

## Article

# The Diatomic Diversity of Two Mediterranean High-Elevation Lakes in the Sibillini Mountains National Park (Central Italy)

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**Abstract:** Temporary high-elevation lakes represent vulnerable and unstable environments strongly threatened by tourism, hydrogeological transformations and climate changes. In-depth scientific knowledge on these peculiar habitats is needed, on which to base integrated and sustainable management plans. Freshwater diatoms, thanks to their high diversity and their particular sensitivity to the water chemistry, can be considered powerful ecological indicators, as they are able to reflect environmental changes over time. The aim of the present study was to analyze the diatomic diversity of the Pilato and Palazzo Borghese lakes, two small temporary high-mountain basins, falling in a protected area within the Apennine mountains chain (central Italy). Diatoms data were collected, at the same time as 12 physicochemical parameters, through six microhabitat samplings, from 17 June to 30 August 2019. In both lakes, a total of 111 diatomic species and varieties were identified. The most species-rich genera were *Gomphonema*, *Navicula*, and *Nitzschia*. The Pilato Lake showed a diatomic community dominated by few species, favored by more stable and predictable environmental conditions than the Palazzo Borghese Lake, which hosted a more diversified community, guaranteed by greater spatial and temporal heterogeneity. Both lakes were characterized by the presence of diatomic species typical of good quality waters. The occurrence of numerous aerial species reflected adaptation strategies adopted to colonize environments subjected to extended drought periods. Endangered diatomic species of particular conservational interest were recorded, confirming the need to preserve their habitats.

**Keywords:** diatom assemblages; Apennine lakes; Pilato lake; Palazzo Borghese lake; biodiversity conservation; climate change



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## 1. Introduction

Freshwater diatoms play an important ecological role in the overall functioning of aquatic systems [1]. Being at the base of the trophic network and starting point of food chains, they support primary productivity, producing oxygen, as well as contributing to ecosystem metabolism in terms of energy flows and nutrient cycling [2–5]. As a consequence, changes in diatoms community composition and species abundances trigger changes in other biotic communities as well, such as zooplankton, macrophytes, and fish fauna [6].

In addition to this, freshwater diatoms can also be considered powerful bioindicators, such as macro-benthic communities, able to promptly reflect environmental changes occurring over time, thanks to their high diversity, their short generation time and their particular sensitivity to the physico-chemical characteristics of the ecosystem in which they live [7–9]. For this reason, these organisms are often used to monitor aquatic systems [10]. Moreover, the analysis of fossil diatom assemblages in paleontological studies allows one to trace past environmental changes in aquatic systems [11,12]. The European Water Framework Directive (WFD 2000/60/EC, European Commission 2000), transposed

by Italian legislation with the D.Lgs. 152/2006, considers diatoms as valid indicators of ecological quality, and proposes specific indexes for both running and lacustrine waters. These diatoms potentially acquire particular relevance in the assessment of temporary high-elevation lakes' ecological status [5]. In fact, such isolated ecosystems are particularly vulnerable to biodiversity loss, as a consequence of the negative effects of various anthropogenic pressures, including intensive summer tourism and climate changes [12]. The composition of diatomic communities, based on the different tolerance of species to water chemistry, and their ability to respond promptly to multiple stressors, can provide important ecological information on these unstable environments, the survival of which is strongly linked to hydrological and climatic processes.

Pilato and Palazzo Borghese lakes are small temporary high-mountain basins located in the Apennine chain (central Italy). Both are of particular importance from a naturalistic point of view, as each of them hosts an endemic species of fairy shrimp: *Chirocephalus marchesonii* Ruffo and Vesentini, 1957 is endemic to the Pilato Lake, while *Chirocephalus sibyllae* Cottarelli and Mura, 1975 is endemic to the Palazzo Borghese Lake. These unstable biotopes, which are characterized by extreme physical conditions (total drought and freezing periods), as well as representing biodiversity hot-spots, can be considered effective sentinels able to witness the effects of environmental changes over time [13,14]. For the central Apennines, a recent study highlighted some effects of global climate change in terms of rising temperatures and prolonged drought periods, which threaten the survival of these biotopes and, therefore, their biocoenoses [15]. However, despite the great ecological interest of diatom assemblages [8], there are no specific studies related to the actual diatomic diversity of the Pilato and Palazzo Borghese lakes, which is currently totally unexplored. With the aim to fill this gap, the present research focused on the investigation over the different diatom species inhabiting the two environments, with special reference to their ecological preferences and conservational value.

## 2. Materials and Methods

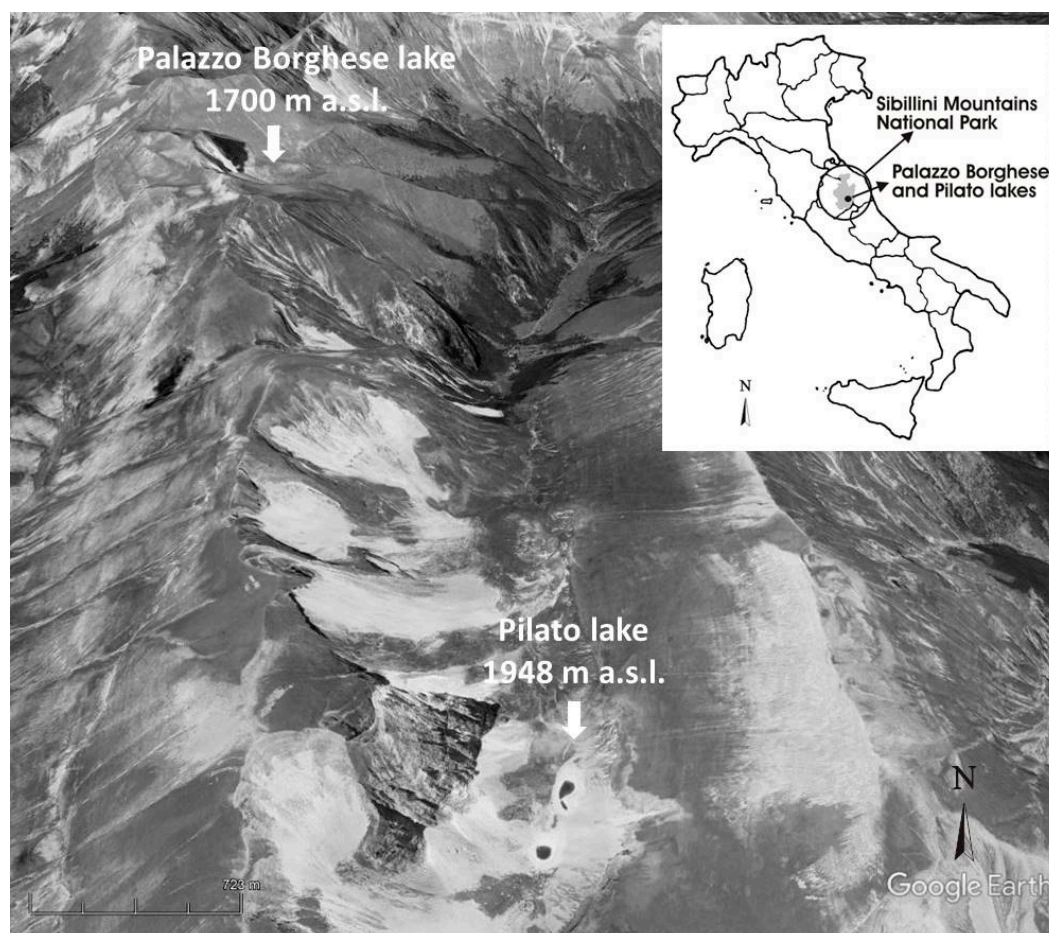
### 2.1. Study Area

The Pilato and Palazzo Borghese lakes are located in the Apennine chain, within the territory of the Sibillini Mountains National Park (central Italy) (Figure 1). Both lakes are of karst-glacial origin [16], and are enclosed in a morain cirque [17]. They are separated by an air-line distance of 5 km.

The Pilato Lake (42°49'35" N; 13°15'54" E) is a small mountain basin located at 1948 m a.s.l., with a surface area of 32,000 m<sup>2</sup>. During winter and early spring (i.e., from December to April) it is covered by ice and snow. At the end of the snowmelt, which usually coincides with the end of June, the lake reaches its maximum hydrometric level, with a depth of 9 m and a water volume of 57,000 m<sup>3</sup> [15]. In summer, following the progressive decline of water level, due to infiltration and evaporation processes, the lake splits into two ponds connected by an isthmus. These two ponds usually freeze in late autumn, but total drying may occur already in July, especially in occasion of poor winter snowfall and/or high air temperatures. In the present paper we do not analyze the climate change effects in terms of rising temperatures and decreasing precipitations occurring in the study area, because this aspect has already been described in Carosi et al. [15]. In addition to climate changes, also the seismic events that have affected the area in recent years, and the related hydrogeological transformations, in terms of water-table changes, could have influenced the hydrological dynamics of the lake [18,19].

The Palazzo Borghese Lake (42°52'11" N; 13°14'33" E) is a temporary pond located at 1700 m a.s.l., in a pasture land. It fills each spring with water from snowmelt, and it generally dries up after about one to two months. At the end of the snowmelt, which usually coincides with the end of May, the lake reaches its maximum hydrometric level, with a depth of 2.5 m and a water volume of 5000 m<sup>3</sup>. On the basis of these characteristics, it can be classified as a "predictable melt pond" *sensu* Belk [20], showing a temporary but periodic and fairly predictable hydrological cycle, compared to temporary pools that

receive water exclusively by rain. However, the hydrological cycle of the Palazzo Borghese Lake, as well as that of the Pilato Lake, is extremely variable and strictly connected to the climatic conditions. This environmental dependence, resulting in the deep abiotic factor changes occurring throughout the seasons, implies that there are significant repercussions on the adaptive strategies adopted by colonizing species [21].



**Figure 1.** Study area location and elevation of the Pilato and Palazzo Borghese lakes (photo taken on 29 June 2020).  
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Both lakes are an attraction for tourists, especially in summer; tourism is appreciated for the local economy but, as reported also for other mountain lakes, the high presence of hikers can represent a stress for these delicate ecosystems, in terms of shoreline erosion [13,22] and trampling of the banks [15], even if they are often located within a protected area [23].

## 2.2. Data Collection

For both lakes, samples for water chemistry and benthic diatoms analysis were collected in 2019, during the spring and summer seasons. The samplings were carried out from the appearance of the lakes until their drought. In particular, in the Pilato Lake, four samplings were carried out, from June to August (17/06; 08/07; 31/07; 30/08). In the Palazzo Borghese Lake two samplings were carried out, in June (09/06; 23/06), until the water level allowed to collect benthic diatoms (i.e., in the presence of submerged substrates in the euphotic zone). A total of 12 samples (six epilithic and six epiphytic) were collected in both lakes. On each sampling date, 12 physico-chemical parameters that greatly influence the natural distribution of diatoms were measured. In particular, dissolved oxygen ( $\text{mg L}^{-1}$ ), oxygen saturation (%), pH (units), electric conductivity ( $\mu\text{S cm}^{-1}$ ), water tem-

perature ( $^{\circ}\text{C}$ ) were measured in the field, at the same time of diatom samplings (approx. 12:00 p.m.), using electronic meters (YSI, Yellow Springs, OH, USA; Hanna Instruments, Padova, Italy; WTW GmbH, Weilheim, Germany). Water samples were collected in 1 L polyethylene bottles, according to environmental protection agencies (APAT, APHA, AWWA, WEF) specifications [24,25] for laboratory analysis of chlorides ( $\text{mg L}^{-1}$ ), ammonia ( $\text{mg L}^{-1}$ ), nitrites ( $\text{mg L}^{-1}$ ), nitrates ( $\text{mg L}^{-1}$ ), phosphates ( $\text{mg L}^{-1}$ ),  $\text{BOD}_5$  ( $\text{mg L}^{-1}$ ) and COD ( $\text{mg L}^{-1}$ ). Diatom samples were collected according to the ISS/SNPA MG.111/2014 standard protocol [26]. In order to collect epilithic species, diatoms were removed by the surface of solid substratum, as pebbles and stones, using a toothbrush, whereas to sample epiphyte forms, the samples were taken from macrophytes and filamentous algae.

### 2.3. Laboratory Preparation and Techniques

In the laboratory, the water content of chlorides, ammonia, nitrites, nitrates, phosphates,  $\text{BOD}_5$  and COD was quantified by spectrophotometry (Photometer WTW Photolab S12 Manufactured by Xylem Analytics Germany Sales GmbH and Co., Mainz, Germany) using standard methods [24,25].

As regards diatom taxa observations and counts, samples and enduring slides were prepared according to the ISS/SNPA MG.111/2014 standard protocol [26]. All slides were observed using ECLIPSE Ci-L optical microscope manufactured by Nikon, at  $40\times$  and  $1000\times$  magnification (oil immersion objective). Digital images were taken using Nikon DS-Fi3 camera mounted on ECLIPSE Ci-L microscope, and processed with Nikon NIS-Elements D image management software. A water sample of 50 mL, collected on a surface of  $100\text{ cm}^2$ , was split into aliquots of 5 mL, that were used to prepare permanent slides. Diatom frustules were cleaned using hydrogen peroxide and hydrochloric acid; then, the solution was washed with distilled water. For each subsample of 5 mL, at least two reiterations have been observed. The enduring slides were prepared placing a volume of say 0.100 mL of the homogeneous subsample on glass slides ( $25.4 \times 76.2\text{ mm}$  manufactured by Pearl), evaporated to dryness at room temperature, and then mounted in a synthetic resin with high refraction index  $> 1.6$  (Naphrax $^{\circ}$ ). In order to detect the large size species (i.e.,  $>70\text{ }\mu\text{m}$ ), that are often represented by one or a few specimens, the subsamples were appropriately diluted. In this way, it was possible to evaluate the presence of both r-strategist species (i.e., species characterized by high growth rate, fast reproduction, low survival), which usually appear with very high population abundances, and K-strategist species (i.e., species characterized by low growth rate, high survival), which almost always are represented by a small number of individuals. To evaluate the Pollution Sensitivity Index (IPS) [27], the Trophic Index (TI) [28], the Specific Diversity Index (H) [29], and the Dominance Index (D) [30], 400 individuals per slide were counted and identified at species level, using the OMNIDIA $^{\circ}$  6.0/2016 software. The IPS is a diatom-based saprobic index that reflects organic pollution, while the TI is based on diatom species sensitivity to nutrient water content. The count of each diatom taxon was expressed into relative abundance (RA) as a percentage of the total valves counted and identified in each sample. Moreover, to perform the morphological characterization, at least 50 individuals for each species were measured in length ( $\mu\text{m}$ ), width ( $\mu\text{m}$ ), the number of striae in  $10\text{ }\mu\text{m}$  were counted, and the presence of teratological forms *sensu* Falasco et al. [31] was recorded. In particular, modification of the frustule morphology of diatoms cells, in terms of deformities in the valve outline or alterations in the striation pattern, was detected. For the less abundant species, all individuals were measured.

To improve the identification of small or difficult taxa, the scanning electron microscope (SEM) was used. In this case, the images were performed with SEM JEOL JCM-6000-plus, manufactured by JEOL S.p.A; the samples have been metallized in gold with JEOL SMART Coater.

In order to determine diatom species, the following dichotomous keys have been used: Lange-Bertalot and Ruppel [32]; Krammer and Lange-Bertalot [33,34]; Lange-Bertalot and Krammer [35]; Houk et al. [36]; Bey and Ector [37]; Hofmann et al. [38]. Where



information was available, the ecological amplitude of the species was assessed according to the indicator values for pH, salinity, nitrogen uptake metabolism, oxygen requirements, saprobity, trophic state and moisture aerophily reported by van Dam et al. [39]. The Red List status of diatom species was evaluated according to the German Red List of diatoms [40].

### 3. Results

#### 3.1. Environmental Characterization

The results of the physico-chemical characterization of the waters are reported in Table 1. A general oligotrophy condition emerged for both lakes, with particularly low nutrient content, in terms of ammonia, nitrates, nitrites, and phosphates concentrations. The waters were characterized by low temperatures, according to the climatic conditions, and good oxygenation levels. Low values were found for BOD<sub>5</sub> and COD, testifying for good water quality. Sulphates were absent in all samples, while the chlorides never exceeded 23 mg L<sup>-1</sup>. The pH was always alkaline. The salt content, expressed by the electrical conductivity, was low, even if higher values for this parameter were found in the Palazzo Borghese Lake (Table 1).

**Table 1.** *t*-test analysis for physico-chemical parameters and diatomic indices Pollution Sensitivity Index (IPS) [27] and Trophic Index (TI) [28]: comparison between the Pilato and Palazzo Borghese lakes. *p* < 0.05 is in bold.

Environmental Variables and Diatomic Indices	Pilato Lake		Palazzo Borghese Lake		<i>t</i>	<i>p</i>
	Range	Mean ± SD	Range	Mean ± SD		
Water temperature (°C)	11.3–16.8	14.53 ± 2.03	7.8–19.8	13.8 ± 13.8	0.11	0.915
Dissolved oxygen (mg L <sup>-1</sup> )	6.4–8.8	7.95 ± 0.95	8–9.8	8.9 ± 1.27	1.26	0.275
Chlorides (mg L <sup>-1</sup> )	6–19	12.13 ± 3.76	14–23	18.5 ± 6.4	2.15	0.097
Sulphates (mg L <sup>-1</sup> )	0	0	0	0	-	-
Ammonia (mg L <sup>-1</sup> NH <sub>4</sub> <sup>+</sup> )	0.053–0.125	0.08 ± 0.02	0.06–0.063	0.0615 ± 0.002	0.99	0.375
Nitrates (mg L <sup>-1</sup> N-NO <sub>3</sub> )	0.5–1.6	0.75 ± 0.39	1.3–0.6	0.45 ± 0.21	1.23	0.285
Nitrites (mg L <sup>-1</sup> N-NO <sub>2</sub> )	0.05–0.09	0.07 ± 0.01	0.05–0.07	0.06 ± 0.01	0.44	0.685
Phosphates (mg L <sup>-1</sup> P-PO <sub>4</sub> )	0.03–0.10	0.059 ± 0.03	0.09–0.22	0.155 ± 0.092	2.17	0.096
BOD <sub>5</sub> (mg L <sup>-1</sup> )	0.6–4.8	2.1 ± 1.66	0.8–1.8	1.3 ± 0.71	0.36	0.736
COD (mg L <sup>-1</sup> )	3.5–11.6	8.96 ± 2.91	8–13.3	10.65 ± 3.75	0.62	0.568
Conductivity (µS cm <sup>-1</sup> )	132–180	151.38 ± 16.84	197–203	200 ± 4.24	<b>3.73</b>	<b>0.020</b>
pH (units)	7.4–8.5	7.94 ± 0.58	8.28–8.6	8.44 ± 0.23	1.04	0.358
IPS	19.2–19.6	19.4 ± 0.14	12.7–17.2	14.47 ± 2.40	<b>4.80</b>	<b>0.001</b>
TI	1.17–1.34	1.24 ± 0.07	2.26–2.84	2.55–0.29	<b>10.08</b>	<b>0.026</b>

The differences between mean values observed in the Pilato and Palazzo Borghese lakes were statistically significant, according to the *t*-test analysis, only for conductivity (*t* = 3.73; *p* = 0.020).

#### 3.2. Diatom Communities

In both lakes, a total of 111 diatomic species, belonging to 43 genera, were identified (Table 2; Supplementary Materials Figure S1). In particular, 88 different species and varieties have been found in the Pilato Lake, while 54 different species and varieties were determined in the Palazzo Borghese pond. The most species-rich genera were *Gomphonema* (12 species), *Navicula* (11 species), and *Nitzschia* (10 species).

**Table 2.** List of the 111 species of diatoms recognized in the Pilato and Palazzo Borghese lakes in 2019. For the species for which they are available, the values of diatomic indices IPS [27] and TI [28], and the threatened status according to Lange-Bertalot Red List criteria [40] were reported. The different quality classes are indicated with the following colours: light blue = Class I (High); green = Class II (Good); yellow = Class III (Moderate); orange = Class IV (Poor); red = Class V (Bad). 2 = severely endangered (species showing significant population decline or subject to substantial threat caused by human impact); 3 = endangered (species showing a significant population decline or one that is probably threatened by human impact); G = at risk (threatened species, but available information is not sufficient to allow an assignment to categories 1 to 3); Z = not classified; ? = not threatened (species populations have increased, are stable or have decreased slightly); \* = currently not considered threatened V = decreasing (species displaying a substantial population decline but not yet considered as threatened); R = extremely rare (extremely rare species for which there are only one to six records); D = data deficient (information on the distribution, biology or level of threat is insufficient).

Taxon	OMNIDIA Code	Pilato Lake	Palazzo Borghese Lake	IPS Sensitivity	TI Sensitivity	Threatened Status
<i>Achnanthes coarctata</i> (Brebisson) Grunow and Grun. 1880	ACOA	X	X	4.5	0.9	?
<i>Achnanthidium lineare</i> W. Smith 1999	ACLI	X		5.0	1.8	3
<i>Achnanthidium minutissimum</i> (Kützing) Czarnecki 1994	ADMI	X	X	5.0	1.2	?
<i>Amphora inariensis</i> Krammer 1980	AINA	X	X	5.0	2.1	3
<i>Amphora meridionalis</i> Levkov 2009	AMDN	X		2.6		Z
<i>Amphora ovalis</i> (Kützing) Kützing var. <i>ovalis</i> 1844	AOVA	X		3.0	3.3	?
<i>Aulacoseira granulata</i> (Ehr.) Simonsen 1979	AUGR	X	X	2.9		?
<i>Aulacoseira granulata</i> var. <i>angustissima</i> (Ehr.) Simonsen var. <i>angustissima</i> (O.M.) Simonsen 1979	AUGA	X	X	2.8		?
<i>Caloneis silicula</i> (Ehr.) Cleve 1894	CSIL	X		4.5	2.5	*
<i>Cocconeis euglypta</i> Ehrenberg emend Romero and Jahn 1854	CEUG	X		3.6	2.3	?
<i>Cocconeis euglyptoides</i> (Geitler) Lange-Bertalot 2004	CEUO	X		3.5		Z
<i>Cocconeis pediculus</i> Ehrenberg 1838	CPED	X		4.0	2.6	?
<i>Craticula ambigua</i> (Ehrenberg) Mann 1990	CAMB		X	3.0		*
<i>Craticula molestiformis</i> (Hustedt) Lange-Bertalot 2000	CMLF	X	X	2.0	2.9	?
<i>Cyclotella intermedia</i> (Manguin) Houk Klee and Tanaka 2010	CITD	X	X	5.0		Z
<i>Cyclotella meneghiniana</i> Kützing 1844	CMEN	X		2.0	2.8	?
<i>Cymbella excisa</i> Kützing 1844	CAEX	X		4.0		Z
<i>Cymbella excisa</i> var. <i>procera</i> Krammer 2002	CEPR	X		4.0		Z
<i>Cymbella parva</i> (W.Sm.) Kirchner 1878	CPAR	X	X	5.0		Z
<i>Cymbella perparva</i> Krammer 2002	CPPV	X		5.0		Z
<i>Cymbella vulgata</i> var. <i>plitovicensis</i> Krammer 2002	CVPL	X				Z
<i>Cymboplectra yateana</i> (Maillard) Krammer and Lange-Bertalot 1998	CBYA	X		4.8		Z
<i>Denticula tenuis</i> Kützing 1844	DTEN	X		5.0	1.4	*

Table 2. Cont.

Taxon	OMNIDIA Code	Pilato Lake	Palazzo Borghese Lake	IPS Sensitivity	TI Sensitivity	Threatened Status
<i>Diademsis contenta</i> (Grunow ex V. Heurck) Mann 1990	DCOT	X		4.0		?
<i>Diademsis perpusilla</i> (Grunow) D.G. Mann et al. 1990	DPER	X		5.0	1.2	?
<i>Diatoma moniliformis</i> Kützing 1833	DMON	X		4.0	2.0	?
<i>Diploneis krammerii</i> Lange-Bertalot and Reichardt 2000	DKRA	X		4.0		V
<i>Diploneis oculata</i> (Brebisson) Cleve 1894	DOCU	X		4.0	1.0	*
<i>Encyonema auerswaldi</i> Rabenhorst 1853	EAUE	X		4.0	2.1	Z
<i>Encyonema lange-bertalotii</i> Krammer morphotype 1 1997	ENLB	X		4.0		Z
<i>Encyonema minutum</i> (Hilse in Rabh.) Mann and Mann 1990	ENMI	X		4.6	2.6	*
<i>Encyonema ventricosum</i> (Agardh) Grunow et al. 1875	ENVE	X		4.0		*
<i>Epithemia adnata</i> (Kützing) Brébisson 1838	EADN	X		4.0	2.2	?
<i>Epithemia sorex</i> Kützing 1844	ESOR	X		4.0	2.7	?
<i>Eucocconeis laevis</i> (Oestrup) Lange-Bertalot 1999	EULA	X		5.0	1.2	*
<i>Eunotia arcus</i> Ehrenberg 1838	EARC	X		5.0	1.0	2
<i>Eunotia bilunaris</i> (Ehr.) Mills 1934	EBIL	X		5.0	1.7	?
<i>Eunotia minor</i> (Kützing) Grunow 1881	EMIN			4.6	1.5	*
<i>Fallacia insociabilis</i> (Krasske) Mann 1990	FINS	X	X	3.0		*
<i>Fistulifera saprophila</i> (Lange-Bertalot and Bonik) Lange-Bertalot 1997	FSAP		X	2.0	2.6	?
<i>Fragilaria acidoclinata</i> Lange-Bertalot and Hofmann 1993	FACD	X		5.0		G
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst 1864	GANG		X	3.0		*
<i>Gomphonema cuneolus</i> Reichardt 1997	GCUN	X		5.0		Z
<i>Gomphonema cymbelliclinum</i> Reichardt and Lange-Bertalot, 1999	GCBC		X	3.8		Z
<i>Gomphonema drutelingense</i> Reichardt 1999	GDRU		X	3.8		Z
<i>Gomphonema longilineare</i> Reichardt 1999	GLGL		X			Z
<i>Gomphonema micropus</i> Kützing 1844	GMIC	X	X	3.0	2.0	*
<i>Gomphonema minutum</i> (Ag.) Agardh 1831	GMIN	X	X	4.0	2.0	?
<i>Gomphonema olivaceum</i> (Hornemann) Brébisson 1838	GOLI	X		4.6	2.1	?
<i>Gomphonema pumilum</i> (Grunow) Reichardt and Lange-Bertalot, 1991	GPUM	X		4.5	1.6	*

Table 2. Cont.

Taxon	OMNIDIA Code	Pilato Lake	Palazzo Borghese Lake	IPS Sensitivity	TI Sensitivity	Threatened Status
<i>Gomphonema rosenstokianum</i> Lange-Bertalot and Reichardt 1993	GROS		X	5.0		Z
<i>Gomphonema sarcophagus</i> Gregory 1856	GSAR		X	3.2	1.3	V
<i>Gomphonema tergestinum</i> (Grunow) Fricke 1902	GTER	X	X	4.0	1.4	G
<i>Gyrosigma attenuatum</i> (Kützing) Rabenhorst 1853	GYAT		X	4.0	2.6	?
<i>Gyrosigma sciottense</i> (Sullivan and Wormley) Cleve 1894	GSCI	X		4.0	2.7	*
<i>Hantzschia abundans</i> Lange-Bertalot 1993	HABU	X	X	1.5	3.6	?
<i>Hantzschia amphioxys</i> (Ehr.) Grunow and Grunow 1880	HAMP	X	X	1.5	3.6	?
<i>Hantzschia calcifuga</i> Reichardt and Lange-Bertalot 2004	HCAL		X			D
<i>Luticola binodis</i> (Hustedt) M.B. Edlund 2001	LBIN		X			D
<i>Luticola mutica</i> (Kützing) Mann et al. 1990	LMUT	X	X	2.0	2.9	?
<i>Luticola nivalis</i> (Ehrenberg) Mann Crawford and Mann 1990	LNIV	X		5.0	2.9	D
<i>Meridion circulare</i> (Greville) Agardh 1831	MCIR	X	X	4.2	2.5	?
<i>Muelleria gibbula</i> (Cleve) Spaulding and Stoermer 1997	MUGI		X	4.9		G
<i>Navicula capitatoradiata</i> Germain 1981	NCPR	X		3.0	3.3	?
<i>Navicula cryptotenella</i> Lange-Bertalot 1985	NCTE	X		4.0	2.3	?
<i>Navicula densilineolata</i> (Lange-Bertalot) Lange-Bertalot 1993	NDSL	X		5.0		3
<i>Navicula gregaria</i> Donkin 1861	NGRE	X		3.4	3.5	?
<i>Navicula lanceolata</i> (Agardh) Ehrenberg 1838	NLAN	X		3.8	3.5	?
<i>Navicula lundii</i> Reichardt 1988	NLUN	X	X	4.8		D
<i>Navicula microdigitoradiata</i> Lange-Bertalot 1993	NMDG	X		3.0		R
<i>Navicula reichardtiana</i> Lange-Bertalot 1989	NRCH		X	3.6	2.3	?
<i>Navicula tripunctata</i> (Müller) Bory 1822	NTPT	X		4.4	3.1	?
<i>Navicula trivialis</i> Lange-Bertalot 1980	NTRV	X		2.0	3.3	?
<i>Navicula wildii</i> Lange-Bertalot 1993	NWIL	X	X		0.3	3
<i>Neidium bisulcatum</i> (Lagerstedt) Cleve 1894	NBIS	X	X	5.0	0.6	3
<i>Neidium longiceps</i> (Gregory) Ross 1947	NLGI		X	4.0	0.6	G
<i>Nitzschia acidoclinata</i> Lange-Bertalot 1976	NACD		X	5.0	2.3	*



Table 2. Cont.

Taxon	OMNIDIA Code	Pilato Lake	Palazzo Borghese Lake	IPS Sensitivity	TI Sensitivity	Threatened Status
<i>Nitzschia angustata</i> (Smith) Grunow 1880	NIAN	X				*
<i>Nitzschia clausii</i> Hantzsch 1860	NCLA		X	2.8	3.9	?
<i>Nitzschia dissipata</i> (Kützing) Grunow 1862	NDIS	X		4.0	2.4	?
<i>Nitzschia inconspicua</i> Grunow 1862	NINC	X		2.8	3.1	?
<i>Nitzschia linearis</i> (Agardh) Smith 1853	NLIN	X		3.0	3.4	?
<i>Nitzschia pusilla</i> (Kützing) Grunow 1862	NIPU	X		2.0	2.7	?
<i>Nitzschia subtilis</i> Grunow and Grunow 1880	NISU	X		3.0	3.9	*
<i>Nitzschia sociabilis</i> Hustedt 1957	NSOC	X		3.0	2.8	*
<i>Nitzschia supralitorea</i> Lange-Bertalot 1979	NZSU		X	1.5	2.9	?
<i>Pinnularia borealis</i> Ehrenberg 1843	PBOR	X	X	5.0	1.9	?
<i>Pinnularia grunowii</i> Krammer 2000	PGRU	X	X			Z
<i>Pinnularia microstauron</i> var. <i>angusta</i> (Ehr.) Krammer 2000	PMIA	X	X	4.0		Z
<i>Pinnularia obscura</i> Hustedt 1927	POMT		X	3		Z
<i>Pinnularia rupestris</i> Hantzsch 1861	PRUP		X	4.2		G
<i>Pinnularia schoenfelderii</i> Krammer 1992	PSHO	X	X	4.5		G
<i>Placoneis placentula</i> (Ehr.) Heinzerling 1908	PPLC	X	X	4.0	1.6	V
<i>Planothidium frequentissimum</i> (Lange-Bertalot) Lange-Bertalot 1999	PLFR	X		3.4	2.8	?
<i>Planothidium joursacense</i> (Hérilaud) Lange-Bertalot 1999	PJOU	X		3.0		3
<i>Planothidium lanceolatum</i> (Brebisson) Lange-Bertalot 1999	PTLA	X		4.6	3.3	?
<i>Psammothidium daonense</i> (Lange-Bertalot) Lange-Bertalot 1999	PDAO	X		4.5	1.1	G
<i>Pseudostaurosira robusta</i> (Fusey) Williams and Round 1987	PRBS	X		4.8	1.0	Z
<i>Reimeria sinuata</i> (Gregory) Kociolek and Stoermer 1987	RSIN	X		4.8	2.0	?
<i>Rhoicosphenia abbreviata</i> (Agardh) Lange-Bertalot 1980	RABB	X		4.0	2.9	?
<i>Sellaphora atomoides</i> (Grunow) Wetzel and Van de Vijver 2015	SEAT		X			Z
<i>Sellaphora pseudopupula</i> (Krasske) Lange-Bertalot 1996	SPPU	X	X	2.0		Z
<i>Sellaphora saugerresii</i> Wetzel et al. 2015	SSGE	X				?
<i>Stauroneis gracilis</i> Ehrenberg 1843	SGRC	X		5.0		V
<i>Stauroneis reichardtii</i> Lange-Bertalot et al. 2003	SRCH		X			Z
<i>Staurosira construens</i> Ehrenberg 1843	SCON	X		4.0	1.4	?
<i>Staurosira venter</i> (Ehr.) Cleve and Moeller 1881	SSVE	X	X	4.0		?

Table 2. Cont.

Taxon	OMNIDIA Code	Pilato Lake	Palazzo Borghese Lake	IPS Sensitivity	TI Sensitivity	Threatened Status
<i>Staurosirella lapponica</i> (Grunow) Williams and Round 1987	STLA	X		5.0		Z
<i>Tryblionella apiculata</i> Gregory 1857	TAPI		X	2.4	2.8	?
<i>Ulnaria acus</i> (Kützing) Aboal 2003	UACU	X	X	5.0	1.2	*
<i>Ulnaria ulna</i> (Nitzsch) Compère 2001	UULN	X		5.0	1.2	*

The diatom community of the Pilato lake was characterized by the dominance of a few species, represented by a high number of individuals (Specific Diversity Index (H) mean value = 1.66; Dominance Index (D) mean value = 0.60). High relative abundances of *Achnanthes minutissimum* (Kützing) Czarnecki 2008, *Cyclotella intermedia* (Manguin) Houk Klee and Tanaka 2010 and species belonging to the *Cymbella* C. Agardh 1830, and *Navicula* JBM Bory de St. Vincent 1822 genera, were observed (Figure 2a). Contrastingly, in the Palazzo Borghese pond the diatomic community was characterized by a greater diversity of species, with a high mean value of Shannon Diversity Index (Specific Diversity Index (H) mean value = 2.47; Dominance Index (D) mean value = 0.33). The community was characterized by stable and dominant populations of *Meridion circulare* CA. Agardh 1824 and some species of *Gomphonema* and *Navicula* genera (Figure 2b). Additionally, in this case, 15 diatom species belonging to the *Gomphonema* and *Pinnularia* genera were observed.

In the Pilato Lake, for the species *Cymbella excisa* var. *procera* Krammer 2002 and *Pseudostaurosira robusta* (Fusey) Williams and Round 1987, the individuals were characterized by small sizes in comparison to the standard measurements reported in the literature [37,41] (Figure 3). As regards the morphological characterization of the diatoms occurring in both lakes, smaller sizes than the norm were detected for *Craticula molestiformis*, *Neidium bisulcatum*, *Gomphonema micropus* and *Staurosira venter* (Ehr.) Cleve and Moeller 1881 (Figure 3).

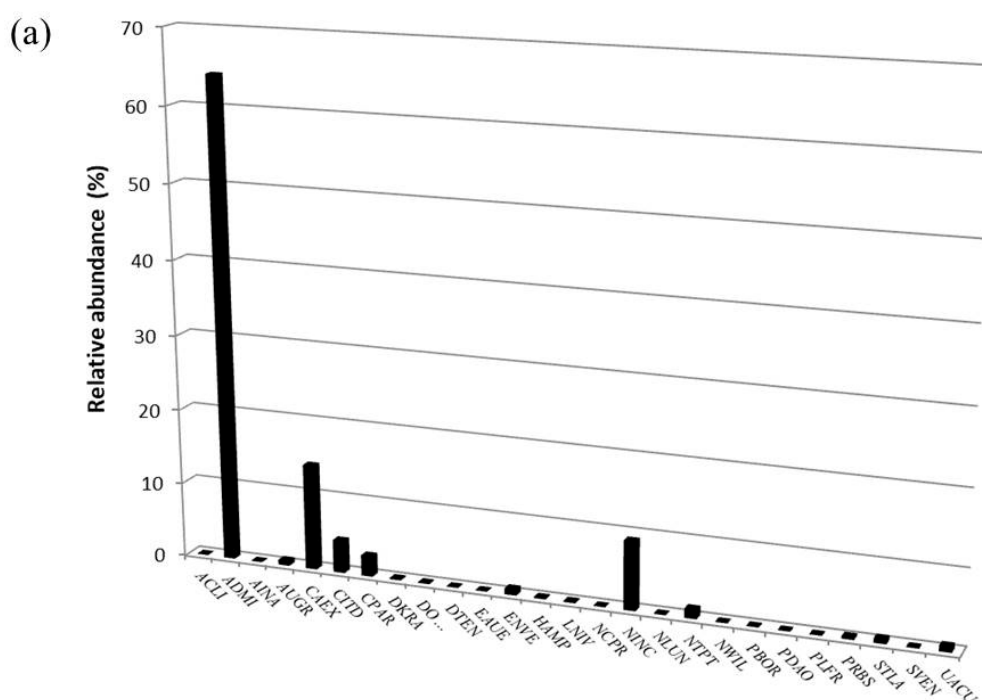
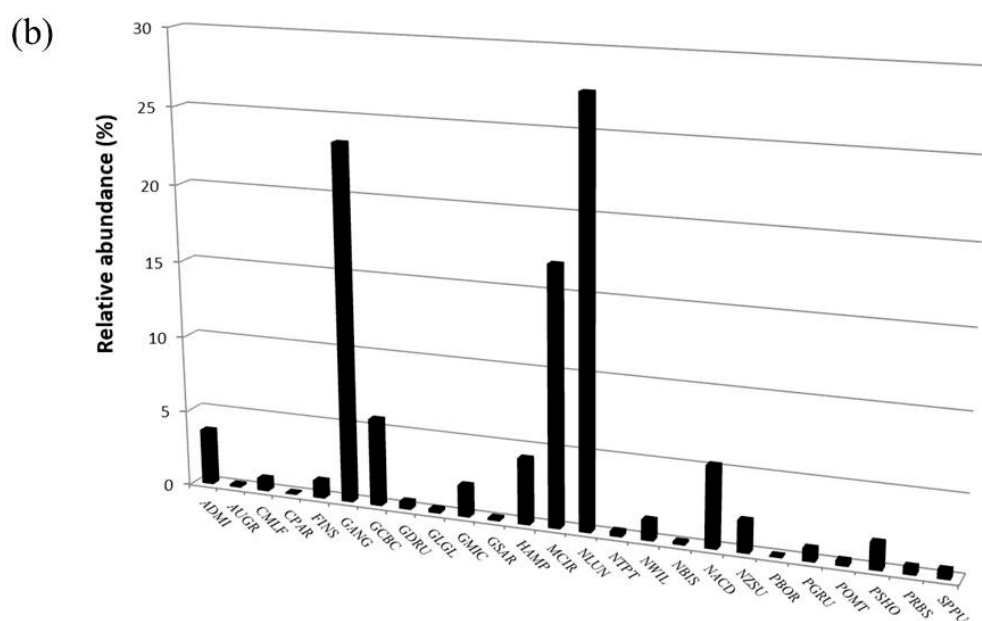
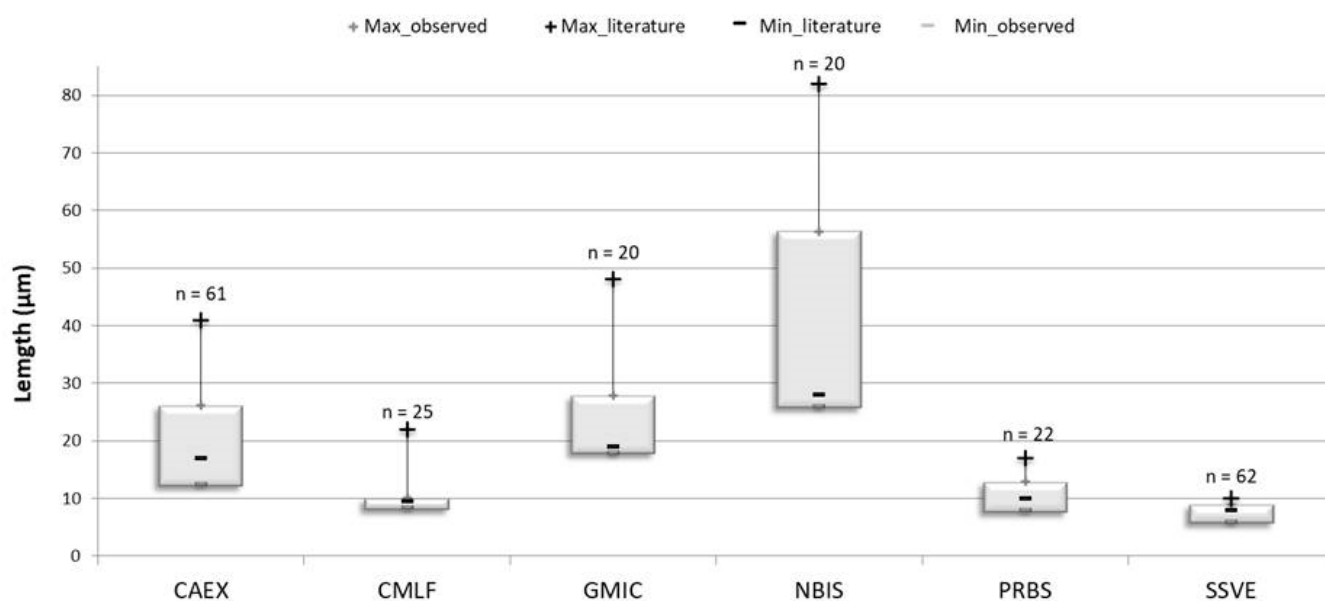


Figure 2. Cont.



**Figure 2.** Relative abundance of the diatom species found at least once in one sample for: (a) Pilato lake; (b) Palazzo Borghese Lake.



**Figure 3.** Mean length values for six diatomic species detected in the study area, and comparison with the length ranges reported in the literature. The bold dash represents the mean value of the minimum lengths of the species reported by the author of its description. The gray dash indicates the mean value of the minimum lengths of the species measured in the present study.

According to the Lange-Bertalot Red List of rare and/or threatened diatomic species [40], in the Pilato Lake was detected the presence of 12 species of particular conservation interest (Table 2). Six species—*Achnantheidium lineare* W. Smith 1999, *Amphora inariensis* Krammer 1980, *Navicula densilineolata* (Lange-Bertalot) Lange-Bertalot 1993, *Navicula wildii* Lange-Bertalot 1993, *Neidium bisulcatum* (Lagerstedt) Cleve 1894, and *Planothidium joursacense* (Hérubaud) Lange-Bertalot 1999—were listed as “endangered”. *Eunotia arcus* Ehrenberg 1838 was considered “severely endangered”. Four species—*Psammothidium daonense* (Lange-Bertalot) Lange-Bertalot 1999, *Fragilaria acidoclinata* Lange-Bertalot and

Hofmann, *Gomphonema tergestinum* (Grunow in Van Heurck) Schmidt in Schmidt et al. 1902, *Pinnularia schoenfelderi* Krammer 1992—were listed as “at risk of extinction”. Particular attention must be paid to *Navicula microdigitoradiata* Lange-Bertalot 1993, which was reported among the “extremely rare” species.

Additionally, in the Palazzo Borghese pond, the presence of the following threatened species (equal to 14.8% of the total) was detected: *Amphora inariensis*, *Navicula wildii*, and *Neidium bisulcatum* were listed as “endangered”. *Gomphonema tergestinum*, *Muelleria gibbula* (Cleve) Spaulding and Stoermer 1997, *Neidium longiceps* (Gregory) Ross 1947, *Pinnularia rupestris* Hantzsch in Rabenhorst, and *Pinnularia schoenfelderi* were considered “at risk of extinction”.

### 3.3. Ecological Preferences

In the Pilato lake the analysis of diatomic indexes showed a high percentage of species belonging to the high and good classes (68.2% for IPS and 35.2% for TI), indicating a high sensitivity to pollution and trophic levels (Table S1). The presence of many aerial or subaerial species was testified by a high percentage of species (38%) which are also adapted to terrestrial environments or temporarily dry places [39]. The ecological characterization of the recognized species based on the standard ecological requirements reported in Van Dam et al. [39], showed the dominance of alkaline (52%) and circumneutral species (32%); high percentages of species that require a high (22%) and good (25%) quantity of oxygen has been observed. Poorly tolerant  $\beta$ -mesosaprobia species (37%) prevailed over oligosaprobia species (12%); species characteristic of eutrophic environments (36%) were more abundant than oligotrophic ones (16%), although the Pilato Lake showed marked oligotrophic characteristics, typical of high mountain lakes.

In the Palazzo Borghese Lake, the diatomic indexes analysis showed a fairly evenly distributed presence of species belonging to the different classes, (IPS: High 24.1%, Good 22.2%, Moderate 18.5%, Poor 14.8%). The presence of aerial or sub-aerial species was represented by 23 species, equal to 43% of the total species found in the lake. According to Van Dam et al. [39], the ecological characteristics of the recognized species showed that: alkaline and neutrophilic species were dominant (63%) representing the majority in the diatom community; the prevalence of species requiring a good (22%) and moderate (16%) quantity of dissolved oxygen was observed; poorly tolerant  $\beta$ -mesosaprobia species (30%) prevailed over oligosaprobia species (11%); the eutrophic species, typical of nutrient-rich environments, were dominant (30%), in contrast with the oligotrophic characteristics of the pond.

The differences between mean values of IPS and TI observed in the Pilato and Palazzo Borghese lakes were statistically significant, according to the *t*-test analysis, for both indices (IPS:  $t = 4.80$ ;  $p = 0.001$ ; TI:  $t = 10.08$ ;  $p = 0.026$ ) (Table 1).

In both lakes, the incidence of teratological forms was equal to 0.5%. Deformities in the valve outline or in the frustule morphology were observed for: *Achnanthes minutissimum*, *Cocconeis euglypta* Ehrenberg emend Romero and Jahn 1854, *Cymboplectra yateana* (Maillard) Krammer and Lange-Bertalot 1998, *Fistulifera saprophila* (Lange-Bertalot and Bonik) Lange-Bertalot 1997, *Gomphonema* spp., *Meridion circulare*, *Navicula* spp., *Nitzschia* spp., *Stauroneis reichardtii* Lange-Bertalot et al. 2003 and *Staurosira venter* (Figure S2). These abnormal forms were more frequent in the epiphytic samples of the Palazzo Borghese lake. In most cases, the malformations resulted in a decrease in the cell surface, with constrictions involving one or both sides of the cell. No mixed situations were observed, in terms of simultaneous alterations on the same valve, nor changes on frustules in connective view.

## 4. Discussion

The analysis of water chemistry in Table 1 did not reveal the existence of substantial differences compared to what is reported in the literature for either of the two lakes [21,42], whose chemical characteristics are typical of high-altitude oligotrophic lakes. The Palazzo Borghese Lake showed significantly higher conductivity values than Lake Pilato: this

result may reflect the presence in the catchment area of a non-negligible grazing intensity. In fact, being located in a pasture land, the Palazzo Borghese lake is subject to a greater supply of nutrients due to surface run-off water during snow-melting. Certainly further investigations are required to be able to draw comprehensive conclusions on this issue; however, our results did not highlight the presence of strong impacts on the water quality, resulting from the grazing of domestic animals in the basin and in the surrounding areas. Except for the conductivity, the other physico-chemical parameters of the water were comparable with those measured in the Pilato Lake.

As reported for other Mediterranean high-mountain temporary ponds [5], both lakes harbored a high diatom diversity, including many rare and endangered species. In agreement with Falasco et al. [43], our findings showed that the diatom assemblages of the lakes were rich and complex, despite the extreme environmental conditions they have to face.

Both lakes showed overall diatomic communities typical of good quality waters, but they also differ in terms of species diversity and dominance. The higher species richness in the Pilato Lake confirmed the condition of a larger and more durable lake. It offers a greater number of niches than those naturally occurring in the Palazzo Borghese pond, which usually persists only for one to two months, from the snow-melting process in early spring until the beginning of June. Here, the high Shannon Diversity index ( $H = 2.47$ ) showed that the dominance was less concentrated. Therefore, on the one hand, the Pilato Lake was inhabited by more species, but few were dominant and many of them were rare; in the small lake of Palazzo Borghese, on the other hand, there are fewer species but better subdivided into the different microhabitats.

As could have expected in the case of high-altitude lakes with calcareous bedrock, the ecological characteristics of the diatom species found in the investigated area showed a high percentage of species typical of waters with pH close to neutrality, well oxygenated, slightly saline, autotrophic tolerant, oligotrophic. The hydrological regime that characterizes the astatic environments represented by the two lakes implies some important repercussions at the level of adaptive strategies adopted by the colonizing species. The presence of aerial and subaerial diatomic species was detected in both lakes; these species are typical of aquatic environments subjected to summer drying, and therefore they are able to survive in extreme physical conditions. This ability to colonize environments subjected to drought periods appeared particularly advantageous in the Pilato and Palazzo Borghese lakes, given the close link between their hydrological characteristics and climate conditions, with special reference to winter snowfall as limiting factor.

#### *Future Perspectives and Management Implications*

The morphological characterization of the species whose dimensions have been found to be smaller than those reported in the literature, deserves further studies. The small size observed for *Cymbella excisa* var. *procera*, *Pseudostaurosira robusta*, *Craticula molestiformis*, *Neidium bisulcatum*, *Gomphonema micropus* and *Staurosira venter* could be hypothetically related to the climate change effects in terms of water warming, which seem to favor small-sized diatom cells [44]. Some studies highlighted the decline in diatom cell size, linking it to rising water temperatures in lake ecosystems associated to climate changes [45,46]. Small-sized diatoms, thanks to their high surface area to volume ratios, have a greater efficiency in nutrient uptake in the presence of light, and divide faster than large-sized cells [47], and thus they can be advantageous under water warming conditions.

The present study also addressed the incidence of teratological forms, which in such fragile environments represented an important added value to algal characterization and a significant prospect of study for the future. In fact, the presence of diatom teratologies could represent a response to environmental stress in terms of water pollution, eutrophication, heavy metal contamination, salinity, drought conditions and so on [48]. Nevertheless, as highlighted by Falasco et al. [43], the teratological forms are not included in the calculation of the indices contemplated by the WFD, thus representing a limit to the assessment of the ecological status of surface waters. In our study, we considered appropriate to highlight



their presence, albeit with low percentages (0.5%), because were several recognized species showing malformations of the frustule, especially evident for some individuals belonging to the genus *Gomphonema* and *Navicula*. In particular, the occurrence of individuals affected by teratology in the epiphytic samples of the Palazzo Borghese Lake, could be reasonably related to the physical stress (drought conditions) caused by the temporariness of the aquatic system. Moreover, recent studies conducted in marine Mediterranean systems [49] evidenced a relevant incidence of salinity on the teratological forms in planktonic foraminiferal assemblages. Falasco et al. [48] reported high conductivity and salinity among the causes of teratology for many freshwater diatoms. According to these findings, it is possible to hypothesize that the higher incidence of teratological forms found in the lake of Palazzo Borghese could reflect conductivity differences, but no specific studies shedding light on this aspect are available in the literature. Further investigations are needed to support this hypothesis.

## 5. Conclusions

The presence in both lakes of a total of 15 species reported in the Red List means that the two biotopes should be considered unique environments to be protected. As with other high-altitude lakes, Pilato and Palazzo Borghese lakes are also rapidly changing under the influence of climate change [15], confirming the role that these environments play in witnessing the effects of global warming. Lake Pilato, in particular, is undergoing increasingly frequent early drying events [15] which will inevitably have repercussions on its biocoenoses. The data collected in the present study provide a starting point for understanding how the diatomic community will modify in the future in relation to these changes. Moreover, for 24 species (21.6% of the total) it was not possible to evaluate the conservation status as they are not classified in the Red List compiled by Lange-Bertalot and Steindorf [40], which was developed for the diatom species occurring in Germany. Therefore, in accordance with what wished by Falasco et al. [43], in our opinion, there is the need to draw up specific catalogues for the Mediterranean Alpine region, integrating data available in the literature with new information. Finally, a discrepancy in the indicator values of some diatoms species was observed. In particular, the presence in the community of many diatom species classified as having a high-nutrient optimum, did not reflect the real oligotrophic conditions of the investigated environments; this result suggests the need to deepen and update, through new research, the ecological preferences of the species showing a poor predictive performance for the ecological status of high-elevation lakes.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/environments8080079/s1>, Figure S1: Illustrations of the 111 species of diatoms recognized in the Pilato and Palazzo Borghese lakes. All bars correspond to a length of 10 µm. Figure S2: Teratological forms observed for *Achnantheidium minutissimum*, *Cocconeis euglypta*, *Cymbopleura yateana*, *Fistulifera saprophila*, *Gomphonema* spp., *Meridion circulare*, *Navicula* spp., *Navicula tripunctata*, *Nitzschia acidoclinata*, *Nitzschia supralitorea*, *Stauroneis reichardtii*, *Staurosira venter*, *Birafidea*.

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