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Assessing the Fish Stock Status in Lake Trichonis: A Hydroacoustic Approach

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Abstract: Fish stock monitoring is an important element for the sustainable management of inland water resources. A scarcity of data and the lack of systematic monitoring for Lake Trichonis precludes an up-to-date assessment. To assess the current status of pelagic fish stock, a hydroacousting survey was conducted for the first time in Lake Trichonis, Greece. In October 2019, the lake was acoustically surveyed with two, horizontally and vertically mounted, 120 kHz transducers during day and night. A decreasing gradient in pelagic fish density from the western to the eastern shores of the lake was observed. Fish density was significantly higher in the intermediate layers of the water column, in the eastern region, compared to the western region. The lake appears to host primarily communities of small-sized fish (TL: 0–5 cm), whereas larger fish (TL: 5–50 cm) are a small minority of the total fish stock. The overall average estimated fish length was approximately 2.4 cm. The adoption of routine inland fish stock monitoring through hydroacoustic methods could be a promising step in the effort to improve the understanding of unique inland water ecosystems with minimum impact on endemic species, as well as to mitigate human impact and achieve long-term sustainable management.

Keywords: acoustics; fish density; fish distribution; Mediterranean lake

1. Introduction

Within Europe, in order to eliminate or mitigate freshwater degradation, the Water Framework Directive (WFD) calls for all the natural aquatic ecosystems to achieve a "good" ecological status. To date, inland fisheries management is focused on improving the aquatic environment for biodiversity, allowing the sustainable exploitation of the resources and verifying conservation and protection of fish and fisheries [1]. However, the methodologies adopted by researchers for the monitoring of freshwater fish stocks, such as gillnetting, are often unable to support an ecosystem–based management approach [2], as they fail to comprehensively address the spatiotemporal variability of fish stocks [3], require high effort [4] and often result in a reduction of fish biomass [5].

In this context and considering the proposed WFD goals, there is a strong demand for standardized, reliable, reproducible and non-invasive methods for routine monitoring programs. Satellite-based Earth Observation data provide safe and cost-effective approaches for the systematic monitoring of water bodies [6] and fish environmental preferences but fail when it comes to the direct detection of fish [7]. On the other hand, hydroacoustic methods have been applied in all kinds of aquatic ecosystems in order to acquire detailed information about aquatic fauna, and especially about fish [8].

In freshwater ecosystems, hydroacoustic methods are used for assessing fish biomass, fish behaviour, stock abundance and fish size distribution [8]. The most widely used scientific echosounders in freshwaters operate at single frequencies of 38, 70, 120 and 200 kHz [9]. The main advantage of hydroacoustics is the capability to remotely sample large volumes of water in a relatively short amount of time, providing high spatial resolution in both the horizontal and the vertical domain. In addition, acoustic measurements are non-invasive and non-extractive [8,10]. Moreover, the technological improvements in this field and their increased precision have contributed to their extensive use in both marine and freshwater ecosystems [11–13]. Although there are several limitations of current hydroacoustic methods, e.g., lack of species identification, difficulties in shallow waters, and limitations in noisy environments, and despite the considerable need for further research, the application of hydroacoustics is based on a well-established, standardized methodology for inland fishery production assessment and management [14].

Geographically, hydroacoustics research in freshwater ecosystems has so far been concentrated in North America and Northern and Central Europe (83% of the studies reviewed in [15]). Although the Mediterranean Basin is found to be globally important for its freshwater biodiversity [16], few studies have been published with respect to the application of hydroacoustic methods (e.g., [17]). In Greece, hydroacoustics research has so far been applied in marine systems, mostly focusing on the application of acoustic techniques to monitor marine fisheries resources (e.g., [18,19]). Nevertheless, hydroacoustic research on Greek inland waters has not been conducted before.

Inland fishery production in Greece is impacted by overfishing and habitat alteration, as well as pollution [20]. Professional fisheries are operated primarily in large lakes. Lake Trichonis, the largest natural lake in Greece, bears very high economic, cultural and ecological significance. The lake is important for its fish fauna since 20 species have been recorded in its waters, 11 of which are endemic (see detailed list in [21,22]), among which there are unique species such as *Economidichthys trichonis*. The species present in the lake are classified into 8 different systematic families, which, ecologically, span all available ecological niches (herbivores, benthivores, planktivores and carnivores). Among them, there are carnivorous species such as *Anguilla anguilla* and *Silurus aristotelis* that feed at night, predominantly on fish [23,24]. The majority of fish species inhabiting Lake Trichonis belong to Cyprinidae and spawn during spring to early summer [25–28]. Moreover, small gobies are reproducing in the same period [25]. Additionally, the lake belongs to one of the most important fishing attractions of the country. In fact, a land-locked, not overexploited population [29] of *Atherina boyeri* is also present in the lake, which offers, with the exploitation of other species such as *Rutilus panosi* and *Scardinius acarnanicus*, economical support for professional fishers. According to the local Fisheries Department, the primary catch composition for the period of 1989–1998 is made up of small species. In particular, in October 1989, *A. boyeri* (L_{max} = 13.5 cm [30]; L_m = 5.8 cm [31]) comprised approximately 64%, while *Tropidophoxinellus hellenicus* (L_{max}= 9.3 cm [31]) and *Scardinius acarnanicus* (L_{max} = 35.4 cm [32]; L_m = 14–18 cm [31]) contributed each 17% of fisheries production (calculated as the percentage, per weight, of all catches). In 1990, 47% of the production consisted of *T. hellenicus* (L_{max} = 9.3 cm [31]), while 22% of production was *R. panosi* (SL = 12.0–14.0 cm [31]). In 1998, 56% of the annual production of the lake consisted of *Atherina boyeri* while 20% of the production was *Scardinius acarnanicus* (L_{max} = 35.4 cm [32]; L_m = 14–18 cm [31]). Other species (*Silurus aristotelis*, *Luciobarbus albanicus, Carassius gibelio, Tropidophoxinellus hellenicus*) comprised approximately 3% of the total production. A similar species composition was found to occur during the contemporary fishing catches (local professional fishermen, pers. comm).

Despite its significance, because of limited staff, management authorities are having a hard time monitoring fish harvests of individuals for the prevention of illegal fishing in Lake Trichonis. Furthermore, the lack of comprehensive long-term studies has resulted in the limited availability of information about fish stock status in the lake. Although historical data exist as a result of research projects carried out by universities, institutes and organizations, it is scarce and, in practice, the status of inland fisheries is largely unknown. Taking all the aforementioned into account, it is important to adopt a standardized, effective and non-invasive method for the assessment and monitoring of inland fish stocks.

Hydroacoustic techniques could serve as a non-destructive tool for inland fish monitoring. The purpose of this study is to use a hydroacoustic method for obtaining a detailed overview of the freshwater fish stock status in Lake Trichonis and highlight the hindrances and improvements that need to be considered, in order to adopt this methodology as a common practice in Greek lakes. In particular, we aimed to (i) quantify freshwater fish biomass and fish density, (ii) assess fish size distributions, and (iii) study the variation of fish vertical distribution in relation to water abiotic parameters. Therefore, despite ongoing progress in hydroacoustics, the present work comprises a first-step towards the attempts that should be made for fish stock assessment in larger biogeographical scales [15], and especially in the Mediterranean countries, where high levels of endemicity are observed.

2. Study Area

Lake Trichonis, located in the central-western part of Greece, has a surface area of 96.9 km^2, a maximum length of 19 km, and is the deepest (maximum depth of 58 m, average depth of 30 m) natural lake in Greece (Figure 1). The trophic status of the lake is characterized as oligotrophic [33]. Lake Trichonis belongs to the Natura 2000 network established under the Habitats Directive 92/43/EEC and is characterized as an area with high potential research value [34]. Responsible for the protection, management and monitoring of the lake is the Management Authority of Messolonghi Lagoon.

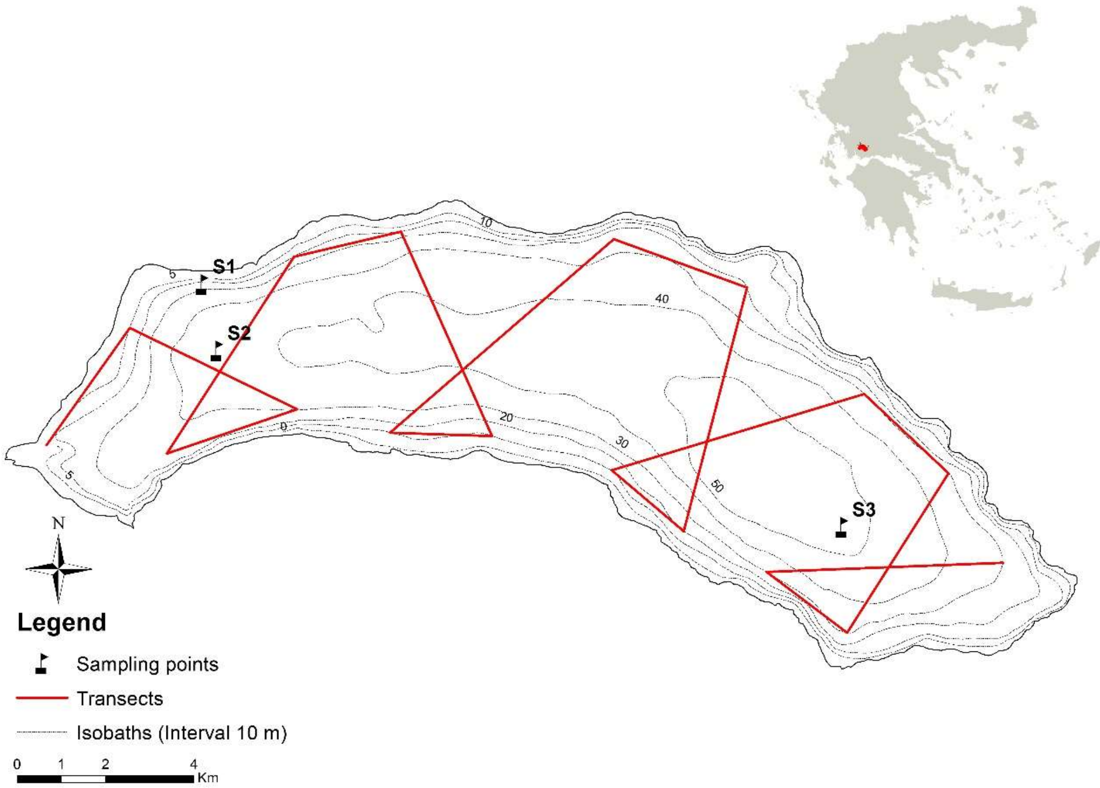

Figure 1. Depiction of the study area and lake bathymetry (derived from the digitization of 1:50,000 scale topographic maps, which constitute an appropriate reference source for such geomorphometric information [35,36]) with the hydroacoustic survey and the sampling points overlaid.

3. Materials and Methods

3.1. Hydroacoustic Survey Design

The hydroacoustic survey was carried out in Lake Trichonis during the period of 3–10 October 2019. The pattern of the survey transects was designed in such a way, as to include fish communities along areas of identical or similar depths (i.e., along depth contours) (Figure 1). Horizontal and vertical recordings were carried out simultaneously using a suitable arrangement.

In order to assess the variation in diurnal estimates of fish biomass and distribution, the survey was conducted during both day and night [37]. The day survey commenced one hour after local sunrise, whereas the night survey commenced one hour after local sunset. Considering the high sensitivity of horizontal recording to the noise introduction into the hydroacoustic records, the survey was carried out on windless days. In total, two hydroacoustic surveys were needed to cover the whole lake; one during daytime and one during nighttime. The total length of each survey was ~40 km, resulting in a coverage coefficient of 4 per survey [38,39]. Surveys were grouped per time of day, into daytime and nighttime surveys.

A Simrad EK60 echosounder with a frequency of 120 kHz, equipped with two simultaneously operating transducers (SIMRAD ES120-7C), was used to collect the acoustic data. The transducers were mounted at a depth of about 1 m, at the front of the boat. The first transducer was vertically oriented and the second one horizontally oriented, enabling fish detection in the surface zone. The horizontal transducer was oriented perpendicularly with respect to the vertically oriented transducer and was facing towards the side of the vessel (perpendicular to the sailing direction). The echosounder was driven by the Simrad ER software. The ping interval was set to 0.2 s. Each transducer transmitted 2–3 pings per second, alternating between transducers (known as multiplexing), for a total ping rate of 5–6 pings per second. During surveying, the boat maintained a constant speed of 6 km/h and a volume of 3.7×10^6 m^3 of water was explored. For the full calibration of the system, a standard copper sphere of 23 mm in diameter was used and gains were calculated according to Demer et al., 2015 [40]. The sphere was placed at approximately 9.4 m of depth from the transducer, which was aimed vertically towards the sphere. The calibration was performed before starting with the first day survey. The effect of background (passive) noise was removed from all files by subtracting passive listening data collected before the survey. The background noise was checked by stopping pulse transmission and putting the equipment into a passive listening mode. Passive listening was performed on a windless day before the acoustic survey.

Survey positioning was assisted by a Garmin GPSMAP 60CSx GPS receiver (Geolocation/horizontal accuracy of 3–5m—95%).

3.2. Hydroacoustic Data Processing

Acoustic raw data were converted to compatible formats and processed using the Sonar5 Pro software (CageEye A/S, Oslo, Norway). Fish density values (number of individuals per hectare) were calculated using the S_V/TS scaling method [8], which uses volume back-scattering strength, S_V, and the mean target strength (TS) to calculate fish density.

Acoustic biomass was expressed as the volume backscattering strength (S_V), which is the equivalent logarithmic measure of the volume backscattering coefficient (s_V) obtained through echo-integration [8]. S_V has been found to be a good expression of biomass distributions, especially for small targets [8].

Mean target strength (TS) was estimated based on single echo detections (SED) [41]. In order to express TS into total length measurements (cm) rather than dB, empirical TS-length relationships were used. Equations for a 120 kHz echosounder given by [42] in the case of vertical and [43] in the case of horizontal recordings were applied. Fish Total Length distribution was categorized into 50 classes by 1 cm step, starting from zero.

During the data post-processing, the threshold for target strength (TS) was set to −65 dB and for S_V to −71 dB, in order to cover the entire possible size spectrum of fish targets [44]. The collected data were visually examined to remove subsets with noise and non-fish targets, in order to improve the signal-to-noise ratio of the dataset. A layer of 0.5 m above the detected bottom layer was excluded in order to eliminate the effects of bottom back-scattering. A surface layer of 4 m was applied in horizontal and vertical recordings in order to exclude the near field. Each transect in both the horizontal and the vertical survey was divided into 200-m segments. In order to assess the vertical distribution of fish, the water column recorded in the vertical survey was divided into 1-m thick depth layers. The vertical data were processed up to a depth limit equal to the maximum depth up to the bottom, depending on the position of the segment within the transect. The horizontal data were processed up to a range equal to the maximum depth of the lake. The surface layer ranging from 0–4 m was covered by the horizontally-aimed transducer and compensates for the lack of data in the near field of the vertical transducer. Data recorded horizontally were processed using deconvolution procedure. Deconvolution is a procedure employed in the processing of horizontally recorded data and is especially used for the determination of target size. It is based on stochastic assumptions of random aspect orientation [45].

The data were taken along the transect, which was divided in segments. In charts of vertical distribution, the horizontal layer was taken to describe the entire surface layer and is represented by a single depth range. The rest of the vertical layers have been accordingly matched and aligned, below the horizontal layers. The overall arrangement represents a division of the total insonified lake volume in cells, each of which spans 200 m × 1 m (along-transectx vertically), with a variable athwart dimension for each depth, as a result of the conically-shaped beam. Exceptionally, the cells of the surface layer were sized 200 m × 4 m (along-transect x vertically). The data were either summed or averaged vertically (for vertical analyses) and averaged or summed horizontally (i.e., along the transect) for horizontal or lengthwise analyses.

Due to the potential effects of multiple scattering and acoustic shadowing caused by very dense aggregations of fish on TS and the consequent density estimates, additional measures were checked and examined. These include the share of single echoes (the ratio between volume backscattering strength resolved as single echoes and the total volume backscattered strength) and the Sawada index N_V (the number of fish per acoustic sampling volume where TS is estimated) [46] (see Supplementary Materials).

3.3. Environmental Parameters

In situ measurements of physico-chemical parameters were conducted during the day survey in Lake Trichonis at three sampling stations (Table 1 and Figure 1). In order to estimate the temperature (°C) and dissolved oxygen (DO) (mg/L) vertical profiles, the measurements were carried out at 1-m depth intervals using a YSI multiparameter probe (YSI Incorporated, Ohio, USA).

Table 1. Locations of the 3 sampling stations in Lake Trichonis.

Lake Location	Latitude (deg)	Longitude (deg)	Sampled Depth Range (m)
Sampling point S1	38.58625	21.48000	22
Sampling point S2	38.57250	21.48301	34
Sampling point S3	38.53671	21.61033	50

3.4. Statistical Analysis

The calculated parameters were mapped along the transect, as well as depth-wise, and were explored for differences between day and night. Mean S_V (dB), share of single echoes (%) and fish density (inds./ha) were averaged per layer, mapped by depth and compared for differences between day and night. Cross-correlation analysis was performed on the average fish density depth-wise distributions using depth-lag to determine the mean depth difference between day and night fish density distributions. Fish density was explored for spatial distribution differences, while it was also color-mapped both along transect and depth-wise, in the West-East direction, in order to visualize the variation in both dimensions at the same time, where transect mileages were matched to lake regions based on the transect path. Finally, fish density was also summed depth-wise at each segment and interpolated spatially to create total-water-column fish density maps for day and night.

To study fish density in combination with size composition, the targets were separated in two classes, ≤5 cm (small fish) and >5 cm (large fish) and density was re-calculated for small and large fish, respectively, using the corresponding target count ratios. The target count proportions for small and large fish were also mapped independently as stacked absolute values vs. depth (excluding layers with a total of <100 targets), separately for day and night, to study variations due to time of day, depth and size class simultaneously. Targets that were classified in 1 cm sized classes were separated by orientation (surface layer vs. vertical data layers) and time of day (daytime vs. nighttime) and were weighed by average class size, to calculate weighted average fish sizes. Specifically, the middle of each class was multiplied by the total target count of the class; the products were summed and divided by the total targets from all classes.

The segments of the transect closest to each sampling point were determined, and fish density was determined by depth for each of those segments (summing fish density values for all layers). Thereafter, the average fish density based on these segments was determined. A circle of specific radius was used to locate the closest segments, and segments were included wherever they intersected this circle. For two of the points, a radius of 1 km was used, whereas a radius of 2.5 km was used for the third point, because no segment was close enough to intersect a circle of 1 km radius around that point.

Data parsing and post-processing was carried out using custom scripts in "R" (R Development Core Team, 2020), as well as LibreOffice Calc and Microsoft Excel. Thematic maps were extracted using ARCGIS and QGIS. Interpolation for the creation of Raster Grids from the scattered processed hydroacoustic data was carried out using Multilevel B-splines [47]. Comparisons between series were made using pairwise t-tests (or 1-sigma confidence intervals where noted), and distributions were compared using Kolmogorov–Smirnov tests. For all tests, a significance level a = 0.05 was used. In some results, marginal hypothesized differences were also explored as the mean difference leading to a p-value approximately equal to a.

4. Results

4.1. Acoustic Biomass and Fish Density

Acoustic biomass detected per sampling unit (Figure 2) did not exhibit diurnal heterogeneities. During the day, the average S_V per depth layer was −65.9 ± 9.1 dB, ranging from −87.6 dB to −49.4 dB, while during the night, the average S_V per depth layer was −66.1 ± 10.1 dB, ranging from −83.8 dB to −49.3 dB. No significant difference was found ($p = 0.92$). Concerning the vertical distribution, during the day and night survey, the highest biomass was observed in the layer 16–30 m, (average value −55.8 dB in day survey and −55.6 dB in night survey). The coefficient of variation (CV) revealed greater heterogeneity in the vertical distribution during night rather than during the day survey (CV = 15% against 13%).

The share of single echoes did not exhibit large fluctuations comparing between acquisition times (Figure 3). Specifically, the day average was 17 % ± 20.2 %, ranging from 0 % to 91.9 %, while the night average was 15 % ± 11.3 %, ranging from 0 % to 44.8 %. Day-to-night difference was not statistically significant ($p = 0.39$). Most of the values were relatively low, typically less than 40% in most layers, during both day and night. The layers close to the surface (<8 m depth) were exceptions to this observation, with the night measurements exhibiting a share of single echoes up to approximately 45% at night versus approximately 90% during the day for those layers.

Fish density layer average was approximately 1226 ± 1608 inds./ha, ranging from 8 to 7984 inds./ha during the day. During the night, the fish density layer average was 3473 ± 3994 inds./ha, ranging from 20 to 16,384 inds./ha. The vertical distribution of fish density was significantly different between day and night ($p < 0.001$). An assumed mean difference of 1250 inds./ha less during the day produces a *p*-value of 0.05 for the t-test, meaning that this is a marginal difference that could be statistically accepted. Moreover, average fish density is more than double at night than during the day in the surface layer (0–4 m) and 5 times higher at 15 m (Figure 4). Application of a cross-correlation model between day and night vertical distributions and using depth as the lag parameter, reveals an average depth-wise offset of ~9 m between the two distributions, with a maximum correlation coefficient of 0.724 at dh = 9 m (Figure 5). This means that the distributions are very similar but translated with respect to each other by approximately 9 m of depth.

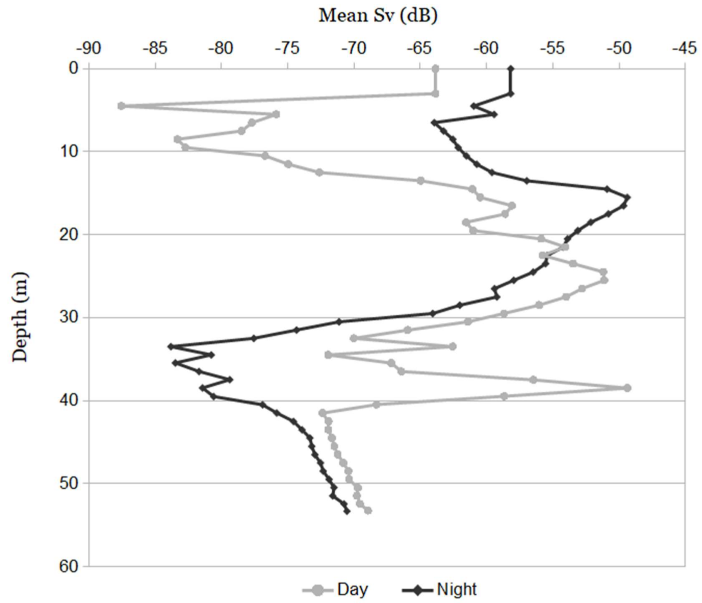

Figure 2. S_V (dB) vertical distribution during the day and night survey in Lake Trichonis.

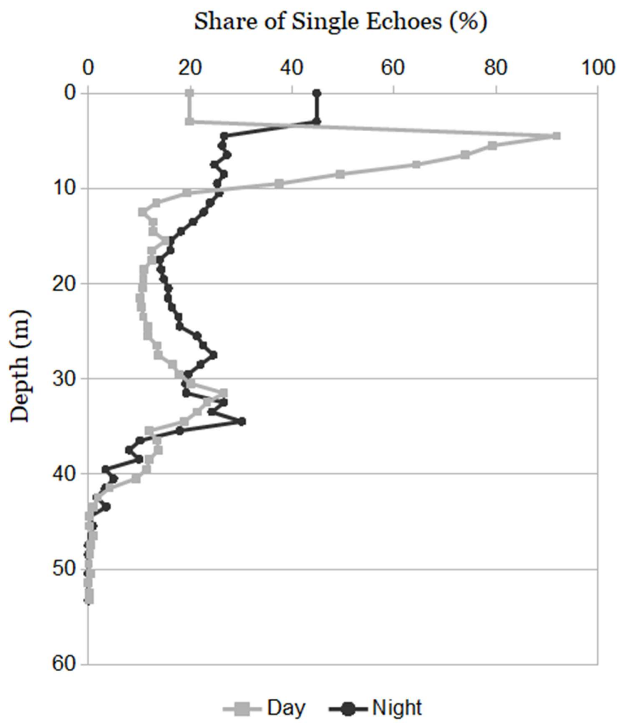

Figure 3. Differences in share of single echoes (%) between different layers as well as measurement acquisition time (daytime, nighttime).

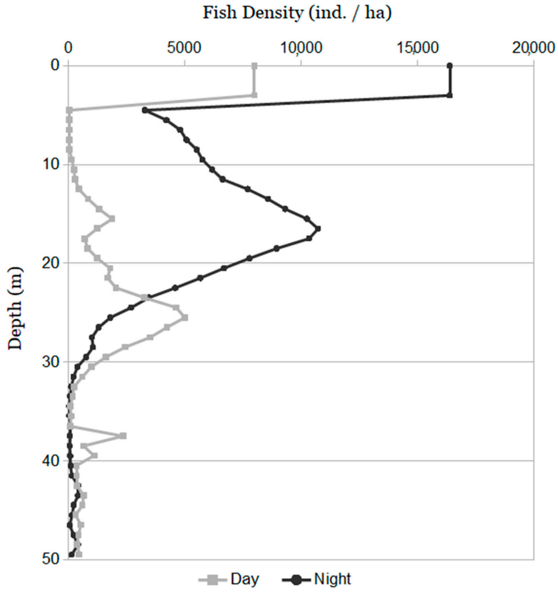

Figure 4. Vertical distribution of average density (inds./ha) for each water column layer during day and night surveys.

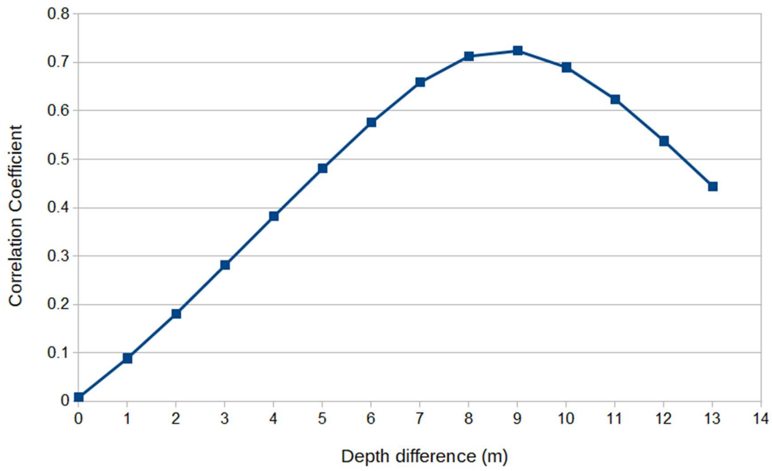

Figure 5. Cross-correlation between day vs. night, using depth (h) as the lag parameter. A depth difference of dh = ~9 m produces the maximum correlation between day and night fish density distributions.

Total water column fish density exhibited significant differences between the shallow and the deep areas of the lake, separating at a longitude decimal degree of 21.55 ($p < 0.0001$, Figure 6). Average fish density during the day was 41,925 ± 18,735 inds./ha in the shallow areas (western side of the lake) and 32,424 ± 13,548 inds./ha in the deep areas of the lake (eastern area) (total water column sums). During the night, fish density was 174,986 ± 69,521 inds./ha in the western region and 127,930 ± 79,934 inds./ha in the eastern region (total water column sums). An assumed mean day fish density difference of 8880 inds./ha between regions produces a marginal value of $p = 0.05$, while for the night, a marginal $p = 0.05$ value is produced by an assumed mean night fish density difference of ~45,750 inds./ha. The fish density depth-wise and spatial distribution along the transect, as well as the differences between day and night are shown in detail in Figures 7 and 8. A horizontally interpolated spatial distribution of fish density is shown in Figures 9 and 10.

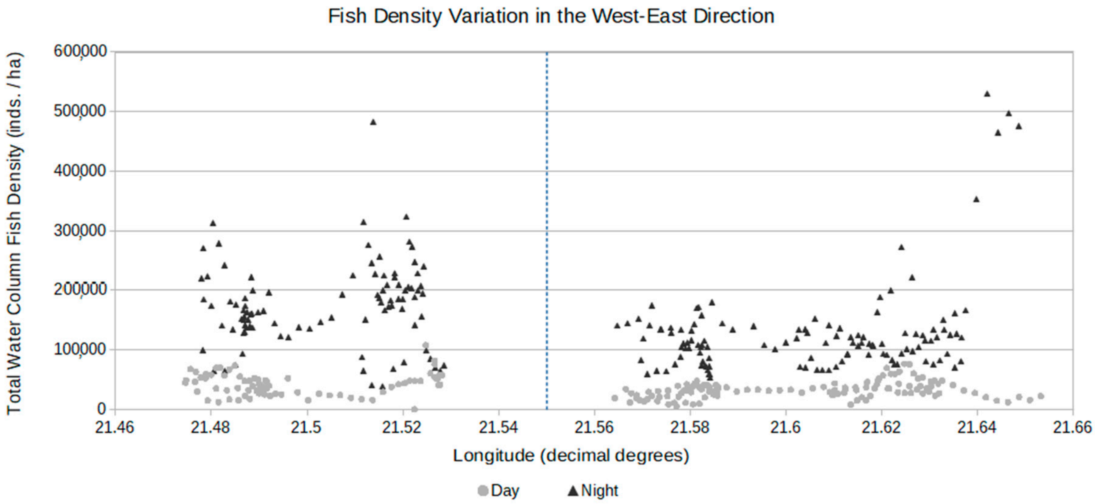

Figure 6. Total water column fish density vs. longitude of averaged-cell midpoint. Significantly different fish density clusters are visible between the regions for both day and night.

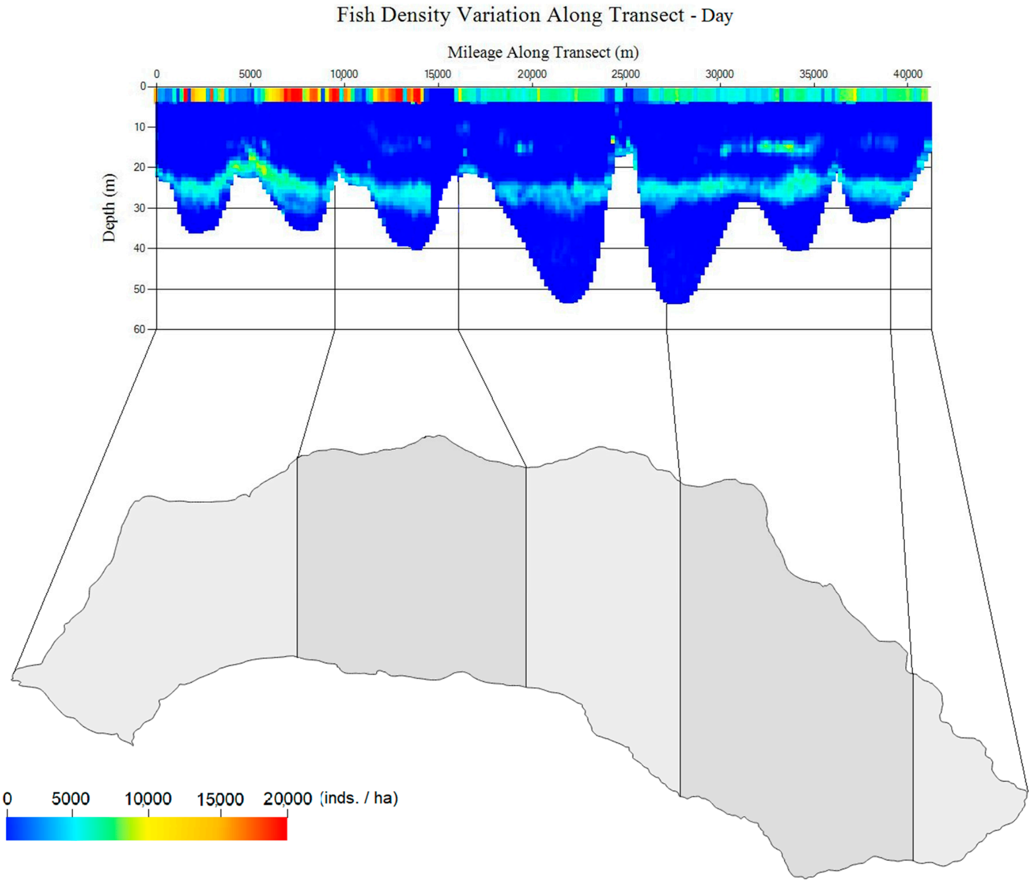

Figure 7. Color map of daytime fish density depth-wise and along transect variations. Figure is spatially referenced with respect to the lake.

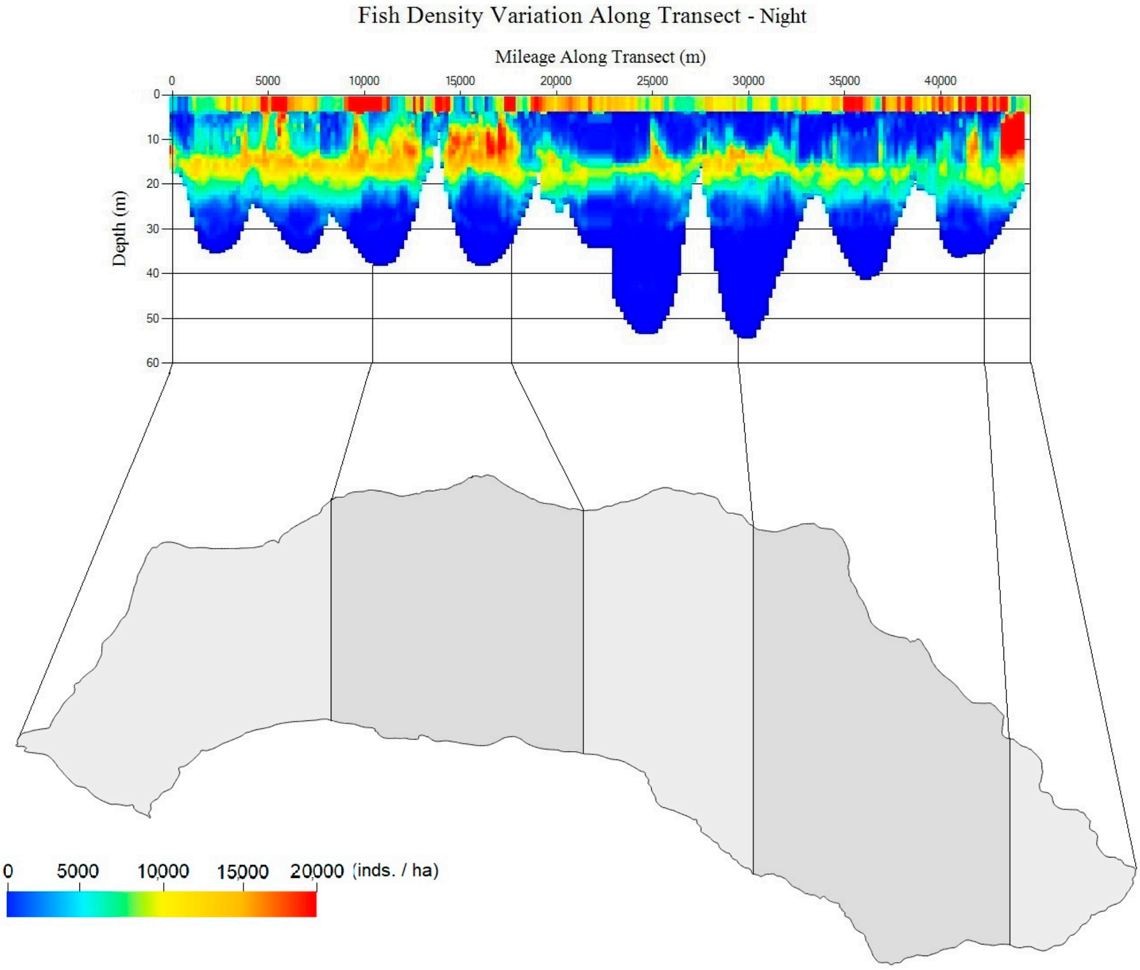

Figure 8. Color map of nighttime fish density depth-wise and along transect variations. Figure is spatially referenced with respect to the lake.

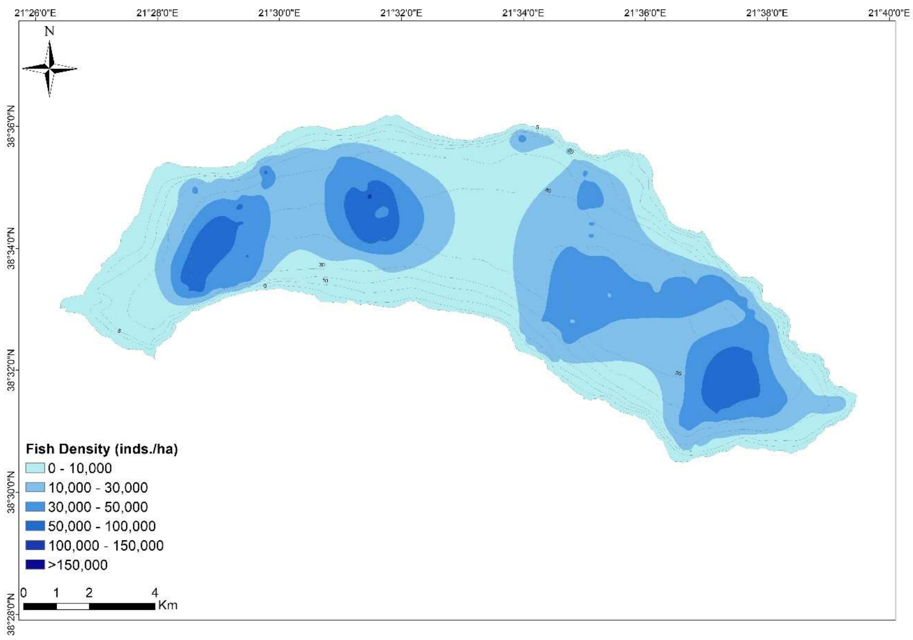

Figure 9. Fish density (inds./ha) distribution during the day survey in lake Trichonis.

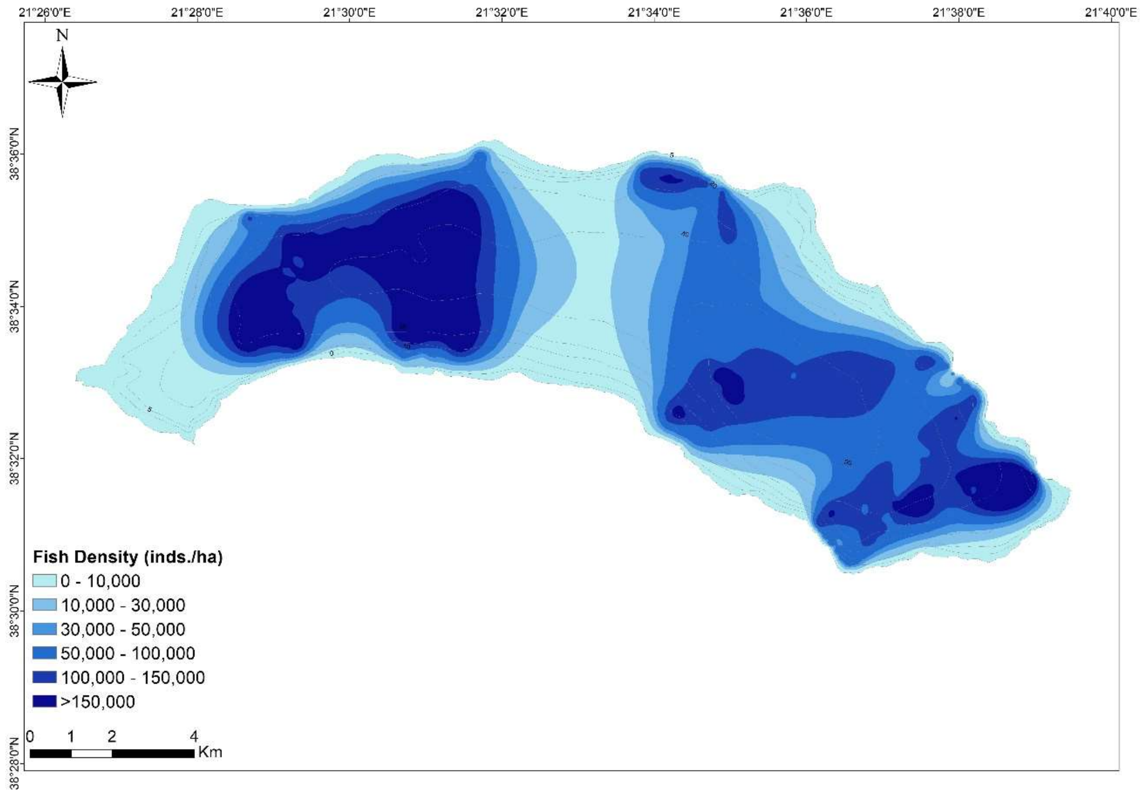

Figure 10. Fish density (inds./ha) distribution during the night survey in lake Trichonis.

4.2. Fish Size Composition and Distribution

Individual fish lengths, converted from TS data, ranged up to 50 cm. The length of most of the detected fish is not larger than 5 cm (Figures 11 and 12). Smaller fish, with lengths less than 5 cm dominate the total counts and observed fish density diminishes as target size increases. After separating by orientation (surface layer vs. vertical data layers) and time of day and weighing for class size, weighted mean fish size for the surface layer was ~1.6 cm for the day survey and ~2 cm for the night survey, while for the layers from 4 m to the bottom, mean fish size was ~4.5 cm for the day survey and 2.9 cm for the night survey.

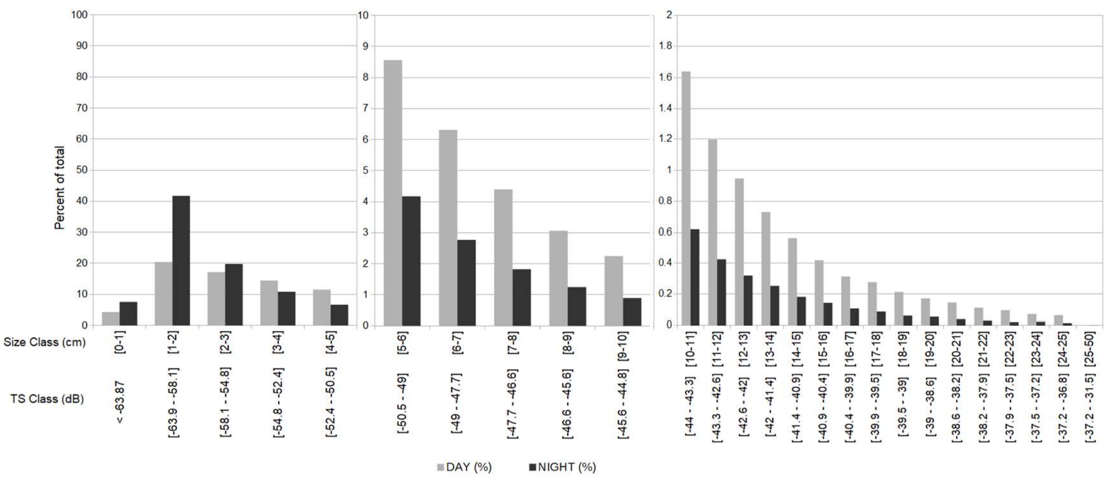

Figure 11. The size (total length, cm) and Target Strength (dB) distribution of pelagic fish recorded during night and day vertical surveys in Lake Trichonis.

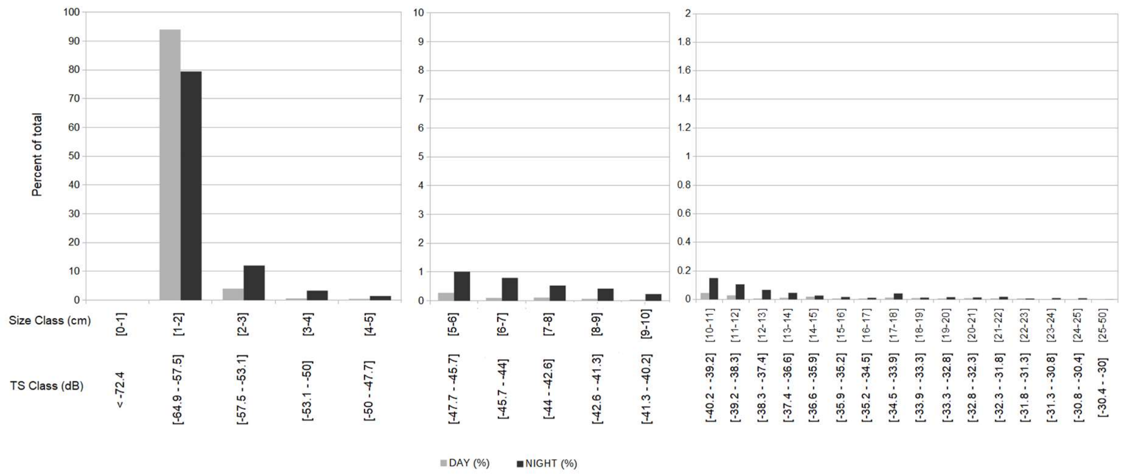

Figure 12. The size (total length, cm) and Target Strength (dB) distribution of pelagic fish recorded during night and day horizontal surveys in Lake Trichonis.

Small fish density during the day was 922 ± 1324 inds./ha, while during the night, it was 3068 ± 3496 inds./ha. Correspondingly, large fish density during the day was 304 ± 463 inds./ha, whereas during the night, large fish density was 405 ± 808 inds./ha. Small fish density was significantly larger during the night ($p < 0.001$), with a marginal $p = 0.05$ being produced by an assumed mean difference equal to 1220 inds./ha (of size < 5 cm). Large-fish density distribution by depth was not significantly different between day and night (Figure 13). Horizontal layer fish density exhibits fish density values more than an order of magnitude higher than the vertical layers, for both day and night (Figure 14).

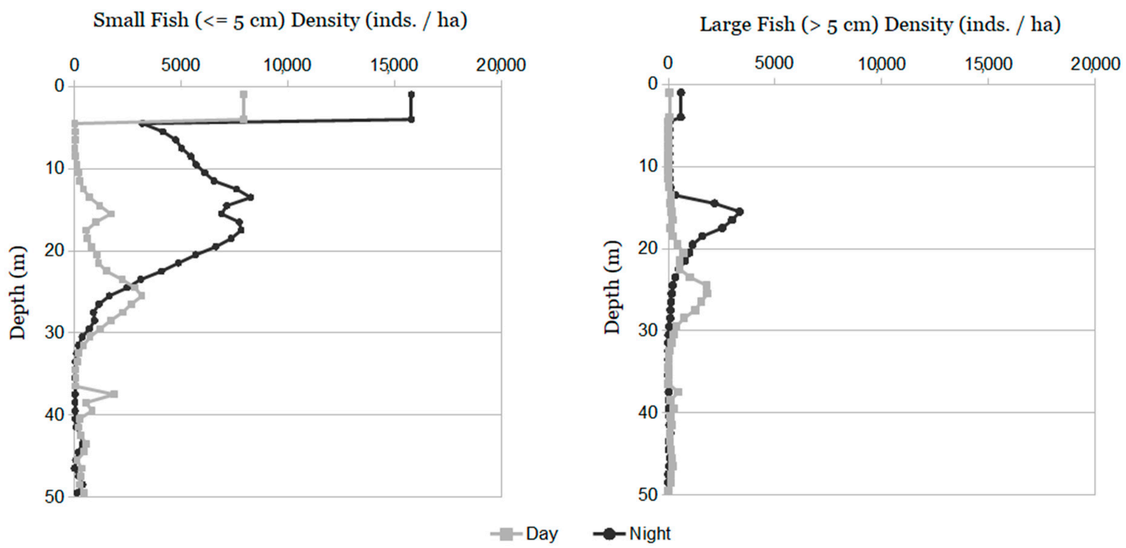

Figure 13. Vertical distribution of density (inds./ha) of the detected target size classes (small: <5 cm, large: 5–50 cm) during the day and night surveys in Lake Trichonis.

4.3. Temperature and Oxygen Stratification

The water temperature in the surface layer varied between 23.5 and 24.7 °C in the three sampling points. In the eastern region (deepest point), a thermocline at around 18–28 m was recorded, while temperature was almost constant in the hypolimnion (11.6–11.9 °C). During the survey, differences in temperature and oxygen profiles were observed between the western and eastern region (Figure 15). In the deep point of the western region, the thermocline was recorded at around 15–31 m, while the temperature in the hypolimnion was 12.7 °C. In the shallow point of the western region, temperature was almost constant up to a depth of 14 m, after which it exhibited a noticeable gradual drop, reaching its lowest value (14.3 °C) at 22 m.

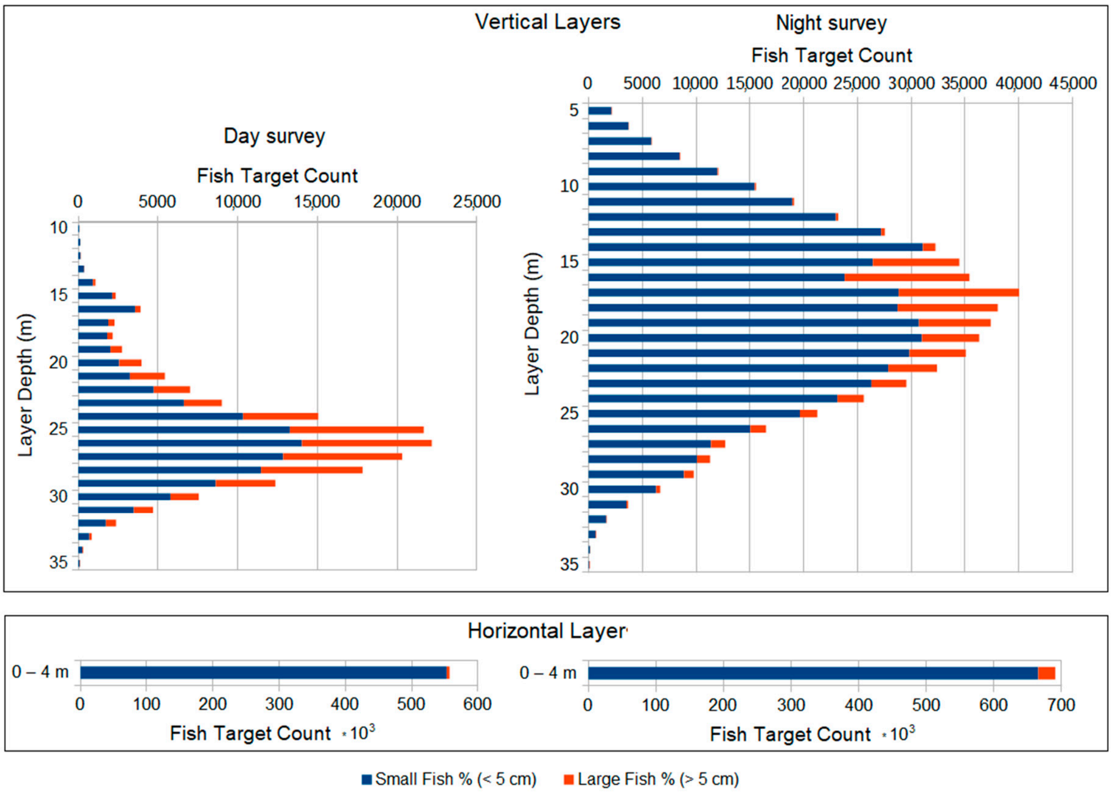

Figure 14. Proportional fish distribution by size, depth and time of day. Layers with <100 detected individuals excluded for clarity.

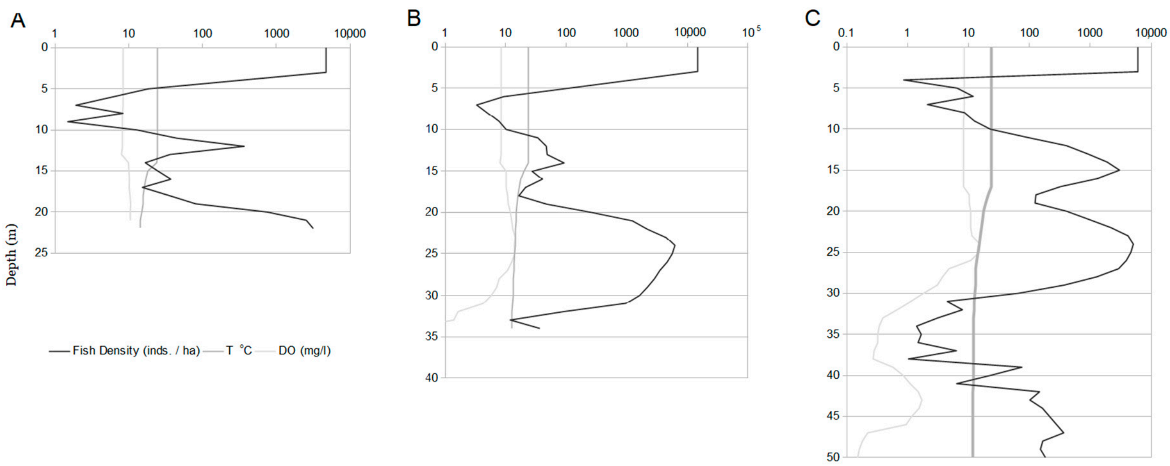

Figure 15. Variations of density (inds./ha), dissolved oxygen (mg/L) and water temperature (°C) with depth (m) at the three different sampling locations of Lake Trichonis ((**A**). Sampling point S1 (**B**). Sampling point S2 (**C**). Sampling point S3).

The horizontal variations of DO concentrations in the epilimnion from the eastern to the western region of the lake were weak. In the eastern region (deepest point) the DO concentration remained at approximately 8.3 mg/L in the upper 17 m mixing layer and significantly increased to 10–11 mg/L at a depth of 18–26 m. An anoxic layer was recorded after a depth of 30 m, as DO concentration fell below 2 mg/L [48]. Namely, the recorded DO values ranged from 1.82 to 0.15 mg/L. In the shallow point of the western region, the DO concentration was higher (approximately 10.5 mg/L) at around 16–22 m of depth than in the surface layer (approximately 8.3 mg/L). In the deep point of the western region, the highest concentration of DO (approximately 11.9 mg/L) was recorded at a depth around 15–27 m. An anoxic layer was also recorded after the depth of 32 m.

The vertical distribution of fish during the day survey varied according to thermal stratification and seemed to roughly follow the vertical variations of DO concentrations (Table 2). Based on the results of correlations between fish density and dissolved oxygen, it appears that as the depth of the sampled water column increases, a strong correlation manifests as the relationship between these two parameters. An apparent rise in correlation coefficient absolute values was observed between fish density and dissolved oxygen, especially around the area of the deepest point of the lake, whereas anoxic conditions occurred in the bottom layers.

Table 2. Correlation coefficients between fish density (inds./ha) and DO (mg/L)/Temperature (°C) for the three sampling points in Lake Trichonis.

	Sampling Point S1		Sampling Point S2		Sampling Point S3	
	Temperature	Dissolved Oxygen	Temperature	Dissolved Oxygen	Temperature	Dissolved Oxygen
R	−0.56	0.54	−0.52	0.53	0.018	0.61
R^2	0.31	0.29	0.27	0.28	0.00032	0.37
***p*-value**	0.013	0.018	0.0026	0.0023	0.91	5.9×10^{-6}
DF	17	17	29	29	45	45

5. Discussion

Volume backscattering strength (S_V), was used in this study as a proxy variable for fish biomass [8]. The calculation of biomass based on acoustic recordings requires knowledge of an overall fish SL–W (Standard Length to Weight) relationship, particularly for a mixed-species community, as well as TS estimates for fish fauna [49]. Given that this was the first hydroacoustic survey in Lake Trichonis, it was not feasible to obtain the aforementioned values. S_V values were compared among the different layers of the water column [50]. The average S_V values in Lake Trichonis −66.1 dB in the night and −65.9 dB in the day were slightly lower than in other 18 European lakes (on average −62.8 dB) [51]. The recorded range of S_V values in the different layers was also comparable with values of monomictic alpine lakes with similar morphology (ranges from −62.3 to −67.4 dB in different layers and lakes) [9] and with the results of Cech et al. [52], who studied distributions of juvenile fish (range of S_V was measured from −71.3 to −45.7 dB). Similarly to this last study, in Lake Trichonis, fish formed more or less dense layers; the type of aggregations is probably dependent on the kind of species.

Fish density was found to be lower in the deep areas (eastern area) than the shallow areas (western area) of the lake. Additionally, fish density was higher in intermediate depth locations, indicating that fish targets are generally distributed in the water column at larger depths. An offset between day and night was observed in the vertical distribution of fish, estimated at approximately 9m (fish were located deeper during the day). Fish were more closely aggregated during the day and more dispersed at night. Vertical migration behavior with diel periodicity is well described [53] and is linked to predation avoidance and reduction of competition [54].

A statistically significant difference was observed in vertical distribution between day and night in the pelagic zone. The overall recordings revealed a relatively high degree of fish aggregation (low share of single echoes), especially during daytime, which, in turn, makes it harder to discern and count single targets and decreases the reliability of density estimations. According to Appenzeller and Leggett [55], the estimation of the number of targets can be biased towards smaller values when fish form dense aggregations, due to acoustic shadowing. Precise determination of fish target counts should coincide with the time of their maximal dispersion [37]. The share of single echoes did not present significant changes in depth distribution between day and night. Nevertheless, during the night, significantly larger fish densities were observed at smaller depths, thereby observing a wider size spectrum of fish living in the lake. In this respect, it may suggest that night hydroacoustic surveys in Lake Trichonis could be more suitable. Nonetheless, we recommend that diel differences be further investigated by carrying out additional day-night comparisons, also using fish catches. The use of hydroacoustics in a future setting over the same study area would be useful to investigate the possible bias and aggregation-related behavior and simultaneously also the true size composition by the use of appropriate fishing gear.

In relation to higher fish densities, values of the Sawada index were also taken into account to consider reliability of the density estimates. In most of the analyzed cells, values of N_V were below the commonly accepted limit of 0.1 by Parker-Stetter et al. [56], only exceeding 0.1 in the layers with the densest aggregations, with a few or no single echo detections. Nevertheless, TS distribution was examined. There was no statistically significant difference in the two distributions (TS for all N_V and TS only for $N_V < 0.1$) in the lake, neither for the day (K-S D-value = 0.2143, $p = 0.9205$), nor for the night (K-S D-value = 0.167, $p = 0.9985$) (for details, see Supplementary Materials).

The majority of all detected targets have a small size, while larger targets only comprise a minimal fraction. The presence of small-sized fish [21] is also a reason for the threshold used in this study, which may seem to be lower than other hydroacoustic studies using a frequency of 120 kHz. The lake hosts 20 fish species [21], most of which are of small size, such as the big-scale sand smelt *Atherina boyeri*, which occupies the pelagic regions of the lake and its population is extremely abundant, supporting a valuable commercial purse-seines fishery [29]. It is important to note that the targets observed could not be other than fish, as no big zooplankton, which could overlap with small fish, was observed in the lake [22], nor invertebrates [57] could be expected in this autumn time. Nevertheless, we cannot exclude the possibility of juvenile fish being mixed into the composition of the recorded populations. However, due to the lack of catch data in the present study, as well as due to the lack of corresponding historical data, it is not possible to identify the smaller fish target species, thus precluding a conclusive interpretation of the results. Consequently, provided that the detected fish target species composition, biology and behavior are not known, further investigation into the variations in acoustic parameters across an annual cycle is warranted [58]. It is also necessary to use control catches to enable proper interpretation of acoustic targets.

A general estimation of the fish length, even roughly, is crucial to the understanding of an aquatic ecosystem [9]. Fish length from TS data can be calculated based on an equation that expresses this relationship [8]. The most important factor affecting acoustic estimates appears to be the appropriate relationship between the fish length and TS for a given population. Equations of Love [42] and Frouzova et al. [43] were derived from different fish species and size ranges, and therefore, the estimation of fish length based on those equations ought to be further assessed with data from catches regarding their use for the endemic species of the lake.

The vertical distribution of the fish in the lake appears to have been primarily driven by water temperature and oxygen stratification. In the present study, the surface layer density does not seem to be correlated to dissolved oxygen and water temperature, but other factors, such as predation risk, may play an important role. Similarly to Doulka and Kechayias [59], Lake Trichonis showed a minimum saturation value of dissolved oxygen in the lower hypolimnion in autumn. However, in contrast to the study of Doulka and Kechayias [59], in the present study anoxic conditions were recorded in the lake's hypolimnion, rendering the DO concentration a limiting factor for the vertical dispersion of fish. As a result, in the vertical domain, fish distribution was highly correlated with oxygen stratification, i.e., it decreased as the dissolved oxygen also decreased. This correlation between density and dissolved oxygen is well established, while the relation can be explained, in part, by the fact that there is a natural threshold of oxygen, beyond which survival very quickly becomes non-viable [60]. It is also important to note that the presence of larger abundances of large fish in intermediate layers is in agreement with the results of Breitburg et al. [61], according to which the response of predators and prey to oxygen distribution variations strongly influenced the spatial focus of trophic interactions.

Consequently, the metalimnion in Lake Trichonis is the most productive depth stratum, due to the intense variation of abiotic and biotic variables [59].

The methodology presented and the results obtained through this research were the first step to present and analyze the challenges for optimizing a hydroacoustic approach for monitoring freshwater fish stock in Greek lakes. This methodology could be incorporated in the decision-making process towards the improvement of the implementation of the EU Water Framework Directive 2000/60. The use of hydroacoustic techniques can eliminate some distortions traditionally contained in censuses and studies of fish fauna in lakes. Lake Trichonis is traditionally exploited by commercial fishermen, most of which use purse-seine fishing. The knowledge of feeding and nursery grounds where fish are gathered during their vertical and horizontal movements through the day will help and support local authorities and contribute to decision making in applying restrictions for a more sustainable fisheries management. The potential adjunct usage of common fishing gear might not be able to adequately reveal the potentially essential role of small fish in the open waters of Lake Trichonis. As a result, the proposed methodology can provide new insights for enhancing fisheries monitoring.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/6/1823/s1. Figure S1: Empirical distribution functions for TS, categorized in 2-dB bins separately for day and night, as well as for the sets of all sampled cells, and of only cells with $N_V < 0.1$. Figure S2: Sawada N_V index vertical distribution with 1-sigma confidence intervals (left) and day-night difference, i.e., day values minus night values (right).

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