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Trophic State Evaluation of a Large Mediterranean Lake Utilizing Abiotic and Biotic Elements

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ABSTRACT

The trophic state of a freshwater ecosystem reflects its environmental quality. This is why several trophic indicators have been developed for such water bodies based on chemical, physical and biological parameters. Apart from that, there are several biotic elements which can be used in accessing the environmental condition of a freshwater ecosystem. Zooplankton organisms are important elements of the structure and function of lakes and are considered useful indicators of alterations in their trophic dynamics and ecological state related to changes in nutrient loading and climate. In accordance to the above, the present study is an attempt to assess the trophic condition of the largest lake in Greece (Lake Trichonis) through the investigation of the physicochemical elements, along with the biotic indications provided by a three-year study of the lake's zooplankton. The present results, compared with previous studies conducted between 15 and 25 years before, showed that there was an increase in the maximum values of the concentrations of chlorophyll-a and nutrients, while there was a decrease in water transparency. The implementation of Carslon's trophic state index (TSI) revealed that Lake Trichonis still remains an oligo-to mesotrophic ecosystem as it was in the past. However, although the zooplankton investigation showed several features that are common in oligotrophic lakes, there are certain eutrophic characteristics of the zooplankton community (e.g. abundance variation pattern, indicator species, seasonal succession of cladocerans) pointing out a different state of the ecosystem in comparison to the past. In conclusion, the use of a biotic element like zooplankton revealed that Lake Trichonis is experiencing a transitional condition towards the eutrophic state and points out the necessity for constant inspection and monitoring of this ecosystem.

KEYWORDS

1. Introduction

Eutrophication of freshwater ecosystems such as natural lakes and reservoirs is a major environmental problem. Generally, eutrophication is the result of nutrient overenrichment of the water originated from natural processes such as decomposition of organic matter and sediment nutrient release, or to land-based activities and external nutrient loading (e.g. agricultural activities, sewage and industrial discharges, atmospheric releases from fossil fuel combustion, etc.). Agricultural activities are considered as the major provider of nutrients in lakes, especially in the Mediterranean region, where agricultural sector comprises an important economic factor [1]. Eutrophication involves a change in a lake's status from a macrophyte-dominated clear water state to a phytoplankton-dominated turbid state, with severe effects to the ecosystem like changes in the structure and trophic interactions among phytoplankton, zooplankton and benthic communities.

The growing need for the control of nutrient enrichment and pollution leads the European Union to the establishment of the Water Framework Directive (2000/60/

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EC) in order to achieve a "good quality" in all European water bodies. Several indicators, indexes and models have been developed to assess eutrophication and water quality in aquatic ecosystems based on chemical, physical and biological parameters. Carlson's trophic state index (*TSI*) is the most widely used in freshwater bodies [2]. The index uses phytoplankton biomass estimated indirectly by chlorophyll-α pigment concentrations, water transparency (by the use of Secchi depth) and total phosphorus concentrations. The TSI index ranges from 0 to 100, although theoretically there are no lower or upper boundaries. *TSI* values less than 40 correspond to oligotrophic conditions, while between 40 and 50 for mesotrophic and between 50 and 70 for eutrophic. Finally, index values greater than 70 are associated with hypertrophic conditions.

Except from direct measurements of physicochemical and biological elements, there are several biotic indicators which can be used in accessing the environmental quality of a water body [3]. Thus, in contrast to the instant evaluation of the state of an ecosystem provided by the measurements of physicochemical parameters, the biotic indicators have the advantage to show the influence of the chronic affection of the environmental disturbance to the structure of the aquatic communities, as it comes from the conditions existed before the measurements. In this way, the use of biotic indicators can be more useful in prediction of the evolution of the quality of an aquatic ecosystem. Zooplankton organisms are important elements of the structure and function of lakes, as they occupy a critical position between phytoplankton and higher consumers like fishes within the trophic web [4]. Its strategic position in the aquatic ecosystems, as well as its sensitivity to both man-made and natural changes, makes zooplankton quite suitable for biological monitoring of water quality [5]. Zooplankton is still not included as a biological quality parameter for aquatic ecosystems according to the implementation of the EU Water Framework Directive, though there are several studies that have shown its usefulness as an indicator of alterations in the trophic dynamics and the ecological state of lakes related to changes in nutrient loading and climate [6].

Lake Trichonis is the largest natural lake in Greece, having a surface area of 98.6 km^2, a catchment area of 421 km^2 and a potential water volume of approximately 2.868 km^3 [7]. It is located in the prefecture of Aitoloakarnania in the western part of the country (38°18'N - 38°51'N, 21°01'E - 21°42'E) at an altitude of 18 m a.s.l. It is a deep (Z_{max} = 57 m, Z_{mean} = 29 m) and warm monomictic lake, exhibiting a long period of thermal stratification. There are various surface water supplies (e.g. seasonal streams), as well as groundwater inflows (approx. 30% of the total annual water inflows), which

provide an adequate quantity of water and resulted to positive water balance [7]. The excess of the lake's water is discharged through a sluice gate canal to the adjacent Lysimachia Lake to avoid potential flooding. From the biodiversity point of view, Lake Trichonis has great ecological importance and was included in the Natura 2000 protection network due to the priority habitat of calcareous fens (*Cladium mariscus*). Moreover, it has also great economic importance in respect to the use of water for irrigation purposes of the nearby agricultural areas, as well as to the commercial fishery of *Atherina boyeri* which is the dominant species in the fish community [8]. Around the lake there are several villages with a total population of about 35,000 people and the main economic activities are agriculture, livestock breeding and olive-oil refinery manufacturing. In the cathcment area of the lake there are 150 km^2 of agricultural land (mainly tobacco, olive trees, cotton and corn), while there are also 33 operating olive-oil refineries that dispose their wastes untreated in septic tanks or directly in local streams. All the above activities, in combination with the lack of a wastewater treatment plant in the catchment area, imply significant organic pollution loads for the lake [1].

The environmental elements of Lake Trichonis and its trophic condition have been documented by several studies [1,9-11] in the past and according to them the lake has been classified as oligotrophic to mesotrophic. At the more recent report of Bertahas *et al.* [1], which compare data from two periods within a decade (1990-1991 and 2001-2002), the trophic state of the lake seemed to have altered from mesotrophic in the former period to oligotrophic in the latter, although the nutrients' concentrations have been greatly increased during this decade. This paradox was attributed to the particular hydrology of the lake by the above authors, who concluded that its trophic status is mainly hydrologically dependent and thus unpredictable [1]. On the other hand, there have been recent reports of the presence of zooplankton species in Lake Trichonis which are considered indicators of eutrophic conditions in other European lakes [12,13]. Consequently, all the above reports give a puzzling icon of the environmental state of the lake and, along with the given ecological and economic importance of this ecosystem, make the need for further investigation imperative.

Considering the above, through a three-year investigation (between 2003 and 2006), the present study aims to: 1) provide the most recent analytical data of the physicochemical environment and of the trophic state of the lake; 2) compare these recent data with the previous studies to assess its environmental state progress; 3) make an effort to combine abiotic and biotic (e.g. zooplankton) data in order to draw a more accurate picture of the present and possibly the future environmental

quality of this ecosystem.

2. Materials and Methods

2.1. Zooplankton Sampling and *in Situ* Measurements

Three sampling stations (A, B and C) with depths of 48, 35 and 25 m respectively, were selected for data collection in Lake Trichonis (**Figure 1**). The overall sampling was carried out at monthly intervals during a three-year period from September 2003 to August 2006. Zooplankton samples were taken from all the sampling stations during the first two periods (September 2003-August 2005), while due to the lack of differences among the three stations (see bellow) and for logistic reasons, the samples were taken only from the deepest station A in the third period (September 2005-August 2006). The zooplankton was collected with a conical plankton net (40 cm in diameter, 100 cm in length, 50 µm mesh size), which was modified to be a closing net with the addition of a second rope and a releasing trigger, analogous to those of the WP-2 closing net. Vertical hauls were conducted at 10 m depth intervals from the surface down to 40 m, to 30 m and to 20 m for the stations A, B and C respectively. The net was towed at a speed of approximately 0.5 m·sec^{-1}. All samples were taken in the morning and were preserved in plastic bottles filled with 4% neutralized formalin solution.

In situ measurements of the physicochemical parameters were taken during the second sampling period (September 2004-August 2005) from all the three stations, while from just station A during the third period (September 2005-August 2006). Temperature, dissolved oxygen concentration (DO), pH and conductivity measurements were taken at 2 m intervals from the surface down to a maximum depth of 40 m, using WTW portable instruments. Water transparency was measured with a Secchi disc.
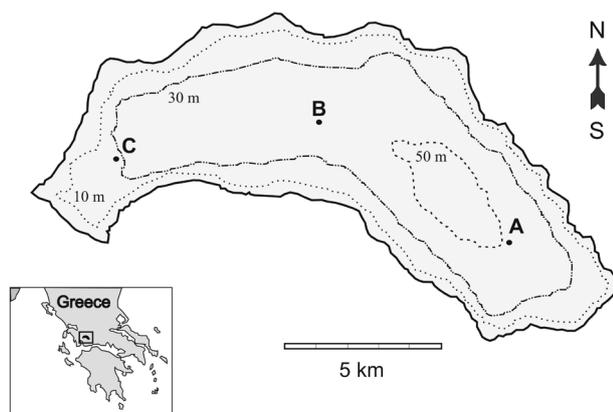
Figure 1. Lake Trichonis with the three sampling stations (A, B and C).

2.2. Chemical Parameters and Chlorophyll-*α*

For the estimation of total phosphorus (*TP*), phosphates (PO$_4$), nitrates (NO$_3$), nitrites (NO$_2$) and ammonia (NH$_4$), water samples were collected at all stations (September 2004-August 2005) and at the deepest station A (September 2005-August 2006) from 0, 10, 20, 30 and 40 m with a 5 l Hydrobios water sampler. Analyses of all chemical parameters were performed according to APHA, AWWA & WPCF [14]. For the determination of chlorophyll-*α* (*Chl-α*), 1500 ml of the water samples taken from the above depths was filtered through a Whatman GF/A glass fibre filter shortly after collection. Pigment extraction was made in 90% acetone and concentrations were determined spectrophotometrically [14]. Due to technical problems, no *Chl-α* measurements were taken in the period between September 2004 and February 2005.

2.3. Trophic Classification

The trophic classification of the lake was estimated using Carslon's [2] trophic state index (*TSI*) which utilizes chlorophyll-*a* (*Chl-a*), Secchi disk transparency (*SD*) and total phosphorus concentrations (*TP*). In particular, the TSI index for the three different quality variables such as Secchi depth TSI_{SD}, chlorophyll-*a* TSI_{Chl-a} and total phosphorus TSI_{TP} was calculated according to the equations:

$$TSI_{SD} = 60 - 14.41 \times \ln(SD)$$

$$TSI_{Chl-a} = 30.6 + 9.81 \times \ln(Chl-a)$$

$$TSI_{TP} = 4.15 + 14.42 \times \ln(TP)$$

For TSI_{Chl-a} and TSI_{TP}, the average epilimnetic (0 - 10 m) concentrations of *Chl-a* and TP (both as µg·l^{-1}) were used, while for the TSI_{SD}, the values of the Secchi disk (m) in each sampling station was used. The overall Carlson's *TSI* index was calculated as the average value of TSI_{SD}, TSI_{Chl-a} and TSI_{TP} in the three stations as follows:

$$TSI = \frac{TSI_{SD} + TSI_{Chl-a} + TSI_{TP}}{3}$$

2.4. Zooplankton Analysis

In the laboratory, the zooplankton specimens were examined microscopically and were identified to the lowest taxonomic level possible, using the keys of Rylov [15], Ruttner–Kolisko [16], Korovchinsky [17], Alonso [18] and Benzie [19]. For the abundance analysis, three counts of 1.5 ml sub-samples from each sample were made on a Sedwick-Rafter cell having a total volume of 100 ml [13]. The non-parametric Kruskal-Wallis test and the Mann-Whitney test (U-test) were used for assessing differences in the environmental parameters and in the

abundance of zooplankton species and groups among sampling stations and sampling periods.

3. Results and Discussion

The temporal variations of the physicochemical parameters recorded in station A in Lake Trichonis during a two-year period (September 2004-August 2006) are presented in **Figures 2** and **3**. Station A is selected to be the most representative for the changes monitored along the entire water column of the lake, since it is the deepest station and considering the absence of statistically significant differences among the three sampling stations for almost any of the above parameters (Kruskal-Wallis test, $p > 0.05$).

3.1. *In Situ* Measurements

Lake Trichonis is a deep, warm monomictic lake where
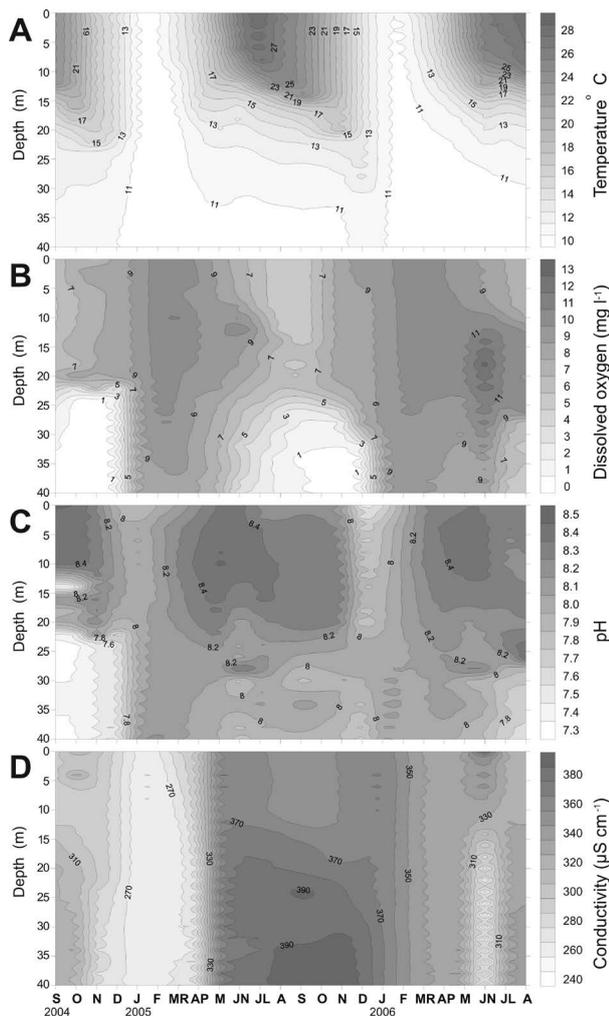
Figure 2. Vertical profiles of temperature (A), dissolved oxygen (B), pH (C) and conductivity (D) measured *in situ* in the deeper sampling station A of Lake Trichonis during September 2004 - August 2006.
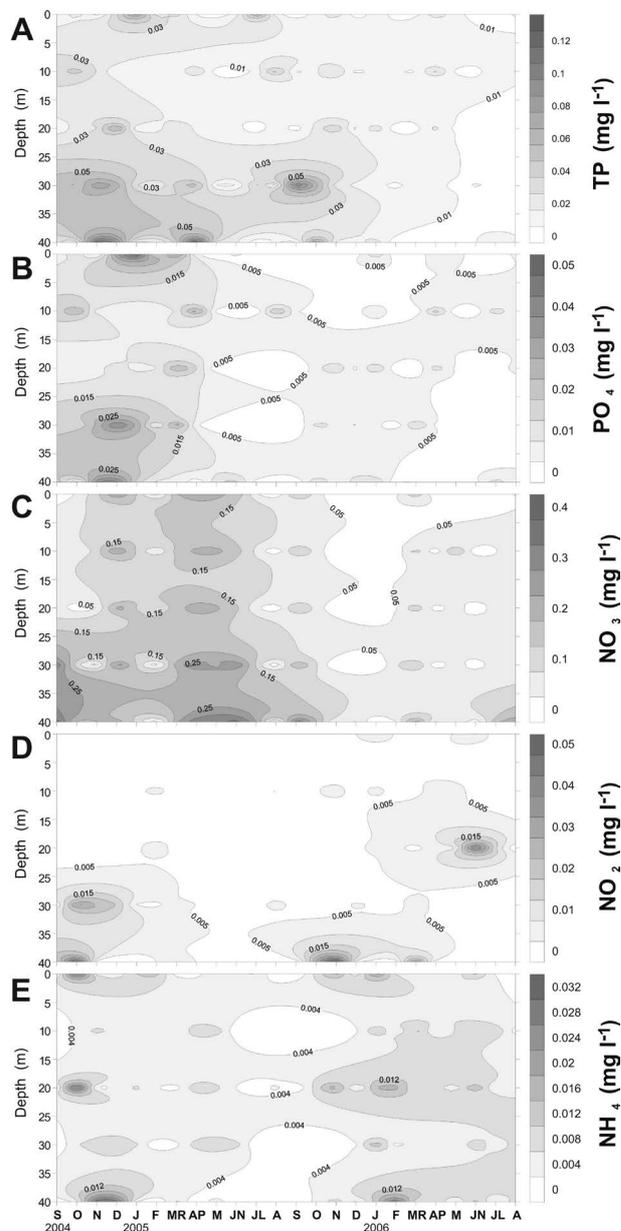
Figure 3. Vertical profiles of the concentrations of total phosphorus (A), phosphates (B), nitrates (C), nitrites (D) and ammonia (E) in the deeper sampling station A of Lake Trichonis during September 2004 - August 2006.

the temperature variation leads to the development of a seasonal thermocline, causing highly stable water stratification within the lake. Temperature fluctuated between 10°C and 29.7°C (**Figure 2**) and there were no differences between the sampling periods and among the three sampling stations (Kruskal-Wallis test, $p > 0.05$). In comparison to the previous studies of Tafas *et al*. [11], Koussouris *et al*. [10] and Overbeck *et al*. [9] the maximum temperature value was greater by 2°C, 3.7°C and 5°C respectively (**Table 1**). Stratification of the lake started in April and the thermocline, which was created

Table 1. Comparisons of the variation of the physicochemical parameters recorded in Lake Trichonis during the present study with analogous reports from previous studies.

Parameter	Present study	Tafas *et al.*	Koussouris *et al.*	Overbeck *et al.*
	(2004-06)	(1997)	(1993)	(1982)
Temperature (˚C)	10 - 29.7	9.5 - 27.5	11.1 - 28.0	10.0 - 25.0
Dissolved oxygen (mg·l^{-1})	0.3 - 14	3.7 - 12.0	0.5 - 12.2	2.0 - 12.0
pH	7.37 - 8.55	7.6 - 8.5	7.7 - 8.8	8.1 - 8.5
Conductivity (µS·cm^{-1})	247.5 - 398.2	195 - 320	230 - 270	267 - 301
Transparency (m)	4 - 13	4 - 11	4 - 12.5	5.7 - 13.9
Chlorophyll-α (µg·l^{-1})	0.2 - 9.4	2.2 - 8.2	2.3$^{(1)}$, 4.3$^{(2)}$	0.5 - 0.8
TP (µg·l^{-1})	1.5 - 131	<7	15$^{(1)}$, 66$^{(2)}$	22 - 112
PO$_4$ (µg·l^{-1})	0 - 95.2	<20 - 70	1 - 14	2 - 40
NO$_3$ (mg·l^{-1})	0 - 0.718	0.05 - 0.45	0.003 - 0.125	0.02 - 0.232
NO$_2$ (mg·l^{-1})	0 - 0.058	0 - 0.005	0.0013 - 0.018	0.001 - 0.005
NH$_4$ (mg·l^{-1})	0 - 0.035	<0.01 - 0.09	0.005 - 0.13	0 - 0.062

$^{(1)}$: Average value, $^{(2)}$: Max value.

within the 10 - 20 m layer, lasted until November. Turnover started to appear in December where the metalimnetic layer sinks to about 25 m and this gradual mixing of the water column lasted until the end of February. During turnover, and especially in January and February, temperature had a uniform distribution from the surface to the bottom of the lake (**Figure 2**).

The water transparency fluctuated between 4 and 13 m and presented higher values in the summer and lower in winter. The lowest transparency value of 4 m was equal to the respective value reported by Tafas *et al.* [11] and Koussouris *et al.* [10], and was recorder in winter at the shallowest station C, which is influenced by the high amounts of water drainage entering into the lake in this period due to increased precipitation.

The water stratification in Lake Trichonis resulted to the presence of greater dissolved oxygen concentrations within the metalimnion, a feature common in deep stratified lakes [20]. The high transparency of the water during the summer period permitted the photosynthetic activity in the metalimnion, where the phytoplankton growth was benefited by the high nutrient concentrations released by decomposition processes. Indeed, the maximum concentration of dissolved oxygen was recorded in June 2006 (13.93 mg·l^{-1}) within this layer as a result of oxygen produced by algal populations. During the stratification period the DO in the hypolimnetic layer decreased leading to hypoxic conditions (DO < 2 mg·l^{-1}) under the depth of 30 m in autumn. In contrast, during the winter turnover the lake was well oxygenated (DO between 8.30 and 10.85 mg·l^{-1}) throughout the entire water column (**Figure 2**). In comparison to the previous studies in the lake (**Table 1**), the maximum DO concen-

tration recorded in the present study was higher and the minimum DO value was lower.

There was little variation in the pH values which fluctuated between 7.37 and 8.55 during the entire study period, while there were no significant differences with the previous studies (**Table 1**). In the vertical axis, higher pH values were recorded in the epilimnion during the stratified period, while they were almost constant in the whole water column during the mixing period (**Figure 2**).

Conductivity fluctuated between 247.5 and 398.2 µS·cm^{-1}, with higher values in the hypolimnion during the stratification period, while during the mixing period there was little conductivity variation with depth (**Figure 2**). It must be pointed that, the values in the third sampling period (September 2005-August 2006) were considerably greater than the second one (September 2004-August 2005), although this was not statistically significant (U-test, p > 0.05). Considering the previous studies of Tafas *et al.* [11], Koussouris *et al.* [10] and Overbeck *et al.* [9], there was an increase of the highest value of conductivity by 24.4%, 47.5% and 32.3%, respectively (**Table 1**).

3.2. Chemical Parameters and Chlorophyll-α Concentrations

The concentration of total phosphorus (*TP*) presented the highest value of 0.131 mg·l^{-1} in April 2005 in 40 m (station A). Statistically significant differences among the three sampling stations were found (Kruskal-Wallis test, p < 0.05), with station C presenting higher values. This station is in the part of the lake that is close to agricultural land and activities, which probably were responsi-

ble for the greater enrichment of the water with phosphorus. A statistically significant difference (U-test, p < 0.05) in the TP concentrations between the two sampling periods was recorded, with higher values in the second period (September 2004-August 2005) in comparison to the third one (September 2005-August 2006). The same was also true for the concentrations of phosphates (PO_4) and also the nitrates (NO_3) between the two periods (U-test, p < 0.05), while no differences among stations concerning these two nutrients were found (Kruskal-Wallis test, p > 0.05). The phosphates in the water fluctuated between undetected values to 0.095 mg·l^{-1} (**Figure 3**). Nitrates (NO_3) and nitrites (NO_2) showed intense temporal variation and their concentrations fluctuated between undetected values to 0.718 and 0.058 mg·l^{-1} in July 2005 (station B, 30 m) and in November 2005 (station A, 40 m), respectively. The concentration of ammonia (NH_4) generally presented low values except in October 2004 when high values reaching up to 0.035 mg·l^{-1} were observed in the three sampling stations. There were no statistically significant differences between the two sampling periods for ammonia as well as for nitrates and nitrites (U-test, p > 0.05). Most of the above parameters presented considerably higher concentrations in comparison to the previous studies of Overbeck *et al*. [9], Koussouris *et al*. [10] and Tafas *et al*. [11] (**Table 1**). In particular, there was a remarkable increase of the maximum concentration of nutrients, such as TP (17.0% - 1771%), phosphates (36.0% - 580.0%), nitrates (59.6% - 474.4 %) and nitrites (222.2% - 1060%). In addition, the present values for the average concentration of *TP*, nitrates and nitrites in the water column are higher than the respective values reported by Bertahas *et al*. [1] in their survey conducted in 2001-2002, while the average concentrations of phosphates and ammonia are lower. Ammonia has been increased by 43.4% in comparison to Overbeck *et al*. [9] (1982), but considerably decreased by 157.1% and 271.4% in comparison to Tafas *et al*. [11] and Koussouris *et al*. [10], respectively.

In the vertical axis, the general trend for most of the above chemical parameters was to present lower values in the epilimnion and higher in the meta-or/and the hypolimnion during the stratified period, while their concentration was more uniform during turnover (**Figure 3**). The higher *TP* values in the hypolimnion were recorded during stratification and especially in the period of oxygen depletion, while during the mixing period higher concentrations were recorded just close to the bottom of the lake. The reason for this is probably the greater production of phosphorus due to decomposition of organic matter within the hypolimnion at the end of the stratification period (autumn), or/and to phosphorus release from the bottom sediment.

The concentrations of *Chl-α* fluctuated between 0.227

μg·l^{-1} in July 2006 at the surface to 9.37 μg·l^{-1} in April 2006 in the 20 m of station A. *Chl-α* was always higher in the metalimnetic layer during the stratification period, while it was having a uniform distribution during the mixing period (**Figure 4**). No differences among the three stations were found (Kruskal-Wallis test, p > 0.05). The minimum *Chl-α* concentration reported in the present study was lower than the respective values in all the previous studies, while the present maximum value overcomes these of the past (**Table 1**).

3.3. Trophic Classification

The application of Carlson's index (*TSI*) revealed values between 25.7 - 67.5 for total phosphorus (TSI_{TP}), 23.0 - 40.0 for transparency (TSI_{SD}) and 15.2 - 47.5 for *Chl-α*, (TSI_{Chl-a}) considering all sampling stations (**Figure 5**). No statistically significant differences among the three sampling stations for TSI_{TP}, TSI_{SD} and the total *TSI* were found, although the TSI_{Chl-a} values for station C were significantly higher that of the other two stations (Kruskal-Wallis test, p < 0.05). The results from the three sampling stations showed that Lake Trichonis is classified to the oligotrophic lakes according to the transparency measurements, while according to *Chl-α*, it is classified as oligotrophic to mesotrophic, when the use of TSI_{TP} classifies the lake to mesotrophic and sometimes eutrophic level. Finally, the overall Carlson's *TSI* index fluctuated between 24.3 and 45.4 having an average of 36.4 which classifies Lake Trichonis to the oligotrophic level.

3.4. Zooplankton Composition and Abundance

The mean integrated abundance of the total zooplankton in the water column of the three sampling stations varied between 2.55 to 131.9 ind l^{-1}. Statistically significant abundance differences among the three sampling periods were found (Kruskal-Wallis test, p < 0.05), with the first period (September 2003-August 2004) presenting the lowest mean value. In contrast, no differences among the three stations (at the 0 - 20 m depth range) for any of the
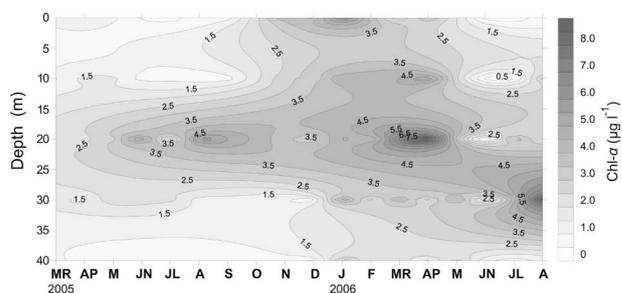
Figure 4. Vertical profiles of the concentrations of chlorophyll-*α* in the deeper sampling station A of Lake Trichonis during March 2005 - August 2006.

sampling periods were recorded (Kruskal-Wallis test, p > 0.05). This was possibly due to the strong hydrological homogeneity along the horizontal axis of the lake, as a result of currents, surface seiche, water inflows and outflows [7,21]. Generally, the abundance values recorded in the present study were greater compared to other great oligotrophic lakes of the southern Europe, such as Lake Ohrid [22], Lake Tavropos [23] and Lake Bracciano [24]. On the other hand, the zooplankton abundance is lower than other eutrophic lakes of Greece like Lake Volvi [25], Lake Mikri Prespa [26], Lake Pamvotis [27], as well as the nearby eutrophic Lake Lysimachia [28] and the mesotrophic Lake Amvrakia [29]. The temporal variation of the total zooplankton was characterized by a decrease of abundance after September with lowest values in winter, while a first increase in spring due to rotifers and a second one in summer due to copepods. This variation did not seem to follow the monoacmic pattern (only one peak of abundance in summer) which is characteristic of the oligotrophic lakes according to the PEG model [30].

The zooplankton community in Lake Trichonis was consisted of the groups of rotifers, copepods, cladocerans and the larvae of molluscs (**Figure 6**). In particular, the three-year investigation revealed 25 species of rotifers, three copepod species, seven cladocerans and the larvae of the mollusc *Dreissena blanci* [8,13]. Copepods, and especially the calanoid *Eudiaptomus drieschi*, prevailed in the zooplankton community accounting for an average proportion of 50.0%, followed by rotifers (24.0%), cladocerans (15.6%) and the mollusc larvae (10.4%). *E. drieschi* also dominated the crustacean community (copepods and cladocerans) accounting on average for 65.6%, while the contribution of cladocerans and the cyclopoid copepods varied between 1.5% to 67.7% and 0.1% to 14.9%, respectively (**Figure 7**). The domination of calanoida among the crustaceans, which was observed during the three-year of investigation in Lake Trichonis, is characteristic of oligotrophic lakes [31-33]. In comparison to the past, however, the percentage of calanoida

in the crustacean community seems to have been decreased from 50% - 100% [10] to 22% - 88% in the present study, while cladocerans increased their contribution. In addition, the present species composition within the crustacean community is different compared to the reports of Koussouris *et al.* [10], with the presence of only one calanoid species, instead of three [10]. Furthermore, considering that increased contribution of cladocerans and cyclopoid copepods in the crustacean communities is characteristic of eutrophic lakes [34], the
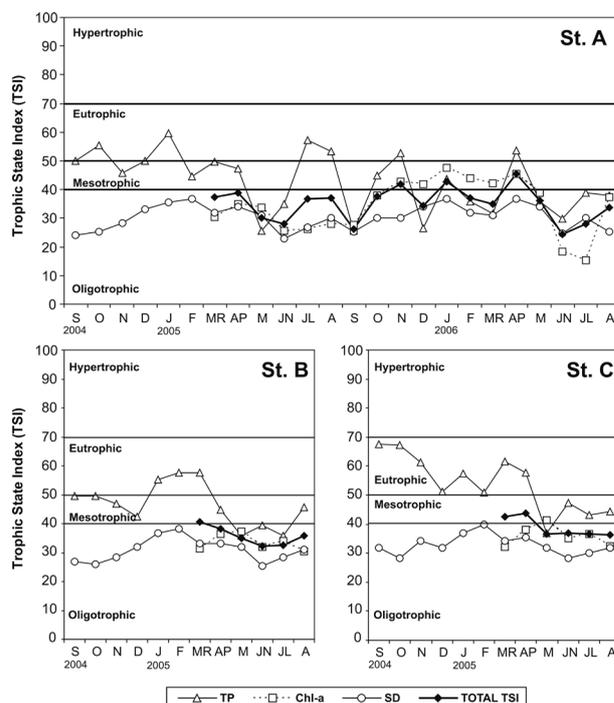
Figure 5. Monthly variation of the values of Carlson's TSI index for total phosphorus (TSI_{TP}), chlorophyll-a (TSI_{Chl-a}) and transparency (TSI_{SD}), and also the overall TSI index (total *TSI*) in Lake Trichonis during September 2004 - August 2006 for station A, and during September 2004 - August 2005 for stations B and C.
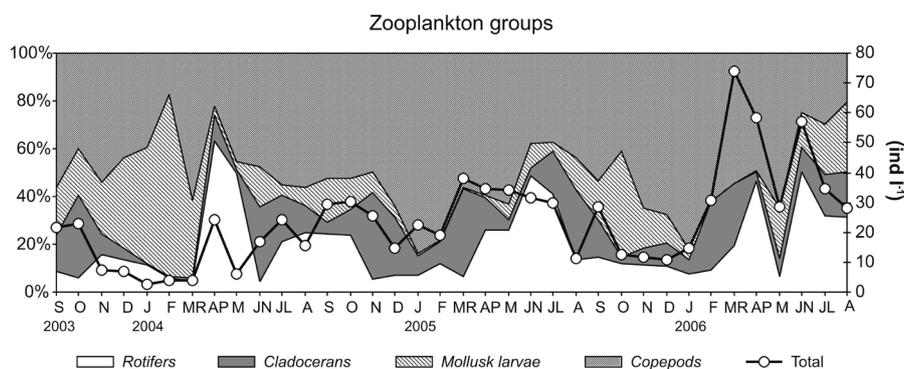
Figure 6. Monthly variation of the mean integrated (0 - 40 m) abundance of the total zooplankton (ind l^{-1}) and percentage contribution of the main zooplanktonic groups (rotifers, cladocerans, copepods and mollusc larvae) to the total abundance recorded during September 2003 - August 2006 in station A of Lake Trichonis.

decrease in the proportions of the calanoid copepods and the increase of cladocerans in Lake Trichonis could possibly indicate an alteration in its trophic condition.

Among the cladocerans, *Diaphanosoma orghidani* prevailed, accounting for an average of 50.9% in the community, and followed by *Bosmina longirostris* (25.6%) and *Daphnia* sp. (22.2%), while *Ceriodaphnia pulchella*, *Alona* sp. and *Leptodora kindtii* were found in small numbers and sporadically in the samples (**Figure 8**). A seasonal succession between *B. longirostris* which prevailed in the colder periods, and *D. orghidani* which prevailed in the warmer ones, was recorded. This type of succession in the community of cladocerans is similar to the situation described by Geller and Müller [35] for eutrophic lakes. However, this was apparent only during the first year, while in the second and third year two other cladocerans were added to the community and the succession of species was altered.

Among the zooplankton taxa found in Lake Trichonis during this extensive investigation, there were certain species which are considered indicators of either oligotrophic or eutrophic conditions (**Table 2**). Thus, the rotifers *Brachionus calyciflorus*, *Filinia longiseta*, *Hexarthra mira*, *Keratella quadrata* and *Pompholyx sulcata*,

along with the cladocerans *Bosmina longirostris* and *Daphnia cucullata* are indicators of eutrophic conditions [36-39]. On the other hand, *Kellicottia longispina* and *Ploesoma hudsoni* of the rotifers and the cladocerans *Daphnia galeata* and *Leptodora kindtii* are indicators of oligotrophic conditions [38]. It must be pointed that, the percentage contribution of all the oligotrophic indicator species in the zooplankton of the lake ranged between 0.6% to 8.9% in the three sampling periods, while the respective range for the eutrophic species was 9.5% to 13.6%. This resulted to an average of 7.5% for the oligotrophic and 10.6% for the eutrophic indicators in the overall sampling period. Furthermore, it is interesting to take a closer look to the presence of the cladocerans of the genus *Daphnia* sp., among which *D. cucullata* was the dominant species, while the abundance of *D. galeata* was an order of magnitude lower and it was found sporadically in the samples. The two species can hybridize and produce intermediate forms [19] and that is why we refer to them as *Daphnia* sp. Both species had not been found in Lake Trichonis according to the previous studies of Koussouris [40,41] and Koussouris *et al*. [10] conducted in 1975-1977 and in 1988-1989, respectively. Indeed, Kehayias *et al*. [12] were the first to report their
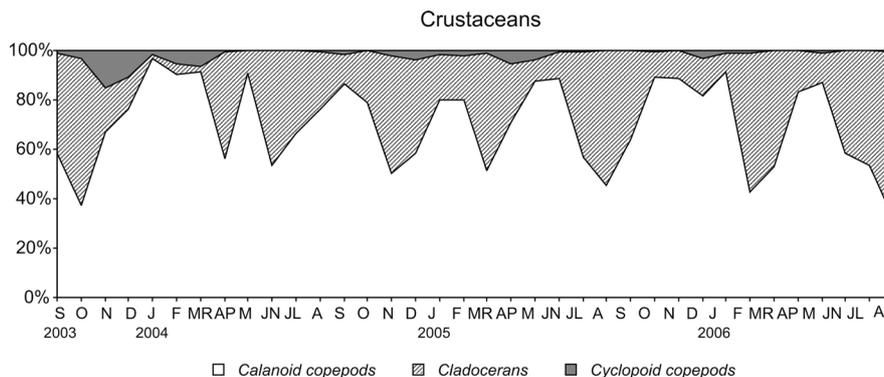
Figure 7. Average percentage contribution of the calanoid copepods, cladocerans and cyclopoid copepods to the crustacean community in Lake Trichonis (station A) during September 2003 - August 2006.
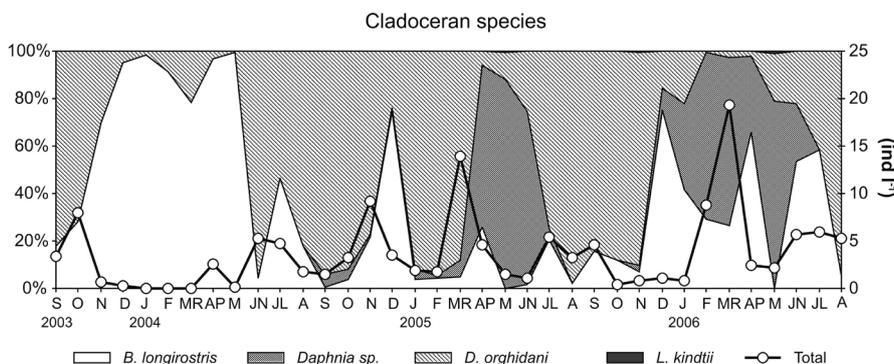
Figure 8. Monthly variation of the mean integrated (0 - 40 m) abundance of the total cladocerans (ind l^{-1}) and percentage contribution of the most important species to the cladoceran's community recorded during September 2003 - August 2006 in station A of Lake Trichonis.

Table 2. Indicator species of oligotrophic and eutrophic conditions [36-39] in Lake Trichonis.

Species	Oligotrophic indicator	Eutrophic indicator
ROTIFERA		
Brachionus calyciflorus (Gosse 1851)		x
Filinia longiseta (Ehrenberg 1834)		x
Hexarthra mira (Hudson 1871)		x
Kellicottia longispina (Kellicott 1879)	x	
Keratella quadrata (Müller 1786)		x
Ploesoma hudsoni (Imhof 1891)	x	
Pompholyx sulcata (Hudson 1885)		x
CLADOCERA		
Bosmina longirostris (O.F. Müller 1785)		x
Daphnia cucullata (G.O. Sars 1864)		x
Daphnia galeata (Sars 1864)	x	
Leptodora kindtii (Focke 1844)	x	

presence in zooplankton samples taken just one year before the present investigation (2002-2003). *D. cucullata* is considered a typical representative of eutrophic lakes in Europe [39], while *D. galeata* is an indicator of oligotrophic conditions [38]. The characteristic body form and shape of *D. cucullata* makes almost impossible its misidentification if it had been caught in the samples during the previous studies. Thus, if the possibility of an accidental transfer of this species to the lake by human activities is excluded, the only reasonable explanation for its absence from the species lists in the past was that actually it was present in the lake but in undetected numbers, while during the last years there was an increase in its density. One reasonable explanation for its increase of abundance during the last decades could be the alteration of trophic condition of the lake towards higher levels, which favoured the development of a denser population. On the other hand, there is also a possibility of a prey-predator relationship, in which the diminishing of the predation pressure on this species by its predator *Atherina boyeri* has resulted to prey recovery. According to the recent investigation of Doulka *et al*. [42], *A. boyeri* is the main zooplanktivorous predator in Lake Trichonis, where it exercises selective predation and *D. cucullata* is among its highly selected prey. Thus, a greater population of *A. boyeri* in the past could have diminished the population of *D. cucullata*, while the decrease of the former within the lake could result to the increase of the abundance of the latter. Though, there are not enough data to support this theory and more investigation on this issue is required.

4. Conclusions

The present investigation showed that Lake Trichonis

seems to hold the main physicochemical features observed in the previous studies of Overbeck *et al*. [9], Koussouris *et al*. [10] and Tafas *et al*. [11], conducted between 15 to 25 years before the present investigation. However, in comparison to the above studies, there was an increase in the maximum values of most of the physicochemical parameters recorded in the present study, although their patterns of variation in the water column were similar. In particular, the maximum values of temperature, DO, conductivity and chlorophyll-α have been increased by 6.1% to 18.8%, 14.8% to 16.7%, 24.4% to 47.5% and 14.6% to 1075%, respectively. Moreover, there was a considerable increase of the maximum concentration of nutrients, such as *TP*, phosphates, nitrates and nitrites. The lowest value of transparency in the present study was similar to the respective values reported by Tafas *et al*. [11] and Koussouris *et al*. [10], but was lower by 29.8% than the reports of Overbeck *et al*. [9].

Considering that Lake Trichonis is situated in an agricultural area, the elevated values of nutrients in the present study were probably related to the inflow of irrigation and drainage waters rich in residual fertilizers, which were used in the extensive cultivations during the past years [43]. The present higher values of *Chl-α*, and as a by-product the increased DO in the metalimnetic layer, could be also the result of this chronic influx of nutrients into the lake. In accordance, the elevated productivity of the basin could be also among the reasons for the increase of conductivity [20], and this could have resulted to the lower values of water transparency in comparison to the oldest previous study of Overbeck *et al*. [9]. According to Bertahas *et al*. [1], phosphorus is the key nutrient that regulates eutrophication in Lake Trichonis as the limiting nutrient for algae growth. However, it seems

that it is not the quantities of phosphorous entering the ecosystem but its hydrological regime which affects in a great extent its trophic status. In particular, the quantities of rainfall precipitation and water recharge are responsible for regulating the water outflow from the lake and consequently the phosphorus flush out of the system [1]. In this sense, the *TP* fluctuation among years found in the present study could be originated to the variation of these hydrological elements in the lake's catchment area. On the other hand, the strong decrease in the concentrations of *TP*, phosphates and nitrates in the period of September 2005 - August 2006 in comparison to September 2004 - August 2005, could be related to the termination of the tobacco cultivations during the former period. Tobacco cultivation was developed in the prefecture of Aitoloakarnania at the end of the 19th century and this part of Greece became the country's major producing area of tobacco. However, from 1/1/2006 with the CAP reform in relation to tobacco cultivation, the overall production presented a significant reduction of about 80% in Greece and, specifically, 100% reduction in the whole prefecture of Aitoloakarnania [44]. As a result, tobacco producers either turned to other cultivations, or in most cases left their fields uncultivated. Although this pause in the agricultural activities has not been documented in details for the area, it is suggested that it could be among the reasons of the nutrients' decrease in the lake observed a year after this action.

Nevertheless, the nutrients' concentrations have been increased from the past the trophic level of the lake has not altered dramatically. Thus, although the previous investigators [1,9-11] did not use *TSI* index to have direct comparisons, they accounted Lake Trichonis as an oligo- to mesotrophic aquatic ecosystem. The reason for this stability of the lake implies its large water volume and its positive water balance, as previously mentioned. On the other hand, the anthropogenic impacts on Lake Trichonis can be considered as low, in the sense that there are no heavy industrial units around or close to the lake. However, the existence of a large number of olive-oil refineries, as well as of several creameries and small livestock facilities like sheep and pig-farms, can be considered a constant thread for the increase of organic pollution entering the lake. In addition, the absence of biological treatment facilities for most of the surrounding villages, which probably result to undefined quantities of urban wastewater constantly dumping into the lake, are serious reasons of a future increase in lake's trophicity.

Consequently, the indications concerning the physicochemical elements of Lake Trichonis and its trophic state during the last thirty years point out a general stability and a strong resistance of the lake to trophic elevation. However, the present zooplankton investigation reveals a puzzling icon of the environmental state of the ecosystem, accumulating several indications. Thus, the total zooplankton abundance, the dominance of the calanoid copepods (instead of the cyclopoids or/and the rotifers) in the zooplankton community as well as among crustaceans, and the presence of certain indicator species, point out an oligotrophic character of the lake. On the other hand, the pattern of the temporal zooplankton variation and the seasonal succession pattern among cladoceran species resemble those reported from eutrophic lakes of Europe. Moreover, the appearance of "new" species, like *D. cucullata*, along with the disappearance of calanoid copepod species, the increase of the proportions of cladocerans among crustaceans in comparison to the past, and the increased proportions of indicator species of eutrophic conditions within the zooplankton community, may suggest that certain biological parameters have been altered from the past and indicate possibly a different ecological status of the lake. The bottom line could be that Lake Trichonis is probably experiencing a transitional condition towards the eutrophic state and thus, the need of constant inspection and monitoring not only of the abiotic parameters but also the biotic ones in the frame of a general management plan for this ecosystem, is indispensable.

Acknowledgements

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