A Monte Carlo experiment for measuring acoustic properties of macroalgae living tissue

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Abstract: A methodology is developed to measure *ex situ* ultrasonic velocity of submerged aquatic vegetation tissue, in particular, macroalgae, in a nondestructive and efficient manner. An entire thallus is submerged in artificial seawater-filled tank through which many ultrasonic pulse-echo measurements are recorded while thallus parts are randomly displaced. Average sound speed of tissue is estimated from normal fit to extracted travel times given measured total volume fraction of tissue and travel time in water alone. For species *Ecklonia radiata* the resulting values for sound speed 1573.4 ± 4.8 m s^{-1} and adiabatic compressibility $3.134 \times 10^{-10} \pm 1.34 \times 10^{-11}$ Pa^{-1} at 18 °C agree with more laborious and destructive methods.

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1. Introduction

With recent advances acoustics enables monitoring of marine biotopes over a range of scales.[1] Multibeam echosounder and sidescan sonar surveys generate backscatter data that can be processed to extract textural and morphological features and material properties (acoustic facies) facilitating the mapping of benthic communities. Work is in development to assess the use of propagation data to monitor aggregate primary production in kelp beds and forests.[2] Regardless of the acoustic sensing approach bridging physics and biology is required to more effectively transform acoustic observations into ecologically meaningful information. This letter addresses the measurement of sound speed and compressibility of macroalgae living tissue as key inputs to future models that will describe the frequency-dependent response of individual and assemblages. As soft biological media, macroalgae tissues are viscoelastic solids with nonhomogeneous and anisotropic properties. Acoustic investigations are further complicated due to the morphology of most species with complex geometry and high variability between thickness and surface area of the leaf-like flattened (blade) and stem-like (stipe) structures. Several approaches have been followed to overcome these difficulties, each with their own specificities. Large pieces were placed in a resonator tube to investigate their "low-frequency" behavior in accounting for both tissue structure and elastic properties.[3] Ultrasonic techniques[4] were adapted to directly measure intrinsic properties of distinct tissue types.[5] For both measurement types, suspensions were prepared with well blended tissue and artificial seawater (ASW) to form a "soup"-like heterogeneous mixture from macroalgae[5] or seagrass;[6] however, acoustic properties could potentially be transformed through breakdown of cell structure. To prevent this eventuality, measurement could be made on a piece of intact tissue immersed in seawater but accuracy would be severely limited by uncertainty in measuring a loosely defined

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and relatively small thickness of undulated or corrugated blade shapes. Accuracy requires labor-intensive preparation of samples of sufficient size, e.g., by "millefeuille"-like layering several tens of blades[5] to obtain a thickness of a few centimeters and vacuuming air and extraneous water entrapped between the layers. Depending on species, such stacks may be difficult to assemble and their surface too uneven to ensure proper coupling with contact probes. Also, release of mucous when blades are cut at ambient temperature may change tissue composition. A colder temperature will reduce this effect but may change acoustic behavior in addition to the sound speed decrease strictly related to high water content. But the major drawback of these procedures is their destructive nature not enabling return of the samples to the collection site and follow-up of an individual. Age, season, and nutrient availability will all effect growth, cell packing, and concentration of cell contents such as alginates and as such individual acoustic properties will vary over time, therefore, nondestructive techniques are advantageous. This paper details a method developed that preserves living tissue and is applicable to a wide range of macroalgae species. First, the principle of the measurement is explained. An experiment is then described on a key habitat-forming species of shallow water reef environment in temperate Australia [Fig. 1(a)]. Results are then discussed.

2. Principle of measurement

An entire macroalgae sample is submerged in a cuboid tank filled with seawater [Fig. 1(b)]. Sample and water volumes are accurately measured. Ultrasound pulses are emitted perpendicularly through one vertical face. Echoes from the opposite face are received back at the emitter. For a given "snapshot," the volume of tissue traversed by a collimated beam will depend on the position and orientation of every part of the algal body (thallus) at that instant. Pulse-echo data are recorded while continuously displacing the whole thallus by slow random movements of rotation about the stipe and translation in the three directions. Each snapshot involves different pieces of tissue and angles of incidence. The travel time (TT) will depend linearly on the unknown linear lengths of water and tissue along the direct path, and on the respective measured and unknown sound speeds. A single measurement is similar to those made on krill[7] but in reflection mode and for smaller volume fraction of biomass ($<10\%$). When averaged over a large number (N) of snapshots, the fraction of tissue traversed by the beam will converge to the ratio of entire sample volume and total volume. TT data are statistically modeled by a normal probability density function (Pdf) whose parameters provide an estimate of the tissue average sound speed. This is justified by the central limit theorem which states that the sum of independent samples from any

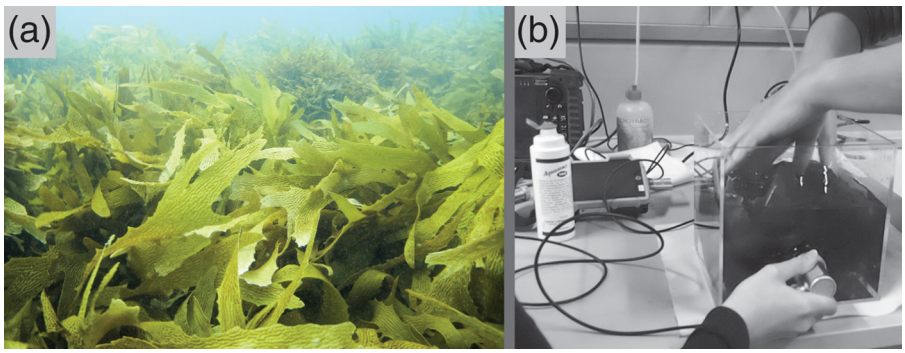

Fig. 1. (Color online) Monte Carlo experiment for measuring ultrasonic velocity of macroalgae living tissue. (a) Investigated species *E. radiata* at the sample collection site of Fortescue Bay, Tasmania, June 2012. (b) Experimental arrangement showing a whole thallus submerged in a tank filled with artificial seawater and fitted with an ultrasonic transducer and a temperature logger. An operator randomly displaces the thallus by slow continuous movement of rotation and translation while a large number of pulse-echo sequences are recorded.

distribution with finite mean and variance converges to the normal distribution as the sample size goes to infinity. To obtain high accuracy and precision, the thallus part positions must be randomized as much as practically possible and N must be sufficiently large. The proposed method is then roughly equivalent to a scan of the entire wet volume along N evenly distributed paths between pairs of opposite faces. The measurement is modeled as a bulk compression wave propagating in a (random) succession of water and tissue media. Shear waves are neglected since in soft biological tissue they are highly attenuated at ultrasonic frequencies. Surface waves are ignored because the wavelengths ($\lambda > 0.7$ mm) are not sufficiently smaller than the blade thicknesses. No physical or chemical alteration of the living cells is expected if the power levels are comparable to those of medical ultrasound (<1 W cm^{-2}).

3. Pilot experiment

Mature individual *E. radiata* macroalgae were collected from Fortescue Bay, Tasmania (−43.12574°S, 147.96068°E), in June 2012 [Fig. 1(a)], and they were transported moist in a cooled unit to Brussels, Belgium. On arrival, the samples were submerged and left for 24 h in a bath of aerated ASW to rejuvenate. The ASW was prepared from Instant Ocean sea salt mixture giving a solution with the same concentrations of calcium (4×10^{-2} g l^{-1}) and magnesium (1.32 g l^{-1}) as natural seawater. Specific gravity (relative density $\rho_w = 1.026$, salinity 35 ppt) were the same as at the collection site. A precision-manufactured cubic tank made of acrylic glass (Plexiglas) was dimensioned to host an entire adult specimen submerged in water with no amassing of tissue at the walls [Fig. 1(b)]. Side length and wall thicknesses measured with Vernier calipers (10 μm resolution) were respectively $200 \pm 2 \times 10^{-2}$ mm and $4 \pm 2 \times 10^{-2}$ mm. A water volume just sufficient to contain the whole alga was taken from the storage bath. It was first degassed in a vacuum chamber since bubbles including those generated by the aeration system would cause signal transmission loss and sound speed dispersion, depending on their amount and size distribution relative to ultrasound frequency. Since a main objective was to investigate the sample in natural healthy condition and to enable the return of the sample to nature in the same condition, great care was taken to not alter chemistry or hasten degradation or decomposition of the tissue. Undissolved air was removed while preserving dissolved oxygen (DO) content. Vacuuming duration was determined by repeating the operation on fresh samples for increased time periods and measuring DO of each processed sample with an immersed optode. DO concentration began to decrease rapidly after 15 s from 180 μM l^{-1} to 50 μM l^{-1} in the next 40 s, corresponding to the time when bubbles of visible size ceased to come out. An ultrasonic transducer (General Electric Company, Germany, GE B2S) with a low (2 MHz) nominal frequency and a large (23.1 mm) diameter crystal was selected to have a high echo-to-noise ratio. In seawater, the beam has a diameter of 5.9 mm and −6 dB divergence angle of 1.9°. The transducer was placed on a vertical wall, 5.5 cm above the tank bottom at mid distance between the side walls, and tightly coupled to its outer surface with protective membrane removed and gel couplant. Ultrasonic testing (UT) equipment was used with pulser/receiver electronics to send electrical pulses to the transducer at controlled times and record the raw echoes. The signal was digitized with a resolution of 8 bit (255 levels) and a sampling frequency of 25 MHz. A MATLAB code was written to automatically determine TT with the highest accuracy. An algorithm was developed to minimize timing error due to variation of trigger point in presence of a wide range of peak amplitudes. TT data were extracted with 4-ns resolution and an estimated accuracy better than 10 ns. A calibrated sensor immersed in the tank logged temperature during the whole experiment. Sound speed of ASW alone was measured with the 2-MHz and a 6-MHz probes at four temperatures in the range 16.5 °C–18.4 °C. A linear fit to the data agreed well with thermodynamic equation of seawater–2010 (TEOS-10) prediction ($\delta < 0.2\%$). The sample was weighted with electronic balance (366 ± 6 g) and then completely immersed. Two operators carried out UT collectively recording $N = 3 \times 10^3$ pulse-echo

sequences with an average repetition rate of 5 Hz (200-ms interval) over a total period of 10 min. During that period the water temperature increased by 0.5 °C from 17.6 °C; the predicted mean value of ASW sound speed equals to 1515.3 m s^{-1}. After UT, the volumes of water (3268 ± 15 ml) and tissue (284 ± 6 ml) were determined by carefully weighting the quantities necessary to fill the tank up to the level marks placed before and after alga submersion. The resulting tissue density ρ_a and volume fraction ϕ_a are $1.289 \pm 4.9 \times 10^{-2}$ g cm^{-3} and $8.0 \times 10^{-2} \pm 2 \times 10^{-3}$. The tissue sound speed c_a is determined by

$$c_a = (\phi_a d_t)/[\tau'_{w+a} - (1 - \phi_a)\tau'_w], \quad (1)$$

where $\tau' = \tau - d_g/c_g$, τ are the one-way TT between wall-water to wall-air interfaces for ASW alone and submerged alga; d_t is the tank inner dimension; d_g is the wall thickness; c_g is the acrylic glass sound speed (2750 m s^{-1}); and ϕ_a is the tissue volume fraction. The sound speed contrast is given by $h = c_a/c_w$. In Eq. (1), a small temperature difference ΔT_w with respect to ASW measurement is accounted for as $\tau'_w = d_t/(c_w + \Delta c_w)$ where Δc_w is the predicted difference of water sound speed. The tissue sound speed was assumed to increase with the same coefficient as water (2.9 m s^{-1} °C^{-1}) due to the high water content (70%–90%) of brown seaweed species; this was verified through direct measurement on *E. radiata* stipe.[5] From additional measurement of density ρ_a, the tissue adiabatic compressibility κ_a is obtained: $\kappa_a = 1/(c_a^2 \rho_a)$.

4. Results and discussion

A two-dimensional histogram of relative attenuation vs reduced TT data ($N_1 = 2747$) is shown in Fig. 2(a). The TT corresponds to Eq. (1) denominator for $\phi_a = 0$. The attenuation (dB) is calculated from the peak echo amplitude relative to its amplitude in water only. Some echoes (3% of N) were not detected because of high attenuation. As expected from the measurement principle, the data are scattered but the histogram shows a single central region of higher density. As the alga is moved, TT varies with the relative path lengths in water and tissue, a value close to zero corresponding to virtually no tissue intersecting the beam. Attenuation is due to energy absorption in the tissues as well as specular and diffuse scattering from their surfaces. For each snapshot, the reflection and transmission coefficients depend on orientation of each individual surface with respect to beam axis. In Fig. 2(b), the histogram of c_a, as computed by Eq. (1) for $\phi_a = 8.0 \times 10^{-2}$, is bell-shaped and has a value range of 1515–1635 m s^{-1} [Fig. 2(b)]. The tails correspond to rarer instances where the beam traversed a tissue fraction much smaller or larger than ϕ_a. Outliers (5% of N), due to operator's fingertips incidentally intercepting the beam, clustered in a distinct peak which was readily excluded. A fit with a normal Pdf results in c_a mean value 1573.0 ± 4.8 m s^{-1}

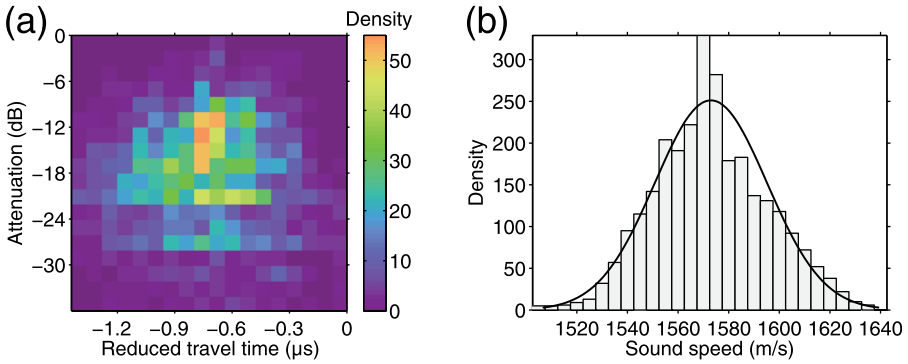

Fig. 2. (Color online) (a) A two-dimensional histogram of relative attenuation vs reduced travel time data. (b) Histogram of sound speed calculated by Eq. (1). The mean 1573.0 m s^{-1} and SD 22.2 m s^{-1} of the fitted normal density (solid line) provide an estimate of average tissue sound speed of the *E. radiata* sample.

and standard deviation of 22.2 m s^{-1} with respective confidence intervals [1572.2, 1573.8] m s^{-1} and [21.58, 22.75] m s^{-1}. The c_a error is given for the combination of length and volume measurement errors that yield the largest value. Given the transducer nominal frequency and fractional bandwidth (52.5%) the longest wavelengths (≈1 mm) in tissue are comparable to the shortest path lengths (≈1–2 mm blade thickness). The sound speed corrected for a summer temperature of 18 °C at the collection site was 1573.4 m s^{-1} which corresponds to a sound speed contrast of 1.038 and a compressibility κ_a of 3.134×10^{-10} Pa^{-1}. The results are in excellent agreement with those obtained by three complementary (destructive) measurements on the present and other samples.[5] Transmission measurements with contact probes directly applied on blade tissue stacks gave a value of 1572.5 ± 4.0 m s^{-1}. The lower sound speed (≈1541 m s^{-1})[5] of stipe medulla does not significantly reduce the thallus-average value since its volume represents less than 2% of thallus; the medulla is ≈25% of stipe that is in turn ≈7% of thallus. Accuracy was mainly determined by the number of TT measurements and their statistical independence as well as temperature control (within 0.1 °C) and accuracy of length, volume, and weight measurements. Good agreement of c_w measurement and prediction at two distant frequencies suggests no significant dispersion due to resonant scattering of bubbles in water and vacuum treatment effectiveness. Given sample storage in a darkened tank and low-light laboratory condition, formation of gas bubbles in medullary tissue as observed on some species at very high photosynthetic levels could be definitely excluded. An alternative measurement procedure would entail the alga statically suspended and a probe being displaced on vertical walls to systematically scan the entire volume, with a grid spacing comparable to beam width. However, small variation in wall thickness or imperfect parallelism of the faces due to assembly or hydrostatic pressure can introduce additional uncertainty. Close proximity of the beam to boundaries may result in additional reflection and echo time spreading. By accounting for morphological and anatomical characteristics, the experimental setup can be adapted to many brown algae species such as—*Laminaria* spp.—enabling characterization of genus- and species-specific tissue structure and composition, e.g., density of chloroplast and concentrations of alginate and other carbohydrates. In spite of high frequency, kelp species with discrete gas-filled bladders such as—*Macrocystis pyrifera*—with a pneumatocyst at the base of each blade can also be investigated. This requires a larger number of snapshots since those involving specular reflection at tissue-gas interface need to be excluded. Some species such as—*Fucus* spp.—that develop elongated gas-filled regions within the lamina itself may be more problematic. For these species as well as seagrasses with gas-filled channels within the tissue, the (destructive) "soup" method[5,6] is better suited. The small leaf thickness relative to ultrasonic wavelengths is a limiting factor. Being nondestructive and minimally invasive, the proposed method enables return of healthy samples at the collection site and repeated measurement on the same individual at later time, e.g., to study environmental, seasonal, and age dependence of tissue elasticity in relation to cell contents. An apparatus could be developed to perform the measurement *in situ*.

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