Phytoplankton as an indicator of Eutrophication

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ABSTRACT

In modern days the major problem faced during aquaculture including pisciculture and prawn culture is nutrient pollution. The Nitrate and Phosphate ratio, normally present in pond, with respect to O_2 is 106:16:1, i.e. the red field ratio. As a result of increasing nutrients, aquatic algae, especially the major population of phytoplankton is richly supplied with nutrients. Therefore, it leads to a huge amount of their growth those results in reduction in BOD level of aquatic ecosystems like pond, lake, coastal sea and estuaries leading to eutrophication. This event can easily be understood through the presence of phytoplankton in water. High amounts of phytoplankton are present in eutrophic water. Therefore, we can say that phytoplankton intensity is the indicator of Eutrophication.

Introduction

In recent days the production of fish has gradually decreased in India, especially in West Bengal (Dutta *et al.,* 2016). Various causes are responsible for that such as increasing toxicity of water, reduction of salinity etc (Chislock *et al.,* 2013). Eutrophication is the most important cue for fish production reduction (Talbot and Hole, 1994). Phytoplanktons account for much of primary production in aquatic ecosystems (Lizotte, 2008). The ratio of oxygen to carbon to nitrogen to phosphorus in the tissue of algae is approximately 212:106:16:1, roughly proportional to the atomic weight of carbon and nitrogen and phosphorus atoms. This ratio is called the Redfield Ratio (Takahashi *et al.,* 1985; Ptacnik *et al.,* 2010; Redfield, 1934). Phytoplanktons are assumed to assimilate nitrogen and phosphorus from the environment in a ratio roughly 16:1 (Ricklefs and Miller, 1999). The N:P ratio in the environment then is an indicator of relative availability of Nitrogen and Phosphorus to phytoplankton. Values of N:P less than 16:1 suggest that nitrogen is limiting whereas N:P greater than 16:1 indicate that there is less phosphorus per unit of nitrogen thus that phosphorus is limiting (Ricklefs and Miller, 1999). Biological indicators are species used to monitor the health of an environment or ecosystem (Thingstad and Mantoura, 2005). They are any biological species or group of species whose function, population, or status can be used to determine ecosystem or environmental integrity (Lavogina *et al.,* 2019). An example of such a group of copepods and other small water crustaceans present in many water bodies (Van Donk *et al.*, 2011). Such organisms are monitored for changes (biochemical, physiological, or behavioural) that may indicate a problem within their ecosystem. Bio indicators can tell us about the cumulative effects of different pollutants in the ecosystem and about how long a problem may have been present, which physical and chemical testing cannot perform. In general, the Redfield ratio for the freshwater lakes is

greater than 16:1 and phosphorus is considered the limiting nutrient in such ecosystems (Edmondson, 1970, Schindler, 1977, Seitzinger *et al.*, 2002). The production of most stream ecosystems is also thought to be limited by phosphorus, although streams in arid and semi-arid regions appear to be exceptions. Most estuaries and open-water marine ecosystems have very low N: P ratios and therefore nitrogen limited. Importance of phosphorus as a limiting nutrient in lake ecosystems was established by a number of classic whole-lake fertilization studies conducted in lakes on the Canadian Shield in the 1970s.(Schindler, 1977). In these studies, small lakes fertilized with phosphorus showed a dramatic increase in productivity whereas the addition of nitrogen had no effect. This experiment demonstrates the crucial role of phosphorus in eutrophication. The near basin, fertilized with carbon (in sucrose) and nitrogen (in nitrates), exhibited no change in organic production. Indeed several different lines of investigation involving whole fresh water lakes show reduction of phosphorus an effective means of controlling eutrophication. For instance whole-lake experiments and case-histories show that several lakes of different depth or area have recovered after phosphorus inputs were reduced (Schindler, 2012). Laurentian Great Lakes were found to recover to a great extent with reduced algal biomass following control of phosphorus inputs from household detergents and waste water treatment plants in the U.S. and Canada. N:P ratios too was observed to remain well above Redfield Ratios (Dove and Chapra, 2015).Lack of control of nonpoint sources of phosphorus contamination and intensified use of agricultural practices involving phosphorus containing fertilizers and high run-off has been found to be the reason of eutrophication in Lake Erie (Michalak, 2013), while agricultural input has also contributed to increased bioavailability of phosphorus (Baker, 2014). Moreover extensive hypoxia at the central basin of this lake leads to internal recycling of phosphorus and re-eutrophication (Scavia, 2014). Studies in some European and US lakes have shown that total phosphorus level should come down to a threshold level of <100ug/l for reduction of algal blooms and cyanobacterial growth (Carvalho, 2013, Müller, 2014). Chemical treatments like addition of iron alum or bentonite clay inhibit phosphorus recycling from sediments and effectively reduce phosphorus with concomitant decline in algal chlorophyll concentration (49 to 6.5 µg/l) (Wolter, 2010) or

tend hypereutrophic condition to transform to oligo-mesotrophic (Lürling 2013). Although this is an expensive control method for larger lakes yet it proves the fact controlling phosphorus is key to reduction of eutrophication. Interestingly, addition of nitrates helps to maintain a high redox level at the mud water interface that prevents recycled phosphorus from entering the system, resulting in reduced algal blooms (Schindler, 2012). Experimental addition of different nutrients in lakes separated by vinyl sea curtains generated different responses in terms of phytoplankton biomass and species composition. response was 10 folds greater in parts receiving nitrogen, carbon and phosphorous and twice greater in parts receiving nitrogen and carbon compared to unfertilized compartments (Findlay, 2011).

The process by which a body of water becomes enriched in dissolved nutrients (such as phosphates) that stimulates the growth of aquatic plant life usually result in the depletion of dissolved oxygen. The increased production resulting from phosphorus addition to an aqueous system is referred to as Eutrophication.

Effect of Eutrophication

Much of the interest in phosphorus as a limiting nutrient grew from an application of eutrophication in lake ecosystem function. The release of material from raw sewage into rivers and lakes creates what is called Biological Oxygen Demand (BOD) due to oxidative breakdown of detritus by microorganisms. Inorganic components such phosphorus stimulate the production of organic detritus, adding to BOD. In worst manifestations, this type of pollution can deplete the surface water of its oxygen leading to the suffocation of fish and other obligate aerobic organisms. Eutrophication is clearly the most severe environmental problem in many lakes and marine coastal areas, leading to hypoxia and anoxia in bottom waters, nuisance algal blooms and changes in species composition of phytoplankton. Plankton biomass, species composition and community structure are commonly monitored in surface water, However these parameters, except biomass, have seldom been applied as classification elements in assessing different eutrophication levels. The shallow nearshore and archipelago areas are especially threatened by eutrophication. This is partly due to natural high productivity of these areas and partly because they serve as recipients of different land effluents. Clear changes on ecosystem level can already be

seen in many areas. Fast growth algae (i.e. plankton and green algae) are among the first components of an ecosystem to respond to differences in water nutrient levels. The biomass and production of epiphytes on the head surfaces and under water vegetation have clearly increased in coastal areas during the last years. Phytoplankton dynamics are dependent on physiological and biochemical factors which have been shown to inhibit pronounced seasonal variation due to climate factors. The short-lived phytoplankton reacts drastically due to the annual life cycle. In the Baltic Sea , the seasonality in phytoplankton growths differ between the east-west and north-south gradient. Therefore in assessing the eutrophication level it is important to identify appropriate time periods in which eutrophication indicators are most sensitive and significant. Characterization of the phytoplankton community is multidimensional, interference between nutrient and salinity dependencies lead to combined changes in phytoplankton composition and abundance requiring different classifications schemes for each. Moreover, different classification schemes are hampered by the limitation of the number of data sets in which there are similar climatic and salinity conditions but different eutrophication levels. The role of nutrients in regulating the seasonal and regional abundance of phytoplankton, their blooms, species successions, and carbon production (primary production) is a classical problem in biological oceanography. The natural fertility of marine waters varies greatly because the amount of nutrients supplied through natural processes to support photosynthesis and growth of the phytoplankton varies regionally and seasonally. The Sargasso Sea, for example, is well known for its poor nutrient supply and oligotrophic nature, in contrast to the high productivity and fisheries yield of nutrient-rich upwelling regions. Nutrient enrichment of oligotrophic waters can benefit food chains, but coastal ecosystems, which are intrinsically productive because of natural nutrient recycling processes, can become degraded through excessive enrichment with anthropogenic nutrients (Ærtebjerg *et al.*, 2003; Cloern, 2001; Jørgensen *et al.*, 1996). Analyses and mitigation of the impact of eutrophication on coastal phytoplankton behaviour require an understanding of the photosynthesis-nutrient relationship. This coupled physiology regulates the basic, cellular responses of the phytoplankton to nutrient enrichment, while the population that develops is modified by food web processes.

This physiology, and eutrophication as a process will be reviewed briefly before describing some specific responses of the phytoplankton to nutrient enrichment.

The phytoplankton (primary producers) require four primary inorganic macronutrients (NH_4, NO_3, PO_4, SiO_2) and five micronutrients (Fe, Cu, Zn, Mn, Mo) to fix carbon during photosynthesis. The photosynthesis equation can be written as an oxidation-reduction reaction having the general form : (Equation 1)

$$2H_2O + CO_2 + Light \rightarrow (CH_20) + H_2O + O_{2 \text{(EQUATION 1)}}$$

with the reaction mediated by the pigment chlorophyll a. Light energy is used to oxidize water yielding gaseous molecular oxygen (Falkowski *et al.*, 1980). The CO_2 reduced during photosynthesis is temporarily fixed to an organic molecule (CH_20) which serves as the substrate used in subsequent biosynthetic reactions to manufacture amino and nucleic acids, lipids, proteins, enzymes, etc. required for cellular growth and reproduction. This biosynthesis requires the concurrent assimilation of essential nutrients (nitrogen, phosphorus, micro-nutrients, etc.) during photosynthesis. The rate of nutrient assimilation is a function of its concentration, and increases hyperbolically with it. There are two kinetic characteristics of the nutrient uptake curve which vary among species and influence their responses to nutrient enrichment (or limitation) in competition with other species (Smayda, 1997). The maximum velocity of cellular uptake (Vmax) and the concentration of nutrient (Ks) at which uptake is one-half V_{max}.

The half-saturation constant, K_s, is particularly important since it determines the efficiency with which species take up nutrients at low concentrations. The higher the Ks constant, the less able the species in question to assimilate nutrients at low concentrations. On this basis, it might be expected that eutrophication would favor species less efficient in nutrient uptake.

Numerous experiments have shown that at increased rates of nutrient uptake cellular growth is stimulated, and an increase in population abundance results. Similar to the nutrient uptake curve, the population will increase up to an asymptotic level and remain relatively constant irrespective of further increases in nutrient concentration. The population at this upper (non -grazed) level corresponds to the carrying capacity for that particular

species growing in response to the nutrient being supplied. The carrying capacity of a given nutrient level is not constant; it varies with nutrient type, concentration, accompanying growth factors, and among species. Excessively high nutrient levels, particularly NH4, can be inhibitory (Thomas *et al*., 1980). The amount of light (Eq. 1) available influences the effect of nutrients on photosynthesis at the cellular (individual) level, a relationship found also for natural populations. Photosynthesis when deprived of light ceases, or decreases when nutrients are in short supply. During these conditions, the reverse reaction in Eq. 1 predominates or is favoured; i.e. oxygen is consumed (respiration) leading to the production of CO_2.

If the respiration pathway is prolonged and the ratio of photosynthetic oxygen production to respiration drops below 1.0, the decreasing oxygen level will push the water body towards hypoxia or, in extreme cases, to anoxia. Thus, nutrient loading can oxygenate and de-oxygenate a nutrient-enriched water mass (Rabalais, and Turner, 2001).

In fact BOD has shown a linear positive relationship with Chlorophyll a, the predominant chlorophyll in green plants and algae and also an indicator of phytoplankton abundance and biomass. This study, aimed at developing a numeric relationship between chlorophyll *a* fluorescence and BOD for a eutrophic urban lake that may represent lake water conditions in the subtropical southern USA, showed the relationship to be stronger with the 10-day BOD (*r* 2=0.83) than with the 5-day BOD (*r* 2=0.76). Decline in chlorophyll a showed concomitant BOD decrease which suggested that the die-off phytoplankton caused the major consumption of oxygen (Xu 2015). Similar relationship was obtained between Chlorophyll a and BOD in stagnant urban lake basin in Danang (Phu, 2014) with a linear gain coefficient of $R^2 > 0.95$

In coastal Gulf of Finland studies demonstrate that hypoxia or anoxia driven by anthropogenic eutrophication caused difference in zoobenthos assemblages indicating critical influence of oxygen on individual species, as some were less adapted to low oxygen conditions(e.g., *Monoporeia affinis*) than others (e.g., Chironomidae) (Rousi, 2019).

In another study, conducted at several bays of Poland, the highest biomass concentration and maximum abundance of phytoplankton were recorded at the stations under the strongest anthropogenic influence. At these stations there was also a dominance of opportunistic species that reproduced quickly and occupied the ecological niche, thus reducing the diversity of the phytoplankton community. This was an indicator of the upset of the balanced ecosystem . Diatoms were the most abundant group of the phytoplankton community in all three bays studied. Species that were highlighted as significant for the specific area in this study were S. marinoi in Šibenik Bay, *L. minimus* in Kaštela Bay and the genus Chaetoceros spp. in Mali Ston Bay. Dinoflagellates were the second most significant group. A noticeably larger abundance of dinoflagellates was recorded in the Kaštela Bay area, characterized as the most influenced by anthropogenic pressure (Bu•anèiæ, 2016).

Eutrophication indicator

For tracking the level of eutrophication in waterland the main tool generally used is the amount of chlorophyll content in water. European Union Water Framework Directive (WFD) was examined to analyse the quality and planktonic biomass of coastal water of the Archipelago Sea, where they usually found that total phosphorus (TP) and total nitrogen (TN) alone accounted for 87% and 78% of the variation in Chlorophyll, respectively (Kauppila and Pirkko, 2007). This chlorophyll is generally found in most of the planktonic biomass in marine coast area. Among chlorophyll pigments found in planktonic biomass chlorophyll a (Chla) is considered the principal variable to use as a trophic state indicator (Steele, 1962). This is established through planktonic primary production and algal biomass, and algal biomass is an excellent trophic state indicator. Eutrophic status was assessed in the state of the Florida Bay ecosystem selecting Chl a as an indicator as its concentrations reflected the integral effect of many water quality factors that were altered by restoration activities and also because it was sensitive to ecosystem drivers (stressors, especially nutrient loading) and was feasible to monitor (Boyer *et al*., 2009). It has been seen that this green pigment chlorophyll a is a significant component of all kinds of algae that is the main constituent of the phytoplankton. Phytoplankton, i.e algae are the main basis of most aquatic food webs. Algae also help to purify water by absorbing nutrients and also heavy metals. Due to good sensitivity with changes in pH, nutrient or temperature, algae can be also used as good tools for detecting the quality of water in lakes. Depending on nutritional quality lakes are

of four types : Oligotrophic (Unproductive lakes, low N & P; low productivity; very clear waters, high hypolimnetic O_2, high species diversity), Mesotrophic (Intermediate level of productivity; clear water lakes with submerged aquatic plants and medium levels of nutrients), Eutrophic (Productive with high nutrient supply, high productivity, algae blooms – fish kills) and Hypereutrophic (very nutrient-rich and productive lakes (eg. >100 ug P/L) – severe algal blooms; low transparency.(< 1m)). In eutrophic ecosystems, response to the addition of nutrients, especially N and P, is crucial. This can be natural (due natural nutrient deposition, rate being very slow) or may be artificial (due to anthropogenic activity, results in fast and persistent organic pollution). Algal bloom is the significant visible tool for the identification of eutrophication. The relationships between eutrophication and phytoplankton biomass are more evident than the effects of anthropogenic nutrient enrichment on individual phytoplankton species (Riegman *et al.*, 1992). Field studies have detected changes in species composition and abundance in a variety of nutrient-enriched environments, including the Dutch Wadden Sea (Cadee and Hegeman, 1986), the Baltic Sea (Kahru, *et al.*, 1994. Olli, 1996), the Black Sea (Bodenau, 1993, Bodenau and Ruta 1998) and New York Bay (Mahoney, *et al.*, 1977). As already pointed out, the increase in nutrients during the initial phase of eutrophication leads to higher phytoplankton biomass through yield-dose kinetics. During these initial stages, particularly during the winter-spring period, the indigenous flora (most often diatoms) usually increases in abundance without novel changes in species behaviour occurring. The Si-requiring diatoms are generally considered to be the 'most desirable' bloom species with regard to grazer suitability, trophic value and water quality. If nitrification continues to increase, a change in the species composition and the size structure of the phytoplankton community may result and potentially affect energy flow in the impacted ecosystem. In extreme cases, the impacts of altered phytoplankton species composition, abundance, size structure and bloom events in response to nutrient enrichment cascades through upper trophic levels and reform food web structure (Turner, 2001). This latter (secondary) effect tends to blur the primary responses (i.e. phytoplankton behavior) to eutrophication and mask the effect. The nutrient-induced reformation of ecosystems is an extremely complex and poorly understood ecological process.

Phytoplankton as Eutrophication Indicator

The shift in phytoplankton species that has attracted the most interest is the shift in abundance (blooms) from diatoms to other non-motile species and flagellates — a functional group shift. Functional groups and their shifts are of interest because of significant differences in their physiology and ecological impacts. There is special interest in the diatom: flagellate ratio as a potential indicator of eutrophication since the global increase in harmful microalgal blooms (HABs) is primarily a flagellate species phenomenon. It has been hypothesized that the diatom: flagellate ratio should decrease with increasing nutrient enrichment, and consequently might serve as an indicator of eutrophication status.

There is some supporting evidence for this from Kastela Bay, Croatia, where a progressive, long-term increase in anthropogenic nutrient has been accompanied by a 10-fold decrease in the ratio of diatom to flagellate abundance (Maresovic and Pucher-Petkovic, 1991). The primary nutrient expected to regulate the shift in functional groups from diatoms to flagellates is silica, which is required by diatoms but not by other microalgal groups exclusive of silicoflagellates (Officer and Ryther, 1980; Smayda, 1990). Silica concentrations and ratios with N and P are altered by eutrophication, with the degree and pattern of change influenced by the chemical nature of the waste water being discharged (Officer and Ryther, 1980; Conley *et al.,* 1994). Silica is assimilated by diatoms stoichiometrically in the Redfield Ratio in atomic proportions of 1:1 with N, and 16:1 with P. At Si:N supply ratios of <1:1, diatoms will be Si-limited, and N-limited at Si:N supply ratios >1:1. Smayda (Smayda, 1990), based on an evaluation of long-term blooms and nutrient conditions in various regions, has suggested that anthropogenic enrichment of N and P has led to long-term declines in the ratios of Si:N and Si:P which potentially favor non-diatom blooms in such impacted regions. Mesocosm experiments led by Egge *et al*. (Egge and Aksnes, 1992) suggest that there is a threshold of approximately 2 μM Si, below which "diatoms, as a group, are outcompeted by the 'flagellate group'. The merit of the Si ratio and threshold concepts as eutrophication switches that result in species shifts and altered community abundance is still under investigation. But it is clear that the species-specific responses to these proposed Si effects are under multifactorial

control rather than are simple linear responses. For example, Sommer's (Sommer, 1994) experiments showed that diatoms became dominant at Si:N ratios >25:1, while flagellates were superior competitors at lower ratios. Although irradiance did not significantly influence the competition between diatoms and flagellates in these experiments, it was important in the competition among diatom species.

There has been much greater interest in N:P ratios since N and P have been the primary nutrients focused on by phytoplankton ecologists. Niemi (1987) was among the first to invoke N:P ratio control of cyanobacterial blooms of Nodularia and Aphanizomenon species in the Baltic Sea. In his view, these N-fixing species were able to capitalize on the elevated phosphorus levels occurring then, leading to their competitive advantage over other functional groups. Similar N:P regulation of Nodularia spumigena in a P-enriched Australian estuary has been reported (Lukatelich and McComb, 1986).

In Tolo Harbour, Hong Kong, a long-term increase in harmful algal and red tide blooms has accompanied eutrophication associated with a marked increase in the human population (Smayda, 1990). Hodgkiss and Ho (Hodgkiss and Ho, 1997) report that the annually averaged N:P ratio decreased from 20:1 to 11:1 over a seven year period, during which the number of dinoflagellate blooms increased. In the Dutch Wadden Sea, a long-term increase in abundance of Phaeocystis pouchetii and its dominance of the annual phytoplankton cycle has occurred in response to nutrient enrichment via riverine discharge (Lancelot *et al.*, 1987). Altered nutrient ratios appear to have played an important role in this exploitation. Riegman *et al.* (Riegman *et al.*, 1992) have shown that the average annual dominance of Phaeocystis was inversely related to the average N:P ratio during its growth season (April - September), and that the ratio of $NH_4 : NO_3$ influenced life cycle stage. In laboratory experiments, Riegman (Riegman, 1995) found that Phaeocystis in competition against other species became dominant at N:P molar ratios of <= 7.5 and approached monospecific bloom formation at N:P ratios of 1.5. The successful competition of Phaeocystis against diatoms in achieving Wadden Sea preeminence was also linked to lower Si concentrations (Egge and Aksnes, 1992). The catastrophic bloom of Chrysochromulina polylepis in the Kattegat and Skagerrak during 1988 has been linked to N:P ratio

effects on bloom magnitude and toxicity (Maestrini and Granéli, 1991). Although the role of nutrients in this bloom remains controversial. The effects of nutrient ratios on phytoplankton species were also highlighted by Anderson (Anderson *et al.*, 2002).

Apart from marine ecosystems, algae are often used as biological indicators to measure freshwater nutrient levels. Phytoplankton populations have a positive, linear correlation with phosphorus increase, and the Redfield ratio can be particularly useful to determine whether nutrients are available in adequate levels for growth (Soballe and Kimmel, 1987). However, this relationship between phytoplankton biomass and nutrient concentration has been found to be less strong in ponds, because of submerged vegetation and activity of large zooplankton (Teisser, 2012). Knowing the species makeup of the algal community is more telling, because the presence and abundance of certain groups can indicate different environmental conditions. Cyanobacteria, green algae, and diatoms all flourish under different chemical parameters. Cyanobacteria's capacity for nitrogen fixation allows them to dominate freshwater systems when N:P ratios are low (Schindler, 1977). Heterocyst formation is negatively correlated with dissolved inorganic N in the water, so they are easily able to outcompete diatoms and green algae that cannot fix their own nitrogen (Smith, 1983). However, cyanobacteria have no competitive edge in phosphorus competition, so when N:P ratios are high there is generally more equal balance of all phytoplankton groups (Smith, 1983). Species composition is also useful in studying the health of a pond, because different species flourish under different conditions. Palmer identified certain species of algae to be indicative of clean water supplies, including Staurastrum and Pinnularia (Palmer, 1959). He associate other species, such as Euglena (Euglenophyceae), Oscillatoria (cyanobacteria), Anabaena (cyanobacteria), and Microcystis (cyanobacteria), with polluted waters (Palmer, 1959). There have been many other species and genera of algae, since Palmer, that have been identified as common to ponds, lakes, eutrophic bodies, oligotrophic bodies, acidic waters, etc. (Wehr and Robert, 2003). If these species are present in a body of water, they can provide an indication of environmental conditions. Freshwater plankton communities also vary with seasonal succession (Hutchinson, 1967; Wetzel, 2001). Succession is largely driven by temperature, light penetration,

and nutritional concentration (Hutchinson, 1967). Algae vary in their optimum range for these conditions, and so with changing conditions, different species can proliferate. Nevertheless, algae are not the only biological component of pond ecosystems and, therefore, should not be the sole indicators of ecosystem health. Especially because studies have shown ponds, specifically, to have a lesser association between nutrient levels and algae biomass, a whole-ecosystem, community structure evaluation is critical to assessing the state of a pond (Teisser 2012; Shubert, 1984). To create a standard method for surveying pond health (Menetrey, 2005) identified five groups as principal: plants, Gastropoda (snails and slugs), Coleoptera (beetles), Odonata (dragonflies and damselflies), and Amphibia (amphibians). These groups are representative because they occupy different trophic levels within the pond, demonstrate a variety of dispersal techniques, and have some degree of information known about their environmental tolerance (Menetrey, 2005). Ephemeroptera (mayfly) larvae have been used in eutrophication studies as well, because they are known to be sensitive to low dissolved oxygen levels (Menetrey, 2008). Aside from using biological indicators such as these to determine the health of the ecosystem, it is also important to understand the various aspects of pond biology because any action taken to address eutrophication will inevitably impact other aspects of the food web.

Operationally, two different approaches have been applied in analyzing the effects of eutrophication on phytoplankton: a mass balance approach and an organismal approach. The eutrophication classification schemes discussed above, i.e. the increased rate of supply of organic carbon to an ecosystem (Nixon, 1995); the ratio of particulate to dissolved organic carbon, the soluble amino acid turnover times (Seki and Iwami, 1984); winter dissolved inorganic nitrogen levels and summer chlorophyll (= biomass) levels (CSTT) are mass balance approaches. These classifications emphasize the abundance (yield) of phytoplankton that develops in response to added nutrients (dose). Conceptually, the mass balance approach applies the photosynthesis-respiration reaction Eq-1 ($2H_2O + CO_2 + Light \rightarrow (CH_2O) + H_2O + O_2$) in combination with the stoichiometric relationships (Redfield Ratio) that occur between nutrients (nitrogen, phosphorus) and the photosynthesis and respiration of organic matter. In this relationship, nitrogen (N) and phosphorus (P)

are bound and released during the synthesis and respiration of organic carbon (C) which, in turn, result either in the production or utilization of oxygen (O): O:C:N:P .

The quantities of the four elements processed in this biochemistry, known as the Redfield Ratio, can be expressed in terms of atoms ($2H_2O + CO_2 + Light \rightarrow (CH_2O) + H_2O + O_2$), or by weight ($2H_2O + CO_2 + Light \rightarrow (CH_2O) + H_2O + O_2$): Equation 2

O:C:N:P=212:106:16:1
O:C:N:P=109:41:17.2:1

Thus, during photosynthesis (primary production) for every atom of P assimilated, 16 atoms of N will be assimilated and 106 atoms of C fixed into organic matter (from CO_2 – on Eq-1 $2H_2O + CO_2 + Light \rightarrow (CH_2O) + H_2O + O_2$), liberating 212 atoms of O. This assimilation of N and P leads to phytoplankton growth (i.e. biomass = carbon, chlorophyll, etc.) and oxygenates the water mass. Ideally, from a knowledge of the amount of N and P available, for example the DAIN concentrations recommended by CSTT (Comprehensive studies for the purposes of Article 6 of DIR 91/271 EEC, the Urban Waste Water Treatment Directive, 1994), one can estimate the amount of C that would be produced to evaluate potential eutrophication effects. And, from the biomass level, i.e. the summer chlorophyll (= biomass) levels applied by CSTT as an index of eutrophication status, one can estimate the amount of N and P needed to produce and sustain that biomass level. In that case, one needs to know the C:Chlorophyll a ratio, which varies from 40:1 to 150:1 (by weight) and depends on the phytoplankton group present and their nutritional status. The Redfield Ratio, as in Eq 1, describes a reversible process, i.e. the respiration or decomposition of biomass. The breakdown of C consumes O2 and liberates N and P. A good example of such mass balance behavior with adverse effects is provided by blooms of the dinoflagellate genus Ceratium which sometimes result in hypoxic or anoxic events. Blooms of Ceratium species can be stimulated by nutrient enrichment (Braarud, 1945). Hypoxic and anoxic Ceratium blooms have been reported in the Kattegat (Granéli *et al.,* 1989), German Bight (Dethlefsen and Westernagen, 1983) and New York Bight (Falkowski *et al.,* 1980) among other areas. During such events, the Ceratium species respond in yield-dose behavior to available nutrients, with the population increasing until essential nutrients become exhausted. Nutrient exhaustion then prevents further growth and is even unable to sup-

port the existing biomass. The population becomes physiologically stressed; the cells lose their motility and sink to the bottom sediments where they rot. During their decomposition, O_2 is utilized ($2H_2O + CO_2 + Light \rightarrow (CH_2O) + H_2O + O_2$) which decreases in situ concentrations and pushes bottom water towards hypoxia or anoxia. Such occurrences and the degree of deoxygenation depend upon the amount of Ceratium biomass being decomposed and the degree to which the bottom waters are ventilated by physical oceanographic processes ($2H_2O + CO_2 + Light \rightarrow (CH_2O) + H_2O + O_2$). Regions of bottom water stagnation, particularly during summer-autumn when Ceratium blooms predominate, are predisposed to hypoxic blooms. Anoxic Ceratium blooms are also facilitated by reduced grazing pressure on Ceratium species because their large cell size leads to predator-prey mismatches. Anoxic events can accompany blooms of other dinoflagellate species (Jones, 1982; Legovic, 1994) silicoflagellates, cyanobacteria (Galat and Verdin, 1989), Phaeocystis (Rogers and Lockwood, 1990) and diatoms (Delafontaine and Flemming, 1996). However, it is often difficult to find a definitive link between the hypoxia (anoxia) developing during phytoplankton blooms and nutrient enrichment.

Summary

Nutrient pollution is a great problem for both marine and freshwater bodies in the aquatic ecosystem. Cultural eutrophication associated with nutrient pollution can lead to unhealthy ecosystems with a lack of oxygen i.e anoxia and reduction of biodiversity. The water becomes turbid and reaches a phytoplankton dominated state. Additionally this type of water has high accumulation of nutrients like nitrogen and phosphate that act as the food resource for the phytoplankton trophic level. As a result of obtaining an excessive amount of nutrient resource, phytoplankton multiplies at an enormous rate that in turn causes a change in the plankton population, causing a general increase in water turbidity and greater fluctuation in oxygen levels. Therefore we can consider phytoplankton population intensity as the indicator of eutrophication. This is of immense significance as in recent days eutrophication of pond ecosystems result in death of aquatic beneficiary animals like fish, prawn, mussel etc that result in great economic loss. Therefore phytoplankton can be used as a valuable tool to determine the level of eutrophication. This will be very useful in determining eutrophication for fishermen and simultaneously they can take necessary measures for relief from that problem. Also from this indicator one can easily understand the nutrient accumulation (i.e N and P) in water that come from excessive use of fertilizer in cultivation fields. This will also make the farmers aware of the quantity of fertilizer to be used and encourage a sustainable ecosystem.

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Conflict of Interest

All authors declare that there is no conflict of interest other than publication of this review paper.

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