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The storage products of diatoms, *under nutrient limitation*

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1.0 Abstract

In the present study, the effect of nutrient limitation on the storage products of diatoms will be assessed. The major storage products for diatoms are carbohydrates and lipids. Diatoms increase their total lipid content under nitrate, phosphate and silicate limited conditions. The triacylglycerols (TAGs), important for biofuel production, are increasing most under nutrient limited conditions. The longer fatty acids as (Sidhu, 2003) eicosapentaenoic (EPA, C20:5) and docosahexaenoic (DHA, C22:6), also known as fish oils, are decreasing in nutrient limited conditions. This is because the diatom is switching from longer chains of fatty acids to shorter chains that can be stored as neutral lipids in the vacuole. The carbohydrates increase under nitrate and phosphate limited conditions. The amount of extracellular carbohydrates and cellular changes between species and is not dependent on nutrient limitation. The increase in carbohydrates takes care of an increases in their sinking rate. They are able to sink out of the euphotic zone, when conditions are unfavourable. They sink below the nutricline and stay there in a resting state. They re-enter the euphotic zone due to vertical mixing or replenishing of the limited nutrients, were they use the carbohydrates as an energy source to accumulate the nutrients. With silicate limitation the amount of carbohydrates is decreased. The sinking rate is increased by a change in morphology under silicate limitation. They use lipids for their energy storage. When returned to favourable conditions, lipids are used for growth.

In conclusion the total amount of lipids increases under nutrient limitation and the total amount of carbohydrates increases under nitrate and phosphate limitation but not under silicate limitation.

2.0 Introduction

In the present study the effect of the nitrate, phosphate and silicate will be reviewed. When these nutrients are limited algae change their metabolism. Carbohydrates and lipids are the storage products and in this study the focus is on how they will change in amount during nutrient limited conditions. This is in an ecological perspective an interesting study, because in oceans and freshwaters the availability of nutrients is not constant and equally divided. In most cases it is patchy and it depends on the season when it will be abundant. Diatoms bloom in early spring when silicate is present and sink out when silicate is limited. The amount of carbohydrates and lipids determine the nutritional value of the phytoplankton for higher trophic levels and is probably changing when nutrients get limited. Therefore the nutrients in the oceans can be a predictor for which species will thrive where. In this way it is possible to determine high quality spots, where lots of phytoplankton will bloom and the nutritional value is high and the low quality spots and monitor the changes in time. However there is not only an ecological importance for this study. The amount of lipids and carbohydrates are also important for human applications of phytoplankton. The lipids in microalgae could be an alternative to vegetable and fossil oils. The advantages in cultivating microalgae for oils are in the first place; they can be cultivated on non-arable land. Second, when they are cultivated in closed photo bioreactors, they require less fresh water than terrestrial plants. Furthermore, the production is not season limited (Gouveia et al, 2008). Triacylglycerols (TAGs) are the preferred lipid class for most applications. The bulk edible oils are composed of TAGs, and TAGs are preferred for biodiesel production. TAGs have a high fatty acid content because they have a glycerol backbone with three fatty acids attached to it and no other chemical constituent besides glycerol; this makes them ideal for biofuel production (Breuer et al, 2012). TAGs are not only preferred for biofuel but also for the production of fish oils. Fish oils play an important role in enhancing human health and preventing human diseases as diabetes and cancer(Sidhu, 2003). Eicosapentaenoic (EPA, C20:5) and docosahexaenoic (DHA, C22:6) are formed by microalgae and can be an alternative for fish oils, this is advantageous because they don't have the peculiar taste, odour and stability problems that are associated with the current fish oils(Wen et al, 2002).

In aquacultures most species live on phytoplankton and a higher amount of carbohydrates and lipids in phytoplankton will support the growth of the species(Muller-feuga, 2000).

Diatoms are chosen for this study, because they are a dominant group in the phytoplankton composition in open oceans and play an important role in the marine foodweb. Diatoms are abundant algae in fresh and oceanic water, about 20 to 25 percent of all the carbon fixation on earth is done by diatoms. Oceanic diatoms produce about 40% of the total oceanic primary production. They are food for marine and freshwater microorganisms and animal larvae.

All diatoms have a cell wall made of silica, the frustule, that is also what makes them a unique group in the world of phytoplankton. Diatoms divide when the conditions are favourable. For most diatoms this is in early spring. The diatoms divide at a rapid tempo to quickly dominate the phytoplankton community. After this enormous bloom in early spring, conditions in the water column changes, mostly because of depletion of nutrients. When the nutrients get depleted diatoms increase in sinking rate and enter waters with low light conditions and less nutrients. They stay in a resting phase in the deeper water column until they sink out to the ocean floor or re-enter the upper mixed layer by vertical mixing, which also brings new nutrients into the water column, which favours the growth again. For most diatoms this is an annual cycle (Round et al 1990).

The storage products of diatoms contain great amounts of carbon. Light is the key incentive to accumulate carbon. In the chloroplast the carbon is fixated and glucan biosynthesis and lipid biosynthesis are up regulated in the beginning of the light period. At the end of the light period the tricarboxylic acid cycle (TCA) cycle and lipid β-oxidation are up regulated to form lipids and carbohydrates. In the night period a decrease is seen in the amount of lipids and carbohydrates in the cell, probably due to catabolic utilization of the energy reserves and mitosis (Chauton et al , 2013).

Lipids are formed due to carbon getting incorporated in acetyl-CoA and this is transformed in fatty acids. Short fatty acids with few double bonds are combined with glycerol on the endoplasmetic reticulum (ER), and are stored as neutral lipids in the cytosol or chloroplast. The fatty acids that are elongated and desaturated are combined with phosphorus or carbohydrates into complex phospholipids or glycolipids to provide polar membrane material. In general fatty acids contain more energy than carbohydrates. However it takes a longer time to produce fatty acids than carbohydrates (Lancelot & Mathot, 1985). The lipid fraction is also higher than the carbohydrate fraction in the diatom cell; this is because the lipids probably serve as a fixed carbon reservoir for protein biosynthesis at night and at least partially as long term storage product. This reservoir seems to be retained until carbohydrates are exhausted(Stehfest et al, 2005).
For the optimal production of lipids that can be used for fossil oils, but also for fish oils, algae have to be selected on their lipid content. Many screening studies are done to see which algae contain what kind of lipids. In diatoms the amount of lipids generally lies between the 3.0-15.6 $\mu g\ \mu m^{-3} \times 10^{-8}$(Lynn et al, 2000). This amount is measured in non limited diatoms. In ash free dryweight this is about 5% to 20% lipids (Brown et al, 1997)
Carbohydrates are another form of storage, in diatom mainly in the form of chrysolaminarin (1,3β-D-glucan). In the light period chrysolaminarin is incorporated into glucan through gluconeogenesis. Glucan is stored in the vacuole and in the dark period the glucans provide energy for nutrient assimilation and carbon skeletons for the synthesis of other biomolecules through glycolysis and the tricarboxylic acid (TCA) cycle (Granum et al, 2002).
Carbohydrates are not only important for the growth of diatoms and for human applications as aquacultures, but also to control the buoyancy of the diatoms in the water column. They are non-motile, so to stay in the same place in the water column and not sink out, they have to control their buoyancy. In previous studies is observed that when cultures are severally stressed through prolonged dark treatment, diatoms are able to minimise the sinking rate, by dividing rapidly and decrease the amount of carbon in the cell. The decrease in the amount of carbon per cell happens faster than the minimising of the cell volume by dividing. Therefore carbon probably drives sinking rate changes (Waite et al, 1997). This effect is however not observed in all the diatoms. (Waite et al., 1997). The amount of carbohydrates in diatoms lies between 3.5-9.02 $\mu g\ \mu m^{-3} \times 10^{-8}$ (10% to 50% in ash free dryweight)(Lynn et al., 2000; Brown et al., 1997)
Both carbohydrates and lipids can vary in amount due to nutrient limitation. The major nutrients for diatoms are nitrate, phosphate and silicate. The general mechanisms of uptake and metabolism differ between the nutrients.
Phosphate is an important part of the DNA in microalgae cells, but also structures on the membrane, called phospholipids, contain large amount of phosphorus. Phosphate is primarily taken up in the light period. In research is observed, that in phosphate limiting condition the diatoms are still dividing for at least two days. This is possible because the phospholipids on the membrane can be replaced by other lipids. The phospholipids are recycled and phosphorus is used to build new DNA for dividing. The phospholipids on the membrane serve as a storage for phosphate, because when conditions turn to phosphorus replete conditions the phospholipids are rebuild on the membrane in large amounts, so that again in phosphate limited conditions they can use this as a source for phosphate to still be able to divide (Martin et al, 2011).
Nitrogen is an important component of proteins. Nitrate is taken up by diatoms and processed in the cell by nitrate reductase in nitrite and by nitrite reductase in ammonium during the light period. Ammonium is also taken up by diatoms and is transformed by glutamate dehydrogenase into glutamate in the cytosol, mitochondrium and chloroplast. Glutamate is a an amino acid and a building block for proteins(Valenzuela et al., 2012). When nitrate is limiting the cell is also able to take up urea, which can serve to effectively replenish compounds needed for growth. In nitrogen deplete conditions the cells also express an up-expression of transcripts for nitrogen transport and assimilatory genes to really try to take up all the nitrogen that is left in its surroundings. (Breuer et al., 2012)

Silicate especially is an essential nutrient for diatoms, because the cell wall is made of silicate. The diatoms take up dissolved silicic acid from the water and precipitate opaline silica to form their cell wall. The uptake can happen during night an light periods (Martin-jézéquel et al, 2000). The cell wall requires less energy to synthesize than organic cell walls, which is a saving on the overall energy budget. The cell wall probably has an effect on the ph buffering, facilitating the conversion of biocarbonate into dissolved CO_2, which is easier to take up in the cell. (Milligan & Morel, 2002). When silicate gets depleted in the open ocean, the diatom bloom ends in most cases and diatoms sink in deeper waters. Other phytoplankton species are then able to take over. So solely for diatoms silicate is the limiting nutrient for growth in the oceans. The use of silicon by diatoms is probably the key to their ecological success, when silicon is available in large amounts, diatoms will also be abundant in most of the cases. Diatoms already represent more than 70% of the phytoplankton community when the silicon concentration are larger than 2 mmol m^{-3}(Fig. 1) (Egge & Aksnes, 1992). Silicate favours growth of diatoms and thus leads to higher primary production(G. Li et al., 2013) and silicate is required for the initiation of DNA synthesis in diatoms(Vaulot et al, 1987).

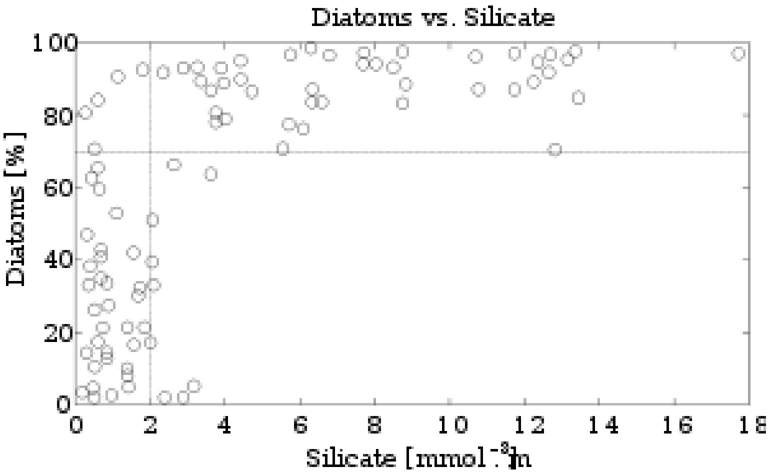

Figure 1, The amount of diatoms compared to the amount of silica in the water(Egge & Aksnes, 1992)

3.0

In the present study the effect of the three different nutrient limitations are assessed separately.

Lipids

3.0 Under N limitation

Many studies are done on the effect of nitrogen (N) limitation on the amount of lipids in diatoms. Most studies found that the lipid content increases during N limitation. Lipid biosynthesis appears to become the alternative sink for the excess electrons derived from the photosynthesis when nitrogen is limited, because nitrogen limitation reduces the ability of microalgae to use photosynthetically fixed carbon for protein synthesis. In *Thalassiosira pseudonana* a marine centric diatom an increase in fatty acids is seen. Under N-replete conditions the cell contains about 12.9 % fatty acids and in N-deplete conditions 20.3 % (Jiang et al, 2012). *Callitris muelleri* a marine/freshwater diatom showed a seven times higher amount of neutral lipids in N limited conditions than in N replete(Mcginnis et al, 1997).

For *Phaeodactylum tricornutum* a marine pennate diatom there was almost a twofold increase seen in the amount of the lipid/amide ratio in the N-deplete conditions compared to the N-replete conditions(Valenzuela et al, 2012; Frada et al, 2013; Breuer et al, 2012).

The neutral lipid content in *P. tricornutum* per cell increases 2-4 fold under nitrogen limitation. Transcriptional profile analyzed by RNA-Seq showed that expression levels of 1213 genes (including key carbon fixation, TCA cycle, glycerolipid metabolism and nitrogen assimilation genes) increased under nitrogen limitation and the light harvesting complex genes are down-regulated(Valenzuela et al., 2012).

But not all fatty acids show an increase when N is limited. Eicosapentaenoic (EPA, C20:5) and docosahexaenoic (DHA, C22:6) show a decrease in N limited conditions (Mendoza Guzmán et al, 2010; Breuer et al, 2012). These are the fish oils that are important for humans but also in aquacultures. In normal conditions *P. tricornutum* has around 24% of EPA and in N-limited conditions this reduces to 13 %, almost a twofold decrease. The loss of EPA is counterbalanced by an increase in saturated and mono-unsaturated fatty acids. The diatom is switching form poly-unsaturated fatty acids, as EPA, to shorter chains(Breuer et al, 2012) . These shorter chains can be stored in the cytosol or chloroplast and are the neutral lipids. In favourable conditions they can be used as an energy source.

In a study of Larson & Rees (1996) a culture of *P. tricornutum* was nitrogen limited, this culture still divided and had an increase in lipids. After 5 days of limitation nitrogen was added to the cultures. The lipid content of this cultures decreased again due to an increase of the activity of the glyoxylate cycle enzyme, isocitrate lyase, that catabolised lipids. The conclusion to draw from this research is that after a phase of nitrogen limitation the cells can re-enter a growth phase again by using the stored lipids (Larson & Rees, 1996). The neutral lipids are thus an important storage product that is formed under nitrogen limited conditions, and is used again in favourable conditions.

3.1 Under P limitation

Diatoms have a storage of phosphorus in the form of phospholipids. So when P is limited they are still able to grow for some time and use phosphate to build DNA and RNA. In total limited conditions the growth stops and the cells are not able to divide anymore, because new DNA cannot be formed(Davey et al., 2008). But the cell is still alive and can accumulate carbon to build fatty acids. In the study of Stehfest et al (2005) on *P.tricornutum*, previously discussed with N limitation, also some cultures were phosphorus limited. In the N limited culture, the amount of lipids increased as said before. In the phosphorus limited cultures the amount of lipids increased as well and after a re-addition of phosphorus to the culture, the amount of lipids remained high for a week. In contrast to the N-limited cultures were the amount of lipids after a nitrogen addition decreased immediately (Stehfest et al, 2005). Why the decrease in fatty acids after a phosphate addition is slower than with a nitrate addition is still unknown.

Gong et al (2013) observed the total amount of fatty acids under P limitation and N limitation in *P.tricornutum.* The amount of fatty acids at day 5 of P limitation was around 250 mg/g dry cell weight and under N limitation this was around 100 mg/g dry cell weight. However in the same research a

Chaetoceros sp., marine planktonic diatom, showed a different result. In P limitation after 5 days the amount fatty acids was around 200 mg/g dry cell weight and under N limitation 350 mg/g dry cell weight. The amount of EPA in the cells decreases in phosphate limited cultures for both species.(Gong et al, 2013).
In *T. pseudonana* the amount of the two fatty acids 20 : 5ω3 and 22 : 6ω3 (DHA and EPA) was also decreased under P limitation but under N limitation this decrease was not visible(Bienfang et al, 1982).
In *S. minutulus* the same effect as in *P.tricornutum* is observed; the amount of lipids is greater in phosphate limited conditions than in nitrogen limited conditions. The composition of the lipids was also different under different kind of limitations. The amount of phospholipids was smaller in the phosphate limited conditions compared to the nitrogen limited conditions, but the galactolipids counterbalanced this effect(Fig. 2). Galactolipids are membrane lipids and replace the phospholipids in phosphorus limited conditions. (Lynn et al, 2000).
Therefore, diatom responses to N and P limitation are not uniform. Overall they all show an increase in lipids when phosphate or nitrate is limited. The major fatty acids that are increasing under N and P limitation are TAGs(Lynn et al., 2000).

3.2 Under Si limitation

Diatoms require silica to produce their cell walls (frustules). So when silicate gets limited the cell is not able to divide anymore because they cannot form a new frustule. A higher amount of silicate is positively correlated with carbon fixation and thus favours growth leading to a higher primary production (G. Li et al., 2013). Cell grown under silicate limitation show an increase in carbon on a per cell volume basis (Harrison et al, 1977)
In *Cyclotella cryptica* lipids accumulate under silicon deficiency. Within 4 hours after the cells become limited there is a reduction in the percentage of carbon that is transformed in chrysolaminarin and an increase in the percentage of carbon that is partitioned into lipids. The enzymes that have a role in this process are up regulated. The UDPglucose:β-(1-> 3)-glucan-β-3-glucosyltransferase (chrysolaminarin synthase) is reduces by 31% during the first 4 hours of silicon deficiency and the activity of acetyl-CoA carboxylase increased about 2-4 fold after 4 hours. As a result of an increase in activity the amount of lipids are increased(Roessler, 1988).
In *Stephanodiscus minutulus* the lipid content is increased all most twofold during silicate limitation. The same species under nitrate or phosphate limitation shows an increase in lipids as stated before, but those increases were not as big. In the control in stationary phase the amount of lipids was 6,47 $(\mu g*\mu m^{-3})*10^{-8}$, in Si- this was 15,6$(\mu g*\mu m^{-3})*10^{-8}$, in N- 7.69$(\mu g*\mu m^{-3})*10^{-8}$ and in P- 12,7$(\mu g*\mu m^{-3})*10^{-8}$ (Lynn et al., 2000). However looking at the composition of the fatty acids observed is that under phosphate limitation the cell has less lipids in total compared to the total lipids under silicate limitation, but is richer in TAGs(Fig. 2).
The amount of polyunsaturated fatty acids (PUFA's) decreases when cells get silicate limited (EPA, DHA) and mono-unsaturated fatty acids (MUFA's) increase during the limited period. This is consistent with the observations under phosphate and nitrate limitation(Diekman et al, 2009).

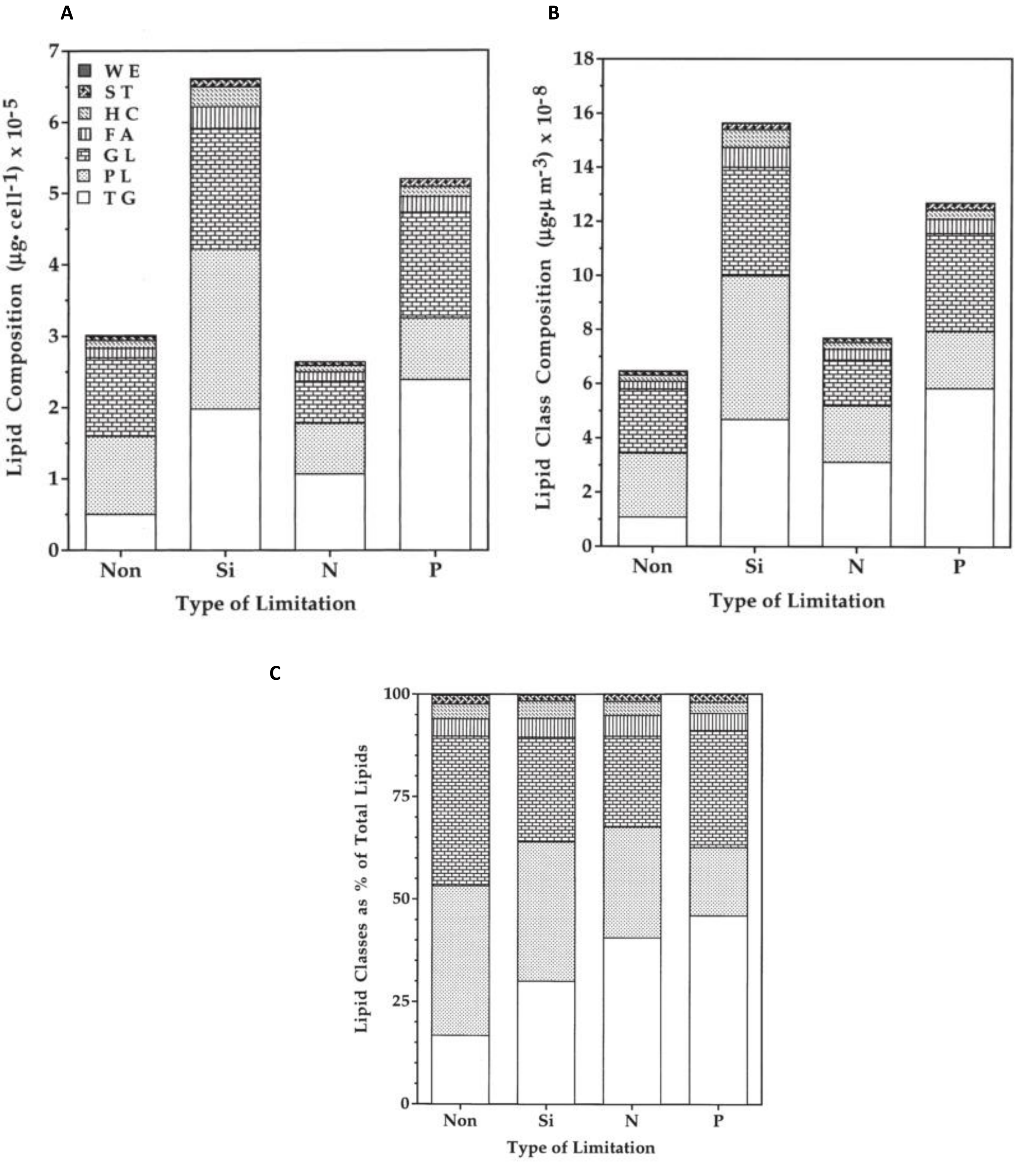

Figure 2, The major lipid classes for *S. minutulus*,
growing without limitation, with silicon limitation (Si), nitrogen limitation(N) or phosphorus limitation (P), as the composition (A) per cell, (B) per volume, and (C) as a percentage of total lipids. FA, fatty acids; GL, galactolipids; HC, hy- drocarbons; PL, phospholipids; ST, sterols; TG, triglycerides; WE, wax ester(Lynn et al., 2000).

4.0 Carbohydrates

4.0 Under N limitation

Carbohydrates are synthesized in the light period and consumed during the dark period; this is different in fatty acids, that do not show pronounced differences during light and dark periods. (Stehfest et al., 2005; Chauton et al., 2013). In most studies an increase in carbohydrates is observed when N is limited. Both in *Achnanthes brevipes* and in *Stephanodiscus minutulus* the increase in carbohydrates is seen (Lynn et al., 2000; Guerrini et al, 2000). However in some species of diatoms no differences are observed between the amount of carbohydrates in N replete and N deplete medium. For example in *T. pseudonana* an increase in lipids is visible during N depletion, but not in carbohydrates(Jiang et al., 2012). So N limitation can cause an increase in carbohydrates but not in all cases.

Algae have cellular production of carbohydrates, but also extracellular production. This extracellular production is a significant part of the algal primary production and carbohydrates are the major part of extracellular production (Granum & Myklestad, 2002). The physiological mechanisms that cause the excretion of carbohydrates are poorly known. The carbohydrates that are excreted are often heteroglycans and often form gelatinous mucilages. The amount of extracellular carbohydrates differs between diatoms. In the research with *A. brevipes* an increase in carbohydrates was seen. This were the cellular carbohydrates, the extracellular carbohydrates did not change under N limitation. The carbohydrate that increases most under N limitation is cellular β-1,3-glucan. In *S. costatum* in 3 to 4 days after limitation the cellular β-1,3-glucan stabilized to 75 to 80% of the cellular organic carbon. This is probably the maximum level. In the same experiment the protein levels declined rapidly because of the lack of nitrogen. The combined glucan accumulation and sharp reduction in protein synthesis makes the protein:glucan ratio a sensitive indicator of the nutritional status in phytoplankton. When glucan is increased it probably can have an effect on the buoyancy of the algae. Algae seem to use glucan to induce vertical mixing. The algae that are in the euphotic zone can accumulate glucan under nutrient depleted conditions. The accumulation of glucan causes an increased cellular density, what leads to sinking out of the euphotic zone into the lower water columns, below the nutricline, were the algae stay in resting phase. When nutrients get replenished again glucan can be mobilized, which causes a reversed effect and the cells rise to the euphotic zone again(Granum et al, 2002). Another example of this is *C. pseudocurvisetus*, marine diatom, the total cellular organic carbon under N limitation increased to 70 %(Kuwata et al, 1993). So nutrient depletion can probably cause an increase in sinking rate by the accumulation of glucan in some species.

4.1 Under P limitation

Under phosphate depletion the carbohydrates tend to increase as well. In the research of Lynn et al (2000) the amount of carbohydrates increased under phosphate limitation. However under N limitation the carbohydrates increased more. Another study showed that under phosphate limitation the diatom *A. brevipes* had an increase in carbohydrates, this happened also under N limitation. However under N limitation no increase in extracellular carbohydrates were found and with P limitation there was an increase in extracellular carbohydrates. Results of this research showed that activities of pyruvate kinase, phosphorylating NAD-dependent 3-phosphate-glyceraldehyde dehydrogenase, and 3-phospho-glycerate kinase were reduced. So P limitation favours extracellular carbohydrates and inhibits carbohydrate catabolism. This was not found under N limitation(Guerrini et al., 2000).

Still not all studies state this. In a study of Penna and Berluti (1999) three different diatoms were studied*, Chaetoceros sp., Skeletonema costatum* and *Nztzschia closterium.* Under P limitation *N. closterium* showed an increase in carbohydrates and the extracellular carbohydrates made up for 50 % of the total carbohydrates. In the two other species this effect was not observed. *Chaetoceros sp.* and *S. costatum* showed an increase in extracellular carbohydrates under N limitation. Therefore the excretion of carbohydrates in this case is species specific, but nutrient limitation has an effect on it (Penna & Berluti, 1999).

4.2 Under Si limitation

In the study of Penna & Berluti (1999), stated above, silicate limitation did not have an effect on the release of extracellular carbohydrates.
In a research with the diatom *Cyclotella cryptica* the chrysolaminarin synthase is reduced 31 % after 4 hours of silicon defiency, so less carbon gets allocated to carbohydrates. The percentage of carbon that gets allocated to lipids is higher in silicon deficiency in this case(Roessler, 1988).
However not all species show a decline in carbohydrates under silicate limitation. In *T. weissfloggii* an increase is seen of carbohydrates under silicate limitation(Diekmann et al., 2009).
In aquacultures *Chaetoceros calcitrans* and *T. pseudonana* are important food for bivalve larvae. When growth was limited by silicate in these species no differences in carbohydrates were seen. Under N limitation and P limitation an increase was seen in carbohydrates in both species (Bienfang et al, 1982).
When silicate starved cells were reintroduced with silicate the soluble glucan increased again. This mechanism is different in N and P starvation, were glucan increases under starvation and decreases in replenished conditions. So the control in sinking rate is different under silicate limitation(Li & Volcani, 1985). But the diatoms appear to sink faster under silicate limitation (Harrison et al, 1990)
In some studies it is stated that diatoms under silicate limitation increase their sinking rate by a change in morphology. The reduction of the spines that can be on the call wall of diatoms, called setae and the rods joining diatoms together are shorter under silicate limitation. These changes in morphology can increase the sinking rate of diatoms (Smayda & Boleyn, 1966).
In a research of Harrison et al (1977) under silicate limitation the diatom *S. costatum* showed the reduction of length of the rods and the reduction of spines was shown in *C. debilis*. *T. gravida* showed many elongated cells with a malformed mucilaginous thread(Harrison et al., 1977).
Biprotoplastic cells are also observed in silicate starved cultures, were due to a limited amount of silicate the duplication of cells cannot occur. The cell organelles can be duplicated but just before the formation of the daughter cell the cell cycle stops. Biprotoplastic cells have a higher sinking rate, because of their higher volume and greater size. (Li & Volcani, 1985; Coombs et al, 1967).
Overall under silicate limitation energy is not stored in carbohydrates but primarily in lipids, which have a higher energy amount. The sinking rate is controlled by the morphology of the cells and not the accumulation of glucan in most species.

5.0 Conclusion

The major storage products of diatoms are carbohydrates and lipids. They can be used as an energy source for the diatom. Carbohydrates contain less energy but can be metabolized faster compared to lipids. Lipids have a high energy content and are primarily used as an energy source when the carbohydrates are exhausted. The lipid content in diatoms is around 5% to 20% in ash free dryweight and carbohydrates are around 10% to 50% in ash free dryweight (Brown et al., 1997).
The content of carbohydrates and lipids changes under nutrient limitation.
Lipids tend to increase under N limitation in most cases. Lipid biosynthesis can be an alternative sink for the excess in electrons generated in the photosynthesic reaction, because the lack of nitrogen causes a inhibition in the production of proteins. This energy is then allocated to lipids. In most diatoms an enormous increase is seen. In *P. tricornutum* the neutral lipid content is increasing around 2-4 fold. But not all lipids tend to increase under nitrogen limitation. The poly-unsaturated fatty acids are decreasing; this is counterbalanced by an increase in saturated and mono-unsaturated fatty acids. So EPA and DHA, important fish oils, do not increase during N limitation. If you want to enhance the production of fish oils in diatoms, nitrogen limitation will not have a beneficial effect. On the other hand TAGs in general, important in biofuel production, are increasing under nitrogen limitation.
When cells of diatoms get in contact with nitrogen again after a limited period, the stored neutral lipids decrease. They are used as an energy source to metabolize new products to enhance dividing and growth again. So diatoms in the oceans accumulate lipids under nitrogen limitation and use this in favourable periods.
With phosphate limitation the diatoms also tend to increase the amount of lipids in their cells. Compared to nitrogen limitation this increase can be higher or smaller, this is species specific in most diatoms. Diatoms normally have phospholipids on their membrane, but under phosphate limitation the phospholipids are recycled to phosphate en galactolipids are placed on the membrane instead of phospholipids. The amount of fish oils in phosphate limited conditions is also decreasing. So overall diatoms increase their amount of neutral lipids in phosphate and nitrate limitation. With silicate limitation this effect is stronger. The accumulation of carbon into lipids increases under silicate limitation. However the composition of lipids is different. Under phosphate limitation a large amount of the lipids in the cells are TAGs, under silicate limitation this is not the case. So even if under silicate limitation the amount of total lipids is more than in phosphate or nitrate limited cultures, the amount of TAGs is less. So for biofuel production silicate limitation is probably not the most effective way to generate large amount of TAGs. EPA and DHA decrease in silicate limited cultures as well. For fish oil production from algae, limitation of nutrients has a negative effect on the total production.
In ecological perspective silicate is the limiting nutrient for diatoms in most cases. When silicate gets limited diatoms sink out of the euphotic zone. When silicate is abundant diatoms will be the dominant phytoplankton group.
In carbohydrates the effect of nitrate, phosphate and silicate is more species specific. But in general under nitrate limitation the amount of carbohydrates increases. Especially the amount of glucan (chrysolaminarin) increases. The amount of proteins synthesized decreases due to the lack of nitrogen. So the glucan accumulation and reduction in protein synthesis makes the protein:glucan ratio a sensitive indicator of the nutritional status in phytoplankton. When glucan increases it also affects the buoyancy of the algae. Glucan increases the sinking rate of the algae, so when this is accumulated in nitrate limited conditions, the diatom can sink out of the euphotic zone, below the nutricline. They can re-enter the euphotic zone by vertical mixing but also when the limited nutrient is replenished. They use glucan as energy source and float to the euphotic zone again. Under phosphate limitation there is an increase in carbohydrates as well but not as strong as under nitrate limitation. But under phosphate limitation the glucans are accumulated as well and this also affects their sinking rate, the same way as in nitrate limitation.
However under silicate limitation something different happens. Carbohydrates decrease; the enzymes that generate chrysolaminarin show less activity and the enzymes that take care of the

production of fatty acids are increased. The sinking rate of diatoms under silicate limitation is higher. They do not use the accumulation of glucans to control their buoyancy, but something that works faster. They change their morphology under silicate limited conditions. This change in morphology takes care of the increase in sinking rate. So when silicate gets limited diatoms sink out of the euphotic zone faster than in nitrate or phosphate limited conditions. Under silicate limitation they use lipids as the reserve energy source. They probably do not need carbohydrates to increase their sinking rate, so they make lipids. Lipids contain more energy, so they have higher energy storage.

In conclusion lipids are important for biofuel production and fish oils. Biofuel production is positively affected by nutrient limitation and fish oil production is not. Total lipid content is increasing under all three limitations. Carbohydrates are increasing in nitrate en phosphate limited conditions and are important for buoyancy control. In silicate limitation carbohydrates are decreasing and lipids are increasing, they control their buoyancy by changes in morphology and use lipids as an energy source. So under limitation the nutritional value is increased in most cases but the sinking rate is also increased, so for higher levels in the food chain, the time to arrive at a diatom bloom is probably best in the beginning and middle of a bloom. Near the end the nutritional value of the diatoms is higher but they also sink out of the euphotic zone, so less diatoms are available.

6.0 References

Bienfang P.K., Harrison P.J., Quarmby L.M.(1982). Sinking rate response to depletion of nitrate, phosphate and silicate in four marine diatoms. *Marine Biology, 67,* 295-302

Breuer, G., Lamers, P. P., Martens, D. E., Draaisma, R. B., & Wijffels, R. H. (2012). The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains. *Bioresource technology*, *124*, 217–26

Brown, M. R., Jeffrey, S. W., Volkman, J. K., & Dunstan, G. . (1997). Nutritional properties of microalgae for mariculture. *Aquaculture*, *151*(1-4), 315–331

Chauton, M. S., Winge, P., Brembu, T., Vadstein, O., & Bones, A. M. (2013). Gene regulation of carbon fixation, storage, and utilization in the diatom Phaeodactylum tricornutum acclimated to light/dark cycles. *Plant physiology*, *161*(2), 1034–48

Coombs,J., Darley, W.M., Holm-Hansen, O., Volcani, B.E. (1967). Studies of the biochemistry and fine structure of silica shell formation in diatoms. Chemical composition of *Navicula pelliculosa* during silicon-starvation synchrony. *Plant Physiology, 42,* 1601-1606

Davey, M., Tarran, G. A., Mills, M. M., Ridame, C., Geider, R. J., & LaRoche, J. (2008). Nutrient limitation of picophytoplankton photosynthesis and growth in the tropical North Atlantic. *Limnology and Oceanography*, *53*(5), 1722–1733

Diekmann, a. B. S., Peck, M. a., Holste, L., St John, M. a., & Campbell, R. W. (2009). Variation in diatom biochemical composition during a simulated bloom and its effect on copepod production. *Journal of Plankton Research*, *31*(11), 1391–1405

Egge, J., & Aksnes, D. (1992). Silicate as regulating nutrient in phytoplankton competition. *Marine Ecology Progress Series*, *83*, 281–289

Frada, M. J., Burrows, E. H., Wyman, K. D., & Falkowski, P. G. (2013). Quantum requirements for growth and fatty acid biosynthesis in the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae) in nitrogen replete and limited conditions. *Journal of Phycology*, *49*(2), 381–388

Gong, Y., Guo, X., Wan, X., Liang, Z., & Jiang, M. (2013). Triacylglycerol accumulation and change in fatty acid content of four marine oleaginous microalgae under nutrient limitation and at different culture ages. *Journal of basic microbiology*, *53*(1), 29–36

Gouveia, L; Marques, AE; da Silva, TL; Reis, A 2009. *Neochloris oleoabundas* UTX#1185: a suitable renewable lipid source for biodiesel production. *Journal of Industrial Microbiology Biotechnology, 36,* 821-826

Granum, E, Kirkvold, S., & Myklestad, S. (2002). Cellular and extracellular production of carbohydrates and amino acids by the marine diatom Skeletonema costatum: diel variations and effects of N depletion. *Marine Ecology Progress Series*, *242*, 83–94

Granum, Espen, & Myklestad, S. M. (2002). A simple combined method for determination of β-1 , 3-glucan and cell wall polysaccharides in diatoms. *Hydrobiologia 37,* 155–161.

Guerrini, F., Cangini, M., Boni, L., Trost, P., & Pistocchi, R. (2000). Metabolic responses of the diatom *Achnanthes brevipes*(Bacillariophyceae) to nutrient limitation. *Journal of Phycology*, *36*(5), 882–890.

Harrison, P. J., Conway, H. L., Holmes, R. W., & Davis, C. O. (1977). Marine diatoms grown in chemostats under silicate or ammonium limitation. III. Cellular chemical composition and morphology of Chaetoceros debilis, Skeletonema costatum, and Thalassiosira gravida. *Marine Biology*, *43*(1), 19–31

Harrison , P.J., Thompson, P.A., Calderwood, G.S. (1990). Effects of nutrient and light limitation on the biochemical composition of phytoplankton. *Journal of Applied Phycology, 54,* 70-7

Jiang, Y., Yoshida, T., & Quigg, A. (2012). Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. *Plant physiology and biochemistry : PPB / Société française de physiologie végétale*, *54*, 70–7.

Kuwata, A. (1993). Ecophysiological characterization of two life forms, resting spores and resting cells, of a marine planktonic diatom, *Chaetoceros pseudocurvisetus*, formed. *Marine Ecology Progress Series*, *102*, 245–255

Lancelot, C., & Mathot, S. (1985). Biochemical fractionation of primary production by phytoplankton in Belgian coastal waters during short- and long-term incubations with 14C-bicarbonate. *Marine Biology*, *86*(3), 219–226

Larson, T. R., & Rees, T. A. V. (1996). Changes in cell composition and lipid metabolism mediated by sodium and nitrogen availability in the marine diatoms *Phaeodactylum tricornutum* (Bacillariophyceae). *Journal of Phycology*, *32*(3), 388–393

Li, C.-W., & Volcani, B. E. (1985). Studies on the biochemistry and fine structure of silica shell formation in diatoms. *Protoplasma*, *124*(1-2)

Li, G., Lin, Q., Shen, P., Ni, G., Song, X., Wang, S., Fan, Y., et al. (2013). Variations in silicate concentration affecting photosynthetic carbon fixation by spring phytoplankton assemblages in surface water of the Strait of Malacca. *Acta Oceanologica Sinica*, *32*(4), 77–81

Lynn, S. G., Kilham, S. S., Kreeger, D. A., & Interlandi, S. J. (2000). Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). *Journal of Phycology*, *36*(3), 510–522

Martin, P., Van Mooy, B. A. S., Heithoff, A., & Dyhrman, S. T. (2011). Phosphorus supply drives rapid turnover of membrane phospholipids in the diatom *Thalassiosira pseudonana*. *The ISME journal*, *5*(6), 1057–60

Martin-jézéquel, V., Copernic, P. N., Plouzane, F.-, & Brzezinski, M. A. (2000). Review silicon metabolism in diatoms: implications for growth. *Journal of Phycology 840*, 821–840.

Mcginnis, K. M., Dempster, T. A., & Sommerfeld, M. R. (1997). Characterization of the growth and lipid content of the diatom *Chaetoceros muelleri. Journal of Applied Phycology 9,* 19–24.

Mendoza Guzmán, H., Jara Valido, A., Carmona Duarte, L., & Freijanes Presmanes, K. (2010). Analysis of interspecific variation in relative fatty acid composition: use of flow cytometry to estimate unsaturation index and relative polyunsaturated fatty acid content in microalgae. *Journal of Applied Phycology*, *23*(1), 7–15

Milligan, A. J., & Morel, F. M. M. (2002). A proton buffering role for silica in diatoms. *Science (New York, N.Y.)*, *297*(5588), 1848–50

Muller-feuga, A. (2000). The role of microalgae in aquaculture : situation and trends.*Journal of Applied Phycology 12:* 527–534.

Penna, A. & Berluti, S. (1999). Influence of nutrient ratios on the in vitro extracellular polysaccharide production by marine diatoms from the Adriatic Sea. *Journal of plankton research*, *21*(9), 1681–1690.

Roessler, P. G. (1988). Changes in the activities of various lipid and carbohydrate biosynthetic enzymes in the diatom Cyclotella cryptica in response to silicon deficiency. *Archives of Biochemistry and Biophysics*, *267*(2), 521–528

Round, F.E. and Crawford, R.M. (1990). *The Diatoms. Biology and Morphology of the Genera.* Cambridge University Press, UK.

Smayda T.J., Boleyn B.J. (1966). Experimental observations on the flotation of marine diatoms. II. *Skeletonema costatum* and *Rhizosolenia setigera. Limnology Oceanography, 11,* 18-34

Sidhu, K. S. (2003). Health benefits and potential risks related to consumption of fish or fish oil. *Regulatory toxicology and pharmacology RTP*, *38*(3), 336–344

Stehfest, K., Toepel, J., & Wilhelm, C. (2005). The application of micro-FTIR spectroscopy to analyze nutrient stress-related changes in biomass composition of phytoplankton algae. *Plant physiology and biochemistry : PPB / Société française de physiologie végétale*, *43*(7), 717–26.

Valenzuela, J., Mazurie, A., Carlson, R. P., Gerlach, R., Cooksey, K. E., Peyton, B. M., & Fields, M. W. (2012). Potential role of multiple carbon fixation pathways during lipid accumulation in Phaeodactylum tricornutum. *Biotechnology for biofuels*, *5*(1), 40

Vaulot, D., Olson, R. J., Merkel, S., & Chisholm, S. W. (1987). Cell-cycle response to nutrient starvation in two phytoplankton species, Thalassiosira weissflogii and Hymenomonas carterae. *Marine Biology*, *95*(4), 625–630

Waite, A., Fisher, A., Thompson, P., & Harrison, P. (1997). Sinking rate versus cell volume relationships illuminate sinking rate control mechanisms in marine diatoms. *Marine Ecology Progress Series*, *157*, 97–108

Wen, Z., Jiang, Y., & Chen, F. (2002). High cell density culture of the diatom *Nitzschia laevis* for eicosapentaenoic acid production : fed-batch development. *Process Biochemistry 37*, 1447–1453.