Ariola BACU*, Fatbardha BABANI*

THE DYNAMICS OF THE AMOUNT OF TOTAL PHYTOPLANKTON DNA THROUGH THE YEAR PROMISES A NEW POSSIBLE INDICATOR OF THE LEVEL OF TROPHY IN THE LAKE OF SHKODRA

Përmbledhje: Liqeni i Shkodrës, pjesa Shqiptare, përfaqëson një nga ekosistemet më të rëndësishëm në vend. Të katër stacionet e kampionimit gjatë vitit 2009 reflektuan një nivel të trofisë midis ultraoligotrofisë dhe oligotrofisë. Qëllimi kryesor i studimit ishte përdorimi i sasisë së ADN totale të fitoplanktonit për ndërtimin e një korelacioni të mundshëm me parametrat e tjerë si temperatura e ujërave, pH, klorofili a, që janë indikatorë të nivelit të trofisë. I rëndësishëm është fakti që shpërndarja e sasisë së acidit nukleik të fitoplanktonit ndjek të njëjtin rend si klorofili a, duke dhënë në këtë mënyrë sugjerimin e fuqishëm se ky parameter biologjik mund të ketë përdorim real si indikator i gjendjes trofike. Gjithashtu, për herë të parë, ne patëm si qëllim të hetojmë praninë e cianobakterit Syneccococus, një nga dy pikocianobakteriet kryesorë në Liqenin e Shkodrës, bazuar në amplifikimin PCR të fragmentit të ADN ribosomale. Mostrat nga të katër stacionet përmbanin fragment me interes, që u riprodhua me sukses.

Fjalë kyçe: *klorofili a, pigmente fotosintetikë, acide nukleike, fitoplanktoni, gjendja trofike*

Abstract: The Lake of Shkodra, Albania represents one of the most important ecosystems in the country. The four stations of sampling, during 2009, reflected a level of trophy between ultraoligotrophy and oligotrophy. Main goal of the study was the use of the amount of the total phytoplankton DNA for the establishment of an possible correlation with the other parameters as temperature of the waters, pH, chlorophyll a, which are indicators of the level of trophy. Important is the fact that the phytoplankton nucleic acid content distribution follows the same order as the chlorophyll a, giving this way the strong suggestion that this biological parameter might be of real use as indicator of the trophic state. Also, for the first time we aimed to investigate the presence of cyanobacteria Syneccococus, one of the two main picocyanobacteria, in the Lake of Shkodra based on the PCR amplification of a ri-

* Ariola Bacu, Fatbardha Babani, Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania

bosomal DNA fragment. Samples from the four stations contained the fragment of interest, which was reproduced successfully.

Key words: *chlorophyll a, photosynthetic pigments, nucleic acids, phytoplankton, trophic state*

INTRODUCTION

The ecosystem of Lake of Shkodra is an important ecosystem for Albania and the region. Many researches were conducted before 90 s on the quality of its waters regarding the physical-chemical content mainly. These data showed that the waters of the lake were being polluted with microelements, which originated from the mines of Fe and Cu and were brought to the lake from the Drin River (Babani *et al*., 2010). Later on, Albania and Montenegro aimed to preserve the values of the lake through a better understanding of the level of trophy. Thus, the evaluation of the level of trophy based on chlorophyll *a* content was conducted for the first time in 2009 (Babani *et al*., 2010).

Trophic state of the water bodies are classified by four levels (Hakanson & Bryhn, 2008) from the lower to the higher level of the biological productivity: *Oligotrophic* (clear and blue water, with very low levels of nutrients and algae), *Mesotrophic* (slightly green water, still clear, moderate levels of nutrients and algae), *Eutrophic* (green and murky water, with higher amounts of nutrients and algae) and *Hypertrophic* (supersaturated in phosphorus and nitrogen, excessive phytoplankton growth, poor water clarity). Chlorophyll *a,* total phosphorus, total nitrogen and water clarity are generally used as indicators to measure „biological productivity" of the water bodies or their capacity to support life. The indicators system is useful for the creation of a clear view on the situation of the lakes and lagoons which are ecosystems with high biodiversity, as well as for the assessment of the dynamics of environmental changes (progressive or regressive). It is already reported a direct relationship between phosphorus concentrations, chlorophyll *a* (algal biomass), and clarity of the waters because, phosphorus drives algal growth, which then affects water clarity (Sanders *et al.,* 2001; Vidal *et al*., 1999). Among the widely used parameters for the study of the trophic state and for a better understanding of its dynamics, are also the temperature and pH of the waters. Usually the higher the temperatures and pH values, the higher the level of trophy.

In this paper we report the use of the phytoplankton nucleic acid content as a possible new indicator of the level of trophy in freshwater systems (lakes and lagoons). Total phytoplankton DNA was extracted, analyzed for the purity and quantity, and the data were used to evaluate the relationship with the rest of the indicators. Thus, use of the DNA amount might also help to understand differences in phytoplankton biomass in the water bodies (Bacu & Babani, 2010). Another aspect of this study was the preliminary evaluation for the first time of the presence of the main component of the small phytoplankton of the freshwater systems, picophytoplankton species named *Synechococcus*, in the Lake of Shkodra. The molecular anal-

ysis was based on the amplification of 16 S–23 S cyanobacterial ribosomal DNA, which is reported to be successful on the identification of cyanobacteria in different locations in the world (Honda *et al*., 1999; Robertson *et al*., 2001). The presence of this species in the lake waters were discussed in the terms of the variable environmental conditions included nutrient content and level of trophy.

MATERIALS AND METHODS

The Lake of Shkodra was monitored from March to October 2009. Coordinates of the stations were (GPS): St. 1 – N 41° 47' 33.0", E 019° 36' 72.3"; St. 2 – N 41° 47' 08", E 19° 36' 62"; St. 3 – N 41° 47' 10.0", E 019° 36' 31.4"; St. 4 – N 41° 48' 21.4", E 019° 36' 85.0".

Water samples were collected monthly from April to October 2009, at four stations. Samples were collected in a volume of three liters. Two water subsamples (1 l) were directly filtered through Whatman GF/F filter. The material retained on the filters was used for pigment analysis, and DNA extraction in a 1: 1 ratio.

Content of chlorophyll „a", „b", and „c" was determined according to the acetone trichromatic methods using the equations based on the absorption maxima for each component respectively (with coefficients of Jeffrey & Humphrey). All absorbance values are corrected taking in consideration the turbidity of acetone extracts (Jeffrey & Humphrey, 1975; Lorenzen, 1967; Ston & Kosakowska, 2000).

The phytoplankton DNA was extracted according to Paul, 2008. The purity of the DNA was evaluated based on Sambrook *et al*., 1989.

PCR for the determination of the presence of *Synechoccocus* was run on Eppendorf Master Cycler machine. Primers used to amplify cyanobacterial rDNA were 16 S–1247 F and 16 S–241 R according to Rocap *et al*., 2002. PCR was run in a 20 ml solution. Sigma taq polymerase (0.5 U), $MgCl_2$ (2 mM,), buffer (1 X), deoxynucleoside triphosphates, and 100 pmol of each primer. Temperature cycle was: 94 C° for 4 min, followed by 40 cycles of 94 C° for 1 min, 52 C° for 1 min, and 72 C for 3 minutes. Final step was a 10 min stretch at 72 C°. DNA was checked by a 1% agarose gel.

RESULTS AND CONCLUSIONS

The main objective of the present work was to represent the total phytoplankton DNA as a possible new indicator of the trophic state in freshwater systems. In order to achieve this, a comparative study on the use of different physical and biological parameters for the evaluation of the level of trophy in the lake of Shkodra, Albania was conducted. As previous studies show, the chlorophyll a, total phosphorous, total nitrogen, and water turbidity are generally used as indicators to measure „biological productivity" of the water bodies or their capacity to support life. However, research is conducted for more than half a century trying to find other possible bioindicators of the level of trophy, among which the total plankton DNA.

It has been hypothesized that particulate DNA concentrations could be used to provide reasonable estimates of living biomass (Holm-Hansen *et al*., 1968). Indeed, cell DNA was found to be linearly related to cell carbon for a variety of phytoplank-

ton species (Holm-Hansen, 1969). In contrast to other cellular constituents, which vary widely with growth conditions, DNA content of a given cell type varies within a narrow range, typically 2-fold in extent for eukaryotes, with extremes reached at the beginning (*G 1* phase) and end *(G 2* phase) of the cell (Boucher *et al*., 1991). The failure of initial attempts (Holm-Hansen, 1969) to reconcile biomass estimates from DNA, on the one hand, and from particulate carbon, chlorophyll, or ATP, on the other hand, was attributed to the presence of an important fraction of 'detrital' DNA. This assertion was challenged in the case of coastal waters. Field experiments were realized to test the ability of the cell cycle analysis method to estimate species-specific phytoplankton growth rates. The attempt to use the double nucleated cells as a means to check the accuracy of growth rate estimates was not successful. The estimated growth rate fits the observed pattern of phytoplankton succession. Furthermore, research on understanding of the ratio among replicating and non replicating DNA showed that „Non replicating DNA" comprises 70–90% of the total phytoplankton DNA in oligotrophic oceanic waters (Limnology and Oceanography, 1986, 31(3), 637–645). The DNA content appears to be a scaleable cell component covarying with the carbon and nitrogen contents of the phytoplankton cells (Marcel *et al*., 2008). This covariation allows the total DNA content to be used as an accurate, independent estimate of total cell carbon biomass in unicellular pelagic phytoplankton (Sutcliffe, 1968; Marcel *et al*., 2008*).*

With present research we aimed to build a correlation among existing bioindicators of the level of trophy and the total phytoplankton DNA variation during the year in the freshwater system of the lake of Shkodra. Results showed that the amount of total phytoplankton DNA at the four chosen stations increases during the summer months (Fig. 1), which corresponds to the phytoplankton chlorophyll content.

Regarding the last (chl a), the lake of Shkodra belongs to the oligotrophy level (Fig. 2). Taking into consideration the variation of the temperatures and the ph of the waters (Bacu & Babani, 2010), it becomes clear that there is a linear relation between temperature (Fig. 3), pH (Fig. 4), chlorophyll content and DNA amount,

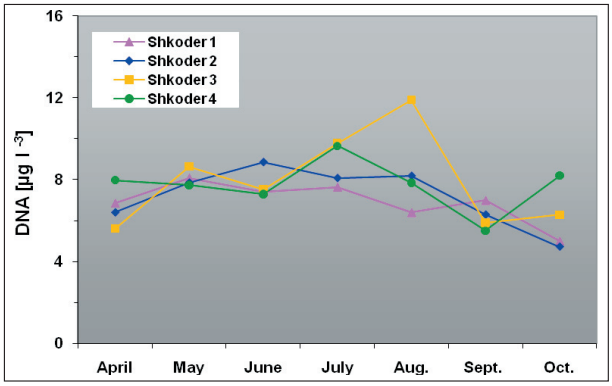

Figure 1. Variation of the amount of total phytoplankton DNA during March-October 2009.

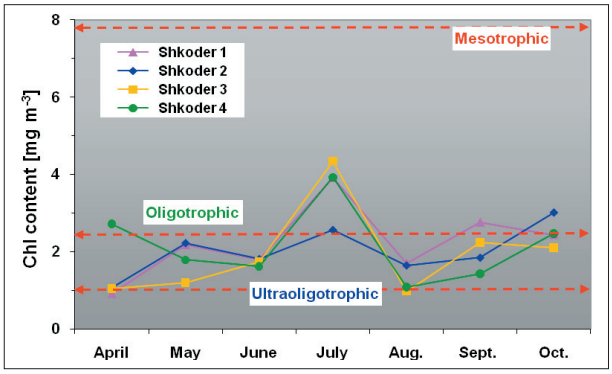

Figure 2. Chlorophyll content at the four stations of the lake of Shkodra

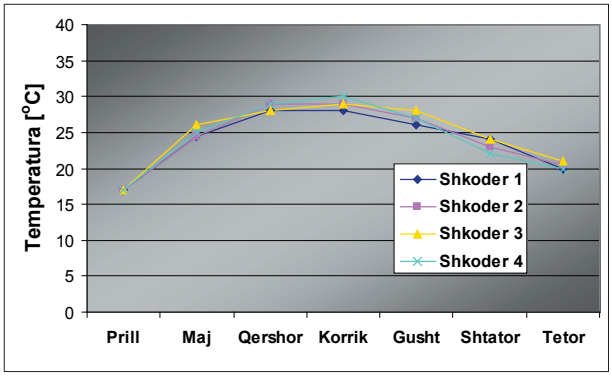

Figure 3. Variation of the temperature of waters at the Lake of Shkodra, March-October 2009

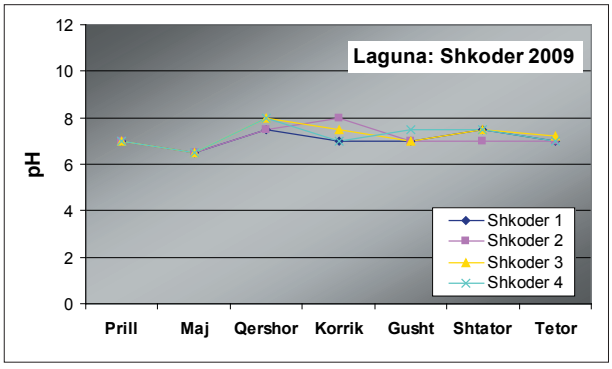

Figure 4. Variation of the pH of waters at the Lake of Shkodra, March-October 2009

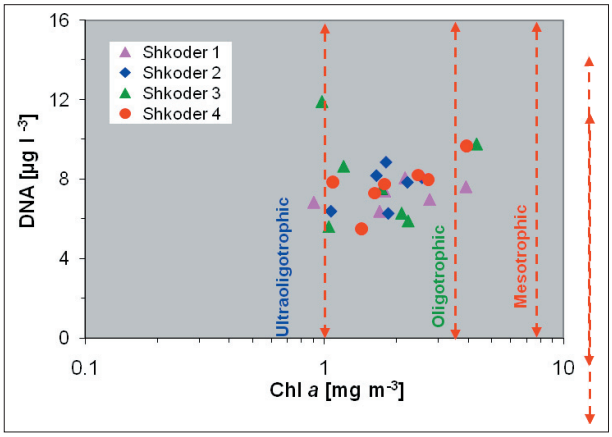

Figure 5. Relation among chlorophyll content and total phytoplankton DNA amount in the four stations of the Lake of Shkodra

which may be described as, the higher the temperatures, the higher the ph, and both the chlorophyll and DNA content. Values of the chlorophyll content and total phytoplankton DNA were used to construct a graphic, which shows this relation (Fig. 5). Based on the results of this work, we believe that the evaluation of the amount of the total phytoplankton DNA is one possible parameter for the evaluation of the phytoplankton biomass, since the amount of DNA per cell of phytoplankton is not variable due to physical environmental conditions. Measurement of this parameter offers information on the amount of phytoplankton cells/unit of volume of the sampled water independently of the intensity of the biological activity of these cells and in right proportion with the real biomass of phytoplankton in specific environmental conditions.

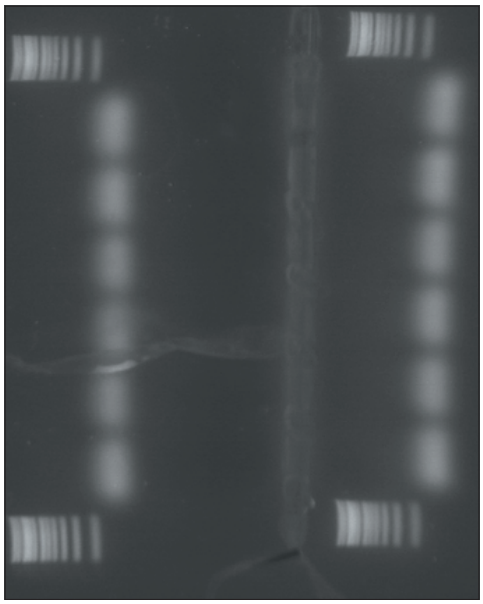

Photo 1. The 450 bp band corresponding to the amplified gene fragment of Synechococcus, starting from the DNA template isolated as total genomic DNA of phytoplankton cells

Molecular tools were used to determine the presence of cyanobacterial picophytoplankton in the waters of the Lake of Shkodra. The reasons for studying the bacteria from marine picophytoplankton are numerous. First of all, these are organisms of great ecological importance; these cells are some of the principal primary producers of the phy-

toplankton, which is responsible for half of the photosynthesis on the planet. These bacteria are therefore prominent actors in global oceanic function, in the carbon cycle, and consequently in the evolution of climate. To our knowledge, there is no previous research regarding the categories of picophytoplankton of the Albanian marine and freshwater systems. This way the present work represents a starting point. The molecular analysis to verify the existence of cyanobacteria *Synechococcus* based on the amplification of 16 S–23 S cyanobacterial ribosomal DNA, has been successful on the identification of cyanobacteria in different locations in the world. Results indicated that this species is present in the waters of the lake of Shkodra, into the four stations of sampling (Photo 1).

The product was about 450 bp and was reproduced successfully. This presence is supported from a number of parameters as, the warm temperatures, which are reported to increase the abundance of *Synechococcus*, the reduced salinity, high nutrient content, which provide optimal conditions for cyanobacterial growth. Generally, in more nutrient rich coastal and estuarine environments *Synechococcus* dominates (Olson *et al*., 2002).

REFERENCES

[1] Babani, F., Bacu, A., Kongjika E., Stamo, I., Ylli, A. 2010. *Monitorimi i gjendjes trofike dhe cilesise se ujrave ne lagunat e ekosistemit Kune-Vain, dhe Liqenin e Shkodres per vitin 2009.* Raport Zyrtar.

[2] Bacu & Babani, 2010. *Preliminary results on the presence of cyanobacteria Synecoccocus in the lagoon waters of Northwestern Albania and Lake of Shkodra*. Balwois Book of Abstracts, vol II, Conference on water observation and information system for decision support. Full paper online.

[3] Bacu A. & Babani, F. 2010. *A comparative study on the efficiency of use of different physical and biological parameters for the evaluation of the level of trophy in the lagoon system of Kune-Vain, Albania*. BENA International Conference on Fishery and Aquaculture, May 2009. In press.

[4] Boucher, N., Vaulot, D. & Frederic P. 1991. *Flow cytometric determination of phytoplankton DNA in cultures and oceanic populations.* Marine Ecology Progress Series. Vol. 71: 75–84.

[5] Hakanson, L. & Bryhn, A. C. 2008. *Eutrophication in the Baltic Sea. Present situation, nutrient transport Processes, Remedial Strategies.* Springer-Verlag Berlin Heidelberg, 7.

[6] Holm-Hansen, 1969. *Determination of microbial biomass in ocean profiles.* Limnol. Oceanogr. 14: 740–747.

[7] Honda, D., Yokota, A. & Sugiyama, J. 1999. *Detection of seven major evolutionary lineages in cyanobacteria based on 16 SrRNA gene sequence analysis with new sequences of five marine Synechococcus strains.* J. Mol. Evol. 48: 723–739., 58.

[8] Jeffrey, S. W. & Humphrey, G. F. 1975. *New spectrophotometric equation for determining chlorophyll a, b, c1 and c2.* Biochemistry of Plant Physiology, 167: 194–204.

[9] Lorenzen C. J. 1967. *Determination of chlorophyll and phaeopigments: spectrophotometric equations,* Limnology and Oceanography. No 12.

[10] Marcel, J. W., Terry L. C., Sieracki, M. C. 2008. *Cellular DNA content of marine phytoplankton using two new fluorochromes: taxonomic and ecological implications.* Journal of Phycology, Volume 33 Issue 3: 527–541.
[11] Olson, R. J., Chisholm, S. W., Zettler, E. R. & Armbrust, E. V. 2002. *Pigments Size, and Distribution of Synechococcus in the North Atlantic and Pacific Oceans.* Limnology and Oceanography. 35: 45–58.
[12] Paul J. 2008. *(Phytoplankton DNA extraction).* Text Book.
[13] Robertson, B. R., Tezuka, N. & Watanabe, M. M. 2001. *Phylogenetic analyses of Synechococcus strains (cyanobacteria) using sequences of 16 SrDNA and part of the phycocyanin operon reveal multiple evolutionary lines and reflect phycobilin content.* Int. J. Syst. Evol. Microbiol. 51: 861–871.
[14] Rocap, G., Distel, D. L., Waterbury, J. B. & Chisholm, S. J. 2002. *Resolution of Prochlorococcus and Synechoccocus ecotypes by using 16 S–23 S Ribosomal DNA Internal Transcribed Spacer Sequences.* Applied and Environmental Microbiology. 68: 1180–1191.
[15] Sambrook, Fritsch, Maniatis, 1998. *Molecular Cloning.* A Laboratory Manual. Cold Spring Harbor Laboratory Press.
[16] Sanders, R., JickellS, C., & Mills D. 2001. *Nutrients and chlorophyll at two sites in the Thames plume and southern North Sea.* Journal of Sea Research, 46: 13–28.
[17] Ston, J. & Kosakowska, A. 2000. *Qualitative and quantitative analysis of Baltic phytoplankton pigments.* Oceanologia, 42: 449–471.
[18] Sutcliffe, W. H. JR., & Sharp, J. 1968. *Algae: Amounts of DNA and organic carbon in single cells.* Science 163: 87–88.
[19] Vidal, M., Duarte, C. M. & Sanchez, M. C. 1999. *Coastal Eutrophication Research in Europe: Progress and Imbalances.* Marine Pollution Bulletin, 38: 851–854.