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Ecological Tools for the Management of Cyanobacteria Blooms in the Guadiana River Watershed, Southwest Iberia

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1. Introduction

Strong water demand for irrigation, energy and drinking water production is responsible for an increasingly regulation of freshwater flow patterns and watersheds. In this context, the construction of dams allows water storage but seriously restricts freshwater flow downstream. Due to scarcity of freshwater resources, reservoir water management often promotes high hydraulic residence. This may cause strong impacts on biological components of aquatic ecosystems, influencing the development of cyanobacteria blooms and aggravating their harmful impacts.

Aquatic cyanobacteria, a group of relatively slow growing photosynthetic organisms, are stimulated by high water residence times as well as increased temperatures and low N : P ratios, conditions that usually limit the growth of other competing phytoplankton groups (Carmichael et al., 1996; Chorus & Bartram, 1999; Kawara et al., 1998; Kononen et al., 1998; Paerl, 2008). Cyanobacteria blooms have been repeatedly associated with eutrophication processes (Berg et al., 1987; Carmichael et al., 1988; Codd, 2000; Chorus, 2005; Druvietis, 1997; Pinckney et al., 1998), but they might also dominate under oligotrophic conditions (Galvão et al., 2008; Havens et al., 2003; Mez et al., 1997; Sivonen & Jones, 1999).

Cyanobacteria blooms management became an emergent priority as a result of worldwide surveys of aquatic ecosystems affected by massive cyanobacteria blooms and their serious health and ecosystem risks (Blaha et al., 2009). Indeed, cyanobacteria are able to produce a wide range of secondary metabolites which are toxic to humans and wildlife, generally referred as cyanotoxins. From a toxicological perspective, cyanotoxins are classified as

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hepatotoxins, neurotoxins, cytotoxins, and dermatotoxins (Wiegand & Pflugmacher, 2005). According to their chemical structure, these toxic compounds are peptides, heterocyclic (alkaloids) or lipidic compounds (Sivonen & Jones, 1999). Effects of toxins on humans can be triggered mainly by direct skin contact or consumption of contaminated water. Furthermore other potential routes of exposure have been documented including aerosol inhalation, contaminated food ingestion and dialysis (Chorus & Bartram, 1999; Dunn, 1996; Jochimsen et al., 1998; Pouria et al., 1998). Additional problems related to cyanobacteria bloom episodes in raw water sources used for drinking water production include noxious effects such as bad taste and odour, due to the presence of geosmin and 2-methylisoborneol (Jähnichen et al., 2011). Cyanobacteria may also produce a wide range of currently unknown toxins with great toxicological significance (Blaha et al., 2009). Thus, cyanobacteria blooms constitute a key concern for drinking water production, and are also relevant for establishing water quality management policies (e.g., Water Frame Directive, WFD; Directive 2000/60/CE of 23 October 2000).

Phytoplankton is recognized as an essential biological element in monitoring programs used to define the ecological quality and health of aquatic environments. In the scope of the WFD, phytoplankton is used to classify trophic state of aquatic ecosystems (Domaizon et al., 2003), as well as to determine the effectiveness of management, restoration programs and environmental legislation (Brierley, et al., 2007). Phytoplankton biomass and composition, along with trophic state indices (TSI) and physical-chemical variables, are essential to establish freshwater ecological status (Carlson, 1977; Reynolds et al., 2002).

The need for translating complex biological information into Multimetric Indicators of Ecological Condition, required by water managers, has led to the development and testing of multiple ecological indices. According to the Evaluation Guidelines adopted by the United States, (Jackson et al., 2000), selected ecological indicators used for ecological classification should: (1) be easily obtained through standardized well-documented methods; (2) provide relevant information in terms of specific management concerns; (3) allow for temporal and spatial variability, without losing discriminant capacity, and (4) maintain reliability. Despite the great effort put into sampling and analytical methods standardization, we consider that indices recently adopted to evaluate ecological status of surface waters are still far from complying with all these criteria.

The Guadiana River watershed (Fig. 1) is the fourth largest river basin in the Iberian Peninsula (67480 km^2), and is located in a semi-arid region with a Mediterranean climate. Annual precipitation averages ca. 500 mm, and the hydrographic regime is torrential, with concentrated rainy periods and a prolonged dry season, usually from May to September. The Mediterranean climate irregularity is also expressed in strong interannual variability, with intense rainy years alternating with years of extended droughts (Daveau, 1987). Managing water availability under such demanding conditions lead to the construction of hundreds of dams, from which almost 90 have a volume capacity over 1 hm^3. Reservoir water management strategies are strongly limited by increasing water demands for irrigation and drinking water production, causing severe restriction of freshwater flow. Recent construction of the large Alqueva dam further increased flow regulation.

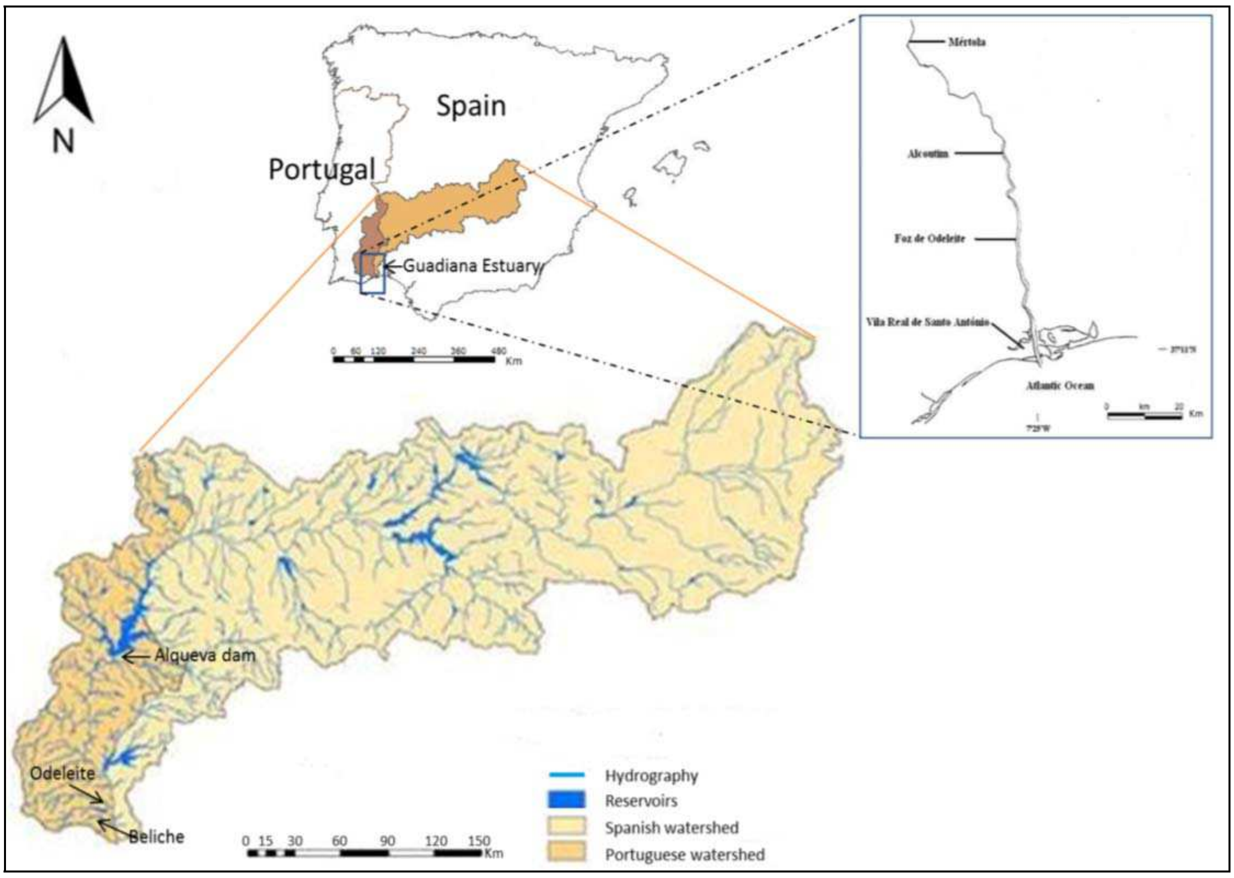

Fig. 1. Guadiana River water basin and location of study sites: Guadiana estuary and adjacent freshwater reservoirs (Alqueva, Odeleite and Beliche)

The main goal of this work is to evaluate recent water management strategies adopted for the Guadiana watershed, comparing different criteria used to classify ecological status and potential. The study is based on long-term ecological data series, and addresses two distinct case studies: (i) the Guadiana estuary (1997-2009); and (ii) adjacent Beliche and Odeleite freshwater reservoirs (2003-2010).

2. Guadiana estuary study

2.1 Study area, sampling strategy and methods

The Guadiana estuary is a mesotidal system (tidal amplitude: 1.3–3.5 m), with a length of 70 km, a maximum width of ca. 550 m, an average depth of 6.5 m, and an average water residence time of 12 days (Domingues & Galvão, 2007; Vasconcelos et al., 2007). The upstream saltwater intrusion is usually located close to Alcoutim (ca. 38 km from river mouth), whereas tidal influence extends to Mértola (ca. 70 km from river mouth; see Fig. 1). The lower estuary ranges from partially stratified to well-mixed, whereas the upper estuary is generally well-mixed (Cravo et al., 2006; Morais et al., 2009; Oliveira et al., 2006; Rocha et al. 2002). A series of dams has severely restricted its freshwater flow (ca. 75 %), and the recent construction of the large Alqueva dam (ca. 150 km upstream from river mouth) increased flow regulation up to 81% of the total catchment area (55 000 km^2) starting in 2003 (Galvão et al., 2008). Since human activity in the Guadiana watershed is mostly agriculture and the main anthropic pressure is associated to dams, the Guadiana is considered one of the best conserved but also most vulnerable estuaries of the Iberian Peninsula (Vasconcelos et al. 2007).

At different stations (see Fig. 1), vertical profiles of water temperature and salinity were determined in situ using a YSI 556 MPS probe. Vertical profiles of photosynthetically active radiation (PAR) intensity were determined using a LI-COR radiometer. Light extinction coefficient (k_e, m^{-1}) was calculated using an exponential function (eq. 1), where I_z represents the light intensity at depth Z (m) and I_0 is the light intensity at the surface:

$$I_z = I_0 e^{-KeZ} \quad (1)$$

Subsurface water samples (ca. 0.5 m) were collected at different sampling stations (Alcoutim and Mértola) for determination of dissolved inorganic nutrients and phytoplankton variables. For nutrient concentration, samples were immediately filtered through cellulose acetate filters (Whatman, nominal pore diameter 0.2 μm) to acid-cleaned vials. Ammonium (NH_4^+), phosphate (PO_4^{3-}) and silicate (DSi) were determined upon arrival to the laboratory, while samples for nitrate (NO_3^-) where frozen (-20ºC) until analysis. All nutrient analyses were made in triplicate, according to the spectrophotometric methods described by Grasshoff et al. (1983), using a spectrophotometer Hitachi U-2000 for ammonium, phosphate and silicate, and an autoanalyzer Skalar for nitrate and nitrite.

Chlorophyll *a* concentration was determined spectrophotometrically using glass fiber filters (Whatman GF/F, nominal pore diameter = 0.7 μm). Chlorophyll *a* was extracted overnight at 4ºC with 90% acetone; after centrifugation, absorbance of the supernatant was measured in the spectrophotometer Hitachi U-2000 at 750 and 665 nm, before and after addition of HCl 1 M (Parsons et al., 1984).

Phytoplankton composition (including cyanobacteria), abundance and biomass were determined using epifluorescence (Haas, 1982) and inverted microscopy (Utermöhl, 1958). Samples for enumeration of pico- (<2 μm) and nanophytoplankton (2 - 20 μm) were preserved with glutaraldehyde (final concentration 2%) immediately after collection, stained with proflavine and filtered (1 - 5 mL, depending on the amount of suspended matter) onto black polycarbonate membrane filters (Whatman, nominal pore diameter 0.45 μm). Preparations were made within 24 h of sampling using glass slides and non-fluorescent immersion oil (Cargille type A), and then frozen (-20ºC) in dark conditions, to minimize loss of autofluorescence. Enumeration was made at 787.5x magnification using an epifluorescence microscope (Leica DM LB). Samples for enumeration of microphytoplankton (>20 μm) were preserved with acid Lugol's solution (final concentration ca. 0.003%) immediately after collection, settled in sedimentation chambers (2 - 10 mL, depending on the amount of suspended matter; sedimentation time = 24 hours) and observed at 400x magnification with an inverted microscope (Zeiss Axiovert S100). A minimum of 50 random visual fields, at least 400 cells in total and 50 cells of the most common genus were counted.

For microcystin – LR (MC-LR) determination, 1.5 to 2 L water samples were filtered through Whatman GF/F filters, which were frozen until extraction with 20 mL 75 % (v/v) methanol. High performance liquid chromatography (HPLC) was carried out in a Dionex Summit equipment with photodiode array detector (PDA) and Chromeleon 6.3 software, using a C18 column (Merck Purospher STAR RP18 endcapped, 3 μm particles, LiChro-CART, 55 mm x 4mmm) kept at 40ºC. As a mobile phase, acetonitrile and Milli-Q water were used containing

0.05% (v/v) TFA (trifluoroacetic acid) in a 25 : 75 ratio. Extract was then evaporated in a rotary evaporator (50-54 ºC). Chromatograms were analyzed between 180 and 900 nm, with main detection at 238 nm for absorption spectrum characteristic of MC-LR. Purified MC-LR (Sigma) was used as standard, and results are expressed in MC-LR equivalents per volume of sample (Meriluoto & Codd, 2005; Ribau-Teixeira & Rosa, 2005; Sobrino et al., 2004). Samples collected during 1999 were analyzed for MC-LR using both ELISA and HPLC techniques in Dr. Wayne Carmichael´s laboratory, Wright State University, Ohio, U.S.A and results confirmed in Prof. Vitor Vasconcelos´ laboratory, Universidade do Porto, Portugal.

It is to be noted that the HPLC technique applied for MC-LR determination was carried out in different years by different technical staff and/or students (Master´s, PhD & post-doctoral fellows) in different specialized laboratories. During 1999, MC-LR was analyzed in parallel in two laboratories (Dr. W. Carmichael, Wright State, U.S.A., and Prof. V. Vasconcelos, Universidade do Porto, Portugal). From 2002 onwards, MC-LR analysis was performed in the Environmental Technology Lab., Universidade do Algarve with Prof. M. J. Rosa (2002-2003) and Dr. M. R. Teixeira (2004-2009). Therefore, although not considered significant in this study, slight variations in the extraction and HPLC methodology existed, as well as some adjustments in the interpretation of chromatograms.

2.2 Results

Monthly mean river flow at Pulo do Lobo (ca. 85 km upstream from river mouth) and total monthly rainfall at Alcoutim measured from 1996 to 2009 (Fig. 2) revealed four distinct river

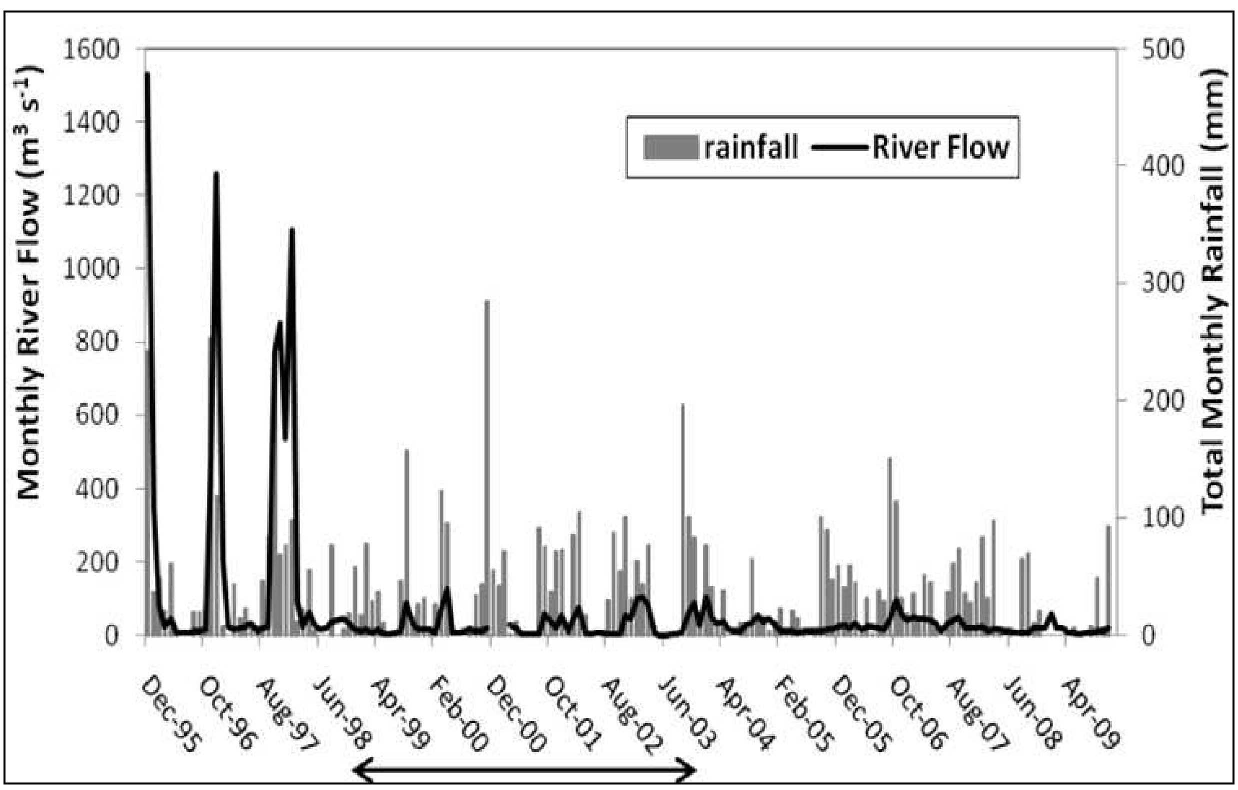

Fig. 2. Monthly mean river flow ($m^3 s^{-1}$) at Pulo do Lobo and total monthly rainfall (mm) at Alcoutim from 1996 to 2009 (data source: http://snirh.pt/). Arrow marks period of dam construction and filling

flow regimes: period before Alqueva dam construction (1996-1998), period during dam construction (1999 – 2000) and filling (2001 - 2003) and severely regulated river flow afterwards. Before Alqueva, river flow fluctuated widely from torrential winters to dry summers typical of Mediterranean flood - drought rainfall regime. However, starting with construction and filling of Alqueva dam, river flow became abruptly restricted particularly during winter months despite heavy rainfall. Mean river flow during summer reached 20 – 25 m^3 s^{-1} during 1997 – 1998 previous to Alqueva, and decreased below 10 m^3 s^{-1} from 1999 to 2003 during Alqueva construction and filling. Afterwards, summer river flow increased to 10 – 15 m^3 s^{-1} during 2004 – 2005 reaching 20 – 25 m^3 s^{-1} during 2007 – 2008, but decreased back below 10 m^3 s^{-1} during 2008 – 2009.

Box plots in Fig. 3A also revealed these oscillations with respect to median values and overall distribution during these periods. Light extinction coefficient (Ke, see Fig. 3C), which is tightly correlated to sediment load, was generally low during 1997- 1998 previous to Alqueva; however, during dam construction in 1999 and part of 2000, light extinction reached maximum values in extremely turbid waters with very high sediment load (Suspended Particulate Material, SPM, peak values of 140 mg L^{-1}; data not shown). After this period of dam construction and extensive soil movement, waters tended to clear with Ke values decreasing from 2000 to 2010, but with wide intra-annual fluctuations due to winter summer oscillations in rainfall and river flow. It is to be noted that the composition of SPM varied markedly between river mouth (Vila Real Sto António) and upper estuary (Alcoutim and Mértola). In the lower estuary, SPM was mainly composed by quartz, which contributed minimally to light attenuation in the water column. On the contrary, in the middle and upper estuarine regions, SPM was mostly dominated by clays (Machado et al., 2007), which usually play an important role in light absorption.

Nitrate concentration, the predominant form of total dissolved inorganic nitrogen (ca. 65% - 89% of total inorganic N), showed a decreasing trend after construction of Alqueva dam (see Fig. 3B). Nitrate annual means during 1996-2001 in Alcoutim ranged between 65.0-73.6 μM, whereas mean NO_3^- was 56.2 μM in 2002, further decreasing to 30.4 ± 17.3 μM in 2005 (Barbosa et al., 2010), and remaining relatively low (32.23 ± 20.7 μM) during 2007-2009 (Domingues, 2010).

As referred in previous studies (Barbosa et al., 2010), silicate concentration was usually correlated with rainfall and river flow, and negatively correlated to chlorophyll *a*. Both DSi and nitrate exhibited seasonality with higher values during winter and lower values between midspring and summer. In contrast with nitrate, DSi exhibited an obvious increase during the period of the Alqueva dam filling (2002–2003) that led to a significant increase in the Si:N and Si:P molar ratios (data not shown), and a subsequent decline after its completion from 2004 to 2010 (see Fig. 3B).

Chlorophyll *a* (Fig. 4A) and total cyanobacteria abundance (Fig. 4B) in the upper estuary revealed a sharp collapse in 1999 during Alqueva construction, increasing during dam filling (2000 – 2001). Afterwards, chlorophyll *a* decreased again markedly from 2002 to 2010. Cyanobacterial abundance since Alqueva dam completion did not recuperate to high values observed previously (1997-1998). Furthermore, potentially toxic species, such as *Microcystis* spp., which were previously abundant exceeding WHO alert level 2 (≥100 000 cells mL^{-1}) during 1997 and 1998, have remained at very low densities if not practically absent from water samples collected in the upper estuary after Alqueva dam completion.

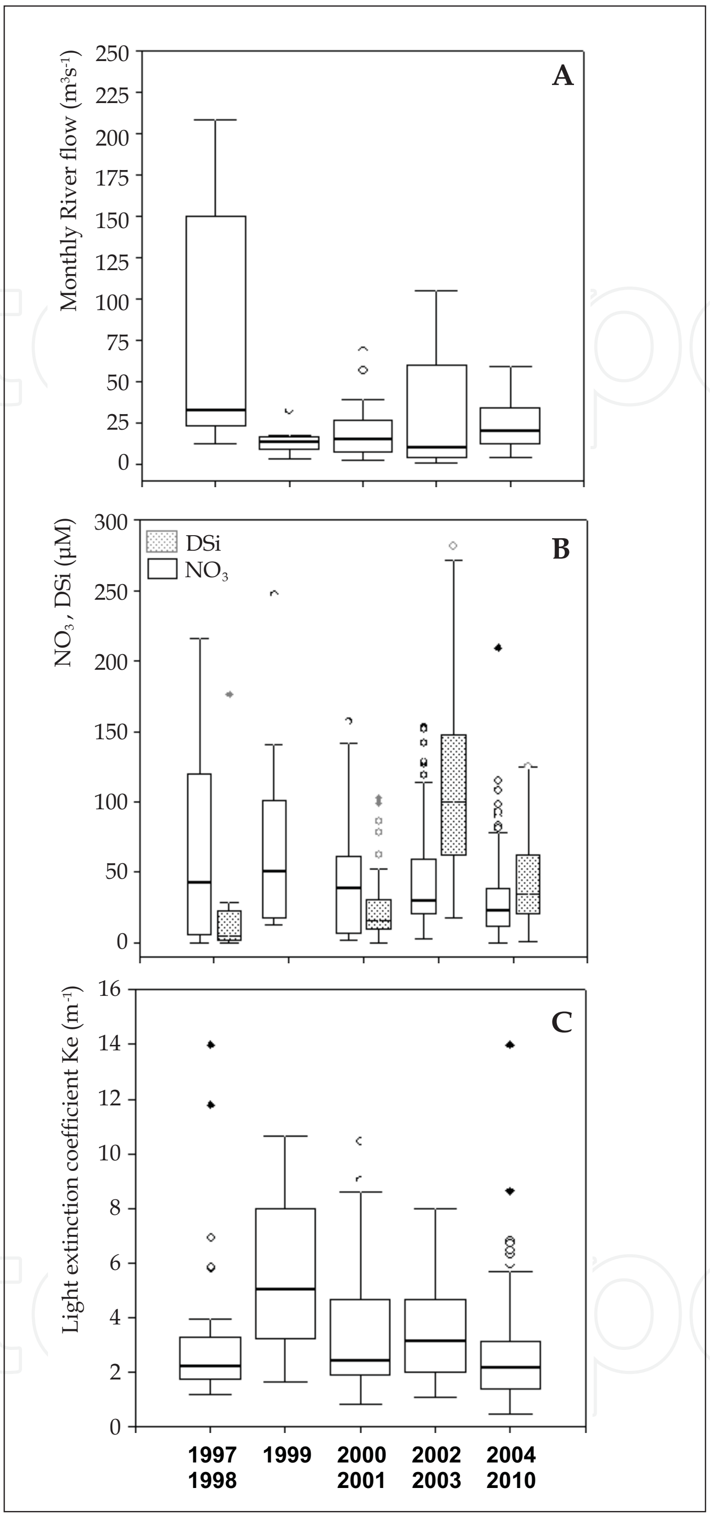

Fig. 3. Box and whisker plots showing the distribution of monthly Guadiana river flow (A), subsurface nitrate (NO_3^-) and silicate (DSi) concentration (B), and light extinction coefficient, Ke (C) in the Guadiana upper estuary, binned into different periods. Median value is represented by the line within the box, 25th to 75th percentiles are denoted by box edges, 5th to 90th percentiles are depicted by the error bars, outliers are indicated by circles, and extreme values by diamonds. Extreme values of monthly river flow (maximum 1258 m^3s^{-1}, year 1997) were omitted for clarity

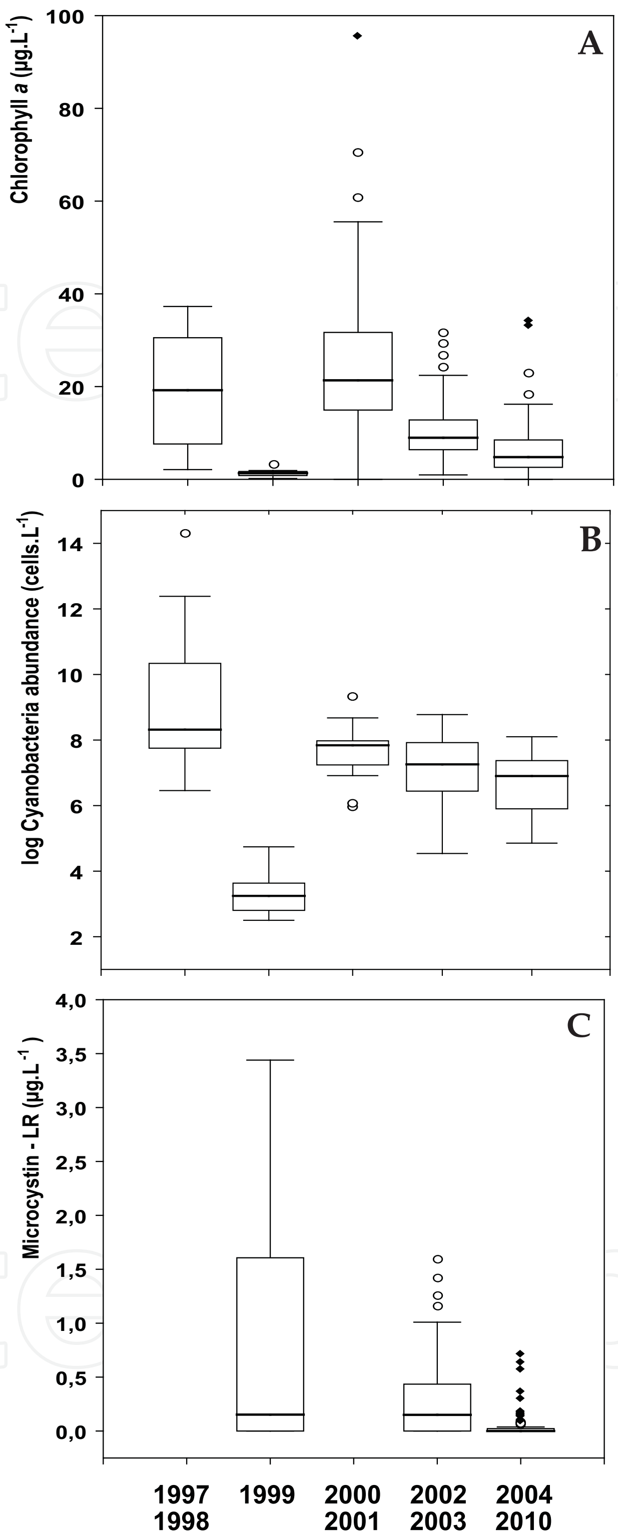

Fig. 4. Box and whisker plots showing the distribution of chlorophyll *a* concentration (A), total cyanobacteria abundance (B), and microcystin-LR concentration (C) in the Guadiana upper estuary, binned into different periods. Median value is represented by the line within the box, 25th to 75th percentiles are denoted by box edges, 5th to 90th percentiles are depicted by the error bars, outliers are indicated by circles, and extreme values by diamonds. An extreme chlorophyll *a* value (216.0 µgL^{-1}, year 2001) was omitted for clarity

Furthermore, the number of taxa observed not only in cyanobacteria, but also in phytoplankton populations has declined significantly. During summer of 1997 and 1998 the following genera were observed in numbers >1000 cells mL^{-1}: *Microcystis, Anabaena, Oscillatoria, Merismopedia, Lyngbya, Gomphosphaeria, Coelosphaerium, Syenchococcus*, and several unidentified species of *Chroococcales.* In contrast, during 2007 – 2009, besides unidentified Chroococcales, only *Planktothr*ix could be occasionally identified.

Total cyanobacterial abundance included abundant small chroococcoid species and more rare large filamentous forms, and was not correlated with chlorophyll a (see Fig. 5).

As for microcystin – LR (MC-LR) concentrations in suspended particulate material (Fig. 4C), distribution in different periods showed highest values during 1999, often surpassing the 1 µg L^{-1} limit for drinking water (WHO 1998 guidelines). Yet, in 1999 both cyanobacteria abundance and chlorophyll *a* reached overall minimum values observed in the study period from 1997 to 2010. MC-LR decreased from 2002 onwards with concentrations frequently below detection limit. In fact, after 2004, MC-LR concentrations in the particulate fraction never surpassed 1 µg L^{-1}.

The variation of MC- LR concentration over time (see Fig. 6) revealed the same decreasing trend as box plots in Fig. 4C. The frequency of samples where microcystins were non-detectable increased over time particularly during 2004 and 2005. Gap years (2000, 2001, 2006 and 2007) are due to lack of funding for regular monitoring in estuarine waters.

Microcystin concentration during the study period was not correlated to total cyanobacteria abundance or chlorophyll *a* (see Fig. 7).

2.3 Discussion

In past published reports dealing with the microbial ecology of the Guadiana estuary (Domingues et al., 2005; Domingues & Galvão, 2007; Rocha et al., 2002), the impact of Alqueva dam construction was predicted to increase eutrophication conditions and possibly promote cyanobacterial blooms and associated cyanotoxins. In fact, this has not been observed during the seven-year period after dam completion. Not only cyanobacteria, but overall phytoplankton abundance, biomass and chlorophyll *a* concentrations have decreased markedly and have remained at low levels even in the upper estuary, where peak chlorophyll maxima usually occurred.

Typical estuarine phytoplankton succession observed in the Guadiana estuary from diatoms in early spring, to chlorophytes and finally cyanobacteria in late summer and fall was driven by nutrient regime with high winter loads of nitrogen and phosphorus discharged downriver, and silica depletion after the spring diatom bloom (Rocha et al., 2002). These authors also referred that cyanobacteria dominated the chlorophyll maximum zone in the upper estuary in late summer- early fall, due to warm waters, reduced sinking and grazing, as well as N limitation with low N:P ratio. Nitrogen limitation during summer increased in the period after Alqueva in the upper estuary (Barbosa et al., 2010). Additionally, nutrient enrichment experiments performed during 2008 clearly demonstrated that phytoplankton growth was nitrogen limited (Domingues et al., 2011).

Contrary to more stringent nitrogen limitation, the improved light regime with lower extinction coefficients should have promoted overall phytoplankton growth from 2003

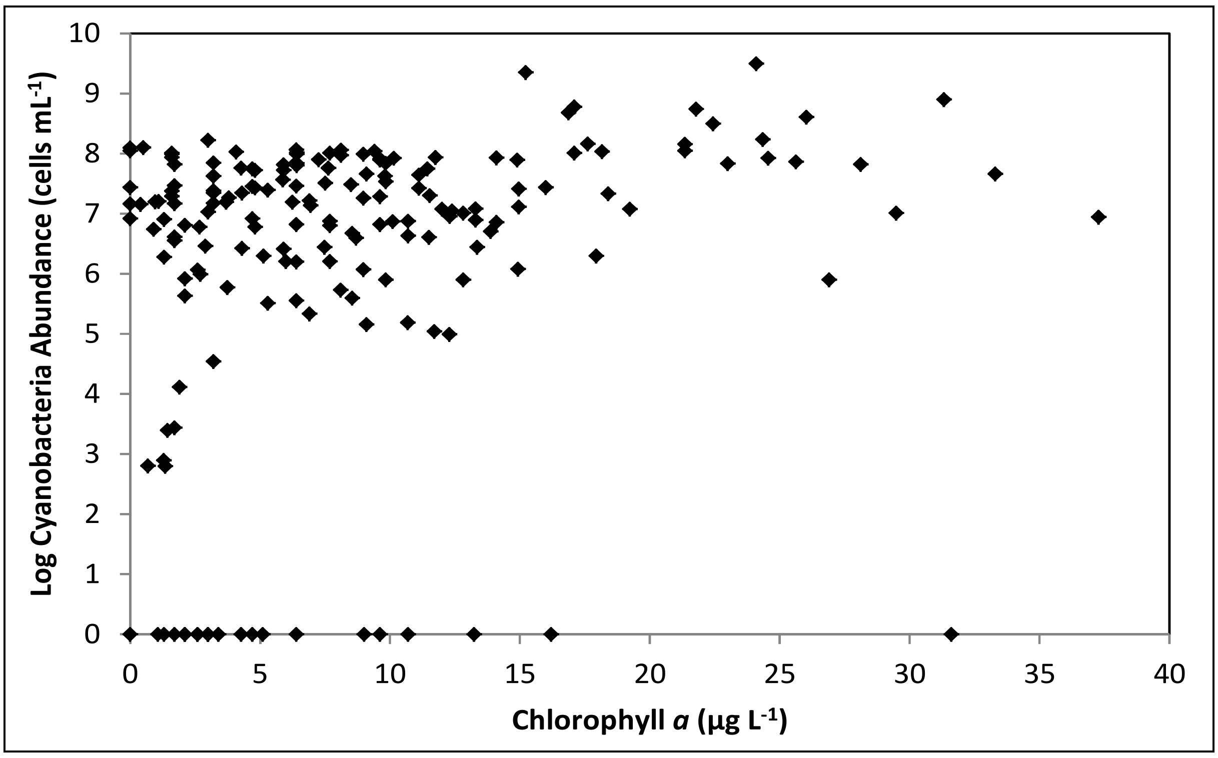

Fig. 5. Log of total cyanobacteria abundance (cells mL^{-1}) and chlorophyll *a* concentration (µg L^{-1}) in upper Guadiana estuary (pooled data from Alcoutim and Mértola) from 1997 to 2009. Zero abundance values not log transformed

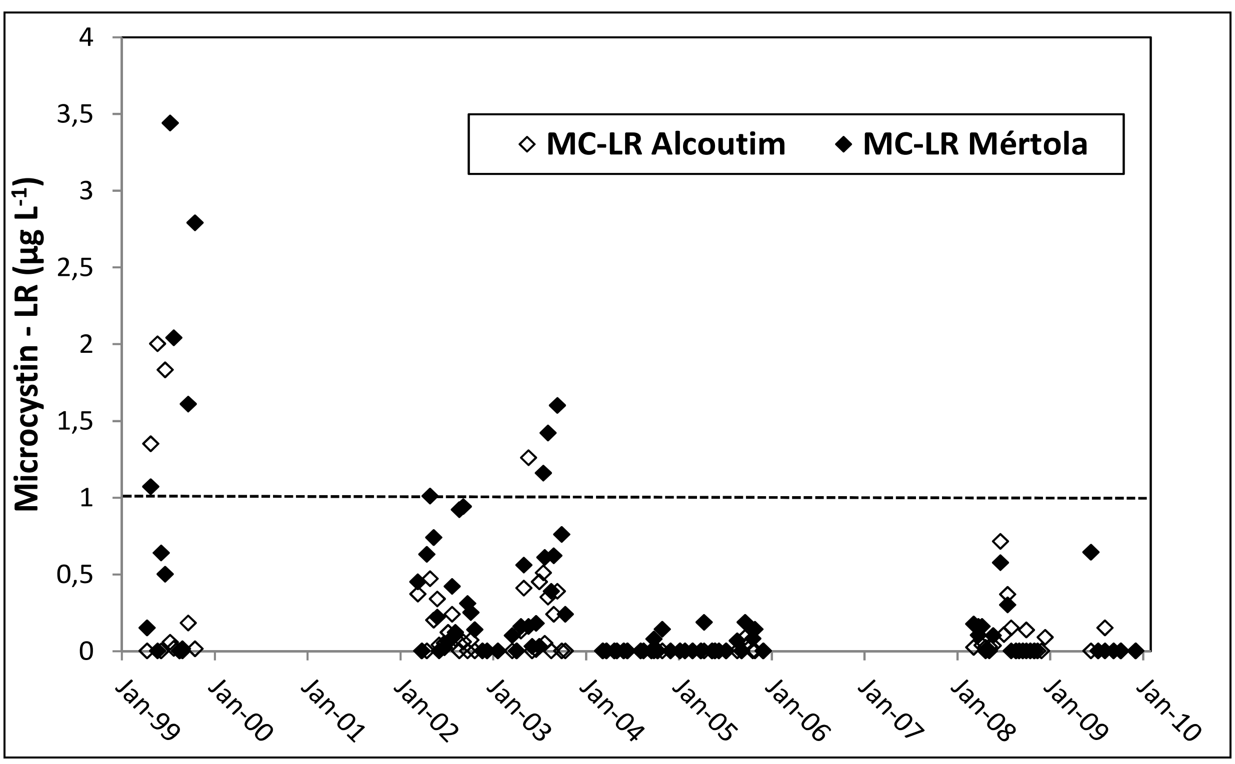

Fig. 6. Microcystin-LR concentration in particulate fraction (µg L^{-1}) over time in upper Guadiana estuary (Alcoutim and Mértola) from 1996 to 2009. Dashed line indicates the 1 µg L^{-1} limit for drinking water (WHO 1998 guidelines)

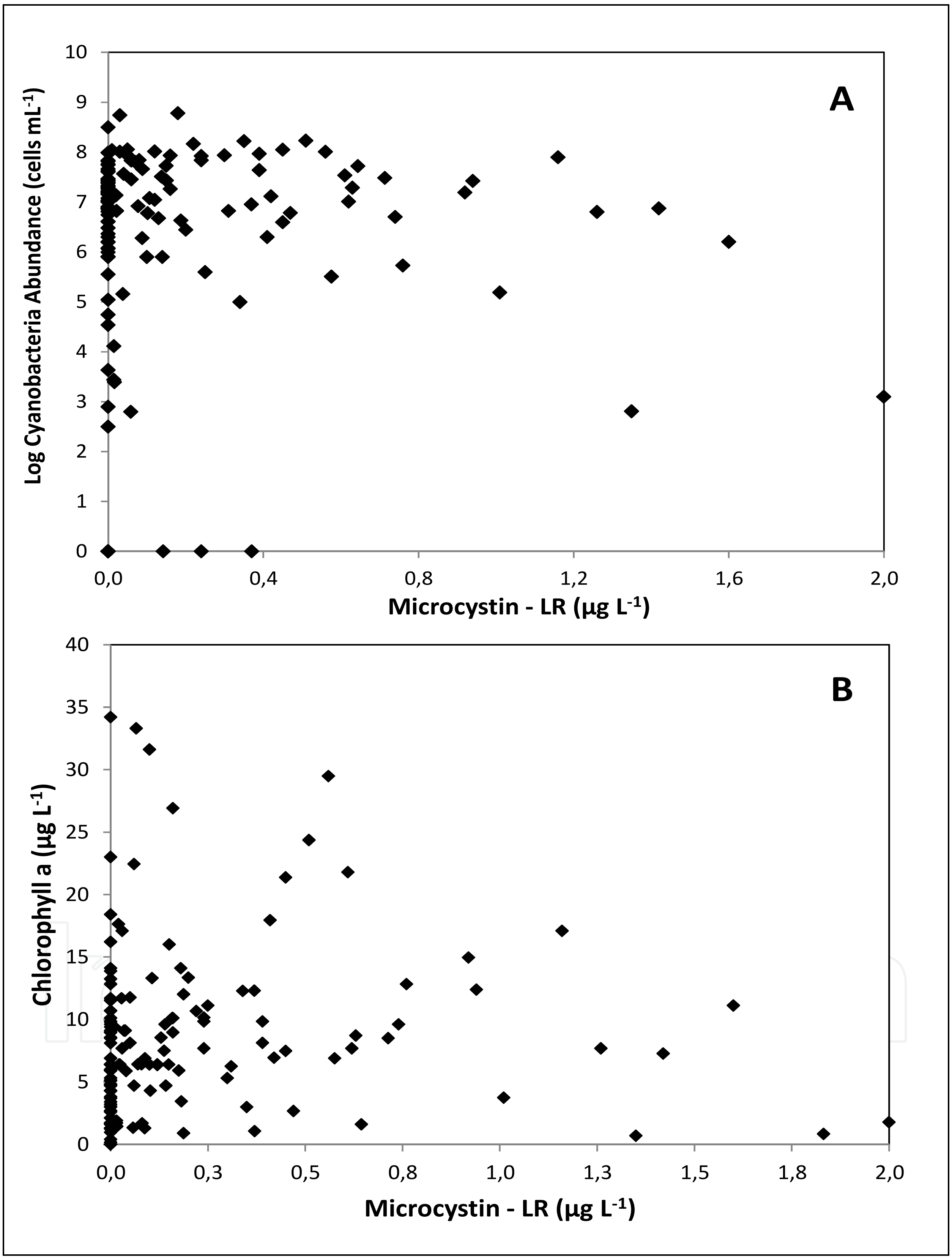

Fig. 7. Log of total cyanobacteria abundance (**A**) and chlorophyll *a* (**B**) versus microcystin concentration in particulate fraction (µg L^{-1}) in upper Guadiana estuary (Alcoutim and Mértola) from 1997 to 2009. Zero values not log transformed

onwards, after Alqueva dam completion. In view of decreasing trend in chlorophyll and overall phytoplankton abundance from 2003 to 2010, nitrogen availability appeared to play a preponderant role rather than light in this turbid estuary. This shift from light to nutrient limitation was probably the most determinant trend for phytoplankton observed after Alqueva (Barbosa et al., 2010). However, since chroococcoid cyanobacteria have higher affinity for nutrients due to small size, and most filamentous forms as well as some chroococcoid species have nitrogen fixing potential, these photosynthetic prokaryotes should have been less affected by lower nitrogen availability than larger non-nitrogen fixing eukaryotic phytoplankton. Freshwater reservoirs created by dams do not retain only water but also suspended particulate material, including planktonic microorganisms. In consequence, not only are nutritional regimes affected downstream, but also freshwater microbial populations with complex life cycles, such as cyanobacteria. Filamentous cyanobacteria in response to environmental forcing can produce different cell types which are adapted to nitrogen fixation, nutrient storage and reproductive strategies such as winter dormancy and dispersal. Thus, freshwater reservoirs by retaining these morphotypes could seriously affect not only bloom formation but also species composition downstream.

The lack of correlation between chlorophyll and cyanobacteria abundance could be simply explained by predominance of small chroococcoid cells with reduced chlorophyll content. Consequently, poor or absent correlation between chlorophyll and microcystin concentrations should also be expected.

As previously described by Galvão et al. (2008), microcystin concentration were generally not correlated with cyanobacteria abundance or biomass in natural waters (freshwater reservoirs and Guadiana river, South Portugal), since different strains and/or species could produce microcystins at different rates depending on cell cycles and environmental conditions, which has also been documented in laboratory analyses (eg. Kameyama et al., 2004; Rapala et al., 1997; Saker et al., 2005).

Furthermore, in all temperate estuaries, cyanobacteria accumulate and thrive in the chlorophyll *a* peak (Cloern, 1987; Pearl et al., 2006; Pinckney et al., 1998), directly upstream from the turbidity maximum. Restricting river flow can cause perturbations of estuarine circulation, particularly in terms of location and intensity of the turbidity maximum, which in turn will affect the chlorophyll *a* maximum in the upper estuary (Cloern, 1987, 1999). Thus, cyanobacteria decline cannot be simply explained by any one environmental driver, but rather in terms of estuarine circulation. Nutrients tend to be regenerated in the turbidity maximum and phytoplankton bloom directly upstream from this zone, benefiting in this interface between nutrient enriched and clear waters. Unfortunately, how seriously the huge Alqueva reservoir has affected estuarine circulation and the turbidity maximum in the Guadiana estuary has not yet been assessed.

The Alqueva dam not only is the largest dam in the Guadiana watershed but due to its location affects most strongly the estuarine section of the river. In spite of efforts by the Alqueva water management authorities to maintain "ecological" river flow this is not compulsory according to existing Portuguese water resources legislation. Ecological river flow can be broadly defined as the flow necessary to conserve and maintain natural aquatic (freshwater) ecosystems. In Portugal, this is very simply calculated as a value > 2.5 to 5% of the modular water flow to be maintained throughout the year, if conditions permit. Different studies have recently challenged this approach proposing more careful analyses of natural river flow variations and applying them to flow regulation by dams (Alves & Bernardo, 1998; Alves & Gonçalves, 1994; Chicharo et al., 2006; Chicharo et al., 2009; Wolanski et al., 2008). Flow Incremental Methodology, and other hydrological or ecohydrological approaches, would ensure that natural variations in freshwater flow would be mimicked by dam discharge, albeit dampened. Finally, monitoring of environmental impact usually considers either endangered or economically important vertebrate species existing in the freshwater zone. Yet, marine and freshwater micro- and macroorganisms need also to be considered in terms of whole ecosystem impact. In fact, microorganisms, such as cyanobacteria, appeared to be sensitive indicators of estuarine ecosystem perturbation in this study. Thus, it is proposed that photosynthetic prokaryotes should be used as indicators of "good" estuarine water quality rather than just "bad".

2.4 Conclusion

This 13-year study of the Guadiana estuary in Southern Portugal, directed towards assessing the impact of dam construction on cyanobacteria populations in the freshwater zone, revealed that phytoplankton abundance, chlorophyll *a* and diversity decreased markedly from 2003 onwards after Alqueva dam completion. This declining trend in phytoplankton could be explained by both light limitation during dam building coupled with more stringent nitrogen limitation after dam completion. Interestingly, cyanobacteria abundance, diversity and microcystin concentration exhibited an even more pronounced decrease, which could not be attributed to any monitored environmental factors, but instead to perturbations in overall estuarine circulation. The collapse in cyanobacteria populations in the upper estuary warrants a more careful approach towards maintaining ecological river flow in dam discharge. Future research in the Guadiana estuary should address not only the impact of restricted river flow on estuarine circulation, turbidity maximum and associated chlorophyll peaks, as well as provide more adequate approaches towards maintaining an ecological river flow, possibly using cyanobacteria as an indicator of good water quality.

3. Guadiana reservoirs management

3.1 Cyanobacteria management in reservoirs

Water management in the Guadiana River watershed is a complex transnational problem and has been object of negotiations between Portugal and Spain for decades now. The last bilateral Agreement assured the integrated management of water and territory, covering quantitative and qualitative features, stipulating minimum flows (under normal rainfall conditions), and foreseeing the permanent exchange of hydrologic and environmental data and information (Mendes, 2010). Environmental laws, in both countries, ensure public access to the monitoring data, allowing for international comparison of water quality in different parts of the watershed.

Normal and drought conditions in the Guadiana catchment have been modelled in multiple hydrologic studies (e.g. Brandão & Rodrigues, 2000), allowing for better management in terms of water availability. Nevertheless, water quality concerns have been mostly ignored in water reservoir management decisions. Impaired sewage treatment and agro-industrial mal-practices have been repeatedly blamed for water quality deterioration in the Guadiana river basin, both in Portugal and Spain. Official reports for the Portuguese part of the watershed specifically accuse illegal sewage discharges from pig production farms and olive mills of being responsible for high nitrate concentrations (PBH, 2001) As generally accepted, the mere existence of a new dam contributes to water quality degradation, since new populations and activities are attracted to the watershed. The main water user in the Guadiana watershed is agriculture, using 90 to 95% of the consumed water (PBH, 2001). It is thus expected that newly introduced crops after the start of the Alqueva dam irrigation system, in particular the new intensive olive tree orchards, will have strong impacts in future water quality.

A diagnosis of the actual ecological status of the catchment based on reliable methods and classification indices is therefore crucial. In Spain, the Confederácion Hidrográfica del Guadiana (CHG) and, in Portugal, the Administração da Região Hidrográfica do Alentejo, conducted a diagnostic snapshot classification, based on monitoring surveys from 2005 and 2006 for the Spanish part of the catchment, and on 2009-2010 data for the Portuguese watershed. Classification results, based on multiple indices, are available online (CHG, 2006, 2007-2008, 2009; ARH Alentejo, 2011).

According to these official reports, Guadiana reservoirs fall into diverse typologies, but the majority of them behave as warm monomyctic lakes, that remain stratified during the dry season and mix the water column in winter. As expected in result of high hydraulic residence time and elevated temperatures, these freshwater reservoirs are dominated, at least in the summer, by potentially toxic cyanobacteria from the genera *Pseudanabaena, Anabaena, Planktothrix, Oscillatoria., Geitlerinema., Aphanizomenon, Merismopedia, Microcystis, Woronichinia, Synechocystis,* and *Aphanocapsa*. (CHG, 2009) Toxic species of these cyanobacteria may reach high densities forming harmful algal blooms (HABs). In fact, the term water bloom originally referred to surface scums of cyanobacteria, but has since been applied to almost any planktonic population (not even necessarily algal) with densities significantly above the normal (Reynolds, 2006).

Managing these cyanobacteria harmful algal blooms (CHABs) has become a major concern in view of the potential health impacts both through drinking water or farming products consumption (Edwards et al., 1992; Hoegar et al., 2005).

CHABs management might involve prevention actions and/or mitigation solutions. Numerous techniques have been developed for these purposes, but as stated by Perovich et al. (2008) most of them have not been explicitly evaluated and optimized for use in the control of CHABs, particularly when toxins are present.

Prevention techniques rely on CHAB association with eutrophication processes and aim to control CHAB through nutrient limitation or decreasing hydraulic residence time. These techniques include watershed protection or restoration, through adequate sewage treatment implementation, promotion of farming good practices, particularly in the use of fertilizers and pesticides; erosion control; stimulation of margin riparian vegetation as well as controlled surface water discharges. Nutrient input reduction by controlling point sources has had success in several CHABs managing cases (Piehler, 2008), but it is now acknowledge that restoration efforts seldom bring aquatic communities back to the diversity and composition they used to bear before suffering human impacts (Jacquet et al., 2004)

Mitigation enforcement, by control and removal of an installed bloom, might rely on techniques such as the addition of algicides, the introduction of fish schools, surface scums elimination or water column mixing (Piehler, 2008). Such techniques often bring about unexpected results (Jacquet et al., 2004).

The only technique used both for prevention as well as mitigation of CHABs is the reduction of water residence time, through surface water discharges. It was well known in the 1960s (Odum, 1971) that the type of water discharges, and specially the height of water column, at which they were performed, strongly influenced plankton assemblages both up- and downstream from a reservoir. While surface release mainly exports warmer water and their plankton communities, bottom discharge introduces downstream cold, nutrient enriched water, keeping the warmer plankton rich waters inside the reservoir (Wright, 1967). This means that in reservoirs with bottom water flow, slow growing picoplankton, including cyanobacteria, is given the opportunity to develop blooms, instead of being rapidly flushed downstream. Water extraction for drinking water production tends to use water at a medium height of the water column, avoiding both the surface plankton, and the bottom metal enrichment. As observed in the Algarve reservoirs (Reis, unpublished data) withdrawing cold water from the hypolimnion and maintaining a floating inoculum of warm temperature selected cyanobacteria at the surface is transforming reservoirs into bioreactors like structures, favoring the occurrence of prolonged summer blooms. This water management technique tends to enhance stratification, delaying water column mixing.

As acknowledged by increasing awareness for the need of establishing an ecological flow, reservoir water management plays a key role in downstream river ecology, but also in upstream ecology.

Rapid changes in the water level in response to summer increased water demand seriously hinders the installation of riparian vegetation, challenging some prevention techniques. Thus, caution should be taken when applying the same ecological criteria to reservoirs as for lakes, as advocated by the European WFD. In fact, in most natural lakes excess water overflows into effluent streams, exporting phytoplankton and accumulating nutrients in bottom colder water. On the contrary, in a semi-arid region most reservoirs managers seldom let water level rise enough to cause superficial overflow, and regulate water flow by smaller continuous discharges at mid-height of the dam wall.

While classifying reservoirs as Heavily Modified Water Bodies (HMWB) the WFD allows for hydro-morphological pressures upon their ecological status, pressures to which natural lakes are not subjected. Reference conditions for establishing the ecological potential of the HMWBs should be given by reference conditions for the ecological status of natural lakes of the same eco-region, but reference conditions bearing natural lakes in a semi-arid region are scarce. In fact, there are no natural lakes in Southern Portugal.

3.2 Ecological tools foreseen in the European Water Frame Directive

In the scope of the WFD implementation, the Guadiana watershed is included in the Mediterranean Region. The Geographical Intercalibration Group for this region (Med GIG) was responsible for establishing boundary values for the Med GiG Member State classification systems. Submitted values were adopted through the European Commission Decision of 30 October 2008 (2008/915/EC).

For their intercalibration exercise, the Med GIG agreed on using chlorophyll *a* and total biovolume as phytoplankton biomass indicative parameters, and elected three phytoplankton composition metrics, namely the contribution of cyanobacteria to total phytoplankton biovolume, the General Algal Index (GAI - Catalàn et al. 2003) and the Mediterranean Phytoplankton Trophic Index (MedPTI - Marchetto et al. 2007). Although recognizing strong limitations of the dataset used and the fact that not all tipologies of Mediterranean lakes and HMWBs were covered, actual law enforcement stipulates that ecological potential of the reservoirs in the Guadiana watershed should be classified, according to the proposed metrics. However, application of such phytoplankton composition metrics to CHABs management has yet to be assessed.

The following case study constitutes an effort to evaluate the Med GIG selected ecological indicators when applied to water management strategies for the Guadiana watershed. Different phytoplankton metrics determined for two reservoirs and compared in order to assess their usefulness in CHAB management, taking in consideration the EPA Guidelines for Evaluation of Ecological Indicators (Jackson et al., 2000)

3.3 Study area: Beliche and Odeleite reservoirs

Beliche and Odeleite reservoirs are located on two small affluent streams to the Guadiana estuary (Fig. 8), and were built for purposes of drinking water production. They are interconnected by an underground water channel, with sluices that are operated by the managing authorities, whenever they need to transfer water from Odeleite to Beliche reservoir. Together these reservoirs constitute the raw water source for 230,000 inhabitants of eastern Algarve, a province on the south coast of Portugal. Since Algarve constitutes an important national and international tourism destination, summer population more than

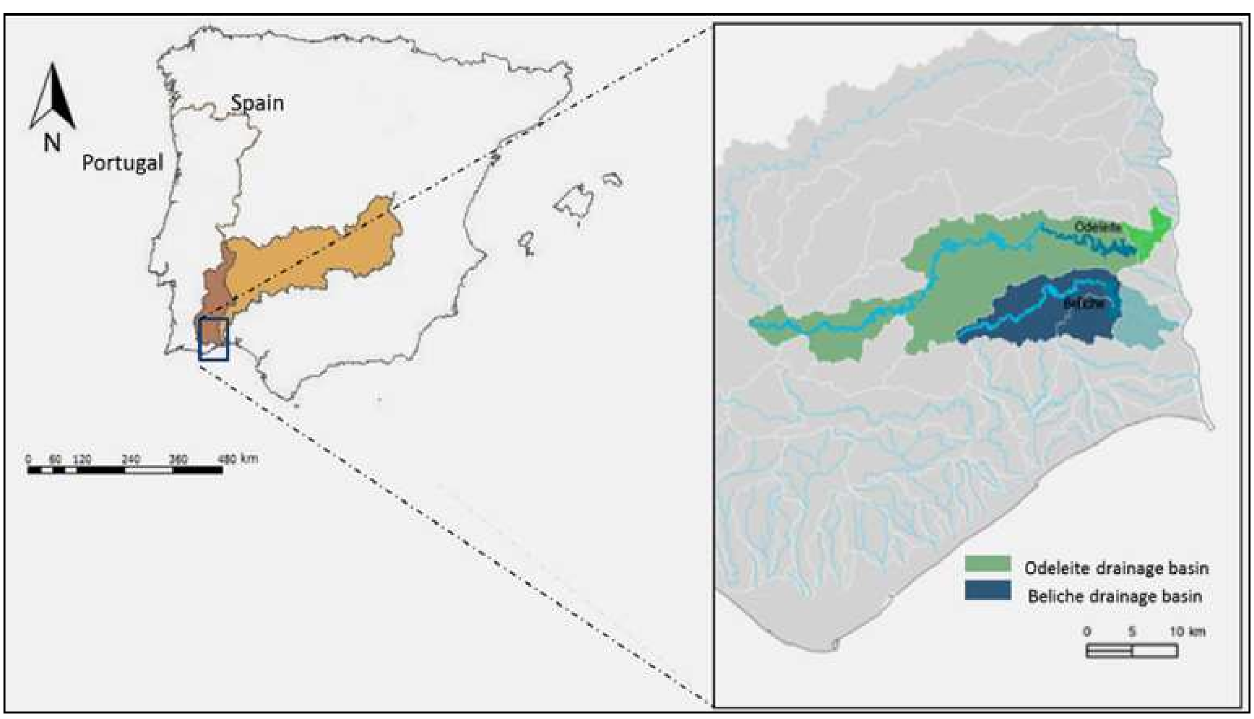

Fig. 8. Location of Beliche and Odeleite drainage basin

doubles and puts water demand at its peak during the high season (June through September), coinciding with low precipitation and high atmospheric temperature. Table 1 presents important features of these two reservoirs, where the most notorious aspects are the ones relative to their catchment area. While other reservoirs from the Guadiana watershed contributed to extensive development of irrigation (eg. Alqueva), promoted by the use of EU subsidies that encouraged high value intensive crop production (Varela Ortega et al. 1998, Varela-Ortega et al. 2003), the catchment areas of these two streams do not support any significant economic activity (Fig. 8).

Historical reasons linked to deforestation and farming mal-practices, back in the 30s of the XX century, led to extensive soil erosion, agriculture relinquishment and human desertification. Indeed, these catchment areas are the poorest counties in Portugal, with population densities around 20 habitants /Km^2. Human settlements are small villages concentrated downstream of the catchment area. In order to rehabilitate the landscape there has been a large investment in replanting pine woods with the objective of developing new soil and future stimulation of natural vegetation. Apart from some small goat herds in the Odeleite watershed and extensive cropping of sparse almond trees, there are no human impacts, no sewages, no pig style farms, no intensive or extensive farming. From a CHAB prevention point of view, it is difficult to point out what could be improved for the protection of their drainage basins.

Reservoir	Beliche	Odeleite
Stream	Ribeira de Beliche	Ribeire de Odeleite
Watershed	Guadiana	Guadiana
Catchment area (km^2)	98	347
Latitude (mean)	37º 16' 35''	37º 19' 52''
Longitude (mean)	-7º 30' 33''	-7º 29' 11''
Year of closure	1986	1996
Max. water column height (m)	30	30
Total volume (x 10^6 m^3)	48	130
Flooded surface (ha)	292	720
Mean annual precipitation (mm)	644	722
Min. stored water volume (x 10^6 m^3) / Date	7 / Sept 2006	40 / Nov 2005
Max. stored water volume (x 10^6 m^3) / Date	46 / May 2010	130 / May 2010

Table 1. Beliche and Odeleite reservoirs location and some main features. (Data source: http://snirh.pt/)

3.4 Methods

These reservoirs have been monitored for standard physical, chemical and microbiological water quality, including, since 2003, determination of phytoplankton biomass and composition. Monthly surface and bottom samples were taken, from 2003 to 2009, at Choça Queimada tower for Odeleite reservoir, and at the extraction tower of the Beliche reservoir. During 2009 and 2010, a new sampling site in the middle of the lake, 500m upstream from the dam wall, was added according to new guidelines from the European Med GIG (INAG, 2009). At each of these sampling sites, vertical profiles were determined *in situ* using a YSI

650 MDS probe, for water temperature, dissolved oxygen, pH and conductivity. Nutrient concentrations analysis were performed at the accredited (EN 17025) water analysis laboratory from Administração da Região Hidrográfica do Algarve (ARH Algarve), who was also responsible for all the sampling campaigns. Phytopigments including chlorophyll *a* were analyzed at Huelva University (Forján et al., 2008) through HPLC, according to Young et al. (1997). Microcystin detection and quantification was performed according to Carmichael & An (1999) using the micro-ELISA kit Microcystin Plate Kit from Adgen – Agrifood Diagnostic. Phytoplankton composition was determined by the same methods referred in 2.1. Phytoplankton biovolumes following European guidelines were calculated on the basis of predefined 3-dimensional shapes and their respective stereometric formulas as recommended by Edler (1979a, 1979b) and Hillebrand et al. (1999), according to the CEN/TC230/WG2/TG3 N108 Water Quality and Olenina et al. (2006).

Berger Parker dominance index was determined by calculating the proportion of the most abundant species over the total phytoplankton cell density (Magurran, 1988). Carlson Trophic State Index (TSI) was calculated for chlorophyll *a* values and both for total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations, according to Carlson (1977). Contribution of cyanobacteria to total phytoplankton is given by the percentage of total biovolume attributed to cyanobacteria. Catalán Index for Algal Groups (InGA) was determined by using biovolume proportions of colonial and non-colonial algal groups (Catalán et al., 2003). The MedPTI index was calculated according to Marchetto (2009).

3.5 Results and discussion

3.5.1 Hydrometric features

Monitoring data for the last 7 years included a severe 18 months drought from 2004 to 2006, and an exceptional rainy year in 2010. As seen in Fig. 9, water level at Odeleite reservoir had to be lowered in November 2006 for maintenance works, originating intense bottom and surface discharges. Surface overflow was released through the stream bed into the Guadiana estuary, but bottom discharged water flowed through the underground channel into Beliche reservoir, causing more surface and bottom discharges at this reservoir. Water volumes discharges at surface and at bottom of both reservoirs are enlisted in Table 2, revealing how water level regulation in both reservoirs is interconnected. Apart from water extraction for municipal consumption, no water outflow occurred from February 2004 to March 2006, during the drought (see Table 2). In fact, there is no ecological flow stipulated for these streams, such that, downstream from the dam walls, only estuarine water flows in during high tide.

Bottom discharges from Odeleite to Beliche, through the underground channel, induced mixing of water column, with ressuspension of sediment and nutrients. Indeed, both reservoirs did not behave as warm monomyctic lakes, but rather as artificially polimyctic, since water column mixed in the winter and also partially, whenever channel sluices were opened. Water level regulation and Beliche water withdrawal for drinking water were seemingly the main impacts on the water quality of these reservoirs. Nevertheless, sharp shifts in water level also affected margin vegetation, contributing to increased nutrient leaching from soils.

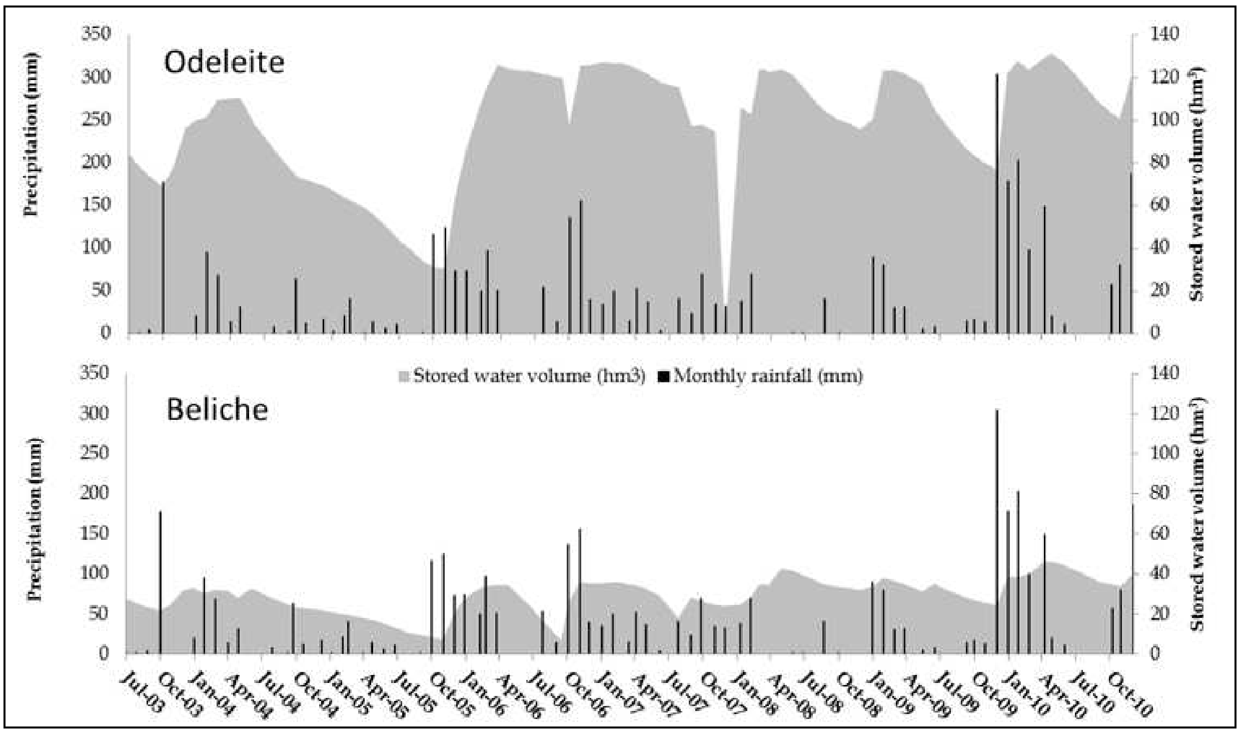

Fig. 9. Evolution of stored water volume (hm^3) and mean monthly rainfall from July 2003 to November 2010 in Odeleite and Beliche reservoirs. Data source: http://snirh.pt/ and http://www.drapalg.min-agricultura.pt/

Reservoir	**Odeleite**		**Beliche**	
Month/year	**Bottom discharge (dam^3)**	**Surface discharge (dam^3)**	**Bottom discharge (dam^3)**	**Surface discharge (dam^3)**
Jul/2003	0	0	13	0
Aug/2003	0	0	26	0
Sep/2003	0	0	13	0
Oct/2003	0	0	1	0
Nov/2003	1234	8722	1818	0
Dec/2003	3894	9821	382	0
Feb/2004	6648	0	1562	0
Mar/2006	5970	0	1409	0
Nov/2006	15196	19868	3536	1776
Dec/2006	8685	0	543	0
Feb/2007	2364	0	0	0
Aug/2007	74	0	0	0
Mar/2008	0	261	0	0
Apr/2008	6095	18388	0	1012
Feb/2009	8495	0	0	3502
Dec/2009	0	22450	0	5105
Dec/2010	0	29821	40	6714
Jan/2011	22528	0	0	2186
Mar/2011	50139	0	0	12577

Table 2. Surface and bottom water discharged from Odeleite and Beliche since 2003 (Data source: http://snirh.pt/)

3.5.2 Nutrient dynamics

Despite these hydrographical fluctuations, no nutrient accumulation or eutrophication trend was detected. Yearly turn-over of Dissolved Inorganic Nitrogen (DIN) and Soluble Reactive Phosphorus (SRP) was clear in Fig. 10, where water temperature at the surface can be used as reference for seasonal changes in nutrient dynamics in Beliche reservoir.

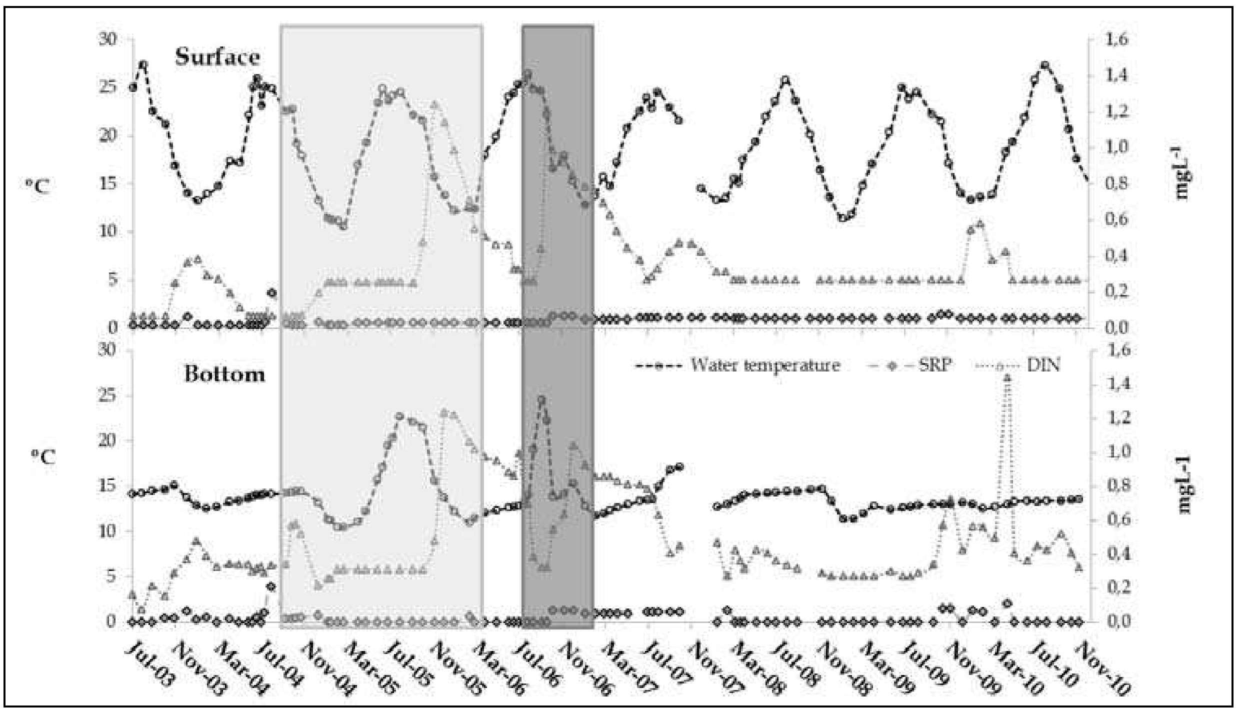

Fig. 10. Dissolved Inorganic Nitrogen (DIN; mg*L^{-1}), Soluble Reactive Phosphorus (SRP; mg*L^{-1}) and water temperature (ºC) during 2003 -2010 in Beliche reservoir. Drought months are highlighted in light grey. Months were unusual Odeleite to Beliche discharges occurred are highlighted in darker grey

The increase in DIN levels during the drought years was fictitious (see light grey box Fig. 10), since water level was so low that surface and bottom samples were almost undistinguishable. Shallower depth allowed for oxygen diffusion to the bottom inhibiting deep summer denitrification. Unusual surface and bottom DIN levels occurred in November 2006 due to exceptional water transfer from Odeleite to Beliche. As stated in Galvão et al, 2008, management of the underground channel between the two reservoirs has been associated with conditions favoring blooms through bottom sediment and nutrient resuspension. Consequent water column mixing was revealed by similar bottom and surface temperatures. Comparing Fig. 9 with Fig. 10 also indicated that the increase in DIN concentration in March 2010 was linked to high precipitation levels. Overall low nutrient concentrations in both reservoirs were associated with oligotrophic conditions. In spite of phosphorus limitations and low median and mode values for DIN:SRP ratios, high average N:P ratios were observed, due to outlier values observed in 2005, 2006 and March 2010.

3.5.3 Phytoplankton dynamics

In terms of cell abundance, more than 80% of monthly water samples during last eight years from Odeleite and Beliche reservoirs, were dominated by cyanobacteria (Fig. 11), but data gathered during 2009 and 2010 showed diatom (Bacillariophycea) dominance in terms of biovolume proportion (Fig. 12) in at least 50% of the samples.

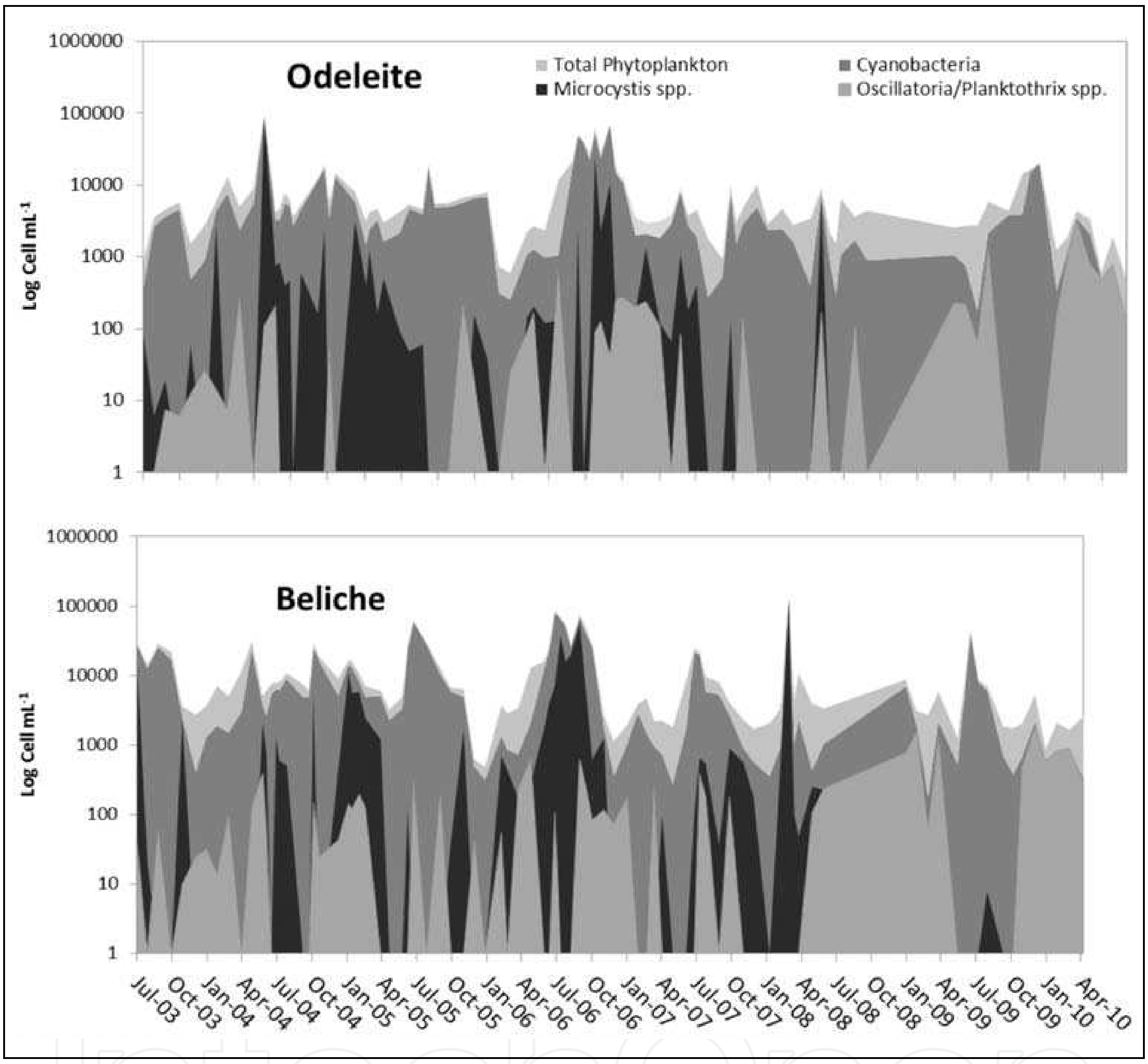

Fig. 11. Cyanobacteria dynamics in Odeleite and Beliche reservoirs. Total phytoplankton abundance (Log cells mL^{-1}) compared with total Cyanobacteria, *Microcystis* and *Oscillatoria/Planktothryx* spp. abundances

In terms of cell abundance, *Microcystis* spp. dominated both reservoirs until spring 2008, but Oscillatoriales dominated in terms of biovolume. Cyanobacteria cell densities above WHO alert level 1 of 2000 cells mL^{-1}, occurred in 62 to 63% of all samples, with episodes of *Microcystis* blooms in June 2004 for Odeleite and July 2004 for Beliche. In summer 2006, a *Microcystis* spp. bloom was toxic with microystin concentrations at the bottom of Beliche reservoir reaching 3.5 µg L^{-1}. Despite high cyanobacteria abundances, no significant levels of microcystins were detected under other bloom situations.

Biovolume proportions for main algal groups (Fig. 12) confirmed cyanobacterial dominance from August to October 2009 in Beliche and from October to December 2009 in Odeleite.

Summer bloom absence in 2010 could be linked to high water discharges in consequence of an exceptional rainy winter and spring. (see Fig. 9 and Table 2).Thus, during the study period both Beliche and Odeleite reservoirs were susceptible to CHABs.

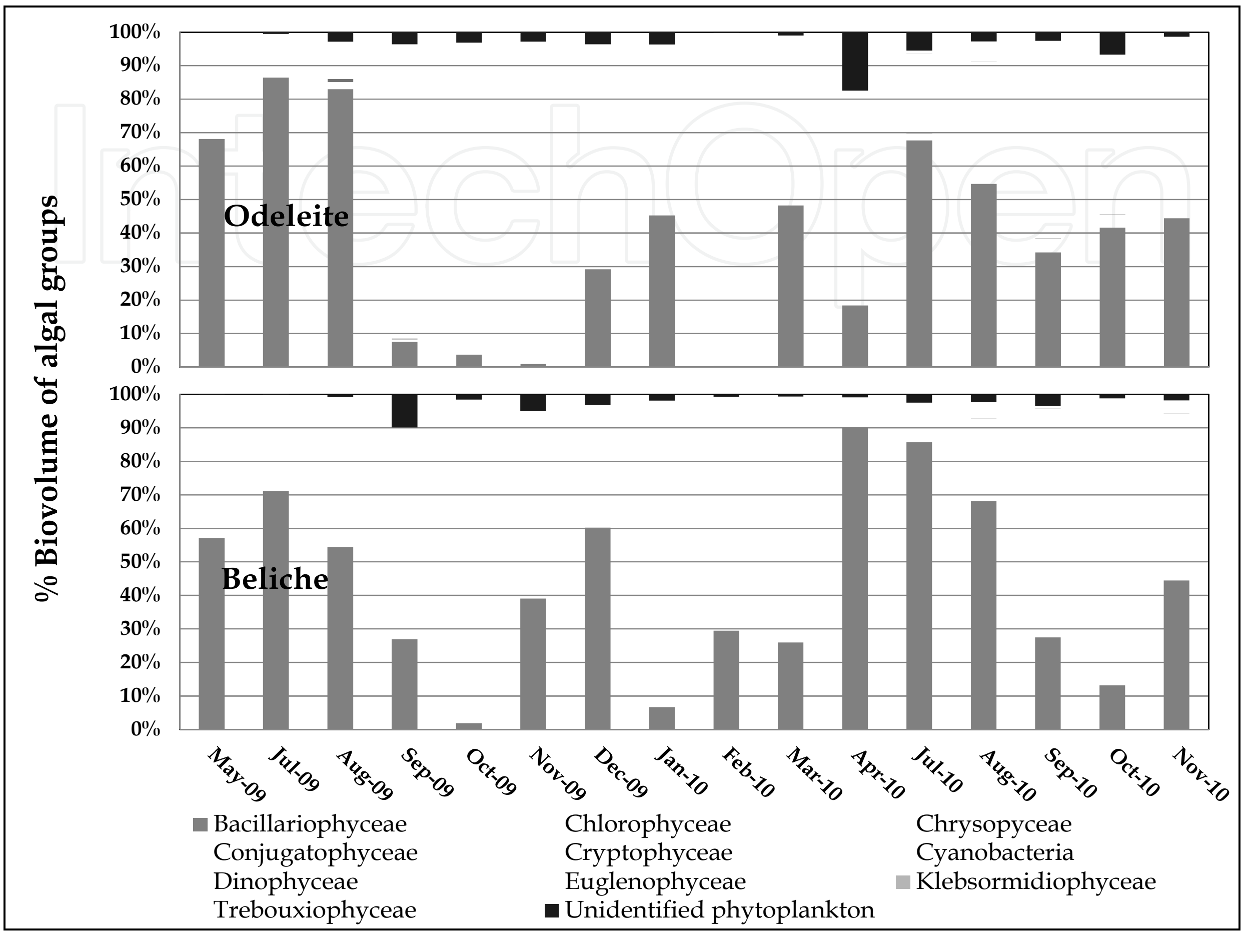

Fig. 12. Relative contribution (%) of main algal groups to total phytoplankton biovolume

3.5.4 Ecological indices

Table 3, compiles Carlson Trophic State Indices (TSI) calculated for Beliche and Odeleite based on Chlorophyll *a* (Chl-a) and on total phosphorus (TP) as well as for phytoplankton ecological index (MedPTI) proposed by Marchetto et al. (2009) for Italian deep lakes in the Mediterranean region. This index is based on the proportion of biovolumes of species, listed in Italian lakes, and should not be applied in situations where the biovolume of listed existing species does not exceed 70% of total phytoplankton biovolume. Since the contribution of Marchetto species to total phytoplankton biovolume reached 77% in Beliche during 2010 and 71% in Odeleite, the MedPTI index was also calculated for comparison. TSI based on transparency measured by Secchi depth was not calculated, since it has long been established that torrential hydrographic regimes promote high values for this index without any correlation with eutrophication. Total Phosphorus concentrations were also misleading, since bottom sediment resuspension promoted by artificially induced polimyctic behavior, released organic phosphorus unavailable for phytoplankton into the water column. Low chlorophyll *a* values in spite of high cellular cyanobacteria abundance, as referred previously, was due to low chlorophyll content of cyanobacteria.

Monthly values for the MedPTI index are illustrated in Fig. 13, with open circles and boxes representing non-valid values based on lower than 70% species contribution to total phytoplankton biovolume. This figure constituted a test to the robustness of the MedPTI index applied to Beliche and Odeleite.

A)

Reservoir/Index	TSI (Tota P)	TSI (Chl-a)	MedPTI
Beliche	30	47	3,05
Odeleite	19	47	2,90

B)

TSI classes	<30	30-40	40-50	50-60	60-70
MedPTI classes and upper limits	excelent	high – good (<2.77)	good – moderate (<2.45)	moderate – poor (<2.13)	poor – bad (1.81)

Table 3. A) Determined values for Carlson Trophic State Index (TSI) based on total P concentrations and on chlorophyll a content, and for phytoplankton composition MedPTI index. B) Classification boundary values for TSI and MedPTI with a color code to facilitate interpretation

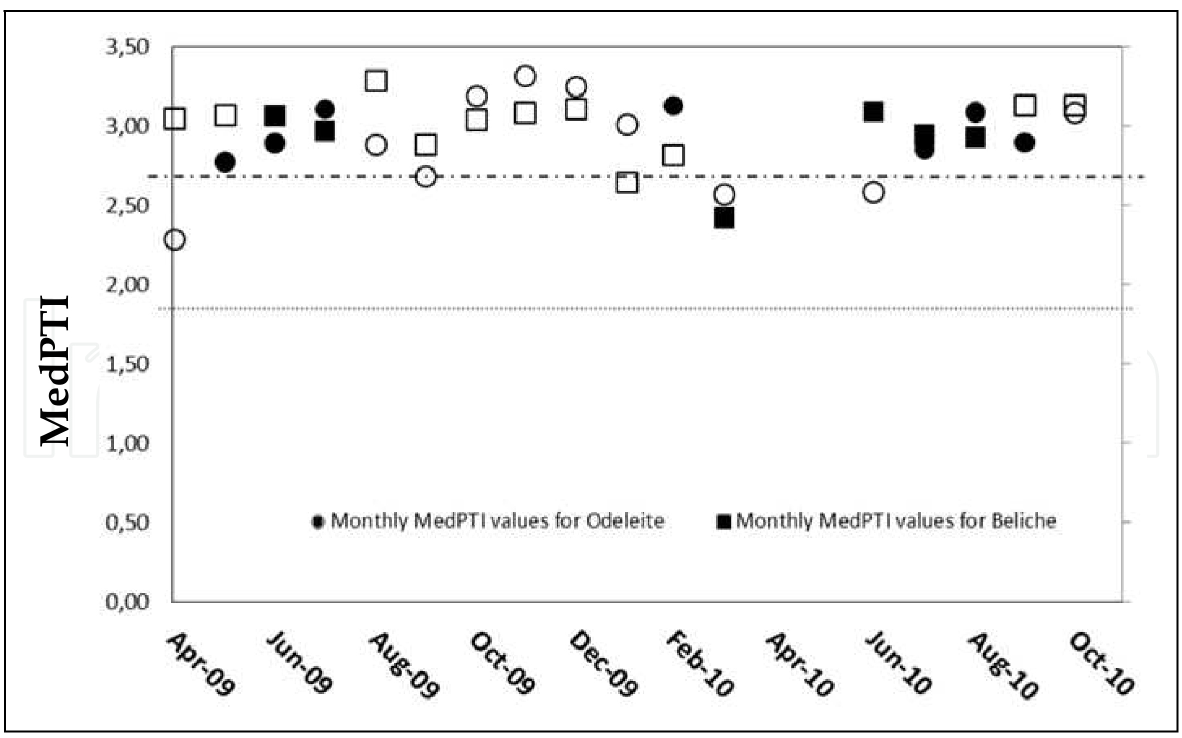

Fig. 13. Monthly values for MedPTI. Dashed lines indicate lower boundaries of "excellent" and "good" classifications. Open circles and boxes correspond to non-valid values for Odeleite and Beliche, respectively. See text for explanation

Since MedPTI was specifically developed for deep natural lakes in Italy, this index should probably not be applied to reservoirs without adjustments to existing species lists. Nevertheless, MedPT1 classifications obtained in this study seemed more consistent than those obtained using General Algal Group Index (InGA) proposed by Catalàn et al (2003).

Table 4 compiles values for InGA Catalàn et al. 2003). These authors recommended that the use of this multi-metric index of phytoplankton composition for ecological status classification should be calculated for late summer and fall samples. Trophic state metrics, as recommended by several water authorities in Portugal and in Europe, apply a color code for easy comparison of different classifications, which was used in Tables 3 and 4. In the case of Beliche and Odeleite reservoirs, fall values represented a worst case scenario and artificially attributed a good or moderate classification to waters that would otherwise be recognized as very good. Fig. 14 illustrates monthly variability of Catalàn InGA values.

Period	Beliche	Odeleite	InGA limits	InGA classes
2009-2010	0.167	0.237	>0.1	very good
2009	0.098	0.067	0.01-0.1	good
2010	0.196	0.403	0.005-0.01	moderate
October 2009	0.022	0.008	0.003-0.005	poor
October 2010	0.536	1.864	<0.003	bad

Table 4. Catalàn InGA values determined for Beliche and Odeleite reservoirs for several groups of samples

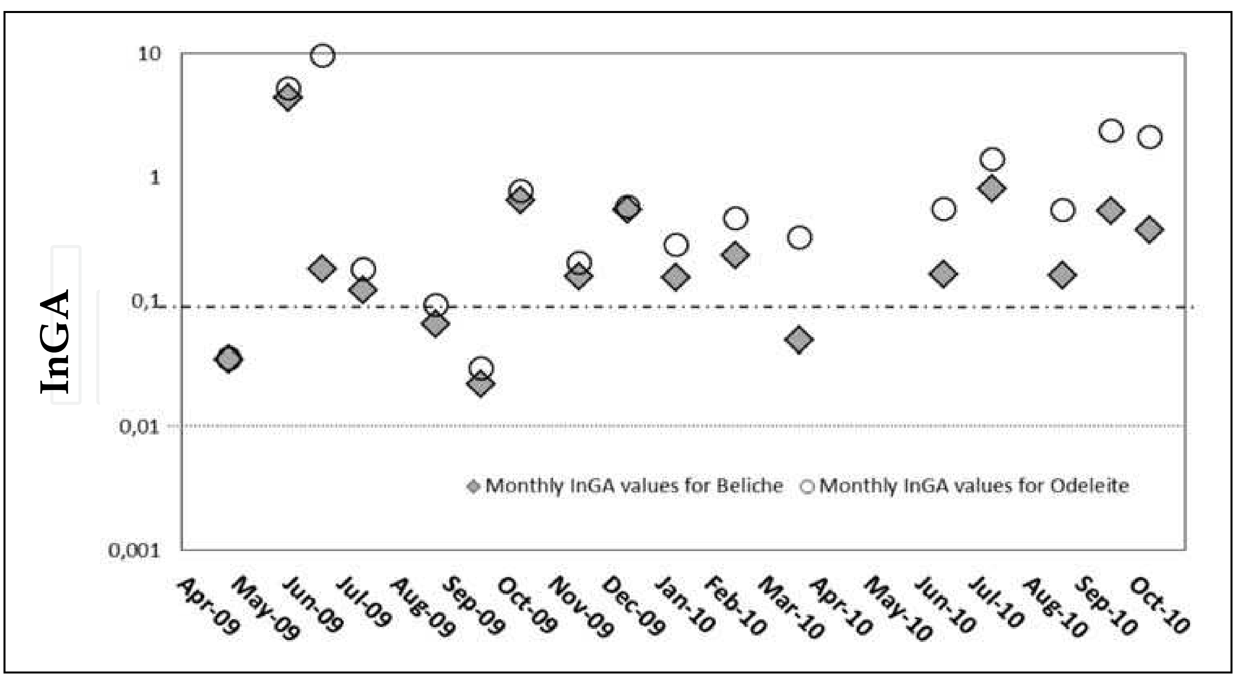

Fig. 14. Monthly values of the General Algal Groups Index (InGA) determined for Beliche and Odeleite Reservoir 2009-2010 data. Dashed lines indicate the lower limits for Very Good (0,1) and Good (0,01) classification

As discussed by Jackson et al. (2000), an index that does not allow for temporal and spatial variability, might lose reliability. The decreased InGA value calculated for summer 2009 (Fig. 14), reflected the weight of 4 given to cyanobacterial biovolumes in the index formula (Eq. 2)

$$\mathrm{InGa} = \frac{1 + 2 * (\mathrm{D} + \mathrm{Cnc}) + \mathrm{Chnc} + \mathrm{Dnc}}{1 + 0.1 * \mathrm{Cr} + \mathrm{Cc} + 2 * (\mathrm{Dc} + \mathrm{Chc}) + 3 * \mathrm{Vc} + 4 * \mathrm{Cia}} \quad (2)$$

D	Dinophyceae	Cc	Chrysophyceae colonial
Cnc	Chrysophyceae non colonial	Dc	Bacillariophyceae colonial
Chnc	Chlorococcales non colonial	Chc	Chlorococcales colonial
DnC	Bacillariophyceae non colonial	Vc	Volvocales colonial
Cr	Cryptophyceae	Cia	Cyanobacteria

In fact, whenever a lake or reservoir has a cyanobacterial bloom, InGA value will indicate it, but so will simply calculating the relative contribution of cyanobacteria to total phytoplankton biovolume.

Another problem emerging from the application of multi-metric indices is the loss of interpretability. With InGA, high contributions to total biovolumes of Dinophyceae and non- colonial phytoplankton groups are assumed to improve the ecological status, whereas colonial forms and cyanobacteria worsen it. Thus, high biovolume proportions of non-toxic Chroococcales, are given the same negative weight as toxic filamentous cyanobacteria.

It is well known and accepted that different metrics applied to the same ecological condition can attribute different classifications. This is obviously linked to the information provided by the variables selected in each metric analysis. Criteria for the selection of these metrics should consider the prerequisites previously mentioned (see section 1.), such as: (i) obtainability, (ii) relevance in term of specific objectives, (iii) discriminant capacity, (iv) allowance of natural variability and (v) reliability.

Results obtained for dominance (Fig. 11) and cyanobacterial contribution (> 9%) to total phytoplankton biovolume, (Fig. 12), indicate eutrophication under WFD guidelines (JRC EC, 2009). Considering the oligotrophic state of both reservoirs, the reliability of such indicators should be reevaluated, at least for warm semi-arid regions.

Solimini et al. (2006) considered the contribution of cyanobacteria to total phytoplankton biomass, as a reliable and simple indicator of trophic state, based on the following assumptions: (i) most cyanobacteria species show a strong preference for eutrophic conditions, (ii) due to toxicity of some taxa, blooms can pose serious water quality, animal and human health risks, as well as environmental problems, and finally, (iii) the large contribution of cyanobacteria blooms to phytoplankton biomass.

This study contradicts the first assumption, since the genera and species typically linked to eutrophication were found associated to oligotrophy. The second assumption was partially verified, but toxin production seemed to be limited in oligotrophic conditions, despite high cell abundances. Potentially toxic cyanobacteria do not always produce cyanotoxins, so toxicity needs to be confirmed. Finally, the last assumption which links eutrophication to large contribution of cyanobacteria to phytoplankton biomass, should be re-assessed in Mediterranean regions, where, even under oligotrophic conditions, cyanobacteria are favoured in naturally warm waters.

3.6 Conclusion

This case study applied ecological multi-metric indices recommended by the European Commission (2008/915/EC) to two reservoirs of the Guadiana watershed, which have repeatedly developed cyanobacteria blooms in the summer, in association with high hydraulic retention. Values for the ecological potential measured by these indices ranged from Bad (> 9% contribution of cyanobacteria biovolumes) to Good (InGA) and Very Good (InGA and MedPTI). However, these indices do not provide insight on appropriate CHAB prevention and mitigation measures. Instead, long term monitoring of ecological data, was necessary to propose appropriate countermeasures. In fact, in these two reservoirs the only effective measure to prevent or mitigate CHABs was to reduce water residence time by discharging surface water.

4. Final considerations

The long-term study of the Guadiana estuary revealed unforeseen impacts in the aquatic microbial ecology after completion of the large Alqueva dam, causing in particular the collapse of natural cyanobacteria populations in the upper estuary. The sharp decline in photosynthetic prokaryotes, as well as in the phytoplankton community, could be attributed to overall perturbations in estuarine circulation, rather than any single or combined environmental drivers. Thus, regulation of dam discharges to maintain ecological river flow is essential to maintain estuarine primary productivity, using such ecological tools as abundance and diversity of cyanobacteria as sensitive indicators of "good" estuarine water quality. However, national environmental agencies and water resource authorities have yet to apply adequate ecohydrological approaches to river flow and dam discharge management, while policymakers seemingly lack the political will to enforce ecological river flow in Portuguese legislation.

On the other hand, monitoring of Beliche and Odeleite freshwater reservoirs assessed the usefulness of different ecological indicators. Aquatic ecologists have long presented a plea (e.g. Margaleff, 1974; Reynolds, 2002) for a better understanding of phytoplankton composition and dynamics in ecological studies. Multi-metric phytoplankton indices, such as recommended by the European Commission (2008/915/EC), attempt to translate complex biological information into user-friendly ecological classifications. These EC metrics might be useful for water policy purposes, but do not seem to have any utility in CHABs management. Ecological tools should clearly indicate the need for prevention or mitigation measures for CHAB management, which multi-metric indices fail to do. Instead, adequate ecological tools should rely on long-term multi-variate studies, which address the complexity of aquatic ecosystem function and dynamics.

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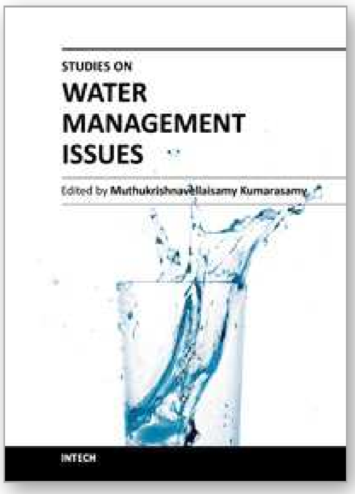

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This book shares knowledge gained through water management related research. It describes a broad range of approaches and technologies, of which have been developed and used by researchers for managing water resource problems. This multidisciplinary book covers water management issues under surface water management, groundwater management, water quality management, and water resource planning management subtopics. The main objective of this book is to enable a better understanding of these perspectives relating to water management practices. This book is expected to be useful to researchers, policy-makers, and non-governmental organizations working on water related projects in countries worldwide.

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