Article

Comparing hydroacoustic fish stock estimates in the pelagic zone of temperate deep lakes using three sound frequencies (70, 120, 200 kHz)

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Abstract

Several decades of research have led to the acceptance of hydroacoustics as a reliable measurement method to monitor fish population in lakes, but full standardisation and intercalibration are still lacking. The aim of this study was to investigate the effect of sound frequency on acoustic parameters, such as volume backscattering strength, target strength, and the estimation of fish abundance. Data were recorded *in situ* using 3 frequencies (70, 120, 200 kHz) simultaneously in 2 different lakes. The results among the frequencies were compared and statistically tested. Data from the 70 and 120 kHz frequencies yielded similar results, but the 200 kHz echosounder estimates in temperate lakes seemed different, especially in cases of high fish abundance, which is typical of eutrophic ecosystems. This work indicates that the abundance estimates of fish populations in temperate lakes based on 200 kHz frequency may differ from results obtained using lower frequencies, and that further study is needed.

Key words: hydroacoustics, multifrequency, standardisation, target strength, WFD

Introduction

Lakes are impacted by many anthropogenic uses of water resources (Hermoso and Clavero 2013) that alter their ecological function. There is a need to monitor water volumes using bioindicators. Fish, due to the variety of trophic positions of fish communities and their longevity compared with other components of the aquatic biocoenosis, have been recognised as particularly appropriate bioindicators (Karr 1981, Argillier et al. 2012). Hydroacoustics have been increasingly used in recent years and provide a wide range of information on aquatic ecosystems, from distribution and abundance of fish populations to bottom characteristics such as bathymetry and sediment classifications, but also on other biotic communities such as zooplankton and macrophytes (Trenkel et al. 2011). Several decades of research at sea and in freshwaters have led to the acceptance of hydroacoustics as a reliable measurement method, both in marine and lake ecosystems (Simmonds and MacLennan 2005, Rudstam et al. 2012). Instrumentation has matured to be used routinely in scientific studies and monitoring surveys in various freshwater ecosystems (Godlewska et al. 2004, Winfield et al. 2007, Djemali et al. 2009, Sotton et al. 2011), but full standardisation and intercalibration are still lacking. The Study Group on Fisheries Acoustics in the Great Lakes has guided research toward measurement standardisation (Rudstam et al. 2009) and has developed a standard operating procedure (Parker-Stetter et al. 2009). In Europe, the Comité Européen de Normalisation (CEN) standard, Water Quality – Guidance on the Estimation of Fish Abundance with Mobile Hydroacoustic Methods (CEN 2009), has recently been accepted (Hateley et al. 2013). The effect of collection parameters on results must

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be examined, and, if necessary, parameters must be standardised. Sound frequency is considered a primary parameter because the acoustic backscatter strength from fish is frequency-dependent (Horne 2000).

The use of different frequencies is increasingly popular to differentiate among species, especially in marine environments (Korneliussen and Ona 2002, 2003, Gauthier and Horne 2004). In freshwater ecosystems, multifrequency approaches were primarily used to separate backscattering of fish from that of mysids (*Mysis relicta*; Axenrot et al. 2009) or chaoborus (*Chaoborus flavicans*; Eckmann 1998, Knudsen et al. 2006), which are common in many lakes. Despite the increasing number of hydroacoustic surveys, however, systematic studies on the effects of frequency on total echo-energy, fish target strength (TS), or the subsequent evaluation of fish abundance performed *in situ* in freshwater are few. Recent work includes our previous reports on the use of 2 frequencies, 70 kHz and 120 kHz (Guillard et al. 2004, Godlewska et al. 2009).

The aim of this study was to broaden our previous knowledge on the effects of sound frequency on fish responses. We applied a 200 kHz frequency system to the previously tested 70 and 120 kHz frequencies; the 200 kHz frequency is commonly used by scientific teams to monitor and study fish populations in freshwaters (Rowe 1993, Comeau and Boisclair 1998, Krumme and Saint-Paul 2003, Jones et al. 2007, Winfield et al. 2009). We recorded *in situ* data by simultaneously using the 3 frequencies in 2 lakes and then compared the results among the frequencies to determine the effect of frequency choice, its significance in monitoring fish populations in temperate lakes, and whether standardisation is required.

Study site, materials, and methods

The experimental work was conducted in September 2009 in Lake Annecy and Lake Aiguebelette, located in the French Alps. Lake Annecy (45°51′24″N; 06°10′20″E) is a deep, oligotrophic monomictic lake, with a maximum depth of 69 m, length of 14.6 km, width of 2 km, and total area of 27.4 km^2 (Perga et al. 2010). Lake Aiguebelette (45°33′28″N; 5°48′5″E) is a smaller, mesotrophic monomictic lake, with a maximum depth of 71 m, length of 4.2 km, width of 2.8 km, and total area of 5.45 km^2. Similar to other lakes in temperate areas, fish populations in both lakes show vertical distribution in autumn strongly related to the thermocline (Guillard et al. 2006a, Yule et al. 2013; Fig. 1). Using the "TS versus Range" function of the applied post-processing software Sonar5-Pro (Lindem Data Acquisition, Norway), we chose 15 m as the depth separating fish populations above and below the thermocline. In the upper pelagic habitat, juveniles of roach and perch school during daylight (Guillard et al. 2006b) and disperse at sunset to feed (Masson et al. 2001). The lower layers below the thermocline are inhabited by salmonids, particularly whitefish (*Coregonus lavaretus*), mostly >15 cm in length (Mehner et al. 2010, Yule et al. 2013).

Hydroacoustic measurements, georeferenced by GPS connected to the echosounder, were performed at a sailing speed of ~8 km h^{-1} along regular transects in the pelagic areas of both lakes, covering the main part of the lakes. Surveys were conducted at night, starting ~1 hour after sunset, to sample fish when they dispersed.

Three Simrad EK60 echosounders, one for each frequency, were used for the survey. A PC running Simrad ER60 software controlled the three EK60 and ensured simultaneous pinging and recording. The applied split beam transducers were ES70-7x7, ES120-7x7, and ES200-7x7 (i.e., with the same 7x7° half-power opening angle). The transducers were mounted as close as possible to each other on a special frame aimed vertically downward with the transducer faces at a 0.5 m depth. For all 3 frequencies, the pulse durations were set to a medium pulse length (0.256 ms; Godlewska et al. 2011) using a 5 Hz ping repetition rate. Sonar5-Pro post-processing software was applied for data analysis using multifrequency tools.

Elementary sampling distance units (ESDU) were set to ~1000 pings (i.e., ~500 m) to extract the total mean volume backscattering strength (S_v) in decibels (dB) and the mean TS in dB (MacLennan et al. 2002). In Lake Annecy, 2 layers were analysed from the surface (at a depth of ~3 m to avoid surface noises) to the thermocline (15 m) and from the thermocline to the bottom (1 m above

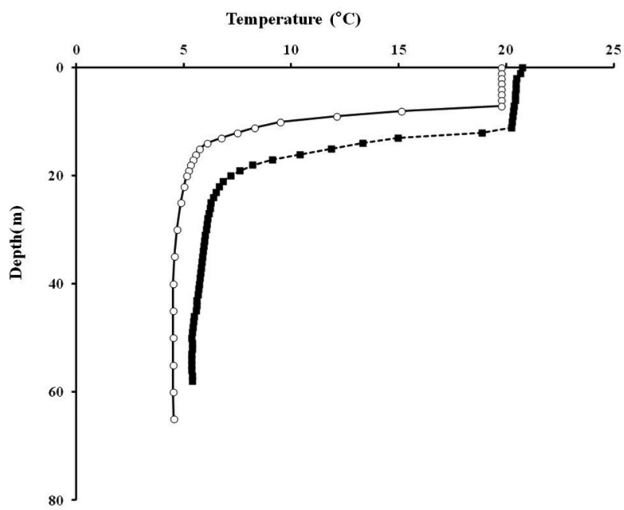

Fig. 1. Temperature profiles in Lake Annecy (black squares) and Lake Aiguebelette (white circles), September 2009 (data from SOERE OLA).

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the bottom to avoid false echoes). In Lake Aiguebelette, the number of single echo detections (SED) by ESDU was low, generally <20; therefore, to increase the number of targets by ESDU, only one layer, from the surface to the bottom, was analysed.

Because we were interested in comparing the output at 3 frequencies rather than quantitatively estimating fish abundance in both lakes, this conservative procedure seemed appropriate. For all 3 frequencies, the TS threshold was set to −55 dB, which for a frequency of 70 kHz corresponds to targets >3.5 cm according to the Love (1971) equation (Emmrich et al. 2012). In autumn, most of the young-of-the-year (YOY) fish sizes in both lakes exceed this length (Decelière-Vergès et al. 2009, Guillard et al. 2011); therefore, using this threshold, all YOY were included in the analysis, even if there were differences among the frequencies for TS values. The Sv threshold was set 6 dB lower, to −61 dB, according to Parker-Stetter et al. (2009). The criteria used to differentiate among individual targets (i.e., SED) were set to 0.8 and 1.3, accordingly (the minimum and maximum returned pulse width relative to the transmitted pulse duration). The maximum gain compensation was 3 dB (one way) with a maximum phase deviation of 0.3 degrees. A visual inspection of the echograms was performed to remove noises on all 3 echograms using Sonar5-Pro's manual inspection and erasing tools. The Sawada index (Sawada et al. 1993) was calculated to ensure that conditions were suitable for *in situ* TS estimation, which means the Sawada index was >0.1 for all ESDU (Godlewska et al. 2011). Acoustic data (i.e., mean Sv and mean TS) from each ESDU were computed and analysed. To estimate fish abundance, the Sv/TS scaling method (Balk and Lindem 2011) was applied according to the Forbes and Nakken (1972) equation. The transducers' calibration was tested in a tank according to the procedure recommended by Foote et al. (1987). *In situ* calibration with a full survey setup was performed with Simrad's Lobe software prior to the surveys in each lake.

A major axis estimation procedure was used for a comparison in pairs (2 frequencies at a time). Linear regression (LR) and major axis (MA) estimation are both least squares methods in which the line is estimated by minimising the sum of squares of residuals from the line. The difference between the methods is how the errors that produce the line are estimated. For LR, the errors are found normal to the x-axis, whereas for MA, they are estimated normal to the output line. The choice of the best line-fitting method depends on the purpose. Regression is useful when a line is desired for predicting one variable (Y) from another variable (X). If the statistic of primary interest is the slope, then MA is more appropriate than LR (Warton et al. 2006). MA assumes that the error variance is equal for X and Y, often a reasonable assumption when checking if 2 methods of measurement agree, as in our case. When the methods of measurement are unbiased, the true values of the subjects are known to lie on the line Y = X, the (1:1) line. Statistical tests described in Warton et al. (2006) were performed to compare the slopes of the major axis against a slope on the (1:1) line.

Nonparametric ANOVA Friedman tests were performed (using Statistica 9.0 software) to determine whether differences in the mean Sv, mean TS, and mean fish abundance (averaged over the ESDU) among the 3 frequencies were statistically significant. Additionally, we calculated the Kendall's coefficient of concordance, an extension of the Spearman Rho correlation procedure for more than 2 groups. Kendall's coefficient of concordance is a nonparametric statistic; it makes no assumptions regarding the nature of the probability distribution and can handle any number of distinct outcomes, is a normalisation of the statistic of the Friedman test, and can be used for assessing agreement among raters. Kendall's coefficient ranges from 0 (no agreement) to 1 (complete agreement).

The 3 frequencies of fish size distributions (TS in 3 dB classes) were computed by pooling data from all transects for the entire Lake Aiguebelette and for the 2 layers in Lake Annecy. Distributions were compared and tested using the nonparametric Friedman ANOVA test.

Results

S_v and TS comparisons

Surveys conducted on Lake Annecy provided data on 27 ESDUs in 2 layers (27 × 2 = 54 analysis cells) and on 12 ESDUs in 1 layer (12 analysis cells) on Lake Aiguebelette. Sv and TS comparisons were performed in pairs: between 70 and 120 kHz, 70 and 200 kHz, and 120 and 200 kHz. Tests performed according to Warton et al. (2006) indicated that in both lakes, results for all 3 frequencies showed relationships close to the (1:1) line (Fig. 2). For Sv and TS data, the slopes of the major axis estimation were not significantly different from 1 at the level $p = 0.05$, except in 3 pairs (Fig. 2) from the lower layer of Lake Annecy: TS between 120 and 200 kHz ($p = 0.037$), Sv between 70 and 200 kHz ($p = 0.015$), and Sv between 120 and 200 kHz ($p = 0.006$).

Statistical nonparametric ANOVA Friedman tests performed on the whole dataset (based on mean values) showed that differences between the basic acoustical parameters (Sv and TS) at the 3 frequencies were statistically significant (Table 1) in all cases. In the case of Sv, Kendall's coefficient of concordance was ~1, signifying that values at all 3 frequencies were highly proportional.

Table 1. ANOVA Friedman test for the 3 frequencies (70, 120, and 200 kHz) tested for the S_v and TS values. In Lake Annecy, 2 layers were analysed, lower and upper; in Lake Aiguebelette, 1 layer is analysed (total). Kendall's coefficient of concordance (KCC) is indicated in bold.

	Annecy upper layer		**Annecy lower layer**		**Aiguebelette**	
	KCC = 0.794		**KCC = 0.861**		**KCC = 1.000**	
	***p* = 0.0000**		***p* = 0.0000**		***p* = 0.0000**	
	mean	**std**	**mean**	**std**	**mean**	**std**
S_v 70	−62.29	2.70	−67.89	2.00	−65.49	2.68
S_v 120	−62.37	2.66	−67.43	1.87	−64.94	2.65
S_v 200	−64.13	2.92	−68.86	2.48	−67.47	2.34
	KCC = 0.413		**KCC = 0.848**		**KCC = 0.583**	
	***p* = 0.0000**		***p* = 0.0000**		***p* = 0.00009**	
	mean	**std**	**mean**	**std**	**mean**	**std**
TS 70	−47.14	1.35	−36.18	1.08	−36.87	0.89
TS 120	−46.43	1.34	−35.47	0.82	−36.48	0.98
TS 200	−46.44	1.30	−37.18	1.04	−37.92	0.74

Although the difference between Sv values at all frequencies was small, the probability to observe them was high; therefore, the test indicated a statistically significant difference. Kendall's coefficient was more variable for TS than for Sv, indicating that the data were less correlated, which is not unexpected considering the high variability of TS, even for individual fish.

The statistical significance of differences computed from statistical tests does not mean that these differences were large. The differences between 70 and 120 kHz were low for both parameters (the difference between mean values was <0.55 dB for Sv and <0.71 dB for TS; Table 1). Using 200 kHz data introduces much larger differences in most cases (the difference between mean values reached 2.53 dB for Sv and 1.71 dB for TS). In one case for the upper layer of Lake Annecy, the mean TS in the data from the 120 kHz system was nearly identical to the results from the 200 kHz system.

TS distributions

Although covering roughly the same sampling volume, the 3 frequencies recorded a different number of single targets: the 70 kHz transducer detected 2253 single echoes, the 120 kHz transducer detected 2882 single echoes, and the 200 kHz transducer detected 3137 single echoes. The number of single echo detections differed among the 3 frequencies in the lower layer of Lake Annecy and in the single layer of Lake Aiguebelette, where the difference in the results from the 70 kHz system were statistically significant from the other 2 frequencies (Table 2). Thus, to facilitate comparison, percentages rather than numbers are used on the y-axis of the frequency distributions (Fig. 3), where substantial differences seem to exist between size distributions from the 200 kHz system versus the distributions from the other systems. This difference, however, is not statistically significant according to an ANOVA Friedman test (Table 2).

Fish abundance

Fish abundances were calculated from Sv and TS data. For Lake Annecy (Fig. 4), the slopes differed significantly from the (1:1) line for all frequency pairs ($p < 0.05$) with one exception, the 70 and120 kHz pair in the lower layer of Lake Annecy ($p = 0.61$). In Lake Aiguebelette (Fig. 4), the relationships between frequency pairs were not significantly different from the (1:1) line ($p > 0.05$).

The nonparametric ANOVA Friedman test showed that all fish abundances computed by echosounding, independent of the applied frequency, were significantly different (Table 3).

Again, differences among the 3 frequencies, although statistically significant, were not large, especially when the fish abundance was low. The fish abundances estimated in Lake Annecy below the thermocline were similar, with mean values of 262, 249, and 280 fish per ha, respectively, for the 70, 120, and 200 kHz systems (Fig. 5; Table 3). For the abundance levels found in Lake Aiguebelette, the difference between the 70 and 120 kHz systems was also low (570 and 588 fish per ha, respectively), but definitively lower for 200 kHz (458 fish per ha). In areas of high fish abundance, such as in the upper layer of Lake Annecy, the results from all the frequencies largely differed (4764, 3944, and 2668 fish per ha for the 70, 120, and 200 kHz systems, respectively).

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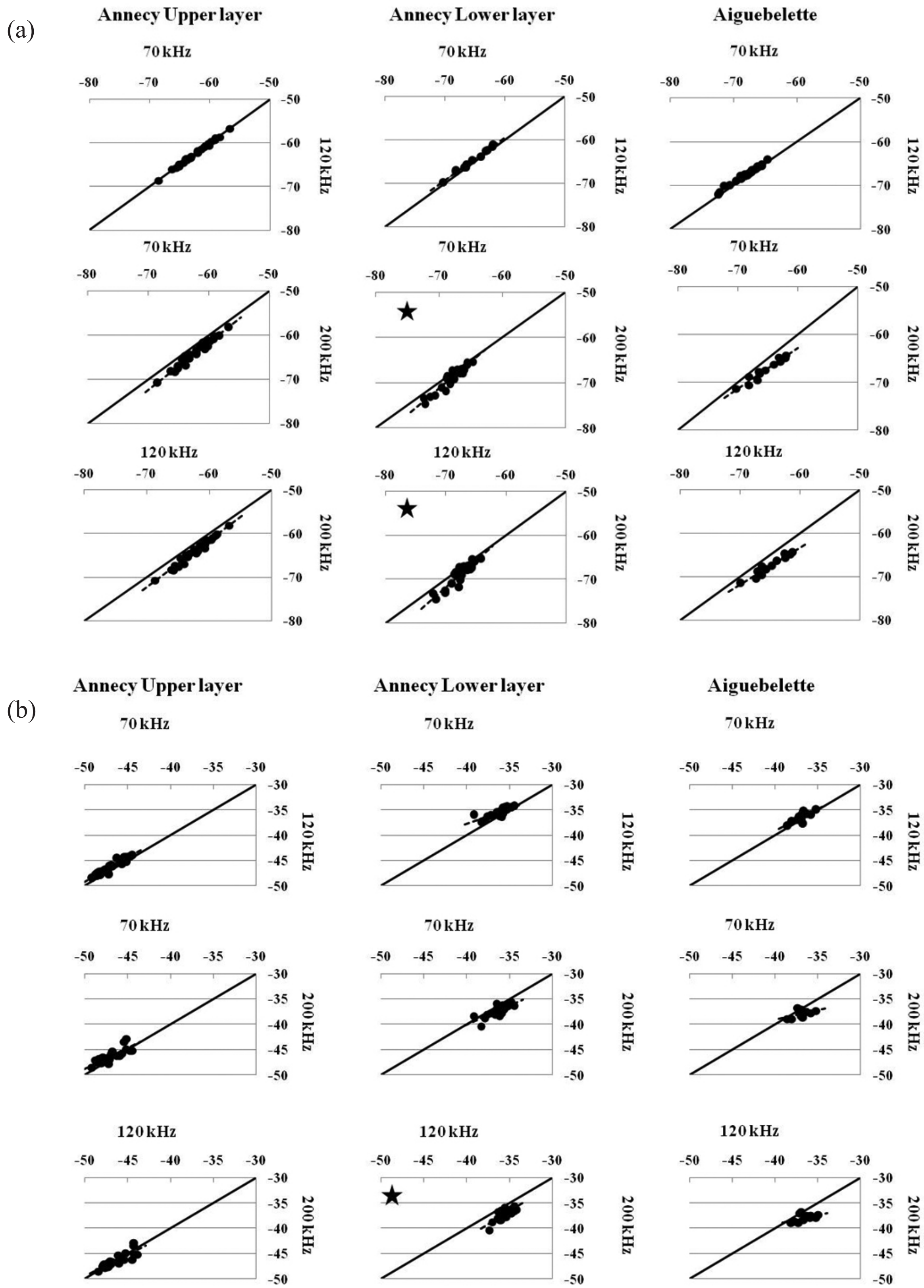

Fig. 2. Relationships between (a) S_v and (b) TS data for the 3 frequency pairs from lakes Annecy and Aiguebelette. Dashed line = major axis; black line = (1:1) line. Star indicates a significant difference ($p = 0.05$) between the major axis and the (1:1) line.

Table 2. ANOVA Friedman tests for the TS distributions (frequencies) as numbers (N) and %. Kendall's coefficient of concordance (KCC) is indicated in bold. SED = single echo detections.

	Annecy upper layer		Annecy lower layer		Annecy total		Aiguebelette	
	KCC = 0.033		**KCC = 0.433**		**KCC = 0.187**		**KCC = 0.300**	
	***p* = 0.7165**		***p* = 0.0131**		***p* = 0.0131**		***p* = 0.0498**	
	mean	**std**	**mean**	**std**	**mean**	**std**	**mean**	**std**
70 kHz SED number	567.80	993.49	241.50	258.80	809.30	944.91	132.30	81.74
120 kHz SED number	644.20	984.65	307.20	308.45	951.40	910.20	104.40	69.20
200 kHz SED number	565.70	722.06	340.80	317.99	906.50	663.52	102.90	63.34
	KCC = 0.413		**KCC = 0.012**		**KCC = 0.008**		**KCC = 0.030**	
	***p* = 0.7165**		***p* = 0.8948**		***p* = 0.8948**		***p* = 0.7408**	
	mean	**std**	**mean**	**std**	**mean**	**std**	**mean**	**std**
70 kHz TS dist %	10.00	17.49	11.11	10.74	10.00	11.68	10.00	6.18
120 kHz TS dist %	10.00	15.28	11.10	9.99	10.00	9.57	9.99	6.62
200 kHz TS dist %	10.00	12.76	11.11	9.17	10.00	7.32	10.00	6.16

Table 3. ANOVA Friedman test of differences among 3 frequencies (70, 120 and 200 kHz) on fish abundance. Kendall's coefficient of concordance (KCC) is indicated in bold.

	Annecy upper layer		Annecy lower layer		Aiguebelette	
	KCC = 0.931		**KCC = 0.150**		**KCC = 0.757**	
	***p* = 0.0000**		***p* = 0.0177**		***p* = 0.0001**	
	mean	**std**	**mean**	**std**	**mean**	**std**
Fish abund. 70 kHz	4764	3669	262	95.25	570	208.11
Fish abund. 120 kHz	3944	2971	249	98.93	588	203.09
Fish abund. 200 kHz	2668	2001	280	129.24	458	186.44

Discussion

In previous studies by Guillard et al. (2004) and Godlewska et al. (2009), comparisons of the most common frequencies (70 and 120 kHz) for fish studies in temperate lakes have shown a satisfactory correspondence among the results found for Sv, TS, and fish abundance when using the same pulse length of 0.256 ms (Godlewska et al. 2011). In this study, the results for these 2 frequencies were also in satisfactory accordance with previous results, especially for instances of low fish abundance as observed in the lower layer of Lake Annecy and in Lake Aiguebelette. This work emphasises that 70 and 120 kHz transducers can be used for acoustic data and fish abundance comparisons in ecosystems where the fish abundance is not high, in our case <600 fish per ha. Small but statistically significant differences were found in this survey between these frequencies. The differences were not sufficiently large to concern fisheries management, however, and the 2 frequencies can be regarded as being practically similar.

We have extended our previous work by testing at 200 kHz, a frequency often used in fish population surveys (Krumme and Saint-Paul 2003, Lilja et al. 2003, Winfield et al. 2007, 2009). The results from the 200 kHz study were less correlated with the 2 other frequencies, and, in general, the study resulted in lower fish abundance, which agrees with findings by Wanzenböck et al. (2003), who performed a comparison at 120 and 200 kHz, using 2 different systems (Simrad and Biosonics). The systems differed in many parameters (e.g., pulse length, beam opening, software); furthermore, the transducers were mounted on 2 sides of the boat, so that the sampling volumes overlapped only partly, mainly in deep water, and therefore estimating to what extend the observed differences were due to frequency was not possible.

In our studies, fish abundance estimates from the 200 kHz system differed from the results obtained by the 70 and 120 kHz systems for fish abundances of ~600 fish per ha, similar to Wanzenböck et al. (2003) data for Irrsee. Differences were augmented in high abundance

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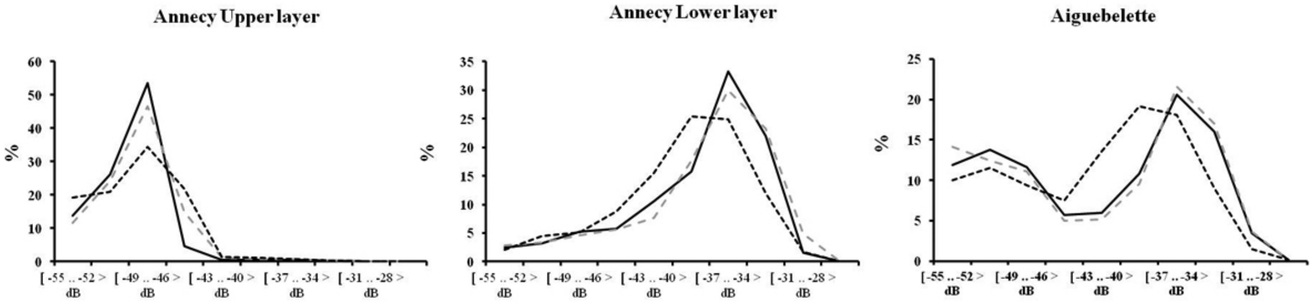

Fig. 3. TS distributions in the 3 dB classes for the 70 kHz (black line), 120 kHz (grey dotted line), and 200 kHz (black dotted line).

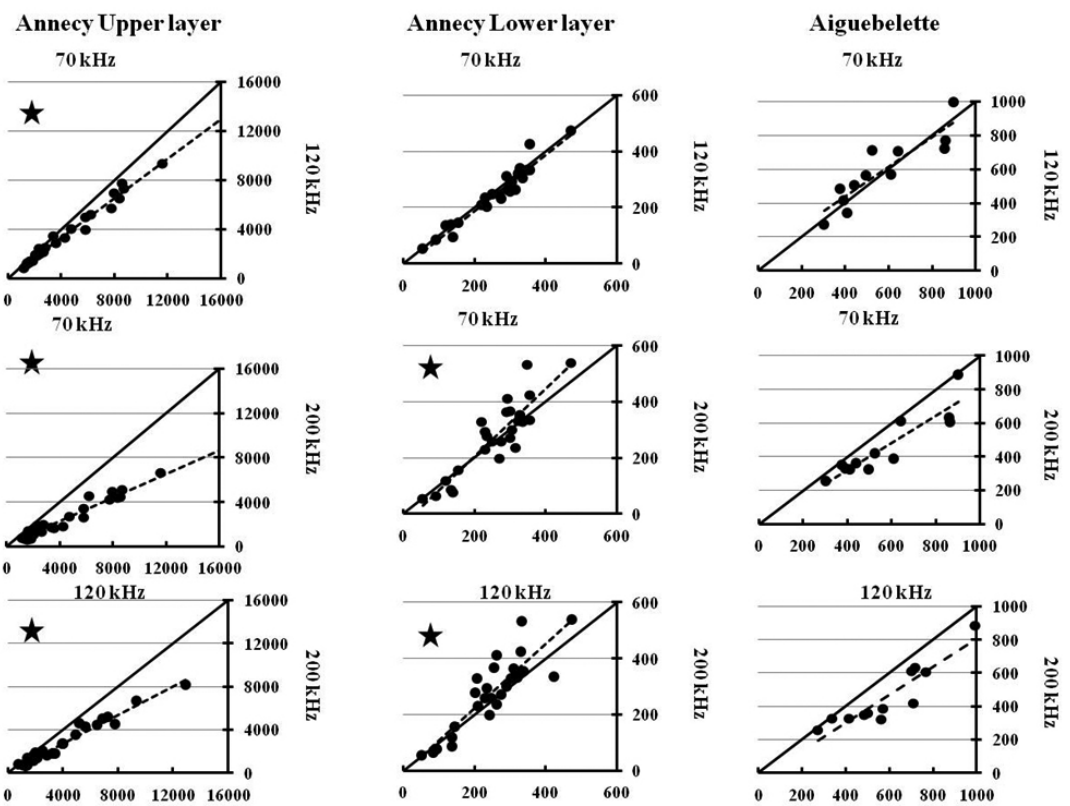

Fig. 4. Relationships among fish abundance for the 3 frequencies pairs from lakes Annecy and Aiguebelette. Dashed line = major axis; black line = (1:1) line. Star indicates a significant difference between the major axis and the (1:1) line.

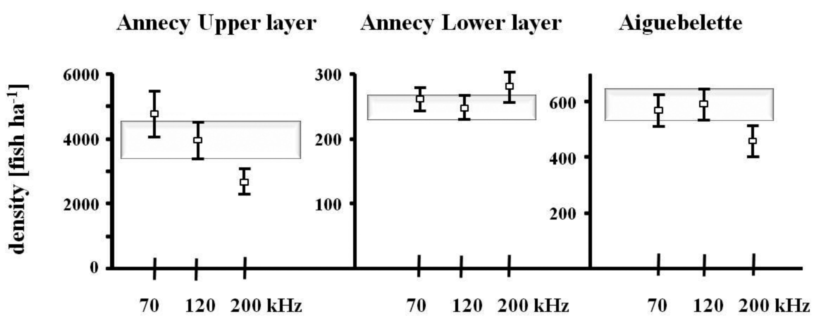

Fig. 5. Mean fish abundance computed for the 3 frequencies, 70, 120, and 200 kHz, from Lake Annecy's upper and lower layers, and Lake Aiguebelette. Open squares = mean values; error bars = standard deviations; white box = range of 120 kHz estimates, based on the calculation of the confidence interval.

conditions, which are usually found in eutrophic lakes (Gerdeaux et al. 2006), but also in the upper layers of oligotrophic lakes with a high number of juveniles (Guillard et al. 2006b). At low fish abundance, as observed in the lower layer of the oligotrophic Lake Annecy, we computed 262 and 280 fish per ha using the 2 most distant frequencies, 70 and 200 kHz, respectively. This difference is small from a fisheries perspective but was statistically significant according to an ANOVA Friedman test. In the upper layer, where YOY were numerous (Guillard et al. 2006a), fish abundances were estimated to be 4764 and 2668 fish per ha for 70 and 200 kHz, respectively. At this high fish abundance, these 2 estimates proved to be significantly different according to an ANOVA Friedman test, and from a fisheries perspective the estimates would most likely also be considered to be different.

In the study of essential parameters, such as the frequency on the analysis of results, *in situ* work is important because the object of research, the lake with the fish, is directly included, which is not possible in other types of controlled experiments. The problem with *in situ* studies is, however, that they include additional variables that can be difficult to control, which may influence the results in unknown ways and thus reduce verifiability. The environment adds sources of variability such as weather and sea state changes, target behaviour, unwanted targets, and other noise sources. On small boats, as in the present experiment, the researchers themselves may cause variability through movements in the boat, influencing the transducer tilting, which also impacts the results.

Abundance estimates are based on the mean fish TS, which can vary more than 10 dB, even for the same individual fish recorded with the same transducer due to changes in movements and inclines (Dawson and Karp 1990, Gauthier and Rose 2002, Godlewska 2004, Henderson et al. 2008), and thus could obviously vary with different transducers. Fish behaviour, such as change in fish orientation and position relative to the sound beam, deeply increases this variability and, consequently, differences in results. The reflection lobes from fish are frequency dependent and become more rugged and narrow with increasing frequency. Variation in fish aspects and observation angle will therefore have a greater impact on the resulting TS observed with 200 kHz versus 70 kHz (Horne 2000).

Echosounders using 70 and 120 kHz frequencies are the most common devices used to monitor and study fish populations in freshwater. We reemphasise that *in situ* results from these 2 frequencies produce similar results if the recommended parameters are used (CEN 2009, Parker-Stetter et al. 2009), but that it is not the same when using a 200 kHz echosounder instead of 70 or 120 kHz echosounders. Fish abundance estimates in temperate lakes with high fish abundance based on a 200 kHz echosounder seem different from the fish abundance estimates obtained with 70 and 120 kHz echosounders. Although 200 kHz is an attractive frequency because of the small size of the transducer, this work shows that fish population abundances in temperate lakes based on this frequency can be lower than results obtained with lower frequencies, a finding in accordance with acoustic theory (Simmonds and MacLennan 2005). More *in situ* and caged fish experiments with different frequencies, different fish species, and different densities are still needed to improve our understanding of frequency responses from fish populations in temperate lakes, our knowledge of the fish abundance effect, and reliability of the hydroacoustic method as a monitoring tool in such environments.

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