ECOLOGICAL STOICHIOMETRY IN AQUATIC ECOSYSTEMS

Dedmer B. Van de Waal
Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 25750, Bremerhaven, Germany

Maarten Boersma
Alfred Wegener Institute for Polar and Marine Research, Biologische Anstalt Helgoland, Postbox 180, 27483 Helgoland, Germany

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Summary

This chapter focuses on the balance of the elements carbon, nitrogen and phosphorus in ecological interactions and processes of aquatic ecosystems. Primary producers stand at the base of the food web, transforming inorganic nutrients, using light energy, into organic biochemicals. These biochemicals are characterized by a specific elemental composition, differing in the relative content of carbon, nitrogen and phosphorus. As the biochemicals are related to specific cellular functions, shifts in primary producer C:N:P stoichiometry may alter its growth, and affect the phytoplankton community composition. Subsequently, such changes at the base of the food web may cascade throughout the entire aquatic ecosystem.

In aquatic ecosystems, the main primary producer is phytoplankton. The elemental C:N:P ratio of phytoplankton typically reflects the so-called Redfield ratio of 106:16:1, but can also greatly vary. This variation is caused by differences in availability of inorganic carbon, light energy, and nutrients, which will influence resource allocation to biochemicals, and affect phytoplankton growth. Typically, phytoplankton carbon:nutrient stoichiometry increases with 1) decreasing growth rates as result of enhanced nutrient limitation, 2) decreasing nutrient availability relative to other non-limiting nutrients, and 3) enhanced light and CO₂ availability, in particular under nutrient limited conditions. Changes in phytoplankton growth and elemental composition may have consequences for the entire food web, as it determines the quantity and quality of food available for herbivorous zooplankton. In contrast to the elemental ratios of phytoplankton, zooplankton elemental ratios are more constraint, which is referred to as elemental homeostasis. This constraint is due to a fixed
allocation of elements to structural biochemicals, and due to the lack of efficient storage mechanisms.

Taking the variable elemental stoichiometry of phytoplankton into account, there is often a mismatch in elemental composition between autotroph and heterotroph. Typically, phytoplankton is more carbon rich as compared to zooplankton, resulting in higher carbon:nutrient ratios. Zooplankton developed several mechanisms to account for this mismatch, including selective feeding, enhanced turnover rates, and selective respiration or discharge. The extent to which a zooplankton species is affected by a stoichiometric mismatch with the available food will further depend on its elemental composition, its growth rate, and its size. The stoichiometric mismatch between phytoplankton and zooplankton may subsequently cascade to higher trophic levels.

Eutrophication and climate change will alter the availability of nutrients, carbon and light in aquatic ecosystems. These anthropogenic-driven changes in resource availability will likely affect phytoplankton C:N:P stoichiometry. This will have implications for consumer growth and reproduction, and thus may alter the structure and functioning of entire aquatic food webs.

All in all, the field of ecological stoichiometry provides a very useful framework to couple inorganic elements with ecosystem structure and functioning.

1. Introduction

All organisms are built from the same basic set of elements. Apart from hydrogen (H) and oxygen (O), predominant elements in life are carbon (C) and nitrogen (N). These four elements constitute up to 99% of living biomass. Many other elements are also important in cellular processes, such as for example magnesium in photosynthesis, or iron in oxygen transport through the body. Of these ‘lesser’ elements, phosphorus (P) plays the most prominent role as it is essential for both the storage and transfer of genetic information, and the energy metabolism within cells.

Primary producers link the non-living world with the living world by converting inorganic carbon and nutrients into organic compounds, mostly with the help of light as energy source. Where macrophytes and macro-algae may dominate in more shallow systems, microalgae (phytoplankton) are the key primary producers in most pelagic aquatic systems. They are the main prey of herbivorous grazers such as ciliates, rotifers and crustaceans (zooplankton). These primary consumers are in turn consumed by secondary consumers, for instance by fish (Stibor and Sommer 2004). Growth and reproduction of consumers obviously greatly depends on the quantity of their food, typically determined by the availability of carbon (i.e. energy). However, also the quality of the food is of importance, especially when the quantity of the food is so high that energy requirements of maintenance processes are met. In the stoichiometric context of this chapter food quality will be mainly determined by the relative availability of carbon and nutrients.

The balance of carbon and nutrients in phytoplankton depends on the availability of light and resources in the environment, which in turn affects the production of organic
compounds. Zooplankton consumes phytoplankton, and thereby obtains energy and nutrients that were accumulated by the algae. The ratios between the elements available in phytoplankton do not always match the requirements of the consumers. The field of ecological stoichiometry studies the balance between these elements among organisms and their interactions with the environment. As such, the field of ecological stoichiometry incorporates ‘first principles’ like mass balance, and can be applied to a variety of processes from the cellular to ecosystem level.

For this chapter, we focus on aquatic ecosystems only, even though ecological stoichiometry obviously applies to all interactions between producers and consumers. Furthermore, we will focus on the elements carbon, nitrogen and phosphorus. These elements, apart from oxygen and hydrogen (mainly present in water), typically show the highest relative abundance in living organisms compared to the non-living world. As such, these elements have the highest potential to be limiting biological production. For detailed information on the complete field of ecological stoichiometry we refer to the seminal book of Robert W. Sterner and James J. Elser (2002).

2. Linking Biological Functions to Elemental Stoichiometry

Primary producers typically take up inorganic carbon and nutrients from their environment by using light energy (photosynthesis), and transfer inorganic elements with the obtained energy into organic biochemicals (Fig. 1). Hence, these biochemicals are the first step in coupling elemental composition of the non-living world with the stoichiometry of life. As the various biochemicals consist of different elements in different ratios, they depend differently on the elements that are acquired. Moreover, also the machinery necessary to synthesize these substances has different elemental requirements. The synthesized biochemicals are then used to build cellular compounds, with specific cellular functions. Can these cellular functions be coupled with a specific C:N:P stoichiometry? To answer that question, the acquisition and assimilation of carbon, nitrogen and phosphorus from inorganic elements towards structural compounds will be discussed in this paragraph.

2.1. Conventions about Element Ratios

In literature associated with ecological stoichiometry the relative content of an element in an organism is expressed in different sorts of units (e.g. as percentage of dry weight, or as atomic ratios). Here we aim to structure these units in line with Sterner and Elser (2002). Where we discuss elemental content, amounts are expressed as mass of an element relative to the raw dry-mass (i.e. including inorganic and organic compounds) of the whole organism (e.g. %C by mass). Preferentially, however, units are converted to atomic ratios of elements (e.g. C:N), as chemical reactions are also described by atom-to-atom interactions, and when we think in building blocks of different organic compounds, ratios are the measure of choice. Elemental ratios are useful in describing the relative contribution of one element to another. Ratios however, do not give information about the absolute amount of respective elements. For instance, a high C:P ratio may indicate a relatively high amount of carbon, relative to the phosphorus content, but gives no information on the absolute amount of both carbon and phosphorus in the organism, which may be low or high.
2.2. Nutrient Acquisition

Phytoplankton obtains its carbon from dissolved CO$_2$ and bicarbonate (HCO$_3^-$) during photosynthesis. Therefore, the availability of both inorganic carbon and light plays an important role in carbon acquisition of phytoplankton. The inorganic carbon is converted into carbohydrates, which can be used for synthesis of polysaccharides, amino acids and nucleotides, or converted into fatty acids and lipids (Fig. 1). Nitrogen is taken up as nitrate (NO$_3^-$), nitrite (NO$_2^-$) or ammonium (NH$_4^+$). Several cyanobacteria species can fix nitrogen from N$_2$, and other species can take up nitrogen in the organic form, for instance as urea. Once taken up, inorganic nitrogen is incorporated into a carbon-backbone and assimilated to amino acids (Fig. 1). Phosphorus is usually taken up as ortho-phosphate, which includes phosphate (PO$_4^{3-}$), hydrogen phosphate (HPO$_4^{2-}$), and dihydrogen phosphate (H$_2$PO$_4^-$), but a few examples also exist of uptake of organic phosphorus compounds. Cellular inorganic phosphorus (P$_i$), together with amino acid derived nucleobases, is incorporated into a carbon-backbone forming nucleotides (Fig. 1).

![Schematic diagram](image)

Figure 1. Schematic diagram of carbon, nitrogen and phosphorus uptake and assimilation in phytoplankton. C$_i$, N$_i$ and P$_i$ refer to cellular inorganic carbon, inorganic nitrogen, and inorganic phosphorus, respectively.

2.3. From Inorganic Nutrients to Organic Compounds

Inorganic elements taken up by primary producers are assimilated into biochemicals and cellular structural compounds. The key biochemicals are carbohydrates, lipids (consisting of fatty acids), proteins (consisting of amino acids), and nucleic acids (consisting of nucleotides). The different biochemicals comprise varying elemental ratios. Hence, changes in the relative composition of these biochemicals will affect the C:N:P stoichiometry of organisms. As the biochemicals fulfil different cellular functions, the C:N:P stoichiometry of the cell as a whole may be coupled to these functions.
2.3.1. Carbohydrates

Glucose is the most commonly known carbohydrate and it plays a central role in the energy metabolism of organisms. Carbohydrates also serve as important carbon and energy storage compounds, for instance as polysaccharides such as starch and glycogen. In addition, polysaccharides are also part of the structural biomass, for instance as cellulose in plants, as peptidoglycan in bacterial cell membranes, and as chitin in fungi and invertebrates.

Cellulose is a chain of glucose and consists of water and carbon, i.e. it does not contain nitrogen or phosphorus (Table 1). Peptidoglycan and chitin also contain nitrogen, resulting in a structural atomic C:N ratio of 2.6 and 5.9 respectively (Sterner and Elser 2002). Thus, increases in relative glucose content of cells will enhance the C:N ratio of the cell. Increases in peptidoglycan and chitin content will increase the cellular carbon content, but the nitrogen content as well, thereby enhancing the cellular N:P ratio.

2.3.2. Lipids

Lipids are biomolecules used amongst others for storage of energy and as structural compounds in cell membranes, but also as potential signaling compounds (e.g. sterols). Typical lipids used for energy storage are the tricylglycerols, which consist of three fatty acids linked to a glycerol molecule. These tricylglycerols have a high carbon content, and are well suited for carbon (energy) storage (Table 1). Fatty acids and glycerols do not contain nitrogen and phosphorus. Hence, tricylglycerols are carbon rich biochemicals, devoid of nitrogen and phosphorus. Another important group of lipids, prominently involved in cellular membranes, are phosphoglycerides. This group of lipids contains a phosphoester or phosphonate linkage between the fatty acids and a variable terminal functional group.

As the functional groups often contain some nitrogen, phosphoglycerides contain carbon, nitrogen and phosphorus. Nitrogen however is only present in minor amounts (Table 1). Hence the compounds contain a relatively high amount of phosphorus. Thus, increases in the relative cellular tricylglycerol content will enhance the cellular carbon:nutrient ratio, while an increase in the relative phosphoglyceride content will enhance the overall carbon content, but reduce the N:P ratio.

2.3.3 Proteins

Proteins are involved in many cellular processes and in particular in the form of enzymes. As a result, proteins play a key role in the cellular metabolism. In addition, proteins are important structural compounds, and are involved in cell signaling. Proteins are comprised of one or more polypeptides, which are typically built from chains of different combinations of 20 proteinogenic amino acids. These amino acids are nitrogen based biochemicals, with nitrogen contents varying between 8% in tyrosine to 32% in arginine (by mass; see also Table 1).

Assuming a protein is comprised from an equal proportion of the 20 amino acids, the average carbon content will be 47% (by mass), the nitrogen content will be 15% (by
mass), and the overall atomic C:N ratio will be 3.6 (see also Table 1). This may deviate if proteins contain more N-rich or N-poor amino acids. Yet, the average nitrogen content of 15% (by mass) seems representative for proteins (Sterner and Elser 2002). Hence, increases in the relative protein content may greatly reduce the C:N ratio, but particularly may increase the N:P ratio.

### 2.3.4 Nucleic Acids

Nucleic acids contain the genetic information of all organisms. DNA and RNA are used to encode, transmit and express genetic information. DNA and RNA are constructed from nucleotides that consist of a phosphate group, a nucleobase and a pentose sugar. The nucleobases that can be incorporated are the purines adenine and guanine, and the pyrimidines cytosine, uracil and thymine. The purines and pyrimidines differ in their elemental composition, but as their contribution to nucleic acids is typically equal (Lehninger et al. 1993), an average has been assumed to estimate their carbon, nitrogen and phosphorus content.

Following this assumption, the nucleic acids comprise an average nitrogen content of 14-15% and an average phosphorus content of 8-9% (by mass; see also Table 1). The nitrogen content is relatively high and comparable to proteins. As the nucleic acids also contain a relative high amount of phosphorus, their N:P ratio is relatively low. Thus an increase in the relative cellular content of nucleic acids will likely cause minor changes in the cellular C:N ratios, but will decrease the cellular N:P ratio.

<table>
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<th></th>
<th>% C (mass)</th>
<th>% N (atomic)</th>
<th>% P (atomic)</th>
<th>C: N</th>
<th>N:P</th>
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Table 1. Relative contribution (by mass) of carbon, nitrogen and phosphorus, and the atomic C:N and C:P stoichiometry, of several key biochemicals.
2.3.5. Biominerals

Some organisms contain large amounts of biominerals such as calcium carbonates, silica and hypoxyapatite. Calcium carbonates (CaCO$_3$) can be found in exoskeletons of crustaceans, but are much more visible in for instance mollusk shells, foramineferal tests and coccolithophorid cell walls. The latter two groups play a key role in the biogeochemical cycling of calcium and carbon. Silica (or opal; amorphous hydrated silica SiO$_2$ . nH$_2$O) is a major constituent of diatom frustules, giving diatoms a unique molar Si:C ratio of ~1 (Sommer 1986). Hence, diatoms play a key role in the biogeochemical silicon cycle. Hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$) is the key component of bones and bony materials (e.g. teeth and antlers) of vertebrates. Bones play an important role in determining the elemental stoichiometry of vertebrates. In particular due to the high phosphorus content, bone content may greatly affect organism C:P ratios (see also 4.3).

2.4. Turning Things Around

From the above it has become clear that the elemental composition of an organism is intricately linked to its biochemical composition. This, we can also turn around. Organisms with high C:N and C:P ratios will have a relative high amount of carbon-based biochemicals such as carbohydrates and lipids, whereas they will have relatively low amounts of nitrogen- and phosphorus-based biochemicals such as proteins and nucleic acids, respectively. Thus, the elemental composition of an organism may indicate its relative biochemical composition, which in turn can be associated to specific cellular functions.

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Biographical Sketches

Dr. Dedmer B. Van de Waal (Wijnjewoude, the Netherlands, 1982) is a postdoctoral research associate at the Marine Biogeosciences Department of the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany. He received his Ph.D. degree (2010) at the Laboratory of Aquatic Microbiology of the University of Amsterdam, the Netherlands, in collaboration with the Department of Aquatic Ecology of the Netherlands Institute of Ecology. During his Ph.D. he investigated climate-driven changes in the ecological stoichiometry and toxin production of harmful cyanobacteria. Currently, he studies the impacts of global change on the physiology, stoichiometry and competitive interactions of harmful and calcifying dinoflagellates.

Prof. Dr. Maarten Boersma (Gouda, the Netherlands, 1964) is a senior research scientist at the Biologische Anstalt Helgoland of the Alfred Wegener Institute for Polar and Marine Research. He received his Ph.D. degree (1994) from the University of Amsterdam, the Netherlands, working on the seasonal dynamics of Daphnia species in a shallow eutrophic lake. Subsequently, he spent five years a research associate at the Max-Planck Institute for Limnology in Plön Germany. Since 2001 he is on the island of Helgoland working on the ecological stoichiometry of phytoplankton-zooplankton interactions.