ASSESSMENT OF ECOLOGICAL STATUS AND PRELIMINARY RESULTS ON REFERENCE CONDITIONS IN ALPINE GLACIAL LAKES (BULGARIA) – A CONTRIBUTION TO THE IMPLEMENTATION OF THE WATER FRAMEWORK DIRECTIVE

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ABSTRACT

The ‘normative definitions’ of ecological water quality classes given by the Water Framework Directive (WFD) are narrative descriptions of the conditions present in water bodies of different qualities relative to reference conditions found in unimpacted sites. Defining the type-specific reference conditions for water bodies and finding biological metrics that sensitively reflect only the anthropogenic deviations from those conditions are the biggest challenges that the ecological status assessment faces. This study aimed at phytoplankton, macrophyte, macro-invertebrates and fish-based assessment in two high mountain glacial lakes and establishing preliminary reference conditions. The lakes are located in Rila and Pirin Mountains, Southwest Bulgaria. Chernoto Lake is ultra-oligotrophic and has abundant submerged macrophytes; Bezbog Lake is also oligotrophic with submerged macrophytes, both with macrozoobenthic communities typical for oligosaprobic conditions. Sampling was carried out from July to September in 2009 and 2011. The biological and water quality parameters in the lakes are discussed. Indices elaborated in a European context (Algae Group Index, Reference Index, adapted biotic index, Pantle & Buck’s saprobic index and German trophic index PETI) were tested on the collected data set. The type-specific taxa for the studied lake type were preliminarily determined.


Keywords: glacial lakes, ecological status, reference conditions, type-specific taxa, WFD

Introduction

For correct evaluation and characterisation of a lake ecosystem it is considered essential to adopt an integrated approach that takes into account physical, chemical and biological metrics (phytoplankton, macrophytes, bottom macro-invertebrates and fish). It is recommended that the above biological indicators and, additionally, a range of supporting hydro-morphological and physico-chemical elements should form the core of any monitoring program on lakes (38).

The algal flora of 33 glacial lakes in Bulgaria, not including Chernoto and Bezbog, was studied (30). Following a mainly taxonomical and floristic publication on phytoplankton (37), phytoplankton studies in glacial lakes have proceeded with occasional data for abundance and vertical distribution at the Seven Rila Lakes (3, 4, 5, 22). An assessment of 78 lakes based on aquatic macrophytes in Bulgaria was carried out in 2009 (15). Determinations of aquatic oligochete fauna found in glacial lakes from Rila (34) and Pirin Mountains (35), including Bezbog and Chernoto lakes, were made from the collections of Prof. Valkanov, which were gathered during his expeditions between 1932 and 1968, devoted to studying life in high-mountain lakes.
guidance) on establishing reference conditions and ecological status for inland surface waters (13).

The sampling points at both lakes (Fig. 1) were selected with a view to be representative for the existing habitat diversity and homogeneity. Biological quality elements (BQEs) together with physico-chemical parameters of lake water were monitored in the period from July to September in 2009 and 2011.

![Fig. 1. Location of the studied lakes: No. 1 – Bezbog; No. 2 – Chernoto Lake.](image)

Water quality parameters (temperature, pH, conductivity, transparency, dissolved oxygen and oxygen saturation) of lake water were measured in situ. Additionally, NH₃-N, NO₂-N, NO₃-N, PO₄-P, total nitrogen and phosphorus, COD, and BOD, were analyzed just after sampling on spectrophotometer NOVA 60 (MERCK) following adopted standards: ammonium nitrogen – ISO 7150/1, nitrite and nitrate nitrogen – EN 26777 and ISO 7890-1, total nitrogen (TN) – EN ISO 11905-1, phosphate phosphorus – EN ISO 6878, total phosphorus (TP) – EN ISO 6878, biochemical oxygen demand – EN 1899-1,2, and chemical oxygen demand – ISO 15705.

Phytoplankton seasonal (summer and autumn) observations were made during the second year. Discrete sampling from the deepest lake zone was carried out with a Ruttner batometer in 5 fixed median horizons: 0 m (surface area), 3.0 m, 7.0 m, 10 m, and 15 m (bottom layer). The general taxonomic scheme of Van den Hoek et al. (36) for phytoplankton was followed. At least 100 individuals were registered for the most numerous species per sample (23). The biovolume was determined following geometric form formulas (24), and was transformed into biomass according to Wetzel and Likens (39). The ecological status assessment was based on 4 main metrics (total phytoplankton biovolume, Algae Group Index, transparency according to Secchi, chlorophyll a) and 3 additional ones (% Cyanobacteria, presence of “bloom” and toxic species). Chlorophyll a concentration was determined spectrophotometrically, after extraction in 90 % ethyl alcohol, following a standard method – ISO 10260:1992. The intensity of the phytoplankton “bloom” was assessed on the basis of total biovolume on a 5-degree scale: I degree ≤ 2.5 mm³·L⁻¹; II degree ≈ (2.5–10) mm³·L⁻¹; III degree ≈ (10–500) mm³·L⁻¹; IV degree ≈ (500–5000) mm³·L⁻¹; V degree (“hyperbloom”) > 5000 mm³·L⁻¹. In calculating % Cyanobacteria, some species/genera for oligotrophic waters were excluded, focusing on toxic species and eutrophic indicators (following WFD Intercalibration technical report, Part 2 – Lakes, Section 3 – Phytoplankton biomass metrics Annexes). The Algae Group Index (AGI, Catálan Index) was calculated for each sample (6).

The interpretation of phytoplankton data based on the above metrics followed the classification system for the oligotrophic lake type in Bulgaria (7).

The taxonomic composition and abundance of aquatic macrophytes were recorded as biological metrics in the normative definitions of the ecological status classification in the WFD, Annex 1.2 (14). All submerged, floating-leaved, helophyte and amphiphyte species (Charophytes, Bryophytes and Tracheophytes) were recorded. Sampling was carried out following the recommendations of Schaumburg et al. (28). The taxonomy of vascular plants followed Flora Europaea (31, 32). The abundance of each species was noted on a five-degree scale (1 = very rare, 2 = infrequent, 3 = common, 4 = frequent, 5 = abundant, predominant) according to Kohler (17). Calculated Reference Index (1, 28) was transformed in EQR, where the value of “1” reflects the best possible ecological status.

An adapted version of the multi-habitat sampling method (8), applying different techniques according to EN 27828:1994 and EN ISO 9391:1995 was used for benthic macroinvertebrate fauna sampling. The assessment was based on an adapted version of the Irish Q-scheme (9, 10), Pantle and Buck’s saprobic index (25) and Feeding type index (29).

Data about the ichthyofauna in the two target lakes and information about fish stocking were obtained by interviews with park staff and fishermen.

**Results and Discussion**

Reference conditions and respectively high ecological status based on water quality parameters and all BQEs were assessed at both lakes (Table 1). A slight deviation from reference conditions was illustrated by AGI during 2011. The transparency was not representatively evaluated due to high transparency – over maximum lake depth, especially at the ultra-oligotrophic Chernoto Lake, with biomass about 0. Chlorophyta, Chrysophyta, Bacillariophyta and Desmidiales were represented with the highest number of species in Chernoto Lake; Desmidiales, Bacillariophyta and Cyanoprokaryota in Bezbog Lake (Table 2). Pyrrhophyta, Chlorophyta, and Cryptophyta dominated at Chernoto Lake in respect to biovolume; Pyrrhophyta, Bacillariophyta and Cryptophyta in Bezbog Lake. These results corroborated previous data for alpine lake biomass (3).

Among the general species richness at both studied lakes, the percent of Desmids and Diatoms was considerable and confirmed previous reported data (3, 37). Vodenicharov and Vodenicharov (37) assumed increasing of Chlorococcales as
## Table 1

Morphometric characteristics, water and biological quality parameters in lakes

<table>
<thead>
<tr>
<th>Lake features</th>
<th>Bezbog</th>
<th>Chernoto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude, a.s.l. (m)</td>
<td>2240</td>
<td>2302</td>
</tr>
<tr>
<td>Latitude</td>
<td>41 43 59.6</td>
<td>42 07 37.8</td>
</tr>
<tr>
<td>Longitude</td>
<td>23 31 24.3</td>
<td>23 27 46.0</td>
</tr>
<tr>
<td>Maximum depth (m)</td>
<td>7</td>
<td>15.5</td>
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</table>

### Water quality indicators

<table>
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<th></th>
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</thead>
<tbody>
<tr>
<td>T, °C</td>
<td>3</td>
<td>7.1</td>
<td>10.4</td>
<td>16.2</td>
</tr>
<tr>
<td>Dissolved oxygen (mg·L⁻¹)</td>
<td>7.9</td>
<td>9.3</td>
<td>7.9</td>
<td>8.2</td>
</tr>
<tr>
<td>Oxygen saturation (%)</td>
<td>76</td>
<td>126</td>
<td>93</td>
<td>111</td>
</tr>
<tr>
<td>pH</td>
<td>7.8</td>
<td>7.3</td>
<td>7.5</td>
<td>7.8</td>
</tr>
<tr>
<td>Conductivity (µS·cm⁻¹)</td>
<td>16</td>
<td>11.5</td>
<td>9.7</td>
<td>9.4</td>
</tr>
<tr>
<td>SD (m)</td>
<td>&gt;7</td>
<td>&gt;7</td>
<td>&gt;15.5</td>
<td>&gt;15.5</td>
</tr>
<tr>
<td>NH₄-N (mg·L⁻¹)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NO₂-N (mg·L⁻¹)</td>
<td>0</td>
<td>0</td>
<td>&lt;0.002</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>NO₃-N (mg·L⁻¹)</td>
<td>&lt;0.2</td>
<td>&lt;2</td>
<td>&lt;0.2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>TN (mg·L⁻¹)</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>PO₄-P (mg·L⁻¹)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TP (mg·L⁻¹)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>COD (mg·L⁻¹)</td>
<td>6.8</td>
<td>5.6</td>
<td>&lt;4</td>
<td>&lt;4</td>
</tr>
<tr>
<td>BOD₅ (mg·L⁻¹)</td>
<td>&lt;1</td>
<td>&lt;1</td>
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### Biological Quality Element Metrics

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<tbody>
<tr>
<td>AGI</td>
<td>0.48</td>
<td>0.92</td>
<td>0.99</td>
<td>0.55</td>
</tr>
<tr>
<td>Total biovolume (mm³·L⁻¹)</td>
<td>0.95</td>
<td>0.43</td>
<td>0.17</td>
<td>0.57</td>
</tr>
<tr>
<td>Chl A (µg·L⁻¹)</td>
<td>&lt;0.2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>% Cyanobacteria</td>
<td>0.74</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blooms</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RI</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Macrophytes - EQR</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MZB - Total quantity (m²)</td>
<td>132</td>
<td>436</td>
<td>154</td>
<td>246</td>
</tr>
<tr>
<td>% Oligochaeta</td>
<td>24</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Biotic index</td>
<td>n.a.</td>
<td>3.5</td>
<td>n.a.</td>
<td>4.5</td>
</tr>
<tr>
<td>Potamal trophic index (PETI)</td>
<td>n.a.</td>
<td>0.99</td>
<td>n.a.</td>
<td>0.83</td>
</tr>
</tbody>
</table>

n.a.: not analyzed
an indication of an eutrophication trend, thus we accepted the Desmids and Diatoms domination in the summer phytoplankton as an indicator of reference conditions.

The results showed deviation of AGI from the reference value in conditions of no clear biomass domination of Dinoflagellates. The specific species composition and percent biomass allocation among divisions in the suited ultra-oligotrophic lakes, defined AGI values around 1, i.e. good status. This, in particular, was due to the equal percent participation of Colonial Chrysophyte and Colonial Diatoms, and, respectively, Chrysophyte and Diatoms not colonial in the total biomass, which took part in the AGI calculation. Thus further data accumulation is recommended towards correction of class boundary between high and good status.

Macrophytes illustrated high status with maximum value of Reference Index (RI). Macrophyte populations typical for alpine lakes were registered; they were dominated by *Isoetes lacustris* (Bezbog), *Subularia aquatica* and *Sparganium angustifolium*. *Subularia aquatica* accounted for almost 90 % of the total abundance observed at Chernoto Lake. Helophytes and bryophytes were scarcely represented. The indicator species abundance was above 90 % and the maximum depth of plant colonization was 1.2 m.

The macrozoobenthic communities gave similar results. Taxa typical for oligosaprobic conditions were registered in Chernoto Lake: *Ecdyonurus eperoides*, *Capnia* sp., *Phylopotamus* sp., *Odontocercum hellenicus*, *Plectrocnemia conspersa*. Reference conditions and high ecological status were assessed at Chernoto Lake, based on the applied metrics (% Oligochaeta and Biotic index). The assessed trophic index characterized the ecosystem as stable and unaffected.

The macroinvertebrates illustrated near referent conditions and good ecological status at Bezbog Lake during the first year of observation. The anthropogenic impact was considered to be reduced based on three indices assessed in 2011, which illustrated high status. Nevertheless, some taxa typical for impacted waters were still registered (two Dipterean larvae: *Chironomus riparius* and *Cryptochironomus defectus*).

Both Bezbog and Chernoto lakes have been repeatedly stocked over the years with Brown trout (*Salmo trutta fario* and, probably accidentally, with the alien species Rainbow trout (*Oncorhynchus mykiss*), similar to many other alpine lakes in Bulgaria. Furthermore, the Chernoto Lake was stocked with Brook trout (*Salvelinus fontinalis*), which is also an alien species of North American origin. In Bezbog Lake numerous

### TABLE 2

Relative abundance of phytoplankton based on taxonomic groups

<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Species number</td>
<td>Biovolume</td>
<td>Species number</td>
</tr>
<tr>
<td><strong>Bezbog Lake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanoprokaryota</td>
<td>9.1</td>
<td>0.7</td>
<td>15.2</td>
</tr>
<tr>
<td>Chlorophyta</td>
<td>18.2</td>
<td>6.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Desmidiales</td>
<td>25.0</td>
<td>0.5</td>
<td>18.2</td>
</tr>
<tr>
<td>Chrysophyta</td>
<td>11.4</td>
<td>4.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Bacillariophyta</td>
<td>29.5</td>
<td>9.5</td>
<td>51.4</td>
</tr>
<tr>
<td>Pyrrophyta</td>
<td>2.3</td>
<td>74.2</td>
<td>6.1</td>
</tr>
<tr>
<td>Cryptophyta</td>
<td>4.5</td>
<td>4.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Total species number</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chernoto Lake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanoprokaryota</td>
<td>7.1</td>
<td>0.2</td>
<td>15.2</td>
</tr>
<tr>
<td>Chlorophyta</td>
<td>25.0</td>
<td>6.0</td>
<td>30.3</td>
</tr>
<tr>
<td>Desmidiales</td>
<td>14.3</td>
<td>0.4</td>
<td>12.1</td>
</tr>
<tr>
<td>Chrysophyta</td>
<td>21.4</td>
<td>5.4</td>
<td>6.1</td>
</tr>
<tr>
<td>Bacillariophyta</td>
<td>14.3</td>
<td>0.4</td>
<td>24.2</td>
</tr>
<tr>
<td>Pyrrophyta</td>
<td>10.8</td>
<td>77.6</td>
<td>9.1</td>
</tr>
<tr>
<td>Cryptophyta</td>
<td>7.1</td>
<td>10.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Total species number</td>
<td>28</td>
<td></td>
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</table>
Although the Minnow is a native species in the waters of Bulgarian alpine lakes, it is considered a non-native one, introduced by coincidence with the trout fry and/or as bait used by anglers. In both of the studied lakes angling (and incidental poaching) is practiced. Thus, both in Bezbog and Chernoto lakes the ichthyofauna is subjected to long-term human impact mainly through artificial stocking (including introduction of alien and non-native species) and, to a lesser extent, by fishing pressure. The available data do not allow defining even retrospectively a referent state of the ichthyofauna. Therefore the lakes are considered unrepresentative for fish-based assessment.

Based on species composition during the studied period, 23 phytoplankton, 3 aquatic macrophyte, 17 macroinvertebrate preliminary type-specific taxa were found (Table 3). Isoetes lacustris is among the most common species in European lakes and is classified as a reference species, which should have very low abundance or be completely absent in impacted lakes (27). Monodominant coenoses of quillwort are widely distributed in Pirin Mountain glacial lakes, with high (70 % to 80 %) projective cover of the above-ground parts (33).

Thus, although restricted to Pirin Mountain lakes distribution, Isoetes lacustris was listed as a type-specific taxa. It has to be emphasized that the attempt to assess reference conditions, even those of the same lake type, needs continuous

<table>
<thead>
<tr>
<th>Scientific name</th>
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<tbody>
<tr>
<td><strong>Phytoplankton</strong></td>
<td><strong>Macrophytes</strong></td>
</tr>
<tr>
<td>Cyanoprokaryota (Blue-green Algae)</td>
<td>Isoetes lacustris L.</td>
</tr>
<tr>
<td>Aphanothece elabens (Bréb. in Men.) Elenk.</td>
<td>Sparganium angustifolium Michx.</td>
</tr>
<tr>
<td>Coelosphaerium subarcticum Kom. et Kom.-Legn.</td>
<td>Subularia aquatica L.</td>
</tr>
<tr>
<td><strong>Chrysophyta (Golden Algae)</strong></td>
<td><strong>Macroinvertebrates</strong></td>
</tr>
<tr>
<td>Chrysococcus minutus (Fritsch) Nygaard</td>
<td>Bivalvia</td>
</tr>
<tr>
<td>Mallomonas tonsurata Teil. var. alpina (Pasch. et Ruttn.) Krieg.</td>
<td>Sphaerium sp. Scopoli 1777</td>
</tr>
<tr>
<td><strong>Bacillariophyta (Diatoms)</strong></td>
<td><strong>Ephemeroptera</strong></td>
</tr>
<tr>
<td>Aulacoseira italica (Ehrenb.) Simonsen</td>
<td>Ecdyonurus epeoides Demoulin 1955</td>
</tr>
<tr>
<td>Diatomas mesodon (Ehr.) Kütz.</td>
<td>Plecoptera</td>
</tr>
<tr>
<td>Tabellaria fenestrata (Lyngb.) Kütz.</td>
<td>Capnia sp. Pictet 1841</td>
</tr>
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<td>Tabellaria flocculosa (Roth) Kütz.</td>
<td>Nemoura cinerea (Retzius 1783)</td>
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<tr>
<td><strong>Chlorophyta (Green algae)</strong></td>
<td><strong>Gastropoda</strong></td>
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<tr>
<td>Chlamydomonas passiva Skuja</td>
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<td>Oocystis apiculata W. West</td>
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<tr>
<td>Radiococcus nimbatus (De-Wild.) Schmidle</td>
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<td>Trochiscia aciculifera (Lag.) Hansg.</td>
<td>Sialis lutaria (Linnaeus 1758)</td>
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<td><strong>Trichoptera</strong></td>
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<td>Halesus digitatus (von Paula Schrank 1781)</td>
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<td>Euastrum bidens (Ehr.) Näg.</td>
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<td>Euastrum didelphes Ralfs ex Ralfs</td>
<td>Odontocerum hellenicus Malicky 1972</td>
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<td>Euastrum verrucosum Ehr. ex Ralfs</td>
<td>Philopotamus sp. Stephens 1829</td>
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<td>Micrasterias rotata (Greville) Ralfs ex Ralfs</td>
<td>Electrocnemia conspersa (Curtis 1834)</td>
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<tr>
<td>Sphaerozosma vertebratum Brébisson ex Ralfs</td>
<td><strong>Diptera: Chironomidae</strong></td>
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<td>Spondylosium lunellii Borge</td>
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<td>Brilia sp. Kieffer 1913</td>
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<td>Xanthidium antilopaeum (Bréb.) Kütz.</td>
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<td>Cricotopus (Cricotopus) algarum (Kieffer 1911)</td>
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<td>Eukifferiella gracilis (Edwards 1929)</td>
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<tr>
<td>Tvetenia calvescens (Edwards 1929)</td>
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</table>

The populations of Minnow Phoxinus phoxinus were presented. Although the Minnow is a native species in the waters of Bulgarian alpine lakes, it is considered a non-native one, introduced by coincidence with the trout fry and/or as bait used by anglers. In both of the studied lakes angling (and incidental poaching) is practiced. Thus, both in Bezbog and Chernoto lakes the ichthyofauna is subjected to long-term human impact mainly through artificial stocking (including introduction of alien and non-native species) and, to a lesser extent, by fishing pressure. The available data do not allow defining even retrospectively a referent state of the ichthyofauna. Therefore the lakes are considered unrepresentative for fish-based assessment.
researches and the list of type-specific taxa needs further evaluation, since no two lakes are alike, and therefore, despite the similar basic conditions, no floras and faunas are alike.

Conclusions
The phytoplankton, aquatic macrophytes, macroinvertebrates and fish investigated together with the main environmental factors illustrated specific ultra-oligotrophic ecosystems with low phytoplankton quantity and scarce macroinvertebrate communities. Fish were found to be completely unrepresentative and were excluded from assessment of national type L1. Additional calibration of AGI is recommended towards specific phytoplankton communities which naturally showed higher index value, minimum biomass and rich species diversity. More data are necessary for class boundary validation of scale for glacial lakes based on phytoplankton.

The preliminary list of type-specific taxa for reference conditions as indicators for high ecological status in glacial alpine lakes was reported to indicate oligotrophic conditions. These taxa should be represented at least by 50 % in waters having a high ecological status.

The overall ecological status assessment demonstrated that both lakes were unaffected with undisturbed reference conditions.

The results obtained increase the knowledge about the glacial lake ecosystems and are basis for future researches. These preliminary reference conditions can be applied, through a comparison with observed BQEs at a site, in the assessments of ecological status. Additional biological quality elements (zooplankton and phytobenthos) should be considered for monitoring under the WFD in further assessment.

Acknowledgements
We would like to thank West Aegean River Basin Directorate – Blagoevgrad, for supporting the research, and the Executive Editor and anonymous peer Reviewer for their contributions to this manuscript.

REFERENCES