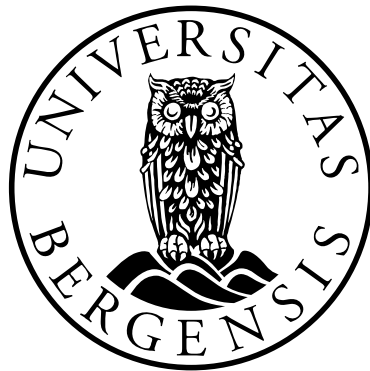# Carbon to nutrient ratios in marine systems: understanding natural variability

*Studies of seasonal, inter-annual and regional variability*

**Helene Frigstad**

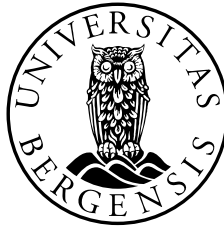Dissertation for the degree philosophiae doctor (PhD)
at the University of Bergen

May 2012

# Carbon to nutrient ratios in marine systems: understanding natural variability

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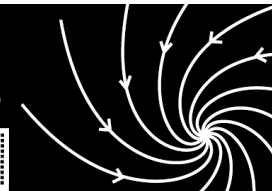
PhD thesis in Chemical Oceanography

Geophysical Institute, University of Bergen

Norway

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My family and friends, here in Bergen, Oslo, Brigthon, Ål and Arendal are very important to me, and I thank you for being who you are and supporting me and what I do. I very much value the time I can spend with you, and look forward to much more of it in the months and years to come. Finally, I wish to thank my husband Erlend, who always lifts my spirits, and keeps me fed, sane and happy.

Helene Frigstad

Bergen, May 2012

# Abstract

Ecological stoichiometry deals with the coupling of the carbon (C), nitrogen (N) and phosphorus (P) cycles at the base of the aquatic food webs. Variation in C:N:P stoichiometry is driven by a multitude of factors, and the aim of this thesis is to assess natural variability and identify the key drivers of seasonal, inter-annual and regional C:N:P stoichiometry.

**PAPER I** focuses on the drivers of seasonal variation in C:N:P stoichiometry in two 20-year time series in the Skagerrak. The main result was that the suspended particulate organic matter (seston) was composed of less than 50% autotrophs at both stations. The sestonic fractions have contrasting C:nutrient ratios, which could result in an apparent Redfield stability over the season.

Light intensity and spectral composition can affect the carbon to nutrient ratios in autotrophs, and uptake of $^{33}$P in three arctic diatoms exposed to ultraviolet radiation (UVR) was tested in **PAPER II**. The results emphasize the species-specific physiological responses of P uptake under UVR stress, and showed in general little support to the hypothesis of increased P uptake during UVR exposure.

**PAPER III** deals with the long-term trends in carbon, nutrients and stoichiometry in the Skagerrak. The nutrient concentrations decreased over the 20-year time period, while the concentrations of C and N in seston, dissolved organic N and the non-autotrophic material all increased significantly and non-linearly between 1998 and 2000. These trends were connected to a reported "darkening" of the coastal waters in Skagerrak, and indicates that a regime-shift took place in the early 2000's.

Over 3600 observations of the C:N ratio in the Arctic and pan-Arctic shelves were analysed in **PAPER IV**, covering an area not included in previous meta-analyses of seston stoichiometry. The C:N ratio in most regions was significantly higher than the Redfield ratio, and showed significant non-constant behaviour, thus violating the assumption of a constant ratio between C and N.

# List of papers

PAPER I:

Frigstad, H., Andersen, T., Hessen, D. O., Naustvoll, L. J., Johnsen, T. M. & Bellerby, R. G. J. 2011. Seasonal variation in marine C:N:P stoichiometry: can the composition of seston explain stable Redfield ratios? *Biogeosciences,* 8**,** 2917-2933.

PAPER II:

Hessen, D. O., Frigstad, H., Faerovig, P. J., Wojewodzic, M. W. & Leu, E. 2012. UV radiation and its effects on P-uptake in arctic diatoms. *Journal of Experimental Marine Biology and Ecology,* 411**,** 45-51.

PAPER III:

Frigstad, H., Andersen, T., Hessen, D. O., Jeansson, E., Skogen, M., Naustvoll, L. J., Miles, M., Johannessen, T. & Bellerby, R. G. J. Long-term trends in carbon, nutrients and stoichiometry in Norwegian coastal waters: evidence of a regime shift. *Manuscript in preparation*.

PAPER IV:

Frigstad, H., Andersen, T., Bellerby, R. G. J., Silyakova, A., & Hessen, D. O. Variation in the seston C:N ratio of the Arctic Ocean and pan-Arctic shelves. *Manuscript in preparation*.

# Contents

# 1.  Background

## 1.1   Carbon and nutrient cycles in the oceans

The atmospheric concentration of carbon dioxide ($CO_2$) is increasing due to combustion of fossil fuels, cement production and land use changes (IPCC, 2007), and despite global policy efforts to reduce emissions the annual rate of increase is currently higher than the most pessimistic IPCC scenario (IPCC, 1995). Oceans have taken up around 50 % of the anthropogenic $CO_2$ since the beginning of the industrial revolution (Sabine et al., 2004), and the land and ocean carbon (C) sinks have thus acted to reduce the effect of anthropogenic emissions of $CO_2$ on the global climate.

Through the biological C pump atmospheric $CO_2$ is fixed into organic matter and the world oceans account for around 50% of the total global primary production (Longhurst et al., 1995).  Photoautotrophs (autotrophs hereafter) utilize light energy to fix inorganic C, nitrogen (N) and phosphorus (P) (collectively called nutrients) to organic matter in the process called photosynthesis. This process is illustrated in the following reaction equation (Anderson, 1995):

$$106 \ CO_2 + 16 \ HNO_3^- + H_3PO_4 + 78H_2O \Leftrightarrow C_{106}H_{175}O_{42}N_{16}P + 150 \ O_2 \qquad (1)$$
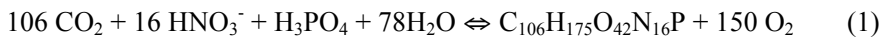
the reverse process is called aerobic remineralization, which is a energy yielding process where organic mater and $O_2$ (oxygen) is consumed and C, nutrients ($NO_3$ (nitrate) and $PO_4$ (phosphate)) and $H_2O$ (water) are released. Organisms are built up of macromolecules and elements in certain proportions, as reflected by Eq. 1.

The stoichiometric ratios of C, N and P in Eq. 1 were described by Redfield (1958), and the so-called Redfield ratio was set to C:N:P = 106:16:1 (atomic ratios). Although the fact that the elemental composition of deep-water chemistry and surface plankton was similar had been known prior to this work, the most revolutionary concept that Redfield set forth was that this similarity was caused by "the plankton

determining the chemical composition of the ocean" (Falkowski, 2000). The stoichiometry of C, N and P will be discussed further in section 1.2.

The autotrophs take up C species dissolved in seawater, and the dissociation of C takes place through several steps:

$$CO_{2(atmos)} \Leftrightarrow CO_{2(aq)} + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{2-} + 2H^+ \qquad (2)$$
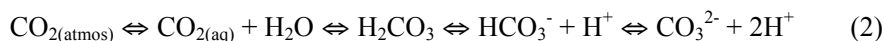
the $CO_2$ molecule combines with water ($CO_{2(aq)}$) after being exchanged over the air-sea interface and forms $H_2CO_3$ (carbonic acid), which rapidly dissociates to form $HCO_3^-$ (bicarbonate) and $CO_3^{2-}$ (carbonate) The sum of all the inorganic C species is called dissolved inorganic carbon (DIC), and the most abundant C species under current oceanic conditions is bicarbonate ($\sim$ 91%) followed by carbonate ($\sim$ 8%) with very low amounts of carbonic acid and aqueous $CO_2$ (combined $\sim$ 1%). Autotrophs rely on a variety of different $CO_2$ concentration mechanisms to increase the $CO_2$ concentration around the cell, and C is normally not regarded as limiting for phytoplankton growth (Giordano et al., 2005). However experimental work has shown that some species might be C-limited at present $CO_2$ concentrations or benefit from increased ambient $CO_2$ (Riebesell et al., 1993, Rost et al., 2008).

The biogeochemical cycles of the two major nutrients, N and P, differ from each other in a fundamental way. Organisms can fix gaseous N ($N_2$) from the atmosphere ($N_2$ fixation) and use nitrate as an oxidant (in place of oxygen as in aerobic remineralization) in the remineralization of organic mater (denitrification). These two processes decouple the N and P cycle, and introduce deviations from a fixed N:P ratio as expected from photosynthesis and remineralization as shown in Eq. 1. Nitrate is classically regarded as the limiting nutrient for phytoplankton growth in the oceans (Ryther, 1969), however, due to the ability of biology to modulate the N cycle, while P must be supplied from external sources (ultimately riverine inputs from terrestrial sources or dust deposition), P can be the limiting nutrient on longer time scales (Broecker and Peng, 1982, Tyrrell, 1999).

The formation of organic matter through photosynthesis takes place in the sunlit upper water column (euphotic zone) in the oceans (see Fig. 1). The organic matter is operationally separated into two pools, the particulate organic matter (seston hereafter) and the dissolved organic matter (DOM). By definition, seston is particulate matter > 0.45 µm, but there are no strict boundaries between these pools, hence for practical reasons seston is treated as matter being retained on a filter (usually with pore size of 0.7 µm), while the dissolved fraction is what passes through. The functional difference between these pools is that the DOM is too small to sink through the water column and is mainly transported with the oceanic currents, while the non-motile seston particles are large enough to sink out of the euphotic zone.
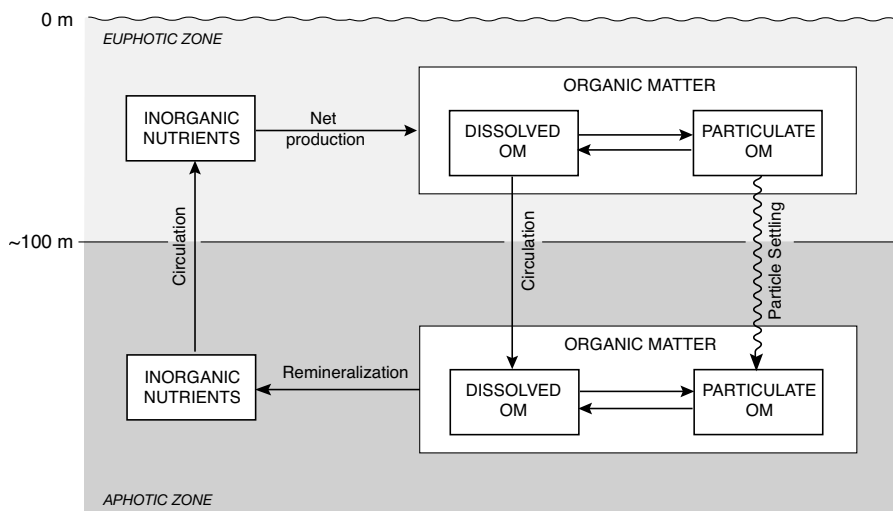
Figure 1. Schematic showing the production and remineralization of organic matter in the ocean. From Sarmiento and Gruber (2006)

Most of the organic material produced in the euphotic zone is remineralized within this layer and resupplies inorganic C and nutrients for continued production, which is called regenerated production. Traditionally this fraction has been calculated from the uptake of $NH_4^+$ (ammonium), which is the preferred N source and is utilized very quickly (Dugdale and Goering, 1967). Around 20 % of the organic matter is transported out of the euphotic zone to the deeper layers (Laws et al., 2000), where it

is remineralized either using oxygen as oxidant where available (as in Eq. 1) or in the absence of oxygen other oxidants might be used (e.g. denitrification, sulphate reduction). The fraction of organic matter leaving the euphotic zone is known as export production, however only a minute but spatially variable fraction of the exported material is buried in the sediments (Hedges and Keil, 1995, Dunne et al., 2007). On longer temporal and spatial scales the material leaving the euphotic zone (export production) has to equal the material brought back to the euphotic zone (new production) for the system to remain in steady-state (Eppley and Peterson, 1979). The new production can be identified as the production in the euphotic zone fuelled by nitrate, as the process of nitrification (nitrifying bacteria that oxidize ammonium to nitrate) is light inhibited, and believed only to occur below the euphotic zone (Dugdale and Goering, 1967). Thereby the new (equal to export production) and the regenerated production of the system can be identified and quantified by using the N cycle and the uptake of the two N species, nitrate and ammonium. Later discoveries have complicated somewhat this picture, with the discovery of atmospheric deposition of both nitrate and ammonium, higher rates of $N_2$ fixation than originally believed and nitrification occurring at the base of the euphotic zone (Sarmiento and Gruber, 2006, Gruber, 2008). In addition, humans are not only perturbing the C cycle, but also the cycles of N and P through industrial fixation of nutrients for fertilizers and the burning of fossil fuels. The human fixation of reactive N (that can be utilized by biology) equalled the $N_2$ fixation in the world oceans over the 1990's (Gruber and Galloway, 2008). The C:N ratio of the autotrophs constitute an important link between the C and N cycles (Gruber and Galloway, 2008), and recent $CO_2$ enrichment experiments indicate that the marine biota could constitute a negative feedback on climate change through a flexible C:N ratio (Ramos et al., 2007, Riebesell et al., 2007).

In the classical Dugdale and Goering (1967) paradigm of new and regenerated production, the heterotrophic prokaryotes (bacteria hereafter) were believed to play a modest role in the consumption and remineralization of C and nutrients. The influencal paper by Azam et al. (1983) introduced the concept of the *microbial loop*

where the bacteria were recognized as significant consumers of DOM and an important food source for higher trophic levels. The consumption of DOM by the microbial loop has been estimated to account for around 50% of the global primary production and the export of DOC to deeper layers to around 20 % of global export production (Hansell et al., 2009). The bacterial utilization of DOM thereby acts to retain nutrients in the euphotic zone and channel energy up to higher trophic levels, which would otherwise be "lost" in classic food web. Bacteria also compete with autotrophs for nutrients, and the balance between carbon and nutrients can affect the outcome of this competition (Thingstad et al., 2008)

The total effect of the biological C pump on the vertical gradients of C and nutrient concentrations in the oceans, is that the surface waters become reduced in nutrients and elevated in oxygen through photosynthesis, while the remineralization of exported organic material cause the deeper and intermediate layers to become enriched in DIC and nutrients and depleted in oxygen. These waters are returned to the surface through upwelling and vertical mixing, which closes the cycle of the organic C pump (upward arrow in Fig. 1).

In addition to the organic C pump, there are two other mechanisms that affect the uptake and distribution of C in the oceans: the solubility pump and the carbonate counter pump (Heinze et al., 1991). The solubility pump is controlled by physical processes, governed by the relative solubility of $CO_2$ in seawater, where cold waters can hold more C relative to warm waters (Weiss, 1974). The carbonate counter pump is driven by the uptake of bicarbonate by organisms (mainly coccolithophorids, foraminifera and pteropods) to create calcium carbonate shells or skeletons. This process also removes DIC from the surface oceans and releases it in the deeper layers, however because the calcification process also releases $CO_2$ it leads to higher partial pressure of $CO_2$ ($pCO_2$) in the surface ocean and consumes alkalinity. Thus the carbonate counter pump has the opposite affect of the organic C pump on the $pCO_2$ in the oceans. The relative effects of the three C pumps can be evaluated with model experiments, and the organic C pump is found to have a dominating influence on the vertical gradients of C and nutrients (70%) over the carbonate C pump (downward

transport of C as calcium carbonate) and the solubility pump (see sect. 8.4 in Sarmiento and Gruber, 2006).

## 1.2   Drivers of variable stoichiometry

The chemical elements and the cycles of C, N and P, as discussed in 1.1 are linked through the production of organic matter by photosynthesis (or by the use of chemical energy as in chemoautotrophs) and the remineralization of this material primarily by bacterial degradation. The proportions in which these elements are utilized is traditionally described by the Redfield ratio of C:N:P = 106:16:1, which links the production and remineralization of organic material and thereby the cycles of C, N and P. Ecological stoichiometry deals with the balance of these elements, and understanding the fundamental processes and interactions responsible for creating the proportions between the elements as can be measured in the field or in a laboratory. It is within this field and in understanding the fundamental processes responsible for coupling the carbon and nutrient cycles this thesis has its main focus.

Ecological stoichiometry can be defined as "the balance of multiple chemical substances in ecological interactions and processes, or the study of this balance" (Sterner and Elser, 2002). The chemical substances, or elements, that can be studied include in principle the entire periodic table, however in the context of ecological stoichiometry the elements C, N and P are of prime interest (even though many marine studies unfortunately do not include P). These three elements dominate the biomass of most organisms due to their role as "major constituents in biological structural molecules" (Sterner and Elser, 2002), yet undoubtedly elements like Si and Fe also play important roles.

Carbon is the backbone of all life on the planet, and its fundamental role is due to this element having the highest binding energy in the periodic table (Williams and Frausto da Silva, 1996). Carbon can form four covalent bonds (with elements including itself, nitrogen, oxygen, hydrogen and sulphur) and therefore allows the organism to form elementally flexible and energy-rich molecules. Biomolecules high in C include: proteins, lipids, carbohydrates and pigments (the elemental composition of biomolecules is based on table 2.2 in Sterner and Elser, 2002). Nitrogen can have a

wide range of stable oxidation states (from +5 in nitrate to -3 in ammonium), which reflects this elements ability to gain or give electrons from or to other elements. Nitrogen in biomolecules thus serve several purposes, both in energy capture and release, and not the least as the key element of proteins (including enzymes), nucleic acids (DNA and RNA) and pigments (for ex. Chlorophyll a). Phosphorus is an important element in the genetic material of all life, even though it is scarce in the non-living world. The importance of P is thought to be related to the stable negative state this element retains in chemical reactions, which is important for the stability and motility of the molecules (Westheimer, 1992). Phosphorus is also present in proteins and lipids, however the highest P content is found in genetic material (DNA and RNA) and in the energy transport molecule ATP.

As described above the elements C, N and P are fundamental to all life and the inorganic forms need to be present for formation of organic material to take place both on land and in the oceans. The Redfield ratio was introduced in section 1.1, and it describes the stoichiometric relationship between C, N and P in dissolved inorganic form (nutrients) and in marine particulate matter (seston). The observation that these two pools had such similar and fixed relationships over the world oceans has had profound implications for biogeochemical studies post-Redfield (for discussion of the Redfield ratio as a paradigm and potential implications of new findings, see section 1.3).

Autotrophs fix C and assimilate nutrients from their surroundings, however the elemental composition of their cells are not merely mirror images of the supply on the outside, i.e. they are not just "what they eat" (Sterner and Elser, 2002, Frost et al., 2005). They can take up C and nutrients at varying rates and have the ability to utilize or store these elements depending on the requirements of the cell, which is a sensible adaption in an environment with highly dynamic nutrient supply and growth conditions. Plant cells frequently have a central vacuole, which allows them to store nutrients and compounds that could otherwise be toxic to the cell in high concentrations. This opens for "luxury consumption" of elements that are in concentrations above what the cell instantaneously needs for growth. For example,

algae can store phosphate as polyphosphate within the central vacuole (Rhee, 1973), when the ambient concentration are higher than the cell's demands and store it for subsequent use. This large flexibility in nutrient uptake makes the autotrophs able to utilize the nutrients available to them, almost regardless of which ratios they might be present in. Therefore, the degree of nutrient limitation is perhaps the single most important determinant of autotroph stoichiometry (Sterner and Elser, 2002).

Goldman et al. (1979) performed culture experiments, where it was found that algae obtained C:N:P ratios close to the Redfield ratio when the growth rate was close to its maximum and the algae did not experience nutrient limitation. Therefore, the observations that seston C:N:P was close to the Redfield ratios meant that oceanic algae must be growing close to their maximum values under nutrient sufficient conditions (e.g. Harris, 1986), and deviations from the Redfield ratios has frequently been used to measure the nutritional status of the autotrophs. However, as noted by Sterner and Elser (2002), this need not be the case, because the autotrophs can be growing under low nutrient conditions as long as the ratio the nutrients are supplied in is close to the Redfield ratio. In addition, other studies have emphasized the contributions of non-algal matter to the C:N:P ratios in seston, and that the seston elemental ratios are not a good indicator of phytoplankton physiological state (Maranon, 2005, Ptacnik et al., 2010, Frigstad et al., 2011).

Light intensity is another important factor for determining autotroph stoichiometry, since the fixation of C is directly linked to the amount of energy available to drive photosynthesis. Therefore, the demand for nutrients will be higher in high light environments, and hence the balance between the dissolved nutrients and light irradiance will have a large effect on the resulting C to nutrient balance in the autotrophs (Sterner et al., 1997). Several experiments have found increasing C:P ratio in algae grown under high light and low ambient P concentrations (Sterner et al., 1997, Hessen et al., 2002). However, not only the intensity of light, but also its spectral composition can affect the C and nutrient acquisition. While high levels of photosynthetically active radiation (PAR, 400 – 700nm) have been shown to increase C:nutrient ratios,  moderate levels of ultraviolet radiation (UVR, 280 – 400 nm) can

yield decreased C:nutrient ratios (Leu et al., 2006, Leu et al., 2007, Hessen et al., 2008). The decreased C:nutrients in response to UVR radiation can either be caused by a lower C fixation through reduced photosynthetic activity (e.g. Neale et al., 2003), or increased phosphate uptake to repair damage to the genetic material (Hessen et al., 1995). The effect of UVR radiation on P uptake is species-specific, however the species-specific physiological role of P under UVR exposure remains unsettled (Hessen et al., 2012).

The intensity of light and concentrations of nutrients follows a predictable pattern in the seasonally stratified regions in the world's temperate oceans. Deep winter mixing redistributes nutrients in the water column, and the stratification due to thermal heating of the water column in spring is traditionally believed to control the onset of the spring bloom (Sverdrup, 1953). At the beginning of the productive season the nutrients are still replete, and the growth of the autotrophic community is not primarily nutrient limited. As the productive season progresses, nutrient limitation becomes more severe, and the intensity of incoming solar radiation peaks around the summer solstice (late June) in the northern hemisphere. On this basis one can hypothesise that the C:nutrient in the high nutrient, moderate light conditions during spring should be lower than during the low nutrient, high light conditions in summer. An increase in seston C:N ratios from spring to summer has been shown for several northern high latitude regions (Daly et al., 1999, Bates et al., 2005, Mei et al., 2005). The growth of autotrophs can also be inferred by the net removal of nutrients and DIC from the euphotic zone by photosynthesis. Several studies have reported a larger drawdown of DIC relative to nitrate compared to the Redfield ratio (Sambrotto et al., 1993, Kortzinger et al., 2001, Falck and Anderson, 2005), and the phenomenon has been termed C overconsumption (Toggweiler, 1993). In Körtzinger et al. (2001) they find an increase in the seston C:N as they move south into more nutrient depleted post-bloom conditions along a transect in the North Atlantic. However, this increase is small compared to the C overconsumption in the inorganic fraction and in the calculated C:N ratios of export production. Similarly, the relatively few studies including several C and N pools (i.e. seston, DOM, nutrients and DIC) point to a

larger C overconsumption in the inorganic relative to the organic fraction (Sambrotto et al., 1993, Banse, 1994, Thomas et al., 1999, Kortzinger et al., 2001). In regions where $N_2$ fixation is not thought to be an important factor, the C overconsumption must be sustained by preferential remineralization of nutrients, either in slowly sinking detritus (Sambrotto et al., 1993, Thomas et al., 1999, Kortzinger et al., 2001) or a build up of C-rich (and N-poor) DOM in the euphotic zone (Williams, 1995, Kahler and Koeve, 2001, Falck and Anderson, 2005). Additionally transparent exopolymer particles (TEP; Alldredge et al., 1993), which have been shown to have high C:N ratios (Engel and Passow, 2001) could represent a route for the C overconsumption in the euphotic zone to the deep ocean (Koeve, 2005). The regenerated nutrients can fuel additional production in the euphtic zone, and consequently estimates of new production based on nitrate might underestimate production rates (Thomas et al., 1999).

The degree to which the C overconsumption in the surface waters reaches the deep ocean, and thus are sequestered on long time scales is important, because it represents a potential negative feedback on atmospheric $CO_2$. Compilation of existing data sets have shown an increasing C:N of sinking material due to preferential remineralization of nutrients (Schneider et al., 2003), and the potential feedback of a depth dependent C:N ratio can influence atmospheric $CO_2$ concentrations by about 20 parts per million (Schneider et al., 2004). However, deep ocean remineralizations ratios of C:N has been shown to be close to the Redfield ratio (Anderson and Sarmiento, 1994), and if the C overconsumption is mainly during summer (Koeve, 2004) and remineralized above the depth of the winter mixed layer, it is questioned that the "extra-Redfield" C is sequestered into the deeper ocean, and therefore influence the oceanic C-budget on longer time scales (Koeve, 2006).The deep ocean remineralization rates of Anderson and Sarmineto (1994) did not include the Atlantic Ocean, because this basin was too complex for the chosen method. A study on the remineralization ratios in the North Atlantic Ocean showed higher than Redfield C:nutrient ratios in the remineralized material in the deeper waters, and thereby a higher C drawdown by the biological C pump than what would be expected from applying Redfield ratios in the formation of organic matter, as in Eq. 1 (Thomas,

2002). Subsequent studies emphasize the efficient biological C pump in the North Atlantic and North Sea, and the large potential these regions have for negative feedbacks on atmospheric $CO_2$, through a "shelf pumping" of C to the deep ocean (Thomas et al., 2004, Bozec et al., 2005, Thomas et al., 2005).

The concept that emerges is one of variable stoichiometry between the C and nutrient elements in space and time (Karl et al., 2001, Michaels et al., 2001, Arrigo, 2005), rather than the constant proportionality described by Redfield. However, the growing literature on various uncouplings of the C:N:P ratios might at times be difficult to reconcile, and even conflicting, and there is an expressed need to better the documentation of natural variability in seston C:N:P ratios in marine environments (e.g. Sterner et al., 2008). Sarmento and Gruber (2005) advocate the continued use of a global constant Redfield ratio, and notes "Much of the difference between the various estimates may be due simply to the spatiotemporal scale of the observations on which they are based". This stance has been criticised and Flynn (2010) writes: "That even the newest text books on the subject (e.g. Sarmiento and Gruber, 2005) still build heavily on Redfield-Monod model types, with scant mention of the importance of variable stoichiometry, is disappointing".

Sterner et al. (2008) tested the proposed effect of spatiotemporal scales on seston stoichiometry, and found the constant Redfield ratio (albeit with higher C content) to be valid on large spatial scales. However the assumption of a constant proportionality between the elements broke when analysing regional scales. These authors proposed the "biogeochemical mosaic hypothesis", where on a global (or sufficiently large) scale all the various mechanisms that might uncouple the elemental ratios are included, while on smaller spatial scales only part of the mosaic is present and may yield uncoupled element ratios.

This idea of the Redfield ratio representing the "average" biogeochemical signal has parallels to the study by Klausmeier et al. (2004a) where it was found that different growth strategies in phytoplankton had distinct N:P ratios, where a typical "bloomer" has a low N:P ratio (due to the high P demands for rapid growth) while the

"survivalist" had a high N:P ratio (due to high N demand for resource acquisition) (Sterner and Elser, 2002, Arrigo, 2005). The Redfield N:P ratio of 16:1 was therefore not the optimal biochemical composition of the autotrophs (e.g. Lenton and Watson, 2000), but rather the average N:P ratio of the different growth types, as also emphasized by Geider and La Roche (2002). A recent modelling study also showed that the observation of N:P close to the Redfield ratio, was the result of "circulation-averaging" by mixing of remineralized matter with non-Redfield N:P ratios (Sambrotto, 2010, Weber and Deutsch, 2010). Even though the Redfield ratio of C:N:P = 106:16:1 was constructed as an average and Redfield and his co-workers was aware of species-specific variations (Redfield et al., 1963, Falkowski, 2000), the "Redfield ratio has sometimes been misconstrued as the universal and constant stoichiometry of phytoplankton" (Klausmeier et al., 2008).

Parameterizing variable stoichiometry in models ads complexity and requires a robust mechanistic understanding of the process to be included (Anderson, 2010, Flynn, 2010), however including variable stoichiometry has been shown improve model representation of important aspects of C and nutrient cycles (Klausmeier et al., 2004b, Christian, 2005). In order for parameterizations of variable stoichiometry in models to actually increase our ability to accurately model the processes at hand, the parameterizations need to be based on detailed mechanistic understanding. For the processes generating variable stoichiometry frequently this understanding is missing, which complicates sound parameterizations.

The concept of C overconsumption has received renewed attention, because mesocosm experiments have shown increased C uptake relative to nitrate in phytoplankton grown under high $CO_2$ (Riebesell et al., 2007, Bellerby et al., 2008). Model simulations including parameterizations of this process have shown that the negative feedbacks on atmospheric $CO_2$ can be substantial, amounting to between 34 to 70 Gt C by the end of this century (Schneider et al., 2004, Oschlies et al., 2008).

Variable stoichiometry in autotrophs also affects higher trophic levels through various predator-prey interactions (Hessen and Andersen, 1992, Sterner and Elser,

2002). In general, autotrophs have rigid cell walls, which are poor in nutrients but high in C, which leads to generally high C to nutrient ratios (even though phytoplankton are far more nutrient-rich that most terrestrial plants that are high in C-rich cellulose). Heterotrophs are more dependent on homeostasis (stable intra-cellular conditions) and have far more rigid elemental requirements (Sterner and Elser, 2002). These organisms often do not have as much structural material, and hence the relatively low C to nutrient ratios of the autotrophs does not necessary meet their nutritional demand. The Liebig "law of the minimum" states that an organisms growth will be limited by the element that is in lowest concentrations in the substrate relative to the requirements of the cell.  Following this concept, one can theorize an elemental ratio of, for example C:N, where the growth of the organism will switch from being limited by for example N to C (i.e. "threshold elemental ratios" in Urabe and Watanabe, 1992). Even if the amount of food available to a consumer is high, the organism can still experience growth limitations if the quality of the food is poor (i.e. high C:nutrient ratio) (Sterner and Hessen, 1994, Andersen et al., 2007). The larger the elemental imbalance between the food and the consumer, the less efficient growth will appear as measured by the element in surplus, in other words the "nutrient use efficiency" with respect to that element will be reduced (Vitousek, 1982, Sterner and Elser, 2002). Moreover, the stoichiometric imbalance between autotrophs and heterotrophs (or food and consumer) can affect the continued growth of autotrophs by the elemental ratios of the recycled matter, which has been termed "consumer-driven nutrient recycling" (Andersen and Hessen, 1991, Elser and Urabe, 1999).  Therefore, the variation in elemental composition in autotrophs is important for understanding the nutritional supply and growth in heterotrophs, food-web dynamics, breakdown of organic matter and ultimately global C cycling and earth's climate.

Human perturbations to carbon and nutrient cycles are continuing and in many aspect increasing in intensity, and there is a urgent need to increase our basal understanding of natural systems and their variability, especially if we are to be able to say anything about how these systems might be responding to human perturbations in the future. The goal of this work was therefore to examine the natural variability in

seasonal, inter-annual and regional seston C:N:P stoichiometry, and to evaluate the most important drivers in shaping this variability. This will improve the knowledge on the couplings between the cycles of C and the major nutrients, N and P, and the biogeochemical setting in which this variability operate.

## 1.3 The Redfield ratio as a paradigm

Does the Redfield ratio constitute a paradigm, and if so does the emergence of variable stoichiometry necessitate a paradigm shift? In the influential work by Redfield (1934) he examines observations of seawater chemistry and the composition of plankton, and notes "it is as though the seas had been created and populated with animals and plants and all of the nitrate and phosphate which the water contains had been derived from the decomposition of this original population". This implies that the ratio between inorganic N and P dissolved in the water column had a remarkable similarity to the organic ratio of these same elements found in plankton. In addition this ratio seemed to be well-constrained and detectable with slight differences in different world oceans. Whether the Redfield Ratio is in a full-fledged paradigm is an open question, however I will argue that it at least has paradigmatic characteristics. Following Chalmers (1999) a paradigm is "the general theoretical assumptions and laws and the techniques for their application that the members of a particular scientific community adopt". This can certainly be said to apply to the Redfield ratio, which is referred to as the cornerstone and key foundation of biogeochemistry (e.g. Sterner and Elser, 2002), According to Falkowski (2000), the Redfield Ratio was quickly adopted by the oceanographic and geochemical communities as "canonical values", and treated as a natural law in similar ways to Avogadro`s number in physics. It has had widespread applications, especially for modelling of ocean biogeochemistry and opened the opportunity for calculating the utilization of one element from another. Recently is has become even more important for global earth-system models and modelling of climate change as a constant relating the capacity for uptake of C per unit of nutrients in the ocean. Many studies have been undertaken to examine the composition of seston (Copin-Montegut and Copin-Montegut, 1983, Li et al., 2000, Schneider et al., 2003, Sterner et al., 2008), and the exact values of the Redfield ratio have been widely debated since their introduction (although relatively few studies report phosphorous data). Although many studies show non-Redfieldian behaviour on a regional basis, most conclude that these are not statistically significant

for the global ocean, and support a fixed global Redfield (or slightly revised) ratio. Therefore, even though there is much discussion on the correctness of a global constant ratio of C:N:P, this is more often than not applied, and the Redfield ratio "remains the paradigm"(Sterner et al., 2008). In this work they reject the constant Redfield model on smaller spatial (and potentially temporal) scales, however they conclude that "our findings do not of course reject the seminal conclusions of Redfield about the oceans, but they do add some subtlety and nuance". They also underline that not enough awareness of the fact that Redfields initial papers did not have extensive datasets on seston and that the data was poorly documented and included zooplankton, is included when applying a fixed Redfield ratio in contemporary models and studies.

In another influential work "The Structure of Scientific Revolutions", Thomas S. Kuhn proposes his theory on the evolution of science (Kuhn, 1996). The concept of paradigms is essential to Kuhn's theories on the evolution of science, and for him shifting from one paradigm to the next through the course of science was not necessarily getting you any closer to the truth, it was just a shift in the way the members of the community regarded the theory in their scientific field. This is in contrast to philosophy of science before Kuhn, where practitioners like Karl Popper believed there was a progression in science towards an unequivocal truth. For Kuhn science progressed according to the following discontinuous path: pre-science - normal science – crisis – revolution – new normal science – new crisis (Chalmers, 1999). In the pre-science period, before the emergence of a paradigm, all facts derived from observations and experiments are "likely to seem equally relevant", and eventually one of the pre-paradigm schools becomes dominant, which "because of its own characteristic beliefs and preconceptions, emphasized only some special part of the too sizable and inchoate pool of information"(Kuhn, 1996). This shows that Kuhn did not necessarily believe that one school was any closer to the truth than the others, it just explained the facts at hand in a way that gained most momentum at the time. As dictated by the framework of the paradigm normal science is the "puzzle solving" activity, and this phase is where most scientists will spend their career, "with the aim of improving the match between it (the paradigm) and nature" (Chalmers, 1999).

During the course of these activities incidents where the paradigm fails will appear, and Kuhn believes that it is these anomalies and the systematic exploration of them that lead to *scientific discoveries* within the realm of the paradigm. A crisis emerges when the failures become so serious that the paradigm is rejected (Chalmers, 1999). Kuhn writes, "failure of existing rules is the prelude to a search for new ones", and this is characterized by "pronounced professional insecurity" before theories laying the foundation for a new paradigm is established (Kuhn, 1996).

Kuhn defines a paradigm shift (or a *scientific revolution*) as the "tradition-shattering complements to the tradition-bound activity of normal science", and whether the insights into the variable nature of elemental stoichiometry can be said to fulfil that criterion is rather doubtful. In addition, there has to be a "switch in visual gestalt", when going from one paradigm to the next, which cannot be said to apply if one moves into an understanding of the non-constant nature and coupling of elements. Kuhn's description of what happens when an anomaly leads to the development of new scientific discoveries seems to fit the current situation in ecological stoichiometry rather well: "the previous awareness of an anomaly, the gradual and simultaneous emergence of both observational and conceptual recognition, and the consequent change of paradigm categories and procedures often accompanied by resistance" (Kuhn, 1996). So it rather seems that ecological stoichiometry is experiencing a scientific discovery, and expanding and refining its still continuing paradigm, rather than a scientific revolution. A scientific discovery is an important part of the progress in science and like the scientific revolution is accompanied by resistance in the community and will take time before it is implemented.

# 2.  Overview of main results

Variation in seston C:N:P stoichiometry seasonally, inter-annually and regionally is driven by an unknown multitude of factors, of which nutrient status, light intensity (and spectral composition) and species composition have been shown to be important. The species composition, growth rate and the amount of light and nutrients available to the algae can be controlled in the laboratory by the use of a chemostat. However, to study the natural variation of C:N:P ratios in the field one must deal with an innately uncontrollable environment and also the heterogeneous nature of the pool that is being measured. The seston will include the autotrophs, which are the aim of the analysis, but also unknown quantities of small heterotrophs (bacteria and protozoa) and detritus of autochotonous and allochotonous origin. The goal of this thesis is to contribute to an increased mechanistic understanding of the processes creating variable stoichiometry in natural environments, which can guide the development of parameterizations of these processes leading to variable stoichiometry in regional and global earth-system models.

In **PAPER I** we used two 20-year time series from the Norwegian Costal Current (NCC) in Skagerrak to document the degree of natural variation in seston C:N:P ratios and investigate drivers behind the observed seasonal variation. By the use of an ordination we found that water mass characteristics (origins of source waters) and bloom dynamics were most important for driving variation in the C:N ratio. While the C:P and N:P ratios were closely linked, and related to nutrient concentrations and productivity levels. A regression model approach was used to determine the fraction of live autotrophs and non-autotrophic material. Both stations had less than 50% live autotrophs, and in order to evaluate the robustness of the estimate the percentage of live autotrophs from the regression models was compared with calculated phytoplankton C. The two independent measures of autotrophic percentage were comparable, and had similar seasonal cycles. The C:nutrient ratios were in general lower than the Redfield ratio in the live autotrophic fraction, while the C:nutrient ratios were higher in the non-autotrophic fraction. This was most likely

due to preferential remineralization of nutrients, and the main difference between the fractions was in the P content. This study emphasized the importance of evaluating the heterogeneous composition of seston, especially in coastal regions. The sestonic C:N:P ratios are not necessarily good indicators of phytoplankton physiological state, and represents a balance of the various sestonic fractions.

The effect of intensity and spectral composition of light for the uptake of $^{33}$P in three arctic diatoms were tested in **PAPER II**. The Arctic ice sheets are thinning, and larger periods of the year are totally ice-free, which is fundamentally altering the underwater light climate. Photosynthetically active radiation (PAR; 400 – 700 nm) and ultraviolet radiation (UVR; 280 – 400 nm) has been shown to have opposing effects on the C:P ratio, where PAR tend to increase the C:P ratio while UVR has been shown to yield decreased C:P. Whether the effect of UVR is due to a decreased efficiency of C-fixation during photosynthesis or an increased uptake of P for cellular repair is unknown. In this study the algae cultures were exposed to either PAR radiation alone or to UVR+PAR radiation, and to evaluate any potential thresholds the cultures were exposed to either moderate or high UVR doses. Most of the investigated species showed decreased P uptake under high UVR exposure, probably reflecting membrane damage or disruption of important cellular processes. However, no indications of increased P uptake during moderate P exposure were found for two of the species. While one species, which had remarkably fast P uptake kinetics, showed a slight increase in P uptake under moderate UVR stress. The results emphasize the species-specific physiological responses of P uptake under UVR stress, and showed in general little support to the hypothesis of increased P uptake during UVR exposure.

In **PAPER III** a 20-year time series from the Arendal station in the NCC was used to examine the long-term changes in hydrography, total and dissolved nutrients, seston concentrations and elemental ratios. The aim was to assess the effect of changes in the NCC due to reduced nutrient loading in continental rivers and inter-annual variability forced by the North Atlantic Oscillation (NAO). We chose to focus on trends in the January to April time period, to include the spring bloom in all

investigated years, and because the advected signal from the North Sea and the effect of the NAO on the circulation in the Skagerrak is strongest in winter and early spring. The nutrient budgets in the North Sea and Skagerrak are dominated by exchange over the marine boundaries, and the inter-annual variation in the NCC was largely controlled by the NAO, which influences the inflow of water and the strength of circulation within Skagerrak. There was a long-term decrease in nutrient concentrations, which by the use of a water mass analysis was connected to the decrease in nutrient loading in continental rivers advected with waters from the southern North Sea and German Bight. In contrast to the nutrient reductions the concentrations of seston, dissolved organic nitrogen and estimated non-autotrophic material underwent an abrupt upward shift between 1998 and 2000. It is hypothesized that the simultaneous thresholds is caused by humic coagulation, which represent a link between the increase in DOM and the non-autotrophic fraction of seston. This is connected to a reported darkening of the Skagerrak and Baltic Sea, which has important consequences for water transparency, depth of the euphotic layer and optimal feeding strategies. Two other studies report large-scale shifts in the marine ecosystem in coastal Norwegian Skagerrak in the same time-period, and together with the thresholds observed in this study this suggests a regime-shift has occurred.

In **PAPER IV** >3600 observations of POC and PON north of 60° were used to evaluate the C:N ratio of seston in the Nordic Seas, Arctic Ocean and pan-Arctic shelves. These areas are among the most productive in the World Ocean and are disproportionally important in the global cycling of C and are expected to undergo large changes to sea-ice extent, stratification and light regimes with climate change. The C:N ratio of 8.3 proposed by Sterner et al. (2008) was only applicable for the North Water and Northeast Water Polynyas west and east of Greenland, respectively. The C:N ratio for all observations was 7.3, and significantly higher than the Redfield C:N ratio of 6.6. Also on a regional basis all pan-Arctic shelves had a C:N ratio significantly above the Redfield ratio, except for the Chukchi and East Siberian Seas, which could be related to the inflow of nutrient-rich Pacific waters to these areas. The Kara and Laptev Seas had relatively high C:N ratios, and were also the regions most

exposed to terrigenous C from rivers and coastal erosion. The C:N ratio peaked under low Chl *a* levels, while no strong latitudinal or day length trends was found. There was significantly variation in the C:N ratio over relatively short distances (e.g. the Atlantic and Arctic influenced sectors of the Barents Sea), while it did not differ in all the primarily Atlantic-influenced regimes (e.g. the Norwegian, Greenland and Atlantic Barents Sea. There was a consistent pattern in all but three regions of decreasing C:N ratio with increasing seston concentrations, thus violating the assumption of a constant ratio between C and N.

# 3.   Future work

In the future I wish to continue the work from my PhD with understanding variability and key drivers in C and nutrient cycles, and there are a number of issues directly related to the four papers included in the thesis that would be interesting to pursue. For example, the two 20-year datasets from the Norwegian Coastal Surveillance Programme represent a unique source of information, both because of the length of the time series and also the amount of variables measured. I would like to perform SMA regressions (or similar techniques) on these time-series, similar to what was done on the Arctic cruise datasets. The wealth of variables present should make it possible to explore if it is primarily the nutrient use efficiency (as proposed by Sterner et al. (2008)) or a shift in the composition of seston (as proposed in PAPER IV) that cause the C:nutrient ratios to decrease with increasing seston concentrations. In addition, the indications of a regime-shift in the coastal waters of the Norwegian Skagerrak should be explored further. Especially, I would like to analyse the 5 stations of the programme along the southern and western coast, to determine if the same thresholds are present in all locations. In addition, it would be interesting to look at longer time series of Secchi depths at multiple locations to see if a decreasing trend would be significant, and examine the link with the observed threshold in suspended material. Concerning the cruise database collected for this thesis from the northern high latitudes, I would like to look more closely into potential drivers for the regional variability (e.g. nutrient limitation, PAR, stratification) where these variables can be made available.

This thesis has focussed primarily on the dynamics of C and nutrients in the seston, however, I would like to extend the scope to the all the pools of C, N and P (particulate organic, dissolved organic, dissolved inorganic and total pools). Only in this way can we understand how elemental imbalances in one pools transfers to the next and the total extent of variability in elemental ratios. Especially, I think it would be interesting to re-examine how C overconsumption in DIC over nitrate and phosphate in the North Atlantic and Arctic transfers to the dissolved and particulate

organic pools and the exported material out of the system. This applies both to responses to nutrient limitations in the field and to $CO_2$ perturbation experiments in mesocosms and in the laboratory.

Most $CO_2$ perturbation experiments are performed with seawater depleted in nutrients, which is manipulated with the desired $CO_2$ concentration and spiked with nutrients (mostly in Redfield proportions) to initiate the bloom. I believe it would be interesting to see how the community responds to increased DIC concentrations under nutrient limitation and C:N:P supply ratios diverging from the Redfield ratios, as also proposed by Sterner and Elser (2002).

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