

# Experimental Biomass Assessment of Three Species of Freshwater Aquatic Plants by Horizontal Acoustics

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## ABSTRACT

Estimation of aquatic plant biomass is a crucial component of successful management of aquatic ecosystems. In shallow water habitats, horizontal acoustics could be a promising approach for assessment of plant biomass, but tools for the interpretation of the acoustic characteristics of plants are needed. We first attempted to study the biomass of submerged aquatic plants by horizontal echosounding, with the goal to describe a basic acoustic feature,  $S_v$  (volume backscattering strength), and relate it to plant biomass. We set up experimental and field studies to describe three common freshwater species: water persicaria (*Polygonum amphibium* L.); Eurasian watermilfoil (*Myriophyllum spicatum* L.); and sago pondweed (*Potamogeton pectinatus* L.). We studied 96 plants (single plants, small and large patches), 64 in the experiment and 32 in a shallow turbid lake (Neusiedlersee, Austria), using a SIMRAD EK60 echosounder, with a circular composite transducer (nominal angle 6.8°). For all species, we found a positive linear relationship between  $S_v$  and dry biomass, describing 67 to 83% of the variability. The slope of the relationship differed statistically between species. The  $S_v$  of species overlapped, irrespective of their biomass; therefore, it was not suitable for species identification. The physiological/morphological states of the plants probably influence  $S_v$ , which was higher for species in the lake versus those in the experiment. Although gas, more than the biomass, is pre-

sumed to cause the plants reflectivity, we suggest an explanation of the relationship between these two variables. This study was a first step toward developing background research to create an operational system for shallow water studies of aquatic plants.

*Key words:* frequency 120 kHz, horizontal beaming, multiple target, shallow turbid lake, sonar.

## INTRODUCTION

Freshwater plants are one of the key elements in aquatic ecosystems (Madsen 1993). Their successful management is required to maintain the desired functions of target water bodies, including water quality, recreation and/or fish production (Orth et al. 2002, Winfield 2004). Along with species structure, abundance (Fortin et al. 1993), and the mapping of aquatic plants (Marshall and Lee 1994), an assessment of biomass (Duarte 1987) is typically the necessary starting point for further analysis (Madsen 1993), shaping the actual management. Classic methods of biomass assessment, based on determining biomass from plant samples collected over a studied area (Madsen 1993), are quite laborious, especially in large water bodies. To meet increasing requirements for large-scale information, new approaches to biomass assessment have been emerging (Maceina et al. 1984, Duarte 1987).

Shallow waters are typical habitat for many submerged freshwater plants, and horizontal echosounding has recently emerged as a potential research tool for such waters (Kubec-ka 1996, Duncan et al. 1998, Mulligan 2000). This tool, widely used for the detection and biomass assessment of fish (Simmonds and MacLennan 2005), has rarely been employed to study plants, although they commonly occur next to fish in the acoustic records. To date, acoustic studies of

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macrophytes have focused mainly on vertical echosounding in deep waters and seas, developing plant detection and species identification approaches (Thomas et al. 1990, Sabol et al. 2002, Faghani et al. 2004). Attempts to study macrophytes and/or their biomass by horizontal acoustics are rather scarce (Sabol et al. 1997). In addition, Sabol et al. (2002) argued that, compared to vertical acoustics, data from horizontal acoustics lack explicit means of interpretation (e.g., quantifying vegetation height and density); however, their records result in a greater acoustic cross section of vertically oriented plants. This clearly indicates a need for tools to interpret horizontal acoustic characteristics of aquatic plants. To read from and understand the acoustic records of aquatic plants, an unbiased conversion is needed, from the acoustic parameters to those more familiar to field biologists (such as biomass). As an initial step, we made a set of experimental acoustic measurements of both single plants and patches of three common freshwater aquatic species: water persicaria (*Polygonum amphibium* L.), Eurasian watermilfoil (*Myriophyllum spicatum* L.), and sago pondweed (*Potamogeton pectinatus* L.), and compared them with field data collected in a shallow turbid lake, Lake Neusiedlersee, Austria.

We aimed the recording at the basic acoustic parameters of the study plants; consequently, we related them to the plant biomass. In addition, we looked at the differences between the study species. The field data were collected in a large shallow turbid lake to determine if macrophytes could be detected in field conditions. We used a frequency of 120 kHz, a value widespread in freshwater fish studies and even recommended in underwater acoustics due to its suitable resolution in shallow water conditions (Duarte 1987, Kubecka and Wittingerova 1998, Drastik and Kubecka 2005, Frouzova et al. 2005). The acoustic features of our freshwater study plants had not been previously described for this or any other frequency.

## MATERIALS AND METHODS

Three species of aquatic macrophytes, water persicaria, Eurasian watermilfoil, and sago pondweed (Figure 1) differ markedly in their morphology. Persicaria usually has leafless stems with a raceme of apical leaves floating on the water surface; milfoil stems are often branched and covered throughout by fine short leaves; and pondweed stems are thin, often branching from about the middle of the stems, with long thin leaves. Single plants of persicaria and milfoil used in the study had 0 to 3 main branches; pondweed usually formed multiple branches. The average stem diameter of persicaria was 0.4 cm, milfoil 0.3 cm, and pondweed 0.1 cm. Leaf length in milfoil was 0.10 to 0.15 cm, width 0.2 cm; pondweed leaves were up to 7 cm long and 0.1 to 0.2 cm wide.

We used a fixed-located SIMRAD EK60 echosounder, operating at the frequency of the 120 kHz, with an ES 120-7C circular composite transducer, with a nominal beam angle of 6.8° and low side lobes (two-way maxima being -28 dB). The transducer was mounted on a remote-controlled pan-tilt rotator (Subatlantic Co.), enabling horizontal and vertical rotation. The echosounder recording setup was: pulse length 0.128 ms, emission interval 0.02 s, power 50 W, and TVG 20logR. The noise level of the amplitude echo detector was

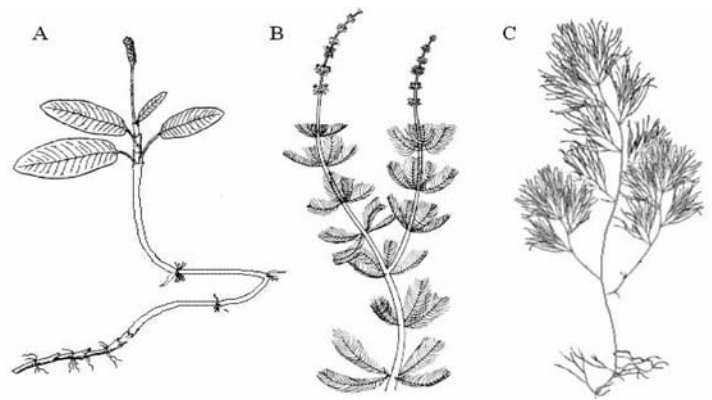


Figure 1. The species studied: A. Water persicaria (*Polygonum amphibium* L.); B. Eurasian watermilfoil (*Myriophyllum spicatum* L.); C. Sago pondweed (*Potamogeton pectinatus* L.).

set to the value of -100 dB. For further details and settings of the equipment used, see the Simrad (2004) manual. The echosounder was calibrated on a standard target (copper sphere) with a diameter of 23 mm and TS of -40.8 dB (Foote et al. 1987).

Compared to individual plants, described as single targets (Nealson and Gregory 2000), plant patches were considered as multiple targets. Because we employed single plants in addition to plant patches in the study, we used  $S_v$  volume backscattering strength (in dB) as the basic acoustic variable for multiple targets.

## Experimental and Field Horizontal Echolocation of Aquatic Macrophytes

The experiments were carried out July 5-19, 2005, in a 13 by 8 by 2 m outdoor concrete pool (Figure 2), where average water temperature was 21 °C and the water was mixed 3 to 4 times a day to prevent microstratification. Plants for the experiments were collected, including roots, from shallow ponds of the Trebonsko area (Czech Republic), immediately placed into transport boxes without exposure to the air to avoid disturbance, and transferred to the pool where they were kept continuously in water throughout the experiment. The plants collected represented a range of different types of single plants and patches (Table 1). Within species, the following categories of plants were recorded: single plants,

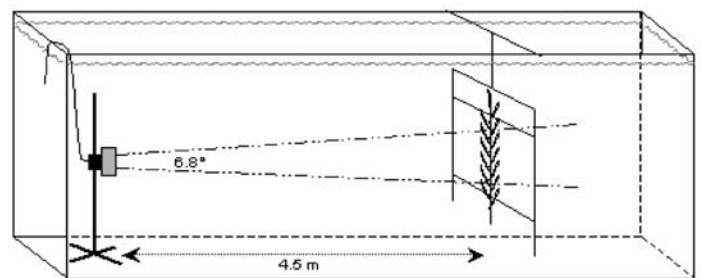


Figure 2. Schematic view of an experimental set-up of echosounding of aquatic macrophytes in a pool (13 by 8 by 2 m). Distance ratios do not reflect the actual.

TABLE 1. BASIC CHARACTERISTICS OF THE STUDIED PLANTS SPLIT INTO PLANT CATEGORIES (SINGLE PLANTS, SMALL PATCHES, AND LARGE PATCHES) AND BY SITE (EXPERIMENTAL POOL, LAKE NEUSIEDLERSEE). COLUMN NOTE 'N' IS THE NUMBER OF PLANTS IN EACH CATEGORY FOR THE POOL OR LAKE; 'K' IS THE NUMBER OF REPLICATION OF ACOUSTIC MEASUREMENTS OF PLANTS/PATCHES (THEY ARE RELATED TO ACOUSTIC VARIABLES). SPECIES INCLUDE *POLYGONUM AMPHIBIUM*, *MYRIOPHYLLUM SPICATUM*, AND *POTAMOGETON PECTINATUS*.

Plant category	Single plants			Small patches			Large patches		
	Species		n	k	n	k	n	k	
<i>P. amphibium</i>	12	3	6	3	7	3-6			
<i>M. spicatum</i>	12/4	3/1-3	6/5	3/1-3	7/4	3-6/1-3			
<i>Pot. pectinatus</i>	6/7	3/3	-/5	-/3	8/5	3/3			

Dry biomass in beam [g]	Mean	SD	Min-Max	Mean	SD	Min-Max	Mean	SD	Min-Max
Experimental pool									
<i>P. amphibium</i>	0.78	0.25	0.46-1.36	2.45	0.48	2.01-3.31	24.53	9.44	17.18-45.16
<i>M. spicatum</i>	0.24	0.06	0.17-0.37	0.74	0.09	0.58-0.84	13.06	6.14	8.16-26.46
<i>Pot. pectinatus</i>	0.23	0.24	0.06-0.68				10.79	7.81	2.24-25.65
Neusiedlersee Lake									
<i>M. spicatum</i>	0.80	1.07	0.27-2.40	3.63	1.59	1.72-5.54	44.77	37.38	11.51-83.53
<i>Pot. pectinatus</i>	0.59	0.40	0.28-1.22	4.76	1.86	2.35-7.25	38.71	8.95	31.25-53.44

small patches (3 single plants together) and large patches (22 to 102 stems, depending on the species, to a maximum patch width of 20 cm, or a maximum of 10 cm to either side of the beam axis; Figure 2, Table 1). The field records were collected in large, shallow Lake Neusiedlersee, Austria (average depth 1.6 m) in August 2005 and 2006. Here, watermilfoil and pondweed were studied in categories: single plants, small patches (2 to 13 plants), and large patches (11 to 125) to a maximum width of 45 cm.

For recording in the pool, all plants were lightly fastened with their tops and root parts placed between nylon threads in a fixed position on a portable aluminum frame, to ensure as natural position of the plants in the water column as possible. Although nylon threads do not reflect in a sound beam, they were carefully placed outside of the beam, as was the aluminum frame. The frame with plants was placed 4.5 m in front of the transducer. The lake setup was identical, with the difference that, where possible, the plants were recorded at their growth place. In dense surrounding vegetation, the chosen plants and patches were uprooted, mounted to the frame, and moved to an adjacent open water area for recording.

The main factors considered when choosing the distance of plant from the transducer were: the diameter of the beam (i.e., largest possible cross section of plant to be ensounded); water depth (to avoid bottom and surface reflections of sound); meaningful applicability in the field; and feasibility of testing in the experimental pool. The results of this study are valid for a distance of 4.5 m from the transducer. Due to the negligible side-lobes of the transducer (Simrad 2004) and the strong echoes of the plants, side-lobes were not considered to have an affect on the records. Because side lobes can potentially affect the final results, at the start of each experiment we mapped the beam with a calibration sphere when recording the empty water column and determined the side lobes did not impact the results.

The beam from the transducer was aimed horizontally across the pool. Aided by a standard target suspended in the desired position, the horizontal axis of the beam was placed

so that it hit mid-distance between the nylon threads. A plant was then installed between the threads on axis of the beam indicated by the standard target to ensure that the desired, and in all cases the same, middle part of the installed plants would be recorded. This setup simulated conditions in shallow lake water, where it was also used. There, the beam was set to the middle distance between the bottom and water surface. The size of the recorded part of an installed plant (55 cm, "L55"; range 54-57 cm) was related to the diameter of the sonar beam at a distance of 4.5 m from the transducer (Figure 2).

For each plant or patch, a stable record was made of the plant in the beam (about 2 min), and repeated at least 3-times (Table 1). Between any two records, the plant/patch was slightly changed in the orientation of the stems (slightly turned around their axis) while remaining centered. The goal was to record a maximum echo from the plant/patch. In the lake, one to three records were taken, depending on the weather conditions. The lengths of the plants were then measured, and the recorded middle portion (L55) of each plant was cut off. The plants were then dried (42 h, 105 C) and weighed to obtain dry biomass (Westlake 1965).

## Data Analysis

Raw sonar data were processed by Sonar5 (Balk and Lindem 2005) and further analyzed in Microsoft Excel, Statistica 5.0, and GraphPad Prism. A threshold for the amplitude echogram in Sonar5 was set to -70 dB. Values of  $S_v$  were read from an oscilloscope set in mean ping mode, giving mean  $S_v$  from all pings in a selected part of the plant record on the echogram. The pulse volume (Burczynski 1979, Simmonds and MacLennan 2005) of the sampled plants, under the conditions described above, was 42 L. The echosounder extrapolates the  $S_v$  obtained for plants in the pulse volume to 1 m<sup>3</sup> (1000 L), assuming that the level of backscattering cross-section area stays the same within the whole volume. This was not true for the plants; thus, the  $S_v$  obtained was considered valid for the 42 L sampled, and then manually recalculated

to 1 m<sup>3</sup>. We used reciprocal proportion for the volume and the S<sub>v</sub>, used in its linear form: s<sub>v</sub> (volume backscattering coefficient in m<sup>2</sup>/m<sup>3</sup>; Simmonds and MacLennan 2005):

$$s_{v1000} = \frac{42L * s_{v42}}{1000L}$$

where the numerical values of s<sub>v</sub> refer to the volume related to it. After recalculation, the logarithm of s<sub>v</sub> was applied to obtain S<sub>v</sub>: S<sub>v</sub> = 10 \* log<sub>10</sub> \* s<sub>v</sub>. To assess the relationship between acoustic values and dry biomass, the weight of the plants' recorded portion (L55) was used (i.e., the mass of plants within the 42-L pulse volume).

S<sub>v</sub> values entering the analyses were the mean values of repeated measurements of each plant/patch, and were log-transformed. We compared mean S<sub>v</sub> values between species using nested ANOVA with the levels: 1, Species; 2, Plant; and 3, S<sub>v</sub> values. ANOVA was also used to compare the slopes of linear regressions of mean S<sub>v</sub> to dry biomass. Tukey HSD for unequal numbers of observations was used as post-hoc test for ANOVA where appropriate.

## RESULTS

### Description of Species

We studied 94 plants/patches, 64 in the experiment and 30 in the lake (Table 1). Overall weights of dry plant biomass in the beam did not overlap between categories in any of the species (Table 1) used in the experiment; the corresponding S<sub>v</sub> however, did overlap. For example, in persicaria, overall S<sub>v</sub> values of small and large patches overlapped slightly (Table 2), although the plants of these two categories differed substantially in weight. In contrast, S<sub>v</sub> of single plants and small patches did not overlap, although they were much closer in weight to each other (Tables 1 and 2). In the lake, the range of plant weights and S<sub>v</sub> also differed in their overlap between plant categories (Tables 1 and 2).

A significant positive linear relationship was found between S<sub>v</sub> and dry biomass for all species, both in the experiment and the lake, when looking across all categories (Table 3; Figure 3). Within categories in the experiment and the lake, the S<sub>v</sub>-biomass relationships were mostly weak and showed variable slopes.

No obvious difference was found between the reflections of those species with leafless single stems (persicaria) and those with branched stems covered with leaves (watermilfoil and pondweed). Although S<sub>v</sub> in single plants differed between the species, the values approached one another with increasing weight (i.e., they did not retain any constant difference that could be related to the character of the stem).

In watermilfoil, it seemed that the S<sub>v</sub> and biomass relationship showed no further increase of S<sub>v</sub> with weight in the heaviest patches (Figure 4). Under certain conditions, a threshold of the biomass can probably be reached where no further increase of reflection occurs.

### Comparison Between Species

Despite the overlap of S<sub>v</sub> between the species used in the experiments, the sets of S<sub>v</sub> values showed between-species differences as well (nested ANOVA across all categories: F = 8.91, df = 2, p = 0.016). Moreover, a significant difference was detected between pondweed and watermilfoil (Tukey p = 0.03). The same was found for single plants (NA: F = 16.09, df = 2, p = 0.004), when pondweed differed from the other two species (Tukey: from watermilfoil p = 0.03; from persicaria p = 0.047). The S<sub>v</sub> values appeared to be higher in pondweed, but because of the lower number of observations and high variability in this species, these results may not be accurate. In the lake, the sets of S<sub>v</sub> values for watermilfoil and pondweed widely overlapped and thus did not differ statistically; the same was true for the single plants.

In the experiment, the linear relationships between mean S<sub>v</sub> and dry biomass (Table 3; Figure 3) differed in slope between the species when looking across all categories (ANOVA: F = 9.74, df = 2; 58, p < 0.001). This was mainly due to the steeper slope of persicaria (Table 4; Figure 3). Slopes of the S<sub>v</sub>-biomass relationship and their mutual position were also similar for the watermilfoil and pondweed studied in the lake (Figure 3).

Slopes of the S<sub>v</sub>-biomass relationship for the same species in the experiment and the lake did not differ (ANOVA). In contrast, the elevation of the intercept differed significantly (ANOVA pondweed: F = 11.07, df = 1; 28, p = 0.0025; watermilfoil: F = 11.42, d = 1; 35, p = 0.002), and showed higher S<sub>v</sub> values for the lake plants of the same weight range as those used in the experiment (Figure 4).

TABLE 2. MEAN, STANDARD DEVIATION (SD), AND RANGE (MINIMUM-MAXIMUM VALUES) OF S<sub>v</sub> (VOLUME BACKSCATTERING STRENGTH) RECALCULATED TO 1 M<sup>3</sup>, FOR EACH PLANT CATEGORY OF THE STUDIED SPECIES. FOR THE SPECIES ABBREVIATIONS, REFER TO TABLE 1.

Mean S <sub>v</sub>	Single plants				Small patches				Large patches			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Experimental pool												
<i>P. amphibium</i>	-27.48	1.84	-29.63	-24.00	-19.32	1.66	-21.63	-17.40	-14.49	2.22	-18.70	-12.77
<i>M. spicatum</i>	-27.77	3.21	-32.30	-21.83	-21.52	1.49	-22.90	-18.73	-17.33	1.79	-19.63	-14.30
<i>Pot. pectinatus</i>	-23.36	2.43	-25.80	-20.87					-16.86	1.79	-19.77	-13.97
Neusiedlersee Lake												
<i>M. spicatum</i>	-22.13	2.98	-25.17	-18.97	-15.26	1.56	-17.27	-13.07	-13.77	0.91	-14.77	-12.57
<i>Pot. pectinatus</i>	-19.54	2.54	-22.63	-15.87	-15.24	0.92	-16.23	-14.20	-13.47	0.72	-14.23	-12.50

TABLE 3. REGRESSION COEFFICIENTS AND PARAMETERS OF LINEAR RELATIONSHIPS ( $\alpha$ -INTERCEPT,  $\beta$ -SLOPE) BETWEEN  $S_v$  AND DRY BIOMASS FOR EACH SPECIES, ACROSS ALL PLANT CATEGORIES:  $S_v = \alpha + \beta \cdot \text{LOG}(W)$  (ALL RELATIONSHIPS ARE SIGNIFICANT AT  $P = 0.001$ ).

	Mean $S_v$	Slope ( $\beta$ )	Intercept ( $\alpha$ )	n
Experimental pool				
<i>Polygonum amphibium</i>	$R^2 = 0.82$	8.399	-25.361	25
<i>Myriophyllum spicatum</i>	$R^2 = 0.72$	5.947	-23.182	25
<i>Potamogeton pectinatus</i>	$R^2 = 0.76$	3.567	-20.325	14
Neusiedlersee Lake				
<i>Myriophyllum spicatum</i>	$R^2 = 0.67$	4.064	-19.174	13
<i>Potamogeton pectinatus</i>	$R^2 = 0.83$	3.427	-18.279	17

## DISCUSSION

We found that  $S_v$  in horizontal echosounding is a meaningful acoustic parameter in the description of submerged aquatic plants. The descriptive features of this parameter vary, depending on which feature we examined. Ranges of  $S_v$  values overlapped between species, suggesting that if a random record of a plant is chosen, we cannot identify the species using  $S_v$ . Similarly, because of the overlap of  $S_v$  between plant categories of different weights, it would be difficult to identify the category (single plant, patch) when the  $S_v$  of the random record falls within the overlapping range. The same was true for the lake data.

Conversely, we revealed a significant positive linear relationship between  $S_v$  and dry biomass for all species when assessing across all plant categories. The slope of this relationship differed slightly between the species, suggesting that  $S_v$  could be a potential predictor of plant biomass for a particular species. Although the relationship explained around 75% of the variability in each species, it is not yet sufficient for  $S_v$  to be used as an accurate predictor (Sabol et al. 2002).

Within the categories of each species, diversity of slopes of the  $S_v$ -biomass relationships (from significantly positive to negative), as opposed to the strong positive relationships found across all categories within a whole species, might be caused by a number of factors. One possible factor is the low number of observations within each category, which did not allow for a stronger result. Another possible factor is limited sensitivity of the equipment to subtle differences of weight between the recorded plants. In addition,  $S_v$  as a stochastic variable naturally shows a certain level of variability (Simmonds and MacLennan 2005). The same reasoning could explain the variance of  $S_v$  between repeated records for each plant and the variance within the whole species studied. Within all plants, it is also questionable whether  $S_v$  variation is caused only by the stochasticity of  $S_v$  or perhaps also by inconsistent reflectivity of the recorded plants. In the latter case, this phenomenon may coincide with the same one known for fish (Frouzova et al. 2005), whose reflectivity differs with the angle at which they are oriented in the beam (e.g., side aspect, tail aspect). Although most aquatic plants usually have round stems (so that the angle should not matter), they are usually

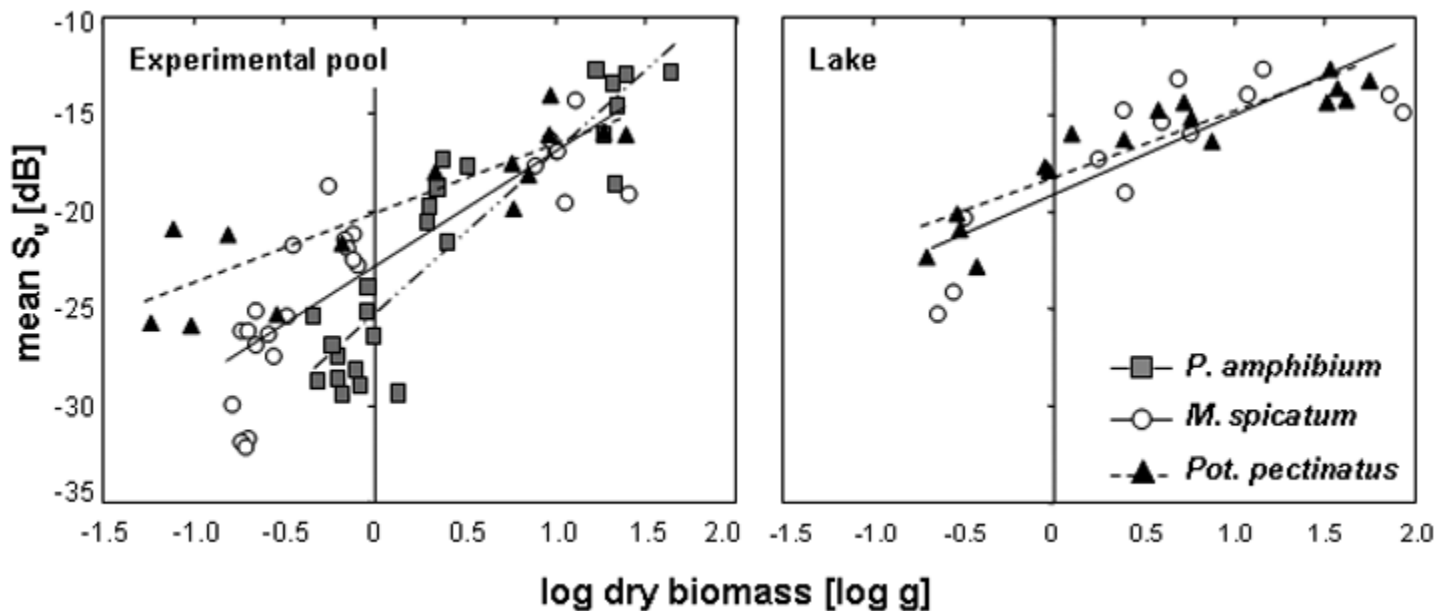


Figure 3. Scatterplot of mean  $S_v$  and dry biomass of recorded plants, with trendlines of linear relationship for water persicaria (*Polygonum amphibium*), Eurasian watermilfoil (*Myriophyllum spicatum*), and sago pondweed (*Potamogeton pectinatus*), in both the experiment and the lake. For equation parameters of the relationships, see Table 3.

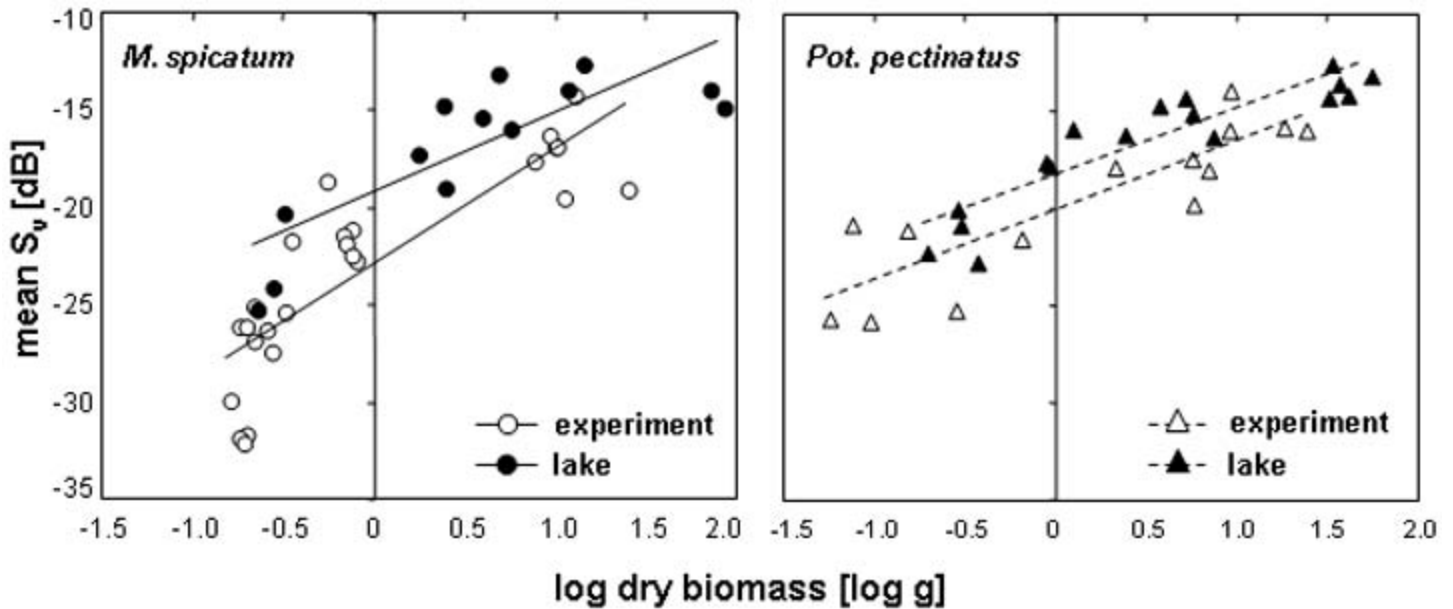


Figure 4. Comparison of relationships of  $S_v$  and biomass for Eurasian watermilfoil (*Myriophyllum spicatum*), and sago pondweed (*Potamogeton pectinatus*), studied in both the experiment and the lake.

not straight and have nodi or other structures, whose presence and orientation might influence the plant reflectivity.

In the case of watermilfoil, the  $S_v$ -biomass relationship showed no further increase of  $S_v$  with weight in the heaviest patches. One possible explanation could be that when a certain biomass is attained (or a factor causing the reflectivity is accumulated to this level, as a result of biomass accumulation), the patches do not further increase their reflection level. This effect can be the result of acoustic shadowing (Røttingen 1976, Simmonds and McLennan 2005). To learn more about this, patches of higher biomass will have to be studied.

Although its exact phenomenological basis has not yet been established, the acoustic impedance, which results in reflectivity, is thought to result primarily from the gas within the plants (Sabol et al. 2002). This assumption is supported by the observation that the more buoyant species (i.e., those with more gas) are more acoustically reflective (Sabol and Burczynski 1998). The positive linear relationship found in our study between a measure of acoustic reflection ( $S_v$ ) and the plant biomass suggests that in case the plant biomass is not the main basis of plant reflectivity, a positive relationship of the biomass to the basis of reflectivity can be expected. For such a relationship we suggest that the biomass of the plant structures, mainly those containing the highest amount of gas in the plant (e.g., stems), corresponds with the amount of gas these structures contain. Here, the thickness of the stem walls (or of the other structures) and the inside diameter of the particular structure would play a role. We did not find a significant difference between the species with bare stems and those with leafy stems; in the studied species, the stems themselves probably played the main role in the reflectivity (i.e., those structures containing the most gas and most biomass). It would be interesting to compare our findings with Sculthorpe's (1967) treatment of buoyancy in aquatic vascular plants.

The hypothesis above could also explain the finding that in both species studied in the lake, the  $S_v$  values were higher than those for the plants of similar weight range in the experiment. Since there was no difference in weight between the plants, the explanation might be found in the inside diameter of their stems, and thus the reflective area of gas within. Visually, the plant stems were slightly thicker in the Lake Neusiedlersee plants, but no direct relevant records were taken. In this case, the stems of larger diameters did not differ in weight from those with a smaller diameter, which possibly had thinner walls. Frouzova et al. (2005) concluded that in fish, it is not the volume of the swimbladder (presumably the most reflective and gas-filled structure in the fish body) that is the best indicator influencing the measure of fish reflectivity (TS-target strength), but the swimbladder backscattering area. We suggest that in plants, the inside diameter of their stem, and thus the area of gas within the stem could be an indicator.

Our experiment was intended as an initial step toward developing a complex background for an operational system for use in shallow water plant studies (biomass assessment, mapping). The first goal, to find the meaningful relationship between acoustic and real biomass of plants, was successfully met.

Although a higher frequency may resolve finer details, by using a frequency of 120 kHz we intentionally employed one

TABLE 4. RESULTS OF NESTED ANOVA FOR COMPARISON OF SLOPES ( $\beta$ ) OF THE LINEAR RELATIONSHIPS BETWEEN  $S_v$  AND DRY BIOMASS BETWEEN SPECIES IN THE EXPERIMENT. IN THE PARENTHESES ARE DEGREES OF FREEDOM (DF). \*SIGNIFICANCE AT  $P = 0.05$  AND \*\*\*AT  $P = 0.001$ . FOR THE SPECIES ABBREVIATIONS, REFER TO TABLE 1.

Nested ANOVA, F-values	<i>M. spicatum</i>	<i>Pot. pectinatus</i>
<i>P. amphibium</i>	4.76* (df = 1; 46)	22.21*** (df = 1; 35)
<i>M. spicatum</i>		5.50* (df = 1; 35)

of the very common frequencies used in freshwater fish studies (Kubecka and Wittingerova 1998, Frouzova et al. 2005, Drastik and Kubecka 2005). This enables potential future comparisons of fish and plant data at the same frequency; additionally, using one frequency (i.e., one piece of equipment) for assessing plants as well as fish will have an economic benefit.

The dimensions of the beam used were suitably applicable in the shallow waters studied, and we did not encounter bottom or surface reflections interfering with plant echoes, either in the experimental pool (minimum depth 1.5 m) or in the lake (minimum depth of plant records was 0.8 m), at the distance studied. In the lake, this set-up was most suitable for use in windless and rainless conditions, when the water surface was more-or-less still. In that case, the surface and bottom reflections on the echogram showed 0.3 m or more beyond the recorded plant, a sufficient distance to acoustically separate echoes (Simmonds and MacLennan 2005). Records affected by steady and/or strong rain and wind were not used for analysis.

The study showed positive potential for plant detection and biomass assessment using horizontal echosounding. The next steps toward an operational boat-based system application include: identifying the range of distances between echosounder and plants in which the interpretation of acoustic and biomass attributes is both possible and meaningful; studying other acoustic characteristics and their potential to describe acoustic behavior, biomass reading and/or other assessments of aquatic macrophytes.

## ACKNOWLEDGMENTS

The project was supported by a postdoc grant of the Grant Agency of the Czech Republic, No. 206/04/P092. We would like to thank Martina Ctvrtlikova, Tomas Juza, Lubos Pialek, Oldrich Jarolim, and Michal Tuser for their invaluable help in the field. We greatly thank Simona Polakova for her advice with the statistics. For useful suggestions concerning the data and text, we also thank Jan Kvet, Frantisek Zemek, Peter R. Lemkin and Jiri Nedoma.

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