Study of surface layers of water bodies using hydroacoustic method

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Study of surface layers of water bodies using hydroacoustic method

Ph.D. thesis

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Annotation

This Ph.D. thesis is focused on the new developed mobile upward-looking system, which allows to study with non-destructive method invertebrates and fish near the water surface. In the first part, we studied behaviour of a horizontal sound beam, using a standard target at a known depth. The second part, I compared the newly developed upward-looking system with fry trawl to possibly monitoring juvenile fish whether the recorded data correspond to reality. In the third part, we used two different frequencies to differentiate *Chaoborus* larvae from the smallest juvenile fish. In last part, I compared new system and a very used passive gillnets method. This work provides an initial step towards the improvement of study water surface layer.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

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České Budějovice, 18. 6. 2020

Mgr. Roman Baran

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Paper I.

Balk, H., Søvegjarto, B.S., Tušer, M., Frouzová, J., Muška, M., Draštík, V., Baran, R., Kubečka, J., 2017. Surface-induced errors in target strength and position estimates during horizontal acoustic surveys. Fish. Res. 188, 149–156. https://doi.org/10.1016/j.fishres.2016.12.017

Roman Baran participated in data acquisition, processing and in writing manuscript.

Paper II.

Baran, R., Jůza, T., Tušer, M., Balk, H., Blabolil, P., Čech, M., Draštík, V., Frouzová, J., Jayasinghe, A.D., Koliada, I., Mrkvička, T., Muška, M., Ricard, D., Sajdlová, Z., Vejřík, L., Kubečka, J., 2017. A novel upward-looking hydroacoustic method for improving pelagic fish surveys. Sci. Rep. 7. https://doi.org/10.1038/s41598-017-04953-6

Roman Baran was responsible for sampling arrangement, hydroacoustic survey, data processing, statistical analysis and writing manuscript.

Paper III.

Baran, R., Tušer, M., Balk, H., Blabolil, P., Čech, M., Draštík, V., Frouzová, J., Jůza, T., Koliada, I., Muška, M., Sajdlová, Z., Vejřík, L., Kubečka, J., 2019. Quantification of chaoborus and small fish by mobile upward-looking echosounding. J. Limnol. https://doi.org/10.4081/jlimnol.2018.1837

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Paper IV.

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Contents

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Introduction

Acoustic instruments which transmit and receive sound waves can be used to detect fish or other objects far beyond the range of vision. The pulse travels through the water environment and is scattered by the objects with different homogeneities than that the surrounding medium. Consequently, a backscattered sound, called an echo, returns back and is detected by a receiver of the sonar. The received signal contains information about the ensonified objects. Acoustic technology has had a major impact on research of fish at environment where it is the only method capable of surveying of large volumes of water. The information provided by sonars and echosounders is also an important factor in the efficiency of modern fishing operations (Simmonds and MacLennan, 2005).

Historically, the greatest progress and development of underwater acoustics took place in marine environment mainly due to the military activities in First and Second World War, (Simmonds and MacLennan, 2005). Acoustic methods of fish abundance estimation were started in the 1950s. Initially these were based on simple ideas of counting individual echoes. The calibration methods of the time were imprecise, and the target strength of fish was uncertain. Intensive theoretical and experimental investigations in the 1970s and 1980s led to a better understanding of what acoustic techniques could and could not do (Keiner and Rozwadowski, 2007).

Open water represents the largest volumes in larger lakes and reservoirs. Hydroacoustics is an obvious option covering large areas without disturbing fish. The most commonly used acoustic approach for study open water is downward-looking transducers, which beam from the surface to the bottom. This principle is well usable for sampling of seas or unstratified lakes. However, there is a blind zone at the surface created by the depth of the deployed transducer (at least several cm) and the physical near-field where the acoustic beam is not fully formed. Additionally, near the transducer, the sampling volume is very low and provides a very limited coverage of the near-surface layers (Simmonds and MacLennan, 2005) (Fig.1)

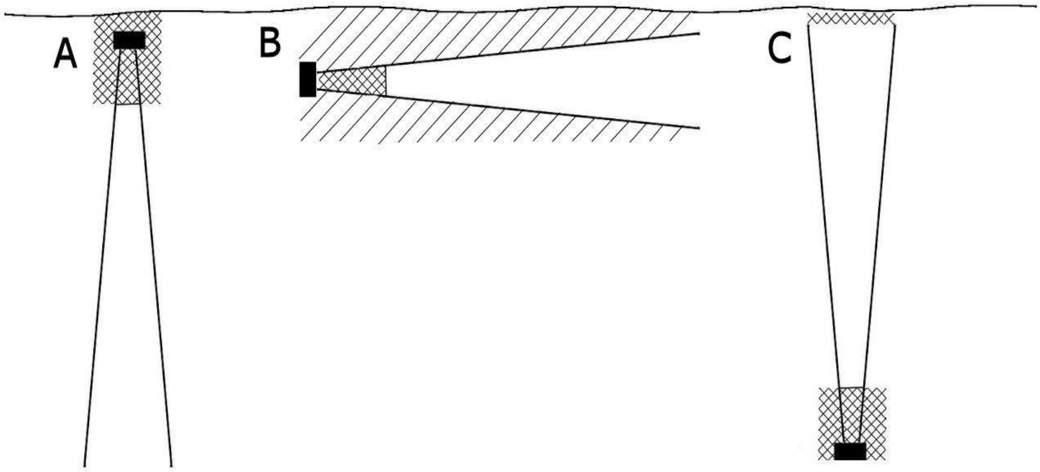

Fig. 1 Blind zones near the water surface during three types of sonar beam orientation.
A) vertical down-looking beaming, b) horizontal side-looking beaming, c) vertical upward-looking. Double hatching - blind zones due to transducer deployment, nearfield and phase boundary, single hatching – underestimated volume above and below the beam during horizontal beaming.

In stratified lakes and reservoirs the fish are predominantly near the surface(Bohl, 1979; Godlewska and Jelonek, 2006; György et al., 2012; Hrabik et al., 2006; Prchalová et al., 2003; Vašek et al., 2009; Yule et al., 2013) especially at night. Fish may occur only a few metres under the surface (Vašek et al. 2008) and, for this reason, the downward-looking approach does not provide reliable data near the surface. A suitable solution could be to operate the transducer horizontally (Kubečka and Wittingerová, 1998). Horizontal echo sounding, also called horizontal beaming, covers the surface layers well. This method proved to be very suitable and therefore it was used to explore fish in stratified reservoirs or lakes(Godlewska et al., 2012; Muška et al., 2013;

Tátrai et al., 2009; Yule, 2004). However, each technique has advantages and disadvantages and horizontal echo sounding is no exception.

The most critical problem is that the estimated size of fish changes at different orientations relative to the transducer axis, the so-called side aspect (Frouzová et al., 2005). It is obvious that while in the horizontal plane the fish may be visible at all angles during one turn of its body around its dorso-ventral axis, during vertical observations only narrow range pf aspects close to well-defined dorsal aspect are recorded. The difference between different fish body orientations at horizontal plane is up to 30+ decibels (Frouzová et al., 2005, Fig. 2) and when the target strength was converted to size and then to the biomass, the differences were striking (Boswell et al., 2008).

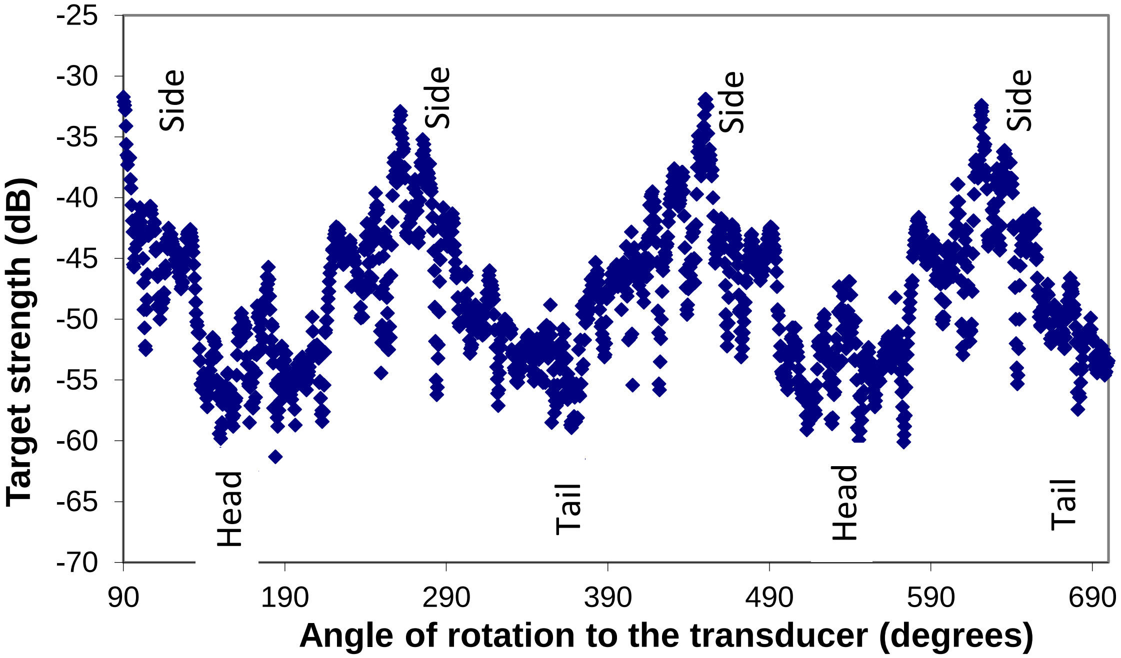

Fig. 2 The relationship between body aspect and target strength for all aspects of fish horizontal plane (data kindly provided from Frouzová *et al.*, 2005).

The determination of size is often performed using deconvolution (Kubečka et al 1994), which is based on stochastic assumptions of random aspect orientation that may not be entirely true (Tušer et al., 2009). So, the uncertainty about the aspect and TS interpretation in terms of real fish size still remains a major difficulty connected with fish assessment by horizontal sonar.

Higher acoustic noise levels due to reverberation and lower signal-to-noise ratio also complicate the detection of mainly small fish with horizontal beaming (Kubečka, 1996). Under the conditions of thermal stratification, the acoustic beam can also bend due to the effect of water temperature on the speed of sound on the edge of beam at different temperature layers (Trevorrow, 2001). In this case it is very important to observe the summer stratification and if there is a big difference between the temperature layers, then it is better to use another date of sampling. Alternatively, it may be feasible to shorten the surveyed range thus reducing the sampling volume and limiting major advantage of acoustic sampling.

Still, this is not the end of horizontal beaming problems list. New findings on the multipath signals in the horizontal beam show strong interference near the surface (Balk *et al.*, 2017, Paper I). This phenomenon discovered during the course of my PhD study significantly reduces the possibility of correct fish size and depth detection near the surface. The error arises mainly in determining the proper depth of targets and target strength. This difficulty together with all other mentioned above caused the decision to abandon originally designed PhD topic of Seasonal changes in the spatial occurrence of fish in reservoirs as this was planned with using horizontal beaming as the main sampling tool. All problems with horizontal beaming together would lead to a great uncertainty in estimation of observed fish size and depth and we faced a real risk of accurate analysis of highly inaccurate data. The errors can be as large as over 30 dB and this is hardly compatible with reliable fish analysis.

All identified shortcomings of horizontal echo sounding do not allow us to obtain accurate data from sampling lakes or reservoirs. For sampling fish near the surface, it was necessary to develop a different suitable acoustic principle. A potential solution to address the aforementioned disadvantages horizontal echo sounding beaming is an upward-looking system, where the transducer is oriented vertically, but the direction of beaming is from the water column towards the surface. This arrangement makes it possible to record fish in the near surface layer (Fig. 1) and to accurately determine their size and depth. So far, this type of system has been mostly restricted to stationary locations where the transducer is fixed to the bottom of the water body and continually samples the same place (Arrhenius et al., 2000; Čech and Kubečka, 2002; Jarolím et al., 2010). In the case of stationary upward-looking, we observe only one location and do not take advantage to survey the large volume in a short time. Fish records with stationary upward-looking is to a large extent a passive method that depends on the movement of fish. This dissertation offers a method how to overcome this limitation.

Results

This dissertation is composed of four original papers – three of them already published (Papers I, II and III) in impacted international scientific journals, one is in a form of unpublished manuscript (Paper IV).

Paper I

Balk, H., Søvegjarto, B.S., Tušer, M., Frouzová, J., Muška, M., Draštík, V., Baran, R., Kubečka, J., 2017. Surface-induced errors in target strength and position estimates during horizontal acoustic surveys. Fish. Res. 188, 149–156. https://doi.org/10.1016/j.fishres.2016.12.017

New findings on the multipath signals in the horizontal beam show strong interference near the surface. Errors in target strength up to 10 dB and depth position up to 0.5 m were observed. Simulations suggested that multipath signal propagation interfered with the direct path of that signal. When standard target (calibration copper sphere) was moved away from the transducer at fixed depths, the estimated target strength and depth of the target (1) stayed as it should be, (2) started to oscillate as a function of range. The amplitude increased with increasing range. The frequency decreased from the oscillation start until (3) at certain range the oscillation stopped (Fig. 3). The first zone (about 5-10 m) is free from interference and it is possible to use it for monitoring. This zone can be maximized by shortening the pulse length and lowering the transducer. However, the target depth does also influence on the range of the zone. Since the range of useable zone get shorter when targets approach the surface, the shallowest targets will limit the possible range to be surveyed. Lowering the transducer will help, but at the same time reduce the observable part of the surface zone. These results significantly limit the possibility of exploration near the water surface using horizontal beaming.

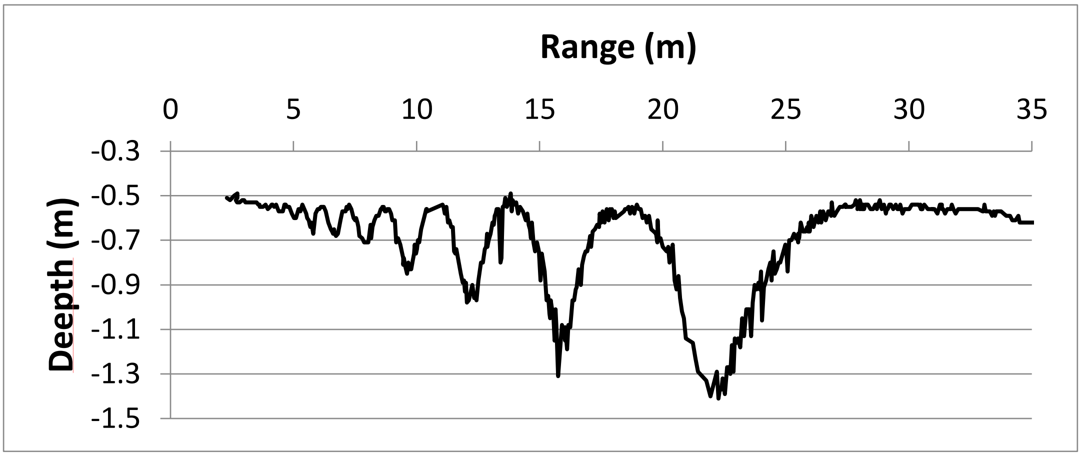

Fig 3: Estimated depth of standard target (real depth= 0.5 m) from different ranges from the transducer.

Paper II

Baran, R., Jůza, T., Tušer, M., Balk, H., Blabolil, P., Čech, M., Draštík, V., Frouzová, J., Jayasinghe, A.D., Koliada, I., Mrkvička, T., Muška, M., Ricard, D., Sajdlová, Z., Vejřík, L., Kubečka, J., 2017. A novel upward-looking hydroacoustic method for improving pelagic fish surveys. Sci. Rep. 7. https://doi.org/10.1038/s41598-017-04953-6

Information about fish distribution and abundance in the upper water column is often fundamental. However, this information is extremely hard to obtain using classical hydroacoustic methods. A new rigid frame system was developed for pushing upward looking transducers of the scientific echo sounder (38 and 120 kHz) in front of the research vessel. No statistically significant differences in the estimated abundance of juveniles were found between the two sampling methods. The comparison of abundance estimates gathered by the two frequencies were also not significantly different. The predicted mean lengths from acoustic sampling and the trawl catches differed by less than 10 mm in all comparisons. The new acoustic system circumvents the known disadvantages of horizontal and downward-looking hydroacoustic transducers when sampling above the thermocline. Mobile upward-looking hydroacoustics is a promising fish-friendly method for further quantitative studies of pelagic upper layer fish communities, which are of great importance in many aquatic ecosystems where fish inhabit productive surface layers.

Paper III

Baran, R., Tušer, M., Balk, H., Blabolil, P., Čech, M., Draštík, V., Frouzová, J., Jůza, T., Koliada, I., Muška, M., Sajdlová, Z., Vejřík, L., Kubečka, J., 2019. Quantification of chaoborus and small fish by mobile upward-looking echosounding. J. Limnol. https://doi.org/10.4081/jlimnol.2018.1837

Chaoborus larvae inhabit frequently the water column of lakes, when they can be mistaken for small fish. Because larvae ascend up to the blind zone of downward-looking echo sounding at night, quantitative acoustic estimation of them is possible only with upward-looking approach. In the target strength range of invertebrates (smaller than -59 dB), the 38 kHz echosounder recorded only a small proportion of targets while the 120 kHz echosounder recorded distinct peaks corresponding to high densities of Chaoborus (target strength, range -70 to -60 dB, average -66 to -64 dB). Data obtained with the 120 kHz frequency echosounder confirms that this frequency, primarily used to study fish, is capable of studying Chaoborus as well. Using the lower frequency of 38 kHz offers the potential separation of a very small cohort of fish (6-20 mm TL) from Chaoborus larvae when the investigation of such extreme application is needed. The study demonstrates the applicability of the mobile upward-looking hydroacoustic system to survey Chaoborus.

Paper IV

Baran, Blabolil, P., Čech, M., Draštík, V., Frouzová, J., Holubová, M., Jůza, T., Koliada, I., Muška, M., Peterka, J., Prchalová, M., Říha, M., Sajdlová, Z., Šmejkal, M., Tušer, M., Vejřík, L., Kubečka, J., New way to investigate fish density and distribution in the shallowest layers of the open water, manuscript

While paper 2 deals with young of the year fish detection, this manuscript assess the usability of mobile up-looking system to study larger fish. It also focuses on the very surface layers where it is extremely hard to obtain reliable quantitative records using conventional hydroacoustic methods. For this reason, the mobile hydroacoustic upward-looking system (38 kHz split-beam echosounder) in combination with a passive sampling method (gillnets) was tested to investigate the fish community (fish larger than 8 cm total length) in the upper 3 m of water column. Most fish are located in the depth layer closest to the surface down to 1 m – 50-78 % by acoustics (layer 0.3 – 1 m) and 55-71 % by gillnets. The size structure of both methods was generally similar, but the acoustic results contained a higher proportion of small fish (< 12 cm SL). It was found most fish occur very close to the surface and these would be mostly missed by down- or side-looking acoustic sampling. Comparison with gillnets showed that upward-looking records provided similar fish size distribution.

Discussion

The presented dissertation contributes to the development new system of hydroacoustic research for the surface layer. New research has shown that the used horizontal beaming method has quite a few shortcomings and quantitative data interpretation is very complicated. The most commonly used down-looking method for physical reasons cannot record the layer near the surface at all, for this reason, data from a depth of more than two meters below the surface are used (Emmrich et al., 2012; Yule et al., 2009).

The smooth surface can introduce errors when acoustics is applied to monitor fish horizon-tally in the surface layer, even when the beam is tilted away from the surface. The influence of the surface depends not only on the depth and tilt of the transducer but also on the depth of the target. A scientist using horizontal echo-sounder applications near the surface should be very careful surveying water bodies when the surface is smooth. Earlier, a smooth surface has also been regarded as optimal for horizontal surveys since it provides better stability for the beam and lower noise (Trevorrow, 2001). Our results showed that the mirror reflections can be more serious than the noise from a slightly wavy surface. On the other hand, even quite small ripples on the surface were sufficient to remove the interference problem and stop the oscillations. The researchers using horizontal beaming applications to cover the surface layer must pay attention to interference and seek to avoid surveying when the surface is smooth. For this reason, it is necessary to set the transducer deeper from the surface and then it is not possible to view the surface layer in a holistic perspective (Fig. 1).

Mobile upward-looking is based on the principle of stationary upward-looking, which had very good results in record fish near the water surface (Arrhenius et al., 2000). There are two ways to convert a stationary upward-looking mobile – towed system for back the ship and pushing the system in front of the ship. However, towed system was had very poor manoeuvrability (Guihen et al., 2014). For this reason, the second option was used, not dragging, but pushing the system in front of the ship (Paper II). The mean lengths of trawl-caught fish and those predicted by the upward-looking method differed by less than 10 mm. In all synoptic comparisons fish sizes predicted from acoustic data usually have a wider spread (higher variance) when compared with direct catch measurements (Emmrich et al., 2010; Mason et al., 2005). Newly developed mobile upward-looking hydroacoustic system is a promising fish-friendly method for further quantitative studies of pelagic upper layer fish communities, which are of great importance in many aquatic ecosystems where fish inhabit productive surface layers.

On the other hand, not only fish, but also aquatic insects and other invertebrates such as *chaoboridae* live near the water surface, which may cause a certain error during the study of juvenile fish near the surface (Knudsen et al., 2006; Malinen et al., 2005). For this reason, invertebrates had to be partially selected from the upward-looking record to reduce the error in juvenile fish research (Paper III). The 120 kHz frequency can efficiently record *Chaoborus* larvae or pupae, which can bias the hydroacoustic estimates of fish in waterbodies. The peak of *Chaoborus* was recorded between -70 and -60 dB target strength with the top between -64 dB and -65 dB. These values correspond to other published results for pelagic invertebrates (Knudsen et al., 2006; Prchalová et al., 2003). Observations with an echosounder using a higher frequency of 200 kHz suggest slightly higher range of modal TS -64 to -60 dB (Bezerra-Neto et al., 2012; Jones and Xie, 1994). We confirmed earlier findings that the frequency of 38 kHz does not record *Chaoborus* larvae (Jones and Xie, 1994; Knudsen et al., 2006). For this reason, if we use multiple frequencies, fish can be reliably distinguished from *Chaoborus* larvae.

In (Paper IV) study demonstrates that mobile upward-looking acoustic surveys have potential to be a reliable tool in fish community assessment other epilimnion of stratified lentic waters. It was found that fish occur in the largest numbers and biomass only 1 m from the surface. These results correspond to earlier studies (M. Prchalová et al., 2009; Řiha et al., 2015; Vašek et al., 2008) but with finer depth resolution. Clumping of fish in the surface layers seems to be a common feature especially in eutrophic and strongly thermally stratified waters (Bohl, 1979; Eckmann, 2007; Quinn et al., 2012; Yule et al., 2013). However, for the gillnets, the effective sampling volume of gillnets is not known (Deceliere-Vergès et al., 2009; Prchalová et al., 2011a). For this reason, only the comparison of the size composition of the captured fish and the reconstructed size from the acoustics could be used. The results were similar indicating that both approaches sample similar fish communities.

Each sampling method has its advantages and disadvantages, and mobile upward-looking is no exception.

1. The most fundamental advantage of the new system is the ability to obtain data by non-destructive method near the water surface up to a depth of 10 m. With the combination with down-looking, the entire water column can be sampled.
2. Upward-looking system provides reliable data when compared to the active fry trawl method as well as the passive gillnets method. When comparing size and abundance, similar results were found with the active and passive method.
3. The new hydroacoustic system allows sampling of both juvenile to adult fish.
4. New upward-looking system allows to use combination of several transducers with several frequencies. This system records data in greater detail and therefore it is possible to study fish and *Chaoborus* larvae at the same time.

On the other hand, upward-looking system in front of the ship also has some drawbacks.

1. Upward-looking system cannot survey in shallow lakes or reservoirs or shallow areas of deeper waterbodies, because the carrying construction needs certain depth (certainly more than 5 m) and there is a danger of damage by various structures at the bottom (such as stones, branches, aquatic plants etc.).

2. The upward-looking device is quite large and requires a larger research vessel. Extensive lever which has to be driven through the water efficiently slows the device down to the speed of 4 km/hours. However, this functional prototype can be significantly modified with the modern materials to reduce weight and size. For this reason, it is not possible to explore a large area or volume quickly. Lifting of sampling grab to a smaller depth would increase the speed but decrease the sampling volume. The use of several wide transducers in upward-looking system can be a way forward.

3. Day period is not suitable because of sinusoidal movement of planktivorous fish (Čech and Kubečka, 2002, Jarolím et al. 2010). In day sampling, escape reactions before the upward-looking system were recorded (Baran unpubl. data). On the other hand, in the recordings that were recorded at night, these reactions do not occur (Baran *et al.*, 2017,2020).

Conclusions

Upward-looking system is a non-destructive method that has promising results and makes it possible to explore fish or invertebrate near water surface. Thanks to this acoustic system, it is possible to explore surface layers the lakes and reservoirs which has been neglected or examined by sidelooking horizontal beaming. The surveying is limited to nights, depths >5 m and the speed is slow. The recording fish near the surface is an undisputed advantage that was not possible with other the non-destructive method. By further developing and investigating shortcomings, we can more easily handle or largely eliminate them.

Perspectives

The upward-looking system does not allow data acquisition at shallow depth, mainly due to the large size of the existing system. The size can be adjusted using lighter and stronger materials such as carbon fibers. However, this device will still be limited by the possibility of exploration at shallow depths. Autonomous underwater vehicles (AUVs) can be a possible solution for this problem. In many respects, AUVs are ideal platforms for acoustic surveys. They can be directed to a variety of depths in the water column and can therefore be positioned at sufficient distance so as not to have an effect on the natural behavior of the resource they are to survey (Fernandes et al., 2003; Stoner et al., 2008). If we use wireless data transmission and modern navigation systems AUVs work very well and you can get high quality data from any depth (Benoit-Bird and Waluk, 2020; Moline et al., 2015).

Classifications based on acoustic methods ideally rely on accurate knowledge of the organisms responsible for the backscattering responses. This is usually obtained from dedicated and concurrent sampling using capture devices such as nets, optical methods, or other forms of physical sampling (Fernandes et al., 2016). It is possible to distinguish fish from invertebrates by using several acoustic frequencies (Knudsen, Larsson and Jakobsen, 2006; Axenrot *et al.*, 2009, Paper III). Moreover, recently emerged broadband acoustic systems transmitting frequency modulated (FM) signals, typically linearly-frequency modulated signals, for characterizing fish and other marine organisms (Lavery, Chu and Moum, 2010; Stanton et al., 2010) can increase power of taxonomic resolution of acoustic targets. This setting is called “wideband” refers to a system that combines multiple transducers, each with different broadband or narrowband signals and capabilities, to span a range of frequencies larger than can be achieved with a single transducer (Lavery et al., 2017).

We developed mobile rigid system using the principle of upward looking acoustic which allows researchers to study fish near the surface. This principle can add further interesting insights into the occurrence and behavior of fish in stratified lakes and reservoirs. Results of our studies (Paper II and IV) showed that comparing this method with active and passive sampling methods give similar results and the data obtained by this principle are reliable. In paper III we used two different frequencies to distinguish juvenile fish and *Chaoborus* larvae. This shows that the use of modern knowledge and methods can eliminate the shortcomings of this system. In the future, AUV will make it possible to study even small depths and larger volume. The broadband system may make it possible to gradually distinguish individual taxonomical groups of fish.

Another promising perspective lies in the application of multibeamy systems combining the information from many beams into three-dimensional picture (acoustic cameras). Systems like DIDSON and ARIS revolutionized fish migration and behavior studies (Jůza et al., 2013; Rakowitz et al., 2012a) and have the possibility to overcome the directivity and multipath scattering. Early models had too low sensitivity towards small fish and weaker aspects (Tušer et al., 2014) but this limitation is likely to be surpassed by further signal-to-noise improvements. So there is a good hope that ultrasonic systems will provide quantitative fish results in notoriously difficult environment like stratified reservoirs with the bulk of fish stock close to the surface.

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Research papers

Paper I

Surface-induced errors in target strength and position estimates during horizontal acoustic surveys

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Surface-induced errors in target strength and position estimates during horizontal acoustic surveys.

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ABSTRACT

Horizontally-aligned, fixed and mobile, transducers are routinely deployed at depths of 0.5–0.75 m to survey the surface layer of waterbodies for fish. However, simulations and measurements demonstrate that a smooth surface can cause serious errors in the target strength (TS) and split-beam angular position estimates.

Errors in TS up to 10 dB and depth position up to 0.5 m were observed. Simulations suggested that multi-path signal propagation interfered with the direct path of that signal. Furthermore, when a standard target (calibration copper sphere) was moved away from the transducer at fixed depths, the estimated TS and depth of the target started to oscillate as a function of range. The amplitude increased with increasing range. The frequency decreased from the oscillation start until a certain range where the oscillation stopped. The region of oscillation depended on both the transducer and target depth. Horizontal observations of known fish echoes behaved similarly. Experiments in a lake showed that the influence from the surface disappeared when the surface became rippled due to wind.

1. Introduction

Hydroacoustic methods are well established for monitoring fish in water bodies (Simmonds and MacLennan, 2005). The methods work for the pelagic zone, but the surface and bottom zones are challenging. The surface layer can be monitored with bottom-up (Čech and Kubečka, 2002) or horizontally mounted transducers (Kubečka and Wittingerová, 1998). Mobile bottom-up methods are technically more challenging and do not work in areas too shallow to give a sufficient sampling volume. Thus, the horizontal method often remains as the only option for hydroacoustic surveying of shallow layers.

During horizontal acoustic surveys, the transducers are often mounted at depths of 0.5–0.75 m below the surface, panned sideways, and tilted so that the upper half-power edge of the beam is parallel with the surface. According to our experience this setting allows observing ranges from 4 to something around 30 m away from the boat depending on the surface state and water depth.

The surface layer, however, poses some problems with the horizontal application (Simmonds and MacLennan, 2005). First, even slight rolling of the boat can easily cause the beam to strike the surface, which can act as a strong acoustic reflector. Second, temperature depth gradients inside the surface layer can be strong and cause refraction of the propagating sound (Medwin and Clay, 1998). Third, sound multi-pathing induced by non-ideal beam patterns and reflections from a smooth surface can cause interference.

Generally, the horizontal setup with the beam well-aligned below the surface relies on the assumption that outside the half-power beam there is too little sound energy to cause any interaction with the surface. The background for the assumption is that the acoustic intensity drops off very quickly outside the half-power beam and that the side lobes for the commonly applied transducers are well damped.

The main aim of this paper is to investigate whether this assumption is correct or not, and how failure of the assumption may influence on the echo-sounder's ability to estimate the target's vertical positions and target strengths. The influence on the echo-sounder is demonstrated with in situ experiments, and the theoretical explanation is supported with simulations.

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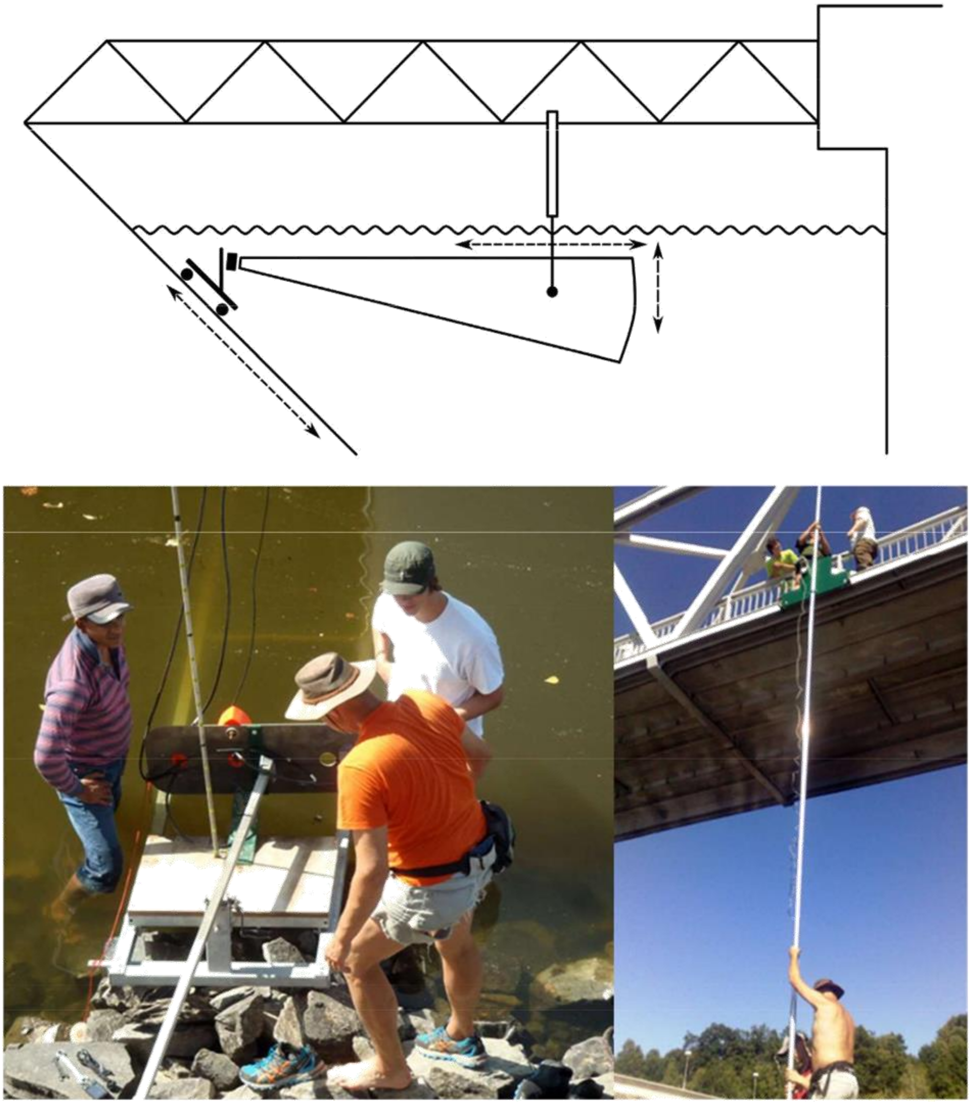

Fig. 1. Experimental setup: Upper: A scheme showing the deployment of a transducer and standard target below the bridge to the Římov reservoir control tower. Dashed arrows indicate directions of movement of the transducer's stand and the acoustic test target. Lower left: The transducer stand with the attached elliptic transducer ES120-4x10. The long bar connected to transducer's plate controlled the tilt, the vertical pole determined the depth of the transducer. An electronic Attitude and Heading Reference System (AHRS) (not seen) measured the tilt. The stand was situated on rails enabling depth adjustment. Lower right: The monorail with a long pipe sheltering and guiding a monofilament line holding the standard target to the surface of the reservoir.

2. Material and methods

2.1. In-situ experiments

We mounted standard targets at fixed depths and moved them away from the fixed positioned transducer. The main experiments were conducted in the Římov reservoir (Czech Republic) under the bridge leading to the water outlet and power control tower. The reservoir-bed is steep at this site (Fig. 1).

The acoustic recordings were done with a Simrad EK60 echosounder equipped with an ES120-4x10 transducer. The transducer was an ordinary tonpilz transducer with 108 weighted elements operating at 120 kHz, and with a 4 × 10° opening angle. The elliptical opening angle has made this transducer popular for horizontal mobile lake and fixed river counting applications. We used it in the most common way with the 4° axis marked on the transducer as the along ship axis pointing vertical in the water. The EK60 was set up with a pulse-duration of 0.128 ms, and a power of 100 W. With this setup, the pulse consists of about 15 cycles. With a sound speed of 1487 m/s, the wavelength λ is approximately 1.23 cm.

The transducer was mounted on a railcar running on a platform going down into the water along the steep reservoir bed. The platform was equipped with rails, legs and a winch. The legs facilitated deployment on the reservoir bed, and the winch enabled us

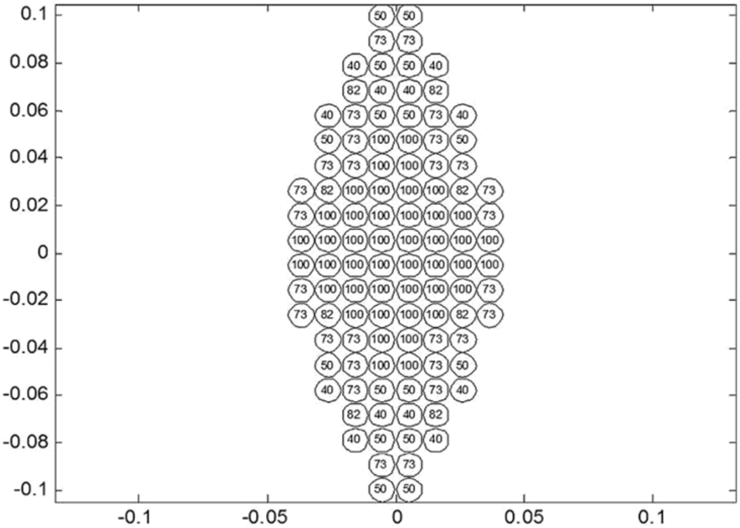

Fig. 2. Transducer design: The ES120-4x10 transducer consists of 108 elements, each positioned with a distance of 1.05 cm from each other centres, and weighted from 100 to 40% as indicated in element's circle.

to lower the railcar down to the correct water depth (Fig. 1). The transducer tilt was adjusted manually and locked to the required tilt for each experiment. A waterproof Attitude and Heading Reference System (AHRS; Søvegjarto, 2015) was mounted on the side of the transducer to constantly monitor and record the tilt. The output from the AHRS was verified with a Leica DISTO™ 5D laser range finder for each trial before the railcar was lowered down into the water. Transducer depth was measured on a vertically mounted mechanical ruler mounted on the railcar starting at the level of the transducers centre.

Deployment of the target was done using a monorail arrangement mounted on the bridge (Fig. 1). Monofilament fishing line designed for a low degree of stretching (thickness 0.35 mm) held the target. The line was lowered down from the bridge to ease depth adjustments. The line was protected against wind with an aluminium pipe leading from the bridge and ending just above the water surface. As a target we deployed a SIMRAD standard copper target (23 mm in diameter). Using the actual water temperature at the site, the standard target had TS of −40.2 dB for the 120-kHz transducer frequency. In addition to the standard target we also tested echoes from an anesthetized fish (common bream, *Abramis brama*, 340 mm standard length) The fish was mounted so that it was observed in the side aspect. The mounting was done in a way similar to Nakken and Olsen (1977).

The echo-sounder was set up with a ping repetition frequency (prf) of about 5.5 s^{-1}. The targets were moved with a speed of about 3.6 cm.s^{-1} giving 20 pings cm^{-1}.

Post-processing of the data was done with the Sonar5-Pro package (Lindem Data Acquisition, Oslo, Norway). The system was set up for single-echo detection and tracking. Transducer depth and tilt was put into Sonar5-Pro for each experiment, enabling the software to directly plot the tracked targets depth, uncompensated target strength (TSu), beam pattern compensated target strength (TSc), and angular positions as functions of range relative to the surface. The data from the tracked targets was exported to Microsoft Excel for further studies and for producing the figures presented here. No ping to ping averaging was applied in the analysis of the oscillations, but for the presented figures we applied a running mean window to reduce noise. The length of the running window was set to cover 20 cm of the targets path estimated from the recording ping repetition frequency and target velocity.

2.2. Simulations

To compare the theory and measurements, we wrote a simple ray-based simulator in Pascal language. We described the geometry with the transducer, surface and a target in a xy–coordinate system where y describes the depth and x the distance along the surface and looped the calculations for increasing range. In this 2D world, we allocated point positions for emission reflection and receiving sound. For the sonar equations we applied the point source, point target and plane wave assumptions. Assuming specular reflection and using the law of reflection enables calculation of reflection points at the surface for the rays between the transducer and the target points. For the surface reflection points, we applied a simple constant damping, 180° phase shift and pressure release model.

The transducers beam pattern was included through a model of the transducer, providing simulated angular dependent intensity for the outgoing and incoming rays. The ES120-4x10 transducer is designed with 108 weighted elements equally spaced 1.05 cm apart from each other on the transducer surface (Fig. 2). We estimated the resulting sound intensity with a resolution of 3600 × 3600 points and down sampled the obtained beam pattern to 3600 points along the central vertical axis. The simulated beam pattern is compared with the measured beam pattern for the applied transducer in Fig. 3. The basis for the code for this transducer model was originally provided by Helge Bodholt from SIMRAD and can be found in Kjølerbakken (2003).

To find the received Echo Level and angular position of the target we allocated three points on the transducer face, one central point for emission and for detecting the amplitude, and two points 5 cm up and down from the centre point to find the angular target position. Each of these three points received the echo from the four rays that hit them according to:

$$p(t) = e_1(t, t_i)a_1\cos(\omega t - kR_1) - e_2(t, t_i)a_2\cos(\omega t - kR_2)$$
$$-e_3(t, t_i)a_3\cos(\omega t - kR_3) + e_4(t, t_i)a_4\cos(\omega t - kR_4) \qquad (1)$$

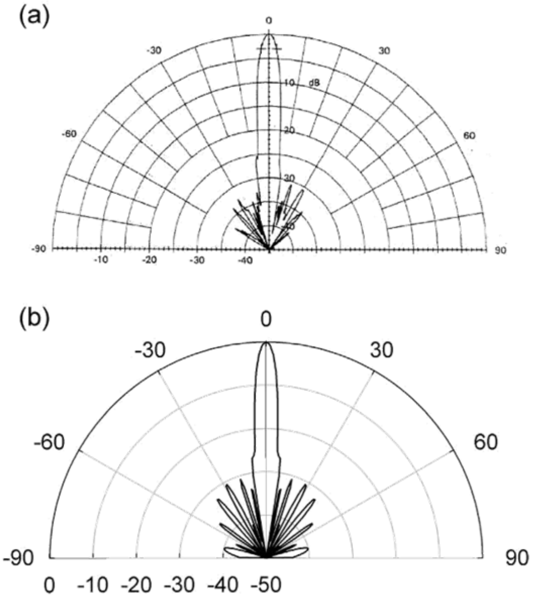

Fig. 3. Comparing measured and simulated beam patterns: Beam patterns of the elliptic ES120-4x10 transducer seen in the y-axis domain (along-ship domain according to Simrad's terminology), (a) measured by the manufacturer and (b) simulated.

where *p* is the received pressure in Pascal at any instance of time t. The angular frequency and the wave number are denoted *k* and *ω* respectively, while *R* is the range or distance that the ray has travelled on the way to and from the target. The e_1 to e_4 are the envelope functions described below and a_1 to a_4 are the peak amplitudes of the pressure for the four ray combinations hitting each receiver point. These amplitudes depend on the source level, the outgoing and incoming ray angles relative to the tilted transducer's beam pattern, the transmission loss along the ray paths, and the surface reflection coefficient. Index 1 indicates the direct ray while the rest are surface reflected rays as seen in Fig. 4. The plus and minus signs occur due to whether the ray has been shifted 0, 180 or 360° by the surface.

For a pulsed system, each incoming ray will have a limited duration. To simulate the returned echo pulse, we sampled, normalized and linearized echoes from the recorded data to obtain a "standard" echo pulse. A sinusoidal model was fit to this pulse and applied so that the half power of the sinusoidal had the same duration as our standard pulse. This gives the following envelope function e_i

$$e_i\left(t'\right) = \begin{cases} \sin^{1.7}\left(\dfrac{t'}{u}\cdot\pi\right) & t' \in \quad [0..\ \ u] \\ \\ 0 & t' \notin \quad [0..\ \ u] \end{cases} \tag{2}$$

where t' is a clock variable starting at zero upon the arrival time of the first ray and then running until all rays have arrived and passed. The variable u is a scaling factor ensuring that the envelope function gets the same pulse width at its 50% value as the duration of the standard pulse at the same level. The subscript i is the index of each of the four rays that hit a point at the transducer surface (Fig. 4).

The sum of the four rays, p(t) in Eq. (1), arriving at the simulator's three receiving points were handled by peak detectors. The peak value from the transducers centre receiving point was applied for estimating the received echo level (EL) and target range. The timing of the peak of the echoes obtained for the upper and lower receiving points were applied for estimating the targets off-axis angles in the simulated y-domain. TSu and TSc are found according to the classical point source sonar equation (Urick, 1983):

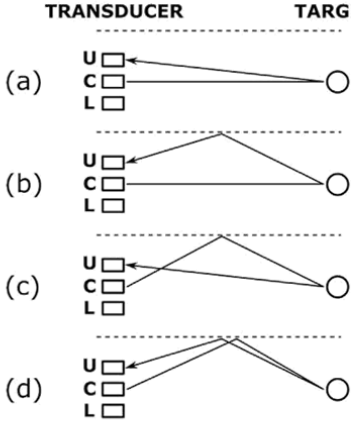

Fig. 4. Four paths between transducer and target: Schemes depicting four scenarios of multi-path signal propagation for a single ray when emitted by the central element (C) and received only by the upper element (U) of a transducer. Each transducer's element receives rays travelled along four different paths: (a) direct paths for both emitted and reflected signals, (b) only a direct path for the emitted, (c) only a direct path for the reflected, and (d) no direct paths. In the case of three elements with the transducer's central element being the only emitter, 12 variant rays have to be simulated. The rays emitted and received by the central element are applied to estimate the echo level. The rays received by the upper and lower (L) elements are applied for phase detection.

$$\begin{aligned} TSu &= EL - SL + 2TL(R) \\ TSc &= TSu + 2GC(\varphi) \end{aligned} \tag{3}$$

were subscript c and u is applied to distinguish between off-axis compensated and not off-axis compensated TS respectively. TL is the one way Transmission Loss, associated with a time variable gain function (TVG). It includes both geometric spreading and absorption loss. GC is the gain compensation function as applied by Simrad in their EK60 echo-sounder. GC takes the angles obtained by the phase detector and applies an approximated Bessel function to find the off-axis compensation (Kieser and Ona, 1988).

From the fixed transducer depth and transducer tilt, and from the simulated target range and measured off-axis angle φ, we can find a simulated estimate for the target depth according to

$$TaD = TrD + R \cdot \tan(TrT - \varphi) \tag{4}$$

where TaD is the target depth estimate, TrD the transducer depth, R the estimated distance to the target, all expressed in meters. TrT is the transducer tilt applied in the simulation.

3. Theory

3.1. Surface reflection

If the sound from the transducer hits a smooth surface, then nearly all sound will be reflected back to the water, independent of the grazing angle. The reflected sound will have a phase shift of 180°.

Due to the difference in acoustic impedance between water (Z_W) and air (Z_A), the surface forms a near perfect pressure release boundary. For air we have $Z_A = \rho c = 420 \quad Pa \cdot s/m$ while for water $Z_W = 1500000 \quad Pa \cdot s/m$ (ρ is density in $\text{kg}\,\text{cm}^{-3}$ and c is sound speed in $\text{m}\,\text{s}^{-1}$). According to Blackstock (2000), the proportion of reflected and propagated sound can be calculated from the impedances in the following way:

$$s_r = \frac{(Z_W - Z_A)^2}{(Z_W + Z_A)^2}, s_p = \frac{4Z_A Z_W}{(Z_A + Z_W)^2} \tag{5}$$

where s_r denotes sound reflected back and s_p sound propagated into air. Applying Z_A and Z_W for water and air, we see that nearly all sound is reflected ($s_r = 0.998$) and very little sound propagates into the air ($s_p = 0.001$).

For a pressure release boundary, the sum of incident and reflected sound pressure is zero ($p_i + p_r = 0$). Eckhart (1953) used this approximation to develop equations for estimations of water surface back-scattering under various conditions. The approximated equation shows that the phase of the reflected pressure wave will be shifted by 180° relative to the incident wave.

Reflection is also a function between sea state and grazing angle. Urick (1983) uses the Rayleigh parameter Ry and describes the surface as perfect reflector if Ry « 1. Ry is defined as:

$$Ry = kH\sin(\theta) \tag{6}$$

where k is the wave number, H the root mean square value of the wave height (crest to trough in meters) and θ is the grazing angle in degrees. We see that Ry goes to zero and becomes independent of the grazing angle when H goes to zero. According to the law of reflection, the angle of the reflection will be equal to the sound's incident grazing angle.

When Ry increases, the water surface will start to scatter and send incoherent sound in all directions. Fortuin (1969) provides a good summary of 87 cited references regarding surface reflection and scattering. Most are theoretical works, but Chapman and Scott (1964) followed Eckhart's work and found practical equations. Chapman and Scott's equation takes the grazing angle and the average slope of the waves as input. Moreover, Cox and Munk (1956) provide a link between the wind speed and the wave slope utilizing the sun glitter. Furthermore, Marsh (1961), Marsh et al. (1961) and Marsh (1963) also made considerable contributions to modelling the water surface scatter under various conditions.

3.2. Multi-path and interference pattern

Interference patterns for waves following a direct and an indirect path were first investigated by Humphrey (1831). Humphrey did his work on light demonstrating that light could be described as waves and that interference patterns in the form of cancellation points would occur when two light rays 180° out of phase met.

Young (1947) and Urick (1983) examined this for underwater sound. Young tested frequencies between 0.2 and 22.5 kHz with a hydrophone moved away from the source and reported observed interference pattern for the lower frequencies. He also developed a model for the interference including the refraction. Urick referred to experiments done during the Second World War. In these experiments, a sound field from omni-directional sources placed near the surface was measured with hydrophones moving away from the source. Strong variations in sound intensity were observed as a function of distance to the source.

Hence, surface interference by multi-pathing is a well know phenomenon. What was not clear to us was whether and to what extent the surface would influence on the returned echoes from small targets when strongly directive split-beam transducers were mounted under the surface, and aimed nearly horizontally, slightly tilted down to avoid hitting the surface (usual deployment in horizontal fishery applications).

If multi-pathing occurs, the transducer would receive an echo directly from the target and a delayed echo from the surface. Arrival time, amplitude and phase would depend on geometry and the transducers directivity. Waves arriving at overlapping time will be summed and result in a signal modified in amplitude and phase.

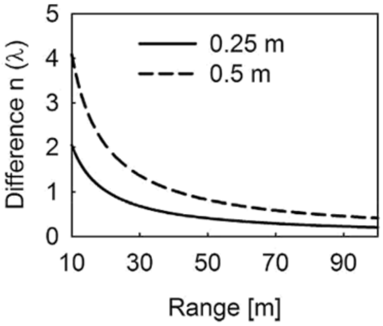

Fig. 5. Difference in ray lengths: Range-dependent differences between travelled distances of direct and indirect sound rays, measured in a number of wavelengths (n), for the target at depths of 0.25 and 0.5 m below the surface. The transducer was located 0.5 m below the surface.

With multi-pathing, a target moved away from the transducer in the surface layer can theoretically pass through three zones depending on the pulse duration and the transducer and target depth: 1) Near the transducer, the surface reflected echo will arrive as a weak signal after the direct echo. No interference will be seen. 2) With increasing distance, the two echoes will start to overlap and cause increasing interference. Constructive and destructive interference will occur with increasing range until n in Eq. (7) below becomes less than 1/2. 3) Further away the travelled distances for the two waves will be more and more equal causing slowly increasing destructive interference.

$$R_i = R_d + n\lambda \qquad \Leftrightarrow \qquad n = \frac{R_i - R_d}{\lambda} \tag{7}$$

Here R_i is the length of the indirect ray while R_d is the length of the direct ray. λ is the wave length, and n is the difference in travelled distance measured in wave lengths. Fig. 5 shows the factor n as a function of range for two pair of depths for transducer and target. The intensity of the surface reflected rays will, close to the transducer pass in and out of the transducers side lobes while at long range it will approach the side of the main lobe depending on the tilt of the transducer.

3.3. Interference influence on the split-beam positioning system

When sinusoidal waves with the same frequency, but with different amplitudes and phases are mixed, they cause a resulting sinusoidal wave with the same frequency, but with modified phase and amplitude. A split-beam transducer receives the signal at an upper and lower receiver face and applies the phase difference to detect the target's position relative to the acoustic axis. Even small contribution from a surface reflected wave can cause a phase shift that will corrupt the split-beam positioning system. For the horizontal application, this leads to incorrect target strength and target depth estimates. The off-axis angle estimate is applied in the echosounders beam compensation function and will thereby also lead to incorrect off-axis compensation of the measured target strength.

4. Results

4.1. The three signal zones

Both experiments and simulations showed that a smooth surface had a significant influence on the echo-sounder's ability to estimate the correct TS and target depth both for standard targets

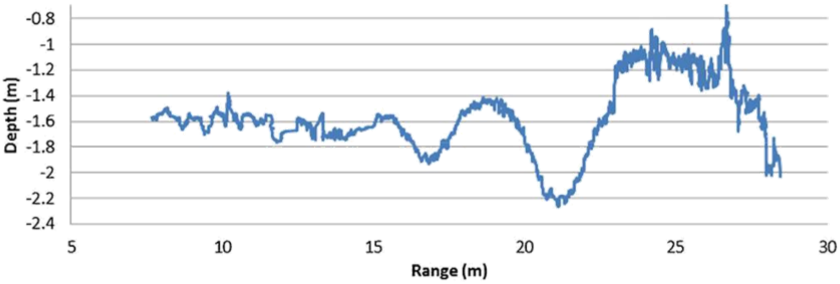

Fig. 6. Observed fish depth vs range. Split-beam along ship angles converted to depth for an anesthetized common bream of 340 mm standard length. The fish was mounted in the side aspect at a fixed depth of 150 cm and moved away from the transducer.

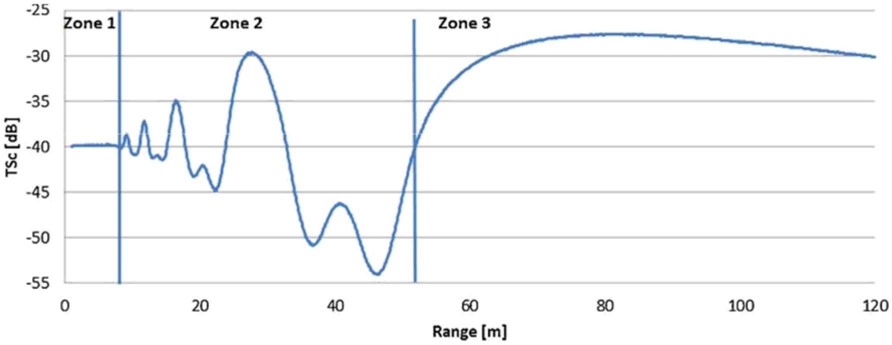

Fig. 7. Three zones with different modification of the signal: Simulation of the off-axis compensated target strength (TSc) for the standard target (−40.2 dB) demonstrating the three different signal zones. The transducer was mounted 0.5 m below the surface and tilted 2 deg. down. The target was moved away from the transducer at a constant depth of 0.5 m.

and for the tested fish (Figs. 6 and 7). The target moving away from the transducer at a fixed depth past the three theoretically expected zones. In Fig. 7 we see zone 1 close to the transducer with correctly estimated TS and depth, zone 2 where TS and depth oscillates, and finally zone 3 with incorrect TS and depth, but without oscillations.

For the experiments, the errors disappeared when wind stirred up the surface. In the simulator, the phenomenon disappeared when the surface reflection coefficient was set to zero.

4.1.1. Zone 1 near the transducer

Zone 1 is characterized with correct and non-oscillating TSc and correct off-axis angle φ. According to the theory we should see a strong echo from the direct path followed by a weak echo from the surface reflection arriving after the main echo. We could see this on the simulations, but not on the recorded data. We assume the signal was too weak to be detected by the echo-sounder.

4.1.2. Zone 2, the oscillation zone

Both measurements and simulations show the same trends in this zone. The start of the zone can be characterized by small oscillation amplitude with a relatively high oscillation frequency. When moving the target away from the transducer, the amplitude increases while the frequency decreases until a final cycle marks the end of the oscillation zone. Both the target and the transducer depth influenced on the start and the extent of this zone increasing with increasing depth. Fig. 8 demonstrates this for a target and transducer simulated at various depths. With increasing depth, the end of zone 2 increased more than the start, resulting in an elongation of zone 2 relative to shallower mounted transducers and targets.

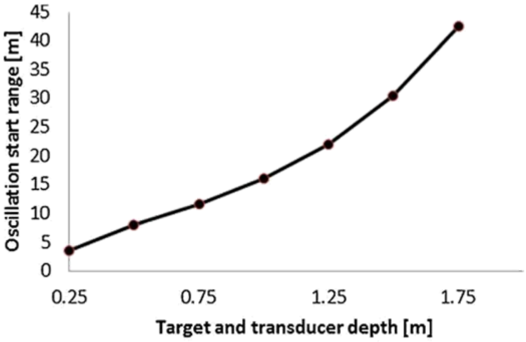

Fig. 8. Oscillation start range: Simulated start range of the oscillation versus increasing transducer and target depth. Start range is defined as the distance between the transducer and the first significant peak in the in simulated target strength.

4.1.3. Zone 3, relaxation zone

In zone 3 the, target strength and angle estimates should still be influenced by interference from the surface, but without oscillations. From a maximum value at the start of the zone we should expect slowly declining TS with increasing range. In the beginning of the zone TS would be too high due to constructive interference. When the indirect path becomes ¼ of a wavelength longer than the direct path destructive interference will occur and TS will continue to drop, now below the correct value.

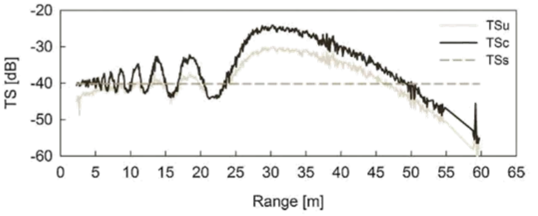

Fig. 9. Measured oscillation of compensated and uncompensated TS: In-situ measurements of the acoustic target strength: uncompensated (TSu) and compensated (TSc). TSs indicates the theoretical value of the standard target. The transducer was placed at a depth of 0.5 m and tilted two degrees down. The target was moved away from the transducer at a constant depth of 0.5 m.

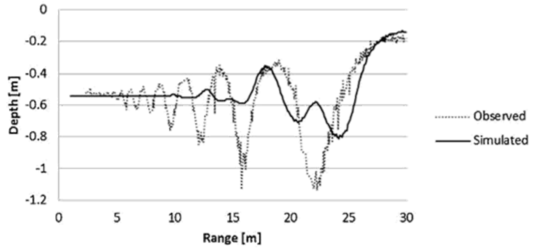

Fig. 10. Comparing simulated and measured target depths: Observed and simulated depth of the standard target with the transducer placed at a depth of 0.5 m and tilted 1.2° down. The target was moved away from the transducer at a constant depth of 0.5 m.

Zone 3 was difficult to measure with the echo-sounder due to noise. We tempted to record data out to 60 m but in general the target disappeared in noise short after a range of 30 m range. In Fig. 9 it may look like the TS peaks for the last time at about 30 m from the transducer, but according to the simulations in Fig. 7 there should be one more peak around 80 m.

4.2. *Influence on the split-beam position estimate*

Applying the phase detector to the simulated signals received at the upper and lower part of the transducer face results in angular target position estimates. From the applied transducer depth, tilt and off-axis angle φ, we can estimate the target depth. Fig. 10 shows simulated and observed target depths for a particular case. Although the target is physically located at a fixed depth of 0.5 m, both the measurement and simulations show oscillating target depths with range. Moving the target from 17 to 23 m causes the simulator to estimate a fluctuation in estimated target depth of about 35 cm. The experimental data show even higher fluctuation. Compared with the height of the beam (120 cm at 20 m range) we see that this fluctuation is significant. The results are ruled by the geometry in the setup. Changing depths or transducer tilt will result in other values.

Since the split-beam echo-sounder uses the detected angles to compensate TS for the off-axis loss, TSc can end up more incorrect than TSu. For example, if the target actually is in the centre of the beam while the phase detector incorrectly positions it above or below, then compensation will add incorrectly to the measured TS. This is demonstrated in Fig. 9 where, at certain ranges such as the ~19 m, the TSu is correct while TSc is about 3 dB too high.

5. Discussion

This exercise demonstrated clearly that a smooth surface can introduce errors when acoustics is applied to monitor fish horizontally in the surface layer, even when the beam is tilted away from the surface. The theory predicts that three zones should exist. The in-situ experiments showed two of the zones while the simulator showed all three. The experimental data did not have sufficient range to clearly show the last zone. Hence it does not contradict the results from the simulator. The results indicate that our understanding of the problem is correct.

The influence of the surface depends not only on the depth and tilt of the transducer but also on the depth of the target. A scientist using horizontal echo-sounder applications near the surface should be very careful surveying water bodies when the surface is smooth. Smooth surfaces are common for many narrow lakes especially during night time when such surveys often are conducted. Moreover, a smooth surface has also been regarded as optimal for horizontal surveys since it provides better stability for the beam and less acoustic surface noise. The implications of significant (up to +/−10 dB) biases in TS can be serious, particularly if the TS is used to estimate fish length through some kind of empirical relation (e.g. TS = A*log[length] + B). While there are also clear depth biases, a few decimetres error in fish depth is less likely to be of concern. The same possibly applies to echo integration as the amplitude bias is both increasing and decreasing thus compensating each other.

Some discrepancies were seen between the results from the simulator and the experimental data with respect to the start of the zones, the frequency in the observed TS and the TS and depth oscillation amplitudes. Discrepancies were expected due to the simplicity of the simulator such as the applied point source, point surface reflection and point target assumptions.

In the presented simulations we applied a fourth ray being reflected by the surface two times. This fourth ray arrives later than the other rays and it has the same phase as the direct wave. If we look at the results in Fig. 10 we see that the simulated target position rises in the water at 22 m range while the experimental data show the opposite. Turning off the forth ray in the simulator resulted in a similar dip as seen in the measurement at this range. This may indicate that the fourth ray does not arrive as we assume.

Refraction is another effect not included in the simulator. Temperature profiles were measured and ray tracing showed that the sound beam slowly bent down in the water. This bending will cause the echo-sounder to observe that a target moved to longer range also will appear with a slow rise in the water. Refraction does also misshape the beam to some extent and thereby the assumption of spherical spreading. The ray tracing showed a slight reduction in the beam with. This fools the echo-sounder to apply a too large transmission loss TL. According to Eq. (4) this lead to an overestimate of TS. We did see indications of this effect in some of the experimental data.

The purpose with the simulator was not to fully recreate reality, but to assist in verifying the theory and link it to the observations. Although simple, it demonstrated the three zones and predicted the effect of varying transducer and target depth and transducer tilt.

Calm weather has been assumed superior to rough weather for the surveys with horizontal sonar (Trevorrow, 1998; Simmonds and MacLennan, 2005). Our experiments showed that the mirror reflections can be more serious than the noise from a slightly wavy surface. Even quite small ripples on the surface were sufficient to remove the interference problem and stop the oscillations. Very calm weather with a mirror surface should therefore be avoided for the surveys. When the surface is very calm, it may be tempting to think that one would be able to get around the phenomenon by stirring up the surface mechanically. A simple way would be to align the sound beam under the boats bow wave, to disturb the surface with other boats or to spray water on it. It remains to test if this can work and to test if this will influence on the fish behaviour during the monitoring. Applying transducers with better side lobe suppression than ES120 4x10 transducer should also be tested.

The first zone is free from interference and it is tempting to think that this may be applied for monitoring. The zone can be maximized by shortening the pulse length and lowering the transducer. But the target depth does also influence on the range of the zone. Since the range of zone get shorter when targets approach the surface, the shallowest targets will limit the possible range to be surveyed. Lowering the transducer will help, but at the same time reduce the observable part of the surface zone.

6. Conclusions

First, researchers using horizontal beaming applications to cover the surface layer must pay attention to interference and seek to avoid surveying when the surface is smooth.

Next, more experimental work is needed in order to give guidelines for horizontal beaming under smooth surface conditions. Different transducers with extreme side lobe suppression should be tested. It is also important to test various sea state situations to find when it is safe to use the application. Experimenting with mechanical solutions for rippling the surface should also be tested, such as mounting the beam under the boats bow wave or letting other boats stir up the surface.

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Paper II

A novel upward-looking hydroacoustic method for improving pelagic fish surveys

(Original pages 1-12)

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A novel upward-looking hydroacoustic method for improving pelagic fish surveys

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For ethical reasons and animal welfare, it is becoming increasingly more important to carry out ecological surveys with a non-invasive approach. Information about fish distribution and abundance in the upper water column is often fundamental. However, this information is extremely hard to obtain using classical hydroacoustic methods. We developed a rigid frame system for pushing upward looking transducers of the scientific echo sounder (38 and 120 kHz) in front of the research vessel. The efficiency of the new approach for monitoring juvenile fish at night was investigated by comparing the results with a quantitative fry trawl in the Římov Reservoir in the Czech Republic. The experimental setup enabled comparisons for the 0–3 m and 3–6 m depth layers, which are utilized by almost all juvenile fish in summer. No statistically significant differences in the estimated abundance of juveniles were found between the two sampling methods. The comparison of abundance estimates gathered by the two frequencies were also not significantly different. The predicted mean lengths from acoustic sampling and the trawl catches differed by less than 10 mm in all comparisons. Results suggest that mobile hydroacoustic upward-looking systems can fill the methodological gap in non-invasive surveying of surface fishes.

Pelagic layers often represent the biggest volumes of large waterbodies. Surface layers (epilimnion) receive the most sunlight and are in contact with the atmosphere[1]. Very often, it is the most productive layer of the water column, and unlike the deeper layers, contains most phytoplankton, zooplankton and fish[2,3]. Freshwater fish dominance near the water surface mainly at night can be found almost everywhere in the world, for example, Europe (The Czech republic, Germany, France, Hungary, Norway, Poland, United Kingdom, Switzerland etc.)[4–11], North and South America (United States of America, Canada, Argentina)[12–14] or in tropical areas (Sri Lanka, Thailand)[15,16].

Because of their high abundance, juvenile fish play an essential role in food webs and can indicate the future development of the fish stock as a whole[17]. In lakes and reservoirs, juvenile fish often hide in littoral habitats or use benthic refugia during the day and spread to the open water at dusk to utilize pelagic food resources[5,9,18]. During the growing season, most fish use the upper layers in mesotrophic and eutrophic waters, which are the warmest, the most productive and unlike the deeper layers have no limits with respect to dissolved oxygen levels[4,19]. Juvenile fish also occur in the upper layers of oligotrophic lakes at night to follow vertical migration of zooplankton and to reduce their vulnerability to piscivorous fish[13,20,21]. Up until now, the only established quantitative method to study small fish in the upper waters was night trawling[22]. However, this method is labour-intensive, disrupts fish in their environment and may injure or kill juvenile fish.

Hydroacoustic methods are becoming increasingly popular because they can sample large volumes of water relatively quickly, are non-invasive and are non-lethal to the aquatic organisms being studied. The most commonly used acoustic approach is using downward-looking transducers, which beam from the surface to the bottom (i.e. from a boat or other platform to the deepest point). However, there is a blind zone at the surface created

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survey cruise	depth 0–3 m	depth 3–6 m
38 kHz (night 1–3)	390/497/550	39/82/43
120 kHz (night 2 and 3)	437/704	15/21
Fry trawl (night 1–3)	488/1096/550	24/164/93

Table 1. Numbers of acoustically detected fish tracks and fish captured by the fry trawl. The results for individual nights are separated by /.

	depth 0–3 m				depth 3–6 m			
	Upstream		Downstream		Downstream		Upstream	
Sampling volume (m^3)	38 kHz	120 kHz[a]	38 kHz	120 kHz[a]	38 kHz	120 kHz[a]	38 kHz	120 kHz[a]
Counted as wedge	52,700	17,300	54,800	18,400	33,700	11,200	33,800	9,200
Trawl	85,500	85,500	NA	NA	74,300	74,300	NA	NA

Table 2. Sampling volume of each cruise. [a]Only night 2 and 3, NA – not applicable.

by the depth of the deployed transducer (at least several cm) and the physical near-field where the acoustic beam is not fully formed[23]. Additionally, near the transducer, the sampling volume is very low and provides a very limited coverage of the near-surface layers. In stratified lakes, fish often occur only a few centimetres to a few metres under the surface and, for this reason, the downward-looking approach does not provide reliable data near the surface and is often replaced by horizontal echo sounding[24, 25].

Horizontal echo sounding, also called horizontal beaming, covers the surface layers well but has several major shortcomings. The most critical problem is that the estimated size of fish changes at different orientations relative to the transducer axis, the so-called side aspect[26, 27]. The determination of abundance and size is often performed using deconvolution[25], which is based on stochastic assumptions of random aspect orientation that may not be entirely true[28]. Moreover, upon the establishment of thermal stratification, the acoustic beam can bend due to the effect of water temperature on the speed of sound on the edge of beam at different temperature layers[29], thus complicating the definition of sampled volume. New findings on the multipath signals in the horizontal beam show strong interference near the surface[30]. Higher acoustic noise levels due to reverberation also complicate the detection of mainly small fish with horizontal beaming[28].

A potential solution to address the aforementioned disadvantages of both downward-looking and horizontal beaming is an upward-looking system, where the transducer is oriented vertically, but the direction of beaming is from the water column towards the surface. This arrangement makes it possible to record fish in the near surface layer and to accurately determine their size. So far, this type of system is mostly restricted to stationary locations where the transducer is fixed to the bottom of the water body and continually samples the same volume[31–33]. One of the earliest mobile uses of an upward-looking system was by Probst[34] who used a towed upward-facing transducer to study juvenile fish. However, towed systems are very sensitive to direction change and reduce the manoeuvrability of the towing vessel; in their case, a radius of approximately 250 m was required to change the direction of the vessel. It is also possible to use Remotely Operated Vehicles[35], but their costs and the risk of collision with irregular bottom are still high. To circumvent these disadvantages, we developed a rigid upward-looking system located in front of the survey vessel to overcome all the sampling shortcomings described above. The aim of this study was to compare difference in abundance and size distribution between a new mobile upward-looking acoustic system and quantitative night fry trawling. On the acoustic side of the experiment we employed a frequency commonly used to detect small fish with narrow beam (120 kHz) side by side with the frequency mostly used for large fish with wider beam (38 kHz). Pros and cons of the two approaches are being compared.

Results

The number of fish captured in the two sampled layers depends on the true depth distribution of fish (Table 1). Based upon trawling the number of fish in the 0–3 m layer was about eight times higher than in the 3–6 m layer. With acoustic data this ratio is even higher due to the beam morphology which is wider in the shallowest layer (larger sampling volume, Table 2). During one cruise the acoustic wedge volume was smaller than the volume sampled by the 3 × 3 m trawl. However, the echosounder sampled both layers at a time while the trawl only sampled one. The volume sampled by the 120 kHz system was considerably smaller due to its smaller beam dimension and the fact that it was only used during two sampling nights.

The dominant trawling catch species in the 0–3 m depth layer were roach (*Rutilus rutilus*), common bream (*Abramis brama*) and bleak (*Alburnus alburnus*) while perch (*Perca fluviatilis*), pikeperch (*Sander lucioperca*) and bream dominated in the 3–6 m depth layer (Supplementary Table S2).

Fish length frequency distribution. The new hydroacoustic system was able to record many sizes of fish (Supplement Fig. S1). Selecting a lower threshold was difficult on night 1 (19/20 August 2013) because of the high occurrence of newly-hatched fry measuring 15–25 mm (Fig. 1). For 2014 data it was easier to define troughs in the size frequency distributions because the YOY fish were considerably larger (30–50 mm).

When all YOY fish lengths were compared using both methods (upward-looking hydroacoustic system at a 38 kHz frequency and fry trawl), the observed size overlap was generally high (Fig. 1). The size distributions differences were statistically significant from zero for the 0–3 m depth layer but mostly not significant for the 3–6 m

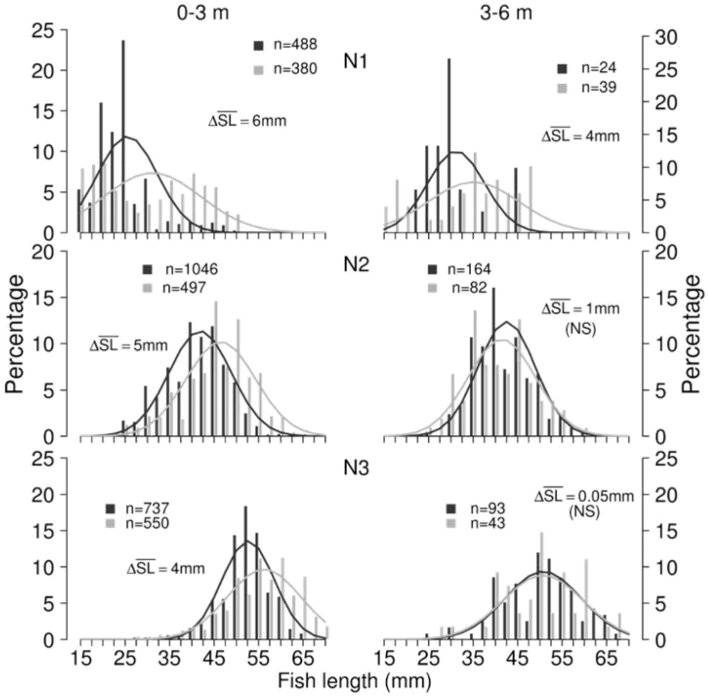

Figure 1. Length frequency distribution of juvenile fish on each night. Numbers of fish in the trawl catches (black) and tracked fish in the 38 kHz acoustic records (grey) are provided for each night and each depth layer. ΔTL stands for the difference between average acoustic and trawl length. NS – nonsignificant difference.

depth layer (Table 3). When comparing the length frequency distribution on different nights, we found that mean length of juvenile fish in the trawl were significantly smaller than from the hydroacoustic method in the 0–3 m layer, (night 1:6 mm mean difference, night 2 and 3:5 and 4 mm mean difference respectively; Fig. 1). In the 3–6 m layer the mean length of juvenile fish was significantly smaller in the trawl only in one case (night 1:4 mm mean difference, other nights' sample the differences were negligible, Fig. 1, Table 3).

In 2014, the juvenile pelagic community was also investigated using a 120 kHz echo sounder. When comparing the length frequency distribution by the trawl to the one reconstructed from the 120 kHz frequency acoustic results we can see reasonable overlap again. The difference between the trawl and the acoustic results was quite similar to that obtained with the 38 kHz system (acoustics record bigger fish in night 2:1 mm and night 3:4 mm, for depth 0–3 m and d 7 and 10 mm for 3–6 m, with the limitation of smaller numbers, Fig. 2). Despite the high overlap of the two distributions, the differences were statistically significant.

Fish abundance. In the 0–3 m depth layer, abundance estimates obtained using both methods (acoustic frequency 38 kHz and trawl) in all zones during all sampling dates showed similar trends with lower density in the dam area (Fig. 3), and no significant differences were found between both methods (Table 3). Abundances estimated by acoustics and trawl were close to 1:1 line but in zones with higher densities of fish, the acoustic track counting tended to underestimate the abundance in the 0–3 m depth layer (Fig. 4). In the 3–6 m depth layer, differences between the two sampling methods were more common (Fig. 1 and Table 2), but in general, the slope of the relationship was not different from the 1:1 line (Fig. 4 and Table 3).

Upstream and downstream acoustic cruises again showed similar trends in fish abundance (Fig. 5). No statistical difference in abundance was found between these two cruises (Table 3).

TS distribution. Comparison of TS distribution from the two acoustic frequencies showed a rather similar pattern especially in N3 depth of 0–3 m (Supplementary Fig. S2). However, TS distribution was still significantly different (KS test $p < 0.001$, for N2 and N3 in depth 0–3 m). TS frequency distributions in depth 3–6 m were not significantly different (KS test $p > 0.05$). Fish length predicted from 120 kHz records were on average several mm

Compare	Test	Df	p-value*
0–3 m slope — 1	slope estimate	15	< 0.001
3–6 m slope = 1	slope estimate	12	>0.025
0–3 m abundance	Ks.test	17;17	>0.025
3–6 m abundance	Ks.test	14;14	>0.025
0–3 m abundance upstream and downstream cruise	Ks.test	17;17	>0.025
3–6 m abundance - downstream and upstream cruise	Ks.test	17;17	>0.025
N1_0–3 m length dist.	Ks.test	488; 380	<0.008
N1_3–6 m length dist.	Ks.test	24; 39	<0.008
N2_0–3 m length dist.	Ks.test	1046; 497	<0.008
N2_3–6 m length dist.	Ks.test	164; 82	>0.008
N3_0–3 m length dist.	Ks.test	737; 550	<0.008
N3_3–6 m length dist.	Ks.test	93; 43	>0.008
N2_0–3 m length dist. 120 kHz	Ks.test	1046;437	<0.013
N2_3–6 m length dist. 120 kHz	Ks.test	164;15	<0.001
N3_0–3 m length dist. 120 kHz	Ks.test	737;704	<0.001
N3_3–6 m length dist. 120 kHz	Ks.test	93;21	<0.001

Table 3. Statistical results for juvenile abundance and size comparison between methods. N1, N2 and N3 refer for night 1, night 2 and night 3, Ks.test refers to Kolmogorov – Smirnov test, dist. – distribution, *Bonferroni correction (0.025, 0.0125, 0.0083, respectively 2, 4, 6 same test).

smaller than from 38 kHz records (Supplementary Fig. S6) and therefore they are a bit closer to the sizes of trawl caught fish (Figs 1 and 2).

Discussion

Results show that our novel upward-looking system and fry trawling provided comparable estimates of YOY fish abundance, as well as similar size distributions. Acoustic detection of small fish is to a great extent a question of a) signal to noise ratio (SNR) and b) of "linearity" in the regression function between log fish length and TS toward the lower end of the fish size spectrum[36]. When sampling a wind protected lake on calm nights at short ranges 0–6 m, the acoustic environment was extremely clean (noise level < -75 dB). The system could reliably detect the smallest fish in the water column at both frequencies tested.

The majority of acoustic data were collected with a 38 kHz transducer, a device that is scarcely used in freshwater systems. The most important reason for applying this frequency was the transducer's opening angle of 12 degrees, which was, the widest split-beam transducer available at the time. Because even wider 38 kHz transducers are developed (Frank Knudsen, Kongsberg – Simrad, personal communication), these kinds of transducers are very promising for upward looking applications in the near future. The sampling volume when compared to the common 7-degree transducer is about three times larger (Table 2). 38 kHz has not been tested extensively for detecting small fish. That we could see fish well with this low frequency can be a bit surprising since theory states that targets should be generally bigger than the wavelength[36]. For 38 kHz the wavelength is about 40 mm, which is more than double the length of the commonly observed 15 mm fish, and much longer than the swimbladder for these fishes. Much of the theory describing this is, however, related to solid rigid spheres stating that targets smaller than the wavelength are in the Rayleigh scatter zone[37]. Fish are, however, not fixed, rigid, spheres. The swimbladder is shaped more like an ellipsoid than a sphere and according to Medwin *et al.*[38] we can calculate a radius for an equivalent sphere. Doing this for fish as small as 15 mm, we find that we stay well within the safe zone for detecting small fish with the 38 kHz transducer[38]. We also did a trial with a 120 kHz system running in parallel with the 38 kHz system and verified that the two systems gave similar results. When Love[39] did his work on TS regression for 38 kHz he also included fish down to 15 mm, and reported detecting them without problems.

The mean lengths of trawl-caught fish and those predicted by the upward-looking method differed by less than 10 mm. In all synoptic comparisons fish sizes predicted from acoustic data usually have a wider spread (higher variance) when compared with direct catch measurements[10–12]. Our results show this trend only to a small extent (Fig. 1). The largest potential source of error for predicting fish size from acoustic records is the TS-length regression. We have used two published regressions available to predict length. One was 38 kHz general multispecies regression for a dorsal aspect[39] and the other 120 kHz regression for perch[43]. Several millimetres differences between predicted lengths for acoustic sampling and trawl-caught fish lengths (Figs 1 and 2) supports the need for more aspect-, frequency- and species-specific regressions between TS and fish size in the future. More attention should be paid to the experimental conditions (free swimming fish should be preferred to tethered and stunned individuals) which could influence the regressions[23]. The tank experiments of free-swimming larvae that would include repeated measurements of larvae and juveniles as they grow through time would be the ideal way to develop better models relating TS to length.

We did not find any significant differences in the abundance estimates obtained from the acoustic method and from the trawl. Furthermore, the regression curve correlating them was not significantly different from 1:1 in the 3–6 m depth layer. However, we have found that the slope was significantly different from 1:1 in the 0–3 m depth

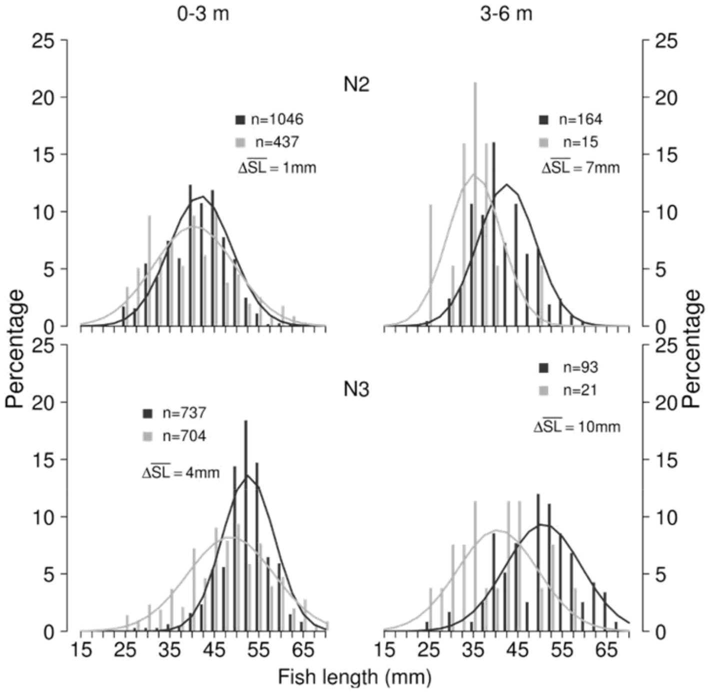

Figure 2. Length frequency distribution of fish from 120 kHz echo sounder and trawling. Numbers of fish in the trawl catches (black) and tracked fish in the 120 kHz acoustic records (grey) are provided for each night and each depth layer. ΔTL stands for the difference between average acoustic and trawl length.

layer. This difference was probably caused by high densities of fish that were not recognized as single targets in the acoustic tracks. This result can be caused by overlapping echoes that do not satisfy SED criteria and cannot be tracked[23,44]. So even at relatively small sampling volumes, there may be a need to use echo integration in addition to single target analysis to estimate the total abundance in dense fish communities. This is supported by higher values of Sawada index in some observations (Supplementary Table S3)[45]. Echo integration gave abundance estimate that were higher but not significantly different from the trawl (Supplementary Fig. S3, KS. test $p > 0.05$ for both depth). However with echo integration of small targets another challenge emerges with the need of laborious and potentially subjective removing of fish larger than the targeted YOY group[46]. Therefore, we consider the track counting to be generally more accurate for estimating larval fish abundance.

Although fish distribution had a relatively simple longitudinal pattern in the Římov reservoir (steady increase in fish abundance from the dam towards the tributary, Figs 3 and 5), this pattern was not identified over all surveys. The discrepancies between hydroacoustics and trawling results can be caused by the fact that despite the two sampling boats following very similar trajectories, the trajectories were not completely identical in space and time. Disturbing effect of passing boats during the survey at night is less likely[22,47,48], but cannot be excluded completely[49].

As described in the introduction, horizontal beaming has a number of limitations and also does not provide reliable estimates of juvenile fish. Mobile upward-looking hydroacoustic transducers largely overcomes the drawbacks of both horizontal beaming and downward-looking transducers when the goal is to survey the upper layer of a water body. In addition, the results obtained using our mobile upward-looking method were comparable with quantitative juvenile trawling. Still, the mobile upward-looking method has two obvious limitations:

1. The presented set-up has two 12 m long holding arms that can only be used on relatively large research vessels (Fig. 6). Nevertheless, it is possible to reduce the length of the arms if surveys were limited to shallower layers. In such case, it would be possible to use the equipment in shallower lakes but with the trade-off of significantly reduced sampling volume. Potential larger sampling volumes can be achieved by using a transducer with a wider beam (transducers with a wider are now being sold (Frank Knudsen, Simrad Inc., Kongsberg – personal communication).

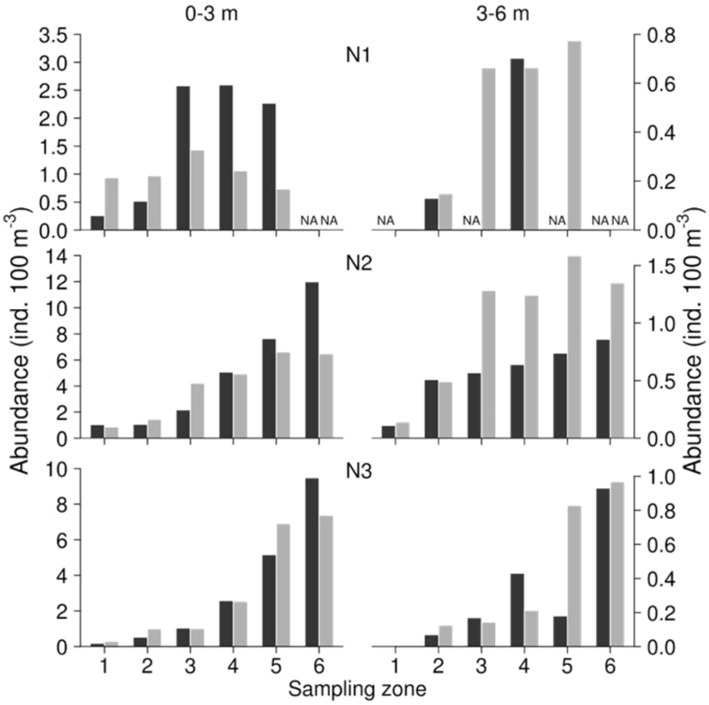

Figure 3. Abundance of juvenile fish on each night (N1–N3, 38 kHz). In each zone (1–6) and in 0–3 m (upstream cruise) and 3–6 m (downstream cruise) sampled by trawling (black) and upward-looking (grey). NA – not sampled.

2. Currently, our results are only for juvenile fish. Our acoustic records indicated many larger fish present in our studied reservoirs (Supplementary Fig. S1). However, no real-time capture methods for larger fish were available during this study such as pelagic trawling with a large trawl. The direct suitability of the proposed mobile upward-looking hydroacoustic method for studying the yearling and older portion of the fish stock should be verified in future studies.

The new sampling method circumvents the known disadvantages of horizontal and downward-looking hydroacoustic transducers when sampling above the thermocline. It is possible to enumerate late summer larval and juvenile fish community with both 38 and 120 kHz acoustic systems. Mobile upward-looking hydroacoustics is a promising fish-friendly method for further quantitative studies of pelagic upper layer fish communities, which are of great importance in many aquatic ecosystems where fish inhabit productive surface layers.

Material and Method

Study area. This study was conducted in the Římov Reservoir, Czech Republic (48°50′N, 19°30′E, 471 m a.s.l., Fig. 7), which was constructed on the Malše River in 1978. It is a canyon-shaped reservoir with a total length of 12 km, a maximum volume of 33 × 10^6 m^3, a surface area of 2.1 km^2, and an average and maximum depth of 16 m and 45 m, respectively. The trophic state of the reservoir is mesotrophic to eutrophic with the dominance of common bream, roach and bleak in both juvenile and mature fish communities[19, 50]. Due to the strong temperature and oxygen vertical gradients during summer months, both juvenile and adult fish inhabit the water layer above the thermocline[4, 19, 32].

Vertical profiles of temperature and dissolved oxygen were measured during all surveys (Supplementary S4) from the surface to 10 m depth in zone 1 using a calibrated thermistor YSI 556 MPS probe. Surveys were performed on 19/20 August 2013 (night 1), 23/24 July 2014 (night 2) and 8/9 August 2014 (night 3).

Acoustic system. The acoustic part of the study was executed using a newly-developed method based on a mobile upward-looking acoustic system[51]. To implement this approach, an epoxide laminate research vessel 11

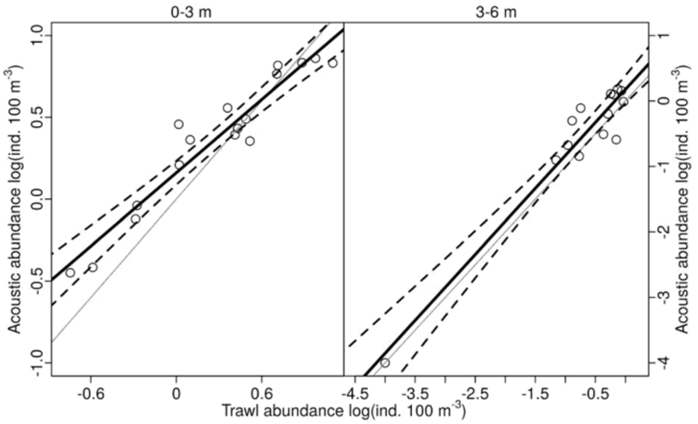

Figure 4. Linear regression model between upward-looking hydroacoustic abundance and trawl abundance (38 kHz). Each dot represents a separate sampling event. The regression line is displayed in solid black and the 95% confidence intervals are displayed in dotted lines. The 1:1 line is displayed in solid grey. The regression equation for each depth layer ($y = 0.092 + 0.691x$; $r^2 = 0.75$ and $y = 0.15 + 1.029x$; $r^2 = 0.92$, respectively for the 0–3 m and 3–6 m depth layers).

m long and 3 m wide with a 210 HP engine was equipped with two 12 m lifting arms on either side that held a platform with adjustable tilt and transducer(s) (Fig. 6). During the acoustic survey, the lifting arms submerged the platform to a depth of 8 m, approximately 5 m in front of the vessel. The platform was tilted so that the transducer faced the surface and was beaming upwards perpendicularly (the exact vertical position of the acoustic beam was verified using an electronic clinometer RIEKER H5A1-90).

Acoustic measurements were collected using primarily a Simrad EK60 split-beam echo sounder operating at a frequency of 38 kHz (circular transducer SIMRAD ES38-12 with a nominal angle of 12 deg.). This echo sounder was chosen for three main reasons. First, this frequency has minimal sensitivity to aquatic invertebrates such as *Chaoborus* larvae, which could potentially interfere with small fish echoes[52]. The second reason was the need to maximize the sampling volume at short range. The Simrad ES38-12 transducer employed had the widest opening angle of all split-beam transducers that were commercially available at the time of the survey. Thirdly, 38 kHz transducer is the relatively low sensitivity of TS to small changes in fish tilt and the generally smaller TS variability of 38 kHz compared with higher frequencies[23]. In 2014 an additional echo sounder operating at a frequency of 120 kHz (circular split-beam transducer SIMRAD ES120-7G with a nominal angle of 7 deg.) was also used on the same platform. The operating power of the 38 kHz echo sounder was set to 100 W with 0.05 ms pulse interval (20 ping s^{-1}) and the pulse length was set to 256 μs. The 120 kHz EK 60 echo sounder was set to 100 W with 0.05 ms pulse interval (20 ping s^{-1}) and the pulse length was set to 128 μs (higher frequency has sufficient number of waves in shorter pulse allowing thus for higher spatial resolution and shorter blind zone). Before each survey, both transducers were calibrated in down looking position of the platform using a 60 mm diameter copper sphere for 38 kHz and 33.8 tungsten sphere for 120 kHz as per methods described by Foote[53].

Raw acoustic data were converted and analysed using the Sonar5Pro post-processing software (Lindem Data Acquisition, Oslo, Norway). Beyond the theoretical blind zone with transducer ringing signal (half of the pulse width, 19 cm for 38 kHz, 8 cm for 120 kHz), we defined a safe margin of 0.1 m below so the surface echo was safely excluded from data processing prior to data analysis. The acoustic data were divided into two depth layers; to correspond to the layers sampled by the trawl (0.1–3 m, further called 0–3 m and 3–6 m below the surface). Data from both layers were recorded at the same time (Table 1).

Fish total length (TL) was calculated from target strength using regression parameters derived for various fish species at a 38 kHz frequency[39] and for juvenile perch at a 120 kHz frequency[43] (better agreement than[39] for 120 kHz):

$$\mathrm{TL} = 10^{\left(TS - \frac{63.38}{19.13}\right)} \quad \text{frequency} \;\; 38\,\text{kHz} \tag{1}$$

$$\mathrm{TL} = 10^{\left(TS - \frac{86.41}{20.79}\right)} \quad \text{frequency} \;\; 120\,\text{kHz} \tag{2}$$

where TL is fish total length[39,43] in cm and TS is the target strength in dB.

Signal to noise ratio (SNR) can be seen by looking at the echoes from the smallest fish and compare them with background noise level composed of the background noise reverberation level and the echosounders electric

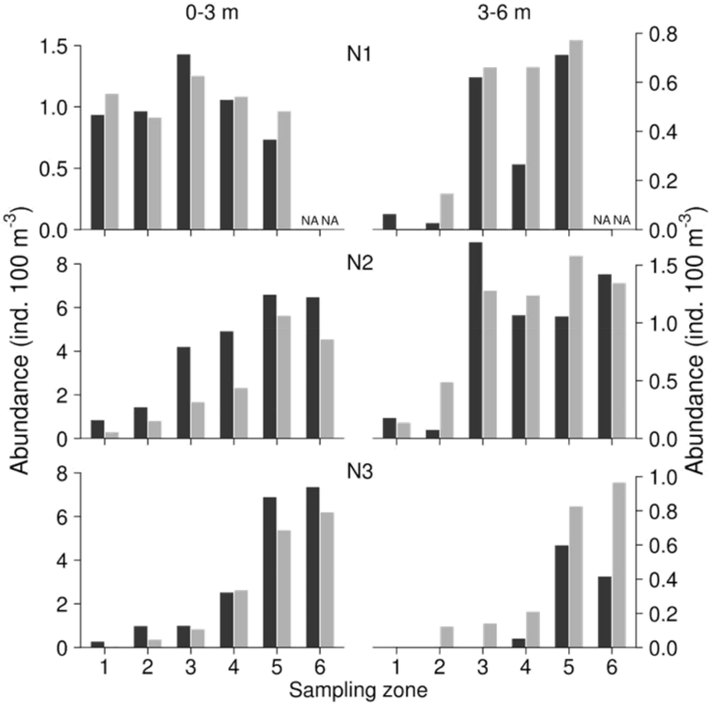

Figure 5. Replicability of abundance results of the two subsequent acoustic surveys (38 kHz). Abundance estimates obtained from the upward-looking hydroacoustic system during the three night of sampling (N1, N2 and N3) in each of the six sampling zones and in both the 0–3 m (left column) and 3–6 m (right column) depth layers. The estimates obtained during the upstream cruise are shown in black and those obtained during the downstream cruise are shown in grey. NB: in 3–6 m layer occasionally no fish of relevant size occurred. NA – not sampled.

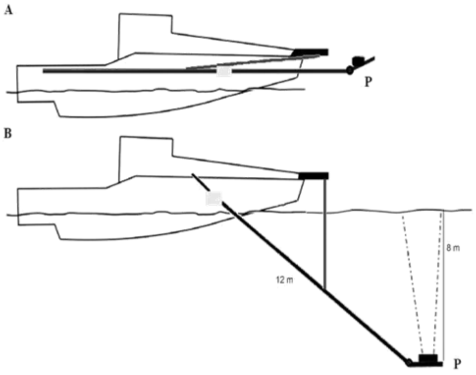

Figure 6. Schematic representation of the upward-looking acoustic system. (**A**) transport position and (**B**) operational position. P - tilltable platform holding the transducers, see details[51].

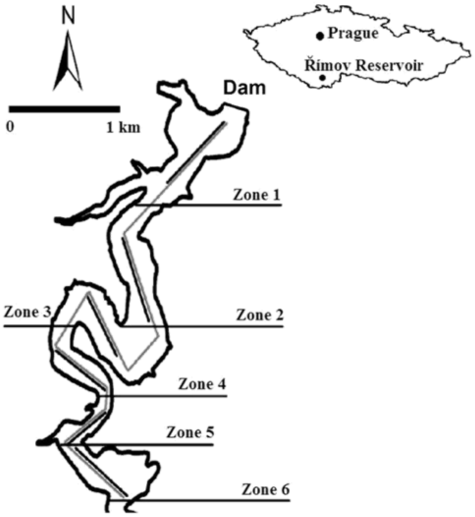

Figure 7. A map of the Římov Reservoir and location in the Czech Republic. The grey line shows the trajectory of the mobile upward-looking survey, and the black line indicates trawl sampling. Six sampled zones are also displayed (indicator lines show the southern end of the sampled zone). Figure was created by ArcMap 10.2.

noise. In our acoustic recorded data, we determined that the noise levels were lower than −75 dB. With the smallest fish targets having an off-axis compensated target strength of −60 dB we have an SNR of more than 15 dB for targets in the centre of the beam and still more than a 9 dB SNR for targets at the edge of the beam.

Acoustic surveys were performed in straight-line transects at a constant speed of 1 ms $^{-1}$ following the original river valley (Fig. 7). To avoid striking the bottom with the submerged platform, only the deepest part (depth >10 m, 6 km long zone from the dam) of the reservoir was sampled (zone 1–6, Fig. 7). To determine the depth between the bottom and the platform, a downward-looking single-beam transducer (Simrad ES200 with nominal angle 9°) was mounted on the underside of the platform. Acoustic recordings started from the dam one hour after sunset (at approximately 22:30 p.m. in zone 1, Fig. 7) and finished in the middle part of the reservoir (finished approximately at 0:30 a.m. in zone 6, downstream, Supplementary Table S1). Recording was stopped at the end of zone 6, the boat was turned around and after a half hour waiting period (to avoid bubbles made by the boats propellers), the same transects were sampled in the opposite direction (from zone 6 towards zone 1 finished at approximately 3:45 a.m., upstream, Supplementary Table S1). The GPS location of the vessel was measured using a Garmin GPSMAP 60CSx GPS throughout the survey.

An automatic single echo detection (SED) primary threshold of −70 dB was used to define targets of interest. A fish track was defined as having at least three subsequent echoes of the same target, separated by a maximum of one missing ping within a 0.1 m vertical range. All tracks were manually checked and the tracks outside the nominal beam (between −6 and 6 or −3.5 and 3.5 degrees for 38 and 120 kHz system, respectively) were removed. The minimum and maximum acoustic thresholds for juvenile fish were set into the troughs of size frequency distributions[54] accepting the targets of interest consistent with YOY fish. These limits were as follows for targets: TS −61.5 to −50 dB and corresponding to fish 15–50 mm in the trawl catch during night 1, (TS −55.8 to −47.8 dB and −55.7 to −45.60 dB, respectively for 38 and 120 kHz) corresponding to fish 25–65 mm in the trawl catch during night 2 and (TS 55.8 to 47.2 dB and 55.7 to 44.8 dB, respectively for 38 and 120 kHz) corresponding to fish 25–70 mm in the trawl catch during night 3.

Fish abundance was calculated according to the trace counting method[23,54]:

$$(f/m^3)_{\text{tracks}} = tracks/\mathrm{v_w} \tag{3}$$

where *tracks* stands for the number of tracks in a given transect and it is divided by the sampled wedge volume $\mathrm{v_w}$ in m^3 (based on the equivalent beam angle and sailing distance). Fish abundance was reported as the number of fish in 100 m^3 of sampled water (*f/100 m³*) for the 0–3 and 3–6 m depth layers.

The second method of Sv/TS scaling method was used to analyse recorded upward-looking data by echo integration (to compare with tracked fish densities, which can fail under high target densities). Echograms were analysed using the same threshold restrictivity as for track-counting. Fish bigger than −47.5 dB were erased by a special function of SONAR 5 software. In-situ fish tracks were used for estimating mean TS. Only targets of TS between −55.8 and −47.8 dB were used for night 2 and targets of TS between −55.8 and −47.2 dB were used for night 3.

Direct fish sampling. Pelagic habitat sampling was performed using a 3 × 3 m fixed-frame fry trawl. The trawl body was 10.5 m long with a knot-to-knot mesh size of 6.5 mm in the main belly and 4 mm in the cod end (for details see ref. 22, Supplementary Fig. S5). The trawl was towed for 10 min approximately 100 m behind the second research vessel (trawler) at a speed of $1\,\mathrm{m\,s^{-1}}$. Samplings occurred at two depth layers (0–3, 3–6 m), the shallower depth was sampled during the upstream cruise and the deeper depth during the downstream one (Supplementary Table S1). During night 1, zone 6 was not sampled due to extremely low water levels in 2013. Trawling data from the layer 3–6 m from zones 1, 3 and 5 were also missing for this year. All trawling tows began approximately 20 minutes after the acoustic survey and had parallel trajectories (Fig. 7).

Fish caught by the trawl were immediately euthanized using a lethal dose of MS 222 and were subsequently preserved in a 4% formaldehyde solution. In the laboratory, fish were identified to the species level[55], counted and TL was measured to the nearest mm. For each trawl tow, the sampled water volume was calculated based on the tow distance measured by GPS, and the CPUE (catch per unit effort) of the trawl tow was expressed as catch per $100\,\mathrm{m}^3$ of water sampled.

Animal treatment was performed under permission from the Experimental Animal Welfare Commission under the Ministry of Agriculture of the Czech Republic (Ref. No. CZ 01679). All methods were performed in accordance with project protocols approved by a named institutional and national committee (Ref. No. 77/2013).

Statistical analyses. Differences in the abundance estimated by trawling and hydroacoustics sampling were compared using a Kolmogorov – Smirnov paired test for both depth layers separately. Additionally, the abundance estimates for both methods were regressed against each other, and we determined if the slope parameter was significantly different from unity[56]. Length frequency distributions of caught fish and predicted length frequency from tracked fish were tested by Kolmogorov – Smirnov paired for comparisons. Statistical analyses were carried out using the R language[57].

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Author Contributions

R.B., T.J., M.T., P.B., M.C., V.D., A.D.J., I.K., M.M., Z.S., L.V. and J.K. participated in the field work. R.B., T.J., M.C. and J.K. designed the study. H.B. helped with processing the data and theoretical calculations. R.B., T.M. and D.R. conducted the statistical analysis. R.B., T.J., M.T. and J.K. wrote the manuscript. All authors contributed substantial comments during manuscript preparation.

Additional Information

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Paper III

Quantification of chaoborus and small fish by mobile upward-looking echosounding

(Original pages 60-70)

ORIGINAL ARTICLE

Quantification of *Chaoborus* and small fish by mobile upward-looking echosounding

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ABSTRACT

Chaoborus larvae inhabit frequently the water column of lakes, when they can be mistaken for small fish. Because larvae ascend up to the blind zone of downward-looking echo sounding at night, quantitative acoustic estimation of them is possible only with upward-looking approach. For this reason, the mobile hydroacoustic upward-looking system (120 and 38 kHz split-beam echosounder) in combination with a direct catch method (trawling) was tested to investigate the night community of invertebrates and juvenile fish in the surface layer of the Římov reservoir (Czech Republic). In the target strength range of invertebrates (smaller than -59 dB), the 38 kHz echosounder recorded only a small proportion of targets while the 120 kHz echosounder recorded distinct peaks corresponding to high densities of *Chaoborus* (target strength, TS range -70 to -60 dB, average TS -66 to -64 dB). At 120 kHz frequency, the TS distribution of smaller cohort of juvenile fish (<25 mm in length) overlapped the TS-distribution of *Chaoborus*. The number of these smaller juvenile fish was so small compared with the number of *Chaoborus* that they did not seriously bias acoustic *Chaoborus* estimate. The correlation between the density of *Chaoborus* with small contamination of juvenile fish larvae from trawling and acoustic recording made with the 120 kHz echosounder was high (R^2=0.88), but the acoustic densities from trace counting appeared to underestimate *Chaoborus* abundance when the density was >1.5 ind.m^{-3}.

INTRODUCTION

Hydroacoustics is a well-established method for assessing the parameters of fish stock in the sea and in fresh water (Simmonds and MacLennan, 2005). Even small fish can be detected (Frouzová and Kubečka, 2004) but problems arise when larvae of phantom midge (*Chaoborus* sp.) and juvenile fish occur in the same habitat (Malinen *et al.*, 2005; Knudsen *et al.*, 2006). In stratified lakes, targets of interest often occur only a few centimeters to a few meters under the surface and, for this reason, the downward-looking approach does not provide reliable data near the surface and is often replaced by horizontal echo sounding. Upward-looking system makes it possible to record small targets in the near surface layer and to accurately determine their size (Baran *et al.*, 2017).

The phantom midge *Chaoborus sp.* (Diptera, family Chaoboridae) spend most of its life cycle in water (Burrows and Dorosenko, 2014). *Chaoborus* larvae eliminate the risk of predation from planktivorous fish by performing diel vertical migrations, spending the day in the hypolimnion or sediment and at night ascending to the epilimnion (Voss and Mumm, 1999; Lagergren *et al.*, 2008). For buoyancy regulation, *Chaoborus* larvae have two pairs of air sacs (Teraguchi, 1975), which acoustically produce similar echoes as strong as juvenile fish (Eckmann, 1998). Consequently, the night time co-presence of *Chaoborus* larvae with small fish may cause significant errors in acoustic estimates of juvenile fish (Eckmann, 1998; Vinni *et al.*, 2004; Malinen *et al.*, 2005).

The simplest and most widely used approach is to consider the echoes from small targets as reverberation and to eliminate their contribution to the total echo integral by thresholding (Simmonds and MacLennan, 2005). For poor signal-to-noise conditions, this principle was improved considerably by Eckmann (1998) based on stepwise thresholding and the allocation of echo-integrator output to acoustically smaller *Chaoborus* and larger fish. This method can be used in dense *Chaoborus* aggregations when co-occurring targets are of clearly distinct sizes. However, use of target strength (TS) where possible (in lower densities when single targets can be

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Key words: Římov reservoir; juvenile fish; invertebrate; trawling; hydroacoustics; target strength.

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distinguished) should provide a more direct distinguishing of *Chaoborus* and fish.

The bias produced by the inclusion of *Chaoborus* in fish estimates is apparently dependent on echosounder frequency. Jones and Xie (1994) reported that the strongest echo of *Chaoborus* can be recorded by an echosounder using a frequency of 225 kHz, due to better sensitivity of higher frequencies. Similarly, Knudsen *et al.* (2006) found that the best frequency for studying of *Chaoborus* larvae is 200 kHz.

Our work was concentrated on 120 kHz, a very common frequency for studying fish in lakes and reservoirs (Simmonds and MacLennan, 2005; CEN, 2014; Draštík *et al.*, 2017). The aim of this study was to disentangle the acoustic record of the pelagic invertebrate community dominated by *Chaoborus* mixed with small fishes using two contrasting echosounder frequencies of 120 and 38 kHz. The paper aims to fill the knowledge gap on target strength of *Chaoborus* specifically for the 120 KHz frequency widely used in fisheries surveys. We analyzed size distributions and abundances of non-fish and fish targets and compared acoustic results with a direct capture method.

METHODS

This study was conducted in the Římov Reservoir (48°50'N, 19°30'E, 471 m asl, Fig. 1), 170 km south of Prague, Czech Republic. The reservoir was constructed on the Malše River in 1978. It is a canyon-shaped reservoir with a total length of 13 km, a maximum volume of 33 x 10^6 m^3, a surface area of 2.1 km^2, and an average and maximum depth of 16 m and 43 m, respectively. The trophic state of the reservoir is mesotrophic to eutrophic with well-developed thermal stratification during the summer. Dominant fish are common bream (*Abramis brama* Linnaeus, 1758), roach (*Rutilus rutilus* Linnaeus, 1758) and bleak (*Alburnus alburnus* Linnaeus, 1758). These species frequently occur in open water of the reservoir during the first year of life (Jůza *et al.*, 2009, 2013). Chaoborus larvae are not abundant (Říha *et al.*, 2015) and were observed to form scattering layers in the hypolimnion during the day ascending to the surface at night (Čech and Tušer, *personal communication*).The pelagic habitat of the reservoir was investigated using mobile hydroacoustics and trawling over the course of two nights, 23/24 July (N1) and 8/9 August (N2) in 2014

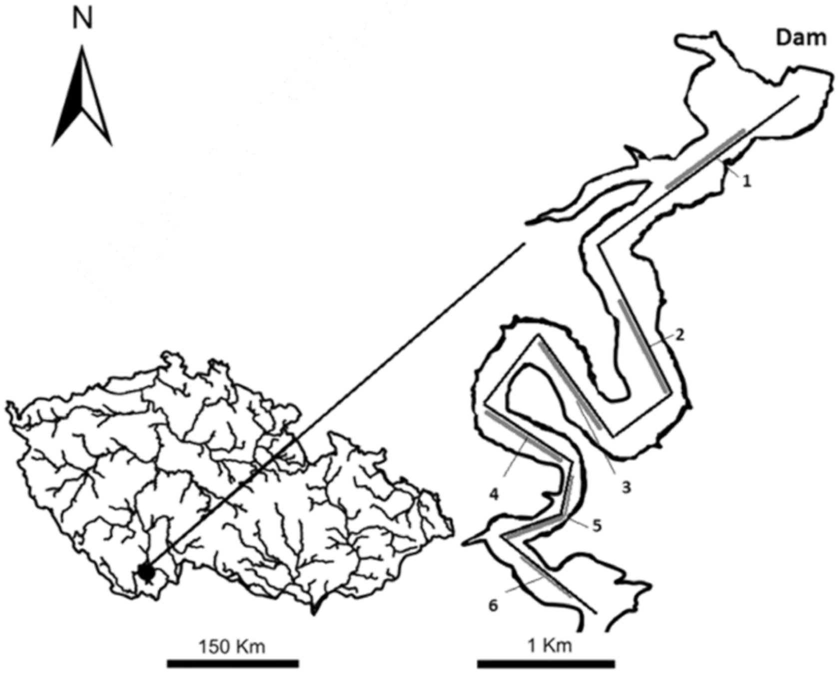

Fig. 1. A map of the Římov Reservoir and its location in the Czech Republic. The black line shows the trajectory of the mobile upward-looking survey, and the gray line indicates the trawl sampling in depth 0-2 m (upstream) and 3-5 m (downstream).

with slightly different stratification conditions (Fig. 2).

The acoustic part of the study was performed using a newly developed method based on a mobile upward-looking acoustic system (Baran *et al.*, 2017). A research vessel (11 m long with a 210 HP engine) was equipped with two 12 m long submersible arms on either side that held a tiltable platform with attached transducers between their front ends. During the acoustic survey, the arms submerged the platform to a depth of 8 m with transducers emitting towards the surface. An exact vertical position of the acoustic beam was measured using an electronic clinometer, the RIEKER I15A1-90 (RIEKER Inc. USA).

Frequencies of 120 kHz (circular split-beam transducer SIMRAD ES120-7G with a nominal angle of 7 degrees) and 38 kHz (circular split-beam transducer SIMRAD ES38-12 with a nominal angle of 12 degrees) were used in the study. The operating power of the 120 and 38 kHz echosounder was set to 100 W with 0.05 s pulse interval (20 ping s^{-1}) and the pulse length was set to 128 μs and 256 μs for 38 kHz. Before each survey, both transducers were calibrated using a 33.2 tungsten-carbide sphere for 120 kHz and a 60 mm diameter copper sphere for 38 kHz calibration as described by Foote *et al.* (1987).

The acoustic survey was performed in straight-line transects at a constant speed of 1 $m.s^{-1}$ following the original river valley (Fig. 1). To avoid striking the bottom with the submerged platform, only the deepest part (depth >10 m, 6 km long zone from the dam) of the reservoir was sampled. Acoustic recordings started from the dam one hour after sunset (approximately at 22:30) and finished upstream in the middle part of the reservoir (approximately at 0:30). Recording was then stopped in the middle part of reservoir, and after a half hour waiting period (to avoid bubbles made by the boat propellers), the same transects were sampled downstream (from the middle part to the dam finished approximately at 3:45 a.m.). The GPS coordinates of the survey cruise was measured using a Garmin GPSMAP 60CSx GPS handheld connected to an external antenna for better reception of the signal.

Raw acoustic data were analyzed using the Sonar5-Pro post-processing software (Lindem Data Acquisition, Oslo, Norway). Beyond the theoretical blind zone (half of the pulse length from the phase boundary – water surface), we defined a line of 0.1 m and 0.2 m, for the 120 kHz and 38 kHz echosounders, respectively, below the detected surface so the surface echoes were safely excluded from data processing prior to data analysis. The acoustic data were divided into two depth layers according to layers sampled by trawl depths (0-2 m and 3-5 m below the surface, without defined line 0.1 or 0.2 m). An automatic single echo detection was set up to accept targets between lower and upper thresholds for -70 to -49 dB (corresponding to a theoretical fish length of about 0.5-60 mm for small perch calculated for the frequency 120 kHz using the TS length relationship of Frouzová and Kubečka (2004). The same regression was used to convert captured fish lengths to TS from the 120 kHz echosounder. To convert the captured fish size to the TS from the 38 kHz echosounder the regression by Love (1977) was used. A valid track was defined as at least two subsequent single echoes from the same target, separated by a maximum of one missing ping within a 0.1 m vertical range gate.

Acoustic tracks abundance was calculated according

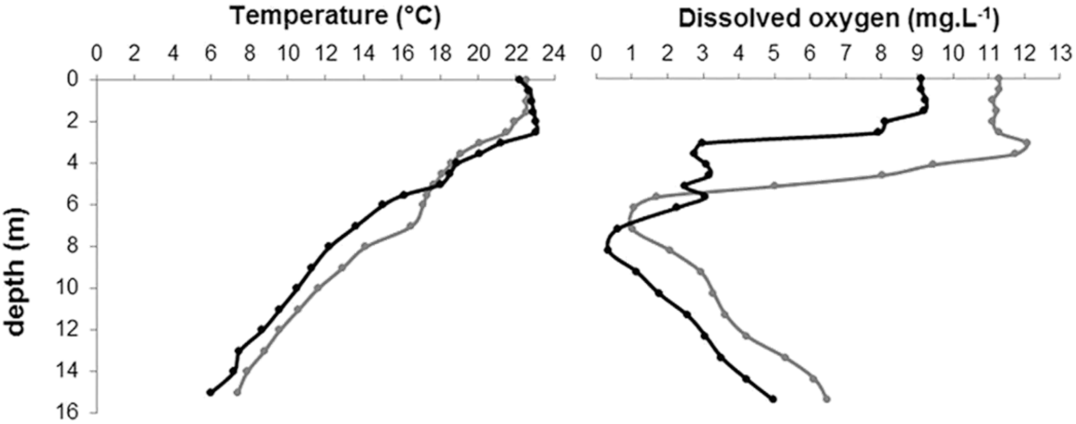

Fig. 2. Vertical profile of water temperature and dissolved oxygen. The gray lines indicate measurements for night 1 and black for night 2.

to the track counting method (Simmonds and MacLennan, 2005; CEN, 2014):

$$(f/m^3)_{tracks}=tracks/v_w$$

where *tracks* stands for the number of tracks in a given transect and it is divided by the sampled wedge volume v_w in m^3. Abundance was separately expressed as the number of tracks in 100 m^3 of sampled water (f/100 m^3) for the 0-2 and 3-5 m depth layers. The tracks larger than -55 dB were considered to represent fish only while targets within the TS range of -70 to -55 dB were considered a mixture of air containing invertebrates and the smallest fish from summer spawning

Sampling of the pelagic habitat was performed using a fixed frame ichthyoplankton trawl (mouth opening 2x2 m, mesh size 1 mm x 1.35 mm) with the collecting bucket at the end (Jůza *et al.*, 2010). The trawl was towed for 5 min approximately 100 m behind a research vessel (with 15 HP engine power) at a speed of 1 m s^{-1}. Sampling was performed in two layers differing by oxygen and thermal conditions (0-2 m and 3-5 m, Fig. 2). The shallower depth was sampled during the upstream cruise and the deeper depth during the downstream cruise. All trawling tows began approximately 10 min after the acoustic survey and were hauled in parallel trajectories with the acoustic trajectories, with a total of six ichthyoplankton tows made per one depth layer (Fig. 1). For each trawl tow, the sampled water volume was calculated based on the trajectory tow distance measured by GPS, and the CPUE (catch per unit effort) of the trawl tow was expressed as catch per 100 m^3 of water sampled.

Samples were immediately euthanized using a lethal dose of MS 222 and then preserved in a 4% formaldehyde solution. In the laboratory, samples were examined under a stereoscope (Lomo MBC-10) to categorize the caught objects into *Chaoborus* larvae, *Chaoborus* pupae, other invertebrates (*Chironomidae* larvae, *Chironomidae* pupae, *Hydracarina*, *Branchiura*) and juvenile fish. *Chaoborus* larvae and pupae were counted, and body lengths (excluding the anal papillae) of 120 random individuals of each night and depth of both groups were measured to the nearest mm. Other invertebrates were also counted and in 60 randomly chosen individuals body length of each category was measured to the nearest mm. The resulting size structure was used for all counted individuals in the same depth and night. juvenile fish were counted and total length (TL) was measured to the nearest mm.

Differences in size structures of *Chaoborus* were used in a paired *t*-test. Differences in the abundance estimated by ichthyoplankton trawling and hydroacoustics sampling were separately compared using the Kolmogorov - Smirnov paired test for both depth layers. Additionally, the abundance estimates for both methods were regressed against each other to determine if the slope parameter was significantly different from 1 (Taskinen and Warton, 2013). Statistical analyses were carried out using the R software (R Development Core Team, 2015).

RESULTS

Upward-looking hydroacoustics record

During acoustic surveys, the 120 kHz frequency echosounder recorded 8672 and 1068 tracks in the 0-2 m and 3-5 m layer, respectively (sampling volume 12.514 and 8046 m^3), while the 38 kHz frequency observed 766 and 486 tracks for the given layers (sampling volume 20,837 and 13,382 m^3).

During both nights, a distinct peak of all tracks between -70 and -60 dB TS was recorded by the 120 kHz system especially at the 0-2 m depth range, peaking at -64 dB at night 1 and -65 dB at night 2 (Fig. 3). Using the regression from Frouzová and Kubečka (2004), this corresponds to the theoretical fish length (TL) of 10 and 9 mm respectively. At the 3-5 m depth range the targets were less abundant. The peak of TS at the depths of 3-5 m was -66 dB for night 1 and -67 dB for night 2, which correspond to the theoretical fish length 9 and 8 mm respectively.

On the contrary, the 38 kHz echosounder recorded much fewer tracks without a distinct peak as expected from the previous results of the 120 kHz frequency (-70 to -60 dB, Fig. 4). At night 1 in the 0-2 m depth the most abundant size group of tracks was recorded in the range of juvenile sizes -51 to -49 dB. The situation was similar for night 2, again at 0-2 m depth, when tracks -50 and -49 dominated. In the 3-5 m depth layer the track abundance was lower, and TS-distribution was bimodal two peaks were clearly visible in the frequency distribution for both nights (Fig. 4).

Ichthyoplankton trawl catch

In twelve hauls during two different nights we caught 18.119 and 4683 invertebrates at the depth of 0-2 m and 3-5 m respectively (sampling volume 12,928 and 14,982 m^3). *Chaoborus* larvae showed a much higher density by one or two orders of magnitude compared to fish (Tab. 1). The total catch of juvenile fish in 12 hauls was 498 and 159 individuals for the two depth strata respectively. *Chaoborus* larvae and pupae dominated at the surface representing more than 90 percent of the whole catch, while at the deeper layer they only constituted around 50-70 percent of the total catch (Tab. 2). At the 3-5 m depth, a significantly higher number of Hydracarina and Chironomidae larvae were recorded for night 2 (N 2, Tab. 2).

The mean size of *Chaoborus* larvae at the surface was 9.5 mm for N 1 (Tab. 3) and 9.0 mm in all other samples.

The *Chaoborus* larvae were significantly smaller for N 2 (paired *t*-test, df=119, $P<0.05$) at the surface and also at the 3-5 m depth (paired *t*-test, df=119, $P<0.05$). *Chaoborus* pupae were the same sizes among nights and layers (6.0 mm) with the exception of slightly larger individuals in the deeper layer for night 1 (6.5 mm; Tab. 3). The *Chironomidae* larvae were about 9.0 mm long on N 1, but in the deeper layer and on N 2, they were smaller (8 mm, Tab. 3). Fig. 5 illustrates the size structure of the entire catch of invertebrates in the ichthyoplankton trawl.

The size distribution of juvenile fish can be divided

Tab. 1. Abundance of trawl catch (N.100 m^{-3}).

	Chaoborus larvae				Fish			
	N1		N2		N1		N2	
	0-2 m	3-5 m	0-2 m	3-5 m	0-2 m	3-5 m	0-2 m	3-5 m
Zone 1	69.2	67.3	156.6	3.9	2.6	1.6	1.1	0.2
Zone 2	177.7	75.3	79.8	5.2	4.0	0.7	0.6	0.6
Zone 3	210.0	6.0	77.7	7.7	5.9	1.9	1.2	0.7
Zone 4	84.1	4.6	138.7	3.6	3.9	1.1	2.5	1.0
Zone 5	52.2	5.0	36.5	4.5	10.9	1.5	3.0	1.1
Zone 6	39.6	10.7	46.1	1.1	7.1	1.6	7.5	0.9

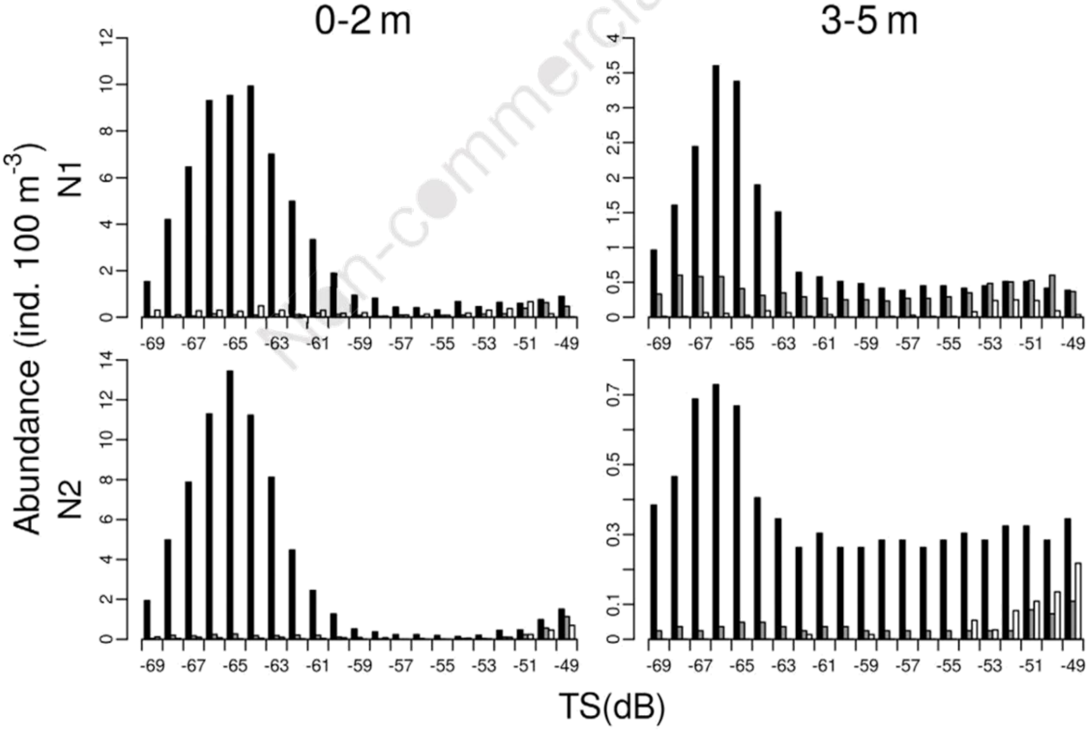

Fig. 3. TS distributions by 120 kHz echo sounder (black), TS distribution by 38 kHz echo sounder (grey) and TS distributions of fish converted into TS for the frequency of 120 kHz. from trawl catch (white). All values are average values over the six zones in Tab. 1. N1, night 1; N2, night 2.

into two cohorts. The first (6-20 mm) consisted most probably of fish from late summer spawning of bleak and perch. The other (30-50 mm) presumably corresponds to roach and bream from the ordinary spring spawning (Fig. 6). The smallest fish were primarily recorded near the surface during night 1. During night 2 there were significantly fewer numbers in the first cohort, while the second cohort of juvenile fish grew by 10 mm (40-60 mm). At the 3-5 m depth, the number of juvenile fish in the first cohort was lower than at the surface, and during night 2 only a few individuals of the smaller cohort were recorded.

Tab. 2. Percental proportion of invertebrates and fish contribution in the trawl catch. Total number of invertebrates and fish 11.980 and 6298 at N1 and N2.

Category	Night 1				Night 2			
	Depth 0-2 m		Depth 3-5 m		Depth 0-2 m		Depth 3-5 m	
	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD
Branchiura	0.7	1.2	3.1	4.05	1.0	1.0	4.6	1.5
Chaoborus-L	73.0	14.7	53.4	19.67	78.0	12.9	36.1	19.5
Chaoborus-P	19.6	9.4	18.8	6.97	15.2	8.2	12.4	8.1
Chironomidae-L	0.1	1.0	3.7	4.87	0.2	0.4	24.1	14.0
Chironomidae-P	0.0	0.0	1.3	2.01	1.9	2.8	3.0	4.3
fish 5-60 mm (TL)	3.0	3.7	2.6	2.94	2.1	3.6	6.2	4.2
Hydracarina	3.6	4.0	17.2	11.52	1.7	1.0	13.6	7.5

L, larvae; P, pupae.

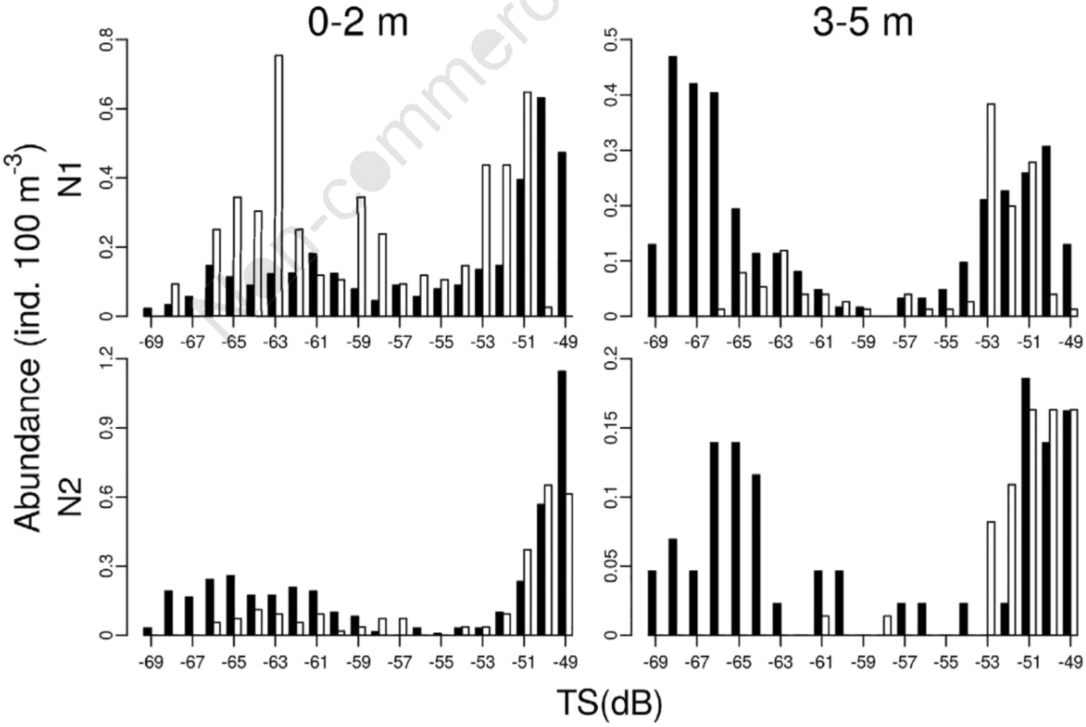

Fig. 4. TS distribution of the acoustically detected targets and fish catch of trawl. Acoustic 38 kHz (black histograms) and fish sizes captured by the trawl converted into TS (white histograms) at the 38 kHz frequency.

Comparison between acoustic and trawling results

The logarithmic abundance estimates of *Chaoborus* by both methods (120 kHz echosounding and trawling) were not statistically different when using a Kolmogorov - Smirnov paired test (df=24, P>0.05). However, inspecting the 1:1 regression revealed the estimated acoustic abundance to be lower, especially in the zones where the abundance of trawl catch was the highest (Fig. 7).

Tab. 3. Mean size (mm) and standard deviation (SD) of the invertebrates and fish caught by the trawl.

Category	Night 1				Night 2			
	Depth 0-2 m		Depth 3-5 m		Depth 0-2 m		Depth 3-5 m	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
Branchiura	6.0	0.4	6.0	0.5	5.0	0.3	5.0	0.3
Chaoborus-L	9.5	0.4	9.0	0.4	9.0	0.3	9.0	0.3
Chaoborus-P	6.0	0.6	6.5	0.2	6.0	0.1	6.0	0.4
Chironomidae-L	9.0	0.0	8.5	0.4	8.5	0.2	8.0	0.1
Chironomidae-P	0.0	0.0	6.0	0.3	5.5	0.8	5.5	0.1
fish 5-60 mm (TL)	23.5	7.5	29.0	10.5	32.0	13.8	44.0	4.8
Hydracarina	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0

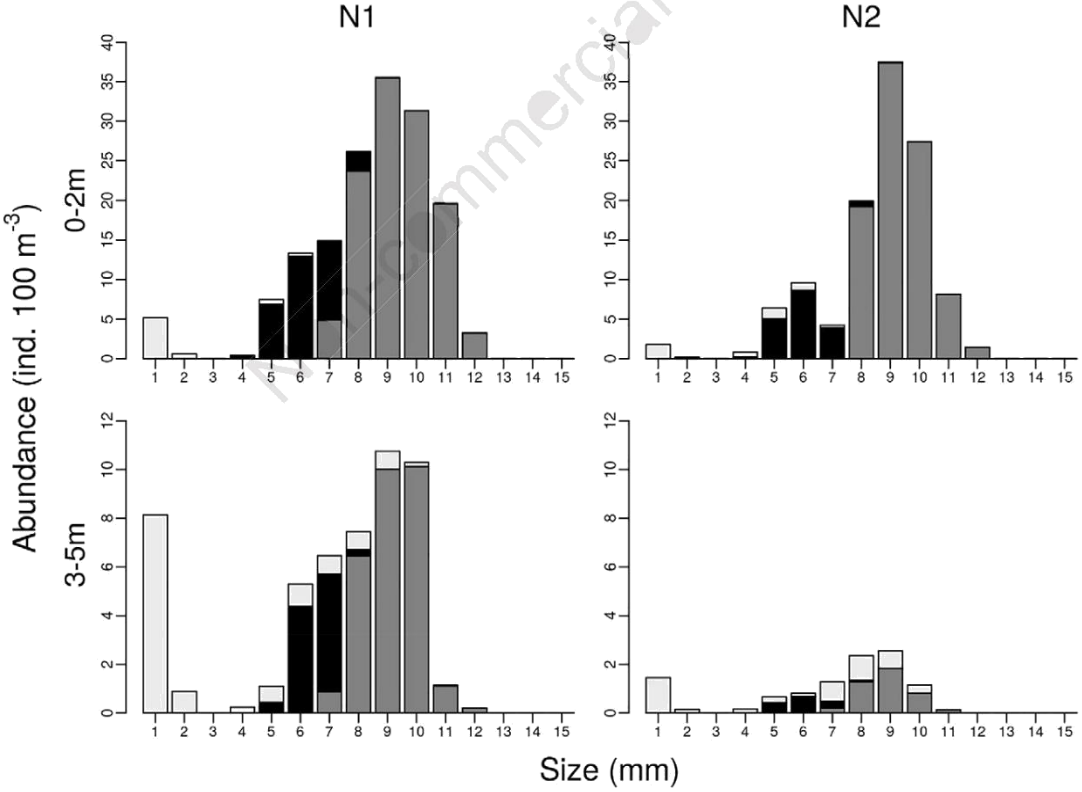

Fig. 5. The size distribution of invertebrates caught by ichthyoplankton trawl. Gray histograms indicate *Chaoborus* larvae, black *Chaoborus* pupae and light grey "other invertebrates".

DISCUSSION

The mobile upward-looking echosounding methodology can record *Chaoborus* and juvenile fish near the water surface reasonably well. The 120 kHz frequency can efficiently record *Chaoborus* larvae or pupae, which can bias the hydroacoustic estimates of fish in waterbodies. The size distributions of the ichthyoplankton trawl catch and 120 kHz acoustic records showed a similar peak corresponding to the size of *Chaoborus* – the most abundant pelagic animal reflecting the echoes with good signal-to-noise ratio of some 10 dB

Chaoborus larvae dominated night trawl samples mainly in the upper surface layer and very similar densities were recorded in 120 kHz acoustic results. Knudsen *et al.* (2006) found that using the 200 kHz frequency *Chaoborus* larvae had a TS of about -65 dB Another group of targets with an overlapping TS range

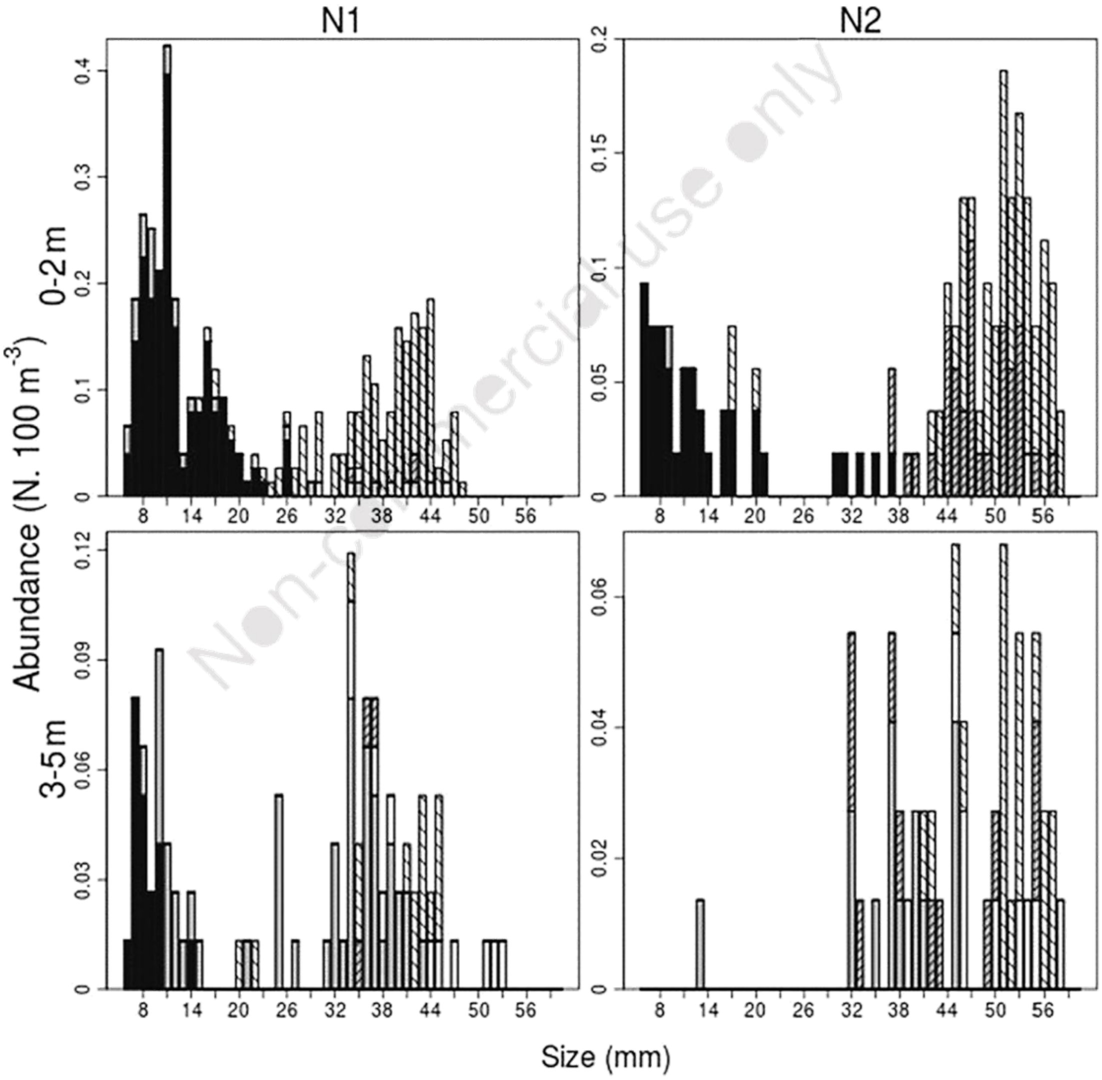

Fig. 6. Size and species structure of fish catch of the trawl. Black indicate *Alburnus alburnus*, grey *Perca fluviatilis*, white stripes *Rutilus rutilus*, grey stripes *Abramis brama*, white *Stizostedion lucioperca*.

are *Chironomids* pupae with a TS range of -77 to -65 dB (Kubečka *et al.*, 2000). They have a complex of tiny gas hollows (thoracic horns) with hydrostatic functions (Langton, 1995). However, in the ichthyoplankton trawl, we recorded the proportion of *Chironomids* as only about 2-3 percent of all caught invertebrates, especially at the deeper layer. For this reason, *Chironomids* pupae hardly influence the results.

Chaoborus larvae showed a reduction in their average size (about 0.5 mm) between two sampling nights. Size reduction from spring to autumn is also reported by other authors (Eckmann, 1998; Knudsen *et al.*, 2006). The reason behind decreased size is most likely the growth of very small I-II instar larvae to observable size between surveys and possible emergence of large IV instar larvae. Prchalová *et al.* (2003) found that *Chaoborus* larvae and pupa have a TS in the range between -70 to -64 dB in the 120 kHz transducer. This partly corresponds to our results. However, our TS range is larger here probably because the mentioned study was conducted in tropical Thailand in February and only small individuals could occur or different species of *Chaoborus* may have been present. Several observations with an echosounder using a higher frequency of 200 kHz suggest slightly higher range of modal TS -64 to -60 dB (Jones and Xie, 1994; Knudsen *et al.*, 2006; Bezerra-Neto *et al.*, 2012). A higher frequency is likely to be more sensitive for recording small targets. so the TS of *Chaoborus* may be higher with a 200 kHz echosounder compared to a 120 kHz one.

From Fig. 3 the acoustic size range of *Chaoborus* is considerably smaller than that of the older YOY fish cohort. The size of the smaller fish cohort from summer spawning (reported by Hladík and Kubečka, 2003; Čech *et al.*, 2012), however, overlaps with the size of *Chaoborus* both physically and acoustically in the 120 kHz frequency. It is only possible to distinguish the large cohorts of juvenile fish with the recommended threshold -55 dB or about 30 mm fish length based on the TS.

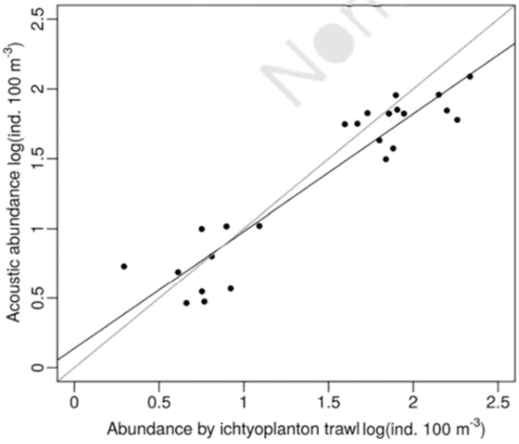

Fig. 7. The relationship between *Chaoborus* abundance estimates by the trawl and by 120 kHz echo sounder (targets from -70 to -55 dB). The fitted linear regression equation was $y=0.8420 * x + 0.1381$, coefficient of determination $R^2=0.88$.

At 38 kHz, which is not sensitive to *Chaoborus* (Jones and Xie, 1994; Knudsen *et al.*, 2006), we recorded a reasonable agreement between the acoustic and trawling densities (Fig. 4) of the cohort of fish fry larger than -55 dB The smaller size groups apparently contained a mixture of small fish from later spawning, invertebrates and possibly other targets. These were mainly present in the deeper 3-5 m layer (Fig. 4). Invertebrates other than *Chaoborus* such as parasitic *Branchiura, Chironomidae* or water mites *Hydracarina* were more abundant in the deeper layer.

Ichthyoplankton trawl with the mesh size 1*1.35 mm is not a traditional method for sampling *Chaoborus*. It was found rather quantitative for sampling fish larvae (Jůza *et al.*, 2010). In our case it was used because the presence of targets of -70 to -60 dB was recorded vastly in up looking records in the year previous to the survey (Baran *et al., unpublished data*). We expected to find fish larvae in the open water of the reservoir and this was the reason for the trawl selection. Some smaller slim invertebrates might have been lost through the meshes of the trawl. These losses are unlikely to be significant as many even smaller invertebrates were retained (Fig. 5). Also our mean sizes of *Chaoborus* are similar to sizes reported in similar studies (Eckmann, 1998; Knudsen and Larsson, 2009). On the other hand, 2x2 m trawl is a robust sampling tool which greatly reduces the chances of sampled invertebrates to escape.

The acoustically estimated abundance of *Chaoborus* sized tracks was lower than trawl catch, especially in places where both methods recorded the highest abundance of *Chaoborus* larvae. The low abundance of *Chaoborus* larvae allowed us to use a trace counting method that required well detected traces of target individuals. Differences between density estimates by echo sounding and trawling can occur for at least two reasons. First, the method used to process the acoustic data by track counting does not enable the distinguishing between multiple overlapping targets that may occur at a higher abundance (Kocovsky *et al.*, 2013; Baran *et al.*, 2017). Second, some discrepancies between hydroacoustics and trawling results can be caused by the fact that despite the two sampling boats following very similar trajectories, it was not possible to concurrently sample the same volume of water by the two methods and horizontal distribution of *Chaoborus* larvae and fish was not homogenous. Possible solution of the first problem is the echointegration of entire invertebrate signal. The main problem with this approach is the need to use "upper threshold" to eliminate all fish targets from the record. Integrating any fish echo into the invertebrate record would lead to huge overestimation of density and can bias the results heavily. At this stage the application of "upper threshold in echo-integration" is not used routinely and is rather subjective so we decided to base our results on track counting. The advantage of track counting is that we are relatively sure that everything counted were the targets of interest.

CONCLUSIONS

The present study demonstrates the applicability of the mobile upward-looking hydroacoustic system to survey *Chaoborus*. The indisputable advantage of this system is the monitoring of juvenile fish and *Chaoborus* near the surface in stratified artificial lakes or natural lakes in the same record. Data obtained with the 120 kHz frequency echosounder confirms that this frequency, primarily used to study fish, is capable of studying *Chaoborus* as well. Using the lower frequency of 38 kHz offers the potential separation of a very small cohort of fish (6-20 mm TL) from *Chaoborus* larvae when the investigation of such extreme application is needed. Later in the ontogeny it is possible to use also the 120 kHz frequency, however, the TS thresholds over -60 dB are needed to distinguish fish from *Chaoborus* larvae. In our case, the estimation juvenile fish of over 30 mm was easy and safe.

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Paper IV

New way to investigate fish density and distribution in the shallowest layers of the open water

(submitted manuscript)

New way to investigate fish density and distribution in the shallowest layers of the open water

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Abstract

Information about fish distribution and abundance in the upper part of the water column are often fundamental for both research and management. However, this information is extremely hard to obtain using conventional hydroacoustic methods. For this reason, the mobile hydroacoustic upward-looking system (38 kHz split-beam echosounder) in combination with a passive sampling method (gillnets) was tested to investigate the fish community (fish larger than 8 cm total length) in the upper 3 m of water column of Římov Reservoir (Czech Republic) during the growing season. We found most fish located in the depth layer closest to the surface down to 1 m – 50-78 % by acoustics (layer 0.3 – 1 m) and 55-71 % by gillnets. The size structure of both methods was generally similar, but the acoustic results contained a higher proportion of small fish (< 12 cm SL). The hydroacoustic and gillnet sampling recorded similar number of fish per one-night sampling.

The upward-looking system is a promising approach to study the fish community in the neglected surface layer, but more studies of its efficiency for large fish monitoring are needed.

1. Introduction

Open water represents often the largest volumes of lakes and reservoirs. Quantitative fish sampling of these volumes still represents a challenge (Kubečka et al., 2012). Hydroacoustics is an obvious option covering large areas without disturbing fish and causing sampling-related mortality. However, most fish utilize shallow water closest to the surface (Jarolím et al., 2010; Kubečka and Wittingerová, 1998; Vašek et al., 2009) and it is difficult to obtain sound estimates with conventional downward-looking acoustics due to narrow beam width close to water surface. Only a small proportion of the fish stock can be assessed with downward-looking echosounding in eutrophic waters (Kubečka and Wittingerová, 1998). Another option for pelagic fish assessment, the horizontal beaming, seems to be rather difficult and influenced by unwanted interference of multipath reflection (Helge Balk et al., 2017). Therefore, reliable quantitative assessments of fish community in the shallowest layers of the open water are rare.

An alternative approach of mobile upward-looking surveying was developed to mitigate this unsatisfactory situation. This approach was showed to provide very clear data recordings of small fish and even invertebrates (Baran et al., 2017 and 2019). Upward-looking echosounding has been used for surveying of fish populations at fixed location (Čech and Kubečka, 2002; Jarolím et al., 2010), but rarely in mobile mode (Probst et al., 2009).

Upward-looking surveys are more reliable when provided at night (Baran et al. 2017 and 2019). During the surveys of Baran et al., (2017 and 2019) it was observed, that at daytime, fish are very likely to react to the survey vessel in front of it as was previously recognized by Rakowitz et al., (2012) and Muška et al., (2013). Daytime is also not a suitable period for surveying because most reservoir fish perform sinusoidal swimming to search the zooplankton more efficiently (Čech and Kubečka, 2002; Jarolím et al., 2010).

While performing sinusoidal swimming, the fish body aspect exposed to the upward-looking transducer is very difficult to define as the fish can have any aspect within the range of + 30 to –30 degrees tilt (Čech and Kubečka, 2002).

In this study, we explored whether night mobile upward-looking represents a reliable tool for community assessment of yearling-and-older fish. During the summer season in the Římov Reservoir (Czech Republic), we used the upward-looking acoustics and CEN multimesh gillnets simultaneously to enable comparison of size distributions obtained from the two gears. CEN multimesh gillnets (CEN, 2015) are relatively free of size selective biases for a wide range of fish sizes larger than 8 cm (Prchalová at al., 2009) and smaller than 30 cm standard length (Šmejkal et al., 2015). Further, we analyzed vertical micro-distribution of fish within the uppermost 3 m of the water column. We assumed high overlap between fish size distributions from the two gears as an indication of absence of avoidance behavior of fish being surveyed by upward-looking.

2. Material and method

2.1 Study area

This study was conducted in the Římov Reservoir (48°50'N, 19°30'E, 471 m above sea level., Fig. 1), 170 km south of Prague, Czech Republic. The reservoir was constructed on the Malše River in 1978. It is a canyon-shaped reservoir with a length of 12 km (on original riverbed), a maximum volume of 33 x 10^6 m^3, a surface area of 2.1 km^2, and an average and maximum depth of 16 m and 43 m, respectively. The trophic state of the reservoir is mesotrophic to eutrophic with well-developed thermal stratification during the summer. Dominant fish are common bream (*Abramis brama (*Linnaeus, 1758)), roach (*Rutilus rutilus (*Linnaeus, 1758)) and bleak (*Alburnus alburnus* (Linnaeus, 1758).

2.2 Fish sampling

The fish community was investigated in the pelagic habitat of Římov Reservoir over the course of three nights - 19/20 August 2013 (night named N1), 21/22 May 2014 (N2) and 8/9 August 2014 (N3). Two localities (Fig. 1) with similar stratification conditions during sampling nights (Fig. 2) were surveyed by the two methods.

2.2.1 Acoustic sampling

The acoustic part of the study was performed using a newly developed approach based on a mobile upward-looking acoustic system (Baran et al., 2017 and 2019). A research vessel (11 m long with a 210 HP engine) was equipped with two 12 m long submersible arms on either side that held a tiltable platform with attached transducers between their front ends. During the acoustic survey, the arms submerged the platform to a depth of 8 m with transducer emitting towards the surface in front of a research vessel. An exact vertical position of the acoustic beam was measured using an electronic clinometer, the RIEKER H5A1-90.

A frequency of 38 kHz (circular split-beam transducer SIMRAD ES38-12 with a nominal angle of 12 degrees) was used in the study. The operating power of the echosounder was set to 100 W with 0.05 s pulse interval (20 pings s^{-1}) and the pulse duration was set to 256 μs. Before each survey, the system was calibrated using a 60 mm diameter copper sphere as described by Demer et al. (2015).

The acoustic survey was performed using straight-line transects (1 km long) at a constant speed of 1 $m.s^{-1}$ near the gillnet set (Fig. 1). Two localitions were studied; L1 near the reservoir dam, and L2 in the middle part near the town of Velešín. Sampling occurred at depths greater than 10 m to avoid striking the bottom with the submerged platform. The GPS coordinates of the survey cruise were measured using a Garmin GPSMAP 60CSx GPS handheld unit connected to an external antenna for better reception of the signal.

Raw acoustic data were analyzed using the Sonar5-Pro post-processing software (CageEye A/S, Oslo, Norway). Beyond the theoretical blind zone (half of the pulse width from the phase boundary – water surface), we defined a surface line 0.3 m below the actual water surface so that surface echoes were safely excluded from data processing prior to data analysis (surface blind zone). The acoustic data were analyzed only to depths of 270 cm below the defined surface line. The acoustic system sampled the depth range of 0.3-3 m;

the gillnets 0-3 m. Automatic single echo detection (SED) threshold boundaries were set from -45.5 dB (corresponding to a theoretical fish length of about 80 mm). No maximum TS threshold was applied. To convert the captured fish size to the target strength (TS) from the 38 kHz echosounder we used the TS-to-length regression of Love (1977). A valid track was defined as at least two subsequent echoes from the same target, separated by a maximum of one missing ping within a 0.1 m vertical range gate.

Fish abundance (A) was calculated according to the track counting method (CEN, 2014; Simmonds and MacLennan, 2005):

$$\mathrm{A} = tracks/v_w * 100$$

where *tracks* stands for the number of tracks in a given transect, divided by the sampled wedge volume v_w in m^3. Fish abundance was expressed as the number of fish in 100 m^3 of sampled water (fish 100 m^{-3}).

Length-weight relationship to convert recorded fish of known length to biomass was used from the gillnets catch from the reservoir without regard to species (general length-weight relationship for all species captured by pelagic gillnets).

2.2.2 Gillnet sampling

Standard CEN pelagic gillnets were used 3 m high x 30 m long, having twelve 2.5m long panels with the following mesh sizes:5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43, 55 mm, knot to knot; CEN, 2015). Additionally, this gang was extended with large mesh gillnets (3 m high x 40 m long with 10 m long panels having 70, 90, 110 and 135 mm, knot to knot (Šmejkal et al., 2015). The large mesh panels ($\geq$ 70 mm) had four times higher effort than the small mesh panels (< 70 mm). Therefore, the catches of large mesh gillnets were divided by four to standardize the length of each panel to 2.5 m for all meshes.

The gillnets were set in a straight line approximately parallel to the shore over maximum depths (30-40m at L1 and 20-25m at L2). Three gillnets of each type were set as a basic effort at each location each night (3x90 m^2 and

$3x120m^2$ for CEN gillnets and large mesh gillnets, respectively). The gillnets were set two hours before sunset (approximately at 18:30) and pulled two hours after sunrise (approximately at 7:00) (Prchalová et al., 2010). The total gillnet effort was 36 nets (2 locations x 3 nights x (3+3) nets/night).

The catch was sorted by species. For each captured individual, the right-angled distance from the floating upper line representing the water surface was measured with the accuracy of 5 cm (=depth of fish capture). Total length (TL) to the nearest 0.5 cm and weight (g) was measured for each individual fish.

2.3 Statistics

Differences in fish sizes from gillnet catches and sizes predicted from the acoustic sampling were compared using the Kolmogorov-Smirnov paired test for all fish larger than 8 cm TL. Only fish and acoustic data from the 0.3-3 m depth layer 0.30 were compared. Statistical analyses were carried out using the R software (R Core Team 2019).

3. Results

3.1 Comparison of total catches and records

A total of 699 fish larger than 8 cm were caught in gillnets. The 36 gillnets altogether caught 451 bleak, 84 common bream, 120 roach, 26 European perch (*Perca fluviatilis*) and 18 individuals of other species. During hydroacoustic surveys, 463 targets bigger than 8 cm (-45.5 dB) were recorded and 15,526 m^3 of water was sampled. The number of captured fish and recorded targets were similar except N2 at L2, when gillnets caught more fish than were recorded with the hydroacoustic survey (Fig. 3).

3.2. Vertical fish distribution

In pelagic gillnets we captured between 55 to 71 percent of all caught fish in the topmost 1 m from the surface (Fig. 3). The percentages of acoustic targets detected in the 0.3 to 1 m depth layer represented 50 to 78 percent of all targets sampled in the top 3 m (Fig. 3). Depth distribution compared by the two methods was not statistically different except Night 2, L. 2 when too few fish were recorded at the surface by acoustics. With more than 50% of all catches, the dominant species was bleak, occurring most often at a depth of less than 1 m (Fig. 4). In N3 bleak dominated all depths down to 2.5 m. The second most common species was roach, which occurred mainly at the site 2 at depths >1 m below the surface. Common bream was found at any layer of the studied depth range. The size structure was distributed as follows - bleak dominated in the range 8-22 cm, roach was most abundant at sizes 24-38 cm and common bream dominated the largest size groups (Fig. 5).

3.3. Fish and target sizes comparison

Mean length of caught fish was usually greater than the estimated mean size from acoustic targets (Table 2, Fig. 6). Two times (N2 at L2 and N3 at L1) the estimated fish lengths from acoustic targets were significantly smaller than the caught fish from gillnets (Table 2). However, on N1 at L1 the mean length of the estimated acoustic sizes were bigger than the mean length of caught fish, but these differences were not statistically significant (Table 2).

Mean weight fish captured by gillnets was in four cases bigger than the reconstructed mean weight of acoustic targets (Table 3). However, only once was the average reconstructed weight significantly lower than that of fish caught in gillnets (Table 1). In two cases, the average weight of fish reconstructed from acoustic targets was higher than the weight of fish caught by gillnets.

Both approaches measured frequency peak corresponding to bleak and small roach (Fig. 5). Size resolution of gillnets was higher to dominant group for bleak 12-14 cm. The two pooled length distributions of overall sample on Fig. 5 were not significantly different between the gillnets and acoustic records Ks- test (>0.05, df 18).

4. Discussion

The current study demonstrates that mobile upward-looking acoustic surveys have potential to be a reliable tool in fish community assessment other epilimnion of stratified lentic waters. All studied parameters of the acoustic assessment including size distributions, overall recorded fish/targets, and vertical distributions corresponded well with the same parameters obtained by gillnets.

Both methods revealed that fish had an affinity to the topmost 1 m of the water column. Given this vertical distribution, it is practically impossible to obtain representative samples of the fish community by both classical down-looking and side-looking horizontal beaming (Fig.7). With both these approaches we underestimate the organisms living in the topmost surface layer. Side-looking horizontal beaming covers the surface layers, however, the most critical problem is that the estimated size of fish changes at different orientations relative to the transducer axis, the so-called side aspect (Frouzová et al., 2005; Rudstam et al., 2003). The determination of abundance and size is often performed using deconvolution (Kubečka and Wittingerová, 1998), which is based on stochastic assumptions of random aspect orientation that may not be entirely true (Tušer et al., 2009). The newly-discovered problem of the so-called mirror effect (Helge Balk et al., 2017) significantly affects the actual depth of targets and their target strength. Down-looking precludes sampling of about the topmost 3 m, due to transmitter deployment depth, blind zone and the very small sample volume near transducer (Simmonds and MacLennan, 2005). For this reason, down-looking is used for a depth of more than 2 m below the surface (Emmrich et al., 2012; Guillard et al., 2014; Knudsen et al., 2006; Yule et al., 2009). Alternative ways of surveying, such as upward-looking system, should be considered for waters with abundant surface-oriented fish, which appear to be rather frequent in many kinds of waters (Busch and Mehner, 2011; Helland et al., 2007; Jarolím et al., 2010; Marie Prchalová et al., 2009; Vašek et al., 2009).

Acoustic data were collected with a 38 kHz transducer with opening angle of 12 degrees which was the widest split-beam transducer available at the time of our survey work. A 38 kHz transducer, operating with the pulse duration of 256 μs, have nearly a 20 cm blind zone from the phase boundary, where fish echoes are obscured by much stronger reflections from the surface (Simmonds and MacLennan, 2005). However, the benefit of large sampling volume at the surface prevails over the loss of data near the surface (Fig.7).

The blind zone in the near surface layers with the upward-looking system is still much smaller in comparison to vertical or horizontal acoustics.

Both gillnet and acoustic surveys sampled similar numbers of recorded fish except during N2 at L2. The N2 data collection was in May when the gillnet catch may have been influenced by enhanced swimming activity connected with spawning. Fish swimming activity is one of the major factors affecting gillnet catch (Prchalová et al., 2010) and a general increase in activity connected with spawning can lead to might cause extraordinarily high catch. Another explanation for the differences is the fact that the gillnets integrate 12 hours of effort and some of the fish captured by the gillnets may be absent in the open water at night when the acoustic sampling took place in mid night. Outside of the spawning season, upward-looking system had reasonable agreement with the gillnet catches. We have to keep in mind that while sampling volume of acoustic sampling is defined by ultrasonic beam parameters, the effective sampling volume of gillnets is not known (Deceliere-Vergès et al., 2009; Prchalová et al., 2011b). Therefore, it is not possible to compare absolute abundance or biomass estimates directly. In this respect, the comparison of upward-looking system with an active sampling gear like a pelagic trawl with known efficiency should be considered in the future.

The estimated average sizes from up-looking were generally smaller than the average sizes of the fish caught by the gillnets. Two times the predicted lengths and one time for the weight there was a were significantly smaller than the average sizes of fish caught by gillnets. These differences could be due to several reasons:

1. TS-length regression is one potential source of error for predicting fish size from acoustic records. We have used 38 kHz generalized (multispecies) regression for vertical aspect (Love, 1977). However, this regression is not created for upward-looking and was not developed using European freshwater fish. On the other hand, the regressions of Love, 1977 has been found to provide realistic fish sizes in many freshwater studies (Boswell et al., 2008; Eckmann and Engesser, 2019; Frouzová et al., 2005) so we may assume that it did not cause most differences we observed.

2. Even if performed at very close locations on the same night, the two methods may not record the exact same fish. Peak sampling times are different. The fish are mostly caught by the gillnets when they swim intensely before sunset and then at sunrise (Millar, 2011; Prchalová et al., 2010). Acoustic surveys took place during the middle of the night when only a portion of larger fish reside in the open water (Muška et al., 2013) while many small fish migrate to the open water (Říha et al., 2015). These distributions could explain why smaller fish were recorded by the acoustic approach, and reinforces the need for the comparison of instantaneous results obtained with active sampling gear like a pelagic trawl or purse seine.

3. Although the fish reactions at night are limited (Rakowitz et al., 2012b), we cannot fully exclude the possibility of avoidance behavior of larger fish from the upward-looking system. This phenomenon should be studied by assessing the fish behavior directly in front of the sampling boat or by comparing with a robust active sampling method (see above). In the same time, we should keep in mind that the gillnet have generally lower efficiency in capturing small fish due to their lower inertia when entering the mesh (Marie Prchalová et al., 2009). Therefore, the proportion of fish under 150 mm length could be slightly underestimated in the gillnet catch.

Conclusions

The new sampling method circumvents the disadvantages of horizontal and downward looking hydroacoustic transducers when sampling near the surface (upper 3 m) to a large extent. We found most fish very close to the surface and these would be mostly missed by down- or side-looking acoustic sampling. Comparison with gillnets showed that upward-looking records provided similar fish size distribution. Evaluation of upward-looking mobile echo sounding could be enhanced by the comparison with quantitative active sampling gears or by a detailed assessment of fish behavior in front of the research vessel.

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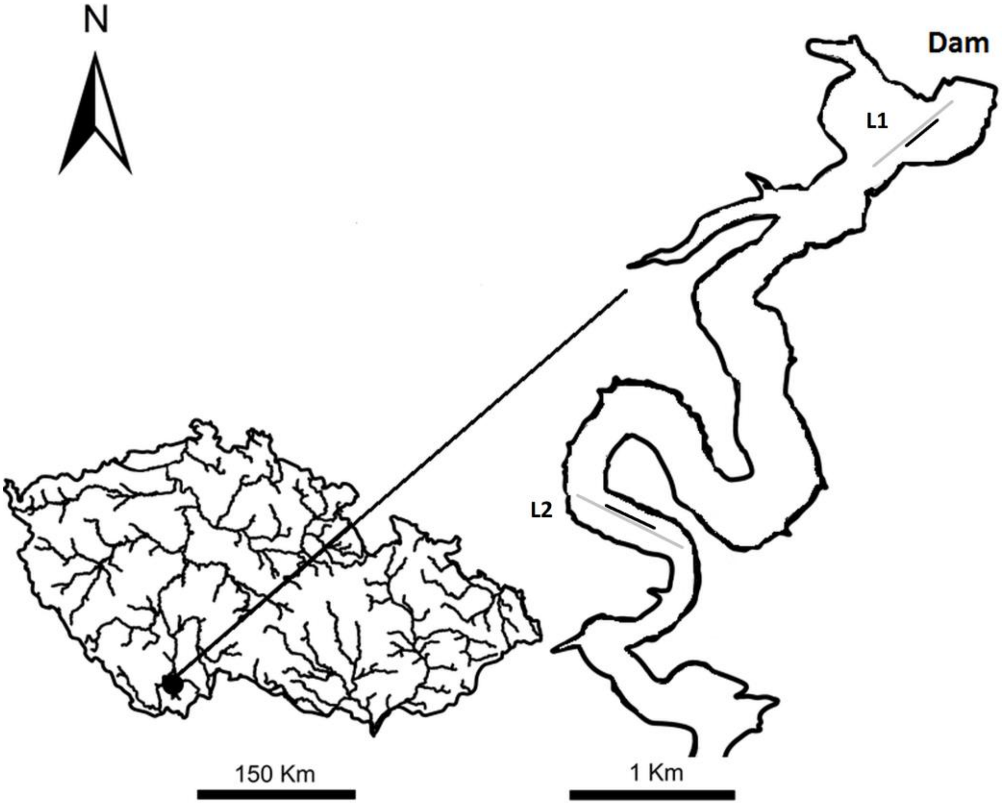

Fig. 1 A map of the Římov Reservoir and sampling locations (L1 and L2) in the Czech Republic.

The black lines show the location of pelagic gillnets and the gray lines indicate the acoustic transects.

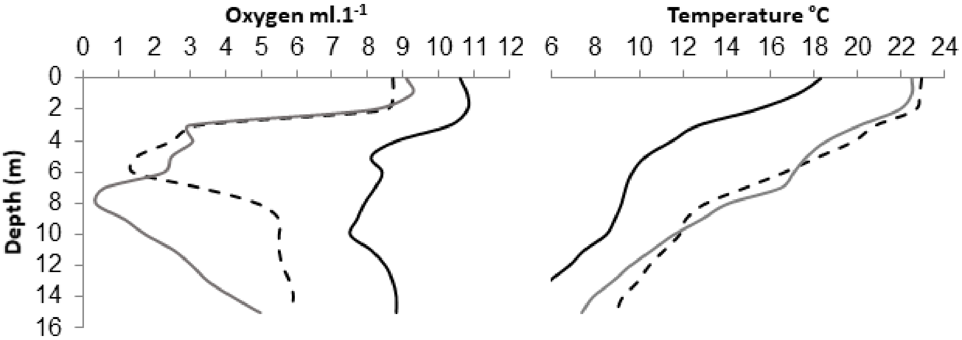

Fig. 2 Dissolved oxygen and temperature vertical profiles measured during each sampling night. Dashed black lines indicate N1 (19/20 August 2013), solid black N2 (21/22 May 2014), and the dark grey N3 (8/9 August 2014).

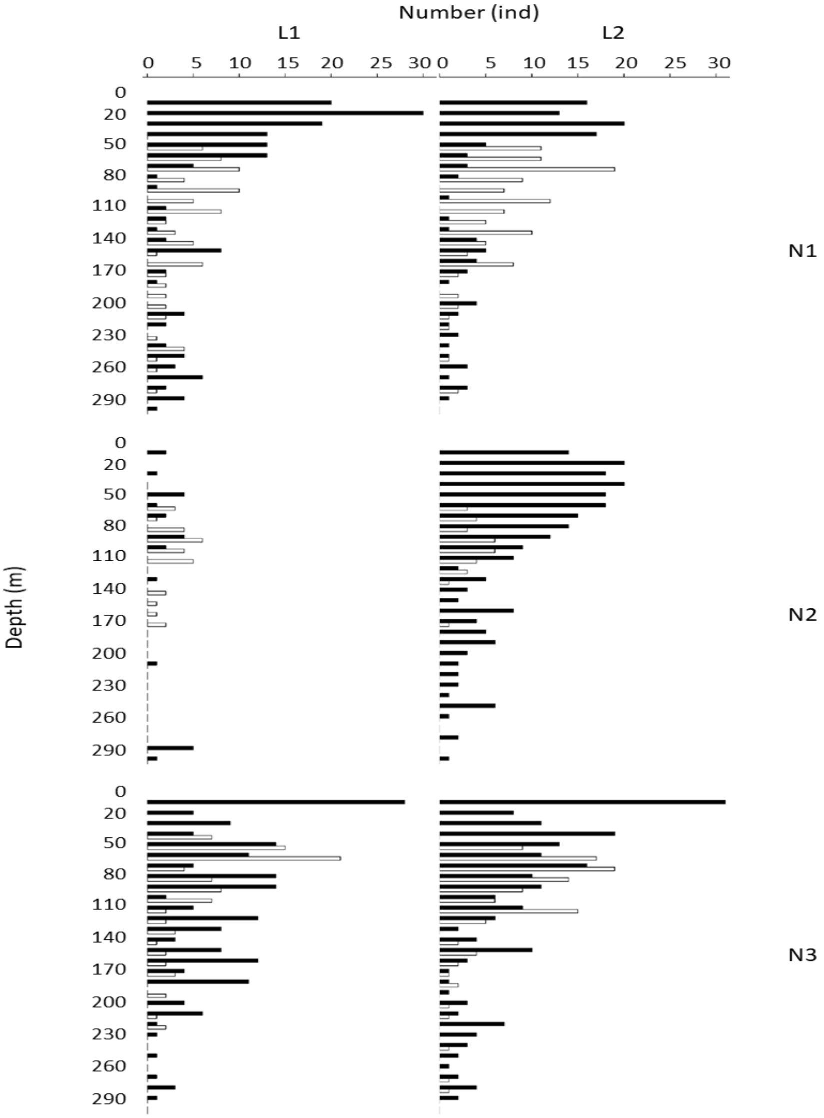

Fig. 3 Number of fish caught by gillnets and predicted lengths estimated from acoustic sampling by depth below the lake surface at the two locations (L1 and L2) over three nights (N1, N2 and N3 (see methods for details). Black bars indicate size distributions measured by gillnets and white bars estimated with acoustic sampling.

The acoustics did not record data at a depth of 0-30 cm from the surface.

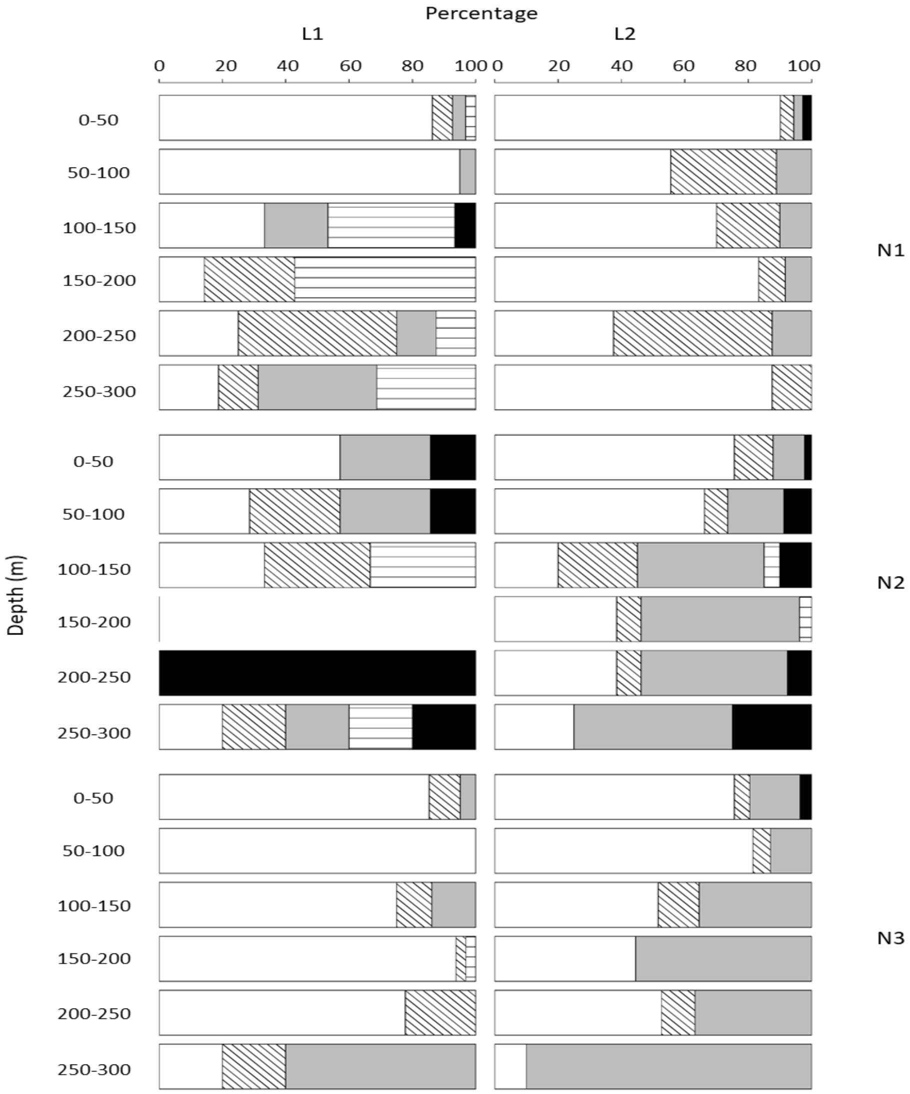

Fig. 4 Species composition of fish species captured in different depths by the gillnets.

White indicates bleak (*Alburnus alburnus*), oblique striped indicates common bream (*Abramis brama),* grey indicates roach (*Rutilus rutilus*), horizontal striped indicates European perch (*Perca fluviatilis*) and black indicates other species. N stands for nights, L for localitions.

Table 1. Statistical comparisons of depth distributions shown in Fig. 3. N1, N2 and N3 are night 1, night 2 and night 3, respectively; L1 and L2 are location 1 and location 2, respectively.

* Bonferroni correction (0.0083 for 6 same test); df were 27 in all test

Compare	**p-value***
Vertical distribution N1 – L1	>0.008
Vertical distribution N1 – L2	>0.008
Vertical distribution N2 – L1	>0.008
Vertical distribution N2 – L2	**<0.001**
Vertical distribution N3 – L1	>0.008
Vertical distribution N3 – L2	>0.008

Table 2. Mean total length of caught fish and estimated mean total length from acoustic targets

N1, N2 and N3 refer night 1, night 2 and night 3; L1 a L2 refer locality 1 and locality 2

* Bonferroni correction (0.0083 for 6 same test); df were 27 in all test

Sampling night	**Locality**	**Mean length (cm)**	**Reconstructed mean length of target (cm)**	**P-Value***
		Gillnets	**Acoustic**	
N1	**L1**	**17.05**	**22.42**	>0.008
N1	**L2**	**22.05**	**17.45**	>0.008
N2	**L1**	**18.84**	**12.77**	>0.008
N2	**L2**	**20.34**	**10.07**	**<0.001**
N3	**L1**	**15.28**	**7.96**	**<0.001**
N3	**L2**	**16.03**	**9.53**	>0.008

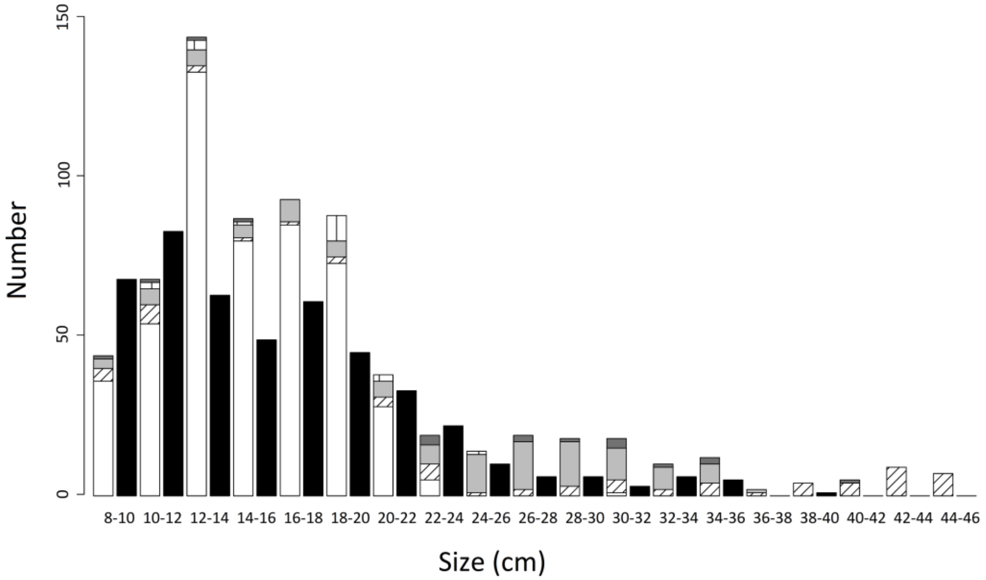

Fig. 5 Length frequency distribution of caught fish and recorded targets (all three nights and both sites pooled)

Black indicate hydroacoustic, second column caught fish - white indicates bleak (*Alburnus alburnus*), oblique striped indicates common bream (*Abramis brama),* light grey indicates roach (*Rutilus rutilus*), vertical striped indicates European perch (*Perca fluviatilis*) and dark grey indicates other species. N stands for nights, L for localitions.

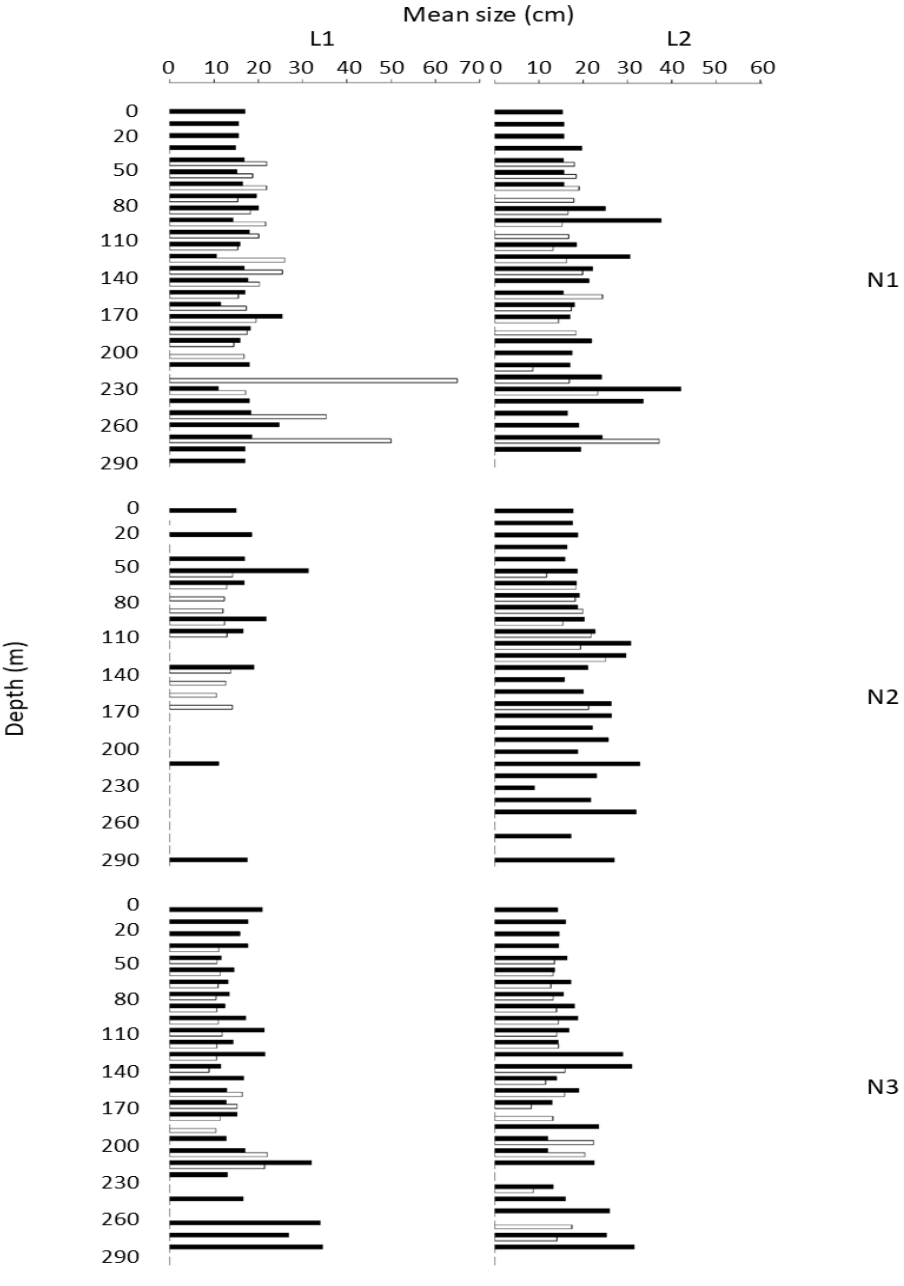

Fig. 6 Mean total length of caught fish and recalculated size of targets in individual nights and depth intervals.

Black indicates gillnet catch and white acoustic measurements. The acoustics did not record data at a depth of 0-30 cm from the surface

Table 3. Mean weight of caught fish and reconstructed mean weight of acoustic targets

N1, N2 and N3 refer night 1, night 2 and night 3; L1 a L2 refer locality 1 and locality 2

* Bonferroni correction (0.0083 for 6 same test); df were 27 in all test

Sampling night	**Locality**	**Mean weight (g)**	**Reconstructed mean weight of target (g)**	**P-Value***
		Gillnets	**Acoustic**	
N1	**L1**	**67.58**	**105.46**	>0.008
N1	**L2**	**59.11**	**95.28**	>0.008
N2	**L1**	**74.24**	**51.68**	>0.008
N2	**L2**	**154.56**	**64.68**	**<0.001**
N3	**L1**	**101.14**	**60.79**	>0.008
N3	**L2**	**145.69**	**69.56**	<0.008

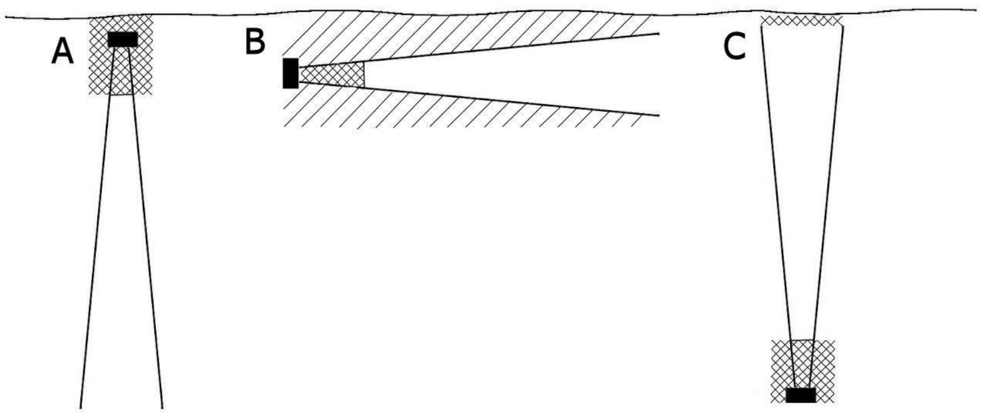

Fig. 7 Blind zones near the water surface during three types of sonar beam orientation.

A) vertical down-looking beaming, b) horizontal side-looking beaming, c) vertical upward-looking. Double hatching - blind zones due to transducer deployment, nearfield and phase boundary, single hatching – underestimated volume above and below the beam during horizontal beaming.

Research papers (not included in this Ph.D. thesis)

Publications in journals with IF:

Žák, J, Jůza, T, Blabolil, P, Baran, R., Bartoň, D., Draštík, V., Frouzová, J., Holubová, M., Ketelaars, H. A. M., Kočvara, L., Kubečka, L., Mrkvička, T., Muška, M., Říha, M., Sajdlová, Z., Šmejkal, M., Tušer, M., Vašek, M., Vejřík, L., Vejříková, I., Wagenvoort, A.J. (2018) Invasive round goby *Neogobius melanostomus* has sex-dependent locomotor activity and is under-represented in catches from passive fishing gear compared with seine catches. *J Fish Biol*. 2018; 93: 147– 152. https://doi.org/10.1111/jfb.13646

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Šmejkal, M., Ricard, D., Vejřík, L., Mrkvička, T., Vebrová, L., Baran, R., Blabolil, P., Sajdlová, Z., Vejříková, I., Prchalová, M., Kubečka, J., (2017) Seasonal and daily protandry in a cyprinid fish. *Sci Rep* **7,** 4737 (2017). https://doi.org/10.1038/s41598-017-04827-x

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Baran, R., Kubečka, J., Kubín, M., Lojkásek, B., Mrkvička, T., Ricard, D., Rulík, M. (2015). Abundance of Cottus poecilopus is influenced by O2 saturation, food density and brown trout in three tributaries of the Rožnovská Bečva River, Czech Republic. Journal of Fish Biology 86, 805–811.□doi:10.1111/jfb.12565

Conferences

Oral presentations (Czech)

Baran, R., Balk, H., Čech, M., Draštík, V., Frouzová, J., Muška, M., Ricard, D., Tušer, M., Kubečka, J., Mobile uplooking (lower acoustic view) a new method of hydroacoustic survey rezervoars. Zoologické dny, 6-7 February 2014, Ostrava, Czech Republic

Baran, R., Hydroacoustics, or a modern survey of fish stocks. IV. Setkání mladých limnologů, 12-14 April 2013, Lužnice, Czech Republic

Baran, R., Rulík, M., Kubín, M., Factor influencing occurrence of sculpin (*Cottus poecilopus).* XIII. Česká ichtyologická konference, 24-26 October 2012, Červená nad Vltavou, Czech Republic

Poster presentations

Baran, R., Tušer, M., Balk, H., Blabolil, P., Čech, M., Draštík, V., Frouzová, J., Jůza, T., Koliada, I., Mrkvička, T., Muška, M., Ricard, D., Sajdlová, Z., Vejřík, L., Kubečka, L. A novel upward-looking hydroacoustic method for improving pelagic fish surveys. 10 Symposium for European Freshwater Science 2017, 2-7 June, Olomouc, Czech Republic

Baran, R., Tušer, M., Balk, H., Blabolil, P., Čech, M., Draštík, V., Frouzová, J., Jůza, T., Koliada, I., Muška, M., Sajdlová, Z., Vejřík, L., Kubečka, L. Quantification of *Chaoborus* by mobile upward-looking echosounding. 10 Symposium for European Freshwater Science 2017, 2-7 June, Olomouc, Czech Republic

Baran, R., Frouzová, J., Jayasinghe ,U. A. D., Jůza, T., Tušer, M., Kubečka, J. The lunar cycle influence of juvenile fish in pelagic area of the Římov reservoir. Ecology of Fish in Lakes and Reservoirs 2014, 8-11 september 2014, České Budějovice, Czech Republic

Curriculum vitae

Date of birth:

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Education and academic employment:

since 2012 Ph.D. student at the Faculty of Science University of South Bohemia in České Budějovice, Czech Republic (Study of surface layers of water bodies using hydroacoustic method, supervisor: Prof. RNDr. Jan Kubečka, Ph.D.)

2009-2012 MSc. student at faculty of science, field of study – Hydrobiology of Palacký University Olomouc, Czech Republic (Faktors influencing occurrence sculpin *Cottus poecilopus,* supervisor: Doc. RNDr. Martin Rulík, Ph.D.)

2006-2010 BSc. student at faculty of science, field of study - Applied ecology of University of Ostrava, Czech Republic (Habitat preferences of fish in heavily affected by anthropogenic stretch of the river Ostravice, supervisor: doc. RNDr. Bohumír Lojkásek, CSc.)

2012-2017 part-time job as a student assistant worker at the Institute of Hydrobiology, Biology Centre Czech Academy of Sciences, České Budějovice - participation in sampling of lakes, reservoirs and rivers in Czech Republic, Ireland and the Netherlands, some of them repeatedly

Teaching:

Lectures at Faculty of Science University of South Bohemia: Agriculture Zoology, Field Practice I and II, Hydrobiology - Field

Special awards:

3rd place in poster section for student in Ecology of Fish in Lakes and Reservoirs 2014

Language skills:

Czech (mother tongue), English (B2, certificate TOEFL ITP), German (passive)

Computer skills:

MS Windows, MS Office, ArcGIS, MISYS, statistical software: R, Canoco, Statistica

Cover photos:
Front cover: Up-looking acoustic sampling by the research vessel Thor Heyerdahl. Underwater parts of the system are drawn schematically.

Back cover: Our new up-looking system can be also used as down-looking when the transducer holding frame is lifted near surface and the transducers are turned down.