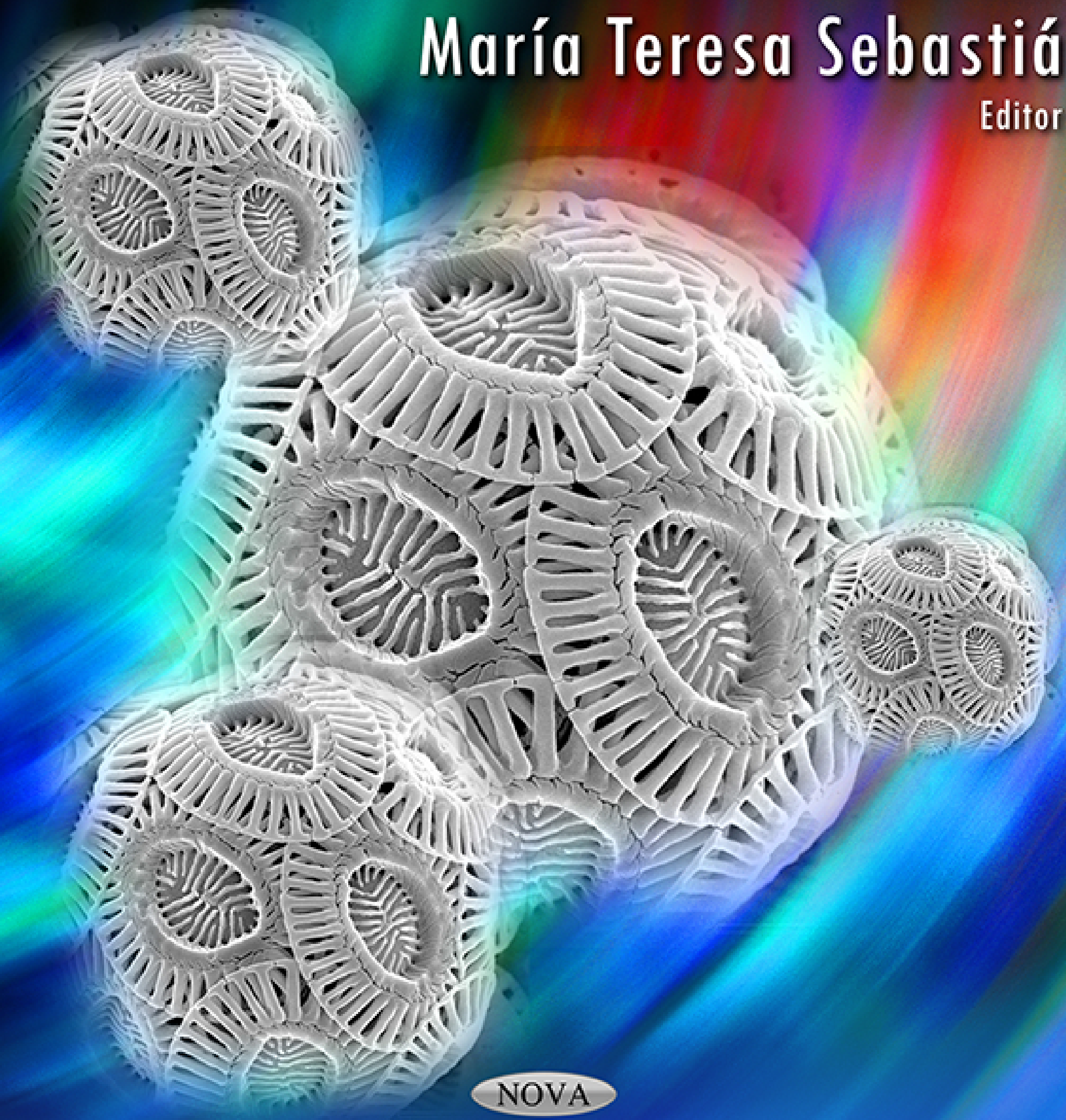
Oceanography and Ocean Engineering

Phytoplankton

Biology, Classification and Environmental Impacts

María Teresa Sebastiá
Editor



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MARÍA TERESA SEBASTIÁ
EDITOR

New York

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Contents

Preface

This book is a new volume of the “Environmental Science, Engineering and Technology” Series which is part of the Nova Science Publishers, Inc. publishing program. The preparation of this book came from Nadya Gotsiridze-Columbus invitation and her editorial team. The realization of this volume has been made possible thanks to the valuable contributions of the authors.

Phytoplankton plays a key role in aquatic ecosystems where it is the major biomass producer. Phytoplankton is characterized by a high time-space variability which is determined by abiotic and biotic factors. In this book, the role of abiotic factors (light, temperature, nutrients, wind, hydrodynamics, CO_2 and UV radiation) and biotic factors (bacteria, zooplankton, macrophytes and fish) is discussed. Anthropogenic pressure can alter those environmental factors causing undesired changes in the composition and biomass of phytoplankton. This book emphasizes effects on water quality, but bottom sediment is also analyzed. The effectiveness of the management measures to restore impacted ecosystems is reviewed and ecological modeling is used as a prediction tool. In this book, the authors describe case studies in different systems such as natural lakes, reservoirs, marine systems and aquatic microcosm systems; covering a wide range of geographic areas from African tropical lakes and Brazilian subtropical lakes to peri-alpine European lakes. The book also includes a chapter about the toxic effects of phytoplankton consumption on *Sarpa salpa*, a fish used for human consumption, and two chapters which study algae removal from the water supply network.

In summary, this book shows how the analysis of phytoplankton composition can serve as an excellent indicator of environmental changes; without forgetting the objective analysis of the results of the implementation of management measures. I hope you will find it truly interesting.

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Chapter I

Phytoplankton Productivity in a Changing Global Climate

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Abstract

Phytoplankton, the major biomass producers in aqueous ecosystems play an important role in regulation of global climate. Several abiotic factors such as light, temperature, CO_2 concentration, acidification and ultraviolet radiation have been found to exert pronounced effects on their productivity. Differential sensitivity of individual species to such interactions may lead to alterations in the phytoplankton composition in a particular ecosystem.

Phytoplankton possesses the enzyme carbonic anhydrase which allows them to increase the internal CO_2 concentration, thereby augmenting the productivity. Similarly, phytoplankton have evolved certain tolerance mechanisms against UV-B radiation, which include avoidance, ROS (reactive oxygen species) scavenging by non-enzymatic and enzymatic antioxidants, the synthesis of UV-absorbing/screening compounds such as the mycosporine-like amino acids (MAAs), scytonemin and sporopollenin and the repair of UV-induced DNA damage and the re-synthesis of damaged PS II proteins. In this review, we describe the range of interactions between phytoplankton with several abiotic factors.

Keywords: Acidification, phytoplankton, global climate change, solar UV-B radiation, UV-absorbing substances, CO_2 concentration

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Introduction

Fossil fuel burning and tropical deforestation have increased the CO_2 concentration in the atmosphere from about 280 ppm, before the onset of the industrial revolution, to a current value exceeding 400 ppm (Etheridge et al., 1996, NOAA, 2013). Additional drivers of global climate change are release of large quantities of CO_2 as well as methane from the Arctic permafrost areas which thaw due to fast rising temperatures (Cory et al., 2013). Judging from the amounts of CO_2 anthrophogenically released every year (currently about 9.5 billion metric tons of CO_2) an even more pronounced increase in the atmospheric concentrations should be expected, which indicates that part of this CO_2 disappears in global sinks.

In addition to terrestrial sinks, where CO_2 is stored in wood and plant litter, the oceans have a major share in the uptake; they are estimated to absorb about one million tons of CO_2 per hour (Gao et al., 2012a) which sums up to about 50 Gt carbon per year (Falkowski, 2002, Sabine et al., 2004). Though the standing crop of phytoplankton accounts for only about 1% of that in terrestrial habitats, marine productivity rivals that of all terrestrial ecosystems taken together because of phytoplankton's faster growth and turnover rates (Falkowski et al., 1997). After being dissolved in the top layers of the oceans, the CO_2 is taken up by phytoplankton to drive photosynthesis resulting in biomass production. As the phytoplankton decays, part of the CO_2 is released again to the water, but part of it sediments directly or as fecal pellets which result from grazing by primary and secondary consumers on the primary producers. This organically bound CO_2 can be trapped experimentally when it is descending in the water column as “oceanic snow” to the deep sea sediments which contain about 5 times more CO_2 than all estimated fossil fuels combined. This process of removal of CO_2 from the top layers of the oceans is called biological pump (Behrenfeld et al., 2006, Falkowski et al., 2000). Once deposited in the sediments the CO_2 is stored there for hundreds of thousands of years (Archer et al., 1994). By this mechanism the oceans play a critical role in limiting global warming.

Increase in Atmospheric CO_2 and Ocean Acidification

The atmospheric CO_2 concentration is a limiting factor of photosynthesis in terrestrial plants (Ziska et al., 2006). For instance, most greenhouse cultures of vegetable crops are given externally applied CO_2 to increase productivity (Jones, 2008). Therefore, one would expect that the increased CO_2 concentration in the top layers of the oceans augments phytoplankton photosynthetic activity and thus productivity at the basis of the oceanic food webs (Hutchins et al., 2007, Riebesell et al., 2011, Schippers et al., 2004). However, most prokaryotic (cyanobacteria) and eukaryotic phytoplankton have developed CO_2 concentration mechanisms based on the enzyme carbonic anhydrase which allows them to increase the internal CO_2 concentration which mitigates the limitation (Aizawa et al., 1986, Bozzo et al., 2000, Sültemeyer, 1998). E.g., the coccolithophorid *Emiliania huxleyi* has been found to increase its intracellular inorganic carbon concentration about 10fold compared to the outer medium (Hayakawa et al., 2003); however, this could not be confirmed in this and another coccolithophorid (Israel et al., 1996, Nimer et al., 1992). Due to effective CO_2 concentration mechanisms increasing concentrations in the water have only a small augmenting effect on productivity. In a phytoplankton sample dominated by diatoms no increase in net photosynthesis was found during a cruise in coastal Pacific waters when the CO_2 concentration was increased (Tortell et al., 2000). In diatoms the CO_2-concentrating mechanism is even down-regulated by increased CO_2 concentrations (Hopkinson et al., 2011, Wu et al., 2010). In other diatoms elevated CO_2 concentrations have been found to enhance photosynthesis; however this was partially compensated by increased respiratory and photorespiratory losses (Gao et al., 2012b, Wu et al., 2010). Therefore it is still controversial whether or not increased atmospheric CO_2 concentrations may enhance phytoplankton productivity on a global scale (Riebesell et al., 2011).

As a result of increasing uptake of CO_2 into the oceans the seawater is acidified because it combines with water to form carbonic acid, which dissociates to bicarbonate, releasing protons (Doney et al., 2009).

$$CO_2 + H_2O \rightarrow H_2CO_3$$
$$H_2CO_3 \rightarrow H^+ + HCO_3^-$$
$$HCO_3^- \leftarrow H^+ + CO_3^{2-}$$

The pH of seawater is well buffered in the range of 7.5 to 8.4. The uptake of more and more CO_2 has lowered this value by about 0.1 units which does not seem much, but indicates an increase in the H^+ concentration by 30 % (Caldeira et al., 2003). Assuming a scenario of increasing CO_2 emissions (such as in the IPCC A1F1 model) (Houghton et al., 2001) an atmospheric concentration of 800 – 1000 ppm is expected by the end of the current century (Hein et al., 1997). This will correspond to a pH reduction of 0.3 – 0.4 in the ocean indicating an increase in H^+ ions in the euphotic zone of the water column by 100 – 150 % (Gao et al., 2012a). Ocean acidification has not only effects on biological processes but also affects the uptake and availability of iron (Shi et al., 2010) and ammonium (Beman et al., 2011, Hutchins et al., 2009).

Many unicellular and multicellular primary producers incorporate calcium in the form of calcium carbonate, a process known as calcification (Zondervan et al., 2001).

$$2\ HCO_3^- + Ca^{2+} = CaCO_3 + CO_2 + H_2O$$

Often these incrustations occur in the form of an endo- or exoskeleton protecting the cells from mechanical damage, predators as well as solar UV radiation. The same is true for many animals such as mollusks, worms, corals and echinoderms. Ocean acidification is known to corrupt calcification. Coccolithophorids are major unicellular primary producers in the oceans. They protect themselves by calcium carbonate plates known as coccoliths (Figure 1). At increased atmospheric CO_2 concentrations, resulting in lowered water pH, biosynthesis of these coccoliths is reduced (Beaufort et al., 2011, De Bodt et al., 2010, Delille et al., 2005, Guan et al., 2010). Similar effects were found in other marine photosynthetic organisms (Israel et al., 1996, Riebesell et al., 2011, Riebesell et al., 2000). Also marine macroalgae, such as the intertidal *Corallina*, incorporate calcium carbonate, and ocean acidification affects this calcification (Gao et al., 1993, Gao et al., 2010, Langer et al., 2006). Likewise calcification is decreased by ocean acidification in calcifying animals, such as mollusks and corals (Anthony et al., 2011, Fine et al., 2007, Melzner et al., 2011, Sinutok et al., 2011).

Since calcified exoskeletons are effective absorbers and scatterers of UV radiation any decrease in calcification causes a higher exposure of the organisms to solar UV radiation, indicating a synergistic detrimental effect of solar UV and ocean acidification (Häder et al., 2011). Solar UV-B irradiation (280 - 315 nm) decreases photosynthetic carbon fixation (Gao et al., 2007a, Häder et al., 2011, Helbling et al., 2003), causes DNA (Buma et al., 2006, Gao et al., 2008) and protein damage (Bouchard et al., 2005a) and alters the morphology (Wu et al., 2009) of photosynthetic organisms.

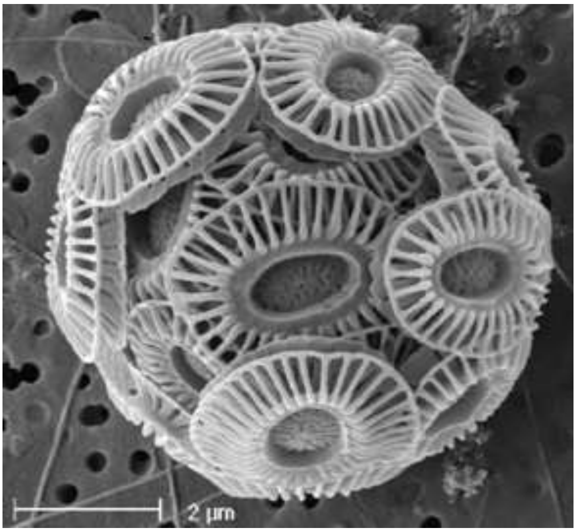

Figure 1. Scanning electron micrograph of the phytoplankton coccolithophore *Emiliania huxleyi* covered with coccoliths. Courtesy of Kunshan Gao, Xiamen, China.

The coccoliths of the Prymnesiophyceae *Emiliania huxleyi* have been measured to reduce solar UV radiation by 26 % (Gao et al., 2009). Water acidification reduces the coccolith thickness, and simultaneously applied UV radiation further decreases calcification. Likewise it was found that solar UV and acidification synergistically reduce calcification in the coralline macroalga *Corallina sessilis* (Gao et al., 2010). When grown under ambient CO_2 concentrations, UV stimulates the calcification of *E. huxleyi*, which can be interpreted as a defense mechanism against elevated levels of solar short-wavelength radiation. However, this protection comes at the expense of a reduced growth rate (Guan et al., 2010). Coralline macroalgae also accumulate UV-screening compounds which partially offsets the effects of detrimental solar UV-B radiation (Gao et al., 2010), but this is not sufficient to counteract the combined effects of both environmental stressors. Therefore a decrease in the vegetation of these reef-building macroalgae can be expected in the wake of global climate change. Water acidification has also been demonstrated to augment the sensitivity of phytoplankton to solar UV-B (Sobrino et al., 2008). In the Prymnesiophyceae *Phaeocystis globosa* acidification resulted in lower photochemical yield and growth rate which was further reduced by simultaneously applied UV-B (Chen et al., 2011).

substantial decline in dinoflagellates between 1960 and 2009, believed to be linked to ocean warming and altered wind conditions (Hinder et al., 2012).

In the Arctic temperature increases have been extreme over the last few decades. This is due to a feedback mechanism: sea ice and snow reflect most of the incoming solar radiation back into space. When the ice and snow melt, water and soil absorb most of this radiation causing a substantial additional warming (Deser et al., 2000). In fact, summer melting occurs earlier and freezing later in the year, and the ice-covered area in the summer has decreased by 49 % from the average between the years 1979 and 2000 (http://nsidc.org). This significant temperature increase augments phytoplankton productivity. Even tropic plankton (radiolaria) have recently been found to invade Arctic waters (http://earth.columbia.edu). The thinning of the ice, earlier melting and later freezing increases the exposure of the phytoplankton populations to solar visible and UV radiation; however, this is partially offset by increased terrestrial runoff with low transparency due to high DOM (dissolved organic matter) (Carmack, 2000). When the ice melts it forms pools on the surface which function like “skylights” or greenhouse windows, which cause an explosion of phytoplankton blooms underneath (http://earth.columbia.edu). This was also found in Antarctica where in spring large blooms of phytoplankton occur under the ice seeded by cells trapped in the ice during winter (Kang et al., 2001, Lancelot et al., 1993). Freshwater from thawing sea ice and glaciers dilutes the sea water and changes salinity with far-reaching effects for the biota. Melt water runoff from glaciers in Polar Regions also decreases the thickness of the upper mixing layer. Stronger stratification exposes the photosynthetic organisms to higher PAR (Photosynthetically active radiation) and UV radiation as well as limits nutrient availability (Steinacher et al., 2010).

Even though the external factors of temperature, pH, CO_2 supply, PAR and UV irradiances as well as mixing depths are known to be primary variables driving photosynthesis and production (Figure 3), their interaction has not been investigated to a large extent (Gao et al., 2012a). This interacting web can only be disentangled by multifactorial analysis (Boyd, 2011, Häder, 2011). In addition, most studies have been carried out in the laboratory in short-term experiments under controlled conditions. In order to reveal the effects in real nature with its fast changing temperature, solar radiation and availability of nutrients, studies need to be carried out in the open ocean (Gao et al., 2012a). This is difficult and time-consuming regarding the vast areas to be covered and the low concentrations of cells in the water column. In order to evaluate the effectivity of physiological and genetic adaptation to the changing growth conditions under a climate change scenario induced by anthropogenic activities, long-term studies and multi-generation experiments need to be devised to understand how phytoplankton will cope with the various stress factors and take advantage of favorable conditions (Hays et al., 2005). Different taxa of primary producers have different abilities to adapt to changing growth conditions such as temperature (Huertas et al., 2011) which will result in changing species composition of future assemblages of primary producers with extensive consequences for the whole marine food webs.

The effects of unknown feedback mechanisms on marine primary producers still need to be disentangled. Do higher temperatures of the oceans result in denser cloud covers? This could result in lower exposure of the phytoplankton to solar UV and PAR. Many phytoplankton and macroalgae are known to produce dimethylsulfoniopropionate (DMSP), an organosulfur compound $(CH_3)_2S^+CH_2CH_2COO^-$, as an osmolyte. This zwitterionic metabolite is excreted and partially broken down to dimethylsulfide (DMS, CH_3SCH_3; DMS) (Karsten et marine cyanobacterium *Synechococcus* had a higher growth rate under increased temperatures and CO_2, while in the related *Prochlorococcus* it did not change, giving the former species an ecological advantage in a climate change scenario (Fu et al., 2007). In the cyanobacterium *Trichodesmium* growth and nitrogen fixation were substantially enhanced by a 4° C temperature increase (Hutchins et al., 2007, Levitan et al., 2010). Also in *E. huxleyi*, isolated from the Sargasso Sea, growth and productivity were augmented by a temperature increase as well as by higher CO_2 concentrations, but both factors did not operate synergistically (Feng et al., 2008).

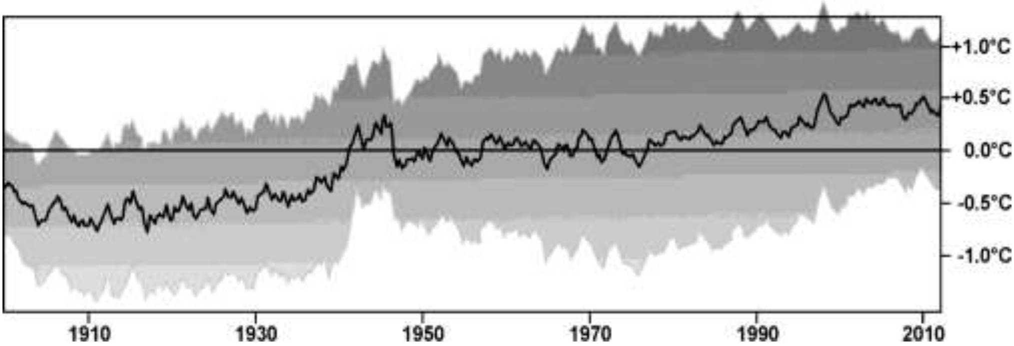

Figure 2. Global ocean temperatures relative to the average from 1900 to 2012 in the top 1 m based on data compiled by Marinexplore (Sunnyvale, Calif). Redrawn from Fischetti (2013). The gray area indicates the margin of error. The pronounced higher temperatures in the 1940s are an artefact due to the unavailability of data from Russia and Europe.

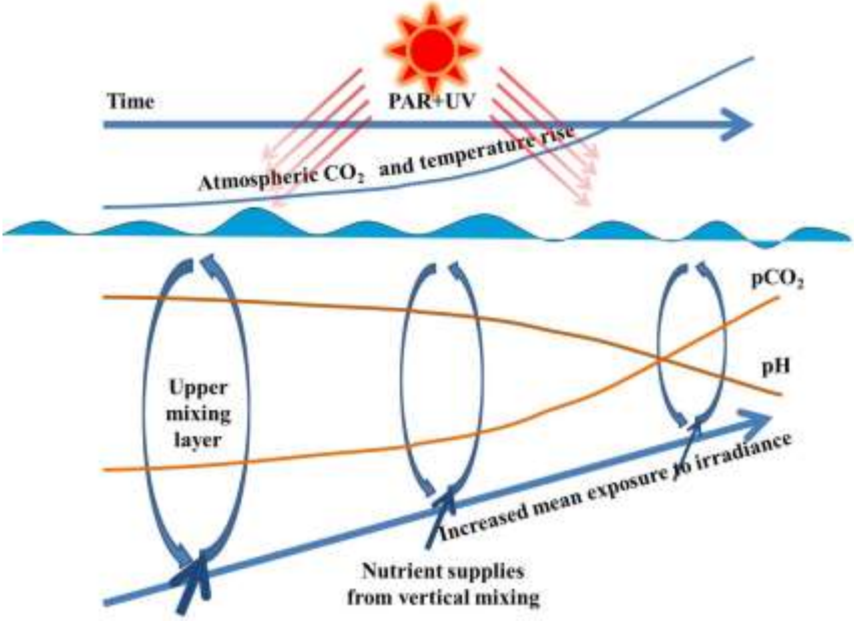

Figure 3. Anthropogenically controlled environmental factors including atmospheric CO_2, ocean acidification, temperature increase with shoaling of the upper mixing layer and resulting increased exposure to solar UV and PAR will be responsible for changes in future phytoplankton growth and marine primary production (from Gao et al., 2012a).

Higher temperatures reduced calcification in another strain of the same species (De Bodt et al., 2010) as well as in the coccolithophorid *Syracosphaera pulchra* (Fiorini et al., 2011). A temperature increase augmented the growth in a mixed natural North Atlantic spring bloom assemblage only when CO_2 was increased simultaneously (Feng et al., 2009); at the same time, calcification was significantly lower. Also in the calcified green macroalga *Halimeda* the combination of increased temperature and acidification reduced chlorophyll content, photochemical yield and calcification (Sinutok et al., 2011). In the bloom-forming raphidophyte *Heterosigma akashimo* maximal photosynthetic carbon fixation was augmented only when both CO_2 and temperature were increased simultaneously while in the dinoflagellate *Prorocentrum minimum* only increased CO_2 was effective (Fu et al., 2008).

These obvious physiological differences are the reason for species shifts in the natural phytoplankton communities in the wake of global climate change. In incubation studies on board a ship during a cruise in the Bering Sea simulated increased CO_2 and temperature caused a shift from diatoms to nanoflagellates (Hare et al., 2007); however, this effect seems to be mainly due to the increased temperature. This was also confirmed in a study on the abundance of diatoms in a North Atlantic bloom (Feng et al., 2009) and a shift from diatoms to dinoflagellates in Daya Bay (China) caused by heating of the water from a nuclear power plant (Li et al., 2011). The observed effects may be due to the increased temperature directly or due to temperature-induced changes in the organic nutrient cycling in the mixing layer (Wohlers-Zöllner et al., 2011).

Solar UV-B radiation affects a wide spectrum of cellular targets in phytoplankton. In addition to damage to elements of the photosynthetic machinery, such as the D1 protein in the electron transport chain in PSII (Bouchard et al., 2006, Bouchard et al., 2005b, Wängberg et al., 1996), energetic short-wavelength UV radiation damages DNA, either directly by the induction of cyclobutane dimers (Klisch et al., 2005, Sinha et al., 2001) or by the formation of detrimental ROS (Reactive Oxygen Species) (Ferreyra et al., 2006, Frost et al., 2002, Pelletier et al., 2006, Roncarati et al., 2008). Blue light or UV-A photons are effective to break the cyclobutane dimers using the enzyme photolyase (Buma et al., 2001, Helbling et al., 2001, van de Poll et al., 2001). This repair mechanism occurs mainly during passive cycling of the phytoplankton in the upper mixed layer. When the cells are close to the surface, UV-B-induced dimerization prevails, and the bonds are broken again when the cells are transported to deeper depth within the mixing layer (Helbling et al., 2008). As all enzymatic reactions, the photolyase-controlled repair of cyclobutane dimers in DNA is augmented by increasing temperature (Gao et al., 2008, Häder et al., 2007a).

Higher temperatures also moderate UV-induced damage of the photosynthetic machinery (Gao et al., 2008, Halac et al., 2010, Helbling et al., 2011, Sobrino et al., 2007), but on the downside they enhance respiratory carbon losses. Temperature-induced changes are already observed in major phytoplankton groups such as dinoflagellates (Cloern et al., 2005, Hallegraeff, 2010, Peperzak, 2003), diatoms and coccolithophorids (Hare et al., 2007, Mericoa et al., 2004), with obvious changes in abundance and habitats. This was confirmed in a mesocosm experiment: higher temperatures augmented growth and resulted in significant changes in the species composition of the phytoplankton and zooplankton assemblage (Lewandowska et al., 2010). The picture becomes more complicated for coastal waters since there both CO_2 concentration and temperature show a pronounced daily cycle affecting phytoplankton productivity. In the northeast Atlantic and North Sea Hinder et al. found a substantial decline in dinoflagellates between 1960 and 2009, believed to be linked to ocean warming and altered wind conditions (Hinder et al., 2012).

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Underwater Light Climate

Light is the single most important factor for photosynthesis in primary producers both in terrestrial and aquatic habitats. Terrestrial plants are adapted to the daily light and dark cycle and can manage the variations induced by changing cloud cover. In addition, they are adapted to various light regimes such as shade or sun conditions and the different light intensities resulting from different solar angles depending on the latitude.

Macroalgae are also fixed in their position and thus have to operate with the ambient light conditions. In addition, there is a modulation of the light climate due to the tidal rhythm which does not coincide with the daily light-dark cycle (Jiménez et al., 1998). Furthermore, the intensity of the transmitted light depends on the transparency of the water column. Most macroalgae are adapted to low light conditions and shut down their photosynthetic quantum efficiency using the mechanism of reversible photoinhibition which regulates the amount of energy funneled into photosynthetic electron transport (Häder et al., 2001, Helbling et al., 2010).

Non-sessile phytoplankton, in contrast, can adapt to the prevalent light conditions by choosing the vertical position in the water column. Some rely on active motility such as flagellates (Häder et al., 1988) and others on changing the buoyancy of their cell bodies, such as cyanobacteria and diatoms (Walsby, 1987). Often motile phytoplankton use positive phototaxis at low light intensities to move to the surface (Matsunaga et al., 2003). This is supported by negative gravitaxis which also brings the cells closer to the surface (Eggersdorfer et al., 1991, Lebert et al., Roberts, 2006). At excessive light intensities – and detrimental UV exposure – the cells often switch to negative phototaxis to move into deeper waters (Josef et al., 2005, Lenci et al., 1983, Matsuoka, 1983). While these mechanisms often work adequately in freshwater habitats, the vertical migration and orientation mechanisms are often overthrown by the action of wind and waves in marine ecosystems (Barbieri et al., 2006, Helbling et al., 2005) where the cells are passively transported within the mixing layer (Yoshiyama et al., 2002). However, even then, often vertical distribution patterns of phytoplankton can be observed in the water column since the active movement superimposes the passive mixing (Piazena et al., 1995).

The irradiance of solar radiation in the water column is governed by many variables in the atmosphere and the properties of the water (Boss et al., 2007), which affect the light levels and the wavelength distribution in the water column (Häder et al., 2007b, Smith and Mobley, 2007). The transparency of the water is determined by dissolved substances, which can be either organic (dissolved organic matter, DOM) (Vione et al., 2009) or inorganic (dissolved inorganic matter, DIM) (Xie et al., 2009), or by particulate matter, which again can be either organic (particulate organic matter, POM) (Mayer et al., 2006) or inorganic (particulate inorganic matter, PIM) (Vahatalo et al., 2007). POM includes also the phytoplankton cells, and the transparency correlates with their concentration (Bracchini et al., 2006, Sommaruga et al., 2006). All these materials absorb and/or reflect the incoming solar radiation. The light attenuation can be neutral, i.e., the same fraction at all wavelengths, or it can be more pronounced at specific wavelengths; e.g., some of the DOM specifically absorbs at shorter wavelengths, therefore this fraction is called colored or chromophoric DOM (CDOM) (Osburn et al., 2009).

The absorption by DOM is highest in eutrophic freshwater systems and lower in oligotrophic habitats. Most of the DOM reaches the water as runoff from the surrounding terrestrial areas and is produced from organic material of decaying plant residues (Boyle et al., 2009). Likewise most of the DOM found in coastal marine ecosystems stems from terrestrial runoff (Day et al., 2009), supplemented by material from decaying macroalgae which are almost exclusively limited to coastal habitats (Hulatt et al., 2007). DOM concentrations are much lower in open oceanic ecosystems and are derived from decaying phytoplankton (Behrenfeld et al., 2009, Vahatalo et al., 2007) with the exceptional addition of decaying swimming macroalgae, as in the Sargasso Sea (Bailey et al., 2008).

In the hyper-oligotrophic waters of the South Pacific Gyre without hardly any DOM extremely high UV-B and UV-A transmissions have been measured (Tedetti et al., 2007); 1 % of the 325 nm radiation incident on the surface penetrates 84 meters down in the water column. For comparison 320 nm radiation is attenuated to 1 % at 27 m in some of the clearest lakes such as the Lake Tahoe (California-Nevada, USA) (Rose et al., 2009) and to 62 m in Crater Lake (Oregon, USA) (Hargreaves et al., 2007).

Measurements of solar radiation in the water column can be either done with broadband radiometers or with spectroradiometers; however the second class of instruments requires a much higher technological effort. In addition, chemical dosimeters based on chemical reactions or damages in DNA can be used (Schouten et al., 2007, 2009). These measurements can be quite accurate provided the instruments are carefully calibrated (Schouten et al., 2008). In order to systematically evaluate erythemal UV irradiances and ozone concentrations in various climate zones, ship-borne measurements were performed during a transect from Bremerhaven (Germany) to Cape Town (South Africa) (Wuttke et al., 2007).

In polar (both Arctic and Antarctic) fresh and marine waters light availability is modified by dissolved and particulate organic and inorganic material which is introduced into the water by runoff during melting (Schindler et al., 2006). Due to the massive increases in temperature in the Polar Regions by climate change, dramatic changes in the transparency and timing of the melting will occur. In an attempt to predict future changes in Arctic and Subarctic small lakes due to global warming, models have been developed using environmental factors such as DOC concentrations, weather and water acidification affecting the transparency and thus the development of organisms (Keller et al., 2006, Paterson et al., 2008).

Colored dissolved organic matter (CDOM) is a driving factor modulating optical properties of freshwater and marine habitats. Biogeochemical cycles are directly and indirectly affected by the concentration of CDOM (Fernandes et al., 2008, Zepp et al., 2007). Since CDOM is produced by the degradation of organic material from phytoplankton and macroalgae – as well as from terrestrial plants for freshwater and coastal habitats – its concentration varies seasonally depending on the availability of organic material as well as on rainfall activity (Suhett et al., 2007). Due to a lower terrestrial input of organic material, high mountain lakes located above the tree line have lower CDOM concentrations and as a result a higher transparency (Rose et al., 2009). CDOM concentrations also depend on the level of solar UV radiation (Hayakawa et al., 2008). Alpine lakes at higher altitudes are affected by intense solar UV, which breaks down CDOM and also affects the species composition in the water. Microbial activity is important for the generation of DOM either inside the water column or on land before the material is washed into the water (Hudson et al., 2007). CDOM has a strong absorption in the UV. UV-B breaks the double bonds in these humic materials. CDOM levels decreased by 18 – 55 % when exposed to solar UV-B, as monitored during a cruise in the Bering Sea at some of the stations (Brooks et al., no year), while at others CDOM absorption was found to increase which was interpreted as the result of decreased microbial activity.

The relationship between DOM and solar UV-B radiation has been analyzed in predator-free rivers in the Northern U.S.: due to the high UV-B absorption of DOM, its concentrations have a large effect on biomass productivity and species composition of the phytoplankton (Frost et al., 2007). Dissolved free amino acids are degraded in the presence of DOM due to the generation of singlet oxygen ($^1O_2$) as has been observed in water samples from Pony Lake, Antarctica as well as from several lakes and rivers in the Northern U.S. (Boreen et al., 2008). CDOM is bleached and photodegraded when exposed to solar UV (Feng et al., 2006, Tzortziou et al., 2007, Zhang et al., 2008). Photodegradation is the main process for mineralization and carbon recycling of DOM (Anusha et al., 2008, Feng et al., 2006, Wang et al., 2009). This increases the transmission of solar UV-B in the water column and results in detrimental effects on aquatic organisms (Feng et al., 2006). However, degradation of plankton and macroalgae releases nutrients such as phosphorus and iron (Bastidas Navarro et al., Navarro et al., 2009, Shiller et al., 2006).

CDOM absorption can be used as a proxy for UV penetration into the water as well as for the water quality; this has been shown for Lake Taihu, China, which is an important drinking water reservoir (Zhang et al., 2007). Seasonal changes in the UV transparency are suggested to be more realistic indicators for environmental changes than in the visible spectrum as found in the subalpine Lake Tahoe, California-Nevada (Rose et al., 2009). When DOM is broken down into smaller fragments these are utilized as food source for bacteria, further decreasing the DOM concentration (Piccini et al., 2009); it also changes the composition of bacterioplankton.

Effects of UV-B Radiation on Phytoplankton

The depletion of the stratospheric ozone layer due to anthropogenic emissions of chlorinated fluorocarbons has aroused major scientific and public concerns due to increases in solar ultraviolet-B (UV-B, 280-315 nm) radiation on the Earth’s surface (Crutzen, 1992). In addition to the Antarctic ozone hole, ozone depletion has been reported to increase and spread in a broader range of altitudes and latitudes throughout the world since the late 1970s (Hoffman et al., 1991). UV-B radiation can penetrate into the water column to ecologically significant depths (Fleischmann, 1989, Jerlov, 1950, Smith et al., 1992) and may lead to the damage of biological systems including natural phytoplankton communities (Meador et al., 2009).

Phytoplankton are unquestionably the major biomass producers in marine ecosystems thus providing an essential ecological function for all aquatic life. They account for nearly half of the total production of organic matter on Earth *via* photosynthesis. In addition to playing a major role in the food web, many genera of phytoplankton produce certain volatile substances such as dimethylsulfide (DMS) that serve as an antecedent of cloud condensation nuclei (CCN) and neutralize the greenhouse effect (Charlson et al., 1987). The cumulative effect of marine biota in the reduction of CO_2 concentration and emission of DMS has been estimated to lower the atmospheric temperature by up to 6°C (Watson et al., 1998). They are also a rich source of dimethylsulfoxide (DMSO) (Simó et al., 1998) having a potential role as cryoprotectant and osmoregulator (Lee et al., 1999). Phytoplankton organisms have been reported to play an important role in regulation of global climate change. The distribution of phytoplankton in the oceans depends upon various factors such as the incidence of solar radiation and nutrient availability. Therefore, a dense phytoplankton biomass has been reported to be present in the euphotic zone and near the coast because of higher nutrient availability due to upwelling of nutrient-rich water from the deep sea and terrestrial influxes.

The obligatory requirement of phytoplankton communities for solar radiant energy for photosynthesis, growth and survival exposes them to deleterious UV-B radiation. It has been reported that UV-B radiation could alter the morphology, impair motility and photo-orientation, damage proteins and DNA, inhibit growth, nitrogen metabolism, pigmentation, enzymatic functioning, fatty acid composition and photosynthesis of various microalgal species (He et al., 2002, Kottuparambil et al., 2012, Rastogi et al., 2010a). Thus, the effects of these ecological consequences on production rates and community structure of primary producers in aquatic ecosystems are of growing concern in recent days (Häder et al., 2011). Biologically effective doses of UV-B have been detected down to a depth of 70 m in the water column (Smith et al., 1992) and may thus affect the aquatic ecosystems (Häder et al., 1998). The variables in the atmosphere and the water that affect the amount of UV radiation and wavelength distribution determine the transmission of solar UV radiation into the water column (Häder et al., 2007b, Smith and Mobley, 2007). The penetration of UV-B into the water depends on the optical properties of the water column. CDOM, generated through microbial degradation of organic material from macroalgae and plankton as well as terrestrial plants, is a major factor controlling optical characteristics of freshwater and coastal habitats (Hulatt et al., 2007).

UV-B radiation can cause either dynamic or chronic photoinhibition in the phytoplankton (Bracher et al., 2000). Dynamic photoinhibition involves the down-regulation of the photosynthetic apparatus and is associated with the dissipation of excess energy as heat; whereas chronic photoinhibition involves photodamage of the PS II reaction center D1 leading to a reduction in the number of functional PS II centers (Hanelt, 1996). Kottuparambil et al. (2012) reported a significant decrease in the photosynthetic performance, motility and increased cellular concentration of reactive oxygen species (ROS) in the unicellular flagellated photoautotroph *Euglena agilis* following UV-B irradiation. In chloroplasts, ROS is reported to cause inactivation and degradation of Rubisco and other components of the Calvin cycle (Patsikka et al., 1998) as well as lipid peroxidation, which results in the disruption of photosynthetic pigments and ultimately results in oxidative stress (Patsikka et al., 2002). UV-B radiation has also been reported to increase the net loss of D1 protein pools in natural phytoplankton (Bouchard et al., 2005a). Severe impairment in motility, orientation and moving velocity after UV-B exposure have been reported in a number of motile microalgae, including *Chlamydomonas, Cryptomonas, Euglena, Gyrodinium* and *Peridinium* spp. (Hessen et al., 1997). UV-B mediated impairment of motility and/or swimming velocity would, therefore, limit the capacity of motile phytoplankton to adapt to the surrounding light environment, thus reducing photosynthesis and growth (Rai and Mallick, 1998). UV-B generates reactive oxygen species (ROS) through endogenous photosensitization reactions in photosynthetic microorganisms (Rastogi et al., 2010c). Severe oxidative stress inside cells can cause DNA damage, impair photosynthetic efficiency and alter the orientation of photosynthetic microalgae (Richter et al., 2003, Vincent et al., 2000). UV affects the cell morphology of phytoplankton in various ways. Decreased growth rates and increased cell volume are common in diatoms under chronic UV exposure (Karentz et al., 1991). These authors also observed elongated cells under UV radiation in various Antarctic marine diatoms, caused by arrested cell division. Behrenfeld et al. (1992) found generally smaller cell volumes in the diatom *Phaeodactylum tricornutum* grown when ambient UV-B was screened off, compared with cells exposed to UV-B.

Certain diatoms (*i.e., Skeletonema costatum*) are very sensitive and did not survive for more than three days, whereas others (*e.g., Amphora coffeaeformis* and *Odontella aurita*) were able to acclimate to UV stress, although through different processes, when exposed to UV radiation (Fouqueray et al., 2007). The concentration of CO_2 seems to have a role in conditioning the sensitivity of a diatom (*Thalassiosira pseudonana*) as this species was more sensitive to UV radiation when acclimated to high CO_2 than under atmospheric CO_2 levels.

Studies conducted by Fritz et al. (2008) revealed that the open-ocean phytoplankton in Antarctic were more sensitive to UV radiation than coastal assemblages, the latter having higher rates of repair. UV radiation induced photoinhibition of natural post-bloom diatom-dominated phytoplankton assemblages from temperate latitudes of Patagonia. However, when samples were dominated by chlorophytes, the inhibition was decreased (Villafañe et al., 2008). Gao et al. (2007b) reported that the tropical phytoplankton assemblages from a coastal site of the South China Sea were significantly inhibited by UV radiation (mostly by UV-B) during sunny days. However, during cloudy days, while small cells (pico- and nanoplankton, <20 mm) were still inhibited by UV radiation, the larger cells (microplankton, >20 mm) used UV-A radiation as a source of energy for photosynthesis.

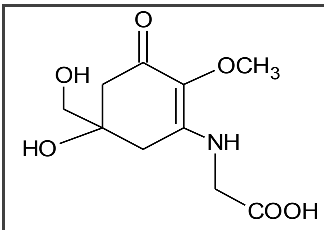
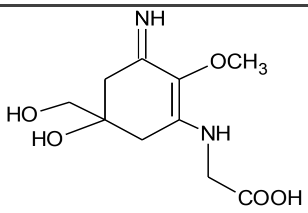
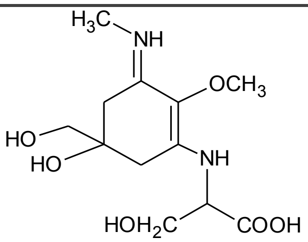
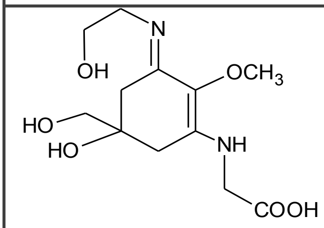
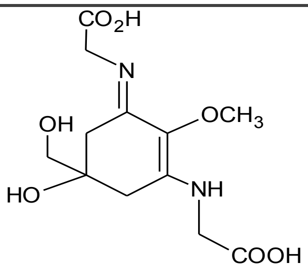
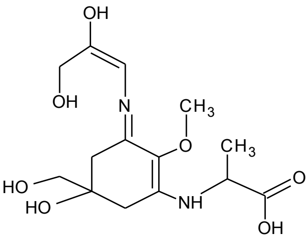
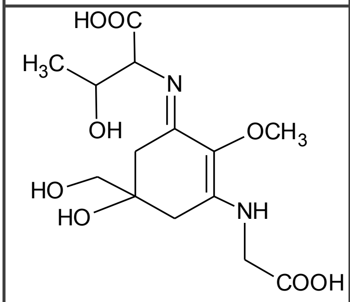
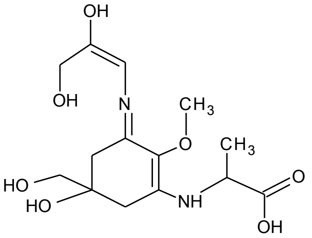
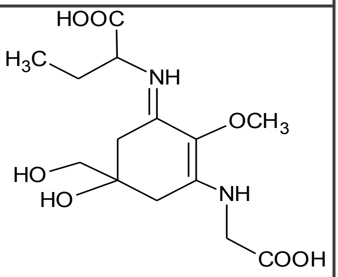
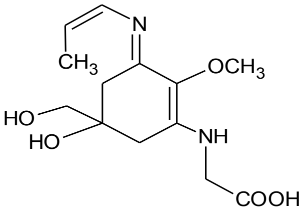
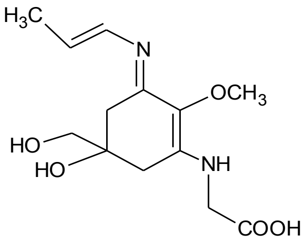
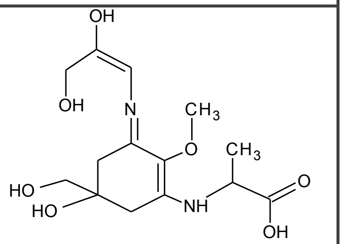
As compared with the oceanic and coastal species, estuarine diatoms are more flexible in terms of photoprotection, and thus have less photoinhibition when exposed to excess light (Lavaud et al., 2007). UV radiation has been reported to reduce the cellular C:P (and N:P) ratios in phytoplankton, thus changing food quality in aquatic food-webs, as well as affecting biogeochemical cycling (Hessen et al., 2008). The UV sensitivity of phytoplankton to UV radiation is affected by the light history of the cells (MacDonald et al., 2003), which in turn is influenced by the mixing regime prevailing in the water column. Studies conducted by Barbieri et al. (2006) showed that the fluctuating radiation regime resulting from vertical mixing had a pronounced beneficial effect on *Heterocapsa triquetra*, whereas *Dunaliella salina* was affected by both high and low solar irradiances, and *Thalassiosira weissflogii* was inhibited only by high solar irradiances. Helbling et al. (2008) reported the significant inhibition of photosynthesis but no DNA damage in three dinoflagellate species under static and mixing conditions during the austral spring. Increasing mixing speed increased UV-induced inhibition of carbon fixation in *Gymnodinium chlorophorum* and *Heterocapsa triquetra*, but not in *Prorocentrum micans*. Most of the loss in carbon fixation in *G. chlorophorum* was due to UV-B radiation, while in *H. triquetra* it was due to UV-A radiation. UV-B radiation was found to have inhibitory effects on the growth of sub-polar phytoplankton under static and mixed conditions, but the synthesis of mycosporine-like amino acids (MAAs) helped the cells to cope with UV radiation by changing the species composition towards the more tolerant ones (Hernando et al., 2006). Helbling et al. (2013) suggested that under ambient nutrient conditions there is a synergistic effect between vertical mixing and UVR, increasing phytoplankton photosynthetic inhibition and excretion of organic carbon (EOC) from opaque lakes as compared to algae that received constant mean irradiance within the epilimnion. Garde and Cailiau (2000) reported the reduction in the growth rate and increased cell volume of *Emiliania huxleyi*, whereas inhibition of the electron transport and decreased maximum quantum yield of photosystem II (Fv/Fm) following UV irradiation was investigated by Marwood et al. (2000). Harada et al. (2009) found that UVR reduced chlorophyll *a* in cultures of *Amphidinium carterae* and *Thalassiosira oceanica*, reduced Fv/Fm and the growth rate of *T. oceanica*. The results of Larkum and Wood (1993) confirmed that phytoplankton are highly sensitive to UV-B radiation. Similar results have been reported by Häder and Häder (1991) while working with a marine cryptoflagellate, *Cryptomonas maculata*. Lesser (1996) reported the significant decrease in photosynthetic performance, chlorophyll content, and Rubisco activities of the dinoflagellate *Prorocentrum micans* following UV-B exposure. Ekelund (1990) observed that the growth of the motile dinoflagellates *Gyronidium aureolum,* and *Prorocentrum minimum* was more sensitive to UV-B radiation than that of the non-motile diatoms *Dityhim brightwellii* and *Phaeodactylum tricornutum*. He also reported that in addition to growth, swimming speed of the dinoflagellates *G. aureolum* and *P. minimum* were affected by UV-B radiation, while the diatoms were nearly unaffected. Prolonged irradiation time even resulted in complete loss of motility in the dinoflagellates. UV-B radiation had dramatic effects on rates of photosynthesis, motility and on absorption spectra in four studied phytoplankton species (Ekelund, 1994). Photosynthesis of *Euglena gracilis* and the diatom *Phaeodactylum tricomution* was more sensitive to UV-B inhibition than that of the dinoflagellates *Heterocapsa triquetra* and *Prorocentrum minimum*. It was observed that the swimming speed of *H. triquetra* decreased more after low visible light and UV-B radiation compared to high visible light and UV-B radiation.

The meta-analysis conducted by Llabrés et al. (2013) demonstrated that the mortality rates in marine biota including microalgae increases rapidly in response to elevated UV-B radiation. Karentz et al. (1991) studied twelve species of Antarctic diatoms to assess UV sensitivity in relation to cellular and molecular aspects of DNA damage and repair and concluded that a wide range of interspecific UV sensitivity was present among the diatoms, emphasizing the ecological implications of changes in natural UV regimes. Skerratt et al. (1998) studied the effects of UV-B radiation on the fatty acid, total lipid and sterol composition and content in three Antarctic marine phytoplankton and found them to be species-specific.

UV-Absorbing Compounds

Phytoplankton organisms have evolved certain tolerance mechanisms against UV-B radiation, which include avoidance, ROS scavenging by non-enzymatic and enzymatic antioxidants, the synthesis of UV-absorbing/screening compounds such as mycosporine-like amino acids (MAAs), scytonemin and sporopollenin, repair of UV-induced DNA damage and the re-synthesis of damaged PS II proteins (Carreto et al., 2011, Rastogi et al., 2010a, Singh et al., 2010b). The vertical migration within the water column allows the organisms to adjust the impinging radiation to a level that is suitable for photosynthesis and to avoid excessive doses of visible as well as UV radiation (Häder, 1988).

Table 1. Molecular structure and corresponding absorption maxima of certain phytoplanktonic MAAs

 Mycosporine-glycine (310 nm)	 Palythine (320 nm)	 Mycosporine-methylamine-serine (327 nm)
 Asterina-330 (330 nm)	 Mycosporine-2-glycine (334 nm)	 Shinorine (334 nm)
 Porphyra-334 (334 nm)	 Mycosporine-glycine-valine (335 nm)	 Palythenic acid (337 nm)
 Usujirene (357 nm)	 Palythene (360 nm)	 Euhalothece-362 (362 nm)

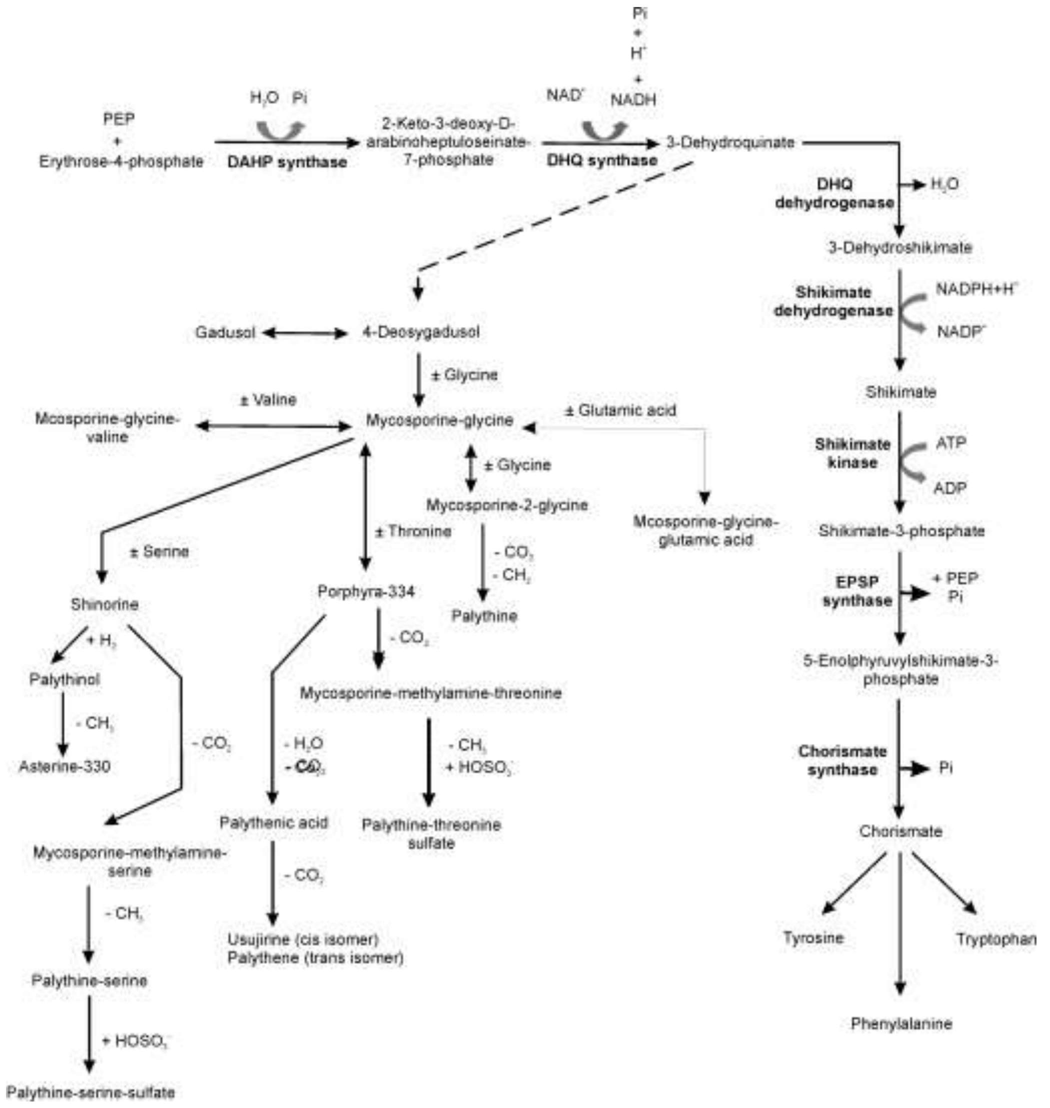


Figure 4. Shikimate pathway for MAAs biosynthesis (adapted from Singh et al. (Singh et al., 2008).

MAAs constitute a diverse family of water soluble, colorless natural products that share a 5-hydroxy-5-hydroxymethyl-cyclohex-1,2-ene ring and have a methoxy-substituent in C2 (Table 1).

They are always substituted in C3 with an amino compound and in C1 with either an oxo or an imino moiety (Gao et al., 2011). Each MAA has a unique retention time when analyzed by HPLC (High-Performance Liquid Chromatography), an absorbance between 310 and 362 nm and a high molar extinction coefficient ($\varepsilon = 28{,}100\text{-}50{,}000\ M^{-1}\ cm^{-1}$) which favors them as potent photoprotectants.

They rely on electronic transitions in the conjugated enone or enamine part of the cyclohexene core for absorption. The exact biosynthetic pathway for MAAs is still unresolved. However, it is considered that 3-dehydroquinate, a six carbon ring formed in the center of the shikimate pathway, serves as a precursor for the synthesis of fungal mycosporines and MAAs via gadusols (Shick et al., 2002, Singh et al., 2008) (Figure 4). The primary MAA mycosporine-glycine, synthesized in the shikimate pathway, undergoes chemical or biochemical transformations to yield other secondary MAAs (Callone et al., 2006). The synthesis of MAAs occurs in fungi, bacteria, cyanobacteria, phytoplankton and macroalgae (red, brown and green algae) but not in animals, because they lack the shikimate pathway. Studies have shown that animals derive MAAs from their algal diet or symbiotic associations (Rastogi et al., 2010b).

The occurrence of MAAs has been reported in marine as well as freshwater phytoplankton organisms (Sinha et al., 2007, Xiong et al., 1999). MAAs have been reported to occur predominantly in members of the Dinophyceae, Bacillariophyceae and Haptophyceae (or Prymnesiophyceae). Lower levels were reported in diatoms, chlorophytes, euglenophytes, eustigmatophytes, rhodophytes, some dinoflagellates and some prymnesiophytes (Jeffrey et al., 1999).

The genes derived from both photosynthetic and heterotrophic lineages may account for differences in the biosynthesis, accumulation and conversion of MAAs among marine algae. For instance, in the dinoflagellates *Heterocapsa triqueta* and *Oxyrrhis marina*, a dehydroquinate synthase (DHQS) similar to YP_324358 in *Anabaena variabilis* PCC 7937 (Singh et al., 2010a) has been reported to be present in the chloroplast and to be fused to O-methyltransferase (Waller et al., 2006). In the dinoflagellates *H. triquetra, O. marina* and *K. micrum* both genes have been reported to be transferred from cyanobacteria via a prokaryote-to-eukaryote lateral/horizontal gene transfer event during evolution (Singh et al., 2010a, Waller et al., 2006).

Dinoflagellates

The occurrence of UV-absorbing compounds in marine phytoplankton was first noted in the dinoflagellates (Balch et al., 1984).

The commonly occurring natural symbiotic dinoflagellate *Symbiodinium* sp., regardless of the clade identity, can synthesize MAAs, namely mycosporine-glycine, shinorine, porphyra-334 and palythine, in contrast to the cultured form (Banaszak et al., 2006). Some photosynthetic free-living species of dinoflagellates such as *Prorocentrum minimun* (Sinha et al., 1998) and *Woloszynskia* sp. (Jeffrey et al., 1999) also contain several MAAs whereas in *Amphidinium carterae* only mycosporine-glycine was found (Hannach et al., 1998). Higher MAA concentrations in *Prorocentrum micans*, adapted to UV radiation did not completely mitigate the detrimental effects of UV on photosynthesis (Lesser, 1996). Red tide dinoflagellates such as *Gymnodinium sanguineum*, *Alexandrium* sp. and *P. minimum* have been reported to contain high concentration of MAAs (Cardozo, 2007, Laurion et al., 2009, Neale et al., 1998).

Recently, Laurion and Roy (2009) reported the presence of seven MAAs in two strains of *A. tamarense* and *H. triqueta*. They also observed two novel MAA-like compounds with distinct and previously unreported absorption maxima in the dinoflagellates *Gymnodinium galatheanum*, *Gymnodinium veneficum* (λ_{max} = 342 nm) and *Prorocentrum micans* (λ_{max} = 352 nm).

Prymnesiophytes

High concentrations of MAAs have been reported in several prymnesiophyte species, especially *Phaeocystis pouchetti* from Antarctica (Marchant et al., 1991), which was found to be induced under PAR and PAR+UV irradiation. *P. pouchetti* predominately contained mycosporine-glycine-valine, shinorine and mycosporine-glycine (Newman et al., 2000). The bloom-forming coccolithophorid *Emiliania huxleyi* has been reported to contain a variety of MAAs (Hannach et al., 1998). Of the three species of prymnesiophytes examined by Llewellyn and Airs (2010), *Isochrysis galbana* and *E. huxleyi* contained MAAs at low concentrations, while *Phaeocystis globosa* contained no detectable MAAs.

Raphidophytes

High levels of the unusual secondary MAA asterina-330 have been reported in *Heterosigma carterae* and *Fibrocapsa* sp. (Llewellyn et al., 2010). *Chattonella marina*, an Australian strain of the raphidophyte has been reported to produce five times more MAAs (mycosporine-glycine; mycosporine glycine-valine and shinorine) and grew 66% faster than a Japanese strain of the same species under inhibiting UV-B radiation (Marshall et al., 2002). Increased MAA production under high irradiances was also observed in other Australian strains of *Chattonella*, but was not noted in other Japanese strains suggesting ecophenotypic adaptation due to differing environmental conditions (Marshall et al., 2002).

Diatoms

Generally lower levels of MAAs have been reported in cultured diatoms compared to dinoflagellates (Llewellyn et al., 2010). Porphyra-334 with lesser amounts of shinorine has been reported to be present in nine bacillariophyte species studied (Riegger et al., 1997) and one additional mycosporine-glycine in one species, *Porosira pseudodenticulata*. In Antarctica, high *in vivo* absorption at wavelengths indicative of MAAs was characteristic of assemblages by the chain-forming diatom *Thalassiosira gravida* (Ferreyra et al., 1994). Generally, diatoms have been reported to contain primary MAAs, but the presence of the secondary MAAs palythine and palythene has been reported in *Corethron criophilum* (Helbling et al., 1996). Carreto et al. (2005) reported the presence of the unusual mycosporine-taurine for the first time in a diatom, *Pseudo-nitzschia multiseries*.

Scytonemin-Like Compound

A UV-absorbing compound with similar absorption spectra and chromatographic behavior to that of scytonemin was reported in a phytoplanktonic bloom of the Icelandic Basin (Sinha et al., 1998). However, due to the lack of chemical characterization of this pigment, there is no direct evidence for the presence of scytonemin or similar pigments in eukaryotic phytoplankton (Llewellyn et al., 1997).

Sporopollenin

Xiong et al. (1997) have reported the occurrence of sporopollenin or sporopollenin-like substances (algenans), biopolymers of variable composition, in certain green freshwater microalgae, for example *Scenedesmus communis* (Blokker et al., 1999) and *Chlamydomonas nivalis* (Gorton et al., 2003) that probably plays an important role in screening of UV radiation. These almost undegradable macromolecules are either bound to the algal cell wall or present in zygospores. They absorb from the ultraviolet to the blue region of the spectrum and thus, are probably responsible for the observed UV-resistance of these organisms (Pescheck et al., 2010, Van Winkle-Swift et al., 1997, Xiong et al., 1997). Potentially, sporopollenins may be present in green macroalgae as well. Whatever the screening compound may be, its screening ability due to absorption may be enhanced by light scattering caused by the macromolecular structure of the compound itself and the cell walls. It has been shown that in light-scattering suspensions the absorbance of chromophores is strongly enhanced (Butler, 1962).

Thus, increased levels of UV-B radiation resulting from depletion of stratospheric ozone are likely to induce changes in community structure which might have consequences for the aquatic food webs and thus on the entire ecosystem. However, the action of defense mechanisms in organisms partially mitigates the inhibitory effects of UV-B. Species differ greatly in their sensitivity to UV-induced damage. This review provides compelling evidence favoring the role of MAAs as photoprotectants in various phytoplankton. The reports of specific distributions of absorption due to MAAs in the water column indicate a role of these compounds in protection against UV radiation, but there are many reports showing that the presence of MAAs in an organism is no proof that this organism is UV-tolerant (Xiong et al., 1997). More work needs to be done to unravel the exact role of photoprotective compounds in the sustainable growth of phytoplankton in water bodies.

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Chapter II

Associations between Climate, Water Environment and Phytoplankton Production in African Lakes

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Abstract

This chapter is a review of phytoplankton dynamics in African lakes with an emphasis on climate and subsequently water quality and how these affect phytoplankton biomass and production in these lentic systems.

Much research has been conducted on phytoplankton communities in African lakes and this has generated debate around whether climate change, among other factors is impacting phytoplankton communities and consequently fish production in these lakes. A few reviews of phytoplankton production, seasonality, and stratification in tropical African lakes, which also considered the effects of nutrient enrichment and the potential impacts of air and water climate warming on phytoplankton production and composition, have been previously presented.

The chapter seeks to update these reviews and also further explore the climate warming question. More specifically, the chapter fills the gaps on how, if at all elevated ambient temperatures are translated to the physical and chemical environment of the lakes and consequently phytoplankton biomass and production as well as seasonality; and subsequently fish production. This has potential, serious implications on human livelihoods that are depended on aquatic ecosystem services that these important lakes provide.

Keywords: Phytoplankton, African lakes, changing environment

Introduction

African lakes (location of the major lakes is shown in Figure 1) are havens for diverse endemic fish fauna of immense ecological and scientific importance that are dependent on sustained phytoplankton seasonality and stratification. Lake Malawi for instance, contains the highest species diversity of fish of any lake in the world and hosts the largest endemic fish population with just over 500 fish species that are unique to the lake. Lakes Victoria and Tanganyika possess ~300 and 140 endemic fish species respectively. Furthermore, many African lakes are a major source of livelihoods through their provision of food in the form of fish and aquatic flora and fauna; fresh water for agriculture, household use, irrigation; as well as means of transportation, navigation and communication. It is estimated that lakes in Africa support 16-17% of inland fisheries (Sithole, 2000; FAO, 2004). Lake Malawi for instance, provides over 60% of the animal protein consumed by Malawi’s entire population. Many African lakes are also used to generate hydro-electric power and as a source of livelihood and revenue from fishing and eco-tourism and many other ecosystem goods that are derived from them.

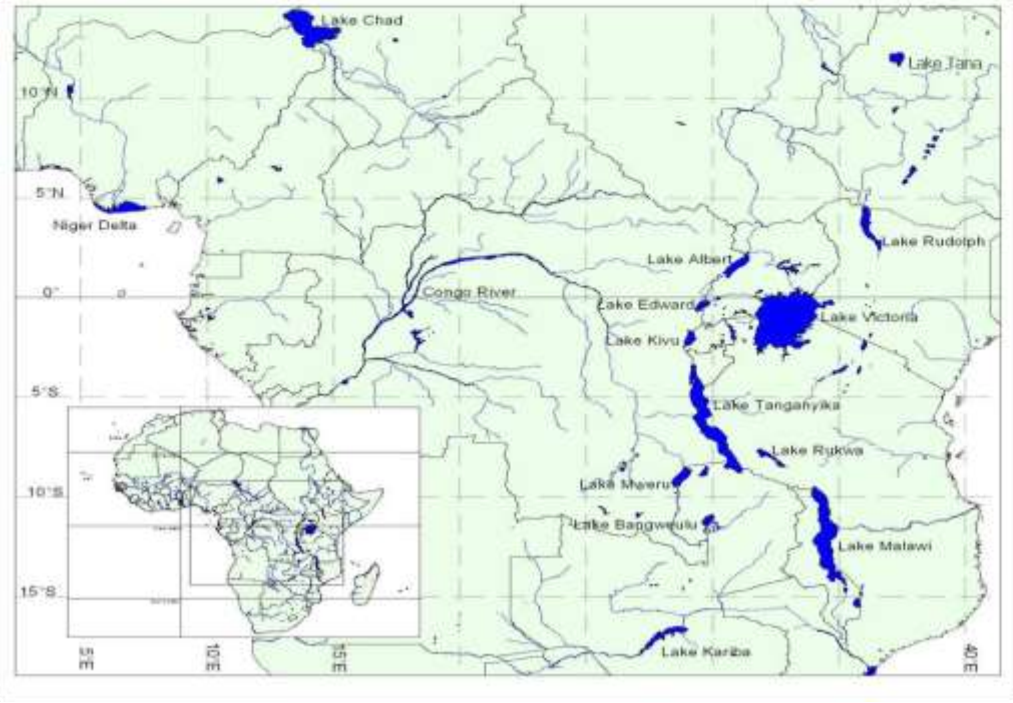

Figure 1. The location of some major lakes in Africa.

Initially, the majority of studies focusing on phytoplankton communities in African lakes merely centred purely on the phytoplankton dynamics, limnology and the water environment. Therefore, these previous reports were predominantly taxonomic focused, with some considering phytoplankton composition and production in specific lakes as these reflect their overall health (Talling and Talling, 1965; Talling, 2001). However, African lakes are known to be potentially highly sensitive to climate change (Johnson and Odada, 1996; Odada et al., 2006; IPCC, 2007; Olaka et al., 2010), as small variations in climate cause wide fluctuations in the thermocline over a narrow range of high water temperature (O'Reilly et al. 2003; Stenuite et al., 2007; Moss, 2010; Ndebele-Murisa et al., 2010). Debate around the potential or observed impacts of climate warming pertaining to biological productivity in these lakes has emerged. Such information is especially pertinent since many tropical African lakes are already experiencing deterioration in water quality and declines in fish abundance and diversity, which are likely to be exacerbated by climate change, and increased anthropogenic pressures (Magadza, 1994; Mohammed and Uraguchi, 2013).

Phytoplankton Biomass and Primary Production

African lakes display a considerable range in phytoplankton biomass and primary production (Table 1), which often reflects their trophic state (Talling and Talling, 1965). One of the least productive lakes on the continent is Lake Kainji which is a typical example of a nutrient poor (oligotrophic) African lake with primary production as low as 0.3 g C m^{-2} h^{-1} (Karlman, 1973).

Adeniji et al. (2001) supported this, stating that the primary production in other African reservoirs is between two and ten times higher than Lake Kainji. In fact, Adeniji et al. (2001) found that production in the upper and central basins of Kainji in 1996 was lower (1.4 g 0_2 m^{-2} d^{-1} than recorded by Karlman (1973) in 1971 (2.3g 0_2 m^{-2} d^{-1}). At the upper, eutrophic end of the production scale is the nutrient rich Lake Nasser whose net primary productivity has been reported to average 4.48 g C m^{-2} h^{-1} and up to 24.8 mg C m^{-2} h^{-1} (Samaan, 1972; Abd El-Monem, 2008).

One of the main reasons for the inadequate representation of biodiversity in development planning processes is a widespread lack of readily available information on the status and distribution of inland water taxa. Pollution and sedimentation, as well as the introduction of invasive species, pose the greatest threat to the aquatic biodiversity of Africa's lakes. On a basic level, activities that influence aquatic environments and biological systems, like forest clearing, the burning of fossil fuels, agricultural pollution and soil loss, lead to greater emissions of carbon dioxide and an increase in global warming. The impacts of climate change are projected to accelerate aquatic and animal population losses and the extinction of a wide range of species and ecosystems. Increased nutrient inflows resulting from expanding urbanization and agriculture have elevated primary production levels and reduced biodiversity in many African lakes.

This was evident in Lake Mokolo (South Africa) where thresholds of 40 P mg L^{-1} indicated decreases in phytoplankton species diversity (Oberholster et al., 2012). Another good example is Lake Chivero whose primary production levels have increased by over ten orders of magnitude from its previously reported range of 1.64- 6.03 g C m^{-2} h^{-1} in 1979 up to a range of 18.5- 140 g C m^{-2} h^{-1} (Robarts, 1979; Ndebele and Magadza, 2005). Likewise, in the morphometrically similar Hartbeespoort dam primary production levels, which normally range between 0.40 and 30.90 g C m^{-2} h^{-1}, have risen to as high as 185 g C m^{-2} h^{-1} (Robarts, 1984).

These elevated primary production levels correspond with algal blooms, and are a direct consequence of high nutrient inflows which overburden the lakes' natural purification systems (Robarts, 1979, 1984; Mugidde, 1993; Ndebele and Magadza, 2005; Mhlanga et al., 2006; Ndebele, 2009; Haande et al., 2011).

Associated with eutrophication changes in Chivero is a progressive decrease in phytoplankton species diversity with the Cyanobacterial species *Microcystis aeruginosa* dominating and *Aulacoseira* spp. (=*Melosira)* co-dominating the phytoplankton community (Ndebele and Magadza, 2006; Mhlanga et al., 2006).

In deeper African lakes, nutrient inflows also exert prominent effects on primary production. Examples include Lake Malawi where primary production levels of 0.24 to 1.14 g C m^{-2} h^{-1} have risen to range between 14.04 and 26.20 g C m^{-2} h^{-1} in recent years (Guildford et al., 2007) and Lake Victoria whose eutrophication caused an eight fold increase in primary production of 3.30- 13.50 g C m^{-2} h^{-1} to increase to as high as 234 g C m^{-2} h^{-1} and more recently, 4 764 mg g C m^{-2} h^{-1} (Mugidde, 1993; Kling et al., 2001; Silsbe et al., 2006).

Table 1. Mean range in phytoplankton biomass (Chloro *a* µg L^{-1}) and primary production per unit area (Σ^A PP in mg C m^{-2} d^{-1}) of some African lakes

Lake	Country	Year	Chlorophyll *a*	Σ^A PP	Source
Cleveland	Zimbabwe	2005- 2006	8.2	120	Ndebele, 2009
Kariba	Zambia/Zimbabwe	2007-2009	10.6	206	Ndebele-Murisa et al., 2012
Tanganyika	Tanzania	2002-2003	6.7	434	Stenuite et al., 2007
Malawi	Malawi	1997- 2000	4.0	463	Guildford et al., 2007
Baringo	Kenya	2003	55.0	480	Schager and Oduor, 2003
Kivu	DRC/Rwanda	2003- 2005	2.2	620	Sarmento et al., 2009
Ziway	Ethiopia	2005	39.2	1 060	Girma and Ahlgren, 2010
Chamo	Ethiopia	2005	29.9	1 590	Girma and Ahlgren, 2010
Nakuru	Kenya	2000-2003	16.0	1 960	Ballot et al., 2004
Naivasha	Kenya	2000	130.0	2 000	Hubble and Harper, 2002
Chivero	Zimbabwe	2002- 2003	43.0	2 330	Ndebele and Magadza, 2005
Victoria	Kenya/Uganda	2001-2002	72.0	2 906	Silsbe, 2004
Awassa	Ethiopia	2005	18.7	3 160	Girma and Ahlgren, 2010
Nasser	Egypt	2005	230.8	3 193	Abd El-Monem, 2008

Lake Water Quality, Thermal Dynamics and Stratification

Environmental factors such as water pH, conductivity, dissolved oxygen, nutrient concentration and heat content and light intensity influence lake water quality and therefore primary production and these in turn are affected by thermal stratification, which is a common feature of tropical African lakes. Stratification is a result of thermal differences in the upper warm layer of water, the epilimnion, and the lower cold layer, the hypolimnion, separated by a zone of steep temperature gradient, the thermocline, and is usually created during periods of high summer temperatures. Stratification is characterized by vertical profiles of water temperature, conductivity, dissolved oxygen concentration and pH. Lake Kariba, like many deep African lakes is typically monomictic with one turnover event per annum, stratifying for at least 10 months per year with turnover occurring in late July or August each year (Coche, 1974; Ndebele-Murisa et al., 2013a). This contrasts with deeper tropical African lakes such as Albert, Kivu, Malawi, Niger, Tanganyika, Turkana and Victoria, which are typically meromictic with infrequent turnover events, the incidence of complete mixing varying with the depth and shape of the lake basin (Baxter et al., 1965). In meromictic Lake Tanganyika, a fairly stable stratification is established during summer, which is generated by increased water temperatures and diminished wind intensities (O'Reilly et al., 2003). However, currents and internal waves, as well as coastal jets and return flows, do cause localised upwelling, which may partly disrupt this stratification (Chale, 2004). In addition, deep monomictic lakes contrasts with many shallower, naturally saline African lakes such as Naivasha (Hubble and Harper, 2002), Nakuru (Raini, 2007; Okoth et al., 2009), Crescent Island Crater, Oloiden and Winam Gulf (Melack, 1979; Osumo, 2001), which are typically polymictic with several turnover events per annum. In the polymictic African lakes, density stratification is a natural feature, which is disrupted by strong winds that influence water circulation, thereby preventing the development of a consistent thermocline (Langenberg et al., 2002).

Stratification is prevalent in many deep tropical African lakes, such as Albert, Cabora Bassa, Chad, Kariba, Malawi, Niger and Turkana, often influencing the seasonality and abundance of phytoplankton. The general annual cycle of thermal stratification in these lakes includes a short phase of partial vertical mixing which is often accompanied, or immediately followed, by a peak of algal abundance to which Bacillariophyceae make a major contribution (Chale, 2004). A second peak dominated by Cyanophyceae often develops after re-stratification (Chale, 2004). In Lake Ogelube in Nigeria, phytoplankton biomass during the rainy season (April-October) is much higher than that during the dry season (November-March). Chlorophyceae (mostly desmids) are most abundant during the rainy season followed in decreasing order of abundance by Cyanophyceae, Bacillariophyceae, Euglenophyceae, Dinophyceae, Cryptophyceae, Chrysophyceae and Xanthophyceae (Nweze, 2006). This order changes in the dry season, when the relative abundance of Bacillariophyceae is greater than that of Cyanophyceae, and Dinophyceae greater than that of Euglenophyceae. A similar seasonal pattern is found in other African lakes, such as Kariba, Malawi, Tanganyika and Victoria where Cyanophyceae algae dominate during summer stratification with Bacillariophyceae tending to dominate in winter at turn-over when stratification breaks down (Hecky and Kling, 1987; Cronberg, 1997; Descy et al., 2010; Ndebele-Murisa et al., 2012).

Density stratification is also a natural feature of many shallow, naturally saline African lakes such as George (Ganf, 1975; Wood et al., 1976), Naivasha (Hubble and Harper, 2002) and Nakuru (Vareschi, 1982; Raini, 2007; Okoth et al., 2009). However, this stratification can be disrupted by strong winds, which influence water circulation thereby preventing the development of a consistent thermocline (Langenberg et al., 2002). This feature has been observed in the four Kenyan lakes Naivasha, Crescent Island Crater, Oloiden Lake and Winam Gulf which display complete vertical mixing and well oxygenated water throughout the water profile during periods of strong winds (Melack, 1979; Hubble and Harper, 2002). Stratification in some shallow African lakes traps nutrients from riverine inflows contaminated by effluent discharges. This is apparent in Lake Chivero during hot summer months (Ndebele, 2003) with stratification breakdown during winter months resulting in the temporary up-welling of nutrients and localized algal blooms (Mhlanga et al., 2006).

In general, stratification has a negative impact on primary production as it confines nutrients to deeper waters of greater density beyond the euphotic zone (Spigel and Coulter, 1996). Therefore, phytoplankton species diversity and total biomass is elevated only in nutrient rich waters at river inflows (Vuorio et al., 2003). As a result, the highest primary production normally occurs in the first few meters of the water column, is often inversely correlated with water transparency, and is usually depressed at the water surface particularly where temperatures exceed 30°C (Robarts, 1979). In most African lakes, strong thermal gradients often develop from daytime warming in the upper layers. These gradients frequently trap photosynthetic oxygen to form well-defined maxima, which demarcate deeper, oxygen-poor layers (Talling et al., 1973). In some lakes, the de-oxygenation layer is so deep that oxygen disappears from the hypolimnion and hydrogen sulphide is produced. The epilimnion maintains a high oxygen concentration throughout the year, except during partial stratification, which may develop during the day causing a gradient of decreasing oxygen concentration with depth. Some features of stratification in artificial African lakes are also influenced by the positioning of turbines. For instance, Lake Kainji's rapid deepening of the epilimnion during stratification has been attributed to discharges from the lake as the levels of the spillways and turbine intakes are situated below the thermocline (Henderson, 1973), whilst a substantive level of nutrients is drawn from Lake Kariba whose turbines are situated below the thermocline (Chifamba, 2000; Tumbare, 2008). Also, when stratification is distrupted such as by overturn, oxygen depletion and release of toxins caused by algal bloom die-off during this period may lead to massive fish mortalities, as reported in Lake Chivero (Moyo, 1997; Mhlanga et al., 2006). In this lake, the dissolved oxygen concentrations following an algal bloom die-off ranged from 2 mg L^{1} at 5 m depth to 3.9 mg L^{-1} at the lake surface. These oxygen concentrations were lower than those (3.2- 4.8 mg L^{-1}) measured in the Nyanza Gulf of Lake Victoria following the collapse of a phytoplankton bloom and associated with high mortalities of Nile tilapia (*Oreochromis niloticus*) (Ochumba and Kibaara, 1989).

Phytoplankton production in tropical African lakes is often low at or near the water surface due to photo-inhibition; therefore, the optimal habitat for phytoplankton is in the first few meters of the water column. Elsewhere, it has been reported that phytoplankton growth is limited at photosynthetic active irradiances above 615 µE m^{-2} s^{-1} (Domingues et al., 2011), yet in Lake Kariba photosynthetic active irradiances exceeding 2 000 µE m^{-2} s^{-1} have been frequently recorded in the surface waters (Ndebele-Murisa, 2011). Photo inhibition of phytoplankton photosynthesis at solar irradiances exceeding 2 000 µE m^{-2} s^{-1} have also been observed in the surface waters of Lake Tanganyika, this reflected in an increased fraction of light-acclimated pico phytoplankton in the phytoplankton biomass (Stenuite et al., 2009). Similarly, in Lake Malawi, small-sized (<2 µm) light-acclimated phytoplankton in the lake surface water account for 30-50% of the total phytoplankton chlorophyll (Guildford et al., 2007). Likewise, at such high irradiances, photo inhibition of phytoplankton photosynthesis has been observed in surface waters of Lake Awassa (Ethiopia), where the vertical distribution of phytoplankton is typical of light-inhibited photosynthetic activity on all but overcast days (Kifle and Belay, 1990). In contrast, Schager and Oduor (2007) found no photo inhibition of phytoplankton in Lakes Bogoria, Nakuru and Elmentaita in Kenya, where phytoplankton photosynthesis is likely more severely inhibited by the high water salinities. In general, phytoplankton production tends to decrease with depth until light becomes the limiting factor after the euphotic depth in African lakes (Mugidde, 1993; Silsbe *et al.*, 2006). This is reflected in Lake Chivero where light is the primary limiting factor to the growth of phytoplankton whereas conductivity and trophic status (measured as chlorophyll *a*) are the most important environmental variables influencing the distribution of ciliate species in this and other African lakes (Robarts and Southall, 1977; Yasindi and Taylor, 2003). This is compliant with a classification of 17 East African lakes on the basis of water conductivity and associated phytoplankton species (Talling and Talling, 1965).

The Influence of Nutrients and Trophic Status

African tropical lakes are classified into three trophic classes based on their total ionic concentrations and conductivity levels (Talling and Talling, 1965). Class I lakes have low total concentrations of ions (alkalinity of less than 6 mg L^{-1}) and conductivities of less than 600 µS cm^{-1}, and derive their water from direct surface runoff or rivers with little salt. Examples of Class I lakes include Cleveland Dam, Lakes George, Kariba, Malawi, Malombe and Victoria (Ndebele, 2009; Lindmark, 1997; Guildford et al., 2007; Haande et al., 2011). Class II lakes have higher total ion concentrations (alkalinity of about 6 to 60 mg L^{-1}), and conductivities of between 600 and 6 000 µS cm^{-1} caused by inflows rich in solutes, particularly sodium carbonate and bicarbonate, drainage from alkaline lavas, and high evaporation in their closed basins (Talling and Talling, 1965). They include Lakes Albert, Edward, Kivu, Tana, Tanganyika and Turkana (Chale, 2004; Lorke et al., 2004; De Wever et al., 2008). Class III lakes are highly saline, with alkalinities mostly above 60 mg L^{-1} and conductivities above 6 000 µS cm^{-1}, and often contain solid mineral deposits such as trona. They include Lakes Eyasi, Katwe, Magadi, Manyara, Nakuru, Natron and Naivasha, which are mostly shallow, endorheic, and exhibit significant density stratification despite their shallowness (Melack, 1979; Hubble and Harper, 2002; Okoth et al., 2009). However, despite these classifications, the trophic status of many tropical African lakes and impoundments is changing, due to rising global temperatures, reduced river inflows, fertiliser runoff, and effluent discharges resulting from increased anthropogenic activity in lake catchments (Ogutu-Ohwayo and Balirwa, 2006). These factors have contributed to increased lake eutrophication, with consequent negative effects on phytoplankton and fish production (Ndebele-Murisa et al., 2010).

Depending on the trophic level, different phytoplankton assemblages may be expected. In the tropics and at mid-latitudes, phytoplankton are typically nutrient-limited, and reduced biological productivity, phytoplankton biomass and growth have been linked to increased water temperatures and reduced nutrient supply (Bergamino et al., 2007). Nitrogen (N) and phosphorous (P) have been identified as the major nutrients governing primary production and phytoplankton biomass in tropical African lakes (Talling and Talling, 1965; Viner, 1977). Indeed, in many African lakes nitrogen-fixing taxa such as *Anabaena* and *Cylindrospermum* spp. proliferate due to the limiting concentrations of this nutrient for other taxa in their waters (Talling and Talling, 1965; Ganf, 1975; Hubble and Harper, 2002; Chale, 2004; De Wever *et al.*, 2008). The low phytoplankton compositional variability of Lake George in Uganda has been attributed to both N and P limitation and the extreme predominance of algal production over that of other biota (Ganf, 1975; Moreau et al., 1993). Similarly, in Lake Kariba it has been demonstrated that the waters are principally P-limited for most of the year with a possibility of N co-limiting at other times (Moyo, 1991; Magadza, 1992). In this case the ability to fix nitrogen becomes an added advantage and may explain the dominance of nitrogen-fixing taxa in this lake (Cronberg, 1997; Ndebele-Murisa, 2011).

The observed dominance of Cyanophyceae in Lake Kariba (Ndebele-Murisa et al., 2012) is consistent with earlier studies (Thomasson, 1980; Ramberg, 1984, 1987; Cronberg, 1997). Cyanophyceae dominance is a common feature in several other deep tropical African lakes such as Lakes Kivu, Malawi, Tanganyika and Victoria (Hecky and Kling, 1987; Talling and Lemoalle, 1998; Chale, 2004). This likely due to the nitrogen-fixing capability of many Cyanophyceae species, which provides them with a competitive advantage over other phytoplankton taxa, particularly in systems where N has been shown to be limiting for phytoplankton growth such as in Lakes Kariba, Malawi, Naivasha and Tanganyika (Hubble and Harper, 2002; Chale, 2004; Moyo, 1991; Guildford et al., 2003*;* De Wever et al., 2008). Lake Kariba's Cyanophyceae species complement resembles that in Lake Victoria where the low total nitrogen to total phosphorus molar ratio (Gikuma-Njuru and Hecky, 2004) is indicative of N-limitation that favours heterocystous N-fixing Cyanophyceae (Mugidde, 2001; Mugidde et al., 2003). This evident also in Lake Kariba where heterocysts and specialised nitrogen-fixing akinetes (thick-walled dormant or resting cells derived from the enlargement of a vegetative cell) occur on the dominant Cyanophyceae *Cylindrospermopsis raciborskii*, (John et al., 2002) whose concentrations also correlate highly with temporal variations in biological nitrogen fixation (Moyo, 1997; Ndebele-Murisa, 2011). In Lake Naivasha, N is more limiting than P with an algal preference for ammonium over nitrate (Hubble and Harper, 2002). In Lake Tanganyika, the addition of N, P and iron (Fe) stimulates total phytoplankton production, based on both High Performance Liquid Chromatography (HPLC) pigment analysis and cellular counts using epifluorescence microscopy (De Wever et al., 2008). Iron enhances production of mainly prokaryotic pico-phytoplankton; N and P increase Chlorophyceae and in some cases Bacillariophyceae production and Fe in combination with N and P improves pico-Cyanophyceae production (De Wever et al., 2008). Silica (Si) is known to increase Bacillariophyceae production because of their siliceous shells and this has been demonstrated in mesocosm experiments (De Wever et al., 2008). Guildford et al. (2003) concluded that N was the major limiting nutrient in Lakes Malawi and Victoria whilst the combination of N and P, with Fe resulted in the highest increase in chlorophyll *a* suggesting that, after N and P, Fe is the next most likely nutrient to limit phytoplankton growth in these lakes.

The observed increased concentrations of Cyanophyceae and decreased concentrations of Bacillariophyceae in Lake Kariba concur with similar phytoplankton compositional changes observed in other African lakes. A typical example is Lake Victoria, where the Bacillariophyceae species *Aulacoseira* and many Chlorophyceae species are presently rare, whilst heterocyst-forming Cyanophyceae, such as *Anabaena* and the cosmopolitan *Microcystis,* are proliferating due to eutrophication (Haande et al., 2011). Similarly, increased concentrations of the Cyanophyceae species *Microcystis aeruginosa* and *Anabaenopsis arnoldii* in the alkaline Lake Turkana have been attributed to nutrient enrichment, (Mageed and Heikal, 2006), with *Microcystis* forming a dominant component of other hyper-eutrophic lakes such as Chivero and Victoria (Ochumba and Kibaara, 1989; Magadza, 2003; Mhlanga et al., 2006), due to its ability to rapidly exploit available nutrients (Harding, 1997). Similarly, in Lake Malawi the Cyanophyceae species *Anabaena* and *Cylindrospermopsis* have recently become more pervasive, due to nutrient enrichment caused by increased anthropogenic activities in the lake's catchment (Hecky and Kling, 1987; Bootsma and Hecky, 1993; Patterson and Katchinjika, 1995). Conversely, Bacillariophyceae concentrations and phytoplankton primary production rates have decreased substantially in Lake Tanganyika (O'Reilly et al., 2003; Stenuite et al., 2007).

The composition and abundance of phytoplankton often reflect the nutrient status of African lakes with, for example, the low Euglenophyceae biomass in Lake Ogelube proposed as indicative of low organic pollution and the predominance of desmids indicative of oligotrophic conditions (Nweze, 2006). Low nutrient contents have also been observed in the normally oligotrophic Lakes Kariba, Kivu and Malawi, yet despite this many African lakes are now prone to nutrient enrichment from anthropogenic activities and this has led to eutrophication and associated problems. Classical examples of eutrophic African water bodies are Lakes Chivero and Victoria and the Hartbeespoort Dam, with recorded N concentrations of over 50 mg L^{-1}. Elevated nutrient levels in Lake Chivero, caused by discharge of partially treated sewage and industrial effluent into the lake, has resulted in an increase in phytoplankton biomass by a factor of more than ten and corresponding reductions in water transparency (Mhlanga *et al.*, 2006). Algal toxins caused by algal bloom die-off have also been previously detected in Lake Chivero and in tap water emanating from the lake (Johansson and Olsson, 1998; Ndebele and Magadza, 2006; Mhlanga et al., 2006), but it has not been established whether these are toxic to humans and other fauna (Moyo, 1997). However, toxic strains of *Microcystis* have been identified in other African fresh water bodies as well as in fresh water systems (Robarts, 1984); elsewhere in the world (Chen et al., 2009).

Cyanobacterial blooms have also been reported in nutrient enriched South African impoundments, namely the Hartbeespoort Dam (Robarts and Zohary, 1986, Van Ginkel et al., 2001) and the Erfenis and Allemanskraal Dams (Van Ginkel and Hohls, 1999) as well as the Orange River (Janse van Vuuren and Kriel, 2008). Similarly, nutrient-rich inflows into the shallow Yaoundé Municipal Lake in Cameroon have resulted in a massively increased phytoplankton biomass of 225 µg mL^{-1} and chlorophyll *a* concentration of 566 mg L^{-1} dominated by Euglenophyceae and Chlorophyceae (Kemka et al., 2006). In Lake Victoria, comparison of historical limnological observations with modern conditions in Ugandan waters of the lake indicates that the present lake is quite different from what it was in the 1950s and 1960s. Oxygen conditions have been altered, with surface waters now being supersaturated with oxygen, while hypolimnetic oxygen concentrations have fallen (Hecky et al., 1994). Phytoplankton productivity has increased by at least a factor of two, and biomass by as much as a factor of four to five, both inshore and offshore (Mugidde, 1993). The increases in productivity have been accompanied by qualitative changes in phytoplankton composition, with greater representation of Cyanophyceae, especially the filamentous nitrogen fixing *Cylindrospermopsis*, *Anabaena*, *Lyngbya*, and *Microcystis* (Balirwa, 1998), indicating a change in the nitrogen/phosphorous ratio, which may be favouring nitrogen-fixing forms. The cause may be a combination of nitrogen mobility from the degraded landscape and atmospheric deposition from biomass burning.

Changes in diatom dominance in Lake Victoria from *Melosira* to *Nitzchia* have coincided with increased depletion of soluble-reactive silicon, which is about ten-fold lower than recorded in the 1960s (Hecky and Bugenyi, 1992; Hecky, 1993). However, the relatively higher silica content in interface habitats at the shore (Balirwa, 1998) in comparison to samples from the 1960s (Hecky and Bugenyi, 1992) may explain the differences in horizontal diatom distribution patterns. The differences could also be a result of unique patterns in ecological structure of the previously unsampled wetland-dominated habitats. In addition, changes in grazing pressure, as shown in studies of benthic communities (e.g., Steinman, 1996), could also alter algal species diversity. Changes in diatom and Cyanobacterial counts may therefore be a result of changes in ratios of several nutrients that may be selectively limiting. The decrease in the lake's transparency (or increase in turbidity) is probably due to a higher chlorophyll concentration, which has resulted in a shallower euphotic depth, causing in turn a loss of the photosynthetic zone (Mugidde, 1992). Higher biomass levels, accompanied by more frequent algal blooms, can result in high oxygen demand during decomposition, which has often resulted in fish kills (Ochumba and Kibaara, 1989) and increasing de-oxygenation of the hypolimnion (Hecky et al., 1994).

Several challenges such as seawater intrusion resulting from overexploitation of groundwater resources in coastal areas, pollution and eutrophication due to increasing populations and increased water demand have been noted in Africa (Darwall *et al.*, 2009; Mwendera, 2010). Eutrophication tends to impact shallow lake systems more than deeper tropical lakes, such as Lakes Malawi and Victoria, with incidences of localized pollution only occasionally reported in the deeper lakes such as Kariba (Feresu and Van Sickle, 1989; Magadza and Dhlomo, 1996). In fact, in Lake Kariba the water has changed from an initial nutrient rich (eutrophic) state to that of its current nutrient poor (oligotrophic) state with P limiting primary production (Moyo, 1991). Improved phytoplankton photosynthetic rates and growth in African waters have been attributed to an increased nutrient supply during the rainy season (Melack, 1979), with peak phytoplankton biomasses in the Wetlands Lakes of North Africa corresponding with elevated phosphorus levels (Fathi et al., 2001). In fact, phytoplankton community composition is affected by nutrient supply in several African lakes. In Lake Victoria, for example, increased concentrations of heterocyst-forming Cyanophyceae such as *Anabaena* and *Microcystis*, and decreased concentrations of Bacillariophyceae such as *Aulacoseira*, have been reported in response to elevated nutrient levels (Haande et al., 2011). Also, increased concentrations of the Cyanophyceae *Microcystis aeruginosa* and *Anabaenopsis arnoldii* in response to nutrient enrichment, have been reported in the alkaline Lake Turkana (Mageed and Heikal, 2006), with *Microcystis* also forming a dominant component of the hyper-eutrophic Lakes Chivero and Victoria (Ochumba and Kibaara, 1989; Magadza, 2003; Mhlanga et al., 2006).

Infact, most artificial African lakes such as Lakes Volta, Weija and Kpong Reservoirs (Ghana), Lakes Chivero and Kariba have undergone different phases of nutrient/trophic status and productivity levels often associated with the filling up of the lakes and consequent stabilisation. When these lakes were formed the formerly riverine waters that once ran through begin to deposit their nutrient rich sediment in the recently formed lakes. In addition, increased water retention behind the dam begins to reduce washout of plankton populations. Soils, newly waterlogged, release nutrients and terrestrial vegetation now inundated, rots and also releases nutrients. Oxygen levels can also fall as a result of decomposition and this can further increase the release of soil bound nutrients from mineral particles. After a while deltas form at the lake periphery and sediment is deposited there whilst submerged soils no longer leach nutrients and terrestrial vegetation has all been broken down. Once the lake has reached capacity nutrient loss via outflow streams will remove a considerable amount of the nutrient load. But in the initial flooding phase there can be a considerable loading of nitrogen and phosphorus compounds within the lake (Moss, 1991). This was the typical case of Lake Kariba, created in the 1950s. Initially, when the lake was formed, large forested areas were inundated, and nutrients leached from the soil and decomposing organic matter increased the fertility of the lake's water. The lake's initial eutrophic status was characterised by continuous blooms of large algae (*Microcystis* and *Volvox* spp.), the proliferation of the aquatic fern (*Salvinia molesta*), and the de-oxygenation of the hypolimnion. However, the lake's initial eutrophic state in the 1960s subsequently declined to an oligotrophic state, which coincided with a concomitant decline in *S. molesta* and algal populations to the current oligotrophic-mesotrophic state (Magadza, 1992; Ndebele-Murisa, 2011). The degree of de-oxygenation in the lake hypolimnion has also declined since the 1960s, with high concentrations of hydrogen sulphide now restricted to deep waters (>30 m) towards the end of the annual stratification period. In Tanzania the dam reservoir Nyumba ya Mungu similarly went through an initial eutrophic phase upon completion and filling of the reservoir (Taylor, 1993). However, in some cases the initial phase of eutrophication does not always occur. For instance in Lake Nasser, Egypt and Sudan, the basin, comprising largely of rock and sand, showed no initial signs of eutrophication and to date the lake is still free of any major infestations of invasive weeds and algal blooms (Fayad, 1999; Abd El-Monem, 2008).

Climate Warming, Phytoplankton Seasonality and Production

Most African lakes have a well-established seasonality in phytoplankton abundance governed mainly by climate and the stratification/mixing cycle (Talling, 1966, 2001; Schargel and Oduor, 2001; Ndebele-Murisa et al., 2010; Macintyre et al., 2012). The general annual cycle of thermal stratification in African lakes is associated with a particular phytoplankton dominance pattern which was described earlier in this chapter. Although this pattern in dominance may differ, it is prevalent in lakes such as Kariba, Kivu, Malawi, Tanganyika and Victoria, where Cyanophyceae dominate during summer stratification and Bacillariophyceae during winter at turn-over (Hecky and Kling, 1987; Bootsma and Hecky, 1993; Patterson and Katchinjika, 1995; Cronberg, 1997; Cocquyt and Vyverman, 1994, 2005; Descy et al., 2005; Sarmento et al., 2006, 2007, 2008). For instance, in the largely oligotrophic and monomictic Lake Kyaninga (Uganda), the phytoplankton community is dominated by Cyanobacteria and Chlorophyta with Bacillariophyta (diatoms) contributing only a minor part of total phytoplankton biomass in both the wet and dry seasons, being characterized by an assemblage of *Urosolenia* and small *Nitzschia* species (Cocquyt et al., 2010). Similarly, in Lake Tanganyika the larger phytoplankton community is largely made of Chlorophyceae and Bacillariophyceae (*Nitzschia* spp) with large blooms of diastrophic filamentous Cyanophyceae periodically observed during September to November at the onset of the rainy season (Salonen et al., 1999; Cocquyt and Vyverman, 2005). However, some recent changes have been noted with Cyanophyceae being observed less as a major contributor to the phytoplankton biomass (Descy et al., 2005; De Wever et al., 2008). In addition, different pico-Cyanophyceae and Bacillariophyceae concentrations have been observed as a result of changes in water column stability at the southern and northern ends of the lake (Hecky and Kling, 1981; Vuorio et al., 2003).

Similarly, Lakes Edward, Kivu and Malawi also have a seasonal succession of major algal groups comparable to that in Lake Tanganyika. These naturally oligotrophic lakes have low phytoplankton biomass and species diversity (Hecky and Kling, 1987). Bacillariophyceae are the dominant group in Lake Kivu during episodes of deep mixing in the dry season with filamentous, diastrophic Cyanophyceae and pico-Cyanophyceae forming a substantial fraction of the autotrophic biomass under conditions of reduced nutrient availability in the stratified water column during the rainy season (Sarmento et al., 2006). Lake Kivu's phytoplankton species assemblage is somewhat intermediate in composition between the oligotrophic Lakes Malawi and Tanganyika and the more eutrophic Lake Victoria, though the dominant Bacillariophyceae of Lake Kivu (*Urosolenia* sp., *Nitzschia bacata*, *Fragilaria danica*) are normally associated with oligotrophic, phosphorous-deficient African lakes (Sarmento et al., 2006, 2007, 2008). Likewise, in the artificial, oligotrophic Lake Kariba Cyanophyceae (*Cylindrospermopsis*, *Anabaena* and *Pseudoanabaena*) dominate and Chlorophyceae used to co-dominate when the phytoplankton biomass would reach its peak (up to 1.52 mg L^{-1} chlorophyll *a*) during the rainy season; with Bacillariophyceae dominating at turnover during the dry season (Ramberg, 1987). However, a comparison of the phytoplankton composition in this lake between 1959 and 1964 with that between 1986 and 1990 showed a change from a riverine-dominated species component rich in desmid flora and large algal species to that dominated by small-celled chlorococcal Chlorophyceae, mainly due to the apparent stabilization of the lake over time (Cronberg, 1997). More recent studies have revealed a further shift in phytoplankton dominance in Lake Kariba with cyanobacteria dominating even more during the turn-over period while Chlorophyceae though more diverse, has diminished in its contribution to total phytoplankton biomass (Ndebele-Murisa et al., 2012).

Inter-annual variations in phytoplankton composition and biomass in temperate regions are known to reflect changes in climate (Lehman et al., 1998). Reduced depths of light transparent mixed zones, due to upward shifts in thermoclines in response to elevated temperatures, could potentially adversely affect primary production in deep African lakes such as Kariba, Malawi, Tanganyika and Victoria. This bears potential cascading effects on the aquatic ecosystems by reducing available nutrients in the upper, mixed waters and often limits biological production (Magadza, 2006, 2011; Ogutu-Ohwayo and Balirwa, 2006; Bootsma and Jorgensen, 2006; Verburg and Hecky, 2009; Hecky et al., 2010; Ndebele-Murisa et al., 2010; Tierney et al., 2010, Urama and Ozor, 2010). Elevated water temperatures as a consequence of elevated air temperatures attributed to global warming have been reported in African lakes. The upper waters in Lake Kivu (above a depth of 250 m) for example increased by 0.5°C between 1937 and 2002, seemingly in response to elevated air temperatures (Halbwachs et al., 2002; Odada et al., 2006). Other examples include Lake Malawi where a 70-year (1930 to 2000) record shows that the mean temperature of water above a depth 300 m increased by 0.78°C due to reduced cold-water intrusions associated with milder winters (Vollmer et al., 2002, 2005). This has led to decreased primary production rates which are attributed to the increasing temperatures, causing a warmer lake and consequently a shallower, more stable thermocline, and restricted nutrient fluxes from the hypolimnion to the surface mixed layer (Vollmer et al., 2002, 2005; Guildford et al., 2007). These findings are corroborated by geochemical records obtained from sediment cores of changes in Lake Malawi over the last 730 years, caused by natural climatic forcing and anthropogenic activities (Branchu et al., 2010). Similarly, Marshall et al. (2008) found that the water temperatures in Lake Victoria rose by 0.9°C between 1960 and 1990 with the deeper waters warming up more (1.3°C) than the upper (1.0°C) from 1927 to 2000. Likewise, mean water temperatures in Lake Tanganyika above a depth of 100 m increased by an average of 0.34°C between 1946 and 1994 with evidence also of elevated water temperatures at depths of down to 300 m (Plisnier, 2000; Tierney et al., 2010) and a reduction in the depth of the thermocline from 68 m to 55 m (Plisnier, 2000). A 44% decline in Bacillariophyceae concentrations during the mixing season and an 88% decrease during the stratified season were reported over the period 1975 to 2000-2001 in Lake Tanganyika (Verburg et al., 2003). This due to less mixing between the shallow and deep waters caused by a more intense and prolonged stratification induced by a 0.9°C increase above ambient in epilimnion water temperatures (Verburg et al., 2003). In addition, it has been found that the upper surface waters of Lake Kariba in the Sanyati Basin have warmed up by 1.7°C since the mid 1960s and by 1.5°C since the 1980s (Ndebele-Murisa et al., 2013a). Additionally, there has been a 50% decline in primary production in Lake Kariba from a previously reported average of 0.42 g C m^{-2} d^{-1} (Cronberg, 1997) to the current measured average of 0.21 ± 0.03 g C m^{-2} d^{-1} (Ndebele-Murisa et al., 2012). This decline attributed to an >80% upward shift in the depth of the thermocline associated with the temperature increase and stronger, more stable stratification (Ndebele-Murisa, et al., 2013a) as well as a 50% reduction in the depth of the euphotic zone from previously reported depths of 31.8 m between 1965 and 1966 (Coche, 1974) and 17.9 m between 1986 and 1989 (Magadza et al. 1989), to the current average depth of 2.8 m (Ndebele-Murisa et al., 2013a).These examples cited suggest that moderate warming may be reducing lake water inflows and therefore nutrients, destabilising plankton dynamics, and potentially cascading up higher trophic levels of mainly planktivorous fish in African lakes (Magadza 2008, 2010, 2011; Verburg and Hecky, 2009; Ndebele-Murisa et al., 2011, 2012).

Therefore the concept of stable phytoplankton communities in some African lakes has been questioned due to climate warming and changing nutrient dynamics. Observations of primary production and phytoplankton densities at regular (weekly and fortnightly) intervals in Lake Kinneret (Israel) over a 35-year period (1969- 2004) led to a repudiation of the concept of a stable phytoplankton seasonality (Zohary, 2004; Ostrovsky et al., 2012). These observations demonstrated remarkable stability in algal species composition and abundance in summer and spring algal blooms in the lake during the first 24 years of monitoring. After 1994, however, deviations from the previously predictable annual pattern of algal blooms were apparent. They were characterized by the absence of prevailing spring *Peridium gatunense* blooms, intensification of winter *Aulacoseira granulata* (=*Melosira*) blooms and increased variability in the magnitude of blooms in other species. Also, nanoplanktonic palatable forms in the high summer phytoplankton biomass were replaced by less palatable forms and there was a new appearance and increase in absolute biomass of toxin-producing, nitrogen-fixing Cyanophyceae as well as an increased incidence of fungi attacking the algae *Peridium gatunense* (Zohary, 2004). For a more updated review of Lake Kinneret, please see Kamenir, this volume and Ostrovsky et al. (2012).

In Tanganyika, shifts in phytoplankton seasonality have been detected through the application of Empirical Orthogonal Function Analysis to a 7-year satellite observation record (Bergamino et al., 2007, 2010). The phytoplankton biomass in Tanganyika has declined by 70% between 1975 and 2000 (Verburg et al., 2003), this decline accompanied by substantial changes in phytoplankton composition. The formerly dominant Cryptophyceae and

Chrysophyceae, which comprised 34% of the total phytoplankton biomass in the north basin in 1975, reduced to 3% in 2000 (Verburg et al., 2003). This reduction accompanied by a 44% decline in Bacillariophyceae biomass during the winter season and an 88% decline in the summer season over the 25-year monitoring period (Verburg et al., 2003; Hardy et al., 2005). Pico-phytoplankton is presently dominant in Lake Tanganyika, contributing up to 70% of the total phytoplankton biomass (Descy et al., 2005; Stenuite et al., 2009). This feature was also noted recently in Lake Kivu (Sarmento et al., 2009). These changes in phytoplankton biomass in Lake Tanganyika have been attributed to warming of the upper waters during the past century that have intensified stratification, diminished mixing of shallow and nutrient-rich deeper waters resulting in decreased primary production and an expansion of the anoxic water mass (Descy et al., 2006). Diminished phytoplankton production rates have also been reported in Lake Kivu (Sarmento et al., 2008), whose waters have warmed up by an average of 0.5°C over the 55-year period from 1937 to 2002 as a consequence of elevated temperatures attributed to global warming (Halbwachs et al., 2002). These seemingly cosmopolitan declines in phytoplankton production in African lakes with climate warming bear potentially deleterious consequences for the fish stocks and human livelihoods (Odada et al., 2006; IPCC, 2007; Hecky et al., 2010; Ndebele-Murisa et al., 2010, 2013b).

Not only are nutrients and therefore production being reduced due to climate warming and subsequent stronger thermal stratification in some African lakes, but shifts in phytoplankton seasonality that may be causing adverse effects up the food chain have been suggested. Microcosm experiments have demonstrated that increased water temperatures associated with climate warming can alter phytoplankton community composition towards high temperature-tolerant species that follow a progression from Bacillariophyceae through to Chlorophyceae to Cyanophyceae, which are competitively superior at higher temperatures (De Wever et al., 2008; De Senerpont-Domis et al., 2013). For a more detailed exposition on the use of aquatic microcosm systems in phytoplankton ecology studies, see Karydis (this volume). The dominance of Cyanobacteria has been shown to reduce total zooplankton biomass and fish production (Ghadouani et al., 2006; Finlay et al., 2007; Hansson et al., 2007). In Lake Tanganyika reductions in phytoplankton biomass (Stenuite et al., 2009) have been attributed to an average 0.2°C warming at 1 000 m and 1.3°C of the upper waters during the past century and an average 0.9°C warming across the lake (Sarvala et al., 2006a, b; Stenuite et al., 2007; Verburg and Hecky, 2009). This warming has intensified stratification and diminished mixing of shallow and nutrient-rich deeper waters adversely affecting silica levels in particular, which has apparently lead to decreased phytoplankton biomass and primary production (Verburg and Hecky, 2009, Naithani et al., 2007) and an expansion of the anoxic water mass (Descy et al., 2006). These changes in the phytoplankton and primary production have been reported as a cause for concern associated with a 30-50% decline in the pelagic fishery (Tierney et al., 2010) in this lake and indeed fisheries data from the Lake Tanganyika show significant correlations with climatic (ENSO) data over the last 40 years (Stenuite et al., 2007; Bergamino et al., 2007, 2010). However, other scholars argue that the relationship between climate and fish production in Tanganyika via the food chain is much more complex than is postulated and that there is not enough evidence to pin climate warming and consequent reduced silica levels as well as primary production as the major factors causing the decline in fishery in Tanganyika and rather cite intensified fishing levels as the major cause (Sarvala et al., 2006b). Verburg et al. (2007) agree that, although in their opinion sufficient evidence has been provided for reduced productivity due to warming, available fisheries data do not support the theory that climate warming has reduced primary productivity and therefore fish production in Lake Tanganyika. Therefore, such declines in primary production and shifts in phytoplankton seasonality require further investigation since a re-appraisal of the Lake Tanganyika data seems to have found there was insufficient evidence of the effects of climate change on primary production but rather that reduced water transparency to light was limiting primary production coupled with riverine and land inputs (Sarvala et al., 2006a, b; Langenberg et al., 2008). Similarly, Marshall (2012) also cites overfishing rather than climate warming as the main cause of the declining Clupeid sardine (*Limnothrissa miodon*) catches in Lake Kariba as opposed to the strong link between climate (particularly ambient temperatures) and *L. miodon* (Kapenta) fish catches in Lake Kariba that is postulated by Ndebele-Murisa et al. (2011, 2013b). However, fishing effort in some African lakes like Tanganyika continues to rise and technologies have improved so that landings have increased during the warming period making it difficult to decipher any impact on fish productivity in response to warming. In Lake Victoria, climate warming has been implicated in the eutrophication of the world's largest lake fishery (Fryer, 1997). However, increased phosphorus loading is more likely the direct cause with climate probably playing a smaller role by increasing stability of seasonal stratification and accelerating the onset of hypolimnetic de-oxygenation (Fryer, 1997). Consequences of lake warming for the hundreds of endemic cichlid fishes, especially those occurring in littoral areas of the lake, are speculative (Phoon et al., 2004).

Much of what is happening to lakes across Africa is attributable to years of drought that reduce river inflows and rising temperatures that cause increased evaporative water loss (Arnell, 1999, 2004). However, land use changes also play a major role in altering water inflows into both African rivers and lakes (Vörösmarty et al., 2000, 2005). For instance, in Lake Malawi, increased population growth and land cultivation in its catchment have led to an increased input of sediment and nutrients, and a consequent decline in lake water quality. These factors, together with intensive fishing, have resulted in a loss of endemic fish species and the proliferation of alien aquatic plants (Bootsma and Hecky, 1993; Hecky et al., 1999, 2010).

Another example is Lake Chad, whose change in size has been as much as 95% between 1963 and 2001 (Figure 2) and the lake has continued to decrease in size since then. The reduction of Lake Chad can be attributed to climate change coupled with poor management decisions in the basin in the midst of increased demand for irrigation water upstream and consequently, unsustainable irrigation projects built by Niger, Nigeria, Cameroon and Chad which have diverted water from the lake as well as from the Chari, Logone and Niger Rivers. In addition, major overgrazing in the region has resulted in a loss of vegetation and serious deforestation thereby contributing to a drier climate (Carmouze et al., 1983; Coe and Foley, 2001). Another extreme example is Lake Chilwa which has dried up nine times in the 20th Century at 10-20 year intervals in relation to patterns in rainfall in the lake basin (Njaya et al., 2011).

These are reflected in Chilwa's lake water levels, which are highly correlated with annual flow in rivers and streams and precipitation in the catchment and therefore climate (Njaya et al., 2010). Possible factors for this lake's reduction in the last half decade include climate change and reduced rainfall but moreso the drastic reduction in the flow of rivers that feed the lake has been caused by deforestation, environmental degradation and declining soil fertility in the lake catchment.

Figure 2. Reduction of Lake Chad reflected from 1963 to 2001. Image downloaded from http://blog.lib.umn.edu/pran0024/architecture/sc00072e75.jpg.

Not only do reductions in water levels within these rivers and lakes affect water supply for domestic, industrial and irrigational use across Africa, but this also reduces habitats of aquatic organisms particularly of shoaling fish and adversely affects hydroelectricity production for such reservoirs as Kafue (Zambia), Kariba (Zambia/Zimbabwe), Cabora Bassa (Mozambique), Volta (Ghana) and for stations along the Nile and Shire Rivers in Ethiopia, Uganda and Malawi respectively; the latter depending on the flow from Lake Malawi (Mukheibir, 2007).

The direct impact of drought is that the run-off is reduced and consequently the storage in dams and rivers is negatively affected. Because the duration of droughts cannot be predicted with certainty, it becomes necessary to impose restrictions on the use of water while environmental flows may also be affected (Mukheibir, 2007; Tumbare, 2008).

Conclusion

This chapter explored phytoplankton dynamics in African lakes as they relate to several factors including nutrient dynamics, stratification, thermal and light dynamics and climate warming. Some consequences of climate warming on lake hydrology and limnology through increased lake water temperature and consequent strengthening of stratification and reduced mixing of surface waters seem to be adversely affecting phytoplankton production by reducing both abundance and diversity whilst shifting composition in many African lakes. The changes are reflected in the phytoplankton community as reduced primary production rates and phytoplankton biomass, as well as a shift in the phytoplankton seasonality and abundance, with increased dominance of Cyanophyceae and decreased dominance of Bacillariophyceae and Chlorophyceae. Despite these observed changes, climate change is not the only culprit as other factors namely land use changes as a result of over-extraction of water and diversions from rivers that feed lakes compounded by pollution and sedimentation are also responsible for the changes being observed. More studies are needed to substantiate most of the changes that are occurring. The use of multi-disciplinary, multi-trophic studies, ranging from microbial food to fish communities constituting a baseline of all organisms in African lakes, is recommended in order to devise management strategies in line with the changes in phytoplankton and overall productivity observed across the continent's precious lakes.

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Chapter III

Small- and Large-Celled Phytoplankton during a Strong Disturbance Period

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Abstract

While natural aquatic communities are characterized by high time-space variability, a variety of statistical methods produce highly sophisticated and efficient tools for analyses of ecological problems. Community-level modeling has earned growing attention as an alternative to and enhancement of studies focusing on individual species. Our aim was to compare the size structure of several components of Lake Kinneret (Israel) phytoplankton assemblage, using a long-term monitoring period made up of both stable years and years with great disturbances. Statistical descriptors of specific size classes identify zones of different variability within the lake phytoplankton spectra. While the annual succession change was most evident through the phytoplankton biomass variability, very conspicuous changes also characterized the small-celled part of the assemblage. At the same time that ataxonomic size spectra and allometries have produced very efficient tools of modern ecology, community-level taxonomic models augment such studies with the help of specific taxonomic group analyses providing diagnostically important information on the health of the studied ecosystem. Species abundances and occurrence rates produce common patterns that attract much interest in macroecology and help us derive simple diagnostic estimates for the assemblage disturbance level. Problems of assemblage-component (sensitive-size regions and phyla) selection and processing, that can amplify the diagnostic value of size spectra, are discussed. Such models deserve attention as efficient numerical tools applicable in theoretical ecology and monitoring.

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Keywords: Aquatic community self-maintenance; phytoplankton; size spectra; species abundance distribution; taxonomic structure

Introduction

In order to assess ecosystem stability, we should examine the living community as a whole. Size-spectrum (SS) analysis (Sheldon et al., 1972) is one of the most common and effective tools applied by ecologists to characterize whole communities. All organisms share a common structural and functional basis of metabolism while additional general rules determine how this metabolism is regulated at several levels of organization. The most important of these rules relate to body size of the individual organisms (West and Brown, 2005). Therefore, a vast stock of important quantitative rules (SS and allometry) is found and applied, describing various properties of living organisms and the structure of natural aquatic communities as related to body size of organisms (Sheldon et al., 1972; Platt and Denman, 1978; West and Brown, 2005).

Natural communities tend to exhibit inherent patterns in size distribution of the organisms comprising them, or biomass size spectrum (BSS) and its normalized variant (NBS) (Sheldon et al., 1972; Platt and Denman, 1978). The identification of such typical patterns of size distribution in natural aquatic communities was used to analyze the stable aspects of community structure and deviations from common patterns (Schwinghamer, 1981; Rodriguez and Mullin, 1986; Sprules and Munawar, 1986). At the same time, the small details of community structure are often highly variable (Rojo and Alvarez-Cobelas, 2003; Scheffer et al., 2003).

While BSS and NBS consider size distribution based on only 2 parameters of living particles – volume and abundance, other structural and functional properties of particles can be applied to obtain more sophisticated diagnoses. Much information is accumulated based on taxonomic composition of assemblages. Size-frequency distributions of taxonomic units have been studied for many years. Such distributions also exhibit common patterns (Roy et al., 2000). Common taxonomic patterns can also survive strong environmental stress (Havlicek and Carpenter, 2001). Broad-scale comparisons of invariant size distributions of species have been published for various types of ecosystems (Roy et al., 2000; Smith et al., 2004).

The operational taxonomic-unit (OTU) methodology (Sneath and Sokal, 1973) is applied to analyze species and other entities of taxonomical hierarchy. Therefore, we speak about OTUs and taxonomic size spectrum (TSS). We apply two forms of taxonomic spectra – the traditional taxonomic size spectrum (TTSS) and the frequency-weighted taxonomic size spectrum (FTSS) (Kamenir, 2007). The contribution of each OTU, equal to 1 in TTSS, is weighted in FTSS by its 'frequency rate' (FRj), i.e., the number of sites occupied by the species. Such an occurrence rate obtained ever growing popularity for modern taxonomic descriptors (McGeoch and Gaston, 2002).

We have already applied TTSS and FTSS to study the phytoplankton of subtropical Lake Kinneret (Kamenir, 2007). Later we tried to compare several types of the lake phytoplankton statistical distributions using long-time monitoring datasets contrasting stable years and years of extreme disturbance (Kamenir et al., 2006; Kamenir and Dubinsky, 2012; Kamenir, 2013).

In this chapter, our aim was to review and compare size structure patterns of several components of Lake Kinneret (Israel) phytoplankton assemblage to establish reliable patterns and better understand the mechanisms of the pattern survival under strong disturbances.

Materials and Methods

Site Description

Lake Kinneret, Israel, situated ca. 210 m below mean sea level at 32°45'N, 35°30'E (Serruya, 1978), is a warm monomictic lake with a surface area of 170 km^2, maximum depth of 43 m, and mean depth of 25 m (Figure 1). The lake, used for recreation and fishery, is also the major national source of drinking water. For that reason, a routine monitoring of numerous variables (water temperature, light, hydrodynamics, cell count, chlorophyll, primary production, hydrochemical determination, zooplankton, fish, etc.), describing its environment and living community, has been carried out since 1969 (Zohary, 2004). From the beginning of this monitoring, Kinneret phytoplankton have shown distinct stability in the form of a repeating annual pattern of species succession and biomass dynamics (Berman et al., 1995). Since the mid-90s, several man-induced changes, including the catchment-area reconstruction, led to drastic changes in phytoplankton annual succession patterns (Zohary, 2004). The resulting and most noticeable change in Lake Kinneret was the increased fluctuations and overall reduction of its water levels and, later, the loss of the previously predictable annual succession pattern (Zohary, 2004). Therefore, we chose two subsets, referred to as the stable period (1985-1995) and the period of extreme disturbance (1996-1999). The lake was already subjected to several size-spectrum analyses, including BSS, NBS, TTSS, and FTSS (Kamenir, 2007, 2013).

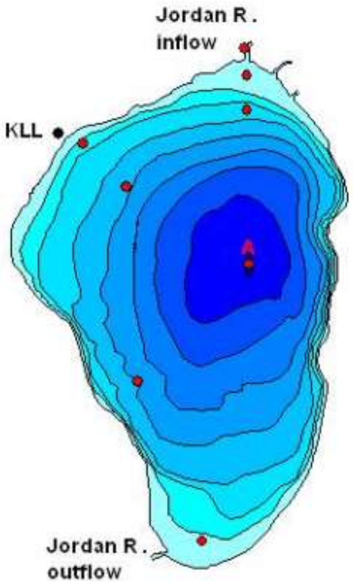


Figure 1. A schematic map of Lake Kinneret, with isobaths of 5 m each and monitoring station A location; after D. Hambright.

Phytoplankton Data Acquisition and Processing

As part of the routine monitoring program, phytoplankton samples were collected at two-week intervals from a fixed pelagic station at the deepest part of the lake, from several discrete depths (0, 1, 2, 3, 5, 7, 10, 15, 20, 30, and 40 m) throughout the water column. Lugol-preserved samples were brought to the laboratory and prepared for microscopic counting using the inverted microscope technique (Lund et al., 1958). All phytoplankton species with individual cells greater than 2 µm in diameter were identified and counted according to species; for species with variable cell size (like *Peridinium gatunense*) – also according to size categories. From the smaller cell range, only the relatively common colony-forming cyanobacteria were included. Sample processing was described in detail in Zohary (2004) and Kamenir et al. (2006).

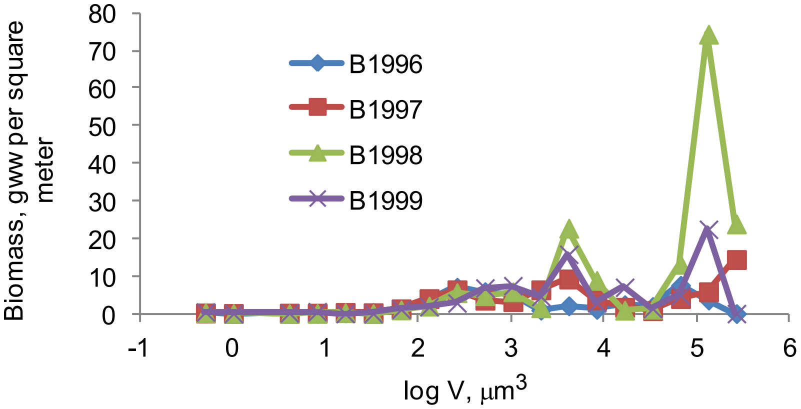
A mean biovolume of the individual cell of each species, or size category for species counted under several size categories, was calculated from linear microscope measurements and the closest geometrical shape. This cell volume was the parameter used for allocating a taxon to a size class. An exception was the filamentous *Aphanizomenon ovalisporum*, in which case the entire filament was used as the size unit. Size classes were created by doubling the cell volume, i.e., by standard increments of the cell size logarithm. The Vxx notation is used throughout this paper for size classes, where xx is the logarithm of the class' right boundary (Kamenir et al., 2006).

The sum of cell volumes of all cells belonging to each size class produces a BSS, while the sum of all cell abundances approximates an NBS (Platt and Denman, 1978). The depth-integrated BSS per unit area was calculated using data from all sampling depths, conducting a linear interpolation between the depths. During stratification, depth integration was only to the mid-thermocline depth.

Count of all OTUs belonging to each size class produces a TTSS, while summation of FRj produces a frequency-weighted taxonomic size spectrum for each phylum (Kamenir, 2007, 2013). Frequency-rate values are plotted along the Y axis of the histogram. These values are the cumulative number of times that all OTUs belonging to a specific size class (*i*) occurred during the year. Using the histogram and crosstab procedures, we calculated TTSS and FTSS as the size-frequency distribution based on all database records for each phylum and for the whole assemblage, for each year from the stable (1985-1995) and extreme-disturbance (1996-1999) periods.

Results and Discussion

The variability of phytoplankton assemblage structure was very high, as can be seen with the help of BSS (Figure 2). The dominant peak height changed several times within just 1-2 years. The horizontal position of the peaks and gaps did not change, but almost 50% of the curve was difficult to decipher as the biomasses of the small-celled ($\log V<2$) algae were low (Figure 2). This part of BSS can be appreciated better if a log transformation is applied for the spectrum Y-axis. Therefore, other variants of spectra can be more helpful, specifically, the log-log transformed BSS and NBS (Figure 3). These types of spectra confirmed the consistency of the horizontal positions of two large-cell ($\log V>3$) peaks. The log-log BSS looked variable at small-cell regions (logV < 2) even for stable years (1985-1995), while the central region (logV=2-3) looked almost the same year after year, even during periods with the most disturbance (Figure 3). Comparison of seasonal BSS confirmed the same distinction between the stable 'nanoplankton plateau' (logV=2-3) and two size-spectrum extremes (Kamenir, 2007).



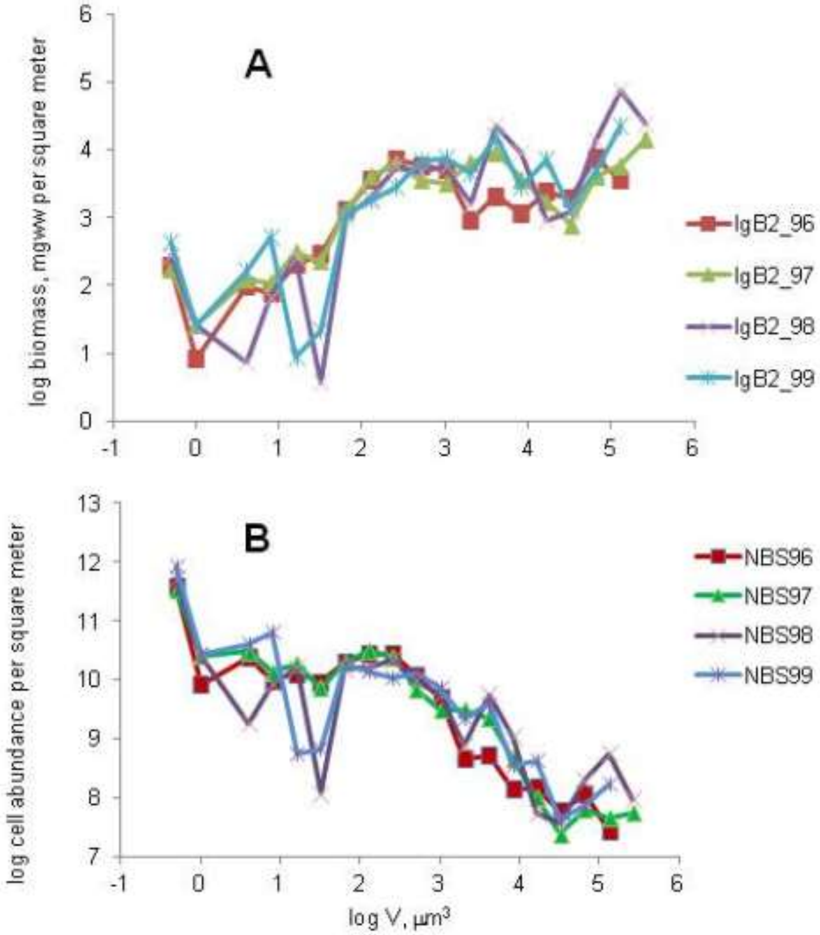
After Kamenir 2007.

Figure 2. Dynamics of biomass size spectrum of Lake Kinneret phytoplankton during disturbance years (1996-1999).

The influence of the assemblage composition can be seen with the help of other types of statistical distributions. Each annual TTSS (Figure 4A) looked like a lognormal distribution or a combination of several lognormal distributions. Distribution of species (TTSS and FTSS) confirmed the difference between the stable central plateau formed by a high number of OTUs, and a small number of OTUs forming variable zones of BSS and NBS (Figures 3-4). Comparison of the taxonomic spectra of separate phyla showed that almost all phyla – Cyanophyta (cya2), Diatomea (dia3), Chlorophyta (chlo4), Dinophyta (din5), Cryptophyta (cry6), and several minute ones – were concentrated near the size-spectrum center, logV2-3 (Figures 4-5). Only 2 phyla inhabited the outlying tails, i.e., logV<1 (cya2) and logV>4 (din5).

The disturbance period (1996-1999) demonstrated a much higher level of variability in large fractions (logV>3). While pronounced disturbances were evident through phytoplankton biomass dynamics (produced mainly by large cells), very conspicuous changes also characterized the small-cell part of BSS and NBS (Figure 3). Some phyla drastically changed (Figure 5) during the disturbance period (Kamenir, 2013). Changes were seen for each phylum, but were especially clearly seen for cya2 (Figure 6).

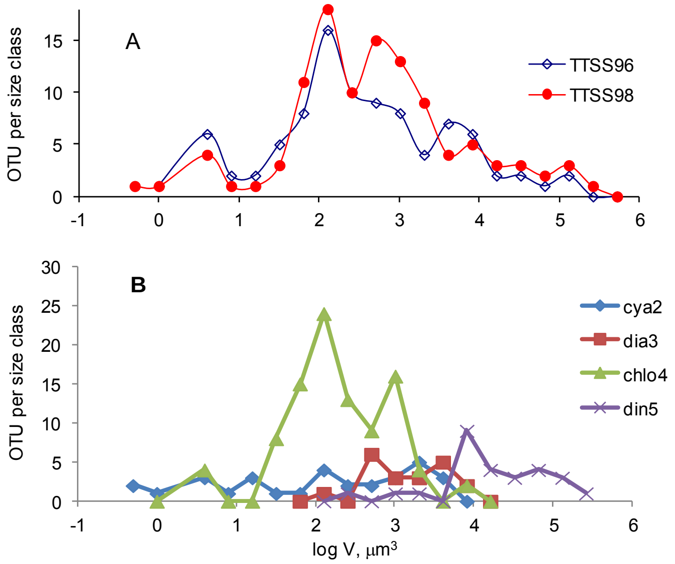
Three parts of phytoplankton could be distinguished with the help of FTSS (Figure 5A and B). The green algae (chlo4) spectrum exhibited the largest stock of species, the small-cell region (cya2) included the cell abundance dominants, and din5 species were champions of the assemblage biomass (Figures 3 and 5).



After Kamenir 2007.

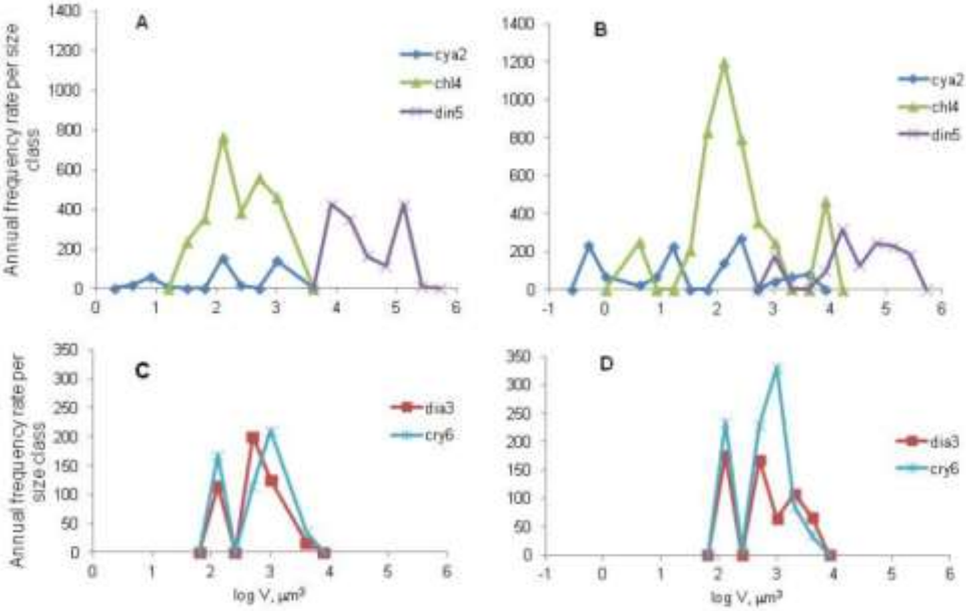
Figure 3. Annual average size spectra of Lake Kinneret phytoplankton assemblage, 1996-1999. The log-log biomass size spectrum (A) and normalized biomass size spectrum (B).

Taxonomical diversity was also supported by several less-active phyla (dia3 and cry6; Figure 5C and D) and several minor phyla producing just several percent of total phytoplankton occurrence counts. Comparison of several types of spectra helped elucidate the role of biodiversity in the assemblage self-maintenance, as the stable nanoplankton-plateau coincided with the highest concentration of species (Figures 3 and 4). Perhaps the law of large numbers is helpful, as it states that the average of a sequence of *n* identically distributed independent random variables tends to their common mean as *n* tends to infinity. This law is important because it certifies stable results for the averages of random events (Grimmett and Stirzaker, 1992). In reality, in both peripheral zones we see pronounced variability of the size-class biomass produced by very few species while numerous species were concentrated inside the much-less variable central zone (Figure 4).



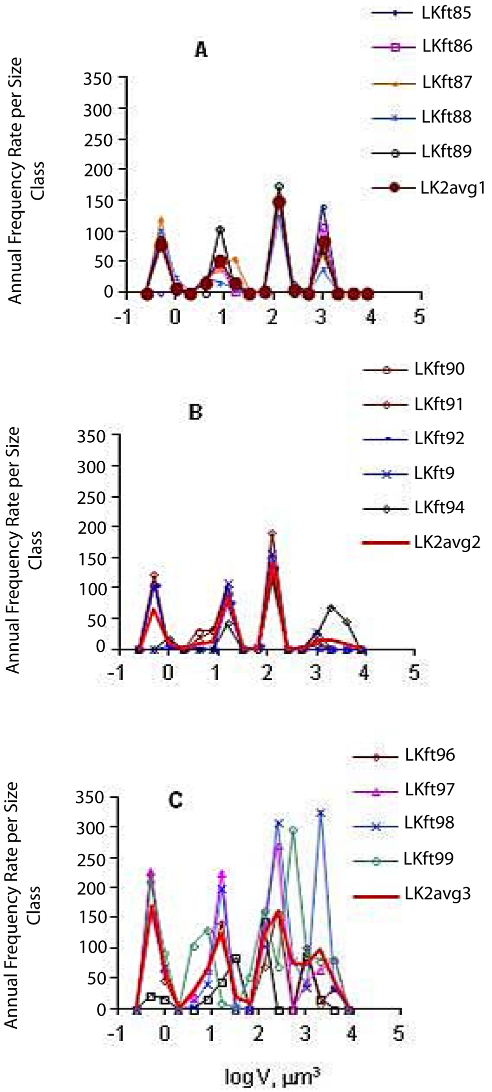
After Kamenir 2007, Kamenir 2013.

Figure 4. Traditional taxonomic size spectra (TTSS). A) Comparison of a contrasting pair (1996 and 1998) of especially strong and weak bloom years; B) main phyla of phytoplankton assemblage (all OTUs found till 1999): Cyanophyta (cya2), Diatomea (dia3), Chlorophyta (chlo4), and Dinophyta (din5).



After Kamenir 2013.

Figure 5. Annual frequency-weighted taxonomic size spectra (FTSS) of main phytoplankton phyla for representative years of stable (A) and extreme-disturbance (B) periods (1985 and 1997, respectively). C, D) Minor phyla of 1985 and 1997. Cry6 is Cryptophyta; names of other phyla are given in legend of Figure 4. Phyla including only 1 size class are not shown.



After Kamenir 2013.

Figure 6. Comparison of FTSS annual patterns (1985-1999) for one phytoplankton phylum (Cyanophyta, cya2) of Lake Kinneret. Three periods of 5 years each (A-C); LK2avg is the 5-year average values.

During each season, phytoplankton in Kinneret and other lakes existed as several phyla inhabiting a set of ecological niches with considerably different nutrient levels as well as stoichiometry, temperature, and light regime (Kamenir and Morabito, 2009; Kamenir, 2013). Hydrodynamic conditions were also very different during stratification and winter mixing (Berman et al., 1995; Zohary, 2004). Nevertheless, annually averaged size spectra demonstrated very high consistency for some types of spectra, even under strong environmentl impacts (Havlicek and Carpenter, 2001; Kamenir et al., 2006).

At the same time that ataxonomic size spectra and allometries have produced efficient tools of modern ecology, community-level taxonomic models augment such studies with the help of specific taxonomic-group analyses providing diagnostically important information on the health of the studied ecosystem. In addition, relative species abundance distribution (SAD) produces specific patterns resembling one of the most-studied macroecology patterns. Application of SAD for Lake Kinneret phytoplankton produced a very consistent pattern composed of 3 parts with considerably different slopes (Kamenir and Dubinsky, 2012). A linear tail at the top of such SAD, where a small group of species were cell-abundance dominants, helped us to construct a numerical index that was very stable during a long period (1985-1992). From 1993, such an index exhibited pronounced changes, and two years later its variability was augmented by notorious variability of the phytoplankton assemblage biomass (Kamenir and Dubinsky, 2012).

Conclusion

We can conclude that both large and small cells demonstrated pronounced variability, and small-celled species pioneered the diagnostically important pattern changes. We can see how heterogeneous was the assemblage composed of species varying some 6 orders of magnitude in cell volume. During each season, the Kinneret phytoplankton were presented by several phyla inhabiting considerably different ecological niches (nutrient levels as well as stoichiometry, temperature, light regime and hydrodynamics). This way, numerous dynamic environmental factors interfere to support many different living forms that can coexist within a relatively small water body. Analysis of phytoplankton size structure pattern and trends of its change under environmental impacts can serve as efficient means for the monitoring of aquatic ecosystems. Some phyla and size fractions are outstandingly valuable for providing important diagnostic information.

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Chapter IV

Phytoplankton Ecological Interactions in Freshwater Ecosystems - Integrating Relationships in Subtropical Shallow Lakes

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Abstract

In this chapter we approach the main aspects of the complex ecological interactions phytoplankton is involved with in subtropical shallow lakes. These systems differ considerably from more studied deep, temperate lakes in the sense that they possess high ratios of littoral: pelagic zones, which in addition to the mild temperatures, allow macrophytes to colonize extensive areas and grow continuously. They are also strongly regulated by wind, which induces patchiness in phytoplankton distribution directly through advection and resuspension and indirectly through nutrients upwelling. Macrophytes play a central role in these systems by controlling the physical and chemical environment through reduced water motion, shading, high competition for nutrients and release of allelopathic substances. They keep phytoplankton biomass low in areas of high plant coverage, resulting in high bacterioplankton:phytoplankton biomass, which potentially increases the competition for inorganic nutrients between bacteria and algae.

The zooplankton find poor refuge inside the plant beds due to high fish density and hence their cascading effect on water transparency is potentially low. Fish densities are higher and reproductive phases are longer and/or continuous and they present an important link of energy and nutrients between benthos, littoral and pelagic zones; they also present a higher share of omnivores than temperate lakes, which increases the resilience of these systems. Recently, ecological modelling has been applied to the largest lake of the subset analyzed (Mangueira lake) through the development of the IPH-TRIM3D-PCLake model. This model incorporates complex ecological (physical/chemical/biological) interactions (e.g., hydrodynamics, spatial heterogeneity, grazing, functional groups, among many others) and has been used as a research and management tool to predict potential effects of shifts in water level, increase in turbidity and climate changes in the lake ecology.

Introduction

The research on freshwater lentic ecosystems has traditionally focused on deep lakes, but recent efforts have emphasized the numerous functions, values, and ecological contributions of wetlands and shallow lakes, including flood abatement, water quality improvement and habitat availability for aquatic and semi-aquatic species (reviewed by Zedler 2003; Hansson et al., 2005; Potthoff et al., 2008; Crossetti et al., 2013). Trophic interactions in shallow lakes have been studied extensively in the north temperate regions (Jeppesen et al., 1997; Jeppesen et al., 2000; Jeppesen et al., 2010), while few studies have examined these interactions in subtropical systems in the southern hemisphere (Meerhoff et al. 2007b; Texeira de Mello et al., 2009; Sosnovsky et al., 2010; Rodrigues et al., 2013). While the last have received less attention, the few studies available so far point out that they present distinct ecological patterns when compared to other lakes, which can be related to their origin, large and permanent influence of macrophytes and strong control by the wind.

In this chapter, we discuss aspects of the phytoplantkon interaction with other biological and environment components in freshwater systems, a topic that has been mostly approached by considering its isolated aspects in the literature. We propose a more holistic view about the complex interactions involving phytoplankton, environment, bacteria, zooplankton and macrophytes, as these interactions largely determine patterns of diversity and biogeochemical pathways in freshwater ecosystems. A more thorough understanding on this topic is important for more precise determination of elements budgets and a more complete understanding of phytoplankton patterns in freshwater ecosystems.

As a background for the discussion of this theme, we cover general aspects of the ecological interactions involving phytoplankton in freshwaters emphasizing 11 subtropical shallow lakes located in the coastal plain (22,740 km^2) of southern Brazil. These shallow lakes (maximal depth: 11 m) were originated in the quaternary as a consequence of a post-glacial marine regression and as a result they are very numerous, covering together approximately 63% of the total coastal plain area (Schwarzbold and Schäfer, 1984; Schäfer, 1988; Holz, 1999). These lakes vary over a wide range of size (0.13 - 808 km^2), distance between lakes (< 1 km to > 500 km) and contribution of macrophyte coverage (Ferreira, 2009; They et al., 2010) but have in common the wind as a strong driving force in the whole coastal plain (Figure 1; Table 1). Many of the lakes have their main axis oriented in a NE-SE direction, which is coincident with the predominant orientation of the winds. This condition maximizes the fetch and has many consequences for biological communities, since it promotes patchiness of phytoplankton, thus resulting in a mosaic of ecological situations mainly in larger lakes like Mangueira (Fragoso et al., 2008) and Itapeva. In the last, several studies have demonstrated that the wind promotes gradients of limnological variables, which causes strong effects on phytoplankton and zooplankton (Cardoso and Motta-Marques, 2003; Cardoso and Motta-Marques, 2004b; Cardoso and Motta-Marques, 2007).

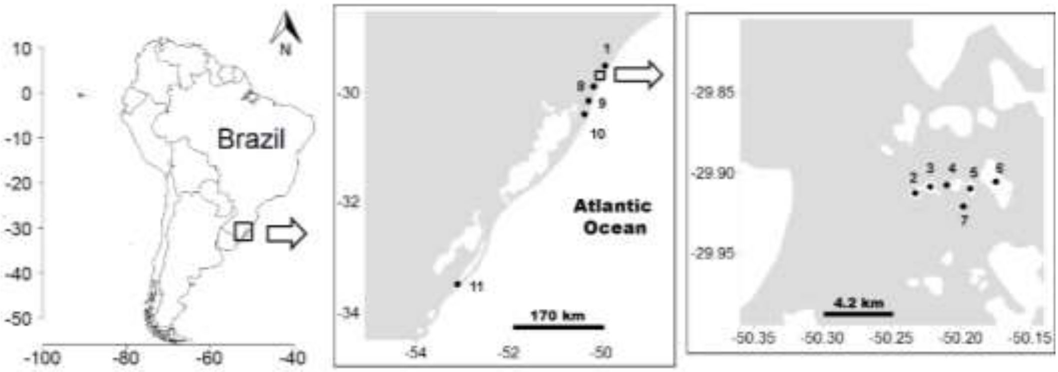

Figure 1. Location of the 11 subtropical shallow lakes approached in this chapter in the coastal plain of southern Brazil. Lakes: 1 - Itapeva, 2 - Horácio , 3 - Inácio, 4 - Rincão, 5 - Passos, 6 - Veados, 7 - Ipê, 8 - Suzana, 9 - Lake 10, 10 - Lake 02, 11 - Lake Mangueira (marked at its southernmost part).

Table 1. General characteristics of the 11 subtropical shallow lakes in the coastal plain of Rio Grande do Sul state (Brazil) discussed in this chapter

Lake	Location	Area (km^2)	Mean depth (m)
Ipê	29º55’17” S / 50º11’55” W	0.13^a	0.80^a
Inácio	29º54’33” S / 50º13’21”W	0.30^a	1.37^a
Lake 02	30º33’08”S / 50º25’60”W	0.36^a	1.81^a
Horácio	29º54’47”S / 50º13’59”W	0.58^a	1.09^a
Rincão	29º54’37”S / 50º11’37”W	0.60^a	0.76^a
Passos	29º54’29”S / 50º12’37”W	0.69^a	1.20^a
Suzana	30º09’57”S / 50º16’53”W	0.97^a	1.02^a
Lake 10	30º24’44”S / 50º21’43”W	1.92^a	1.10^a
Veados	29º54’21”S / 50º10’30”W	2.22^a	1.22^a
Itapeva	29º28’34.27” / 49º54’1.47”	125^b	2.23^c
Mangueira	33º31’22”S / 53º07’48”W	808^a	2.60^d

a - They et al. (2010)
b - Cardoso and Motta-Marques (2003)
c - Schwarzbold and Schefer (1984)
d - Fragoso Jr. et al. (2008)

Because of their shallowness and dominance of winds, these lakes display a tendency to polymixy, with occasional stratification at most a few hours a day (Lewis Jr., 1983). Also, given the benign climatic conditions, there is no period of ice cover. This favors the continuous growth of aquatic macrophytes, with profound impacts on the ecological functioning of these lakes, as will be discussed later in this chapter.

These lakes are in general oligo-mesotrophic, according the OECD classification based on chlorophyll *a* values (OECD, 1982 *apud* Tundisi and Tundisi, 2008) and also to modifications of the Carlson Trophic State Index (Schäfer, 1988). In general, the shallower lakes of this coastal plain have a tendency to more eutrophic states, whereas deeper lakes have a tendency to more oligotrophic states (Schäfer, 1988). Particularly, mesotrophic conditions occur in the spring and summer in Lake Mangueira when it suffers from a notable water withdrawal for irrigation of rice crops (approximately 2 L s^{-1} ha^{-1} during 100 days), as well as a high input of nutrients loading from its watershed (Fragoso et al., 2008). This affetcs the hydroperiod, which is composed of low- (generally in summer) and high-water (generally in winter) phases. Considerable spatial heterogeneity is found in these lakes, specially the larger ones. In the northern and southern part of Lake Mangueira, for example, the lake makes interface with wetlands, presenting large areas covered and infested of emergent (northern) and submerged (southern) macrophytes.

Phytoplankton and Abiotic Scenario

The structuring and dynamics of phytoplankton assemblages in freshwater ecosystems are driven by relationships of phytoplankton with chemical, physical and biological parameters within the ecosystem (Naselli-Flores, 2000). These relationships affect from rates of primary productivity to phytoplankton adaptive strategies to cope with water movements and elementary nutrient availability.

In shallow lakes in general, the phytoplankton communities are driven and regulated mainly by the variability in the physical environment, whereas the selection played by chemical factors, competition and trophic interactions appear to be relatively weaker (Reynolds et al., 1994). This is more critical for large shallow lakes, where wind-induced mechanisms such as resuspension of solids and availability of nutrients and light (Bachmann *et al.*, 1999; Cardoso et al., 2012) are generally driving factors leading to both spatial and temporal heterogeneity of phytoplankton. Diel variations in wind-induced mixing have a profound effect on the growth of phytoplankton and their seasonal succession in lakes (Reynolds, 1984; Peeters et al., 2007). These physical variations cover a wide range of temporal scales (Lewis et al., 1984) and influence the development of different functional groups by regulating their sinking rates, modulating their light regime and facilitating the internal cycling of nutrients (Reynolds, 1997; Reynolds, 2006). Thus, no appreciation of the phytoplankton ecology can ignore the physical characteristics of the suspending medium and the ways in which liquid water is moved about, especially within those 'turbulent' or actively mixed shallow-water or flowing environments (Reynolds, 1994). In this sense, we adress some aspects in which phytoplankton can be influenced by the abiotic scenario in wind-exposed subtropical shallow lakes in southern Brazil.

Phytoplankton and Water Movements

Given that phytoplankton populations live in suspension in water bodies and that only very particular environmental conditions allow effective active movements of the organisms, the phytoplankton community is crucially dependent on the movement of water masses. This physical factor also influences directly the availability of light and nutrients, the two most important resources for algal growth (Reynolds, 1980; Reynolds, 2006; Naselli-Flores, 1998). Thus, water movements govern the selection of species adapted to live in each particular condition, as well as their variations in composition and biomass. Phytoplankton communities generally consist of a diverse number of species with different ecophysiological and morphological characteristics, resource requirements, growth rates, and sinking velocities (Hutchinson, 1967; Reynolds, 2006). The traditional supposition that the survival strategy of phytoplankton centers on the ability to minimize sinking is carefully updated in the context of pelagic motion. Extended residence time in the upper water layers remains the central requirement at most times, which is attained, in many instances, by maximizing the entrain ability of the plankter within the motion. Viewed at a slightly larger scale, many phytoplankters optimize their ‘embedding’ within the surface mixed layer (Reynolds, 2006).

Other physical factors that influence water movements are also of importance, like water level changes, winds, macrophytes and water-column stability. Changes in water column in lentic systems related to the water-circulation patterns (advection and convection) determine the path of the particles in suspension (Serra et al., 2003; Reynolds, 2006). While convection is the source of vertical mixing that governs the dynamics of suspended material, advection is the mechanism of horizontal transport of particles (MacIntyre et al., 2002; Serra et al., 2007). Water-level fluctuations and variable flushing rates have been recognized to condition phytoplankton structure and dynamics (Naselli-Flores, 2000; Naselli-Flores and Barone, 2000; Crossetti et al., 2013), while mixing regime has also received some attention (Berman and Shteinman, 1998; Becker et al., 2009; Becker et al., 2010).

The winds may be the dominant factor controlling phytoplankton patchiness in lakes (George, 1981; Webster, 1990; Cardoso et al., 2012), producing advective downwind movements of surface waters (Marcé et al., 2007). For large shallow lakes, it can be a dominant factor leading to both spatial and temporal heterogeneity of phytoplankton (Carrick et al., 1993). As a consequence of resuspension, the concentration of particulate matter in the water column increases, leading to reduced light penetration that can ultimately promote biological adjustments. In particular, changes in the light climate in shallow lakes can potentially lead to shifts in the biological communities, which evolve from being macrophyte-dominated to being plankton-dominated (Scheffer, 1998).

Shallow lakes will inevitably support a phytoplankton community fitted to their particular conditions and hydrodynamic factors tend to be even more important for plankton distribution in these lakes (Cardoso et al., 2012). Also, these systems are often influenced by water-level variations and may show alterations in the phytoplankton structure, because of a primary effect on abiotic conditions such as light and nutrient availability (Crossetti et al., 2007). Water-level fluctuations emerged as the decisive element of the hydrology, especially in shallow lakes embedded in wetlands that are particularly sensitive to any rapid change in water level and input (Coops et al., 2003), as is the case of Mangueira Lake (Crossetti et al., 2013). Accordingly, several studies have demonstrated the influence of water level variations on the structure of phytoplankton (e.g., Huszar and Reynolds, 1997; Garcia de Emiliani, 1997; Izaguirre et al., 2004; Crossetti et al., 2007; Wang et al., 2011).

Wind-induced currents can also resuspend sediment particles and release phosphorus to the overlying water (Wetzel, 2001), but other processes such as diffusion, wind-induced turbulence, perturbation, attached algae/cyanobacteria and rooted aquatic plants can also play a part. During windy periods, the water column may interact directly with the sediment surface layer, especially in shallow lakes, where the resuspension of materials is favored. In lakes Itapeva and Mangueira, water turbulence induced by wind is apparently the single most important mechanism transporting phosphorus from sediment to the water-column (Becker and Motta-Marques, 2004; Becker et al., 2004; Crossetti et al., 2013).

In areas extensively covered by plant beds, the water is relatively calm, lacking the vigorous mixing of the pelagic zone of larger, deeper lakes. Life forms fitted to such conditions include small and motile species, which have low net sinking rates (Moss et al., 2003). Phytoplankton communities that develop when submerged plant communities are displaced from shallow lakes vary greatly, depending on the balance of nitrogen and phosphorus availability, the retention time of the water mass and the degree of mixing (Moss et al., 2003).

The stability of the water column is also important in determining the size and shape of phytoplankton (Naselli-Flores et al., 2007). Physical variables such as water column stability affect the phytoplankton directly by selecting shapes depending on the intensity of the turbulence and indirectly by modifying nutrient availability (Becker et al., 2008; Becker et al., 2010). Diatoms, for example, tend to be favored by greater mixing (Moss and Balls, 1989; Reynolds, 1997), whereas green algae are favored by very high nutrient concentrations (Jensen et al. 1994) and cyanobacteria by moderately high phosphorus levels, stability of the water mass and long water retention times (Shapiro, 1990; Reynolds, 2006; Becker et al., 2009). The water column stability is thus an important condition for development of cyanobacterial species, being fundamental to the success and formation of blooms (Reynolds, 1984; Paerl, 1988; Becker et al., 2009). In Itapeva Lake, cyanobacteria species were dominant among species forming blooms, probably due to resuspension of (dormant) cells from sediment as the result of the turnover water dynamics caused by wind (Becker et al. 2004). Nostocalean blue-green such as *Anabaena* and *Aphanizomenon* produce akinetes wich can survive on sediments for long periods (Livingstone and sorki, 1980) and eventually form blooms when the proper conditions occur.

A change in the intensity or duration of stratification has a direct impact on turbulent diffusion and phytoplankton cell sedimentation, which are the major mechanisms causing vertical displacement of non-motile cells (Huisman et al., 2006). Stratification and vertical mixing therefore have an immediate influence on phytoplankton sinking velocities, which increase non-linearly with cell size (Smayda, 1969), giving smaller species an advantage in an enviroment where turbulence is not present to ressuspend all planktonic species (Boop et al., 2005).

The water movement in aquatic systems is a key factor that drives resource distribution, resuspend and carries particles, reshape the physical habitat and makes previously unavailable resources available. Phytoplankton assemblages change following those processes creating patterns in time and space (Cardoso et al., 2012).

Phytoplankton Sinking and Floating

The vertical distribution of phytoplankton will depend on the intrinsic ability of the organisms to prolong suspension in the euphotic zone. Reynolds (1984) differentiated three algal groups according to their response to water density and stability: i) non-motile, negatively buoyant algae and high sinking velocities; iii) positively buoyant organisms and iii) neutrally buoyant and motile buoyant species.

Non-motile, negatively buoyant algae and high sinking velocities, such as diatoms, will be favoured in well-mixed waters (Reynolds, 1984), because increasing rates of turbulent mixing maintain the algae in suspension and entrain cells from deeper, darker levels in water column (Huisman et al., 2002).

In case of positively buoyant species, such as cyanobacteria, reduced rates of turbulence can lead to the development of surface scums that form dense patches when they accumulate along a downwind shore (Moreno-Ostos et al., 2009). This group will tend to float in stable layers due to the presence of intracellular gas vesicles that allow them to regulate their position in the water column (Reynolds, 1984). These “functional species” have a distinct competitive advantage at reduced vertical mixing (Huismain et al., 2004; Reynolds, 2006).

The group of neutrally buoyant and motile organisms includes small green algae and mucilage colonial forms with low sinking velocities, and flagellates that can migrate through the water column (Reynolds, 2006). Consequently, under the same hydrodynamic conditions, algal groups with different suspension adaptations can show contrasting vertical distributions (Marcé et al., 2007). Some larger, motile organisms are successful plankters by virtue of adaptations that are antithetical to increasing entrainability. Large, motile species of *Microcystis, Volvox, Ceratium* and *Peridinium* combine relatively large size, motility and shape-streamlining to be able to escape moderate-to-low turbulent intensities in order to perform controlled migrations, at rates of several meters per day. However, reducing sinking rate is far from being a unique or universal adaptation qualifying microorganisms for a planktonic existence (Reynolds, 2006). In the subtropical shallow lakes in southern Brazil the resident phytoplanktonic assemblages have adapted not only to fluctuations in water level, but principally to the set of abiotic variables that comprise the principal force functions (Becker et al., 2004; Crossetti et al., 2007; Crossetti et al., 2013). These factors and biological capabilities can create important patterns by leading to alterations in the local biological communities (Crossetti et al., 2007) and wind-driven hydrodynamics can lead to changes in the whole phytoplanktonic assemblage (Cardoso and Motta Marques, 2003, Cardoso and Motta Marques 2004a). As a result, these systems are dominated by cyanobacteria and microplanktonic diatoms. Diatoms are dependent on water mixture and capable to tolerate constant turbulence induced by wind. On the other hand, cyanobacteria blooms can follow events of disturbances produced by wind or periods of low wind and higher stability (Becker et al., 2004). The strategies of different phytoplankton groups as they explore vertical space in a circulating lake can result in different horizontal distributions in response to wind forcing. Positively buoyant species, accumulating near the surface, should tend to concentrate downwind. Negatively buoyant species aggregating in the deep layers should be displaced upwind, and neutrally buoyant cells should be randomly distributed (Vidal et al., 2010). Therefore the vertical distribution of algae is controlled not only by their buoyancy strategy but also by physical processes in the water column.

Phytoplankton and Light

In shallow lakes, light conditions may depend upon local features, especially wind activity. The impact of vertical mixing on plankton distribution also sets a limit upon the concentration of cells that can be supported by the light energy available (Reynolds, 1997). Algae subjected to active mixing will experience an environment characterized by high-frequency in light fluctuations (Reynolds et al., 1994) associated to up-and-down trajectories through the light gradient, which results in a probabilistic exposure to the underwater light field (Reynolds, 1994).

In intensively mixed systems, such as wind-stirred shallow lakes, resuspension from the sediment or loading from the catchment significantly increases inorganic turbidity and light attenuation. Under those conditions, the potential for high production is not accomplished (Dokulil, 1994) because the received day-time light dose is aggregately depressed and the cells need to undergo photoadaptation accordingly (Reynolds, 1994).

In those environments, Reynolds (1994) describes a phytoplankton community dominated by 'Limnothrix-type' assemblages. This solitary, filamentous cyanobacteria is a good light-antennae, being among the algae having the highest adaptive capacities to support low average insolation. They also maintain approximately zero buoyancy sufficiently well to remain in suspension, while being of a size that renders most of them non-ingestible to the zooplankton (Reynolds, 1988).

In Mangueira Lake, the wind action has been identified as one of the main important features promoting low transparency (Crossetti et al., 2013). In continuously wind-mixed shallow lakes in general, the relative constancy of the mixed environment lead to high turbidities due to resuspension of fine particles from the lake bottom and, even though when the wind energy is not constant, the residual motion and convection within the basin prevents the stability of water column (Reynolds, 1994). *Planktolyngbya limnetica* (Lemmermann) J.Komárková-Legnerová and G.Cronberg and *P. contorta* (Lemmermann) Anagnostidis and Komárekare are dominant in this lake, representing the shade-adapted populations in this turbid environment, which is also inhabited by large diatoms, stirred up from the sediment bottom. In this case, continuous high turbidity may select only adapted species.

Phytoplankton and Nutrients

Several elements are essential as vital compounds of phytoplankton cells. Among them, carbon, nitrogen, phosphorus and silicate are commonly related to limit their growth (Reynolds, 1997). The phytoplankton obtain nutrients from the external medium but they can only be taken up if they occur as soluble compounds present as diffusible ions or as non-dissociated small molecules such as silicic acid (Padisák, 2003).

Several studies have attempted to elucidate the effect of nutrient dynamics within the trophic spectrum on phytoplankton organization (e.g., Reynolds et al., 2000; Rojo et al., 2000). In this sense, it has been pointed out that the influence of nutrients on the structure of the phytoplankton assemblages might be higher in the lower part of the trophic spectrum (Naselli-Flores, 2000), showing that nutrient limitation in oligotrophic lakes may be one of the driving forces shaping community structuring. Besides trophic typology, latitudinal differences can play a role in nutrient limitation. Overall observations point out that primary producers appear to be frequently phosphorus-limited in temperate lakes and nitrogen-limited in tropical regions (Lewis, 2000). One of the most accepted explanations is that the denitrification increases with temperature, thus raising nitrogen losses.

For kinetics and energetic reasons, phosphate uptake at very low ambient concentrations is impaired, since gross uptake rates are compensated by leakage or cell efflux rates. Therefore, growth will cease before the external concentration declines to zero (Padisák, 2003). Phosphorus thresholds for phosphorus-deficient algae usually fall into the nanomolar range, although some studies have reported the exhaustion of the soluble reactive phosphorus fraction at $< 10^{-8}$ M (Reynolds, 1997). This situation is not expected in the Brazilian shallow lakes, since total phosphorus concentrations range from around 0.2 to around 3.6 µM (average: 1.3 µM) and thus above this limiting threshold (They et al., 2010; They et al., 2013a). Crossetti et al. (2007) reported one situation when phosphorus supply eventually raised leading to the replacement of phytoplankton species previously adapted to low nutrients availability and transparency by *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju which is a shade adapted nitrogen fixing cyanobacteria.

Nitrogen is the second element whose relative scarcity impinges upon the ecology of phytoplankton (Reynolds, 2006). Of the sources of nitrogen potentially available to algal uptake, nitrate, nitrite and ammonium ion are by far the most important (Reynolds, 1997). The phytoplankton is generally capable of active uptake of dissolved inorganic nitrogen from external concentrations as low as 0.2–0.3 µM (Reynolds, 2006). In the Brazilian shallow lakes, concentrations of total nitrogen can be found ranging from around 2.0 to 65.0 µM (average around 19 µM) (They et al., 2010; They et al., 2013a) and hence far above limiting concentrations.

However, Mangueira Lake can be marginally nitrogen limited depending on local conditions. As stated in previous studies, there is a spatial heterogeneity in the lake driven by nutrients availability, especially nitrate concentrations, which drives a patchy distribution of phytoplankton (Fragoso Jr. et al., 2008; They et al., 2013a; Crossetti et al., 2013). Since phytoplankton species differ in nutritional requeriments, it is expected that physiological and morphological adaptations would condition species dominance under nitrogen deficiency. Some of them include nitrogen fixation, which although typical of cyanobacterial group Nostocales, can be induced in non heterocytic genera (Padisák, 2003). Among the most dominant species in Mangueira Lake are some *Synechococcus*-type, which were previously mentioned as having some nitrogen fixing strains (Bergman et al., 1997), although it has not been described for Mangueira lake.

Silicate is a key element for mainly diatoms (Egge and Aksnes, 1992) and is intimately associated to terrigenous sources. Hence, in lakes, especially wind-stirred shallow ones, silicate limitation is not expected to occur. In fact, concentrations of silicate in Mangueira Lake frequently exceeds 100 µM (They et al., 2013a), a concentration far above those reported to be limiting for diatoms (2 µM) in lakes (Egge and Aksnes, 1992).

The synergy between the mixing regime and nutrients might shape the phytoplankton communities as well (Becker et al., 2009), specially in ecosystems with great hydrodynamic processes. In these systems, the resuspension of nutrients from the sediment due to wind action may also benefit phytoplankton, as reported in some studies in Mangueira Lake (Crossetti et al., 2007; Crossetti et al., 2013).

Phytoplankton and Bacteria

Interdependence

Phytoplankton and bacteria display a tight interdependence relationship, a classical view that has long been a paradigm for many aquatic ecologists. This view states that phytoplankton is the major primary producer in aquatic ecosystems, supplying much of the bacterial carbon demand (BCD, the sum of bacterial production and bacterial respiration). Bacteria, at their turn, are among the decomposers and mineralizers that fuel algae with the inorganic nutrients they need to sustain primary production. As pointed by Fouilland and Mostajir (2010), this understanding was borrowed from terrestrial ecology, with plants being replaced by phytoplankton. Similarly, it was assumed that all organisms in the water would be directly or indirectly dependent on phytoplankton. The work of Cole et al. (1988) was among the first ones to hypothesize that bacterial production depended strictly on the organic matter supply, including the primary production of phytoplankton. They found out that bacterial production was predictable from algal primary production and chlorophyll *a* and could account for the cycling of more than 60% of the primary production in lakes and oceans.

However, the recognition that bacteria assimilate organic carbon at several scales in time and space (Cherrier et al., 1999; Fouilland and Mostajir, 2010) but mainly from other autotrophs (e.g., macrophytes) and allochthonous sources has thrown this classical view into debate. Moreover, it is also known that the relationship between phytoplankton and bacteria changes (higher/lower coupling) along gradients of primary productivity and can be modulated by other organisms such as viruses, grazers (Cotner and Biddanda, 2002) or, in the case the subtropical coastal shallow lakes in southern Brazil, also macrophytes. This is an ecological situation not completely elucidated, but a strong effect of macrophytes is also expected to occur in lakes with similar climate conditions and coverage of plants. In the case of the brazilian lakes, in spite of the recognized importance of macrophytes, there is evidence in support of dependence of bacteria on phytoplankton, as observed by the marginally significant correlation between bacterial biomass and chlorophyll *a* in the ten lakes data set (all lakes except Itapeva) (r = 0.41; P-value = 0.071) and in Mangueira lake (r = 0.70; P-value = 0.080) (Figure 2).

The primary production is composed of particulate and dissolved components, with the particulate form frequently being the highest fraction (Baines and Pace, 1991). The most common way of measuring it is via the addition of radioactively labeled compounds (e.g., $H^{14}CO_3$) and their uptake by phytoplankton during short incubations (ca. hours). The samples are then filtered and the production is split into particulate (cells retained) or dissolved (filtrates) primary production (PPP and DPP, respectively) (Fouilland and Mostajir, 2010). Since bacteria can only directly assimilate dissolved compounds, the DPP is the fraction of primary production of most interest when considering bacterial consumption. The DPP is highly variable, ranging from 4-70% of the total carbon fixed by photosynthesis (Chróst and Faust, 1983). Much has been discussed in terms of what regulates the DPP and it has been pointed out mainly its dependence on the primary production (amount of carbon fixed available) (Baines and Pace, 1991). In general the DPP increases with the primary production, but systematic differences are found between freshwater and saltwater ecosystems.

In freshwater, DPP increases with primary production following a curvilinear pattern with an asymptote after a certain level of primary production (Baines and Pace, 1991), whereas in marine environments the increase in linear.

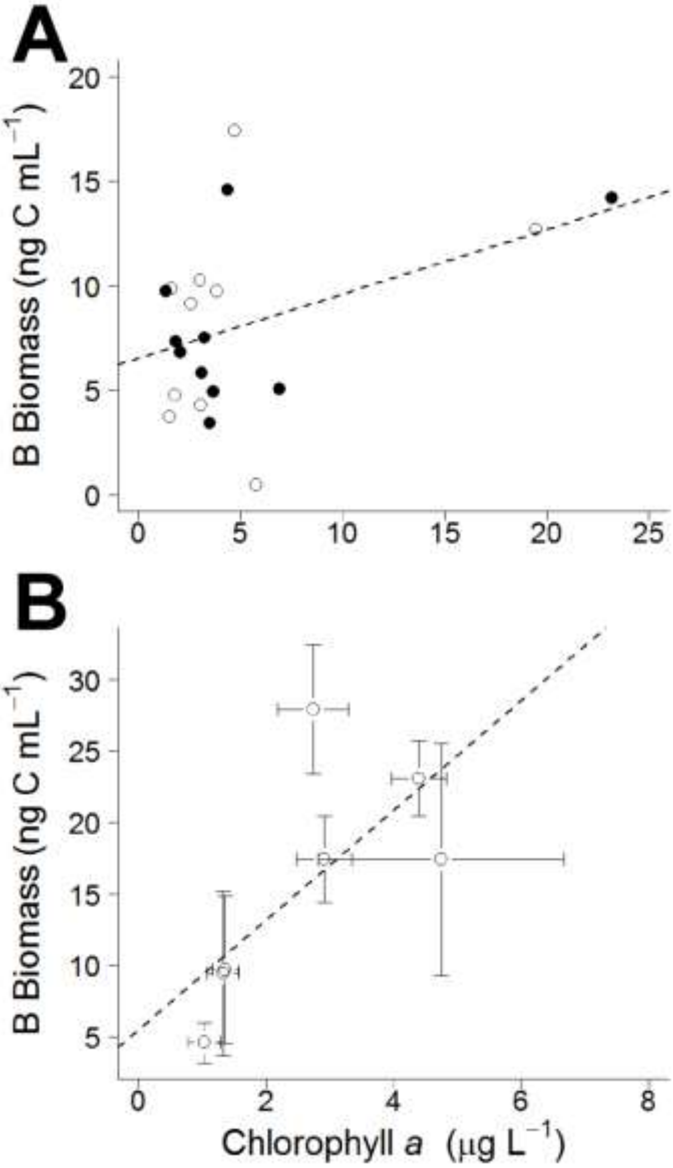

Figure 2. Relationship between bacterial biomass and chlorophyll a in A) 10 coastal subtropical shallow lakes (all lakes covered in this chapter except Itapeva) (They et al., 2010) and B) a seven points transect (3,275 m) ranging from the littoral to the pelagic zone of lake Mangueira, the largest coastal lake of all (They et al., unpublished results). For the lakes data set, the black dots refer to samples taken at littoral zones and white dots refer to samples taken at the pelagic zones. For Mangueira lake, at each point of the transect it was collected a total of three samples. In this figure and all the subsequent ones, the linear relationships were fitted according to model I regressions. The association between x and y was tested through Pearson´s correlation with P-values computed through permutation in R 3.0.0 (R Development Core Team, 2013).

The fraction of the bacterial production that can be sustained by the DPP can vary from 18-77% and in lakes this contribution tends to decrease in more productive systems (Chróst and Faust, 1983; Baines and Pace, 1991). The DPP is composed of different size classes of molecular weight and a considerable fraction (around 20%) is of low molecular weight (< 500 Da) (Chróst and Faust, 1983), smaller than bacterial porins (600 Da) (Weiss et al., 1991) and hence directly assimilable by bacteria. When considering also the respiration, the DPP may account for less than 50% (Baines and Pace, 1991) or from 7-104 % of BCD (Chróst and Faust, 1983; Morán et al., 2002).

Even though DPP is a more direct assessment of the carbon available to bacterial consumption, the most frequently form of primary production measured is the PPP. The measurement of DPP is not trivial because considerable consumption of carbon by bacteria can occur during incubation, thus causing underestimation of DPP (Sell and Overbeck, 1992).

Fouilland and Mostajir (2010) have found, comparing oceanic, coastal, transitional and freshwaters, that the PPP explained significantly the bacterial production only in freshwaters (positive log-log relationship). It is important to stress that this study only took into account paired determinations of bacterial and primary production, differently from other studies (e.g., Cole et al., 1988), which estimated them separately. However, it has been pointed that the apparently high coupling between bacterial and phytoplankton communities in freshwaters could be derived from their high synchronicity, which is stronger than in marine and coastal systems (Kent et al., 2007; Fouilland and Mostajir, 2010).

In Lake Mangueira only PPP has been estimated. Considering primary and secondary production, the bacterial:particulate primary production ratio (BP:PPP ratio; r = -0.60, P-value = 0.153) seems to decrease with PPP, whereas the bacterial production (r = -0.10, P-value = 0.823) do not change along the gradient of PPP (Figure 3). This is suggestive that the importance of the primary production in comparison to the bacterial production increases with increasing trophic status, in accordance to the common pattern found in freshwaters (Cotner and Biddanda, 2002; Fouilland and Mostajir, 2010).

Bacterial production in Lake Mangueira averages 21% (range: 3-55%) of particulate primary production, which is slightly higher than the 16 % average found by Cole et al. (1988) in their cross-system review. The bacterial production does not seem to change with particulate primary production in Lake Mangueira, which indicates that the BP is not affected by low PPP in Lake Mangueira (Figure 3). This is in contrast with cross system analyses, where BP is much higher than PPP at low PPP (Fouilland and Mostajir, 2010). This indicates that BP is sustained by other sources of carbon at low PPP, a situation found in littoral zones of Lake Mangueira. In these zones the high macrophyte coverage is associated to low phytoplankton biomass (They et al., 2013a) and low PPP (They et al., *unpublished results*). This topic is discussed deeper in the subsection phytoplankton and macrophytes interaction. Moreover, the low ratio of BP:PPP (< 5%) is far below the median and interquartiles (30, 19-38) reported for 94 freshwater ecosystems reviewed by Fouilland and Mostajir (2010), which is another evidence that other sources of carbon sustain bacterial production at low PPP, which disrupts the coupling between phytoplankton and bacteria in this lake under oligotrophy.

In general the few data available on these lakes support the coupling between bacterial and phytoplankton biomass, but not between their production rates. This could be the result of a lack of or low synchronicity between bacterial and algal production rates, differently from what is commonly found in freshwaters (Fouilland and Mostajir, 2010).

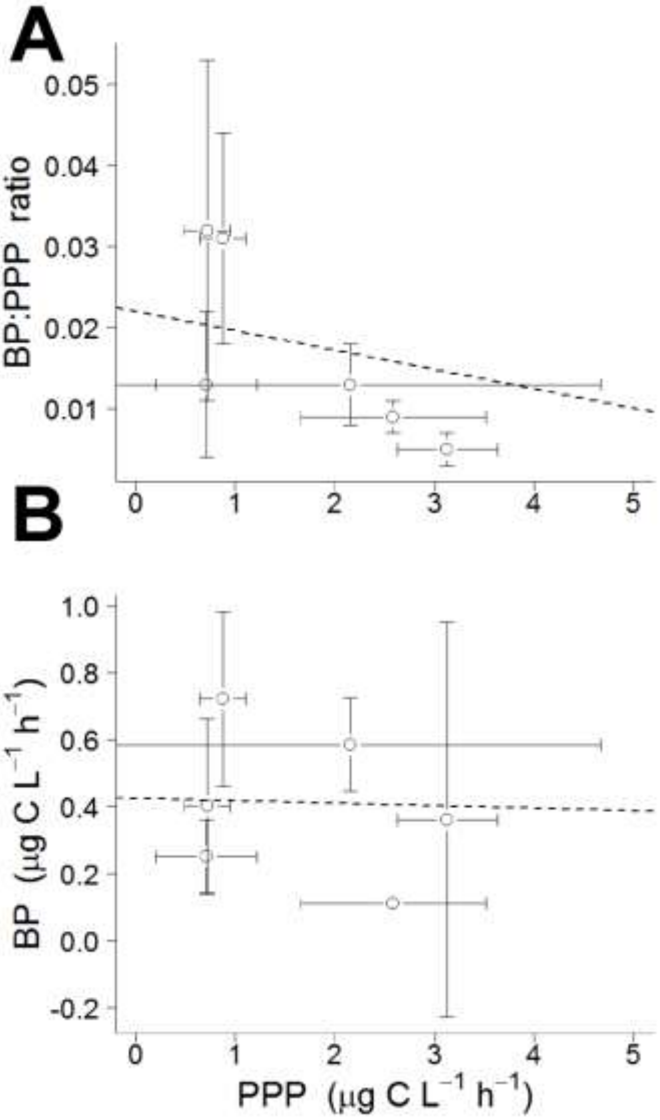

Figure 3. Relationship between A) bacterial production (BP) : particulate primary production (PPP) ratio and particulate primary production (PPP) and B) bacterial production (BP) and particulate primary production (PPP) along a seven points transect (3,275 m) ranging from the littoral to the pelagic zone of lake Mangueira, the largest coastal lake (They et al., unpublished results). At each point of the transect n = 3.

Competition for Nutrients and Trophic Gradients

The common ecological role attributed to heterotrophic bacteria is the decomposition and mineralization of nutrients. However, these bacteria uptake actively inorganic nutrients (Kirchman, 1994) to the point that they become strong competitors of phytoplankton under oligotrophy (Cotner and Biddanda, 2002).

As summarized by Cotner and Biddanda (2002), in less productive systems bacteria become strong competitors for nutrients because of their smaller size; consequently, the ratio bacteria:phytoplankton biomass is high. Conversely, in high productive systems, the high amount of inorganic nutrients favors phytoplankton over bacteria. The overall result is that the system goes from osmotrophy to phagotrophy with increasing trophic status, with a prevalence of organisms consuming particles and other organisms in more productive waters. Other factors also contribute to a diminished role of bacteria in eutrophic systems, like increasing viral lysis and bacterivory (Cotner and Biddanda, 2002).

Trophic gradients have a great impact on bacteria, both in community composition and its role within the trophic web. Gradients of primary productivity have been associated to changes in bacterial diversity (Horner-Devine et al., 2003) which can be partly explained by changes in the release of organic carbon from algae, since it is known that bacteria can develop completely different phylogenetic compositions when growing on different sources of carbon, even though deriving from the same inoculum (Covert and Moran, 2001). The favoring of other secondary producers with increasing trophic status has implications for the whole trophic web, since the bacterial production becomes relatively smaller within the total secondary production (Fouilland and Mostajir, 2010).

At the low end of the productivity gradient (more oligotrophic), there is a relatively higher phytoplankton extracellular carbon release (ECR), which has been associated to nutrient (scarcity) and light stress (supersaturating and low conditions) (Bjørnsen, 1988). This has been a paradox, since this situation favors bacterial competitors in oligotrophic environments, as pointed by Bjørnsen (1988). Even though relatively higher, the total phytoplankton primary production (particulate + dissolved) does not meet the BCD in low productive waters, suggesting that in these ecosystems other sources of labile carbon or even refractory DOC (Dissolved Organic Carbon) are used by bacteria (Fouilland and Mostajir, 2010). Whatever the carbon source used, the equations relating the log of the dissolved primary production vs the log of the particulate primary production in corrected and uncorrected bacterial DOC uptake during experiments revealed indirectly that bacteria remove more DOC in oligotrophic environments (Morán et al., 2002; Baines and Pace, 1991).

The extent to which bacteria rely on phytoplankton-derived carbon is variable. It has been pointed out that there is a high coupling between bacteria and phytoplankton when PPP rates cover all BCD, whereas a low coupling occurs when PPP rates only satisfy less than 10% of BCD (Fouilland and Mostajir, 2010). In low productive waters the primary production does not support entirely the bacterial carbon demand (Fouilland and Mostajir, 2010) and hence one would expect a higher decoupling between bacteria and phytoplankton.

In the subtropical shallow lakes, there is a tendency of more oligotrophic conditions in the littoral zones because of high assimilation of nutrients by macrophytes and inhibition of phytoplankton (see subsection phytoplankton and macrophytes), thus likely promoting higher competition of algae and bacteria for nutrients. This situation could also be expected in small lakes with high ratios of littoral:pelagic zones, which turn them functionally as if they had only littoral zones (completely covered by macrophytes).

More recently, the tight relationship between phytoplankton and bacteria has been revised. It has been argued that this apparently tight relationship may be a common response to extrinsic factors like nutrients (Fouilland and Mostajir, 2010). Decoupling between bacterial production and phytoplankton biomass increases has been associated to the stimulation of phosphorus addition (that alone seems to increase only bacterial production but not chlorophyll *a*) (Le et al., 1994; Zohary et al., 2005) and nitrogen limitation (Le et al., 1994). This have led Fouilland and Mostajir (2010) to the conclusion that a direct carbon based dependency of bacteria on phytoplankton is not supported by their literature survey, since bacteria are able to attain high biomass and activity even in low phytoplankton productive waters. This points out to their new aquatic ecology concept of non-dependency of bacteria on phytoplankton.

Phytoplankton and Macrophytes

Aquatic macrophytes are important elements in the structuring of subtropical shallow lakes (Meerhoff et al., 2003) and can be used as indicators of their ecological quality (Søndergaard et al., 2010). They change chemical and physical conditions of the water, generating mosaics of environmental conditions in small water bodies (Joniak et al., 2007), whereas in large water bodies they frequently induce littoral to pelagic gradients (Wetzel, 1992). These plants strongly impact the whole trophic web, but perhaps their greatest impact is on the maintenance of low phytoplankton biomass in association with macrophyte beds (Søndergaard and Moss, 1997). In general, several mechanisms are involved in their antagonistic relationship with phytoplankton, including shading (Scheffer and Jeppesen, 1997), strong competition for nutrients, especially phosphorus (van Donk et al., 1993; Huss and Wehr, 2004; Lürling et al., 2006) and release of allelopathic substances (Gross et al., 2007; Mulderij et al., 2007). These substances may include polyphenolic compounds with strong inhibitory activity against cyanobacteria, diatoms and chlorophytes (Gross et al., 1996), with overall reduction in phytoplankton biomass lying between 50-80% (Mulderij et al., 2007). The range of macrophyte coverage with detectable allelopathic effect is highly variable, ranging from 3% to 100% (Hill and Gross, 2008).

These described effects can be associated to the macrophyte typology, since the structural tissues and ecological traits of each class (submersed, emergent or floating) influence how they will impact the lake ecology (Søndergaard and Moss, 1997). Moreover, within each class, different species have been characterized by dissimilar physiology and chemical compositions, which would result in the release of different types of substrates (Hempel et al., 2009).

Submersed macrophytes are associated to shading or reduced light conditions, but at the same time reduction of sediment resuspension (Barko and James, 1998), with reduction of water turbidity. The chemical effects include reduction in the internal load of phosphorus (Søndergaard and Moss, 1997; Horppila and Nurminen, 2003) and release of allelopathic substances (Gross et al., 2007; Mulderij et al., 2007). All these mechanisms interact and are dependent on the percentage of underwater coverage (or percentage of volume infested, PVI[11]) (Søndergaard and Moss, 1997)[1], as gradients of PVI can largely explain the variation in phosphorus levels, phytoplankton biomass, respiration and planktonic bacterial production (Rooney and Kalff, 2003a).

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[1] Percentual of volume infested: measurement used for submersed aquatic macrophytes and estimated by multiplying the visual coverage of the plant(s) by its(their) length and dividing it by the local depth (Canfield, 1984).

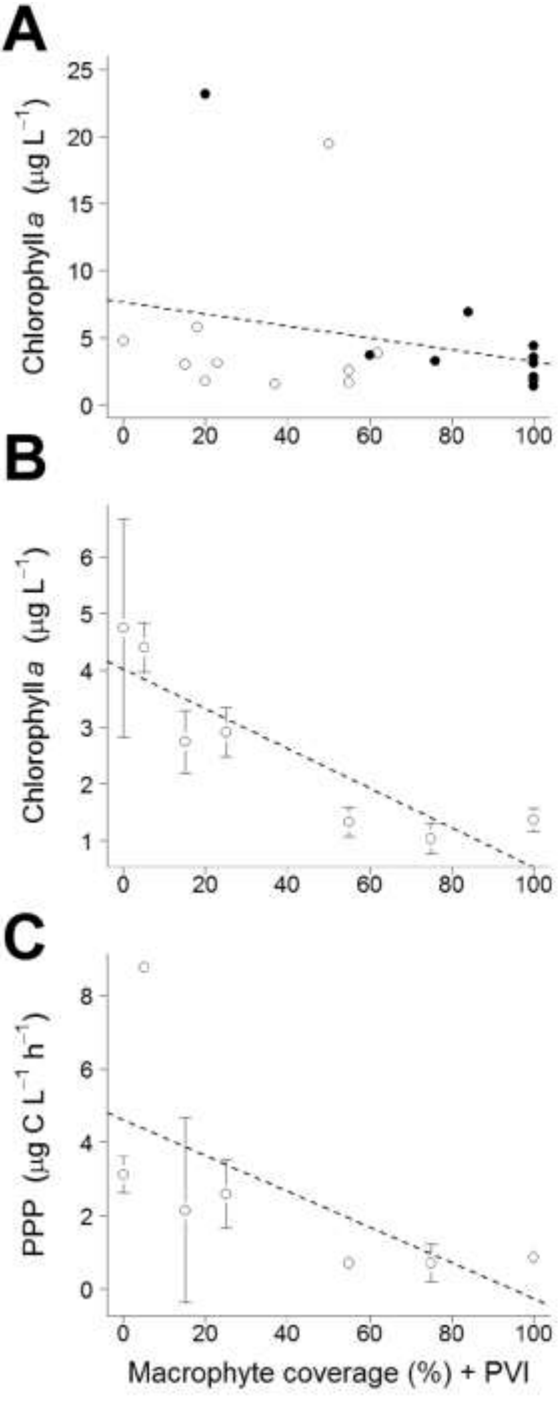

Figure 4. Relationship between A) chlorophyll a and macrophyte coverage plus percentual of volume infested (PVI) in 10 coastal subtropical shallow lakes (They et al., 2010) and B) along a seven points transect (3,275 m) ranging from the littoral to the pelagic zone of lake Mangueira, the largest coastal lake of the 10 lakes data set (They et al., unpublished results). C) Relationship between particulate primary production (PPP) and macrophyte coverage plus percentual of volume infested (PVI) along the Mangueira lake transect. For the lakes, the black dots refer to samples taken at littoral zones and white dots refer to samples taken at the pelagic zones. At each point of the transect n = 3.

Emergent macrophytes are usually rich in lignified and cellulosic tissues and their greatest impact usually is through the release of their decomposing material rich in polyphenolic compounds of difficult decomposition (Wetzel, 1992). They also regulate changes across the aquatic and terrestrial ecosystems (Mitsch and Gosselink, 1993) and their presence causes direct effects on biotic communities (Jeppesen et al., 1998).

Floating macrophytes, at their turn, also contribute with refractory substances (Wetzel, 1992), but their greatest impact is on shading. When they reach extensive coverage, they can lead to anoxic conditions, accumulation of nutrients and a shift in the algal community to myxotrophic species, cyanobacteria and diatoms with low light requirements (Izaguirre et al., 2004).

In Lake Mangueira, the high coverage and metabolism of submerged macrophytes influence the water chemistry and nutrient regime, affecting negatively the phytoplankton community, with significantly phytoplankton biomass reduction and assemblage structure variation in response to the macrophyte presence (Ferreira, 2009). In all the ten lakes this can be visualized by the negative impact of the macrophyte coverage on the chlorophyll *a* levels (not significant for the ten lakes: $r = -0.26$, P-value = 0.254; significant for the Mangueira lake transect: $r = -0.89$, P-value: 0.008) (Figure 4 A-B). This negative relationship was also found for particulate primary production (marginally significant for Lake Mangueira transect: $r = -0.65$, P-value = 0.114).

The water level fluctuation observed in shallow systems influences the presence and distribution of macrophytes (Motta Marques et al., 2002; Wagner and Falter, 2002; Beklioglu et al., 2006; Engelhardt, 2006; Beklioglu et al., 2007; Leira and Cantonati, 2008; Lawniczak et al., 2010). Moreover, because of the intense wind driven hydrodynamic regimes, the macrophyte coverage have stronger effect on the dynamics and structure of phytoplankton community, since these two aspects can drive the spatial heterogeneity of biotic components (Cardoso and Motta Marques, 2003; Crossetti et al., 2007, Cardoso and Motta Marques, 2009; Fragoso Jr. et al., 2008).

The effects of macrophytes on trophic dynamics and water transparency are related to the occurrence of alternative states in shallow temperate lakes (Scheffer et al., 1993). According to the nutrient levels, lakes may alternate between two states, a clear and a turbid water state. The clear water lakes are characterized by high PVI of submerged macrophytes and low chlorophyll *a* during summer. By contrast, turbid lakes present absence of submerged macrophytes and high levels of chlorophyll *a*, especially high during summer (Jeppesen et al., 1999). The macrophytes stabilize the clear water state (Jeppesen et al., 1998), whereas planktibenthivorous fish seem to stabilize the turbid state (through the predation of algae-feeding zooplankton). In the first case, the mechanisms of submerged macrophytes in maintaining water clarity are attributed to supression of phytoplankton growth by removal of nutrients from the water by assimilation (Lürling et al., 2006) or enhanced denitrification (Weisner et al., 1994) and release of allelopathic substances (Gross, 2003; Gross et al., 2007). Other indirect effects include higher grazing pressure on phytoplankton inside than outside the macrophyte beds, and an additional consequence of macrophyte presence for decreasing phytoplankton biomass is that this effect seems to extend beyond the border of the macrophyte beds (Jürgens and Jeppesen, 1997; Jeppesen et al., 1998).

Changes in density and coverage of macrophytes associated with predation pressure could lead to a positive change in the biological structure and water quality in shallow eutrophic lakes (Schriver et al., 1995), strongly affecting their species richness (Kruk et al., 2009). If at one hand the type of macrophyte, e.g, the submersed macrophyte, can affect phytoplankton biomass and community structure increasing predation, on the other hand macrophytes also affect zooplankton by reducing their growth or modifying their life strategies (Wetzel and Søndergaard, 1997; Burks et al., 2000). The reduction in the growth of zooplankton and bacterioplankton may be indirect, due to an allelopathic effect exerted by macrophytes impacting directly the phytoplankton and epiphytic algae (Körner and Nicklisch, 2002; Mulderij et al., 2005; Hilt et al., 2006; Cerbin et al., 2007; Gross et al., 2007; Hilt and Gross, 2008).

Effects of Macrophytes on Phytoplankton and Bacteria Interactions

The presence of macrophytes is a conspicuous feature of subtropical shallow lakes. These plants thrive favored by relatively shallow mean depths (< 2 m) and benign climate conditions, colonizing extensive areas with continuous growth throughout the year. Because of their high primary production, macrophytes usually contribute with large amounts of organic matter to the lake, surpassing in many cases the algal production (Wetzel, 1992; Lauster et al., 2006). In some lakes, they may sustain a large part of the bacterial production (Stanley et al., 2003).

Since macrophytes affect both phytoplankton (see subsection phytoplankton and macrophytes) and also bacteria (They et al., 2013a; They et al., 2013b) in these subtropical shallow lakes, it is expected that they will affect the interaction between phytoplankton and bacteria. In fact, the increase in the macrophyte coverage (emergent + floating + submersed) is positively associated to the ratio between bacterial and phytoplankton biomass in the ten lakes (Figure 5A) ($r = 0.45$, P-value = 0.049) and in the lake Mangueira transect (Figure 5B) ($r = 0.77$, P-value = 0.041). This means that with the increase in macrophyte presence, there is higher bacterial biomass in comparison with phytoplankton biomass, which is likely derived from the strong negative impact macrophytes have on phytoplankton biomass, as discussed in the previous subsection. The ratio between bacterial production to particulate primary production is also positively affected along the Mangueira lake transect (Figure 05C) ($r = 0.69$, P-value = 0.128). These results point out that the difference in biomass is impacted from the differences in production rates, but the effects of grazing still need to be assessed.

At sites with high macrophyte influence and low phytoplankton biomass and production, the bacterial production is likely to be sustained by senescent parts or exudates from macrophytes (Wetzel and Søndergaard, 1997; Rooney and Kalff, 2003b). This shift in carbon source type plays an important role in the patchiness of abundance (Huss and Wehr, 2004) and composition of bacterioplankton communities (Wu et al., 2007). In a study performed in mesocosms using different macrophyte biomass levels of *Potamogetum illinoensis*, the bacterial density and biomass were not correlated with chlorophyll *a* concentration, but to carbon availability and macrophyte biomass. Hence, the DOC concentration utilized by bacteria probably originated from both *P. illinoensis* and its peryphytic community, since submerged macrophytes represent an important source of organic carbon, favoring the macrophyte-periphyton complex (Canterle, 2011).

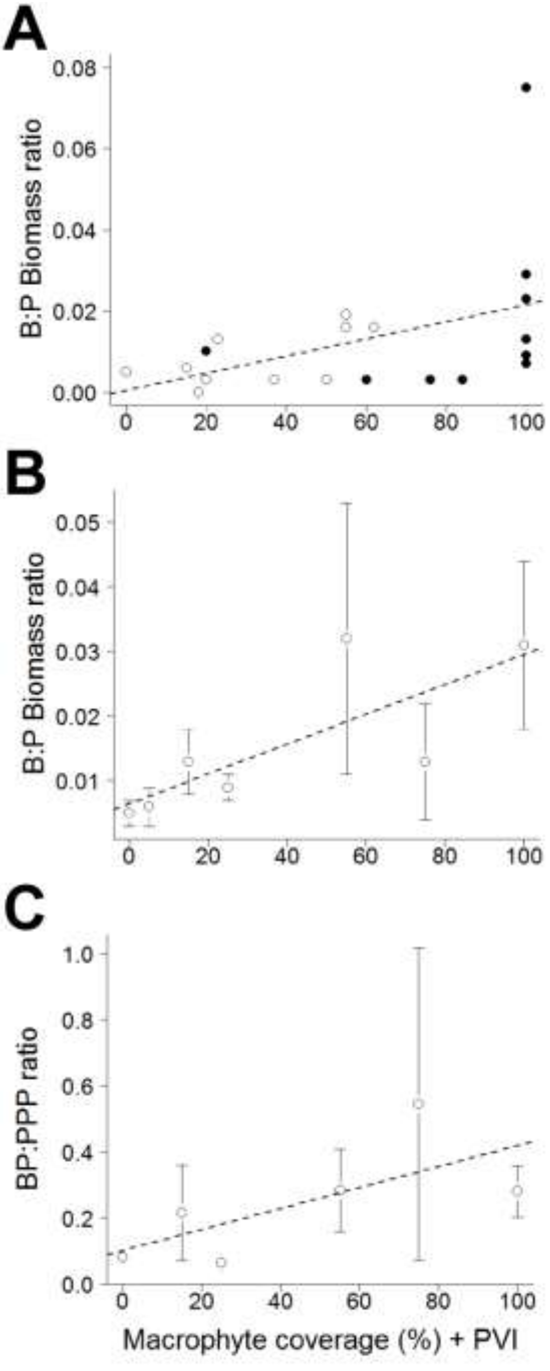

Figure 5. Relationship between bacterial:phytoplankton (P) biomass ratio and macrophyte coverage plus percentual of volume infested (PVI) in A) 10 coastal subtropical shallow lakes (They et al., 2010) and B) along a seven points transect (3,275 m) ranging from the littoral to the pelagic zone of lake Mangueira, the largest coastal lake of the 10 lakes data set (They et al., unpublished results). C) Relationship between bacterial production (BP):particulate primary production (PPP) ratio and macrophyte coverage plus percentual of volume infested (PVI) along the Mangueira lake transect. For the lakes, the black dots refer to samples taken at littoral zones and white dots refer to samples taken at the pelagic zones. At each point of the transect n = 3.

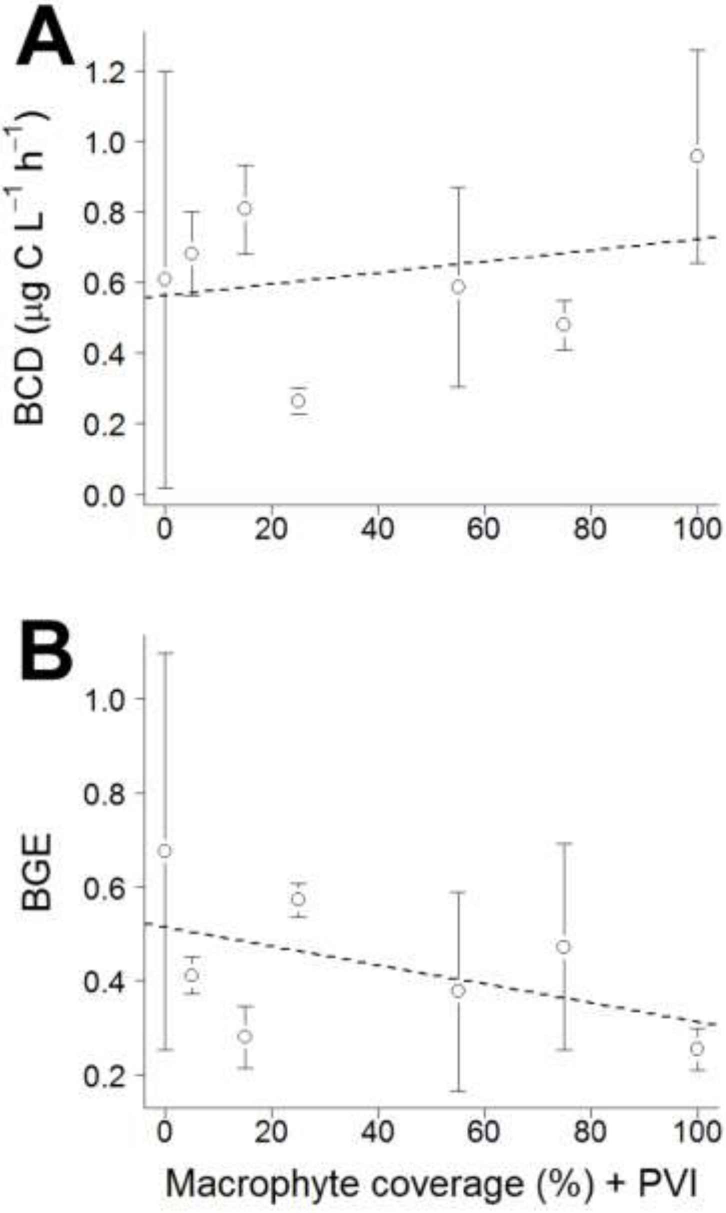

Figure 6. Relationship between A) bacterial carbon demand (BCD) and B) bacterial growth efficiency (BGE) and macrophyte coverage plus percentual of volume infested (PVI) along a seven points transect (3,275 m) ranging from the littoral to the pelagic zone of lake Mangueira, the largest coastal lake of the 10 lakes data set (They et al., unpublished results). At each point of the transect n = 3.

Bacterial activity is affected by the increase in macrophyte coverage. Even though the bacterial carbon demand (BCD) (r = 0.27, P-value = 0.558) did not change, the bacterial growth efficiency (BGE) tends to decrease (but not significantly) (r = -0.50, P-value = 0.253) with the increase in macrophyte coverage (Figure 6). This is suggestive that, as the macrophyte presence increases, the bacteria shifts their source of carbon to macrophyte's derivatives, but this change causes the BGE to decrease because this carbon tends to be more refractory (Bracchini et al., 2006). This low-availability of carbon derived from macrophytes is one possible explanation for the low bacterial respiration detected in littoral zones of Lake Mangueira when compared to pelagic zones (They et al., 2013a). This has consequences for the interaction between phytoplankton and bacteria, since low rates of respiration suggests lower rates of mineralization and a negative feedback in the supply of inorganic nutrients to phytoplankton. Another consequence is a decoupling between bacterial and primary production, which is expected in systems with high supply of allochthonous organic matter (Morán et al. 2002). At last, at higher bacterial: phytoplankton biomass ratio, bacteria are in competitive advantage in taking up inorganic nutrients (Cotner and Biddanda, 2002). This last effect is amplified by the high competitive advantage of the macrophytes in the absorption of inroganic nutrients (Lobão, 2009; They et al., 2010, Canterle, 2011) All these cases are expected to occur in the smaller lakes entirely colonized by macrophytes or in the littoral zones of larger lakes.

Part of the heterogeneity in bacterial metabolism and diversity may be due to indirect effects of macrophytes like reduced wind-driven resuspension of sediment particles into the water column in macrophyte-absent areas (Simon et al., 2002) or by direct effect of macrophyte communities on phytoplankton composition with secondary, cascading effects on bacterioplankton (Lindström, 2001; Eiler and Bertilsson, 2004). Another factor to be considered is the possible allelopathic effect exerted by macrophytes on bacterial abundance similarly to that exerted on other planktonic components (Körner and Nicklisch, 2002; Hilt et al., 2006; Gross et al., 2007; Hilt and Gross, 2008). This effect was demonstrated in a series of experiments conducted by Canterle (2011), in which the presence of the macrophytes *Zizaniopsis bonariensis*, *Schoenoplectus californicus* and *Myriophyllum* sp., a submersed macrophyte with recognized allelopathic effects (Leu et al., 2002, Hilt et al., 2006, Cerbin et al., 2007, Hilt and Gross, 2008), led to low concentrations of chlorophyll *a* and low bacterial density in mesocosms in comparison with controls without macrophytes.

Phytoplankton Ecological Interactions and Subtropical Coastal Lakes Trophic Webs

All freshwater lentic ecosystems (e.g., lakes, lagoons, ponds, wetlands) are home to various life forms, often collectively referred to as food webs. In these systems, many sources of energy and matter can be recognized, like phytoplankton, biofilms (periphyton), macrophytes, suspended particulate matter (SPM) and sediment organic matter (Lamberti and Moore 1984; Frost et al., 2002). In many cases, though, phytoplankton is not the base of the food web and hence understanding the ecological interactions that affect phytoplankton is key to understand the whole ecosystem functioning. In the Mangueira Lake food web, primary consumers reflect autochthonous production, mainly from periphyton and macrophytes (Garcia et al., 2006; Rodrigues et al., 2013). The periphyton (biofilm), which covers a wide range of submerged surfaces, is a key factor for the Mangueira lake food web (Rodrigues et al., 2012; 2013), since it plays a fundamental role in nutrient cycling and storage (Azim and Wahab, 2005), and freshwater fish can use it as a food source in either direct or indirect pathways (Petr, 2000).

Several studies have dealt with isolated aspects of the interaction between phytoplankton and other ecosystem components in the subtropical shallow lakes in southern Brazil. This approach, however, provides a limited picture of the functioning of these systems as it does not take into account how all components (particularly strong drivers like wind and macrophytes) interact and how this interaction shape food webs. This is not an exclusive issue of these lakes, however. While freshwater ecology has often included landscape factors, food webs in freshwaters have been approached mostly at small spatial scales. Studies that carried out experimental approaches or utilized natural gradients remain rare, but will be vital to untangle causal relationships between changing environmental conditions and food-web structure and dynamics (Thompson et al., 2012).

In this context, four key components are clearly distinguished in terms of functioning and control of food webs in these lakes: hydrodynamics, macrophytes, zooplankton and fish.

Hydrodynamics

Hydrodynamic processes and biological changes occur over different spatial and temporal scales in lakes Mangueira and Itapeva. In Itapeva Lake, the hydrodynamic response to wind is faster because of its smaller size (1/3) when compared to lake Mangueira and on a time scale of hours, we can see the water movement from one end of the lake to the other (e.g., from N to S during a NE wind and in the opposite direction during a SW wind). Because of this rapid response, the plankton communities show correspondingly rapid changes in composition and abundance, especially phytoplankton when the resources (luminosity and nutrients) shift in response to wind action (Cardoso et al., 2012). This interaction between wind on a daily scale (hours) and the elongated shape of Itapeva Lake is a determining factor for the observed changes of phytoplankton assemblages composition (Cardoso and Motta Marques, 2003) and spatial distribution of plankton communities (Cardoso and Motta Marques, 2004a, 2004b, 2004c). The rates of change in phytoplankton are very high mainly in spring, when the environmental changes are most intense and rapid. Marked changes in the spatial and temporal gradients of the plankton communities occur along the seasons of the year also as a response to resuspension events induced by wind (Cardoso and Motta Marques, 2009), which are more intense at sites where the fetch is longest. As a result, population replacements in the plankton communities are observed: the resuspension renders diatoms and protists dominant in the system, whereas in calmer waters these organisms are replaced by cyanobacteria and rotifers (Cardoso and Motta Marques, 2003, 2004a, 2004b). Thus, diatoms and protists and eventually benthonic species are indicators of lake hydrodynamics because of their fast responses in their spatial distribution.

The lake Mangueira is also marked by a longitudinal gradient, with spatial heterogeneity and food web driven by environmental variables, especially hydrodynamics. Predominant winds from northeast to southwest can move surface water trough southern Mangueira Lake and provide a lot of energy to suspend fine sediments, as a result of a fetch that can reach 90 km. At the same time, fine sediments and associated nutrients are transported to north by bottom conter-currents, resulting in a southern sandy bottom, clear water, and lower chlorophyll *a* concentration, with consequences to the whole trophic cascade.

Macrophytes

Environments with tendency to oligotrophy like these coastal lakes in southern Brazil (particularly Mangueira Lake) are of special interest for the investigation of food sources and survival strategies. In these lakes, there is a high degree of patchiness of phytoplankton which is related to the presence of macrophytes in littoral zones (Fragoso Jr. et al., 2008). In fact, phytoplankton distribution and abundance in shallow lakes without macrophytes are different from those in lakes with macrophytes (Moss, 1990; Jasser, 1995) and the phytoplankton response depends on the periodicity and extent of macrophyte dominance (O'Farrell et al., 2009). Macrophyte dominance in shallow oxbow lakes, for example, significantly influences phytoplankton species structure but does not automatically result in clear water (Krasznai et al., 2010).

The different macrophyte ecotypes (floating, emergent or submersed) can affect distinctly the interaction between different trophic levels, for example, the composition of bacterial communities in shallow lakes (Canterle, 2011; Zeng et al., 2012). The presence of floating plants can strongly affect the trophic web through direct and indirect effects on different communities such as plankton, invertebrates and fish that inhabit both littoral and pelagic zones (Meerhoff and Mazzeo, 2004). Submersed macrophytes have an impact on the pelagic food web and represents a potential source of substrates for bacteria, influencing ciliates and flagellates (Wetzel and Søndergaard, 1997).

Besides the direct effects on phytoplankton, macrophytes have an overall impact on the whole trophic web. Schriver et al. (1995) reported the influence of zooplankton on phytoplankton in dense macrophyte vegetation but noted the presence of other habitat structuring factors as well. Thus, the habitats created by macrophytes depend on their composition, coverage, growth form, and seasonal dynamics, with subsequent diverse responses of phytoplankton. These responses can even lead to neither strictly clear nor strictly turbid water states in shallow lakes (O'Farrell et al., 2009, Krasznai et al., 2010, Sayer et al., 2010).

In Mangueira Lake, Rodrigues (2009) analyzed limnological and biological data in two sampling points in the littoral zone (northern and southern parts) and found highest chlorophyll *a* concentration in the northern and highest DOC concentration in the southern part. Crossetti et al. (2013) also observed in the same lake that the phytoplankton biomass was highest in the northern part of Mangueira Lake and decreased towards south. Southern Mangueira presented larger biomass of submerged and free floating macrophytes, and this is one of the hypotheses for the higher DOC concentration at this site, along with inhibition of phytoplankton production.

Macrophytes may also secrete allelochemicals that will affect the trophic web through supression of the growth of phytoplankton, bacterioplankton (Mulderij et al., 2006; Hilt et al., 2006; Cerbin et al., 2007; Gross et al., 2007; Wu et al., 2007; Hilt and Gross, 2008), epiphytic algae (Hilt, 2006) and specific zooplankton grazers (Jürgens and Jeppesen, 1997; Cerbin et al., 2007). The allelopathic effects of macrophtes on trophic webs are only beginning to be understood and it is possible that they account for a significant part of variation of the carbon budget of these lakes.

Zooplankton

It is well know that zooplankton forms a central link in the food-web of freshwater in temperate, tropical and subtropical lakes. The efficiency of the food chain can be determined by the capacity of zooplankton to utilize phytoplankton production and its suitability as food for fish. But, in some cases, phytoplankton is not efficiently grazed by zooplankton and enters the detrital food chain directly (Zingel and Haberman, 2008).

Recent studies suggest that the biomass of zooplankton is lower in subtropical than in temperate lakes, particularly when considering phytoplankton biomass (Havens et al., 2007, 2009; Jeppesen et al., 2007), resulting in a much lower zooplankton biomass and zooplankton : phytoplankton biomass ratio (Meerhoff et al., 2007b; Teixeira-de-Melo et al., 2009). Besides this, the refuge capacity of aquatic plants for zooplankton in subtropical systems seems weak, with consequent weak or no cascading effects on water transparency (Iglesias et al., 2007). Moreover, there is little knowledge about grazing inside and outside macrophyte beds in subtropical lakes, in which these plants are a poor refuge for zooplankton because of high fish density within the stands (Meerhof et al., 2007a,b).

Since 80’s, several studies focusing on zooplankton in subtropical lakes from Florida USA were cited by Havens et al. (2011) concerning the composition of zooplankton and its relationship with trophic state, seasonal dynamics, responses to extreme climate events, grazing on phytoplankton and food web dynamics. These studies indicated that temperature may play a role in limiting the size and seasonality of crustacean zooplankton. However, less is known about microzooplankton (protists and rotifers) in subtropical shallow lakes (Cardoso and Motta Marques, 2004b, 2009) and the zooplankton-phytoplankton relationship.

In most studies only crustaceans and rotifers are focused as zooplankton. However, planktonic ciliates also have an important role in aquatic food webs not only in controlling bacteria (Beaver and Crisman, 1982), but also in grazing particles that can be ingested by crustacean and rotifer zooplankters (Sanders et al., 1989). There is clear evidence that planktonic ciliates are an important food resource for larger crustacean zooplankton (Gifford, 1991). It is now known that ciliates form an important trophic link between picoplankton and nanoplankton on one hand, and metazoans on the other (Zingel and Haberman, 2008).

Fish

It is well recognized that top predators can influence the whole food-web structure and biomass, down to the primary producers. Mechanisms of top-down effects, classically referred to as trophic cascades, have received much attention during the last two decades and community-wide effects have mainly been recognized for aquatic temperate ecosystems.

Other important aspect related with phytoplankton distribution and abundance in shallow lakes is the abundance and composition of piscivorous, plankti-benthivorous and omnivorous fish in system/region. Fish play a key role in the trophic web and functioning of shallow lakes. Due to their mobility and flexible feeding behaviour, they provide important linkages between littoral, benthic and pelagic zones (Vander Zanden and Vadeboncoeur, 2002). Fish may affect nutrient translocation and predator–prey interactions in all habitats, as many species depend on both benthic and littoral prey communities (Schindler and Scheuerell, 2002). Via their predation on zooplankton grazers, planktivorous fish may promote trophic cascading effects, leading to increased water turbidity and phytoplankton biomass in the pelagic zone (Carpenter and Kitchell, 1993). In addition, fish may affect water turbidity by trophic cascading effects occurring in the littoral zone.

In particular, the reduction of zooplanktivorous and omnivorous fish often lead to decrease in phytoplankton total biomass. This occurs indirectly through the alleviation of the predation pressure on herbivorous zooplankton. Those effects are either due to a reduction of zooplankton biomass (Carpenter and Kitchell, 1993; Brett and Goldman, 1996; Bertolo et al., 2000) or to an alteration of the specific composition of zooplankton communities without any change in zooplankton biomass (Bertolo et al., 1999; Okun et al., 2008; Danger et al., 2009).

Meerhoff et al. (2007b) have found that littoral fish communities differed markedly in subtropical lakes, where community structure and behavior exerted a potentially stronger impact on littoral trophic dynamics than in temperate lakes. Fish communities in warmer climates are characterized by a higher share of omnivorous species (Winemiller, 1990; Branco et al., 1997) and multiple or frequent reproduction. Some key differences in the structure of the fish community between temperate and warm shallow lakes have been summarized by Lazzaro (1997) and Jeppesen et al. (2005). High abundance, continuous or frequent reproduction (Mazzeo et al., 2003), widespread omnivory (Winemiller, 1990), and low abundances of strictly piscivorous fish (Quirós, 1998) seem common features in warm lakes in different regions of the world. Also, large invertebrate omnivores, such as shrimps, are abundant in the subtropics. Fish are considered the main determinants in community structure in shallow lakes, through, among other mechanisms, their positive cascading effects over periphyton via invertebrates (Jones and Sayer, 2003).

Rodrigues (2009) identified important differences in fish communities (functional feeding guilds) between the extremes of Mangueira Lake. Mainly invertebrate eaters were the dominant feeding guild in both Northern and Southern sampling sites in Mangueira Lake. North/South frequencies of omnivorous fish were quite similar in the 19-21% range, while planktivorous fish presented low captured biomass in both sites. However, it is interesting to note that the dominant main invertebrate eaters were different in both sides of lake. In the northern sampling site, *Oligosarcus jenynsii* (Characidae) was the dominant invertebrate eater, feeding mainly on crustaceans (*Aegla* sp. and *Palaemonetes argentinus*). In contrast, the dominant invertebrate eater of southern sampling site was *Odontesthes humensis* (Atherinopsidae), consuming mainly mollusks (Heleobia sp. and Corbicula sp.). It was also observed an inversion related to frequencies of detritivorous and mainly fish eaters. Muddy sediments in the Northern site may explain the relative abundance of detritivorous (28%), whereas sandy sediments and clear waters could be related to increased frequency of mainly fish eaters in the South, supposing easier prey detection.

Ecological Modelling

In this subsection we discuss general aspects of ecological modelling and present some examples of its utilization in Lake Mangueira. These models have been developed by the Ecotechnology and Applied Limnology research group from the Federal University of Rio Grande do Sul (Institute of Hydraulic Research) and have allowed the analysis of complex ecological interactions, mainly involving phytoplankton directly.

The shared management of natural resources, based on specific knowledge, is the best form of promoting environmental conservation. In aquatic ecosystems, the conservation of water resources depends on specific understanding and management of limnological variables. However, the wide range of physical, chemical and biological processes and factors, and their interactions, makes the quantitative analysis of the aquatic ecosystems very difficult. In addition, management of aquatic ecosystems is, by their characteristics, a field of multidisciplinary action, where there are a great number of alternatives on planning and forecasting, considering their uses, availability and preservation (Tucci, 1998). Thus, the diversity of methodological approaches available to quantify processes are essential to acquire higher understanding of the dynamics in natural systems and promoting tools for accurate decision making. One of these approaches is the mathematical ecological modelling applied to limnology.

Models focused on ecological themes are *thinking pad* at the limnologist’s disposal. It allows formulating questions and obtaining answers considering the pre-established outline conditions. It also allows formulating and testing hypotheses, as well as developing, consolidating or refuting theories.

A model is the representation of some object or system in a language easily accessible and usable, with the means of understanding and searching for answers according to different inputs. In order to better represent reality, a model must simulate the highest possible number of processes that occur in nature. The larger the number of interactions involved, the more complex the systems and consequently the more challenging and necessary are the models. In limnology, a model is a tool developed in order to assist the understanding of an aquatic ecosystem, considering the involved (direct or indirect) drivers, and in different scales, including the anthropic (e.g., social, economical) and natural (e.g., climatic, chemical, biological, hydrographic basin, hydrology, hydrodynamic) factors, and their interactions and processes. Ecological models can be applied on limnology, e.g., for: (a) evaluation responses of trophic cascading interactions before changes in nutrient concentrations (Janse, 2005; Jeppesen et al., 2002; Jakobsen et al., 2004); (b) prediction of phytoplankton blooming (Fragoso Jr et al., 2008, Lucas et al. 1999a, 1999b); (c) determination of the trophic state in aquatic ecosystems (Kishia et al., 2007); (d) investigation of ecological concepts in limnology such as the theory of alternative stable states (Van Nes et al., 1999; Fragoso Jr. et al., 2007); (e) assesment of biomanipulation impacts (e.g., fishing or alteration of trophic interactions) on the system (Carpenter & Kitchell, 1993; Hansson et al., 1998; Meijer et al., 1994); (f) estimative of gross primary production, community respiration and net production of the ecosystem in terms of carbon (Mukherjee, et al., 2002; Sandberg et al., 1998); (g) evaluation of pollution levels.

A model must not be considered as an objective, but as a tool for reaching a determined goal. It may be utilized with the purpose of prediction, understanding processes, filling the variables of interest within a period without survey and generating hypotheses, which may be tested experimentally or *in situ*. It is worthy to emphasize that the modelling should be utilized in partnership with experimental, laboratorial and monitoring works, otherwise its potentiality of application may not be fulfilled.

The application of models for scientific questions is almost compulsory if we want to understand a complex system as an aquatic ecosystem. It is not simple to investigate all the components and their interactions in the ecosystem without using models as a synthesis tool. The use of modelling has advantages and has been revealing gaps in our knowledge. Perhaps the main contribution provided by a model may be the establishment of research priorities, which may reveal properties of the system from scientific hypotheses generated by the model itself. Therefore, the models that simulate interactions in the aquatic ecosystem not only generate results that may be compared with in situ or experimental observations, but also may serve as a thinking base for important scientific questions.

The first and simplest modelling approaches were steady-state models that were often empirical (Schindler, 1975; Canfield and Bachmann, 1981; Mueller, 1982). In these models, chlorophyll a concentration was related to nutrient concentrations in the ecosystem. Ecological water quality models gained strength when they first addressed both biological and chemical factors dynamically (Duckstein et al., 1979; Thoman and Segna, 1980; Jørgensen, 1983). The next step was to incorporate 1D and 2D dimensional hydrodynamic processes like advection and diffusion (Imberger et al., 1978; Somlyody, 1982; Orlob, 1983; Hamilton and Schladow, 1997). This more realistic representation broadened our knowledge of cascading trophic interactions in relation to spatial heterogeneity. Pushed by computation advances, recent ecological approaches were able to represent the entire food-web dynamics coupled with a three-dimensional hydrodynamic model (Skogen et al., 1995; Delhez, 1998; Robson and Hamilton, 2004; Romero et al., 2004; Schrum et al., 2006).

Most complex dynamic aquatic ecosystem models were developed in order to describe the dynamics of marine or ecosystems that were located in temperate zones. Currently such models are still lacking for subtropical ecosystems that have a milder seasonal cycle. Subtropical aquatic environments differ in many aspects from temperate ecosystems, for instance (a) no seasonal die-off of submerged vegetation; (b) more complex food webs with a large number of omnivores; (c) higher diversity of organisms and macrophytes and (d) lack of large zooplankton which produce a bottleneck on top-down energy flux.

In Mangueira Lake, the application of ecological models has helped to elucidate several questions concerning spatial patterns, potential drivers of changes in alternative states and potential effects of climate change:

Fragoso Jr. et al. (2008) identified patchiness in chlorophyll *a* distribution in littoral and pelagic zones and in nothern and southern parts of the lake. Also, it indicated that phytoplankton growth in summer-autumn is not limited by nutrients and light, but possibly by grazing, temperature and respiration;

Ferreira et al. (2008) simulated the impact of drastic reductions in water level. They found that these events could lead to massive mortality of macrophytes and liberation of large amounts of nutrients (PO_4^{-3}) that would result in a change of a clear to a turbid state (threshold of 0.05 mg L^{-1} of PO_4^{-3}). This state could be resultant of either blooming of cyanobacteria or floating macrophytes;

Fragoso Jr. et al. (2010) have found out that effects of increasing light attenuation coefficients are able to induce a change to a turbid state (coefficient of light attenuation > 1.1 m^{-1}), with a collapse of the submersed macrophyte community and dominance of phytoplankton under a chaotic regime (no seasonal cycles);

Fragoso Jr. et al. (2011) simulated scenarios of climate change and its effects on nutrients and chlorophyll *a*. The results revealed a high resilience of the system at a short-term (1.5 year) and a tendency of increasing water transparency with warming. The higher temperatures were associated to increased rates of mineralization in spring, advances in phytoplankton growth and positive effect of water transparency in summer. Other important factors involved in high transparency were the meso-oligotrophic state of the lake (with tendency to nutrient limitation in summer) and the continuous growth of macrophytes, which uptake nutrients constantly. These studies were carried out with the latest version of the model IPH-TRIM3D-PCLAKE and previous, less complex versions. In the next subsection, the latest version is presented in greater detail.

Table 2. State variables in IPH-TRIM3D-PCLake. Abbreviations: s- = state variable. Adapted from Janse (2005)

Description	Unit	on water	Dry weight (DW)	Phosphorus (P)	Nitrogen (N)	Silicate (Si)	Oxygen (O_2)
Hydrodynamic components							
Water depth	[m]	sDepthW	-	-	-	-	-
Water velocity	[m s^{-1}]	sVelW	-	-	-	-	-
Abiotic components in water column							
Inorganic matter	[g m^{-3}]		sDIMW	-	-	-	-
Detritus	[g m^{-3}]		sDDetW	sPDetW	sNDetW	sSiDetW	-
Inorganic nutrients	[g m^{-3}]		-	sPO4W/SPAIMW	sNH4W/sNO3W	sSiO2W	-
Oxygen	[g m^{-3}]		-	-	-	-	sO2W
Abiotic components in sediment							
Inorganic matter	[g m^{-2}]		sDIMS	-	-	-	-
Humus	[g m^{-2}]		sDHumS	sPHumS	sNHumS	-	-
Detritus	[g m^{-2}]		sDDetS	sPDetS	sNDetS	sSiDetS	-
Inorganic nutrients	[g m^{-2}]		-	sPO4S/SPAIMS	sNH4S/sNO3S	sSiO2S	-
Phytoplankton in water column[a]							
Diatoms	[g m^{-3}]		sDDiatW	sPDiatW	sNDiatW	(oSiDiatW)	-
Green algae	[g m^{-3}]		sDGrenW	sPGrenW	sNGrenW	-	-
Cyanobacterias	[g m^{-3}]		sDBlueW	sPBlueW	sNBlueW	-	-
Settled phytoplankton:							
Diatoms	[g m^{-2}]		sDDiatS	sPDiatS	sNDiatS	(aSiDiatS)	-
Green algae	[g m^{-2}]		sDGrenS	sPGrenS	sNGrenS	-	-
Cyanobacterias	[g m^{-2}]		sDBlueS	sPBlueS	sNBlueS	-	-
Vegetation[b]	[g m^{-2}]		sDVeg	sPVeg	sNVeg	-	-
Animal groups[c]							
Zooplankton	[g m^{-2}]		sDZoo	sPZoo	sNZoo	-	-
Zoobenthos	[g m^{-2}]		sDBent	sPBent	sNBent	-	-
Juvenile omnivorous	[g m^{-2}]		sDOniJv	sPOniJv	sNOniJv	-	-
Adult omnivorous	[g m^{-2}]		sDOniAd	sPOniAd	sNOniAd	-	-
Juvenile planktivorous	[g m^{-2}]		sDPlankJv	sPPlankJv	sNPlankJv	-	-
Adult planktivorous	[g m^{-2}]		sDPlankAd	sPPlankAd	sNPlankAd	-	-
Piscivorous	[g m^{-2}]		sDPisc	sDPisc	sDPisc	-	-

[a] Phytoplankton can be lumped into one group, sDPhyt.

[b] Several groups of macrophytes may be defined, such as non-rooted (sDCera), rooted (sDElod), floating leaved plants (sDNymp) and emergents (sDHelo).[c] Aquatic animals, optionally, can be left out.

IPH-TRIM3D-PCLAKE

IPH-TRIM3D-PCLake is a three-dimensional complex dynamic model (Fragoso Jr. et al., 2009) which allows assessing cascading trophic effects in subtropical aquatic ecosystems. Here we present the mathematical structure of this model and its graphical user interface. IPH-TRIM3D-PCLake (also called IPH-ECO) is a complex dynamical model for aquatic ecosystems (available to download at https://sites.google.com/site/iphecomodel/).

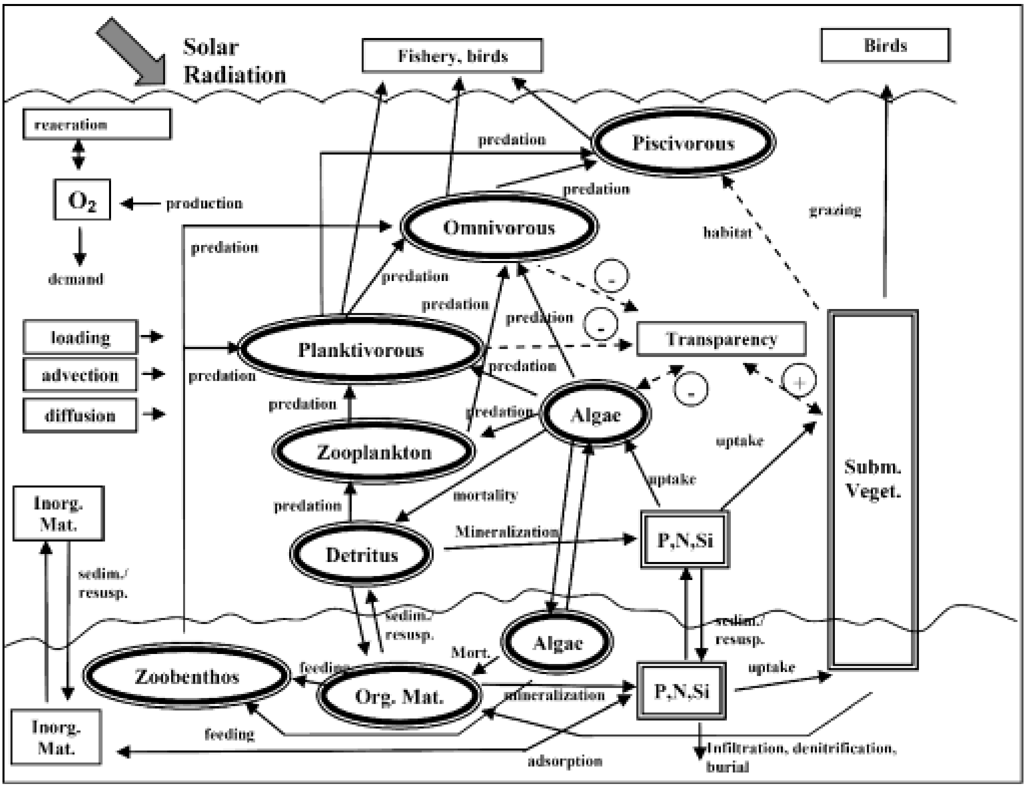

Figure 7. Simplified scheme of the model structure utilized in the Lake Engelsholm. The double blocks are modeled through, at least, three compartments: dry weight and nutrients (P, N and Si). Three functional groups of phytoplankton were defined as cyanobacteria, diatom and green algae. The planktivorous fishes have two life stages: juvenile and adult. The solid arrows represent mass fluxes and hatched arrows indicate empirical relations for indirect effects (minus signal would be a negative influence in water transparency, otherwise positive) (Adapted from Janse et al., 1998).

This model consists of a three-dimensional hydrodynamic module, TRIM3D, coupled with the ecological model PCLake (i.e., chemical kinetics coupled with trophic interactions) (Janse, 1997; Janse, 2005). IPH-TRIM3D-PCLake differs algorithmically from PCLake in the treatment of: (a) horizontal spatial heterogeneity into aquatic system at cell level (e.g., lake and wetland); (b) stratification over water column for several states variables (e.g., temperature, water density, P, N, Si, phytoplankton and zooplankton); (c) more functional groups of fishes, including omnivores. In summary, the model describes the most important hydrodynamic, biotic and abiotic components in the water and sediment of an aquatic ecosystem (see Table 2 and Figure 7). The model was implemented in Visual FORTRAN which is considered one of the most popular languages in the area of high-performance computing. Below we will give a short overview of the model structure, but for a complete mathematical description of the water quality model we refer to Janse (2005).

Hydrodynamic Module

The three-dimensional hydrodynamic equations are derived from Navier-Stokes equations, which describe a three-dimensional free-surface flow and dynamical under the simplifying assumption that the pressure is hydrostatic. Such equations reproduce the physical principle of conservation of volume, mass and momentum. The momentum equations have the following form:

$$\frac{\partial u}{\partial t}+u\frac{\partial u}{\partial x}+v\frac{\partial u}{\partial y}+w\frac{\partial w}{\partial z}=-\frac{1}{\rho_0}\frac{\partial p}{\partial x}+\mu\nabla^2 u+\frac{\partial}{\partial z}\left(\upsilon\frac{\partial u}{\partial z}\right)+fv \tag{1}$$

$$\frac{\partial v}{\partial t}+u\frac{\partial v}{\partial x}+v\frac{\partial v}{\partial y}+w\frac{\partial v}{\partial z}=-\frac{1}{\rho_0}\frac{\partial p}{\partial y}+\mu\nabla^2 v+\frac{\partial}{\partial z}\left(\upsilon\frac{\partial v}{\partial z}\right)-fu \tag{2}$$

$$\frac{\partial w}{\partial t}+u\frac{\partial w}{\partial x}+v\frac{\partial w}{\partial y}+w\frac{\partial w}{\partial z}=-\frac{1}{\rho_0}\frac{\partial p}{\partial z}+\mu\nabla^2 w+\frac{\partial}{\partial z}\left(\upsilon\frac{\partial w}{\partial z}\right)-\frac{\rho}{\rho_0}g \tag{3}$$

where $u(x,y,z,t)$, $v(x,y,z,t)$ and $w(x,y,z,t)$ are the water velocity components in the horizontal (x and y) and vertical (z) directions; t is time; $p(x,y,z,t)$ is the pressure; g is the gravitational acceleration; ρ and ρ_0 are the water density and the constant reference density respectively; f is the parameter of Coriolis; $\nabla=\partial/\partial x\cdot\vec{i}+\partial/\partial y\cdot\vec{j}$ is a vector operator in the plane x-y; μ and υ are the coefficient of horizontal and vertical eddy viscosity respectively;

The volume conservation can be expressed by incompressibility condition:

$$\frac{\partial u}{\partial x}+\frac{\partial v}{\partial y}+\frac{\partial w}{\partial z}=0 \tag{4}$$

Integrating the continuity equation (4) over depth and assuming a kinematic condition at the free-surface obtains to free surface equation:

$$\frac{\partial \eta}{\partial t}+\frac{\partial}{\partial x}\left(\int_{-h}^{\eta}u\,dz\right)+\frac{\partial}{\partial y}\left(\int_{-h}^{\eta}v\,dz\right)=0 \tag{5}$$

where $\eta\ (x,y,t)$ is the water surface elevation relative to the undisturbed water surface; $h(x,y)$ is the water depth measured from the undisturbed water surface. Thus, the total depth is computed as $H(x,y,t) = \eta\ (x,y,t) + h(x,y)$ (Figure 8).

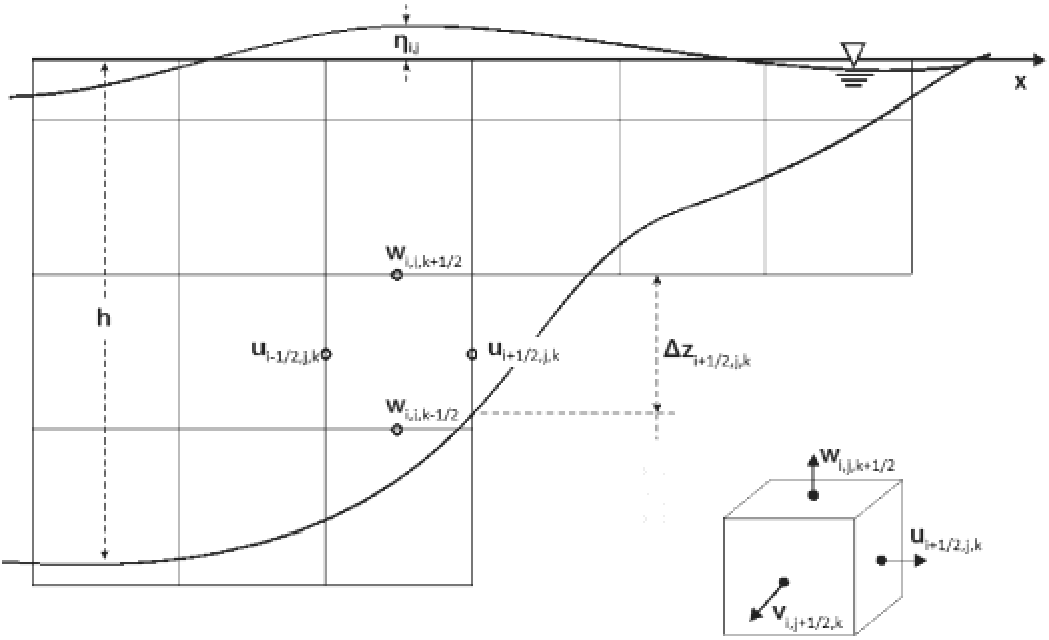

Figure 8. Schematic diagram of the computational grade utilized in the hydrodynamic module. Adapted from Casulli and Cheng (1992).

The boundary conditions for the momentum equations at the free-surface are computed by the wind stress components $\left(\tau_x^w, \tau_y^w\right)$ given by (Wu, 1982):

$$\upsilon \frac{\partial u}{\partial z}\bigg|_{z=\eta} = \tau_x^w\ ,\ \upsilon \frac{\partial v}{\partial z}\bigg|_{z=\eta} = \tau_y^w \tag{6}$$

At the bottom the boundary conditions can be expressed in terms of the water velocities components from layer adjacent to the sediment-water interface $\left(u_b^2, v_b^2\right)$. The bottom stress can be estimated through Manning-Chezy formula written as (Daily and Harlerman, 1966):

$$\upsilon \frac{\partial u}{\partial z}\bigg|_{z=h} = \frac{g\sqrt{u_b^2 + v_b^2}}{Cz^2} u_b\ ,\ \upsilon \frac{\partial v}{\partial z}\bigg|_{z=h} = \frac{g\sqrt{u_b^2 + v_b^2}}{Cz^2} v_b \tag{7}$$

where C_z is the Chezy friction coefficient (Chow, 1959) which is related with Manning's coefficient (n) by:

$$Cz = \frac{H^{1/6}}{n} \tag{8}$$

An efficient numerical semi-implicit Eulerian-Lagrangian finite differences scheme was used in order to assure stability, convergence and accuracy of the solution (Casulli and Cheng, 1992; Casulli and Cattani, 1994; Casulli and Zanolli, 2007). The computational grid and the notation scheme used for hydrodynamic variables are illustrated in Figure 8. Further information about the numerical method used to solve the partial differential equations numerically may be found in (Casulli and Cheng, 1992; Casulli and Stelling, 1998).

Water Quality Module

The chemical and biological interactions, processes and state variables in our model were based on the PCLake model (Janse, 2005), but adapted for subtropical ecosystems and embedded in the hydrological three dimensional model. The abiotic module describes the overall nutrients cycles for nitrogen, phosphorus and silica as completely closed, except for external flows and for loss processes as denitrification and burial. This implies that most components are modeled in at least three units (as dry weight (DW), carbon, nitrogen, phosphorus, detritus and silicate, see Table 2).

Thus, the nutrient-to-dry-weight ratios are not fixed in the model. Moreover, the model can evaluate the amount of organic and inorganic matter, both as the portion of detritus in the water and in the sediment. All biota are modeled as functional groups. Apart from mass fluxes (e.g., food relations, preference for food, etc) the model also contains some empirical relations, to represent some indirect effects between two aquatic communities, such as the fish and macrophytes impacts on resuspension.

Most substances and organisms in the water except fish groups, and macrophytes are subject to advection and diffusion processes, inlet and outlet loading and transition processes. Thus, the general advection-diffusion equation for these state variables might be written as:

$$\begin{aligned}&\frac{\partial(HC)}{\partial t}+\frac{\partial(uCH)}{\partial x}+\frac{\partial(vCH)}{\partial y}+\frac{\partial(wCH)}{\partial z}=\\&\frac{\partial}{\partial x}\left(K_h\frac{\partial(HC)}{\partial x}\right)+\frac{\partial}{\partial y}\left(K_h\frac{\partial(HC)}{\partial y}\right)+\frac{\partial}{\partial y}\left(K_v\frac{\partial(HC)}{\partial y}\right)+source/sink\end{aligned} \tag{9}$$

where C is the average concentration of a given substance or organism in the water column; and K_h and K_v are the horizontal and vertical scalar diffusivity. In Table 3, we listed the main biochemical source/sink processes considered by the model.

Table 3. Some biochemical differential equations in IPH-TRIM3D-PCLake model. All state variables in the water also include source of inflows, loss via outflows, burial correction and are subject to advection and diffusion by the hydrodynamic driver (except for fishes)

$$\frac{\partial IM}{\partial t} = \underbrace{f_{DIM}^{EF}(Sed)}_{\text{erosion flux}} + \underbrace{f_{DIM}^{SF}(T,DO,W)}_{\text{sediment flux}}$$

$$\frac{\partial Det}{\partial t} = \underbrace{f_{Det}^{SF}(T,DO,W)}_{\text{sediment flux}} + \underbrace{f_{Det}^{MIN}(T,DO,Det)}_{\text{mineralization}} + \underbrace{f_{Det}^{MORT}(Phyt,Veg,Zoo,F)}_{\text{biological mortality}} + \underbrace{f_{Det}^{BEG}(Zoo,F,Bird)}_{\text{biological egestion}} + \underbrace{f_{Det}^{CON}(Zoo)}_{\text{consumption}}$$

$$\frac{\partial NH_4}{\partial t} = \underbrace{f_{NH4}^{BU}(Phyt,Veg,T,NH4)}_{\text{biological uptake}} + \underbrace{f_{NH4}^{MIN}(T,DO,Det)}_{\text{mineralization}} + \underbrace{f_{NH4}^{SF}(T,DO,W)}_{\text{sediment flux}} + \underbrace{f_{NH4}^{NIT}(T,DO,NH4)}_{\text{nitrification}}$$
$$+ \underbrace{f_{NH4}^{MORT}(Phyt,Veg,Zoo,F)}_{\text{biological mortality}} + \underbrace{f_{NH4}^{BEX}(Phyt,Veg,Zoo,F)}_{\text{biological excretion}} + \underbrace{f_{NH4}^{BEG}(F,Bird)}_{\text{biological egestion}}$$

$$\frac{\partial NO_3}{\partial t} = \underbrace{f_{NO3}^{BU}(Phyt,Veg,T,NO3)}_{\text{biological uptake}} + \underbrace{f_{NO3}^{SF}(T,DO,W)}_{\text{sediment flux}} + \underbrace{f_{NO3}^{NIT}(T,DO,NH4)}_{\text{nitrification}} + \underbrace{f_{NO3}^{DNIT}(T,NO3,B,DO)}_{\text{denitrification}}$$

$$\frac{\partial PAIM}{\partial t} = \underbrace{f_{PAIM}^{SF}(T,DO,W)}_{\text{sediment flux}} + \underbrace{f_{PAIM}^{SF}(IM,DO,PO4,PAIM)}_{\text{Sorption flux}}$$

$$\frac{\partial PO4}{\partial t} = \underbrace{f_{PO4}^{BU}(Phyt,Veg,T,PO4)}_{\text{biological uptake}} + \underbrace{f_{PO4}^{MIN}(T,DO,Det)}_{\text{mineralization}} + \underbrace{f_{PO4}^{SF}(IM,DO,PO4,PAIM)}_{\text{Sorption flux}} + \underbrace{f_{PO4}^{SF}(T,DO,W)}_{\text{sediment flux}}$$
$$+ \underbrace{f_{PO4}^{MORT}(Phyt,Veg,Zoo,F)}_{\text{biological mortality}} + \underbrace{f_{PO4}^{BEX}(Phyt,Veg,Zoo,F)}_{\text{biological excretion}} + \underbrace{f_{PO4}^{BEG}(F,Bird)}_{\text{biological egestion}}$$

$$\frac{\partial O2}{\partial t} = \underbrace{f_{O2}^{MIN}(T,DO,Det)}_{\text{mineralization}} + \underbrace{f_{O2}^{NIT}(T,DO,NH4)}_{\text{nitrification}} + \underbrace{f_{O2}^{PP}(Phyt,Veg,T)}_{\text{primary production}} + \underbrace{f_{O2}^{RES}(Phyt,Veg,T,F)}_{\text{respiration}}$$
$$+ \underbrace{f_{O2}^{SD}(MIN_{sed},NIT_{sed})}_{\text{sed. demand}} + \underbrace{f_{O2}^{SD}(T,W)}_{\text{reaeration}}$$

$$\frac{\partial Phyt}{\partial t} = \underbrace{f_{Phyt}^{PPH}(Phyt,T,N,P,I)}_{\text{production}} + \underbrace{f_{Phyt}^{RES}(Phyt,T)}_{\text{respiration}} + \underbrace{f_{Phyt}^{MORT}(Phyt)}_{\text{mortality}} + \underbrace{f_{Phyt}^{SF}(T,DO,W)}_{\text{sediment flux}} + \underbrace{f_{Phyt}^{GRA}(Phyt,Zoo,T)}_{\text{grazing}}$$

$$\frac{\partial Veg}{\partial t} = \underbrace{f_{Veg}^{PPH}(Veg,T,N,P,I)}_{\text{production}} + \underbrace{f_{Veg}^{RES}(Veg,T)}_{\text{respiration}} + \underbrace{f_{Veg}^{MORT}(Veg)}_{\text{mortality}} + \underbrace{f_{Veg}^{GRA}(Veg,Bird)}_{\text{bird grazing}}$$

$$\frac{\partial Zoo}{\partial t} = \underbrace{f_{Zoo}^{GRA}(Phyt,Det,Zoo,T)}_{\text{grazing}} + \underbrace{f_{Zoo}^{RES}(Zoo,T)}_{\text{respiration}} + \underbrace{f_{Zoo}^{MORT}(Zoo)}_{\text{mortality}} + \underbrace{f_{Zoo}^{FP}(Zoo,F,T)}_{\text{fish predation}}$$

$$\frac{\partial Bent}{\partial t} = \underbrace{f_{Bent}^{GRA}(Phyt_{sed},Det_{sed},Bent,T)}_{\text{grazing}} + \underbrace{f_{Bent}^{RES}(Bent,T)}_{\text{respiration}} + \underbrace{f_{Bent}^{MORT}(Bent)}_{\text{mortality}} + \underbrace{f_{Bent}^{FP}(Bent,F,T)}_{\text{fish predation}}$$

$$\frac{\partial F_{Jv}}{\partial t} = \underbrace{f_{F_{Jv}}^{FP}(Zoo,F_{Jv},T)}_{\text{juvenil fish predation}} + \underbrace{f_{F_{Jv}}^{REP}(F_{Ad})}_{\text{reproduction}} + \underbrace{f_{F_{Jv}}^{AGE}(F_{Jv})}_{\text{ageing}} + \underbrace{f_{F_{Jv}}^{RES}(F_{Jv},T)}_{\text{respiration}} + \underbrace{f_{F_{Jv}}^{MORT}(F_{Jv})}_{\text{mortality}} + \underbrace{f_{F_{Jv}}^{FP}(F,T)}_{\text{fish predation}}$$

$$\frac{\partial F_{Ad}}{\partial t} = \underbrace{f_{F_{Ad}}^{FP}(Zoo,Bent,F,T)}_{\text{adult fish predation}} + \underbrace{f_{F_{Ad}}^{REP}(F_{Ad})}_{\text{reproduction}} + \underbrace{f_{F_{Ad}}^{AGE}(F_{Jv})}_{\text{ageing}} + \underbrace{f_{F_{Ad}}^{RES}(F_{Ad},T)}_{\text{respiration}} + \underbrace{f_{F_{Ad}}^{MORT}(F_{Ad})}_{\text{mortality}}$$

Phytoplankton can optionally be split in diatoms, green algae and cyanobacteria according to their different effects on the ecosystem. The effective growth rate is dependent on factors such as day length, Photosynthetically Active Radiation (*PAR),* under-water light (light attenuation), water temperature, individual fitness of the species (maximum growth rates), dissolved nutrients in the water, internal P, N (Si) content of the species, respiration, settling, resuspension and mortality. The combined growth rate equation for a given phytoplankton species (*Phyt*) can be described as:

$$\mu_{ef}(z) = G_{max}\ f_p\ \mu_{PP}(Phyt, T, Nut, L)\ Phyt - \mu_{LL}\ Phyt \tag{10}$$

where G_{max} is the maximum algal growth rate at 20ºC; f_p is the photoperiod; $\mu_{PP}(T, N, L)$ is the primary production rate as a function of temperature ($T(z)$), nutrients ($Nut(z)$), and light ($L(z)$); μ_{LL} is the loss rate due respiration and mortality. The temperature effect on primary production (μ_T) was assumed to be a Gaussian function that is widely used in phytoplankton models (e.g., Eppley, 1972; Canale and Vogel, 1974), which has only two parameters:

$$\mu_T(z) = e^{-\frac{0.5}{\sigma^2}\left[\left(T(z)-T_{opt}\right)^2-\left(20-T_{opt}\right)^2\right]} \tag{11}$$

Where σ is the sigma coefficient of the Gaussian function; and T_{opt} is the optimum temperature to phytoplankton growth. The temperature effect is normalized to 1.0 at a temperature of 20 ºC.

We used the well-known Droop function to model nutrient limitation of phytoplankton (Riegman and Mur, 1984):

$$\mu_P(z) = \left(1 - \frac{cPDPhytMin}{rPDPhyt(z)}\right) \cdot \frac{cPDPhytMax}{cPDPhytMax - cPDPhytMin} \tag{12}$$

where *cPDPhytMin* (gP g^{-1} DW) and *cPDPhytMax* (gP g^{-1} DW) are the minimum and maximum P content of the cells respectively; *rPDPhyt* is the instantaneous P-to-dry-weight ratio in the phytoplankton. The equations to compute the growth limitation by nitrogen (μ_N) and silicate (μ_{Si}) are analogous. We used Liebig's law to combine these limitation functions:

$$\mu_{Nut}(z) = min(\mu_P, \mu_N, \mu_{Si}) \tag{13}$$

where μ_{Nut} is the effect on growth rate due nutrient limitation.

Phytoplankton can be assumed heterogeneously distributed over the water column. Light attenuation (α_1) with increasing depth is described by the well-known Lambert-Beer law:

$$\alpha_1(z) = f_{PAR} \cdot (1 - f_{ref}) \cdot I_a \cdot e^{-k_e \cdot z} \tag{14}$$

where f_{PAR} is the fraction of *PAR*; f_{ref} is the light fraction reflected at the surface; I_a is the light intensity above the water surface (W m^{-2}); and k_e is the light attenuation coefficient (m^{-1}).The dependence of the growth rate of phytoplankton on light (μ_L), integrated over a layer (from z_1

to z2), was approached by an optimum function, incorporating photo-inhibition under high light levels.

$$\mu_L = \frac{e \cdot \left(e^{\frac{-\alpha 1(z1)}{I_S \cdot \mu_T}} - e^{\frac{-\alpha 1(z2)}{I_S \cdot \mu_T}} \right)}{k_e \cdot H} \tag{15}$$

where I_S is the optimum light intensity for growth (W m^{-2}). We also assumed that light attenuation coefficient was linearly related to the amount of inorganic matter (*aExtIM*), detritus (*aExtDet*), phytoplankton (*aExtPhyt*) and submerged macrophytes (*aExtVeg*) present in the water:

$$k_e = k_e^{'} + aExtIM + aExtDet + aExtPhyt - aExtVeg \tag{16}$$

where $k_e^{'}$ is the light attenuation (m^{-1}) due particle-free water and color. The presence of submerged macrophytes gives a positive effect on water transparency through various mechanisms such as reductions of wave resuspension, allopathic impact on algal community and provision of shelter for zooplankton and fish (Scheffer, 1998; Van Nes *et al.*, 2003).

The considered processes contributing to the loss rate of phytoplankton are respiration and mortality. They are usually modeled as a single first-order decay being respiration and mortality temperature dependent as:

$$\mu_L = \mu_R + \mu_M \tag{17}$$

being

$$\mu_R = k_{re} \cdot \mu_T \tag{18}$$

$$\mu_M = k_M \cdot \mu_T \tag{19}$$

where μ_L is the total phytoplankton loss rate (day^{-1}); k_{re} is the respiration and excretion rate; and k_M is the mortality rate.

Aquatic macrophytes can be split in several functional groups as rooted, non-rooted, emergent and floating leaved plants. They are modeled in a similar way as phytoplankton (except for the light effect on growth) and, optionally, grazing by herbivorous birds and fishes. No distinction is made between different parts of the plants, for instance roots and leaves. It is also assumed that the nutrients are homogenously distributed over the whole plant. The rooted and emergent plants may extract nutrients from both water and sediment pore water, thus plant biomass was divided in under-ground (roots) and over-ground (shoots) fraction. The light attenuation function only is applied to submerged macrophytes, however floating (*Float*) and emergent (*Emerg*) plants produce a light interception by surface coverage computed mathematically through a limitation factor on under-water light:

$$f_{cover} = min\left[1.0, max\left(\frac{Float}{MaxFloat}, \frac{Emerg}{MaxEmerg}\right)\right] \tag{20}$$

where *MaxFloat* is the biomass of a single layer of floating leaves plants (g DW m^{-2}); and *MaxEmerg* is the carrying capacity of emergent plants standing (g DW m^{-2}). The shading factor of emergent macrophytes can take a maximum value of 1 which means a total surface coverage. The light limitation for submerged plants is modelled as a Monod-type P-I curve, integrated over shoot length (i.e., from top to bottom of the plant) in the water column (Jørgensen, 1994):

$$\mu_L = \frac{1}{k_e \cdot H} \cdot log\left(\frac{1 + \frac{-\alpha 1(L_1)}{h_{sv} \cdot \mu_T}}{1 + \frac{-\alpha 1(L_2)}{h_{sv} \cdot \mu_T}}\right) \tag{21}$$

where L_1 is the distance measured from water surface to top of plant (m); L_2 is the distance measured from water surface to bottom of plant (m); and h_{sv} is the half-saturation for vegetation production at current temperature (W m^{-2}). Macrophytes growth rate also is combined with a density-dependent correction represented by carrying capacity of the environment (Scheffer, 1998).

The animal groups (i.e., zooplankton, zoobenthos and omnivorous, planktivorous and piscivorous fishes) are modelled as logistic growth combined with food limitation function and a correction to assure a closed mass balance. Zooplankton and zoobenthos can both be included only as one functional group. They can feed on phytoplankton and detritus with a certain preference level through different factors for each kind of food, thus a selectivity rank may be defined. The filtering rate has been described through Monod's function that has decreased hyperbolically with the enhancement of seston concentration (i.e., detritus and phytoplankton). Zooplankton and zoobenthos also have growth rates depending on their density in the environment. The fish module includes three main categories – piscivorous, omnivorous and planktivorous – this distinction was made in order to capture the fish diversity in subtropical ecosystems. All fish predation processes are modeled as a so-called 'type II' response (Holling, 1959), this means the predation rate depends on prey density according to a sigmoid function. The omnivorous fish was divided in two distinct live stages: juvenile and adult. This community has a selective preference for its preys and may feed on phytoplankton, zooplankton, zoobenthos and juvenile fish. Planktivorous fishes, also divided in young and adult phase, have a preference to feed on algae, zooplankton and zoobenthos, and similar rules of selectivity attributed to omnivorous fish are applied. It is worth emphasizing that an indirect effect of omnivorous and planktivorous fish in the water transparency was assumed given by their search for food in the sediment (increasing resuspension).

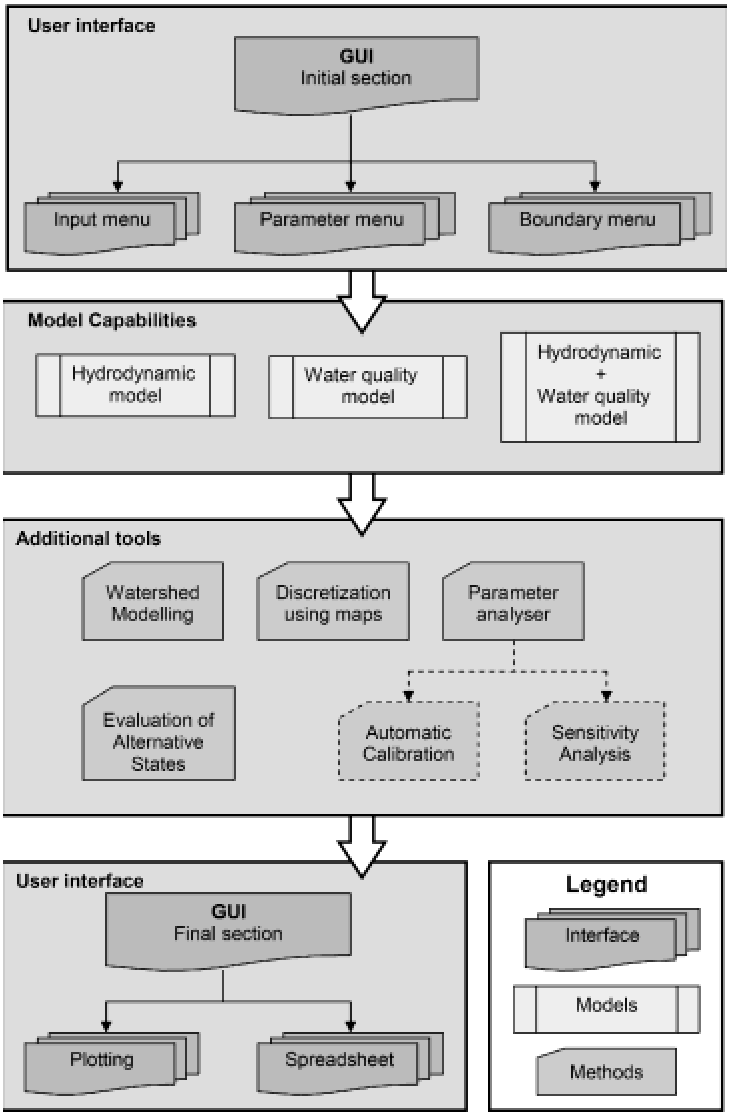

Figure 9. IPH-TRIM3D-PCLake is composed of three main blocks: graphical user interface (initial and final sections); model capabilities and additional tools. The GUI front-end let the user define the problem, then the type of model is specified and, optionally, the selected methods can be invoked. Adapted from Checchi et al. (2007).

Piscivorous fish growth also is assumed to be dependent on the presence of vegetation. Respiration, nutrient excretion and mortality of aquatic animals are all defined as first-order processes.

Graphical User Interface (GUI)

For an extended model like IPH-TRIM3D-PCLake it is important to think about how to control complexity while dealing with numerous parameters. An effective way to control complexity is to implement flexibility in the model design in such a way that it is possible to switch between different modes of complexity (van Nes and Scheffer, 2005). Therefore IPH-TRIM3D-PCLake includes a graphical user-friendly interface for MS Windows environment with a flexible design to vary the complexity of the model. The GUI was developed in Visual Basic which is an event-driven programming language with an integrated development environment (IDE) for its Component Object Models (COM), making it easier the building of interfaces for applications in Windows. As the simulation model was implemented in Visual FORTRAN, we connected the two modules through a FORTRAN Dynamic Link Library (DLL).

The model is organized in three main parts, as shown in Figure 9: Graphical User Interface (initial and final sections), model representations and additional tools. The user is taken through a logical path: first the problem is defined (i.e., initial and boundary conditions, parameters, dimension, numerical approach), the type of simulation (i.e., hydrodynamic, water quality or both), then the simulation run is performed, and, alternatively, the special analysis tools can be used (i.e., watershed modelling, evaluation of alternative steady states, domain discretization, automatic calibration of the parameters and sensitivity analysis). Also the availability of different ways to analyse the results and the application of a sensitivity analysis and various plotting tools, helps to control the complexity (Van Nes and Scheffer, 2005). In the next sections we will describe these parts of the GUI in more detail.

Preprocessing

The first step in the modeling process of an aquatic ecosystem is to gather its physical features (i.e., system boundaries, bathymetry, bottom roughness and distinction between open zones and wetlands). Usually, this information is available in a Geographical Information System (GIS) and the coupling with the model speeds up the model implementation significantly (Campbell, 1996; Burrough and McDonnell, 1998). Basically, the user is required to make, in advance, five maps. All maps are raster files that should have the same resolution as the model. The first map is a mask map that represents a Boolean grid indicating cells inside (code equal to 1) and outside (code equal to 0) the system. This map can also easily be computed from an area delimited by a polygon that represents the system's boundary. The second map, the bathymetry map (real format) is also called Digital Elevation Model (DEM). It may be derived from field campaigns or by remote sensing (e.g., Shuttle Radar Topographic Mission (SRTM) available freely on Web) (Burrough and McDonnell, 1998). The bottom roughness map (real format) allows attributing the Chezy friction coefficient spatially heterogeneous as a function of depth according to Equation 8. The last two maps define wetland areas, as these differ from open zones (e.g., the capacity to promote efficient recycling of nutrient resources and to reduce wind stress on water surface) (Wetzel, 1996). We carefully considered its proprieties through two maps. The first one (Boolean), named Wetland map, allows identifying Wetland regions and then biotic or abiotic environmental factors and self-regulating factors are treated in a different way within model structure. And the second one (real format) assigns values between 0.0 and 1.0 in order to represent the wind stress reduction on water surface for wetlands areas. Usually open zones do not have wind stress reduction (code equal to 1.0), unlike from wetland areas that has values range between 0.1–0.4 depending on stand features.

Input Data

The complexity of IPH-TRIM3D-PCLake model can be adapted depending on study purpose and on data availability. Thus biological state variables were designed to be flexible in the model structure (i.e., the user might lump, split or leave out certain state variables). A private window is provided in order to define the external variables of the system such as water temperature, solar radiation, wind direction, wind speed, rainfall and evaporation. There are several ways to define their values: (a) a constant value can be assumed for whole simulation time; (b) sinoidal patterns for water temperature and solar radiation; (c) equidistant or non- equidistant time series can also be inserted through *Add values* option; and (d) optionally water temperature can be modelled through a heat budg*et al*gorithm. For a complete description of the heat budg*et al*gorithm we refer to Chapra (1997). Initial conditions are added to the ecosystem by using the *Initial Condition* command in the *Input* menu. For all computational cells are computed the initial value for each state variable. The main parameter display window allows the user to edit all parameters which were arranged by parameter groups (i.e., hydrodynamic, abiotic and biotic parameters). This window is reached using the menu option "*Input* > *Parameters*" from the main IPH-TRIM3D-PCLake window. To know about each parameter and their features the user just needs to click on a "*Quick Help*" button nearby its edit box. GUI provides a flexible and simple way of introducing boundary condition to be used in the model simulations. The user can interact with ArcGIS Shape files (*.shp) and include discharge and water quality values. Geoprocessing tools are also organized in a toolbox which can be initiated individually or in combination to perform many GIS tasks including pan, zoom in, zoom out, zoom extent and pen to insert boundary conditions.

Model Capabilities

IPH-TRIM3D-PCLake model consists fundamentally of two main simulation modules: (a) a hydrodynamic module for free water surface; and (b) a water quality module, which encloses nutrient transport mechanisms, chemical kinetics/aquatic food-web interactions. By *Settings* command on *Run* menu the user can choose individual simulations using hydrodynamic module, water quality module or both. The vertical discretization may also be defined in the same window. A consistent horizontal one or two-dimensional model can be derived from the three-dimensional model as a particular case (setting number of layers equal to 1).

The hydrodynamic model predicts water surface elevations, velocities, and, optionally, water temperature. Water temperature can be included in the hydrodynamic calculations because of its effect on water density, otherwise the fluid is considered incompressible and water density is defined as a constant (by default 1000 kg m^{-3}).

Water quality can be updated less frequently than hydrodynamics thus reducing computational requirements. Water quality module is also decoupled from the hydrodynamics (i.e., standalone code for hydrodynamics and water quality where output from the hydrodynamic model is stored on disk and then used to specify advective fluxes for the water quality computations). Storage requirements for long-term hydrodynamic output to drive the water quality model are prohibitive for anything except very small grids. Additionally, reduction in computer time is minimal when hydrodynamic data is used to drive water quality are input every time step.

Additional Tools

Among additional tools available in the model we highlight: (a) the parameter analyzer including Monte Carlo sensitivity analysis and automatic calibration of the parameters; (b) watershed modelling (water quantity and water quality); and (c) domain discretization using map files.

A Monte Carlo multivariate sensitivity analysis can be applied to evaluate which parameters have the strongest effect on model outcomes (Klepper et al., 1994). The basis for this method consists to repeat a certain simulation many times for different sets of parameters, drawing all parameters randomly and independent from uniform probability distributions within ranges around the default values. Sensitivity coefficients are defined by linear regression between the parameter values. A cluster analysis (average linkage) can be used to form groups of parameters with same or opposite effects on the qualitative model output.

After the model is formulated, it is also necessary to calibrate and validate the model. This is performed by adjusting the parameters so that the model outcome approximates to the observed data. Often the parameters are stepwisely and manually adjusted within realistic ranges based on published works. The stepwise manual calibration continues until the model error could no longer be improved for most model outcomes. Automatic calibration is a good alternative to minimize the time effort of this modelling task. Therefore we implemented an efficient global search algorithm based on the Shuffled Complex Evolution developed at University of Arizona (SCE-UA) for parameter estimation (Duan et al., 1992; Duan et al., 1994). To converge efficiently toward the local optimum, the algorithm needs a sample population dependent on the number of parameters to be calibrated. Therefore we recommend only using this tool when the model performs water quality simulations (i.e., decoupled from the hydrodynamic module) working as a lumped model.

The data contained in the maps files (see Preprocessing section) may be combined into a unique *.txt file to be used as input file of the model. The GUI provides an algorithm (menu option: Tool > Discretization using IDRISI® maps) to generate a detailed data-cell network from IDRISI® raster files.

A watershed module also is available in GUI. The watershed modelling works with two main blocks: (a) hydrological module; and (b) water quality module (Figure 09). The hydrologic module is analogou to IPH2 model whose structure is basically composed by the following algorithms (Tucci, 1998): (a) soil water balance; (b) evapotranspiration; and (c) surface and ground water flow in the catchment. The water quality model methodology is based on the buildup-washoff algorithm (Neitsch, 2005). This algorithm works according to the followings rules: (a) consecutive dry days (when there is not runoff) are counted to compute the buildup; (b) at those time steps when runoff occurs, buildup is calculated based on the number of previous dry days and then washoff is computed according to the runoff volume; (c) the dry-days counter is recalculated based on the remaining built up load that was not washed off.

Outputs

The model allows the user considerable flexibility in the type and frequency of outputs. Output is available for the screen, hard copy and plotting. The user can specify the model outcomes and record them during the simulation according to a given frequency. The GUI includes a graphical pre- and postprocessor for plotting/visualization of times series of model outcomes.

Perspectives and Future Directions

The subsections above have demonstrated a great potential for the integration of phytoplankton interactions in subtropical shallow lakes. These systems have been subject of study only recently and many questions remain unanswered. For instance:

i. the determination of the dissolved organic carbon molecular weight and quality spectra produced by phytoplankton and macrophytes;
ii. the quantification and quality of the allelopathic substances produced by macrophytes, besides a more complete understanding of the species that produce them and which environmental factors trigger/modulate their production;
iii. the effect of specific allelopathic substances on phytoplankton, bacterioplankton and zooplankton species;
iv. the specific bacterial species associated exclusively to phytoplankton and macrophytes stands;
v. the *in situ* functions of microbes and their interactions with other organisms and their effects on ecosystem stability;
vi. the monitoring of uncultured microorganisms patterns and the understanding of how these patterns are influenced by changes in abiotic and biotic variables;
vii. an improved understanding of linkages between microbial community dynamics and macroorganisms inside macrophytes beds and environmental conditions;
viii. rates of zooplankton grazing on phytoplankton inside and outside macrophyte beds;
ix. a better understanding of the role of epiphytes, benthos, birds, amphibians, reptiles and mammals in these ecosystems;
x. the role of lake size, distance among lakes and connectivity (biogeography) versus local factors;
xi. more accurate global carbon balance of the lakes considering the horizontal heterogeneity (littoral - pelagic) of phytoplankton and bacterioplankton abundance and activity.

Many of the papers produced in Lake Mangueira have been developed under a Long Term Ecological Research Program (PELD-Taim), thus there is a great potential for the use of the data gathered with time series analysis and the identification of long term trends in the lake associated to changes in land use and climate.

Another promising perspective is the refinement of the current ecological models with the inclusion of parameters like competition for nutrients between bacteria, epiphytes, macrophytes and phytoplankton, release of allelopathic substances and the inclusion of more functional groups within bacterioplankton, phytoplankton, zooplankton, macrophytes, macro invertebrates and fish.

Conclusion

The phytoplankton in subtropical shallow lakes interacts with many ecosystem components - hydrodynamics, bacteria, macrophytes, zooplankton and fish - in different ways when compared to deep, temperate lakes.

The hydrodynamic in these lakes is dominated by winds, which promote patchiness in phytoplankton distribution through advection and changes in community composition due to resuspension of nutrients and organisms and selection of species better adapted to turbulence (transiently or locally). There is a high potential for bacterial competition for nutrients with phytoplankton, especially in littoral zones where the phytoplankton biomass is low and the ratio bacterioplankton:phytoplankton biomass is high. Macrophytes play a central role in these lakes by promoting low phytoplankton biomass in areas where they present high coverage (littoral zones or whole small lakes). In these areas, they have an important role in stabilizing the clear water state through physical (water column stabilization and shading) and chemical effects (competition for nutrients and release of allelochemicals). These plants liberate large amounts of dissolved organic carbon and sustain the bacterial production in littoral zones where the phytoplankton primary production is lower. The zooplankton plays a central role in these systems, particularly ciliates, which act as a link between the pico- / nanoplankton and metazoans. The zooplankton face a poorer refuge in macrophytes stands of subtropical lakes when compared to temperate lakes because of higher fish density, which imparts a lower potential for cascading effects on water transparency. Fish communities in subtropical lakes have higher abundance, frequent or permanent reproduction, a larger share of omnivores and lower share of piscivores than in temperate lakes. Due to their mobility, they impact the transport of energy and nutrients between the benthos, littoral and pelagic zones. The ecological model IPH-TRIM3D-PCLake has been developed by the research group in Ecotechnology and Applied Limnology and has been applied in Mangueira Lake. This model takes into account many complex ecological, physical and chemical interactions, for instance: spatial heterogeneity, effect of hydrodynamics (wind and water level), temperature, light, nutrients, growth and mortality of phytoplankton, zooplankton, zoobenthos, macrophytes and fish, among others. Many of these biological groups can be split into functional groups like phytoplankton (diatoms, green algae, cyanobacteria), macrophytes (emergent, floating and submersed) and fish (piscivores, omnivores, planktivores). The application of these models have helped to identify spatial patchiness in phytoplankton distribution and to predict the potential effect of changes in water level, increasing turbidity and climate change on lake functioning.

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Chapter V

Composition and Dynamics of Phytoplanktonic Communities in 3 Large and Deep Western European Lakes: An Outline of the Evolution from 2004 to 2012

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Abstract

This chapter details the evolution over the last decade of the phytoplanktonic community biomass and diversity for three large natural lakes in Western Europe (e.g., Lakes Annecy, Bourget and Geneva). Such a comparison has never been proposed before for these major ecosystems. It is shown that these lakes which have been restored or are still in a process of re-oligotrophication, display different phytoplanktonic populations, structure and succession while species (Shannon) diversity recently reached the same level. The last 9 years of the lake survey (2004-2012) has been particularly interesting since the phytoplanktonic structure changed abruptly, especially for Lake Bourget and both its biomass and (class) diversity tend to mimic what is now observed in Lake Annecy. However, the Brettum trophic state index based on phytoplankton composition or the proportion of nano- vs. microphytoplankton forms still classify Lake Bourget as mesotrophic (like Lake Geneva) whereas all parameters define Lake Annecy as oligotrophic. This is explained by species assemblages that remain very different between each ecosystem with typically a larger proportion of small cells and mixotrophs in the latter but also, probably to index pitfall. One of the main drivers for such differences between the 3 lakes, situated in a same ecoregion, seems to be the phosphorus concentration although it is also likely that many other factors intervene (e.g., other nutrients, grazing and parasitism, light availability, etc.).

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Introduction

Phytoplankton is represented by small free-floating autotrophic organisms, composed either by individual cells, colonies or filaments. They play a key role in the functioning of aquatic ecosystems as primary producers (i.e., through their photosynthetic activity that produce oxygen and use carbon dioxide) and food for higher trophic levels including zooplankton, fish or mollusk larvae, and benthic macro-invertebrates (Wetzel 2001, Reynolds 2002).

A variety of factors intervene in the regulation of the dynamics and diversity of the phytoplankton as recently highlighted by the revised Plankton Ecology Group (PEG) model (Sommer et al. 2012). Both the phytoplankton biomass and seasonal succession are a complex function of many factors including inorganic nutrient availability, lake morphology and physical conditions encountered in the upper lit layers, and biotic interactions such as zooplankton grazing, viral lysis, eukaryotic or bacterial parasitism (Banse 1994, Reynolds 2002, Brussaard 2004, Mayali & Azam 2004, Chambouvet et al. 2008).

Studying phytoplankton in lakes is very informative to have a better insight on the ecosystem functioning but also because algal species or assemblages may provide a useful indicator of the trophic state of the ecosystem (which nowadays corresponds to a strong societal demand) and of its response (rapid or delayed) to environmental fluctuations (Reynolds et al. 2002, Padisak et al. 2009). For instance, when lakes suffer from eutrophication (i.e., an excess of nutrients like phosphorus), an important biomass of phytoplankton is generally recorded and some harmful species such as toxic cyanobacteria can bloom (Smayda 1997, Chorus & Batram 1999, Reynolds 2006). Also, it has recently been shown that phytoplankton constitutes a very sensitive indicator of climate variability so that both this biological compartment and lakes in general provide some of the most compelling evidence that species and ecosystems are being influenced by global environmental change and can in turn be considered as sentinels of these changes (Straile 2002, Winder & Schindler 2004, Wagner & Adrian 2009, Williamson et al. 2009, Gallina et al. 2011).

Despite of the existence of studies that have been carried out about the phytoplankton in Lake Geneva (Anneville & Pelletier 2000, Anneville & Leboulanger 2001, Anneville et al. 2002, 2004, Rimet et al. 2009), Lake Bourget (Vinçon-Leite et al. 2002, Jacquet et al. 2005, submitted) and Lake Annecy (Domaizon et al. 2003), there is not yet any reference that aimed at comparing these three ecosystems except for Jacquet et al. (2012). The present chapter attempts to compare the inter-annual dynamics over the period between 2004 and 2012 of the different phytoplankton classes, functional groups and diverse indexes in these three lakes located in the same eco-region (Savoie and Haute-Savoie), in order to highlight the existence of a link between community structure and lake trophic status.

Materials and Methods

The principal characteristics of Lakes Annecy, Bourget, and Geneva are summarized in Table 1 and Figure 1 provides to the reader a geographic situation and map of the study area.

Table 1. Main characteristics of Lakes Bourget, Annecy, and Geneva

	BOURGET	**ANNECY**	**GENEVA**
Maximum length (km)	18	14.6	72.3
Maximum width (km)	3.4	3.1	13.8
Surface, Area (km^2)	44.5	26.5	580.1
Altitude (m)	231.5	447	372
Maximum depth (m)	147	65	309
Mean depth (m)	80	42	152.7
Total volume (km^3)	3.6	1.13	89
Watershed area (km^2)	560	278	7975
Water time residence (year)	8.5	3.5	11.5

The environmental monitoring of the peri-alpine lakes is carried out at reference stations, which are located where the lakes are at their deepest, and several kilometers away from their main tributaries. These sampling stations are regarded as being characteristic of the pelagic area and little influenced by terrestrial contributions and local disturbances related to certain human activities.

They therefore provide a relatively reliable picture of the water mass and associated biota status, as well as their response to more global disturbances. Samplings were carried once each month during winter and twice-monthly in spring, summer, and autumn. Between 15 and 22 campaigns are realized each year.

Concentrations of nutrients are measured in samples taken from a series of known depths between the surface and the bottom of the lakes. Among these nutrients, phosphorus is a key element and its concentration is measured after mineralizing the sample by adding ammonium persulfate and sulfuric acid and pressure-sealing. Colorimetric analyses involved adding a reagent (molybdate of ammonium, sulfuric acid, ascorbic acid, antimony, and potassium) and assaying spectrophotometrically (VARIAN). These analyses are carried out according to French standardized protocols (AFNOR).

Raw water samples were taken in the 0-18 m layer using an integrating water sampler developed by Pelletier & Orand (1978). After collection, the water samples used for phytoplankton analysis were immediately fixed with Lugol's solution. 25 mL of each sample were tipped into an Utermöhl (1931) counting chamber and left form a deposit for at least 12 hours, away from light and heat. The count was then carried out using reversed microscopy (Zeiss) to perform a qualitative and quantitative examination of the phytoplankton. The abundances found were converted into biomass (expressed in µg/L) starting from the biovolumes of each species (Druart & Rimet 2008). Species measuring less than 20 µm and with a biovolume of less than 10.000 µm^3 were assigned to the nanoplanktonic class. Those over 20 µm in length and/or with a biovolume of more than 10.000 µm^3 were classified as microphytoplankton.

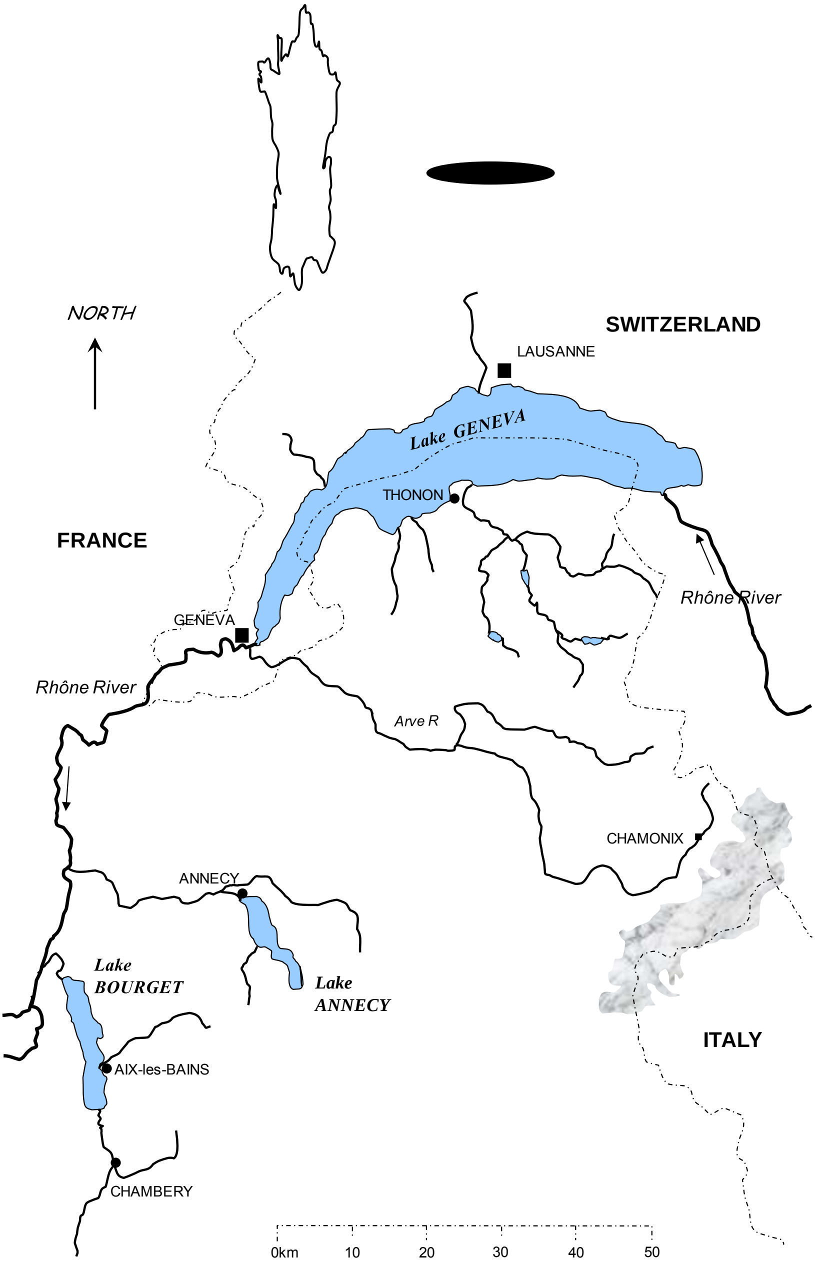

Figure 1. Geographical location in France and map of the 3 peri-alpine lakes situated in Région Rhones-Alpes.

Different biotic indexes based on the phytoplanktonic composition are reported. The Brettum index is related to the trophic level and it has been tested on the three lakes presented in this chapter (Anneville & Kaiblinger 2009, Kaiblinger et al. 2009). It is based on the probability of phytoplankton taxa occurrence along a gradient of total phosphorus divided in 6 trophic classes. For each class, a first index is calculated as follows:

$$I_j = \frac{\sum_{i=1}^{n} v_i x_{ij}}{\sum_{i=1}^{n} v_i}$$

where v_i is the biovolume of the taxon i and x_{ij} is the score of this taxon in the trophic class j

At last, BI is calculated as:

$$BI = \frac{\sum_{j=1}^{6} I_j T_j}{\sum_{j=1}^{6} I_j}$$

where T_j is the weight of each index I, (T1 = 6, T2=5, T3=4, T4=3, T5=2, T6=1).

The Shannon index (1948, H) is also used to assess the change in diversity, according to the following formula:

$$H = -\sum \frac{n_i}{n} \ln\left(\frac{n_i}{n}\right)$$

where n_i and n are the biomass of the taxon i and of the total phytoplankton.

We also used the functional groups as defined by Reynolds et al. (2002) where phytoplanktonic traits such as rapid or low growth rates, high or low requirement of nutrients, light or water column stratification, etc. allow to regroup species, define tolerance to environmental factors and typical habitats.

Results and Discussion

Evolution of the Biomass and Phytoplankton Classes

Figures 2 and 3 reveal the inter-annual changes (from yearly averaged values) in the biomass and the proportions of the main phytoplankton classes between 2004 and 2012 in the three lakes.

For the 3 lakes, it appears that annual phytoplankton biomass decreased during the last decade and became relatively comparable during the last years. For Lake Annecy, the values of phytoplankton biomass were relatively low (<2 mg/L) throughout the period examined. During the last 4 to 5 years, the biomass remained at relatively constant values of about 1 mg/L and the lowest value was reported in 2010. The dominant phytoplanktonic classes in this lake are the diatoms and Chrysophyceae. For this last group, mixotrophic species (e.g., *Dinobryon* spp), which are characteristic of oligotrophic ecosystems, can display relatively high biomasses (up to 200-300 µg/L in 2004-2005). These mixotrophic taxa use osmotrophy or phagotrophy to obtain nutritive elements under conditions in which resources are limited, and this trait has been suggested recently to be a more common strategy than previously imagined (Hansen 2011). In oligotrophic systems or periods of phosphorus limitation (for example in the epilimnion in summer), mixotrophy offers a considerable competitive advantage to these photosynthetic microalgae, giving them a two-fold system of nutrition (Stickney et al. 2000, Domaizon et al. 2003). Contrary to these mixotrophic taxa, other taxa sensitive to grazing, such as the Chlorophyceae, show a disappearing trend over years, as do the taxa indicating richer environments (Domaizon et al. 2011).

In Lake Bourget the annual phytoplanktonic biomass increased from 2004 to 2006, reaching 4.3 mg/L, and then the biomass reduced only slightly until 2008. Since 2009, a marked reduction has been recorded, and the lowest biomasses have been indeed measured during these last years. If one compares 2006 to 2010 or 2011 for example, the estimated biomasses have been reduced by a factor of 4. This pattern is explained very largely by the disappearance of the cyanobacterium *P. rubescens*, which was present in large numbers until the end of the summer 2009, but which then entirely disappeared in response to a conjunction of factors, including significant phosphorus reduction (Jacquet et al. 2012, submitted). In parallel, the proportions of diatoms, Cryptophyceae and Chrysophyceae increased markedly, which followed the same pattern as was observed in Lake Annecy. The increase of the mixotrophs observed during the last years in Lake Bourget confirmed this lake constitute a changing environment where the community adapts with successions of strictly autotrophic to mixotrophic groups and with increasing proportions of species that do not rely on just one resource but can bring the gap between periods of high resources and periods where resources are scarce.

Figure 2. Inter-annual changes in the average phytoplankton biomass between 2004 and 2012.

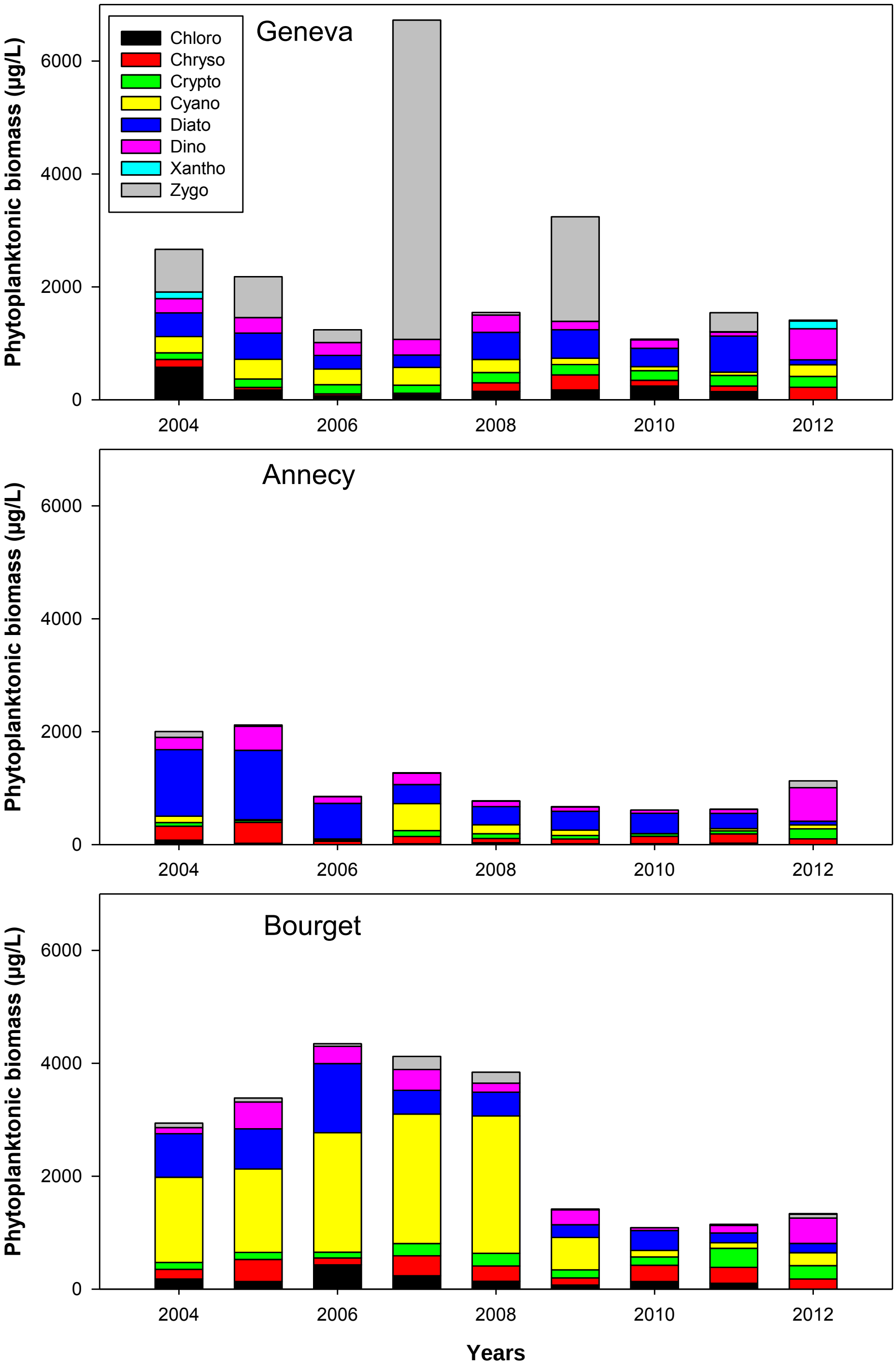

Figure 3. Inter-annual changes in the proportions of the phytoplankton class biomass between 2004 and 2012.

In Lake Geneva, patterns recorded for the phytoplankton biomass appear more chaotic and there is not a clear trend as compared to the other two ecosystems. 2007 was marked by an important phytoplankton peak that was only due to one particular Zygophyceae species, i.e., *Mougeotia gracillima*. The peak observed in 2009 was also due to the development of this species (see below). As for the two other lakes, annual biomass remained below 2 mg/L since 2010.

Evolution of Different Indexes Based on Phytoplankton Biomass or Species

The size of phytoplankton cells constrains many of their physiological rates (e.g., nutrient uptake, growth rate), biotic interactions (e.g., grazing) and behavior (e.g., sinking speed) so that cell size plays a key role in determining the diversity and relative abundance of competing phytoplankton species as well as their transfer to higher trophic levels (Raven 1998).

The high proportion of nanoplanktonic forms in Lake Annecy compared to the larger forms (63.3 ± 20.3%; average value on the time record) corroborates the general view of an oligotrophic ecosystem (Figure 4). Indeed, larger cells are more common under high nutrient supply (Ward et al. 2012) what was confirmed when considering the two other lakes (see below). Changes in the Brettum index for Lake Annecy (e.g., IB = 4.46 ± 0.17; average value over the period 2004-2012 and oscillating between 4.20 and 4.70) indicates that the trophic quality of the lake has been very good since the end of 1990 (Figure 5). It is noteworthy, however, that if between 2004 and 2008, the proportion of the nanophytoplanktonic biomass was indeed dominant in Lake Annecy, after 2008 the proportion of the microphytoplankton increased. In 2012 for instance, the nanophytoplankton represented only 32% of the annual biomass and this percentage was the lowest ever recorded. Moreover, the Brettum index of Lake Annecy decreased markedly in 2012, typically because of the increase of species such as *Scenedesmus* spp, more related to eutrophic environments.

Despite the biomass in Lake Bourget was considerably reduced during the last 4 years, the Brettum index remained low and relatively stable (average IB = 3.50 ± 0.38, oscillating between 3.05 and 3.92) compared to that observed for Lake Annecy, showing that the species in these two ecosystems are still very different. This is also corroborated by the predominance of micro- over nanophytoplankton in Lake Bourget (nanophytoplankton = 17.1±10.1% over the entire chronicle). It is noteworthy, however, that the nanophytoplankton size proportion increased to app 40% since 2009 in this lake. In 2011 and 2012, the relative proportions of these forms in Lakes Annecy and Bourget were in fact comparable.

In Lake Geneva as in the other lakes the biomass was also the lowest in 2010. In this lake, there was a clear downward trend and the Brettum index, although lower on average than in the other two lakes (IB = 3.19 ± 0.32) increased regularly. This indicates an improvement of the water quality, as revealed by the increase in the proportion of the functional groups characteristic of low-nutrient ecosystems (see below). The value in 2012 for the Brettum index in Lake Geneva was even the highest ever recorded but still classified the lake as meso- to moderately eutrophic. The relative proportion of the nanoplanktonic forms is low in Lake Geneva, and fairly similar to that in Lake Bourget (22.5 ± 14.6%).

The difference still observed between Lake Annecy Brettum index and the other lakes is clearly associated to differences in the specific composition. In Lake Annecy, species such as *Kephyrion* spp. or *Chrysolykos planktonicus* which are typical of oligotrophic conditions are still nearly absent from the other two ecosystems. Moreover, some species such as *Scenedesmus* spp, *Aphanothece* spp or *Aphanocapsa* spp still observed in Lakes Bourget and

Geneva (especially in 2012 for the former) indicate that these lakes are still higher nutrient-rich ecosystems compared to Lake Annecy.

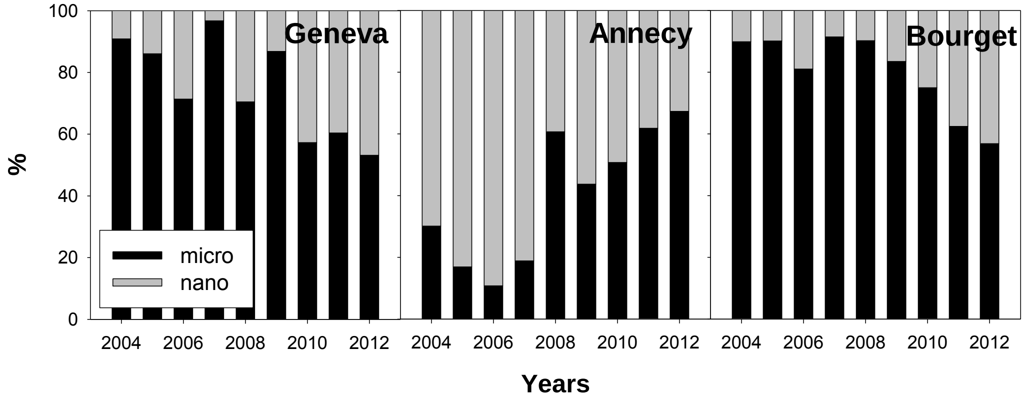

Figure 4. Inter-annual changes in the proportions of the micro- (in black) vs nano- (in grey) size classes.

Figure 5. Inter-annual changes in the Brettum index between 2004 and 2012.

Diversity can indicate nutrient level and/or quality of aquatic ecosystems and some relationships have already been reported (Russel-Hunter 1970, Schelske & Stoermer 1971, Reynolds et al. 2002, Padisak et al. 2009). The Shannon diversity index was used to observe the differences in phytoplanktonic biodiversity and changes over time in the 3 lakes (Figure 6). It also reports the richness and the relative proportion of taxa and appears to be comparable in Lakes Geneva and Bourget, which have higher values than Lake Annecy. However, this diversity seems to have fallen during the last decade in the first two lakes and since 2008, phytoplanktonic diversity has been comparable in all 3 ecosystems. This similarity may be linked to the relative homogenization of the chemical characteristics of these 3 lakes (their P contents in particular), but may also be attributable to the same impacts of climate in these lakes, which are located in the same ecoregion. In 2012, the Shannon index calculated for Lake Geneva was quite similar to the previous 5 years and it was lower than that observed before 2007. The significant decrease recorded in 2007 and 2009 could be directly associated to *Mougeotia gracillima* blooms.

Because of strong competition within the phytoplankton community, quick response of this community to external and internal forcing but also compensatory dynamic (Jochimsen et al. 2013), the composition appears to be a more sensitive indicator than integrative parameters such as total biomass (this is well exemplified with Lake Geneva). However, our data clearly indicate important changes in the composition, and the increase in the abundance of better adapted species to new environmental conditions (e.g., phosphorus depletion in Lake Bourget since 2009).

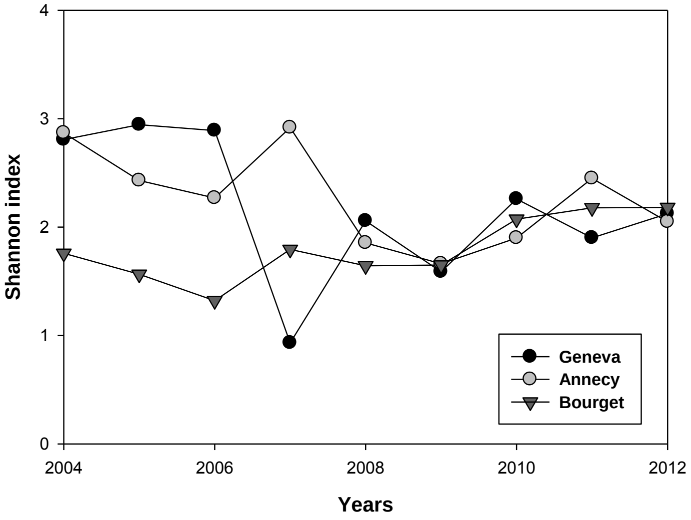

Figure 6. Inter-annual changes in the Shannon index between 2004 and 2012.

From the structure of freshwater phytoplankton assemblages, Reynolds et al. (2002) grouped the various species according to their particular ecological characteristics. Thus, a functional group corresponds to a whole of species having the same ecology. These groups gather taxa living for example in the same trophic levels, same turbulence conditions or the same limnetic and make it possible to better appreciate the factors influencing the phytoplankton and the quality of the lake. Figure 7 presents the annual dynamics of the functional groups of Reynolds.

For Lake Bourget, two main periods could be discriminated. Between 2004 and 2009, the group R was dominant and corresponded to species living in the metalimnion of stratified lake, enable to growth in low light conditions and relatively rich environments (e.g., *Planktothrix rubescens*). From 2010, the group R decreased and the biomass was largely represented by the group E, corresponding to mixotrophic taxa (e.g., *Dinobryon* spp.), typical of oligotrophic conditions. It is noteworthy, however, that 2012 was quite different from 2010-2011 since groups J and K became important and are indicators of relatively nutrient-rich environments. A possible explanation here was that winter 2012 was particularly cold and nutrients from the bottom could reach up the upper lit layers, resulting in the development of taxa characteristics of eutrophic ecosystems (e.g., *Scenedesmus acutus*).

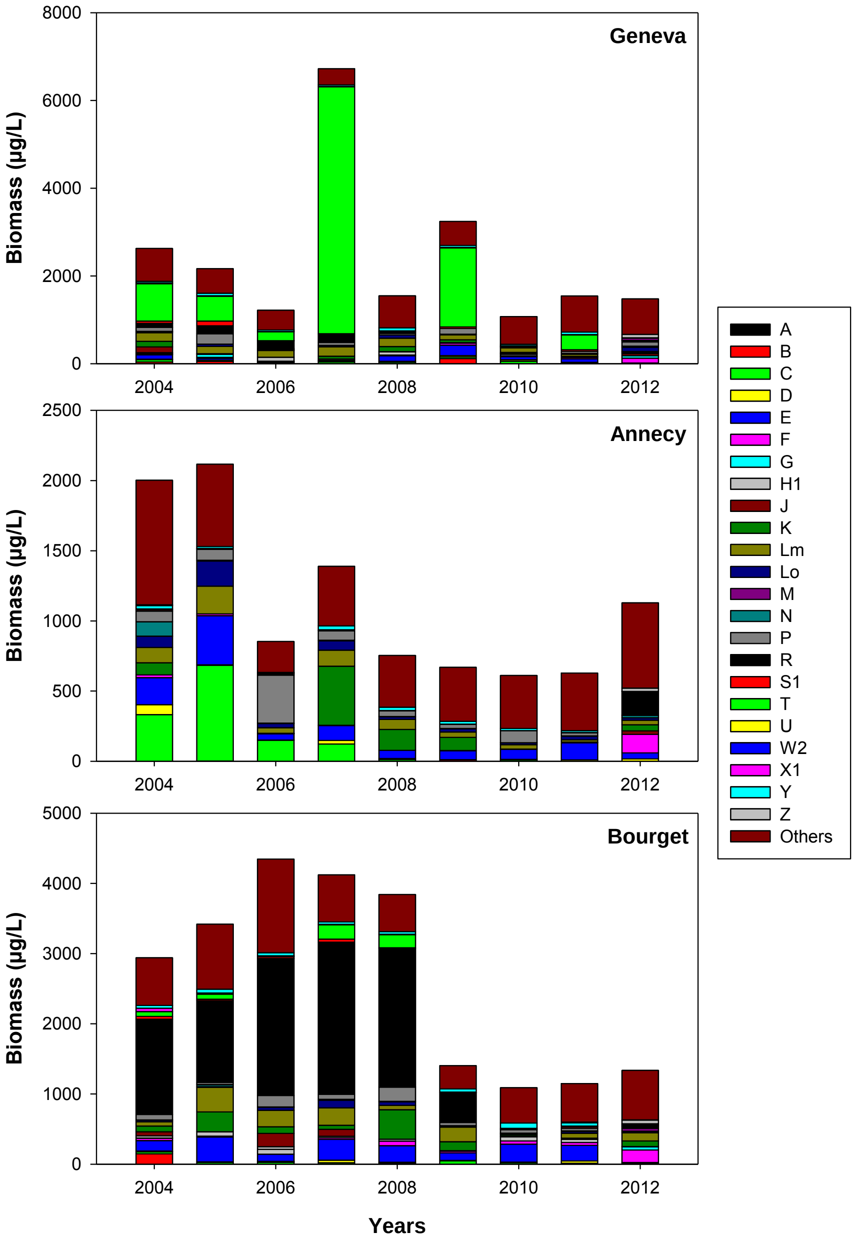

Figure 7. Inter-annual changes in the functional groups between 2004 and 2012.

In Lake Geneva, with the exception of the years when *Mougeotia gracillima* bloomed, all years during the last decade displayed relatively similar biomass. The proportions of the different algal classes varied only slightly despite species composition could change dramatically. In 2012, large quantities of A*chnanthidium catenatum*, *Tabellaria flocculosa*, *Aphanizomenon flos-aquae*, and of Chlorophyceae such as *Chlamydomonas* spp., *Pandorina morum* were measured. During recent years the group E, regrouping taxa characteristics of oligotrophic environments, increased, while eutrophic species became rarer, indicating the process of re-oligotrophication.

For Lake Annecy, the group E (regrouping mixotrophic taxa) increased significantly between 2006 and 2011. In 2012, however, the proportion of this group was lower while the group J with species like *Scenedesmus spp.* and *Pediastrum spp.* (i.e., chlorophyceae more characteristics or eutrophic conditions) increased. The presence of these “eutrophic” species and higher biomass observed in 2012 compared to previous years were likely due to the strong mixing event recorded during winter (February) 2012 that resulted in higher nutrient levels in surface waters that favored the development of the phytoplankton.

Clearly, the high proportion of the group E now observed in both lakes Annecy and Bourget may suggest that these (and other) functionally similar morphotypes exhibit similar dynamics. This has been recently highlighted in another temperate deep and large lake, e.g., Lake Constance (Rocha et al. 2011).

Relationships between Variables

Among key factors likely to explain both biomass and diversity of the phytoplankton, phosphorus is known as determinant in freshwaters. Figures 8 and 9 show the relationship between the annual biomass or the Brettum index with this nutrient.

As expected, these figures reveal a positive relationship between total phosphorus and phytoplankton biomass ($r=0.56$, $n=27$, $p<0.05$) while a negative relationship is observed between total phosphorus and the Brettum index ($r=-0.72$, $n=27$, $p<0.01$). They translate that long-term changes in both the abundance and composition of the phytoplanktonic communities are strongly associated to changes in this resource supply. It is noteworthy however that biomass decline in response to P reduction is not immediate and the ecosystem must reach a relatively low P level before phytoplankton biomass reduces significantly. This was clearly observed for Lake Bourget for which phytoplanktonic biomass was reduced by a factor 3 to 4 before and after 2009 when total phosphorus and phosphates winter concentrations) reached and maintained below 17 and 14 µg/L, respectively (Jacquet et al. submitted).

This phosphorus originates in the catchment area and has various sources (agriculture, industry, domestic); however domestic pollution was identified as having made a major contribution to the phenomenon of eutrophication observed in the years 1970-80. The efforts which have been made to reduce the contributions of the catchment area have been important and relatively similar between Lake Geneva and Bourget, leading to quite similar reductions of the external load in these two ecosystems over the last two decades (Jacquet et al. 2012).

Although our result highlights the critical role of phosphorus availability, this does not exclude the importance of other environmental factors such as the physical structure of the water column, other nutrients and biotic interactions including grazing by zooplankton, infection and lysis by viruses or fungi (Anneville et al. 2004, Sommer et al. 2012). Anneville et al. (2005) or Thackerey et al. (2008) showed indeed that the long-term changes observed in a variety of lakes in the biomass and phenology of the phytoplankton are the result of both nutrient enrichment/limitation and climatic variability.

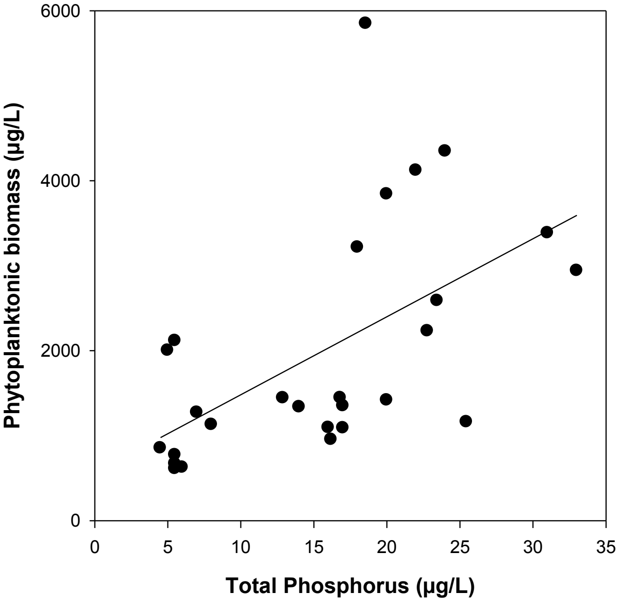

Figure 8. Relationship between total phosphorus and total biomass.

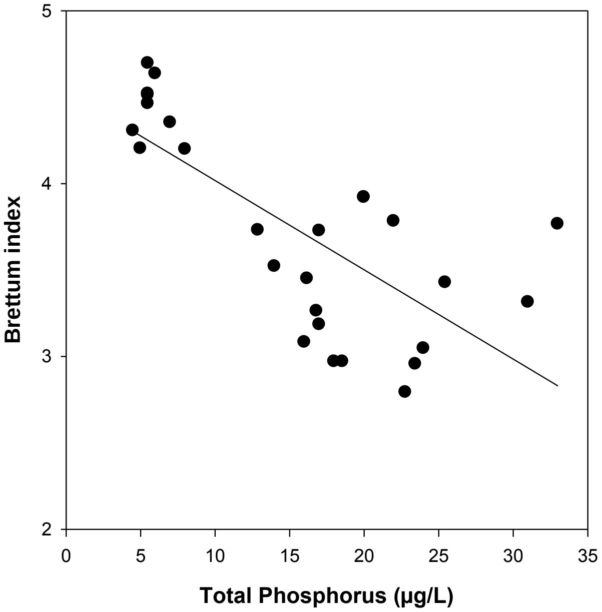

Figure 9. Relationship between total phosphorus and the Brettum index.

Conclusion

In recovering ecosystems such as Lakes Bourget and Geneva, even with constant nutrient concentrations, the inter-annual phytoplankton dynamics may still vary greatly from one year to another suggesting that many other factors (including typically meteorological forcing, grazing, parasitism, etc) can be of importance to explain patterns and diversity evolution. However, for Lake Bourget, it was clear that important reduction of phosphorus observed during recent years led to the disappearance of the cyanobacterium *Planktothrix rubescens* and that a new phytoplanktonic assemblage is settling. The pluri-annual monitoring study proposed here was mainly qualitative and highlighted either the absence of important changes or dramatic modifications in phytoplankton biomass and groups that could be at least related to P evolution in the different ecosystems. Further studies are now necessary in order to find a reliable quantitative description of the inter-annual variability of the phytoplankton assemblage and to incorporate them into ecological models. A first step could be to detail inter-annual dynamics of the whole community, main population and various species or morphotypes and compare such dynamics between the 3 lakes and the PEG (Plankton Ecology Group) model.

This is critical when one knows that aquatic ecosystems are expected to change markedly over the next century in response to anthropogenic stressors. It is expected indeed that the increase of air temperature will probably impact dramatically surface waters where phytoplankton develops. Water will become warmer (and this will be more marked in winter), the column stratification will increase and this will lead to enhanced light exposure. All together, such changes will thus result in a longer phytoplankton growing season but in another way, stronger stratification will also limit the vertical supply from deep waters of nutrients. Obtaining long-term series of phytoplankton abundances and diversity in conjunction with a large set of environmental parameters constitute an important step to be able to predict and assess plankton shifts and their consequences in food webs and biogeochemical cycles. Indeed, models will need to capture the ecology of diverse phytoplankton communities and assess how community structure varies under various environments to make in fine accurate predictions of the consequences of any anthropogenic change.

Acknowledgments

This chapter is a contribution to SOERE GLACPE (Système d'Observation et d'Expérimentation pour la Recherche en Environnement, Grands Lacs Péri-alpins) dealing with the environmental monitoring of the peri-alpine lakes Annecy, Bourget and Geneva. We thank CIPEL, the international commission for the protection of Lake Geneva (Commission Internationale pour la Protection des Eaux du Léman), SILA, the inter-syndical organisation protecting Lake Annecy waters (Syndicat mixte du Lac d'Annecy), and CISALB, the inter-syndical commission protecting Lake Bourget (Comité InterSyndical pour l'Assainissement du Lac du Bourget) that allowed us to use the dataset presented. Monika Ghosh is acknowledged for correcting the English.

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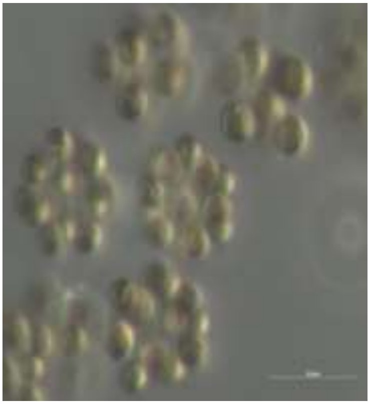
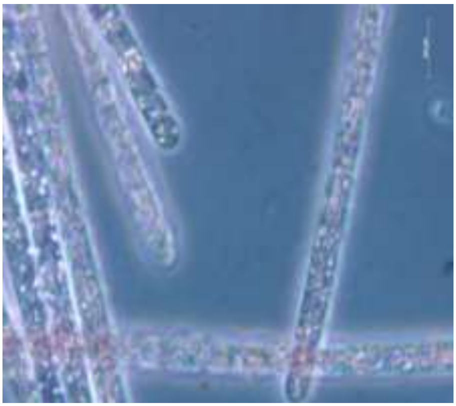
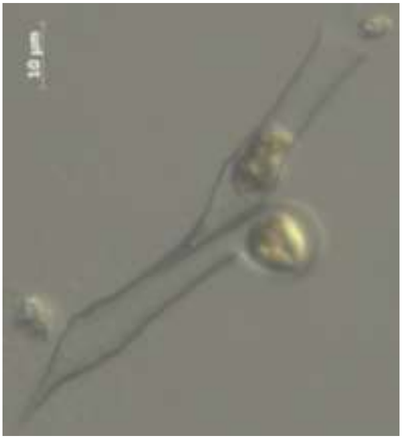
Annex

Annex: A Few Taxa from Peri-Alpine Lakes

Sphaerocystis schroeteri 	***Planktothrix rubescens*** 
Mougeotia gracillima 	***Dinobryon divergens*** 

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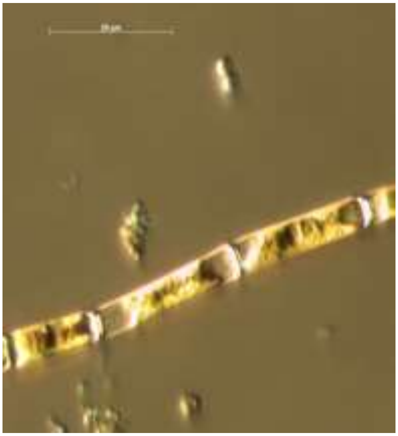
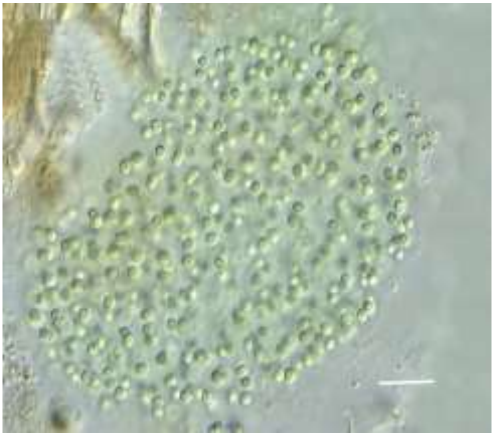
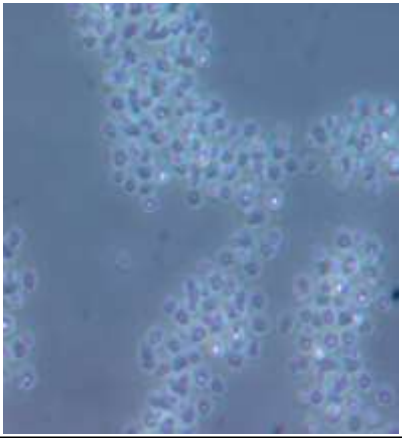
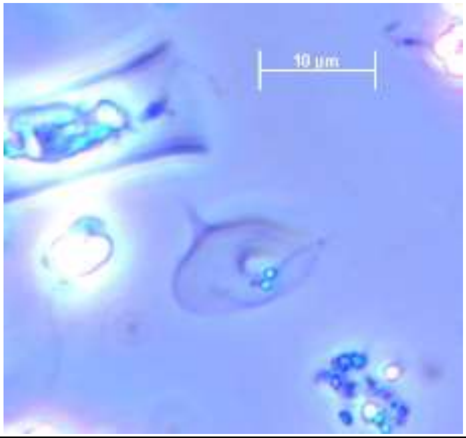
Tribonema ambiguum 	***Aphanothece delicatissima*** 
Aphanocapsa holsatica 	***Chrysolykos planktonicus*** 
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Chlorella vulgaris 	***Rodomonas minuta*** 
Ceratium hirundella 	***Aphanizomenon flos aquae*** 

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Chapter VI

The Phytoplankton Role in Formation of Bottom Sediment Productivity in a Large Reservoir in the Years with Different Temperature Conditions

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Abstract

The aim of research was to estimate the role of phytoplankton as a major contributor to primary production in formation of productivity of benthic biotopes in a large man-made water body (the Rybinsk Reservoir on the Volga River, Russia). The main approach was a comparison of phytoplankton primary production and chlorophyll concentrations in water column with concentrations of chlorophyll and its derivatives in bottom sediments in spatial and temporal aspects. Basic research method was a spectrophotometry of total acetone extract of pigments from plankton and bottom sediments. The data of long-term observations in the ice-free periods from May to October were analyzed. The ratio between contents of pigments in water and in the layer of bottom sediments accumulated for a year was calculated and factors affecting the ratio were considered. The influence of phytoplankton on bottom sediment productivity in the years of different temperature regimes was estimated. The delayed effect of temperature was found. It was concluded that the relationship between phytoplankton primary production and productivity of bottom sediments are predominantly influenced by abiotic conditions.

Keywords: Phytoplankton pigments, chlorophyll *a*, Volga River, benthic biotopes

Introduction

Phytoplankton is the main source of autochthonous organic matter in the large water bodies and the material and energy basis of their biological productivity (Vinberg, 1960; Alimov, 2004). The data on phytoplankton structure and abundance are used to estimate the water quality and ecological state of aquatic ecosystems, because algae respond rapidly to changes of environmental conditions. In recent years special attention in phytoplankton ecology has been called to the role of climatic changes and, above all, temperature which is one of main factors of growth and development of plants along with light and nutrients. In global warming the adaptabilities of phytoplankton and whole aquatic ecosystems to temperature increases must be clarified.

The unexpected abnormal heat in the summer of 2010 has been followed by the many studies of the effects of high temperatures on organisms and ecosystems. It was shown in water bodies of Russia that the raises of temperature affect the communities of aquatic organisms differently (Kopylov et al., 2012a; Lazareva et al., 2012, 2013; Litvinov et al., 2012; Sigareva and Timofeeva, 2012). Despite the research of all levels of life organization, there is not integral conception of effects of extreme heat on the whole ecosystem including the pelagic and benthic zones.

The ideas of productivity of bottom subsystem in different water bodies are usually got on the basis of data on productivity of the pelagic subsystem, assuming a positive direct relationship between these subsystems (Swain, 1985; Möller and Scharf, 1986). However, this relationship is not always found. So, there is not a clear relationship between spatial and temporal dynamics of productional indicators of plankton and benthic communities (Guilizzoni et al., 1983; Leavitt and Findlay, 1994; Sigareva, 2010). Search of causes of these discrepancies is needed to formalize the regularities of relations between components of water ecosystems. In this aspect, convenient objects to be studied are the reservoirs of the Volga River because they are shallow and influenced by highly variable abiotic factors.

Among the biomarkers connected with all links of a trophic chain a special place belongs to plant pigments and, first of all, chlorophyll *a* (chl-*a*) as a pigment being vital for photosynthesis of higher and lower plants. Chl-*a* as indicator of productivity is widely used in the studies of phytoplankton because the content of this pigment correlates with the intensity of photosynthesis and biomass of algae (Vinberg, 1960; Bulyon, 1983, 2005; Elizarova, 1993; Mineeva, 2009). In a number of hydrobiological papers the terms of "chl-*a* concentration" and "algal biomass" have the same meaning. Available instrumental methods of chl-*a* determination enabled the data on productivity of inland waters and the World Ocean to have been obtained. Worldwide distribution of chl-*a* and its relation with the productivity of plant communities is the basis of the Earth study from space. In recent years the studies of pigments in bottom sediments (chl-*a* derivatives, chlorophylls b, c_1, c_2, β-carotene and xanthophylls) have been intensified (Punning and Leeben, 2003; Szymczak–Żyła and Kowalewska, 2009; Reuss et al., 2010; Sigareva and Timofeeva, 2011a,b; Sigareva, 2012; Sigareva et al., 2013).

The aim of research was to estimate the phytoplankton role in formation of bottom sediment productivity in a large reservoir in the years with different temperature conditions, using the data on the spatial and temporal distribution of pigments in water and bottom sediments in the Rybinsk Reservoir on the Volga River, Russia.

Site Description

Rybinsk Reservoir (the coordinates are 58°22′30″N 38°25′04″E) was created in 1941 in the Upper Volga. Its volume is 25.4 km^3, the area at the normal maximum operating level is 4550 km^2, the average depth is 5.6 m, the maximum depths is 30.4 m, and the rate of water exchange is 1.9 cycles per year. The reservoir was divided into four parts: the lake-like central main part (Glavnyi reach) and three river parts: Mologa, Sheksna and Volga river reaches. (Litvinov et al., 2001) Ecological monitoring has been carried out in the main part and Volga river reach (Figure 1). The reservoir is characterized by considerable seasonal fluctuations of the water level caused by the human-induced flow regulation. Their amplitude over the period of reservoir operation averaged 3.3 m. Three typical periods are distinguished within the annual cycle of level variation: spring (filling), summer–autumn (relatively steady level or insignificant drawdown), and winter (drawdown) (Litvinov and Roshchupko, 2007). The averages of Secchi depth for vegetation periods of many years do not exceed 1.5 m. According to nutrient concentrations (nitrogen and phosphorus), phytoplankton biomass, primary production and chl-*a* content, the reservoir is mesotrophic or eutrophic (Bylinkina, 2001; Korneva et al., 2001; Mineeva, 2009; Pyrina et al., 2006). The total biomass of phytoplankton is mainly formed by diatoms and blue-greens (Korneva et al., 2001). As a rule, there is not thermal stratification of water column in the reservoir. The trend of increase in the average temperatures of the surface water layer for periods from May to October during 1947–2008 has been found (Zakonnova and Litvinov, 2010). The large bottom area is occupied by coarse-grained sediments. According to investigation carried out in 1992, the bottom sediments are presented by soils (17% of total area), sand and silty sand (55%), sandy and clayey silts (17%), peat-generated silt (2%), peaty silt (6%), peat and macrophyte deposits (3%) (Zakonnov, 1995). The thickness of sediments varies due to complicated bottom relief, averaging 14.8 cm. The sediment accumulation rate in the reservoir decreased from 9.2 mm/year in 1941–1955 to 2.9 mm/year in 1992–1994 (Zakonnov, 2007). Phytoplankton primary production amounted to 56–124 gC/(m^{2}year) in 1969–1995 and 103–200 gC/(m^{2}year) in 2009–2011 (Pyrina et al., 2006; Kopylov et al., 2012b).

Material and Methods

The results of pigments monitoring in the water column and bottom sediments of the Rybinsk Reservoir in the ice-free periods (usually from May to October) of 2009–2011 were used. The sampling was performed at two-weeks intervals at six permanent stations of the Institute for Biology of Inland Waters, Russian Academy of Sciences, within the Volga reach (st. 1, 2) and lake-like main part (st. 3–6) of the reservoir (Figure 1). Most of the sampling stations are located within the river channels of Volga (st. 1, 2), Sheksna (st. 5) and Mologa (st. 6) rivers. Among them, station 2 is ecoton that is a site in area of merge of waters of the Volga reach and main part of the reservoir. The mean depths at permanent stations 1–6 correspondingly amounted to 11.5, 12.8, 7.0, 6.3, 11.6, and 12.2 m that exceeded the thickness of photosynthesis zone (it in average extends from the surface to 4 m depth). The water temperature was measured in the surface water layer. Water transparency was determined using Secchi disc.

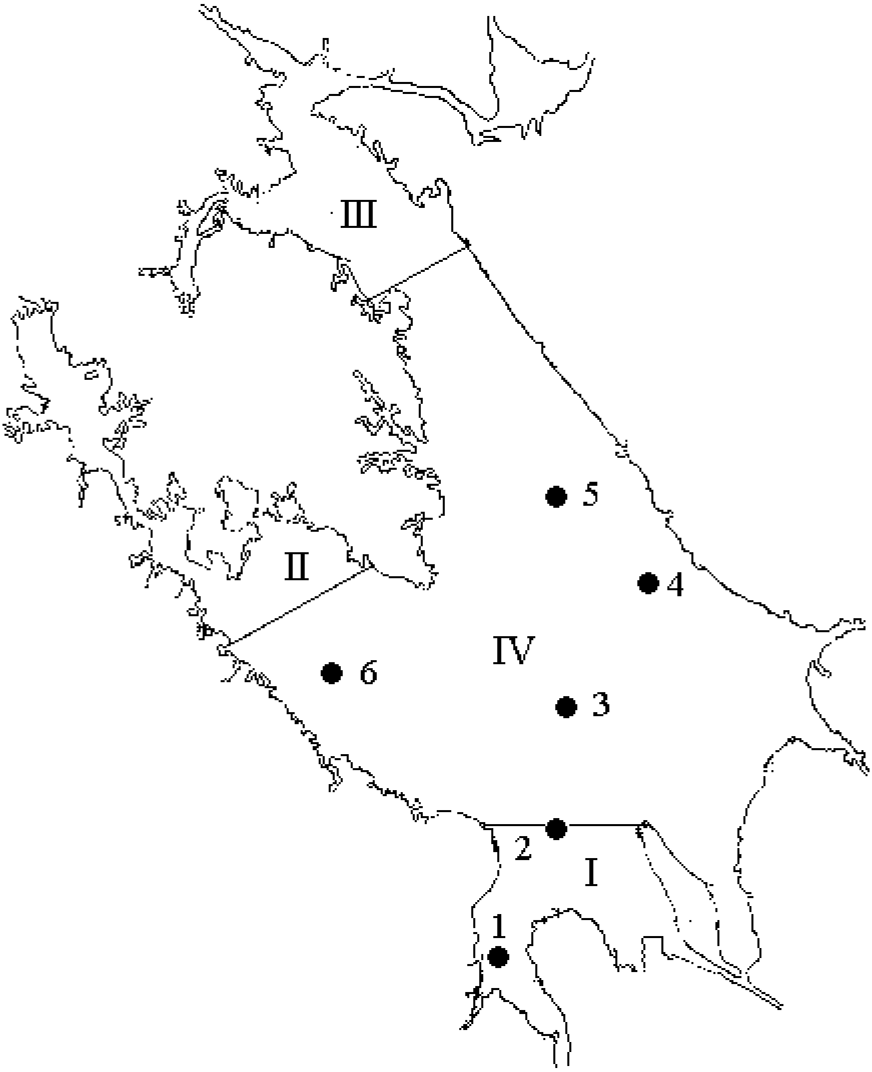

Figure 1. Rybinsk reservoir and the distribution of sampling stations: (I, II, III, IV) correspond to the Volga, Mologa and Sheksna river reaches and the lake-like main part of the reservoir, respectively.

The water samples were taken using a 1 meter long plastic sampler of 4 m volume totally from water columns 0–2 m (the main part of the euphotic zone), 2–6 m (the layer subjected to the strongest mixing and having the maximal volume), and 6 m from the bottom. The water samples were concentrated by direct filtration through Vladisart membrane filters with pore diameters of 5 µm. The bottom sediments were sampled with device, equipped with plexiglas tube of diameter 3 cm and length 34 cm; the top layers (0–2.5 and 2.5–5 cm) were analyzed. The pigment samples were kept at –20°C until analysis.

Pigments from phytoplankton and bottom sediments were analyzed by spectrophotometric method in the total 90% acetone extract using Lambda–25–spectrophotometer (Perkin Elmer). The pigments were extracted by mechanical grinding. Double extraction of pigments from phytoplankton or bottom sediments into acetone was made. The concentration of chl-*a* in phytoplankton was calculated by Jeffrey and Humphrey equations (Jeffrey and Humphrey, 1975); the sum of chl-*a* and pheopigments (Ph) in bottom sediments was calculated using C. J. Lorenzen equations (Lorenzen, 1967). The water content in bottom sediments was evaluated from water loss at drying at 60° C. The volume weight of the bottom sediments was calculated from the equation derived for the surface deposits in the Upper Volga reservoirs (Sigareva and Timofeeva, 2004). The average chl-*a* concentration in the water column was calculated taking into account the thickness of the water layers and chl-*a* concentrations in each water layer.

To estimate the role of phytoplankton in productivity of bottom sediments two indicators were used. The first indicator was a ratio between contents of the chl-*a* in the whole water column (mg/m^2) and chl-*a*+Ph in bottom sediment layer of 1 mm thickness ($mg/(m^2mm)$). The pigment content in bottom sediment layer of 1 mm thickness was calculated taking into account the pigment content in layer of 0–2.5 cm (µg/g dry matter) and air-dried volume mass of deposits (g/cm^3). The second indicator was a ratio (B/PP, %) of assumed algal biomass (B) (calculated from chl-*a*+Ph) in bottom sediment layer, accumulated for a year, to primary phytoplankton production (PP). The assumed algal biomass calculated from sedimentary pigments is a biomass corresponding to chl-*a* concentration in functioning phytoplankton from euphotic water layer. Its calculation is required to compare the content of sedimentary pigments with the primary production correctly. The algal biomass (in units of carbon) in bottom sediments was calculated assuming that chl-*a* content of the algal fresh weight is equal to 0.25% (Elizarova, 1993), the dry matter content of fresh weight amounts to 25.6% and the carbon content of dry matter amounts to 33.5% (Behrendt, 1990). Primary phytoplankton production in the years of observation was calculated by V.V. Bulyon equation (Bulyon, 2005), based on the mean chlorophyll concentration in plankton and mean water transparency.

Statistical data processing was made using standard PC programs. The correlation analysis was used to demonstrate a degree of the relationship between characteristics. The differences between the mean values of characteristics were analyzed using Student’s t-test.

Results and Discussion

Weather conditions in the years of observations were as follows: the seasons of 2009 were slightly warmer than normal, the year 2010 was characterized by an extremely cold winter, a record hot summer and extremely warm autumn, and the year 2011 was characterized by a cold winter, warm spring and summer (Roshydromet, 2010, 2011, 2012). The surface water temperatures throughout the observation periods in the Rybinsk reservoir differed by 3.5, 10.3, and 3.4 times in 2009, 2010, and 2011, respectively (Table 1). Average water temperature in 2011 was higher than that of the previous two years. The water temperature reached 21.6, 27.9 and 24.4 ^{o}C in July and 22.8, 26.4 and 23.2 ^{o}C in August of 2009, 2010 and 2011, respectively. The maximum values of water temperature in July and August were 3–4 °C above those during previous observation period (1947–2008) (Zakonnova and Litvinov, 2010). The temperature optimum for phytoplankton photosynthesis in the Rybinsk Reservoir is considered to be 25°C (Mineeva, 2009). The water temperatures seem to be about the optimum in 2011 and below and above the optimum in 2009 and 2010, respectively. The values of water transparency were similar to most frequently observed values (Pyrina et al., 2006) (Table 1).

Table 1. Pigment concentrations in water and upper (0–2.5 cm) layer of bottom sediments, depths, water temperature and transparency at the permanent stations 1–6 of the Rybinsk reservoir in different years

Parameter		Years		
		2009	2010	2011
Depth, m		5–15 10.4±0.4	5–14 9.9±0.4	5–15 10.5±0.4
Water temperature, °C		6.5–22.8 17.1±0.6	2.7–27.9 17.0±1.0	7.1–24.2 18.2±0.6
Water transparency, m		1.0–2.7 1.3±0.05	0.5–1.9 1.1±0.03	0.6–2.1 1.0±0.03
chl-*a* in water	in the layer 0–2 m, μg/L	0.4–134.4 14.3±2.6	0.8–144.2 18.8±3.1	2.9–88.2 24.8±2.4
	0 m–bottom, μg/L	0.4–58.0 9.9±1.3	0.7–53.7 12.2±1.6	3.6–45.7 19.4±1.6
	0 m–bottom, mg/m^2	4.1–497.8 98.4±11.8	5.0–598.9 122.7±18.8	32.5–639.9 203.0±19.5
chl-*a*+Ph in bottom sediments	mg/m^2 per 1 mm of wet layer	0.5–47.3 21.9±1.9	0.1–59.0 20.2±1.9	0.5–80 30.6±3.1
	μg/g dry matter	0.4–267.6 90.0±9.2	0.1–286.9 93.8±10.8	0.3–431 153.0±17.0
Pigment ratio*		4.5	6.1	6.6
Coefficient of correlation between chl-*a* in the water column (mg/m^2) and chl-*a*+Ph in deposits (μg/g dry matter)		0.21	0.28**	0.40**
Number of observations		52	53	54

Note: Value limits are on top; the mean and standard error are on the bottom; * ratio between concentrations of chl-*a* in the water column (mg/m^2) and chl-*a*+Ph in bottom sediments (mg/m^2 per 1 mm of wet layer), ** significant correlation coefficients ($r_{0.05} = 0.27$).

The phytoplankton role in the formation of productivity of bottom sediments should be assessed comparing the productional characteristics of pelagic and benthic zones under different conditions. The response of the plankton community to increase in temperature is caused not only by the direct influence of this factor on metabolism of phytoplankton and other organisms (bacteria, zooplankton), but also it depend on other environmental factors that vary with weather conditions. For example, the abnormal rise in temperature is accompanied by a prolonged calm weather and the weakening of hydrodynamic activity that leads to stratification of the water column which is not typical of water bodies, mixed in normal years.

Some studies dealt with the analysis of functioning and the identification of potentially negative responses of plankton communities of the Volga reservoirs to extremely high water warming in 2010. It was shown that the content of chl-*a* and primary production of phytoplankton in summer of 2010 were much higher than those in years with usual thermal conditions. Moreover, it was noted a strong bloom in the reservoirs caused by the abundance of blue-green algae. It was concluded that eutrophication of the reservoirs increased (Kopylov et al., 2012a; Lazareva et al., 2012; Litvinov et al., 2012).

To study the effects of warming, the long-term observations are useful (Pyrina et al., 2006; Litvinov et al., 2012). In this chapter the results of monitoring during ice-free periods of years with different temperature conditions are considered. The maximum concentration of chl-*a* in phytoplankton (144.2 µg/L) was found in the euphotic water layer in 2010, while the chl-*a* concentration averaged for the ice-free period was maximal in the year (2011) following the extreme year (2010) (Figure 2, Table 1). The concentration of chl-*a* in the whole water column was directly related to that in water layer 0–2 m, but the degree of the relationship decreased in warmer years: the coefficient of determination (R^2) equaled to 0.86, 0.58 and 0.54, respectively in 2009, 2010 and 2011 (Figure 3, Table 2). The direct correlation between the concentrations of chl-*a* in the layers of the water column seems to be disturbed by increasing heterogeneity of vertical distribution of the suspended plankton matter containing the phytoplankton pigments.

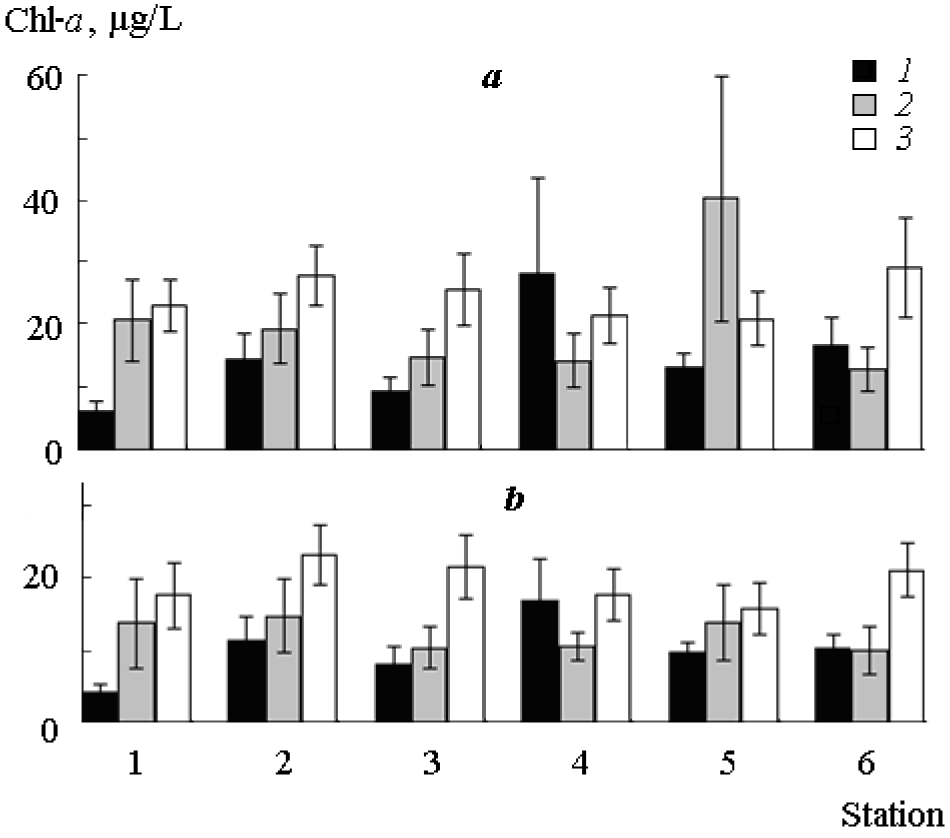

Figure 2. The average chl-*a* concentrations (µg/L) in (*a*) the photosynthetic water layer 0–2 m and (*b*) the whole water column (0 m–bottom) at the stations 1–6 in the Rybinsk Reservoir for observational periods of 2009–2011: (*1, 2, 3*) correspond to 2009, 2010, and 2011, respectively.

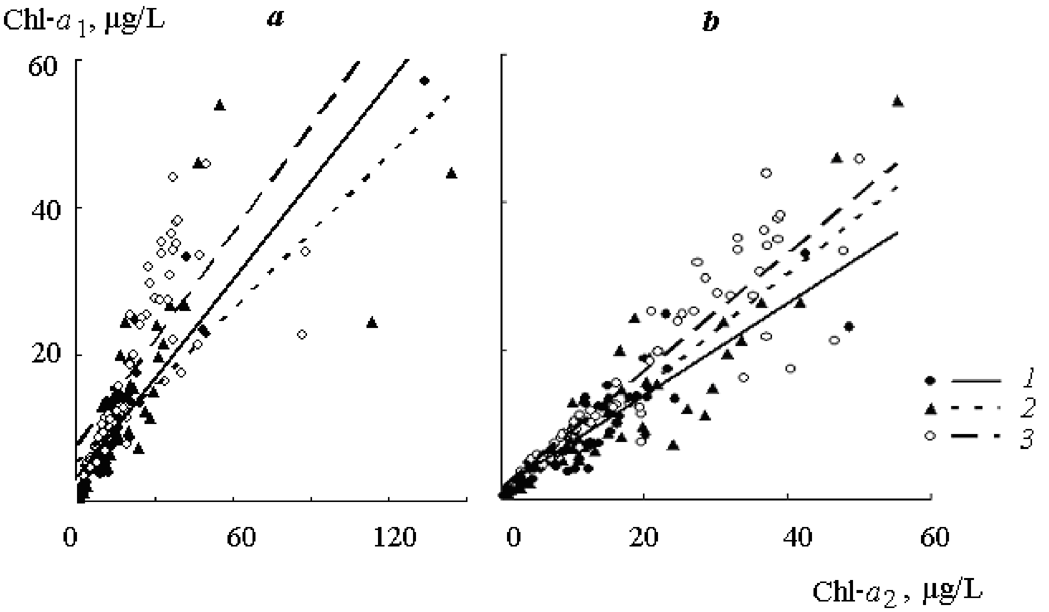

Figure 3. The relationship between chl-*a* concentrations in the whole water column (chl-a_1) and the photosynthetic water layer 0–2 m (chl-a_2) in different years: (*a)* for the entire data set and (*b)* without concentrations over 60 µg/L, occurred in the layer 0–2 m; (*1*, *2*, *3*) correspond to 2009, 2010, and 2011, respectively.

Table 2. Equations describing a relationship between chl-*a* concentrations in the whole water column (Y) and in the water layer 0–2 m (X) in different years, including (I) and excluding (II) high concentrations (>60µg/L)

Year	I		II	
	Equation	R^2	Equation	R^2
2009	Y=0.445X+3.43	0.86	Y=0.622+1.42	0.80
2010	Y=0.347X+5.31	0.58	Y=0.767–0.40	0.83
2011	Y=0.482X+7.46	0.54	Y=0.792X+1.35	0.78

To assess the trophic state of the reservoir, the mean chl-*a* content in phytoplankton for ice-free period averaging the spatial and seasonal phytoplankton variations should be used. According to trophic scale based on phytoplankton chl-*a* content (Vinberg, 1960), the reservoir is classified as eutrophic in years of observations. In abnormal year (2010) the mean chl-*a* content is not statistically different from that in the previous year (2009), and it is significantly lower ($P < 0.05$) than in the subsequent year (2011) (Table 1). Thus, the trophic state of the reservoir was not changed under extreme conditions of 2010.

Interannual changes of chl-*a* content in the layers of water columns in river and lake-like parts of the reservoir differed. In the river Volga reach the chl-*a* concentrations in the separate water layers and the whole water column increased from year to year (Figure 1, 2). In lake-like part the interannual chl-*a* dynamics in water layer 0–2 m were similar to that in Volga reach. However, chl-*a* concentrations in the lower layer 2–6 m are not statistically different in 2009 and 2010 and they increased only in 2011.

The response of the benthic zone to increase in water temperature was comparable to that of pelagic zone. The average concentration of sedimentary green pigments, mainly represented by chl-*a* degradation products, for vegetation period in extreme year (2010) did not differ significantly from that in previous year, and it was essentially less than in the subsequent year (2011) (Table 1, Figure 4). There was a direct correlation between the pigment concentrations in layers of bottom sediments of 0–2.5 and 2.5–5 cm. Higher pigment concentrations most often occurred in the upper (0–2.5 cm) layer.

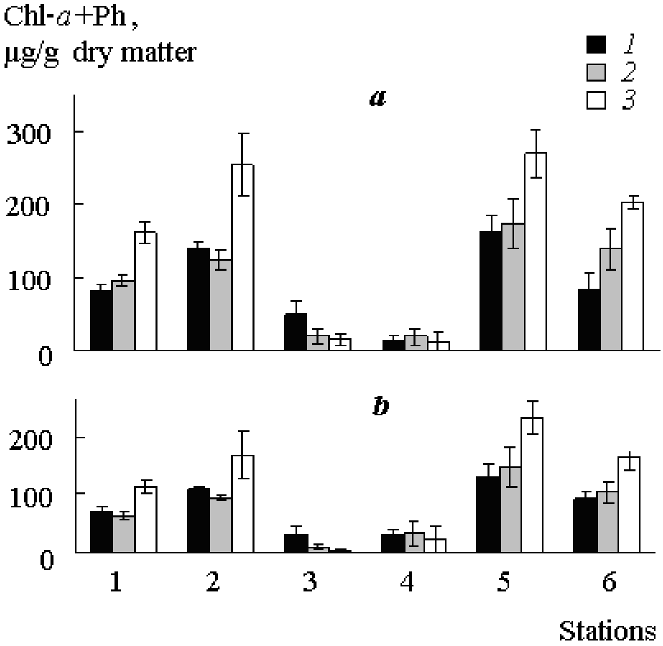

Figure 4. Concentrations of chl-*a*+Ph (µg/g dry matter) in the bottom sediment layers of 0–2.5 cm (*a*) and 2.5–5 cm (*b*) at the stations 1–6 in the Rybinsk Reservoir in different years: (*1, 2, 3*) correspond to 2009, 2010, and 2011, respectively.

The spatial distribution of pigments in the bottom sediments was more heterogeneous than in water column because of different conditions of phytoplankton sedimentation. Low concentrations of chl-*a*+Ph occurred at the central stations (stations 3 and 4) located on the flooded river flood-lands and subject to eroding activity of waves. These stations are characterized by mosaic distribution of bottom sediments with predominance of sandy deposits. High concentrations of sedimentary pigments are typical of the river bed sites occupied by silts: ecotone station 2 in Volga reach and station 5 in the lake-like part (Figure 1, 4). Similar patterns of distribution of sedimentary pigment due to the heterogeneity of the bottom sediment structure were found in years with different temperature conditions.

Pelagic and benthic zones in a shallow reservoir of mixed water masses are related quite closely. The main factors influencing the relation of phytoplankton with benthic biotopes are the depth and sedimentation rate. In lakes of the North-West of Russia the sedimentation rate of suspended matter was shown to equal about 1 m/day (Umnova, 1999). Consequently, it takes chl-*a* from the euphotic water layer some time to get the bottom (about a week in the Rybinsk Reservoir with the average depth of 5.6 m). This fact will lead to delayed effects of increase in water temperature on bottom sediments. The relationships between the characteristics of the bottom sediments and the depth of the water column at the sampling stations were more pronounced in warm years, that is confirmed by the values of correlation coefficient: 0.50–0.64 in 2009, 0.50–0.76 in 2010 and 0.61–0.86 in 2011 (Figure 5). The fact that the average depths at the stations in different years were similar (Table 1) suggests that the main reason for the increasing dependence of the pigment concentrations on the depth was more significant differentiation of benthic biotopes because of reducing of hydrodynamic activity in warm years.

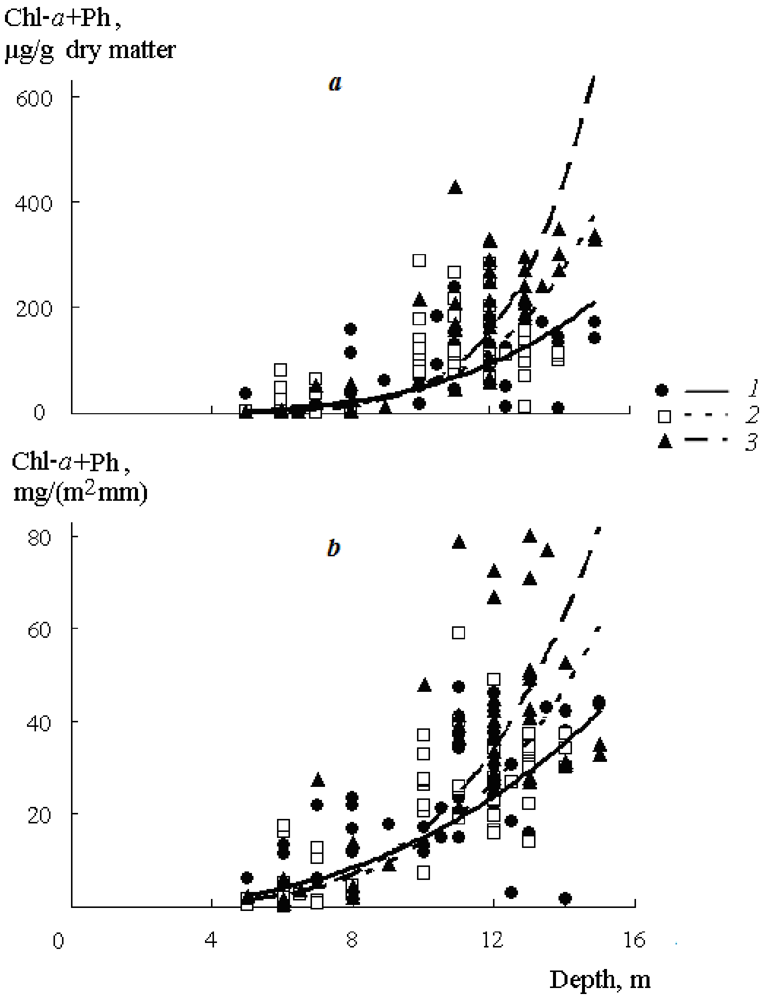

Figure 5. Relationship between concentrations of sedimentary chl-*a*+Ph and depth in the Rybinsk Reservoir: for pigment concentration calculated (*a*) for dry sediment, μg/g dry matter and (*b*) fresh sediment, mg/(m^2·mm); (*1, 2, 3*) correspond to 2009, 2010, and 2011, respectively. The coefficients of determination (R^2) for upper figure are 0.40, 0.58, and 0.85, and they for the lower figure are 0.39, 0.60, and 0.78 in 2009, 2010 and 2011, respectively.

The pigment concentrations in bottom sediments (μg/g dry matter) depended on those in the whole water column (mg/m^2), but the degree of the relationship was different in the observational years. Coefficients of correlation between these indicators increased in 2010 and 2011 (Table 1).

The ratios between the pigment concentrations in the water column and bottom sediments at the sampling stations differed significantly. The mean values of the ratio for ice-free periods amounted 4.5, 6.1, and 6.6 in 2009, 2010 and 2011, respectively (Table 1). Thus, they increased in warm years. To consider only the zones of active sedimentation of suspended matter, the mean pigment ratios amounted 2.9, 5.8 and 5.3 in the Volga reach and 9.8, 6.1 and 6.5 in the lake-like part of the reservoir in 2009, 2010 and 2011, respectively. It should be noted that the pigment ratios were similar in the river (5.3–5.8) and lake-like (6.1–6.5) areas of the Rybinsk Reservoir in abnormally hot years, but the values of this indicator were slightly higher in the lake-like part. It was shown for the Rybinsk Reservoir previously that the mean ratio between the pigment concentrations in the water column and bottom sediments coincides with the mean rate of sedimentation. Taking this into account, the sediment accumulation rate is likely to increase in hot years. This suggests that the role of phytoplankton in formation of bottom sediment productivity increases as a whole in warm years, but the influence of planktonic algocenoses at a separate site depends on environmental conditions: it increases in the riverine reach, but it decreases in lake-like part of the reservoir.

The ratios of assumed algal biomass in bottom sediment layer, accumulated for a year, to primary phytoplankton production (B/PP), calculated using the mean sediment accumulation rate of 2.9 mm/year (Zakonnov, 2007), amounted about 2% for the years of observations. If the values of ratio of pigments in the water column and sediment layer of 1 mm thickness (Table 3) be taken as sediment accumulation rates, the B/PP ratios will amount 3.4, 3.8 and 5.2% in 2009, 2010 and 2011, respectively. Consequently, most of phytoplankton primary production is utilized in the water column, and only a small part of it is deposited, increasing in warming conditions. Obtained values of B/PP correspond to the ratio between the content of organic matter in bottom sediments to primary production in freshwater bodies, but they are significantly higher than the average ratio for the biosphere (0.8%) (Romankevich et al., 2009).

The relationship between the pigment concentrations and water temperature of the surface water layer is weak in all the years. That is confirmed by low correlation coefficients: 0.21, 0.02 and 0.28 for pelagic and 0.14, 0.09, -0.05 for benthic in 2009, 2010 and 2011. Weak correlations between these indicators are due to the seasonal dynamics of phytoplankton in pelagial and heterogeneity of spatial distribution of deposits in benthic, as well as the influence of other abiotic factors (e.g., hydrodynamic activity) on the productional features of the water ecosystem.

Conclusion

The chapter demonstrates an approach to estimate the influence of phytoplankton on the productivity of bottom sediments. Pigment concentrations measured synchronously in the metabolically active layers of bottom sediments and water column were compared. In order to reliably estimate the effects of temperature on the productional properties of water ecosystems, the spatial and temporal dynamics of chl-*a* concentrations should be taken into account. Responses of the pelagic and benthic zones to rises in water temperature were similar: the pigment concentrations increased in these components of ecosystems in warm years. The role of phytoplankton in formation of the productivity of bottom sediments also increased in warming.

Table 3. Parameters used to estimate the role of phytoplankton in formation of productivity of bottom sediments in the Rybinsk Reservoir in the years with different temperature conditions

Parameter	Years		
	2009	2010	2011
Chl-*a* in the water layer 0–2 m, µg/L	14.3	18.8	24.8
Mean water transparency for ice-free period, m	1.3	1.1	1.0
Primary production (PP)*, gC/(m^{2}year)	100.4	111.7	133.9
Assumed algal biomass in the bottom sediments layer, accumulated for a year (B), gC/m^2	2.18** 3.37	2.01** 4.21	3.04** 6.96
B/PP ratio, %	2.2** 3.4	1.8** 3.8	2.3** 5.2

Note: * calculated according to V.V. Bulyon equation (Bulyon, 2005), using the average concentrations of phytoplankton chl-*a* and water transparencies and taking the duration of the ice-free period of 180 days. ** The values calculated for sedimentation rate of 2.9 mm/year (Zakonnov, 2007) are on top; the values calculated for sedimentation rates of 4.5, 6.1 and 6.6 mm/years (for 2009, 2010 and 2011, respectively), which are assumed equal to the ratio of pigments in the water column and sediment layer of 1 mm thickness, are on the bottom.

In general, the temperature increase in the limits of 20–27°C affected the ecosystem of a large reservoir ambiguously. Temperature conditions in 2011 were most favorable for phytoplankton productivity: the water temperature reached 24°C in summer, and its average value for ice-free period was higher than in the two previous years. The results suggest a delayed effect of extreme rise in temperature on the productional features of freshwater ecosystems. To understand better the mechanism of action of global warming on aquatic ecosystems, further studies are required.

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Chapter VII

Eutrophication in the European Regional Seas: A Review on Impacts, Assessment and Policy

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Abstract

The environmental conditions of the regional seas are now a cause for concern and are a subject of public policy since the '70s. The tendency of people worldwide to live or work near the seaside as well as tourism and industrial developments along the seashore, have exercised pressures on the marine environment. These include chemical pollution, loss of biodiversity, introduction of alien species, ecosystem degradation, loss of marine mammal population and beach erosion. Enrichment of the marine environment with nutrients, mainly nitrogen and phosphorus, from agricultural activities, marine aquaculture, sewage effluents and airborne pollution, stimulates phytoplankton growth causing a number of problems known as "eutrophication". Eutrophication can affect fisheries and marine biodiversity; it can also lead to phytoplankton blooms sometimes with toxic phytoplanktonic species, algal scum, bacterial blooms, fungi developments, hypoxia and in extreme cases anoxia. Among the side effects of eutrophication, reduced transparency or even change of the sea color impairs the recreational value of coastal waters and it is therefore a serious problem that has economic consequences for coastal states. A number of regional treaties have already been enforced including the problem of eutrophication. The Barcelona Convention for the Mediterranean, the Bucharest Convention for the Black Sea, the HELCOM Convention for the Baltic and the OSPAR Convention for the North Sea are among the regional instruments implemented for a better governance of the marine environment. In this chapter the eutrophication status of the European Regional Seas is examined, the policies for mitigating eutrophication are reviewed and the effectiveness of the measures over the period of implementation is assessed.

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Keywords: Phytoplankton, Environmental Assessment, Marine Governance, Regional Seas Conventions, Impact Mitigation

Abbreviations and Acronyms

ASMO	Assessment and Monitoring Committee (OSPAR)
BARCON	1976 Barcelona Convention for the Protection of the Mediterranean Sea, as amended
BSW	Black Sea Water
CMED	Ionian Sea and the Central Mediterranean Sea
DABLAS	TASK Force aimed to provide a platform for co-operation for the protection of water and water related ecosystems in the Danube and Black Sea region
DIN	Dissolved Inorganic Nitrogen
DIP	Dissolved Inorganic Phosphorus
EAD	Environmental Action Plan
EEC	European Economic Community
EMED	Aegean and Levantine Seas
EQR	Ecological Quality Ratio
EU	European Union
GES	Good Environmental Health
HELCOM	Baltic Marine Environment Protection Commission
The Helsinki Convention:	The Convention on the Protection of the Marine Environment of the Baltic Sea Area
ICES	International Council for the Exploration of the Sea
ICPDR	International Commission for the Protection of the Danube River
MCA	Multi Criteria Analysis
MED POL	The Marine Pollution Assessment and Control Component of MAP
MOU	Memorandum of Understanding
MSFD	Marine Strategy Framework Directive
NBB	National Baseline Budget
OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlantic
SAP	Strategic Action Plan
PRAM	Programmes and Measures Committee (OSPAR)
PP	Primary productivity
PSU	Practical Salinity Units
UNCED	United Nations Conference on Environment and Development
UNEP	United Nations Environment Programme
UNCLOS	United Nations Convention on the Law of the Sea
UWWD	Urban Waste Water Directive
WFD	Water Framework Directive

WMED	Western Mediterranean Sea

1. Introduction: An Overview

The phenomenon of eutrophication has been known in lakes since 1919 as excessive algal biomass due to over enrichment of nitrogen and phosphorus (Hutchinson, 1967). As a result eutrophication impacts on ecosystems were first studied within the field of limnology. Biochemical processes and ecosystem impacts are well understood by now in the fresh water systems. It is only during the past decades that marine eutrophication became a widespread phenomenon and a threat for the quality of the marine environment (O'Sullivan, 1995; Jørgensen and Richardson, 1996; de Jong, 2006). Marine eutrophication can well be the outcome of natural processes. It can be the result of "upwelling" where hydrographic conditions favor upward movements of nutrient rich deep water masses to the surface nutrient poor euphotic zone. Other sources of natural nutrient enrichment are atmospheric processes (UNEP, 2003b). River waters, even in pristine condition, carry nitrogen and phosphorus into the sea either in dissolved form or in the form of suspended particles. Shallow coastal areas, small bays and lagoons, usually show eutrophic trends due to resuspension of sediment. However, the term eutrophication denotes "cultural eutrophication" that is eutrophication caused by anthropogenic activities (Jørgensen and Richardson, 1996). Human activities accelerate eutrophication rates by increasing the availability of nutrients to coastal water bodies. The main land based sources of nutrients are agriculture, wastewater treatment plants, urban runoff and burning of fossil fuels (NRC, 2000). Since the 80's cultural marine eutrophication is addressed as a serious environmental threat (Rosenberg, 1985; Gray, 1992; Nixon, 1995; NRC, 2000; Karydis and Kitsiou, 2012). Scientists and policy makers for many years were relying on coastal water mass exchange with offshore water bodies which meant that dilution was considered as an effective mechanism for maintaining good environmental status.

Periodic loadings of nitrogen, phosphorus and other nutrients combined with high diversity, taxonomic complexity and rapid reproduction rates are poorly understood at the level of planktonic communities although plankton forms the basis of the food web (Howarth, 2008). Eutrophication impact on the food web structure is a combination of physical, geochemical and multiple ecological processes of the primary producers and feeding strategies of the consumers (Livingston et al., 1977). Coastal systems are vulnerable to environmental stresses due to nutrient loading. Many different structural outcomes in coastal communities reflect the large number of combinations of nutrient loading and response mechanisms that change seasonally and locally. Almost all coastal areas seem to face eutrophication problems. Human activities along coastal areas have increasing effects on phytoplankton response both qualitative (community structure) and quantitative (algal blooms). According to Bricker et al. (1999) "nearly all estuarine waters now exhibit some symptoms of eutrophication, though the scale, industry and impact vary widely, the level of nutrient required to produce the symptoms also varies". It has also been reported (Bricker et al., 1999) that among the estuaries studied in USA, 44 estuaries representing 40% of the total estuarine surface areas suffered from "high expressions of eutrophic conditions" whereas the eutrophic condition of an additional number of 40 estuaries was characterized as "*moderate*".

Decision on the limiting nutrient i.e., the nutrient that is having the key role in eutrophication is crucial as regulatory issues on policy making should focus on the limiting nutrient; measures to prevent or mitigate loads of the nutrient characterized as “the limiting nutrient” can be costly and also have an unenthusiastic reception by the stakeholders and the public. Although the key nutrient in freshwaters is phosphorus, the nutrient controlling eutrophication in the marine environment seems to be nitrogen (NRC, 2000). Scientists came to a consensus about nitrogen as the limiting nutrient after a long debate and extensive experimentation: three large scale field enrichments have been carried out in 90’s in the Narragansett Bay (Oviatt et al. 1995), in the Baltic Sea (Graneli et al., 1990) and in the Laholm Bay in Sweden (Rosenberg et al., 1990). All three studies had the same scope and followed the same methodology and all three reached to the same conclusion: nitrogen was the potentially limiting nutrient. The field experiments were considered that had brought enough evidence about the role of nitrogen as the primary regulator in eutrophication and it was therefore decided in USA that nitrogen should be adopted as the limiting nutrient (NRC, 1993; NRC, 2000). However, during the same time studies in the Eastern Mediterranean Sea indicated that phosphorus was the limiting nutrient in the area (Krom et al., 1991). Research on phosphorus continued and there is now adequate evidence that phosphorus is the limiting nutrient in the area (EEA, 1999; Krom et al., 2005; Krom et al., 2010).

The intensity of the phenomenon has been quantified by scaling trophic states. The classification scheme of marine coastal waters refers to oligotrophic, mesotrophic, eutrophic and hypertrophic conditions: it is based on primary productivity (Nixon, 1995), nutrient concentrations (Ignatiades et al., 1992) and chlorophyll values (Kitsiou and Karydis, 2000). Most coastal marine systems are classified either as mesotrophic or eutrophic systems. Oligotrophy occurs in limited places: the Sargasso Sea is a good example of an oligotrophic marine system; the reason is that the water column is permanently stratified and therefore nutrients concentrated in subsurface waters cannot trigger algal growth as light does not reach this nutrient rich water mass (Jørgensen and Richardson, 1996). The main water mass of the Mediterranean Sea is another example of oligotrophy: surface Atlantic water, low in nutrient concentrations flows through the Straits of Gibraltar and moves eastwards. During this movement, nutrient concentrations decrease even more due to nutrient uptake by phytoplankton so that the Eastern Mediterranean basin is considered among the most oligotrophic areas in the world (Krom et al., 1991; Turley, 1999). Unimpacted coastal areas are usually characterized as mesotrophic due to land inputs, coastal circulation and sediment resuspension. Mesotrophic waters in addition to higher plankton productivity are characterized by higher fish production compared to oligotrophic seas. Eutrophic conditions are in most cases the result of human activities. Estuaries carrying nutrient loads from the watershed as well as hot spot areas near big coastal cities are typical examples of eutrophication.

Eutrophication does not only affect ecosystem quality but is also having effects on human health and economic activities such as recreation and fisheries. Manifestation of these effects include visible bacteria blooms (Aubert, 1988), fungal development and blooms of toxic algae. Reduced transparency is an unpleasant situation for bathers, impairing recreation. Contact with algal material combined with high pH causes dermatitis, or even conjunctivitis; ingestion of sea water by bathers under blooming conditions has been reported to cause diarrhea (Vollenweider, 1992). Decaying of organic material consumes dissolved oxygen causing hypoxia and in extreme cases anoxia. The effects of eutrophication are spaced out over a wide range of symptoms extending from a slight increase of phytoplankton and macrophyte biomass to anoxic conditions. Although the decline of water quality due to eutrophication proceeds gradually, Gray (1992) has indicated five phases describing adverse effects. The onset of eutrophication (1^{st} phase) is characterized as the “enrichment phase” (Table 1). Greater plant biomass, being the response to increased nutrient concentrations, forms a source of food for benthic organisms increasing benthos biomass. This in turn may increase fish biomass. It has been reported that the increase in fisheries yield observed in coastal areas over the last two centuries is the result of anthropogenic eutrophication (Larsson et al., 1985; Tatara, 1991). Eutrophication is not characterized only by quantitative changes at ecosystem level but is also affecting the communities at qualitative (species composition) level (2^{nd} phase). These changes have been observed in both benthic as well as phytoplanktonic community structures (Table 1). Changes in phytoplankton community structure can have a potential effect on the mass and energy flow through the entire ecosystem. Information on the effects of eutrophication on phytoplankton community structure is rather limited (Tsirtsis et al., 2008) and possible effects on the food web need to be investigated. The onset of eutrophic symptoms has been characterized by Gray (1992) as ‘initial effects”. During the “secondary effects phase” (3^{rd} phase) reduction in light penetration affecting photosynthetic activities of the benthic flora, behavioral effects of marine animals and hypoxia are the main symptoms. The most serious threat among the secondary effects is the algal blooms that are rapid growth and high biomass formation of phytoplankton. The blooms are not always an outgrowth of toxic algae. Toxic algae may be present in the marine environment without forming blooms; on the other hand the dominant species of algal blooms may not be toxic. Development of toxic microalgae and accumulation of toxins in fish and shellfish is a threat to human health (Hallegraeff et al., 2003). Extreme effects (4^{th} phase) are characterized by mass growth of macroflora: dense macrophyte and microalgal formations clog channels, lagoons and shallow coastal areas reducing the water exchange and impairing navigation and fisheries (Vollenweider, 1992). Frequent occurrence of toxic blooms and mortality of species is also among the symptoms of the extreme effects. During the final phase (5^{th} phase) of eutrophication (ultimate effects) the impacts result from hypoxia that is oxygen concentration much lower that the saturation levels. Total absence of dissolved oxygen is characterized as “anoxia” (zero oxygen concentration). In addition to animal mortality, undesirable substances such as CO_2, CH_4, H_2S and NH_3 are formed as well as organic acids, toxins and bad smells in the area due to organoleptic (taste and odor) compounds.

The scientific background on eutrophication issues has formed a sound platform for taking legal action and implementing policy on eutrophication. According to Krebs (1972) ecology supports policy in a twofold way: increases the understanding of the natural system and provides a basis for practical actions concerning policy making, legislation and management. Implementation of eutrophication policy assumes information on cause-effects relationships, direct or indirect effects at ecosystem level as well as possible impact on the society (quality of life) and the economy. Knowledge of science especially on cause - effect relationships in marine eutrophication and the information referring to direct / indirect impact on ecosystems, society and economy, are the necessary keys for implementing eutrophication policy.

This policy consists of three phases (de Jong, 2006). The first phase, known as the discovery phase is characterized by an effort for the problem to be recognized. It is not possible at this phase to set the problem of eutrophication to the right dimensions or suggest any measures. If the eutrophication issue is accepted by the authorities and at the same time there is public awareness, then the objectives of 1st phase have been addressed successfully. The second phase is known as political or decision making phase: negotiations start among the stake holders and scientists for solutions that will be feasible combining environmental quality criteria, economic interests and the consensus by the society. The third phase known as management phase focuses on political decisions and management practices that are developed and applied.

This approach assumes strong interlinks between ecologists on the one side and policy makers / managers on the other side. These interlinks became possible as ecologists have been “politicized” by getting involved more and more in decision making panels and at the same time policy makers have been “ecologized” by moving from specific policies such as elimination of nutrient loads and species protection (de Jong, 2006) to the need for an integrated approach (Kitsiou and Karydis, 2011). Within this rational many international organizations like WHO (WHO/UNEP, 1995), OSPAR (1998), UNEP (2003a), HELCOM (1998), EC (2000), EC(2008) and EPA (2001) have taken actions towards the mitigation of the problem of eutrophication.

Table 1. Phases of eutrophication according to Gray’s classification scheme (1992)

Phase of eutrophication	**Effects**	**References**
Enrichment Phase	Increase in macroalgae biomass	Raven and Taylor (2003)
	Increase in phytoplankton biomass	Livingston (2001)
	Increase in bethos biomass	Pearson and Rosenberg (1978)
	Increase in fish biomass	Tatara (1991), Larson (1986)
Initial effects	Changes in species composition	Philippart et al. (2007), Kimor (1991)
Secondary effects	Reduction in light transparency	Ignatiades (1998)
	Hypoxia	Diaz and Rosenberg (2011)
	Toxic algal blooms	Anderson et al. (2002)
	Behavioral effects	Vaquer-Sunyer and Duarte (2008)
Extreme effects	Mass growth of macroalgae	Cardoso et al. (2008)
	Toxic effects	Hallegraeff et al. (2003)
	Species extinction	Lotze et al. (2011)
Ultimate effects	Anoxia	Naqvi et al. (2010)
	Mass mortality	Kollman and Stachowitsch (2001)

The problem of eutrophication in European Waters is now addressed through the coordination of four environmental components: (a) the research community: universities, research centers and industry. They provide the necessary scientific information and propose technical solutions and management practices (b) Regional agreements: there are already regional agreements for the Mediterranean, the Baltic, the Black Sea and the North Sea (c) the European Union (EU): a number of Directives adopted by the EU related to different aspects of the marine environment and (d) the States of Europe: they are responsible for nutrient reductions and protection of the marine environment. The totality of the activities deriving from the Conventions and Directives mentioned above that seek to provide tools for effective management of the seas is known as “governance” (DiMento and Hickman, 2012). More specifically Lemos and Agrawal (2006) define governance as “the set of regulatory processes, mechanisms and organizations through which political actors influence environmental actions and outcomes”. It must be emphasized that in environmental issues there is not a formal definition for governance yet. However, since global governance (Birnie et al., 2009) has been defined as a “continuous process through which conflicting of diverse interests may be accommodated and cooperative action may be taken. It includes formal institutions and regimes empowered to enforce compliance as well as informal arrangements.”

The main objectives of the present work are: (a) to present the eutrophication problem in the European Regional Seas (b) to present the collection of regimes, initiatives and activities of the European Regional Seas that have been designed to provide effective management in mitigating marine eutrophication and (c) to discuss the effectiveness of measures in maintaining good environmental status by reducing nutrient loads in the marine environment.

2. Definitions of Eutrophication

Although the definition of eutrophication seems to be a fairly simple matter, referring to increased nutrient loads mainly of phosphorus and nitrogen, it is a long going story that still continue. The first definition of eutrophication appeared in literature in 1919 for lakes by Naumann: eutrophication was then simply defined as “the increase of nutritive substances in a lake especially phosphorus and nitrogen” (Hutchinson, 1967). The term eutrophication is of Greek origin: “eu” means “well” and “trophi” means “nourishment” (Karydis, 2009). From the etymological point of view eutrophication can be “the process of changing the nutritional status of a given water body by increasing the nutrient resources” (Jørgensen and Richardson, 1996). However, the first widely accepted definition of eutrophication was given much later by Steele (1974): “eutrophication is the increase of the growth rate of algae, following a faster rate of nutrients in the marine environment as well as the consequences”. It must be noticed that it was the first time that “consequences” of eutrophication were mentioned in the definition. These consequences were “specified” in the definition of eutrophication given by Vollenweider in 1992: “eutrophication – in its more generic definition that implies to both fresh and marine waters – is the process of enrichment of waters with plant nutrients, primarily nitrogen and phosphorus that stimulates aquatic primary production and its more serious manifestations lead to visible algal blooms, algal scums, enhanced benthic algal growth of submerged and floating macrophytes” (Vollenweider, 1992). The emphasis of Vollenweider’s definition is on the impact of eutrophication in the environment and the most common effects are mentioned. Gray (1992) has given a shorter definition focusing on the biological aspects of eutrophication: eutrophication occurs “when nutrients are added to the body of water they load, provided they are not toxic compounds and provided that there is sufficient light to increased autotroph growth and also to increased heterotroph growth”. A short definition of eutrophication given by Nixon (1995) was the turning point in defining eutrophication as it did not mention causes-effects and focused on eutrophication as a process leading to biomass production: “eutrophication is an increase in the rate of supply of organic matter to an ecosystem.”

In addition to the definition given by individual scientists, international bodies have defined eutrophication focusing on practical aspects. According to the European Environmental Agency (EEA, 2001), “eutrophication means enhanced primary production due to excess supply of nutrients from human activities independent of the natural productivity level for the area in question”. The term “excess” has to be defined in every case study to provide a basis for data evaluation and characterization of the water body. However, the definition of eutrophication varies in European Union Directives: the Urban Waste Water Treatment (UWWT) Directive places emphasis on nitrogen and phosphorus enrichments: “the enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the water balance of organisms present in water and to the quality of the water concerned” (EC, 1991a). The same definition was also provided by the Directive on pollution of nitrates from agricultural sources (EC, 1991b). According to UNEP (2003) “eutrophication is defined as an environmental disturbance caused by excessive supply of organic matter”. Within the OSPAR Convention (OSPAR, 1998) an ad hoc definition of eutrophication is given (Appendix 1) explicitly “for the purpose of the strategy”: “eutrophication means the enrichment of water by nutrients causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned and therefore refers to the undesirable effects resulting from anthropogenic enrichment by nutrients”. It must be noticed that all the above definitions set by international authorities use the term “disturbance” to summarize the adverse effects from excessive nutrient loading. This characterization means in practical terms that these authorities consider eutrophication as a reversible process which implies in turn that remediation is less costly than “pollution”. The Marine Strategy Framework Directive MSFD although it does not provide a formal definition of eutrophication, it refers directly (Annex I5) to the impacts: “especially adverse effects thereof, such as losses of diversity, ecosystem degradation, harmful algal blooms and oxygen deficiency in bottom waters.”

3. Classification Schemes of Marine Eutrophication

The classification of water masses into trophic levels, oligotrophic, mesotrophic, eutrophic and hypertrophic has been an issue in the quantification of eutrophic impacts since the 1970s. The various classification schemes proposed so far have been based on cause (nutrient) or effect variables (chl-*a*, primary productivity) variables, on bioindicators used for coastal management (cyanobacteria and macroalgal species) or on ecological / environmental indices (Karydis, 2009). In spite of the difficulties encountered in classifying water masses according to trophic conditions, classification schemes are rather useful because they support communication between scientists, water managers, policy makers and the public using objective criteria (Hakanson, 2006; Kitsiou and Karydis, 2011). The shortcomings in scaling are due to a number of reasons (a) it is sometimes difficult to define baseline concentrations that is chl-*a* and nutrient concentrations occurring in pristine waters. The pristine state refers to unimpacted or almost unimpacted environments. The definition of pristine state is “the insignificant impact of pressures on ecosystem functioning and thus approximation of the natural environment” (Thieu et al., 2010). However, pristine sea water conditions are unrealistic in Western European Countries. This is because in many areas field measurements and monitoring projects usually start after the phase of “problem recognition” (Nielsen et al., 2007). There are no time series of data to get an idea of the initial conditions. Scientists to get over this problem use mass balanced models so they can get some indications on historical changes (Larsson et al., 1985) (b) as the variables measured (nutrients, chlorophylls) are system’s variables, it is difficult to partition the origin of nutrient concentrations into natural and anthropogenic (c) nutrient and phytoplankton dynamics is sometimes particularly intense in terms of seasonality.

Seasonal trends result into an overlapping between variable distributions from water masses of different trophic status (Ignatiades et al., 1992; Giovanardi and Tromellini, 1992) (d) indigenous eutrophication: some areas due to their topography (shallow basins), water circulation and sediment composition may show eutrophic trends even without any form of anthropogenic influence. The points mentioned above indicate that there is not a universal scale characterizing eutrophication.

Many indicators have been used over the last thirty years to characterize eutrophic conditions. According to Ferris and Humphrey (1999): “as indicator may be defined a characteristic that when measured repeatedly demonstrates ecological trends and a measure of current state quality in an area”. As an indicator requires robustness in environmental fluctuations, scientists tend to use complex indices incorporating a number of variables. This is the case with the multimetric TRIX index (Vollenweider et al., 1998; Primpas and Karydis, 2011). Ecological indices have also been used in eutrophication studies though only a limited number is suitable to quantify eutrophic conditions (Karydis and Tsirtsis, 1996; Tsirtsis and Karydis, 1998; Karydis, 2009). Shannon’s index can be an example because, in spite of the popularity this index is enjoying in ecology (Digby and Kempton, 1987), it does not respond to different trophic levels (Karydis and Tsirtsis, 1996).

Table 2 shows eutrophication scaling based on nutrient / chlorophyll concentrations and primary productivity rates. In addition, Secchi disk measurements have been used for characterizing eutrophic status. Hakanson (2006) has proposed a transparency scale for the Baltic Sea.

Secchi disk depth greater than 11 m characterizes oligotrophy, 6-11 m mesotrophy, 2-6 m eutrophic conditions and less than 2 m hypertrophic conditions. According to ICES chl-*a* concentrations is a useful indicator for characterizing eutrophic conditions and should be included in the suite of indicator variables (OSPAR, 2005c). Chlorophyll has also been proposed by the Task Group 5 of the MSFD as a main indicator to assess eutrophication status in European waters (Ferreira et al., 2011).

Scaling on chl-*a* concentrations showed generally high concentrations for the Baltic Sea (Wasmund et al., 2001; Hakanson, 2006) whereas the chl-*a* concentrations proposed for a scale in the Mediterranean (Ignatiades, 2005) accounted for 10-25% of the chlorophyll concentrations in the Baltic for the same level of eutrophication. The same trends were observed with primary productivity measurements (Nixon, 1995; Wasmund et al., 2001; Ignatiades, 2005).

Table 2. Eutrophication classifications schemes based on chl-α, Primary Productivity values and nutrient concentrations

Scale	**Oligotrophic waters**	**Mesotrophic waters**	**Eutrophic waters**	**Hypetrophic waters**
(a) Chl-α concentrations (μgl^{-1})				
Hakanson's (2006) scaling (1)	<2	2-6	6-20	>20
Wasmunds' et al. (2001) (2)	<0.8	0.8-4	4-10	>10
Ignatiades' scaling (2005) (3)	<0.5	0.5-1.0	>1.0	
Karydis' scaling (2009) (4)	<0.1	0.1-0.4	0.4-2.21	>2.21
(b) Primary Productivity				
Nixon's (1995) scaling (5)	100 gCm^{-2}y^{-1}	100-300 gCm^{-2}y^{-1}	300-500 gCm^{-2}y^{-1}	>500 gCm^{-2}y^{-1}
Wasmunds's scaling (2001) (2)	<100 gCm^{-2}y^{-1}	100-250 gCm^{-2}y^{-1}	250-450 gCm^{-2}y^{-1}	>450 gCm^{-2}y^{-1}
Ignatiades's (2005) scaling (3)	<1.5 mgCm^{-2}h^{-1}	1.5-3.0 mgCm^{-2}h^{-1}	>3.0 mgCm^{-2}h^{-1}	
(c) Nutrients (μM)				
Wasmunds's scaling (2001) (2)	PO$_4$: <0.2 DIN: <2.0	PO$_4$: 0.2-0.8 DIN: 2-10	PO$_4$: 0.8-3.0 DIN: 10-60	PO$_4$: >3.0 DIN: >60
Ignatiades's scaling (1992) (6)	PO$_4$: <0.02 NO$_3$+NO$_2$: <2.0	PO$_4$: 0.02-0.09 NO$_3$+NO$_2$: 0.09-0.33	PO$_4$: >0.34 NO$_3$+NO$_2$: 0.33-0.52	PO$_4$: >0.34 NO$_3$+NO$_2$: >0.52

(1) Hakanson (2006), (2) Wasmund et al. (2001), (3) Ignatiades (2005), (4) Karydis (2009), (5) Nixon (1995), (6) Ignatiades et al. (1992)

4. Marine Monitoring and Data Analysis

A wide spectrum of data analysis methods have been applied for processing and analyzing sets of data to assess marine eutrophication: statistical methods, remote sensing, spatial analysis, mapping, modeling and multiple criteria analysis are among the most widely used methods for quantitative assessment of eutrophication. Data sets are acquired during marine monitoring surveys which should be properly designed to provide the maximum information about the study area for the available resources (Karydis aand Kitsiou, 2013). Table 3 shows the variables used as descriptors for the quantitative assessment of eutrophication. In EPA (2000) a presentation of different types of sampling designs is presented along with guidance on how to organize the most appropriate sampling design depending on the set objectives. As science is the basis for the management of the marine environment, most international conventions for the protection of the marine environment include a monitoring component for data collection.

Table 3. Variables used for eutrophication assessment (UNEP 2003a)

Physical variables	**Chemical Variables**	**Biological variables**
Temperature (˚C)	pH	Chlorophyll *a* (μgL^{-1})
Transparency	Orthophosphate (μg-at PO_4l^{-1})	Phytopl. Abundance (cellsL^{-1})
Salinity (psu)	Total phosphorus (μg-at Pl^{-1})	Phytopl. Major groups (cellsL^{-1})
Dissolved oxygen (mLL^{-1})	Silicate (μg-at SiO_2L^{-1})	Phytopl. dom. species (cellsL^{-1})
	Nitrate (μg-at NO_3L^{-1})	
	Nitrite (μg-at NO_2L^{-1})	
	Ammonium (μg-at NH_4L^{-1})	
	Total Nitrogen (μg-at NL^{-1})	

One- and multi-dimensional statistical analysis methods are widely applied to detect impacted sites and assess marine eutrophication trends. One-dimensional statistical analysis includes: (a) Descriptive statistics: it summarizes data sets and estimates central tendency and variability. It is considered as the first step in data handling before the application of any further analysis. (b) Detection of outlying values that characterize extreme conditions referring to eutrophic levels (Karydis and Ignatiades, 1992; Karydis 1994) (c) Analysis of Variance (ANOVA) to test the effect of one or more factors to the trophic status of sampling stations. ANOVA is a powerful statistical tool provided that there is no violation of the statistical assumptions and the method is not abused (Kitsiou and Karydis, 2011). An excellent review on the application of ANOVA techniques in marine biology and ecology has been given by Underwood (1981) (d) Correlation and regression to consider possible association among the variables related to marine eutrophication (Estrada et al., 1993; Ignatiades et al., 1985).

Multi-dimensional statistical analysis is often applied in marine eutrophication studies due to the multi dimensional character of environmental analysis. The textbook by Legendre and Legendre, (2003) on numerical ecology is a wide-ranging presentation of multivariate methods in environmental applications Mutli-dimensional methodology includes: (a) Principal Component Analysis (PCA): it is mainly used to reduce the dimensionality of the initial data set (Gauch, 1989; Primpas and Karydis, 2010), (b) Cluster Analysis (CA) to assess the resemblance between sampling sites based on the measured variables and their grouping according to their similarity (Everitt et al., 2001; Kitsiou and Karydis, 2011) (c) Discriminant Analysis (DA) to classify sampling sites in groups based on several variables (Tsirtsis and Karydis, 1999). The outcome of a multivariate statistical application can be used either as the "end product" of a data analysis procedure or as the input for more complex computations and further analysis, such as multiple criteria choice methods, remote sensing techniques and spatial analysis.

The contribution of remote sensing to assess eutrophication trends is important since observations, especially at large spatial scales, are not always possible if they are based only on “ground” measurements. Assessment of eutrophication based on processing and analysis of satellite data from various sensors focuses on the estimation of abundance of phytoplankton chlorophyll in the seawater to provide surface patterns of phytoplankton biomass distribution at a large spatial scale (Oguz et al., 2002; Garcia et al., 2005). Several studies are found in literature where data sets acquired from optical and radar satellite images are used for the analysis of ecological conditions in the marine environment and the trophic status (Andreeva, 2013).

Spatial analysis methods are the means for the description of the spatial data structure and the heterogeneity of data sets. Their application can be performed in the framework of a GIS where accurate spatial databases can be developed and analysis of data sets can be carried out (Jankowski, 2009). In marine eutrophication studies, spatial pattern analysis which refers to the study of spatially continuous phenomena is applied to study the degree of variation of the data collected through sampling at specific sampling sites (Bagheri et al., 2012). Correlograms and variograms are commonly used for the study of spatial patterns, the detection of anisotropies and the determination of the degree of homogeneity in sea water (Vázquez et al., 2005) in order to assess different trophic levels. The spatial structure of variables related to marine eutrophication can be visualized via maps. The creation of surfaces from irregularly spaced point data is achieved by application of interpolation methods based on the conversion of the fragmented information collected during sampling surveys (Burrough and McDonnell, 2000). Further classification of sea water masses according to their trophic level is often performed to the production of thematic maps based on specific eutrophication scales.

In marine eutrophication studies, various models can be found in the literature which have been developed and applied to simulate the interactions among physical, biogeochemical and biological processes and to predict the response of marine ecosystems to stresses related to anthropogenic and physical origins (Lessin et al., 2009; Soetaert and Middelburg, 2009). Zaldivar et al. (2009) proposed a model for the prediction of regime shifts in shallow ecosystems and the definition of threshold levels that cause the regime shifts. The level of detail describing the process of eutrophication differs in the currently used models. In addition, several limitations are reported of integrated models, as far as their degree of integration and their capability of performing efficiently at a variety of conditions. In marine eutrophication studies, it is not realistic to create a single model that will address with accuracy all the interactions and variability in the marine environment (Tett et al., 2003); therefore modeling efforts should focus on rather partial approaches. This way a number of stresses represented by specific variables and the impact the trophic status of sea water will be assessed (Passy et al., in press).

Multiple Criteria Analysis (MCA) aims at the ranking of a number of hypotheses or scenarios (alternative choice possibilities) based on multiple criteria and conflicting objectives (Nijkamp and Voogd, 1986; Kitsiou and Karydis, 2011). MCA methods have been applied for assessing marine eutrophication trends (Moriki and Karydis, 1994; Kitsiou et al., 2002; Ananda and Herath, 2009) and seemed to be effective in discriminating trophic levels in the marine environment. Criteria can be both metric and non-metric and their selection is of high importance for the evaluation of the results. In addition, the assignment of weights to the criteria makes possible the representation of their relative importance, either in cardinal or ordinal units. Extended studies on marine eutrophication (Duarte, 2009; Lloret et al., 2008) have indicated that marine eutrophication should be considered as a component of global change; it is therefore advisable to take into account interactions with the various system components that is, pressures, impacts, socio-economic factors as well as possible effects deriving from the climatic change. As management and policy making takes into account environmental, social and economic aspects of eutrophication, integrated approaches are strongly recommended (Cloern, 2001; Duarte, 2009). A relatively recent review article (Kitsiou and Karydis, 2011) presents a wide range of data analysis methods used for assessing eutrophication and discusses their potentiality and possible shortcomings.

5. The Framework

5.1. The UNCLOS

The Geneva Convention on the law of the Sea in 1958 (UNCLOS, United Nations Convention of the Law of the Sea) did not give special importance on environmental issues (Birnie et al., 2009). Only articles 21 and 25 of the High Seas Convention mentioning the need of the signatory parties to protect the marine environment from oil pollution, oil pollution sources such as ships, pipelines and seabed operations were explicitly included. In addition, the High Sea Convention required from the states to prevent radioactive pollution. As the need for a more detailed legal framework covering a wide range of aspects of the marine environmental protection emerged, a number of regional agreements were signed for the protection of the marine environment of regional seas. Table 4 shows the main international conventions and protocols concerning marine environmental protection.

5.2. UNEP: The Voice of the Marine Environment

The core of this effort was UNEP's regional sea programme initiated in 1974. UNEP was the main environmental component of UN and was described as "*the voice for the environment within the United Nations System*" (Desai, 2006). The Barcelona Convention for the Protection of the Mediterranean Sea is the oldest of the UNEP's Regional Seas Agreements (Frank, 2007). Apart from the Mediterranean Sea, UNEP's Regional Seas Programme includes the Black Sea, the West and Central Africa Seas as well as the Seas of the Wider Caribbean Region. Although some Regional Sea's programs are not administered by UNEP (DiMento and Hickman, 2012), they are still coordinated by UNEP but run separately. These include the North East Atlantic Region (the OSPAR Commission's area of responsibility includes the North Sea) and the Baltic Sea. In spite of the fact that UNEP is only a program, it has acted as a catalyst in coordinating environmental programs within the United Nations System than initiate action itself. Among UNEP's activities is the promotion of treaty implementation, coordination of a number of treaty secretariats as well as the coordination of meetings among the contracting parties and research teams. Above all UNCED (United Nations Conference on the Environment and Development) has called on UNEP to promote cooperation for policy making, monitoring and assessment and give emphasis on environmental impact assessment, promotion of regional cooperation and dissemination of information.

Table 4. International conventions and protocols for implementing eutrophication policy

Legal Documents	**Main objectives**	**Objectives related to eutrophication**
Barcelona Convention	Pollution reduction and environmental protection in the Mediterranean	Mitigation of nutrient loads from terrestrial sources
The Mediterranean Action Plan (MAP)	Sustainable Development and Integrated Coastal Management	Monitoring of nutrients and phytoplankton
OSPAR	Reduction of toxic, persistent and bio-accumulating substances. Reduction of nutrient discharges	Reduction of nutrients by 50%. Monitoring programs.
HELCOM	Pollution mitigation in the Baltic	Nutrient reduction in all sectors including industry, municipal waste water and agriculture
The Gothenberg Protocol	Abatement of acidification, Eutrophication and Ground-level Ozone	Emphasis on the role of nitrogen oxides and ammonia in eutrophication
The Convention of Long Range Transboundary Air Pollution (CLRTAP)	Measures to mitigate ozone, smog and other airborne pollutants. Monitoring and modeling transport and deposition of air pollutants. Impact studies	Monitoring and modeling of airborne reactive nitrogen. Impact assessment at ecosystem level
The Sofia Protocol	Environmental Impacts of sulfur emissions. Stabilization of NO_x emissions	

5.3. The Barcelona Convention

The Barcelona Convention for the Protection of the Mediterranean Sea against pollution (BARCON) adopted in 1976 and entered in force in 1978 is chronologically the first of the Regional Sea's agreements under the auspices of UNEP (Frank, 2007). The objective of the Convention is to "reduce pollution in the Mediterranean Sea, protect and improve the marine environment in the area, thereby contributing to its sustainable development". The operational tool of BARCON is the Mediterranean Action Plan (MAP). Among MAP's objectives was "to promote the conclusions of a framework convention, together with related protocols and technical annexes for the protection of the Mediterranean Environment" (DiMento and Hickman, 2012). Later in 1995 the MAP was replaced by the "Action Plan for the Protection of the Marine Environment and the Sustainable Development of the Coastal Areas of the Mediterranean (MAP Phase II)". The present Barcelona legal framework is accompanied by seven protocols adopting measures against pollution from Land Based Sources, from Ships and Aircraft, from oil and other harmful substances, from pollution resulting from Exploration and exploitation of the Continental Shelf, the Seabed and its subsoil. The last protocol is devoted to Integrated Coastal Zone Management in the Mediterranean. Land Based Sources include all types of discharges into the marine environment including nonpoint pollution through runoff. Discharges of terrestrial origin include also all sorts of sources containing nutrients. These include municipal waste, agricultural activities (fertilizers and manure handling) and certain types of industrial effluents. Some of those discharges reach the marine environment through the rivers.

5.4. The OSPAR Convention

Semi enclosed areas of the North-East Atlantic such as the North Sea and the Irish Sea require special protection due to socioeconomic factors exercising environmental pressures in the area. Inflows from many European rivers like Elbe and Rhine carry nutrients and toxic compounds into the marine environment. In addition, shipping traffic, very intense in the area, as well as oil rigs on the continental shelves of Denmark, Germany, the Netherlands, Norway and the United Kingdom form a source of pollution for the North Sea.

The Convention for the protection of the Marine environment of the North-East Atlantic, known as the OSPAR Convention was the response of the North Sea States to the environmental threats in the area. The Convention was signed by fifteen contracting parties in 1992 entering into force in 1998; it replaced the Oslo Dumping Convention (1972) and the Paris Convention (1974) on Land Bases Pollution. The OSPAR Convention establishes a framework for the protection of the marine environment from all sources causing degradation of the marine environment excluding fishing, atmospheric and vessel sources of pollution because they are regulated by the existing legislation. Contracting parties are "*required to take measures*" (Article 2.1a) to "*eliminate marine pollution*" and "*any other effects of human activities*" on the marine environment including ecosystems and to restore wherever possible impacted areas (OSPAR, 2007).

Policy on eutrophication is explicitly stated in the Convention: "The Contracting parties to the Convention for the Protection of the Marine Environment of the North-East Atlantic adopt the following objective and strategy for the purpose of directing the work of the Commission with regard to combating Eutrophication". Objective 1.1 refers exclusively to eutrophication: "In accordance with the general objective, OSPAR's objective with regard to eutrophication is to combat eutrophication in the OSPAR maritime area, in order to achieve and maintain a healthy marine environment where eutrophication does not occur". The strategy to combat eutrophication will comply with the following principles: (a) the precautionary principle (b) preventive actions (c) rectification of the environmental damage at source if possible and (d) the polluter should pay.

5.5. The Helsinki Convention

The Baltic Sea is particularly vulnerable to pollution due to limited communication of the Baltic with the North Sea through the Skagerrak and Kattegat Straits. The Helsinki Convention (1974), after two decades of operation did not succeed in controlling marine degradation in the Baltic Sea (Fitzmaurice, 1998). Failing to restore good environmental status the Baltic States decided to implement new approaches and strengthen the legal regime. The new Helsinki Convention was entered into force in the year 2000. There are numerous similarities with the OSPAR Convention: among the objectives (Articles 1 and 5) the "*ecological restoration*" of the Baltic, the "*elimination of pollution*" and the reduction of "*adverse impacts of human activities*" are included. The major sources of pollution are nutrients, hazardous substances and maritime activities. HELCOM is the first Convention for the marine environment where eutrophication has received specific attention (Boesch et al., 2000). A Commission is responsible for the implementation of HELCOM. Annual meetings attended by senior officials from environmental ministries of the member states, accompanied by national experts and representatives of the European Commission (DG ENV) assess the status of the environment (Frank, 2007).

HELCOM is the first international convention with specific strategic goal the mitigation of marine eutrophication (de Jong, 2006). The ecological objectives related to eutrophication include nutrient concentrations close to natural levels, water of high transparency, natural oxygen levels, natural levels of algal blooms and natural distribution of plants and animals (HELCOM, 2007). Land based sources of nutrients are of industrial, municipal and agricultural origin. Following the EU agricultural policy trends, enlargement of the agricultural sector is expected over the next few years and therefore the efforts should be particularly focused on agriculture. Annex III of the Convention declares that "contracting parties shall endeavor to attain the goals and apply the criteria and measures enumerated in this Annex in order to control and minimize land based pollution". Nutrient fluxes could be addressed by minimizing nutrient fluxes from agricultural systems (animal feeding, manure handling, crop cultivation practices) and Member States had agreed to a quota on nutrient reductions (Table 20).

5.6. The Bucharest Convention

The Convention for the Protection of the Black Sea Against Pollution (BSC, 2009) was signed in Bucharest in 1992 by six states (Bulgaria, Georgia, Romania, Russian Federation, Turkey, Ukraine) and came into force in 1994. It is also known as the "Bucharest Convention" and forms the basic framework of agreement and three specific protocols: (a) the control of land-based sources of pollution (b) dumping of waste and (c) joint action in the case of accidents (such as oil spills). The main objective is "to substantiate the general obligation of the Contracting Parties to prevent, reduce and control the pollution in the Black Sea in order to protect and preserve the marine environment and to provide legal framework for co-operation and concerted actions to fulfill this obligation."

5.7. The European Legal Framework for the Marine Environment

Although Europe is surrounded by seas i.e., the Mediterranean Sea, the Black Sea, the Baltic Sea, the North Sea and the Irish Sea, EEC started action on environmental policies in 1972. An Environmental Action Plan (EAP) was adopted in the summit of the heads of the nine European States and covered the period 1973-1974 (de Jong, 2006). The objectives of

EAP aim at preventing, eliminating and reducing pollution. In addition to the EAP, it was recognized that "of the forms of pollution, marine pollution is undoubtedly one of the most dangerous due to the effects it has on fundamental biological and ecological balances" (Johnson and Corcelle, 1989). The community legislation is set at the level of frameworks so that they would be helpful "in particular of coordinating and harmonizing the rules for implementing international conventions and of implementing projects to combat land-based marine pollution". A number of Directives on the protection of various aspects of the marine environment have been produced by the EU. Directives with an eutrophication component are presented in Table 5. However, it has been supported the view that "*the protection of the marine environment has traditionally played a secondary role within the EC law*" (Kramer, 1997). The protection of the marine environment was implemented through different environmental policies until the 90's. The first common water policy (EU, 2000) that is the Water Framework Directive (WFD) was mainly focusing on freshwater bodies: the WFD 2000/60/EC extends to coastal waters up to one nautical mile from the baseline (Article 27). It is only after the sixth EAP which set the guidelines for the period 2001-2010 that emphasis was placed on the protection of the marine environment. The emphasis was placed on the need to understand the marine environment and ecosystems so as to minimize the impact from human activities and implement a sustainable management policy (Frank, 2007). European legislation on environmental issues although it contributes to an integrated European Legal Framework and encourages the conclusion of "pure" EU agreements as the most effective tool to harmonize common objectives, causes problems when EU Member States have signed multilateral agreements including states that are non-EU members. The BARCON, OSPAR and HELCOM are good examples of the phenomenon known as good examples of what is known as "mixity" (Frank, 2007).

European Union has adopted a number of Directives aiming on environmental protection of the sea but they were all related to specific aspects of the marine environment. These include the Urban Waste Water Treatment Directive (UWWT), the Nitrate Directive, the Habitat Directive, the Shellfish Directive, the Bathing Water Directive and the Water Framework Directive (Karydis and Kitsiou, 2012). However, it was considered that the marine environment as a precious heritage should be protected through a framework aiming at an overall protection of marine ecosystems and taken into account all human activities that have an impact on the marine environment. In 2008 a Directive on Marine Strategy was adopted to encourage development of strategies and measures for good environmental status (EC, 2008).

The MSFD also integrates environmental objectives of the Directives mentioned above as well as the Directive on Biological Diversity (EEC, 1993). It is stated explicitly in the MSFD Directive that the framework should "contribute to coherence between policies and foster the integration of the environmental concerns into other policies such as the Common Fisheries Policy, the Common Agricultural Policy and other relevant Community Policies" (paragraph 9 of the introductory section of the MSFD. There is also a strong concern for the conservation of biological diversity, mitigation of biodiversity loss and sustainable use of marine biodiversity.

Table 5. EC Directives encompassing a eutrophication component

Law / European Union Directive	**Main objective(s)**	**Objectives related to eutrophication**
The Urban Waste Water Directive (91/271/EEC)	To protect the environment from the adverse effects of waste water discharges	The Directive is targeting to reduce phosphorus by 80% and 70-80% for nitrogen
The Nitrates Directive (91/676/EEC)	Protection of waters against pollution caused by nitrates	Member States should define sensitive and vulnerable areas where good agricultural practices will be implementing in order to protect coastal waters from eutrophication
Water Framework Directive (2000/60/EC)	To achieve “good water status” for all European waters by 2015	Prevention and elimination of pollution of the marine environment with the “ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances”
The Marine Strategy Framework Directive (2008/56/EC)	“The directive establishes a framework” and is “designed to achieve good environmental status in the marine environment”	According to the Task Group 5 on eutrophication “Human induced eutrophication should be minimized, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen efficiency in bottom waters”

The MSFD also recognizes the specific characteristics i.e., specific hydrographic, oceanographic and biological features of the European Regional Seas and has therefore recognized four marine regions (Article 4): (a) the Baltic Sea (b) the North East Atlantic Ocean (c) the Mediterranean Sea and (d) the Black Sea. In particular the Mediterranean Sea is divided into four subregions: (a) the Western Mediterranean Sea (b) the Adriatic Sea (c) the Ionian and Central Mediterranean Sea (CMED) and (d) the Aegean – Levantine Sea (EMED). The overall target is the "*Good Environmental Status*" (GES) in European marine waters by 2021 (Article 1).GES means that marine waters should be clean, healthy and productive within their intrinsic conditions. The MSFD does not explicitly define GES but relies on the Member States to develop appropriate criteria for their marine waters which will be developed by the Commission in a second stage. For each marine region or subregion Member States are required to develop "*Marine Strategies*" aiming at integrated management of all human activities (Article 4). Each marine strategy plan should contain (a) assessment of the current state (b) environmental impact of human activities (c) a definition of GES (d) clean environmental targets and (e) monitoring programs. Monitoring and assessment are fundamental operational tools and it is required by the Member States to standardize the methods of monitoring. Monitoring programs should be compatible within marine regions and subregions. Transboundary forms of pollution should also be taken into account (Article 11).

The MSFD encourages also public participation of existing management bodies or structures responsible for Regional Seas Conventions, Scientific Advisory Bodies and Regional Advisory Councils (Article 19). In addition, results from monitoring programs should be made available to the European Environment Agency (EEA) and evaluation reports should be published (Article 20). Although the MSFD is a holistic approach on the marine environment, eutrophication is considered as a priority issue (ECOSTAT, 2004). In Annex I of the MSFD is stated that "Human – induced eutrophication is minimized, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms and oxygen deficiency in bottom waters". This means that information on the structure and function of the ecosystem is required at least for phytoplankton. Nutrient enrichments are mentioned among pressures and impacts (Annex III): "(i) inputs of fertilizers and other nitrogen – and phosphorus rich substances (e.g., from point and diffuse sources, including agriculture, aquaculture and atmospheric deposition) and (ii) inputs of organic matter (e.g., sewers, mariculture and riverine inputs)". Eutrophication has been characterized by the European Environmental Agency as a major environmental problem in Europe (EEA, 1995).

The problem of eutrophication as an environmental dimension in marine water quality appears in a number of EU Directives as mentioned above. The Urban Waste Water Directive (EEC, 1991a) aimed at reducing nutrients in surface waters, in particular nitrates and phosphates from urban waste water to mitigate eutrophication (Kramer, 2000). The Directive applies to both domestic and industrial waste water. The Directive required that by the year 2005 all agglomerations with a population of more than 2,000 people should have sewerage systems. In addition, Member States had to assign sensitive areas characterized by eutrophic conditions (more than 50 mg of nitrates per liter) and apply secondary treatment. The Nitrates Directive (EEC, 1991b), was adopted in 1991 to protect waters against eutrophication due to nitrates. The term "waters" includes the coastal marine environment. It is the first Directive that provided measures against impacts from agricultural activities (Kramer, 2000). Among the measures that should be taken was the necessity of prohibiting the use of certain fertilizes on a seasonal basis and restrictions as to the quantity of manure that could be used per acre per year. In addition, Member States had to adopt good agricultural practices to be implemented by the farmers. These practices should make limited use of nitrates. The Bathing Water Directive (EC, 2006) was initially adopted in 1975 but was updated in 2006. Bathing water is a water mass where bathing is authorized or not prohibited and is practiced by a large number of bathers (Kramer, 2000). Although the Directive is mainly concerned with public health and therefore the emphasis is placed in total coliforms and faecal coliforms, eutrophication is indirectly involved as water transparency is desirable and close coordination with the UWWT Directive and the Nitrates Directive is recommended. The Habitat Directive (EEC, 1992) although it focuses on water quality assurance, a prerequisite for the protection of habitats, it includes nutrient control in industrial and municipal effluents. The Water Framework Directive (WFD) refers to nutrient control in catchment areas and coastal marine systems (EC, 2000). The Directives mentioned above aim at different environmental issues and can act only as indirect drivers for reducing nutrient inputs into the marine environment. A list of EU Directives encompassing an eutrophication component is given in Table 4.

6. The European Regional Seas

6.1. The Mediterranean Sea

6.1.1. Physiography and Environmental settings

The Mediterranean Sea has been characterized a typical “evaporation Basin” (Danovaro, 2003). It is a semi-enclosed sea connected with the Atlantic through the Gibraltar Strait 15 km wide (Figure 1). The Mediterranean Sea is also connected with the Black Sea through the Straits of Dardanelles with a maximum width of 7 km and average depth of 55m and the Sea of Marmaras. The connection of the Mediterranean with the Red Sea through the Suez Canal is dated since 1889 (Karydis and Kitsiou, 2012). The length of the Suez Canal is 190 km (including the Bitter Lakes), the average depth being 55 m and the maximum width about 200 m. The Mediterranean Sea is surrounded by Southern Europe, North Africa and the Middle East coastal zones (Figure 1). The basin is 3,800 km wide from east to west, whereas the North to South distance varies, the widest part (about 900 km) being between France and Algeria (EEA, 1999). The maximum depth of the Mediterranean is 5,267 m, located off the SW coast of Peloponese, Greece and the average depth about 1,500. The surface area of the basin is about 2.5 million km^2, that is equivalent to 0.82% of the total area of the world’s oceans whereas the volume is 0.32% of the total ocean volume. Although the Mediterranean Sea is a rather deep regional sea, the Northern Adriatic Sea is rather shallow, the maximum depth being less than 200 m. There are big islands in the Mediterranean Sea interrupting the water mass: Cyprus, Crete and Rhodes in the Eastern part and Corsica, Sardinia and the Balearic Islands in the Western Part. The Island of Sicily is located between the Western and Eastern sub basins of the Mediterranean.

Figure 1. The four Regional European Seas.

Evaporation in the Mediterranean Sea exceeds the freshwater inputs (river outflows and atmospheric deposits) by a factor of three. The water deficit is replenished by surface water inflows from the Atlantic through the Gibraltar strait. As these water masses move eastwards, salinity increases due to evaporation exceeding 39‰ in the Sea of Levantine. This high density water sinks and returns to Gibraltar as a deep water mass. The circulation pattern in the Mediterranean Sea follows an anticlockwise pattern. Vertical mixing occurs for depths varying between 200 and 600 m. The residence time of the water masses in the Mediterranean Sea has been estimated between 80 and 100 years (Turley, 1999).

The Mediterranean marine ecosystem is characterized by high levels of biodiversity, especially along the coastal areas where many endemic species occur (EEA, 1999). Although the volume of the Mediterranean water mass accounts only for 0.32% of the world’s ocean volume, eight per cent of the known marine species live in the Mediterranean (UNEP/MAP, 2009) and the Mediterranean has been characterized as one of the 25 hot spots for diversity globally. However, there is concern about the sustainability of the marine ecosystem: nineteen per cent of the species have been characterized as endangered and one per cent is already extinct at regional level (UNEP/MAP, 2009). In addition to the diversity decrease, landings of fishing have shown a decline after an increase in catches in mid 1980s. Hake, red mullet, sole, sardine, anchovy and blue fin tuna are constantly overfished. The same applies for many demersal fish populations. Overfishing is not the only cause for fisheries decline. Loss of habitats is another serious reason: France has lost two thirds of its wetlands, most of them over the last thirty years and Morocco lost quarter of its wetlands. Coastal development has also contributed to the loss of habitats in many parts of the world (UNEP, 2008). Invasions of alien species also known as allochthonus or exotic species have invaded into the Mediterranean Sea mainly through the Suez Canal. So far about thousand species have been catalogued as alien species (Zenetos et al., 2010). There are several pressures affecting the Mediterranean marine environment: agricultural wastes, airborne particles and river outflows contribute nutrient loads into the sea, pathogens, heavy metals and toxic organic pollutants are the main sources of disturbance to the marine Mediterranean ecosystems (EEA, 1999; Aquarone et al., 2009).

The Mediterranean Sea is the most affected sea by alien species both in terms of number of alien species detected and the high rate of introduction into the Mediterranean (Zenetos, 2010). Table 6 shows species distribution in the four subregions of the Mediterranean. There is a gradient from EMED to WMED due to the fact that most species come from the Red Sea (Leseptian species). The general feeling is that in spite of the experts work the number of alien species has been underestimated. This is the case mainly with unicellular species where detection, classification and documentation are particularly difficult. Excluding microalgae, alien species have increased species richness in the Mediterranean by 5.9%.

Table 6. Number of alien species in the Mediterranean subregions

Subregion	Alien species	% of the total number (955)	Invasive species
EMED	718	88.4	108
WMED	328	72.8	64
CMED	267	59.3	75
Adriatic	171	56.1	53

Zenetos et al., (2010).

In general Mediterranean marine ecosystems are characterized by an idiosyncratic combination of features that differentiate them from the Northern European Conditions. Critical points accentuating the impact of pollutants in the coastal systems of the Mediterranean are (Danovaro, 2003): (a) high temperatures: the annual temperature range of surface waters varies between 12 and 25˚C. This high temperature regime enhances metabolic rates (b) microtidal regime: the restricted tidal range (up to 50 cm) limits the potential for dilution and dispersion of nutrients from urban waste and agricultural runoff (c) oligotrophy: high water transparency due to the oligotrophic character of the Mediterranean Sea allows photosynthetic activities in deeper water masses (Ignatiades, 1998) (d) Primary Production: nitrogen is the limited factor in primary production in the world oceans (NRC, 2000). However, the limited nutrient in the Eastern Mediterranean is phosphorus (Krom et al., 1991);

in this case eutrophication in EMED depends on phosphorus availability (EEA, 1999) (e) freshwater inputs: limited freshwater inputs combined with high evaporation rates result into low turnover rates. Table 7 shows the drainage area and the average water fluxes of the ten largest rivers of the Mediterranean Sea (f) biodiversity: the Mediterranean Sea is characterized by high biodiversity and a high proportion of endemic species. Benthic assemblages are characterized by low abundance and biomass due to the oligotrophic character of the basin (Karakassis, 2001; Karakassis and Eleftheriou, 1997) and (g) anthropogenic pressure: about 380 million people live around the Mediterranean basin and more than 100 million tourists (EEA, 1999) visit the area every year. Both numbers are characterized by an upward trend. This population leaves many different types of end-products and most of them end, directly or indirectly, into the marine environment.

Table 7. Water discharges of the major rivers in the Mediterranean

River	**Catchment area (km^2)**	**Mean flow (m^{3}s^{-1})**
Ebro	80,093	426
Rhone	98,000	2,300
Po	74,000	1,540
Tiber	17,375	267
Adige	12,100	200
Neretva	10,380	341
Drin	15,540	222
Evros	53,000	
Atlantic		
Atlantic Ocean inflow – outflow = 1,700 km^{3}y^{-1}		
Black Sea Inflow – Outflow = 164 km^{3}y^{-1}		

Source Ludwig et al., 2009

6.1.2. Environmental Pressures and Eutrophication in the Mediterranean

Land based sources of marine eutrophication in the Mediterranean are partitioned into urban sewage (50%), industrial waste (25%) and agriculture (25%). Nitrogen and phosphorus are transported by the runoff water into the sea either in dissolved form (nitrogen) or absorbed by suspended matter. Intensive methods of cultivation are applied to agriculture since 1960 causing an upward trend in the consumption of fertilizers (UNEP, 2003b). The annual minimum agricultural load of nutrients into the Mediterranean water (excluding Croatia, Egypt, Libya, Malta and Slovenia) is estimated to be about 1.6 million t/y^{-1} for nitrogen and 0.8 million t/y^{-1} for phosphorus (EEA, 1999). The contribution of aquaculture to marine eutrophication, although so far is limited and localized (EEA, 1999) showed an increase: a fish production of 78,000 t/y^{-1} in 1984 increased to 248,000 t/y^{-1} in 1966. The nitrogen load due to fish farming activities is about 1.8 millions t/y^{-1} and the phosphorus load 0.21 millions t/y^{-1}. Sewage pollution is another source of eutrophication in the Mediterranean. The existing data are not reliable since information from relatively small cities with population between 10,000 and 100,000 inhabitants is scanty. However, data from 18 Mediterranean countries (UNEP, 1999) provide a rough estimate of nutrient outflows about 250,000 t/y^{-1} for nitrogen and 75,000 t/y^{-1} for phosphorus.

The offshore waters of the Mediterranean have been characterized as oligotrophic. Nutrient values in the Mediterranean Sea subregions are given in Table 8. Oligotrophic conditions are extended over a gradient from the WMED (mesotrophic to oligotrophic) to EMED (oligotrophic to ultra-oligotrophic) following the nutrient distribution pattern along the W to E direction. The oligotrophic character of the Mediterranean is also illustrated through the distributions of chlorophylls. Figures 1-4 show that the Eastern Mediterranean basin is more oligotrophic than the Western basin. Coastal areas, especially near big cities and estuaries seem to be eutrophic. The EMED subregion has been characterized as the largest phosphorus limited water body in the world ocean (Krom et al., 1991). The annual phytoplankton productivity in the EMED varies between 60 and 80 $gCm^{-2}y^{-1}$ (Psarra et al., 2000) and it is much less compared to primary productivity rates of other oligotrophic environments such as the Sargasso Sea (125 $gCm^{-2}y^{-1}$) and the Northeast Pacific (120-130 $gCm^{-2}y^{-1}$). Primary productivity in the WMED is a bit higher compared to EMED. Surface water masses from the Atlantic carry nutrients into the Mediterranean at low concentrations but available for photosynthesis (EEA, 1999). Nutrient maxima in the WMED varied for nitrate between 4.00 to 1.87 μM, for phosphate from 0.26 to 0.05 μM whereas chl-*a* concentrations ranged from 0.96 to 0.39 mgm^{-3} and primary productivity from 0.83 to 0.14 $mgCm^{-3}h^{-1}$. Chlorophyll concentrations and primary productivity values are given in Tables 9 and 10 respectively. Diatom species were more numerous in the Western Mediterranean whereas dinoflagellates and coccolithophores dominated in the eastern areas. Observed changes in nitrogen - phosphorus concentrations and N/P ratios in deep water masses in the WMED as well as differences between EMED and WMED indicate that regime shifts have already occurred changing phytoplankton composition and structure throughout the basin (UNEP/MAP, 2009). There is a shift from the diatom – siliceous species to non- siliceous microalgae. This is possibly the reason of many algal blooms and eutrophic events occurred in coastal waters. It is therefore obvious that there are many consequences on fisheries and tourism. In spite of the oligotrophic character of the Mediterranean, the highly populated coastal zone and the runoffs from a draining area of 1.5 million km^2 induce eutrophic trends in the coastal waters. The drainage area of the Mediterranean as a ratio over the sea surface is high; this means that the contribution of rivers to coastal eutrophication is substantial. This explains why areas of high productivity are often located near estuaries (Bosc et al., 2004). River discharges carry nutrients from agricultural activities and urban effluents. The characteristics of the 10 largest rivers in the Mediterranean according to their annual water discharge are given in Table 7. As river water is either used for irrigation of is retained in dams, river fluxes decrease with time. However, nutrient loads increase due to intensified human activities. A steady rate of increase in nitrate fluxes for Rhone, Po and Ebro rivers has been recorded between 1970 and 1990. Nitrogen used in agricultural applications varied between 30 and 70 $kgNha^{-1}$ (Cruzet et al., 1999). The rate of increase of phosphate fluxes into the Mediterranean was double compared to the rate of increase of nitrate fluxes.

Table 8. Nutrient concentrations in the four sub regions of the Mediterranean Sea

Sub-basin	Area	Nitrate (µM)	Phosphate (µM)	N/P ratio	References
WMED	NW Alboran	1.66 - 0.75	0.90 - 2.01	10 - 13	Mercado et al. (2005)
	Balearic Sea	0.07 - 4.40	0.01 - 0.26	18.0	Ignatiades et al. (2009)
	Ligurian Sea	1.7 ± 1.8 (DIN)	0.10 ± 0.20		Pettine et al. (2007)
	Ligurian Sea	5.00	0.15		Coste (1987)
	Gulf of Lions	1.0-2.00			Cruzado & Velasquez (1990)
	Central part	0.08 - 4.59	0.03 - 0.25	18.5	Ignatiades et al. (2009)
	N. Tyrrhenian	4.9±3.3 (DIN)	0.20 ± 0.20		Pettine et al. (2007)
	Sicily (trans. Wat)	0.005 - 4.35	1.8 - 15.2		Caruso et al. (2010)
	Gulf of Naples	0.75	0.09	2.87	Innamorati & Giovanardi (1992)
	Tuscany Coastal waters	2.96	0.39	7.55	Innamorati & Giovanardi (1992)
Adriatic Sea	N Adriatic	8.0±7.9 (DIN)	0.20 ± 0.20		Pettine et al. (2007)
	NW Adriatic	3.07 ± 1.36(*)	0.09 ±0.05(*)	34.4 ± 9.33	Innamorati & Giovanardi (1992)
	Surface Water (winter)	2.3 ± 0.7	0.05 ± 0.02		Sokal et al. (1999)
	Western Coastal	0.3 ± 0.2	0.02 ± 0.02		Zoppini et al. (1995)
	NW Adriatic (Feb)	6.88 ± 0.76	0.12 ± 0.44		Giordani et al. (1997)
CMED	Sicily (trans. wat)	0.2 - 14.3	4.6 - 23.7		Caruso et al. (2010)
	Surface Water (winter)	1.3 ± 1.5	0.05 ± 0.05		Sokal et al. (1999)
	Northern Ionian (surface layer)	0.5 - 1.5		21 - 25	Civitarese et al. (1998)
	Northern Ionian (50 - 200 m layer)	0.5 - 5.0	0.05 - 0.22		Civitarese et al. (1998)

Table 8. (Continued)

Sub-basin	Area	Nitrate (µM)	Phosphate (µM)	N/P ratio	References
EMED	SE Aegean	0.25 ± 0.13	0.05 ± 0.04		Ignatiades et al. (1995)
	NE Aegean	0.05 - 1.6	0.02 - 0.08		Siokou-Frangou et al. (2002)
	S Aegean	0.05 - 2.5	0.02 - 0.06		Siokou-Frangou et al. (2002)
	S Aegean	0.1 -1.9	≈ 0.05	18 - 24	Souvermezoglou et al. (1999)
	SE of Rhodes	0.04 - 1.87	0.01 - 0.05	26.3	Ignatiades et al. (2009)
	Offshore waters	0.006 ± 0.001	0.004 ± 0.002		Aktan (2011)
	Coastal waters	0.040 ± 0.069	0.015 ± 0.009		Aktan (2011)
	South of Cyprus	0.08 - 1.57	0.01 - 0.04	31.0	Ignatiades et al. (2009)

Table 9. Chlorophyll concentrations and phytoplankton abundance in the four subregions of the Mediterranean

Sub-basin	Area	Chl-*a* (mgm^{-3})	Phytoplankton Abundance ($cellsL^{-1}$)	References
WMED	NW Alboran Sea	- 1.60		Ramirez et al. (2005)
	NW Alboran Sea	2.05 ± 0.89		Mercado et al. (2008)
	W Alboran Sea	>1 - 7.9		Arin et al. (2002)
	Balearic Sea	0.08 - 0.96	$1.5x10^2$ - $7.5x10^3$	Ignatiades et al. (2009)
	Balearic Sea	0.63 ± 0.15		Lopez-Sandoval et al. (2011)
	Catalano-Balearic Sea	0.2 - 1.8		Estrada et al. (1993)
	Ligurian Sea	0.7 ± 0.4		Pettine et al. (2007)
	Ligurian Sea	3.0		Coste (1987)
	N Tyrrhenian	0.6 ± 0.4		Pettine et al. (2007)
	Gulf of Naples	3.78 ± 2.52		Innamorati & Giovanardi (1992)
	Tuscany Coastal waters			Innamorati & Giovanardi (1992)
	Gulf of Tunis	3.9 - 23.8	$3.6x10^7$ - $3.4x10^8$	Turki et al. (2009)

Sub-basin	**Area**	**Chl-*a* (mgm^{-3})**	**Phytoplankton Abundance (cellsL^{-1})**	**References**
Adriatic Sea	SE Adriatic		7.0x10^{4}	Vilicic et al. (2011)
	N Adriatic	1.2 ± 1.5		Pettine et al. (2007)
	Surface Water	0.4 ± 0.3		Sokal et al. (1999)
	Western Coastal	0.45 ± 0.25		Zoppini et al. (1995)
CMED	Southern Ionian	0.23±0.08		Lopez-Sandoval et al. (2011)
	Surface Water	0.3 ± 0.1		Sokal et al. (1999)
	SE of Rhodes	0.08 - 0.39	1.4x10^{2} - 1.1x10^{3}	Ignatiades et al. (2009)
	Offshore waters	0.56 ± 0.40		Aktan (2011)
EMED	SE Aegean	0.07 - 0.27	6.6x10^{3}	Ignatiades et al. (1995)
	Cretan Sea (S Aegean)	4.87		Ignatiades (1998)
	Coastal waters	0.87 ± 0.69		Aktan (2011)
	Cretan Sea (S Aegean)	4.87		Ignatiades (1998)

Table 10. Primary productivity in the four subregions of the Mediterranean. All values are *in situ* ^{14}C measurements

Sub-basin	**Area**	**Primary productivity**	**Units**	**References**
WMED		142.38	mgCm^{-2}d^{-1}	Macias et al. (2009)
	Front area	880	mgCm^{-2}d^{-1}	Lorenz et al. (1998)
	Non-front area	480	mgCm^{-2}d^{-1}	Lorenz et al. (1998)
	Balearic	0.01 - 0.83	mgCm^{-3}h^{-1}	Ignatiades et al. (2009)
	Gulf of Lions	78 - 106	gCm^{-2}y^{-1}	Lefevre et al. (1997)
	Gulf of Lions	140 - 150	gCm^{-2}y^{-1}	Conan et al. (1998)
	Ligurian Sea	240 - 716	mgCm^{-2}(14h)$^{-1}$	Vidussi et al. (2000)
	Gulf of Lions	60 - 120	gCm^{-2}y^{-1}	Cruzado and Velasquez (1990)
	Ligurian Sea	2.00	gCm^{-2}d^{-1}	Coste (1987)
	Ligurian Sea	80.0	gCm^{-2}y^{-1}	Minas et al. (1993)

Table 10. (Continued)

Sub-basin	Area	Primary productivity	Units	References
		398	$mgCm^{-2}d^{-1}$	Moutin and Raimbault (2002)
	Southern Tyrrhenian	273	$mgCm^{-2}d^{-1}$	Decembrini et al. (2009)
Adriatic Sea	Southern Adriatic	97.3	$gCm^{-2}y^{-1}$	Boldrin et al. (2002)
	NE Adriatic	55	$gCm^{-2}y^{-1}$	Justic (1987)
	NW Adriatic	120	$gCm^{-2}y^{-1}$	Justic (1987)
	Whole Adriatic Sea	26 - 70	$gCm^{-2}y^{-1}$	Dugdale and Wilkerson (1988)
	Western Coastal Adriatic	120	$gCm^{-2}y^{-1}$	Zoppini et al. (1995)
CMED		61.8	$gCm^{-2}y^{-1}$	Boldrin et al. (2002)
	Eastern Ionian	285.26	$gCm^{-2}y^{-1}$	Pagou and Gotsis-Skretas (1990)
EMED	Northern Aegean	0.62 ± 0.45	$mgCm^{-3}h^{-1}$	Ignatiades (2005)
	Southern Aegean	0.30 ± 0.21	$mgCm^{-3}h^{-1}$	Ignatiades (2005)
	Cretan Sea	5.73 - 7.98	$gCm^{-2}d^{-1}$	Gotsis-Skretas et al. (1999)
	NE Aegean	221 ± 13	$mgCm^{-2}d^{-1}$	Siokou-Frangou et al. (2002)
	S Aegean	218 ± 63	$mgCm^{-2}d^{-1}$	Siokou-Frangou et al. (2002)
	SE of Rhodes	0.02 - 0.14	$mgCm^{-3}h^{-1}$	Ignatiades et al. (2009)

Additional sources of eutrophication in the Mediterranean that only recently have received attention are the airborne pollutants, nitrogen oxides (NO_x) and phosphorus (UNEP, 2003b). The estimation of N deposition is difficult due to the variety of the gaseous forms of nitrogen present in the atmosphere (N_2, NH_3, HNO_3) and the rainwater (NO^- and NH_4^+). Calculations based on emission data (Vestreng and Klein, 2002), estimated that total NO_x, NH_3 and total N emissions were about 1,800 kt, 2,300 kt and 4,200 kt of nitrogen respectively in 1999. Nitrogen deposition in the Mediterranean subbasins has been calculated by Tarrason et al. (2000). The distribution of nitrogen deposited in the Mediterranean subregions form the atmosphere is about 279 $kt.y^{-1}$ for WMED, 935 $kt.y^{-1}$ for CMED, 196 $kt.y^{-1}$ for the Adriatic Sea and 728 $kt.y^{-1}$ for EMED.

The most eutrophic part of the WMED subregion is the Gulf of Lions. The Gulf of Lions extends over the coastal region between the Spanish – French border and the Rhone Delta. It is the largest marginal system of the Mediterranean Sea with many lagoons. The main supply of freshwater, nutrients and organic matter in the area is due to the Rhone River (Mojtahid et al., 2009). The catchment area is 95,000 km^2 and provides five million tonnes of suspended solids to the marine environment (Leveau and Coste, 1987). Fluxes of inorganic nitrogen have been estimated about 76,000 $t.y^{-1}$ and inorganic phosphorus fluxes about 8,400 $t.y^{-1}$ (Coste et al., 1977; Coste et al., 1985). Primary production in the Gulf of Lions is greatly affected by seasonality being 15 fold at the spring bloom (Lefevre et al., 1997) followed by an oligotrophic summer time where primary production is less than 50 $mgC^{-2}day^{-1}$. Although the average production in the Gulf of Lions is 88 $mgCm^{-2}day^{-1}$, primary production in the upwelling zone due to Minstral is as high as 263 $mgCm^{-2}day^{-1}$. It has been estimated that 50% of the production due to the Rhone River is new production. Primary production data in the Gulf of Lions indicate that there was no increase over the last thirty years and therefore the Gulf of Lions seems to be a balanced system over the last few decades (Lefevre at al., 1997).

The surface of the Adriatic Sea is about 132,000 km^2 which is equivalent to 5% of the entire surface of the Mediterranean but only 0.8% of its volume (Danovaro, 2003). It is elongated from the North to South basin, the length being 800 km whereas the width varies between 100 and 200 km. The Adriatic is connected with the Ionian Sea through the Straits of Otrando 75 km wide. It is a shallow basin with maximum depth 70 m. Nutrient distribution and phytoplankton growth is influenced by water circulation and transport processes. Although there is complete renewal of the Adriatic water during winter time (Franco and Michelato, 1992), stratification of the water masses is the typical pattern for the rest of the year reducing transport processes and mass exchanges. This is particularly pronounced during the summer in the Northern Adriatic when the anti-clockwise circulation is limited; Po river plume extends offshore for several miles establishing vertical stratification, leading to reduction of oxygen concentration (Danovaro, 2003). Due to stratification, anoxic conditions near the bottom have been reported several times (UNEP, 1999). Dystrophic episodes occur often followed by “smelly waters” due to putrefaction processes. There are also manifestations of algal blooms characterized as nuisance. High nutrient concentrations, increased phytoplankton biomass and low water transparency are the main dominant characteristics of the Adriatic Sea with negative effects on recreational activities. Eutrophic conditions seem to prevail in the western part of the Adriatic Sea mainly due to human discharge, accompanied by algal blooms. Algal blooms of *Peridinium ovum* have been observed in the Gulf of Trieste since 1973 causing deaths to benthic organisms. Similar blooms of *Scrippsiela jaeroense* and *Gonyaulax polyedra* were observed since 1983 (Fonda-

Umani, 1985; Cabrini et al., 1988). Eutrophication is not caused only by excessive phytoplankton biomass but also from macroalgae especially in shallow coastal waters (Kitsiou and Karydis, 2011; Zardivar et al. 2008; Zardivar et al., 2009). Excessive growth of the “nitro-philic” species of *Ulva* and *Enteromorpha* in the Venice lagoon, in addition to the nuisance they have raised a general concern related to possible public health problems. Coupling of phytoplankton community with nutrient in the Gulf of Gabes have been described by Drira et al. (2010).

The Northern Aegean Sea communicates with the Black Sea through the Dardanelles and is characterized by the presence of many islands: Thasos, Samothraki, Limnos, Tenedos, Imbros, Lesvos, Chios and Samos are the largest. There are two deep basins, the Samothraki Basin (1600m deep) and the Chios Basin (1160m deep). There also three plateau, Thermatikos, Samothraki and Limnos. There are also many rivers outflowing into the Aegean Sea: Pinios, Axios, Nestos, Strymon and Evros River are the largest. The main water input in the Northern Aegean Sea is from the Black Sea. Surface Black Sea Water (BSW) is characterized by relatively low salinity, varying between 24 and 35 psu. Black Sea surface water masses move westwards into the Aegean Sea and finally southwards along the Hellenic mainland (Theocharis and Georgopoulos, 1993).

The offshore water masses of the Northern Aegean Sea have been characterized as oligotrophic; nutrient concentrations are approximately twelve times lower compared to nutrient concentrations in the Atlantic. Work on nutrient concentrations carried out by Ignatiades (2005) showed that mean concentrations for phosphates were 0.03±0.01 μM for nitrate, 0.45±0.13 μM for nitrite and 0.12±0.05 μM for ammonia. Average chlorophyll-*a* concentration values were 0.32±0.17 μgL^{-1}. Primary productivity values were 0.62±0.45 $mgCm^{-3}h^{-1}$ indicating oligotrophic conditions (Ignatiades, 2005). However, semi-enclosed bays and coastal areas near estuaries show eutrophic conditions. Detailed information on the trophic conditions of bays and estuarine areas along the Northern Aegean coastline have been given by Karydis and Kitsiou (2012).

Eutrophication trends are also illustrated as the spatial distribution of chl-*a* concentrations (mgm^{-3}) in remote sensing imagery. Figures 3 and 4 show seasonal satellite images (2012 - 2013) with 4 km resolution acquired from the Aqua MODIS sensor (NASA Ocean Color website). The Mediterranean Sea and especially the eastern part is the less eutrophic area among all the studied seas. During autumn, spring and summer Eastern Mediterranean Sea is ultra oligotrophic. During winter shows oligotrophic trends with much lower chl-*a* concentrations compared to the western part. The coastal area of the southeastern Mediterranean shows clearly eutrophic trends during all seasons mainly due to the sewage effluents of Kairo and Alexandria. Northern Aegean shows mesotrophic to eutrophic trends explained by the river inputs from northern Greece and the water inflow from Black Sea. The coastal areas in the Mediterranean Sea with permanent eutrophic trends are the Gulf of Lions, the Adriatic, Northern Aegean and the SE Mediterranean (Nile – Levantine).

6.1.3. The Governance

The policy regime of the Mediterranean Sea is rather complex not only because of the large number of Mediterranean States (22 States including Gibraltar home ground of UK) but also because of the different political, economical and legal nature of the bordering countries. The zone of territorial waters is not the same round the Mediterranean coast and some States have proclaimed exclusive economic zones exercising the rights ground by the United

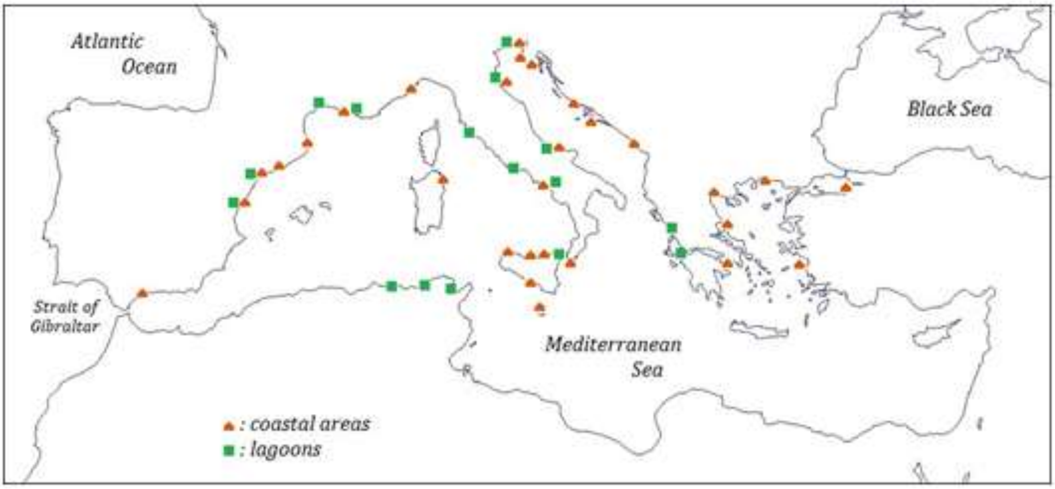
Nations Convention on the Law of the Sea (DiMento and Hickman, 2012). A number of the Mediterranean States have proclaimed zones for ecological purposes. France adopted Law No. 2003-346 which provides that an ecological protection zone may be created. Italy also has adopted legislation for ecological protection zones (Law No. 61, Feb. 2006) as well as Slovenia (Law 4, Oct. 2005). However, there has been good regional cooperation between the Mediterranean States through the UNEP-MAP System composed of the Barcelona Convention, its seven protocols and the Mediterranean Action Plan (MAP).

An important scientific and technical part of MAP is the MED POL program. The emphasis of the MED POL has been placed on oil pollution, heavy metals (mercury, cadmium, copper and zinc), organohalogen compounds mainly PCBs and DDTs as well as organotins (TBTs). However, the assessment of eutrophication has received special attention (UNEP, 2003a). A number of physical, chemical and biological variables for monitoring eutrophication is given in Table 3. Among the biological variables, information from chlorophyll and phytoplankton community structure is required. Remote sensing is also mandatory. The emphasis of MED POL Phase I (1975-1981) was based on the upgrading of the technical infrastructure and scientific personnel training of laboratories involved in the project (Haas, 1990; Karydis and Kitsiou, 2012). The MED POL Phase II (1982-1996) focused on the development of national monitoring programs following the MED POL protocols. The MED POL Phase III (1997-2004) had as main objective pollution control and the last MED POL Phase IV (2005 – to date) is focused on ecosystem quality (UNEP/MAP – Plan Bleu, 2009). The governance of the Mediterranean is also supported by a number of EU Directives presented in section 5.7. Most of the EU Directives adopted for the marine environment targeted only at one sector of the marine environment. A cross sectoral approach is needed for the objectives set by the European Commission and the European Parliament “Towards an Integrated Maritime policy for better governance of the Mediterranean*” to be address successfully*” (Commission of the European Communities, 2009). Harmonization of EU Directives and regional agreements in the Mediterranean is required and therefore there is an effort by both European Policy and Barcelona System to converge as far as their policy and measures are concerned.

6.1.4. Effectiveness of the Measures

The main question of the present section is “how the legal framework and the initiatives taken by the Mediterranean Member States have been?” (DiMento and Hickman, 2012). The answer can be found within successful environmental management practices during the last thirty years. The central data source is the National Baseline Project (NBB) compiled by MED POL. It contains 7,600 records (UNEP/MAP – Plan Bleu, 2009). Each record indicates the emission of a substance for a given industrial sector. Unfortunately the effectiveness in monitoring varies among the different countries, depending on the country size, the level of industrial development, the economy of the States and possible social pressures. The majority of data (77%) was reported by the Northern Mediterranean countries. The NBB inventory classifies 80 different substances into three categories (a) substances of concern, mainly hazardous substances (b) other substances and (c) general parameters to monitor water pollution; Biochemical Oxygen Demand (BOD), Volatile Organic Compounds (VOC) and Total Suspended Solids (TSS). Indicators for nutrient such as nitrogen and phosphorus are grouped within this category. Nutrient data formed the majority of the records.

Nutrient sources are discharged by many industrial sectors including treatment of urban wastewater, organic chemical industry, tanning and dressing of leather. However, the largest emitters for nitrogen are treated urban waste water (31%), livestock farming (19%) and metal industry (11%). For phosphorus, the fertilizers industry accounts for 63% of the phosphorus emissions, livestock farming 20% and treated urban waste water 8%. According to the available data there are many "hot spots", most of them near the cities. These hot spots have been characterized so because of high concentrations of hazardous substances, eutrophication is also present. Athens, Barcelona, Genoa, Marseilles, Naples, Thessaloniki and Toulon are the main eutrophic hot spots along the European coastline in the Mediterranean. In addition, many eutrophic areas occur in the Adriatic Sea and the coastal zone extending from Barcelona to Genoa. There are also eutrophic areas near lagoons (Figure 2).



Source: UNEP/FAO/ WHO (1996) – modified.

Figure 2. Eutrophic sites, coastal areas and lagoons, in the Mediterranean Sea.

The existing data indicate a positive sign for the quality of the marine environment (UNEP/MAP – plan Bleu, 2009): "conservation actions implemented to date have had positive results and some species have already been saved from extinction. Namely from 2001 to date 175 large biodiversity projects have been identified within the Mediterranean region". The improvement of marine water quality is also reflected to the steadily increasing number of beaches monitored within the framework of MED POL. Only 78 per cent of the beaches used for swimming fulfilled the required standards in 1983, whereas this percentage climbed up to 96 per cent in 1987. "Bathing water is improving in a number of Mediterranean Countries, European in particular" (UNEP/MAP – Plan Bleu, 2009). There is also evidence that treatment and disposal of sewage is improving in many Mediterranean countries (Keckes, 1994). An example is the Mediterranean coastal zone of France: in 1980 the treated sewage facilities covered 22.5 of the population. This percentage in 1990 went up to 66.5% and a few years later covered 93% of the population (Keckes, 1994).

6.2. The Black Sea

6.2.1. Physiography Environmental Settings

The Black Sea is a semi-enclosed basin connected with the Mediterranean through the Straits of Dardanelles (Figure 1). The Black Sea area is about 420,000 km^2 (about 460,000 km^2 if the Sea of Azov with an area of 38,000 km^2 is included) and maximum depth approximately 2,200 m. The basin is surrounded by six countries Russia, Ukraine, Romania, Bulgaria, Turkey and Georgia. Due to the river outflow, the Black Sea water basin is influenced by seventeen countries and thirteen capital cities. Danube, Dnieper and Don, being the second the third and the fourth major European rivers drain into the marine environment about one third of the entire continental European area (Bakan and Buyukgungor, 2000). The catchment area and the mean flow of the five largest rivers of the Black Sea are given in Table 11. The total population that lives around the Black Sea, having interactions with Black Sea's marine environment, is estimated to be as high as 170,000 million people; seventeen million that is the 10% of these people live near the coast (BSEI, 2005). The population surrounding the Black Sea region is the third faster growing in the world (Gultekin-Punsmann and Nikolov, 2008). The volume of the Black Sea is about 547,000 km^3 of water, whereas the width of the basin is about 1,200 km. The Black Sea coastline (Ivanov and Belokopytov, 2013) is 4,125 km long (350 the Bulgarian coastline, 225 km the Romanian costline, 1,630 the Ukranian coastline, 475 km the Russian coastaline, 310 km the Georgian coastline and 1,400 km the Turkish coastline). The coastal environment partially is mountainous and surrounded by mountain ranges including the Balkanides, Pontides (the Anatolian mountains), Caucasus and Crimean. The Northern part known as the "*Black Sea Lowland*" (BSL) extends over the low lying Danube Delta. The BSL is surrounded by the Carpathian foreland. The Crimean Peninsula juts out from the Northern Part of the basin dividing the Black Sea into a western and an eastern section.

Table 11. Water discharges of the major Black Sea rivers. The catchment area of the rivers listed below exceeds 50,000 km^2

River	**Catchment area (km^2)**	**Mean flow (m^{3}s^{-1})**	**Reference**
Danube	817,000	6575	Medhycos (2001)
Dniper	470,000	1488	Vorosmarty et al. (1998)
Don	378,000	671	Vorosmarty et al. (1998)
Dnister	66,000	377	Vorosmarty et al. (1998)
Sakarya	56,000	186	Vorosmarty et al. (1998)

The connection to the Mediterranean is via the Bosporus Straits, the Marmara Sea and the Dardanelles. The length of the Bosporus Strait contributes substantially to the formation of the hydrological structure in the Black Sea. The length is about 31 km whereas the width varies between 0.7 and 3.5 km. The depth of the southern and northern sills is about 35 m and 58 m respectively. The Sea of Marmara is a relatively small basin 275 km long and 80 km wide. The Sea of Marmara is connected to the Aegean Sea (Eastern Mediterranean) via the Dardanelles Straits, being 74 km long, 1.3 – 7.5 km wide and 60 m deep. Both the width and the depth of the Straits as well as the sill topography restrict water exchange between the

Black sea and the Sea of Marmara especially the inflow of highly saline Mediterranean water. From hydrographic point of view the Sea of Marmara is a two-layer system. There is a strong interface between the two layers usually formed at a depth ranging between 15 and 25 meters. Brackish Black Sea water outflows through the top layer whereas saline Mediterranean water inflow into the Black Sea though the deeper layer. The outflowing nutrient load into the Mediterranean has been estimated $1.2\text{x}10^4$ t of total phosphorus and $1.9\text{x}10^5$ t of total nitrogen; the inflowing into the Black Sea nutrient loads are $1.0\text{x}10^4$ t and $0.6\text{x}10^5$ t respectively (Polat and Tugrul, 1995).

Black Sea's bottom topography displays an abyssal plain, being the basin area below the isobath of 200 m. It takes 35% of the total area. The continental slopes, confined between the isobaths of 200 m and 2,000 m and covering 40% of the total sea area, are very steep (average slope 5-8° reaching up to 30-40° at some points) except the areas near the Danube and Kerch Fans (Sur et al., 1996) where a wide north-western continental shelf extends from the Crimean peninsula and the west coasts to the W-SW area of the basin, with a shelf break depth of about 200 m which is the depth for the shelf boundary of the World Ocean (Ivanov and Belokopytov, 2013). The Black sea shelf is part of the submerged coastal land and accounts for 25% of the total sea area (112,140 km^2) and 1.5% of the water volume (8,190 km^3). At Sakaya Canyon the depth suddenly increases reaching approximately a depth of 1,500 m near the Anatolian coast of Bosporus. There are numerous canyons intercepting the continental slope throughout the Black Sea.

The climate of the Black Sea is influenced by both the Mediterranean climate and the steppe climate. During the last fifty years the boundaries of the steppe climate shifted far eastward to the Caspian Sea and the temperate climate prevails over most of the area of the Black Sea (Ivanov and Belokopytov, 2013). The evaporation in the basin varies between 332 and 392 km^3y^{-1} exceeding rainfall (225 – 300 km^3y^{-1}). Runoff is about 350 km^3y^{-1}, leading to an excess of freshwater inflow and therefore dilution of the surface seawater masses. There are many estimates in literature concerning the water balance components of the Black Sea; evaluations range between 116 and 520 km^3y^{-1} with an average value of 240 km^3y^{-1} (Simonov et al., 1991). The ratio of the sea volume over the water balance provides an estimate of the period of full water renewal. Assuming an average input of about 800 km^3y^{-1} the residence time is about 700 years. Evaluations based on salt budgets provide a value of 400-500 years (Latif et al., 1991). If however, the Black Sea volume is divided by the volume of seawater inflow from the Mediterranean, the residence time is about 2,500 years. Similar value has been cited in Sverdrup's monograph (Sverdrup et al., 1961). Evaluations using radiocarbon techniques provided a period between 1,000 and 2,000 years (Ivanov and Belokopytov, 2013).

The general pattern of water mass circulation of the basin is characterized as cyclonic. There are two large cyclonic gyres: one in the eastern part and one in the western part of the basin. Although the general circulation pattern in the Black Sea has been developed since the late 19th century, the physical factors that contribute to the Black Sea circulation are not clear as yet (Sur et al., 1996; Ivanov and Belokopytov, 2013). The wind has been recognized as the driving force for the cyclonic surface circulation (Moskalenko, 1976). River runoff was also proposed as an additional reason of cyclonic circulation. In addition, a seasonal thermohaline circulation driven by non-uniform surface fluxes seem to act as a complementary force to the wind driven circulation (Marchuk et al., 1975).

A permanent halocline throughout the Black Sea, located between 100 and 200 m characterizes the vertical pattern of the basin. The stratification is formed by freshwater discharges on the surface coupled with deeper high salinity water mass inflow from the Mediterranean. This pattern favors hypoxic or even anoxic conditions and the Black Sea is the World's largest body of water with anoxic character. Salinity values of the deep water masses are about 22‰ whereas surface salinity values are reduced to 17‰. Apart from the coastal environment, vertical salinity patterns are the same everywhere in the basin.

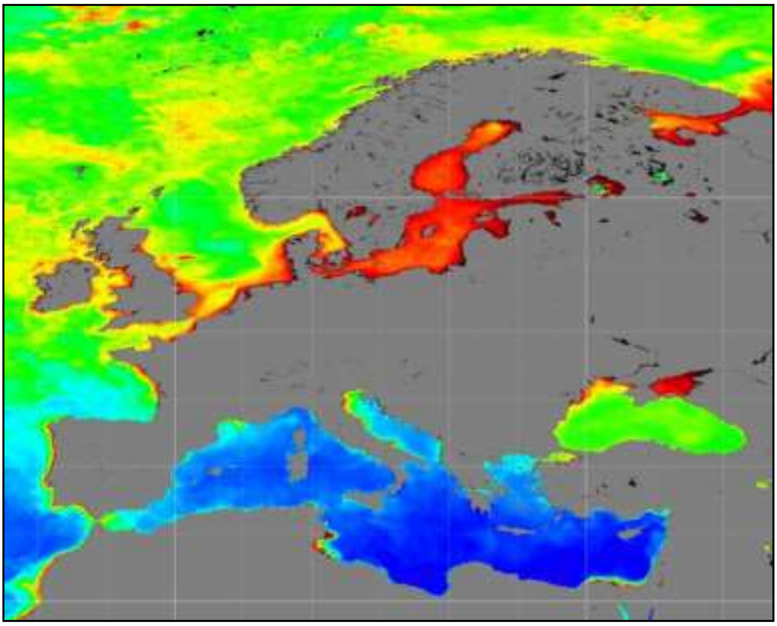
The outflow of the major Black Sea rivers, i.e., Danube, Dniester and Dnieper rivers, into the Black Sea is about 350 km^3. The Danube River alone accounts for the 75% of the outflowing water that is 250 km^3y^{-1} and the Dnieper and Dnister 53 km^3y^{-1} and 9.6 km^3y^{-1} respectively. More information about the catchment area and the outflows of the main Black Sea rivers are given in Table 11. The Danube load on nutrients introduced into the Black Sea before 1992 has been reported to be 80,000 ty^{-1} for phosphorus and 340,000 ty^{-1} for inorganic nitrogen (Mee, 1992). Danube's freshwater bearing nutrients can be transported along the coast following turbulent exchanges and filament structures finally carried into the interior of the basin (Yunev et al., 2002). Estimates on the nutrient load of Turkish rivers during the same period indicate a contribution of about 1,700 ty^{-1} of orthophosphate and 25,000 ty^{-1} of total inorganic nitrogen (Sur et al., 1996).

Land freshwater carries suspended matter, pollutants and nutrients and exercises dynamic control over the basin's water balance, circulation and environmental conditions. As there is an upward trend concerning riverine nutrient inputs, the level of eutrophication has been increasing considerably during the past decades. In addition river outflow has produced permanent column stratification. This type of water mass structure limits vertical movements, characteristic of meromictic basins, favoring hypoxia or even anoxia below the pycnocline located between 100 and 300 m (Lein and Ivanov1991). It has been estimated that 90% of the basin's water is anoxic. Due to excessive freshwater masses inflowing into the Black Sea, the water mass balance is positive and mesohaline water flows into the Mediterranean Sea through the Bosporus and Dardanelle Straits. A number of studies have been published on the freshwater budget of the Black Sea (Jaoshvili, 2002; Algan, 2006). It has been estimated that freshwater inputs from various sources including fluvial discharges, precipitation and inflows from the Azov Sea and the Bosporus Strait vary between 603 and 965 km^3 per year. Part of this water mass evaporates but most of it outflows into the Mediterranean through the Bosporus Strait. There is a two way flow in the Dardanelles Straits: Aegean saline water underflows into the Black Sea whereas brackish Black Sea water outflows as a surface current. Although the number of rivers outflowing into the Black Sea is about 1,000only few of them have catchment areas greater than 50,000 km^2. The Northwestern and northeastern parts of the basin receive most of the water inputs (Table 11).

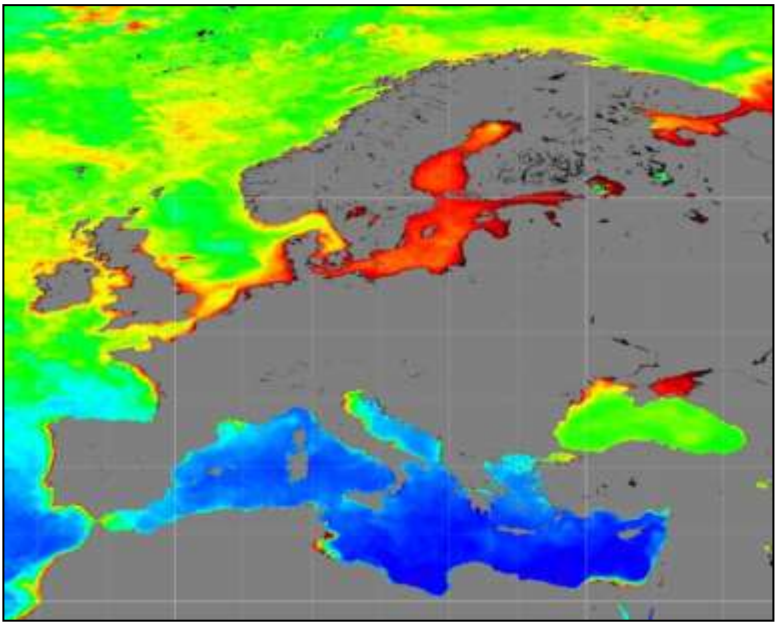
Due to the semi-enclosed character of the Black Sea, the basin is vulnerable to human impacts. As the residence time increases, nutrients and pollutants are retained for longer periods (Doussis, 2006). Nutrient sources include effluents from agriculture, mining and municipal sewage from both cities and coastal settlements. The sources are point and non-point affecting marine eutrophication and fisheries health. Over the last three decades, the Black Sea became a very popular tourist resort, an activity that exercises further pressures to the eutrophic trends of the area and therefore overuses ecosystem services. It has been pointed out that "use of the Black Sea and its tributaries for the disposal of wastes is free (un-priced) and so this ecosystem service is overused, imposing external costs" (BSC, 2008).

6.2.2. Environmental Pressures and Eutrophication in the Black Sea.

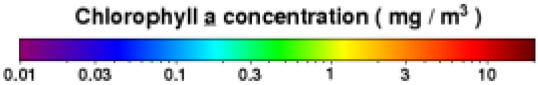
The Black Sea water mass is receiving contaminated urban, industrial and agricultural runoff. As human activities increased over the last four decades, nutrient concentrations, mainly nitrogen and phosphorus have increased (Konovalov and Murray 2001; Osawa et al., 2005). Nutrient inputs into the Black Sea compared to other regional seas of Europe are high. It has been estimated that the total nitrogen in the northern part of the Black Sea during the 90s was six times that to the Baltic Sea and at least twice as much as that of the North Sea (Artioli et al., 2008; Strokal and Kroeze, 2013). In addition the construction of dams mainly on the Danube River has limited the amounts of silicates outflowing into the basin. Increased nitrogen and phosphorus concentrations coupled with low silicate concentrations have changed the phytoplankton community from a diatom dominant community to a community dominated by dinoflagellates, coccolithophores and small flagellates' community (Eker et al., 1999). Table 12 shows the riverine discharges into the Black Sea between 1963 and 1998. Apart from the effects on phytoplankton composition, changes on phytoplankton biomass and bloom intensity have been reported (Bodeanu, 1993). The upward trend in nutrient enrichment that started in the 1960s mainly on the north-western region of the basin has also resulted in a number of impacts beyond phytoplankton (Mousing et al., 2013): loss of shallow water macrophytes (Bologa, 1987), increase in organic matter sedimentation and a decline in benthic invertebrate abundance and diversity have been reported (Zaitsev, 1994). There is also a shift towards smaller and less valuable from the nutritive point of view mesozooplankton groups and opportunistic as well as gelatinous species (Kideys et al., 2000; Oguz, 2005). Excessive organic matter supply intensifies hypoxia affecting many marine invertebrates and fish sensitive to oxygen depletion. The control of mussels on phytoplankton during the 70s was loose due to the decline of the mussel population (Yunev et al., 2007). It has been estimated that nutrient inputs into the Black Sea between 1973 and 1990 have caused losses on living marine resources equivalent to 60 million tonnes. The cost for fisheries and tourism due to eutrophication is about half a billion USD (Strokal and Kroeze, 2013). Nutrient concentrations in the Danube outflow compared during two sampling periods 1980-1985 and 1989-1993 have shown an increase from 2.9 to 4.5 μM for phosphate whereas silicate concentrations showed a decrease from 69 to 47 μM (Lancelot et al., 2002). This silicate concentration decrease followed the decrease of the silicate load outflowing into the Black Sea: an estimated input from the Danube River of $800\text{x}10^3\text{ty}^{-1}$ during the 70s was followed by a reduction down to $(230\text{-}320)\text{x}10^3\text{ty}^{-1}$ during the 80s (Humborg et al., 1997). Nutrient and chl-*a* concentrations values as well as primary productivity values in the Black Sea are given in the Tables 13, 14 and 15, respectively. Mean seasonal satellite values of chlorophylls in the Black Sea are given in Figures 3 and 4. Chl-*a* concentration values observed were constant throughout the year, with a minimum value of 0.3 mg/m^3. The higher concentrations representing eutrophic conditions were observed in the western part, especially in the northwest shelf during the spring period. The offshore waters of the Black Sea show mesotrophic conditions without a fluctuation of chlorophyll concentrations around the year.



Summer 2012

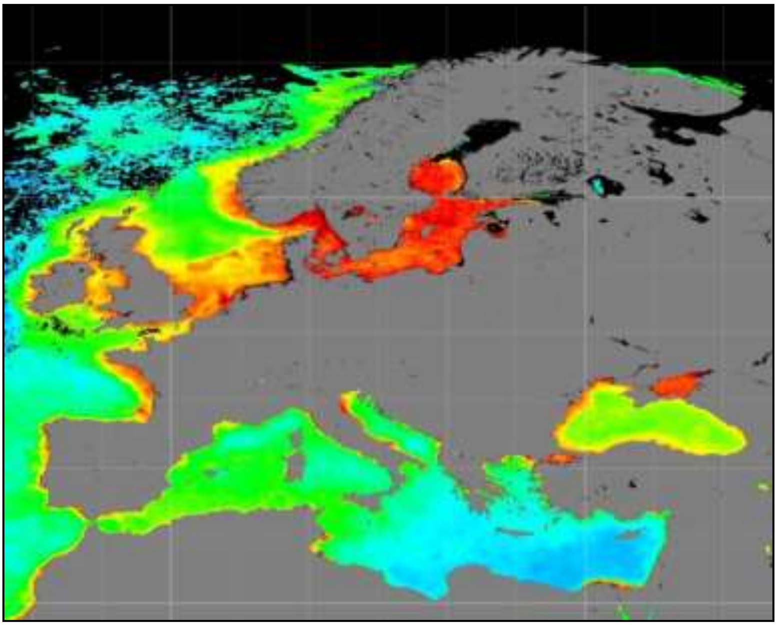


Autumn 2012

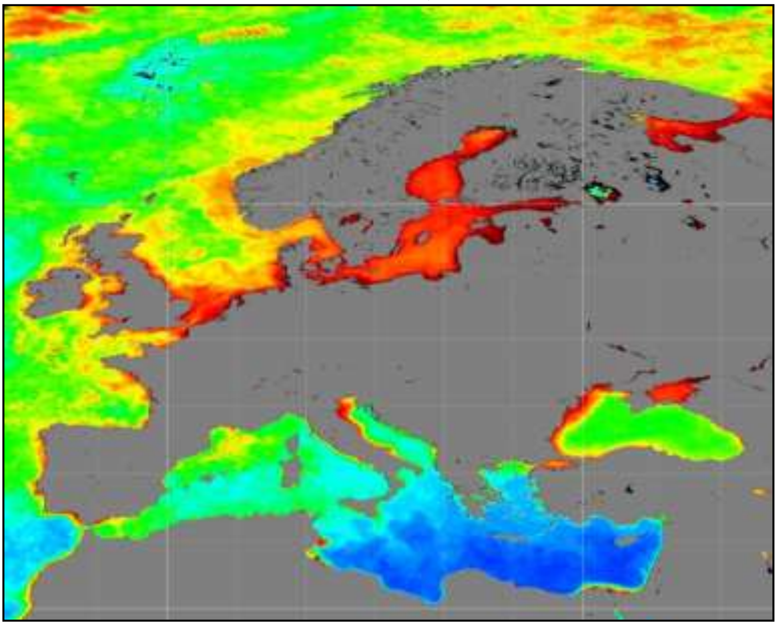


Source: NASA Aqua MODIS.

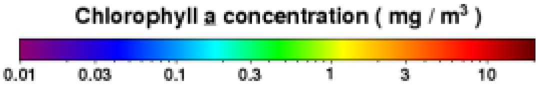
Figure 3. Chl-*a* concentration (mgm $^{-3}$) for (a) Summer 2012, (b) Autumn 2012.



Winter 2012, 2013



Spring 2013



Source: NASA Aqua MODIS.

Figure 4. Chl-*a* concentration (mgm $^{-3}$) for (a) Winter 2012, 2013 and (b) Spring 2013.

Table 12. Riverine nutrient budgets in the Black Sea

Year	Nitrogen ($10^3 t/y^{-1}$)	Phosphorus ($10^3 ty^{-1}$)	Silica ($10^3 ty^{-1}$)	PPR ($gCm^{-2}y^{-1}$)
1963	903	63.4	936	5.65
1968	1213	79.7	1027	7.10
1973	1282	78.5	817	6.99
1978	1456	83.2	927	7.41
1983	1410	78.3	839	6.97
1988	1414	78.3	789	6.98
1993	1227	53.7	758	4.79
1998	1116	55.1	861	4.91

PPR: Potential Primary Production in the sea supported by rivers. Estimates are based on the river load of the limiting nutrient over a period of five years

Ludwig et al., (2009)

Table 13. Nutrient concentrations in the Black Sea

Subregion	Nitrate (µM)	Phosphate (µM)	N/P	Reference
Dardanelles	0.43 ± 0.42	0.23 ± 0.13	1.78 ± 1.00	Turkoglou & Oner (2010)
Samsun Bay	3.14 ± 1.20	0.98 ± 0.55	15.09 ± 9.11	Baytut et al. (2010)
Western sub-basin	4.49 ± 2.83	0.13 ± 0.06		Eker et al. (1999)
Eastern sub-basin	0.13 ± 0.06	0.08 ± 0.07		Eker et al. (1999)
Bulgaria-Varna Bay	159.5 ±74.1*	3.06 ±1.66	52.65±10.66	Moncheva et al. (2001)
Bulgaria-C. Galata	30.16 ± 30.62*	0.25 ± 0.11	78.0±47.68	Moncheva et al. (2001)
Bulgaria-C. Kaliakra	17.89 ±15.01*	0.25 ± 0.10	69.3 ±33.65	Moncheva et al. (2001)
The Bulgarian Coast	1.41 ±1.08	0.08 ± 0.07		Stel'makh et al. (2009)
The NW Part	0.11± 0.05	0.06 ± 0.05		Stel'makh et al. (2009)
The Danube Delta	3.22	0.39		Stel'makh et al. (2009)
The Turkish Coast	0.14 ± 0.04	0.03 ± 0.02		Stel'makh et al. (2009)
The deep sea part	0.13 ± 0.06	0.08 ± 0.08		Stel'makh et al. (2009)
Danube plume(70s)	1.3 ± 0.1	0.11 ± 0.04		Yunev et al. (2007)
Danube plume (90s)	5.3 ± 1.13	0.3 ± 0.06		Yunev et al. (2007)
Central Black Sea (1)	1.2 ± 2.5 (3)	0.28 ± 0.37		Mikaelyan et al. (2013)
Depth> 200m	7.2 ± 0.13 (1)	1.0 ± 0.01 (1)		Mikaelyan et al. (2013)
Depth> 200m	5.2 ± 0.16 (2)	1.5 ± 0.02 (2)		Mikaelyan et al. (2013)
Cent. Part D>1500m	7.4 ± 0.16 (1)	1.0 ± 0.01 (1)		Mikaelyan et al. (2013)
Cent. Part D>1500m	5.3 ± 0.19 (2)	1.5 ± 0.03 (2)		Mikaelyan et al. (2013)
Peripheral deep sea 200-1500	3.37 ± 0.17 (1)	0.3 ± 0.01 (1)		Mikaelyan et al. (2013)
Peripheral deep sea 200-1500	2.45 ± 0.28 (2)	0.4 ± 0.02 (2)		Mikaelyan et al. (2013)

(*) Total Nitrogen (1) Eutrophication Period 1984-1995 (2) Post eutrophication period 1996-2008 (3) in the cold intermediate layer

Table 14. Chlorophyll concentrations and phytoplankton abundance/ biomass in the Black Sea

Sub-region	**Chl-*a* (mgm^{-3})**	**Phytoplankton Abundance (cellsL^{-1})**	**Phytoplankton biomass**	**Reference**
Central BS	0.5-3.0 (1)			Oguz et al. (2002)
Western basin	1.5-2.0 (1)			Oguz et al. (2002)
Eastern basin	1.0-1.5 (1)			Oguz et al. (2002)
Central BS			1.5-41 gm^{-2}	Mikaelyan et al. (2013)
Central BS			19.6 ±2.0 gm^{-2} (2)	Mikaelyan et al. (2013)
Central BS			3.3 ±3.8 gm^{-2} (3)	Mikaelyan et al. (2013)
Dardanelles	1.92 ±0.47	4.1x10^{6} – 3.26x10^{6}		Turkoglou and Oner (2011)
Samsun Bay	0.15-1.30	0.01x10^{6}-1.2x10^{6}		Baytut et al. (2010)
Western basin	0.49 ±0.28		550 ±193 μgl^{-1}	Eker et al. (1999)
Eastern basin	0.10 ±0.05		55 ±14 μgl^{-1}	Eker et al. (1999)
Bulgarian coast	0.52-4.75		1.19 gm^{-3}	Petrova and Gerdzhikov (2008)
Bulgarian coast	2.09 ±1.29			Stel’makh et al. (2009)
NW BS basin	0.90 ±0.56			Stel’makh et al. (2009)
Danube delta	5.50			Stel’makh et al. (2009)
Turkish coast	4.19 ±1.24			Stel’makh et al. (2009)
Deep-sea part	0.49 ±0.08			Stel’makh et al. (2009)
Western part	2.45 ±1.74 (4)			Yunev et al. (2007)
Western part			495 mgm^{-3} (5)	Petranu et al. (1999)
			719 mgm^{-3} (6)	Petranu et al. (1999)
			2244 mgm^{-3} (7)	Petranu et al. (1999)
			4105 mgm^{-3} (8)	Petranu et al. (1999)
NW part	2.4-7.1			Kukushkin et al. (2006)
Danube delta	> 0.68			Aubrey et al. (1996)

(1) Satellite data 1997-1198 (2) 1984-1995 (3) Post eutrophication period 1996-2008 (4) 1980-1995 (5) 1960-1970 (6) 1971-1975 (7) 1971-1980 (8) 1983-1990

Table 15. Primary productivity values in the Black Sea

Sub-region	Primary Prod.	Units	Comments	Reference
Deep water	135 ±30	$gCm^{-2}y^{-1}$	1987-1992$^{(1)}$	Yunev (2011)
Deep water	63 ±18	$gCm^{-2}y^{-1}$	Pre-eutroph. period	Yunev (2011)
Deep water	105	$gCm^{-2}y^{-1}$	1993-2007 $^{(2)}$	Yunev (2011)
Whole basin	169 ±57	$mgC^{-2}d^{-1}$	1980-1985	Yunev et al. (2002)
Whole basin	330 ±140	$mgC^{-2}d^{-1}$	1986-1992	Yunev et al. (2002)
Deep water	170-186	$gCm^{-2}y^{-1}$	1998-2004	Yunev (2011)
Whole basin	271 ±67	$mgC^{-2}d^{-1}$	1992	Yunev et al. (2002)
Deep water	120-170	$gCm^{-2}y^{-1}$	1980s	Yunev (2011)
Deep water	63 ±18	$gCm^{-2}y^{-1}$	Pre-eutroph. period	Yunev (2011)
Whole basin	300	$gCm^{-2}y^{-1}$	In 1980s	Konovalov & Murray (2001)
Whole basin	100	$gCm^{-2}y^{-1}$	In 1960s	Sorokin (1964)
Whole basin	140	$gCm^{-2}y^{-1}$	1978-1992	Konovalov & Murray (2001)
Upper 350m	123 and 210	$gCm^{-2}y^{-1}$	1980s	Karl & Knauer (1991)

(1) Eutrophication period (2) post eutrophication period.

6.2.3. The Governance

The main legal framework for the Black Sea protection is the "Bucharest Convention" signed in 1992 and enforced in 1994, following the signatures of seven member states (DiMento and Hickman, 2012). The Convention was followed by the Black Sea Environment Programme in 1993 and three years later the Strategic Action Plan (SAP) was launched having being accepted by all member nations. The SAP include components concerning the precautionary principle, planning and public participation (UNEP, 2009). Further to the Convention, its four protocols and SAP, the work of the Black Sea Commission is also supported by contingency plans, project work and Memos of Understanding (MOUs).

Although the Black Sea Regional Seas Programme is classified under the UNEP Regional Sea Programme, is not a UNEP administered program. Two out of four protocols are also relevant to marine eutrophication: the first protocol against Pollution from Land Based Sources and the fourth protocol (introduced and ratified eight years later) on Biodiversity and Landscape. In addition to the Bucharest Convention the EU appeared as a new player in the Black Sea Cluster (Frank, 2007), since Bulgaria and Romania became EU members. As EU introduces a new legal and administrative framework, "*mixicity*" is the consequence of "*shared competence*" between the Bucharest Convention and the EU regulations. This problem was recognized by EU officials at an early stage and a document was circulated on "*A Synergy for Black Sea Regional Cooperation – Guidelines for EU*

Intiatives" (Emerson, 2008). This document *inter alia*, is advocated on good governance, connectivity and exchange of information. The EU legal framework is involved with the Black Sea marine eutrophication problem mainly through three Directives (a) Waste Framework Directive (b) Water Framework Directive and (c) Marine Strategy Directive.

6.2.4. Effectiveness of the Measures

Although the current situation of the Black Sea does not satisfy the objectives set by SAP, there is considerable recovery from the 1980's conditions characterizing a serious environmental crisis. A monitoring system was set up with the initiation of BSIMAP on ecosystem health including measurements on the water column, sediment, biota, nutrients and municipal discharges. It has been found the marine eutrophication is under control as "the increased diversity and richness, reduced frequency and magnitude of phytoplankton blooms and thus decrease of total biomass and abundance, reduced frequency of bad/poor EQ classes all point to an improvement of the ecological state of the Black Sea" (BSC, 2008).

A Task Force aimed to provide a platform for co-operation for the protection of water and water related ecosystems in the Danube and the Black Sea region (DABLAS), had successful intervention in the condition of the sea. Waste and wastewater reduction and management, were set objectives by DABLAS and had realistic and successful deliverables in Romania and Bulgaria. More than one hundred projects were materialized in municipalities around the Black Sea Basin to reduce nutrient release into the Black Sea (ICPDR, 2011).

6.3. The Baltic Sea

6.3.1. Physiography and Environmental Settings

The Baltic Sea is a landlocked sea wholly in Europe and among the largest brackish water bodies in the world. It is covering an area of about 400,000 km^2 and is a relatively shallow sea, the average depth being about 50m but the depth for more than half of the area is less. The Baltic Sea is surrounded by fourteen countries with a total population of about 85 million. The catchment area of the Baltic is almost 1,600,000 km^2. It is divided into five deep sub basins connected by narrow sills and channels (HELCOM, 2009): (a) the Baltic Proper (b) the Gulf of Bothnia (c) the Gulf of Finland (d) the Gulf of Riga and (e) the Danish Straits. These sub basins differ in terms of ice cover, salinity, temperature, circulation and residence time of the water. Data on the topographic and hydrological characteristics of the Baltic Sea sub basins are given in Table 16.

North Sea inflows of saline water into the Baltic Sea produce a salinity gradient with high salinity (25 ‰) in the Kattegat area and low salinities (5‰) in the Gulf of Bothnia. These low salinity values are due to river discharges amounting to 436 km^{3}y^{-1} of fresh water. Estimations of precipitations added to the fresh water budget are about 224 km^{3}y^{-1} whereas evaporation / surface water outflow into the North Sea are estimated to be about 184 km^{3}y^{-1} and 947 km^{3}y^{-1} respectively. The water deficit is compensated by 500 km^{3}y^{-1} of saline water inflowing from the North Sea (Reissman et al., 2009). The annual runoff accounts for the two per cent of the Baltic Sea volume and would have raised the sea level of the Baltic by 1.1 m per year if the Baltic was an enclosed sea (Matthaus and Schinke, 1999). There is a permanent halocline throughout the Southern and Central Baltic Sea separating the surface layer from the deep water. Thermocline depth varies in the basins ranging from 35 m to a maximum of 90 m. A thermocline develops during the summer time between 10 and 30 m and disappears in winter where mixing conditions prevail. Due to the high salinity of the North Sea and the Atlantic water (>30‰), there is a permanent near bottom inflow of water from the North Sea and a near surface outflow of brackish water. The inflow events seem to be the driving force of what is known as "the Baltic Conveyor Belt". The water exchange between the Baltic Sea and the North Sea is driven by the river runoff and the meteorological conditions over the North Sea – Baltic Sea area. The mean value of the outflowing water has been estimated about 80,000 m^3s^{-1} (10,000 m^3s^{-1} inter annual variability), whereas mean river run off has been estimated about 14,000 m^3s^{-1} (4,000 m^3s^{-1} inter annual variability) and net precipitation 1,500 m^3s^{-1} (Omstedt et al., 2004). At the same time vertical movements dilute the bottom water and more saline bottom water masses are carried into the brackish surface layer above the halocline. The average residence time of the Baltic deep water is about 20 years.

Table 16. Topographic and hydrological characteristics of the Baltic Sea Gulfs and sub-basins

Sub Basin	**Area (km^2)**	**Max. depth (m)**	**Average depth (m)**	**Residence time (y)**
Gulf of Bothnia (1)	115,516	230	60	34-42
Gulf of Finland (1)	29,600	123	37	24-32
Gulf of Riga (2,5)	16,330	>60	26	30-32
Baltic Proper (3,5)	211,069	459	62	10-28
Danish Straits (4)	42,408	109	19	5-10
Total Baltic Sea	414,893	459	52	

(1) Jurgensone et al. (2011), (2) Kullenberg and Jacobsen (1981), (3) Wasmund et al. (2005) (4) HELCOM (2009), (5) Ronnberg and Bonsdorff (2004)

The water quality of the Baltic Sea changed slowly from a pristine sea in the 19th century into a heavily degraded status today (DiMento and Hickman, 2012). As the Baltic Sea environment has been characterized as unique and fragile, human activities had negative effects on both water quality and the ecosystem. The high population around the Baltic sea Basin, the large drainage area, the small and shallow water body, the limited exchange of the Baltic Sea water masses with the North Sea through the Skagerrak Straits, the intensive agricultural activities as well as industrial activities which discharge high loads of nutrients mainly nitrogen and phosphorus into the Baltic Sea the relatively long residence times and the climatic conditions favor marine eutrophication.

The transition rate has been accelerated since 1950's due to the industrial development and intensified agricultural practices by the neighboring countries. In addition to the limited exchange of water masses with the North Sea, there are no major water inflows in the Baltic, resulting in hypoxia or even anoxia in the central part of the sea. Freshwater inflows in the Baltic form vertical stratification, a feature that increases the vulnerability of the Baltic Sea (Pawlak et al., 2009). Salinity stratification restricts ventilation/oxygenation and finally leads to oxygen depletion. Reduced conditions in the sediments favor phosphorus release into the water column. Land sources of municipal and agricultural origin cause a threefold increase in nitrogen and fourfold increase in phosphorus. Nutrient loads increase biological productivity by two to three times (Saychuk et al., 2008). In addition to land based sources, nutrient also originate from the atmosphere as well as from the atmospheric nitrogen through nitrogen fixation by cyanobacteria (Kirchman, 2000).

During 2001-2006 the water transparency status showed that it was lower in coastal and transitional waters but higher in the open sea. Secchi disk values in the open sea varied between 3.0 and 8.5 m, the median value being 6.0 m (HELCOM, 2009). Transparency status among the sub-basins was satisfactory in the Ankora Basin and the Kattegat but values showed a decrease towards the Gotland Basins and the Gulf of Riga, the lowest values being observed in the Gulf of Finland and the Gulf of Bothnia. In the shallow coastal zone of the North-Eastern Gulf of Riga, the annual mean of the Secchi disk was 1.6 m, in the Vistula plume 4.1 m and in the Oder plume 3.0 m (Wasmund et al., 2001). In these areas the maximum value of the Secchi disk was 15 m. Long term trends during two discreet time periods 1919-1939 and 1969-1991 by Sanden and Hakanson (1996) showed that a Secchi disk decrease by approximately 0.05 my^{-1} was observed during both periods. Transparency changes follow an increase in chl-*a* values about 1% and a primary productivity increase slightly less than 1 per cent per year.

These pressures have also impacts at ecosystem level. Increased phytoplankton biomass, reduces water transparency which in turn limits the colonization depth of benthic macroalgae and seagrasses. These changes also affect species composition and species dominance of both the planktonic and benthic communities. Excessive plant biomass requires large quantities of oxygen during the decomposition phase, adding to the hypoxia and eventually affecting benthic animals and fish (Gray, 1992). Among the effects of limited light penetration to the seafloor, reduction of the area at the bottom of the sea that could be colonized by perennial species, the settlement of opportunistic species characterized by a short life cycle and rapid growth have been reported (HELCOM, 2009).

Nutrient enrichments increase primary production, enhance algal bloom formations and decrease water clarity. It has been reported (Fleming-Lehtinen and Kaartokallio, 2009) that water clarity in the Baltic measured as Secchi disk depth was halved from 1903 to 2009: visibility has decreased from 9 m to 4 m in the main basin and from 8 m to 4 m in the Gulf of Finland.

6.3.2. Environmental Pressures and Eutrophication in the Baltic Sea

Nutrient inputs come from five different sources: (a) direct inputs from domestic effluents (b) direct inputs from industrial wastes (c) indirect inputs from rivers (d) input via atmospheric deposition and (e) via water mass inflow through the Danish Straits (Hakanson and Bryhn). Early studies on nitrogen and phosphorus inputs into the sea have estimated that the total input of nitrogen from domestic and industrial sources was about 50,000 ty^{-1} and the river input about 18,000 ty^{-1} (Kullenberg, 1981). Input of phosphorus from domestic – industrial sources was about 7,500 ty^{-1} and the river output $2x10^5$ ty^{-1}. The net (external) supply of phosphorus was estimated to be within the range of 16 to $40x10^3$ ty^{-1} although uncertainties in the calculations have been reported. Estimates of nitrogen loads in the Baltic Proper/ Gulf of Riga and the Bothnian Bay/ Bothnian Sea showed an upward trend during the 70s and 80s followed by a slight decrease during the 90s. Nitrate loads in the Baltic Proper / Gulf of Riga in the 70s were 246,917 ty^{-1}, in the 80s 275,664 ty^{-1} whereas in the 90s, 218,181 ty^{-1} (Voss et al., 2011). A similar trend was observed in the Bothnian Bay / Bothnian Sea. The nutrient status during 2001-2006 (HELCOM, 2009) has shown that the highest dissolved inorganic nitrogen (DIN) concentrations were observed in the Bothnian Bay; as the Bothnian Bay is phosphorus limited, large amounts of nitrogen may accumulate. Similar situation was also reported for the Gulf of Finland. The high DIN concentrations were attributed to nutrient loads from the Neva River. The highest total nitrogen concentrations were observed in the Gulf of Riga and the Gulf of Finland (26 and 24 μM respectively). There was a gradient of decreasing nitrogen concentrations from the Baltic Proper towards the Danish Straits due to the mixing conditions with the Skagerrak water layer, characterized by low total nitrogen values. Dissolved inorganic phosphorus (DIP) concentrations were high in the Gulf of Riga (0.78 μM) as well as in the Gulf of Finland (0.8 μM) due to river outflows and the sediment resuspension in the Baltic Proper being rich in phosphorus (Pitkanen et al., 2001). Lower DIP concentrations were observed in the Bothnian Sea, the Baltic Proper and the Danish Straits, ranging between 0.35 and 0.47 μM. The lowest DIP concentrations (0.06 μM) were observed in the Bothnian Bay. Total phosphorus concentrations (TP) followed the same trend as DIP, the range being from 0.16 μM (Bothnian Bay) to 0.85 μM (Gulf of Finland). Coastal DIP concentrations in the Bothnian Bay were well correlated with loads of terrestrial origin. Increased N:P ratios were observed due to decreasing phosphorus levels accompanied by an overall excess of nitrogen. These findings support the view that the Bothnian Bay is phosphorus limited. Nutrient concentrations values in the various areas of the Baltic Sea are given in Table 17.

Table 17. Nutrient concentrations in the Baltic Sea

Area	Nitrate (μM)	Phosphate (μM)	Comment	References
Baltic Proper	$0.75 \pm 1.11^{(1)}$	0.10 ± 0.15	year 2001	Wasmund et al. (2005)
Gulf of Riga	$0.78^{(1)}$	0.26	Period 2001-6	Andersen et al. (2011)
Gulf of Finland	$0.84^{(1)}$	0.24	Period 2001-6	Andersen et al. (2011)
Baltic Proper		0.35-0.47	Period 2001-6	Andersen et al. (2011)
Bothnian Bay		0.06	Period 2001-6	Andersen et al. (2011)
Gulf of Riga	$23.3^{(1)}$	0.51-0.77	Period 1976-2008	Jurgensone et al. (2011)
Vistula Lagoon	$205\text{-}212 \pm 4^{(2)}$		1989-2005	Semerova and Smyslov (2009)
Whole Baltic	$37.4 \pm 36.6^{(1)}$	1.0 ± 0.78	1984-2001	Gasiunaite et al. (2005)
Gulf of Riga	7.9 ± 4.2	0.81	1974-2000	Yurkovskis (2004)
Coastal waters	$6.5^{(1,3)}$	$0.9^{(3)}$	1996-1999	Clarke et al. (2003)

(1) DIN; (2) mgm^{-3}; (3) average values (N=70)

Phytoplankton biomass has been described mainly as chl-*a* concentration (HELCOM, 2009). The Ecological Quality Ratio (EQR) was used based on average chl-*a* concentrations and the reference conditions for the respective areas (HELCOM, 2009). The Gulf of Finland, the Gulf of Riga and the Baltic Proper showed the highest deviations from the reference condition values (target EQR value: 0.67), the values being 0.22, 0.35 and 0.23 respectively. EQR values for chl-*a* in the Arkoma Bay and the Gotland Basins ranged from 0.40 to 0.55. Geographical variation was observed in coastal and transitional waters. High chl-*a* concentration values were recorded in the Vistula Lagoon (Semenova and Smyslov, 2009) during 1989-2005. Values as high as 90.9±5.6 μgL^{-1} were registered in August 1997 whereas most values during that period ranged between 39.1 and 130 μgL^{-1}. Chl-*a* data are given in Table 18.

Table 18. Chlorophyll *a* concentrations in the Baltic Sea

Area	**Chl-*a* (μgL^{-1})**	**Comments**	**Reference**
Baltic Proper	1.96±0.72	Upper mixed layer (2001)	Wasmund et al. (2005)
Gulf of Finland	5.4	June-Sept 2001-6	Andersen et al. (2011)
N. Baltic Proper	4.8	June-Sept 2001-6	Andersen et al. (2011)
Gulf of Riga	5.3	June-Sept 2001-6	Andersen et al. (2011)
Open water bodies	1.9-2.7	June-Sept 2001-6	Andersen et al. (2011)
Little Belt (Denmark)	2.5-3.5	2000-2004	Lund-Hansen et al. (2008)
SE Baltic Sea (Russian Sector)	2.9	2003-2007	Kudryavtseva et al. (2011)
Baltic Proper	9.0±1.8	1992-2004	Fleming & Kaitala (2006)
Baltic Proper	13.0 ±3.2	Peak average, 1992-2004	Fleming & Kaitala (2006)
Ankora Basin	7.0 ±0.7	1992-2004	Fleming & Kaitala (2006)
Ankora Basin	10.0 ±2.2	Peak average, 1992-2004	Fleming & Kaitala (2006)
Gulf of Finland	12.0 ±2.2	1992-2004	Fleming & Kaitala (2006)
Gulf of Finland	23.0 ±5.8	Peak average, 1992-2004	Fleming & Kaitala (2006)
Vistula Lagoon	40.0 ±7.9	1989-2005	Semenova and Smyslov (2009)
Gulf of Finland	6.4 ±3.3	1992-1994	Tamminen & Andersen (2007)

Primary Productivity (PP) data from *in situ* measurements are available for many areas of the Baltic Sea (Table 19). Annual gross PP measured in the Gulf of Gdansk during 1993-1997 was 190 gCm^{-2}y^{-1} whereas in the Gulf of Riga during 1991-1997 the average annual production was estimated about 255 gCm^{-2}y^{-1}. Primary productivity measurements have also been carried out in the Russian sector of the Baltic Sea during 2003-2007: the average annual values for PP were 231 gCm^{-2}y^{-1}. Andersen et al. (2011) have classified the eutrophication status in 17 open areas in the Baltic Sea using a multimetric indicator-based assessment tool. This work based on monitoring data has shown that the status was “Bad” in 9 areas, “Poor” in 3 areas, “Moderate” in 3 areas and “Good” only in two out of the 17 areas. None of the areas studied was characterized as “High” as far as the water quality is concerned. Rydberg et al. (2006) have estimated primary productivity over the last 50 years using monitoring data from the Baltic entrance (Kattegat and the Belt Sea). Annual PP values ranged from 50 gCm^{-2}y^{-1}, in the open waters to 500 gCm^{-2}y^{-1} in some Danish fjords during the 1950s. Primary productivity in the Kattegat and the Sound during 1981-2000 ranged between 135-145 gCm^{-2}y^{-1}. Co-variation between nutrient concentrations and primary productivity values were observed after the 1980s. The observed large spatial and temporal variability (Richardson and Christoffersen, 1991) has been explained through the rapid shifting of hydrographic conditions in the sea. In addition, major changes in plankton flora and nutrient concentrations (Andersson and Rydberg, 1988) have also a substantial contribution to the problem of eutrophication.

Table 19. Primary Productivity (PP) in the Baltic Sea

Area	PP	Units	Comments	Reference
Gulf of Gdansk	190	$gCm^{-2}y^{-1}$	1993-1997	Wasmund et al. (2001)
Gulf of Riga	255	$gCm^{-2}y^{-1}$	1993-1997	Wasmund et al. (2001)
Baltic Proper	175	$gCm^{-2}y^{-1}$		Shaffer (1987)
E. Gotland Sea	49	$gCm^{-2}y^{-1}$		Stigebrandt (1991)
Gulf of Gdansk	25.4 ±19.2	$\mu gCdm^{-3}h^{-1}$	1993	Witek et al. (1997)
South Baltic – German Coast	268 ±199	$gCm^{-2}y^{-1}$	1960s and 1970s	Wasmund and Kell (1991)
Little Belt – German Coast	686 - 892	$gCm^{-2}y^{-1}$	2000-4	Lund-Hansen et al. (2008)
SE Baltic Sea – Russian Sector	231	$gCm^{-2}y^{-1}$	2003-2007	Kudryavtseva et al. (2011)
Baltic entrance	50-500	$gCm^{-2}y^{-1}$	1950s	Rydberg et al. (2006)
Kattegat	135-165	$gCm^{-2}y^{-1}$	1981-2000	Rydberg et al. (2006)
NW Kattegat	125	$gCm^{-2}y^{-1}$	1971-1979	Aertejerg et al. (1981)

Spatial variability in the Baltic Sea was observed during 1998 and 2005 using satellite imagery. The average chl-*a* maxima in July-August during 1998-2005 were 2.3 μgL^{-1}. Higher values were observed in the sub basins, chl-α values in the Gulf of Finland being greater than 4 μgL^{-1}. The July-August values in the Gulf of Bothnia were 2.5 μgL^{-1}. The lowest values were observed in the Kattegat (0.5-2.0 μgL^{-1}) during the same period. The general temporal trend of chl-*a* values showed an increase from the 1970s to the present. Chl-*a* values in the Bothnian Bay were increased from 1.1 μgL^{-1} to 3.2 μgL^{-1} from the late 70s until the late 1990s, followed by a decrease to the level of 2.4 μgL^{-1} (Fleming-Lehtinen et al., 2008). Studies on phytoplankton distribution during 1992-2004 (Fleming and Kaitala, 2006) showed that spring blooms were very intensive in the Gulf of Finland (chl-*a* values 23 ±5.84 μgL^{-1}), weakening towards the south in the Ankora basin (10 ±2.23 μgL^{-1}). In addition to maximum chl-*a* concentration values, the bloom lasted longer in the north than in the south. Distinct long changes in the bloom intensity were not observed.

Satellite images in the Baltic Sea show clearly eutrophic to hyper eutrophic conditions during all the year round (Figures 3 and 4), especially during summer, winter and spring, with chl-*a* concentrations higher than 5 mgm^{-3}. The Gulfs of Finland and Riga are hyper eutrophic. In autumn the hyper eutrophic conditions are limited mainly to the Gulf of Riga and the coastal areas of the whole Baltic Sea. The Danish Straits are also eutrophic throughout the year.

6.3.3. The Governance

The Convention on the protection of the Marine Environment of the Baltic Sea Area known as HELCOM started during the cold war. This Convention was signed in 1992 and entered in force in 2000. The main objectives were to take "legislative, administrative or other relevant measures to prevent and eliminate pollution" so that ecological integrity and ecological balance can be finally archived (HELCOM, 2008). The HELCOM Baltic Sea Action Plan adopted by the ministers of the Baltic countries in 2007 had identified as major environmental problems nutrient inflows, hazardous substances and maritime activities whereas as environmental threats biodiversity decline, nature conservation and eutrophication. As eutrophication was considered as the major pollution problem in the area, plan's objectives for eutrophication include decrease of nutrient concentrations close to natural levels in order to achieve good environmental status. It has been estimated that nutrient inputs should not exceed 21,000 tons of phosphorus and about 600,000 tons of nitrogen. As nutrient inputs during the period 1997-2003 were estimated to be about 36,000 tonnes of phosphorus and 737,000 tonnes of nitrogen per year, the amounts of phosphorus inputs should be halved whereas the reduction of nitrogen should be about 20%. Nutrient reductions have been allocated among the signatory countries. The quantities are given in Table 20. Specific measures have been proposed to address these reduction targets (a) wastewater treatment includes removal of phosphorus up to 80-90% as well as substitution of phosphorus in detergents (b) implementation of measures to reduce nutrient inputs of agricultural origin by adopting good practices for manure and fertilization handling and (c) development of relevant programs for nutrient reductions that should include River Basin Management Plans as well. Measures also include monitoring activities and application of appropriate indicators expressing "good quality status" as indicated in the EU Water Framework Directive (EC, 2000).

In addition to the HELCOM regulations EU cooperation is the other environmental regulatory component of the Baltic Sea. All Baltic countries are EU members apart from the Russian Federation. The EU regulates eutrophication through a number of Directives mentioned in Section 5.7. In addition, the Common Agricultural Policy (CAP) and the Common Fisheries Policy (CFP) set rules as to the power of the states in those two activities.

6.3.4. Effectiveness of the Measures

The Baltic Sea seems to enjoy high reputation for both the legal framework and its effectiveness (DiMento and Hickman, 2012), mainly due to comprehensive governance components and the successful implementation of the Baltic Sea Action Plan. The work on improving water quality in the Baltic region has been considered as pioneering. According to Gelpke and Jayarwardene (2004) "the inspiration to initiate conventions for similar-sized semi-enclosed marine areas like the Red Sea, the Persian Gulf, the Mediterranean and others, was drawn from the success of the intergovernmental action in protecting the Baltic Sea".

Among the environmental threats in the Baltic Sea, nutrient inputs have been reported as "one of the essential Baltic problems (Leppakoski, 1980). The visible effects were reduction of water transparency but a decrease in landings of commercial fishes had also been observed. Regarding eutrophication HELCOM Convention focused on urban waste water treatment. Baltic countries constructed biological sewage treatment plants with tertiary treatment. The loads of phosphorus and nitrogen due to urban sewage were reduced by 90% and 40% respectively (Pawlak, 1980). According to Swedish data, the total decrease of phosphorus inputs to the Swedish coastal area was about 50%. Nitrogen inputs to the Baltic of municipal and industrial origin had decreased by 35% and 22% respectively, although there was an overall increase by 10% due to an increased run off by the rivers. It was therefore recognized the need to take further measures to reduce agricultural inputs (HELCOM, 2009).

The monitoring program required by the HELCOM is a powerful operational tool for assessing the effectiveness of the measures. According to the monitoring time series since the mid 1990s, it has been estimated that the amount of 37,535 t of phosphorus in 1995 was reduced to 25,300 t of phosphorus per year in 2008. Similar downwards trends were estimated for nitrogen: 800,960 t of nitrogen in the mid 1990s were reduced to 580,630 t of nitrogen per year. These figures are impressive and it has been assessed that over the last twenty years the annual decrease in phosphorus loads was about 13 per cent (DiMento and Hickman, 2012), a decrease that was found statistically significant. However, this desrease has not avoided criticism. Hakanson (2006) maintains that although nutrient input reduction is a politically correct decision, it may not mean much in practical terms if percentages are used: a fifty per cent reduction in 10 kg of phosphorus is 5 kg, whereas a 50% in 10 t is 5,000 kg (Hakanson, 2006).

Table 20. Nutrient reduction allocations to reach good ecological status agreed by the HELCOM signatory parties

Member State	Phosphorus (t)	Nitrogen (t)
Denmark	16	17,200
Estonia	220	900
Finland	150	1,200
Germany	240	5,600
Latvia	300	2,560
Lithuania	880	11,750
Poland	8,760	62,400
Russia	2,500	6,970
Sweden	290	20,780
Transboundary pool	1,660	3,780
Total	15,016	133,170

HELCOM (2007)

The nutrient reduction planned which is 133,000 t of nitrogen and 15,000 t of phosphorus may not be correct, although is based on the MARE NEST model outputs (Savchuk and Wulff, 1999). These nutrient loads have been allocated as reduction quotas to the different Baltic Sea countries (Table 20). However, the cost differs from one country to another depending on the economy sector that has to contribute most of the nutrient reductions. Phosphorus and nitrogen reduction of some activities can be fairly costly. It has been reported (Hakanson, 2006) that the cost reduction of phosphates in detergents is less than 0.4 €/kg whereas phosphorus reduction in agriculture is between 5 and 100 €/kg. Phosphorus reduction in feed for animals is estimated between 5 and 7 €/kg of phosphorus and cultivation – harvesting of mussels – clams costs 35 €/kg. The final target of HELCOM to remove 133,000 ty^{-1} of nitrogen and 15,000 ty^{-1} of phosphorus seems to be particularly costly; the estimated cost is of the order of 10 billion euros. It has also been reported that the problem of eutrophication differs among the various sub regions of the Baltic Sea. The Gulf of Riga and the Gulf of Finland seem to be "hot spots" in the Baltic which means the more stringent measures should be taken. HELCOM has identified 169 pollution hot spots; this number though includes other forms of pollution. Ninety four out of the 169 hot spots had been cleared by 2011, most of them polluted by waste water treatment plants (DiMento and Hickman, 2012). However, diffuse sources of eutrophication such as agricultural runoff and airborne nutrients were much more difficult to control. It should not pass unnoticed that part of the reductions in 1990s was the result of collapsing of agriculture in the Eastern Block. Further suggestions on mitigating nutrient loadings in the Baltic Sea is focusing on the hot spots and emphasis should be paid on phosphorus as it is known by now that phosphorus is the limiting nutrient in most areas in the Baltic Sea.

6.4. The North Sea

6.4.1. Physiography and Environmental Settings

The North Sea is a marginal sea on the NE Atlantic, surrounded by Germany, the Netherlands, Belgium and France to the South, Denmark and Norway to the East, the Orkney Islands and the east coast of England and Scotland to the West. It is bordered by the Shetland Islands to the North and the main connection with the Atlantic is with the Norwegian Sea on the Northern part. The North Sea is also connected with the Atlantic through the English Channel. The Skagerrak and Kattegat Straits connect the North Sea to the Baltic; the North Sea is also connected with the Atlantic through the Dover Straits.

The area of the North Sea encompasses and area of 575,000 km^2 and volume of 40.3 km^3 (Reid et al., 1988). It is about 970 km long and 580 km wide with the average depth about 90 m. Apart from the Norwegian trench with a maximum depth of 725 m, the North Sea is a relatively shallow Sea (30-200 m). The North Sea receives freshwater from European watersheds as well as from the British Isles. The largest continental watersheds affecting the North Sea are the Elbe and Rhine-Meuse watersheds. In addition to the drainage basins of the bordering countries, the North Sea also receives water masses from the Baltic Sea. The North Sea is used extensively for a variety of purposes and therefore the pressures in the marine environment are remarkable. In addition to the marine traffic, a population of 31 million people lives along the coastal areas and estuaries of the North Sea and 164 million people in the catchment area (Ducrotoy, 1999). The influx from summer visitors and industrial activities, have also to be taken into account. The area receives wastes from coastal cities as well as from the population discharging wastes into a number of rivers: the Rhine, Elbe, Weser, Scheldt, Ems, Thames, Trent, Tees and Tyne (Clark, 1992). It has been assessed that 50% of the total nitrogen input comes from Rhine and Meuse (NSTF, 1994). However, the major influence of the North Sea comes from the Atlantic Ocean through the northern part. Understanding of eutrophication processes requires knowledge of hydrography and water balances. Reviews on the physical oceanography of the North Sea have already been presented (Salomons et al., 1988). Estimates on precipitation – evaporation balance of water masses have indicated that at an average there is an excess of freshwater supply over evaporation. The flow rate of the excessive water mass is 2,700 m^{3}s^{-1}. The precipitation excess is about 70 mm in the southern part and increases towards the north ranging between

200 and 300 mm (ICES, 1983). Precipitation is greater than evaporation during the summer time everywhere in the North Sea encouraging the formation of vertical temperature gradients. Changes in salinity affect the water balance. It has been estimated that during the period 1948-1974 as much as 56% of the annual salinity variation was due to the river flows (Reid et al., 1988).

Temperature and salinity differences affect the vertical structure of the water bodies. Good vertical mixing prevails during winter in most areas, whereas from spring to autumn some areas are well stratified. As a consequence the North Sea encompasses at least two sub systems: a shallow well mixed area in the southern part and a deeper area in the central and northern parts with summer stratification. As the southern part is influenced by river outflows is more liable to eutrophication whereas the northern part is less liable to eutrophic drives (de Wolf and Zijstra, 1988). The boundaries between water masses of different regimes are sharply defined at the surface and they form what is known as “fronts” (Reid et al., 1988). The fronts cause surface agitation, often accompanied by eddies. These water movements have a decisive effect on nutrient dispersion and phytoplankton distribution.

6.4.2. Environmental Pressures and Eutrophication in the North Sea

The increased nutrient discharges in the North Sea during the last few decades causes changes on the European Continental Coastal Ecosystem (De Galan et al., 2004). Those changes are triggered by nutrient loads and they form complex interactions between algal abundance, productivity, light availability and mixing conditions in the water column (Ducrotoy, 1999).

In addition, frequent occurrences of phytoplankton blooms arouse public concern. It has been estimated that the yearly algal blooms during the past three decades has been doubled on average (Ducrotoy, 1999). The intensity of eutrophic symptoms is causing oxygen depletion events especially near the bottom water masses. This is attributed to the decomposition of excessive planktonic biomass produced under eutrophic conditions.

Increased levels of nitrogen and phosphorus had been observed since 1960’s (Table 21). Phosphate concentrations in 1970’s were significantly higher than those reported in 1962 (Folkard and Jones, 1975).

The higher increase of phosphates compared to nitrates and nitrites were attributed to detergents. Postma (1973) attempted a rough calculation of nutrient inputs into the North Sea from the Atlantic and the rivers. The estimations for phosphates were 70,000 tons from the Atlantic and 70,000 tons from the rivers which indicated that both phosphorus sources were equivalent. The estimates for nitrogen were 550,000 tons from the Atlantic and 700,000 tons from the rivers. The Rhine River alone accounted for 28,000 tons of phosphorus and 280,000 tons of nitrogen. Postma (1973) also calculated primary production and found that it varied between 250 gCm^{-2} to 350 gCm^{-2}; these high values were explained by high nutrient loads. Richardson and Heilman (1995) compared measurements of primary production from the 1950s with measurements that had been carried out during the period 1984-1993 and concluded that primary productivity rates increased from 100 $gCm^{-2}y^{-1}$ to about 200 $gCm^{-2}y^{-1}$. More information on primary productivity values are given in Table 23.

Table 21. Nutrient concentrations in the North Sea

Area	**Nitrate (µM)**	**Phosphate (µM)**	**Comment**	**References**
Dover Strait	0.85	0.1	1979-1981	Bently et al. (1993)
Bay of Somme	0.15-30*	>2	1992-2007	Lefebvre et al. (2011)
Cental North Sea	<6	<0.5	1987	Van Beusekom and Diel-Christiansen (2009)
Helgoland Waters	20.0	0.8-1.0	Before 1981	Raabe & Wiltshire (2009)
Western North Sea	1.8±1.9		2004-2005	Suratman et al. (2008)
NE North Sea		0.6**	2001-2005	Eilola et al. (2010)
North Sea (Denmark)	10.0*	0.65**	2001-2005	Eilola et al. (2010)
SE North Sea	8.50*	0.60**	2001-2005	Eilola et al. (2010)
Central North Sea	8.0*	0.60**	2001-2005	Eilola et al. (2010)
SW North Sea	10.0*	0.8**	2001-2005	Eilola et al. (2010)
Dutch Coast (10 miles offshore)	56.5±18.6*	1.35±0.33**		Skogen & Mathisen (2009)
Dutch Coast (70 miles offshore)	7.7±2.1*	0.43±0.10**		Skogen & Mathisen (2009)

*DIN; **DIP

In addition to nitrogen riverine discharges in the marine environment mentioned above that have been estimated to be worldwide about 60 TgNy^{-1}, nitrogen deposition from the atmosphere should also be taken into account. Although the average nitrogen deposition in the tropics has only been doubled over the last few decades, it has increased more than six fold in the temperate zone due to human activities (Holland et al., 1999). Nitrogen deposition through the atmosphere is remarkable throughout Europe and should be taken into account when quantitative aspects of eutrophication are considered (UNEP, 2003b; OSPAR, 2003b).

The result of phosphorus and nitrogen increase was that silicon became the first nutrient to be depleted, whereas silicon 40 years earlier was abundant compared to phosphorus and nitrogen concentrations (Van Bennekom et al., 1975). On the other hand, as phosphorus inputs were increasing at a higher rate compared to nitrogen, it was concluded (Van Bennekom et al., 1975) that phosphate could still function as the limiting factor since the average N/P ration in algae is 15. *Phaeocystis puchetti* seems to consume phosphates, causing temporarily phosphate depletion during the spring blooms. Observations on nutrient concentrations near Helgoland influenced by inputs from the river Elbe (Lucht and Gillbricht, 1978), indicated that the atomic N/P ratio had decreased due to increased phosphorus concentrations during 1968-1974. They concluded that “this result indicates that P will lose its possibly limiting influence upon phytoplankton development if its concentration increases further with respect to N”. It was noticed in the area of Helgoland since 1962 (Hagmeier, 1978) that there was an inverse relation between phytoplankton biomass and salinity indicating the influence of river discharges into the marine environment. Measurements based on Continuous Plankton Recorders (Reid, 1978) showed an upward trend in phytoplankton biomass in the North Sea since 1958. Quantitative differences were also observed: diatom populations decreased whereas biomass changes in *Ceratium* species were minimal. Various possible explanations were examined beyond nutrient enrichments; marine pollution, climatic changes, biological succession, phytoplankton composition and sampling artifacts contributed to the multidimensionality of the North Sea eutrophication problem. The fact that changes concerning both phytoplankton and zooplankton in the North Sea were similar to plankton changes in the North Atlantic seem to support the view that increased phytoplankton biomass and increased primary productivity could be attributed to climatic changes. Reid (1978) urged that lack of time series of phytoplankton production measurements in the North Sea could not be easily explained. As primary production measurements before 1965 were lacking, phytoplankton biomass assessments were based on estimates. Postma (1973) concluded that a 30% increase of the primary production was due to the influence of the Rhine River. It has also been calculated (de Jonge and Postma, 1974) that yearly average concentrations of phosphate and particulate phosphorus were doubled in the Western Wadden and the adjacent North Sea between 1950 and 1970. Increased phytoplankton production in the area probably because of phosphorus availability has also doubled total suspended matter during the same period.

As there are extensive shallow areas in the North Sea, the benthic – pelagic coupling is important in the productivity of the North Sea. Marine plants through their photosynthetic activity use inorganic sea water constituents. Phytobenthic communities in the North Sea occur down to 30 m in the most transparent areas whereas in coastal waters their maximum depth of occurrence is limited between 10 and 15 m. Macrophyte accumulation tends to increase over the last decades. It is estimated that 50,000 tons of plant material is removed from the North Sea beaches every year (NSTF, 1992). Estimates of the cover density of macroalgal species belonging to Chlorophyta and Ulvae were carried out during 1990-1992 by means of aerial surveys on the German Coasts. At least 15% of the area is covered by algae every year whereas sometimes the macroalgal carpet covers between 30 and 60% of the tidal flats (Ducrotoy, 1999).

The North Sea satellite images show mesotrophic to eutrophic characteristics (Figures 3 and 4). The eutrophic trends are mainly observed to the entire southeastern continental coast of the North Sea and they are extended quite offshore especially during winter and spring. The northern offshore area shows mesotrophic trends throughout the year. Chlorophyll data are given in Table 22.

6.4.3. The Governance

The present cluster in the North Sea contains three major types of international components: (a) the International Council for the Exploration of the Sea (ICES) that is also involved in marine pollution issues (b) the Convention for the Protection of the Marine environment of the North East Atlantic (OSPAR) and (c) the EU Directives on the marine environment. The OSPAR Convention is the result of merging the Oslo and Paris Conventions; this merging took place in 1992 in a ministerial meeting of the Oslo and Paris Commissions. Among the priorities set as political initiatives, was the agreement to eliminate various pollutants (toxic persistent and bio-accumulating compounds) into the marine environment. The second priority was referring to the reduction of nutrients “where the inputs are likely to cause eutrophication”. Following the OSPAR Convention a number of working groups was formed for assessment and monitoring. The group on the development of the Strategy to Combat Eutrophication was co-ordinated by the Programme and Measures Committee (PRAM) whereas questions of marine scientific nature were addressed by the Environmental Assessment and Monitoring Committee – ASMO (de Jong, 2006). The Committee's objectives included the development and adoption of common practices for addressing eutrophication problems in the North Sea, including a science background on nutrient input levels, N/P ratios, ecosystem quality, local differences and seasonal variations. The first target of the common identification procedure was to establish criteria to discriminate between eutrophic and non-eutrophic conditions. This work lasted for five years between 1993 and 1998. The second target includes the development of ecological objectives regarding eutrophication. The efforts of ASMO to come to a proposal adopting common procedures ended in 1994 to a draft report on "*Common procedure for the identification of the Eutrophication Status of the Maritime Area of the Oslo and Paris Conventions*". This report was finally adopted with the reservations of France, Spain and the United Kingdom (ASMO, 1995). The eutrophication status was classified into three categories: (a) Eutrophication Problem Areas: when there is strong evidence of impacts on marine ecosystem quality due to enrichments by nutrients (b) Potential Eutrophication Problem Areas: when the status of eutrophication is unknown. This uncertainty may be due to: (i) insufficient scientific information as to classify the conditions of the sea and (ii) there is evidence that nutrient levels, trends of fluxes can cause disturbance to the marine ecosystem and (c) the European Union Policy: although the EU started environmental policies as early as 1972 (as European Economic Community – EEC then) and a number of Directives followed related to marine environmental issues, the first Directive exclusively devoted to the marine environment, the "Marine Strategy Framework Directive" (MSFD)appeared relatively recently (EC, 2008). This Directive recognizes the North Sea Atlantic area which also includes the North Sea, as a distinct ecoregion.

Table 22. Chlorophyll *a* concentrations in the North Sea

Area	**Chl-*a* (μgL^{-1})**	**Comment**	**Reference**
Bay of Somme	58.5	Max values 1992-2007	Lefebvre et al. (2011)
Western North Sea	1.0±0.7	2004-2005	Suratman et al. (2008)
North Sea (Denmark)	3.0	2001-2005	Eilola et al. (2010)
SE North Sea	2.0	2001-2005	Eiola et al. (2010)
SW North Sea	3.0	2001-2005	Eilola et al. (2010)
W North Sea	7.5	2001-2005	Eilola et al. (2010)
NE North Sea	3.0	2001-2005	Eilola et al. (2010)
Dutch Coast (10 miles offshore)	9.4 ±2.7		Skogen & Mathisen (2009)
Dutch Coast (70 miles offshore)	1.1 ±0.2		Skogen & Mathisen (2009)

Table 23. Primary Productivity (PP) in the North Sea

Area	**Primary Prod.**	**Units**	**Comments**	**Reference**
Central North Sea	150-250	$gCm^{-2}y^{-1}$	1980s	Joint & Pomroy (1993)
Coastal North Sea	300-400	$gCm^{-2}y^{-1}$	1980s	Joint & Pomroy (1993)
Northern North Sea	70-90	$gCm^{-2}y^{-1}$	1965-1970	Steele (1974)
Southern Bight (Belgium)	320	$gCm^{-2}y^{-1}$	1970s	Joint et al. (1993)
English Channel	100-300	$gCm^{-2}y^{-1}$	1964-1974	Boalch et al. (1978)
Whole North Sea	250-350	$gCm^{-2}y^{-1}$	1960s	Postma (1973)
Whole North Sea	115	$gCm^{-2}y^{-1}$	1988	Skogen et al. (2004)
Central North Sea	0.91-153	$gCm^{-2}d^{-1}$	1981-1983	Reid et al. (1990)
Open North Sea	0.45-3.2	$gCm^{-2}d^{-1}$	1983	Reid et al. (1990)

6.4.4. Effectiveness of the Measures

The main objective for mitigating eutrophication in the North Sea is the reduction of nutrients by 50% compared to input levels in 1985. These inputs refer to phosphorus and nitrogen in water bodies and it is likely to cause disturbance (OSPAR, 2009). Within twenty years since the implementation of the measures that is in the year 2005, most of the OSPAR Member States reported success for the 50% reduction target concerning phosphorus (OSPAR, 2008a). On the contrary there was not success for the target value referring to nitrogen: only Denmark succeeded to reduce nitrogen inputs by 50%. Referring to the different sources of nutrient inputs in the Greater North Sea a total reduction of about 12% was recorded concerning riverine inputs and direct discharges of nitrogen during 1990-2001 (OSPAR, 2005a). A remarkable decrease in the total direct discharges of phosphorus (about 33%) was observed but the riverine inputs did not show any significant downward trend. Riverine discharges carry nutrients mainly from agricultural activities; agriculture accounts of 61% of nitrogen being the largest source and 32% for phosphorus being the second largest source (OSPAR, 2008b). Assessment on atmospheric transmissions showed that there was no change between 1990 and 2002 according to the Comprehensive Atmospheric Monitoring Programme (OSPAR, 2005b) whereas model results from the co-operative programme for monitoring and evaluations from the Long Range Transmission of Air Pollutants in Europe (EMEP), indicated a total reduction of 16% in the deposition of nitrogen in the North Sea (OSPAR, 2007b). However, in various smaller areas of the North Sea, upward trends of nutrient inputs have been locally observed. Increased atmospheric nitrogen deposition was also observed locally, possibly due to emissions from growing ship traffic. According to the EMEP model calculations, atmospheric nitrogen deposition accounts for the 1/3 of all nitrogen inputs in the North Sea. OSPAR has classified the marine areas into problem, potential problem and non-problem areas. Out of 204 areas assessed, 106 areas were classified as problem areas, 25 as potential problem areas and 73 as non-problem areas. Recent assessment has indicated that three offshore problem areas could now be classified as non-problem areas, whereas many other areas showed changes in the “good direction” with respect to eutrophication (OSPAR, 2009). The Swedish “offshore Skagerrak” and the Danish offshore “Skagerrak Open Area” have also been characterized by now as non-problem areas. The same happened with the Dutch “Offshore Grounds” (OSPAR, 2003a). However, many

North Sea States doubt about the effectiveness of the measures. This is because there is a time lag between the implementation of the measures and measurable improvement in the problem area. This lag period varies between 10 and 30 years according to some estimates (OSPAR, 2009). Some States maintain that a 50% reduction target will be effective to improve water quality and increase the number of non-problem areas, whereas Belgium supports the view that a 90% reduction will be necessary. As there is transboundary nutrient pollution due to nitrogen and phosphorus transportation from a long distance, many countries express their reservation about the effectiveness of nutrient reduction at the source. Unless nutrient deposition material originates from sources far away from the problem area, nutrient reduction at a local scale will not be an adequate measure to mitigate eutrophication.

Climatic variations and climatic change can also introduce uncertainty about the effectiveness of the measures. Unusually wet years increase the amount of runoff of nutrient into rivers and finally nutrient discharges into the marine environment. Changes in sea temperatures, nutrient availability as well as changes in the seasonal stratification affect phytoplankton species composition and biomass. An algal bloom of the toxic microalga *Dinophysis* was reported after a period of heavy rainfall (OSPAR, 2009). Higher atmospheric concentrations of carbon dioxide increase the acidity of the seawater. This can have effects on primary productivity and possibly on the calcification of the cell walls of some groups of microalgae. Continuation of the implementation of the measures and assessment of their effectiveness through monitoring project and modeling, will provide information on whether they are in the “good direction”. A decision therefore could be made in future on whether the adopted measures will continue or a more flexible management scheme should be adopted.

7. Discussion

Eutrophication was first recognized as an environmental problem in freshwater systems a long time ago (Naumann, 1919; Hutchinson, 1967); however, marine eutrophication has been recognized as a problem just since the 50s (NRC, 1969). This delay is possibly due to the belief that hydrodynamic conditions in the marine environment are adequate for nutrient transportation and diffusion. In addition, incorporation of nutrient inputs through chemical and biological processes would take place without noticeable adverse effects on the ecosystem and the marine environment in general. The impact of eutrophication was more noticeable along the coastal zone which is a very important part of the global system: although the coastal zone is less than 20% of the Earth’s surface, it sustains 90% of the fisheries and 25% of the world’s biological production (BSPC, 2006).

International agreements such as the Barcelona Convention for the protection of the Mediterranean Sea and the Helsinki Convention for the protection of the Baltic Sea were signed in 1976 and 1974 respectively (DiMento and Hickman, 2012); measures were proposed to mitigate among other environmental threats the problem of eutrophication. However, the biogeochemical processes concerning eutrophication were not well understood. This is not simply a matter of scientific interest but above all a practical problem. There has been a long debate on the limiting nutrient (Dugdale, 1967, NRC, 2000). It was well established that in most cases nitrogen was the limiting nutrient. However, it is known by now that there are sub basins in the European Regional Seas where phosphorus is the limiting nutrient like the Eastern Mediterranean and part of the Baltic Sea. The relationships between causative factors (nutrient inputs, elevated nutrient concentrations, change in the Redfield ratios) and indirect effects on zooplankton, benthic invertebrates and fish were understood and quantified in the late 80s. All international conventions and EU Directives seem to converge to the same objectives concerning the handling of eutrophication: high water transparency, oxygen levels, less algal bloom episodes and ecosystem health are the main goals. Those objectives require measures that are both costly and also fairly often cause conflicts in the society and the stakeholders.

The exponential increase of eutrophic events observed since the 1970s in many parts of the world was correlated with increased nitrogen supply due to anthropogenic activities (Boesch, 2002). As a result, decision makers in USA realized that nitrogen control was necessary to address coastal eutrophication especially in Tampa Bay and Chesapeake Bay (NRC, 2000). At that time in Europe there was no focus yet on eutrophication problems and consequently on the limiting nutrient. However, the Nitrate Directive (EC, 1991b) required from the Member States to reduce nitrogen discharges from agricultural sources. Although the main objective of the Nitrate Directive was to protect groundwater quality, coastal waters were also included. The fact that it was already known that the limiting nutrient in lakes was phosphorus, introduced a degree of uncertainty about the limiting nutrient in the sea until some fundamental biogeochemical differences between freshwater and the marine environment were understood: (a) the nitrogen fixation process is more intensive in the freshwater systems due to the abundance of blue-greens (b) denitrification takes place at high rates in the continental shelf of the marine environment compared to lakes (c) there is higher availability of phosphorus in the marine environment compared to freshwater systems; ions in saline waters tend to induce desorption of phosphate ions from suspended matter, increasing therefore the bioavailability of phosphorus to photosynthetic organisms (Howarth and Marino, 2006).

Nutrient reduction outflows may sound as an easy and effective solution; apart from the fact that the measures are costly, a number of principles should be taken into account (Ducrotoy and Elliot, 2008): (a) environmental sustainability, concerning habitat protection and ecological integrity (b) economic viability: changes on industrial production or adoption of good agricultural practices should be planned within an economically feasible framework (c) technological feasibility: engineers should be able to produce systems environmentally friendly at competitive prices (d) the measures should be socially tolerable (e) the measures should be legislatively permissible, administratively achievable and politically acceptable. All the problems mentioned above assume good environmental governance. However, at an international level there are complexities concerning governance due to “the number of organizations and laws, miscommunications, policy gaps, capacity deficiencies, competition for funding and waning political will” (DiMento and Hickman, 2012). The various initiatives and regimes aim at a specific environmental objective known as the “cluster”. In environmental governance the term clusters “describes the aggregation of attempts to improve regional seas” (DiMento and Hickman, 2012). Cluster components do not necessarily function in a harmonic way; there are activities in the cluster that due to bureaucratic or territorial reasons do not communicate with other cluster components and therefore do not integrate with them. Within this framework, international conventions and management practices should be reexamined.

The success in improving environmental quality through a cluster of initiatives and practices is assessed as “effectiveness”. The effectiveness (DiMento and Hickman, 2012) “denotes a solution of the environmental problem that brought together international actors to confront it”. It includes physical parameters i.e., whether marine water quality and ecosystem have improved but also effectiveness is related to environmental law, environmental management, environmental plans and programs. Finally effectiveness is achieved by good relations among the “players”: environmental governance cannot be effective if there are conflicts among the participants. As natural ecosystems and human activities are complex, decisions made rely on imperfect knowledge of the problem and therefore a continuous and systematic assessment of the effectiveness of the measures is necessary.

Conflicts within the cluster for marine environmental protection of the European Regional Seas are partly due to the effect of a strong institutional framework regarding marine environmental issues. Different bodies may be responsible for marine environmental legislation: among these bodies is the Environmental Council (Working Group on the Environment), the Transport Council (Transport Working Group) or the Fisheries Council (External Fisheries Group), all of them dealing directly or indirectly with environmental issues (Frank, 2007). Oceans and seas are dealt with by different committees in the European Parliament apart from the Committee for the Environment: Public Health Committee, Food Safety Committee, Agriculture and Fisheries Committee, Transport and Tourism Committee are among the committees with environmental interests. Administrative fragmentation also exists at national level: competent authorities on environmental matter are spread among Ministries of the Environment, Transport and Fisheries as well as with lower rank administrative units. The number of EC directives including eutrophication components that is the Urban Waste Water Directive, the Nitrates Directive, the Bathing Water Directive, the Shellfish Directive and the Water Framework Directive indicate the sector-by-sector responsibility of EU on environmental matters.

In addition to the institutional fragmentation mentioned above, there is often conflict of interests among the Member States. Member States can be divided into three groups according to their economic interests (Frank, 2007): (a) countries with strong maritime interests: Cyprus, Denmark, Greece and Malta and partly Belgium, the Netherlands and the UK (b) countries with strong fisheries interests: Ireland, Italy, Spain and Portugal (c) economically oriented countries: Germany, Finland, Sweden and to some extent the Netherlands. These diversified interests are expressed within the different institutions of the European Union. The adoption of marine environmental measures is limited because of the limited jurisdiction of the European Parliament in the environmental decision making process.

All conventions concerning the European Regional Seas have also been signed by States that are not EU Members. The Barcelona Convention has been signed by 22 Mediterranean States but only seven of them are EU members. In the Convention for the Black Sea only Bulgaria and Romania are member states whereas in the HELCOM Convention for the Baltic Sea, Russia is not an EU member state. This phenomenon is known as “mixity”. A direct legal consequence of mixity is what is known as “shared competence”. Conventions signed in the past among Member States did not necessarily agreed with the subject matter of EC Legislation (Frank, 2007). This kind of mixed agreement is defined by Schermers (1983) as “any treaty which an international organization, some of its member states and one or more third states are parties and for the execution of which neither the organization nor its member states have full competence”. The mixity problems have influenced EU external affairs. Many countries in the past especially from the Eastern Block did not accept EU as an International legal entity and they had chosen to negotiate directly with each partner. Although the matter seems to be smoother for the present time, mixity can still be a problem affecting the integrity of the common marine environmental policy.

The first integrated marine environmental policy framework is the MSFD. The main policy of MSFD is to protect “nature and biodiversity”. The Directive goes beyond the “sustainable exploitation of renewable resources” and requires reconsideration of the “Common Fisheries Policy and other relevant Community Policies (paragraph 9 from the introductory section). The ambitions of the MSFD go further than the protection of European Waters and aim “at the protection and sustainable development of oceans and their biodiversity throughout the world, focusing not only on European waters but also on adjacent seas such as the Barents Sea and the Arctic Ocean and other international seas” (Frank, 2007). The Directive is structured into thematic fields; the 5th field is dealing with the mitigation of eutrophication: “human-induced eutrophication is minimized, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms and oxygen deficiency in bottom waters”. However, member states of HELCOM and OSPAR agreements have expressed their fears about the future function of Regional Conventions concerning European Waters as they maintain that their conventions is more suitable framework for protecting the marine environment. The Directive recognizes the diverse conditions due to different hydrological, oceanographic and biogeographic characteristics of the seas. It is therefore necessary to adopt specific approaches concerning planning of policy and implementation of measures. European marine waters have already been divided into regions (article 4) so that member states and regions can develop “marine strategies” suitable for the region and in an integrated way encompassing all human activities (article 3). Criteria for the quality descriptors referring to eutrophication has been given by a group of experts (Ferreira, et al., 2011). Their work includes criteria for the descriptors (water transparency, primary production, oceanic decomposition and algal community structure), pressures, monitoring and research on specific topics.

Conclusion

The governance for the protection of European Regional Seas is well developed but with a degree of overlapping between regional conventions, EU Directives and national legislation. The implementation though shows heterogeneity: some states can react to the implementation of the measures more effectively than other “slow reacting” states. Although progress concerning integration has been noticed more efforts should be done within the EU Member States but also between EC States and non EU state partners. More flexible management schemes should be adopted as each regional sea is characterized by local geographic, oceanographic and ecosystemic conditions as well as by different kinds and intensity of human pressures. Monitoring and assessment should be carried out on a continuous basis as it will provide a good feedback to the cluster of environmental policy and management.

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Chapter VIII

Use of Aquatic Microcosm Systems in Phytoplankton Ecology Studies: Objectives, Limitations and Applications

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Abstract

Ecological microcosm systems are used as tools for examining, understanding and manipulating ecosystems processes as well as for assessing human impact on coastal marine ecosystems. Multiple species experimental systems act as interlinks between the natural environment and single-membered algal laboratory cultures. Microcosms can form a relatively controllable system to study phytoplankton ecology, primary productivity, phytoplankton-bacteria associations, food webs, nutrient dynamics, water-sediment interactions and biogeochemical processes. They can also be used to understand environmental problems such as nutrient inputs, eutrophication, species invasions, harmful algal blooms (HAB) and climate change. In addition, subjects related to ecological aspects like hot springs, temporary ponds and hypersaline lagoons can be investigated using microcosms. However, the artificial boundaries of the containers, the isolation of the experimental systems from the natural environment and unsatisfactory simulation of the physical conditions have to be taken into account when objectives are set and experiments are designed. The duration of a microcosm experiment is limited to the time period characterized by the system's steady-state conditions. Nutrient enrichments have to be well balanced to keep the steady state conditions as long as possible. The size of the initial community (inoculum) plays an important role in the maturity and replication of the systems and therefore it has to be considered very carefully. Once the objective(s) in a microcosm experiment is set, the particular system has to be standardized so that results from different experimental runs can be compared.

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Knowledge of scaling effects and artifacts of enclosure are of fundamental importance for obtaining results that can be extrapolated from experimental systems to natural ecosystems. In the present chapter physical, chemical, biological and ecological factors are reviewed. Experimental limitations due to spatial and temporal factors are discussed and applications referring to nutrient dynamics, phytoplankton ecology, eutrophication, modeling and climate change are presented.

Keywords: Nutrients, Eutrophication, Climate change, Phytoplankton ecology, Modeling

1. Introduction

Marine ecosystems are complex systems both in structure and in function. As systems, they are arrays of compounds that interact and result into matter, energy and information flow (Heath, 1979). Ecosystem functions and processes can be studied from various points of view. The ecosystem can be described through the biodiversity of its species. Furthermore, changes in biodiversity due to the availability of matter and energy result into the succession of species. A different approach can be the study of metabolic processes such as photosynthesis, respiration, decomposition of organic matter and nutrient cycles. Biogeochemical cycling of chemical elements form a major part of ecosystem functioning. Field work on the processes mentioned above although provides valuable information on the status of the ecosystem, it does not always provide information and understanding on the system dynamics or the ecological mechanisms.

However, it is not always possible either to experiment on environmental impact under field conditions or proceed to hypothesis testing. Hazardous chemicals and radiotracers require a controlled environment. This is provided by the microcosms where multispecies experiments can be performed and researchers can manipulate variables and design experiments. Microcosms are defined “as samples from natural ecosystems housed in artificial containers and kept in a laboratory environment. These systems are generally initiated by taking whole samples from ecosystems into the laboratory” (NAS, 1981). As microcosms can generally accommodate lower trophic levels, a great deal of work on toxic effects has been carried out on plankton organisms since the 1980s (Giddings et al., 1981; Cairns, 1986; Meador et al., 1993; Cairns and Niederhehner, 1995; Crane, 1997; Karydis, 2011). It was also realized fairly early (McConnell, 1962) that microcosms formed ideal experimental units for phytoplankton ecology and eutrophication studies. There are many microcosm studies on nutrient dynamics and phytoplankton (Brito et al., 2010; Estrada et al., 2008), on eutrophication (Tsirtsis and Karydis, 1997; Edwards et al., 2005) and phytoplankton ecology (Floder et al., 2006; Pasternak et al., 2009; Narwani et al., 2009). As there is a concern about climate change possibly due to anthropogenic enhancement of the greenhouse effect (Kerr, 2000), many scientists try to carry out experiments on scenarios of climate impact on eutrophication; they study the effects on aquatic chemistry and phytoplankton in the marine environment as well as in shallow lakes where short term effects are expected if temperature increases (Admiral, 1977; McKee et al., 2000; Feuchtmayr et al., 2009). In spite of the mounting number of publications on climate change, the number of papers on the possible effects of temperature increase in the aquatic environment are rather limited but with an upward trend (Moss et al., 2003; Domis et al., 2007; Trochine et al., 2011). Special topics in phytoplankton such as toxic phytoplankton and possible HABs (Estrada et al., 2008), saline lakes (Ramirez-Olvera et al., 2009), shallow ecosystems (Roessink et al., 2008) and hypertrophic lakes (Sommer, 1989) have also been published.

Microcosm systems can be either natural microcosms inoculated by field samples (Beyers and Odum, 1993) or synthetic microcosms set up by inocula from laboratory cultures (Meador et al., 1993). Microcosms are usually kept under laboratory conditions so it is easier to control and manipulate the system (Estrada et al., 2008) but they can also be placed in the field i.e., in situ microcosms (de la Broise and Palenik, 2007; Sanderson et al., 2009). Irrespective of the experimental set up (microcosm volume, number of replicates, type of enclosure, initiation and experimental conditions) the multi-species tests should be repeatable, reproducible and more interpretable than field work (Sanderson, 2002). They seem to form interlinks between single species experiments and field work. As the experimental designs are planned to meet specific objectives, these studies are highly specialized and therefore is not easy to apply standardized protocols. The degree of repeatability and reproducibility depends on the experimental conditions: high “ecological realism” in designing the experiment means reduced the replicability and reproducibility and vice versa (Kraufvelin, 1999).

The simplicity is not necessarily a disadvantage: ecosystems are characterized by complexity, comprised of many interacting components; it is difficult to understand ecosystem processes due to the noise of the system. This noise masks functioning and ecosystem behavior (Cushing et al., 2003). On the contrary, the simplicity and manipulability in microcosm studies allows the development of clear-cut cause-and-effect relationships (Giesy and Allred, 1985). It is not therefore difficult to realize why laboratory microcosms have been attractive for ecological research; they have been considered suitable for hypothesis generation and testing in ecology, in spite of the shortcomings that have been indicated by various authors a long time ago (Giesy, 1980; Beyers and Odum, 1993): wall effects, short term experimental designs and the limited number of trophic levels restricting the “top down” effects are the main problems. Light, temperature, turbulence and depth rarely simulate field conditions. In addition, secondary effects due to specific treatments (introduction of alien species, nutrient enrichment) cannot be detected in short term multi-species experiments. In spite of these shortcomings concerning the realism of microcosm systems, the number of applications using laboratory microcosms has increased exponentially over the last two decades. In the present chapter the microcosm applications referring to nutrient effects on phytoplankton, eutrophication, modeling, phytoplankton competition and succession as well as microcosm experiments on climate change over the last twenty five years, are presented. Methodological potentialities and constraints of microcosms as systems for hypothesis generation and testing in phytoplankton ecology are also discussed.

2. Aquatic Microcosms: System Description and Properties

2.1. Experimental Objectives

Aquatic microcosm systems have significantly contributed to the development of contemporary biological and ecological thought (Benton et al., 2007). From the modeling of competition experiments (Gause, 1934) to testing community theory (Drake et al., 1996), microcosms have formed an indispensable tool for understanding and modeling natural processes (Wilson and Botkin, 1990). However, there are limitations concerning the replication of natural conditions mainly due to the size (Ives et al., 1996; Bertolo et al., 1999), the complexity of species (Roelke et al., 2003), the boundary conditions (Chen and Kemp, 2004) and the physical – chemical variables (McKee et al., 2003). It is therefore important that the shortcomings deriving from laboratory conditions should be taken into account when experiments are designed.

Table 1. Methodological aspects in microcosm systems

Description	**Reference**
(a) Experimental design	
Pulsed nutrient inflows to standardize the system	Roelke et al. (2003)
Comprehensive description of a microcosm experiment	Estrada et al. (2008)
In situ microcosms: description of the underwater system	de la Broise & Parenik (2007)
Balancing between ecological accuracy and statistical precision. Standardization procedures	Sanderson (2002)
On board experiments on nutrient limitation	Zohary et al. (2005)
Use of continuous cultures in nutrient enrichment experiments	Edwards et al. (2005)
Description of a 2x2x2 factorial experimental design	Burgmer & Hilleband (2011)
Standardization of a microcosm system for eutrophication studies	Tsirtsis and Karydis (1997)
Chemostat competition experiments comparing continuous nutrient flow versus discontinuous nutrient enrichments	Sommer (1985)
Different initial mixtures of phytoplankton inocula were tested until convergence in succession was achived	Sommer (1991)
(b) Variation/detectability	
Analysis of variance and detectability of changes in the community	Sanderson et al. (2009)
	Sanderson (2002)
(c) Scaling	
Microcosm system scaling: the effects of radius on nutrient cycling	Chen & Kemp (2004)
(d) Wall effects	
Micro/mesocosms experiment to study the effects from periphyton	Chen & Kemp (2004)

The origin of a microcosm is crucial throughout the experiments. Microcosms deriving from natural ecosystems represent field conditions at the beginning of the experiment. However, these experiments are not reproducible since each field sampling is unique as far as the species composition and the relative abundance is concerned. Alternatively, Standardized Aquatic Microcosms (SAM) have specific species composition at a predefined relative abundance, establishing identical conditions at the beginning of the experiment (Meador et al., 1993). This approach allows identical community structure in successive experiments. The placement of different organisms from laboratory cultures in the same system also known as “*multiple seeding*” allows the system to establish its own species interactions (Beyers and Odum, 1993). This process is also known as self-organization. “Self organization occurs when a pattern consistently develops in excess of that which could be predicted based on a summary knowledge of initial conditions” (Kauffman, 1993; Drake et al., 1996).

There are three criteria that should be taken into consideration when designing or handling microcosm experiments (Beyers and Odum, 1993): (a) the boundaries of the experimental units (b) the restrictions in energy and matter exchange as well as the restrictions in species dispersal through the artificial boundaries and (c) the multiple species character of the system.

2.2. Boundary Conditions

The volume of microcosms used for plankton studies can be from a few liters (Tsirtsis and Karydis, 1997) to 80-100 L (Giddings and Eddlemon, 1979); in some microcosms, volume sometimes exceeds one cubic meter (McKee et al., 2000). It is obvious that the size of a microcosm system varies greatly but short term experiments lasting a few days can be accommodated in volumes about a liter of even less (Floder et al., 2006; Hitchcock et al., 2010). Microcosm experiments designed to run for more than two weeks require volumes exceeding 10 L (Tsirtsis and Karydis, 1997; Edwards et al., 2003; Estrada et al., 2008). They are usually glass aquaria, glass conical flasks or polycarbonate containers. Larger volumes have been used ranging between 1000 and 3000 L, although this seems to be in the interphase between microcosms and mesocosms (Sanderson et al., 2009). The boundaries between microcosms and mesocosms are not clear. There is a tendency for containers more than one cubic meter to be characterized as mesocosms (Berlzile et al., 2006), although some authors characterize them as micro/ mesocosms (Sanderson et al., 2009) or even as microcosms (McKee et al., 2000). Microcosms are usually kept under laboratory conditions but microcosm tanks can be placed in the field, usually in the back yard of the laboratories. However, even underwater *in situ* microcosms have also been reported (de la Broise and Palenik, 2007); a frame made of PVC tubing was immersed and 24 bottles about 1 L volume each were used as microcosms and attached to the frame. This way light intensity and temperature changes were simulated successfully.

A usual “boundary effect” is the settling of organisms on the container walls; these periphyton communities (bacteria, algae, protozoa and may be metazoans) are considered as “artifacts” that complicate the extrapolation of the results from the laboratory to nature. Biomass growth on the container’s wall can dominate over the algal biomass and the primary production of the microalgal biomass in the water mass (Chen and Kemp, 2004). Nutrient dynamics can also suffer from a disproportionate influence due to a large surface to volume ratio, characterizing microcosms of small volume (Durzik et al., 1979). Wall cleaning proposed as a standard procedure (Petersen et al., 1999), seems to be a dubious practice. Removal of wall communities might lead to substantial loss of nutrients from a closed system (Dudzik et al., 1979). High demand for nutrient sources may lead to nutrient exhaustion. Ecological properties on such a microcosm system can be distorted and make the objectives of the study pointless. On the other hand keeping the wall community during the experiment, it might affect the outcome by releasing algal and organic material. Nutrient cycling and pollution studies can be greatly influenced by the wall and bottom effects. In addition, some larger species might be stressed due to narrow space which will affect both species behavior and physiology.

The spatial scale in microcosms is a crucial step in the experimental design: patterns and processes become evident when the scale increases beyond a critical level of microcosm size, exceeding threshold conditions (Wiens, 2001). In addition scaling patterns seem to be non-linear (Kemp et al., 2001). Thresholds can differ for the various components and functions of the microcosm system. Nutrient cycles, ecological properties, phytoplankton community abundance, benthic production and behavior of small animals require different spatial scale if the results are going to be extrapolated to natural systems.

Temporal scaling seems to be more difficult to control due to a tendency of phytoplanktonic communities to show a temporal pattern characterized as the "*bloom phase*" followed by a low algal biomass phase or "*bust*" phase (Tsirtsis and Karydis, 1997; Petersen et al., 2009). This pattern implies that unless the experiment has been designed on a short term basis (1-2 weeks duration), it is not easy to statistically detect possible treatment effects compared to the control systems. It has been observed in experiments with zooplankton a two weeks lag phase behind the phytoplankton bloom. Taking into account the limited period of time for a "healthy" phytoplankton growth, temporal constraints should be considered when experiments on phytoplankton – zooplankton relationships are planned or when results are interpreted. Experiments with macrofauna species such as fish, large crustacea, macrophytes and macroalgae are not recommended at a microcosm level due to the required spatial and temporal scaling.

2.3. Ecological Factors and Experimental Constraints

Light

The objective with light sources in laboratory microcosms is to simulate the sunshine. Light sources are the main source of energy supporting primary productivity; it also affects the behavior of animal species. Primary production in the microcosm systems forms the basis for the study of ecological food webs and mineral cycles. There are numeral types of light sources: tungsten lamps, fluorescent tubes, metal halide and mercury vapor lamps. None of them simulates satisfactorily the sunlight spectrum. Use of fluorescent tubes is a common practice although a light intensity decline of about 15-20% has been reported over a three months period (Petersen et al., 2009). When an experiment is planned, light intensity, light quality as well as light absorption and attenuation by the walls of the container should be taken into account.

Temperature

Temperature is an important factor in affecting growth rates of the organisms. As the volume of microcosms is limited they respond quickly to temperature fluctuations. It is therefore necessary to for the microcosms to be kept in constant temperature rooms where both light conditions and temperature can be accurately controlled.

Incorporation of Sediments

Although the incorporation of sediments in microcosms increases the complexity of the system and makes the experimental conditions more realistic, it may have some negative effects by introducing unwanted organisms. However, the addition of sediment is necessary under specific experimental planning: (a) when biogeochemical cycles are included (b) when benthic organisms are studied and (c) pollution studies on non-polar substances that tend to accumulate in the sediment such as hydrocarbons. It must be taken into account that the interaction between sediment and water column in the microcosm is not always a simulation of natural conditions. Stratification of the water column in coastal waters and lakes disengages benthic activities from processes in the water mass. As it is difficult to establish a thermocline in a laboratory microcosm or realistic mixing, ecosystem simulation is far from realistic if sediments are included.

Depth

The shallow depth of a laboratory microcosm can successfully simulate a shallow pond or a lagoon but it is an unrealistic approach for a coastal marine environment. The impact from the vertical irradiance will be negligible due to the small depth; for depth reasons vertical diel migration of zooplankton cannot be studied. Phytoplankton sinking and phytoplankton decomposition have to be treated and interpreted with caution in nutrient cycling studies and ecological modeling (Dudzik et al., 1979).

Ecological Complexity

Ecological complexity can refer to the physical complexity of the environment (number of habitats, degree of connection between habitats), food web complexity, species assemblage structure, diversity and levels of ecological organization (Petersen et al., 2009). It relies on the experimenter to decide on the degree of complexity of the microcosm.

2.4. Microcosm Systems: Laboratory Manipulation

System Initiation

The initial conditions have to be clearly defined when planning the experiments: temperature, salinity, water flow, water mixing, light, addition of nutrients and species to be introduced should be designed in detail.

Replicability

Replicability can be used by the authors in many different contexts but the following definition seems to be the most appropriate for microcosms (Giesy and Allred, 1985): “Replicability means, the establishment of more than one individual experimental unit within a particular experimental treatment. Statistically this is a measure of within-treatment variance. To assess replicability of the system is to determine the similarity of replicate experimental units of an experimental treatment at a given point in time and space that, by definition and design, are meant to be identical”. Due to the relative complexity of microcosms and variation of the community within the system, replicability cannot lead to identical replicates. However, well planned microcosm experiments are meant to bridge the gap between single species laboratory bioassays and real environmental status (Sanderson, 2002).

As a microcosm inoculum represents a sub-system of the natural aquatic environment, a microcosm is allowed to establish its own trophic and species interactions. This process already mentioned as self organization results into the development of unique properties. When several identical microcosm systems are initiated, ecosystems emerge with different species composition (Beyers and Odum, 1993; Roelke et al., 2003). This phenomenon seems to be a problem as far as the replicability of indentical treatments is concerned. On the other hand the basic feature of an ecosystem is self organization, adaptation and maintenance. This seems to be a problem for the experimenter who tries to combine replication and a natural ecosystemic expression. The problem of replicability of microcosms has been studied by various authors a long time ago (Abbot, 1966; Isensee, 1977; Griddings and Eddlemon, 1979). The variation between replicates in microcosm experiments seemed to vary between 10 and 30%, a percentage that is generally acceptable for this scale of biological work. However, it has been reported that coefficients of variation varied between 40 and 80% (Giddings and Eddlemon, 1979). This percentage is rather high and may be due to the dynamic conditions encountered in nutrient recycling and the number of cycles that take place in such an experiment. Coefficients of variation have also been reported for primary production and respiration in 80 L and 8 L microcosm systems: in the first case the coefficient of variation for primary production (PP) was 15% and for respiration (R) 11%. In the 8 L containers the coefficients of variation in PP and R were 24 and 21% respectively. Although PP and R showed acceptable replication, species composition seems to deviate considerably (Roelke et al., 2003).

3. Field Work versus Laboratory Experiments

During the last thirty years there has been an enormous increase in data collection from the marine environment; many researchers are focusing on the elucidation of complex interactions observed in community studies which is a prerequisite to understand ecosystem function and propose measures for environmental protection and management. Information resulting from the natural environment is characterized by “ecological realism” but at the same time researchers cannot “experiment” directly with nature by “manipulating variables” and therefore their “brilliance” is limited to what is known as “sampling design”. There are more problems that can be identified with field data: (a) the amount of noise enclosed in the sets of data (b) the multidimensional character of nature and (c) the inter-correlation among the variables. Luckily, the increasing availability of cheap computing power that allows marine ecologists to analyze mounts of data concerning community ecology as well as the number of textbooks on analysis of environmental/ ecological data (Gauch, 1989; Legendre and Legendre, 2003; Pielou, 1984; Dingby and Kempton, 1987; Jongman et al., 1987; Everitt et al., 2001; Romesburg, 2004), make data analysis a relatively easy job. However, the expected end product, describes current conditions. Scenarios for future trends cannot be easily set up and mechanisms concerning interactions among system components of the physiological base of ecological processes cannot be easily approached.

Living systems, single species, communities and ecosystems are characterized by organized complexity. This complexity can be understood through the analysis of the structural components of the system and the various functions within or between those components. Ecosystems can rarely maintain steady state conditions as driving forces, abiotic and biotic conditions continuously change. In addition, species assemblages are highly indeterministic being sensitive to fluctuations of environmental variables. This explains why it is difficult to test hypothesis by designing field studies.

On the other hand, laboratory studies offer to researchers a friendlier working environment. They provide information on the physiology and biochemistry of phytoplankton (Stewart, 1974; Werner, 1977). The term “laboratory studies” characterizes any kind of experiment carried out under controlled laboratory conditions (Ives et al., 1996). Although the standard methodology was based on single species systems, single species tests have been criticized for poor contribution to the understanding of ecological phenomena and environmental pressures (Kimball and Levin, 1985; Odum, 1984). Two-membered cultures seem to have ecological significance as far as algal competition is concerned under different environmental conditions: effects of toxic compounds (Dayton and Lewin, 1975), nutrient limitation (Tilman, 1977; D’Elia et al., 1979; Lovstad, 1984), light limitation (Nelson, et al., 1979) and interactions (Rijstenbil, 1989; Uchida et al., 1999). Further development of the two-membered cultures is the microcosms. Microcosms simulate part of the ecosystem because: (a) they do not usually include higher trophic levels (b) they do not “mimic” successfully the physical factors (light and circulation) and (c) there are many constraints reflected to the biogeochemical properties due to boundaries and wall effects; however, they provide information of ecological significance.

Both approaches, field work and laboratory experiments are necessary but need different handling by the researchers: field information needs simplification to understand the behavior of the various ecosystem components whereas the complexity in the laboratory experiments should be increased to be more realistic than the single species systems: in other words researchers should try “to simplify nature and upgrade the structure of laboratory experiments”. A conceptual approach is given in Figure 1 in the form of a flowchart; data collected during field work should undergo through exploratory data analysis. Assumptions can be checked and the noise can be removed. The next step should be data preprocessing: ecological and environmental trends will evolve (if any) and all preliminary requirements for statistical applications at the final level will be known. Parallel to the field data work, laboratory experiments can be improved in complexity. Two membered species experiments can provide useful information on species competition and prey predator relationships. Further complexity requires species assemblages at one or more trophic levels. At this stage, information from microcosms can be interchanged with information resulting from data analysis of the field data (Kitsiou and Karydis, 2011). Field information can generate further hypotheses that can be tested at microcosm level whereas conclusions from microcosm experiments can contribute to the understanding of specific ecological processes and functions of the real system. Questions that could be addressed through this “field – microcosm association”, include interspecific competition, species succession, species invasions, species extinction, biogeochemical cycles, sediment – water column interactions, global climate changes, nutrient inputs, HAB’s mechanisms and nutrient limitation studies. In addition to the understanding of ecosystem functioning, microcosms can also provide information useful for understanding environmental pressures and for designing coastal policy, in spite of the fact that multispecies tests have been criticized by some authors as not "decisive" and therefore unsuitable for regulatory frameworks (Mount, 1985; Tebo, 1985).

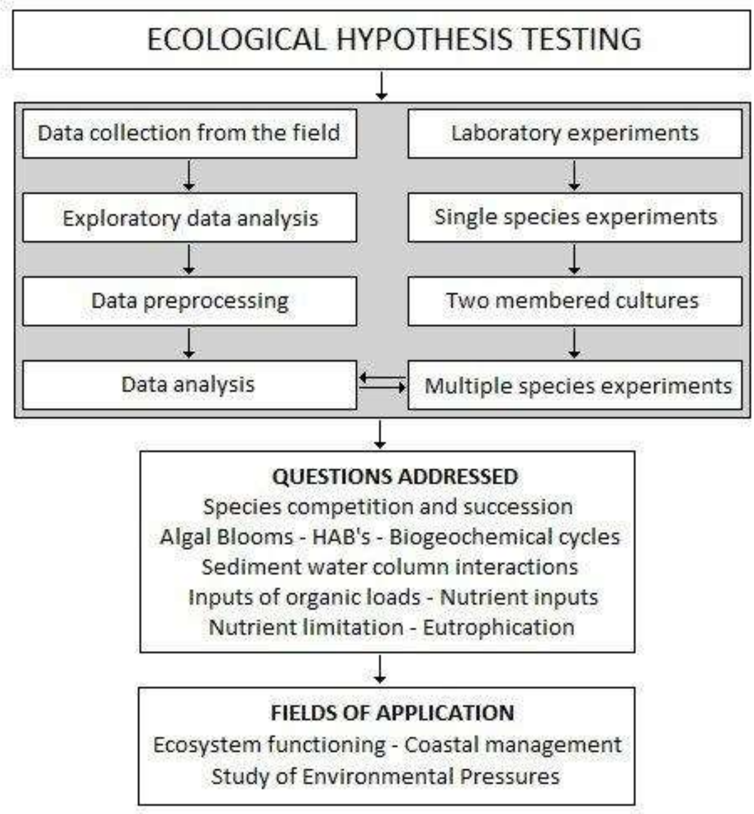

Figure 1. Flow chart indicating the steps in ecological hypothesis testing using both field data and laboratory systems. The objectives as well as the fields of application are indicated.

4. Scaling in Aquatic Experimental Ecology

Microcosms are designed to scale in relation to the natural environment. The term scale in microcosms means "the spatial or temporal dimension of an object or process, characterized by both grain and extent" (Turner and Gardner, 1991). Microcosm scales are depended on spatial and temporal aspects: the spatial scale refers to container's dimensions (length, width, height, volume, microcosm wall surface area) as well as shape and nature of the internal physical structure (sediment, periphyton); the temporal aspect includes the duration of the experiment (days, weeks, months), sampling frequency, water exchange rates and rates of biogeochemical processes (Gardner et al., 2001). Microcosm walls are physical structures usually from metal, glass or plexiglas that confine the system and make it controllable and manipulable. However, these enclosures introduce two types of enclosure artifacts (Sanderson, 2002): (a) the internal surfaces that usually accommodate bacteria, animals and photosynthetic organisms (microalgae and plants) affecting processes such as the ecology, metallic rates and nutrient cycling (b) the solid boundaries that limit the flow of material and energy to and from the environment (nutrients, organic carbon, light, temperature exchange). The term “enclosure artifact” means “the departure of a specific ecological property measured in an experimental system from that observed in nature” (Gardner et al., 2001). Wall effects decrease as the dimensions of the container increase due to the increase of volume to surface ratio. Artifacts due to microcosm walls are not standard for a given volume/ surface ratio but tend to increase as the duration of the experimental run increases.

Decision on the duration of a microcosm experiment is a critical step of the experimental design. The experimenter should always bear in mind that the purpose of a microcosm experiment is to reduce the uncertainty and variability when extrapolating from unialgal cultures to a multispecies experimental system (Sanderson, 2002). Scaling should not only refer to the volume of the microcosm but should also consider the linear dimension of the enclosure. For example microcosm volume of about 1 m^3 can be accommodated either in a container having linear dimension 1x1x1 m or in a container having a square base 0.7x0.7 m and 2 m height; although these microcosms have both the same volume, there is an additional scaling effect due to the microcosm depth. Experiments conducted at the University of Maryland’s Multiscale Experimental Ecosystem Research Center (Petersen et al., 1997), have shown that gross primary productivity was also related to the depth scale, but the form of scaling was depended on nutrient and light conditions: primary productivity rates were proportional to the microcosm surface under light limited conditions but proportional to the volume under nutrient limited conditions. There is always a question depending on the experimental objectives to know to what extent the microcosms in hand are “analogs” to the natural systems. This also depends on the kind of processes under study. Ecological phenomena like emigration, immigration and transport are not suitable at a microcosm scale due to communication constraints between the microcosm and the natural environment. Concepts such as competition, succession and equilibrium, although they have been studied with the use of aquatic microcosms, experimenters should bear in mind that competition and succession phenomena are more intensive in microcosms than in the open sea (NRC, 1995). On the contrary, primary productivity, biogeochemical cycles, nutrient limitation and some aspects of eutrophication can be handled in microcosms and extrapolated to the marine environment on a rather legalistic basis.

Solving the problem of extrapolation of the results from enclosed experimental ecosystems to larger scales in the aquatic environment, is always a critical step. Scale reduction in time, space and complexity of physical and biological processes may lead to unrealistic ecological conclusions. A technique usually used by engineers to create scale models, known as dimensional analysis, has been proposed to be applied in small experimental ecosystems (Petersen and Hastings, 2001). Legendre and Legendre (2003) maintain that ecologists are not used with dimensional analysis although the method is relatively simple and at the same time forms a powerful tool. However, literature on dimensional analysis for natural phenomena appeared as early as the mid twentieth century (Langhaar, 1951). Application of dimensional analysis in ecology becomes feasible because many fields in ecological research use equations such as population dynamics and ecological modeling. Legendre and Legendre (2003) provide a number of applications in dimensional analysis at ecosystem level, showing that insights from differential equations can be obtained without solving them and quantitative predictions about complex processes could be made.

5. Applications Based on Aquatic Microcosms: A Few Paradigms

5.1. Nutrient Studies

Nutrient status and nutrient competition have been studied in Elenmeyer flasks using phytoplankton communities (Sommer, 1989). The results showed that in most cases nitrogen was the limiting nutrient, phosphorus was the next but silicate limitation on three diatom species occurred once only during the experiments. The Monod equation was found suitable to describe nutrient limitation for most of the abundant individual species. The entire phytoplankton assemblages could be described by a version of a Droop equation. Microcosm experiments with natural phytoplankton communities were carried out where one nutrient (phosphorus) or two key nutrients (phosphorus and silicon) were added to the laboratory system (Sommer, 1985). Deviation from steady state was found to increase the number of coexisting species whereas pulsed nutrient supply showed to be an advantage for green algae and disadvantage for diatoms.

Microcosm bioassays were used to study nutrient limitation of phytoplankton growth in the Baratania Basin near the Louisiana coast. Nutrient enrichments (N and P) were carried out in 10 L containers under different N/P ratios (Ren et al., 2009). The bioassays showed that phytoplankton growth was N-limited but also phosphorus co-limitation occurred sometimes, meaning that phosphorus acted as a secondary limiting nutrient. Nutrient loading is also affecting species composition, enhancing growth of undesirable freshwater cyanobacteria. This work suggested that microcosm bioassays may provide the necessary information to understand eutrophication processes in specific systems like the Baratania Basin before measures on ecosystem recovery are decided and implemented.

Remirez-Olvera et al. (2009) using 1 L microcosms, studied the role of nitrogen and phosphorus in tropical saline lakes in connection with phytoplankton growth. The results showed that the two nutrients were alternating their roles as limiting nutrients in phytoplankton growth of the Lake Alchichica (Central Mexico). This rotating scheme between the two nutrients was attributed to natural causes which can be a recent supply of new volcanic material rich in phosphorus as well as anthropogenic impacts deriving from phosphorus fertilizers from specific crop cultures.

The response of phytoplankton and planktonic bacteria to both organic (glucose) and inorganic nutrient sources (N and P) was studied using 1.25 L microcosm systems (Hitchcock, et al., 2010). The results showed that the presence of dissolved organic carbon was inducing heterotrophic bacterial biomass without affecting chl-*a* concentrations. These microcosm experiments showed the role of dissolved organic matter in heterotrophic bacterioplantkon growth.

Interactions between nutrients and toxicants have not received enough attention although the possible mechanisms are already known (Karydis, 1978; Roessink et al., 2008). Oil toxicity on algal cultures of *Cyclotella cryptica* was reduced when extracellular products were added from older cultures (Karydis, 1980). The nutritional state of phytoplankton is crucial for the resistance of microalgae to a number of factors exercising stress (Werner, 1977). When hydrocarbon toxicity was studied in relation to nutrient limitation (Karydis, 1981) on the marine diatom *Skeletonema costatum*, it was found that the cells in phosphorus limited media were far more sensitive to the toxicant. Similar views have also been expressed by Pieters et al. (2005) studying the tolerance of the phytoplankton grazer *Daphnia magna*. It was found that under low nutrient status, therefore low phytoplankton status, *D. magna* was 2-3 times more sensitive to an insecticide compared to a high nutrient status.

Roessink et al. (2008) using microcosms to study the effects of some toxic organic chemicals (triphenyltin, polycyclic aromatic hydrocarbons and polychlorinated biphenyls), observed that *Ephoron virgo* and *Chronomus riparius* could compensate possible adverse effects from the organic pollutants when the two species were kept under a high nutrient status. Nutrient interactions (N and P) during their removal from the secondary treated municipal wastewater were investigated using Vertical Free Surface (VFS) flow wetland microcosms (Zhang et al., 2008). It was found that an optimum N/P ratio is required to attain a maximum growth rate of the plant material.

Different N/P ratios were used in 30 L microcosms (Estrada et al., 2008) to study possible interactions between microalgae and copepods. The results have indicated the potential key role of N/P ratios in controlling communities and should be taken into account when coastal management practices are implemented.

Physical processes are not often taken into account when chemical factors such as nutrient are interfacing with osmotrophic organisms such as bacteria and unicellular algae. It has been pointed out by Mann and Lazier (1991) that at a small scale, in boundary layers around phytoplankton cells, flow speed decreases and can be as low as zero. The site of microscopic turbulence eddies in the water decreases to zero at the boundary. This phenomenon creates problems in nutrient transportation through the cell walls as well as the removal of cellular waste material away from the organism. Arin et al. (2002) attempted to study the effects of nutrients to bacteria and phytoplankton under conditions of turbulence using microcosm systems. Twelve 15 L cylindrical plexiglass containers were divided into three groups (four replicates each): (a) control: no nutrient addition (b) nitrogen addition (N/P ratio: 1:160) and (c) balanced nitrogen and phosphorus addition (N/P ratio: 1:16). Slight turbulence was generated with vertically oscillating grids. The results showed that although there was no clear effect in the unenriched media, there were noticeable differences in the treated containers. This experiment shows the need to consider hydrodynamic conditions in microcosm experiments with phytoplankton.

5.2. Eutrophication

Microcosms (10 L in volume) were used to obtain information useful in models for predicting eutrophication (Edwards et al., 2003). The chl-*a* yield (q) from Dissolved Available Inorganic Nitrogen (DAIN) was calculated as the ratio of cumulative increase in chl-*a* in the microcosm to cumulative decrease in dissolved organic nitrogen during the sampling period (Edwards et al., 2005). Phytoplankton response to enrichments events showed that the yield q did not get a single value but varied due to ecophysiological responses of phytoplankton to different nutrient regimes. The bloom q_{max} value (2.35 μg chl-*a* ($\mu molN^{-1}$)) as well as the post bloom q_{eq} (0.95 μg chl-*a* ($\mu molN^{-1}$)) values were calculated. The authors suggested that the q_{eq} value is suitable for assessing coastal eutrophication due to continuous nutrient inflows. On the other hand the use of q_{max} was proposed for enrichment events that can trigger algal blooms.

A microcosm system was also developed (four replicates, 30 L sea water each) to model eutrophication processes at both physical level (natural microcosms) as well as at the modeling of the eutrophication processes that could be parameterized in the laboratory microcosm. The whole function of the system was satisfactory and it could form an experimental platform to study at a scale effects from sewage effluents, effluents rich in plant nutrients as well as from non-point sources, i.e., fertilizers (Tsirtsis and Karydis, 1997)

A eutrophication assessment based on the yield of chlorophyll from nutrients in the presence of benthic microalgae was performed by Brito et al. (2010), using laboratory microcosms about 4 L in volume. This system was rather complex and nutrient fluxes were affected by enriched seawater, the growth of microphytobenthos (MPB) and the exchange of nutrients between the water column and the sediment. The results indicated the vital role of the MPB community and at the same time set a number of questions for future work; the role of denitrification processes as well as grazing activities of microheterotrophs.

5.3. Phytoplankton Ecology

The changes in species composition of phytoplankton assemblages under grazing pressure as well as under resource competition have been studied using microcosm systems (Sommer, 1988). Microcosm volume was 2 L and the polycarbonate containers were set up in pairs: the light reactor contained phytoplanktonic species and the dark reactor was enriched with zooplankton (*Daphnia* sp.). Excretion of phosphorus and nitrogen from zooplankton and silicate assimilation by phytoplankton resulted into decrease of Si:N and Si:P ratios. These were the main variables affecting the stability of the system. Silicate assimilation by diatoms decreases the Si:P ratio and favors the growth of other groups of phytoplankton species. In this experiment grazing influenced the outcome of competition and has shown that diatoms needed higher Si:P ratios to be dominant. A number of questions of ecological interest concerning succession from diatoms, possible tendency towards a steady state or towards oscillations and the possibility for a stable ("climax") species composition have been addressed.

The convergence in succession of phytoplankton communities was studied using microcosm systems (Sommer, 1991). Microcosm volume was 12 L. Different inocula were used by mixing plankton samples from different lakes and different proportions. Zooplankton species such as *Daphnia* were present in the system. It was observed that zooplanktonic communities (*Daphnia* and copepods) were replaced by smaller species (*Chydorus, Bosmina*, rotifers); all cultures were limited by the same nutrient and finally, after 7-9 weeks the phytoplankton composition of the different microcosms converged. The experiment showed that community composition is influenced by the "*top down*" (grazing) and "*bottom up*" (temperature, nutrients) effects and not from the size of the inoculums, therefore suggesting that microcosm systems are efficient to study ecological phenomena and extrapolate the results to field conditions.

The effect of different N:P ratios on phytoplankton composition and growth of the toxic flagellate *Alexandrium catanella* was examined in microcosms (Estrada et al., 2008). Three different treatments were performed: N:P Redfield ratios, high N:P ratios and low N:P ratios. The copepod *Acartia grani* was added. The results showed that P had a positive effect on the growth of *A. grani* possibly due to increased phytoplankton biomass. The role of phosphorus for bacterial growth was clarified.

A competition experiment between benthic and pelagic phytoplankton assemblages has been performed using microcosm systems 2.5 L in volume (Pasternak et al., 2009). Phosphorus concentrations were adjusted to simulate both eutrophic and oligotrophic conditions. The results showed that chl-*a* concentrations were significantly affected as well as the particulate phosphorus of the system. In all experiments benthic microalgal community seemed to dominate in all the experiments. An assumption tested that nutrient additions would lead to a loss of benthic communities was not verified by the results.

The hypothesis testing on “whether the internal competitive dynamics of a phytoplankton assemblage or externally generated resource variability (disturbance) were more effective at maintaining species diversity” was performed by employing controlled microcosms (Narwani et al., 2009). The authors found that in undisturbed microcosm systems species diversity was effectively maintained due to system’s internal dynamics. It was also found that phytoplankton community species composition had played a crucial role in determining species diversity in the disturbed system.

A system of six phytoplankton strains formed the pelagic phytoplankton assemblage and more than ten species from the phytoplankton benthic community (Floder et al., 2006). The experimental design was a cross-classified two factorial experiment (phosphorus, light) with four replicates (2x2x6=16 units) per experiment that is 48 units in total. Each unit was a 250 Erlenmeyer flask and the duration of the experiment was six days. The results indicated a strong interaction between the pelagic and benthic community with a benefit for the pelagic assemblage due to light availability. Grazing rates of crustacean zooplankton on phytoplankton was studies in 4 l microcosms mimicking Canadian Subarctic lakes and ponds (Symons et al., 2012).

5.4. Modeling

A simulation model was constructed (Bartleson et al., 2005) to simulate how the initial conditions, rates of nutrient supplies and the presence of grazing zooplankton affected macrophyte-algal competition. The data source was based on results from 10 L microcosm systems. This approach showed that combination of simulation models with microcosm ecosystem experimentation is an advantageous approach because: (a) allows setting of the conditions (b)some variables non easily measurable in the field can be measured under laboratory conditions and (c) the experiments can be repeated with changes / improvements on the laboratory system and/ or the simulation model itself.

A numerical model describing competition among five phytoplankton species for three nutrient limiting factors under continuous inflow and pulsed inflow was developed (Roelke et al., 2003). Model simulation described successfully the outcomes of the laboratory experiments. Large hydraulic disturbances and long period between disturbances moved the system to a steady-state condition that was clearly a function of inflow events. The model suggested that management approaches should not rely on individual species but rather on community behavior.

Phytoplankton succession under different climate scenarios was studied by developing a competition model (Domis et al., 2007). Changes of the limiting nutrients, green algae abundances, cyanobacteria and diatoms were included in the model. Quantification of the effects of different climate scenarios on the model parameters was performed by fitting the model to the time series data of the experiment. Experimental and model data showed the role of nutrient limitation in species succession. Furthermore, possible explanations were given based on phytoplankton physiology (Bienfang et al., 1982; Reynolds and Wiseman, 1982).

5.5. Climate Change

Although the number of papers published on climate changes over the last few decades is about 80,000 articles (source: ISI Web of Science), studies on the impact of temperature increase on phytoplankton community structure are only a few (Weyhenmeyer et al., 1999; Weyhenmeyer 2001; Moss et al., 2003). There are some indications that different algal groups respond differently to climate warming under phosphorus limitation. In addition, the most sensitive response in temperature rising is observed with cyanobacteria, compared to diatoms or green algae (Domis et al., 2007). In spite of the fact that laboratory microcosms can hardly mimic the different aspects of climate change, they can be useful in indicating trends in phytoplankton community structure and composition. A series of microcosm experiments were carried out to test the effects of three spring scenarios of climate change on marine phytoplankton communities (Domis et al., 2007). Temperatures ranged from 9 to 13˚C in the cold scenario, 9 to 19˚C in the medium scenario and 9-25ºC in the warm spring scenario. Experimental data showed that low availability of silicate measured in microcosms caused a decline of the diatom community and as a consequence the cyanobacterial and green algal population showed an exponential growth and reached a peak between the 20th and the 30th day of the experiment.

UV irradiance is affecting marine organisms either directly or indirectly: (a) directly: it can damage the DNA of the cells causing mutations or cell mortality; it also breaks down the chlorophylls (photo-oxidation) decreasing photosynthetic rates. UV radiation can also damage some enzymes with groups sensitive to UV radiation (b) indirectly: it can oxidize many dissolved or suspended compounds especially of organic origin. This UV action is very important in case of a climate change. As most of the suspended compounds are finally assimilated by the food chain, it is likely that implications on carbon and the nutrient cycling will occur. In addition, some toxic compounds, such as crude oil can be photo-oxidized producing ketones and aldehydes; these products of photo-oxidation are characterized by higher toxicity compared to their parent compounds as well as higher bioavailability due to their high polarity. Belzile et al. (2006) review the effects of UV on freshwater and coastal marine ecosystems from information collected from experimental units. Although the authors characterize outdoor enclosures with volume greater than 1 m^3 as mesocosms, most of them are in fact in the interface between microcosms and mesocosms. The authors maintain that there are some shortcomings in these experiments: (a) the UV enhancement used in most experiments is unrealistic and (b) the UV impact on planktonic organisms may have poor predictive values due to scaling problems, to absence of higher trophic levels or because of poor representation of key environmental factors in the microcosm. However, this approach helps to predict future impacts due to climate changes. The effect of UV radiation on the dynamics of alpine phytoplankton has also been studied (Williamson et al., 2010).

Table 2. Examples of microcosm applications on phytoplankton

Field of Research	**Reference**
(a) Nutrients	
Nutrient limitation studies in tropical saline lakes	Ramirez-Olveira et al. (2009)
Microcosm bioassays at different N/P ratios to evaluate nutrient limitation	Ren et al. (2009)
Nutrient effectiveness to bacteria and phytoplankton under a small scale turbulence in microcosm systems	Arin et al. (2002)
Chlα experiments in microcosms with sediments	Brito et al. (2010)
Nutrient pollutant interactions studies using microcosm systems	Roessink et al. (2008)
Study of possible impacts of different N/P ratios on interactions between microalgae and copepods	Estrada et al. (2008)
Responses of bacteria, phytoplankton and zooplankton to dissolved organic carbon and nutrient additions	Hitchcock et al. (2010)
(b) Eutrophication	
Development of a microcosm system to model eutrophication processes	Tsirtsis and Karydis (1997)
Evaluation of the Chlα yield from dissolved inorganic carbon as an estimator for assessing eutrophic conditions	Edwards et al. (2005)
Microcosm studies on the dynamics between nutrient and phytoplankton biomass as a tool to assess eutrophication	Edwards et al. (2003)
(c) Phytoplankton Ecology	
Competition between benthic and pelagic microalgae	Pasternak et al. (2009)
Hypothesis testing: is the internal competition dynamics of phytoplankton assemblages more effective at maintaining species diversity overtime?	Narwani et al. (2009)
Study of phytoplankton dynamics based on nutrient pulsed inflows into microcosms	Buyakates and Roelke (2005)
Competition between pelagic and benthic assemblages using microcosms	Floder et al. (2006)
Nutrient competition in phytoplankton	Sommer (1991)
Interactions between algae and bacteria	Liu et al. (2012)
(d) Modeling	
A simulation model describing dynamics in the abundance of cyanobacteria, diatoms and green algae under nitrogen limitation	Domis et al. (2007)

Table 2. (Continued)

Field of Research	Reference
Development of a competition model in multispecies microcosm systems	Roelke et al. (2003)
Modeling the effect of nutrient supply rates on macrophytes and epiphytic algae	Bartleson et al. (2005)
Development of a simulation model including rates of phytoplankton, bacterial biomass, DOC, NO_3, NH_4^+ and SiO_2	Tsirtsis and Karydis (1997)
(e) Climate change	
Study of three warming scenarios on succession using three algal groups	Domis et al. (2007)
Possible impact of UV and climate change in the marine ecosystem	Belzile et al. (2006)
Effects of warming trends on lake phytoplankton communities in microcosm systems	Moss et al. (2003)
Study of allelopathy between filamentous green algae and phytoplankton when the climate gets warmer	Trochine et al. (2011)

Elevated water temperatures were also used in microcosm systems to test possible effects from climate warming on encouraging phytoplankton growth and triggering algal blooms (McKee et al., 2003). Experiments in containers mimicking enriched ponds have been carried out to study possible effects between the Filamentous Green Alga (FGA) *Cladophora* sp. and natural phytoplankton communities (Trochine et al., 2011). The results indicated that the control of FGA on phytoplankton can be significant if climate gets warmer as FGA growth is generally favored at higher temperatures and nutrient enrichments from the lakes.

The scenarios of different warming trends on the ecology of phytoplankton were tested on 48 experimental open air microcosms (Moss et al., 2003). In addition to sediment, submerged plants and plankton community, each unit was stocked with a standard community resembling to the communities of the shallow lakes. There were three temperature levels: non-heating (control), 3˚C above the ambient temperature all the year round and 3˚C above ambient temperature of the summer season (April-September). The results suggested that in this type of shallow unstratified type of lake simulated in the above mentioned experimental units, temperature increase enhanced cyanobacterial growth.

6. Discussion

Microcosm systems seem to bridge the gap between chaotic conditions in the field and single species laboratory experiments. The former, apart from the complexity, does not “produce” reproducible results whereas the latter (controlled experiments) seem to partially contribute to the understanding of the community responses under experimental conditions. The extrapolation of quantitative microcosm outcome into natural conditions is also a point of debate as ecological systems does not seem to have a single characteristic scale and ecological work may consider a number of different scales included in the microcosms (Levin, 1992). A question arises: is actually the microcosm a compartment of a natural ecosystem studied under controlled conditions characterized by increased species complexity? The relevance of microcosm experiments to community and ecosystem ecology has been a matter of concern among many ecologists (Carpenter, 1996; Ives et al., 1996). Experimental microcosms seem to suffer from a “credibility gap” as those small systems may be sometimes inappropriate to understand larger scale processes (Benton et al., 2007). Some authors (Peters, 1991) supported the view that microcosms are weak analogies to natural systems as their structure is far less complex compared to field conditions and therefore poor as experimental tools to generate and test ecological hypotheses.

Some authors (Drake et al., 1996) maintain that microcosms are easily replicated and therefore provide a precise control over spatial heterogeneity and environmental factors as well as a basis to manipulate experimental conditions. Replication seemed to be among the methodological objectives of various researchers working on laboratory microcosms (Abbott, 1966; Dudzik et al., 1979; Giddings and Eddlemon, 1979; Beyers and Odum, 1993). However, the need for replication in microcosm experiments may be due to the narrow minded concept of researchers influenced by the protocols of laboratory experiments. Ecology as a field science cannot be deterministic but influenced by stochastic processes; the microcosm approach can be therefore considered as the interlink between field conditions and laboratory experiments. In addition, it can be used in hypothesis generation and testing.

Microcosm experimentation allows the ecologist to gradually increase the system complexity until the compilation of the information exceeds a “critical value” and system’s behavior can then be revealed. In that context, although microcosms are simplified, that is natural models compared to the real system, they can be useful in exploring and testing ecological processes and phenomena.

Stochastic processes are driven from the ecosystem tendency to be self-organized. Self organization is a continuous process in natural systems due to the changes of ecosystem factors. Species succession for example is recognized as a self organization process in the natural environment (Beyers and Odum, 1993). Self organization in microcosms takes place at two different stages (a) upon the onset of the experiment and (b) at a later stage. Self organization following microcosm “seeding” is dominated by competition among the different species. Driving forces for self organization during the mature stages of microcosms are processes like nutrient cycling, grazing, animal control of plants, accumulation of organic exudates, bacterial growth and the key role of species in maintaining diversity. The replacement of species assemblages in microcosms is predictable in many cases. It is also known that conditions generated by a kind of organism, determine to a large extent the conditions that favor other organisms. Dormant organisms can also revive and affect the composition of the community. The opposite can also happen: as experimental conditions differ from the field conditions, mortality can be caused to a number of species. Interspecies relationships can also be developed in a microcosm. The blue green alga *Anabaena flosaque* and the green alga *Chlorella vulgaris* were grown in microcosms in the presence of multispecies bacterial assemblages (Rodgers and De Pinto, 1981). Interspecies reinforcement was found as a result of the combination of nitrogen fixing and soluble nitrogen produced through recycling.

Microcosms as experimental units for testing ecological hypotheses have substantially contributed to the development of contemporary ecological thought (Tilman, 1977; Drake et al., 1996). Among their advantages are the precise control over abiotic factors (temperature, light, nutrient sources), the manipulability of the system, the control over spatial heterogeneity but above all the experimental designs that can be “tailored” to the objectives. In spite of the obvious “merits” of laboratory microcosms there is criticism including simplification, scaling problems, environmental effects and limited chance to elucidate secondary effects. Laboratory microcosms being weak analogues to natural systems they cannot be used to test or support ecological theories (Peters, 1991). However, their contribution in ecology is vital provided that the experimenter will realize that microcosms are only models of the natural system and the experiments are designed to address specific questions. Microcosms are not realistic in terms of species composition and relative abundance but functions such as succession, metabolic balance and homeostasis can provide valuable information about the functioning of the real system (Ferens and Beyers, 1972; Beyers and Odum, 1993).

The problem of scaling has been a point of debate for both field studies and laboratory experiments designed to study cause - effect relationships over the last two decades. In both cases there is a need for experimental designs that will allow extrapolations to nature. In either case there are three essential steps of experimentation (Gardner et al., 2001) and include (a) clearly stated objectives (b) an experimental design suitable for rigorous hypotheses and (c) analysis of results leading to acceptance or rejection of the objectives As experiments are controlled, replication and replicability is expected and microcosms seem to be the answer. On the other hand realism is required so that the conclusions of the experiment should be ecologically valid. By the term realism we are referring to similarity of a microcosm to natural ecosystems. This can be feasible up to a certain point as there are several variables that cannot simulate natural systems under controlled conditions. These include light intensity, fluctuations and spectral quality; temperature variations, stratification, turbulence and mixing. Depth and water – sediment area ratios are also important as well as productivity ratios and community structure (Giesy and Odum, 1980). It has been reported that in the field of limnology there are many examples indicating discrepancies between microcosms and natural systems (Carpenter, 1996). The researcher has always to compromise between control and realism as these two meanings exhibit an inverse relationship (Kemp et al., 1980). These authors emphasized that experiments on small aquatic systems with clearly defined physical boundaries are subject to the same scaling constraints as “bottled experiments” (Petersen et al., 1999). So the challenge remains to understand limitations and potentiality of scaling so as to be able to know to what extent the results can be extrapolated from the laboratory to nature (Frost et al., 1988). Otherwise artifacts may appear; by the term artifact “we mean the departure of a specific ecological property measured in an experimental system from that observed in nature” (Frost et al., 1988). It has been reported (Kemp et al., 2001) that container effects related to wall growth and reduced exchange tend to decrease exponentially as the size of the container increases. This is because the volume: area ratio increases as the linear dimensions of the container increase. Effects due to containment tend to increase with the duration of the experiment. As the system is maintained under controlled conditions there are no perturbations that would help the system to stabilize (Beyers and Odum, 1993; Roelke, 2003) and therefore small differences at the beginning of the experiment (initial conditions) may be magnified and play an important role in system’s shaping.

Scaling patterns in laboratory microcosms are not realistic when sediment is involved. N-cycling / N-input rates follow an inverse exponential pattern in coastal systems. However, wall effects, particularly growth of periphyton, seem to affect the vertical distribution, being more uniform in the microcosm system (Threlkeld, 1994). Scaling artifact should be taken into account when researchers decide about the size of organisms that will be accommodated in the laboratory system. This is always a delicate point and may explain the fact that most of microcosm work has been focused on organisms characterized by small size and short generation times. Experiments with bacteria (Hitchcock et al., 2010), phytoplankton (Pasternak et al., 2009), zooplankton (Symons et al., 2012) and protists (Zohary et al., 2005) are very popular among aquatic ecologists. The fish trophic level is a matter of debate in microcosm systems (Harte et al., 1980). It is rather simple to manipulate bacteria, protists, phytoplankton and zooplankton in microcosm than fish populations in tanks. It is therefore advisable when ecological hypotheses are tested to use the simplest systems and allow many generations to be developed during the experiment (Benton et al., 2007). A formulation for calculating the minimum container size needed for experiments, including the fish trophic level, has been given by Kemp et al. (2001). However, as a minimal requirement of community structure in a microcosm, the inclusion of decomposers, producers and consumers is recommended (Nixon, 1969). This view has also been pointed out by Odum (1977) stating that disturbances at ecosystem level cannot be studied efficiently at lower levels of biological organization. The situation is more indefinite when time scales are involved. Time scales in natural ecosystems are considerably longer than in microcosms. The “ecological age” of a water mass in an area tends to be inversely proportional to the residence time of that area (Kemp et al., 2001). Time scaling in experimental systems of “batch” type should not exceed a period of two or three weeks (Tsirtsis and Karydis, 1997) whereas if water residence time is regulated in the form of a continuous culture, the time scale of the experiment can be much longer but both design and interpretation of the results should be considered carefully as artifacts may appear and distort the information provided by the microcosm experiment. Skepticism on the ecological value of the results from microcosm experiments has also been expressed by Carpenter (1996). The author argues that microcosms are simply an indirect way to understand ecological phenomena and suggests that only studies at appropriate scales can provide reliable microcosm results.

Microcosm scaling seems to affect replication among experimental units or otherwise the size of the variance. Maximum replication means for the experimental ecologist increased statistical power for detecting effects among the treatments (Kemp et al., 2001). Some authors maintain that microcosms are easily replicated (Drake et al., 1996). The replicability of microcosms has been a subject of concern since the very early day of microcosm research (Abbott, 1966). The reason was that assumptions of replication were not accepted by many authors (McIntire et al., 1964; Beyers, 1964; McConnell, 1962; Whittaker, 1961). Experiments based on measurements of metabolism and physiology (Abbott, 1966), have shown reasonable reproducibility in photosynthetic rates, the coefficient of variation being between 10 and 30% was considered acceptable compared to typical laboratory experiments. Similar experiments where total dissolved solids, alkalinity, Na, Ca, Mg and Cl concentrations were measured, it was found that their coefficients of variation were under 20% (Giddings and Eddlemon, 1979). On the contrary respiration values did not show good reproducibility and the variability was interpreted in a later work (Abbott, 1967) as an indeterminate error attributed to biomass variation and grazing by herbivorous zooplankton.

Replication at community levels seems to be rather unlikely. Two reasons have been reported concerning the “philosophy” behind microcosm requirements and microcosm research (Isensee, 1976). The first reason is that microcosms are not ordinary “sampling units” but contain populations of different species. In such a complex system, slightly different responses of some individuals to a variable or a stressor can lead in each replication to a totally different outcome as far as the community structure is concerned (Roelke et al., 2003). The second point concerns the degree of precision required by the experimenter. This point is usually connected with the requirements of the statistical method applied for data analysis.

Statistical procedures are a powerful tool in microcosm studies as the experimental design can be “tailored” to specific statistical methods. Analysis of variance is a popular and versatile tool for laboratory experiments. Many types of ANOVA have been developed to fit all kinds of experimental designs in laboratory biological (or ecological) experiments: one way, two way, three way, multifactor ANOVA, nested design and split plot design. There is an excellent paper on ANOVA techniques in experimental marine biology and ecology (Underwood, 1981). A recent review article (Kitsiou and Karydis, 2011), has been published on statistical methods used in the field for assessing eutrophication. However, most of them can be applied in microcosms, especially if community structure is examined. As laboratory experiments are carried out in a controlled environment, statistical concepts, constraints, assumptions and potentiality of the methods are well defined a priori. In addition, ecological and biological experimentation is replete of information on replication, controls, confounding and scales (Underwood, 2007). Apart from statistical methods, ecological indices can be used as estimators of ecological responses in microcosms. The use of diversity, biotic and similarity indices in water quality studies is known a long time ago (Washington, 1984). However, there are reservations about their effectiveness in quantifying responses of aquatic communities to stressors both in field conditions (Karydis, 2009) as well as in multispecies tests accommodated in laboratory microcosms (Pontasch et al., 1989).

Simulation models in laboratory microcosms provide the mathematical formulation that allows understanding of natural processes. As microcosm systems are less complex and less noisy than natural systems, a modeling approach can be the preliminary step for a more complex model that could describe processes in the natural environment. This way simulation models can be useful in closing the gap between field work and laboratory experiments (Adams and Giddings, 1982). The advantages in modeling on microcosms have been reported by Wilson and Botking (1990): (a) the species list is controlled (b) calculations on mineral cycling are simplified and (c) the small size of microcosms eliminates heterogeneity facilitating understanding of ecological processes. The modes were founded on the ground of physiology, demography and biogeochemical cycling. In addition to models expressing matter flow, a model of energy for maintaining diversity has been proposed (Beyers and Odum, 1993).

Microcosm experiments can be designed with clear cut cause-and-effect relationships and this is why they seem to be attractive for regulatory decision making. However, some authors (La Point and Perry, 1989) maintain that microcosms may be unsuitable for predicting environmental impacts in two cases (a) when the concentration or quantity of a stressor does not reflect field conditions and (b) secondary effects result in the loss of a food web component, or keystone species or even when some of the sensitive species are replaced by more resilient organisms. Carpenter (1996) has been more critical about the phenomena that "can be bottled": the clarity of the process taking place in the bottle and the advantage of statistical documentation are not efficient to counterbalance the realism of the field work. In addition, decision makers do not seem to be concerned with high statistical significance as political opportunities and pressures are a matter of priority (Lee, 1993). This gives an idea of the limitations of microcosm systems. They are useful to understand specific processes or the function of ecological subsystems but not reliable to draw conclusions at ecosystem level, field work being indispensable in knowing and understanding the real world.

Conclusion

There are many obvious advantages in using microcosm systems in ecological and environmental quality studies: nutrient biogeochemistry, eutrophication, phytoplankton ecology, climate change and modeling are among the scientific fields using the microcosm approach. Enclosures of multi-species assemblages manipulated under laboratory conditions set by the experimenters, contribute to the understanding of complex processes. However, once the inoculum has been taken from the natural environment, the aquatic community is self-organized; physical, chemical and biological conditions prevailing in the enclosure as well as boundary effects, determine to a large extent the type of the "bottled" community. In addition, the understanding of succession, biogeochemistry, metabolic balance and homeostasis of the system should not encourage researchers to attempt any kind of "linear" extrapolation of the microcosm output into the natural environment. Dimensionality including factors like depth, light, nutrients, boundary effects and periphyton should be taken into account during the transition from the laboratory to the "realism" of the natural environment. Researchers have to put up all the time with the dilemma between controlled conditions and realism as these two meanings are linked with an inverse relationship: system control increases reproducibility but loses in realism and vice-versa. As the degree of complexity of a microcosm is set by the experimenter, microcosm systems seem to be ideal to develop and build up simulation models. Physical complexity will be decided (and gradually expanded) upon the progressive model performance to simulate natural processes.

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Chapter IX

Toxicity of *Sarpa salpa*, as Related to Phytoplankton Consumption

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Abstract

The aim of this chapter was to investigate for *Sarpa salpa* the seasonal trend in the food sources and the oxidative stress in the organs. In addition, the toxicity was assessed by mouse bioassay of extract of the fish's organs collected in autumn, the peak of occurrence of hallucinatory syndrome. The toxicity was further studied for compounds present in epiphyte collected from the sea at the end of spring and in summer that are digested by the *S. salpa* in these seasons. We observed a higher lipid peroxydation in different tissues of *S. salpa* compared to the control fish *Diplodus annularis*. We noticed a significant correlation ($p < 0.05$) between the total amount of toxic dinoflagellates and the level of thiobarbituric acid reactive substances (TBARS) in the liver, the brain and the muscle, this for all seasons and all sizes. The lethal dose (LD50%) determined for crude ciguatoxin (neurotoxins) extracts of viscera, liver, brain and muscle of *S. salpa* were as follows: 1.217, 2.195, 14.395, 18.645 g/kg mouse, respectively. Moreover, the cytotoxic effect observed for epiphytes extract confirms the transfer of toxins originating from toxic dinoflagellates, which live as epiphytes on *Posidonia oceanica* leaves, to the fish by grazing. In addition, we studied the cytotoxicity of extracts of epiphytes found in the stomach content of *S. salpa* collected in summer. We tested these fractions in human hepatic cell line HepG2. We observed a significantly decreased cell viability of HepG2 at a dilution (1/40) of epiphyte extracts from stomach contents of two fish we had collected. Our chapter indicates that, toxic phytoplanktons are responsible for the increase of oxidative stress in the organs of *S. salpa*. Hence, the edible part of *S. salpa*, especially the

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viscera and liver, can cause a threat to human health, and consumption should, for this reason, be dissuaded.

Keywords: *Sarpa salpa*; Diet; Neurotoxins; Toxic dinoflagellate; TBARS; Mouse Bioassay; HepG2

Introduction

Sarpa salpa, also known as salema porgy, is a species of bream which is an herbivorous sea fish that preferentially feeds on the seagrass *Posidonia oceanica* through the year (Peirano et al. 2001; Prado et al. 2008) and is used for human consumption in the Mediterranean region. Due to its low cost, this fish is predominantly on the menu of the lower income classes. The consumption of the *S. salpa* is, however, inadvisable in certain periods of the year because it causes a hallucinatory syndrome and central nervous system disorders. Most poisonings involving *S. salpa* consumption have been reported in spring and summer (Chevaldonne 1990; Raikhlin-Eisenkraft et al. 1989). An important observation in this context is the presence of ciguateric species that live as epiphytes on *Posidonia oceanica* leaves (Ben Brahim et al. 2010) and are co-ingested by the *S. salpa* as part of their diet (Velimirov 1984).

The Gulf of Gabes is a threatened biotope mainly due to the pressure of anthropogenic expansion and dumping of large quantities of phosphogypsum and other chemical products which severely impacted benthic habitats (Hamza-Chaffai et al. 1999). It has been shown that epiphytes of seagrass are sensitive to environmental changes (Giovannetti et al. 2010). For example, various studies reported increases in epiphyte biomass parallel with nutrient enrichment (Armitage et al. 2006), eutrophication (Frankovich et al. 2009) and water quality criteria) for nutrients and biostimulatory substances. (Meric et al. 2005). This has led to a substantial proliferation of microalgae and particularly of toxic dinoflagellates in the Gulf of Gabes (Turki et al. 2006). Such proliferation of undesirable microalgae has been shown to result in increasing problems in both coastal and estuarine environments (Leong and Taguchi 2005; Smayda 1997). For instance, ciguatera food poisoning increases due to the presence in fish of a toxin produced by the benthic alga *Gambierdiscus toxicus* and other coral microalgae, most of them belonging to these genera: *Prorocentrum*, *Ostreopsis* and *Amphidinum*. Another less common form of poisoning is ichthyoallyeinotoxism that is characterized by the development of central nervous system disturbances, especially hallucinations and nightmares (Chateau-Degat 2003 Halstead 1988). Several of the toxins increase to dangerous levels for humans during their transmission through herbivorous and carnivorous fish (Vaillant et al. 2001).

It is known for a wide range of environmental pollutants such as heavy metals and ciguatoxin (neurotoxins) that they can induce oxidative stress in aquatic animals including fish. The generation of reactive oxygen species (ROS) is commonly associated with cellular injures due to alterations in DNA, proteins and membranes (Leonard et al. 2004). Lipid peroxidation estimation has been found to have a high predictive value as a biomarker of this effect (Guilherme et al. 2008). Also, antioxidant enzymes have been proposed as biomarkers of contaminant-mediated oxidative stress in a variety of marine organisms, and their induction reflects a specific response to pollutants or toxins (Cossu et al. 1997). Because of, on one hand, the possible effects on the fish and, on the other hand, in relation to consumption of the fish by humans; it is relevant to study the oxidative stress in fish.

The objective of this chapter was to specify the oxidative stress in fish *S. salpa* organs.

Moreover, toxicity was investigated by mouse bioassay of compounds present in the epiphytes collected from the Island of Kerkennah at the end of spring, in epiphytes digested by *S. salpa* in summer and in the extract of fish organs collected in autumn.

Therefore, to assess the cytotoxic compounds that is present in the extracts of epiphyte digested by *S. salpa* in summer. To carry out an in vitro study model, human hepatic cell lines HepG2 was used.

Materials and Methods

Study Area

The Gulf of Gabes is located between 35° N and 33° N and extends from "Ras kapoudia" at the 35° N parallel level to the Tunisian-Libyan border. It shelters various islands (Kerkennah and Djerba) and lagoons (Bougrara and El Bibane). The climate is dry (average precipitation: 210 mm year^{-1}) and sunny with strong easterly winds. The tide is semidiurnal, with a maximum range of about 2 m.

Specimen Collection

The study was carried out off the Island of Kerkennah (Figure 1) (Gulf of Gabes; Southeast Tunisia). This archipelago is characterized by extensive *Posidonia oceanica* seagrass meadows (Hamza et al. 2000).

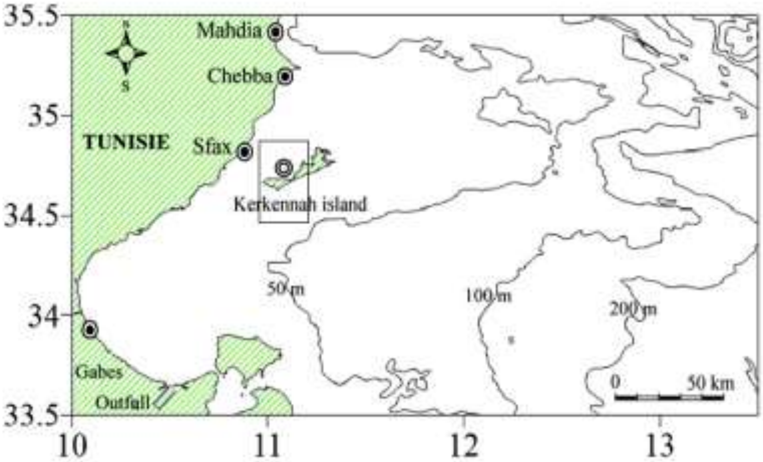


Figure 1. Geographical map focusing on sampling location in the Gulf of Gabes.

Specimens of *S. salpa* were collected between January 2006 and January 2007; 59 specimens in winter, 57 in spring, 57 in summer and 55 in autumn. The total lengths (TL) of the fish were measured to the nearest 0.1 cm and weight to the nearest 0.1 g. Their sizes ranged from 12.8 to 28 cm, and the fish were divided into three classes according to Pallaoro et al. (2008): adults (3–7 years, large size) TL>20 cm; subadults (2 years, medium size) 17 cm<TL<20 cm. Immediately after capture, the fish were dissected, the guts were removed and preserved in a 4 % formalin solution. Also, the liver, brain and muscles (including dark muscles) were removed, rinsed with ice-cold saline and stored at -80 °C until further analysis. In the laboratory, prey identification was carried out to the lowest possible taxonomic level. Species abundance and wet weight were recorded to the nearest ±0.001 g after removal of surface water by blotting paper. As control, we collected the annular seabream, *Diplodus annularis* (Linnaeus 1758) which belongs to the same biotope and the same family and is a highly valuable commercial fish in Tunisia between January 2006 and January 2007. Fifty specimens of *D. annularis* were collected in winter, 48 in spring, 51 in summer and 52 in autumn.

Additional batches of *S. salpa* and *D. annularis* were collected in the same region for toxin extraction from stomach content, for mouse bioassay and in vitro study batches.

Collection of Epiphyte from Marine Ecosystems and Toxin Extraction

Epiphytes were collected from four different locations in a *Posidonia oceanica* meadow from depths of 5-15 m at the end of spring of 2006. *Posidonia oceanica* leaves were put in a beaker, 250 mL of sea water was added, the mixture was shaken and filtered by vacuum filtration onto a Whatman GF/F glass fiber filter (diameter, 0.45 μm). Filters were immediately stored at −80 °C.

For toxin extraction, the filter was cut into three equal parts, each part was separately shaken for 3 h at 25°C in the dark with 3 mL of one of the following solvents: ethanol, acetone and chloroform to extract the toxins. The solvent was collected and the extraction repeated with the same solvents; the combined extracts were subsequently dried at room temperature under flux (N_2). Finally, dried extracts were suspended in 2 mL 0.9 % saline+1 % Tween 60 and were sonicated for 5-10 min.

Collection of Epiphyte from Stomach Contents of S. salpa and Toxin Extraction

For this experiment, 20 specimens of *S. salpa* were collected from different locations around the Island of Kerkennah during the summer of 2006. After the fish were dissected, their stomach content was put in a beaker, 250 ml of sea water was added, and the content was shaken and filtered by vacuum filtration onto whatman GF/F glass fiber filter (diameter, 0.45 μm). Filters were then immediately stored at -80°C. The extraction of the toxins in the stomach contents of *S. salpa* was done using the same procedure as described for the toxins in epiphytes from marine environment (see above).

Seasonal Variation in Cellular Stress and Marine Toxins Accumulated in Tissue of the Different Organs of *S. salpa*

Biochemical Assays in Fish Organs

The frozen organs (1 g), i.e., the liver, brain and muscle were homogenized (Ultra Turrax T25, Germany) in an ice-cold buffer (1/2, w/v; TBS, 50 mM Tris; 150 mM NaCl; pH 7.4) and centrifuged (5,000 g, 30 min, 4 °C), the supernatants were frozen at (-80 °C).

Lipid peroxidation was estimated by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Esterbauer (1993).

The protein content of tissue extracts was determined using the method of Lowry et al. (1951) using bovine serum albumin as reference standard.

In Vivo Study

Lipid-Soluble Extracts Preparations from Fish Organs and LD50 Determination

Samples for toxicity assay were prepared as follows: the fish muscle (including dark muscle) or organs (50–100 g) was thawed and cooked at 70 °C for 15 min in a water bag to denature proteins to enhance extraction efficiency during homogenization. Samples were cooled to room temperature, minced and extracted twice with acetone (3 L/kg flesh) using an explosion proof homogenizer. The acetone filtrate was dried in a rotor evaporator at 55 °C water bath and 556 mbar vacuum, re-dissolved in 90 % of aqueous methanol (0.5 L/kg flesh) and extracted twice with hexane (1:1, v/v) to remove impurities from the aqueous methanol phase. The aqueous methanol portion was dried in rotor evaporator at 55 °C water bath and 337 mbar vacuum, re-dissolved again in 25 % of aqueous ethanol (0.5 L/kg flesh) and extracted with diethyl ether (1:1, v/v) three times. The extraction of lipophilic toxin from fish tissues was performed by subsequent liquid-liquid partitioning (separator funnel) as described by Lewis (2003). Diethyl ether extracts were concentrated by using rotor evaporator re-dissolved in a known volume of chloroform-methanol (97:3, v/v) for quantification and was dried under nitrogen gas. The protein extracts were stored at -80°C prior to testing.

In order to study the toxicity of marine toxin expected to be present in the samples, lipid-soluble extracts of the samples were analyzed using a mouse bioassay that was previously described by Vernoux (1994) and Lewis (1995, 2003). Mouse bioassay experiments were carried out using seven groups of male mice weighing 18–22 g (eight animals per group) purchased from the Central Pharmacy of Tunisia (SIPHAT, Tunisia). Animals were housed in a controlled environment (22±3°C, 54–56% humidity, a 12-h/12-h light–dark cycle). Mice were fed with a commercial balanced diet (SICO, Sfax, Tunisia) and drinking water was offered ad libitum. The body weight of the mice at the start of the experiment was measured. The ether-soluble extract was suspended in 1% Tween 60/0.9% saline at different concentrations, sonicated at 37°C for 5–10 min, 0.8 mL (0.04 mL/1 g of mouse) was injected intraperitoneally (i.p) and assayed in duplicate. Control mice were administered the same volume of 1 % Tween 60/0.9 % saline only. The mice were closely monitored at 1h interval for 3-5 h after sample injection. Symptoms of intoxication including hypothermia (rectal body temperature below 33°C), diarrhea, reduced locomotor activity and time of death of the mice (if this occurred within the first 24 h) were recorded. Symptoms or signs of intoxication in mice, other than the abovementioned, were rejected in this experiment to avoid subjective bias (Hoffman et al. 1983; Lewis 1995). The diethyl ether extract containing marine toxin was quantified using the principle of the dose versus time-to-death relationship equation log (MU) = 2.3 log (1+1/T), where MU is the number of mouse units (one MU=LD50 dose for a 20 g mouse) and T is survival time in hours of each mouse (Lewis and Sellin 1993; Lewis 1995, 2003).

In Vitro Study

Cell Culture and Growth Inhibition Studies

Human hepatoma cell line HepG2 was obtained from American Type Culture Collection (HB-8065, ATCC, Rockville, MD). Cells are grown in 75 cm^2 on flasks using William's Medium E supplemented with 10 % fetal calf serum, 2 mM L-glutamine, 20 mU/ml insulin, 50 nM dexamethasone, 100 U/ml penicillin, 100 µg/ml streptomycin, 2.5 µg/ml fungizone, 50 µg/ml gentamycin, and 100 µg/ml vancomycin. The cells were subsequently incubated at 37°C in a 5 % CO2 incubator. The cells were seeded at a density number of 2×10^4 per well in 96-well culture dishes (cat. 6005181; Packard BioScience, Meriden, CT). The cells were treated with various concentrations of toxic extract epiphyte from stomach contents for 72 h followed by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2Htetrazolium-5-carboxanilide (XTT) assay.

XTT Assay

Colorimetric assay (XTT based) for the nonradioactive quantification of cell proliferation and viability (Roche catalog) was used. The assay is based on the cleavage of the yellow tetrazolium salt XTT to from an orange formazan dye by metabolic active cell. Cells were cultured for the indicated period. Afterwards, the medium was replaced with 100 µl of XTT solution (sodium 3′-[1-(phenylamino- carbonyl)-3, 4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate) added per well (XTT, Roche, Mannheim, Germany). Cells were again placed in a CO_2 incubator for 4 h. After this period, the conversion of XTT to soluble formazan was determined spectrophotometrically in a plate reader (Bio-Rad Model 3550, measurement 490 nm, reference 655 nm, Hercules, CA). The percentage of viable cells was calculated against cells in the same experiment that were not exposed to organ extract with DMSO as control.

Statistical Analysis

For the biochemical assays in fish organs, data are presented as average ± standard deviation. The calculations were performed on groups of five animals each, and the differences were examined by a two-way analysis of variance (fixed factors: size and season), followed by the Fisher test (Stat View) and the significance was accepted at $^*p<0.05$.

Also, for the inter-seasonal correlation between the total toxic algae dinoflagellates and the markers of oxidative stress in different organs of *S. salpa*, correlation coefficients (R) were calculated for all sizes and all seasons together using the Pearson correlation.

For the study in vitro and in vivo, data are presented as average ± standard deviation for four experiments. The differences were examined by one-way ANOVA followed by the Fisher test (StatView), and the significance was accepted if $p<0.05$.

Results

Seasonal Variation in Cellular Stress And Marine Toxins Accumulated in Tissue of the Different Organs of *S. salpa*

Seasonal Variation of Lipid Peroxidation for the S. salpa

The levels of lipid peroxidation (TBARS) in different organs and compartments of the *S. salpa* specimens were studied. The results were compared with those found for the control fish, the annular seabream *Diplodus annularis*. The organs were selected on the basis of functional criteria, which made them preferential targets, i.e., xenobiotic metabolism (liver) and the known neurotoxic effect of toxic dinoflagellates ingested by *S. salpa* (brain) and muscle being the preferred part of the fish used for human consumption. We noted several differences: in winter, no significant difference was found for the TBARS in the medium and large size classes of *S. salpa* compared to *Diplodus annularis* of the same size class (Figure 2a).

In spring, there was only a significant increase found of the TBARS in the liver of the large-sized *S. salpa* to its control (Figure 2b). In summer, increased levels of TBARS were found in the livers of both large- and medium-sized *S. salpa*.

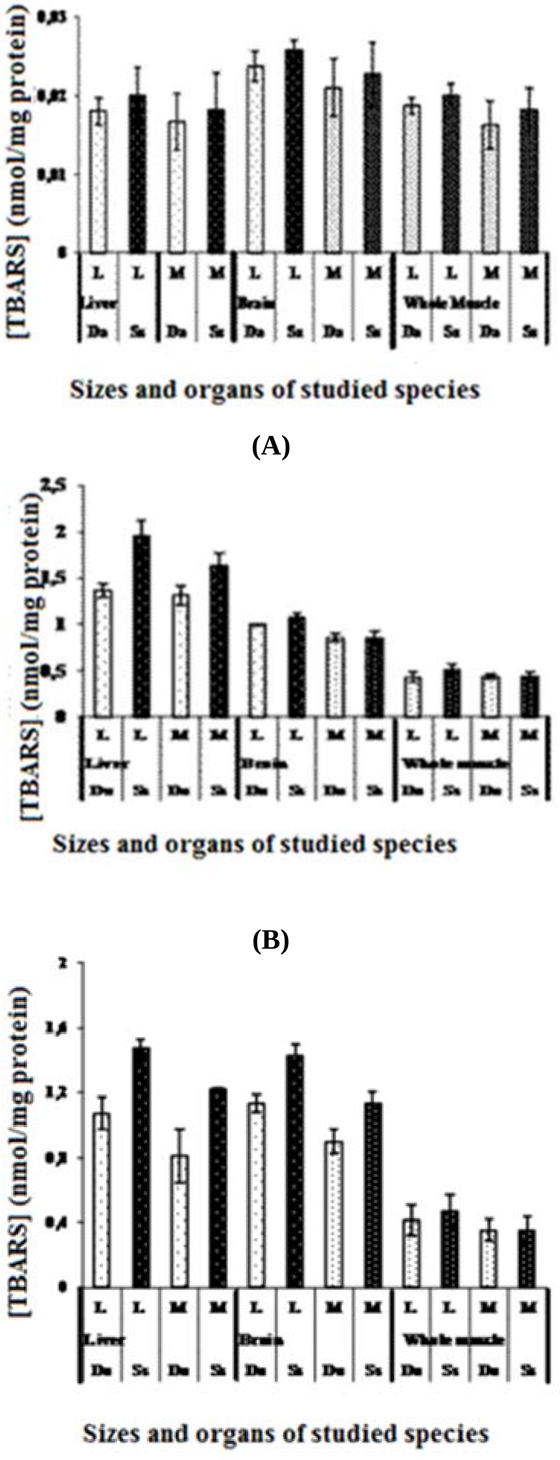
In addition, we observed a significant increase of the TBARS in the brain for the large size class (Figure 2c). In autumn, we observed significantly higher levels of lipid peroxidation in the liver, brain and muscle (including dark muscle) of large size class *S. salpa* and in the liver and brain of medium size class *S. salpa* compared to their size matched *Diplodus annularis* controls (Figure 2d).

In Vivo Study

LD50 Determination of Marine Toxin Accumulated in the Organs of S. salpa

After extraction with diethyl ether, we obtained the crude ciguatoxins (neurotoxins) from 50 g of fish sample: viscera (viscera except liver) 1.16 ± 0.20 g (mean ± SE); liver 1.02 ± 0.15 g (mean ± SE); brain 0.5 ± 0.11 g (mean ± SE); muscle (including dark muscle) 0.69 ± 0.20 g (mean ± SE).

For the LD50 determination, we used six experimental groups and one control group, each with eight mice. Affected mice exhibited typical signs of neurotoxicity disorders including hypothermia (rectal body temperature <33 °C; trembling), a significantly reduced locomotor activity during the first 2 h and failure breathing and no evident signs of gastrointestinal problems (e.g., diarrhea). Results are given in Table 1.

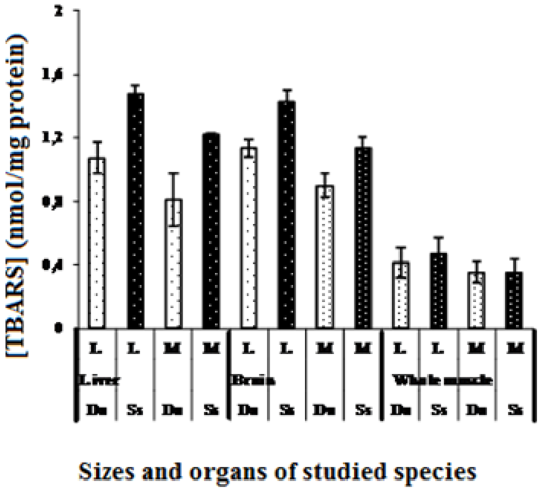


(A)

(B)

(C)

Figure 2. (Continued).



(D)

Figure 2. The level of TBARS in the liver, brain and muscle (including dark muscle) during winter (a), spring (b), summer (c) and autumn (d) for the *S. salpa* compared to the control fish *D. annularis* for the large (3–7 years) (TL>20) and medium (2 years) (TL<20 cm) size classes. The values represent the average of 5 measurements ± SD. *$p \leq 0.05$ indicates significant difference between *S. salpa* (Ss) and control fish, *D. annularis* (Da).

The difference between toxin concentrations of the liver, viscera and brain extracts was significant as compared to muscle extract, also a significant difference ($p<0.05$) between the liver and viscera organ since concentrations of toxins in organs are defined in ascending order: muscle, brain, liver and viscera. Additionally, after extraction of epiphyte from the aquatic environment (different stations from the Island of Kerkennah) and stomach contents of *S. salpa*, we obtained the crude microalgae toxins from 250 ml epiphyte water, respectively: 300 ± 2.88 mg; 295 ± 4.08 mg (mean ± SE). The difference between toxin concentrations of epiphyte extracts from *Posidonia oceanica* and stomach content of *S. salpa* confirms the transfer of toxins originating from toxic epiphytic phytoplankton that live on the *Posidonia oceanica* leaves to the fish organs by grazing (Table 2).

Table.1. Concentration of toxicity expressed in mouse units per 100 g tissue and micrograms per 100 g tissue, estimated in fish organs of *S. salpa* collected from the Island of Kerkennah (Gulf of Gabes; South East Tunisia) during autumn (2006)

	Concentration of toxicity (MU/100 g tissue)	**Concentration of toxicity (µg/100 g tissue)**	**DL50% g/kg of mouse**
Liver extract	24 ± 0.40	481.75 ± 94.46	2.195
Viscera extract	48.25 ± 0.85	792 ± 147.8	1.217
Brain extract	2.87 ± 0.025	65.75 ± 7.4	14.395
Muscle extract	0.75 ± 0.004	13.62 ± 1.13	18.645

Values are average ± SE; n=8.

Table 2. Toxicity concentration of epiphytes extract from *Posidonia oceanica* collected during spring (2006) and epiphytes extract of stomach contents collected from the Island of Kerkennah (Gulf of Gabes; South East Tunisia) during summer (2006) expressed respectively in mouse units per 100 ml of epiphyte water and in mouse units per stomach contents

	Concentration of toxicity (MU/100mL of epiphyte water; MU/stomach contents)
Epiphyte extract from *Posidonia oceanica*	0.46 ± 0.0025 MU/ 100 mL of epiphyte water
Epiphyte extract from stomach contents	1.05 ± 0.0062 MU/stomach contents

Values are average ± SE; n=8.

Inter-seasonal Correlation between the Total Toxic Algae Dinoflagellates and the Markers of Oxidative Stress in Different Organs of S. salpa

The level of lipid peroxidation (TBARS) in *S. salpa* shows a cumulative effect over the consecutive seasons, beginning in spring with a maximum effect during autumn and affecting an increasing number of organs, first the liver, then the brain, and finally the muscle. This effect further increase with the size of the animal, which might be related to the amount of *Posidonia oceanica* leaves consumed that are enriched with toxic epiphytic phytoplankton in a similar seasonal pattern. To justify and consolidate our precedent observations, we started a series of tests to find a correlation between the total toxic dinoflagellates in the stomach contents of *S. salpa* and the profiles of oxidative stress in fish organs. Pearson correlation analysis is listed in Table 3. There were significant positive correlations between the total toxic dinoflagellates and the level of TBARS measured in the liver, the brain and the muscle for all seasons and all sizes together.

Table 3. Correlation matrix (Pearson test) between the total toxic algae dinoflagellates in stomach contents of *S. salpa* and the level of TBARS for all seasons and all sizes together in fish organs

	Total toxic dinoflagellate in stomach contents of *S. salpa*
Total toxic dinoflagellate in stomach contents of *S.salpa*	1
Level of TBARS in liver	0.263*
Level of TBARS in brain	0.646**
Level of TBARS in muscle	0.734**

In Vitro Study

Inhibition of HepG2 Cell Growth by the Epiphytes Extracted from Six Stomach Contents of S. salpa

The epiphyte extracts of six stomach contents of *S. salpa* were collected during summer around the Island of Kerkennah and tested on HepG2 cells for cytotoxic activity. We observed a significant decrease of mean cell viability of HepG2 at the dilution (1/40) of the epiphyte extract only for two specimens tested (Figure 3).

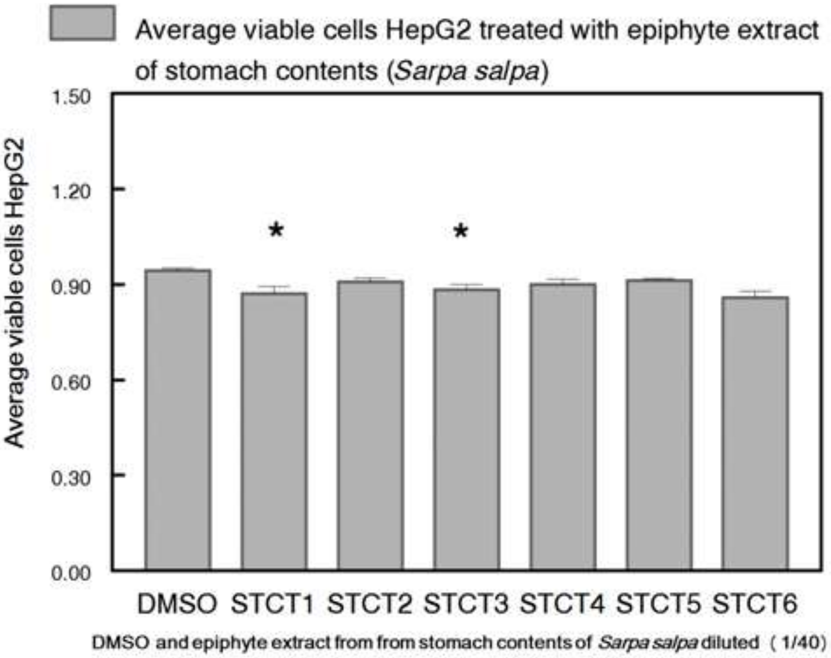


Figure 3. Cytotoxic effect induced after 72 h exposure to varying concentrations of epiphyte extract present in the stomach contents of different species of *S. salpa* collected in the summer, against human liver HepG2 cells. Data are expressed in percentage of unexposed controls. Experiments were performed three times and in duplicate per concentration. The significance level observed is *p<0.05 in comparison to control group values.

Discussion

Seasonal Variation in Cellular Stress in the S. Salpa as a Result of Fluctuations in their Diet

Oxidative stress is related to the formation of ROS, which are continuously generated endogenously during normal metabolism and as by-products of biotransformation reactions of toxins or xenobiotics. Cellular antioxidant status is used to evaluate the ability of organisms to resist an environmental stress such as those induced by some marine pollutants (Frenzilli et al. 2004). Looking at the feeding behavior of *S. salpa*, it would seem that the low TBARS levels in winter are due to the absence of toxic epiphytic dinoflagellate on *Posidonia oceanica* leaves consumed. In spring, the increase in the percentage of *Posidonia oceanica* as the food source for the large-sized fish could explain the difference found between the TBARS level in the livers of *S. salpa* vs. *Diplodus annularis*. Moreover, we noticed that an increase in the TBARS level is certainly due to the presence of toxic algae dinoflagellate in the stomach contents of *S. salpa* with a percentage equal to 1.14 %. It should be noted that many other factors can contribute to the formation of the TBARS, particularly the age of the organisms and the type of food (Bocquené and Galgani 1998). In summer, *Posidonia oceanica* leaves showed greatest colonization by epiphyte owing to the fact that the surrounding water was calmer. *S. salpa* had maximum periods of grazing on *Posidonia oceanica* leaves during summer and autumn. This has an effect on the TBARS level in the livers and also in the brain of both large and medium-sized *S. salpa*. The brain is very susceptible to oxidative damage due to the high content in cellular membranes containing unsaturated lipids and to the high rate of oxygen metabolism (Giuffrida-Stella and Lajtha 1987). However, the brain is not particularly enriched in antioxidant enzymes (Benzi and Moretti 1995). In autumn, fish from both size classes consumed *Posidonia oceanica* leaves as the preferential food source (>50 %). The percentage of the ciguateric species, compared to the other seasons, was the highest (5.26 %) and this reflected the rate of TBARS level with significantly higher levels for *S. salpa* in all tissues investigated. These observations enabled us to conclude that in autumn there was a peak in toxicity (oxidative stress) in *S. salpa* and that the toxicity is now even present in the muscle of the large-sized fish.

Methodology Used

Mouse bioassay (AOAC 1980; Lewis 2003) remains the accepted regulatory method for detection and quantification of many marine toxins in suspect samples, so as to protect the health of the public. The assay may also quantify lethal and sublethal doses of marine toxins that are found in coral fish extracts (Louzao et al. 2004).

These toxins (especially neurotoxins, e.g., ciguatoxins, brevotoxin) structure cannot be destroyed through cooking, refrigeration and weak acid treatments (Bruslé 1997; Guzmán-Pérez and Park 2000). It is also well known that even the application of temperature up to 120 °C would not reduce the toxicity of ciguateric fish (Pottier et al. 2002).

In our chapter, standard laboratory criteria include hypothermia (rectal body temperature below <33°C, trembling), symptoms of intoxication (reduced locomotors activity, respiratory failure). These are objective parameters to determine the presence of toxin in fish organs also both epiphyte from stomach content and *Posidonia oceanica*, especially for those in sublethal amounts.

Three clinical reports were published about possible ciguatera poisoning in humans after consumption of fish caught in the eastern Mediterranean. One case involved the *S. salpa* (Bentur and Spanier 2007; Spanier et al. 1989), two other reports, one recently published, involved rabbitfishes, *Siganus* sp. (Herzberg 1973; Raikhlin-Eisenkraft and Bentur 2002). Within a few hours, specific signs of poisoning occur including delirium, visual and/or auditory hallucinations (often involving animals), depression and feelings of impending death with reactive tachycardia and hyperventilation and disturbed behavior. If they are able to sleep, patients classically report terrifying nightmares (De Haro et al. 2003).

The interaction between herbivores and seagrass can be mediated by epiphytes (Tomas et al. 2005; Young et al. 2005), at least in part, because seagrasses do not appear to be an attractive food source (Bulleri et al. 1999; Hereu 2006) as the presence of phenolics in them proves to be a source of chemical deterrents (McMillan 1984). Ciguatoxins are produced by *Gambierdiscus toxicus* epiphytic dinoflagellates living on macroalgae and other substrates in tropical areas. Other dinoflagellates have also been suspected (e.g., *Amphidinium carterae*, *Coolia monotis*, *Prorocentrum lima*, *P. concavum*, *P. rhathymum* and *Ostreopsis siamensis* (Swift and Swift 1993).

We noticed that the toxicity in the muscle and brain of *S. salpa* were lower than the toxicity in the viscera and liver. Shellfish exhibiting any detectable level of toxicity by mouse bioassay are considered potentially unsafe for human consumption. In practice, a value of 20 MU/100 g (USFDA 2005) is considered the guidance level at or above which shellfish are prohibited from harvest. Moreover, we noticed that the cytotoxic compounds present in different organs of this fish can pose a threat to human health and is a source of intoxication especially in the visceral part. This chapter demonstrated the effectiveness of the mouse bioassay to determine the edibility of the studied fish.

Inter-seasonal Correlation between the Total Toxic Algae Dinoflagellates and the Marker of Lipid Peroxidation for the *S. salpa*

The induction of the increase in lipid peroxidation response was a logical answer to the exposure to toxic substances generated by toxic dinoflagellates. Algal toxins represent a major global hazard to public health. Ensuring seafood contains safe concentrations of these toxins, many of which can induce toxic effects such as neurotoxicity, is one of the major challenges to the shellfish/aquaculture industries as well as to regulatory authorities. The metabolism of toxic compounds frequently results in the formation of ROS, which significantly contribute to their toxicity (Chovanec et al. 2003). A positive correlation was observed between the total toxic dinoflagellates and the TBARS level at the side of the liver, the brain and the muscle for all seasons and all sizes together.

Inhibition of HepG2 Cell Growth by the Epiphytes Extracted from Six Stomach Contents of *S. salpa*

The epiphyte extracts of six stomach contents of S. *salpa* were collected during summer around the Island of Kerkennah and tested on HepG2 cells for cytotoxic activity.

We observed a significant decrease of mean cell viability of HepG2 at the dilution (1/40) of the epiphyte extract only for two specimens tested. This cytotoxic effect was allotted to the important number of toxic dinoflagellates co-ingested by these fish as part of their diet. Hence, ciguatoxins are transferred to *S. salpa* originating from toxic dinoflagellates which live in epiphyte on the leaves of *Posidonia oceanica* to fish organs by grazing. Indeed, the concentration in the marine environment of these toxins present in animal organs depends therefore on the quantity of introduced food containing *Posidonia oceanica* leaves rich in toxic epiphytic phytoplankton of a seasonal nature. Factors influencing the concentration of ciguatoxins that accumulate in fish include the rate of dietary intake, the efficiency of absorption, the degree and nature of any toxin biotransformation, the rate of excretion, and the growth rate of fish (Lewis and Holmes 1993).

Conclusion

We noticed a significant correlation between the total toxic dinoflagellates in the stomach contents and the TBARS level at the side of the liver, the brain and the muscle for all seasons and all sizes together. In addition, we noticed that the origin of toxicity in fish organs correlates with the rate of dietary intake containing *Posidonia oceanica* leaves which are rich in toxic epiphytic phytoplankton of a seasonal nature. The cytotoxic effect of epiphytes extract confirms the transfer of toxins originating from toxic epiphytic phytoplankton that live on the *Posidonia oceanica* leaves to the fish organs by grazing.

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Chapter X

Tannin-Based Coagulants in the Control of Algae Proliferation: New Perspectives of Consumption-Water Treatment

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Abstract

Algae removal from natural waters by coagulation with a new tannin based coagulant called *Tanfloc* has been investigated in the laboratory by using the Jar Test technique. The influence of variables such as coagulant dosage, initial concentration of algae, temperature, pH and agitation period has been studied. Standard agitation stage was adjusted to 30 minutes (30 rpm) followed by 1 h of sedimentation. The increase of the treatment efficiency is verified for water with high contamination of several different algae species, while pH and temperature were found not to be very influential factors. Algae were removed from aqueous solutions up to 98% through coagulation/flocculation process.

1. Introduction

Algae are photosynthetic, aquatic plants that use inorganic nutrients such as nitrogen and phosphorus (Manaham, 2000). In freshwater, the term "algae" is referred to microscopic organisms, mainly unicellular, some of them form colonies visible to the naked eye as tiny green particles. In general, these bodies are finely dispersed throughout the water and, at high densities, can produce a significant turbidity (Divakara and Sivasankara, 2002). The "cyanobacteria" are organisms that have characteristics of both algae and bacteria. On one hand, they present a similar size to algae, while on the other hand, they contain blue-green or green pigment and, therefore, perform photosynthesis. Therefore, they are also called "blue-green algae." Many species of cyanobacteria can be accumulated on the foam surface, usually called "blooms" with a very high cell density (Gregor and Marsak, 2005).

The planktonic algae proliferation is a problem because it can affect different stages in the process of purification and distribution in the supply network (Whitehead et al. 1997; Steynberg et al. 1998, Pan et al. 1999). Thus, interferences arise at different levels:

- Reduction of the filtration efficiency and increased cleaning and operational costs. As a result of the low efficiency in the sedimentation process, they increase the amount of sediment that reaches the filters. This requires the use of high quantities of water to clean them and, sometimes, to reduce their effective life, so increasing the operation costs and maintenance of water treatment.
- Modifications on the disinfection efficacy process, reduction of the residual chlorine and additionally appearance of trihalomethane species. This is due to the fact that some species of algae are resistant to chlorine, so they do not disappear with the chlorination process. Consequently, the ammonia remnant (residuals of algae activity) may interact with chlorine and produce chloramines (Henderson et al., 2008). On the other hand, those algae that may release their organic content once the cellular wall is destroyed may increase the risk of trihalomethane formation because of the interaction with free chlorine in aqueous medium.
- Increase the water pH level. The photosynthetic activity involves a displacement of carbonate-bicarbonate in the water due to catchment of carbon dioxide. As a consequence, the concentration of H^+ ions gets lower and the pH level of the water increases. The harder water more readily facilitates the formation of deposits by sedimentation of calcium and magnesium, which are responsible for corrosion problems in metal pipes.
- Presence of algae in the supply network. Mixotrophic species (capable of carrying out photosynthesis and assimilating organic compounds as an energy source) can survive inside the pipe, because they do not strictly require the presence of light. Some algae such as *Chorella* can form biofilms that adhere to the interior of the pipeline. When a certain thickness is reached, it can be washed away by water flow, so increasing the turbidity.
- Alteration of organoleptic properties such as odour and taste. The presence of compounds resulting from the metabolism of algae constituents of biofilms produces odours and flavors reminiscent of mould. Some of them can be attributed, for

example, to the release of phenols and their reaction with chlorine to generate 1,2,3 trichlorophenol.

Occurrence of toxic compounds for animals and people in the event of cyanobacteria blooms. Among these compounds there are neurotoxins like anatoxin, produced by some representatives of the genera *Anabaena*, and hepatotoxins like microsystin and microcystin, produced by *Microcystis* and *Oscillatoria* respectively. The standard way for solving the appearance of these species in water is the use of methyl 2-isoborneol and geosmin, but this treatment causes a bad taste in drinking water.

Therefore, the removal of algae from water intended for human consumption and recreational use is an important and costly task to undertake in water treatment plants. It requires that algae are, preferably, removed from drinking water during the initial stages to ensure minimal impact on subsequent processes (Henderson et al., 2008).

Among the various methods that have been suggested for algae separation are autoflocculation, centrifugation, chemical treatment, filtration, flotation, sedimentation and ion exchange (Lee et al., 2012).

Sedimentation by gravity is the first method for algae removal in wastewater treatment systems (Smith and Davis, 2013). The surface charge of algae is also possible by flocculation with flocculants such as $Al_3(SO_3)_2$ and polyelectrolytes, although in many cases this is not very effective.

Ives (1959) studied the coagulation/flocculation and demonstrated experimentally that the algal cells carry a negative electric charge. He postulated that the mechanism of algal removal by chemical coagulants was the result of charge neutralization of the negatively charged algae by the positively charged metal and hydroxyl-metal ions and subsequent agglomeration and sedimentation.

Although harvesting of algae by passage through ion exchange columns is uneconomical, there are studies that reveal much about their surface properties. Golueke and Oswald (1970) found that pond algal culture passed through strong and weak anion exchange resin columns did not result in algal removal. However they found that algae could be removed by passing an algal suspension through a column either of a strong or weak cation exchange resin. The mechanism of removal apparently involved flocculation that resulted from a change in surface charge of the algal cells brought about by the charge of the resin.

There are other studies of *Chlorella* cells elimination with synthetic organic polyelectrolytes which show a good flocculation with cationic polyelectrolytes (Henderson et al., 2008), however the same results were not observed with anionic.

All algal cells have a negative zeta potential at natural water pH. This arises as a consequence of the dissociation of the functional groups at the cell surface, particularly carboxylic acid groups present in the cell wall (Northcote et al., 1958).

Under tannins denomination there are lots of chemical families. Tannins have been used traditionally for tanning animal skins, but it is possible to find several products that are distributed as flocculants. Tannins come from vegetal secondary metabolytes (Schofield, 2001), such as bark, fruits, leaves, etc. Tannin-rich barks come from trees such as *Acacia sp.*, *Castanea sp.* or *Schinopsis sp*. However, it is not needed to search for tropical species: *Quercus ilex*, *Quercus suber* o *Quercus robur* also have tannin-rich bark. This is relevant along the entire Mediterranean area.

Tanfloc is a trademark that belongs to TANAC (Brazil). It is a tannin-based product, which is modified by a physico-chemical process, and has a high flocculant power. It is obtained from *Acacia mearnsii de Wild* bark. This tree is very common in Brazil and it has a high concentration of tannins. The production process is under intellectual patent law, but similar procedures are widely reported as Mannich base reaction (Tramontini, 1994). The specific industrial process for Tanfloc is referred by US patent number 6,478,986 B1 (Lamb and Decusati, 2002). It involves tannin polymerization by addition of formaldehyde (37%), ammonium chloride and commercial hydrochloric acid. The product so obtained under certain temperature conditions has a viscous appearance with 36% of active material. The scientific literature refers to a reaction mechanism that involves a tannin mixture, mainly polyphenol tannins whose structure may be similar to flavonoid structures such as resorcinol A and pyrogallol B rings.

Similar products have been studied as general flocculants previously (Graham, 2008; Beltrán-Heredia et al., 2009). Tanfloc has been tested as flocculant in wastewater (Caselles, 2007; Beltrán-Heredia et al., 2009) and its results are promising. Regarding special pollutant removal, Tanfloc has also been tested (Sánchez-Martín et al., 2009)

The target of this research is to evaluate the use of natural coagulants/flocculants in algal removal from surface water under a qualitative point of view. After a preliminary screening on algae removal capacity of several natural agents, which involved eight coagulants, the study has centered on Tanfloc. It has studied the influence of variables such as coagulant dosage, pH, temperature, and agitation time on the coagulation/flocculation process and chlorophyll *a* removal as an indicator of algal biomass. Further studies must be carried out for statistical confirmation of the feasibility of such water treatments.

2. Materials and Methods

The trials were carried out with water from the Guadiana River in Badajoz (southwestern Spain) and were enriched with different algae solutions (*Chlorella vulgaris, Scenedesmus quadricauda, Oocystis solitaria*) grown in the laboratory. The basic characteristics of this water are shown in Table 1.

2.1. Natural Coagulants

Eight types of natural coagulants products were tested in a preliminary screening, with the purpose to obtain information about their capacity. They were prepared in the following way: Cationic starch was supplied by Cargill (USA). It is used as an authorized alimentary supplement. It is presented as powder.

Apart from TANFLOC, three other products with the same nature have been provided by Silvateam, SA (Italy). This is the case of SILVAFLOC and AQUAPOL C1 and AQUAPOL S5T. The differences between SILVAFLOC, TANFLOC, AQUAPOL C1 and S5T lay on tannin nature (*A. mearnsii* to AQUAPOL and TANFLOC and *Schinopsis balansae* for SILVAFLOC) and on chemical modification, which is under copyright law. TANFLOC and AQUAPOL C1 are presented as powder, while SILVAFLOC and AQUAPOL S5T are presented as a dense solution. *Moringa oleifera* extraction was done as described in Okuda (1999) and recently in Beltrán-Heredia and Sánchez-Martín (2008). Seeds were obtained from SETROPA, Holland, The extraction process was carried out in the following way: seeds were ground to powder in a domestic mill. A 1M NaCl (PANREAC) solution was prepared and 5 g of *Moringa oleifera* powder was put into 100 mL. The NaCl solution with powder was stirred for 30 minutes at pH 7 (room temperature). Then, the extract was filtered twice: once through commercial filter paper on Büchner funnel and once again through a fine filtering *millipore* system (45 μm glass fiber). The result was a semi-transparent, milky-like liquid.

Opuntia ficus-indica mucilage was obtained in the following way: pods of *Opuntia ficus-indica* were cut and external layer was removed manually. 100 g of this fraction was dried in an oven at 60ºC for 24 h. Subsequently it was micronized in a domestic grinder (Braun) and was used directly as a coagulant. Aluminium sulphate $Al_2(SO_4)_3 \cdot 18\ H_2O$ was supplied by Panreac (Spain).

2.2. Algae Cultures

Algae cultures were incubated at 25º C under white light with a photoperiod of 12:12 in a culture medium supplied by Fluka (Algae Culture Broth). These inocula were provided by the Department of Botany, University of Coimbra (Portugal).

2.3. Flocculation Test

The investigation was carried out in batch process according to the standardization Jar Test with a single agitation stage. Water treatment was carried out in the followings way: 500 ml of water was put into a beaker. It was subjected to the Jar Test (VELP Scientifica JLT4 apparatus) without addition of flocculant (30 rpm for 30 minutes) as an experimental control. The sample was kept standing for 1 h and the concentration of chlorophyll *a* was determined as an indicator of algal biomass in the water. With this procedure it is possible to determine the initial concentration of chlorophyll *a*, with the exception of algae that have a natural sedimentation. Subsequently the same procedure was performed for the different variables.

2.4. Chlorophyll *a* Analysis

Chlorophyll *a* is a photosynthetic pigment present in all species of phytoplankton, including eukaryotic organisms (algae) and prokaryotic (cyanobacteria) and is commonly used for the determination of phytoplankton biomass (Gregor, Marsalek, 2004). Most of the methods of quantification are based on chlorophyll pigment extraction by the disintegration of cells in an organic solvent such as methanol, ethanol or acetone and its subsequent determination by spectrophotometry (Richards and Thompson, 1952; Parsons and Strickland, 1963). The method of analysis used for determination of chlorophyll *a* in water samples has been the spectrophotometric technique. (Standard Methods for the Examination of Water and Wastewater, 1992). The procedure was as follows: filtered a known volume of sample through a glass fiber filter (Whatman GF / C). Filters are placed in a freezer for 24 hours to facilitate the rupture of cell walls and release of pigment. Subsequently, the filters are placed in test tubes cut lined aluminum foil and add 10 mL of extraction solvent (methanol). It is left to stand in darkness for 2 hours to facilitate the extraction of the pigments. After that time, the absorbance is measured in a spectrophotometer at 665 and 750 nm. In the same cuvette, one drop of 1N HCl is added and after a minute, read the absorbance at both wavelengths. The concentration of chlorophyll *a* can be calculated according to the expression 1:

$$[Clorophyll\ a] = F \cdot \left[\left(Abs_{665}^{a} - Abs_{750}^{a}\right) - \left(Abs_{665}^{b} - Abs_{750}^{b}\right)\right] \cdot K \cdot v \tag{1}$$

Where chlorophyll *a* is expressed in μg/L.
Abs^{a} is absorbance before acidification.
Abs^{b} is absorbance after acidification.
F = 2.72 for methanol
K is specific absorption coefficient, 11.62 for methanol.
V is volume of extract in mL/L filtered water

3. Results and Discussion

3.1. Raw Water Characterization

Surface water was selected to work on. It was taken from the Guadiana River, at Badajoz (Southwest of Spain) and was enriched with algae solutions. It is intended with this decision to study the problem from a real point of view. The characteristics of this river water are shown in Table 1.

Table 1. Raw water characterization data

Parameter	Units	Value
Conductivity	μS cm^{-1}	400
Suspended solids	mg l^{-1}	15
Total solids	mg l^{-1}	452
Turbidity	NTU	123.3
Calcium	Ca^{2+} mg l^{-1}	37.7
Hardness	$CaCO_3$ mg l^{-1}	152
Ammonium	N mg l^{-1}	1.81
Nitrate	NO_3^- mg l^{-1}	5.3
Nitrite	N mg l^{-1}	0.033
Chloride	Cl^- mg l^{-1}	40.4
$KMnO_4$ oxidability	O_2 mg l^{-1}	19.3
Phosphate	P mg l^{-1}	0.044
Total phosphorus	P mg l^{-1}	0.064
Total coliforms	Colonies per 100 ml	800
Fecal coliforms	Colonies per 100 ml	400
Fecal streptococcus	Colonies per 100 ml	140

3.2. Preliminary Screenings

Several trials of algae removal were performed with different natural agents to obtain information about their efficiency in the process of coagulation/flocculation. Most of them were based on polysaccharides (starches and mucilage) and others were based on modified tannin as flocculant agents (Tanfloc, Silvafloc and Acquapol). Figure 1 shows the percentages of *Chlorella* removal that were carried out with various flocculant agents. Standard dosage of 10 mg/L of flocculant agent and 175 µg/L of chlorophyll *a* was fixed. The experiments were conducted at pH 7 and 20º C as standard testing Jar Test of 30 minutes at 30 rpm. As can be appreciated, all the products have a good removal ability. Those considered polysaccharides (cationic starch and *Opuntia sp* mucilage) show a significant algal biomass removal. In the case of starch, this percentage is about 50%, while mucilage is up to 60%. The use of these two agents in water treatment has been studied previously for the removal of turbidity (Merta, 1995). Coagulating capacity is linked with the electrostatic destabilization of negatively charged colloids, such as the algae cell membrane.

Regarding the *Opuntia* sp mucilage, it has also been studied as a coagulant agent (Young, 2005), but in this case the chemical nature of this product is more complex and the coagulation process is not yet understood.

Another coagulant agent widely studied and with great potential in the field of water treatment is the *Moringa oleifera*. As Figure 1 depicts, this plant extract removes a high percentage of algal biomass. The protein nature of the principle of the *Moringa* coagulant induces the formation of networks that catch the negatively charged particles and cause sedimentation (Okuda, 2001).

Referring to tannin-based flocculant, Tanfloc reaches a higher percentage of algae removal, about 97%. Then come Acquapol S5T and Acquapol C1. The last of these coagulants, according to algae removal, is Silvafloc. Although there are no marked differences (the range of effectiveness is between 78% and 97%), the slight variations in efficacy may be due to the tannin base and degree of polymerization, which is different in each case.

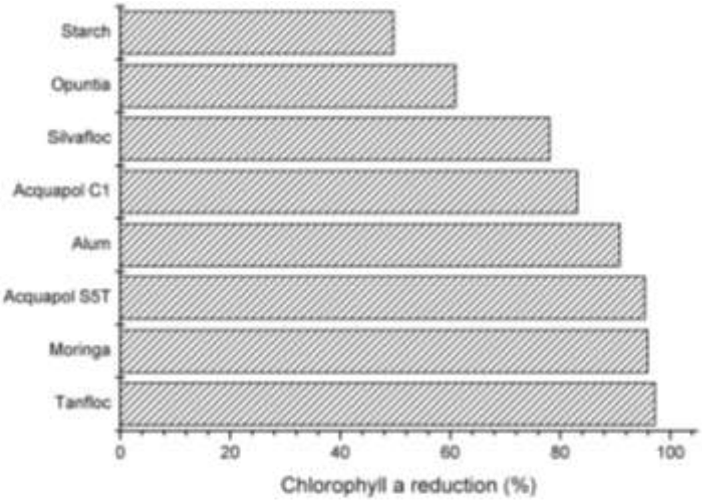

Figure 1. Preliminary screening for *Chlorella* removal.

Aluminum sulphate was used in order to compare results from natural and from synthetic coagulant agents. As can be seen, the levels of algae removal are of the same order in each case.

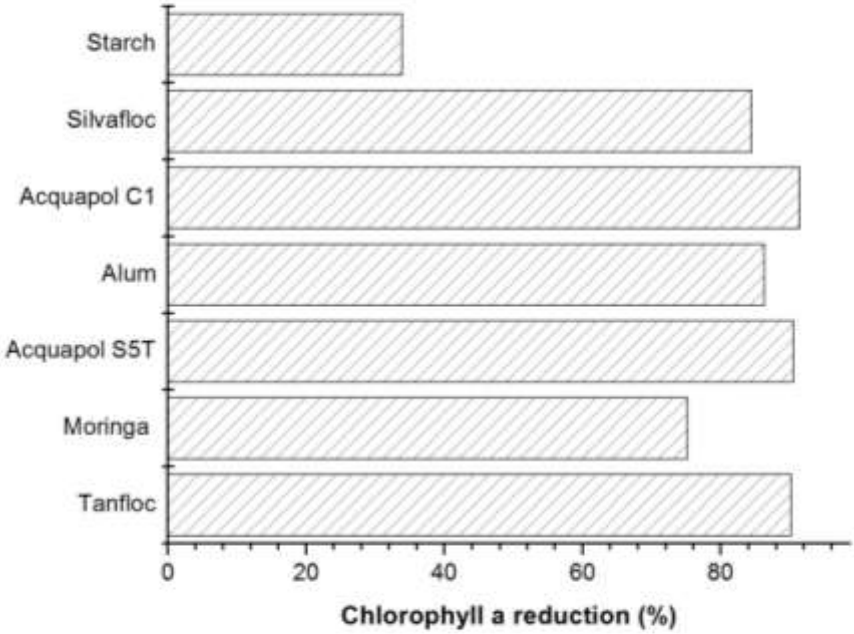

Figure 2. Preliminary screening for *Scenedesmus* removal.

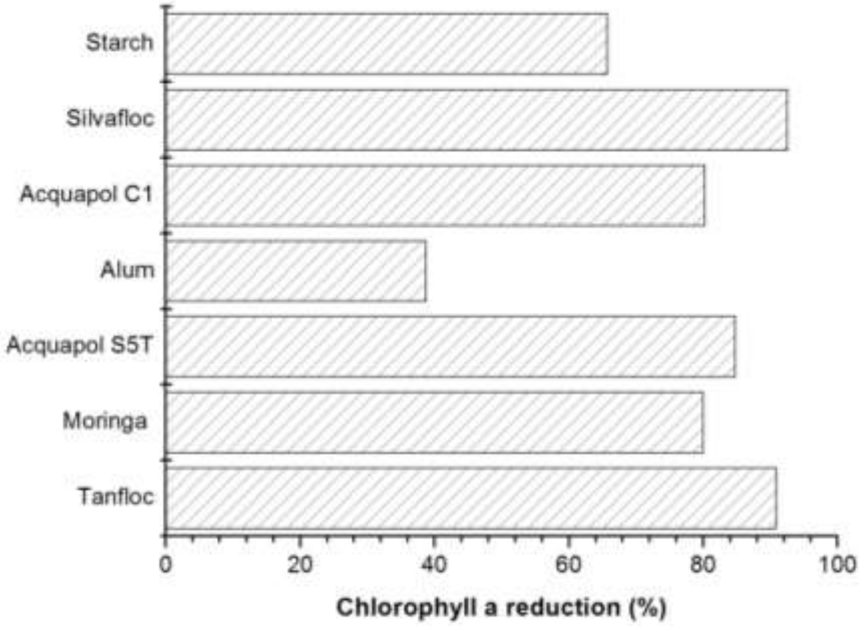

Figure 3. Preliminary screening for *Oocystis* removal.

In same way as *Chlorella*, Figures 2 and 3 show the results of screenings conducted with the flocculant agents for *Scenedesmus* and *Oocystis*, respectively. Again all tannin flocculants (Acquapol C-1, Acquapol S5T, Tanfloc and Silvafloc) showed high removal efficiency of algae, greater than 80%. *Moringa oleifera* also achieves high removal percentages, close to 80%, while starch is the worst flocculant, with algae reductions between 40 and 60%. For its part, alum (aluminum chloride) shows a low efficacy for *Oocystis* and high for *Scenedesmus*.

3.3. Influence of Variables

Tanfloc Dosage

A series of experiments were made in order to determine the effect of coagulant dosage on algae removal. A fixed algae concentration of 383 μg/L was used with different doses of Tanfloc: from 2 to 12 mg/L. As can be appreciated in Figure 4, the final concentration of *Chlorella* tends to decrease as Tanfloc dose increases. However, it is observed that process efficiency peaks at a maximum and higher doses of coagulant do not achieve lower chlorophyll *a* concentrations. The reduction percentage in chlorophyll *a* is very high, reaching values around 90% from 6 mg/L of coagulant. There is a residual chlorophyll *a* concentration that it not possible to remove by coagulation and it seems to be about 10 μg/L.

Figures 5 and 6 show the influence of Tanfloc dosage on the percentage reduction for *Scenedesmus* and *Oocystis*, respectively. The trend is similar in both cases to that observed for *Chlorella*.

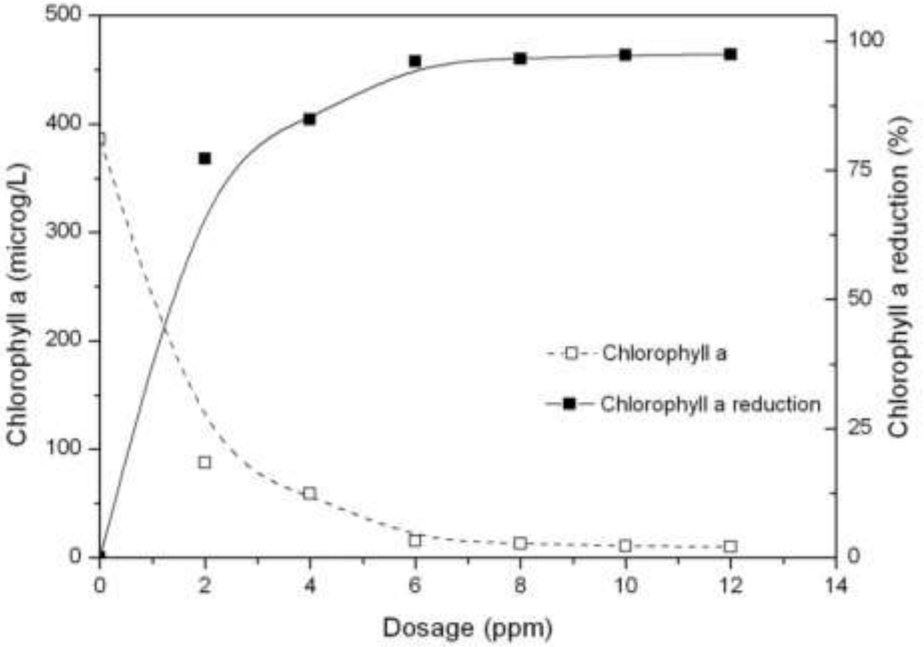

Figure 4. Tanfloc dosage influence for *Chlorella* removal.

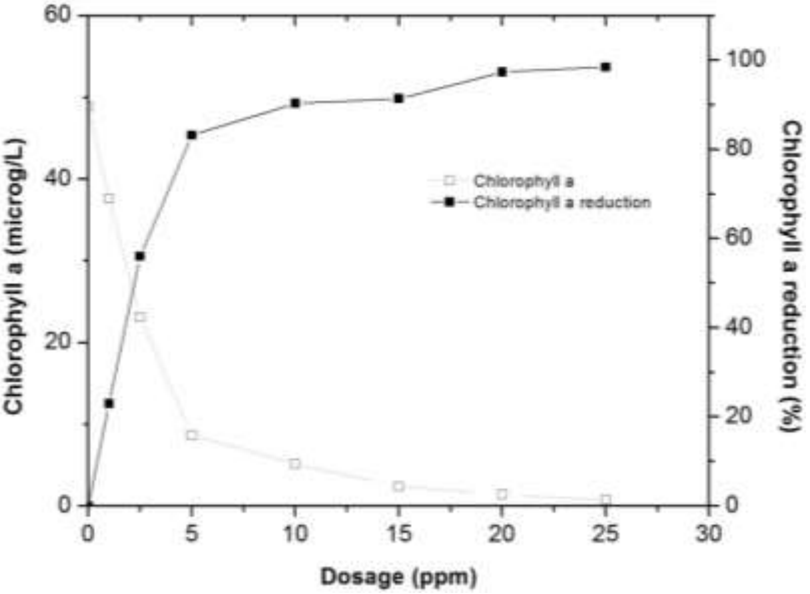

Figure 5. Tanfloc dosage influence for *Scenedesmus* removal.

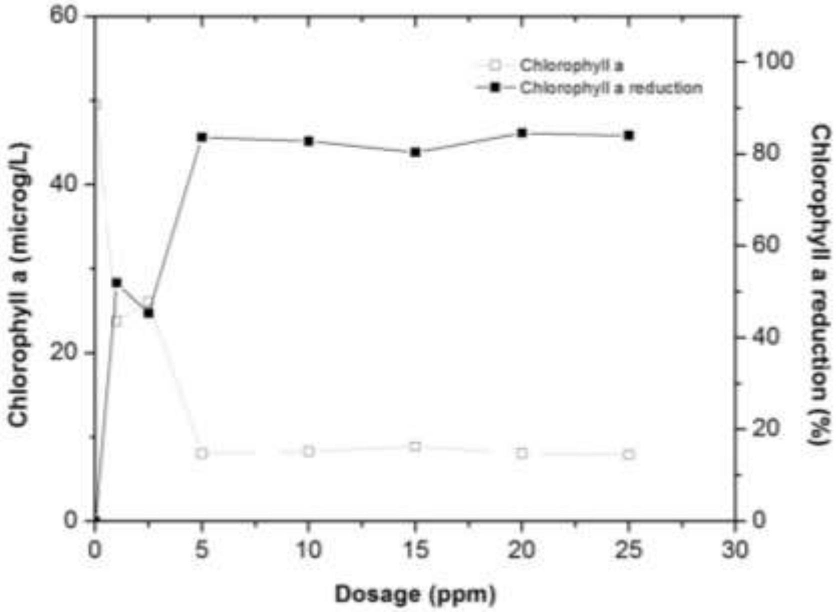

Figure 6. Tanfloc dosage influence for *Oocystis* removal.

Initial Concentration of Algae

In order to evaluate the effect of increasing the initial concentration of *Chlorella*, a fixed dosage of 10 mg/L of Tanfloc was applied. Initial concentration of algae was varied between 100 and 500 μg/L. The results are shown in Figure 7, which represents the reduction percentage in chlorophyll *a* and residual chlorophyll at the end of the coagulation/flocculation process. The reduction rate is very high, arriving in most cases at a value higher than 95%. It can also be seen that residual chlorophyll increases by increasing initial concentration of algae, for the same dose of Tanfloc. Figure 10 shows the coagulant capacity, where it is appreciated to be directly proportional to the initial concentration of algae.

Similarly, the influence of the initial algae concentration has been studied for *Scenedesmus* and *Oocystis*. The results are shown in Figures 8 and 9. It can be seen that there is a positive trend in the percentage reduction of algae in both species.

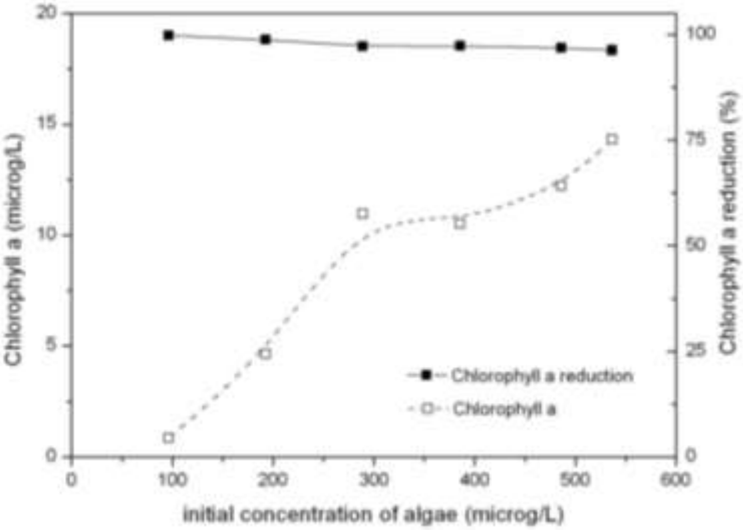

Figure 7. Initial *Chlorella* concentration influence.

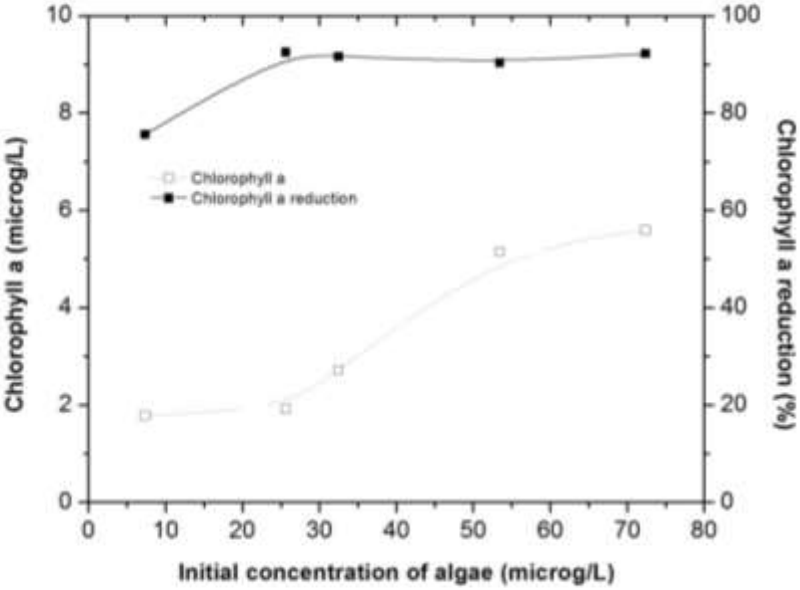

Figure 8. Initial *Scenedesmus* concentration influence.

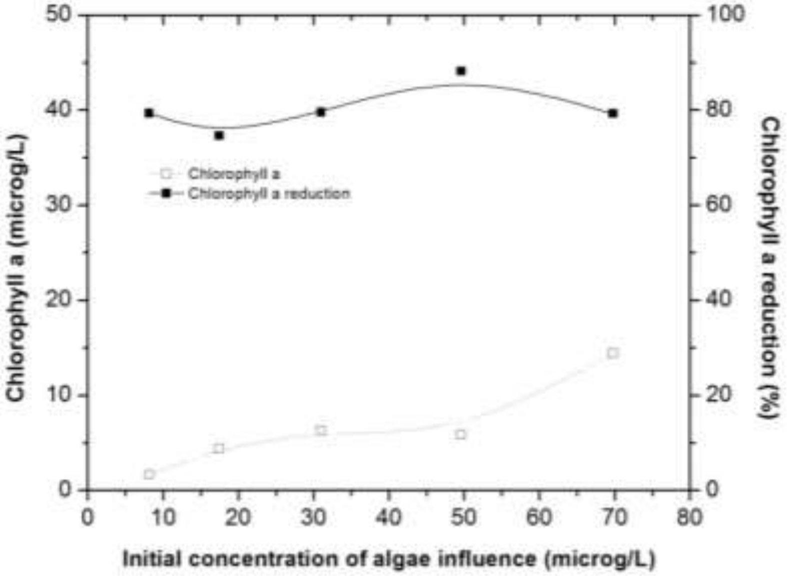

Figure 9. Initial *Oocystis* concentration influence.

When comparing capacity vs. initial algae concentration, one can depict a figure such as Figure 10. There, coagulant capacity (q) is calculated from equation 2:

$$q = \frac{C_O - C}{D} \qquad (2)$$

Where q is coagulant capacity, μg/mg,

C_o is initial concentration of algae, μg/L,
C is final concentration of algae μg/L and
D is coagulant dosage, mg/L.

As Figure 10 depicts, capacity decreases by increasing coagulant dose for a given concentration of algae. This is a normal behavior that is in agreement with other previous studies (Sanghi, et al., 2006).

Temperature

In order to observe the influence of the temperature on the *Chlorella* removal process by coagulation/flocculation, a series of experiments was carried out over a range of temperatures. Experiments at 10, 20, 30 and 40 ºC were performed, with an initial concentration of algae of 385 μg/L and a dose of Tanfloc of 10 mg/L. The temperature influence is minimal in the range studied, obtaining percentages of algae removal around 98% (Figure 11). In general, the temperature does not seem to be significantly important in this process, except for tests performed at 10 °C, with a slightly lower algae removal (93%). This is also shown in Figure 12 where coagulant capacity is lower than in the experiment conducted at 10 ºC and there are not significant differences between the rest of the experiments.

pH

pH values were varied between 4 and 9, in order to determine its influence on *Chlorella* removal. Figure 11 represents the percentage reduction of chlorophyll *a*, obtaining high values (87-97%). Figure 12 shows the coagulant capacity versus pH and it can be appreciated that pH level is not significantly important in the algae removal process.

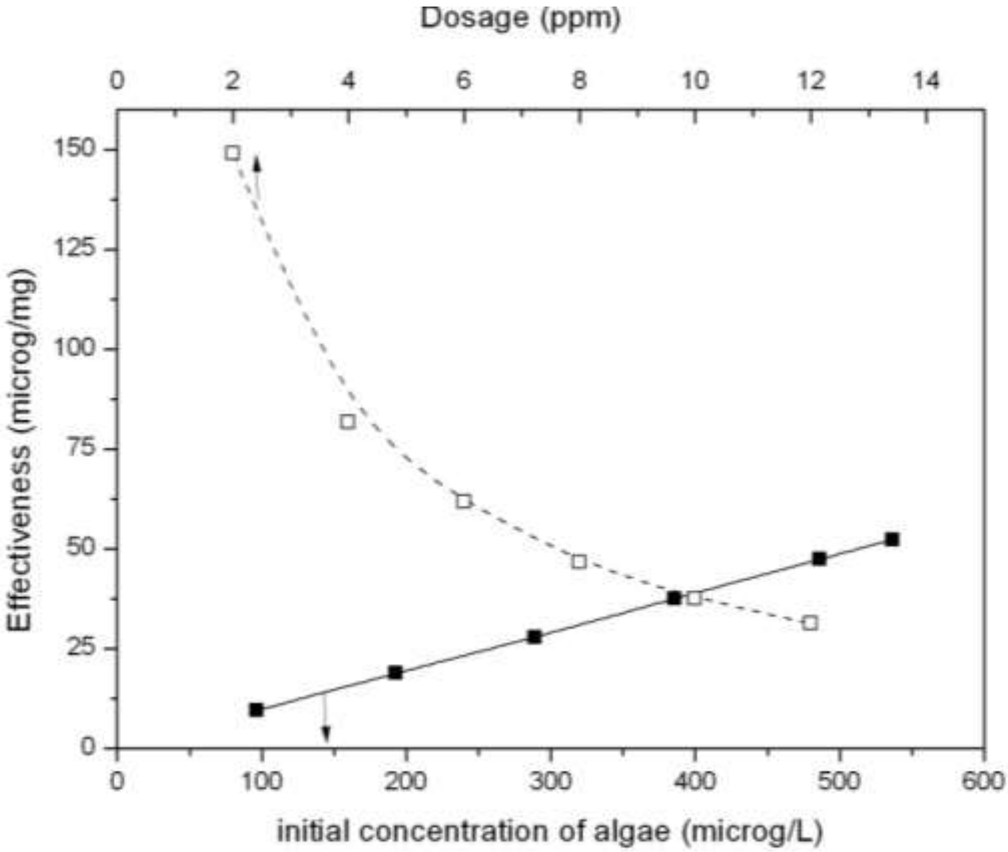

Figure 10. Coagulant capacity vs. Tanfloc dosage and initial concentration of *Chlorella*.

Agitation Time

Experimental series were carried out in order to evaluate the influence of agitation time on *Chlorella* removal. Experiments at 10, 20, 30, 40, 50 and 60 minutes were performed with an initial concentration of algae of 385 μg/L and Tanfloc dosage of 10 mg/L. Figure 11 shows the reduction percentage of chlorophyll *a* versus agitation time. It can be seen that this variable does not significantly affect the algae removal process, reaching percentages around 95% in all experiments. Figure 12 represents the coagulant capacity, showing that the capacity is slightly lower at 10 minutes of agitation. No differences were observed from 20 min and above.

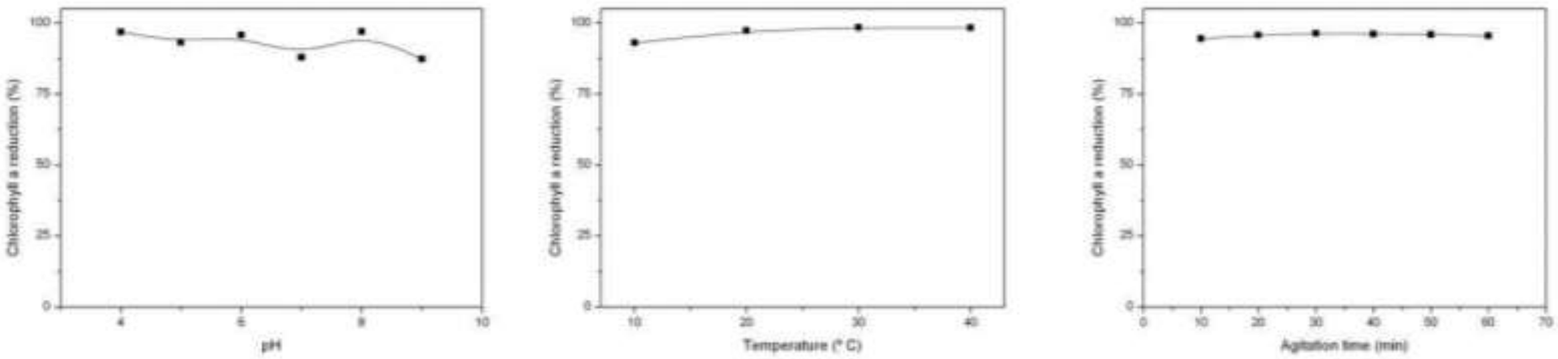

Figure 11. pH, temperature and agitation time influence for *Chlorella* removal.

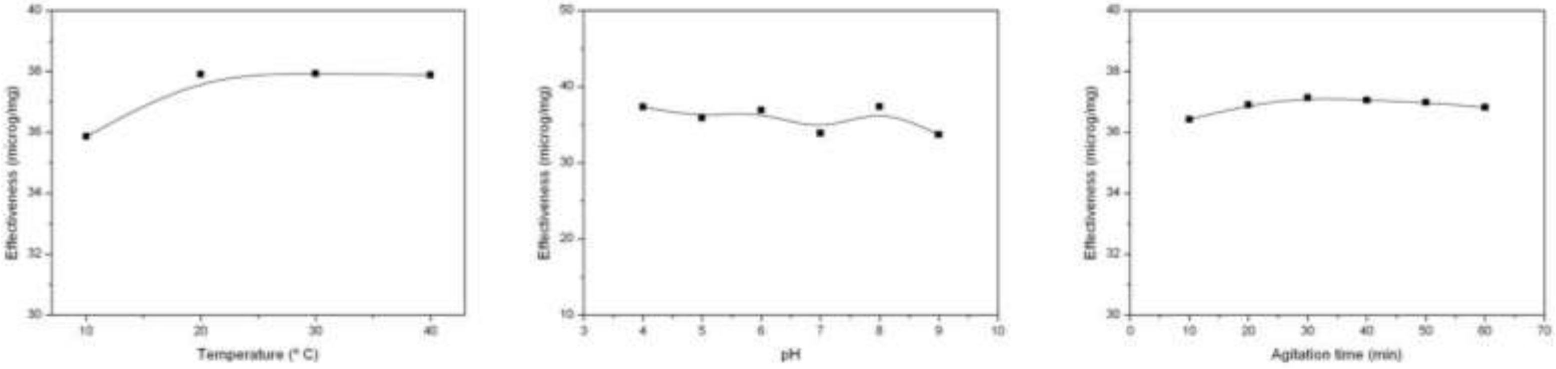

Figure 12. pH, temperature and agitation time influence for *Chlorella* removal effectiveness.

Likewise, the influence of agitation time on the flocculation test has been studied for *Scenedesmus* and *Oocystis*. Figures 13 and 14 show that a stirring time of 20 to 30 minutes is sufficient to achieve the equilibrium between algae and flocculant.

Conclusion

This research has the following conclusions:

- Tanfloc is very effective in algae removal by coagulation/flocculation. The study has revealed algae removal around 90% in most of cases.
- The capacity of the elimination algae process by Tanfloc depends on the coagulant dose and the initial concentration of algae.
- From a dose of 6 mg/L of Tanfloc the percentage reduction of chlorophyll *a* reaches values higher than 90%.
- Temperature, pH and agitation time do not significantly affect to algae removal by coagulation/flocculation. High levels of algae reduction are achieved across the range of parameters that were studied.

The use of these kinds of coagulants must reduce the impact of algal blooms either in water supply facilities and in free water masses. This is an important advantage if compared with the traditional water treatment agents. Further studies must be carried out for optimizing the specific application for several water and wastewater samples.

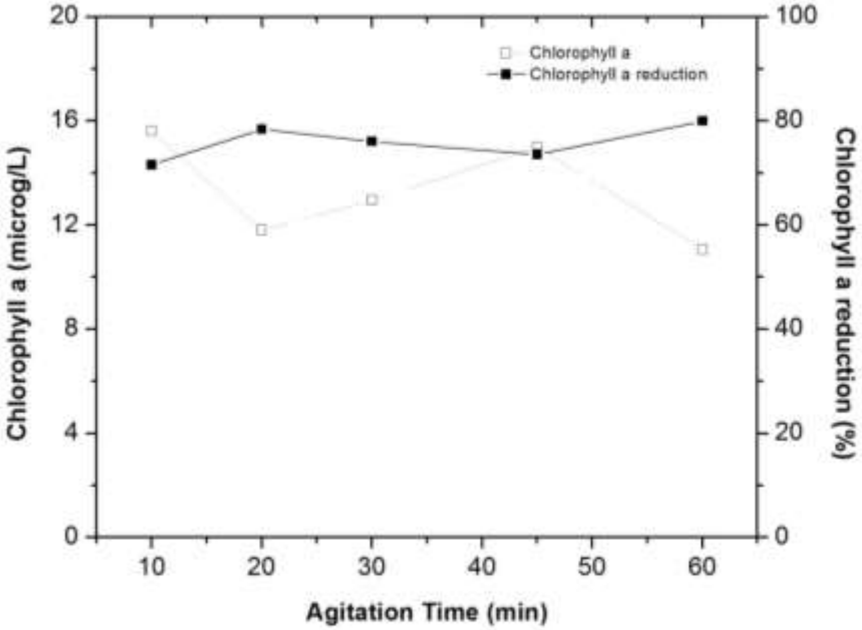

Figure 13. Agitation time influence for *Scenedesmus* removal.

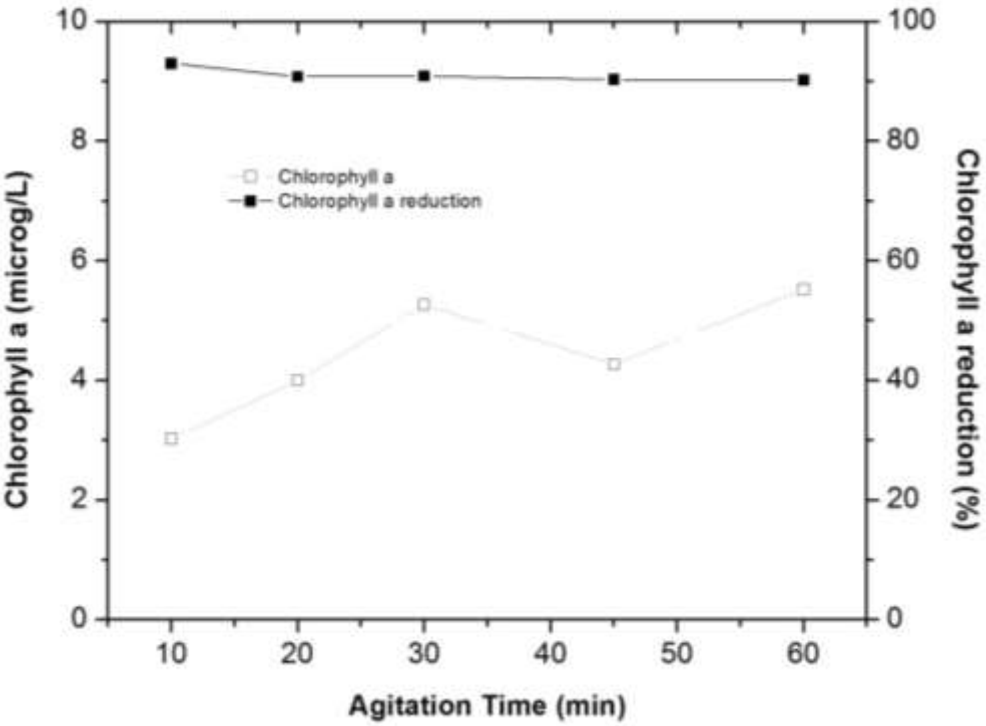

Figure 14. Agitation time influence for *Oocystis* removal.

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Chapter XI

Algae Removal with Natural Coagulants: The Case of *Moringa oleifera* Seed Extract

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Abstract

Algae removal capacity by coagulation/flocculation process with natural products has been investigated in the laboratory using the Jar Test technique. *Moringa oleifera* seeds extract has been tested for algae removal from surface water. The influence of variables has been studied in this process, including operating parameters such as coagulant dosage, initial concentration of algae, temperature, pH, agitation time and agitation speed. Test periods were adjusted to 30 minutes of slow agitation (30 rpm) followed by 1 h of sedimentation. The increase in the treatment capacity is verified for water with high contamination of several algae species, while pH and temperature were found to be not very important factors in the removal efficiency. Algae were removed from aqueous solutions up to 98% through the coagulation/flocculation process.

Keywords: Algae, natural coagulants agents, flocculation, *Moringa oleifera,* chlorophyll *a*

1. Introduction

Algae are considered to be a loose group of organisms that have all or most of the following characteristics: aquatic, photosynthetic, simple vegetative structures without a vascular system, and reproductive bodies that lack a sterile layer of protecting cells. In addition, there is a wide range of vegetative morphologies, including the following: unicells, colonies, pseudofilaments and filaments, amongst others (Wehr, D.J. and Sheath, G.R., 2003). Generally, these bodies are finely dispersed throughout the water and, at high densities, can produce a considerable turbidity (Divakara and Sivasankara, 2002).

Algae in drinking water supply can cause significant disturbances including taste and odour, production of disinfection by-product (DBP), obstruction to coagulation, clogging of filters and assimilable organic carbon (AOC) for growth of biofilm (Chen and Yhe, 2005).

The state and evolution of freshwater ecosystems are affected by a variety of biotic and abiotic factors, as well as by natural and human-induced processes that may differ both in duration and intensity (Kagalou et al., 2008). Algae content in aquatic systems can be an important and representative parameter that must be taken into account, not only for the environmental implications for these kinds of ecosystems, but also because of the fact that its presence is a significant economic factor in the consideration of water processing techniques (Alfafara et al., 2002).

The surface water quality, especially in lakes and reservoirs, is often affected by the eutrophication phenomenon, which, according to the definition adopted by the Organisation for Economic Co-operation and Development (OECD), is an enrichment of nutrients in the water which leads generally to symptomatic changes such as increased algae production and other aquatic plants, degradation of fisheries and deterioration of water quality in general.

Therefore, the algae removal in water is an important and costly labour in treatment plants producing drinking water for human consumption and for recreational use. It requires that algae are removed from drinking water, preferably during the initial stages to ensure minimal impact on subsequent processes (Henderson et al., 2008).

The multiple problems that the presence of algae in drinking waters present must be faced through specific physico-chemical techniques. These are always based on the inactivation of the microorganism life and amongst them one can find coagulation, flotation, filtration and many others. Advanced Oxidisation processes (AOPs) are also placed in this group of recently-developed techniques: ozonation (Plummer and Edzwald, 2001) and potassium permanganate addition (Wang et al., 2013).

Talking specifically about oxidisation processes, it has been observed that the reactive dose of total inactivation depends on the algae type and the oxidising agent, but, in general, reagent consumption is usually high. This is obviously a remarkable drawback when using these techniques because of the presumable formation of trihalomethane due to the high organic matter inside the cellular wall (Wachter and Andelman, 1984).

Other inactivation techniques studied are electrochemical treatment (Alfafara et al., 2002; Wenyan et al., 2005) and by ultrasound (Zhang et al., 2006).

Within the physical-chemical treatments, the most widely studied is the process of coagulation/flocculation using reagents commonly used in water treatment: iron salts, aluminum salts, natural polysaccharides, etc. (Henderson et al., 2008). The effectiveness of these reagents is based on the nature of the outer layer of algae, which have an electric charge in its outer membrane between - 10 and - 40 mV (zeta potential). This negative electrical charge naturally favours the action of the cationic coagulant.

It has sometimes been considered that the combination of these processes with a pre-oxidant (Chen and Yen, 2009), such as chlorine (Plummer and Edzwald, 2002), ozone (Plummer and Edzwald, 2002), potassium permanganate (Chen et al., 2008), potassium ferrate (Ma and Liu, 2002), ultrasound (Shi et al., 2008), etc., results in improvements in the algae removal by coagulation/flocculation. This fact is justified by the oxidation of organic matter associated with the outer wall of the algae, usually a type of glucuronic polysaccharide whose oxidation produces many acid groups that can easily link with coagulants, in addition to increasing the negative zeta potential of the algae.

Particularly, some clay materials have positive charges on their surface and thus may interact with algae (Avnimelech et al., 1982; Han and Kim, 2001), several authors have proposed the use of different clays to remove phytoplankton through subsequent sedimentation/filtration/flotation (Beaulieu et al., 2005; Sengco and Anderson, 2004).

Based on the gravity-difference techniques, some studies presented an air-flotation process for algae removal (Liu et al., 1999; Chen et al., 1998). It is also possible to combine this well-known process with with oxidizing pretreatments, such as the use of potassium permanganate, ozone or potassium ferrate. (Ma et al., 2012; Chen et al., 2009). The combination of these improves the effectiveness of the entire process.

Regarding the shape and size differences, filtration mechanisms can be applied for the removal of algae. These techniques can be based on sand or on polymeric membranes. Some studies already present the elimination of phytoplankton (El-Taweel and Ali, 2000; Al-Malack et al., 1998) and can also include some kinds of post-treatment processes such as coagulation and/or flotation (Ma et al., 2007; Kunicane et al., 1986).

Coagulation technique on water and wastewater treatment is a widespread purification process that can be based either on man-made coagulants or natural ones. These last ones (starches, seed derivatives, tannin-based coagulants or clays) present many advantages if compared with traditional ones (such as aluminum sulphate or ferric chloride). Specifically, there are some previous works that have pointed out the ability of *Moringa oleifera* seed extract in removing algae from water. Mwangi et al. (2008) studied the direct application of *Moringa* extract as a primary coagulant in stabilisation ponds with eutrophication problems. More specifically, some studies of adsorption were carried out by Warhurst et al. (1997) about the removal of hepatotoxin, this time through activated carbon based on *Moringa oleifera* pods.

Moringa oleifera is a tropical tree from the genus *Moringaceae* that comes from sub-Himalayan valleys and is currently distributed throughout the world, in the tropics and subtropics. It is a multi-purpose tree. Its multiple properties have been known for a long time (Fuglie, 2001) and several authors have referred to the importance of *Moringa oleifera* in human (Makkar and Becker, 1996) and animal nutrition (Richter et al., 2003), pharmacology (Cáceres et al., 1991), cosmetics (Armand et al., 2003), etc

The utilisation of *Moringa oleifera* as a water treatment agent is perhaps one of its most interesting usages. Although there are previous papers investigating its utilisation as natural adsorbent for special pollutants removal (Kumari et al., 2006; Mataka et al., 2006; Warhurst et al., 1997), the seeds of this tropical tree have a high level of proteins that work like cationic polyelectrolytes once they are added to raw water (Ghebremichael et al., 2005; Okuda et al., 2001). Proteins causing colloids destabilisation and removal by sedimentation are those which act as cationic polyelectrolytes and neutralise suspended materials, as most of them are negatively charged, like algae, which have a negative electrical charge in their cell walls, (Ives, 1959; Golueke and Oswald, 1970).

Due to this fact, characterising the flocculant process in drinking water is a very important task. The interest in *Moringa oleifera* in this aspect has been pointed out by institutions such as the FAO (Food and Agriculture Organization). Its multiple uses have been recommended by the Food and Agriculture Organization (FAO) (Jahn et al., 1986).

The objective of this research is to evaluate the use of natural coagulants/flocculants in algal removal from surface waters from a qualitative point of view. After a preliminary screening on algae removal capacity of several natural agents, which involved eight coagulants, the study has centered on *Moringa oleifera*. It has involved the study of the influence of variables such as coagulant dosage, pH, temperature, agitation time and agitation speed on the coagulation/flocculation process and chlorophyll *a* removal as an indicator of algal biomass.

2. Materials and Methods

The assays were carried out with water from the Guadiana River in Badajoz (southwestern Spain) and were enriched with different algae solutions (*Chlorella vulgaris, Scenedesmus quadricauda*, *Oocystis solitaria*) grown in the laboratory. The basic characteristics of this water are shown in Table 1.

2.1. Natural Coagulants

Eight types of natural coagulants products were tested in a preliminary screening, with the purpose to obtain information about their capacity. They were prepared in the following way:

- Cationic starch was supplied by Cargill (USA). It is used as an authorised alimentary supplement. It is presented as powder.
- Modified tannin was supplied by Tanac, S.A. (Brazil). Its name is TANFLOC and consists of tannins from *Acacia mearnsii* that have been chemically modified in order to introduce a quaternary nitrogen that confers TANFLOC its cationic character. Apart from TANFLOC, three other products with the same nature have been provided by Silvateam, SA (Italy). These are SILVAFLOC and AQUAPOL C1 and AQUAPOL S5T. The differences between SILVAFLOC, TANFLOC, AQUAPOL C1 and S5T lay on tannin nature (*A. mearnsii* to AQUAPOL and TANFLOC and *Schinopsis balansae* for SILVAFLOC) and on chemical modification, which is under copyright law. TANFLOC and AQUAPOL C1 are presented as powder, while SILVAFLOC and AQUAPOL S5T are presented as a dense solution.
- *Moringa oleifera* extraction was done as described in Okuda (1999) and more recently in Beltrán-Heredia and Sánchez-Martín (2008). Seeds were obtained from SETROPA, Holland. The extraction process was carried out in the following way:

seeds were ground to powder in a domestic mill. A 1m NaCl (PANREAC) solution was prepared and 5 g of *Moringa oleifera* powder was put into 100 mL. The NaCl solution with powder was stirred for 30 minutes at pH 7 (room temperature). Then, the extract was filtered twice: once through commercial filter paper on Büchner funnel and once again through a fine filtering *millipore* system (45 μm glass fiber). The result was a semi-transparent, milky-like liquid.

- *Opuntia ficus-indica* mucilage was obtained in the following way: pods of *Opuntia ficus-indica* were cut and the external layer was removed manually. 100 g of this material was dried in an oven at 60ºC for 24 h. Subsequently was it micronised in a domestic grinder (Braun) and was used directly as a coagulant.
- Aluminium sulphate $Al_2(SO_4)_3 \cdot 18\ H_2O$ was supplied by PANREAC.

2.2. Algae Cultures

Algae cultures were carried out at 25ºC under white light with a photoperiod of 12:12 in a culture medium supplied by Fluka (Algae Culture Broth). These inocula were provided by the Department of Botany, University of Coimbra (Portugal).

2.3. Flocculation Test

The investigation was carried out in discontinuous regime according to the standardization Jar Test with a single agitation stage.

Water treatment was carried out in the following way: 500 mL of water was put into a beaker. Jar Test (VELP Scientific JLT4 apparatus) without addition of flocculants (30 rpm for 30 minutes) was performed like experimental control. The sample was kept standing for 1 h, and the concentration of chlorophyll *a* was determined as an indicator of algal biomass in the water. By this technique it is possible to determine the initial concentration of chlorophyll *a*, with the exception of algae that have a natural sedimentation. Subsequently the same procedure was performed for the different variables.

2.4. Chlorophyll *a* Analysis

Chlorophyll *a* is a photosynthetic pigment present in all species of phytoplankton, including eukaryotic organisms (algae) and prokaryotic (cyanobacteria) and is commonly used for the determination of phytoplankton biomass (Gregor and Marsalek, 2004). Most of the methods of quantification are based on chlorophyll pigment extraction by the disintegration of cells in an organic solvent such as methanol, ethanol or acetone and its subsequent determination by spectrophotometry (Richards and Thompson, 1952; Parsons and Strickland, 1963).

The method of analysis used for determination of chlorophyll *a* in water samples has been the spectrophotometric technique using Standard Methods for the Examination of Water and Wastewater (APHA et al. 1992).

3. Results and Discussion

3.1. Raw Water Characterisation

Surface water was selected to work on. It was taken from the Guadiana River, at Badajoz (Southwest of Spain) and was enriched with an algae solution. It is intended with this decision to study the problem from a real point of view. River characteristics of this water are shown in Table 1.

Table 1. Raw water characterisation data

Parameter	Units	Value
Conductivity	μS cm^{-1}	400
Suspended solids	mg l^{-1}	15
Total solids	mg l^{-1}	452
Turbidity	NTU	123.3
Calcium	Ca^{2+} mg l^{-1}	37.7
Hardness	$CaCO_3$ mg l^{-1}	152
Ammonium	N mg l^{-1}	1.81
Nitrate	NO_3^- mg l^{-1}	5.3
Nitrite	N mg l^{-1}	0.033
Chloride	Cl^- mg l^{-1}	40.4
$KMnO_4$ oxidability	O_2 mg l^{-1}	19.3
Phosphate	P mg l^{-1}	0.044
Total phosphorus	P mg l^{-1}	0.064
Total coliforms	Colonies per 100 ml	800
Fecal coliforms	Colonies per 100 ml	400
Fecal streptococcus	Colonies per 100 ml	140

3.2. Preliminary Screenings

Several trials of algae removal were performed with different natural agents to obtain information about their efficiency in the process of coagulation/flocculation. Most of them were based on polysaccharides (starches and mucilage) and others were based on modified tannin as flocculant agents (Tanfloc, Silvafloc and Acquapol). Figure 1 shows the percentages of *Chlorella* removal that were carried out with various flocculant agents. Standard dosage of 10 mg·L^{-1} of flocculant agent and 175 μg·L^{-1} of chlorophyll *a* was fixed. The experiments were conducted at pH 7 and 20° C as standard testing Jar Test of 30 minutes at 30 rpm. As can be appreciated, all the products have a good removal ability. Those considered polysaccharides (cationic starch and *Opuntia* mucilage) show a significant algal biomass removal. In the case of starch, this percentage is about 50%, while mucilage is up to 60%. The use of these two agents in water treatment has been studied previously for the removal of turbidity (Merta, 1995). Coagulating capacity is linked with the electrostatic destabilization of negatively charged colloids, such as the algae cell membrane.

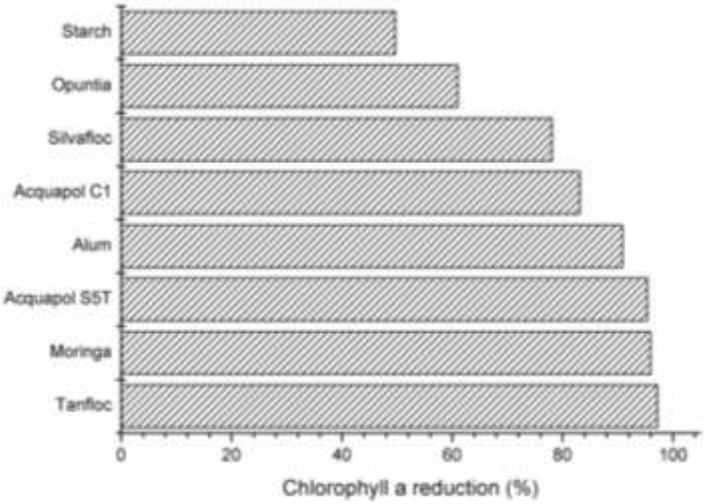

Figure 1. Preliminary screening for *Chlorella* removal.

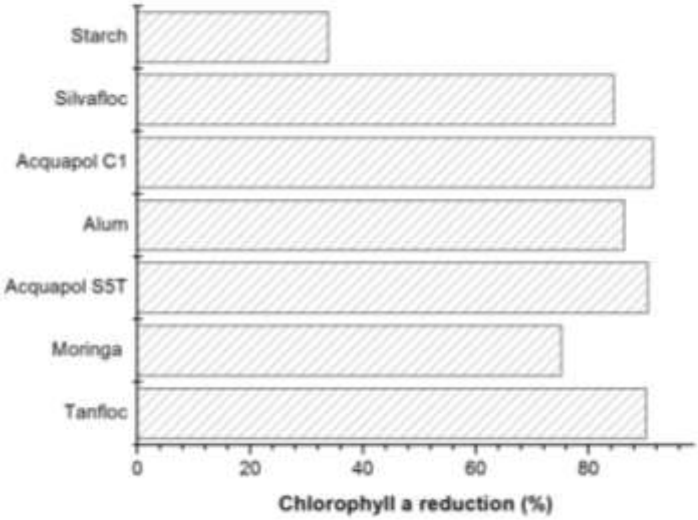

Figure 2. Preliminary screening for *Scenedesmus* removal.

Regarding the *Opuntia* mucilage, it has also been studied as a coagulant agent (Young, 2005), but in this case the chemical nature of this product is more complex and the coagulation process is not yet understood.

Another coagulant agent widely studied and with great potential in the field of water treatment is the *Moringa oleifera*. As Figure 1 depicts, this plant extract removes a high percentage of algal biomass. The protein nature of the principle of the *Moringa* coagulant induces the formation of networks that catch the negatively charged particles and cause sedimentation (Okuda, 1999).

Referring to tannin-based flocculant, Tanfloc reaches a higher percentage of algae removal, about 97%. Then come Acquapol S5T and Acquapol C1. The last of these coagulants, according to algae removal, is Silvafloc. Although there are no marked differences (the range of effectiveness is between 78% and 97%), the slight variations in efficacy may be due to the tannin base and degree of polymerisation, which is different in each case.

Aluminum sulphate was used in order to compare results from natural and from synthetic coagulant agents. As can be seen, the levels of algae removal are of the same order in each case.

In the same way as *Chlorella*, Figures 2 and 3 show the results of screenings conducted with the flocculant agents for *Scenedesmus* and *Oocystis*, respectively. Again, all tannin flocculants (Acquapol C-1, Acquapol S5T, Tanfloc and Silvafloc) showed high removal efficiency of algae, greater than 80%. *Moringa oleifera* also achieves high removal percentages, close to 80%, while starch is the worst flocculant, with algae reductions between 40 and 60%. Regarding alum, it shows a low efficacy for *Oocystis* and high for *Scenedesmus*.

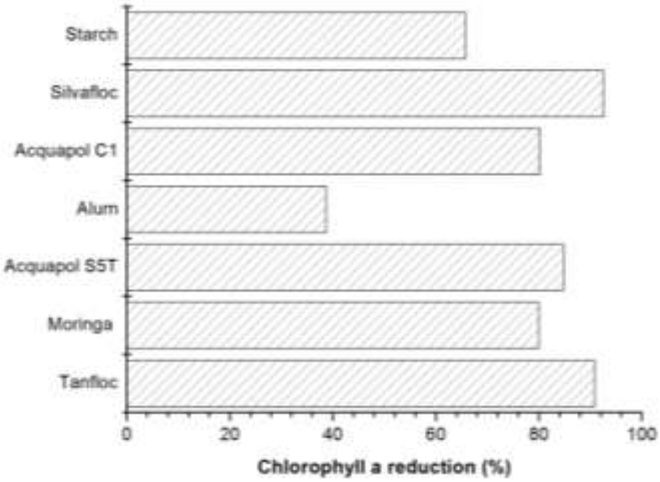

Figure 3. Preliminary screening for *Oocystis* removal.

3.3. Influence of Variables

Moringa oleifera Dosage

A series of experiments was made in order to determine extract dosage influence on algae removal. A fixed algae concentration was used for its removal with different doses of *Moringa oleifera*: from 1.5 to 40 mg·L^{-1}. As can be appreciated in Figure 4, the final concentration of *Chlorella* tends to decrease as the *Moringa oleifera* dose increases. From 5 mg·L^{-1} the percentage of chlorophyll *a* reduction is at the maximum, 98%, and higher doses of coagulant do not achieve lower chlorophyll *a* concentrations.

Figure 4 shows the coagulant effectiveness (q) calculated from equation 2:

$$q = \frac{C_O - C}{D} \tag{2}$$

Where q is coagulant effectiveness, $\mu g \cdot mg^{-1}$,

C_o is initial concentration of algae, $\mu g \cdot L^{-1}$,

C is final concentration of algae $\mu g \cdot L^{-1}$ and

D is coagulant dosage, $mg \cdot L^{-1}$.

As shown in Figure 4, effectiveness decreases with increasing coagulant dose, for the same given concentration of algae.

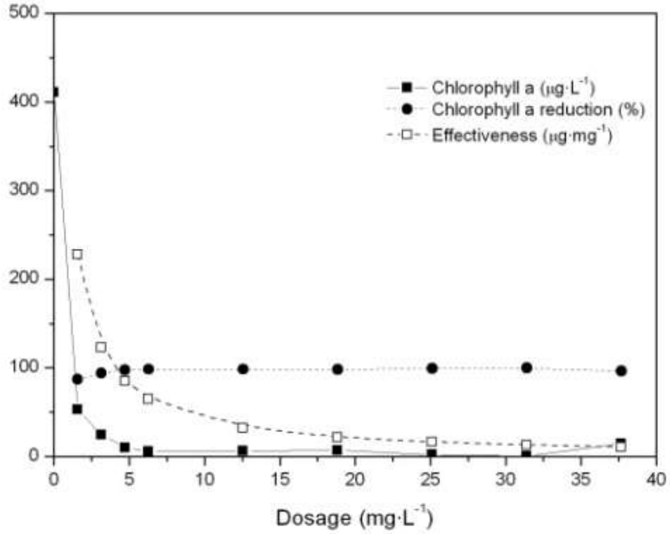

Figure 4. *Moringa oleifera* dosage influence for *Chlorella* removal.

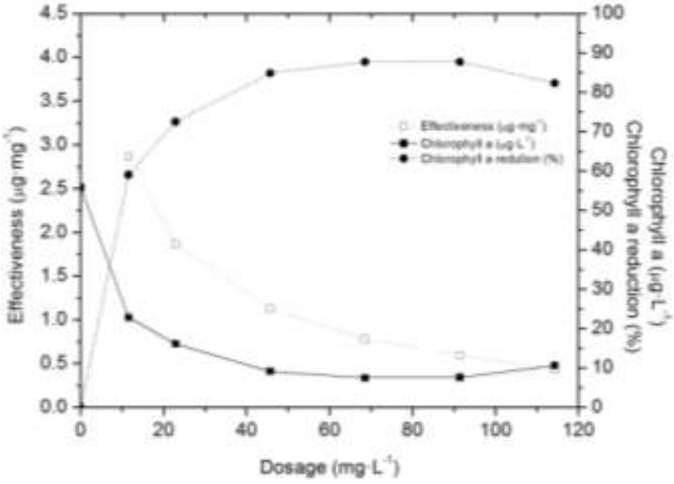

Figure 5. *Moringa oleifera* dosage influence for *Scenedesmus* removal.

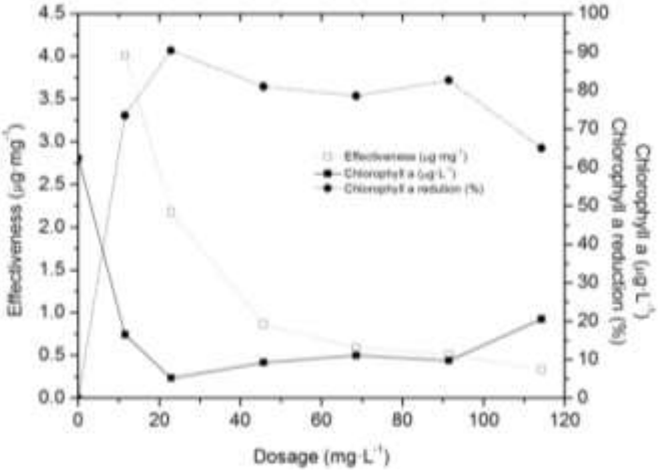

Figure 6. *Moringa oleifera* dosage influence for *Oocystis* removal.

Figures 5 and 6 show the influence of *Moringa oleifera* dosage on the percentage reduction and the effectiveness for *Scenedesmus* and *Oocystis*, respectively. The trend is similar in both cases to that observed for *Chlorella*, although the optimal conditions require higher doses of *Moringa oleifera*, 20 mg L^{-1} for *Oocystis* and 80 mg L^{-1} for *Scenedesmus*.

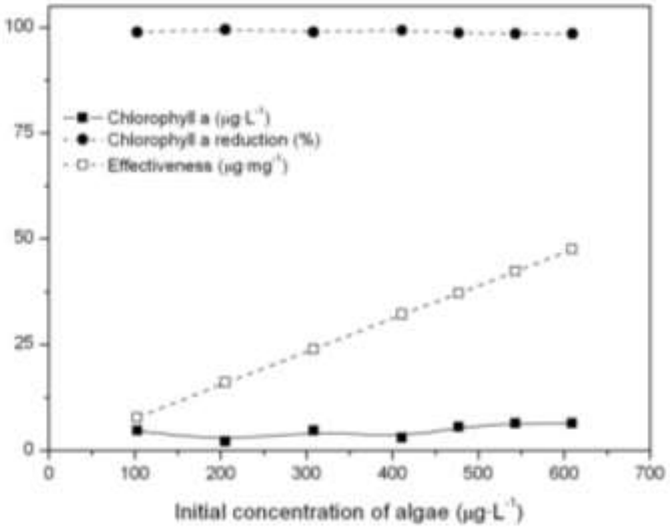

Figure 7. Initial *Chlorella* concentration influence.

Initial concentration of algae

In order to evaluate the effect of increasing the initial concentration of *Chlorella*, a fixed dosage of 16 mg·L^{-1} of *Moringa oleifera* was applied. Initial concentration of algae was varied between 100 and 600 μg·L^{-1}. The results are shown in Figure 7, which represents the reduction percentage in chlorophyll *a*, residual chlorophyll *a* and coagulant effectiveness at the end of the coagulation/flocculation process. The reduction percentage is very high, arriving in all cases at a value around 98%. It can also be seen that residual chlorophyll remains constant while increasing the initial concentration of algae for the same dose of *Moringa oleifera*. It can be appreciated that coagulant effectiveness is directly proportional to the initial concentration of algae. There are residual chlorophyll *a* concentrations that it is not possible to remove by coagulation and this seems to be about 5 μg·L^{-1}.

Similarly, the influence of the initial algae concentration has been studied for *Scenedesmus* and *Oocystis*. The results are shown in Figures 8 and 9. It can be seen that there is a positive trend in the percentage reduction of algae and their effectiveness in both species.

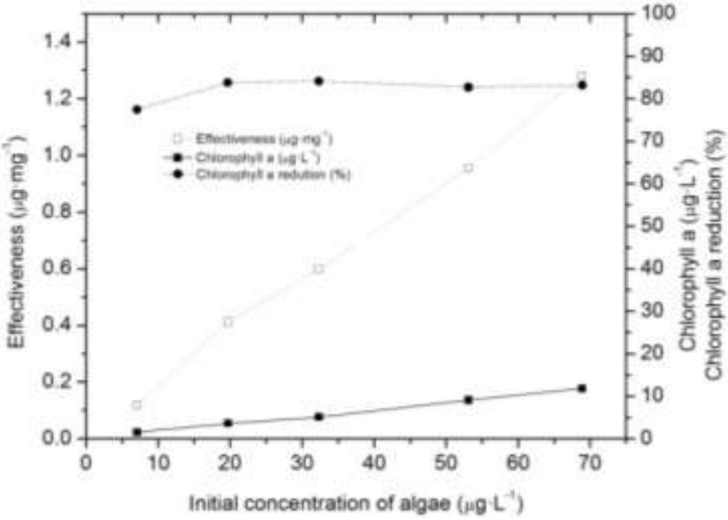

Figure 8. Initial *Scenedesmus* concentration influence.

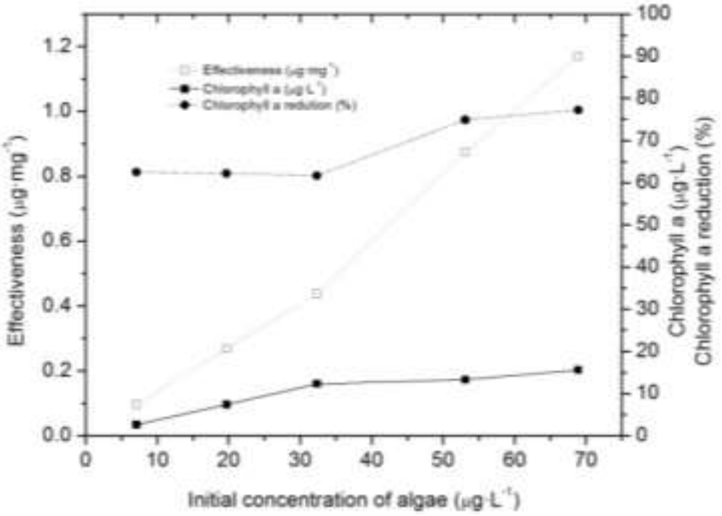

Figure 9. Initial *Oocystis* concentration influence.

Temperature

In order to observe temperature influence on the *Chlorella* removal process by coagulation/flocculation, a series of experiments was carried out over a range of temperatures. Experiments at 10, 20, 30 and 40ºC were performed, with an initial concentration of algae of 400 $\mu g \cdot L^{-1}$ and a dose of *Moringa oleifera* of 16 $mg \cdot L^{-1}$. The temperature influence is minimal in the range studied, obtaining percentages of algae removal between 84 and 98% (Figure 10). In general, the temperature does not seem to be significantly important in this process, except for tests performed at 10°C, with a slightly lower algae removal rate (84%). Looking at residual chlorophyll *a*, it can be appreciated that it is higher at 10ºC than for the other tested temperatures. The coagulant effectiveness is not significantly different between the experiments conducted.

pH

pH values were varied between 4 and 9, in order to determine its influence on *Chlorella* removal. Figure 11 represents the percentage reduction of chlorophyll *a*, residual chlorophyll *a* and coagulant effectiveness for experiments conducted. The chlorophyll *a* percentages are high enough, reaching 97% in all cases. It can also be observed that at a higher pH, the residual chlorophyll decreases slightly. The coagulant effectiveness is constant for all experiments; pH does not seem in general to be significantly important in the process of algae removal.

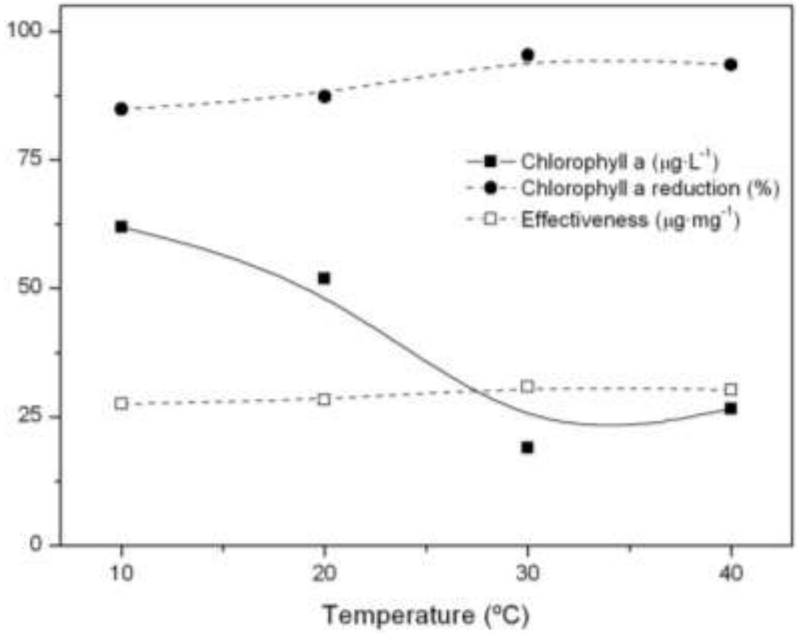

Figure 10. Temperature influence for *Chlorella* removal.

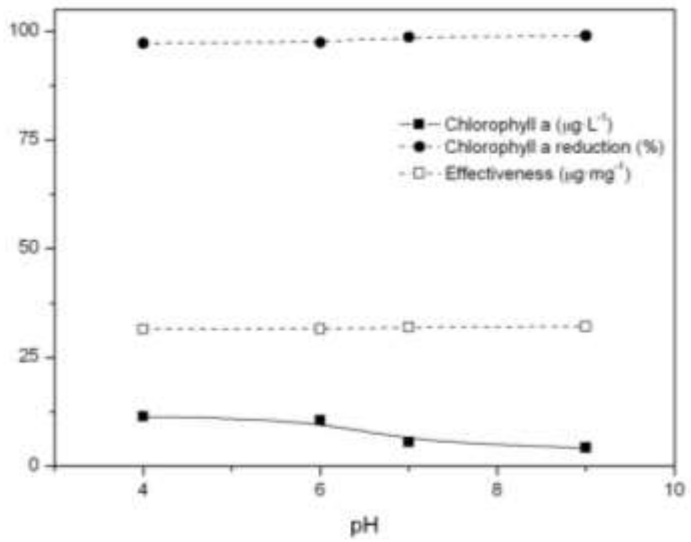

Figure 11. pH influence for *Chlorella* removal.

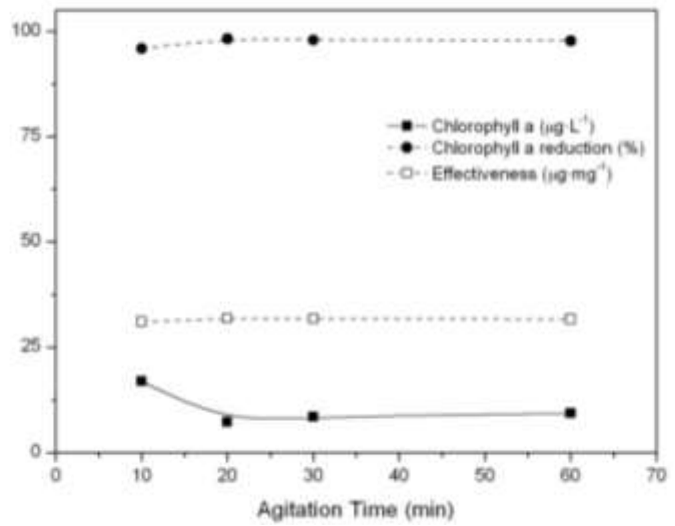

Figure 12. Agitation time influence for *Chlorella* removal.

Agitation Time

A series of experiments were carried out in order to evaluate the influence of agitation time on *Chlorella* removal. Experiments at 10, 20, 30, 40, 50 and 60 minutes were performed with an initial concentration of algae of 400 $\mu g \cdot L^{-1}$ and a dose of *Moringa oleifera* of 16 $mg \cdot L^{-1}$. Figure 12 shows that after 10 minutes of agitation, the percentage reduction of chlorophyll *a* increases slightly (from 95 to 97%), and therefore the residual chlorophyll *a* is lower, showing not significant differences from the 20 minutes of agitation. The coagulant effectiveness remains constant during the times studied.

Likewise, the influence of agitation time on the flocculation test has been studied for *Scenedesmus* and *Oocystis*. Figures 13 and 14 show that a stirring time of 20 to 30 minutes is sufficient to achieve the equilibrium between algae and flocculant.

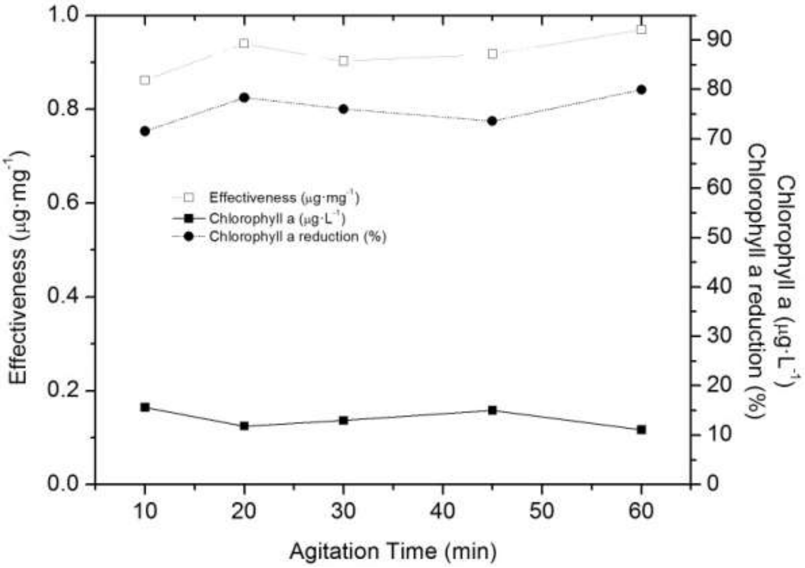

Figure 13. Agitation time influence for *Scenedesmus* removal.

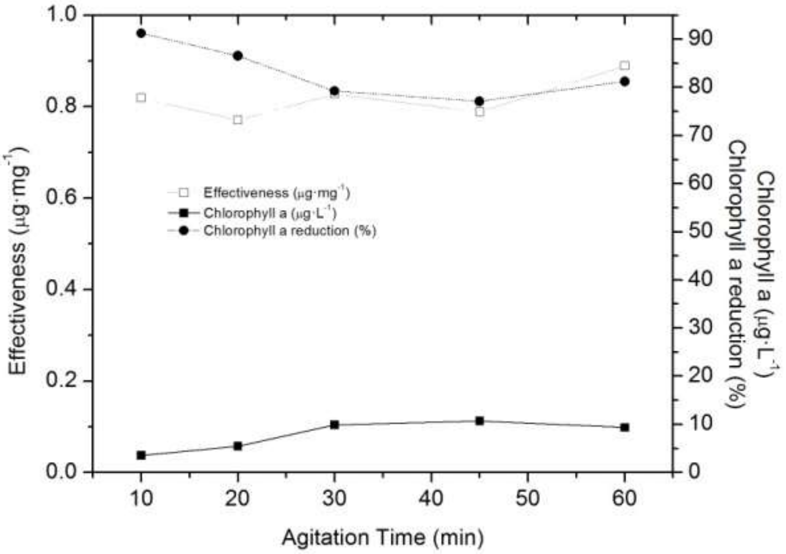

Figure 14. Agitation time influence for *Oocystis* removal.

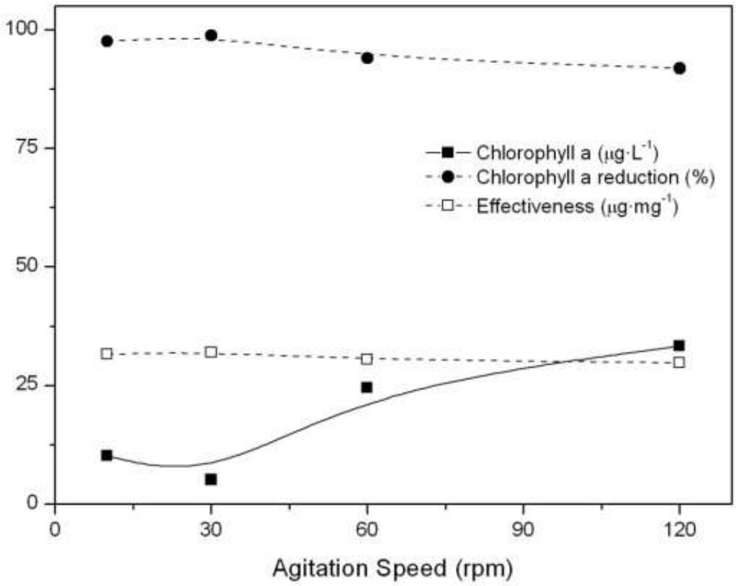

Figure 15. Agitation speed influence for *Chlorella* removal.

Agitation Speed

In order to study the influence of this variable on algae removal, a series of experiments have been carried out at 10, 30, 60 and 120 rpm. Figure 15 represents the percentage reduction, residual chlorophyll *a* and coagulant effectiveness. It can be seen that slightly higher percentages were achieved at lower speeds, with a minimal residual chlorophyll *a* at 30 rpm. The coagulant effectiveness is practically constant in the range tested.

Conclusion

Experimental data has revealed the following conclusions:

- The *Moringa oleifera* extract is very effective in algae removal by coagulation/flocculation. The study has revealed algae removal around 90% in most cases.
- Temperature and pH do not significantly affect algae removal by this process.
- The optimum agitation time is set to 20 minutes. However, lower agitation periods can be applied with lower chlorophyll *a* levels.
- The optimum agitation speed is 30 rpm.

As can be appreciated, the use of this natural coagulant is evidently effective for the general purpose of treating water and/or wastewater. By using these kinds of coagulants, the multiple drawbacks linked to the traditional treatment in Water and WasteWater Treatment Plants (WWTPs) can be passed over. Moreover, many aspects not solved by traditional alum salts can be positively treated with *Moringa oleifera* seed extract, such as the increment of organic matter. Furthermore, the operational costs would be lower and the treatment could be feasible even in less developed areas.

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Index