds.) (2009) Coral Health and Disease in the Pacific: Vision for Action. NOAA Technical Memorandum NOS NCCOS 97 and CRCP 7. National Oceanic and Atmospheric Administration, Silver Spring, MD 314pp.

Gardner TA, Côté IM, Gill JA, *et al.* (2003) Long-term region-wide declines in Caribbean corals. Science 301: 958-960.

Gilliam DS (2011) Southeast Florida Coral Reef Evaluation and Monitoring Project 2010 Year 8 Final Report. Florida DEP Report #RM085. Miami Beach, FL. 42p.

Green EP and Bruckner AW (2000) The significance of coral disease epizootiology for coral reef conservation. Biological Conservation 96: 347-361.

Grimsditch GD and Salm RV (2006) Coral reef resilience and resistance to bleaching. The International Union for the Conservation of Nature and Natural Resources (IUCN). Gland, Switzerland. 52pp.

Heffner RA, Butler MJ, and Reilly CK (1996) Pseudoreplication revisited. Ecology 77(8): 2558-2562.

Hijnen WAM, Beerendonk EF, and Medema GJ (2006) Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. Water Research 40: 3-22.

Hoegh-Guldberg O (1994) Population dynamics of symbiotic zooxanthellae in the coral *Pocillopora damicornis* exposed to elevated ammonium {$(NH_4)_2SO_4$} concentrations. Pacific Science 48: 263-272.

Holmes-Farley R (2002) Chemistry and the aquarium: What is alkalinity? Advanced Aquarist 1(2). http://www.advancedaquarist.com/2002/2/chemistry (accessed December 17, 2012).

Holmes-Farley R (2004) Reef aquarium water parameters. Reefkeeping 3(4). http://www.reefkeeping.com/issues/2004-05/rhf/index.php (accessed Dec. 17, 2012).

Holmes-Farley R (2005) Nitrite and the reef aquarium. Reefkeeping 4(5). http://reefkeeping.com/issues/2005-06/rhf/index.php (accessed Dec. 17, 2012).

Holmes-Farley R (2006) Refractometers and salinity measurement. Reefkeeping 5(11). http://reefkeeping.com/issues/2006-12/rhf/index.php (accessed Dec. 17, 2012).

Houk P and Starmer J (2007) Rapid ecological assessment for Yap, Ngulu, and Ulithi, Yap State, Federated States of Micronesia: quantitative assessments of coral-reef assemblages and macroinvertebrate abundances. Yap Action Community Program. Yap, Federated States of Micronesia. 43p.

Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54(2): 187-211.

Johnson PD and Besselsen DG (2002) Practical aspects of experimental design in animal research. ILAR Journal 43(4): 202-206.

Joshi S (2005) Underwater light field and its comparison to metal halide lighting. Advanced Aquarist 4(8). http://www.advancedaquarist.com/2005/8/aafeature (accessed Dec. 17, 2012).

Joshi S (2010) Quantitative comparison of lighting technologies: Metal halide, T5 fluorescent and LED. Advanced Aquarist 9(2). http://www.advancedaquarist.com/2010/2/aafeature2 (accessed Dec. 17, 2012).

Kilkenny C, Parsons N, Kadyszewski E, *et al.* (2009) Survey of the quality of experimental design, statistical analysis and reporting of research using animals. PLoS ONE 4(11): e7824.

Kinsey DW and Davies P (1979) Effects of elevated nitrogen and phosphorus on coral reef growth. Limnology and Oceanography 24(5): 935-940.

Kinzie RA and Hunter T (1987) Effect of light quality on photosynthesis of the reef coral *Montipora verrucosa*. Marine Biology 94: 95-109.

Kinzie RA, Jokiel PL, and York R (1984) Effects of light of altered spectral composition on coral zooxanthellae associations and on zooxanthellae *in vitro*. Marine Biology 78(3): 239-248.

Kleypas JA, McManus J, and Menez L (1999) Using environmental data to define reef habitat: Where do we draw the line? American Zoologist 39: 146-159.

Koop K, Booth D, Brodie J, *et al.* (2001) ENCORE: The effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Marine Pollution Bulletin 42(2): 91-120.

Korrubel J and Riegl B (1998) A new coral disease from the southern Arabian Gulf. Coral Reefs 17: 22.

Lasagna R, Albertelli G, Colantoni P, *et al.* (2009) Ecological stages of Maldivian reefs after the coral mass mortality of 1998. Facies 56: 1-11.

Lithner D, Larsson, Å, and Dave G (2011) Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Science of the Total Environment 409: 3309-3324.

Lobban CS, Raymundo LM, and Montagnes DJ (2011) *Porpostoma guamensis* n. sp., a philasterine scuticociliate associated with brown-band disease of corals. Journal of Eukaryotic Microbiology 58(2): 103-113.

Luna GM, Biavasco F, and Danovaro R (2007) Bacteria associated with the rapid tissue necrosis of stony corals. Environmental Microbiology 9: 1851-1857.

Marubini F and Atkinson MJ (1999) Effects of lowered pH and elevated nitrate on coral calcification. Marine Ecology Progress Series 188: 117-121.

Marubini F and Davies PS (1996) Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Marine Biology 127(2): 319-328.

Maynard JA, Anthony KRN, Marshall PA, *et al*. (2008) Major bleaching events can lead to increased thermal tolerance in corals. Marine Biology 155: 173-182.

Miller DJ and Ball EE (2000) The coral *Acropora*: what it can contribute to our knowledge of metazoan evolution and the evolution of developmental processes. Bioessays 22: 291-296.

Miller J, Maragos J, Brainard R, *et al.* (2008) The State of Coral Reef Ecosystems of the Pacific Remote Island Areas. pp. 353-386. In: JE Waddell and AM Clarke (eds.), The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008. NOAA Technical Memorandum NOS NCCOS 73. NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team. Silver Spring, MD. 569p.

Millero FJ (ed.) (1996) Chemical Oceanography. 2nd Edition. CRC. Boca Raton, FL. 496p.

Muller-Parker G, McCloskey LR, Hoegh-Guldberg O, *et al.* (1994) Effect of ammonium enrichment on animal and algal biomass of the coral *Pocillopora damicornis.* Pacific Science 48: 273-283.

Mundy CN (1996) A Quantitative Survey of the Corals of American Samoa. Report to the Department of Marine and Wildlife Resources, American Samoa Government. 33p.

Obura DN and Grimsdith G (2009) Resilience Assessment of Coral Reefs - Assessment Protocol for Coral Reefs, Focusing on Coral Bleaching and Thermal Stress. IUCN working group on Climate Change and Coral Reefs. IUCN, Gland, Switzerland. 70p.

Page CA and Willis BL (2008) Epidemiology of skeletal eroding band on the Great Barrier Reef and the role of injury in the initiation of this widespread coral disease. Coral Reefs 27: 257-272.

Pawlowsky E (2008) Current in aquaria, best way to create. pp. 127-132. In: RJ Leewis and M Janse (eds.), Advances in Coral Husbandry in Public Aquariums. Public Aquarium Husbandry Series, vol 2. Arnhem, Netherlands.

Pedersen MF and Borum J (1996) Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Marine Ecology Progress Series 142: 261-272.

Rawlinson KA, Gillis JA, Billings REJ, *et al.* (2011) Taxonomy and life history of the *Acropora*-eating flatworm *Amakusaplana acroporae* nov. sp. (Polycladida: Prosthiostomidae). Coral Reefs 30: 693-705.

Riddle D (2007) Product review: Alkalinity test kit showdown. Advanced Aquarist 6(8). http://www.advancedaquarist.com/2007/8/review (accessed Dec. 17, 2012).

Riegl BM, Bruckner AW, Rowlands GP, *et al.* (2012) Red Sea coral reef trajectories over 2 decades suggest increasing community homogenization and decline in coral size. PLoS ONE 7(5): e38396.

Ritchie RJ, Eltringham K, Salih A, *et al.* (1997) *Plesiastrea versipora* (Lamarck, 1816), the white rat for coral research? pp. 403-408. In: JC den Hartog (ed.), Proceedings of the 6th International Conference on Coelenterate Biology. Nationaal Natuurhistorisch Museum, Leiden, Netherlands.

Rosenberg E, Koren O, Reshef L, *et al.* (2007) The role of microorganisms in coral health, disease and evolution. Nature Reviews Microbiology 5: 355-362.

Rothenberger P, Blondeau J, Cox C, *et al.* (2008) The State of Coral Reef Ecosystems of the U.S. Virgin Islands. pp. 29-73. In: JE Waddell and AM Clarke (eds.), The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008. NOAA Technical Memorandum NOS NCCOS 73. NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team. Silver Spring, MD. 569p.

Seltman HJ (2012) Experimental Design and Analysis. http://www.stat.cmu.edu/~hseltman/309/Book/Book.pdf (accessed Dec. 17, 2012).

Shafir S, Van Rijn J, and Rinkevich B (2006) Coral nubbins as source material for coral biological research: A prospectus. Aquaculture 259: 444-448.

Sheppard CRC, Harris A, and Sheppard ALS (2008) Archipelago-wide coral recovery patterns since 1998 in the Chagos Archipelago, Central Indian Ocean. Marine Ecology Progress Series 362: 109-117.

Siikavuopio SI, Dale T, Christiansen JS, *et al.* (2004) Effects of chronic nitrite exposure on gonad growth in green sea urchin *Strongylocentrotus droebachiensis*. Aquaculture 242: 357-363.

Sokal RR and Rohlf FJ (2012) Biometry: The Principles and Practice of Statistics in Biological Research. 4th edition. W. H. Freeman and Co. New York. 937p.

Sprung J (1999) Corals: A Quick Reference Guide. Ricordea Publishing. Coconut Grove, FL. 240p.

Sweatman H, Cheal A, Coleman G, *et al.* (2008) Long Term Monitoring of the Great Barrier Reef. Status Report, number 8. Australian Institute of Marine Science Townsville. 379p.

Sweet M, Jones R, and Bythell J (2012) Coral diseases in aquaria and in nature. Journal of the Marine Biological Association of the United Kingdom 92(4): 791-801.

Toda T, Okashita T, Maekawa T, *et al.* (2007) Community structures of coral reefs around peninsular Malaysia. Journal of Oceanography 63: 113-123.

USEPA (2006) Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule. Office of Water (4601). EPA 815-R-06-007. 436p.

Veron JEN (2000) Corals of the World. Australian Institute of Marine Science, Townsville, Queensland, Australia. 1382p.

Wobeser GA (1981) Diseases of Wild Waterfowl. Plenum Press, New York, NY. 300p.

Wobeser GA (1994) Investigation and Management of Disease in Wild Animals. Plenum Press, New York, NY. 265p.

Woodley CM, Bruckner AW, McLenon AL, *et al.* (2008) Field Manual for Investigating Coral Disease Outbreaks. NOAA Technical Memorandum NOS NCCOS 80 and CRCP 6. National Oceanic and Atmospheric Administration, Silver Spring, MD. 85p.

Yanong RPE and Erlacher-Reid C (2012) Biosecurity in Aquaculture, Part 1: An Overview. USDA Southern Regional Aquaculture Center No. 4707. 10p.

APPENDIX 1 – Chemical disinfectants commonly used in aquaculture.

Disinfectant type	Products/chemicals	Concentration	Contact time	Specific activity*	Comments
Chlorine	**Sodium hypochlorite** (liquid) **Calcium hypochlorite** (powder, granules, pellets) **Household bleach** Available chlorine content may vary with brand and formulation.	200-500 mg/L available chlorine 5,000-10,000 mg/L available chlorine Typically 10% bleach (v/v) in water yields approximately 5,000 mg/L available chlorine.	10-60 minutes for general disinfection. Higher concentrations and longer contact times may be required for specific pathogens. 10-30 minutes to eliminate some more resistant mycobacteria and spores. When cleaning tanks, disinfect for 24 hours, rinse and allow to dry.	B, EV, NEV, F, ±M, ±S	Can be neutralized with sodium thiosulfate (7 mg to 1 mg chlorine). Irritates mucus membranes, eyes, and skin at high concentrations. Use with caution in poorly ventilated rooms. Corrosive to metals and may damage silicone sealants and rubber gaskets. Do not mix with ammonia or products containing phosphoric acid.
Alcohol	**Ethyl alcohol** (ethanol) and **isopropyl alcohol** (isopropanol)	60-90% (v/v) in water	10-30 minutes for general disinfection.	B, EV, F, M	Most commonly used for hands and counter/work surfaces. Prolonged/repeated use on plastic and rubber may damage these materials. Ethanol is highly flammable; isopropyl alcohol is safer.
Iodine/Iodophor	**Providone-iodine** Contains 9-12% available iodine by weight. Formulations may contain 1-10% providone-iodine which is equivalent to 0.1-1% available iodine.	30-50 mg/L free iodine Dilution depends on product.	10-30 minutes for general disinfection. Prolonged contact time likely for mycobacteria and spores.	B, EV, ±NEV, F, ±M, ±S	Formulations may vary by brand; follow product label instructions carefully. Will stain clothing and skin. Somewhat corrosive to metals.
Quaternary Ammonium Compunds	**Roccal®-D** 10% solution of benzalkonium chloride	250-500 mg/L benzalkonium chloride Roccal dilution of 0.1-1.5% commonly used	10-30 minutes for general disinfection.	B, EV, ±F	Commonly used on floors, footbaths, walls, equipment, and furnishings due to good penetration on porous surfaces. Must be rinsed thoroughly to avoid ammonia toxicity.

*bacteria (B), enveloped viruses (EV), non-enveloped viruses (NEV), fungal/mycotic agents (F), mycobacteria organisms (M), spores (S).
(±) designates variable results documented in the literature. Adapted from Yanong and Erlacher-Reid 2012.

Continued on next page

APPENDIX 1 (continued)

Disinfectant type	Products/chemicals	Concentration	Contact time	Specific activity*	Comments
Phenolic derivatives	***ortho*-phenyl phenol** ***ortho*-benzyl-*para*-chlorophenol** ***para*-tertiary amylphenol** **Lysol®** 1% benzyl-4-chlorophenol-2-phenylphenol	2-5% active ingredient 1% Lysol	10-30 minutes for general disinfection.	B, EV, ±NEV, F, ±M	Some common household products. Skin irritation and absorption can occur. Must be rinsed thoroughly to avoid toxicity.
Virkon® Aquatic	**Virkon® Aquatic** 21.4% potassium peroxymonosulfate and 1.5% sodium chloride	Virkon dilution of 0.5-1% or 50-100 g per 10 liters water	10-15 minutes for general disinfection.	B, EV, NEV, ±F, ±S	Commonly used for footbaths. Do not exceed 30 minutes of contact time on metal objects. Active for 7 days after mixed. Considered environmentally safe, biodegradable.
Chlorhexidine	**Nolvasan®-S** 2% chlorhexidine diacetate **Virosan™** 2% chlorhexidine gluconate Most solutions contain 2% active chlorhexidine.	Nolvasan dilution of 10% (v/v) in water for dis-infection	5-10 minutes for general disinfection.	B, EV, ±F	Commonly used for footbaths because it is more resilient to organic material than chlorine or iodophors. Activity reduced in hard water (e.g., seawater), extreme alkaline or acidic pH, or in the presence of anionic and non-ionic based moisturizers and detergents.
Hydrogen peroxide	**3% hydrogen peroxide** **35% PEROX-AID®**	3-30% (w/w) active ingredient	5-30 minutes for general disinfection.	B, EV, NEV, F, ±M, ±S	As an oxidizer, concentrated solutions may irritate eyes, skin, and mucous membranes. High-strength solutions should be stored in a suitable, vented container to prevent rupture from the buildup of oxygen gas.

*bacteria (B), enveloped viruses (EV), non-enveloped viruses (NEV), fungal/mycotic agents (F), mycobacteria organisms (M), spores (S).
(±) designates variable results documented in the literature. Adapted from Yanong and Erlacher-Reid 2012.

APPENDIX 2 – Equipment list for three experimental design examples

Small-Scale Biological Challenge

- Experimental tanks or containers of appropriate size with lids (transparent acrylic or glass, cut to fit)
- Seawater reservoir (>10 gal) with air supply to provide constant circulation
- Light fixture(s), timers, light meter, and neutral density filters or shade cloth to adjust light intensity
- PVC pipe, 1/2” to 1” diameter for various manifolds
- Thermometers and heaters or temperature controlled room
- Suction cups for supporting tubing and heaters inside each tank
- Various PVC and nylon barbed fittings to attach pipes and tubing
- Vinyl, silicone, or similar tubing of various diameters (e.g., 3/16”, 1/4”, and 1/2”), cut to fit
- Plastic needle, ball, or pinch valves to regulate air, source-, and waste-water flow
- Air pump to supply air manifold and seawater reservoir
- UV light(s) to sterilized source water
- Supplies to ensure biosafety and containment (e.g., 70% ethanol, 10% bleach, lab coats, gloves, foot mat with disinfectant)

Effects of Different Light Sources

- Experimental tanks or containers of appropriate size
- Light fixtures, timers, and light meter
- Powerfilters and/or powerheads for circulation
- PVC, Teflon®, or similar stand for coral fragments
- Thermometers and heaters or temperature controlled room
- Vinyl, silicone, or similar tubing (~1/4” diameter) with valve to siphon waste-water from tanks
- Non-transparent dividers or partitions to block ambient light

Chemical Dosing Experiment

- Experimental tanks or containers of appropriate size (glass or Teflon®)
- Water bath or temperature controlled room
- Water circulator to maintain water bath temperature
- Tubing/hose of various sizes and fittings to plumb water circulator to water baths
- Powerheads/pumps to circulate water within water baths
- Light fixture(s), timers, and light meter
- Teflon® liners to fit into experimental containers (optional)
- Air pumps, gang valves, and 3/16” vinyl or similar airline tubing
- Rigid 1/4” Teflon® tubing to aerate and circulate water in each experimental container connected to airlines by a quick disconnect fitting
- Fitted transparent lids for each experimental container

This page intentionally left blank.

United States Department of Commerce
Rebecca M. Blank
Acting Secretary

National Oceanic and Atmospheric Administration
Kathryn D. Sullivan
Acting Under Secretary of
Commerce for Oceans and Atmosphere
NOAA Administrator

National Ocean Service
Holly Bamford
Assistant Administrator