Freshwater Biology (2008) **53,** 912–923 doi:10.1111/j.1365-2427.2007.01944.x

Echo-sounding can discriminate between fish and macroinvertebrates in fresh water

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SUMMARY

1. Acoustic scattering from fish and macroinvertebrates was studied in a boreal Finnish lake at three echosounder frequencies (38, 120 and 200 kHz). Split-beam transducers with partly overlapping 7° beams were employed. Acoustic, fish and invertebrate sampling were undertaken simultaneously. Vertical gradients of temperature and oxygen concentration were measured during the exercise.

2. At all frequencies, a narrow scattering layer coincided with the thermocline. At 38 kHz, fish were detected well with practically no reverberation from invertebrates while 200 kHz detected both fish and invertebrates.

3. Minor differences in the magnitude of acoustic scattering from fish were found between frequencies and between depth layers, but scattering at different frequencies was correlated at all depths. Acoustic scattering and fish density indices from trawl catches, consisting mostly of smelt (*Osmerus eperlanus*) (97%) and vendace (*Coregonus albula*) (3%), were significantly correlated.

4. Acoustic scattering from invertebrates increased with sound frequency. Correlation analysis suggested that the invertebrate scattering was mostly induced by *Chaoborus flavicans*. A low frequency is recommended for estimating fish abundance without bias from reverberation induced by invertebrate scattering. Although fish and invertebrates can also be successfully discriminated at a single frequency by thresholding and cross filtering, the combination of a low and a high frequency is a more robust tool for effective fish-invertebrate discrimination.

Keywords: *Chaoborus flavicans*, multi-frequency, smelt, trawl, vendace

Introduction

Echosounding is a remote sensing technique with many applications in hydrobiology. It enables rapid sampling of almost the entire water column over large areas and analyses over a wide size range of aquatic organisms. In the hydroacoustic system, an echosounder transmits a pulsed sound beam into the water and subsequently detects and analyses the backscattered echoes. The size of fish can be estimated in decibels from their target strength (TS, dB relative to an area of 1 m^2), which is related to the length and

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species of fish, and their biomass per unit area from the Nautical area scattering coefficient (NASC, m^2 nmi^{-2}) (e.g. MacLennan, Fernandes & Dalen, 2002; Lilja *et al.*, 2004). Hydroacoustic systems are usually deployed from moving vessels, although stationary installations may also be used.

Hydroacoustics has become a well-established method for assessing the size of fish stocks and the sizes of individual fish both in the sea and in fresh water. However, species separation is a major challenge in acoustic measurements and scattering from unwanted targets can bias fish stock estimates. In freshwater lakes, several studies have focused on the influence of larger aquatic invertebrates, such as *Chaoborus* larvae, on acoustic backscattering and on methods aiming at discrimination between fish and aquatic invertebrates (Eckmann, 1998; Malinen, Horppila & Liljendahl-Nurminen, 2001; Malinen, Tuomaala & Peltonen, 2005; Knudsen, Larsson & Jakobsen, 2006). Although scattering from non-fish targets is often considered undesirable, the ability to detect organisms over a wide size spectrum can be used to study ecosystem structure.

The acoustic information obtained from mixed aggregations is sometimes discarded because of an inability to identify properly the fish species or to distinguish between non-fish and fish scatterers. Multi-frequency acoustics could enhance the separation and identification of acoustic targets. In this field, the current trend is to increase the amount of information collected through an increase in frequency bandwidth within a transducer or in the number of transducers with different discrete frequencies (Horne, 2000).

Multi-frequency methods for identifying species have been used in the marine environment to study zooplankton since the late 1970s (Greenlaw, 1979; Holliday & Pieper, 1980) but, as technology has advanced, it has become possible to improve target identification and separation of both fish and plankton based on multi-frequency acoustics. A comprehensive overview can be found in Fernandes *et al.* (2006). Madureira, Everson & Murphy (1993) differentiated between Antarctic krill *Euphausia superba* Dana and other scatterers using 38 and 120 kHz transducers. Simard & Lavoie (1999) separated fish and krill echoes using the difference in backscattering strength at two frequencies (38 and 120 kHz). Korneliussen & Ona (2002) combined data from two or more discrete echo sounder frequencies (18, 38, 120 and 200 kHz) to separate the acoustic scattering from zooplankton and fish in mixed recordings in the ocean. The difference in signal strength at 38 and 120 kHz has been used by Everson, Tarling & Bergström (2007) to distinguish northern krill [*Meganyctiphanes norvegica* (Sars)] from other acoustic scatterers. McKelvey & Wilson (2006) separated fish and euphausiids in scattering layers off the west coast of North America based on recordings from 38 to 120 kHz echo sounders.

In fresh water, only a few studies have been conducted on fish and invertebrates using multi-frequency acoustics, even though the problems are basically the same as in the ocean. Trevorrow & Tanaka (1997) showed that the scattering from the freshwater amphipod *Jesogammarus annandalei* (Tattersall) at 88, 118 and 198 kHz was frequency dependent and could be used to quantify the type and size of the scattering target. Knudsen *et al.* (2006) established the scattering response of larvae of the phantom midge [*Chaoborus flavicans* (Meigen)] and found that it resonated at 200 kHz but it was a very weak scatterer at 38 kHz. Eliminating *Chaoborus* as a bias in acoustic fish estimates could therefore be obtained using a low echosounder frequency.

In this paper, we describe a comprehensive study combining multi-frequency echosounding with pelagic fish and invertebrate sampling in a boreal lake. The aim was to test whether a multi-frequency approach can successfully discriminate between fish and invertebrate acoustic scatterers.

Methods

Study area

Lake Paasivesi (62°10′N, 29°25′E) is a 110 km^2, oligotrophic, mesohumic (water colour 40–50 mg Pt L^{-1}) and deep (mean depth 21 and maximum 75 m) meteorite impact lake in Finland (Fig. 1) (Pesonen *et al.*, 1999). It is deepest in the middle and the lake is open and almost without islands. Temperature stratification of the water column is typically evident from the beginning of June to the end of September, and mean residence time is about 3 months. Smelt [*Osmerus eperlanus* (Linnaeus)], vendace [*Coregonus albula* (Linnaeus)], European whitefish [*C. lavaretus* (Linnaeus)] and perch (*Perca fluviatilis* Linnaeus) are the most common species in the pelagic area of L. Paasivesi (e.g. Jurvelius *et al.*, 2005).

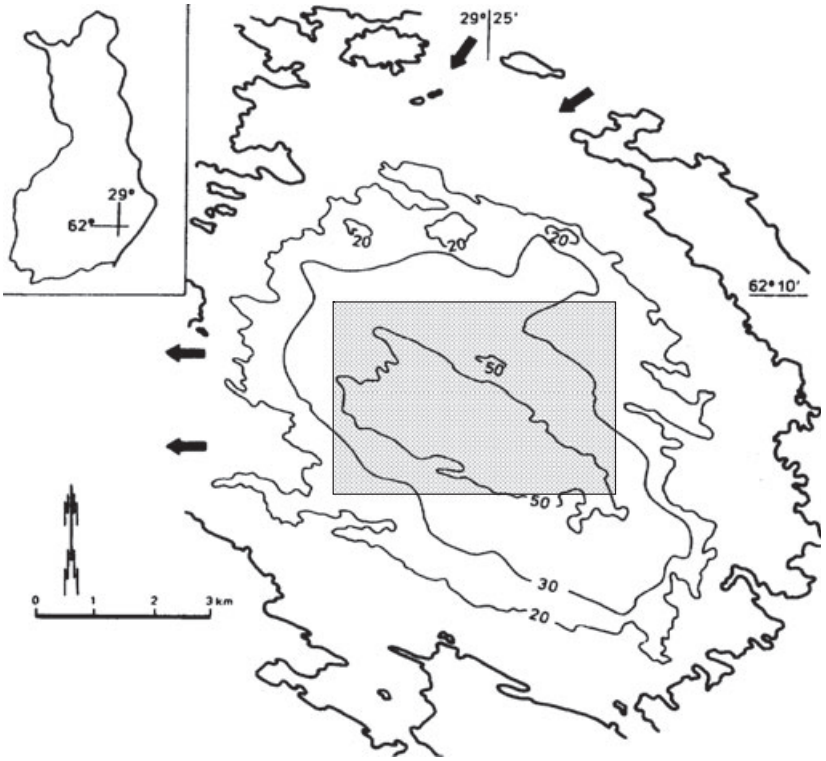

Fig. 1 Map of Finland and a bathymetric map showing depth contours (m) of L. Paasivesi. Study area where echosounding, trawling of fish, sampling of invertebrates and temperature and oxygen profiling took place, is dotted on the map. The thick arrows show the main direction of the water current, and the bar on the lower left corner shows the scale of the map.

Fish sampling

Fish in the echo-surveyed area were sampled by trawling using a 28 m long research vessel (Muikku). The trawl was 13.5 m wide and 15 m high and had a three-storey structure, the height of each storey being 5 m. The cod-end mesh size was 5 mm from knot to knot. Three hauls were taken in the middle of the lake on 11 October 2005 (Table 1). The hauling speed was 1 m s^{-1}, and the durations of hauls were 42, 44 and 28 min respectively. The trawl depth was regulated by altering the length of the ropes from each otterboard to the buoys at the surface. The hauling depth was checked with an echosounder.

In each trawl-haul, the catch (kg) was sorted to species separately from every cod-end. The number of specimens was counted in catches <1 kg, while in larger catches a sub-sample of *c.* 0.5 kg was taken. The length distributions of smelt and vendace were recorded in 5 mm length classes separately for every cod-end. The mean mass of smelt in every cod-end was estimated by dividing the weight of a smelt sub-sample by the number of specimens. This mass was used to estimate the total number of smelt specimens caught. The mass of individual vendace was measured from sub-samples. The catch and yield per swept area [numbers per nautical mile (nmi^{-1}), kg nmi^{-1}], as indices of fish density, were estimated for every cod-end using the catch, the duration and speed of the haul, and the dimensions of the trawl.

Invertebrate sampling

To assess the density of invertebrates, samples were collected using a Multi Plankton Sampler MultiNet® Type Midi (Hydro-Bios GmbH, Germany) with 500 μm mesh size net bags. This mesh size gives an unbiased sample only of macroinvertebrates, such as *C. flavicans*, *Mysis relicta* Lovén and Amphipoda, whose length is typically from several mm to a few cm. Smaller (<1 mm–*c.* 2 mm) planktonic invertebrates (Copepoda, Cladocera, Rotatoria), were also found in the samples. The densities of Copepoda and Cladocera were estimated. Those densities should be considered only minimum estimates of their true density, but the relative differences in their densities between different depths are considered representative.

The sampler has a 50 × 50 cm (0.25 m^2) opening frame and five 2.5 m long nets. The nets can be opened and closed during operation using a personal computer (PC) connected to the sampler with a cable. The

Table 1 Fish catch and catch per swept nmi^2 (numbers and kg) in the trawl hauls and the corresponding mean of Nautical area scattering coefficient (NASC) at 38 kHz in L. Paasivesi on 11 October 2005

			Smelt		Vendace		Other		Catch nmi^{-2}			
										(kg)		
Haul	Start time (hours)	Depth layer (m)	Specimen	kg	Specimen	kg	Specimen	kg	Specimens (1000s)	All Smelt +	vendace	NASC (m^2 nmi^{-2})
3	20:12	0–5	973	4.29	1	0.02	1	0.01	65	292	286	
		5–10	345	1.49	1	0.02	0	0	23	99	99	19.2
		10–15	616	2.84	4	0.11	3	0.86	41	253	196	15.3
1	13:41	10–15	2666	11.11	4	0.01	0	0	239	995	995	69.8
		15–20	459	2.32	0	0	2	1.22	41	317	208	2.6
		20–25	529	3.44	20	0.67	2	0.35	49	399	399	14.3
2	17:02	20–25	591	3.79	56	1.70	2	0.30	61	541	541	15.3
		25–30	407	2.89	118	3.10	1	0.12	49	571	571	54.9
		30–35	368	2.95	16	0.15	4	0.29	36	318	318	25.6
%			97	80	3	13	<1	7				

'Other' consists of pike-perch [*Sander lucioperca* (Linnaeus)], whitefish, burbot [*Lota lota* (Linnaeus)] and lamprey [*Lampreta fluviatilis* (Linnaeus)]. Sunrise took place at 07:35 hours and sunset 18:03 hours.

sampler has an integrated pressure sensor to collect data on the operation depth (accuracy ± 10 cm). In addition, the sampler is equipped with two electronic flow metres, one inside and another outside the frame, to collect data on the volume of water passed through the net bags during operation. Pressure and flow information is available online in the PC on the deck of the research vessel. This enables the desired depth and speed for each net bag to be chosen during the haul.

Samples were collected on 11 October 2005. The first sampling was performed between 13:30 and 13:45 hours from depths of 40, 35, 30 and 25 m and the second between 15:50 and 16:05 hours from depths of 20, 15, 10, 5 and 1 m, in that order. The speed of horizontal hauling was 1.0 m s^{-1} while the length and duration of each haul varied from 118 to 180 m, and from 1 min 58 s to 3 min respectively. Consequently, the volume of water sampled in the haul varied from 31 m^3 to 49 m^3. Invertebrates were stored in 96% ethanol and identified, counted and measured for length in the laboratory. Inverted and stereo microscopes were used for counting.

Temperature and oxygen profiles

Vertical gradients of temperature (°C) and oxygen content (mg L^{-1}) were measured with a CTD meter (SBE 19 Seacat Profiler; Sea-Bird Electronics Inc., Bellevue, WA, U.S.A. Licor LI-193SA Spherical Quantum Sensor, Li-Cor Biosciences, Lincoln, NE, U.S.A.) at two sites in the study area during the exercise.

Echo sounding

In total, five hydroacoustic data sets were collected simultaneously with exploratory trawl hauls (Table 1) and invertebrate sampling on 11 October 2005. Three Simrad EK 60 echosounders with frequencies of 38, 120 and 200 kHz were used. The operating software was ER60 version 2.1.2. It synchronized the pinging and recorded the data in Simrad's raw format. Transducers were spherical split-beams with 7° beam angles. The transducers were mounted onto a bracket in accordance with recent recommendations for collecting acoustic multi-frequency data (Korneliussen & Ona, 2002) and installed at 1.5 m water depth at the side of the ship. All depth data are given relative to lake surface, not to transducer depth. The acoustic beams were overlapping and pointed straight down.

The echosounders were operated simultaneously; pulse duration was 512 μs, pulse interval 0.3 s and transmission power 200 W at all frequencies. Ship speed was 1 ms^{-1}. Prior to the survey, the vessel was anchored in a sheltered bay and the echosounders were calibrated according to recommended methods (Foote, 1982; Foote *et al.*, 1987) using standard targets for the particular frequencies.

Processing of acoustic data

The terminology of MacLennan *et al.* (2002) is followed in this paper. Thus, s_v = volume backscattering coeffi-

cient (m^{-1}), $S_v = 10 \log_{10}(s_v)$ = (mean) volume backscattering strength (dB re 1 m^{-1}), NASC = Nautical area scattering coefficient (m^2 nmi^{-2}) and TS = target strength (dB re 1 m^2) are used. SONAR6-MF post-processing software (Balk & Lindem, 2006) was applied in the data analysis. Each echogram was divided into 10 horizontal segments. Fish and invertebrates were separated as described below and NASC (m^2 nmi^{-2}) for both groups were measured.

When extracting the data for the fish frequency response analysis, the diffuse and weak scattering was removed by thresholding (S_v threshold = −70 dB re 1 m^{-1}). When collecting data on invertebrate echoes, Sonar6-MF's Target Noise Separator (TNS) was set up to mask for targets other than invertebrates. The TNS consists of a series of image analysing operators (Niblack, 1986). We set it up to work on time varied gain 20 log*R*, 38 kHz echograms. A 7 × 7 (number of samples in the range domain × the number of samples in the ping domain) median filter followed by a constant threshold of −88 dB, and a 3 × 3 growing operator was found to form a mask that separated invertebrates from fish echoes. The mask was applied to remove unwanted targets in all frequencies.

Time varied gain 20 log*R* was selected to give a constant threshold with the ability to remove fish and fish schools equally effectively at all ranges. The data from a 38 kHz transducer were applied as source for the mask, because this frequency clearly had the best fish to invertebrate response. The median operator was applied to amplify the weak part of the fish traces. This operator gave a better result than others, such as the dilation operator, especially for the ringing effects seen underneath some fish traces in the 38 kHz echograms. Ringing is an effect where fish continues to reradiate sound after the sound pulse has passed because of oscillation phenomena. The constant threshold was found by trimming until fish and invertebrates were sufficiently separated. The growing operator was applied to widen the mask and thereby reduce the risk of including echoes with fish trace energy in the invertebrate analysis.

Statistical analysis

Frequency response analysis Repeated measures ANOVA was applied to reveal the statistical significance of the differences in NASC at different frequencies. When analysing the fish detections, the data collected simultaneously with trawling were used. The three transects were divided into 10 horizontal segments (see above) and 5 m high vertical layers that corresponded with the sampling depth of different trawl cod-ends. When analysing the invertebrate detections, the data collected simultaneously with invertebrate sampling were divided into 10 horizontal segments (one tenth of transect length) and 5 m high vertical layers from 5 to 40 m.

The segment length was selected with respect to the fish and invertebrate density to maintain a stable average NASC within each segment and at the same time reveal spatial variations in the frequency response. For the fish transects, each segment covered on average 210 m sailed distance, lasted for 3.5 min and contained 693 pings. For the invertebrate recordings each segment covered 90 m, 1.5 min and 297 pings. These segment/depth data cells were used in repeated measures ANOVA as the subject for measurement, sound frequency (38, 120 and 200 kHz) as within-subject factor and depth as between-subject factor. Polynomic contrasting was applied in pairwise comparisons between frequencies and Tukey's test between depths.

Before analysis, NASC observations were log-transformed to normalize the within-cell distributions and homogenize variances. The measured acoustic scattering of the segments in certain individual transects and depth layer was assumed to form a random sample of independent observations because the observations for adjacent segments were typically not strongly positively auto-correlated (average linear auto-correlation −0.08 for fish echo analysis and 0.12 for invertebrate echo analysis).

Comparison of acoustic data with fish and invertebrate samples The abundance index of fish density, the catch and yield per swept area (specimens nmi^{-1}, kg nmi^{-1}) from trawls was compared using regression analysis with the NASC from fish detections at 38 kHz from the 5 m depth layer of acoustic data matching the time, location and depth layer of trawls.

Linear correlation was applied to analyse the association between invertebrate abundance data from the Multi Plankton Sampler, and NASC from invertebrate detections at 200 kHz matching the location and depth (2 m high layers) of the abundance estimate.

Results

The clearly distinguishable objects (red in Fig. 2), that can be interpreted as fish, were visible at every frequency. With an increase in echo-sounding frequency there was an increase in diffuse and weak (blue) scattering. At all frequencies, a narrow scattering layer was detected between 24 and 26 m depending on location. Temperature and oxygen profiles showed that a thermocline was located at a depth of 24–25 m, together with a peak in oxygen concentration (Fig. 3).

Acoustic scattering from fish showed clear, yet minor, frequency dependence. The NASC differed significantly at all three frequencies (all $P < 0.05$), the scattering at 38 kHz being somewhat higher than at 120 and 200 kHz (Fig. 4). Significant differences in scattering were also found between depth layers. The NASC in haul 1 at 15–20 m was significantly lower than in all other layers (all $P < 0.05$) and strongest scattering levels were detected at 10–15 m (haul 1) and 25–30 m (haul 2). The NASC values for different frequencies in different 5 m high layers correlated highly significantly (all $r > 0.98$, $P < 0.001$).

Almost all (97%) of the trawl catch (specimens) consisted of smelt, 3% of vendace and <1% of other species (Table 1). The mean size of smelt increased with depth (Spearman $r = 0.93$, two-tailed $P < 0.001$) (Table 2). The NASCs of different layers correlated positively with their trawl catch per swept area (CSA) (Pearson's r, all one-tailed $P < 0.05$), most significantly at 38 kHz (Fig. 5). The CSA measured in kg correlated more strongly with NASC than CSA in numbers (Fig. 5) as the catch weight takes into account not only the effect of fish number but also their average size on scattering.

The invertebrate scattering (scattering remaining after removal of fish tracks from the echograms), increased with frequency, differences being significant between all three frequencies (all $P < 0.001$). The scattering was weakest at 38 kHz and strongest at 200 kHz (Fig. 6). Significant differences were also detected between depth layers, the layer 25–30 m generally yielding the strongest scattering values, except at 38 kHz where scattering was strongest at 5–10 m because of some transducer ringdown energy left on the 38 kHz below 5 m at low threshold.

The invertebrate scattering matching the water columns of the different invertebrate samples was highest at 25 and 30 m depths (Table 3). The NASC at 200 kHz correlated most strongly with the density of *C. flavicans* but also significantly with *M. relicta*, Amphipoda and Copepoda (Table 4). However, after removal of the effect of *Chaoborus* with partial corre-

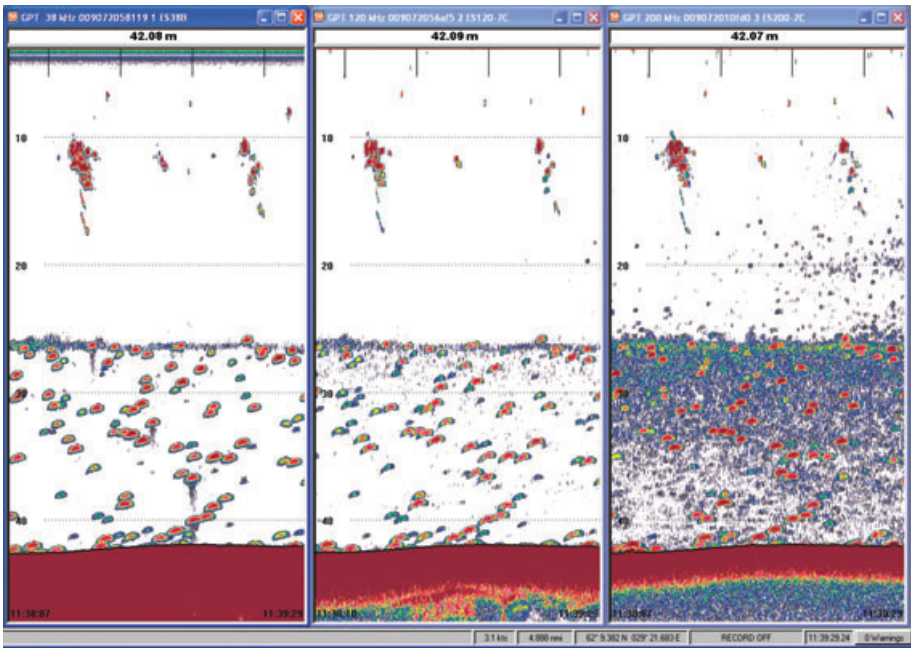

Fig. 2 Examples of echogram recordings at 38, 120 and 200 kHz in L. Paasivesi on 11 October 2005 at noon. S_v threshold −80 dB re 1 m^{-1}.

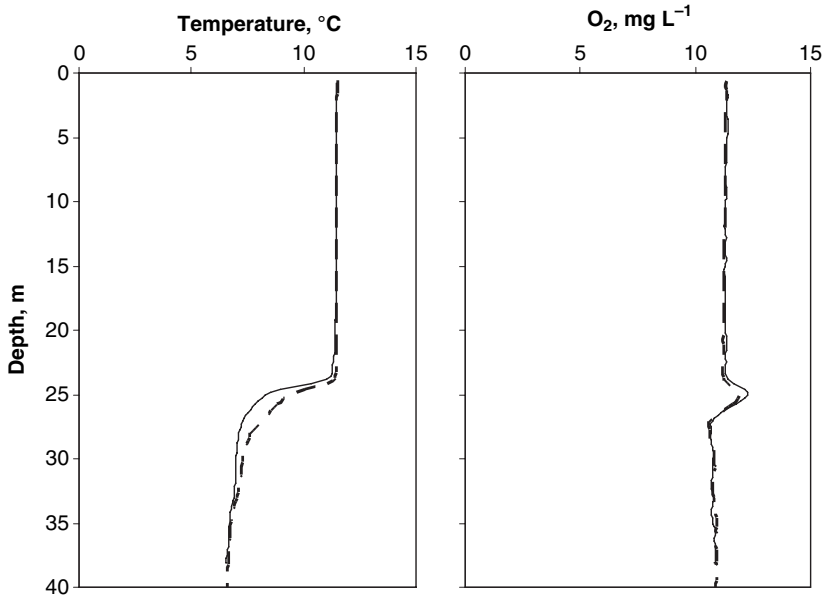

Fig. 3 Temperature and O_2 profiles at two sites in the study area in L. Paasivesi on 11 October 2005.

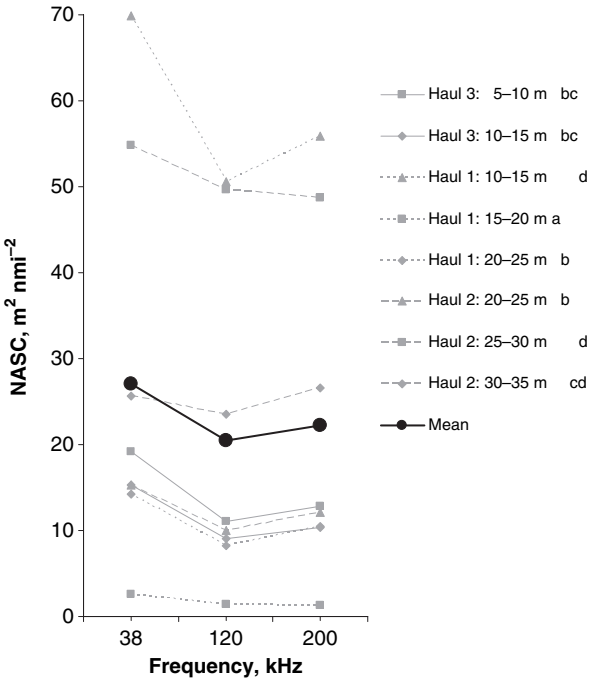

Fig. 4 The frequency response of the Nautical area scattering coefficient (NASC) from fish (S_v threshold = −70 dB re 1 m^{-1}) in different depth layers in L. Paasivesi on 11 October 2005. Letters a–d indicate homogenous subsets according to ANOVA.

lation, none of the other correlations remained significant, suggesting that most of the scattering was caused by *Chaoborus*. The invertebrate scattering at 38 and 120 kHz was found too weak to provide additional information on species-specific scattering.

Table 2 Geometric mean length of smelt and vendace in the exploratory trawling in L. Paasivesi on 11 October 2005

	Length (cm)		
Depth layer (m)	Smelt	Vendace	Both
0–5	9.3	–	9.3
5–10	8.1	–	8.2
10–15	9.6	15.0	9.6
10–15	9.0	12.1	9.0
15–20	10.1	–	10.1
20–25	10.8	15.7	11.0
20–25	10.7	15.7	11.2
25–30	11.1	15.3	12.1
30–35	11.5	15.1	11.7

Discussion

The difference in acoustic scattering of fish between frequencies was low. This is consistent with results from other freshwater species (Trevorrow, 1996; Rudstam *et al.*, 2003; Guillard, Lebourges-Dhassy & Brehmer, 2004) and suggests that all fish targets behaved as geometrical scatterers (where target strength is stable with frequency) for frequencies and water depths investigated. However, backscattering at 38 kHz was somewhat higher than at the two other frequencies. This was probably caused by the reduced sound directivity of fish with decreasing echosounder frequency (Foote, 1985), increasing total backscattering. Similar results have been reported for several marine fish species (Korneliussen & Ona, 2002,

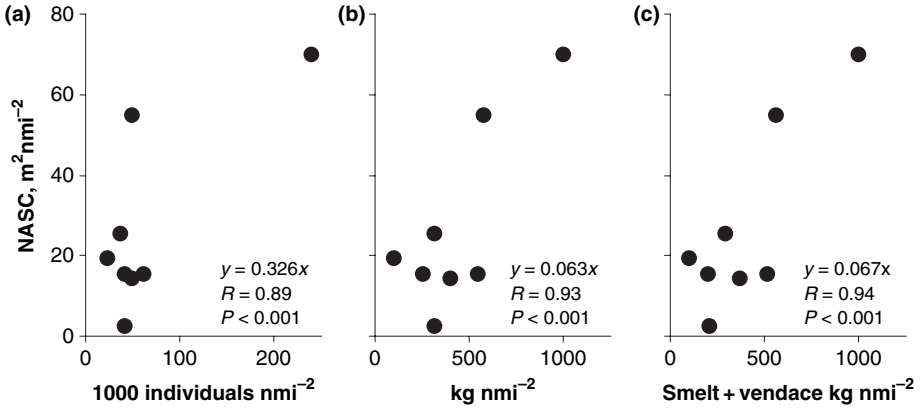

Fig. 5 The regression $y = ax$ of trawl catch (individuals and kg) per swept nmi^2 on NASC at 38 kHz in L. Paasivesi on 11 October 2005.

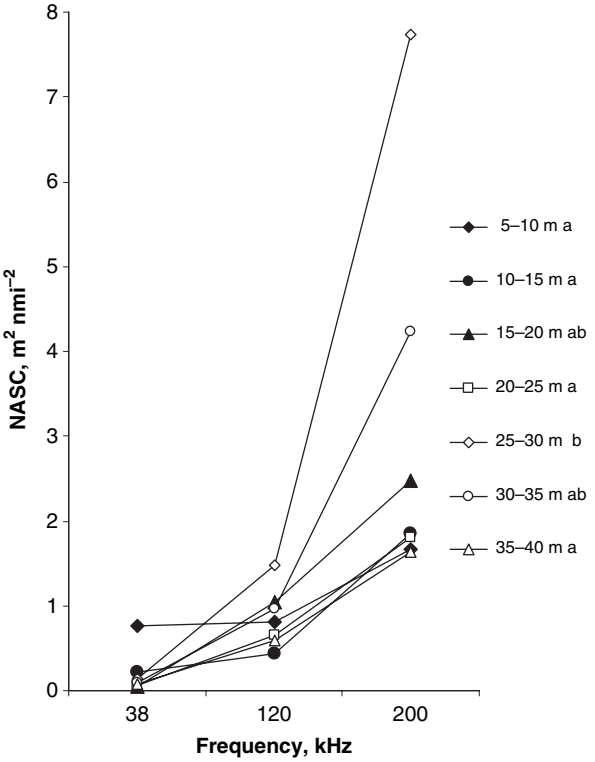

Fig. 6 The frequency response of Nautical area scattering coefficient (NASC) from non-fish targets in L. Paasivesi on 11 October 2005. Letters a–d after depth indicate homogenous subsets according to ANOVA.

2003; Pedersen, Ona & Korneliussen, 2004). Acoustic scattering at 120 kHz appeared somewhat lower than at 200 kHz, which is not consistent with the above explanation. The difference should be within the expected variation in multi-frequency data sets and could be attributed to several factors, such as body versus swimbladder scattering, calibration, nonlinear effects, absorption coefficient, sound speed, noise, etc. (see Fernandes *et al*., 2006). For fish detection, 38 kHz is recommended above higher frequencies due to less target directivity giving more stable target strengths and less noise from plankton scattering. However, a lower frequency will require a relatively larger transducer that can impose a restriction on portable use. A low frequency does also have a poorer vertical resolution and will not detect as small targets as higher frequencies.

The narrow scattering layer observed at all frequencies at about 25 m coincided with both a rapid change in temperature and a peak in oxygen concentration. The temperature change of the water will cause a contrast in acoustic impedance and could explain the reflectivity of the observed layer. Metalimnetic oxygen maxima are events that occur in a large number of lakes (e.g. Wetzel, 1975). If L. Paasivesi was a clear water lake, the peak in oxygen concentration could indicate photosynthetic activity and presence of phytoplankton that also could reflect sound (Vancuyck, Guerinancey & Sessarego, 1993). However, this lake actually has dark water (*c.* 45 mg Pt mL^{-1}) and its productive layer is <5 m (A.-L. Holopainen, pers. comm). Therefore, a more probable explanation for its metalimnetic oxygen maximum is that the high water temperature in the epilimnion reduced the solubility of oxygen in that layer while oxygen consumption exceeds replacement in the hypolimnion.

Although significant correlations were found between fish density indices estimated from trawl

Table 3 Invertebrate density (specimens 100 m^{-3}) and mean Nautical area scattering coefficient (NASC) from invertebrate detections in different depths (m) at 200 kHz (Δd = 2 m) in L. Paasivesi on 11 October 2005

Depth	*Mysis relicta*	*Chaoborus flavicans*	Amphipoda	Copepoda	Cladocera	NASC (m^2 nmi^{-2})
5	0	3	0	335	778	0.29
10	23	9	2	1252	**1172**	1.04
15	53	5	23	4006	985	0.26
20	194	13	13	1968	771	0.79
25	247	136	38	5180	238	2.23
30	**252**	**155**	**114**	**8564**	23	**2.65**
35	235	12	82	5490	10	1.46
40	255	5	55	4527	9	0.68

Maximum values are shown in bold.

Table 4 Pearson's correlation (r) between Nautical area scattering coefficient (NASC) and invertebrate densities (Table 3)

		NASC	*Mysis relicta*	*Chaoborus flavicans*	Amphipoda	Copepoda	Cladocera
NASC	*r*		**0.649**	**0.916**	**0.706**	**0.760**	−0.595
	P		0.041	0.001	0.025	0.015	0.060
M. relicta	*r*	**0.649**		0.530	**0.747**	**0.755**	**−0.876**
	P	0.041		0.177	0.033	0.030	0.004
C. flavicans	*r*	**0.916**	0.530		0.582	**0.715**	−0.485
	P	0.001	0.177		0.130	0.046	0.223
Amphipoda	*r*	**0.706**	**0.747**	0.582		**0.940**	**−0.842**
	P	0.025	0.033	0.130		0.001	0.009
Copepoda	*r*	**0.760**	**0.755**	**0.715**	**0.940**		**−0.768**
	P	0.015	0.030	0.046	0.001		0.026
Cladocera	*r*	−0.595	**−0.876**	−0.485	**−0.842**	**−0.768**	
	P	0.970	0.004	0.223	0.009	0.026	

Correlations significant at $P < 0.05$ are in bold. Significances (P) for correlations that include NASC are one-tailed for H_1: positive association, all other two-tailed. For all cases $n = 8$.

catches and acoustic scattering, there was also considerable random variation. There are various reasons why a perfect match between acoustic estimates and trawl catches are not expected. First, the sampling volume of the acoustic beam and the trawl are different and the trawl is sampling the water column later than the echo sounder. Further, the catchability of fish by the trawl may vary between hauls and depths for several reasons. The fish may avoid the approaching vessel, especially when the trawling is conducted in daylight and/or close to the surface. We tried to avoid this problem by restricting the trawling depth to >10 m during day. It is also possible that some of the smallest smelt escaped through the codend. Thus, trawl CSA must be considered both an underestimate and an imprecise index of the true fish density.

Acoustic scattering from invertebrates increased with sound frequency, suggesting that the targets behaved as Rayleigh scatterers (target strength rises rapidly with frequency). Despite the fact that different invertebrate groups were mixed throughout the water column, our data suggest that the scattering was mainly attributable to *C. flavicans*. It is a strong scatterer relative to other invertebrates and will resonate around 200 kHz because of the paired air sacks in each larva (e.g. Jones & Xie, 1994; Knudsen *et al.*, 2006). *Mysis relicta* may also contribute to scattering. Gal, Rudstam & Greene (1999) have earlier detected its scattering at 420 kHz. Also, Amphipoda may induce some scattering (Trevorrow & Tanaka, 1997) because they were rather large (mean length 8.3 mm) in L. Paasivesi. It has been previously demonstrated by Frouzová, Kubecka & Matena (2004) that Cladocera and Cyclopoda are insignificant scatterers at the frequencies employed and can therefore be neglected. One further scattering candidate is *Limnocalanus macrurus* Sars (Copepoda) since it is relatively large (>2 mm) and numerous with a vertical density distribution in reasonable agreement with the

acoustic scattering in this work. A marine copepod, *Calanus finmachicus* Gunnerus, has similar scattering properties (Korneliussen & Ona, 2000). If a higher frequency than 200 kHz had been used in our study, more information about scattering from *C. flavicans* versus than that from the other species might have been obtained.

Despite the differences in the length distributions of smelt and vendace the present data set may not be optimal to distinguish between fish species. Species-specific differences in the length–TS relationship, and also variations in fish behaviour, may obscure determination of fish species from TS measurements (Lilja *et al*., 2004). However, Gauthier & Horne (2004) discriminated between five fish species [*Mallotus villosus* (Müller), *Clupea pallasii* Valenciennes, *Theragra chalcogramma* (Pallas), *Pleurogrammus monopterygius* (Pallas), *Thaleichthys pacificus* (Richardson)] analysing their target strength differences between pairs of carrier frequencies. In addition, Kloser *et al.* (2002) were able to discriminate three groups of deep water fish (myctophids, morids, macrourids) with amplitude mixing from acoustic recordings at 12, 38 and 120 kHz. The ability to enhance acoustic identification of different fish species is another important application if multi-frequency acoustic systems are available.

Diel vertical migrations of pelagic fish exist in L. Paasivesi and they are strongest between mid-summer and the break down of thermal stratification, beginning usually with decreasing air temperature and autumn winds in September (Jurvelius & Heikkinen, 1988). In a lake comparable to L. Paasivesi, Rahkola-Sorsa & Jurvelius (2001) found that zooplankton migrated from 10–15 m depth to the 0–10 m surface layer during the night. In the present study, the sampling of invertebrates and their acoustic monitoring was undertaken during the day, when the invertebrates were assumed to stay deeper in the water column below the near field of the echo-sounder transducer. The fish sampling was carried out both during the day and after sunset. This does not affect the interpretation of the results because the trawl samples were always compared with the hydroacoustic data collected simultaneously with trawling.

In conclusion, at 38 kHz, there was practically no acoustic scattering from invertebrates and scattering from fish was somewhat higher than at the two other frequencies. A low frequency is therefore recommended for estimating fish abundance without bias from invertebrate scattering, while a high frequency will detect both fish and invertebrates. Although fish and invertebrates can also be successfully discriminated at a single frequency by thresholding and cross-filtering, the combination of a low and a high frequency as used in this study is a more robust tool for effective fish-invertebrate discrimination.

Acknowledgments

We thank the staff of R/V Muikku for patience and good humour in fish and invertebrate sampling. Special thanks for Raimo Riikonen, Heikki Auvinen, Tommi Malinen and Heather Mariash for fish sampling, and Anja Lehtovaara for analysing zooplankton samples. Ministry of Agriculture and Forestry of Finland financed this study. Professor Roger Jones, University of Jyväskylä, kindly checked the English language.

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(Manuscript accepted 3 December 2007)