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ORIGINAL RESEARCH

Including 38 kHz in the standardization protocol for hydroacoustic fish surveys in temperate lakes

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Fisheries acoustics, frequencies comparison, freshwater, hydroacoustics, lake, standardization

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Abstract

Hydroacoustics has become a requisite method to assess fish populations and allows to describe the relationships of fish with other elements of the aquatic ecosystem. This nonintrusive method is currently an integral part of the sampling procedures recommended for fish stock assessment by the Water Framework Directive and has been standardized by the European Committee for Standardization [CEN (2014) CSN EN 15910 - Water quality - Guidance on the estimation of fish abundance with mobile hydroacoustic methods, Category: 7577 Water quality. Biological.]. In Europe, hydroacoustic surveys are performed in freshwater using different frequencies. Consequently, there is a need to evaluate if survey results can be compared. This study aimed to carry out *in situ* comparisons at the 38 kHz frequency (noted f) with two other commonly used frequencies, 70 and 200 kHz. The 38 kHz frequency has seldom been compared with other frequencies in freshwater although it is widely used worldwide, especially in the Great Lakes of North America and in Sweden. In 2016, hydroacoustic data were acquired in Lakes Annecy and Bourget using methods validated in previous studies that compared the frequencies 70, 120 and 200 kHz. This study showed similar density and biomass estimations as a function of frequency, density(f) and biomass(f), between the frequencies studied for low to moderate fish densities. For higher fish densities, the results were more variable and need to be verified. Fish density(f) and biomass(f) estimations sometimes exhibit differences between frequencies, which is not fully in agreement with theoretical calculations. The aim of this study was to evaluate frequency comparisons in practise. However, if the differences on acoustic metrics, density(f) or biomass(f) between frequencies were occasionally statistically significant, the differences were small enough to be considered negligible for fish population management. These analyses led to better knowledge of the responses from fish in temperate lakes for the studied frequencies. Our findings should be considered when revising the CEN standard.

Introduction

Lake ecosystems provide numerous services (Keeler et al. 2012). Therefore, there is an increasing need for knowledge about lakes for use in fisheries management or in monitoring and studying the state of the ecosystem. Hydroacoustics is a useful tool for increasing our knowledge about freshwater ecosystems, and numerous publications focus on the applications of hydroacoustics (Emily et al. 2017; Farrell et al. 2017; Riha et al. 2017).

Hydroacoustics has been developed over several decades and is today recognized as a strong and reliable method (Rudstam et al. 2012; Draštík et al. 2017). It is routinely used in the context of scientific studies and monitoring programmes (Winfield et al. 2008; Samedy et al. 2015; Lian et al. 2017; Tao et al. 2017); however, some standardisations and intercalibrations are still necessary (Guillard et al. 2014). The Study Group on Fisheries Acoustics in the Great Lakes conducted studies to improve the standardisation of operating procedures (Rudstam et al. 2009) and developed a standardised process for the American Great Lakes (Parker-Stetter et al. 2009). In the same manner, the European Committee for Standardization (CEN) adopted a standard for fish abundance estimation in Europe using mobile hydroacoustics (Hateley et al. 2013; CEN, 2014); however, the use of different echosounder settings for the acquisition of hydroacoustic data needs to be further investigated to analyse their impacts on the main metrics recorded during hydroacoustic surveys (Axenrot et al. 2016).

The acoustic frequency (noted f) is one of the most important factors for considering variability, as the acoustic backscattering properties of different fish are frequency dependent (Horne 2000). In Europe, different monofrequency sounders are used, depending on the country: France mostly uses the 70 kHz frequency, Poland uses 120 kHz, England uses 200 kHz and Sweden uses a 38 kHz sounder (Draštík et al. 2017). Therefore, it would be useful to determine if the results from surveys using different frequencies could be compared. Previous studies have highlighted the similarities between 70 and 120 kHz results, while 200 kHz results differed when fish densities were high, or more than 600 fish.ha^{-1} (Guillard et al. 2014).

This study aims to include 38 kHz with the previously compared results from 70, 120 and 200 kHz in the context of the standardisation of freshwater hydroacoustic methods for monitoring fish populations in lakes (Guillard et al. 2004, 2014; Godlewska et al. 2009).

We evaluate the impact of frequency on hydroacoustics results - especially by including the 38 kHz frequency - on the nautical area backscattering coefficient, as defined by MacLennan et al. (2002), using $s_A(f)$ in m².ha^{-1} (Balk and Lindem 2014; Yule et al. 2013), Target Strength (TS (f) in dB re 1 m² (noted dB), MacLennan et al. 2002) and lake managers' metrics as a function of frequency: fish density(f) and biomass(f) (Simmonds and Maclennan 2005). Data were recorded in two lakes using three frequencies simultaneously (38, 70 and 200 kHz), to compare *in situ* data and results at 38 kHz, which is commonly used in some countries, with the two other frequencies.

Materials and Methods

Study site

The data acquisition surveys were performed in 2016 in France in Lakes Annecy (45°51'24''N; 06°10'20''E) and Bourget (45°43'55''N; 5°52'06''E) from September 12th to 15th and 26th to 30th, respectively (Fig. 1).

Similar to other lakes in temperate regions, in late summer, the fish populations in these two lakes showed a vertical structure, linked to thermal stratification (Guillard et al. 2006a; Yule et al. 2013). The thermocline, a region of rapid thermal transition between cold water in the hypolimnion and warm water in the epilimnion (Coloso et al. 2008), separated fish species having different thermal preferences, which was the case in Lakes Bourget and Annecy (Yule et al. 2013). The temperature profiles (data from OLA, Observatory of LAkes (http://www6.inra.fr/soere-ola ©SOERE OLA-IS, AnaEE-France, INRA Thonon-les-Bains, CISALB, SILA, developed by Eco-Informatics ORE INRA Team), (Fig. 2A and C) showed the presence of a strong thermocline in each of the two lakes. Thus, the water column was divided into two parts: an upper layer with warm water and a lower layer with colder water. In the upper layer, juvenile roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) formed schools during the daytime (Guillard et al. 2006b) and dispersed within the same layer after sunset to feed (Masson et al. 2001). In the lower layer, salmonids were dominant (Mehner et al. 2010), especially whitefish (*Coregonus lavaretus*) in both lakes (Yule et al. 2013). Thus, fish populations were specific to each layer, that is, above and below the thermocline (Fig. 2B and D) and therefore have been analysed separately. Based on temperature profiles and echograms, we determined the upper layer to be from a depth of 2 m to 15 m in Lake Bourget and from 4 m to 12 m in Lake Annecy. We excluded hydroacoustic data close to the surface to avoid surface noise and data in the near field (MacLennan and Simmonds 1992).

Hydroacoustics surveys

The hydroacoustic data [data from OLA, Observatory of LAkes (http://www6.inra.fr/soere-ola ©SOERE OLA-IS, AnaEE-France, INRA Thonon-les- Bains, CISALB, SILA, developed by Eco-Informatics ORE INRA Team)] were collected at 38, 70 and 200 kHz using Simrad echo sounders (EK60, ER60) and transducers (ES38-7B, ES70-7C and ES200-7C), all having 7 degrees of half-power opening angles. The transducers were set in a frame to beam vertically and mounted aligned vertically as close as possible to maximize sampling volume overlap. The echo sounders were set to transmit pulses simultaneously. The

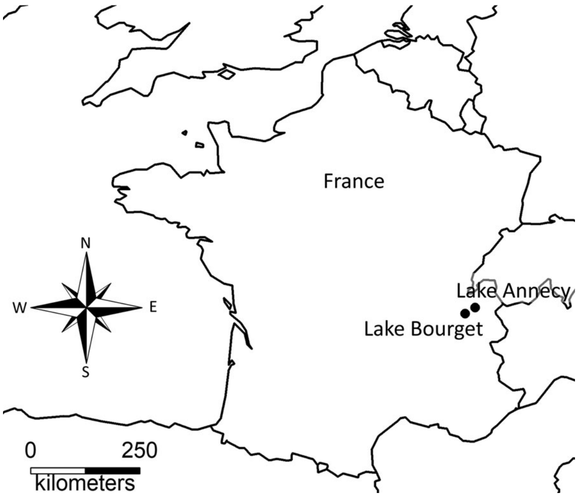

Figure 1. Geographic position of Lakes Annecy and Bourget, the two study lakes.

frame was pole mounted onto the vessel. The transducers were submerged to a depth of 0.70 m. Pulse lengths were set to 0.256 ms (Godlewska et al. 2011) and transmitted at 5 Hz with a transmitting power of 100 W. Calibration was performed for all frequencies according to the standard protocol of Foote et al. (1987) and the manufacturer's manual prior to surveying in both lakes. Calibration results agreed well with previous tank calibrations at Ifremer (Brest, France). Surveys were performed overnight in calm to moderate wind conditions at a speed of approximately 8 km.h^{-1} using zig-zag (Lake Bourget survey) and parallel transect (Lake Annecy survey) designs.

Data were post-processed with the multifrequency tool in Sonar5-Pro software (Balk and Lindem 2014), which allowed to process the three frequencies synchronously. Sonar5-Pro considers the absorptions whose differences are low (Francois and Garrison 1982a,b; Lurton 2002). TS (f) thresholds were set to −60 dB to include juvenile fish at all frequencies (Yule et al. 2013). In temperate lakes in the autumn, juveniles reach sizes corresponding to this threshold at a 70 kHz frequency (equation 3 below, see Love 1971), which we considered as a reference for this study (Emmrich et al. 2012; CEN, 2014; Guillard et al. 2014). The threshold of the mean volume backscattering strength, S_v(f) (in dB re 1 m^{-1}, noted dB), was set 6 dB lower at −66 dB according to Parker-Stetter et al. (2009). Single Echo Detections (SED) were determined using the Sonar5-Pro software with the following settings: a pulse length ratio between 0.8 and 1.3, a maximum gain compensation of 3 dB (one way) and a sample angle standard deviation 0.3 degree (Godlewska et al. 2011; Guillard et al. 2014). The Elementary Distance Sampling Unit (EDSU) was set to 250 m (same as applied by Guillard et al. 2014) to extract the area backscattering coefficient, s_A(f) (MacLennan et al. 2002) and Target Strength (TS (f)) separately in each layer. TS(f) is the mean TS(f) of SED's for each EDSU by layer. Acoustic data (*i.e.*, s_A(f) and TS(f)) were used to calculate fish density(f), fish length(f), and biomass(f), common metrics used by fisheries managers and scientists, using equations 1–3, from the echo-integration method, the integral of backscattered sound energy scaled by mean TS(f) in the linear domain (S_v(f)/TS(f) scaling) (Balk and Lindem 2014). Although Love's equation is generalized and has been used for many years, it is still commonly used and relevant (i.e., Ye et al. 2013; Zenone et al. 2017; Morrissey-McCaffrey et al. 2018).(Love 1971; Rudstam et al. 2012). The fish biomass calculation is done with equation 2 (Carlander 1969).

$$\text{density (fish.ha}^{-1}) = \frac{s_A}{4\pi * 10^{TS/10}} \tag{1}$$

$$\begin{aligned}\text{biomass (kg.ha}^{-1}) &= \text{density} * \text{ mean weight}\\ &= \text{density} * (10^{-2} * \text{Total length}^3)\end{aligned} \tag{2}$$

$$\text{Total Length (cm)} = 10^{\frac{TS - 0.9 * \log_{10}(\text{frequency}) + 62}{19.1}} \tag{3}$$

The Sawada index (Sawada et al. 1993) was examined to ensure that conditions allowed for the *in situ* estimation of TS(f). Only EDSUs with a Sawada index below 0.1 were used in the analyses (Godlewska et al. 2011).

During data post-processing using the selected thresholds, noise in the form of gas bubbles, ghost echoes and electric noise from the echosounder were identically removed for each frequency using the cleaning tool of Sonar5-Pro. In a few areas, echograms at 200 kHz were still very noisy with selected thresholds. Noise was not visible at other frequencies, which confirms that it is not fishes. Since the aim of the study was to compare the responses of fish at different frequencies, EDSUs with too much noise were excluded from the analysis (Fig. 3). Noise subtraction areas of the echograms were not included in the analyses. The bottom was identically detected for all frequencies using the auto-detection tool in Sonar5-Pro, visually checked and manually corrected.

Statistical analysis

For mean TS(f), s_A(f), fish density(f) and biomass(f), metrics for each frequency were compared pairwise by using the Student's parametric t-test. Boxplots illustrate the results of these tests: when 38 or 200 kHz are significantly different from the reference frequency 70 kHz, one

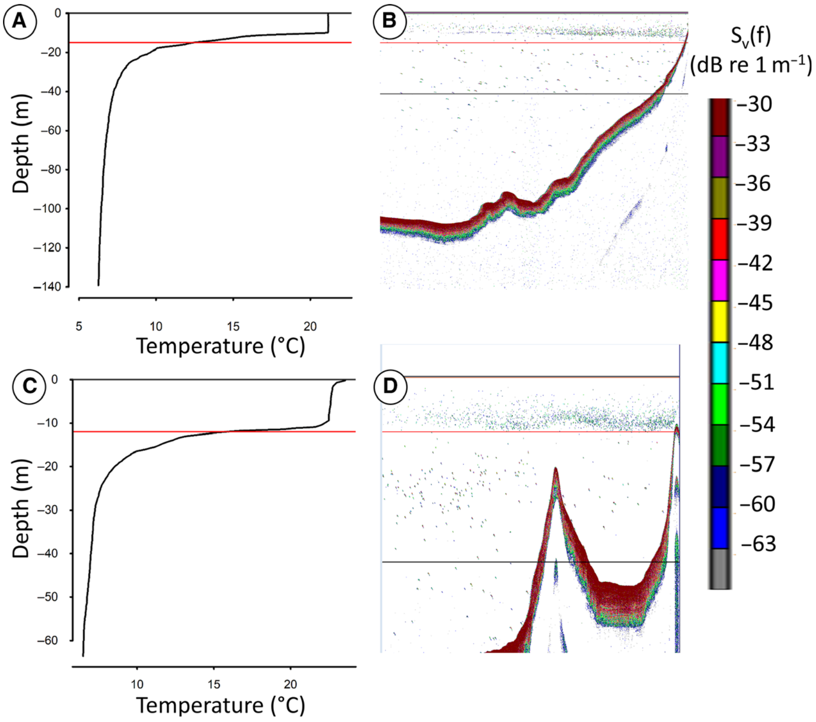

Figure 2. Temperature profiles (A and C) and echogram examples (B and D) in Lakes Bourget (A and B) and Annecy (C and D). The red line represents the limit between the upper and lower layers. The black line represents the lower limit of analyses.

star represents a *P*-value between 0.05 and 0.10 and two stars represent a *P*-value under 0.05. 70 kHz is considered the reference because it is the intermediate frequency and has been studied extensively.

TS(f) mean values were calculated in a linear domain. The major axis procedure can be used instead of linear regression when measurement error is unknown (Warton et al. 2006), which is the case for acoustic metrics. This method was used to compare pairs of s_A(f) and TS(f) from the same EDSU. Thus, the slopes of the major axes of these comparisons were compared with a 1:1 line. The statistic tests are used to evaluate the differences between two frequencies for each metric. Frequencies that are not significantly different are marked 'o' (results are identical), while significant differences are represented by '*' if the *P*-value is between 0.05 and 0.1 (significant) and '**' if the *P*-value is under 0.05 (highly significant). Unless otherwise specified, 0.05 is considered the significance threshold.

The results are presented starting with the lower layer, which corresponds to low densities and should present with more similarities between frequencies, according to previous studies, especially that of Guillard et al. (2014).

Results

Surveys on Lake Bourget recorded 310 EDSUs for the lower layer and 315 for the upper layer, as the depth became too shallow for some EDSUs. For the smaller Lake Annecy, 103 EDSUs were recorded for the lower layer and 112 for the upper layer. Some segments were deleted due to high Sawada index values (above 0.1) or due to the presence of too much noise (Table 1).

Comparisons of s_A(f) and TS(f) in lower layers

A Student's t-test showed that mean s_A(f) for the three frequencies in the lower layer with lower densities were not significantly different within the same lake. Figure 4 (upper panel) presents the boxplots, which allow for a visual comparison between median s_A(f) values obtained at the three different frequencies. Data were also compared by EDSU using the major axis procedure, a statistical test from Warton et al. (2006). In the lower layer of Lake Bourget, the major axis was not different from the 1:1 line for any pairwise comparisons. However, the results for Lake Annecy were significantly different for all frequencies (Fig. 4). All results are summarized in Table 3.

Concerning TS(f) based on SED, a Student's t-test showed that the means of TS(f) for each layer were non-significantly different for Lake Annecy. Mean TS(f) of frequencies 38 and 70 kHz on Lake Bourget were significantly different.

The statistical results from comparing the major axis and the 1:1 line showed no significant differences for the pairs 38–200 kHz and 70–200 kHz from Lake Bourget (Fig. 5); in contrast to the pair 30–70 kHz. For Lake Annecy, only the pair 38–200 kHz was not significantly different.

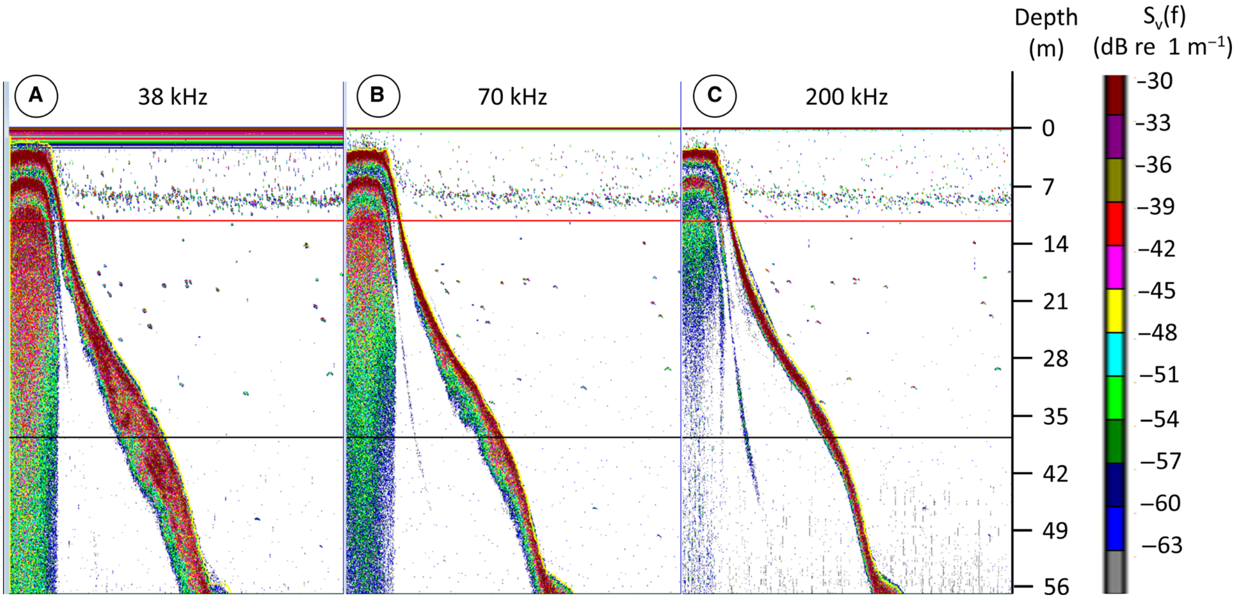

Figure 3. Echograms of the three frequencies (A: 38 kHz, B: 70 kHz, C: 200 kHz) in Lake Bourget. The depth scale represents the depth (in metres) and the coloured scale the mean volume backscattering strength ($S_v(f)$ in dB). The red line represents the thermocline and the black line represents the lower limit of analyses.

Fish density(f) and biomass(f) estimation in lower layers

Fish density(f) and biomass(f) were calculated from $s_A(f)$ and TS(f) using equations (1) and (2). A Student's t-test showed no significant differences between mean densities(f), except for pair 38–70 kHz in Lake Bourget (Fig. 6). For biomass(f), a Student's t-test showed a significant difference between most of the biomass(f) levels estimated by frequencies. Only the pairs 70–200 kHz from Lake Bourget and 38–70 kHz of Lake Annecy were not significantly different (Fig. 6). The means and standard errors of densities(f) and biomass(f) in the lower layers were also calculated (Table 2).

Comparison of $s_A(f)$ and TS(f) in upper layers

In the upper layer, a Student's t-test showed no differences in $s_A(f)$ between different frequencies for Lake Annecy. For Lake Bourget, only the pairwise comparison between 70 and 200 kHz was not significantly different.

When statistics of major axis from Warton et al. (2006) were applied, all major axes were significantly different from the 1:1 line (Fig. 7). All results are summarized in Table 3.

In both lakes, the mean TS(f) values were significantly different, except for the pair 70–200 kHz. Moreover, all major axes were different from the 1:1 line (Fig. 8).

Table 1. Recapitulation of analysed and non-analysed EDSUs (Elementary Distance Sampling Unit).

	Bourget EDSU [EDSU number (%)]		Annecy EDSU [EDSU numbers (%)]	
	Upper layer	Lower layer	Upper layer	Lower layer
Initial number of EDSU	315	310	112	103
Sawada index (% from initial number of EDSU)	21 (6.7%)	35 (11.3%)	3 (2.7%)	0 (0%)
Noise (% from EDSU number with Sawada index suppression)	5 (1.4%)	5 (1.6%)	21 (18.9%)	4 (3.6%)
Total of analysed EDSU	289	270	88	99

Fish density(f) and biomass(f) in upper layers

Mean densities(f) were not significantly different in both lakes (Student's t-test; Fig. 9).

Mean biomass(f) provided from all frequencies were not significantly different in Lake Annecy. In Lake

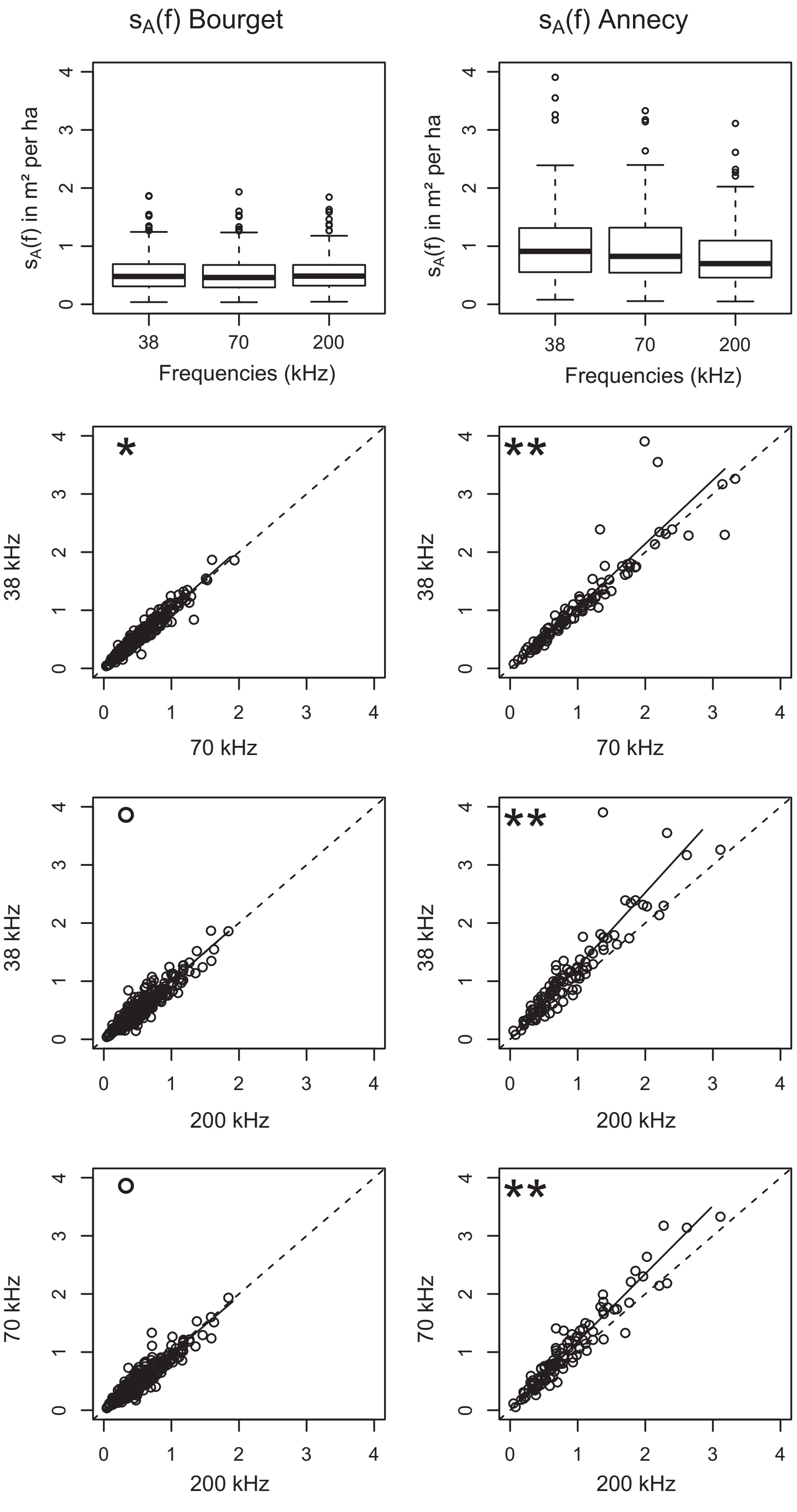

Figure 4. Comparisons of s_A(f) (in m².ha^{-1}) in lower layers of Lakes Bourget and Annecy. Boxplots show the median, first and third quartile for the central box. External lines represent data amplitude (the upper one is the maximum data or the sum of the third quartile and 1.5 times amplitude between first and third quartile). Other points are extreme values. 'o' indicates no statistical difference between results; one and two stars over the graphic indicate a significant difference at the 10% and 5% significance levels, respectively, between the major axis (in black) and the 1:1 line (dotted).

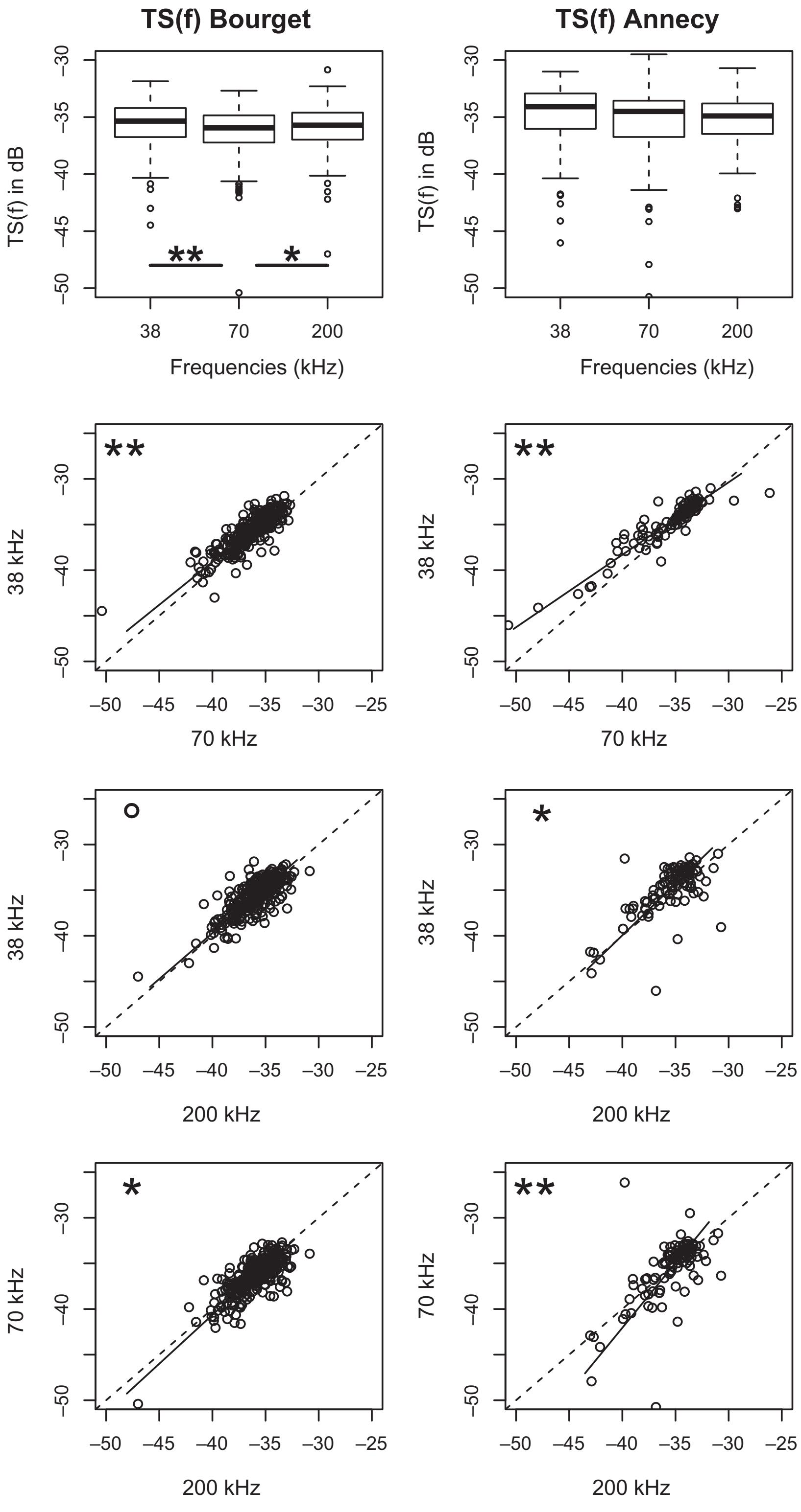

Figure 5. Comparisons of TS(f) (in dB) in lower layers of Lakes Bourget and Annecy. Boxplots show the median, first and third quartile for the central box. External lines represent data amplitude (the upper one is the maximum data or the sum of the third quartile and 1.5 times amplitude between first and third quartile). Other points are extreme values. 'o' indicates no difference; one and two stars over the graphic indicate a significant difference at the 10% and 5% significance levels, respectively, between the major axis (in black) and the 1:1 line (dotted).

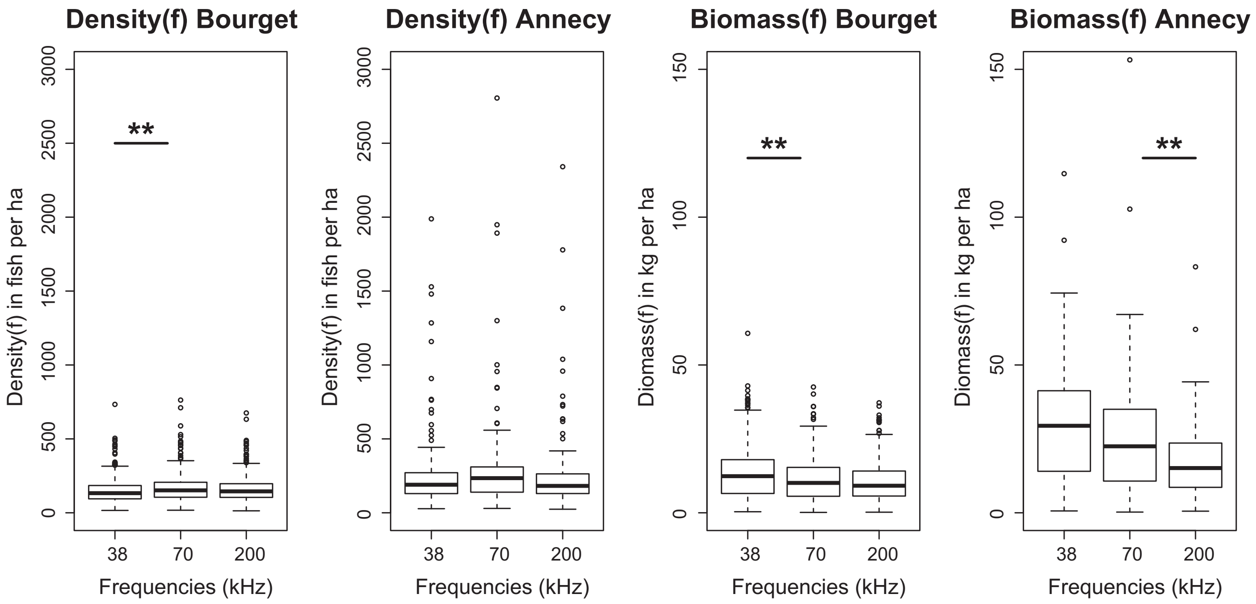

Figure 6. Comparisons of fish densities(f) (in fish.ha^{-1}) and biomass(f) (in kg.ha^{-1}) estimations in the lower layers in Lakes Bourget and Annecy. Boxplots show the median, first and third quartile for the central box. External lines represent data amplitude (the upper one is the maximum data or the sum of the third quartile and 1.5 times amplitude between first and third quartile). Other points are extreme values. Two stars indicate a significant difference at the 5% level.

Table 2. Means and standard errors of density(f) and biomass(f) in the layers of Lakes Bourget and Annecy.

		Lower layer (Mean ± SE)			Upper layer (Mean ± SE)		
Variable	Lake	38 kHz	70 kHz	200 kHz	38 kHz	70 kHz	200 kHz
Density(f) (fish.ha^{-1})	Bourget	152 ± 91	174 ± 107	165 ± 94	3978 ± 3389	4299 ± 3706	3729 ± 3219
	Annecy	290 ± 322	357 ± 492	277 ± 335	3773 ± 3778	4084 ± 4465	3229 ± 3296
Biomass(f) (kg.ha^{-1})	Bourget	13.9 ± 9.3	11.4 ± 7.6	10.7 ± 7.0	6.6 ± 5.1	3.7 ± 3.4	3.1 ± 3.1
	Annecy	31.8 ± 24.1	26.5 ± 22.0	18.2 ± 13.2	5.0 ± 5.0	3.5 ± 3.9	2.9 ± 3.4

Bourget, mean biomass(f) were significantly different for all frequencies except for 70 and 200 kHz (Fig. 9). Means and standard errors of all EDSUs in the upper layers are presented in Table 2.

Discussion

The main aim of this study was to extend the knowledge on the impact of the data acquisition frequency on $s_A(f)$, TS(f) and estimated fish density(f) and biomass(f) using hydroacoustics in freshwater. The frequency 38 kHz has not been included in previous freshwater frequency standardization studies (Guillard et al. 2014; Draštík et al. 2017). Therefore, in this study, the range of studied frequencies included the 38 kHz frequency that is commonly used in the Great Lakes of North America and in Scandinavian countries. The 38 kHz-7 degrees transducer weights approximately 40 kg and its use is normally reserved for large boats, with a suitable installation for the transducer. This study compared 38 kHz with 70 and 200 kHz, with the latter previously compared by Guillard et al. (2014). Therefore, with these new results (Table 3), all frequencies commonly used for studying fish population in freshwater have been compared (Guillard et al. 2014; Draštík et al. 2017).

Stanton et al. (2010) and Lavery et al. (2007) have shown that acoustic data depend on frequency. Nevertheless, for the goal of standardisation, acquired data *in situ* had to be analysed to know if results from surveys using different frequencies could be compared. Previous studies comparing the outputs of several frequencies in freshwater observed significant similarities between 70 and 120 kHz (Godlewska et al. 2009) and 129 kHz (Guillard et al. 2004) in regions with low fish densities, estimated to be

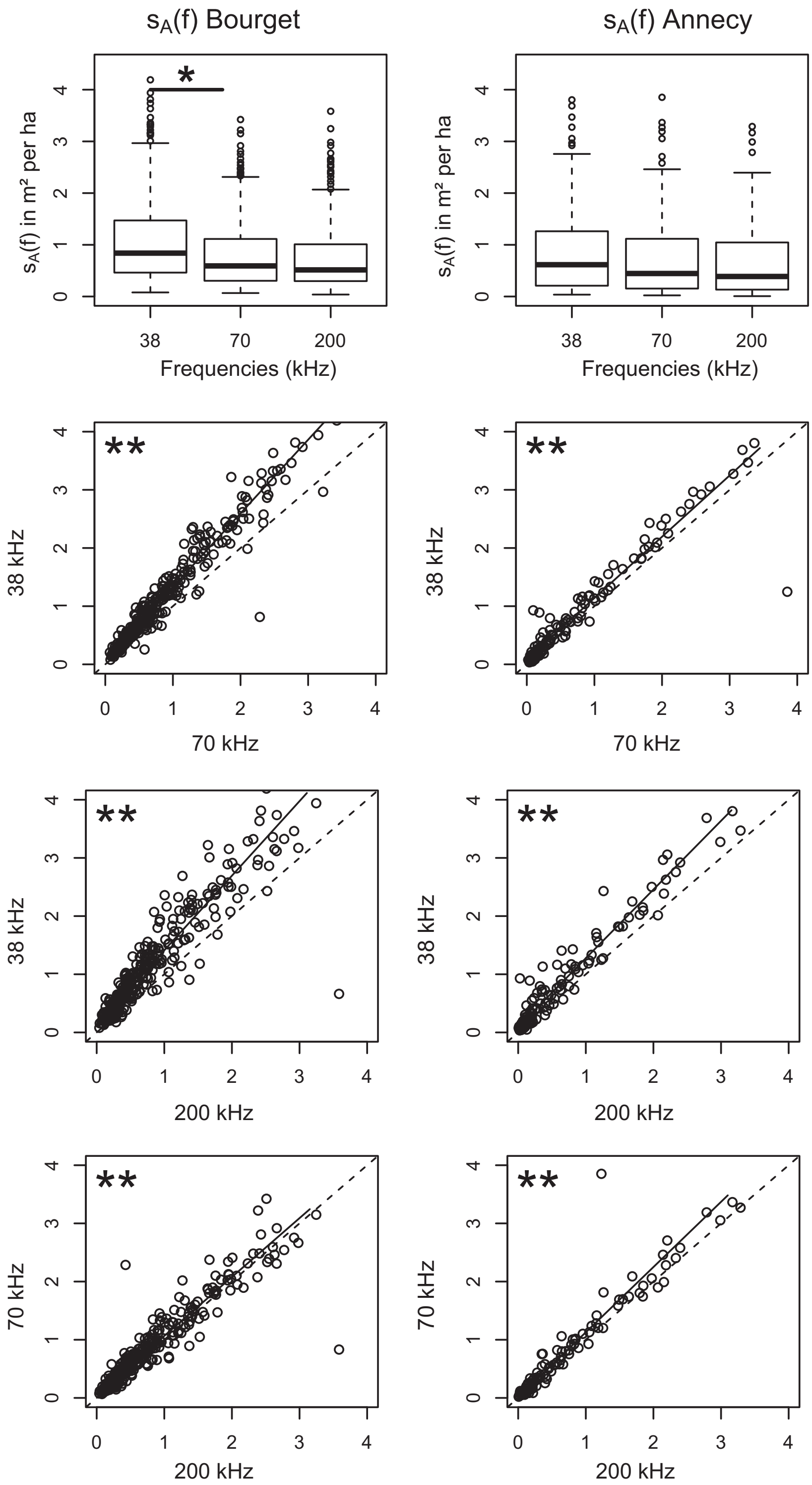

Figure 7. Comparisons of $s_A(f)$ (in m².ha^{-1}) in the upper layers of Lakes Bourget and Annecy. Boxplots show the median, first and third quartile for the central box. External lines represent data amplitude (the upper one is the maximum data or the sum of the third quartile and 1.5 times amplitude between first and third quartile). Other points are extreme values. Two stars over the graphic indicate a significant difference at the 5% significance level between the major axis (in black) and the 1:1 line (dotted). Significant differences are represented by "*" if the *P*-value is between 0.05 and 0.1 (significant) and "**" if the *P*-value is under 0.05 (highly significant)..

less than 600 fish per hectare for one layer (Guillard et al. 2014). Fish density(f) and biomass(f) are calculated using $s_A(f)$ and TS(f) but do not necessarily have the same statistical comportment: differences in $s_A(f)$ and TS(f) can counteract or exacerbate one another. The estimation of biomass(f) appears to decrease when frequency increases. This phenomenon is likely due to an erroneous estimation of total length, which can also decrease at higher

Table 3. Summary of all comparison tests performed in this study. A point indicates a non-significant difference, one and two stars represent differences at the thresholds of 10% and 5%, respectively.

			Lower layer			Upper layer		
Variable	Test	Lake	38–70 kHz	70–200 kHz	38–200 kHz	38–70 kHz	70–200 kHz	38–200 kHz
$s_A(f)$	Comparison of means	Bourget	o	o	o	**	o	**
		Annecy	o	o	*	o	o	o
	Comparison by EDSU pairs	Bourget	*	o	o	**	**	**
		Annecy	**	**	**	**	**	**
TS(f)	Comparison of means	Bourget	**	*	*	**	o	**
		Annecy	o	o	o	**	o	**
	Comparison by EDSU pairs	Bourget	**	*	o	**	**	**
		Annecy	**	**	*	**	**	**
Density(f)	Comparison of means	Bourget	**	o	o	*	*	*
		Annecy	o	o	o	*	*	*
Biomass(f)	Comparison of means	Bourget	**	o	**	**	o	**
		Annecy	o	**	**	o	o	o

The grey shadings highlights the different levels of significance. The darkest grey and "o" symbol indicate a non-significant difference, the light grey and "*" symbol indicate a difference at the threshold of 10%.

frequencies. Since fish biomass(f) should not change depending on frequency, at least one equation is not efficient enough for the full consideration of frequencies. One of the aims of this study was to establish a theoretical frequency comparison in practise. However, if the differences in acoustic metrics, density(f) or biomass(f) between frequencies were occasionally statistically significant and were small enough to be considered negligible for fish population management (Guillard et al. 2014). Our results were therefore analysed in the light of these previous studies. We first analysed 70–200 kHz, which were previously studied, and then we discussed results of comparing 38–70 kHz. We consider 70 kHz as the reference frequency because it is the intermediate frequency and has been studied extensively, and we did not study the pair 38–200 kHz to not be too redundant.

In regions of high fish densities, the frequency 200 kHz was highlighted as significantly different in most cases as compared to the other two frequencies, 38 and 70 kHz. Such high fish densities occur in eutrophic lakes or in the upper layers of alpine lakes, such as Lakes Bourget and Annecy, where seasonal young-of-the-year recruits can be numerous (Guillard et al. 2006b; Yule et al. 2013). This difference is highlighted by the major axis comparison and does not necessarily appear with a Student's t-test. However, the results in this study from comparing 70 and 200 kHz showed similar metrics (TS(f) and $s_A(f)$) at low fish densities. The only significant differences in 70–200 in the lower layer were in Lake Annecy in the case of the major axis comparison. As Guillard et al. (2014) found, our study highlights a more important similarity between 70 and 200 kHz in low fish densities than in high densities. However, for fish density(f) and biomass (f), the results are similar for low and high fish densities, which does not exactly concur with Guillard et al. (2014). The dissimilarity between these two studies highlights the need to improve the comparison, with longer surveys or experiments in natural and/or controlled conditions.

For the 38 kHz frequency versus 70 kHz, there were some significant differences for estimated densities(f) and biomass(f) in Lakes Bourget and Annecy. In the lower layer of Lake Bourget, where fish densities were low, the density(f) estimations differed between 70 and 38 kHz. However, from a fisheries management point of view, this difference could be acceptable: the mean estimation based on all EDSUs in the lower layer of Lake Bourget was 152 ± 91 fish per hectare for 38 kHz and 174 ± 107 for 70 kHz. This difference is on average <13% (38 compared to 70 kHz) with a large standard error due to the high variability in the mean from EDSUs.

The results of our study confirmed earlier findings that fish densities(f) appear to influence the estimates of different frequencies. Indeed, in the lower layers, where fish density was low, all the frequencies used provided almost similar results. However, in the upper layers, where density was high, and sometimes higher than the threshold of 600 fish per hectare proposed by Guillard et al. (2014), results from the different frequencies were more variable, with a majority of cases being significantly different. However, we did not find any relationship between the density(f) estimation and the difference between frequencies. Only complementary experiments in natural and/or controlled environments or in natural environments with a large range of densities and a sufficiently large number of repetitions could more thoroughly explore the impacts of fish density

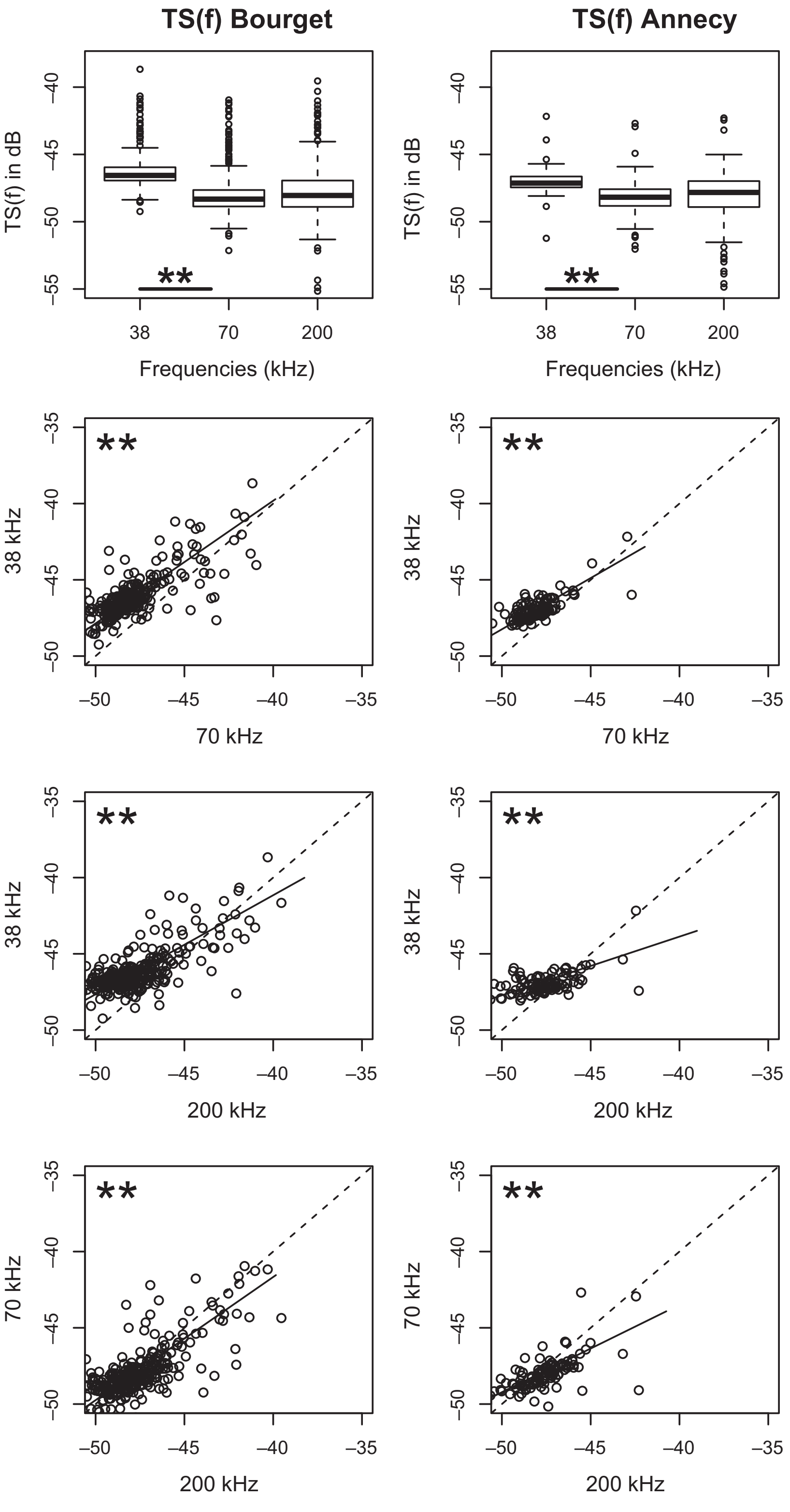

Figure 8. Mean TS(f) (in dB) comparisons in the upper layers of Lakes Bourget and Annecy. Boxplots show the median, first and third quartile for the central box. External lines represent data amplitude (the upper one is the maximum data or the sum of the third quartile and 1.5 times amplitude between first and third quartile). Other points are extreme values. Two stars over the graphic indicates a significant difference at a 5% significance level between the major axis (in black) and the 1:1 line (dotted).

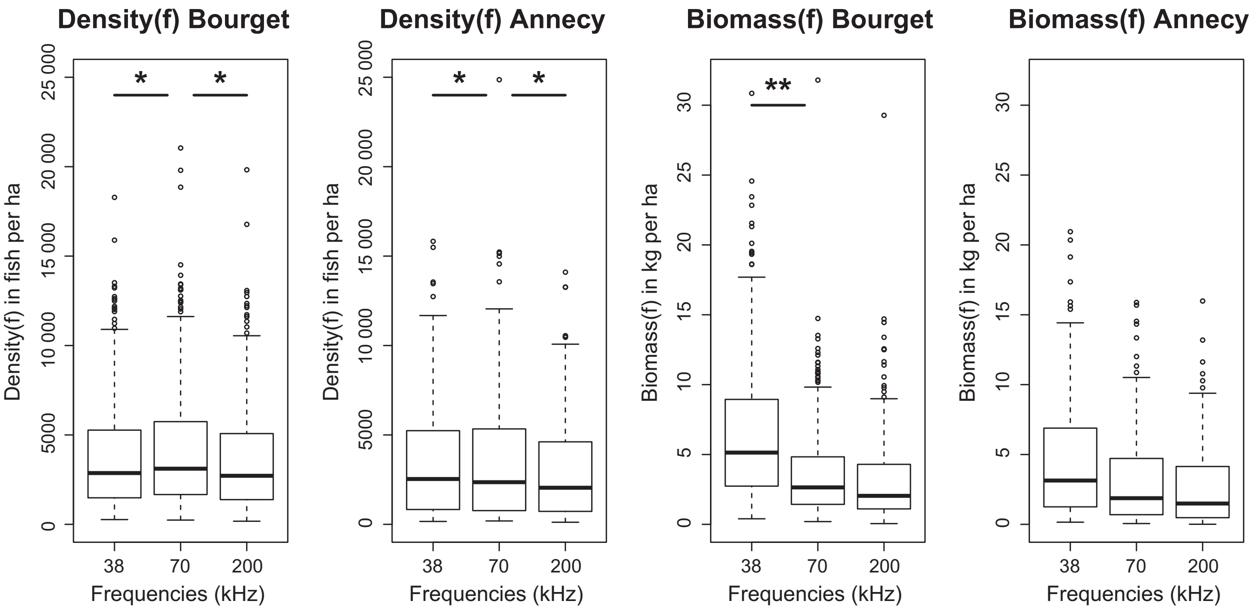

Figure 9. Comparisons of fish density(f) (in fish.ha^{-1}) and biomass(f) (in kg.ha^{-1}) estimations in the upper layers of Lakes Bourget and Annecy. Boxplots show the median, first and third quartile for the central box. External lines represent data amplitude (the upper one is the maximum data or the sum of the third quartile and 1.5 times amplitude between first and third quartile). Other points are extreme values. Two stars indicate a significant difference at the 5% level and one star indicates a difference at the 10% level.

on the measured differences between estimates at various frequencies. Other studies with data recorded at different frequencies allowed for other *in situ* inter-comparisons and so, could improve the use of hydroacoustics and the link with acoustic theory. More data could also highlight systematic biases in comparison of different frequencies.

In the upper and lower layers, fish populations are different, with different swim morphology and swim bladders and consequently, different acoustic properties influencing on the reflection. Moreover, since smaller fish in the upper layer are closer to the surface, they are also closer to the boat, which can lead to avoidance reactions. These behaviours can influence acoustic records: as if they swim downwards, the echo will be more directive, which can accentuate the difference between frequencies. However, this is not the only explanation, and other hypotheses could possibly explain the more important differences between frequencies in upper layers. First, even if the transducers are close to each other, they do not sample exactly the same volume. Therefore, each transducer does not record the exact same targets at close distances. Moreover, not only is the shared volume less in the upper layers than in the lower layers; however, the total sampling volumes are also different. This could explain why measurements are increasingly different in the upper layer. Second, fish populations are different in upper and lower layers. In upper layers, fish are smaller, with less directivity compared to larger fishes in the lower layer (MacLennan and Simmonds 1992). Moreover, deeper fish will be registered by a higher number of consecutive pings, and thus present a smoother measure. In addition, at low densities, the relative variation is higher and certain tests can present more difficulties in highlighting significant differences. Finally, individual TS(f) can vary more than 10 dB, even for the same individual fish (i.e. within one track) (Dawson and Karp 1990; Godlewska et al. 2004). As highlighted by Ona (1999), it is important to work using mean measures, for the better smoothing of variabilities.

Another thing that may influence on the differences between frequencies is the nature of the applied transducers. The 38 kHz transducer was a ton-pilz transducer with much higher Q-factor than the other transducers being built by composite materials. The Q-factor is linked to the transducers resonance properties. A strongly resonant system reacts different to an impulse such as an echo from a fish than a low resonant system does. This will cause different shape of the resulting echoes witch again will influence on the echo length used as one of the criteria in the single echo detector. The 38 kHz transducer is built differently than other transducers; therefore, individual targets could be seen to be not exactly identical with transducers of different nature. This effect could cause some differences in acoustic data. A thorough study of the effect of the coupling between the transducers Q-factor and the resulting echo length from various targets is needed to answer this question.

Conclusion

It is of great importance to know whether acoustic metrics, which are used to estimate fish densities in lakes, can be compared. Sample units must be large enough to smoothen environmental variability. It is also necessary to consider fish density, which could influence estimates and cause differences between frequencies (Guillard et al. 2014). However, our results showed that estimates from surveys conducted with 38 kHz frequency results could be compared with results obtained with 70 kHz for density (f) and biomass(f) estimations, which are the main metrics used by scientists and fisheries managers. The results from the comparison of 70–200 kHz, which were slightly different from Guillard et al. (2014), highlight the need to continue *ex situ* and *in situ* comparisons to improve the reliability of this non-intrusive method. However, considering mean and standard error, the results from these two frequencies might also be considered as similar for density(f) and biomass(f) estimations, depending on study aims and required precision.

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