



reef zlements®



HYBRID 2 PART DOSING SYSTEM™

The Comprehensive Manual





PREFACE

The Reef Zlements Hybrid 2 Part Dosing System™ (H2P™) is the culmination of extensive scientific research of over 270 articles and books, combined with years of experience keeping marine aquariums and growing corals in re-circulating systems (i.e. closed aquariums).

In particular, although we have never met, we would like to thank Professor Frank J. Millero for his lifelong contribution to mankind's knowledge of Chemical Oceanography and also to the many others who contributed and allowed us to learn and, in part, develop our products, reaching the point we currently are at.

Above all, we would also like to thank our great Friend, Mr David Saxby (founder of D-D The Aquarium Solution), for the long-life experiences he shares with us daily and for the most enjoyable time he spends with us.

Finally, we hope that the work we did and continue to do allows others to address a common challenge reef aquarium enthusiasts face: maintaining a thriving reef aquarium.



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OVERVIEW

Welcome to the User Manual for the Reef Zlements Hybrid 2 Part Dosing System. Reef Zlements is excited to introduce you to this cutting-edge solution, which is made to ensure the health and vitality of your marine ecosystem.

The Reef Zlements H2P™ is a holistic system and methodology designed with the ethos that reefers should ensure the best conditions in their aquariums and meet all the nutritional needs of their corals to keep a healthy aquarium. The H2P™ system ensures your corals thrive by providing them with a comprehensive range of nutrients.

The H2P™ Dosing System is capable of independently controlling alkalinity and pH while supplying all other nutrients needed for sustaining a healthy marine biome. The benefits of this ground-breaking system ensure reefers can keep their water chemistry exactly where it needs to be, which means that corals will grow healthier and faster. You can rest assured that your corals are getting a complete, balanced nutritional program with the H2P™ Dosing System. This document gives you all the knowledge required to understand, start, and maintain an H2P™ Dosing System.

Low pH levels and other inadequate parameter levels can lead to numerous issues with corals, affecting their health and growth. Unlike traditional systems that rely solely on bicarbonate or carbonate (or a mix that cannot be controlled), the H2P™ system is formulated to supply the right alkalinity source that promotes ideal pH levels at the right time. The H2P™ system is also formulated to provide all other needed nutrients, ensuring that your corals and aquatic biome receive everything they need.

In this manual, we aim to explain the fundamental elements of scientifically maintaining a successful reef aquarium and to clear any confusion, misconception or incorrectly disseminated information surrounding the levels we maintain in reef aquariums, pH, CO₂, bicarbonate, carbonate, and hydroxide as dosing methods. We will demonstrate the advantages of a dosing system that integrates multiple alkalinity sources, providing a more stable and effective solution for maintaining optimal alkalinity, pH, and



macro and trace element levels in your aquarium.

This manual also explains the importance of the most commonly used elements that have been scientifically proven to benefit marine life and corals. While the statements in this manual are based on scientific evidence, and some sections delve into scientific concepts underpinned by extensive scientific references, we have decided to place these references at the end of the manual. Nonetheless, rest assured that you do not need a background in science or even to read this entire manual to use the H2P™ Dosing System effectively (if you want to access a simplified version, please refer to the H2P™ Quick Start Guide).

Our goal is to simplify its usage and application, making it accessible to everyone whilst exposing the strength of our system based on science rather than marketing and misconceptions and to give you the confidence that what we propose is based on science and our love for the creatures we so much care for rather than profits. We do recommend, however, that particular attention is given to the chapters where we provide an overview of the H2P™ System and the one where we provide a step-by-step implementation guide, other chapters are perhaps aimed at the reefers with particular interest in chemistry and biochemistry.

Because we recognise that it is not just a dosing methodology needed to ensure our reef aquariums' long-term success, before using the H2P™ Dosing System, we suggest fulfilling specific prerequisites. Although these steps are not system-specific, they are crucial for achieving the best results when maintaining a reef aquarium. To help you achieve that, this manual will guide you through some of these prerequisites and provide a straightforward guide to setting up and operating your H2P™ dosing system. We are confident that with the H2P™ Dosing System, you will achieve a long-term, prosperous marine ecosystem with healthy and vibrant corals at its core.



UNDERSTANDING REEF AQUARIUM DOSING

What is a Dosing System?

A reef aquarium dosing system is a critical component for keeping water chemistry in check for the upkeep of sensitive corals and other aquatic life found in a closed reef aquarium system. Dosing can be performed automatically or manually. Nonetheless, it should allow precise quantities of necessary nutrients to be delivered into the aquarium. These include alkalinity, calcium, magnesium, potassium, strontium, trace elements, and other nutrients required for the health and growth of corals and other marine life.

There are different types of dosing systems which include:

All-in-One Systems: Our InOne is an example of an All-in-One system that combines multiple components into a single solution. These systems depend on bacteria metabolising the carbon they contain so that calcium and carbonates are available to corals.

Multi-Part Systems: Our H2P™ Dosing System falls into this category and typically consists of two, three, four, or more parts, each delivering different components.

Alternative Methods: Other means of supplementing a reef aquarium, such as calcium reactors, which, although not traditionally considered a dosing system, effectively supply the aquarium with essential components like carbonates, calcium, and to some extent (if specific media is added), magnesium. For the success of the reef aquarium biome as a whole, these typically need to be supplemented with other macro and trace elements, along with nutrients. Usually, due to the dependence on CO₂, this method tends to depress pH, which, if not counteracted, leads to potentially not-so-



good results compared to the previous methods.

Understanding and utilising a dosing system is crucial for achieving and maintaining optimal water chemistry and promoting a thriving reef environment. This method will help you master our H2P™ Dosing System.

The Reef Zlements H2P™ Dosing System Overview

The H2P™ Dosing System consists of two main components.

Part 1: Hybrid Complete/pHplus

This vital component consists of two interchangeable solutions, Complete and pHplus, each designed to increase alkalinity and enrich your reef aquarium with a comprehensive blend of macro and trace elements. Each litre contains 10,000 dKH units, and a 10 ml dose can raise the alkalinity of 100 litres of water by 1 dKH.

- Complete: Ensures optimal alkalinity while gently elevating pH.
- pHplus: Ensures optimal alkalinity while actively increasing pH.
- Custom Blending: Mix Complete with pHplus to tailor the pH balance to your aquarium's needs. Dose them together or separately for pH adjustment and control.

Part 2: Universal

Universal is the rebranded and essential part of the Reef Zlements H2P™ Dosing System. It contains approximately 72,000 mg/L of Calcium, but Universal is not just about Calcium.

It's a meticulously balanced blend of 13 essential macro and trace elements crucial for the health and growth of your corals.

Each element plays a critical role in creating a stable and thriving



environment for your reef. With Universal, you're not just dosing; you're fostering an ecosystem that closely mimics the natural ocean environment.

H2P™ Implementing the system

Welcome to the implementation section of the H2P™ Dosing System manual. In this section, we will guide you through the essential steps to effectively start and manage the H2P™ system in your aquarium. From initial setup to advanced dosing strategies, you will learn how to harness the full potential of the H2P™ system to maintain optimal water chemistry and ensure the health and vitality of your reef ecosystem.

We will cover everything from the initial setup, including how to start dosing the H2P™ system, to utilising the D-D KH Manager for precise mutual control of alkalinity and pH. Additionally, we will discuss the recommended levels for macro and trace elements, the importance of extra supplementation, and the correct procedures for water changes. Finally, we will touch on professional laboratory testing, including an ICP testing schedule, to ensure the aquarium remains in perfect balance.

By following this comprehensive guide, you will be well-equipped to implement the H2P™ Dosing System effectively and ensure a thriving reef aquarium.

How to start dosing the H2P™ Dosing system?

Step 1

Determine the alkalinity consumption of the tank. This can be done by testing alkalinity, then stopping all dosing and testing alkalinity again exactly 24 hours later. The difference between the alkalinity levels will be the consumption in 24 hours.

For example, if your first test reads 7.0 dKH, and your second test performed



exactly 24 hours later reads 6.7 dKH, your daily consumption would be 0.3 dKH.

Step 2

With the alkalinity consumption determined, the daily Part 1 (Complete, pHplus or a mix of the two) volume can then be calculated assuming that every 10ml of Part 1 will increase the alkalinity in 100 litres of water by 1 dKH.

Adjusting pH at the desired alkalinity level:

- If pH is not monitored, please use Complete as the sole component of Part 1
- If pH is monitored you can dose Complete and pHplus at different times of the day to adjust pH towards to the level you desire:

To raise pH: Increase the proportion of pHplus.

To lower pH: Decrease the proportion of pHplus.

An alternative way to dose is to mix Complete and pHplus to any ratio or either Complete or pHplus on their own.

Make adjustments gradually and monitor the changes.

Step 3

Place the dosing lines over a very high flow/turbulent area or alternatively directly into the return pump (don't submerge the dosing lines and ensure they aren't exposed to any splashes). If you don't have a high flow area in the sump this can be achieved with a small pump placed near the dosing lines. Please ensure that after dosing Part 1 it completely dissolves.

Note: When dosed, if Part 1 doesn't completely dissolve it is likely to settle at the bottom of the sump as a white calcium carbonate "mass", and will not work as intended. This happens, because the localised chemical conditions promote the calcium carbonate precipitation and dissolution doesn't happen. Once pH normalises to aquarium levels and the localised



saturation is reduced, dissolution of the calcium carbonate will happen.

It is also important to note that to not run into a precipitation scenario, that the levels (i.e. pH, alkalinity, calcium, magnesium, phosphate, etc.) are kept within the recommended ranges below and this is true regardless if using bicarbonate, carbonate or hydroxide to increase alkalinity.

Step 4

As good practice, place the dosing containers in a stable and safe place to avoid any spills into the tank (e.g. avoid placing them directly on top of the sump or tank).

If you know your calcium daily consumption you can start dosing Universal knowing that 10ml will increase calcium by 7.2 mg/L in 100L of water.

Alternatively, if you don't know the calcium consumption, your tank alkalinity is within 6.5-7.5 dKH and Calcium lower than 420 ppm, start dosing equal parts of Part 1 and Universal Part 2 based on Part 1 daily volume determined earlier. These doses should be divided into as many small doses as possible and dosed throughout the day (ideally at least one dose of each part per hour). Note: Part 1 and Universal Part 2 should be dosed at least 15 minutes apart to avoid chemical interference.

If your tank alkalinity is above 7.5 dKH, stop the dosing of any alkalinity solutions and allow your tank alkalinity to drop to a value between 6.5-7.5 dKH. Similarly, if your tank's calcium is above 420 ppm stop all calcium addition until calcium drops to a value between 400-420 ppm.

If your calcium is above 420 ppm you should not begin dosing Universal Part 2 until your calcium level is below the recommended value of 420 ppm. However, you can dose Part 1 as needed.

If your alkalinity is within the recommended range and your calcium or other elements are low, please use the individual elements we offer to adjust them to the recommended levels you can find below. Do not attempt to use any of the 2 parts to increase individual elements, e.g. Calcium, Magnesium, etc., Reef Zlements H2P™ dosing system was not formulated for this purpose,



and doing so will result in the likely overdose of other elements.

Step 5

Test alkalinity/pH daily and calcium every 3-4 days for the first 2-3 weeks. Nonetheless, we recommend a continuous and consistent testing regime, as over the years the most successful reefers are those who keep a strong testing regime.

Do not allow alkalinity or calcium to increase or decrease during this time. If necessary to keep alkalinity and/or calcium stable, adjust Part 1 and/or Universal Part 2 dosing volumes independently.

Test salinity weekly and ensure salinity is kept within the recommended levels that you can find below. Please note that high salinity will quickly lead to coral losses.

We recommend that you periodically check the aquarium's water quality using our latest laboratory ICP OES DSOI analysis machine. Testing at least every four weeks using the Reef Zlements 2-Part dosing system will give you the insight and recommendations you need to keep the aquarium in top condition.



MACRO AND TRACE ELEMENTS SUPPLEMENTATION

Elemental and other parameter levels

It wouldn't be far from the truth if one said that maintaining natural seawater elemental concentration would be a good baseline for maintaining our aquariums. However, there might be some advantages in maintaining different levels from what is found in oceanic waters, mainly because an aquarium is, we can say, somewhat different from the oceans and the reefs. As such, although for many parameters, we recommend levels close to natural seawater levels, for others, we recommend slightly different levels. This is based on our experience and observations, which although they have a scientific basis, are primarily based on our empirical observations and experiments.

Base Parameters

Temperature – 24°C and within 23°C and 28°C

Salinity – 34 ppt and within 33.5-35 ppt

Alkalinity – 6.8 dKH and within 6.2-7.0 dKH

pH – 8.2-8.3 and not lower than 8.15 or higher than 8.4

Macro and Micro elements

Boron – 6 mg/L and within 4-10 mg/L

Bromine – 75 mg/L and within 60-100 mg/L

Calcium – 420 mg/L and within 380-480 mg/L

Chloride – 18500 mg/L and within 18150-19500 mg/L

Fluoride – 1.5 mg/L and within 1-1.9 mg/L



Magnesium – 1400 mg/L and within 1300-1440 mg/L

Potassium – 420 mg/L and within 390-500 mg/L

Sodium – 10500 mg/L and within 10200-11000 mg/L

Strontium – 10 mg/L and within 5-12 mg/L

Sulphate – 2695 mg/L and within 2427- 2964 mg/L

Sulphur – 900 mg/L and within 810-990 mg/L

Trace elements

Barium – 15 µg/L and within 10-100 µg/L

Chromium - 0.5 µg/L and within 0.1-1 µg/L

Cobalt - 0.2 µg/L and within 0.1-1 µg/L

Copper - 0.2 µg/L and within 0.1-1 µg/L

Iodine - 60 µg/L and within 60-95 µg/L

Iron - 0.4 µg/L and within 0.2-5 µg/L

Lithium – 200 µg/L and within 180-500 µg/L

Manganese – 2 µg/L and within 0.9 - 4 µg/L

Molybdenum - 15 µg/L and within 15 – 20 µg/L

Nickel - 2.5 µg/L and within 2 – 5 µg/L

Rubidium – 200 µg/L and within 150-500 µg/L

Selenium - 0.2 µg/L and within 0.1- 0.5 µg/L

Silicon – 150 µg/L and below 300 µg/L

Tin – 0 µg/L and below 10 µg/L

Vanadium - 2 µg/L and within 0.5 - 5 µg/L

Zinc – 5 µg/L and within 3 - 12 µg/L



Nutrient levels for SPS dominated:

Nitrates – 5 mg/L and within 4 - 25 mg/L

Orthophosphate – 50 µg/L and within 40 and 80 µg/L

Nutrient levels for LPS dominated:

Nitrates – 8 mg/L and within 5 - 50 mg/L

Orthophosphate – 80 µg/L and within 50 and 120 µg/L

Nutrient levels for Mixed reef:

Nitrates – 6 mg/L and within 5-50 mg/L

Orthophosphate – 60 µg/L and within 40 and 100 µg/L

With the above recommendations for temperature, pH, alkalinity, and calcium, we would also like to note that although higher pH, alkalinity, and calcium levels would accelerate coral growth, the recommended levels balance the benefits to coral growth and calcium carbonate water saturation. This promotes coral growth and health while controlling abiotic calcium carbonate precipitation.



Do I need additional trace elements?

Maintaining stable environmental parameters ensures a thriving aquarium biome, including healthy corals. This includes keeping alkalinity, pH, temperature, macro and trace elements, organic carbon load and others in check. While macro elements generally remain stable in seawater, trace elements are prone to rapid precipitation due to their chemical properties, concentrations, and seawater conditions.

The H2P™ Dosing System simplifies the process of meeting corals' macro elemental needs. However, there may be occasional needs to adjust specific elements. For instance, when coralline algae proliferate, there is typically a higher demand for magnesium, necessitating supplementation. These exceptions are why Reef Zlements provides individual macro elements for such corrections, ensuring targeted adjustments when needed. However, it is important to note that this is not a daily occurrence.

Nonetheless, the scenario is different for trace elements. Although the RZ H2P™ supplies the needed trace elements, these have significantly shorter residency times in seawater compared to macro elements, especially in an aquarium environment. As such, it may be beneficial to supplement with a continuous source of trace elements.

To understand this concept, it is important to understand residency time.

The residency time of an element in seawater (or any other solution) refers to the average time that the element remains in the solution before being removed by processes such as precipitation, biological uptake, mechanical and chemical filtration, or sedimentation.



$$\text{Residency time} = \frac{\text{Total Amount of Element in solution}}{\text{Rate of removal of the Element}}$$

Residency times for different elements in the oceans are not comparable to those we experience in our aquarium closed systems. In oceanic conditions, calcium, for example, has a residency time of approximately one million years, whereas in an aquarium, depending on the kept livestock, it might be only 1-2 months.

On the other hand, Iron has a residency time of around 200 years in the ocean; however, in an aquarium, it has a residency time of merely a few hours, at best a few days.

In addition to this, manufacturers like Reef Zlements face other important challenges, such as different biome elemental requirements, unknown sources of trace elements introduced by reefers, different filtration setups (e.g., the use – or not – of GFO, activated carbon, and other filtration media), and the use of refugia. These variables make it impossible for any manufacturer to create an “off the shelf” solution that fits all aquariums perfectly, except in a marketing scenario twisted from reality and aimed at boosting sales.

Given the above, regardless of our H2P™ Dosing System containing all the macro and trace elements that corals need to thrive, we can start to understand why it is likely beneficial to dose trace elements in small but more frequent doses, over and above our main dosing system.



How to dose the extra elements

Now that the need and reason for dosing extra trace elements have been discussed and demystified, we need to address how to dose these additional trace elements.

Given that the main reason for this need is the residency time of trace elements in aquarium saltwater, the strategy should be to dose these traces as regularly as possible and in the smallest doses possible, to ensure we are keeping them in the most stable concentrations possible.

However, we shouldn't dose these traces without testing and monitoring due to the risk of overdosing or, in fact, not doing enough. Regular ICP testing is required to administer adequate volumes of these elements (more on ICP testing below).

Whilst Reef Zlements does offer individual trace elements element solutions, with the H2P™ Dosing System, there isn't the need to dose single element solutions one at a time; instead, two bespoke solutions can be made up by the user based on the users ICP testing results. The reason Reef Zlements offers individual trace elements is linked to the fact that an off-the-shelf multi-element solution can easily overdose certain traces if one or more of the elements in those solutions are already elevated in the tank.

So, what is the principle by which we should mix the different elements in two different solutions? The principle is simple and is based on the fact that, in general, ions with similar charges (i.e. anions or cations) do not react with each other because like charges repel one another due to electrostatic forces.

To facilitate this, Reef Zlements has created two categories for its elements, i.e. "Type A" for Anions and "Type C" for Cations. So, with this categorisation, we can simply add similarly charged elements to one of the solutions and the opposite charged ones to the other.



But what are the Reef Zlements Type A and C elements? This is a fairly simple question to answer and the following elements can be mixed together:

Type A

- Iodine
- Molybdenum
- Selenium
- Sulphur/Sulphate
- Vanadium

Note that although Sulphur is a macro element, it has been added to the list due to the need to be dosed slowly to avoid microbe problems which can cause STN and RTN in corals.

Type C

- Barium
- Cobalt
- Chromium
- Iron
- Copper
- Manganese
- Nickel
- Rubidium
- Zinc

Now that we know which trace elements can be mixed together, the next step is to mix them in the correct amounts and perform a dilution. This allows us to dose these traces hourly between Parts 1 and Part 2 of the H2P™, ensuring that traces are dosed into the aquarium every 15 minutes.



This is a very easy step. You only need to use the ICP results and mixing suggestions to create these custom mixes (at the time of writing, this is being developed and will be available soon). In the meantime, you can use the calculator available in the ICP portal or contact us for help.

Once these two mixes have been created, you should dose 1ml of each solution (Type A and C) per hour, continuously.

As an important note, we recommend mixing the two solutions to last no longer than the ICP routine schedule to ensure ideal dosing amounts and avoid any overdoses.

Nutrient supplementation

Before proceeding with this section, we need to define nutrients. We can consider everything that nourishes the biome a nutrient. In this context, we will regard carbon, nitrogen/nitrates, phosphorous/phosphate, and all related compounds as nutrients.

Regarding nutrient supplementation, we recommend taking a conservative approach and only dosing the different nutrients when necessary. For example, if both nitrate and phosphate are low, we recommend increasing the concentration of these with both Nitro and PhosPlus, then starting CarboPlus, AminoPlus, and VitaPlus in as little amounts as possible to maintain both nitrate and phosphate.

This will ensure the organic nutrients corals and other microorganisms need whilst minimising their availability in the water column which can lead to potential bacterial and pollution issues.

Ensuring adequate nutrient levels is crucial to maintaining healthy and growing corals.



PROFESSIONAL LABORATORY TESTING

Maintaining a reef aquarium requires precise monitoring and management of water chemistry to ensure the health and growth of corals and other marine life. At Reef Zlements, we like to say that we don't keep corals; we keep the water.

While hobbyists can test for basic parameters like pH, alkalinity, and nitrate at home, testing for most macro and trace elements, such as strontium, potassium, iodine, copper, and many others, presents significant challenges, as home test kits for those elements are extremely inaccurate.

Many trace elements, including iodine, iron, and manganese, are essential for coral, algae, and bacterial health but are difficult or impossible to monitor using conventional home test kits. Testing for these macro and trace elements and other key parameters often requires advanced analytical techniques such as Inductively Coupled Plasma (ICP), Ion Chromatography (IC), and "robotic" Titrations with higher accuracy than what home test kits offer.

These complex methods require specialised equipment and technical expertise, which are not typically available to home aquarium hobbyists.

ICP testing is an analytical method that allows measuring the chemical composition of water. It can measure a wide range of elements, including both macro and trace elements, with high accuracy. This comprehensive analysis helps identify any deficiencies or excesses that can affect the health of the reef ecosystem. Alongside ICP testing, IC testing allows laboratories to measure other ions like nitrate, orthophosphate, fluoride, and sulphate, among others. Automated or robotic titration can measure parameters such as alkalinity, pH, conductivity and others.



By combining different analytical techniques, laboratories can offer comprehensive testing like Reef Zlements offers. The detailed results from these tests provide valuable insights for adjusting dosing regimens. By knowing the concentrations of essential elements, reef keepers can fine-tune their supplementation, ensuring that corals receive the nutrients they need without over-dosing or under-dosing.

Regular ICP testing helps maintain the long-term stability of the reef aquarium. By consistently monitoring and adjusting water chemistry based on precise data, reef keepers can avoid drastic fluctuations that can stress or harm corals and other marine life. Properly balanced water chemistry, achieved through the insights provided by ICP testing, supports optimal coral growth and vibrant colouration. Healthy corals are more resilient to diseases, parasites and environmental stressors.

Our team of application specialists strive to provide the most accurate and reproducible results. For better understanding of the process and care we dedicate to providing the highest quality results possible, here's a description of a typical Reef Zlements ICP OES technician operational day:

At the start of the day, the ICP is run for about 30 minutes to stabilise the plasma temperature. Once the temperature and plasma are stable, the spectra (wavelengths) calibration is initiated using a standard that includes elements with emission lines across the UV/VIS spectrum. A proper wavelength calibration ensures accurate peak centring, maximising instrument sensitivity and ensuring precise spectral interference identification. Wavelength calibration also ensures that any spectral interference identification built into the instrument software is as accurate as possible.

Each analyte is then calibrated for Reverse Osmosis using solutions produced under DAkkS accreditation according to the DIN EN ISO/IEC 17025 certification or NIST SRM 3100 series traceable calibration solutions. The instrument's calibration success is verified by checking each analyte's calibration curve correlation factor. A new sequence is then created and run, including a control standard, three samples of the same water, and another control standard to further confirm the calibration success and results



reproducibility. Customer sample analysis follows.

Whilst these sequences run, and the carbon fibre autosampler probe goes from sample to sample it is rinsed in two separate suprapure acid solutions and a third ultrapure water to ensure as little carryover (cross sample contamination) as possible. Adequate flushing time with both ultrapure water and the sample itself helps to prevent carryover; randomly placed sample blanks also validate this.

Each result is then individually viewed to identify any potential issue and if all is well uploaded to our portal and approved.

The same procedure as above (with the exception of the plasma stabilisation) is then performed for the saltwater samples.

By “running” the Reverse Osmosis samples before the saltwater ones, we ensure that no carryover from saltwater samples happens.

At the end of the day, the instrument is thoroughly flushed and cleaned with suprapure acid solutions, including its torch, cyclonic spray chamber and nebuliser.

Please note that a similarly exhaustive methodology is being implemented for our new ICP MS offering and with that we aim to ensure a comprehensive and robust analytical process across all our laboratory techniques, aiming at offering the most reliable and accurate results in the hobby.

This way we hope that our testing services become an invaluable tool for all reefers to maintain their aquariums. It offers a level of precision and comprehensiveness that standard test kits cannot match, enabling reefers to maintain optimal water chemistry and promote the health and growth of their marine life.



ICP Testing schedule

Before starting to use the H2P™ dosing system, we recommend performing a Reef Zlements Advanced ICP. This will allow establishing a baseline and taking any corrective actions, ensuring the basis for using H2P™ successfully.

After the start of the H2P™ dosing and once all macro elements and Iodine have been corrected, we recommend a second ICP be sent two weeks later. This will serve to determine a daily Iodine, Molybdenum, and other trace element maintenance dose. After that, we recommend performing a routine ICP at least every four to six weeks.

This routine ICP test will allow for minor corrections and address any issues that may arise (e.g., accidental overdosing, unknown introduction of certain trace elements in the tank via unknown sources, etc.). Over time, this routine will allow us to fully understand the aquarium's specific consumption and avert any unexpected issues.

WATER CHANGES

When using the Reef Zlements H2P™ system, with a few exceptions, routine water changes can be avoided, not only because water changes aren't as effective as the H2P™ system maintaining elemental levels, water changes can be a major destabilising factor and as such don't support the stability corals need.

With the above said, we see water changes as a tool to remove unwanted compounds or elements that have increased to undesired levels. These water changes are sometimes necessary as specific pollutants cannot be effectively removed by other means, or they are in a context where the easiest resolution route is to perform a series of water changes; the most



common example we very often see is Tin.

Similarly, given that DOC (Dissolved Organic Carbon) can accumulate to unhealthy levels even with strong filtration, we recommend performing a series of 2-3 large water changes (i.e. around 30%) every six months. This should ideally be performed during spring and summer when airborne pollen is at its highest levels. This will ensure that unhealthy dissolved organic levels in the aquarium remain low and pathogens can't proliferate as easily. During such water changes, we strongly recommend taking the opportunity to syphon the substrate (if there is one) and make sure that any detritus or waste that may have accumulated at the bottom is removed – make sure that the dirt that is being removed from the substrate does not flow freely into the water column to avoid any issues. This is of high importance as organics that accumulate in any anaerobic layers of the substrate may be converted into hydrogen sulphide – one of the truly responsible compounds for the old tank syndrome.

Nonetheless, rigorous ICP and home testing routines and strict maintenance are key to ensuring the implementation of this water change approach.

By maintaining a wise approach to water changes and their reef-keeping methodology, one can keep a balance between no routine water changes and a healthy aquarium environment.

THE D-D KH MANAGER

Whilst the H2P™ dosing system can be successfully implemented using manual dosing schedules and a standalone pump like the D-D P4 Pro dosing pump, an exciting new tool that will revolutionise aquarium dosing automation is soon to be available.

In partnership with D-D The Aquarium Solution, Kamoer, and Reef Zlements, a new functionality for the D-D KH Manager has been developed, taking this



app-controlled device to the next level. The KH Manager not only samples and tests your tank water for KH, maintaining alkalinity levels automatically, but now also offers advanced dosing control to allow you to control your pH.

Most hobbyists are continually chasing the tail of low pH, but by using pHplus as your 'Part 1' solution, you can gradually increase the average pH of your system to an optimum level of 8.2-8.3, which is the ideal range to achieve the best coral growth and health. Once you get to that point you can then use Complete to maintain the elevated level and cycle back to pHplus if the pH starts to gradually fall.

By using a KH Manager, with the new programming, the switch from pHplus to Complete can now be fully automated around a user defined set point, using the pH measurement that the unit already takes when carrying out the normal KH test and adjustment, to maintain a constant elevated 'Average pH'. The additional benefit of this automation is that it can also reduce the normal day to night pH swing that occurs on all aquariums.

Natural diurnal biological activity within the aquarium will act every day to raise and reduce the pH of your system. Photosynthesis within the corals during the day, whilst the lights are on, will raise your pH, and respiration at night, will lower the pH. On most aquariums this daily swing of pH will be between 0.2 and 0.3, depending on stocking and degassing effectiveness of the system.

Using the KH Manager, the programme will enable you to dose Complete during the day whilst the pH in the aquarium is naturally rising, and to dose pHplus at night to counteract the natural fall. This will give you the ability to potentially halve the daily pH swing on your system so that it spends more time in the ideal range.

If you allow the KH Manager to maintain the alkalinity and pH levels, some interesting safeguard measures exist. For example, to prevent the KH Manager from overdosing KH Buffer, the user can set a physical limit to the volume to be dosed. If you don't want the KH Manager to dose more than 10 ml of KH Buffer autonomously, even if it determines that 20 ml is needed to reach the target alkalinity level, it will only dose 10 ml.



Additionally, it can be set to send alerts about abnormal testing results (outside the set range for both KH and pH). If the alkalinity test provides an abnormal result, an auto re-test can also be set. These features give the user confidence that the aquarium is safe.

This technology means that, for the first time, reefers can manage and maintain stable alkalinity and pH levels in real time, ensuring a perfectly balanced and healthy aquarium environment.

PRE-REQUIREMENTS TO SET UP A SUCCESSFUL AQUARIUM

Although there are many ways to keep a reef aquarium, maintaining a successful one involves more than just using the right dosing method and supplements. Ensuring the right environmental conditions is crucial for long-term success. This includes aspects such as filtration, lighting, water flow, nutrient management, rock scaping, RO water, salt used, monitoring and automation, livestock selection, and tank location. While this manual focuses on the H2P™ Dosing System, it's essential to first establish the proper foundations for your aquarium.

Filtration

Proper filtration is an absolute necessity to have great water quality and the healthiest aquarium environments possible. If fresh air is vital to our life, a perfect supply of water is vital to life in a reef aquarium. These filtration systems clean out pollutants while still keeping the ideal balance for coral and fish living in your aquarium. By investing in the right type of filtration, you support yourself in turning your reef aquarium into a thriving and vibrant marine ecosystem filled with all sorts of colourful creatures.

Filter rolls



Filter rolls such as the Clarisea are crucial for maintaining water quality in reef aquariums. They continuously capture and remove particulates from the water as they flows through the filter media.

The Clarisea filter roll utilises a roll of fine fleece material that steadily advances, ensuring that fresh, clean filter media is always in place. This continuous mechanism allows for efficient mechanical filtration without frequent maintenance.

The primary advantage we see of using filter rolls is the early removal of organic matter. This is crucial as it captures uneaten food, fish waste, and detritus before they decompose into pollutants that get released into the water. Filter rolls help to maintain low nutrient levels by averting the breakdown of organic matter. Ensuring this is key for preventing the accumulation of harmful substances in the water and in the substrates that may lead to multiple issues such as the “old tank syndrome”. This condition happens when long-established tanks with a poor level of husbandry experience a decline in water quality and ecosystem stability due to the release of harmful substances like hydrogen sulphide from the substrate into the water.

Moreover, filter rolls contribute significantly to maintaining water clarity. By constantly removing these particulates, the roller filters keep the water clear, enhancing light penetration, which is vital for the health of photosynthetic organisms such as corals and macroalgae. Clear water also improves the aesthetic appeal of the aquarium. Furthermore, by reducing the amount of organic matter, filter rolls lessen the burden on biological filtration systems.

Regular monitoring of the filter roll system is essential to ensure it functions correctly and advances the filter material as needed. Proper installation and integration into the existing filtration setup are crucial for optimal performance.



Skimmer

We strongly advise having a skimmer rated for the number of animals and size of your tank, which we believe every system should have. The protein skimmer is the main filtration component that deals with organic waste suspended in water, as these systems are heavily invested in gas exchange necessary for maintaining excellent water parameters.

A protein skimmer is designed to be proactive, removing dissolved organic compounds from the water before they have a chance to break down. The skimmer does this by producing thousands of air microbubbles which are injected into the water column. Hydrophobic organic compounds attach to the surface of these bubbles and are floated upwards into a collection cup. This proactive process lowers nutrient levels, preventing nuisance algae growth and providing a healthier environment for corals and fish.

Not only is waste removed, but protein skimmers also act as gas exchangers. By continuously mixing air and water, skimmers help increase oxygen levels. On top of this, assuming that the pressure of CO₂ (pCO₂) is lower in the air surrounding the aquarium than in the aquarium water itself, they will help reduce carbon dioxide (CO₂) concentrations in the water.

This process is especially important to keep the pH levels within a “healthy” range. Too much CO₂ will likely cause the pH level to drop, creating an acidic environment that would stress or harm everything from corals to fishes, and the wider biome. However, when there is a high pressure of CO₂ on the environment around the aquarium, the increased gas exchange can introduce more CO₂ into the water, potentially lowering the pH and creating an acidic environment so, it is also important to bear this in mind and if needed to aerate the aquarium room to lower these CO₂ levels.

For this reason, you should choose a protein skimmer of an appropriate size for your tank at peak operation as such, it is important to consider the number of animals, volume size, and environment when the aquarium is fully established.



Refugium

A refugium is often a key component of many thriving reef tanks, offering a sanctuary for beneficial creatures and aiding with nutrient export. An appropriately sized refugium containing macroalgae provides a habitat for beneficial organisms such as copepods, amphipods, and other microorganisms. These organisms contribute to the overall health system by consuming detritus and serving as an important food source for fish and corals.

Refugium macroalgae such as Chaetomorpha are paramount for nutrient export. These plants use the carbon, nitrates, phosphates and trace elements dissolved in the water column to grow, decreasing those nutrients inside your main display tank. This nutrient uptake helps reduce nuisance algae growth in the display tank, remove other waste compounds, and improve general water quality. Macroalgae need nutrients such as carbon, nitrate, phosphate, and other trace elements to perform optimally and contribute effectively. It is essential for the perfect growth and export of nutrients that macroalgae are supplied with all those nutrients; without those, macroalgae will not perform as required. Therefore, consider this in the dosing regimen when planning your refugium.

Being photosynthetic, a macroalgae refugium employed in a light counter cycle to the main aquarium will improve the system's pH stability. During photosynthesis, which occurs in the light cycle, CO₂ is used up, and oxygen (O₂) is a byproduct. So, whilst corals and algae in the main display tank respire, consuming O₂ and releasing CO₂, the macroalgae in the refugium performs photosynthesis and consumes CO₂, releasing O₂. This helps to stabilise the pH around the clock by lowering CO₂ concentration, helping to reduce the nocturnal 'pH drop' common in most reef aquariums.

Refugiums play a significant role in stabilising pH and decreasing nutrients while enhancing biodiversity. They provide a safe haven for many species threatened by larger tank occupants. This biodiversity, fostered by your



refugium, helps make the reef system more resilient and healthy overall. Your refugium can also serve as a nursery for various species, aiding in the natural restocking of your tank's ecosystem. This contribution to the ecosystem should make you proud of your refugium's role in enhancing biodiversity.

The benefits of a refugium can be maximised only if they are maintained well. This includes maintaining great water movement, providing enough lighting, and periodically harvesting some of the macroalgae to remove the old/dead macroalgae from the system. Nonetheless, make sure not to remove healthy macroalgae as you will be reducing the nutrient export capacity of your refugium.

Routine maintenance is not just a task but a responsibility that supports the growth of macroalgae and other beneficial organisms that live within the refugium. By being proactive in your maintenance, you are ensuring the health and vitality of your reef system.

Biological Filtration

Biological filtration is the backbone of keeping a healthy, stable reef aquarium. The process relies on microbes and other microorganisms that colonise the surfaces of everything you place in your tank – i.e., rock, live sand – to break down organic matter as well as waste products. Microbes and other microorganisms convert this waste into nitrogen gas and other substances through a process that is known as the bacterial assimilation in the nitrogen cycle. Adequate quantities of rock and live sand are crucial to provide the necessary surface area for these beneficial microorganisms to populate which will ideally result in a biodiverse and mature biome.

Highly porous rock: The D-D Aquascape Rock offers a large amount of surface area on which these bacteria can grow. It acts as a physical support and aesthetic element in the aquarium, which at some points can act as biological filter too. Live sand, for example will also add to the biological filtration process by expanding surface area and adding beneficial



microorganisms as well as tiny invertebrates.

Effective biological filtration, which contributes to the aesthetics of the aquarium, is largely dependent on how the rocks are placed and their design. This will require some structure design (rock scape) and the making of these structures to maximise water flow allowing all rock surfaces to be well oxygenated ensuring ample areas for beneficial bacteria to colonise. Please see the Rock Scaping portion of this guide for information on rock positioning and design to increase effectiveness in biological filtration. Bio-media – other than the rock and sand mentioned – like ceramic rings, sintered glass or bio-balls can give you more surface for bacteria coverage. Remember; in this case size is important!

Biological filtration is effective only when some basic conditions are satisfied, which allow beneficial bacteria to remain alive and functional. Maintaining stable water parameters is highly important, which means maintaining pH, temperature, and salinity, as well as carbon, nitrate, phosphate, and a range of trace elements. Without these at the correct levels, bacteria will not perform as we expect and may even perish.

Biological filtration is vital for maintaining water quality and supporting the overall health of a reef aquarium. Using an adequate volume of rock and live sand provides the necessary surface area for beneficial microorganisms to thrive, promoting a mature and stable biome.

Sufficient rock and live sand - to achieve the proper surface area for effective development of beneficial micro-fauna, necessary for a mature, stable biome.

Chemical Filtration

One important part of looking after a reef aquarium is the use of chemical filtration to help keep water quality in check. This encompasses the utilisation of chemical media to absorb dissolved organics, and other pollutants that mechanical and biological filtration may not tackle; therefore, maintaining



a stable environment which promotes a healthy biome which of course includes healthy corals and fish. Chemical filtration has many benefits but, like any other type of filter, it can also undesirably have negative impacts, i.e. it can remove elements we want in the water.

Crystal-clear water can be produced by using activated carbon (Reef Carbon) and flocculants, such as Blizzard – these products bind dissolved organic compounds that cause yellowish or cloudy water, and allow them to be easily removed resulting in clearer water and better light penetration. However, there are additional styles of media that can meet other goals. GFO (Granular Ferric Oxide) types like Rowaphos, aluminium-based phosphate removers, or ion- exchange resins for the removal of targeted pollutants, e.g., copper, are popular choices.

Although this section is not intended to detail the different filtration media, due to its impact on water chemistry, we will delve into some detail regarding GFO, given it is widely used in reef aquariums to effectively manage and reduce phosphate levels. Composed of ferric oxide-hydroxide granules, GFO effectively binds phosphate ions due to its high surface area-to-volume ratio. GFO is primarily used for phosphate control, reducing phosphate levels to prevent nuisance algae growth and promote coral health. It is important to note that GFO will also remove other ions like silicon, manganese, iodine, and chromium, among many other trace elements. Therefore, when using GFO, additional trace elements will likely need to be supplemented. Additionally, when added to the aquarium, GFO has an initial alkalinity depletion effect, which needs to be considered when using this type of phosphate removal media.

GFO can be placed in media bags in high-flow sump areas; however, for effective use, GFO is best utilised in a fluidised reactor like the D-D FMR75, ensuring even water flow and maximum contact. To ensure GFO remains effective, the pH should be kept no higher than 8.35 as its efficiency is dependent on pH. Another important aspect of using GFO is that depending on the conditions, it can release iron into the water, which can promote algae and bacterial blooms; furthermore, low-quality, non-virgin (i.e., recycled) GFO previously used in the water treatment industry can, under specific



circumstances, release some of the absorbed pollutants, like arsenic, back into the water.

Therefore, when using this type of media, it is important to select high-quality virgin material like that which is used in Rowaphos.

In conclusion, chemical filtration is a very useful tool for maintaining a healthy reef aquarium. It helps to remove dissolved organics, toxins, and other impurities that are not conducive to the good growth and health of corals, fish, and other marine life.

Ozone

Ozone (O_3) is a powerful oxidising agent used in reef aquariums to increase water quality and clarity by breaking down organic pollutants and killing microbes. While very helpful, this application is a double-edged sword because of its potent oxidative capabilities which can have negative effects on corals.

There are many benefits to using ozone in a reef aquarium. Ozone effectively cleaves the long chains of dissolved organic compounds (DOC) into simpler molecules that can more readily be filtered out by protein skimmers or processed through biofilters, lowering total organics and improving water clarity. It eliminates yellowing compounds, typically created by dissolved organic material, which in turn clears the water and improves the light transmittance in the aquarium. Ozone is also effective at killing bacteria, viruses, and other pathogens that can inhabit the water and pose a threat to corals and other marine organisms, keeping disease outbreaks in check. This can, in turn, lower the number of free-swimming parasites like *Cryptocaryon irritans* (when in the free-swimming stage) and help improve the condition of the aquarium further.

But ozone can be detrimental for corals. Ozone is an oxidative stressor and at high concentrations may damage coral cells, resulting in bleaching or tissue necrosis—even death. To reduce oxidative injury, corals use antioxidant



defences; however, their ability to mount an effective response appears to be insufficient after exposure to high concentrations of ozone under thermal stress. Ozone has also been shown to inhibit nitrifying bacteria—a source of health for the nitrogen cycle in reef aquaria—and thus can disrupt balance within these systems leading to toxic ammonia or nitrite spikes. The leftover ozone can then be toxic to marine life if not properly managed. Further, oxidation can lead to adverse byproducts—bromate is generated when ozone reacts with bromide. To prevent these detrimental impacts, ozone should be degassed from the water before returning it to the aquarium, usually by means of activated carbon and other degassing methods.

The application of ozone in reef aquariums is best controlled with the use of high-quality Ozone Generators that provide accurate production of Ozone to keep the Oxidation-Reduction Potential (ORP) at about 300 to 400mV. These levels remain safe for marine life and are not likely to cause an issue! You must also properly degas and filter your water using activated carbon in order to remove any leftover ozone from the water before it returns back into the aquarium, as well as correctly ventilate around your ozone generator for safely allowing escaping ozone gas.

So, in summary, ozone is potentially a very useful piece of equipment, especially when it comes to both water treatment and sterilizing pathogens that the skimmer cannot remove. Although its use is effective, it must be used in a way that does not negatively impact corals and other marine life.

UV Sterilisers

In reef aquariums, ultraviolet (UV) sterilisers are commonly used to maintain specific aspects of water quality. UV sterilisation is a process used to kill or inactivate bacteria, viruses, mould spores, and fungi by breaking down the very structure of their DNA. UV sterilisers are slightly less aggressive than ozone but must still be used with caution to avoid negative impacts on the aquarium ecosystem.

UV sterilisers are capable of destroying bacteria, viruses, and some types of



parasites in water, which helps reduce the diseases that can pass between fish and corals. They are aimed at the free-swimming stages of parasites like *Cryptocaryon irritans* (marine ich) and *Amyloodinium ocellatum* (velvet) to help control outbreaks. Additionally, UV sterilisers work as a control unit against planktonic algae, which can make your water look green and even cloudy, hence enhancing water clarity.

However, UV sterilisers can have harmful effects on corals. Though UV sterilisers work well in killing off harmful microorganisms, they can also kill beneficial bacteria and phytoplankton necessary for nutrient cycling within your tank to give you a healthy aquarium. Without these beneficial microbes, the microbial balance can be disturbed, resulting in poor nutrient cycling, water quality and even poor coral health. The water flow rate and the turbidity in water limit UV light penetration. Without enough clear water or too fast of a flow, the UV steriliser becomes less effective. UV sterilisers must remain on long enough to allow for sufficient exposure time required for the light waves to adequately kill microorganisms. If the flow rate of water through the steriliser is too fast, pathogens may not be adequately rendered harmless.

Choose a UV steriliser that is correctly dimensioned for the volume of your tank and tailored to what your system requires. Change the steriliser flow rate to give sufficient contact time, noting the manufacturer's recommendations for optimum flows. Replace the UV bulb according to the manufacturer's recommendations, typically every 6-12 months for continuous use, to maintain optimum performance. If the quartz sleeve that houses the UV bulb is dirty, the water might not receive as much UV light, which may reduce the effectiveness of your sterilizer – so cleaning it from time to time is important.

To sum it all up, UV sterilisers have a place in reef aquariums for maintaining water quality and controlling pathogens. When used properly, they provide benefits such as better water clarity, diminished disease occurrence, and improved overall water quality.



Water Flow

Water movement is a cornerstone to reef aquarium health and influences all factors of an entire biome, from coral viability to photosynthesis, growth, feeding, thermal stress tolerance and microbial stability.

Water flow also aids in the export of oxygen from coral tissues, which lowers oxidative stress and improves photosynthetic efficiency. It also shapes corals' growth forms and rates and metabolic activities, with different species having unique responses to flows. In general, good flow = more growth.

In addition, good water movement helps transport prey particles and nutrients and thus increases corals' food intake, leading to healthier, more colourful coral growth. It also helps corals release heat and waste metabolites, which makes the corals less stressed in high temperatures. High flow rates keep everything in balance by not allowing pathogenic bacteria to bloom, especially during scorching days of extreme temperatures.

One of the most ignored requirements is water flow, which can be crucial for systems dominated with SPS species.

Lighting and Spectrum

Light: Almost all corals require light, and a good form of lighting is essential. With their symbiotic relationship with zooxanthellae, the photosynthetic algae living in its tissues, light is essential for corals. Good lighting is vital as it will help with photosynthesis, coral growth, and health. If you look at any modern-day reef tank, more than likely, the display has LED lights; these are highly configurable and powerful lights as such, adjusting them to balance light quality and intensity for optimal coral health is paramount.



Duration

A 10–12-hour photoperiod with a slow (i.e. 1 to 2 hours) increase and decrease in light intensity at the start and end of the photoperiod is recommended and frankly ideal as it mimics nature. The light period of a tank must be set with caution, as an inadequate time will interfere with photosynthesis activity and affect coral metabolism due to decreased irradiation, but at the same time, prolonging it can cause photoinhibition and damage the photosynthetic apparatus, leading to coral bleaching and ultimately death.

Intensity

Light intensity should be tailored to the different types of corals in your tank. Soft and LPS corals require lower intensity (50-250 $\mu\text{mol}/\text{m}^2/\text{s}$), while SPS corals need higher intensity (250-450 $\mu\text{mol}/\text{m}^2/\text{s}$).

Intensity is of particular importance as too much will cause photoinhibition and in extreme cases can cause damage to zooxanthellae leading to coral bleaching. On the other hand, insufficient intensity will starve the corals. As such, we recommend using a PAR/Spectrum meter to set up not only the intensity but the ideal spectrum as per the below.

Spectrum

Studies suggest that the light spectrum impacts corals by influencing antioxidant activity, photosynthesis, oxidative stress, gametogenesis, survival, and calcification rates, with blue light generally beneficial and artificial light at night and red light having negative effects.

UV Light (380-450nm): UV radiation exacerbates thermal stress, leading to increased damage in both host tissues and algal symbionts during bleaching events. Nonetheless, fluorescent pigments stimulated by UV light in corals provide photoprotection by dissipating excess energy and reflecting harmful



light, enhancing resistance to bleaching during heat stress.

Blue Light (450 - 495nm): Blue light (peak at 455nm) enhances coral growth, zooxanthellae density, chlorophyll content, and photosynthesis rates compared to red light, which can harm corals.

Red Light (620 – 750nm): Research has shown that Red light negatively impacts SPS coral health, reducing survival rates, zooxanthellae density, and chlorophyll content. Corals exposed to red light show lower photosynthesis performance and higher oxidative stress.

Green Light (495 - 570nm): Supports photosynthesis but is less effective than blue light.

The light spectrum has a profound impact on coral health and physiology. Blue light generally promotes coral growth, photosynthesis, and antioxidant activity, while red light and artificial light at night can be detrimental, causing oxidative stress and disrupting biological rhythms. UVR can damage corals, with fluorescent pigments providing photoprotection, so it is important to balance corals' exposure to UV and the "promoted" colouration. Efficient light absorption by coral skeletons and enhanced calcification under blue light are critical for coral survival and growth.

Coverage

Ensure all corals receive adequate light by using a combination of spot/puck lights and light bars, such as AI Hydras with AI Blades. This will ensure even coverage of the aquarium.

Moonlight Cycles

Moonlight cycles are crucial for coral reproduction and a range of physiological or behavioural processes. Synchronous moonlight cycles may help by mimicking conditions that normally signal coral spawning and other natural behaviours. Nonetheless, if set it needs to be very cautiously



planned as excessive light can have very negative impacts on corals.

Rock Scape and Sand

Rock placement and sand are essential to creating a successful and sustainable marine aquarium. These elements influence biological filtration, microbial diversity, water flow, and nutrient distribution, all of which contribute to the health and stability of the aquarium ecosystem. As such, ahead of laying down rocks in the aquarium, consider planning and creating a nice and open rock scape.

A well-designed rock scape influences water flow, ensuring efficient nutrient distribution and waste removal, preventing toxic buildup.

Porous rock, like the D-D Aquascape Dry Rock , acts as a biofilter, removing nitrogenous compounds and hosting bacteria that “cycle” these compounds, maintaining water quality.

Diverse microbial communities on rocks and within coral mucus are crucial for coral health and live rock, usually are a sign of good microbial diversity, highly beneficial to the success of the tank. However, if you are considering the use of live rock ensure these are pest free, as pest can certainly take out the enjoyment of the hobby.

Nonetheless, a properly constructed dry rock scape which is seeded with beneficial microorganisms and microbes from the sand and rock of an existing successful aquarium, or high-quality small amounts of live rock can help mimic natural conditions, supporting a stable microbial community and avoiding the risks of using large amounts of live rock.

If the rock is arranged in such a way that blocks water flow, it will impact the corals’ health, as they will be stressed by poor water quality or environmental conditions. Corals benefit from improved water flow and filtration in a well-designed rock scape.

Adequate rock spacing, which allows adequate flow, will help mitigate disease outbreaks by reducing stress and preventing pathogen accumulation.



Reverse Osmosis Water

For several reasons, it is crucial to use high-quality Reverse Osmosis (RO) water instead of tap water in reef aquariums.

Tap water often contains contaminants like chlorine, chloramines, heavy metals, nitrates, and phosphates, which can be harmful to marine life. RO water, being highly purified, ensures that the water added to the aquarium is free from these harmful substances.

RO water provides a stable and controlled environment. It offers a consistent starting point for preparing saltwater, making it easier to maintain desired chemical parameters.

Additionally, by minimising nitrates and phosphates, RO water helps prevent unwanted algae growth.

RO water is essential for the health of sensitive marine life, such as corals and invertebrates, which are highly sensitive to contaminants found in tap water. Furthermore, using RO water allows precise control over water chemistry. Reefers can customise the mineral content, ensuring the ideal water conditions for their reef inhabitants and avoiding unwanted reactions from pollutant-contaminated water. RO water also prevents long-term issues by avoiding the accumulation of contaminants and bioaccumulation in marine organisms, which can lead to chronic health problems and reduce overall health.

In conclusion, using RO water in reef aquariums creates a cleaner, more stable environment that supports the health and growth of corals, fish, and invertebrates while preventing common issues associated with tap water, leading to a thriving reef ecosystem.



Salt

There are many reasons to invest in high quality salt for your reef aquarium grounds. Salt mixes of good quality are designed to replicate the natural constituents of seawater and will include all the essential macro, micro elements as well as trace elements at their correct concentrations which ensures animals receive enough minerals.

A good quality salt supports coral growth and health by providing the right levels, which are critical for the metabolic processes in corals. These salts also ensure the absence of high nutrient levels like nitrate and phosphate, which in high concentrations would be detrimental to the various biological processes in corals and other invertebrates.

Premium salt mixes are manufactured using the purest ingredients, which means that you have a lesser chance of adding contaminants (e.g. heavy metals, nitrates, and phosphates) to your tank system along with bound water in one form or another - all potentially hazardous for marine life. They should be free of impurities or unwanted additives that can harm water quality.

So, to summarise, using a good salt is one of the most important things you can do when starting a reef tank. It sets the basis to keep corals and other marine life healthy, it won't introduce contaminants into the aquarium which is a key aspect of a long-term successful aquarium. Spending on a quality marine salt mix will result in healthier corals, and more stable reef environment which means less ongoing issues further allowing you to enjoy the aquarium long term.



Dosing

Although you can manually dose each of these needed chemicals to your reef aquarium, automation with the help of a dosing pump is recommended. Manually dosing is difficult and time-consuming as consistent, accurate measurement & timing is required for optimum results. Consistency and precision are paramount to keeping the fragile balance of an aquarium's ecosystem intact – automated dosing provides exactly that.

Use a dosing pump which supports at least four channels, like the D-D P4 Pro. These pumps enable auto-dosing of myriad chemicals such as calcium, carbonates, and trace elements, which are essential for maintaining ideal water parameters. Automated dosing ensures that the correct amount of each chemical is dosed at the right time, reducing chances of human error.

When using our H2P™, one crucial thing to have in mind when the time comes for you to select a dosing pump is the need for the pump to be chemical-resistant, enabling you to handle the various chemicals used in the aquarium without degrading or malfunctioning. For example, the D-D P4 Pro is designed to be compatible with the H2P™ dosing system and other chemical components, ensuring longevity and reliability.

Considering fail-safe aspects in dosing pumps is essential to prevent overdosing or underdosing. An overdose can cause serious problems that are dangerous for the life of your aquarium. Auto shutoff, alarms and redundant safety verifications are some of the features that will protect against these risks to provide for secure effective dosing system operation.

Not all dosing pumps are compatible with all chemicals. The D-D P4 Pro is noted for its compatibility with pHplus. This compatibility is due to the pump's chemical-resistant materials, which prevent degradation and ensure accurate dosing. Using a non-compatible pump could lead to inaccurate dosing and potential equipment failure.

Automating the dosing process in a reef aquarium with a high-quality,



chemical-resistant dosing pump like the D-D P4 Pro is essential for maintaining the delicate balance required for a healthy marine environment. Another advantage of the D-D P4 Pro dosing pump is that also integrates with the D-D KH Manager allowing the highest optimisation of the H2P™ Dosing system.

Nonetheless, high quality and chemical resistant pumps provide consistency, precision, and safety, which are crucial for the long-term success of the aquarium.

Livestock Selection

When choosing livestock for your aquarium, research the needs and behaviours of each species to ensure compatibility with your tank's conditions and each other. Reefers often underestimate this aspect, and animal lives suffer, so it is important to select the right type of animal for the type of tank you want.

We recommend choosing “working” animals i.e. animals that have a function in the aquarium, such as eating algae or controlling pests. Very often, beautiful animals can contribute to the tank's long-term success.

Tank Location

Often disregarded, selecting a tank location that avoids direct sunlight to prevent temperature fluctuations and algae growth is key to the aquarium's success. Ensure easy access for maintenance and monitoring. The easier the access for maintenance, the more likely you are to enjoy it and ensure tight husbandry.

By considering and potentially following these foundational guidelines, you can create a thriving reef aquarium that supports the health and growth of your corals and other marine life. The H2P™ Dosing System will have a



higher impact and will be more effective in supporting a successful reef aquarium when used with these best practices for maintaining a stable and healthy reef environment.

Testing and Monitoring

Over the years, we have observed that reefers who consistently and diligently monitor their aquarium parameters tend to be the most successful. Certain key parameters need regular monitoring to maintain a healthy reef aquarium.

Some of these parameters can and should be monitored at home, while others might require the expertise and instruments of a professional laboratory, such as the Reef Zlements ICP Lab (which we discussed above). Therefore, diligently monitoring these parameters is crucial for maintaining a long-term healthy and stable reef aquarium.

While advanced automation is not mandatory, a minimum level of automation is highly recommended. Although using a D-D KH Manager to implement the H2P™ Dosing System is not mandatory, we recommend using it to fully optimise the system's performance. The D-D KH Manager features a dedicated dosing option specifically developed for the Reef Zlements H2P™ Dosing system, which allows users to maintain the most stable alkalinity and pH levels likely ever achieved.

Parameters we should test and monitor at home

Temperature

Temperature is a crucial factor influencing coral health. Research has investigated the various impacts of temperature on corals, examining their ability to acclimate, the effects on gene expression, and the interactions with other stressors. It has also demonstrated that in some coral species,



temperature influences the incorporation of Magnesium in their skeletons.

Corals exposed to fluctuating temperatures show higher thermal tolerance compared to those in very stable thermal environments. This suggests that prior exposure to variable temperatures can enhance coral resilience to heat stress. However, these should not be rapid changes and should not happen over the course of 1 or 2 days, rather they should mimic the seasons of the year with changes of 2-3°C occurring over a few months like what happens in nature.

It is also important to note that short-term exposure to temperatures 3-4°C above normal “summer ambient” can induce bleaching, while long-term exposure to 1-2°C above ambient can impair growth and coral reproduction.

Higher temperatures also impact water chemistry. For example, higher temperatures accelerate calcium carbonate nucleation and crystal growth, which can lead to calcium carbonate precipitation.

It is also important to understand the impact of temperature on other relevant aspects of keeping a reef aquarium, like the impact of temperature on pests (like *Prosthlostomum acroporae* [AEFW] and *Phestilla subodiosus* sp. nov. [Montipora Eating Nudibranchs]) and bacteria. Lower temperatures may help control and eradicate some pests and slow bacterial growth rates when fighting bacterial issues.

Considering the above, we highly recommend the use of a digital temperature monitor and alarms to constantly monitor temperature, aiming to keep it between 23°C and 28°C. We also recommend keeping a lower temperature during your local winter and at a higher temperature during summer. We recommend increasing and decreasing the temperature over spring and autumn to do so. This will mimic the natural environment and will potentially increase coral resilience. Although we recommend the use of a chiller to fully control temperature, this strategy will help to minimise the impact of heat waves during the summer months.

In conclusion, when setting the temperature level in a tank, consider that in a newly set up aquarium, initially running a higher temperature will accelerate the establishment of the microbes that compose the biome. Also, when



facing undesired pests or bacterial issues, running a lower temperature will slow down their growth and reproduction, and a higher temperature will promote an environment where calcium carbonate will more easily precipitate.

Salinity

Over the years, we have concluded that salinity is one of the most overlooked parameters and one that reefers often struggle to measure correctly and accurately.

In our aquariums, three main factors may impact salinity: evaporation/addition of RO, water changes, and dosing. The latter is especially important because most dosing systems will increase salinity due to the chemistry involved.

This said, it is crucial to monitor and control salinity, which should be checked with a refractometer, a densimeter, or a conductivity meter weekly/bi-weekly and kept within an ideal range, this will be discussed further down in a salinity-dedicated section.

pH and Alkalinity

Although pH and alkalinity will have a dedicated chapter below, it is imperative to mention in this section that it is important to monitor pH regularly and alkalinity at least once a day with manual test kits or advanced tools like the D-D KH Manager. The aim is to maintain stable pH and alkalinity levels as much as possible. More on this topic further below in this manual.

Calcium

Like pH and Alkalinity, calcium is a highly important parameter that needs to be monitored closely. Although we will address Calcium in the Macro and Trace elements section, we wanted to mention that it should be monitored



every 3-4 days and kept within the levels recommended above.

Orthophosphate

Orthophosphate levels significantly impact coral health, influencing growth, skeletal density, and symbiotic relationships. While low orthophosphate levels can lead to bleaching and coral death, excessive levels can inhibit skeleton formation and promote algal competition. Managing orthophosphate levels is crucial for maintaining healthy coral reefs and mitigating the impacts of environmental stressors. It is also crucial to monitor nutrient levels at home weekly with test kits or digital checkers or with a professional, comprehensive test like the Reef Zlements Advanced ICP test (via colourimetry); keeping orthophosphate stable within the recommended levels above will ensure the best coral health and colouration. Nonetheless, more on phosphate and orthophosphate below.

Nitrate

Another important parameter to monitor every week is nitrate, this can be done at home with test kits, digital checkers or with a professional, comprehensive test like the Reef Zlements Advanced ICP test (via Ion Chromatography). Effective management of nitrate levels is crucial for coral protection and resilience while controlling undesired pests (like algae or cyanobacteria). We recommend maintaining nitrate in a ratio of around 1:100 with orthophosphate to ensure coral health and mitigate adverse effects. More about nitrate below.



H2P™ KEY PARAMETERS

Reef Zlements H2P™ Dosing System was crafted to provide unparalleled stability for corals, ensuring they thrive and reach their maximum growth and health potential. Therefore, it is important to examine some of the fundamental parameters involved in promoting the ideal environment for them.

Salinity

The basics and its history

Salinity was first defined as the measure of the mass of dissolved salts in a given mass of seawater. Originally, for its determination, the seawater had to be dried and the resultant salts weighed. However, as one can expect, this presents some difficulties, not only because of the process itself but also because of some chemical reactions that happen at the temperatures needed to drive off all the H₂O.

Nonetheless, the history of salinity measurement is a fascinating journey through the history of oceanography and scientific advancements in understanding and quantifying the saltiness of seawater. We will touch on this in a little detail because salinity is an extremely important environmental factor that affects all animals in our aquarium's health, growth, and survival.

In the mid-19th century, Johan Georg Forchhammer made significant contributions to understanding seawater composition. He analysed various seawater samples and identified the consistent ratio of major ions, leading to the concept of "Forchhammer's Principle" or the "Law of Constant Proportions." This principle states that the relative proportions of the major ions in seawater are constant, regardless of the total salinity.

Building on Forchhammer's work, Martin Knudsen further refined the



methods for determining salinity. In 1901, Knudsen developed a practical method for salinity determination using “chlorinity,” which is the concentration of chloride ions in seawater. Knudsen’s tables correlated chlorinity with salinity and density, standardizing salinity measurements for the first time.

The hydrometer, an instrument that measures the specific gravity (relative density) of liquids and was invented at the start of the 18th century by early oceanographers to measure seawater density, had a significant advancement with Knudsen’s work, making it one of the practical methods that we use today to determine salinity.

The refractive index method for measuring salinity dates back to the 19th century. Early refractometers were used in various scientific fields, including chemistry and pharmacy, to measure the concentration of solutions. Seawater’s refractive index (the measure of how much light is bent or refracted when it enters a substance) changes with its salinity. The refractive index increases as the concentration of dissolved salts in seawater increases.

Advances in optics and materials science throughout the 20th century improved the accuracy and reliability of refractometers. Modern refractometers, which we now use in the hobby, often include digital displays and automatic temperature compensation, enhancing their ease of use and precision.

Another method is the Sound Speed Method, which is the speed of sound in seawater affected by salinity, temperature, and pressure. Higher salinity increases the water’s density, which affects the speed at which sound waves travel through the water. Devices called velocimeters or hydrophones measure the time it takes for a sound pulse to travel a known distance in seawater. Then, empirical formulas, such as the UNESCO algorithm, sound speed data, and temperature and pressure readings, are used to estimate salinity.

The development of conductivity meters in the mid-20th century revolutionised salinity measurement. Significant advancements occurred during World War II when the U.S. Navy needed accurate salinity



measurements for submarine navigation and sonar operations.

Researchers developed more reliable conductivity measurement techniques during this period, and in 1978, the Practical Salinity Scale (PSS-78) was introduced, revolutionising salinity measurement. The PSS-78 defines salinity in terms of the conductivity ratio of seawater to a standard potassium chloride (KCl) solution at a specific temperature (15°C) and pressure (1 atmosphere). Today, we use “modern” conductivity meters to measure salinity accurately. This is one of the most precise methods we can use as hobbyists.

Whilst ICP is not a direct method for measuring salinity, it plays a crucial role in determining the concentrations of various ions in seawater, which can be used to calculate salinity.

Nowadays, with the generalisation of ICP testing, hobbyists at home can easily access this technique.

Since the inception of the first instruments to measure salinity, much has evolved. Although there are other methods to measure salinity, like osmometry, capacitance salinometers, laser-induced breakdown spectroscopy, and others, the above includes all of those the general hobbyist can access. Further expansion on this is undoubtedly exciting but entirely outside the scope of this manual.

But why is Salinity important?

Salinity plays a crucial role in the health and survival of coral reefs, influencing their thermotolerance, reproductive success, and overall physiological functions. Understanding the impact of salinity on corals is essential for developing strategies to mitigate the negative effects and other environmental stressors that occur.

Corals are generally sensitive to changes in salinity due to the need for osmoregulation, which in a reef aquarium can vary due to either dosing, the addition of RO water or water changes with different salinity water.



Hypo-salinity (low salinity) exposure can cause extensive bleaching, tissue necrosis, and increased mortality in corals. Studies on *Stylophora pistillata* revealed severe pathomorphological changes and oxidative stress in both the coral host and its algal symbionts under decreasing salinity conditions.

Conversely, elevated salinity significantly impacts coral species like *Stylophora pistillata*, *Acropora tenuis*, and *Pocillopora verrucosa*, leading to partial bleaching, reduced bacterial and symbiotic algae abundance, and decreased calcification rates.

Salinity but, what levels?

Studies have demonstrated that optimal growth and metabolic rates in some small polyp stony (SPS) and large polyp stony (LPS) corals are observed at salinities between 30 and 35 PSU, with significant declines in performance outside this range. Nonetheless, through ICP testing we often observe depleted levels of some critical macro and trace elements at salinities below 33.5 PSU. Monitoring salinity weekly and maintaining it at 33.5 to 35 PSU is a good and important practice.

pH

The basics

Given that the H2P™ dosing system is the first dosing system developed that allows the reefer to control pH in a much more refined way than ever before and revolves around maintaining ideal and stable levels, we thought it important to dedicate a detailed section to this often- overlooked parameter.

The term pH stands for “potential of hydrogen” and represents the concentration of hydrogen ions (H) in a solution. pH measures the acidity or alkalinity of a solution, with 0 to

6.9 being acidic, 7 neutral, and 7.1 to 14 being alkaline. The pH value is



calculated using the formula:

$$\text{pH} = -\log[\text{H}^+]$$

Where $[\text{H}^+]$ is the concentration of hydrogen ions in moles per litre. Because the scale is logarithmic, each whole number change on the pH scale represents a tenfold increase or decrease in acidity. For example, a solution with a pH of 7.9 is almost twice as acidic as one with a pH of 8.2 and over three times more acidic if compared to a solution with a pH of 8.4.

But why is pH important?

pH plays a crucial role in maintaining coral health in aquariums. Lower pH levels significantly reduce coral calcification rates, making it energetically costly for corals to build and maintain their skeletons. This leads to weaker structural integrity, disrupted symbiotic relationships with zooxanthellae, increased vulnerability to diseases, and reduced thermal tolerance, collectively threatening coral survival.

Studies indicate that lowering the pH by 0.15 to 0.3 units from ambient levels can result in a 30% to 56% reduction in calcification rates in some coral species. Acidified saltwater negatively affects the photosynthetic yield and cell density of free-living zooxanthellae, causing significant physiological and morphological damage. Furthermore, lower pH and higher CO₂ levels alter the microbial communities associated with corals, increasing susceptibility to diseases such as yellow band/blotch disease.

Acidification also reduces corals' thermal tolerance by impairing their ability to manage oxidative stress and maintain cellular function under high temperatures, leading to increased bleaching and mortality rates. These combined effects highlight the importance of maintaining optimal pH levels for coral health.

Conversely, maintaining a pH between 8.2 and 8.4 offers significant benefits for corals. Elevated pH conditions generally have a positive effect on coral calcification and growth. Different coral species show varying abilities to



maintain or even increase their calcification rates at these pH levels. For instance, some species thrive at pH levels around 8.2-8.4, indicating a positive response to such conditions.

Corals maintained at a pH between 8.2 and 8.4 also exhibit stable microbial communities, with no significant increase in bacteria associated with diseases or stress. Additionally, elevated pH levels (around 8.4) during the day enhance the photosynthetic rates of zooxanthellae, benefiting coral health and growth.

Nonetheless, highly elevated pH conditions can also impact coral physiology and health in various ways that haven't been extensively researched. As such, it is recommended not to sustain a pH above 8.6 for long periods of time as the long-term effects on corals aren't fully understood yet. With that said, and as we will discuss below, a higher pH can also promote easier calcium carbonate precipitation, so it is important to keep pH at the recommended levels.

In summary, maintaining optimal pH levels in our aquariums is crucial for ensuring the health and longevity of corals. Proper pH management supports coral calcification, maintains beneficial symbiotic relationships, reduces vulnerability to diseases, and enhances thermal tolerance. On the other hand, lower pH levels have detrimental effects, highlighting the importance of regular monitoring and adjustment of aquarium water chemistry for the well-being of coral ecosystems.

Main Factors Affecting pH

Maintaining a stable pH level is crucial for the health and survival of corals and other marine life in aquariums. Several factors can influence pH levels, necessitating careful monitoring and management to ensure an optimal environment.

Photosynthesis significantly affects pH levels during daylight hours. Photosynthetic organisms, such as zooxanthellae within corals, consume CO_2 and release oxygen. This process reduces the concentration of CO_2



in the water, decreasing carbonic acid formation and its partial pressure ($p\text{CO}_2$), and subsequently increasing pH. This diurnal increase in pH is a natural part of the aquarium's daily cycle and highlights the importance of balancing biological activities within the system.

Respiration processes at night counterbalance the effects of daytime photosynthesis. Both corals and zooxanthellae respire, consuming oxygen and releasing CO_2 . This respiration increases CO_2 concentration and $p\text{CO}_2$, which forms carbonic acid when dissolved in water, thus lowering the pH. This nocturnal decrease in pH needs to be managed to prevent harmful fluctuations that could stress marine organisms.

Carbon dioxide (CO_2) levels directly impact pH. When higher atmospheric $p\text{CO}_2$ forces CO_2 to dissolve in seawater (explained by Henry's Law) to form carbonic acid ($\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3$), which dissociates into bicarbonate and hydrogen ions ($\text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+$) it results in a lower pH. Similarly, elevated CO_2 levels, often resulting from poor gas exchange, overstocking of fish or high environmental $p\text{CO}_2$, can lead to sustained low pH levels, which must be countered to maintain a healthy environment.

Acids can enter the aquarium water through various sources, including biological processes, pollution, or the decomposition of organic matter. Amongst other examples, Phosphatase Producing Bacteria and Inorganic Phosphate Solubilising Bacteria are responsible for the production of organic acids, which then increase the hydrogen ion concentration in the water, thereby lowering the pH.

In summary, photosynthesis and respiration are natural biological processes that cause daily fluctuations in pH. Meanwhile, CO_2 levels and the presence of acids from various sources can lead to more sustained changes in pH. Careful monitoring and management of these factors are essential to maintaining a stable and suitable environment for the health and survival of corals in aquariums.



pH, what levels?

Although, on most surface waters in equilibrium with the atmosphere pH is around 8.2, in small water bodies it can actually vary widely. The pH in inshore waters along Ningaloo Reef ranges from 8.22 to 8.64, while offshore waters range from 8.45 to 8.53. The Great Barrier Reef, on the other hand, shows pH values ranging from 7.98 to 8.37 across different habitats. Whilst in Puerto Rico, pH values on Media Luna reef ranged from 7.89 to 8.17.

Clearly, those are just two regional examples; however, looking at the examples, we can clearly say that the pH in coral reefs ranges from 7.9 to 8.6. Coral reefs experience natural fluctuations in pH, influenced by both biological processes and broader oceanographic conditions but, looking at what was previously mentioned, values from 8.2 to 8.6 can bring benefits to corals. Nonetheless, in the context of a reef aquarium high pH whilst beneficial to corals can bring some challenges.

At higher pH levels, the concentration of carbonate ions in the water increases. This is because higher pH levels shift the equilibrium of dissolved carbon dioxide and bicarbonate ions towards the production of carbonate ions. The chemical reactions involved are:



As the pH increases, the concentration of H^+ ions decreases, which favours the formation of CO_3^{2-} . As such, with higher pH, the concentration of carbonate ions increases, and if enough calcium ions are available the water becomes saturated with respect to calcium carbonate. If the product of the concentrations of calcium ions and carbonate ions exceeds the solubility product of calcium carbonate, the solution becomes supersaturated, and calcium carbonate will begin to precipitate out of the solution (more regarding this subject in the Calcium section further down).

Given all the above, by maintaining optimal pH levels between 8.2-8.4 and understanding the factors influencing pH, aquarists can significantly improve the health and longevity of coral reefs in their care. Regular monitoring and proper management are essential for sustaining vibrant and resilient coral ecosystems.



How to achieve the right pH levels

There are multiple ways to manage pH; nonetheless, H2P™ was specifically created to allow reefers to maintain and manage pH levels along alkalinity and using it as instructed above is likely the most reliable and precise way to control pH.

Alkalinity

The basics – what is alkalinity?

Alkalinity is a crucial parameter in the chemistry of water, particularly in aquatic systems such as coral reefs and aquariums. The most rigorous definitions of alkalinity are necessarily based on acid–base equilibria and represent the capacity of water to resist changes in pH by neutralising acids.

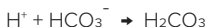
However, rather than beginning with a definition of alkalinity, it is perhaps more intuitive to first consider the changes that take place during the titration of the water with a strong acid, from which point a rigorous definition logically follows.

During a so-called “acidimetric” titration, a strong acid is added to a solution until all aqueous species capable of accepting protons are completely converted to uncharged species. If these “proton acceptors,” or more generally, bases, are present in concentrations in excess relative to the “proton donors” (i.e., acids with relatively large dissociation constants), some of the H^+ added during the titration will be consumed, resulting in a characteristic relationship between the amount of acid added and the resulting pH.

In other words, when the acid is being added, primarily bicarbonate, carbonate and other minor components bind to the H^+ added by the acid and neutralise it. This neutralisation, happens when bicarbonate is converted into carbonic acid, and carbonate into bicarbonate.



Bicarbonate is converted into carbonic acid as follows:



Carbonate is converted into bicarbonate as follows:



From what we are seeing above, alkalinity, is primarily composed of bicarbonate (HCO_3^-) then, carbonate (CO_3^{2-}) ions and to a much lesser extent of other minor contributors like Boric Acid and Borates (H_3BO_3 and BO_3^{3-}), Hydroxide (OH^-), Silicate (SiO_4^{4-}), Phosphates (H_2PO_4^- , HPO_4^{2-} , PO_4^{3-}), Ammonia (NH_3) and Ammonium (NH_4^+), all these, contribute to what is known as Inorganic Alkalinity.

However, there is another form of alkalinity: organic Alkalinity, which is attributed to organic acids like fulvic acid, humic acid, acetic acid, and other carboxylic acids and their conjugate bases. These acids may be found in our aquariums and are derived from the decomposition of organic matter, but they do not contribute as much as the inorganic “counter” parts to the overall alkalinity.

Together, these form what is known as Total (or titration) Alkalinity or what we more commonly know in the hobby as Alkalinity. Looking at these alkalinity contributors and reactions in seawater, typically around a pH of 4.5, these major buffering components have been neutralised. At this point, the amount of acid used to reach this titration endpoint can be used to calculate the total alkalinity of the seawater sample.

This is typically expressed in terms of equivalent calcium carbonate (CaCO_3) in milligrams per litre (mg/L), as milliequivalents per litre (meq/L) or degrees of carbonate hardness (dKH).

This last one is the most common measuring unit amongst aquarists.



Nonetheless, in practical terms, considering the main contributors to alkalinity in aquarium saltwater and to simplify, alkalinity can be expressed by the following formulae:

$$\text{Alkalinity (in meq/L)} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}^-] + [\text{OH}^-] - [\text{H}^+]$$

As very often alkalinity in our hobby is expressed in dKH, to convert alkalinity from meq/L to dKH we can use the following formulae: $\text{dKH} = \text{Alkalinity (meq/L)} / 0.357$ and therefore 2.5 meq/L is the equivalent to approximately 7 dKH of alkalinity.

But why is alkalinity important?

Corals, in particular, absorb calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}) from seawater through their outer cell layer (epithelium). The ions are then transported to the calcifying space (subcalicoblastic space) between the coral's tissue and the existing skeleton. Within the calcifying space, calcium and carbonate ions combine to form calcium carbonate (CaCO_3), which precipitates as aragonite crystals, forming the coral skeleton.

As such, alkalinity is a critical parameter in reef aquariums (and reefs of the world), playing a significant role in maintaining the health and stability of coral reef ecosystems. It influences various biochemical processes, including, as mentioned above, calcification, pH stability, and the overall carbonate chemistry of the water, which are all essential for the growth and resilience of corals and other marine organisms as such. Without carbonate ions, coral growth and, ultimately, their life wouldn't be possible as we know it.

Alkalinity, what levels?

Alkalinity around the world varies due to a number of varied reasons, including seasons which affect the hydrological cycle, the proximity to shore due to land runoff and rivers, depth and geological processes due to hydrothermal vents and sediment interactions, ocean currents and other factors. Nonetheless, according to research on 14 inshore reefs of



the GBR, Total Alkalinity varied between 2069 $\mu\text{mol/kg}$ and 2360 $\mu\text{mol/kg}$ (equivalent to 5.79 and 6.61 dKH) over a two-year period. In the Red Sea, where alkalinity is slightly higher compared to other oceanic regions, ranges from around 2250 $\mu\text{mol/kg}$ to 2500 $\mu\text{mol/kg}$ (equivalent to 6.3 to 7 dKH). Other offshore areas of the Indian Ocean have been shown to vary from around 2200 $\mu\text{mol/kg}$ to 2360 $\mu\text{mol/kg}$ (equivalent to 6.16 to 6.61 dKH). The Pacific Ocean varies between 2200 $\mu\text{mol/kg}$ and 2400 $\mu\text{mol/kg}$ (equivalent to 6.16 and 6.72 dKH) and the Atlantic Ocean 2180 $\mu\text{mol/kg}$ and 2450 $\mu\text{mol/kg}$ (equivalent to 6.10 and 6.86 dKH).

The levels above do show regional (main differences occur at different latitudes) and seasonal variations; however, if we look at the Normalised Total Alkalinity (NTA), which is the parameter used in chemical oceanography to assess and compare the alkalinity of seawater across different regions and conditions, and which accounts for variations in salinity and is represented by the following formula:

$$\text{NTA} = \text{TA} \times \frac{35}{S}$$

Where TA is the measured total alkalinity, and S is the measured salinity. By multiplying the TA by the ratio of standard salinity (35 PSU) to the actual salinity, the alkalinity is normalised, we can observe that the average values are similar between the Indian and Atlantic Oceans, i.e. 2291 $\mu\text{mol/kg}$ (equivalent to 6.41 dKH) and just slightly higher for the average values of the Pacific Ocean with 2300 $\mu\text{mol/kg}$ (equivalent to 6.44 dKH).

Looking at the numbers above, we realise that the alkalinity levels in the reefs and oceans around the world are significantly different from values that our aquariums are often kept at, with the minimum being around 5.79 dKH and the maximum of 7 dKH and if salinity is normalised to 35ppt alkalinity ranges from 6.415 and 6.44 dKH.

With the above said it is safe to say that in nature corals are far from used to the alkalinities we generally maintain in our aquariums. Our approach is to provide corals with the closest environment to nature as possible, whilst offering good pH buffering, good coral growth and health, along with a



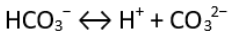
slightly elevated level to account for human and equipment failure.

As such, when using the H2P™ dosing system, we should aim for an alkalinity range between 6.2 and 7.0 dKH. While this range is outside the “normal industry” unnatural standards, managing alkalinity and pH adequately (i.e. within our recommended levels) will ensure optimal long-term health and growth of our corals while avoiding issues like calcium carbonate precipitation.

The different alkalinity sources and pH

As we saw above, maintaining a close-to-nature alkalinity range along with the correct pH in a reef aquarium is crucial for the health and stability of the ecosystem. When deciding on an alkalinity source to increase alkalinity, it is, therefore, essential to consider the impact that the different sources may have on pH.

To understand the relationship that bicarbonate and carbonate have with pH, we need to look at how the carbonate system behaves in saltwater, i.e. we need to understand the chemical equilibria in seawater and, therefore, the primary reactions involved in the carbonate chemistry, which are:



When both bicarbonate and carbonate are added to water, they can act as both an acid and a base because they are amphoteric species. Nonetheless, the pH conditions for the different reactions to happen are significantly different and carbonate would require a pH significantly above 10 to act as an acid, whilst bicarbonate would require a significantly lower pH.

Another well-known alkalinity source that has been used in our hobby for many years is hydroxide. The most well-known is Calcium Hydroxide, i.e. Kalkwasser, which has been used in aquariums for decades. Nonetheless, other forms of hydroxide have also been used. Contrary to the other two alkalinity sources, hydroxide is not amphoteric. For OH^- to act as an acid, a



proton would need to be donated. However, OH^- does not donate protons; instead, it accepts them. Therefore, it can only increase pH, and as such, it is classified as a strong base.

Let's now compare the effects of the different alkalinity sources on pH by looking at some real-life practical examples.

Bicarbonate

Lets assume that we have a 500 L aquarium with a pH of 8.2 with a stable initial concentration of HCO_3^- , CO_3^{2-} and $\text{CO}_2(\text{aq})$ in equilibrium to which we add 250 mmol of bicarbonate.

Adding the bicarbonate will shift the equilibria, and some of the bicarbonate will convert to carbonate and hydrogen ions, which will affect the pH, but let's see how.

To find the resulting concentration of adding 250 mmol of bicarbonate to 500 L of saltwater, we need to perform the following equation:

$$C = \frac{\text{Total mmol}}{\text{Volume L}} \Leftrightarrow C = \frac{250 \text{ mmol}}{500\text{L}} \Leftrightarrow C = 0.5 \text{ mmol/L}$$

So, adding 250mmol of bicarbonate in 500 L of saltwater is equivalent to adding 0.5 mmol/L.

Now, let's use the Henderson-Hasselbalch equation below to understand the effect on pH:

$$\text{pH} = \text{p}K_2 + \log\left(\frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-]}\right)$$



In seawater, the value for the dissociation of bicarbonate to carbonate is approximately 9.1 at 25°C and we can assume the initial concentration of bicarbonate and carbonate in seawater at a pH of 8.2 to be $\text{HCO}_3^- \approx 1.8$ mmol/L (millimolar/L) and $\text{CO}_3^{2-} \approx 0.2$ mmol/L.

With the addition of bicarbonate, its concentration increases by 0.5 meq/L, so the new HCO_3^- concentration is 2.3 mmol/L (i.e. $\text{HCO}_3^- = 1.8$ mmol/L + 0.5 mmol/L).

So, now let us calculate the pH shift in order to maintain equilibrium:

$$\text{pH} = 9.1 + \log\left(\frac{0.2\text{mM}}{2.3\text{mM}}\right) \Leftrightarrow \text{pH} \approx 9.1 + \log(0.087) \Leftrightarrow \text{pH} \approx 9.1 - 1.06 \Leftrightarrow \text{pH} \approx 8.04$$

With the above mathematical exercise, we can clearly demonstrate that the use of 0.5 meq/L of bicarbonate as an alkalinity source in a 500L system with an ideal pH of 8.2 will lower the system pH to a suboptimal level of approximately 8.04.

Carbonate

Let's now see what happens if, instead of using bicarbonate, we use carbonate with the same conditions.

So, if we use the conversion above, adding 250mmol of carbonate to 500L of seawater is equivalent to adding 0.5 mmol/L of carbonate.

Similarly, we use the Henderson-Hasselbalch equation again:

$$\text{pH} = \text{p}K_2 + \log\left(\frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-]}\right)$$



So, given the same original conditions, pK_2 is still approximately 9.1 at 25°C and similarly, we should assume the initial concentration of bicarbonate and carbonate in seawater at a pH of 8.2 to be $\text{HCO}_3^- \approx 1.8 \text{ mmol/L}$ and $\text{CO}_3^{2-} \approx 0.2 \text{ mmol/L}$. With the addition of 0.5 mmol/L of CO_3^{2-} we have approximately 0.7 mmol/L of carbonate therefore:

$$\begin{aligned} \text{pH} &= 9.1 + \log\left(\frac{0.7\text{mM}}{1.8\text{mM}}\right) \Leftrightarrow \text{pH} \approx 9.1 + \log(0.389) \Leftrightarrow \text{pH} \approx 9.1 - 0.41 \Leftrightarrow \\ &\Leftrightarrow \text{pH} \approx 8.6898 \end{aligned}$$

This time, we can clearly see the opposite effect on pH; if carbonate is used, we will observe an increase in pH in the aquarium.

Hydroxide

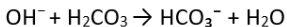
As discussed above, hydroxide is a strong base; as such, it doesn't behave as bicarbonate or carbonate as it can only increase pH. Taking this into consideration, hydroxide can be used if we need to increase the pH in our aquariums significantly. There are, however, some questions regarding it, i.e. when, how much and what will happen if we dose hydroxide instead of bicarbonate or carbonate?

Before demonstrating how much a set dose of hydroxide will increase the pH in the conditions above, it is important to understand why hydroxide is a strong base, and this is easily explained.

When hydroxide (OH^-) is dosed into seawater, it interacts with various components of the seawater, leading to the formation of different species. The primary reactions involve the carbonate system, which includes dissolved carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}); these reactions are as follows:

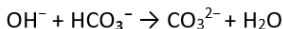


Reaction with Carbonic Acid



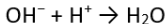
Here, hydroxide reacts with carbonic acid to form bicarbonate and water.

Reaction with Bicarbonate:



Hydroxide reacts with bicarbonate to form carbonate and water.

Reaction with Hydrogen Ions:



Hydroxide neutralises hydrogen ions to form water, increasing the pH of the seawater.

As we can see, when hydroxide is dosed into seawater, it primarily reacts with Hydrogen, Carbonic Acid, Bicarbonate and Carbonate, and there are three resulting species, i.e. Water, Bicarbonate and Carbonate.

So, let's look at what happens when we have a similar scenario to what we discussed previously, where we assumed that we have a 500 L aquarium at 25°C with a pH of 8.2 with a stable initial concentration of $\text{HCO}_3^- \approx 1.8 \text{ mM}$, $\text{CO}_3^{2-} \approx 0.2 \text{ mM}$ and $\text{H}^+ \approx 6.31 \times 10^{-9} \text{ M}$ to which we add 250 mmol (0.5mmol/L) of hydroxide.



Since 1 mmol of OH^- neutralises 1 mmol of H^+ and the amount of OH^- added (0.5 mmol/L) is much greater than the initial H^+ concentration (6.31×10^{-9} M), the initial H^+ will be almost completely neutralised and, in practical terms, the hydroxide concentration will not change:

$$\begin{aligned}\text{New } [\text{OH}^-] &= [\text{OH}^-]_{\text{Added}} - [\text{H}^+]_{\text{Initial}} \Leftrightarrow \text{New } [\text{OH}^-] = 6.31 \times 10^{-9} \text{ M} - 0.5 \times 10^{-3} \text{ M} \Leftrightarrow \\ &\Leftrightarrow \text{New } [\text{OH}^-] = 4.9999369 \times 10^{-4} \text{ M} \Leftrightarrow \text{New } [\text{OH}^-] \approx 0.5 \times 10^{-3}\end{aligned}$$

Since OH^- reacts with HCO_3^- to form CO_3^{2-} and H_2O , we will have a reduction of the concentration of HCO_3^- :

$$\text{New } [\text{HCO}_3^-] = 1.8 \text{ mM} - 0.5 \text{ mM} \Leftrightarrow \text{New } [\text{HCO}_3^-] = 1.3 \text{ mM}$$

Conversely, we will observe an increase of 0.5 mM in the CO_3^{2-} concentration due to the contribution made by the OH^- and HCO_3^- reaction:

$$\text{New } [\text{CO}_3^{2-}] = 0.2 \text{ mM} + 0.5 \text{ mM} \Leftrightarrow \text{New } [\text{CO}_3^{2-}] = 0.7 \text{ mM}$$

Now we can use the Henderson-Hasselbalch equation to find the new pH:

$$\text{pH} = \text{p}K_2 + \log \left(\frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} \right)$$

Given is still approximately 9.1, we have:

$$\text{pH} = 9.1 + \log \left(\frac{0.7 \text{ mM}}{1.3 \text{ mM}} \right) \Leftrightarrow \text{pH} \approx 9.1 + \log(0.538) \Leftrightarrow \text{pH} \approx 9.1 - 0.2688 \Leftrightarrow \text{pH} \approx 8.83$$

Taking the above into consideration, which, although is just an approximation of what really happens in situ, we can clearly state that hydroxide is the alkalinity source, which increases pH the most for a similar amount dosed, followed by carbonate and then bicarbonate, this last one depending on conditions, can actually lower pH.



Alkalinity but, what sources should we use?

Looking at the above, we can clearly start deciding what carbonate sources to use, how to use and when to use them.

So, to answer this question, we need to look at the whole context, look at what we are trying to achieve i.e. what animals are we maintaining and their requirements, and the seawater chemistry i.e. pH, Alkalinity levels, the temperature we maintain, and some other levels which although we haven't touched yet we will discuss further down like the macro elements and nutrient levels.

With the above said, once an alkalinity level is set and achieved using Carbonate or Bicarbonate, one should combine the different alkalinity sources with an intelligent approach in order to achieve the ideal pH.

Let's illustrate this with a simple example that doesn't involve any mathematical calculations; as such, we are only assuming hypothetical pH values which are only meant to illustrate the rationale that should be used when selecting the different alkalinity sources.

Let's assume that we have a 500 L aquarium and that for maintaining an alkalinity of 2.5 meq/L (i.e. 7 dKH), we dose 50 ml of a bicarbonate alkalinity solution hourly over the 24 h.

Given the particular conditions of the aquarium, the pH is 7.8 during the night time and 8.0 at peak during the day. The simple solution (although not necessarily the optimal solution) would be to do a straight swap of the bicarbonate by carbonate or even hydroxide.

So, let's assume that we have replaced the bicarbonate straight by a solution of hydroxide which supplies the same alkalinity amount and has kept the alkalinity level stable at the same level. So, let's hypothetically assume that, with the swap, the pH is now 8.3 at the lowest point (during the night) and 8.7 at peak during the day.



Looking at the pH levels one can say that, from all that was discussed so far, the pH has gone slightly above the ideal levels one should maintain in the aquarium.

Knowing that carbonate has a smaller increase effect on pH than hydroxide, let's now replace the 25 ml of the hydroxide, which is dosed during the 12 h of the day, with 25 ml of a carbonate solution with the same alkalinity concentration. By doing so, the pH will, in theory, decrease but with an emphasis during the day and the end result will be something close to a pH of 8.3 during the night and 8.5 at peak during the day. Now if this pH range is still slightly higher than desired, we can replace or mix part of the carbonate/ with bicarbonate, we should now be able to fine-tune the pH further and achieve a pH between 8.2 and 8.4.

Looking at the above, although, these pH values are just hypothetical values as it wasn't the intention to mathematically prove what values would be achieved, we are able to illustrate how the different alkalinity sources can be used to manipulate pH in our tanks and achieve an ideal pH range. This is something the H2P™ Dosing System does.

Alkalinity sources: do the different sources cause long-term problems?

Recently, several scientifically unsubstantiated claims regarding carbonate and hydroxide have surfaced. These have been associated with “Old Tank Syndrome” (OTS), a term that lacks a clear scientific definition and has only been loosely described. It appears that these claims may have been made for purely commercial reasons, without considering the well- being of aquarium organisms or other relevant factors.

To address these claims, it is important to examine the role of bicarbonate, carbonate, and hydroxide as sources of alkalinity in saltwater aquariums. A review of previous sections reveals that these claims do, indeed, lack scientific merit.

To debunk these claims, let us first understand what is involved in OTS. This term refers to the decline in water quality in long-established aquariums due



to accumulated pollutants and reduced biological filtration efficiency, often resulting from neglected maintenance and husbandry. This decline can lead to unexplained Slow Tissue Necrosis (STN) and Rapid Tissue Necrosis (RTN) in corals (i.e. their slow or rapid tissue death), among other issues.

While OTS lacks a formal scientific definition, we offer an empirical contribution to its understanding. As discussed in the “Pre-requirements” section of this manual, inadequate infrastructure, over time, is likely to result in the accumulation of organic matter and pollutants in the aquarium substrate or the refugium.

This organic material provides a food source for bacteria and other microorganisms. As such, aerobic bacteria decompose organic matter in the presence of oxygen initially, producing carbon dioxide (CO₂), water (H₂O), and other byproducts. However, in areas with poor water circulation, oxygen can become depleted.

When oxygen levels drop, aerobic bacteria cannot survive, and anaerobic conditions develop. Anaerobic bacteria, which thrive without oxygen, take over the decomposition process. In these conditions, sulphate-reducing bacteria (SRB) such as *Desulfovibrio* and *Desulfotomaculum* utilise sulphate (SO₄²⁻) as an electron acceptor instead of oxygen. These bacteria reduce sulphate to hydrogen sulphide (H₂S) as part of their metabolic process, according to the chemical reaction:



Hydrogen sulphide gas (H₂S) is produced as a byproduct of sulphate reduction. This gas can accumulate in the substrate, particularly in areas such as deep sand beds, deeper layers of rock, areas with poor water flow, or in unused (without water flow) media reactors left with used media and detritus. The presence of hydrogen sulphide is often indicated by a characteristic “rotten egg” smell and is extremely toxic to fish, corals, and other aquatic organisms. When disturbed (or when a reactor is turned on without replacing old media and being cleaned), hydrogen sulphide is released, often with catastrophic results.



This issue, combined with excessive organic compounds, nitrate, phosphate, and other pollutants, contributes to what is known as Old Tank Syndrome. It is clear that the different sources of alkalinity, such as bicarbonate, carbonate or hydroxide, have no connection to the Old Tank Syndrome. Suggesting that these substances are dangerous to the aquarium serves only a dubious commercial agenda.

Instead, if reefers maintain clear and rigorous husbandry to keep all areas of the aquarium clear of detritus, Old Tank Syndrome will not occur, not today, not in 10 or 20+ years regardless of the source of alkalinity used.

Final considerations regarding HCO_3^- , CO_3^{2-} and OH^-

Understanding the above is important to deciding what alkalinity source to use. However, additional considerations should still be made.

Using any of these sources is safe, accounting that one uses them adequately and maintains levels compatible with the water chemistry needed to keep the animals we care for.

With this said, whilst using bicarbonate continuously, pH can likely be depressed; using carbonate and hydroxide, in the same manner, will significantly help increase pH, and combining all these sources in an intelligent dosing strategy that can help us maintain the alkalinity and the pH levels we aim for and are best suited for the animals we love.

Macro, Trace elements and nutrients

Bergman defined the earliest chemistry of seawater in 1779; later, in 1819, Marcet suggested that the composition of sea salt is nearly constant, i.e., all species of seawater around the world have the same ingredients with virtually the same proportions. Dana Kester of the University of Rhode Island later called this the first law of oceanography.

Knowing this, elements were classified into Major (macro) elements and Minor elements based on their concentrations and roles within marine



ecosystems; nonetheless, within the Minor elements, we can further divide them into Minor and Trace elements again based on their concentration.

Macro elements are elements found in relatively high concentrations (i.e. >1 mg/L or ppm) in seawater and are essential for marine organisms' biological processes.

These elements are needed in larger quantities and play critical roles in structural and physiological functions. For example, calcium and magnesium are key for the skeletal structure of corals and shellfish, while sodium and chloride are vital for osmotic regulation.

Trace elements are found in much smaller concentrations than macro elements (< 1 mg/L but typically found in much smaller quantities, i.e. <200 $\mu\text{mol/L}$ or ppb), but they are still essential for the health and functioning of marine ecosystems.

Even though trace elements are required in extremely small amounts, they are crucial for the metabolic processes of marine organisms. Deficiencies or imbalances can lead to impaired growth, reproduction, and overall health of marine life.

The Reef Zlements H2P™ Dosing System focus on continuously supplying optimised levels of both macro and trace elements to ensure the best conditions for all animals in the aquarium.

Macro Elements

Boron

Boron (B) is a macro element which is present in seawater as boric acid and borate. Unlike sodium, chloride, magnesium, sulphur, and other macro elements, it has a relatively low concentration of around 4.5 mg/L. As we have seen in the Alkalinity section, Boron in seawater contributes to alkalinity and plays a significant role in the regulation of pH through its speciation between boric acid and borate ion.



Coral species such as *Cladocora caespitosa* and others exhibit a strong pH dependence on boron isotope compositions. This relationship helps corals regulate internal pH levels, critical for calcification processes.

Boron isotope systematics show that corals can up-regulate their internal pH to cope with ocean acidification and lower pH. This process allows corals to maintain an elevated pH in their internal calcifying fluid (pH_{cf}), which is crucial for calcification even in low carbonate ion concentrations.

Experiments with *Acropora* sp. indicate that boron isotopic composition in coral skeletons is influenced by environmental factors such as pCO₂ levels. This suggests that corals use boron to adapt to varying environmental conditions, which is vital for their survival in changing environments.

Boron is involved in stabilising molecules and cell membrane functions, affecting nutrient uptake and overall health. This is crucial for maintaining corals' physiological integrity.

Boron is also involved in bacterial activity playing a role in iron transport in marine life by facilitating Fe³⁺ sequestration by the marine bacterium.

The pH of aquarium water can influence the availability and form of boron. At higher pH levels, boron exists primarily as a borate ion, which is more readily incorporated into coral skeletons and other biological processes. Adjusting pH levels in an aquarium to optimize boron availability is important for maintaining coral health

Nonetheless, while boron is beneficial in the correct amounts, higher concentrations can be harmful. Studies on other aquatic organisms, such as fish, have shown that elevated boron levels can lead to bioaccumulation and impact enzyme activities and lipid peroxidation.

These effects could potentially extend to corals and other reef inhabitants if boron levels are not carefully monitored.

Furthermore, empirical observations have shown that with adequate levels of Boron in the aquarium water SPS will display bright and shiny metallic colours and as such, keeping boron levels around 6 mg/L and between



4 and 10 mg/L in a reef aquarium can help by buffering pH and support coral health by aiding pH regulation within corals and in their metabolic processes. However, it is essential to monitor and maintain appropriate pH levels to ensure boron remains beneficial and does not reach toxic levels.

If the Boron level are lower than the recommended range, at the start of the H2P™ Dosing System use, we recommend using Reef Zlements Boron to bring the level in line with the recommendations.

Bromine

Bromine (Br) in seawater exists primarily in the form of bromide ions (Br^-); however, under certain conditions, bromide can be oxidised to form other bromine species, such as hypobromous acid (HOBr) and bromine gas (Br_2). These species can further react with organic and inorganic compounds, influencing the chemical composition of seawater.

Bromine is essential for the formation of certain biochemical compounds in marine organisms, including corals. It is involved in the synthesis of halogenated organic compounds, which can serve various biological functions, including defence mechanisms and metabolic processes.

Bromide ions play a critical role as cofactors in enzymatic reactions. For example, bromide is required for the enzyme peroxidase to catalyse the formation of sulfilimine crosslinks in collagen IV, which is vital for the structural integrity and development of tissues in marine organisms.

It is also believed to support good LPS/SPS coloration and health, including the fluorescent effect seen in hard corals. Bromine can influence the symbiotic relationship between corals and zooxanthellae (photosynthetic algae). It is thought to assist in the photosynthesis process, which is crucial for the energy supply of the coral.

Low Bromine concentrations will lead to the loss of colour and growth, especially in soft corals, gorgonians, and sponges. Bromine also effects the fluorescent effect seen in hard corals and is significantly important to



blue colouration and also plays a role in the detoxification processes within coral tissues. It aids in neutralising harmful substances that could otherwise accumulate and cause damage to the coral.

The concentration of bromide in seawater is relatively stable and typically around 67 mg/L. This makes bromide one of the more abundant halides in the marine environment, though it is present at much lower concentrations than chloride.

Maintaining an appropriate bromine level is essential for coral reefs' overall health and vitality. Monitoring and adjusting bromine levels in reef aquariums should be part of a broader strategy to ensure a balanced and thriving ecosystem. If the Bromine level is lower than the recommended range, at the start of the H2P™ Dosing System use, we recommend using Reef Zlements Bromine to bring the level in line with the recommendations.

Calcium

Calcium (Ca) is a crucial element in seawater, vital in various biological and chemical processes. It is essential for the formation of skeletal structures in marine organisms, especially corals, molluscs, and certain types of plankton. Calcium also interacts with the carbonate system to regulate the chemistry of seawater.

Calcium levels are critical for the health and growth of hard corals, as they are essential for the process of calcification, where corals build their calcium carbonate skeletons. Variations in calcium concentration can significantly affect coral growth, skeletal density, and overall reef-building capacity.

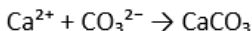
Research has shown significantly decreased growth rates and skeletal density in corals grown in seawater with reduced calcium concentrations. This suggests that calcium is a limiting factor for coral calcification and overall skeletal integrity.

Whilst on the other hand, increased calcium concentration in water significantly enhances calcification and photosynthesis in the coral *Galaxea*



fascicularis. Specifically, an increase in calcium by 2.5 mmol/L resulted in a 30-61% increase in calcium incorporation into the skeleton and an 87% increase in photosynthesis.

Calcium ions (Ca^{2+}) combine with carbonate ions (CO_3^{2-}) to form calcium carbonate (CaCO_3), which precipitates as a solid. This process is fundamental for the growth of coral skeletons and shell formation in many marine organisms.



Nonetheless, the excessive concentration of calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}) in seawater leads to supersaturation which happens when these ions exceed the solubility product (K_{sp}) of calcium carbonate (CaCO_3). This condition favours the precipitation of CaCO_3 , leading to the formation of solid calcium carbonate. As such to avoid precipitation problems is important to understand this concept.

The solubility product for calcium carbonate is expressed as:

$$K_{sp} = [\text{Ca}^{2+}] [\text{CO}_3^{2-}]$$

When K_{sp} reaches a certain level, the solution is supersaturated, and CaCO_3 precipitates.

The saturation state of calcium carbonate in seawater is represented by Ω , defined as the ratio of the product of the concentrations of calcium and carbonate ions to the solubility product of calcium carbonate:

$$\Omega = \frac{[\text{Ca}^{2+}] [\text{CO}_3^{2-}]}{K_{sp}}$$



When:

$\Omega < 1$: Undersaturated (CaCO_3 tends to dissolve).

$\Omega = 1$: Saturated (equilibrium, no net precipitation or dissolution).

$\Omega > 1$: Supersaturated (CaCO_3 tends to precipitate).

However, does $\Omega > 1$ mean constant precipitation?

Well, not necessarily, as the actual precipitation of CaCO_3 is controlled by a complex interplay of biological, chemical, and physical factors. This supersaturation acts as a buffer, ensuring that marine organisms, including corals, can continue to build and maintain their calcium carbonate structures in a stable environment.

In typical ocean conditions, the saturation state (Ω) for aragonite (a form of CaCO_3) is often around 3, and for calcite (another form of CaCO_3), it can be slightly higher i.e. around 5. This supersaturation indicates that seawater has a sufficient concentration of calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}) to support the precipitation of CaCO_3 .

As we can see, higher levels of calcium can be beneficial for corals; however, this is a fine balance between maximising coral health, growth and precipitation. Nonetheless, we will look further into this at the recommended levels section.

In summary, maintaining optimal calcium levels is crucial for the survival and growth of coral in our aquariums and therefore we recommend testing calcium levels weekly either home test kits or ICP testing in order to and keep withing ideal levels. If the Calcium level is lower than the recommended range, at the start of the H2P™ Dosing System use, we recommend using Reef Zlements Calcium to bring the level in line with the recommendations.



Chlorine

Chlorine (Cl) in the form of chloride ion (Cl^-) is the major component in seawater, significantly contributing to its salinity and overall ionic strength. The chloride concentration in ocean water is typically around 19,000 milligrams per litre (mg/L). The concentration can vary slightly depending on the specific location and depth, but 19,000 mg/L is a commonly accepted average value for ocean water.

It interacts with other ions like sodium to stabilise marine chemistry. Elevated chloride levels cause oxidative stress and metabolic disruptions in marine life, including crustaceans, fish and zooplankton. Chloride can bioaccumulate in marine organisms, leading to long-term ecological impacts, particularly in areas with high chloride pollution (e.g. nearshore where road salt discharges to the sea).

Certain marine bacteria exhibit high tolerance to chloride, aiding in biodegrading contaminants and nutrient cycling. Chloride ions interact with other major ions, affecting processes like corrosion and nutrient cycling. Effluents from desalination can increase chloride levels, impacting coral reefs by causing partial bleaching and reducing calcification rates. Chloride affects physiological processes in marine organisms, such as enzyme activity and osmoregulation, and can inhibit carbonic anhydrase activity in marine microalgae, impacting photosynthesis.

Nonetheless, chloride is crucial for osmoregulation, helping marine organisms maintain fluid balance in varying salinity conditions. It plays a role in nutrient cycling, facilitating the transport and availability of other essential nutrients. Chloride helps stabilise the chemical environment of seawater. In corals, chloride ions contribute to ionic balance, aiding in calcification and symbiotic algae function.

High chloride concentrations, however, can be toxic, causing oxidative stress, enzyme inhibition, and disrupted metabolic processes in marine organisms. Elevated chloride levels reduce zooplankton biomass and richness, impacting the food web and overall ecosystem health. Chloride bioaccumulates in marine organisms, leading to long-term ecological



impacts and potential biomagnification through the food web. High chloride levels can cause coral bleaching, affecting symbiotic algae and reducing coral resilience. Chloride can alter microbial community composition and function, affecting nutrient cycling and ecosystem health. Increased chloride concentrations inhibit the growth and photosynthetic activity of marine algae, impacting primary productivity. High chloride levels can also inhibit enzyme activity, affecting critical metabolic processes like glycolysis and protein synthesis.

Chloride is essential for marine ecosystems, influencing salinity, nutrient cycling, and the health of marine organisms. However, it is key to maintain balanced chloride levels dependent on salinity. If the chloride concentration differs from an acceptable range of around 18400 mg/L to 19800 mg/L we recommend to check the salinity and if needed perform water changes with a good quality salt like the D-D Dosing Solution salt to correct the chloride concentration.

Fluorine

Fluorine (F) is a minor element in seawater, playing an important role in marine organisms' physiology and ecology. Fluorine in seawater primarily exists as the fluoride ion (F^-). Its geochemical behaviour is influenced by calcium carbonate precipitation and interactions with other minerals, serving as significant removal mechanisms for dissolved fluoride.

Fluorine compounds, such as potassium fluorosilicate, possess anti-inflammatory properties that may benefit corals. Fluorine might support symbiotic algae (zooxanthellae) within corals, aiding in their metabolic processes and overall health. Fluorine influences nutrient cycling and metabolism in marine ecosystems, potentially supporting coral reef health and productivity. Fluorine has bactericidal properties that can control harmful microbial populations, protecting corals from infections. Additionally, fluorine compounds might modulate stress responses in corals, helping them cope with environmental changes.



Fluoride bioaccumulates in marine organisms' exoskeletons and bones, causing long-term ecological impacts. Marine sponges like *Halichondria moorei* contain significant fluorine levels, providing potent anti-inflammatory properties. Studies show varying fluoride concentrations in marine algae and fish, with differential accumulation and potential ecological impacts.

Fluorine plays a multifaceted role in marine ecosystems, influencing nutrient cycling and the health and protective mechanism of marine organisms, including corals.

Nonetheless, high fluoride concentrations can be toxic to marine organisms, causing oxidative stress and metabolic disruptions. Excess fluoride inhibits the growth and photosynthetic activity of marine algae and corals. Fluoride bioaccumulation in fish can lead to oxidative stress, altered enzyme activity, and disrupted reproductive systems. Low-pH seawater with high fluoride levels can decrease coral calcification and affect the photosynthetic efficiency of symbiotic algae.

Fluoride toxicity varies among aquatic organisms, inhibiting enzyme activity and impacting metabolic processes like glycolysis and protein synthesis.

High fluoride levels induce oxidative stress in marine organisms, including corals, affecting their health and survival. Fluoride ions act as enzymatic poisons, inhibiting enzyme activity and disrupting metabolic processes. Exposure to fluoride causes behavioural changes in fish, indicating neurotoxic effects. Fluoride can alter microbial community composition and function, affecting nutrient cycling and ecosystem health. High fluoride concentrations inhibit marine bivalves' growth and metabolic functions, reducing fitness and increasing susceptibility to stressors.

Maintaining appropriate fluorine levels is therefore, essential for the overall health and vitality of the aquarium inhabitants. In reef aquariums, monitoring and adjusting fluorine levels can be part of a broader strategy to ensure a balanced and thriving ecosystem. If at the start of the H2P™ Dosing System use, fluorine levels are lower than the recommended range, we recommend using Reef Zlements Fluoride to bring the level in line with 1.5 mg/L.



Magnesium

Magnesium (Mg) in seawater plays several important roles in both biological and chemical processes. It is the third most abundant ion in seawater, after sodium (Na^+) and chloride (Cl^-), with a concentration of about 1,350 mg/L.

Magnesium affects the carbonate chemistry of seawater. It can form complexes with carbonate ions, which influences the saturation state of calcium carbonate minerals like calcite and aragonite. This, in turn, affects the processes of calcification and dissolution of carbonate sediments.

Magnesium can inhibit the precipitation of calcium carbonate (CaCO_3) by substituting it into the crystal lattice of calcite, one of the polymorphs of calcium carbonate, making it less stable and more soluble. This process is significant in maintaining the carbonate balance in seawater. When Mg^{2+} replaces Ca^{2+} in the carbonate minerals, it affects the solubility and, consequently, the availability of carbonate ions in the water. This interaction helps to maintain pH stability by preventing excessive precipitation of calcium carbonate, which would otherwise reduce the buffering capacity.

On a biochemical level, Magnesium is a central component of the chlorophyll molecule, which is essential for photosynthesis in marine algae, phytoplankton and corals. Without magnesium, these organisms cannot capture light energy to produce food and oxygen.

Magnesium acts as a cofactor for many enzymatic reactions in marine organisms, including those involved in DNA replication, RNA synthesis, and protein synthesis. It is essential for the proper functioning of enzymes that regulate metabolic processes.

Magnesium also plays a role in the biomineralisation processes of marine organisms. It can influence the formation of calcium carbonate skeletons in corals and shell formation in molluscs. Coralline algae utilise magnesium in their calcitic skeletons with significant variability influenced by temperature and biological control as such due to the growth of these types it is normal to observe changes in the magnesium consumption



rates.

In corals, magnesium can influence the rate of calcium carbonate deposition. While calcium carbonate (CaCO_3) is the primary component of coral skeletons, the presence of magnesium in seawater can inhibit the formation of calcite, one of the polymorphs of CaCO_3 , and promote the formation of aragonite, which is more stable in the marine environment. This affects the overall structure and integrity of corals.

Magnesium is a vital ion in seawater, with multifaceted roles in biological, chemical, geochemical, and ecological processes. It supports the health and functioning of marine ecosystems, influences the carbonate chemistry and mineralization processes, and plays a crucial role in maintaining the stability and productivity of the ocean environment.

Both low and high magnesium concentrations can negatively impact the physiology of marine animals, including algae, fish, anemones and coral. It will impact coral growth, structural integrity, and symbiotic relationships. Maintaining adequate magnesium levels is crucial for the health and stability of corals, and at values of 1000 mg/L or below, growth is greatly reduced or halted, and given its impact on the carbonate chemistry of seawater, it will be difficult to maintain adequate calcium and alkalinity levels. Along with this, coral colouration will be highly impacted, and LPS corals will start to die off.

On the other hand, values above 1600 mg/L can potentially impact on coral growth rates and calcification for reasons already explained above.

Maintaining appropriate magnesium levels is therefore, essential for the overall health and vitality of the animals. In reef aquariums, monitoring and adjusting magnesium levels can be part of a broader strategy to ensure a balanced and thriving ecosystem. If, at the start of the H2P™ Dosing System, the Magnesium level is lower than the recommended range, we recommend using Reef Zlements Magnesium to bring the level to around 1400 mg/L.



Potassium

The potassium (K) concentration in seawater is relatively stable and consistent across the world's oceans. The typical concentration of potassium in seawater is approximately 390 to 400 mg/L.

With the above, potassium plays a crucial role in seawater chemistry and impacts coral health and growth. It is involved in various physiological processes and can influence the overall ecosystem dynamics of coral reefs.

Potassium isotopes in marine biogenic carbonates, including those from corals, show significant variability. This variability is linked to the skeletal potassium phases and indicates biological control over potassium incorporation. This phase control reflects substantial physiological modifications of environmental information recorded in calcifying organisms.

Potassium plays an essential role in the health of corals and their symbiotic relationships with dinoflagellates (*Symbiodinium* spp.). Imbalances can lead to symbiosis dysfunction and coral bleaching. Increased nutrient availability, such as from potassium, can lead to higher symbiont densities, leading to improved coral colouration; however, if substantially above typical seawater levels (i.e. over 500 mg/L), it can suppress the host coral's immune response. This suppression may increase susceptibility to diseases and stress.

Potassium deficiency in seawater can also lead to acute morbidity and mortality in marine invertebrates, fish and corals, impacting their physiological processes such as osmoregulation and membrane potentials. This is critical for maintaining the health of the reef aquarium ecosystem.

Potassium levels influence the activity of crucial enzymes in corals. For instance, changes in potassium concentrations can affect the activity of Na^+/K^+ -ATPase, which is essential for maintaining cellular homeostasis in corals.



Looking at the above, maintaining adequate potassium levels is necessary for optimal growth, nitrogen metabolism, and physiological function.

If, at the start of the use of the H2P™ Dosing System, the potassium levels is lower than the recommended range, we recommend using Reef Elements Potassium to bring the level around 425 mg/L also, please ensure your Potassium levels do not go over 500 mg/L to avoid undesired effects.

Sodium

Sodium (Na) is a major element in seawater, playing a crucial role in the physiological and ecological dynamics of marine organisms. The concentration of sodium in seawater is approximately 10,800 mg/L. This makes sodium the most abundant cation in seawater, contributing significantly to its overall salinity and ionic composition.

Sodium chloride impacts algae and crustaceans by reducing algal growth and affecting crustacean swimming speed and heart rate, indicating toxicity at high concentrations. Sodium chloride also affects carbonic anhydrase activity in marine microalgae, with species specific responses. In cyanobacteria, overexpression of a Na^+/H^+ antiporter gene enhances salt tolerance, enabling growth in high NaCl concentrations. Photosynthetic bacteria like *Rhodobacter sphaeroides* can remove significant amounts of sodium from seawater, showing potential for bioremediation.

Sodium and chloride ions are crucial for osmoregulation in intertidal fish species, maintaining plasma concentration and body water content over varying salinities. In tilapia, dietary sodium chloride aids in saltwater acclimation by influencing plasma osmolality and gill Na^+/K^+ -ATPase activity. Sodium and potassium ions generate osmotic potential in marine fungi, with the hyphal wall selecting potassium over sodium even at high salt concentrations.

Sodium ions are essential for osmoregulation in marine organisms,



including corals, helping them maintain fluid balance and cellular function in varying salinity conditions. Sodium facilitates nutrient cycling and supports various biochemical processes crucial for marine ecosystem health and productivity. Overexpression of sodium-related genes in cyanobacteria enhances their salt tolerance and growth, potentially increasing primary production in marine ecosystems.

High sodium concentrations can be toxic to marine organisms, causing oxidative stress, enzyme inhibition, and disrupted metabolic processes. Elevated sodium levels reduce zooplankton biomass and richness, impacting the food web and overall ecosystem health. Sodium can bioaccumulate in marine organisms, leading to long-term ecological impacts and potential biomagnification through the food web. High sodium levels can cause partial bleaching in corals, affecting their symbiotic algae and reducing coral resilience.

Sodium is a critical ion in marine ecosystems, influencing osmoregulation, nutrient cycling, and the health of marine organisms, including corals. However, high sodium concentrations will impact coral and other marine organisms' survivability and can promote the outspread of cyanobacteria. Maintaining balanced sodium levels between 10,200 and 11,000 mg/L is essential for ecosystem stability and resilience. If the Sodium concentration falls outside this range, we recommend checking the salinity and performing water changes with a good quality salt like the D-D Dosing Solution salt to correct the sodium concentration.

Strontium

Strontium (Sr) levels in ocean waters are significant for understanding marine chemistry, the geological history of the oceans, and the health of marine organisms, including corals.

Strontium levels in ocean waters generally range between 7.2 and 7.8 mg/L, with significant regional variations. These levels are influenced by natural processes such as riverine inputs and hydrothermal activities, which also affect the isotopic composition



of strontium in seawater.

Strontium plays a significant role in coral physiology and skeletal development. Its incorporation into coral skeletons and its interaction with environmental factors such as temperature and light are crucial for understanding coral health and growth patterns.

Strontium uptake in corals is affected by both light and temperature, with higher strontium incorporation occurring under higher light and temperature conditions. This indicates that environmental variables play a significant role in the calcification process and strontium incorporation.

Coral skeletal strontium levels are influenced by physiological factors, not just environmental conditions. For example, different coral genera have varying levels of strontium even under the same environmental conditions, indicating species-specific physiological control over strontium incorporation.

Strontium significantly impacts coral health by influencing their skeletal structure and growth rates. Its incorporation is controlled by both environmental and physiological factors, making it an essential element for understanding coral resilience and environmental adaptations. It has been shown that under elevated strontium levels and high light (up to $400 \mu\text{mol}/\text{m}^2/\text{s}$), incorporation rates of Sr^{2+} into the coral skeleton are significantly higher and that there is a strong correlation between Sr^{2+} uptake and coral growth rates.

In essence, strontium significantly impacts coral health by influencing their skeletal structure and growth rates, and maintaining appropriate strontium levels, somewhat elevated from natural levels, is, therefore, ideal for promoting good and accelerated coral growth.

In reef aquariums, like for other elements, monitoring and adjusting strontium levels should be part of a broader strategy to ensure a balanced and thriving ecosystem. If at the start of the H2P™ Dosing System use, strontium levels are lower than the recommended range, we recommend using Reef Zlements Strontium to bring the level in line with $10 \text{ mg}/\text{L}$ as it will help boosting good growth rates.



Sulphur

Sulphur (S) is a significant element in ocean chemistry, affecting both biological and chemical processes. The concentration and forms of sulphur in the ocean, such as sulphate and organic sulphur compounds, play crucial roles in marine ecosystems and global biogeochemical cycles.

The concentration of sulphur in seawater, when considering all forms of sulphur (including sulphate, sulphide, and other sulphur-containing compounds), is approximately 900 mg/L. Sulphate (SO_4^{2-}) constitutes the majority of this sulphur content, making up about 2,700 mg/L as sulphate ions.

Sulphur is involved in the photosynthesis process, essential for phytoplankton and corals. The availability of sulphur can influence the metabolic and catalytic activities of these organisms, affecting primary production and the overall health of marine ecosystems.

Corals produce high concentrations of dimethylsulphoniopropionate (DMSP) and its breakdown product, dimethylsulphide (DMS), which play crucial roles in coral physiology and stress responses. These sulphur compounds are involved in thermoregulation, osmoregulation, chemoattraction, and antioxidant defence.

Sulphur compounds like DMSP and DMS, however, influence the composition of coral-associated microbial communities. Certain bacteria associated with corals can degrade these compounds, affecting microbial dynamics and potentially playing a role in coral health and resilience.

DMSP and DMS act as scavengers of reactive oxygen species (ROS) in corals, providing an antioxidant defence mechanism that helps corals cope with oxidative stress caused by environmental changes such as increased temperatures and light intensity. As such, sulphur deficiency can weaken the overall health of corals, making them more vulnerable to stressors. The production of DMS by corals during stress events is a significant mechanism for alleviating oxidative stress. Lower sulphur levels can hinder this response,



increasing the risk of coral bleaching and mortality.

Sulphur cycling in marine sediments is primarily driven by sulphate reduction, which is a major energy source for microbes in anoxic environments. This process is tightly interwoven with other element cycles, such as carbon, nitrogen, iron, and manganese. Sulphate-reducing bacteria are essential for the degradation of organic matter and play a significant role in the biogeochemistry of marine sediments.

Sulphate reduction by bacteria in anaerobic conditions leads to hydrogen sulphide (H_2S) formation, which can precipitate as metal sulphides or reoxidise to sulphate. This process is crucial for the sulphur cycle and affects the carbon cycle by outcompeting methanogens for substrates. However, hydrogen sulphide is highly toxic to aquatic organisms like fish and corals even at low concentrations. It can cause acute morbidity and mortality, disrupting physiological processes and leading to severe health issues. As such, it is of the utmost importance that the aquarium substrate is kept clean of organic detritus and sand free from anaerobic areas that can be sources of this toxic gas associated with the “old tank syndrome”.

Compared to natural seawater, both elevated or depleted sulphur levels can severely impact coral health by disrupting sulphur utilisation, impairing antioxidant systems, altering microbial community structures, and reducing the coral’s ability to respond to environmental stressors. It also negatively impacts the microbial biome in aquariums by disrupting biogeochemical cycles, reducing the efficiency of biofilters, and altering microbial community structure.

Maintaining adequate sulphur levels is crucial to ensuring the health and functionality of the microbial communities essential for nutrient cycling and waste removal in the aquarium and for coral resilience and sustainability.

If sulphur levels are lower than the recommended range at the start of the H2P™ Dosing System use, we recommend using Reef Zlements Sulphur to bring the level in line with 900 mg/L. Nonetheless, monitoring and adjusting sulphur levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.



Trace elements

Oceans are giant reservoirs for the elements that are introduced into them, either through the fallout from the atmosphere, influx from rivers or shore or elements that come from the interior of the earth. As such, these reservoirs are "virtually an infinite" supply of these elements.

Trace elements are crucial for the health and sustainability of corals and all animals that live in the oceans. These elements play vital roles in various biological and chemical processes within the aquatic ecosystem, impacting corals and the broader marine life.

However, in our aquariums, we don't have an "infinite" supply of elements as these are limited in quantity and only supplied via contamination or by what the reefer adds to the aquarium. It is, therefore, extremely important that the reefer keeps constant and stable concentrations of those trace elements. However, this is not an easy task mainly due to the extremely limited "residency time" some of these elements have i.e. due to length of time these trace elements remain in the aquarium water before being removed either by precipitation, absorption, oxidation processes or by mechanical filtration export.

Some trace elements, like vanadium, can be removed from the water column in the space of just a few hours, this can be due to both biotic and abiotic processes. As such, it is important to implement a strategy, like the Reef Zlements H2P™ strategy to ensure a stable supply of these traces.

Barium

In the Atlantic Ocean, barium (Ba) concentrations range from 8 to 14 $\mu\text{g/L}$ from surface to deep waters, respectively. In the Pacific Ocean, concentrations range from 8 to 31 $\mu\text{g/L}$, showing a greater increase with depth compared to the Atlantic. Nonetheless, it is agreed that a 10 $\mu\text{g/L}$ level is an acceptable concentration level to maintain in the saltwater aquarium.



Barium is primarily introduced into the ocean through riverine inputs, atmospheric deposition, and submarine groundwater discharge. It tends to accumulate in marine sediments, particularly as barite (BaSO_4), and its distribution is influenced by biological productivity, organic matter decomposition, and upwelling.

Barium in seawater influences coral health and biogeochemical cycles within coral reef ecosystems. It is incorporated into coral skeletons, and its concentration can serve as a proxy for various environmental conditions.

The incorporation of barium into coral skeletons is influenced by light conditions. Under low light, barium incorporation increases, indicating that light availability can affect the Ba/Ca ratios in corals. Coral Ba/Ca ratios are also affected by temperature. Studies show that while temperature influences barium incorporation, calcification rates do not significantly impact Ba/Ca ratios, suggesting that temperature and light are more critical factors.

The residency time of barium in aquaria is influenced by several factors, including its incorporation into coral skeletons, interaction with sediments, and dissolution-precipitation cycles. While specific residency times are not explicitly provided in the studies, the evidence suggests that barium remains active and recycled within the aquaria environment for extended periods, especially in particulate form as barite.

Depleted barium levels may affect coral growth and health by altering the biological processes that control barium incorporation. This can impact the overall resilience of corals to environmental stressors, as barium is involved in nutrient cycling and other vital processes.

Depleted barium levels may enhance these stress responses, leading to further deviations from normal Ba/Ca patterns.

If the barium level is lower than the recommended range at the start of the H2P™ Dosing System use, we recommend using Reef Zlements Barium to bring the level in line with 10 $\mu\text{g/L}$. Nonetheless, monitoring and adjusting barium levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.



Chrome/Chromium

Chromium (Cr) is a trace element in seawater, present in two primary oxidation states: trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)]. Its role in marine environments and its effects on corals are complex, influenced by its chemical behaviour, interactions with marine organisms.

The speciation of chromium affects its bioavailability and toxicity. Cr(VI) is toxic due to its strong oxidising properties, whereas Cr(III) is an essential nutrient in small amounts.

Corals incorporate chromium into their skeletons, where Cr isotopes can reflect the redox state of the surrounding seawater. Trivalent chromium [Cr(III)] is recognised as an essential nutrient for animals, playing a significant role in enhancing metabolic processes. It is involved in lipid metabolism and the maintenance of glucose homeostasis, which are crucial for the health and growth of corals.

Studies on fish have shown that dietary chromium supplementation can enhance immune responses. Although direct studies on corals are limited, it is reasonable to infer that similar mechanisms might improve coral resilience against pathogens and environmental stressors. Enhanced immune function can contribute to better overall health and survival rates in corals.

Trivalent chromium has been shown to promote growth in various aquatic organisms. While specific studies on corals are sparse, it is plausible that appropriate levels of Cr(III) can enhance coral growth by improving metabolic efficiency and nutrient utilisation. This can lead to healthier and more robust coral colonies.

The residency time of Cr(III) in reef aquaria is influenced by a range of factors, including adsorption onto sediments and biological materials, bioremediation processes, and biological uptake by marine organisms. Nonetheless, if dosed correctly in small doses it will deplete from the water rapidly.



In summary, chromium, particularly in its trivalent form, can have several positive effects on corals. It acts as an essential nutrient, enhancing metabolic processes, immune responses, and potentially growth.

If at the start of the H2P™ Dosing System use, the chromium level is lower than the recommended range, it is unnecessary to correct since subsequent supply will be sufficient. Nonetheless, monitoring chromium levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.

Cobalt

Cobalt (Co) is a bio-essential trace element in seawater, playing a crucial role in marine ecosystems. Its influence on corals, along with its biogeochemical cycling, is significant for understanding both environmental impacts and coral health.

Cobalt is scavenged from seawater by manganese-oxidising bacteria and recycled through the remineralisation of organic matter. This process helps maintain the balance of cobalt in the aquarium.

Cobalt is a vital micronutrient for ocean microbes as it is a component of vitamin B12 ($C_{63}H_{88}CoN_{14}O_{14}P$) and various metalloenzymes that catalyse cellular processes. This makes cobalt essential for the growth and metabolism of many marine organisms.

Cobalt is essential for the growth of certain algae and phytoplankton, which uptake it from seawater and recycle it through the marine food web. The biological uptake of cobalt is influenced by the availability of zinc, as certain phytoplankton can substitute cobalt for zinc in their metabolic processes.

Cobalt is essential for the health of symbiotic algae (zooxanthellae) living within coral tissues. These algae perform photosynthesis, providing corals with essential nutrients and energy.

Adequate cobalt levels can enhance the photosynthetic efficiency and overall health of these algae, thereby supporting coral growth.



Cobalt, in the appropriate concentrations, can help mitigate oxidative stress in corals and supports the metabolic processes by contributing to the synthesis of essential vitamins and enzymes. This can promote healthy growth and development, enhancing coral resilience to environmental changes which is particularly important in environments where corals are exposed to various stressors such as increased temperatures and pollution. For example, research on the Pacific white shrimp has shown that appropriate levels of cobalt can enhance antioxidant enzyme activities, which may translate to similar benefits in corals.

Nonetheless, despite its essential nature elevated levels of cobalt can adversely affect coral growth and photosynthetic efficiency. For example, cobalt pollution (i.e. $>5 \mu\text{g/L}$) has been shown to decrease the growth rates of coral species like *Stylophora pistillata* and *Acropora muricata* by 28%, while also affecting their photosystem II efficiency.

In summary, cobalt in seawater plays a crucial role in supporting coral health and growth. It enhances the efficiency of photosynthesis in symbiotic algae, mitigates oxidative stress, supports metabolic processes, and aids in the biogeochemical cycling of nutrients. These benefits underscore the importance of maintaining adequate cobalt levels in marine environments to ensure the health and resilience of coral ecosystems.

Nonetheless, if at the start of the H2P™ Dosing System use, the cobalt level is lower than the recommended range, it is unnecessary to correct since subsequent supply will be sufficient. Nonetheless, monitoring cobalt levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.

Copper

Copper (Cu) is a trace element in seawater that is essential for various biological processes but can also be toxic at elevated concentrations. Its role and effects on marine life, particularly corals, are complex and multifaceted.



Copper is a necessary micronutrient for many marine organisms. It is involved in various enzymatic processes, including those related to respiration and photosynthesis. For instance, copper is a component of cytochrome c oxidase, which is crucial for cellular respiration.

Copper is a vital micronutrient for marine life, including corals. It is a cofactor for essential enzymes such as cytochrome c oxidase and superoxide dismutase, which are crucial for cellular respiration and antioxidant defence, respectively. Proper functioning of these enzymes supports coral health and resilience.

Symbiotic algae (zooxanthellae) within coral tissues require copper for photosynthetic processes. Copper is involved in the photosynthetic electron transport chain, aiding in energy production and contributing to the health and growth of corals through enhanced photosynthesis.

At low concentrations, copper is essential for the proper functioning of antioxidant enzymes. Superoxide dismutase, which requires copper, helps in detoxifying Reactive Oxygen Species (ROS) generated during metabolic processes and environmental stress, protecting corals from oxidative damage.

Copper plays a role in the calcification process of corals by influencing enzymes such as carbonic anhydrase and Ca-ATPase. These enzymes are critical for the formation of calcium carbonate skeletons, essential for coral structure and reef building.

Appropriate levels of copper can enhance the resilience of corals to environmental stressors. For instance, copper can help corals better manage oxidative stress, improving their ability to withstand adverse conditions such as temperature fluctuations and pollution.

Copper has antimicrobial properties that can help protect corals against certain pathogens. This protective role can reduce the incidence of diseases in coral populations, contributing to overall reef health and stability.



Copper influences the symbiotic relationship between corals and their zooxanthellae. Studies have shown that copper can affect the activity of host release factor (HRF) and photosynthesis inhibiting factor (PIF), which regulate algal carbon metabolism and photosynthate release, contributing to a balanced symbiotic relationship.

However, in high concentration copper can be detrimental and exposure to elevated levels of copper induces oxidative stress in corals. This stress can damage cellular structures, including DNA, proteins, and lipids. For example, the coral *Montastraea franksi* showed significant DNA damage and altered gene expression patterns when exposed to copper.

Elevated copper concentrations can also inhibit the settlement and development of coral larvae. Research on *Acropora tenuis* showed that copper concentrations above 42 µg/L significantly reduced larval settlement success.

Copper plays a dual role in marine ecosystems as both a necessary micronutrient and a potential pollutant. While essential for various biological processes at trace levels, elevated copper concentrations can have detrimental effects on corals, including oxidative stress, impaired photosynthesis, reduced reproductive success, and increased susceptibility to bleaching. The interaction of copper with other environmental stressors can further exacerbate these negative impacts. Monitoring copper levels and understanding its effects on corals is crucial for the conservation and management of coral reef ecosystems.

Similarly to other traces with a typical low residency time in the aquarium, if at the start of the H2P™ Dosing System use, the copper level is lower than the recommended range, it is unnecessary to correct since and subsequent supply will be sufficient. Nonetheless, monitoring copper levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.



Iron

Iron (Fe) is a crucial trace element in seawater, playing vital roles in the physiology and ecological dynamics of marine organisms, including algae, corals, and microbes.

Iron is an essential micronutrient for marine life, necessary for processes like photosynthesis, respiration, nitrogen fixation, and DNA synthesis. Its presence in seawater is pivotal for the health and functioning of a wide array of marine species. Iron cycles through various forms in the ocean, influenced by redox reactions, complexation with organic ligands, and interactions with other metals. This cycling affects its availability and bioavailability in marine environments. Iron enters the ocean from various sources, including riverine inputs, atmospheric dust, hydrothermal vents, and coastal runoff. The distribution of iron is influenced by its complexation with organic ligands and particulate matter.

Iron is critical for the growth and photosynthesis of marine phytoplankton. It is involved in the synthesis of chlorophyll and the functioning of photosynthetic electron transport chains. In high-nutrient, low-chlorophyll (HNLC) regions, iron limitation can significantly reduce phytoplankton productivity. The availability of iron can influence the composition of algal communities, as different phytoplankton species have varying iron requirements, thus shaping community dynamics.

Iron is vital for the health of symbiotic algae (zooxanthellae) within corals. These algae require iron for photosynthesis, and iron limitation can reduce photosynthetic efficiency and coral growth. Moreover, iron limitation can exacerbate the effects of thermal stress on corals, potentially contributing to coral bleaching. Iron is essential for the antioxidant defences of corals, and its deficiency can increase susceptibility to oxidative damage. Iron availability also affects the early life stages of corals, including fertilisation success and larval survival.

Marine microbes, including bacteria and archaea, have evolved various mechanisms to acquire iron, such as the production of siderophores—organic molecules that bind and transport iron. These mechanisms are



critical for microbial growth and metabolic functions. Microbes play a significant role in the biogeochemical cycling of iron, influencing its redox state and bioavailability. Iron-oxidising and iron-reducing bacteria are key players in these processes. The production of siderophores by marine bacteria, such as *Alteromonas*, enhances iron acquisition from various sources, including mineral particles and organic complexes. This process supports microbial communities and influences marine iron dynamics.

Nonetheless, iron doesn't only bring benefits as excessive iron can lead to toxicity in algae, affecting growth and metabolic functions. High levels of iron can inhibit photosynthesis and lead to oxidative stress in algae, impacting their overall health and productivity.

Iron can contribute to the formation of harmful algal blooms (HABs), which can produce toxins that are detrimental to life in the aquarium. These blooms can also deplete oxygen in the water, causing dead zones.

Elevated iron levels can exacerbate thermal stress in corals, contributing to coral bleaching. Iron can promote the growth of opportunistic pathogens and harmful algae that outcompete and overgrow corals. High iron concentrations can also negatively impact the photosynthetic efficiency of zooxanthellae within corals. This can reduce the energy available to corals, impairing their growth and resilience.

In summary, iron is a critical element in seawater, essential for the growth and physiological functions of algae, corals, and microbes. Its availability influences primary productivity, community structure, and biogeochemical cycling in marine ecosystems however, in excess can be detrimental. Understanding the role of iron and its interactions with marine organisms is crucial for comprehending the broader dynamics of oceanic health and resilience. Through its fundamental involvement in essential biological processes, iron supports the intricate web of life in marine environments, highlighting the importance of maintaining balanced iron levels for the health of our aquariums.



Similarly to other traces, if at the start of the H2P™ Dosing System use, the iron level is lower than the recommended range, it is unnecessary to correct since subsequent supply will be sufficient. Nonetheless, monitoring iron levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.

Iodine

Iodine (I) is an essential trace element in seawater and likely one of the most important traces, playing significant roles in the physiological and ecological dynamics of marine organisms, including algae, corals, and microbes.

Iodine is crucial for various marine organisms, starting with brown algae, particularly kelps, which are efficient accumulators of iodine.

This element plays a pivotal role in their metabolism and defence mechanisms. In seawater, oxidised forms of iodine, such as iodate, exhibit bactericidal properties, helping control harmful microbial populations.

Although phytoplankton do not significantly alter iodine speciation, iodine is involved in the metabolic processes of some species, supporting their growth and biochemical composition.

Iodine also supports coral health and metabolism, involved in their antioxidant defence mechanisms, protecting them from oxidative stress.

Low iodine levels can also pose significant challenges to marine ecosystems.

Nutrient deficiency due to low iodine levels can impair the growth and metabolic functions of marine organisms, particularly affecting phytoplankton and macroalgae, thus reducing primary productivity. Iodine deficiency can weaken the antioxidant defence mechanisms in corals, making them more susceptible to oxidative stress and bleaching.



Insufficient iodine can negatively affect coral growth and reproduction, leading to weaker skeletal structures and lower reproductive success.

Additionally, low iodine levels can increase the vulnerability of corals and other marine organisms to pathogens, exacerbating disease outbreaks and reef decline.

Iodine deficiency can disrupt the biogeochemical cycles in marine environments, affecting the overall health and balance of the aquarium.

Despite its numerous benefits, high concentrations of iodine can be toxic to marine organisms, including algae and corals, affecting their growth and survival. Excess iodine can disrupt biochemical processes in marine algae, leading to negative impacts on their growth and metabolic functions.

Increased iodine concentrations, can negatively impact coral, leading to bleaching and reduced calcification rates.

Iodine is a critical element, essential for the growth and physiological functions of algae, corals, and microbes. Its availability influences primary productivity, community structure, and biogeochemical cycling in marine ecosystems.

However, the balance of iodine is delicate, with both its deficiency and excess having significant implications for inhabitants of the aquarium. By maintaining appropriate iodine levels, we can support the intricate web of life in our aquariums, ensuring the health and diversity of their inhabitants.

If at the start of the H2P™ Dosing System use, the iodine level is lower than the recommended range, we recommend using Reef Zlements Iodine to bring the level in line with 60 µg/L. Nonetheless, monitoring and adjusting iodine levels should be part of a broader strategy to ensure a balanced and thriving ecosystem. Iodine concentration levels should never be below 40 µg/L with the risk of coral STN and RTN.



Manganese

Manganese (Mn) is an essential trace element in seawater, playing significant roles in the physiological and ecological dynamics of marine organisms, including algae, corals, and microbes.

Manganese is crucial for photosynthesis in marine algae and phytoplankton, playing a key role in the water-splitting reaction during photosynthesis. It also functions as an essential antioxidant, protecting marine organisms from oxidative damage by acting as a cofactor for superoxide dismutase (SOD), an enzyme that mitigates oxidative stress.

In corals, manganese is vital for their health and metabolic processes, supporting growth and skeletal formation. Manganese is involved in nutrient cycling processes carried out by marine microbes, facilitating various redox reactions essential for microbial metabolism.

Additionally, manganese supports the health of zooxanthellae within corals, which are essential for coral energy production through photosynthesis.

Manganese helps in detoxifying harmful metals such as copper by competitive binding, thus protecting marine organisms from metal toxicity. It also induces the proliferation of cells in marine invertebrates, supporting their growth and regeneration processes. Moreover, manganese plays a role in enhancing the immune responses of marine organisms, contributing to their defence mechanisms against pathogens.

Low manganese levels can lead to nutrient deficiencies in marine organisms, impairing their growth and metabolic functions. Phytoplankton and macroalgae are particularly affected.

Manganese deficiency can negatively impact the photosynthetic efficiency of marine algae and coral phytoplankton, leading to decreased energy production.

Insufficient manganese can affect the skeletal formation and structural integrity of corals, leading to weaker and more brittle coral skeletons. It can also weaken the antioxidant defence mechanisms in marine organisms,



making them more susceptible to oxidative stress and environmental damage. Low manganese levels can disrupt the nutrient cycling processes carried out by marine microbes, affecting overall marine biogeochemistry and ecosystem health.

Despite its importance, high concentrations of manganese can be toxic to marine organisms, causing oxidative stress and impairing metabolic functions. Excess manganese can suppress the immune system of marine organisms, reducing their ability to fight off infections and increasing susceptibility to diseases.

Elevated manganese levels can cause neuromuscular disturbances in marine invertebrates, affecting their movement and behaviour. High manganese concentrations can cause tissue damage in corals, leading to sloughing and mortality of coral tissues. Manganese toxicity can disrupt cellular processes in marine organisms, leading to apoptosis, cell cycle arrest, and other detrimental effects.

Excess manganese can impair the bactericidal capacity of marine organisms, reducing their ability to control harmful microbial populations. Understanding the residence time of manganese in seawater, including aquaria, is important for managing its levels. The residence time of manganese is relatively short, ranging from a few hours to a few days depending on environmental conditions and microbial activity. Manganese undergoes rapid cycling between dissolved and particulate forms, influenced by biological and chemical processes.

Manganese is a critical element in seawater, essential for the growth and physiological functions of algae, corals, and microbe as such important that a constant supply to the aquarium is available.

Similarly to other traces, if at the start of the H2P™ Dosing System use, the manganese level is lower than the recommended range, it is unnecessary to correct since subsequent supply will be sufficient. Nonetheless, monitoring manganese levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.



Molybdenum

Molybdenum (Mo) is an essential trace element in seawater, playing significant roles in the physiological and ecological dynamics of marine organisms, including algae, corals, and microbes. Understanding the benefits and negative impacts of molybdenum on these organisms is crucial for comprehending its overall influence on the aquarium ecosystem.

The concentration of molybdenum in seawater is relatively uniform, typically ranging from 9 to 13 $\mu\text{g/L}$. Molybdenum is a central component of nitrogenase, an enzyme crucial for nitrogen fixation in bacteria. This process converts nitrogen into a usable form for marine organisms, enhancing productivity. Additionally, molybdenum is a component of nitrate reductase, an enzyme involved in the reduction of nitrate to nitrite, a key step in the nitrogen cycle.

The availability of molybdenum supports the growth and metabolic functions of phytoplankton, which are crucial for primary production in marine ecosystems. In corals, molybdenum is vital for their health and metabolic processes, aiding in skeletal formation and resilience against environmental stresses. Molybdenum also facilitates various redox reactions essential for microbial metabolism, supporting nutrient cycling processes carried out by marine microbes.

Low molybdenum availability can inhibit nitrogen fixation in bacteria, leading to reduced nitrogen inputs in nitrogen-limited marine ecosystems. Molybdenum deficiency can slow the growth rates of nitrogen-fixing bacteria and phytoplankton, negatively impacting primary productivity. Insufficient molybdenum can disrupt nutrient cycling processes, affecting the overall health and balance of the ecosystem.

Despite its essential roles, high concentrations of molybdenum can be toxic to marine organisms, causing oxidative stress and impairing metabolic functions.

Molybdenum uptake can be inhibited by sulphate, which is abundant



in seawater, potentially limiting its biological availability and impacting processes such as nitrogen fixation.

Changes in the molybdate ratio can affect the redox metabolism and viability of certain microalgae, impacting their growth and survival.

The residence time of molybdenum in seawater, including aquaria, can vary but is generally influenced by its rapid cycling between dissolved and particulate forms.

Environmental conditions and microbial activity also play a significant role in determining its residence time; however, this element doesn't deplete slowly.

In summary, molybdenum is a critical element in seawater, essential for the growth and physiological functions of algae, corals, and microbes. Its availability influences primary productivity, community structure, and biogeochemical cycling in marine ecosystems. However, the balance of molybdenum is delicate, with both its deficiency and excess having significant implications for marine life.

Understanding the role of molybdenum and its interactions with marine organisms is crucial for comprehending the broader dynamics of oceanic health and resilience. By maintaining appropriate molybdenum levels, we can support the intricate web of life in marine environments, ensuring the health and diversity of our oceans.

If at the start of the H2P™ Dosing System use, the Molybdenum level is lower than the recommended range, we recommend using Reef Zlements Molybdenum to bring the level in line with 15 µg/L which albeit being a slightly higher concentration than natural seawater it will contribute to the higher nitrogen reductase more needed in aquaria when in comparison to the ocean.

Nonetheless, monitoring and adjusting iodine levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.



Nickel

Nickel (Ni) is an essential trace element in seawater, playing significant roles in the physiological and ecological dynamics of marine organisms, including algae, corals, and microbes.

Nickel is crucial for various enzymatic processes in marine microbes, aiding in the metabolism of essential nutrients. It supports the growth and metabolic functions of phytoplankton, which are vital for primary production in marine ecosystems. In corals, nickel plays a significant role in their health and metabolic processes, aiding in skeletal formation and providing resilience against environmental stresses.

Low nickel availability can lead to nutrient deficiencies in marine organisms, impairing their growth and metabolic functions. This deficiency particularly affects phytoplankton and macroalgae, reducing primary productivity. Insufficient nickel can also disrupt microbial nutrient cycling processes, impacting the overall health and balance of aquarium ecosystem.

While nickel is essential, high concentrations can be toxic to marine organisms (typically over 5 $\mu\text{g/L}$), causing oxidative stress and impairing metabolic functions. Elevated nickel levels can inhibit photosynthesis in marine algae, reducing primary productivity and affecting the food web. High concentrations of nickel can also cause tissue damage in corals, leading to bleaching and mortality.

The concentration of nickel in seawater varies but is generally found in concentrations $<1 \mu\text{g/L}$, influenced by factors such as water chemistry and biological activity.

Nickel is a critical element in seawater, essential for the growth and physiological functions of algae, corals, and microbes. Its availability influences primary productivity, community structure, and biogeochemical cycling in marine ecosystems. However, the balance of nickel is delicate, with both its deficiency and excess having significant implications for marine life. Understanding the role of nickel and its interactions with marine organisms is crucial for comprehending the broader dynamics of oceanic health and resilience. By maintaining appropriate nickel levels, we can



support the intricate web of life in marine environments, ensuring the health and diversity of our oceans.

If at the start of the H2P™ Dosing System use, Nickel level is lower than the recommended range, we recommend using Reef Zlements Nickel to bring the level in line with 2.5 µg/L which albeit being a slightly higher concentration than natural seawater it will contribute to the metabolic functions of corals. Nonetheless, monitoring and adjusting nickel levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.

Rubidium

Rubidium (Rb) is a trace element found in seawater, playing roles in the physiological and ecological dynamics of marine organisms, including algae, corals, and microbes.

Rubidium is taken up by marine organisms and is involved in their metabolic processes, although its specific functions are not as well-defined as those of other trace elements. The availability of rubidium supports the growth and metabolic functions of phytoplankton, contributing to primary production in the ecosystem. In corals, rubidium is present and may play a role in their metabolic processes and skeletal formation as anecdotally through empirical observation colour changes have been noted.

Low rubidium availability can lead to nutrient deficiencies in marine organisms, impairing their growth and metabolic functions. Phytoplankton and macroalgae are particularly affected, which can reduce primary productivity and impact the entire marine food web.

The concentration of rubidium in seawater is generally around 120 µg/L, with relatively uniform distribution across different oceanic regions and depths. This uniformity helps maintain the stability of marine ecosystems by providing a consistent presence of this trace element.

Rubidium, while not as well-studied as some other trace elements, plays



roles in the growth and physiological functions of algae, corals, and microbes. Its availability can influence primary productivity, community structure, and biogeochemical cycling in marine ecosystems.

Similarly to other traces, if at the start of the H2P™ Dosing System use, the rubidium level is low, it is unnecessary to correct since subsequent supply will be sufficient.

Selenium

Selenium (Se) in seawater plays significant roles in the physiology and ecology of marine organisms like algae, corals, and microbes.

Selenium primarily exists as selenite, selenate, and organic selenide, with organic selenide dominant in surface waters and selenite/selenate in deeper oceanic waters. Coastal waters also have organic forms like seleno amino acids. Methylation by microorganisms creates volatile selenium compounds, crucial for its biogeochemical cycling. Selenium is vital for phytoplankton and zooxanthellae growth. Phytoplankton prefer selenite over selenate, incorporating it into proteins and amino acids.

Selenium enhances antioxidant defences, protecting marine organisms, including corals from oxidative damage. It supports the growth and metabolic functions of kelp, corals and phytoplankton. Selenium in microalgae aids growth and valuable compound production, while its presence in marine biofilms supports microorganism growth.

High selenium concentrations can be toxic, causing oxidative stress and metabolic disruptions in marine organisms.

In summary, selenium is crucial for the growth and metabolic functions of marine organisms, affecting productivity, community structure, and biogeochemical cycling. Maintaining balanced selenium levels is vital for marine ecosystem health and resilience, ensuring the diversity and health of our oceans.

Similarly to other traces, if at the start of the H2P™ Dosing System use, the



selenium level is lower than the recommended range, it unnecessary to correct since subsequent supply will be sufficient – more on this below. Nonetheless, monitoring selenium levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.

Silicon

Silicon (Si) plays a critical role in the physiological and ecological dynamics of marine organisms, especially diatoms and sponges.

Silicon is essential for the growth and productivity of marine diatoms, which significantly contribute to marine primary production and carbon cycling. Diatoms convert dissolved silicon into biogenic silica, influencing oceanic silicon and carbon cycles. The regeneration of biogenic silica is crucial for the supply of dissolved silica to diatoms, with microbial activity playing a fundamental role in this process. Some marine algae accumulate significant amounts of silicon, which may influence competition among phytoplankton species.

Sponge skeletons serve as a significant sink for silicon in the ocean, contributing to the global silicon cycle. Sponges and radiolarians facilitate silicon burial through their siliceous skeletons, with sponges being particularly effective due to their resistance to dissolution.

Silicon enrichment enhances cadmium tolerance in marine diatoms by promoting better silification of cell walls, aiding in managing cadmium toxicity. Silicon also influences the expression of metal transporters, aiding in metal detoxification. The availability of silicon affects metal sensitivity in diatoms, with silicon-starved diatoms showing reduced tolerance to metals like cadmium, copper, and lead.

Silicon supports the growth and structural integrity of corals by contributing to their skeletal formation. It plays a role in nutrient cycling within coral ecosystems, supporting the growth of diatoms and other silicon-dependent organisms crucial for reef productivity. Silicon aids in enhancing the stress resistance of corals by contributing to their



antioxidant defences, helping them cope with oxidative stress and other environmental challenges. Additionally, silicon influences the health of symbiotic algae within corals, supporting their photosynthetic efficiency and overall productivity.

Silicon limitation in seawater impairs the growth and health of diatoms, leading to reduced primary productivity and affecting the entire marine food web. This limitation also makes diatoms more susceptible to pollutants like microplastics and facilitates viral infections, increasing their mortality rates and impacting the silicon and carbon cycles. High concentrations of silicon nanoparticles can be toxic to other marine microalgae, causing growth inhibition and cellular damage. Disrupted silicon availability can alter the metal sensitivity of marine diatoms, making them more vulnerable to metal toxicity.

Silicon is an important element for marine ecosystems, influencing primary productivity, nutrient cycling, and the health of marine organisms. Albeit some silicon being needed in the aquarium, reputable salt brands contain silicon in higher quantities than what is found in the sea, so silicon supplementation is not required, except in particular circumstances which are not within the scope of this manual. Keeping silicon between 100 µg/L and 300 µg/L is recommended for a healthy aquarium ecosystem whilst minimising diatom blooms/outspread.

Vanadium

Vanadium (V) is a significant trace element in seawater, playing an extremely important role in the physiological and ecological dynamics of marine organisms, including algae, corals, and microbes. It is the second most abundant transition metal in seawater, mainly existing as vanadate (VO_4^{3-}). Its levels fluctuate with seasons and redox conditions, affecting its bioavailability. Vanadium undergoes redox cycling between VO_4^{3-} and vanadyl (VO^{2+}), with anoxic conditions promoting reduction to vanadyl.

This element plays a vital role in marine macroalgae, where vanadium-



dependent haloperoxidases facilitate halogenation reactions. Various bacteria use vanadium compounds for nitrogen fixation and bioremediation, contributing to nutrient cycling.

Vanadium enhances antioxidant defences in marine organisms, protecting against oxidative damage including of corals. It supports growth and development, particularly in corals, and plays a role in nutrient cycling, benefiting reef aquariums. Vanadium-dependent enzymes enhance metabolic functions and photosynthetic efficiency in symbiotic algae, aiding coral health. Vanadium compounds are useful in bioremediation, reducing pollutants' toxicity, and enhancing stress responses, tissue repair, immune functions, and symbiotic relationships in corals.

Despite, its benefits in high concentrations, vanadium is however toxic, causing oxidative stress and metabolic disruptions. Exposure to high levels can lead to developmental toxicity in marine embryos, inhibiting essential enzymes, and causing bioaccumulation with long-term ecological impacts. Vanadium can disrupt calcium uptake, affecting biomineralisation and skeletal formation.

Vanadium's residency time in seawater varies with its redox state and conditions. Generally, it has a long residency time, often ranging from several years to decades, due to complex biogeochemical cycling and interactions with particulate matter.

Vanadium is a crucial trace element in the aquarium ecosystem, influencing primary productivity, nutrient cycling, and the health of marine organisms. Its availability and balance are essential for maintaining ecosystem health and resilience.

Similarly to other traces, if the vanadium level is lower than the recommended range at the start of the H2P™ Dosing System use, it is unnecessary to correct since subsequent supply will be sufficient – more on this below. Nonetheless, monitoring vanadium levels should be part of a broader strategy to ensure a balanced and thriving ecosystem.



Zinc

Zinc (Zn) is a trace element that plays crucial roles in the physiological and ecological dynamics of marine organisms, including algae, corals, and microbes.

Zinc in seawater exists primarily as free ions and complexed with organic and inorganic ligands. It is involved in various biogeochemical processes, influencing the chemistry of essential elements like phosphorus and silicon. Zinc speciation includes dissolved and particulate forms, with bioavailability affected by pH, salinity, and organic matter presence. Zinc is essential for phytoplankton growth, acting as a cofactor in enzymes involved in carbon and phosphorus cycles. In oligotrophic waters, zinc exhibits a nutrient-like profile, with concentrations decreasing with depth due to biological uptake and recycling.

Zinc serves as a cofactor for alkaline phosphatase, crucial for phosphorus acquisition in marine organisms including corals. It interacts with other metals like copper and cadmium, influencing their toxicity and bioavailability. Zinc plays a significant role in microbial ecosystems, aiding in the degradation of dissolved organic matter (DOM) and structuring microbial communities. Marine organisms, such as crabs and fish, can bioaccumulate zinc, leading to potential toxic effects.

Zinc enhances antioxidant defences in marine organisms, including corals, protecting them from oxidative damage. It supports coral growth and development by acting as a cofactor for enzymes involved in metabolic processes. Zinc-dependent enzymes support various biochemical reactions necessary for coral health and resilience. Zinc plays a role in nutrient cycling within coral ecosystems, supporting zooxanthellae and can enhance the photosynthetic efficiency of the symbiotic algae, supporting coral energy production and health. Zinc modulates stress responses in corals, aiding in coping with environmental changes and reducing stress impacts. It supports tissue repair and regeneration, enhancing corals' recovery from damage. Zinc enhances immune functions, helping corals resist infections and



diseases. It supports symbiotic relationships between corals and their algae, crucial for coral health and resilience. Zinc-dependent enzymes contribute to corals' resilience and adaptability to changing conditions.

On the other hand, high concentrations of zinc can be toxic to marine organisms, causing oxidative stress and metabolic disruptions. Excessive zinc can inhibit phytoplankton growth and photosynthetic efficiency, impacting primary productivity. Zinc bioaccumulation in marine organisms can lead to toxic effects, including oxidative stress and metabolic imbalances. High levels can induce oxidative stress, affecting health and survival of corals. Zinc can interfere with calcium uptake, disrupting biomineralisation and skeletal formation. It can alter microbial community composition and function, affecting nutrient cycling.

Zinc is, in summary, crucial for the aquarium ecosystems, influencing nutrient cycling, and the health of marine organisms including corals. Its availability and balance are essential for maintaining ecosystem health and resilience.

If at the start of the H2P™ Dosing System use, Zinc levels are lower than the recommended range, we recommend using Reef Zlements Zinc to bring the level in line with 5 µg/L. Nonetheless, monitoring and adjusting nickel zinc should be part of a broader strategy to ensure a balanced and thriving ecosystem.

Nutrients

Nutrients play a critical role in the health and stability of reef aquaria. They influence the growth of corals, algae, and the overall microbial ecosystem. Proper management of nutrients such as dissolved organic carbon (DOC), nitrogen, phosphorus, and trace elements are essential for maintaining a balanced environment that supports the diverse life forms within a reef tank.

Understanding nutrients, their importance and impact is key for allowing the proper nutrient management that supports coral growth, controls algae proliferation, and maintains a healthy microbial balance.



Dissolved Organic Carbon (DOC)

Dissolved Organic Carbon (DOC) is a critical component of the marine nutrient cycle and plays a significant role in reef ecosystems. It consists of organic molecules dissolved in water, originating from a variety of sources, including the decay of plant and animal matter, excretions from marine organisms, and the leaching of organic compounds from terrestrial sources. In the case of aquaria, it originates mainly from food, nutritional and carbon dosing supplements with a smaller amount originating from environmental contaminants that make household dust (i.e. dead skin cells, fibres from clothing and furniture, hair, pollen, and particulate matter from outdoor air).

DOC benefits reef aquariums by serving as a vital energy and carbon source for heterotrophic bacteria and other microorganisms, thus supporting the microbial loop that converts DOC into biomass consumed by higher trophic levels. It enhances coral health by influencing the metabolism of corals and their symbiotic algae, helping corals meet their metabolic needs during low photosynthetic activity. Additionally, DOC contains organic compounds that act as chemical signals, affecting the behaviour and physiology of marine organisms.

However, excessive DOC can have profoundly negative impacts. It can lead to nutrient enrichment or eutrophication, promoting the overgrowth of algae and cyanobacteria that outcompete corals for light and space. High DOC levels can stimulate microbial activity, increasing oxygen consumption and potentially leading to hypoxic conditions detrimental to aerobic organisms. Elevated DOC can also enhance the growth of pathogenic bacteria, raising the likelihood of disease outbreaks in corals. Furthermore, changes in DOC concentrations can alter microbial community compositions, affecting nutrient cycling and overall reef health.

In conclusion, DOC is essential for reef ecosystems, providing nutrients and supporting coral health. Yet, excessive DOC can cause eutrophication, oxygen depletion, and increased disease susceptibility. Therefore, using a moderate carbon dosing regimen and good husbandry is key to maintaining



balanced DOC levels which are crucial for reef aquarium ecosystem health and stability.

Nitrate

While elevated nitrate levels pose significant challenges to coral health, nitrates are also essential nutrients for corals and other marine organisms. In balanced concentrations, nitrates contribute to the overall health and growth of corals by providing a critical source of nitrogen necessary for the synthesis of amino acids and proteins in both corals and their symbiotic algae, zooxanthellae.

Moderate nitrate levels support the metabolic needs of zooxanthellae, promoting photosynthesis and the production of organic compounds that provide energy to corals. Adequate nitrate concentrations help maintain the symbiotic relationship between corals and zooxanthellae, enhancing coral growth and resilience. Furthermore, nitrates can stimulate the growth of beneficial microbial communities within the coral holobiont, contributing to nutrient cycling and overall reef health.

Conversely, environments with excessively low nitrate levels can impair coral health. Nitrate depletion can limit the growth and photosynthetic efficiency of zooxanthellae, reducing the energy available to corals. This can lead to stunted growth, reduced reproduction rates, and increased susceptibility to environmental stressors. In a nitrate-depleted environment, corals may struggle to compete with other organisms for limited nutrients, weakening their overall resilience.

Despite the essential role of nitrates, elevated levels can have detrimental effects on corals. High nitrate concentrations can disrupt the delicate balance necessary for coral health.

Elevated nitrate levels stimulate the growth and density of zooxanthellae. While this can enhance photosynthesis, it leads to an imbalance where zooxanthellae consume more available CO_2 for photosynthesis. This reduces the CO_2 available for coral calcification, leading to decreased skeletal growth and weaker coral structures. High nitrate levels directly interfere with coral calcification. The increased uptake of CO_2 by



zooxanthellae for photosynthesis leaves less CO₂ for corals to use in forming their calcium carbonate skeletons. Over time, this can result in thinner, more fragile skeletons, making corals more susceptible to physical damage and reducing their overall growth rates.

When combined with elevated temperatures, high nitrate levels can exacerbate coral bleaching. Bleaching occurs when the symbiotic relationship between corals and zooxanthellae breaks down, usually due to stress factors like increased temperatures, coral transport, increased light exposure, etc. Elevated nitrate levels contribute to this stress by increasing oxidative stress within coral tissues, reducing their aerobic scope and making them more vulnerable to bleaching. High nitrate levels impact coral physiology by disrupting nutrient cycling within the coral holobiont. This imbalance can interfere with metabolic processes, reduce energy allocation for growth and reproduction, and compromise the coral's ability to regulate its internal environment. These physiological disruptions can weaken corals and reduce their resilience to environmental stressors.

To mitigate the adverse effects of elevated nitrate levels, effective nutrient management strategies are essential. Managing feeding practices to ensure food is consumed quickly and does not decompose in the water helps control nitrate levels. Overfeeding can contribute to excess nutrient levels in the aquarium.

In summary, maintaining optimal nitrate levels is critical for coral health and the overall stability of the reef aquarium and while nitrates are essential nutrients that support coral growth and metabolic functions, elevated levels increase zooxanthellae density, reduce skeletal growth, exacerbate bleaching, and alter coral physiological responses. By implementing effective nutrient management strategies, that keeps nitrate levels within our recommend levels, aquarists can mitigate these adverse effects, promoting healthier and more resilient corals.



Phosphate & Orthophosphate

Phosphate (PO_4) is an ion that consists of one phosphorus atom surrounded by four oxygen atoms in a tetrahedral arrangement.

Phosphate is a crucial nutrient in reef aquariums, predominantly in the orthophosphate form (PO_4^{3-}) or more correctly as soluble reactive phosphorus (SRP), playing a significant role in the health and growth of corals. It is essential for various biological processes and both excessive and insufficient levels can adversely affect coral growth, skeletal density, and overall reef health.

Phosphates are necessary for numerous cellular functions, including the synthesis of nucleic acids and ATP, which are vital for energy transfer and storage in all living organisms. In balanced concentrations, phosphates support the metabolic needs of corals and their symbiotic algae, zooxanthellae, promoting photosynthesis and energy production. However, phosphate deficiency can be as detrimental as excess levels. In corals, phosphate deficiency can lead to bleaching and reduced growth rates. Corals exposed to imbalanced nitrogen-to-phosphorus ratios exhibit severe symbiotic malfunction, loss of biomass, and bleaching, indicating that phosphate is crucial for maintaining healthy symbiotic relationships with zooxanthellae and preventing bleaching.

High phosphate levels inhibit coral skeleton formation by interfering with the process of aragonite CaCO_3 formation, which is crucial for coral skeleton development. Research on juvenile *Acropora digitifera* has demonstrated that elevated phosphate levels inhibit both in vitro and in vivo aragonite formation, leading to weaker and less dense skeletons. High phosphate levels further stress corals when combined with elevated temperatures and low pH.

Studies indicate that such combined stressors reduce phosphate uptake and photosynthesis, exacerbating the negative impacts on coral health. The combined effect of thermal stress and high phosphate levels can lead to increased oxidative stress and reduced calcification rates. Elevated nutrient



levels, including phosphates, can lead to eutrophication, promoting algal blooms that compete with corals for light and space. This competition can reduce coral calcification rates and overall health. Algal overgrowth not only shades corals but also produces allelopathic compounds that inhibit coral growth and survival.

Phosphate stress affects the photosynthesis of symbiotic algae in corals. In *Acropora* species, elevated phosphate levels have been shown to reduce the photosynthetic efficiency and density of zooxanthellae, impacting the overall health and resilience of the coral.

To mitigate the adverse effects of elevated phosphate levels, effective nutrient management strategies are essential. Maintaining optimal phosphate levels is critical for coral health and the overall stability of reef aquarium ecosystems.

In summary, while phosphates are essential nutrients that support coral growth and metabolic functions, elevated levels can inhibit coral skeleton formation, exacerbate stress factors, and promote algal blooms.

Conversely, phosphate deficiency can lead to bleaching and reduced coral growth. By implementing effective nutrient management strategies, aquarists and conservationists can mitigate these adverse effects, promoting healthier and more resilient coral reefs.

Nonetheless, before concluding it is important to understand that, there are several types of phosphate, including orthophosphate, pyrophosphate, polyphosphate and organically-bound phosphate and with that said when using a ICP test to obtain a phosphate measurement we need to note that given that an ICP instrument measures phosphorous which is then mathematically converted into phosphate and not phosphate directly, this represents total phosphate instead of orthophosphate which is the form of phosphate that home test kits are able to measure.

Nonetheless, using a different analytical technique like colourimetry, using a UV-vis spectrophotometer will allow a professional laboratory like the Reef Zlements ICP lab to provide you an accurate measurement of orthophosphate.



Amino Acids

Amino acids are fundamental organic compounds crucial for the health and growth of corals. These building blocks of proteins are essential for various physiological processes within coral tissues and their symbiotic relationships with zooxanthellae.

Amino acids are the basic units of proteins, which are vital for the structure and function of coral tissues. They are involved in skeletal formation, tissue repair, and enzymatic activities, supporting coral growth and development. Amino acids contribute to the growth of coral polyps and overall tissue expansion, playing a role in the production of the organic matrix necessary for calcium carbonate deposition, crucial for skeletal growth.

Amino acids can also be metabolised to provide energy for various cellular processes, particularly when photosynthetically derived energy from zooxanthellae is insufficient. They help corals cope with environmental stressors such as temperature fluctuations, UV radiation, and pollution by synthesising stress proteins and antioxidants, aiding in tissue repair and enhancing resilience to physical injuries and disease.

Amino acids play a critical role in the nutrient exchange between corals and their symbiotic zooxanthellae. Corals provide zooxanthellae with carbon dioxide and nutrients, while zooxanthellae supply corals with glucose, glycerol, and amino acids through photosynthesis.

Corals obtain amino acids from various sources. Heterotrophic feeding on plankton and organic matter provides a rich source of amino acids, especially in nutrient-poor waters. Corals can also absorb dissolved free amino acids directly from the surrounding seawater, facilitated by specific transport mechanisms in their epithelial cells. Symbiotic zooxanthellae produce non-essential amino acids as byproducts of photosynthesis, which are transferred to the coral.

Providing a readily available source of essential amino acids that may be limited in captive environments like an aquarium is proven to be beneficial. However, excessive supplementation of amino acids can lead to nutrient imbalances, promoting undesirable algal growth and negatively impacting



water quality. High levels of dissolved organic compounds, including amino acids, can increase the organic load in the water, necessitating efficient filtration and regular water changes to maintain optimal conditions.

In conclusion, amino acids are indispensable for coral health and growth, serving as the building blocks of proteins, supporting metabolic processes, enhancing stress responses, and facilitating symbiotic relationships. Understanding their sources and roles can help reef aquarists and marine biologists maintain and support thriving coral ecosystems.

Vitamins

Vitamins are essential organic compounds that play vital roles in various physiological processes within corals, such as growth, reproduction, immune function, and overall health. Acting as coenzymes or precursors for crucial metabolic reactions, vitamins are as important to corals as they are to other organisms.

Vitamins significantly contribute to the growth and development of corals. Vitamin C ($C_6H_8O_6$), also known as ascorbic acid, is critical for collagen synthesis, essential for the structural integrity of coral tissues and skeletons, and aids in tissue healing and repair. Vitamin D (D2 known as ergocalciferol $C_{28}H_{44}O$ and D3 also known as cholecalciferol $C_{27}H_{44}O$), though less studied in corals, is believed to be involved in calcium metabolism and skeletal formation, mirroring its role in vertebrates.

In terms of immune function, vitamins are indispensable. Vitamin C acts as an antioxidant, protecting coral tissues from oxidative stress induced by environmental factors such as UV radiation, pollution, and pathogens. Similarly, Vitamin E helps protect cell membranes from oxidative damage, thereby supporting the coral's immune system.

Vitamins also play crucial roles in photosynthesis and symbiosis. Vitamin B12 i.e. cobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) is important for the metabolism of symbiotic zooxanthellae, which perform photosynthesis and provide nutrients to the coral host, supporting the health and growth of these symbionts.



For reproduction, Vitamin A known as retinol ($C_{20}H_{30}O$) is crucial for gamete development and larval growth, and it plays a role in cellular differentiation and proliferation. Folic acid (Vitamin B9 - $C_{19}H_{19}N_7O_6$) is essential for DNA synthesis and cell division, critical during the development and settlement of coral larvae.

Vitamins also aid in stress response and recovery. Vitamins C and E (i.e. α -tocopherol $C_{29}H_{50}O_2$) help corals recover from physical damage and environmental stressors by enhancing antioxidant defences and promoting tissue repair.

Corals obtain vitamins from various sources, naturally, they capture planktonic organisms rich in vitamins and other essential nutrients. They can also absorb dissolved vitamins directly from seawater, which comes from the excretion of marine organisms, decomposition of organic matter, and terrestrial runoff. Symbiotic zooxanthellae within coral tissues can synthesise certain vitamins and provide them to their coral hosts, maintaining a mutualistic relationship essential for their health. Providing a readily available source of vitamins that may be limited in captive environments like an aquarium has proven to be beneficial.

However, over-supplementation of vitamins can lead to nutrient imbalances, promoting undesirable algae growth and affecting water quality. The effectiveness of vitamin supplementation also depends on the formulation and delivery method, as not all vitamins are equally bioavailable in different forms. Like with the other nutrients, high levels of vitamins can increase the organic load in the water, necessitating efficient filtration and maintaining optimal conditions.

In conclusion, vitamins are indispensable for the health, growth, and reproduction of corals. They play critical roles in protein synthesis, immune function, photosynthesis, and stress response. Understanding the functions of vitamins can help reef aquarists and marine biologists support thriving coral ecosystems through balanced nutrition and effective supplementation.



CONCLUSION

The new Reef Zlements H2P™ dosing system signifies a remarkable advancement in aquarium care, providing unparalleled precision and automation.

By combining chemicals that have been formulated based on scientifically proven knowledge and research with robust professional testing and innovative technology, it simplifies the complex task of maintaining optimal water chemistry which is essential to achieving an amazing reef aquarium. The seamless integration of the D-D KH Manager, the P4 Pro dosing pump, and the sophisticated H2P™ system ensures a stable, healthy, and thriving aquarium environment.

Whether you are an experienced reefer or new to the hobby, this innovative system empowers you to achieve and maintain ideal alkalinity, pH and elemental levels effortlessly.

We trust this manual along our professional testing laboratory provides you with the necessary guidance to effectively utilise the H2P™ dosing system in its full capabilities to keep a successful and longstanding reef aquarium. By following the outlined procedures and recommendations, you can ensure the longevity and success of your reef aquarium. Embrace this innovative solution and enjoy the benefits of a meticulously balanced and vibrant aquarium.

On behalf of the whole RZ team, I thank you for choosing and trusting Reef Zlements.

Jose Duarte

Reef Zlements CEO



REFERENCES:

Millero, Frank J.; Editor. Chemical Oceanography, Second Edition. (1996)

Li, Y., Zheng, X., Yang, X., Ou, D., Lin, R., & Liu, X. (2017). Effects of live rock on removal of dissolved inorganic nitrogen in coral aquaria. *Acta Oceanologica Sinica*, 36, 87-94.

Jones, A., & Preston, N. (1999). Sydney rock oyster, *Saccostrea commercialis* (Iredale & Roughley), filtration of shrimp farm effluent: the effects on water quality. *Aquaculture Research*, 30, 51-57.

Hirayama, K. (2007). Studies on water control by filtration through sand bed in a marine aquarium with closed circulating system-VI. Acidification of aquarium water. *Nippon Suisan Gakkaishi*, 36, 26-36.

Morais, A., Abreu, P., Wasielesky, W., & Krummenauer, D. (2020). Effect of aeration intensity on the biofilm nitrification process during the production of the white shrimp *Litopenaeus vannamei* (Boone, 1931) in Biofloc and clear water systems. *Aquaculture*, 514, 734516.

Atwood, H. L., Bruce, J., Sixt, L., Kegl, R., Stokes, A., & Browdy, C. (2005). Intensive zero-exchange shrimp production systems - Incorporation of filtration technologies to improve survival and growth. *International Journal of Recirculating Aquaculture*, 6, 49-64.

Rahman, M., Kadowaki, S., Linn, S., & Yamada, Y. (2012). Effects of protein skimming on water quality, bacterial abundance and abalone growth in land based recirculating aquaculture systems. *Journal of Fisheries and Aquatic Science*, 7, 150-161.

Hoang, D., Tram, D., Hue, N., & Dang, D. (2018). EFFECTS OF SAND AND LIVE ROCK BOTTOM ON WATER QUALITY IN AQUARIUM TANK.

Yuen, Y., Yamazaki, S., Nakamura, T., Tokuda, G., & Yamasaki, H. (2009). Effects of live rock on the reef-building coral *Acropora digitifera* cultured with high levels of nitrogenous compounds. *Aquacultural Engineering*, 41, 35- 43.

Mawi, S., Krishnan, S., Din, M., Arumugam, N., & Chelliapan, S. (2020). Bioremediation potential of macroalgae *Gracilaria edulis* and *Gracilaria changii* co-cultured with shrimp wastewater in an outdoor water recirculation system. *Environmental Technology and Innovation*, 17, 100571.

Lee, W., & Wang, W. (2001). Metal accumulation in the green macroalga *Ulva fasciata*: effects of nitrate, ammonium and phosphate. *The Science of the total environment*, 278 1-3, 11-22

Edworthy, C., Steyn, P., & James, N. (2023). The role of macroalgal habitats as ocean acidification refugia within coastal seascapes. *Cambridge Prisms: Coastal Futures*.

Black, K. D., & Shimmield, G. B. (1996). The environmental impact of marine fish cage culture. *Limnology and Oceanography*, 41(7), 1335-1341. <https://doi.org/10.4319/lo.1996.41.7.1335>



- Caldwell, R. S. (1973). Effect of Ozone on Marine Organisms. *Marine Biology*, 19(3), 204-210. <https://doi.org/10.1007/BF00367985>
- Fisher, N. S. (1999). Effects of Ozone on Marine Life. *Marine Ecology Progress Series*, 17(3), 231-239. <https://doi.org/10.1007/s002270050662>
- Grguric, G., McClure, P. R., & Szabo, J. G. (2000). Effects of ozone and ammonia on nitrifying bacteria in seawater. *Marine Environmental Research*, 50(1-5), 303-317. [https://doi.org/10.1016/S0141-1136\(00\)00051-3](https://doi.org/10.1016/S0141-1136(00)00051-3)
- Langlais, B., Reckhow, D. A., & Brink, D. R. (1991). Ozone in water treatment: Application and engineering. *Environmental Science & Technology*, 25(3), 487-490. <https://doi.org/10.1002/9783527618952>
- Lawrence, A. J., & Soame, J. M. (1980). The effects of ozone on marine invertebrates. *Marine Biology*, 47(2), 157-166. <https://doi.org/10.1007/BF02278013>
- Lesser, M. P. (2006). Oxidative stress in marine environments: Biochemistry and physiological ecology. *Marine Biology*, 149(3), 191-205. <https://doi.org/10.1007/s00338-006-0125-7>
- Moe, M. A. (1992). *The Marine Aquarium Reference: Systems and Invertebrates*. Marine Science Publications.
- Rice, R. G. (1986). Applications of ozone for industrial wastewater treatment—A review. *Ozone: Science & Engineering*, 8(3), 243-258. <https://doi.org/10.1080/01919518608552262>
- Shick, J. M., Lesser, M. P., & Jokiel, P. L. (1996). Effects of ultraviolet radiation on corals and other coral reef organisms. *Global Change Biology*, 2(6), 527-545. <https://doi.org/10.1007/s003380050034>
- Staehr, P. A., Henriksen, P., & Markager, S. (2004). Oxygen dynamics in marine and freshwater environments: a comparison of processes and budgets. *Limnology and Oceanography*, 49(2), 409-420. <https://doi.org/10.4319/lo.2004.49.2.0409>
- Thompson, F. L., & Abreu, P. C. (1991). Water quality in marine aquaculture: Effects of ozone treatment on phytoplankton and bacteria. *Marine Pollution Bulletin*, 22(10), 500-505. <https://doi.org/10.1007/BF02278342>
- Wedemeyer, G. A., & Yasutake, W. T. (1979). Prevention and treatment of nitrite toxicity in juvenile steelhead trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, 36(6), 854-858. <https://doi.org/10.1007/BF00002038>
- Daniels, H. V., & Boyd, C. E. (2007). Biosecurity principles for aquaculture. *Aquaculture Engineering*, 36(2), 159-176. <https://doi.org/10.1016/j.aquaeng.2006.12.004>
- Gatesoupe, F. J. (1999). The use of probiotics in aquaculture. *Aquaculture*, 180(1-2), 147-165. [https://doi.org/10.1016/S0044-8486\(99\)00187-8](https://doi.org/10.1016/S0044-8486(99)00187-8)



Govenar, B., Freeman, M. C., & Berg, H. C. (2004). Ultraviolet radiation as a management tool for aquatic invasive species. *Aquaculture*, 231(1-4), 137-144.

Hoffman, G. L., & Meyer, F. P. (2004). Parasites of freshwater fishes. *Trans. Am. Fish. Soc.*, 90(1), 7-32.

Noga, E. J. (2010). *Fish disease: Diagnosis and treatment*. John Wiley & Sons.

Pelz, O., Chatzinotas, A., & Andersen, N. (2000). Microbial community composition and function in sewage treatment plants. *Water Research*, 34(3), 725-729.

Schumacher, J. (2003). Evaluation of the ultraviolet sensitivity of *Cryptocaryon irritans* theronts using a novel *in vivo* test apparatus. *Journal of Fish Diseases*, 26(7), 381-386.

Timmons, M. B., Ebeling, J. M., & Wheaton, F. W. (2002). *Recirculating aquaculture systems*. Cayuga Aqua Ventures.

Wedemeyer, G. A. (1996). *Physiology of fish in intensive culture systems*. Springer Science & Business Media. Lee, S., Davy, S., Tang, S., & Kench, P. (2017). Water flow buffers shifts in bacterial community structure in heat-stressed *Acropora muricata*. *Scientific Reports*, 7. <https://doi.org/10.1038/srep43600>.

Nakamura, T., Woesik, R., & Yamasaki, H. (2005). Photoinhibition of photosynthesis is reduced by water flow in the reef-building coral *Acropora digitifera*. *Marine Ecology Progress Series*, 301, 109-118.

Sebens, K., & Johnson, A. (1991). Effects of water movement on prey capture and distribution of reef corals. *Hydrobiologia*, 226, 91-101.

Carpenter, L., & Patterson, M. (2007). Water flow influences the distribution of photosynthetic efficiency within colonies of the scleractinian coral *Montastrea annularis* (Ellis and Solander, 1786); implications for coral bleaching. *Journal of Experimental Marine Biology and Ecology*, 351, 10-26.

Fifer, J., Bentlage, B., Lemer, S., Fujimura, A., Sweet, M., & Raymundo, L. (2021). Going with the flow: How corals in high-flow environments can beat the heat. *Molecular Ecology*, 30.

Komorowska-Kaufman, M., Pruss, A., Rzepa, G., & Bajda, T. (2019). Removal of Heavy Metals and Metalloids from Water Using Drinking Water Treatment Residuals as Adsorbents: A Review. *Minerals*, 9(8), 487. <https://doi.org/10.3390/min9080487>

USGS. (2009). Occurrence and distribution of iron, manganese, and selected trace elements in ground water in the glacial aquifer system of the northern United States. *Scientific Investigations Report 2009-5006*.

Health Canada. (2016). *Guidelines for Canadian drinking water quality: guideline technical document-manganese*. Ottawa, ON, Canada.

Hering, J. G., Katsoyiannis, I. A., Theoduloz, G. A., & Berg, M. (2017). Arsenic removal from drinking water:



experiences with technologies and constraints in practice. *Journal of Environmental Engineering*, 143(5), 03117002. [https://doi.org/10.1061/\(ASCE\)EE.1943-7870.0001225](https://doi.org/10.1061/(ASCE)EE.1943-7870.0001225)

Iluz, D., & Dubinsky, Z., 2015. Coral photobiology: new light on old views. *Zoology*, 118 2, pp. 71-8

Brown, B., & Dunne, R. (2008). Solar radiation modulates bleaching and damage protection in a shallow water coral. *Marine Ecology Progress Series*, 362, 99-107

Slagel, S., Lohr, K., O'Neil, K., & Patterson, J. (2021). Growth, calcification, and photobiology of the threatened coral *Acropora cervicornis* in natural versus artificial light. *Zoo biology*, 40 3, 201-207 . <https://doi.org/10.1002/ZOO.21589>.

Kinzie, R., & Hunter, T. (1987). Effect of light quality on photosynthesis of the reef coral *Montipora verrucosa*. *Marine Biology*, 94, 95-109. <https://doi.org/10.1007/BF00392902>.

Mendes, C., Fernandes, C., Moreira, A., Chambel, J., Maranhão, P., & Leandro, S. (2017). Effect of LEDs Light Spectrum on Success of Fragmentation and Growth of Leather Coral *Sarcophyton* spp. *International Journal of Aquaculture*, 7

Lesser, M. (1996). Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. *Limnology and Oceanography*, 41(2), 271-283.

Wijgerde, T., van Melis, A., Silva, C. I. F., Leal, M., Vogels, L., Mutter, C., & Osinga, R. (2014). Red light represses the photophysiology of the scleractinian coral *Stylophora pistillata*. *PLoS ONE*, 9.

Wijgerde, T., Henkemans, P., & Osinga, R. (2012). Effects of irradiance and light spectrum on growth of the scleractinian coral *Galaxea fascicularis*—Applicability of LEP and LED lighting to coral aquaculture. *Aquaculture*, 344, 188-193.

Sorek, M., & Levy, O. (2014). Coral Spawning Behavior and Timing., 81-97.

Bik, H., Alexiev, A., Aulakh, S., Bharadwaj, L., Flanagan, J., Haggerty, J., Hird, S., Jospin, G., Lang, J., Sauder, L., Neufeld, J., Shaver, A., Sethi, A., Eisen, J., & Coil, D. (2019). Microbial Community Succession and Nutrient Cycling Responses following Perturbations of Experimental Saltwater Aquaria. *mSphere*, 4. <https://doi.org/10.1128/mSphere.00043-19>.

Oliver, T., & Palumbi, S. (2011). Do fluctuating temperature environments elevate coral thermal tolerance?. *Coral Reefs*, 30, 429-440. <https://doi.org/10.1007/s00338-011-0721-y>.

Korchef, A., & Touaibi, M. (2019). Effect of pH and temperature on calcium carbonate precipitation by CO2 removal from iron-rich water. *Water and Environment Journal*, 34. <https://doi.org/10.1111/wej.12467>.

Barton, J., Hutson, K., Bourne, D., Humphrey, C., Dybala, C., & Rawlinson, K. (2019). The Life Cycle of the *Acropora* Coral-Eating Flatworm (AEFW), *Prosthodontium acroporae*; The Influence of Temperature and



Management Guidelines. *Frontiers in Marine Science*. <https://doi.org/10.3389/fmars.2019.00524>.

Lüring, M., Eshetu, F., Faassen, E., Kosten, S., & Huszar, V. (2013). Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology*, 58, 552-559. <https://doi.org/10.1111/J.1365-2427.2012.02866.X>.

Riley, J. P., & Skirrow, G. (1975). *Chemical Oceanography*. Academic Press.

Downs, C., Kramarsky-Winter, E., Woodley, C., Downs, A., Winters, G., Loya, Y., & Ostrander, G. (2009). Cellular pathology and histopathology of hypo-salinity exposure on the coral *Stylophora pistillata*. *The Science of the total environment*, 407 17, 4838-51 . <https://doi.org/10.1016/j.scitotenv.2009.05.015>.

Petersen, K., Paytan, A., Rahav, E., Levy, O., Silverman, J., Barzel, O., Potts, D., & Bar-Zeev, E. (2018). Impact of brine and antiscalants on reef-building corals in the Gulf of Aqaba - Potential effects from desalination plants.. *Water research*, 144, 183-191 . <https://doi.org/10.1016/j.watres.2018.07.009>.

Marshall, A., & Clode, P. (2002). Effect of increased calcium concentration in sea water on calcification and photosynthesis in the scleractinian coral *Galaxea fascicularis*.. *The Journal of experimental biology*, 205 Pt 14, 2107-13 .

Iijima, M., Yasumoto, K., Yasumoto, J., Yasumoto-Hirose, M., Kuniya, N., Takeuchi, R., Nozaki, M., Nanba, N., Nakamura, T., Jimbo, M., & Watabe, S. (2019). Phosphate Enrichment Hampers Development of Juvenile *Acropora digitifera* Coral by Inhibiting Skeleton Formation. *Marine Biotechnology*, 21, 291 - 300. <https://doi.org/10.1007/s10126-019-09880-3>.

Godinot, C., Houlbrèque, F., Grover, R., & Ferrier-Pagès, C. (2011). Coral Uptake of Inorganic Phosphorus and Nitrogen Negatively Affected by Simultaneous Changes in Temperature and pH. *PLoS ONE*, 6. <https://doi.org/10.1371/journal.pone.0025024>.

Ye, T. (2008). Effects of phosphate stress on the photosynthesis of symbiotic algae on the hermatypic corals. *Acta Ecologica Sinica*.

Rosset, S., Wiedenmann, J., Reed, A., & D'Angelo, C. (2017). Phosphate deficiency promotes coral bleaching and is reflected by the ultrastructure of symbiotic dinoflagellates. *Marine Pollution Bulletin*, 118, 180 - 187. <https://doi.org/10.1016/j.marpolbul.2017.02.044>.

Marubini, F., & Davies, P. (1996). Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. *Marine Biology*, 127, 319-328. <https://doi.org/10.1007/BF00942117>.

Nordemar, I., Nyström, M., & Dizon, R. (2003). Effects of elevated seawater temperature and nitrate enrichment on the branching coral *Porites cylindrica* in the absence of particulate food. *Marine Biology*, 142, 669-677. <https://doi.org/10.1007/S00227-002-0989-0>.

Galli, G., & Solidoro, C. (2018). ATP Supply May Contribute to Light-Enhanced Calcification in Corals More



Than Abiotic Mechanisms. *Frontiers in Marine Science*, 5. <https://doi.org/10.3389/fmars.2018.00068>.

Holcomb, M., Cohen, A., & McCorkle, D. (2012). An investigation of the calcification response of the scleractinian coral *Astrangia poculata* to elevated p CO₂ and the effects of nutrients, zooxanthellae and gender. *Biogeosciences*, 9, 29-39. <https://doi.org/10.5194/BG-9-29-2012>.

Maier, C., Hegeman, J., Weinbauer, M., & Gattuso, J. (2009). Calcification of the cold-water coral *Lophelia pertusa*, under ambient and reduced pH. *Biogeosciences*, 6, 1671-1680. <https://doi.org/10.5194/BG-6-1671-2009>.

Hill, L., Paradas, W., Willemes, M., Pereira, M., Salomon, P., Mariath, R., Moura, R., Atella, G., Farina, M., Amado-Filho, G., & Salgado, L. (2019). Acidification-induced cellular changes in Symbiodinium isolated from *Mussismilia braziliensis*. *PLoS ONE*, 14. <https://doi.org/10.1371/journal.pone.0220130>.

Lesser, M., & Farrell, J. (2004). Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. *Coral Reefs*, 23, 367-377. <https://doi.org/10.1007/s00338-004-0392-z>.

Venn, A., Tambutté, É., Holcomb, M., Allemand, D., & Tambutté, S. (2011). Live Tissue Imaging Shows Reef Corals Elevate pH under Their Calcifying Tissue Relative to Seawater. *PLoS ONE*, 6. <https://doi.org/10.1371/journal.pone.0020013>.

Meron, D., Atlas, E., Kruh, L., Elifantz, H., Minz, D., Fine, M., & Banin, E. (2011). The impact of reduced pH on the microbial community of the coral *Acropora eurystoma*. *The ISME Journal*, 5, 51-60. <https://doi.org/10.1038/ismej.2010.102>.

Britton, D., Cornwall, C., Revill, A., Hurd, C., & Johnson, C. (2016). Ocean acidification reverses the positive effects of seawater pH fluctuations on growth and photosynthesis of the habitat-forming kelp, *Ecklonia radiata*. *Scientific Reports*, 6. <https://doi.org/10.1038/srep26036>.

Chave, K., & Suess, E. (1970). CALCIUM CARBONATE SATURATION IN SEAWATER: EFFECTS OF DISSOLVED ORGANIC MATTER. *Limnology and Oceanography*, 15, 633-637. <https://doi.org/10.4319/LO.1970.15.4.0633>.

Korchef, A., & Touaibi, M. (2019). Effect of pH and temperature on calcium carbonate precipitation by CO₂ removal from iron-rich water. *Water and Environment Journal*, 34. <https://doi.org/10.1111/wej.12467>.

Novitsky, J. (1981). Calcium carbonate precipitation by marine bacteria. *Geomicrobiology Journal*, 2, 375-388. <https://doi.org/10.1080/01490458109377775>.

Zerveas, S., Mente, M., Tsakiri, D., & Kotzabasis, K. (2021). Microalgal photosynthesis induces alkalinization of aquatic environment as a result of H⁺ uptake independently from CO₂ concentration - New perspectives for environmental applications. *Journal of environmental management*, 289, 112546. <https://doi.org/10.1016/j.jenvman.2021.112546>.



org/10.1016/j.jenvman.2021.112546.

Shick, J. (1990). Diffusion Limitation and Hyperoxic Enhancement of Oxygen Consumption in Zooxanthellate Sea Anemones, Zoanthsids, and Corals.. *The Biological bulletin*, 179 1, 148-158 . <https://doi.org/10.2307/1541749>.

Doney, S., Fabry, V., Feely, R., & Kleypas, J. (2009). Ocean acidification: the other CO₂ problem.. *Annual review of marine science*, 1, 169-92 . <https://doi.org/10.1146/annurev.marine.010908.163834>.

Fabricius, K., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., De'ath, G., Okazaki, R., Muehlehner, N., Glas, M., & Lough, J. (2011). Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nature Climate Change*, 1, 165-169. <https://doi.org/10.1038/NCLIMATE1122>.

Kannapiran, E., & Ravindran, J. (2012). Dynamics and diversity of phosphate mineralizing bacteria in the coral reefs of Gulf of Mannar. *Journal of Basic Microbiology*, 52. <https://doi.org/10.1002/jobm.201100095>.

Cervino, J., Hayes, R., Goreau, T., & Smith, G. (2004). Zooxanthellae regulation in yellow blotch/band and other coral diseases contrasted with temperature related bleaching: In situ destruction vs expulsion. *Symbiosis*, 37, 63- 85.

McCulloch, M., D'Olivo, J., Falter, J., Holcomb, M., & Trotter, J. (2017). Coral calcification in a changing World and the interactive dynamics of pH and DIC upregulation. *Nature Communications*, 8.

Cameron, L., Reymond, C., Bijma, J., Büscher, J., Beer, D., Guillermic, M., Eagle, R., Gunnell, J., Müller-Lundin, F., Schmidt-Grieb, G., Westfield, I., Westphal, H., & Ries, J. (2022). Impacts of Warming and Acidification on Coral Calcification Linked to Photosymbiont Loss and Deregulation of Calcifying Fluid pH. *Journal of Marine Science and Engineering*.

Gagliano, M., McCormick, M., Moore, J., & Depczynski, M. (2010). The basics of acidification: baseline variability of pH on Australian coral reefs. *Marine Biology*, 157, 1849-1856. <https://doi.org/10.1007/S00227-010-1456-Y>.

Gray, S., DeGrandpre, M., Langdon, C., & Corredor, J. (2012). Short-term and seasonal pH,pCO₂and saturation state variability in a coral-reef ecosystem. *Global Biogeochemical Cycles*, 26. <https://doi.org/10.1029/2011GB004114>.

Yang, B., Byrne, R., & Lindemuth, M. (2015). Contributions of organic alkalinity to total alkalinity in coastal waters: A spectrophotometric approach. *Marine Chemistry*, 176, 199-207. <https://doi.org/10.1016/J.MARCHEM.2015.09.008>.

Dickson, A. G. (2023). Alkalinity in theory and practice. *Elements*, 19(1), 7-12. <https://doi.org/10.2138/gselements.19.1.7>

Hohn, S., & Merico, A. (2015). Quantifying the relative importance of transcellular and paracellular



ion transports to coral polyp calcification. *Frontiers in Earth Science*, 2, 37. <https://doi.org/10.3389/feart.2014.00037>.

Falini, G., Fermani, S., & Goffredo, S. (2015). Coral biomineralization: A focus on intra-skeletal organic matrix and calcification. *Seminars in cell & developmental biology*, 46, 17-26. <https://doi.org/10.1016/j.semcdb.2015.09.005>.

Uthicke, S., Furnas, M., & Lønborg, C. (2014). Coral Reefs on the Edge? Carbon Chemistry on Inshore Reefs of the Great Barrier Reef. *PLoS ONE*, 9. <https://doi.org/10.1371/journal.pone.0109092>.

Zvi Steiner, Alexandra V. Turchyn, Eyal Harpaz, Jacob Silverman, (2018). Water chemistry reveals a significant decline in coral calcification rates in the southern Red Sea DOI: 10.1038/s41467-018-06030-6

Ding, D., Patel, A., Singhania, R., Chen, C., & Dong, C. (2022). Effects of Temperature and Salinity on Growth, Metabolism and Digestive Enzymes Synthesis of *Goniopora columna*. *Biology*, 11. <https://doi.org/10.3390/biology11030436>.

Trotter, J., Montagna, P., McCulloch, M., Silenzi, S., Reynaud, S., Mortimer, G., Martin, S., Ferrier-Pagès, C., Gattuso, J., & Rodolfo-Metalpa, R. (2011). Quantifying the pH 'vital effect' in the temperate zooxanthellate coral *Cladocora caespitosa*: Validation of the boron seawater pH proxy. *Earth and Planetary Science Letters*, 303, 163-

173. <https://doi.org/10.1016/J.EPSL.2011.01.030>.

McCulloch, M., Trotter, J., Montagna, P., Falter, J., Dunbar, R., Freiwald, A., Försterra, G., Correa, M., Maier, C., Rüggeberg, A., & Taviani, M. (2012). Resilience of cold-water scleractinian corals to ocean acidification: Boron isotopic systematics of pH and saturation state up-regulation. *Geochimica et Cosmochimica Acta*, 87, 21-34. <https://doi.org/10.1016/J.GCA.2012.03.027>.

Klochko, K., Kaufman, A., Yao, W., Byrne, R., & Tossell, J. (2006). Experimental measurement of boron isotope fractionation in seawater. *Earth and Planetary Science Letters*, 248, 276-285. <https://doi.org/10.1016/J.EPSL.2006.05.034>.

Sanyal, A., Hemming, N., Broecker, W., Lea, D., Spero, H., & Hanson, G. (1996). Oceanic pH control on the boron isotopic composition of foraminifera: Evidence from culture experiments. *Paleoceanography*, 11, 513-517. <https://doi.org/10.1029/96PA01858>.

McCall, A., Cummings, C., Bhave, G., Vanacore, R., Vanacore, R., Page-McCaw, A., Page-McCaw, A., & Hudson, B. (2014). Bromine Is an Essential Trace Element for Assembly of Collagen IV Scaffolds in Tissue Development and Architecture. *Cell*, 157, 1380-1392. <https://doi.org/10.1016/j.cell.2014.05.009>.

Heeb, M., Criquet, J., Zimmermann-Steffens, S., & Gunten, U. (2014). Oxidative treatment of bromide-containing waters: formation of bromine and its reactions with inorganic and organic compounds—a critical



review. *Water research*, 48, 15-42 . <https://doi.org/10.1016/j.watres.2013.08.030>.

Zhang, Y., Liu, J., Shi, D., & Li, Z. (2018). Halogenated Compounds from Corals: Chemical Diversity and Biological Activities.. *Mini reviews in medicinal chemistry*. <https://doi.org/10.2174/138955751866618113124015>.

McCall, A., Cummings, C., Bhawe, G., Vanacore, R., Vanacore, R., Page-McCaw, A., Page-McCaw, A., & Hudson, B. (2014). Bromine Is an Essential Trace Element for Assembly of Collagen IV Scaffolds in Tissue Development and Architecture. *Cell*, 157, 1380-1392. <https://doi.org/10.1016/j.cell.2014.05.009>.

Hain, M., Sigman, D., Higgins, J., & Haug, G. (2015). The effects of secular calcium and magnesium concentration changes on the thermodynamics of seawater acid/base chemistry: Implications for Eocene and Cretaceous ocean carbon chemistry and buffering. *Global Biogeochemical Cycles*, 29, 517 - 533. <https://doi.org/10.1002/2014GB004986>.

Rebello, A., & Moreira, I. (1982). The influence of various seawater components on the buffer capacity for CO₂. *Marine Chemistry*, 11, 33-41. [https://doi.org/10.1016/0304-4203\(82\)90046-9](https://doi.org/10.1016/0304-4203(82)90046-9).

Freundlich, M. (1967). Ion Pairing of Magnesium Sulfate in Seawater: Determined by Ultrasonic Absorption. *Science*, 157, 823 - 823. <https://doi.org/10.1126/SCIENCE.157.3790.823-A>.

Houck, J., Buddemeier, R., & Chave, K. (1975). Skeletal Low-Magnesium Calcite in Living Scleractinian Corals. *Science*, 189, 997 - 999. <https://doi.org/10.1126/science.189.4207.997>.

Evans, D., Millar, Z., Wolvin, S., Pham, P., Lepage, V., & Lumsden, J. (2021). Magnesium concentration influences size and pulse rate in the upside-down jellyfish, *Cassiopea andromeda*.. *Zoo biology*. <https://doi.org/10.1002/zoo.21631>.

Weinbauer, M., Brandstätter, F., & Velimirov, B. (2000). On the potential use of magnesium and strontium concentrations as ecological indicators in the calcite skeleton of the red coral (*Corallium rubrum*). *Marine Biology*, 137, 801-809. <https://doi.org/10.1007/S002270000432>.

Li, W., Liu, X., Wang, K., Fodrie, F., Yoshimura, T., & Hu, Y. (2021). Potassium phases and isotopic composition in modern marine biogenic carbonates. *Geochimica et Cosmochimica Acta*. <https://doi.org/10.1016/J.GCA.2021.04.018>.

Hadfield, C., Clayton, L., Cohrs, D., & Murphy, D. (2012). Acute morbidity and mortality in invertebrates and fish following exposure to potassium-deficient saltwater.. *Journal of fish diseases*, 35 7, 549-53 . <https://doi.org/10.1111/j.1365-2761.2012.01379.x>.

Liu, H., Zhang, X., Tan, B., Lin, Y., Chi, S., Dong, X., & Yang, Q. (2014). Effect of dietary potassium on growth, nitrogen metabolism, osmoregulation and immunity of pacific white shrimp (*Litopenaeus vannamei*) reared in low salinity seawater. *Journal of Ocean University of China*, 13, 311-320. <https://doi.org/10.1007/s11802->



014- 2118-3.

Zhu, C., Dong, S., Wang, F., & Zhang, H. (2006). Effects of seawater potassium concentration on the dietary potassium requirement of *Litopenaeus vannamei*. *Aquaculture*, 258, 543-550. <https://doi.org/10.1016/J.AQUACULTURE.2006.03.038>.

Angino, E., Billings, G., & Andersen, N. (1966). OBSERVED VARIATIONS IN THE STRONTIUM CONCENTRATION OF SEA WATER.. *Chemical Geology*, 1, 145-153. [https://doi.org/10.1016/0009-2541\(66\)90013-1](https://doi.org/10.1016/0009-2541(66)90013-1).

Pasqualetti, S., Banfi, G., & Mariotti, M. (2013). The effects of strontium on skeletal development in zebrafish embryo.. *Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements*, 27 4, 375-9 . <https://doi.org/10.1016/j.jtemb.2013.06.002>.

Reynaud, S., Ferrier-Pagès, C., Boisson, F., Allemand, D., & Fairbanks, R. (2004). Effect of light and temperature on calcification and strontium uptake in the scleractinian coral *Acropora verweyi*. *Marine Ecology Progress Series*, 279, 105-112. <https://doi.org/10.3354/MEPS279105>.

Habicht, K., Gade, M., Thamdrup, B., Berg, P., & Canfield, D. (2002). Calibration of Sulfate Levels in the Archean Ocean. *Science*, 298, 2372 - 2374. <https://doi.org/10.1126/SCIENCE.1078265>.

Fakhraee, M., Hancisse, O., Canfield, D., Crowe, S., & Katsev, S. (2019). Proterozoic seawater sulfate scarcity and the evolution of ocean–atmosphere chemistry. *Nature Geoscience*, 12, 375-380. <https://doi.org/10.1038/s41561-019-0351-5>.

Deschaseaux, E., Jones, G., & Swan, H. (2016). Dimethylated sulfur compounds in coral-reef ecosystems. *Environmental Chemistry*, 13, 239-251. <https://doi.org/10.1071/EN14258>.

Raina, J., Tapiolas, D., Willis, B., & Bourne, D. (2009). Coral-Associated Bacteria and Their Role in the Biogeochemical Cycling of Sulfur. *Applied and Environmental Microbiology*, 75, 3492 - 3501. <https://doi.org/10.1128/AEM.02567-08>.

Yuyama, I., Higuchi, T., & Takei, Y. (2016). Sulfur utilization of corals is enhanced by endosymbiotic algae. *Biology Open*, 5, 1299 - 1304. <https://doi.org/10.1242/bio.020164>.

Zhang, B., Hao, L., Tian, C., Yuan, S., Feng, C., Ni, J., & Borthwick, A. (2015). Microbial reduction and precipitation of vanadium (V) in groundwater by immobilized mixed anaerobic culture.. *Bioresource technology*, 192, 410-7 . <https://doi.org/10.1016/j.biortech.2015.05.102>.

Wolgemuth, K., & Broecker, W. (1970). Barium in sea water. *Earth and Planetary Science Letters*, 8, 372-378. [https://doi.org/10.1016/0012-821X\(70\)90110-X](https://doi.org/10.1016/0012-821X(70)90110-X).

Turekian, K., & Johnson, D. (1966). The barium distribution in sea water. *Geochimica et Cosmochimica Acta*, 30, 1153-1174. [https://doi.org/10.1016/0016-7037\(66\)90035-4](https://doi.org/10.1016/0016-7037(66)90035-4).



Gonneea, M., Cohen, A., DeCarlo, T., & Charette, M. (2017). Relationship between water and aragonite barium concentrations in aquaria reared juvenile corals. *Geochimica et Cosmochimica Acta*, 209, 123-134. <https://doi.org/10.1016/J.GCA.2017.04.006>.

Wei, L., Li, Y., Ye, H., Xiao, J., Hogstrand, C., Green, I., Guo, Z., & Han, D. (2021). Dietary Trivalent Chromium Exposure Up-Regulates Lipid Metabolism in Coral Trout: The Evidence From Transcriptome Analysis. *Frontiers in Physiology*, 12. <https://doi.org/10.3389/fphys.2021.640898>.

Tagliabue, A., Hawco, N., Bundy, R., Landing, W., Milne, A., Morton, P., & Saito, M. (2018). The Role of External Inputs and Internal Cycling in Shaping the Global Ocean Cobalt Distribution: Insights From the First Cobalt Biogeochemical Model. *Global Biogeochemical Cycles*, 32, 594 - 616. <https://doi.org/10.1002/2017GB005830>.

Saito, M., Noble, A., Hawco, N., Twining, B., Ohnemus, D., John, S., Lam, P., Lam, P., Conway, T., Johnson, R., Moran, D., & McIlvin, M. (2016). The acceleration of dissolved cobalt's ecological stoichiometry due to biological uptake, remineralization, and scavenging in the Atlantic Ocean. *Biogeosciences*, 14, 4637-4662. <https://doi.org/10.5194/BG-14-4637-2017>.

Sunda, W., & Huntsman, S. (1995). Cobalt and zinc interreplacement in marine phytoplankton: Biological and geochemical implications. *Limnology and Oceanography*, 40, 1404-1417. <https://doi.org/10.4319/LO.1995.40.8.1404>.

Bunt, J. (1970). UPTAKE OF COBALT AND VITAMIN B12 BY TROPICAL MARINE MACROALGAE 1, 2. *Journal of Phycology*, 6. <https://doi.org/10.1111/j.1529-8817.1970.tb02404.x>.

Marangoni, L., Marques G., Duarte J., G., Pereira, C., Calderon, E., Castro, C., & Bianchini, A. (2017). Copper effects on biomarkers associated with photosynthesis, oxidative status and calcification in the Brazilian coral *Mussismilia harttii* (Scleractinia, Mussidae). *Marine environmental research*, 130, 248-257. <https://doi.org/10.1016/j.marenvres.2017.08.002>.

Reichelt-Brushett, A., & Harrison, P. (2000). The effect of copper on the settlement success of larvae from the scleractinian coral *Acropora tenuis*. *Marine Pollution Bulletin*, 41, 385-391. [https://doi.org/10.1016/S0025-326X\(00\)00131-4](https://doi.org/10.1016/S0025-326X(00)00131-4).

Fonseca, J., Marangoni, L., Marques, J., & Bianchini, A. (2017). Effects of increasing temperature alone and combined with copper exposure on biochemical and physiological parameters in the zooxanthellate scleractinian coral *Mussismilia harttii*. *Aquatic toxicology*, 190, 121-132. <https://doi.org/10.1016/j.aquatox.2017.07.002>.

Fonseca, J., Marangoni, L., Marques, J., & Bianchini, A. (2019). Carbonic anhydrase activity as a potential biomarker for acute exposure to copper in corals. *Chemosphere*, 227, 598-605. <https://doi.org/10.1016/j.chemosphere.2019.04.089>.



Grant, A., Graham, K., Frankland, S., & Hinde, R. (2003). Effect of copper on algal-host interactions in the symbiotic coral *Plesiastrea versipora*. *Plant Physiology and Biochemistry*, 41, 383-390. [https://doi.org/10.1016/S0981-9428\(03\)00034-2](https://doi.org/10.1016/S0981-9428(03)00034-2).

Schwarz, J., Mitchelmore, C., Jones, R., O'Dea, A., & Seymour, S. (2013). Exposure to copper induces oxidative and stress responses and DNA damage in the coral *Montastraea franksi*. *Comparative biochemistry and physiology. Toxicology & pharmacology : CBP*, 157 3, 272-9 . <https://doi.org/10.1016/j.cbpc.2012.12.003>.

Bielmyer-Fraser, G., Patel, P., Capo, T., & Grosell, M. (2018). Physiological responses of corals to ocean acidification and copper exposure.. *Marine pollution bulletin*, 133, 781-790 . <https://doi.org/10.1016/j.marpolbul.2018.06.048>.

Achterberg, E. P., Holland, T. W., Bowie, A. R., Mantoura, R. F. C., & Worsfold, P. J. (2001). Determination of iron in seawater. *Analytica Chimica Acta*, 442(1-14). [https://doi.org/10.1016/S0003-2670\(01\)01168-4](https://doi.org/10.1016/S0003-2670(01)01168-4)

Emerson, D., Roden, E. E., Twining, B. S., & Moyer, C. L. (2012). The microbial ferrous wheel: iron cycling in terrestrial, freshwater, and marine environments. *Frontiers in Microbiology*, 3, 383. <https://doi.org/10.3389/fmicb.2012.00383>

Geider, R. J., & Roche, J. L. (1994). The role of iron in phytoplankton photosynthesis, and the potential for iron- limitation of primary productivity in the sea. *Photosynthesis Research*, 39(275-301). <https://doi.org/10.1007/BF00014588>

Kong, Y., Zou, P., Song, L., Wang, Z., Qi, J., Zhu, L., & Xu, X. (2014). Effects of iron on the algae growth and microcystin synthesis: a review. *Ying yong sheng tai xue bao = The journal of applied ecology*, 25(5), 1533-1540. <https://doi.org/10.13287/j.1001-9332.201405.022>

Leigh-Smith, J., Reichelt-Brushett, A. J., & Rose, A. (2018). The characterization of iron (III) in seawater and related toxicity to early life stages of scleractinian corals. *Environmental Toxicology and Chemistry*, 37(8), 2146-2154. <https://doi.org/10.1002/etc.4200>

Manck, L. E., Park, J., Tully, B. J., Poire, A. M., Bundy, R. M., Dupont, C. L., & Barbeau, K. A. (2021). Petrobactin, a siderophore produced by *Alteromonas*, mediates community iron acquisition in the global ocean. *The ISME Journal*, 16(358-369). <https://doi.org/10.1038/s41396-021-01024-5>

Rädecker, N., Pogoreutz, C., Ziegler, M., Ashok, A., Barreto, M. M., Chaidez, V., Grupstra, C. G. B., Ng, Y. M., Perna, G., Aranda, M., & Voolstra, C. R. (2017). Assessing the effects of iron enrichment across holobiont compartments reveals reduced microbial nitrogen fixation in the Red Sea coral *Pocillopora verrucosa*. *Ecology and Evolution*, 7(6614-6621). <https://doi.org/10.1002/ece3.3214>

Sandy, M., & Butler, A. (2009). Microbial iron acquisition: marine and terrestrial siderophores. *Chemical*



Reviews, 109(10), 4580-4595. <https://doi.org/10.1021/cr9002787>

Shick, J. M., Iglie, K. D., Wells, M. L., Trick, C. G., Doyle, J., & Dunlap, W. C. (2011). Responses to iron limitation in two colonies of *Stylophora pistillata* exposed to high temperature: Implications for coral bleaching. *Limnology and Oceanography*, 56(3), 813-828. <https://doi.org/10.4319/lo.2011.56.3.0813>

Tagliabue, A., Bowie, A. R., Boyd, P. W., Buck, K. N., Johnson, K. S., Saito, M. A., & Whale, S. (2018). The role of external inputs and internal cycling in shaping the global ocean cobalt distribution: Insights from the first cobalt biogeochemical model. *Global Biogeochemical Cycles*, 32(3), 594-616. <https://doi.org/10.1002/2017GB005830>

Wells, M. L. (1999). Manipulating iron availability in nearshore waters. *Limnology and Oceanography*, 44(4), 1002-1008. <https://doi.org/10.4319/lo.1999.44.4.1002>

Agrawal, O. P., Sunita, G., & Gupta, V. K. (1999). A sensitive colorimetric method for the micro determination of iodine in marine water. *Talanta*, 49(4), 923-928. [https://doi.org/10.1016/S0039-9140\(99\)00091-0](https://doi.org/10.1016/S0039-9140(99)00091-0)

Bergeijk, S. V., Hernández, L., Zubía, E., & Cañavate, J. P. (2016). Iodine balance, growth and biochemical composition of three marine microalgae cultured under various inorganic iodine concentrations. *Marine Biology*, 163(1-19). <https://doi.org/10.1007/S00227-016-2884-0>

Butler, E., Smith, J. D., & Fisher, N. S. (1981). Influence of phytoplankton on iodine speciation in seawater. *Limnology and Oceanography*, 26(2), 382-386. <https://doi.org/10.4319/LO.1981.26.2.0382>

Javier, L. H., Benzekri, H., Gut, M., Claros, M. G., van Bergeijk, S. V., Cañavate, J. P., & Machado, M. (2018). Characterization of iodine-related molecular processes in the marine microalga *Tisochrysis lutea* (Haptophyta). *Frontiers in Marine Science*, 5. <https://doi.org/10.3389/fmars.2018.00134>

Leblanc, C., Colin, C., Cosse, A., Delage, L., La Barre, S., Morin, P., Fiévet, B., Voiseux, C., Ambroise, Y., Verhaeghe, E., Amouroux, D., Donard, O. F. X., Tessier, E., & Potin, P. (2006). Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases. *Biochimie*, 88(11), 1773-1785. <https://doi.org/10.1016/J.BIOCHI.2006.09.001>

Prouty, N. G., Roark, E. B., Koenig, A. E., Demopoulos, A. W. J., Batista, F. C., & Kiele, M. (2018). Distribution, speciation, and bioavailability of iodine in deep-sea coral ecosystems of the Gulf of Mexico: Implications for tracking iodine in the environment. *Journal of Environmental Radioactivity*, 187, 122-132. <https://doi.org/10.1016/j.jenvrad.2018.01.003>

Saiz-Lopez, A., & Plane, J. M. C. (2004). Novel iodine chemistry in the marine boundary layer. *Geophysical Research Letters*, 31. <https://doi.org/10.1029/2003GL019215>

Xu, D., Brennan, G. L., Xu, L., Zhang, X. W., Fan, X., Han, W., Mock, T., McMinn, A., Hutchins, D. A., & Ye, N. (2018). Ocean acidification increases iodine accumulation in kelp-based coastal food webs. *Global*



Change Biology, 25(2), 629-639. <https://doi.org/10.1111/gcb.14467>

Binet, M., Reichelt-Brushett, A., McKnight, K. S., Golding, L., Humphrey, C., & Stauber, J. (2023). Adult corals are uniquely more sensitive to manganese than coral early-life stages. *Environmental Toxicology and Chemistry*. <https://doi.org/10.1002/etc.5618>

Burdige, D. (1993). The biogeochemistry of manganese and iron reduction in marine sediments. *Earth-Science Reviews*, 35, 249-284. [https://doi.org/10.1016/0012-8252\(93\)90040-E](https://doi.org/10.1016/0012-8252(93)90040-E)

Burdige, D., & Nealson, K. (1985). Microbial manganese reduction by enrichment cultures from coastal marine sediments. *Applied and Environmental Microbiology*, 50(2), 491-497. <https://doi.org/10.1128/aem.50.2.491-497.1985>

Hansel, C. (2017). Manganese in marine microbiology. *Advances in Microbial Physiology*, 70, 37-83. <https://doi.org/10.1016/bs.ampbs.2017.01.005>

Hernroth, B., Tassidis, H., & Baden, S. (2019). Immunosuppression of aquatic organisms exposed to elevated levels of manganese: From global to molecular perspective. *Developmental and Comparative Immunology*. <https://doi.org/10.1016/j.dci.2019.103536>

Klinkhammer, G. P., & Bender, M. L. (1980). The distribution of manganese in the Pacific Ocean. *Earth and Planetary Science Letters*, 46, 361-384. [https://doi.org/10.1016/0012-821X\(80\)90051-5](https://doi.org/10.1016/0012-821X(80)90051-5)

Oweson, C., & Hernroth, B. (2009). A comparative study on the influence of manganese on the bactericidal response of marine invertebrates. *Fish & Shellfish Immunology*, 27(3), 500-507. <https://doi.org/10.1016/j.fsi.2009.07.001>

Oweson, C., Li, C., Söderhäll, I., & Hernroth, B. (2010). Effects of manganese and hypoxia on coelomocyte renewal in the echinoderm, *Asterias rubens* (L.). *Aquatic Toxicology*, 100(1), 84-90. <https://doi.org/10.1016/j.aquatox.2010.07.012>

Oweson, C., Sköld, H., Pinsino, A., Matranga, V., & Hernroth, B. (2008). Manganese effects on haematopoietic cells and circulating coelomocytes of *Asterias rubens* (Linnaeus). *Aquatic Toxicology*, 89(2), 75-81. <https://doi.org/10.1016/j.aquatox.2008.05.016>

Sköld, H., Baden, S., Looström, J., Eriksson, S. P., & Hernroth, B. (2015). Motoric impairment following manganese exposure in asteroid echinoderms. *Aquatic Toxicology*, 167, 31-37. <https://doi.org/10.1016/j.aquatox.2015.07.016>

Stauber, J., & Florence, T. (1985). Interactions of copper and manganese: A mechanism by which manganese alleviates copper toxicity to the marine diatom, *Nitzschia closterium* (Ehrenberg) W. Smith. *Aquatic Toxicology*, 7, 241-254. [https://doi.org/10.1016/0166-445X\(85\)90042-6](https://doi.org/10.1016/0166-445X(85)90042-6)

Summer, K., Reichelt-Brushett, A., & Howe, P. (2019). Toxicity of manganese to various life stages of



selected marine cnidarian species. *Ecotoxicology and Environmental Safety*, 167, 83-94. <https://doi.org/10.1016/j.ecoenv.2018.09.116>

Sunda, W., Huntsman, S., & Harvey, G. (1983). Photoreduction of manganese oxides in seawater and its geochemical and biological implications. *Nature*, 301, 234-236. <https://doi.org/10.1038/301234A0>

Barros, M.P., Hollnagel, H., Glavina, A., Soares, C., Ganini, D., Dagenais-Bellefeuille, S., Morse, D., & Colepicolo, P. (2013). Molybdate ratio affects redox metabolism and viability of the dinoflagellate *Lingulodinium polyedrum*. *Aquatic Toxicology*, 142-143, 195-202. <https://doi.org/10.1016/j.aquatox.2013.08.012>

Binet, M., Reichelt-Brushett, A., McKnight, K. S., Golding, L., Humphrey, C., & Stauber, J. (2023). Adult corals are uniquely more sensitive to molybdenum than coral early-life stages. *Environmental Toxicology and Chemistry*. <https://doi.org/10.1002/etc.5618>

Burdige, D., & Neelson, K. (1985). Microbial manganese reduction by enrichment cultures from coastal marine sediments. *Applied and Environmental Microbiology*, 50(2), 491-497. <https://doi.org/10.1128/aem.50.2.491-497.1985>

Cole, J., Howarth, R., Nolan, S., & Marino, R. (1986). Sulfate inhibition of molybdate assimilation by planktonic algae and bacteria: some implications for the aquatic nitrogen cycle. *Biogeochemistry*, 2, 179-196. <https://doi.org/10.1007/BF02180194>

Glass, J., Axler, R., Chandra, S., & Goldman, C. (2012). Molybdenum limitation of microbial nitrogen assimilation in aquatic ecosystems and pure cultures. *Frontiers in Microbiology*, 3. <https://doi.org/10.3389/fmicb.2012.00331>

Howarth, R., & Cole, J. (1985). Molybdenum Availability, Nitrogen Limitation, and Phytoplankton Growth in Natural Waters. *Science*, 229, 653-655. <https://doi.org/10.1126/science.229.4714.653>

Klinkhammer, G. P., & Bender, M. L. (1980). The distribution of manganese in the Pacific Ocean. *Earth and Planetary Science Letters*, 46, 361-384. [https://doi.org/10.1016/0012-821X\(80\)90051-5](https://doi.org/10.1016/0012-821X(80)90051-5)

Marino, R., Howarth, R., Chan, F., Cole, J., & Likens, G. (2003). Sulfate inhibition of molybdenum-dependent nitrogen fixation by planktonic cyanobacteria under seawater conditions: a non-reversible effect. *Hydrobiologia*, 500, 277-293. <https://doi.org/10.1023/A:1024641904568>

Bolter, E., Turekian, K., & Schutz, D. (1964). The distribution of rubidium, cesium and barium in the oceans. *Geochimica et Cosmochimica Acta*, 28, 1459-1466. [https://doi.org/10.1016/0016-7037\(64\)90161-9](https://doi.org/10.1016/0016-7037(64)90161-9)

Papadopoulou, C., & Kaniyas, G. (1977). Tunicate species as marine pollution indicators. *Marine Pollution Bulletin*, 8, 229-231. [https://doi.org/10.1016/0025-326X\(77\)90431-3](https://doi.org/10.1016/0025-326X(77)90431-3)

Petersen, K., Paytan, A., Rahav, E., Levy, O., Silverman, J., Barzel, O., Potts, D., & Bar-Zeev, E. (2018). Impact of brine and antiscalants on reef-building corals in the Gulf of Aqaba - Potential effects from desalination



plants. *Water Research*, 144, 183-191. <https://doi.org/10.1016/j.watres.2018.07.009>

Riley, J. P., & Tongudai, M. (1966). Caesium and rubidium in sea water. *Chemical Geology*, 1, 291-294. [https://doi.org/10.1016/0009-2541\(66\)90025-8](https://doi.org/10.1016/0009-2541(66)90025-8)

Smith, R. C., Pillai, K. C., Chow, T. J., & Folsom, T. (1965). Determination of rubidium in seawater. *Limnology and Oceanography*, 10(2), 226-232. <https://doi.org/10.4319/LO.1965.10.2.0226>

Cutter, G., & Bruland, K. (1984). The marine biogeochemistry of selenium: A re-evaluation. *Limnology and Oceanography*, 29, 1179-1192. <https://doi.org/10.4319/LO.1984.29.6.1179>

Wrench, J. (1983). Organic selenium in seawater: levels, origins and chemical forms. *Marine Chemistry*, 12, 237. [https://doi.org/10.1016/0304-4203\(83\)90099-3](https://doi.org/10.1016/0304-4203(83)90099-3)

Cooke, T., & Bruland, K. (1987). Aquatic chemistry of selenium: evidence of biomethylation. *Environmental Science & Technology*, 21, 1214-1219. <https://doi.org/10.1021/ES00165A009>

Vandermeulen, J., & Foda, A. (1988). Cycling of selenite and selenate in marine phytoplankton. *Marine Biology*, 98, 115-123. <https://doi.org/10.1007/BF00392666>

Harrison, P., Yu, P., Thompson, P., Price, N. M., & Phillips, D. (1988). Survey of selenium requirements in marine phytoplankton. *Marine Ecology Progress Series*, 47, 89-96. <https://doi.org/10.3354/MEPS047089>

Gobler, C., Lobanov, A. V., Tang, Y., Turanov, A., Zhang, Y., Doblin, M., Taylor, G., Sañudo-Wilhelmy, S., Grigoriev, I., & Gladyshev, V. (2013). The central role of selenium in the biochemistry and ecology of the harmful pelagophyte, *Aureococcus anophagefferens*. *The ISME Journal*, 7, 1333-1343. <https://doi.org/10.1038/ismej.2013.25>

Trevisan, R., Delapiedra, G., Mello, D. F., Arl, M., Schmidt, É. C., Latini, A., & Dafre, A. L. (2011). Selenium in water enhances the antioxidant defenses induced by copper in gills of the brown mussel *Perna perna*. *Aquatic Toxicology*, 101(1), 64-71. <https://doi.org/10.1016/j.aquatox.2010.09.003>

Yan, X., Zheng, L., Chen, H., Lin, W., & Zhang, W. (2004). Enriched accumulation and biotransformation of selenium in the edible seaweed *Laminaria japonica*. *Journal of Agricultural and Food Chemistry*, 52(21), 6460-6464. <https://doi.org/10.1021/JF040195K>

Zheng, Y., Li, Z., Tao, M., Li, J., & Hu, Z. (2017). Effects of selenite on green microalga *Haematococcus pluvialis*: Bioaccumulation of selenium and enhancement of astaxanthin production. *Aquatic Toxicology*, 183, 21-27. <https://doi.org/10.1016/j.aquatox.2016.12.008>

Mitchell, K., Lima, A. T., & Van Cappellen, P. (2019). Selenium in buoyant marine debris biofilm. *Marine Pollution Bulletin*, 149, 110562. <https://doi.org/10.1016/j.marpolbul.2019.110562>

Gobi, N., Vaseeharan, B., Rekha, R., Vijayakumar, S., & Faggio, C. (2018). Bioaccumulation, cytotoxicity



and oxidative stress of the acute exposure selenium in *Oreochromis mossambicus*. *Ecotoxicology and Environmental Safety*, 162, 147-159. <https://doi.org/10.1016/j.ecoenv.2018.06.070>

Chiarelli, R., Martino, C., Roccheri, M., & Cancemi, P. (2021). Toxic effects induced by vanadium on sea urchin embryos. *Chemosphere*, 274, 129843. <https://doi.org/10.1016/j.chemosphere.2021.129843>

Han, T., Fan, H., & Wen, H. (2018). Dwindling vanadium in seawater during the early Cambrian, South China. *Chemical Geology*. <https://doi.org/10.1016/J.CHEMGEO.2018.05.022>

Kustin, K., Mcleod, G. C., Gilbert, T. R., & Briggs, B. R. (1983). Vanadium and other metal ions in the physiological ecology of marine organisms. *Biometals*, 5, 3-12. <https://doi.org/10.1007/BF0111305>

Meina, E. G., Niyogi, S., & Liber, K. (2020). Investigating the mechanism of vanadium toxicity in freshwater organisms. *Aquatic Toxicology*, 229, 105648. <https://doi.org/10.1016/J.AQUATOX.2020.105648>

Miramand, P., Guary, J., & Fowler, S. (1981). Uptake, assimilation, and excretion of vanadium in the shrimp, *Lysmata seticaudata* (Risso), and the crab, *Carcinus maenas* (L.). *Journal of Experimental Marine Biology and Ecology*, 49, 267-287. [https://doi.org/10.1016/0022-0981\(81\)90076-9](https://doi.org/10.1016/0022-0981(81)90076-9)

Pessoa, J., Garribba, E., Santos, M. F. A., & Santos-Silva, T. (2015). Vanadium and proteins: Uptake, transport, structure, activity and function. *Coordination Chemistry Reviews*, 301, 49-86. <https://doi.org/10.1016/J.CCR.2015.03.016>

Rehder, D. (2013). The future of vanadium. *Dalton Transactions*, 42(33), 11749-11761. <https://doi.org/10.1039/c3dt50457c>

Rehder, D. (2015). The role of vanadium in biology. *Metallomics*, 7(5), 730-742. <https://doi.org/10.1039/c4mt00304g>

Rehder, D. (2019). A role for vanadium in ascidians and in marine algae. *Journal of Oceanography and Marine Research*. <https://doi.org/10.35248/2572-3103.19.7190>

Schiffer, S., & Liber, K. (2017). Toxicity of aqueous vanadium to zooplankton and phytoplankton species of relevance to the athabasca oil sands region. *Ecotoxicology and Environmental Safety*, 137, 1-11. <https://doi.org/10.1016/j.ecoenv.2016.10.040>

Suzuki, S., Kimura, M., Agusa, T., & Rahman, H. (2012). Vanadium accelerates horizontal transfer of tet(M) gene from marine Photobacterium to Escherichia coli. *FEMS Microbiology Letters*, 336(1), 52-56. <https://doi.org/10.1111/j.1574-6968.2012.02653.x>

Barnett, J. P., Millard, A., Ksibe, A. Z., Scanlan, D., Schmid, R., & Blindauer, C. (2012). Mining genomes of marine cyanobacteria for elements of zinc homeostasis. *Frontiers in Microbiology*, 3, 142. <https://doi.org/10.3389/fmicb.2012.00142>



Bielmyer, G. K., Bullington, J., Decarlo, C., Chalk, S., & Smith, K. J. (2012). Effects of salinity on acute toxicity of zinc to two euryhaline species of fish, *Fundulus heteroclitus* and *Kryptolebias marmoratus*. *Integrative and Comparative Biology*, 52(6), 753-760. <https://doi.org/10.1093/icb/ics045>

Corinaldesi, C., Marcellini, F., Nepote, E., Damiani, E., & Danovaro, R. (2018). Impact of inorganic UV filters contained in sunscreen products on tropical stony corals (*Acropora* spp.). *The Science of the Total Environment*, 637-638, 1279-1285. <https://doi.org/10.1016/j.scitotenv.2018.05.108>

Gobi, N., Vaseeharan, B., Rekha, R., Vijayakumar, S., & Faggio, C. (2018). Bioaccumulation, cytotoxicity and oxidative stress of the acute exposure selenium in *Oreochromis mossambicus*. *Ecotoxicology and Environmental Safety*, 162, 147-159. <https://doi.org/10.1016/j.ecoenv.2018.06.070>

Janssen, D. J., & Cullen, J. (2015). Decoupling of zinc and silicic acid in the subarctic northeast Pacific interior. *Marine Chemistry*, 177, 124-133. <https://doi.org/10.1016/J.MARCHEM.2015.03.014>

Jorge, M. B., Silva, K., Wood, C. (2012). Oxidative stress parameters and antioxidant response to sublethal waterborne zinc in a euryhaline teleost *Fundulus heteroclitus*: protective effects of salinity. *Aquatic Toxicology*, 110-111, 187-193. <https://doi.org/10.1016/j.aquatox.2012.01.012>

Kim, T., Obata, H., Gamo, T., & Nishioka, J. (2015). Sampling and onboard analytical methods for determining subnanomolar concentrations of zinc in seawater. *Limnology and Oceanography: Methods*, 13. <https://doi.org/10.1002/lom3.10004>

Niyogi, S., Blewett, T., Gallagher, T., Fehsenfeld, S., & Wood, C. (2016). Effects of salinity on short-term waterborne zinc uptake, accumulation and sub-lethal toxicity in the green shore crab (*Carcinus maenas*). *Aquatic Toxicology*, 178, 132-140. <https://doi.org/10.1016/j.aquatox.2016.07.012>

Seto, M., Wada, S., & Suzuki, S. (2013). The effect of zinc on aquatic microbial ecosystems and the degradation of dissolved organic matter. *Chemosphere*, 90(3), 1091-1102. <https://doi.org/10.1016/j.chemosphere.2012.09.014>

Shaked, Y., Xu, Y., Leblanc, K., & Morel, F. (2006). Zinc availability and alkaline phosphatase activity in *Emiliania huxleyi*: Implications for Zn-P co-limitation in the ocean. *Limnology and Oceanography*, 51(1). <https://doi.org/10.4319/lo.2006.51.1.0299>

Sunda, W., & Huntsman, S. (1995). Cobalt and zinc interreplacement in marine phytoplankton: Biological and geochemical implications. *Limnology and Oceanography*, 40(8), 1404-1417. <https://doi.org/10.4319/LO.1995.40.8.1404>

Wahyono, I. B., Muslim, M., Suseno, H., Suryono, C. A., & Pujiyanto, A. (2023). Bioaccumulation of zinc by *Portunus pelagicus*: Nuclear application techniques that use radiotracer ⁶⁵Zn to study influence of concentration of Zn in seawater. *Maritime Technology and Research*. <https://doi.org/10.33175/>



mtr.2024.266903

Yung, M. M., Wong, S. W. Y., Kwok, K. W., Liu, F. Z., Leung, Y., Chan, W., Li, X. Y., Djurišić, A., & Leung, K. (2015). Salinity-dependent toxicities of zinc oxide nanoparticles to the marine diatom *Thalassiosira pseudonana*. *Aquatic Toxicology*, 165, 31-40. <https://doi.org/10.1016/j.aquatox.2015.05.015>

Carpenter, R. (1969). Factors controlling the marine geochemistry of fluorine. *Geochimica et Cosmochimica Acta*, 33, 1153-1167. [https://doi.org/10.1016/0016-7037\(69\)90038-6](https://doi.org/10.1016/0016-7037(69)90038-6)

Pankhurst, N., Boyden, C. R., & Wilson, J. (1980). The effect of a fluoride effluent on marine organisms. *Environmental Pollution Series A, Ecological and Biological*, 23, 299-312. [https://doi.org/10.1016/0143-1471\(80\)90072-0](https://doi.org/10.1016/0143-1471(80)90072-0)

Camargo, J. A. (2003). Fluoride toxicity to aquatic organisms: a review. *Chemosphere*, 50(3), 251-264. [https://doi.org/10.1016/S0045-6535\(02\)00498-8](https://doi.org/10.1016/S0045-6535(02)00498-8)

Gregson, R. P., Baldo, B., Thomas, P., Quinn, R., Bergquist, P. R., Stephens, J. F., & Horne, A. R. (1979). Fluorine is a major constituent of the marine sponge *Halichondria moorei*. *Science*, 206, 1108-1109. <https://doi.org/10.1126/science.206.4422.1108>

Masoud, M., El-Sarraf, W., Harfoush, A., & El-Said, G. (2006). The effect of fluoride and other ions on algae and fish of coastal water of Mediterranean Sea, Egypt. *American Journal of Environmental Sciences*, 2, 49-59. <https://doi.org/10.3844/AJESSP.2006.49.59>

Mukhopadhyay, D., Priya, P., & Chattopadhyay, A. (2015). Sodium fluoride affects zebrafish behaviour and alters mRNA expressions of biomarker genes in the brain: Role of Nrf2/Keap1. *Environmental Toxicology and Pharmacology*, 40(2), 352-359. <https://doi.org/10.1016/j.etap.2015.07.003>

Chae, Y., Kim, D., & An, Y. (2016). Effect of fluoride on the cell viability, cell organelle potential, and photosynthetic capacity of freshwater and soil algae. *Environmental Pollution*, 219, 359-367. <https://doi.org/10.1016/j.envpol.2016.10.063>

McClymont, A., Arnott, S., & Rusak, J. (2022). Interactive effects of increasing chloride concentration and warming on freshwater plankton communities. *Limnology and Oceanography Letters*, 8. <https://doi.org/10.1002/lol2.10278>

Roy, J. W. (2019). Endobenthic organisms exposed to chronically high chloride from groundwater discharging along freshwater urban streams and lakeshores. *Environmental Science & Technology*. <https://doi.org/10.1021/acs.est.9b02288>

Megaw, J., Busetti, A., & Gilmore, B. (2013). Isolation and characterisation of 1-alkyl-3-methylimidazolium chloride ionic liquid-tolerant and biodegrading marine bacteria. *PLoS ONE*, 8. <https://doi.org/10.1371/journal.pone.0060806>



Petersen, K., Paytan, A., Rahav, E., Levy, O., Silverman, J., Barzel, O., Potts, D., & Bar-Zeev, E. (2018). Impact of brine and antiscalants on reef-building corals in the Gulf of Aqaba - Potential effects from desalination plants. *Water Research*, 144, 183-191. <https://doi.org/10.1016/j.watres.2018.07.009>

Dionisio-Sese, M., & Miyachi, S. (1992). The effect of sodium chloride on carbonic anhydrase activity in marine microalgae. *Journal of Phycology*, 28. <https://doi.org/10.1111/j.0022-3646.1992.00619.x>

Greco, D. A., Arnott, S., Fournier, I., & Schamp, B. (2021). Effects of chloride and nutrients on freshwater plankton communities. *Limnology and Oceanography Letters*, 8. <https://doi.org/10.1002/lol.210202>

Fu, D., Zhang, Q., Fan, Z., Qi, H., Wang, Z., & Peng, L. (2019). Aged microplastics polyvinyl chloride interact with copper and cause oxidative stress towards microalgae *Chlorella vulgaris*. *Aquatic Toxicology*, 216, 105319. <https://doi.org/10.1016/j.aquatox.2019.105319>

Evans, D. (1967). Sodium, chloride and water balance of the intertidal teleost, *Xiphister atropurpureus*. I. Regulation of plasma concentration and body water content. *The Journal of experimental biology*, 47 3, 513-7

Chen, F., Ma, J., Zhong, Z., Liu, H., Miao, A., Zhu, X., & Pan, K. (2023). Silicon limitation impairs the tolerance of marine diatoms to pristine microplastics. *Environmental Science & Technology*. <https://doi.org/10.1021/acs.est.2c09305>

Kranzler, C. F., Krause, J., Brzezinski, M., Edwards, B., Biggs, W. P., Maniscalco, M., McCrow, J., Van Mooy, B. V., Bidle, K., Allen, A., & Thamatrakoln, K. (2019). Silicon limitation facilitates virus infection and mortality of marine diatoms. *Nature Microbiology*. <https://doi.org/10.1038/s41564-019-0502-x>

Lehtimäki, M., Sinkko, H., & Tallberg, P. (2016). The role of oxygen conditions in the microbial dissolution of biogenic silica under brackish conditions. *Biogeochemistry*, 129, 355-371. <https://doi.org/10.1007/s10533-016-0237-1>

Fuhrman, J., Chisholm, S., & Guillard, R. (1978). Marine alga *Platymonas* sp. accumulates silicon without apparent requirement. *Nature*, 272, 244-246. <https://doi.org/10.1038/272244A0>

Maldonado, M., López-Acosta, M., Sitjà, C., García-Puig, M., Galobart, C., Ercilla, G., & Leynaert, A. (2019). Sponge skeletons as an important sink of silicon in the global oceans. *Nature Geoscience*. <https://doi.org/10.1038/s41561-019-0430-7>

Ma, J., Zhou, B., Tan, Q., Zhang, L., & Pan, K. (2019). The roles of silicon in combating cadmium challenge in the marine diatom *Phaeodactylum tricornutum*. *Journal of Hazardous Materials*. <https://doi.org/10.1016/j.jhazmat.2019.121903>

Zhou, B., Ma, J., Chen, F., Zou, Y., Wei, Y., Zhong, H., & Pan, K. (2020). Mechanisms underlying silicon-dependent metal tolerance in the marine diatom *Phaeodactylum tricornutum*. *Environmental Pollution*,



262, 114331. <https://doi.org/10.1016/j.envpol.2020.114331>

López-Acosta, M., Leynaert, A., & Maldonado, M. (2016). Silicon consumption in two shallow-water sponges with contrasting biological features. *Limnology and Oceanography*, 61. <https://doi.org/10.1002/lno.10359>

Kristiansen, S., & Hoell, E. (2002). The importance of silicon for marine production. *Hydrobiologia*, 484, 21-31. <https://doi.org/10.1023/A:1021392618824>

Morse, D. (1999). Silicon biotechnology: Harnessing biological silica production to construct new materials. *Trends in Biotechnology*, 17, 230-232. [https://doi.org/10.1016/S0167-7799\(99\)01309-8](https://doi.org/10.1016/S0167-7799(99)01309-8)

Haas, A. F., Fairouz, M. F., Kelly, L. W., Nelson, C. E., Dinsdale, E. A., Edwards, R. A., Giles, S., Hatay, M., Hisakawa, N.,

Knowles, B., Lim, Y. W., Maughan, H., Pantos, O., Roach, T. N., Sanchez, S. E., Silveira, C. B., Sandin, S., Smith, J. E., & Rohwer, F. (2016). Global microbialization of coral reefs. *Nature Microbiology*, 1(6), 1-7. <https://doi.org/10.1038/nmicrobiol.2016.42>

Bednarz, V. N., Grover, R., & Ferrier-Pagès, C. (2020). Elevated ammonium delays the impairment of the coral- dinoflagellate symbiosis during labile carbon pollution. *Aquatic Toxicology*, 218, 105360.

Cárdenas, A., Neave, M. J., Haroon, M. F., Pogoreutz, C., Rädercker, N., Wild, C., Gärdes, A., & Voolstra, C. R. (2017). Excess labile carbon promotes the expression of virulence factors in coral reef bacterioplankton. *Nature News*. <https://www.nature.com/articles/ismej2017142>

Baird, A. H., Madin, J. S., Álvarez-Noriega, M., & Madin, E. M. P. (2012). Effects of Nutrients and Suspended Sediment on Coral Growth. *Marine Ecology Progress Series*, 461, 235-245.

Kline, D. I., Kuntz, N. M., Breitbart, M., Knowlton, N., & Rohwer, F. (2006). Role of elevated organic carbon levels and microbial activity in coral mortality. *Marine Ecology Progress Series*, 314, 119-125.

Fabricius, K. E. (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Marine Pollution Bulletin*, 50(2), 125-146.

Marubini, F., & Davies, P. S. (1996). Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. *Marine Biology*, 127(2), 319-328.

Wiedenmann, J., D'Angelo, C., Smith, E. G., Hunt, A. N., Legiret, F. E., Postle, A. D., & Achterberg, E. P. (2013). Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nature Climate Change*, 3(2), 160- 164.

Wooldridge, S. A. (2009). Water quality and coral bleaching thresholds: formalising the linkage for the inshore reefs of the Great Barrier Reef, Australia. *Marine Pollution Bulletin*, 58(5), 745-751.

Allemand, D., Tambutté, É., Zoccola, D., & Tambutté, S. (2011). Coral calcification, cells to reefs. *Coral Reefs*:



An Ecosystem in Transition, 119-150. https://doi.org/10.1007/978-94-007-0114-4_8

Falkowski, P. G., Dubinsky, Z., Muscatine, L., & McCloskey, L. (1993). Population control in symbiotic corals. *Bioscience*, 43(9), 606-611. <https://doi.org/10.1126/science.259.5099.187>

Ferrier-Pagès, C., Rottier, C., Béraud, E., & Levy, O. (2010). Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: Effect on the rates of photosynthesis. *Journal of Experimental Marine Biology and Ecology*, 390(2), 118-124. <https://doi.org/10.1016/j.jembe.2010.05.007>

Grover, R., Maguer, J.-F., Allemand, D., & Ferrier-Pagès, C. (2008). Uptake of dissolved free amino acids by the scleractinian coral *Stylophora pistillata*. *Journal of Experimental Biology*, 211(6), 860-865. <https://doi.org/10.1242/jeb.015487>

Houlbrèque, F., & Ferrier-Pagès, C. (2009). Heterotrophy in tropical scleractinian corals. *Biological Reviews*, 84(1), 1-17. <https://doi.org/10.1017/S1464793108004665>

Rodrigues, L. J., & Grottoli, A. G. (2006). Lipid storage changes in response to bleaching events in two Pacific coral species. *Limnology and Oceanography*, 51(1), 595-602. https://doi.org/10.4319/lo.2006.51L_part_2.0595

Sebens, K. P., Vandersall, K. S., Savina, L. A., & Graham, K. R. (1996). Zooplankton capture by two scleractinian corals, *Madracis mirabilis* and *Montastrea cavernosa*, in a field enclosure. *Marine Biology*, 127(2), 303-317. <https://doi.org/10.1007/BF00349100>

Shiah, F. K., & Ducklow, H. W. (1994). Temperature and substrate regulation of bacterial abundance, production, and specific growth rate in Chesapeake Bay, USA. *Marine Ecology Progress Series*, 103, 297-308. <https://doi.org/10.3354/meps103297>

Sorokin, Y. I. (1973). On the feeding of some scleractinian corals with bacteria and dissolved organic matter. *Marine Biology*, 19(4), 329-341. <https://doi.org/10.1007/BF00368023>

Baker, A. C. (2003). Flexibility and specificity in coral-algal symbiosis: Diversity, ecology, and biogeography of Symbiodinium. *Annual Review of Ecology, Evolution, and Systematics*, 34, 661-689. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132417>

Banaszak, A. T., & Trench, R. K. (1995). Effects of ultraviolet (UV) radiation on marine microalgal-invertebrate symbioses. I. Response of the algal symbionts in culture and in hospite. *Journal of Experimental Marine Biology and Ecology*, 194(3), 233-250. [https://doi.org/10.1016/S0022-0981\(95\)80006-8](https://doi.org/10.1016/S0022-0981(95)80006-8)

Benavides, M., Houlbrèque, F., Camps, M., Lorrain, A., Grosso, O., & Bonnet, S. (2018). Diazotrophic symbioses between marine diatoms and bacteria drive biogeochemical cycling. *Nature Communications*, 9(1), 1-8. <https://doi.org/10.1038/s41467-018-04447-1>

Dykens, J. A., & Shick, J. M. (1982). Oxygen production by endosymbiotic algae controls superoxide



dismutase activity in their animal host. *Nature*, 297(5868), 579-580. <https://doi.org/10.1038/297579a0>

Falkowski, P. G., Dubinsky, Z., Muscatine, L., & McCloskey, L. (1993). Population control in symbiotic corals. *Bioscience*, 43(9), 606-611. <https://doi.org/10.1126/science.259.5099.187>

Ferrier-Pagès, C., Rottier, C., Béraud, E., & Levy, O. (2011). Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: Effect on the rates of photosynthesis. *Journal of Experimental Marine Biology and Ecology*, 390(2), 118-124. <https://doi.org/10.1016/j.jembe.2010.12.027>

Harrison, P. L., & Wallace, C. C. (1990). Reproduction, dispersal and recruitment of scleractinian corals. *Ecosystems of the World*, 25, 133-207.

Le Tissier, M. D. A. (1988). The role of heterotrophic nutrition in the physiology of the coral-zooxanthella symbiosis. *Marine Biology*, 98(3), 259-267. <https://doi.org/10.1007/BF00301961>

Sammarco, P. W., & Risk, M. J. (1990). Large-scale patterns in internal bioerosion of Porites: Cross continental shelf trends on the Great Barrier Reef. *Marine Ecology Progress Series*, 59, 145-156. <https://doi.org/10.3354/meps059145>

Shiah, F. K., & Ducklow, H. W. (1994). Temperature and substrate regulation of bacterial abundance, production, and specific growth rate in Chesapeake Bay, USA. *Marine Ecology Progress Series*, 103, 297-308. <https://doi.org/10.3354/meps103297>

Sorokin, Y. I. (1973). On the feeding of some scleractinian corals with bacteria and dissolved organic matter. *Marine Biology*, 19(4), 329-341. <https://doi.org/10.1007/BF00368023>

Szmant-Froelich, A., Reutter, M., & Riggs, L. (1980). Sexual reproduction of *Favia fragum* (Esper): Lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico. *Bulletin of Marine Science*, 30(3), 583-591.

Yamamoto, Y., Hara, K., & Hara, T. (2001). Inhibitory effects of vitamin E on growth of cultured cancer cells: Is the inhibition due to the antioxidant activity? *Cancer Research*, 61(5), 1513-1518.



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