TRITON TECHNICAL PAPER

The TRITON 'REEFKEEPING' RATIOS

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Abstract

The practice of closed system reefkeeping has improved significantly since accurate, cost effective ICP testing of seawater became widely available to marine aquarists. However, the view that reef systems can be understood primarily in terms of the depletion of inorganic elements ignores the complex interplay between organic chemistry and the biology of reef systems: animals, plants, bacteria, cyanobacteria and fungi.

To deal with this some have used biochemical proxies (e.g. Redfield ratio) derived from analysis of plankton in the open ocean as a guideline for the behaviour of seawater in closed reef systems. Here, based on modelling of a large database of closed system reef tank seawater analyses, we can report this assumption to be misguided. Instead we propose a suite of carbon, nitrogen and phosphorus ratios (TRITON Ratios) for use in closed reef systems.

Using N-DOC (CHNS elemental analyser) testing to produce the TRITON ratios it is now possible to infer the presence of various carbon and nitrogen species and their behaviour in previously unmeasured metabolic pathways making it possible to control and manipulate closed system seawater chemistry for increased system stability.

* Note: the phosphorus value used in the TRITON Ratios is supplied by a recent ICP test.

Introduction

Aquarists concerned with the husbandry of coral reef systems have long been aware of the inter-connectedness of chemistry and biological processes where slight variations in chemical elements and compounds have the potential to cause significant biological or biochemical responses.

Likewise the ratios between chemical species also needs to be taken into consideration.
A fundamental concept relating to the organic chemistry of seawater is the Redfield ratio wherein the molar elemental ratio of C:N:P of most phytoplankton (106:16:1) is reported. Superficially, it may appear to be a good idea to match these ratios in closed systems and many aquarists have taken to adding carbon and nitrogen sources without really understanding the cascade of reactions they cause or being able to test for the chemical species actually involved.

In this article we report for the first time on the typical ratios (hereafter referred to as TRITON Ratios) to be found in closed seawater systems under modern reef husbandry techniques.

TRITON Ratios

TRITON Ratios are a useful troubleshooting tool for the modern reef aquarist with access to analytical laboratory testing. When used correctly they allow the aquarist to maintain a more natural balance of the organic compounds found in seawater leading to improved understanding and husbandry outcomes.

The TRITON Ratios are as follows:

- The ratio between *Nitrogen and Nitrogen in the nitrate ion (N : NO_3/N)*
- The ratio between *Nitrogen - Carbon - Phosphorus* (N:C:P)*
- The ratio between *Inorganic Carbon and Organic Carbon (C/i : C/o)*
- The ratio between *Nitrogen - Inorganic Carbon - Organic Carbon - Phosphorus (N : C(i) : C(o) : P)*

Method

A large number of analyses were made over the course of a year at TRITON Lab using a CHNS elemental analyser (N-DOC) and ICP-OES (ICP) with proprietary modifications by TRITON Lab to improve performance with seawater. Dual sample N-DOC / ICP tests were conducted on seawater samples:

a. submitted from our global TRITON user base.
b. supplied by several Public Aquaria.
c. supplied by project partners Cairns Marine and Horniman Museum.

At the time of writing the database of analyses draws on 23000 users and >200 000 tests carried out by TRITON Lab representing the largest analytical data set on the behaviour of seawater in closed reef systems ever assembled.

Samples were handled under Laboratory conditions to minimise contamination. No dilution or acidification was used and all samples were tested within 3 days and again after 5 days to ensure no shift of organic carbon. Sample tubes were tested for suitability and the method validated to run undiluted samples.

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The data were input to a database to calculate the TRTION Ratios (as described below) and visualise the results for comparison. The data were reviewed weekly and matched with biochemical observations from the supplier.

Discussion

Our testing has shown that the ratio of N : NO_3/N is important in understanding the Nitrogen nutrient cycle in seawater aquaria. Typically the marine Nitrogen cycle, as understood by aquarists, focuses on a series of nitrification reactions mediated by bacteria - as per Figure 1.

However in reality other nitrogen bearing organic molecules are also in play and become increasingly influential in smaller closed systems. For example: proteins break down into amino acids, thence Ammonia / Ammonium (NH_4 / NH_3), before finally

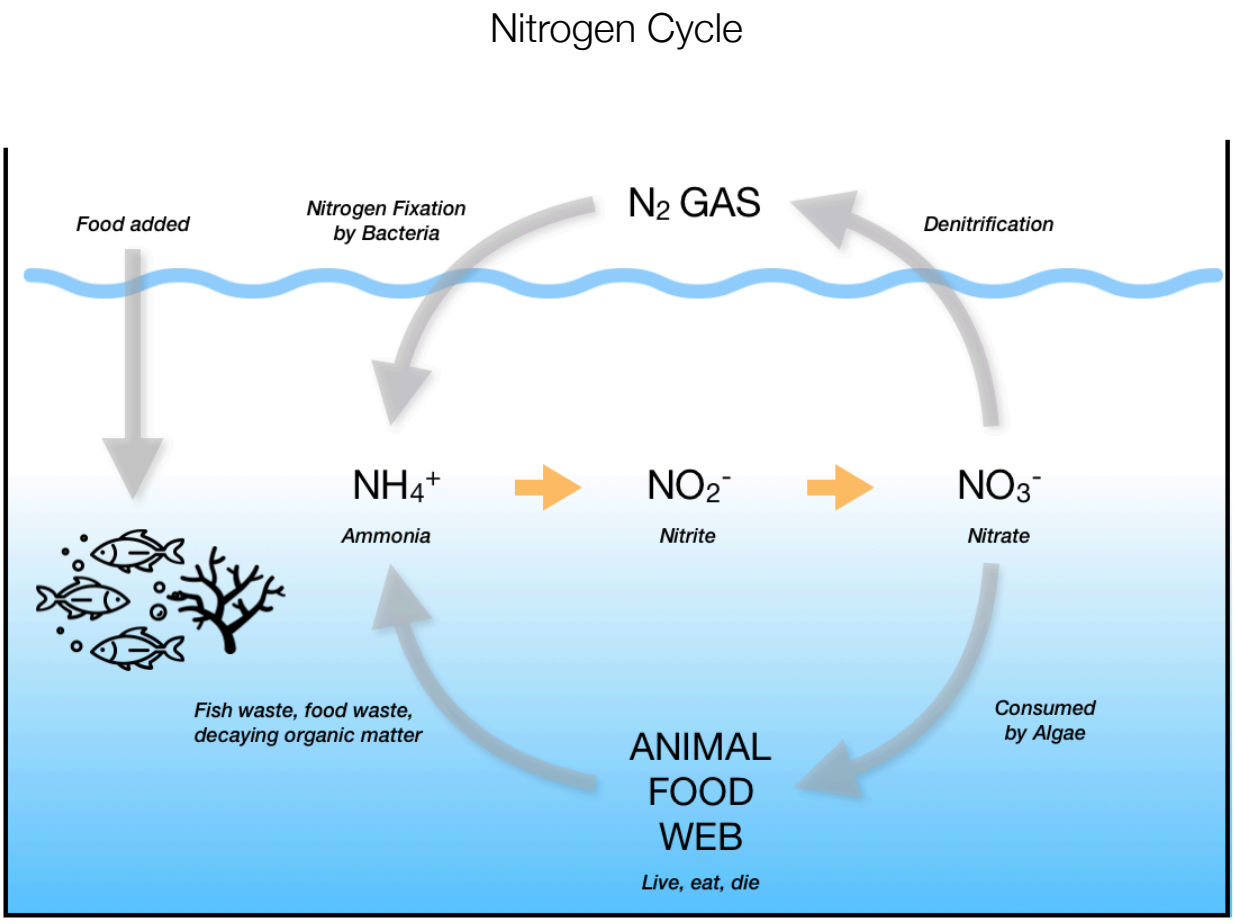

Figure 1 Simplified nitrogen cycle as understood by many aquarists.

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reaching NO_3 through the familiar oxidisation reactions - see Figure 2.

It is important to note that the NH_4 (mid nitrogen cycle) is a more bio-available and energy rich as nutrient for bacteria than the NO_3 at the end. Consequently these organisms / organic reactions have the potential to influence the seawater chemistry of closed reef systems in addition to the better known nitrification bacterial pathway traditionally measured at the end stage NO_3 using droplet test kits. Indeed measuring NO_3 alone is to focus on measuring the 'leftover' N in the organic system after everything else has bio-reacted.

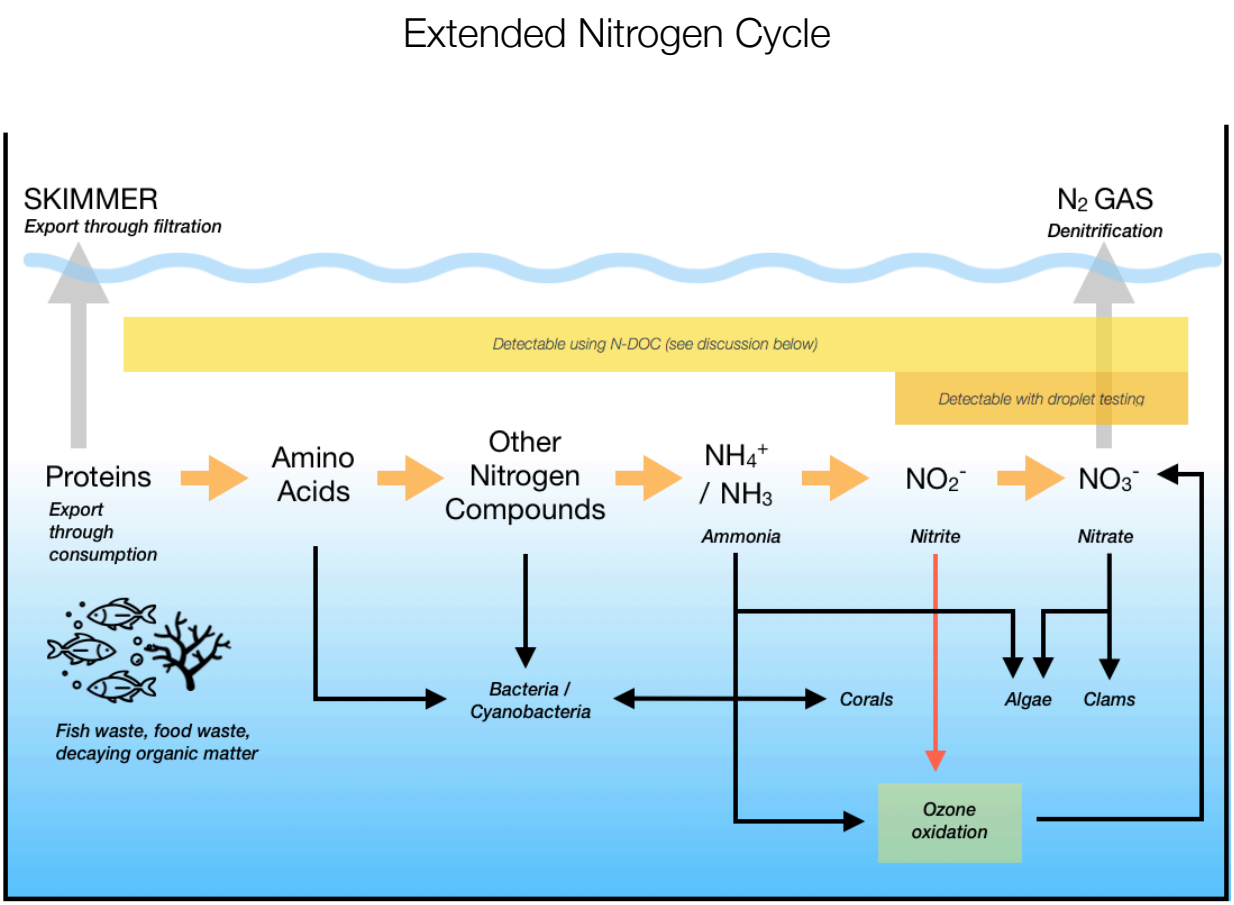

Figure 2 Schematic representation of extended nitrogen cycle with microbial side loop

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It has long been understood that excess organic material leads to high nutrients and problems involving bacteria, cyanobacteria and algae in closed reef aquaria. Traditionally excess organic material such as proteins have been removed using a protein skimmer. However the assumption that by keeping nutrients 'low' in this way we need only measure NO_3 to maintain system health is too simplistic. It represents only part of the story.

Despite the use of protein skimmers the cascade of biochemical reactions seen in Figure 2 continues, to a greater or lesser degree, unseen and unmeasured by the aquarist. It is critical to understand that these reactions are not guaranteed to directly influence NO_3 such that it is possible for a nutrient rich environment to go undetected by NO_3 testing.

TRITON set out to solve this problem by conducting research aimed at understanding nitrogen nutrient pathways in greater detail and modelling how they influenced the behaviour of closed system seawater using our large database of analytical observations.

The end goal being to arrive at 'set points' that allow the aquarist to maintain system stability optimal for coral growth and health while inhibiting the development of unwanted biological responses from bacteria, cyanobacteria and algae.

From this work it was possible to derive the proportions of key measurable organic and inorganic chemical species typical of stable healthy reef systems - the TRITON Ratios.

Nitrogen & Nitrogen in the Nitrate Ion (N : NO_3/N)

TRITON Ratio in mol… 1 : 3

The TRITON Ratio (N— NO_3/N) contrasts how much nitrogen is bound in NO_3 and how much as other nitrogen species. For the aquarist this ratio can be used to:

- assess the performance (size or efficiency) of protein skimmers.
- detect the presence or overabundance of ammonium / amino acids due to poor nutrient control or blind dosing of carbon and nitrogen sources.
- understand the cause of primary producer breakouts: bacteria, cyanobacteria and algae.

Maintaining the TRITON Ratio (N— NO_3/N) at 1 : 3 ensures a competitive balance among primary producers and keeps cyanobacteria 'within bounds' as good nitrogen metabolisers. Better understanding of this ratio has the potential to deliver even more control to the aquarist and is a focus of ongoing research at TRITON.

NItrogen, Carbon and Phosphorus* (N : C : P)

TRITON Ratio in mol… 147 : 12400 : 1

The most widely known 'nutrient ratio' among aquarists and the least understood - especially as it relates to reefkeeping. Many aquarists are aware of the Redfield ratio (1 Mol P : 16 Mol N : 106 Mol C - see Introduction above) and use it as a guideline for closed seawater aquaria. This is an unfortunate and potentially damaging practice promoted by

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misleading marketing and a poor understanding of the science in the wider aquarium industry.

The Redfield ratio is a stoichometry measure derived from analyses of phytoplankton biomass in the open ocean - not seawater itself. Importantly it does not differentiate between inorganic and organic carbon and should not be applied to seawater in closed system reef aquaria as it would deliver a set point of around 0,24 mg for inorganic carbon - around 117 times less than reef seawater. Likewise if applied to organic carbon, it would deliver values about 10 times less than reef seawater.

The Redfield values for nitrogen and phosphorus also need to be re-assessed as they are often in stark variance with low nutrient environments such as coral reefs!

However in reef aquaria the uptake of these nutrients does not need to be balanced as both nitrogen and phosphate can be exported discretely from the aquarium system using denitrification, skimmers or phosphate removers.

Using a modified CHNS elemental analyser TRITON conducted analyses on samples from closed system seawater systems supplied by aquarists from around the world to determine the appropriate set point ratio for N-C-P in closed reef seawater systems. This ratio is a major breakthrough in understanding for modern reefkeeping.

Maintaining the TRITON Ratio for N-C-P is the foundation of balanced water chemistry in reefkeeping and is fundamental to making a tank look good! Departure from this ratio is unfavourable to coral growth and health, and may ultimately lead to problems with bacteria / cyanobacteria even if other measures of chemistry (e.g. ICP analysis) are good.

Inorganic and Organic Carbon (C/i : C/o)

TRITON Ratio in mol… 9 : 1

This novel ratio will be unfamiliar to reefkeepers because until now it has not been possible for aquarists to consider inorganic and organic carbon separately. N-DOC testing provides a new high resolution lens for understanding the behaviour of carbon in reef aquaria.

The primary purpose of this ratio is to gauge the impact of inorganic carbon supplementation by providing a measure of how effectively it is being metabolised.

The values should be kept in balance as inorganic carbon supplementation is a very important, if not the most important parameter in reefkeeping. Not only does it deliver the carbonate ions needed for skeleton formation in stony corals, snails and clams but also CO_2 (as H_2CO_3) for photosynthesis.

Nitrogen, Inorganic Carbon, Organic Carbon and Phosphorus* (P : C(i) : C(o) : N)

TRITON Ratio in mo… 1 : 11150 : 1250 : 147

This TRITON Ratio is an optimised measure for closed reef systems. Once again it is at variance with values from the natural world which are known to vary with latitude, depth and even time of the year.

This ratio again takes advantage of the capacity for N-DOC testing to differentiate between the inorganic and organic forms of carbon providing a powerful troubleshooting tool for specific problems like poor coral growth and coloration.

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For example if C(o) and C(i) are low but N and P are high, then supplementing an alkaline earth metal and organic carbon salt such as calcium acetate may solve the imbalance by lowering the nutrients due to acetate metabolisation and increasing C(i) from H_2CO_3 formed out of CO_2.
Note: this would not be the case if C(o) is high as this may also cause problems.

Achieving and Maintaining TRITON Ratios

Based on the typical variances we see in our N-DOC seawater testing TRITON Lab has developed supplements to assist aquarists to achieve and maintain the TRITON Ratios. This supplementation advise accompanies your test results and dosing is specifically tailored to the size of the aquarium.

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Conclusion

With the advent of N-DOC testing the aquarist finally has access to cost a effective way to detect and measure the presence of key organic chemical species in the reefkeeping system. The development of the TRITON Ratios delivers a powerful new framework of understanding to maintain optimal conditions for coral growth and health while inhibiting undesirable interactions between nutrients, bacteria, cyanobacteria and algae in closed systems.

The TRITON Ratios illuminate hitherto misunderstood and unmeasured nutrient pathways. Together with ICP testing they empower the modern aquarist to participate in a new era of informed, science based, high resolution reefkeeping.

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