

1 Article

# 2 Monitoring of an urban lake in the Mediterranean 3 coast after restoration measures

4 **María-Teresa Sebastiá-Frasquet**<sup>1</sup>, **Jesús Pena-Regueiro**<sup>1</sup>, **José-Andrés Sanchis-Blay**<sup>1</sup>, **Ferran**  
5 **Llario**<sup>1</sup>, **Miguel Rodilla**<sup>1</sup> and **María Pachés**<sup>1,\*</sup>

6 Received: date; Accepted: date; Published: date

7 Academic Editor: name

8 <sup>1</sup> Universitat Politècnica de València; Camino de Vera s/n, 46022 Valencia (Spain)

9 <sup>1</sup> \* Correspondence: mtsebastia@hma.upv.es; Tel.: +34-628-760-811

10 **Abstract:** Urban lakes are artificial systems that accomplish many functions, such as storing rainwater, avoiding flooding  
11 of adjacent urban areas and supporting recreational activities. However, their intrinsic aesthetic value is usually reduced  
12 due to eutrophication problems and anoxia processes. The objective of this study is to present the results of the water  
13 quality monitoring of a small urban lake (11264 m<sup>2</sup> and 1.5 m average depth) in Tavernes de la Valldigna (Valencia, Spain)  
14 during summer 2016. The final aim is to determine the better parameters for monitoring urban lakes having into account  
15 budget restrictions. La Goleta lake has suffered repeated events of fish deaths and bad odors that cause the alarm of  
16 residents and tourists, especially in summer. Municipal authorities undertook a restoration project which first part was  
17 developed during the first semester of 2016. Surveillance monitoring should be financed by the Town Council, so limiting  
18 the monitored parameters to the most appropriate ones is key for guarantying long-term surveillance. The results of this  
19 study show the importance of macrophyte community in determining water quality and maintaining dissolved oxygen  
20 levels. Dissolved oxygen is a key parameter easy to measure and a good indicator of lake water quality evolution.  
21 Analytical methodologies must be adapted to the high organic matter content of these systems to avoid interferences.

22 **Keywords:** storm tank; water quality; nutrients, phytoplankton, macrophytes

23 **PACS:** J0101

24

## 25 1. Introduction

26 Urban lakes have been described as smaller and shallower water bodies than natural lakes, with  
27 a larger ratio of watershed area to lake surface area [1]. This causes a greater exposition of urban lakes  
28 to human impacts. Eutrophication issues have been well studied in natural lakes and the effects of  
29 harmful algal blooms (HABs) have become a growing concern for water resources management.  
30 However, studies focussing on urban lakes are rare and scientists have pointed out the need of a  
31 deeper knowledge of their ecological dynamics to develop effective management strategies [1].

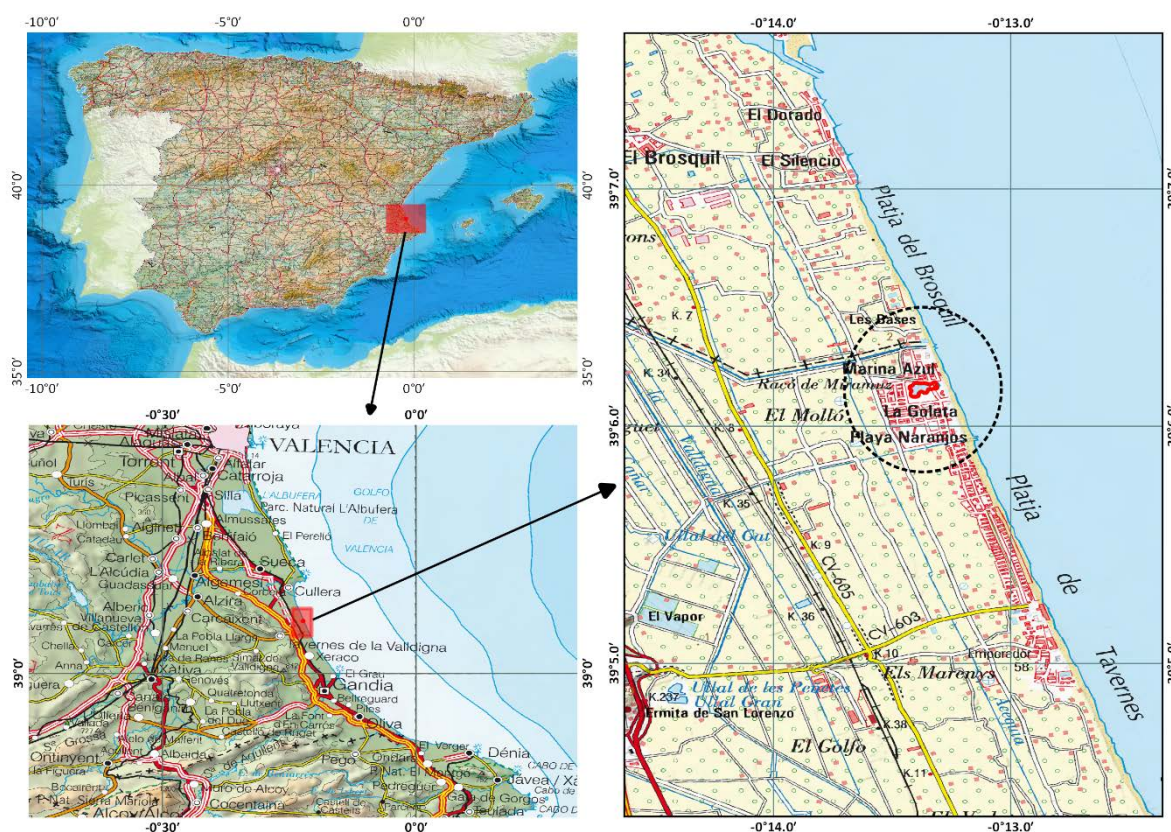
32 Cities benefit from internal urban ecosystems, such as urban lakes thanks to the ecosystem  
33 services that they offer [2]. Direct ecosystem services of urban lakes can be rainwater drainage,  
34 storing rainwater, water supply, recreational and cultural values. The locally generated ecosystem  
35 services have a substantial impact on the quality-of-life in urban areas [2]. To preserve these services  
36 we need to be sure that management strategies are effective, thus, we need effective monitoring  
37 programs able to detect relevant water quality changes.

38 Study area

39 La Goleta Lake is an urban lake located at Tavernes de la Valldigna town (Eastern Spain) (Figure  
40 1). It works as storm tank that collects runoff of nearly 200,000 m<sup>2</sup> of urban area. Its current  
41 dimensions are 11,264 m<sup>2</sup> and 1.5 m average depth. For a complete description see [3]. This town is a  
42 very important touristic destination in the Spanish Mediterranean area. In fact tourism is one of the  
43 main economic activities in the area and is mainly based on residential development [4], it  
44 experiences an important population increase during summer. Since its construction in 1982, the lake

45 has suffered repeated events of fish deaths and bad odours that cause the alarm of residents and  
 46 tourists. So municipal authorities worried by the environmental health risk and the economic impact  
 47 on tourism industry decided to undertake a restoration project. The first phase of the lake restoration  
 48 was developed during the first semester of 2016. A closed circuit for recirculating water was built  
 49 with element such as fountains and waterfalls to increase water aeration. Also, UV clarifiers were  
 50 coupled to the recirculation system. For more details on these restoration measures see [3].

51 During construction of the recirculation system, the water level of the lake was lowered by  
 52 pumping water to the sea. The penetration of sunlight to the bottom of the lake allowed the  
 53 development of a benthic substrate dominated by the green algae *Chara* sp. It has been described that  
 54 Characea occurs in shallow parts of lakes (0.5-2.5m), provided that water quality has sufficiently  
 55 improved and enough light penetrates to the soil [5]. The observed recolonisation process was only  
 56 possible due to very transparent circumstances during works. Other observed changes after  
 57 restoration works was an important increase in the population of the little fish *Gambusia* sp. and the  
 58 disappearance of mosquito larvaes. During this restoration works bigger fishes disappeared from the  
 59 lake.  
 60



61  
 62 **Figure 1** Location of the study area (La Goleta lake, Eastern Spain)

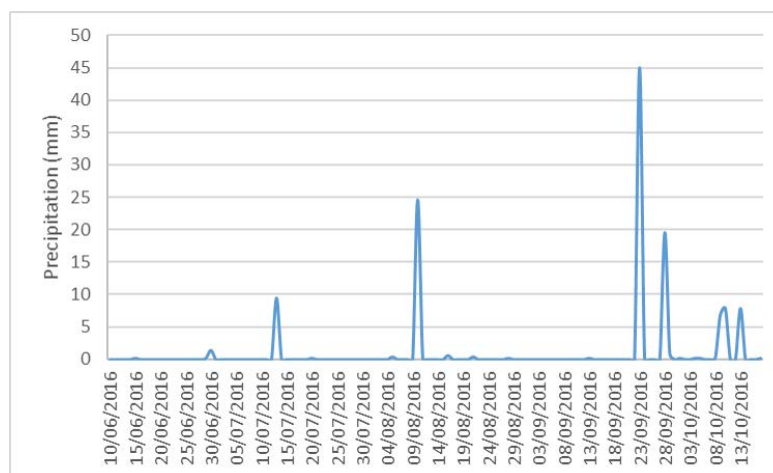
63  
 64 **Aim of the work**

65 The objective of this study is to present the results of the water quality monitoring of La Goleta  
 66 lake (Valencia, Spain) measures from June to October 2016 after the implementation of the first phase  
 67 of restoration. The final aim is to determine the better parameters for monitoring urban lakes having  
 68 into account budget restrictions.  
 69

70 **2. Results**

71 La Goleta lake was usually sampled at the same hour between 9:00 and 10:00h a.m. The sampling  
 72 frequency was biweekly, but was increased after precipitation events because of the well-known

73 effect of the first flush. The first flush phenomenon has been defined as “the initial period of  
 74 stormwater runoff during which the concentration of pollutants is substantially higher than during  
 75 later stages” [6]. The enormous quantity of pollutants discharged in this period into the receiving  
 76 waters has been identified as one of the leading causes of degradation in the quality of receiving  
 77 waters [6]. In figure 2 we can observe the precipitation events during the study period.  
 78



79  
 80 **Figure 2** Precipitation chart during study period. Data source: <http://riegos.ivia.es/datos-meteorologicos>

### 81 2.1. Physical and chemical parameters

82  
 83 Temperature ranged from 30.5 to 22.1 °C, the lowest temperature was observed during the  
 84 October 2016 samplings. Conductivity varied between 2.27 and 5.09 mS, the lowest values were  
 85 observed on October after the cumulative rain of September and October. Average evaporation in  
 86 this area during the study period was 4.3 mm according to meteorological data from the Tavernes de  
 87 la Vallidigna station (source: <http://riegos.ivia.es/datos-meteorologicos>). Dissolved oxygen minimum  
 88 observed value was 1.06 mg/L and the maximum value was 8.32 mg/L. The percentile 40 of dissolved  
 89 oxygen was 4.2 mg/L, meaning that 40% of the measures were lower. Ammonia concentration ranged  
 90 between non-detectable concentrations (< 0.01mg/L) to a maximum of 0.14 mg/L. To avoid toxicity  
 91 problems ammonia concentration should be lower than 0,05 mg/L [7]. This value was exceeded 3  
 92 times on June 14, October 10 and October 17. Dissolved inorganic nitrogen (DIN) ranged from 0.04  
 93 to 0.46 mg/L. Dissolved inorganic phosphorus (DIP) varied between 0.001 and 0.037 mg/L, dissolved  
 94 silicate (DSi) between 0.14 and 1.31 mg/L and total phosphorus (TP) between 0.02 and 1.16 mg/L. In  
 95 order to better define potential nutrient control, we compared nutrient ratios between DIN, DSi and  
 96 DIP concentrations with Redfield ratios (Si:N:P = 16:16:1). DIN was the potentially most deficient  
 97 nutrient for phytoplankton growth during nearly all of the study period. Chemical oxygen demand  
 98 (COD) and Biochemical oxygen demand (BOD<sub>5</sub>) measured on samples taken on October 17 were 36  
 99 and 3 mg/L respectively.

### 100 2.2. Phytoplankton abundance and composition

101 Total chlorophyll *a* (Chl-*a*) concentration ranged from 1.1 to 18.9 µg/L.

102  
 103 In summer campaigns (June to August) the pico and nano phytoplankton composition were  
 104 dominated by *Monoraphidium* sp. and nanoplanktonic flagellates identified as *Cryptomonad* sp. Other  
 105 taxa like *Tetraedron* sp. and *Closterium* sp. belonging to Chlorophyceae were also identified as well as  
 106 *Cyclotella* sp (diatoms) although in a lower cell density. On the other hand, the microplankton  
 107 composition correspond to different genera of dinoflagellates like *Scrippsiella* sp., *Alexandrium* sp.  
 108 *Gonyaulax* sp. and *Gymnodinium* sp. and to centric diatoms such a *Navicula* sp., *Amphora* sp. and some  
 109 pennate like *Pseudonitzschia* sp. The phytoplankton richness and diversity in this season was that  
 110 corresponding to natural ecosystems (Shannon index of 2.5).

111 In autumn campaigns (October) only two taxon were the most abundant in plankton  
112 composition, the chlorococcal species of *Chlorella* (78%) and *Scenedesmus* (17%). Dinoflagellates,  
113 diatoms and Cryptophyceae taxons represented less than 5% with a Shanon index of 1.

### 114 3. Discussion

115 Conductivity was relatively high and was attributed to high evapotranspiration rates (4.3 mm)  
116 and proximity to the sea (sea breeze). Decreases in conductivity occurred after precipitation events.  
117 Dissolved oxygen was a critical parameter of water quality, with concentrations below 4.3 mg/L 40%  
118 of the times studied. The effect of oxygen depletion was observed on fishes living in the lake,  
119 *Gambusia* sp., which were observed staying at the most shallower depth. However, no mortality was  
120 observed. Ammonia levels reached toxic concentrations for fishes three times, but again no mortality  
121 was observed. Previously reported fish mortalities affected big fishes such as *Mugil cephalus*.  
122 *Gambusia* sp. can tolerate a wide range of conditions [8] and that may be the cause of no observed  
123 mortality even after low oxygen and high ammonia conditions. These fish can be highly beneficial to  
124 humans through controlling mosquitoes, which is an important feature given the tiger mosquito  
125 plague that is now expanding on Mediterranean areas. Tough these fish may have negative impacts  
126 on other species with which they interact, such as the *Valencia hispanica* or *Aphanius iberus*, these  
127 species are not present in this artificial water body, so the advantages outweigh the disadvantages of  
128 their presence at La Goleta lake. *Chara* sp. dominant in the benthic substrate prefer low oxygen  
129 waters, and played an important role in DO levels. The lowest oxygen levels were observed early in  
130 the morning due to nocturnal respiration.

131 Nitrogen was the potentially most deficient nutrient for phytoplankton growth during most of  
132 the study period according to Redfield ratio. This Mediterranean area suffers from high nitrate levels  
133 due to agricultural activity [9]. However, the lake watershed is urban [3] so agriculture is not an  
134 important source of nitrate. Phosphorus levels were also high and usually it was not the main  
135 potentially limiting nutrient. Phosphorus is present in first-flush, however, aquatic birds excretion  
136 can be considered the main source of both nitrate and phosphorus. The estimated proportion of  
137 nitrate to phosphorus in aquatic birds faeces is 2.1 [10], which can explain phosphorus not being  
138 limitant. This organic matter accumulated in the sediment can produce a high diffusive flux to the  
139 water column, as it has been observed for both ammonia and phosphorus during summer seasons in  
140 other urban lakes [11]. Silica levels were high because the lake is adjacent to a sandy beach, so silica  
141 potentially limiting circumstances were scarce.

142 Chemical oxygen demand (COD) and Biochemical oxygen demand (BOD<sub>5</sub>) were determined to  
143 measure the amount of organic compounds in water. BOD estimates biodegradable organic matter,  
144 while COD is less specific, since it measures everything that can be chemically oxidized. La Goleta  
145 lake reduced values of BOD<sub>5</sub> indicates that there is an important fraction of non-biodegradable  
146 compounds. This has been related with the source of water from first-flush which is rich in  
147 hydrocarbons and oils [6]. In this area, also we can find pesticides that are used in the in the garden  
148 surrounding the lake. The presence of organic matter in the water column conditioned the choice of  
149 the nitrate analysis method. Brucine methodology was chosen because APHA methods were not  
150 appropriate for waters enriched by organic matter.

151 The dominant phytoplankton groups are characteristic of shallow water bodies, in mixed and  
152 nutrient enriched conditions. In this sensitive Mediterranean ecosystem where the water temperature  
153 is not a limitant factor an increase in nutrient content yields bloom-forming cells reducing the  
154 plankton diversity and performing a bottom-up control. Although during the whole study period  
155 there are species sensitive to forming blooms (*Pseudonitzschia* sp., *Alexandrium* sp., etc), only when  
156 nutrient load increases, for instance after precipitation events, this occurs.

157 In this study we have focused on water quality after the first phase of La Goleta lake restoration  
158 measures. We consider that the most appropriate parameters that should be mid-term monitored are  
159 dissolved oxygen, ammonia and total chlorophyll *a*. These variables are easy to measure and the  
160 information that they give us for water lake management very important. We also think that  
161 monitoring phytoplankton blooms is clue as fish mortalities that could not be explained by oxygen

162 depletion or ammonia toxicity could be due to phytoplankton toxins. [12] monitored cyanobacteria  
163 blooms and the associated cyanotoxin production for 14 months on a monthly basis in an urban lake.  
164 However, we believe that this frequency may be not enough due to the high temporal variability that  
165 characterizes phytoplankton. So, we recommend to the management authorities (the Council in this  
166 case) to analyse phytoplankton when a bloom is detected by high chlorophyll *a* levels. [1] suggested  
167 to monitor chl *a* and TP concentrations as the most critical water quality variables for eutrophic lakes.  
168 We recommend for urban lake monitoring the diagnosis of the main limitant nutrient, because this  
169 can strongly influence the phytoplankton composition and abundance. At present, La Goleta lake  
170 shows a higher potential limitation of nitrate rather than phosphate limitation.

171 Runoff from the watershed exerts significant influence on urban lakes and thus inflow nutrient  
172 reduction is critical for the control of eutrophication [1]. Thus, the second phase of La Goleta lake  
173 restoration measures will target pollutants and organic matter inflow. Sediments are an internal  
174 nutrient source that will be also targeted, the mitigation with macrophytes such as vetiver is planned.  
175 After second phase of restoration measures sediments quality will also be monitored. Degraded  
176 features of water quality include high accumulation rates of oxygen-demanding reduced by products  
177 of anaerobic metabolism on sediments [e.g., methane (CH<sub>4</sub>) and hydrogen sulfide (H<sub>2</sub>S) [13]. These  
178 products can be a net carbon source to the atmosphere with a net effect on greenhouse gas emission  
179 [14]. The disturbance caused to residents by bad odours is one of the recurrent claims to the Council,  
180 as it may happen in any other urban lakes, so we recommend to monitor the levels of this gasses too.  
181

#### 182 **4. Materials and Methods**

183 Water samples were taken at the 2 sampling points shown in Figure 2. These points were selected  
184 after diagnosis sampling of the lake [3]. Only one water sample was collected at 0.05 m depth in each  
185 point, representative of the whole water column due the lake shallowness. Water samples were  
186 collected in 2 L polyethylene bottles. A subsample of 250 mL was removed for phytoplankton cell  
187 counts. Water samples were kept in a cool box (4 °C) and transported to the laboratory. Temperature,  
188 conductivity and dissolved oxygen were determined in situ by means of a data logger PCE-PHD 1.  
189



Figure 3 Sampling points at La Goleta lake

190

191

192

193

194

195

196

197

198

199

200

201

202

203

204

205

206

207

208

209

210

211

212

213

214

215

216

217

218

219

At the laboratory, the water samples were divided into several equal parts, following the conservation procedures suggested by [15]. The samples were filtered through 0.45  $\mu\text{m}$  cellulose acetate membrane filters for nutrient and chlorophyll *a* analyses.

Chlorophyll *a* content was determined with the trichromatic method based on visible spectroscopy [15], using [16] equations to obtain the concentration. Pigment extraction was performed with acetone 90%.

The following nutrients were analysed in all the samples: nitrate, ammonium, dissolved inorganic phosphorus (DIP), dissolved silicate (DSi) and total phosphorus (TP). Nutrients were analysed colorimetrically using the methods of [17]. Nitrate was analysed using the Brucine Method [18]. Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate, nitrite and ammonium. Chemical oxygen demand (COD) and Biochemical oxygen demand ( $\text{BOD}_5$ ) were analysed once to estimate organic pollution of water.

In order to analyse the phytoplankton communities both epifluorescence and Uthermhol microscopic counting methods were used. Epifluorescence was used to identify the pico and nanoplanktonic cells size [19]. Samples contained in 250 mL glass bottles were fixed with glutaraldehyde until reaching a final concentration of 2% [20]. Samples were filtered with Millipore GTPP membranes (pore size 0.2  $\mu\text{m}$ ). Finally, a cover glass was placed on top of the filter [21]. The counts were performed by epifluorescence microscopy [22] with a Leica DM 2500, using the 100 $\times$ -oil immersion objective. A minimum of 300 cells was counted and at least 100 cells of the most abundant species or genera were counted with an error under 20% [23]. Uthermhol was used for micro and macroplanktonic cell size. Phytoplankton samples were fixed with formaldehyde, concentrated according to UNE EN15204:2006, based on [24], and qualitatively examined under a LEICA DM IL inverted microscope. The Shannon-Weaver index ( $H'$ ) was calculated according to [25]

**Acknowledgments:** Authors would like to thank Barbara Ramos, Master's Degree in Assessment and Environmental Monitoring of Marine and Coastal Ecosystems student for helping in phytoplankton analysis and Alba Marí Bachelor's Degree in Environmental Sciences student for helping in physico-chemical analysis.

220 **Author Contributions:** “José-Andrés Sanchis-Blay, Miguel Rodilla and María-Teresa Sebastiá-Frasquet.  
221 conceived and designed the experiments; José-Andrés Sanchis-Blay and María-Teresa Sebastiá-Frasquet  
222 sampled the lake. Ferran Llario and Maria-Teresa Sebastiá-Frasquet performed the analysis; María Pachés  
223 analyzed the phytoplankton samples. All authors contributed to analyze the data; María-Teresa Sebastiá-  
224 Frasquet wrote the paper.”

225 **Conflicts of Interest:** “The authors declare no conflict of interest.”

226

## 227 Abbreviations

228 The following abbreviations are used in this manuscript:

229 Harmful algal blooms (HABs)

230 American Public Health Association (APHA)

231 Dissolved oxygen (DO)

232 Dissolved inorganic nitrogen (DIN)

233 Dissolved inorganic phosphorus (DIP)

234 Dissolved silicate (DSi)

235 Total phosphorus (TP)

236 Chemical oxygen demand (COD)

237 Biochemical oxygen demand (BOD<sub>5</sub>)

238 Chlorophyll *a* (Chl-*a*)

239

## 240 References

- 241 1. Xing, Z., Chua, L.H.C., Imberger, J. Evaluation of Management Scenarios for Controlling Eutrophication in  
242 a Shallow Tropical Urban Lake. *International Journal of Environmental Pollution and Remediation*, **2014**, *2*, 66-  
243 72. DOI: 10.11159/ijepr.2014.xxx. Available online: <http://hdl.handle.net/10536/DRO/DU:30063845>
- 244 2. Bolund, P., Hunhammar, S. Ecosystem services in urban areas. *Ecological Economics*, **1999**, *29*, 293–301
- 245 3. Sebastiá-Frasquet, M.-T.; Sanchis Blay, J.A.; Tormo, J.; Altur, V.; Pena, J. Management plan and surveillance  
246 monitoring for an urban lake in a coastal touristic town. *International Journal of Sustainable Development and*  
247 *Planning*, **2016**, *6* (11), 886 – 896, 10.2495/SDP-V11-N6-886-896. Available online:  
248 <http://www.witpress.com/elibrary/sdp-volumes/11/6/1387> (Accessed on 23/10/2016)
- 249 4. Sebastiá, M.T. & Rodilla, M. Nutrient and Phytoplankton Analysis of a Mediterranean Coastal Area.  
250 *Environ Management*, **2013**, *51*, 225. DOI:10.1007/s00267-012-9986-3. Available online:  
251 <http://link.springer.com/article/10.1007/s00267-012-9986-3> (Accessed on 23/10/2016)
- 252 5. Van den Berg, M.S., M. Scheffer, E. van Nes & H. Coops Dynamics and stability of Chara sp. and  
253 Potamogeton pectinatus in a shallow lake changing in eutrophication level. *Hydrobiologia* **408/409**, 1999,  
254 335-342
- 255 6. Lee, J.H., Bang, K.W., Ketchum Jr., L.H., Choe, J.S. & Yu, M.J. First flush analysis of urban storm runoff. *The*  
256 *Science of the Total Environment*, **2002**, *293*, 163–175. DOI: [http://dx.doi.org/10.1016/S0048-9697\(02\)00006-2](http://dx.doi.org/10.1016/S0048-9697(02)00006-2)
- 257 7. Randall, D.J., Tsui, T.K.N. Ammonia toxicity in fish. *Marine Pollution Bulletin*, **2002**, *45*, 17–23. DOI:  
258 [http://dx.doi.org/10.1016/S0025-326X\(02\)00227-8](http://dx.doi.org/10.1016/S0025-326X(02)00227-8)
- 259 8. Pyke, G.H. A Review of the Biology of *Gambusia affinis* and *G. holbrooki*. *Rev Fish Biol Fisheries*, **2005**, *15*,  
260 339-365. DOI: 10.1007/s11160-006-6394-x
- 261 9. Sebastiá, M.T., M. Rodilla, S. Falco, J.-A. Sanchis. Analysis of the effects of wet and dry seasons on a  
262 Mediterranean river basin: Consequences for coastal waters and its quality management. *Ocean & Coastal*  
263 *Management*, **2013**, *78*, 45-55. DOI: <http://dx.doi.org/10.1016/j.ocecoaman.2013.03.012>
- 264 10. Scherer, N.M., Gibbons, H.L., Stoops, K.B. and Muller, M. Phosphorus loading of an urban lake by bird  
265 droppings. *Lake and Reservoir Management*, **1995**, *11* (4): 317–327. DOI: 10.1080/07438149509354213
- 266 11. Liu, C., Zhong, J., Wang, J., Zhang, L., Fan, C. Fifteen-year study of environmental dredging effect on  
267 variation of nitrogen and phosphorus exchange across the sediment-water interface of an urban lake.  
268 *Environmental Pollution*, 2016, X, 1-10. DOI: <http://dx.doi.org/10.1016/j.envpol.2016.06.040>
- 269 12. Gkelis, S., Papadimitriou, T., Zoutsos, N., Leonardos, I. Anthropogenic and climate-induced change favors  
270 toxic cyanobacteria blooms: Evidence from monitoring a highly eutrophic, urban Mediterranean lake.  
271 *Harmful Algae*, **2014**, *39*, 322–333. DOI: <http://dx.doi.org/10.1016/j.hal.2014.09.002>
- 272 13. Effler, S.W., O'Donnell, S. M., Prestigiacomo, A.R., Matthews, D.A., Auer, M.T. Retrospective Analyses of  
273 Inputs of Municipal Wastewater Effluent and Coupled Impacts on an Urban Lake. *Water Environ. Res.*, **2013**,  
274 *85*, 13-26. DOI: 10.2175/106143012X13415215906690
- 275 14. Barros, N., Mendonça, R., Huszar, V., Roland, F., and Kosten, S. Carbon fluxes in an eutrophic urban lake.  
276 *Geophysical Research Abstracts*, **2014**, *16*, EGU2014-13335
- 277 15. APHA. *Standard methods for the examination of water and wastewater*, 20th ed. American Public Health  
278 Association, Washington, DC American Water Works Association; Water Environment, Federation, 1998.



- 279 16. Jeffrey, S.W., Humphrey, G.F. New spectrophotometric equations for determining chlorophylls a, b and c,  
280 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. PFL*, **1975**, *167*, 191–194.
- 281 17. Aminot, A., Chaussepied, M. *Manuel des analyses chimiques en milieu marin*. CNEXO, Brest, 1983, p. 396.
- 282 18. Jenkins, D. & Medsker, L. L. Brucine Method for the Determination of Nitrate in Ocean, Estuarine, and  
283 Fresh Waters. *Anal. Chem.*, **1964**, *36* (3), 610–612, DOI: 10.1021/ac60209a016. Available online:  
284 <http://pubs.acs.org/doi/abs/10.1021/ac60209a016> (Accessed on 23/10/2016)
- 285 19. Vaultot, D., Eikrem, W., Viprey, M. & Moreau, H. The diversity of small eukaryotic phytoplankton (<3 µm)  
286 in marine ecosystems. *FEMS Microbiol Rev* 2008, *32*, 795–820, DOI: [http://dx.doi.org/10.1111/j.1574-](http://dx.doi.org/10.1111/j.1574-6976.2008.00121.x)  
287 [6976.2008.00121.x](http://dx.doi.org/10.1111/j.1574-6976.2008.00121.x). Available online: <http://femsre.oxfordjournals.org/content/32/5/795> (Accessed on  
288 23/10/2016)
- 289 20. Pachés, M., Romero, I., Hermosilla, Z., Martínez-Guijarro, R. PHYMED: an ecological classification system  
290 for the Water Framework Directive based on phytoplankton community composition. *Ecol. Indic.* **2012**, *19*,  
291 15–23, DOI: <http://dx.doi.org/10.1016/j.ecolind.2011.07.003>. Available online:  
292 <http://www.sciencedirect.com/science/article/pii/S1470160X11002093> (Accessed on 23/10/2016)
- 293 21. Fournier, R., Membrane filtering. In: *Phytoplankton Manual. Monographs on Oceanographic Methodology*.  
294 Sournia, A. (Ed.), UNESCO, 1978, pp. 197–201.
- 295 22. Vargo, G.A. Using a fluorescence microscope. In: *Phytoplankton Manual. Monographs on Oceanography*  
296 *Methodology*. Sournia, A. (Ed.), UNESCO, 1978, pp. 108–112
- 297 23. Lund, J.W.G., Kipling, C., Le Cren, E.D. The inverted microscope method of estimating algal numbers and  
298 the statistical basis of estimations by counting. *Hydrobiologia*, **1958**, *11*, 143–170.
- 299 24. Utermohl, M. Zur Vervollkommnung der quantitative Phytoplankton-Methodik. Mitteilungen  
300 internationale Vereinigung für theoretische und angewandte Limnologie, **1985**, *9*, 1–38.
- 301 25. Danilov, R. & Ekelund, N.G.A. The efficiency of seven diversity and one similarity indices based on  
302 phytoplankton data for assessing the level of eutrophication in lakes in central Sweden. *Science of The Total*  
303 *Environment*, **1999**, *234*, 15–23
- 304



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).