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4pAO5. Complimentary ultrasound methods for the estimation of sound speed in macroalgae.

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Temperate kelp forests are amongst the most productive ecosystems in the world. However, there is mounting evidence that these habitats are in decline, both in range and productivity. Acoustic propagation modelling has been used to identify primary productivity in seagrass beds, and work is ongoing in development as a method of providing large scale measurements of productivity in macroalgae forests. Acoustic predictive models require knowledge of the material properties of interest, yet little is known about the acoustic properties of seaweed species. As a preliminary step towards acoustic modelling of seaweed systems, this study investigates the acoustic properties of *Ecklonia radiata*, a key species in temperate Australian marine systems. Measuring sound speed in macroalgae, as with other biological material, provides unique challenges due to their intrinsic morphological and anatomical characteristics. Using a range of frequencies between 2-10 MHz different methods are proposed to measure sound speed both directly and indirectly. The measurements show a consistent result, with variation according to tissue type. This research provides an important first step towards the development of acoustic propagation models in kelp forest ecosystems.

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INTRODUCTION

Marine ecosystem services are critical for human welfare, with an estimated worth to the global economy of trillions of dollars each year (Costanza *et al.*, 1997). Kelp forests, comprised primarily of brown Laminarian algae, dominate the coastal reefs of the world's cold water marine habitats (Steneck *et al.*, 2002). Kelp forests are amongst the most productive ecosystems in the world and provide essential structure for innumerable associated organisms including marine mammals, fish, crustaceans and molluscs (Steneck *et al.*, 2002). Anthropogenic climate change is now seen as a key threat to biodiversity and ecosystem health in marine systems (Richardson and Poloczanska, 2008; Wernberg *et al.*, 2011). In this paper we investigate the acoustic properties of *Ecklonia radiata*, as a precursor for the development of propagation models as a tool for kelp forest monitoring.

Recent studies have utilised satellite and aerial imagery to map changes in coverage in both seagrass (Ferwerda *et al.*, 2007) and macroalgae (Mount, 2005; Tyberghein *et al.*, 2012) habitats over time. Increasingly, acoustic imaging has also been used in marine environments to create habitat maps and characterise the benthic layer (Lucieer, 2008). This method allows for the mapping of key coastal seaweed habitat and identification of range changes over time. Exploratory researches in seagrass meadows (Hermand, 2004) gives promising results for the possibility of expanding the use of acoustics into measurement of primary productivity. Work thus far has been limited to seagrass communities. However, the high productivity of macroalgae habitats suggests that acoustics also has promise as a technique which will enhance our ability to monitor productivity in kelp forests.

Two important inputs to acoustic models are the ratios of bulk density and compression wave speed of the material to that of the surrounding seawater (Chu and Wiebe, 2005). Several studies have investigated these material properties in zooplankton (Warren and Smith, 2007), copepods and euphausiids (Greenlaw and Johnson, 1982; Chu and Wiebe, 2005; Smith *et al.*, 2010), and seagrass (Wilson *et al.*, 2010). *Ecklonia radiata* is a key habitat-forming seaweed which dominates the shallow water reef environment in southern Australia. As a precursor for acoustic propagation modeling in the shallow-water kelp forests the material properties of *E. radiata* were investigated in the laboratory in June and November 2012. Bulk density and compression wave speed were measured for both stipe and blade tissue using different methods, at nominal frequencies in the range 2–10 MHz. In this paper, we present results for 2.25-MHz and 5-MHz measurements. This study provides a preliminary step towards the development of a geoacoustic model in *E. radiata* kelp beds, and investigates methods for the measurement of intrinsic sound speed in seaweeds and seagrasses.

MATERIALS AND METHODS

Whole plant density measurements were undertaken from 18 individual macroalgae. Additional macroalgae were also used to measure blade and stipe tissue separately, with the blade material from four individuals and stipe from 10 individuals measured. The density contrast (g) was calculated according to:

$$g = \frac{\rho_a}{\rho_w} \quad (1)$$

whereby ρ_w and ρ_a are densities of seawater and algae respectively. Much of the surface area of *E. radiata* (and of other macroalgae) is thin, with blade thickness of less than 2 mm. This thickness varies, depending on the age of the macroalgae, part of the thallus and time of year (Lobban and Harrison, 1994). As such there are difficulties in creating relatively uniform sections of sufficient size for accurate measurement of sound speed through travel time. Therefore, methods used in studies concerning other biological material, such as direct measurement of whole organisms (Warren and Smith, 2007) and *in situ* measurements of large, high-concentration aggregations (Chu and Wiebe, 2005), are not suitable for the measurement of sound speed in many macroalgae species. In this study we use three different methods to determine sound speed through pulse-echo travel time with contact ultrasonic probes. For all measurements, air and seawater temperatures were measured continuously.

One indirect method was used employing travel time measurements through a vessel containing known volume fractions of macroalgae tissue and seawater. This involved the creation of a mixture of blended macroalgae blades and seawater. A high concentration of blade tissue was used for this method. The acoustic model used with this method is based on an effective medium model (Wood, 1930).

Two methods were used to directly measure the sound speed of stipe and blade. For the first method, two stipes from separate macroalgae were used. For both stipes, a long segment from each stipe was measured based on travel time using reflection mode. These sections were then repeatedly cut into shorter segments and the pulse-echo

measurement was repeated on each end at nominal frequencies of 2.25 MHz and 5 MHz. The second method involved the stacking of macroalgae blades. Two stacks were constructed, each containing the blades of two individual macroalgae tightly bounded with minimal cotton. Travel time measurements in transmission mode were then taken both in and out of seawater.

The sound speed contrast (h) was then determined for all sound speed values by:

$$h = \frac{c_a}{c_w} \quad (2)$$

where c_a and c_w are the sound speeds of the macroalgae and seawater respectively. To account for temperature variations during the course of the experiment, sound speed values were corrected to a temperature of 18 °C. Due to the high moisture content of the macroalgae tissue, it was assumed that sound speed increase as a function of temperature would follow that of seawater.

RESULTS

The density of whole macroalgae ranged between 0.97 and 1.31 g/cm³, with an average of 1.14 g/cm³. The stipe tissue gave a markedly lower density than that of whole macroalgae, with a density of 1.07 g/cm³. Blade tissue measured separately showed a slightly higher density than the average of whole plants at 1.26 g/cm³. Corresponding density contrasts are given in Table 1.

The “seaweed soup” gave a corrected sound speed lower than that of the blade stacks and sound speed contrasts of 1.035 and 1.033 (± 0.02) respectively. As seen in Figure 1, we observed a dispersion in non temperature corrected values of 15 m/s and 19 m/s for stipe 1 and stipe 2 respectively. The difference in the average sound speed between stipes (17 m/s) is mostly due to a difference in the average temperature between the two series of measurements. Temperature-corrected sound speeds averaged to 1536 m/s for stipe 1 and 1537 m/s for stipe 2. A summary of sound speed results is given in Table 1.

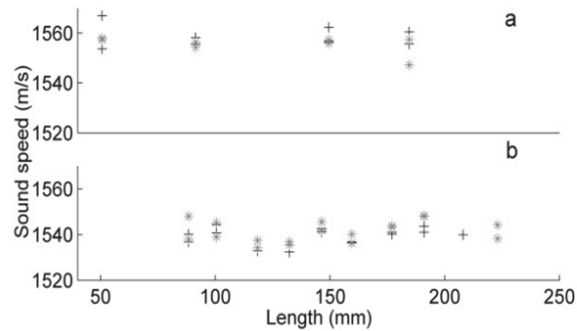


FIGURE 1. Sound speed of *Ecklonia radiata* stipe as a function of cut length from samples 1 (a) and 2 (b), not corrected for temperature. Stars and crosses indicate measurements at 2.25-MHz and 5-MHz nominal frequencies respectively. Stipes 1 and 2 measurements were taken at 23.4 °C and 18.8 °C, respectively.

TABLE 1. Sound speed and temperature measured for artificial seawater and *Ecklonia radiata* tissue types. Note error is calculated based on an assumed maximum of $\pm 5\%$ error in volume unless specified as standard deviation (SD).

Material	Sample preparation	Density contrast g	Temperature-corrected sound speed c (m/s)	Sound speed contrast h
whole algae		1.11 (± 0.10)		
stipe	natural	1.22 (± 0.06)	1536	1.014 (SD ± 0.003)
			1537	1.013 (SD ± 0.003)
blade	stack	1.04 (± 0.04)	1572	1.035
	“soup”		1568	1.033 (SD ± 0.02)

DISCUSSION

A large range of sizes, including very small individuals, were used in density measurements for whole macroalgae and this could account for the lower average density found compared to blade tissue alone, for which measurements focused on adult sized individuals only. This relationship between density and size correlates with values found, for example, with copepods (Chu and Wiebe, 2005). In addition, the large dispersion in values is most likely caused by this variation in size, and the range of stipe:blade ratios apparent in the sample.

The distinct differences in density between stipe and blade correspond with what is known of the morphology and anatomy of macroalgae. The blade tissue in *E. radiata* consists of thin (typically between 1 mm and 2 mm) blades with the epidermal region characterized by densely packed chloroplast cells (Lobban and Harrison, 1994). In macroalgae, this tissue is designed to achieve optimal photosynthesis, and as such chloroplast cells are abundant and tightly condensed (Demes *et al.*, 2011). The cortex and medullary regions containing larger mesophyll cells and loosely packed, vertically elongated medullary cells are relatively small (Figure 2a).

In contrast, the centre medulla and cortex of the stipe tissue (Figure 2b), which contains these loosely spaced cells, is much larger (Lobban and Harrison, 1994; Demes *et al.*, 2011). This tissue contains no photosynthetic cells due to its distance from the epidermis, and takes up a larger part of the internal area. The lower densities found indicate that this pattern in cell packing is reflected in the density of the tissue.

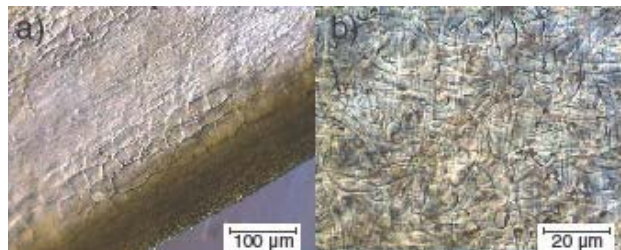


FIGURE 2. Cross section of medulla region and densely packed epidermal layer (bottom right) from blade tissue (a) and loosely packed elongated medullary cells from stipe (b) of *Ecklonia radiata*. Photo courtesy of Suellen Cook, Institute for Marine and Antarctic Studies, Tasmania.

The stipes had a lower sound speed and sound speed contrast than that of blade tissue. This variation is likely a result of tissue differentiation between stipe and blade, and again correlates with what is known of the morphology and anatomy of the algae. The tighter cell packing in blade tissue, and higher cell density, would result in higher sound speed. The sound speed contrast of the stacked *E. radiata* blades was very close to that of the blended blades. The good agreement between the temperature corrected sound speed and sound speed contrasts (which are independent of temperature) found for blended tissue and that of the sound speed measured from the stacked tissue suggests that both methods are an accurate means of measuring the intrinsic sound speed of the macroalgae.

This study shows sound speeds for all *E. radiata* tissue to be higher than that of seawater for the same temperature. This is in contrast to seagrass for acoustic resonator measurement on three species found sound speed values to be lower than that of seawater (Wilson *et al.*, 2010). The intrinsic differences in cell structure between these two groups predict this would be the case. Seagrass cells differ fundamentally from macroalgae cells in the

presence of gas filled channels, or lacuna (Larkum *et al.*, 2006). Macroalgae tissue, with no undissolved gas (in the absence of pneumatocysts) and densely packed cells, creates a material with a higher sound speed.

Sound speed and density contrasts found in this study also exceed those found at high frequency for copepods, salps, jellyfish and gelatinous zooplankton (Chu and Wiebe, 2005; Warren and Smith, 2007; Hirose *et al.*, 2009; Wiebe *et al.*, 2010). This reflects the behavior and life history traits of these organisms and the obvious advantages of neutral buoyancy. In contrast, *E. radiata* is fixed to the benthos, with structurally rigid stipes providing elevation of blade tissue in the water column for increased exposure to photosynthetic light and nutrients.

Another key characteristics of brown macroalgae is a high content of alginates, with reports of up to 30% of dry weight (McKee *et al.*, 1992). Alginates are polysaccharides and it is thought that their primary biological function is structural (Percival, 1979). Sound speed and density increase substantially with reasonably small increases in concentration of alginate in laboratory tests, with sound speeds ranging from 1497 m/s to 1528 m/s for concentrations between 1 and 2 % (Klemenz *et al.*, 2002; Klemenz *et al.*, 2003; Ross *et al.*, 2006). The high sound speed and density found in this research would, in part, be related to alginate content.

CONCLUSION

Ecklonia radiata shows unique acoustic material properties. This study shows variation in both density and sound speed according to tissue type, reflective of the properties of the tissue itself. Different ultrasonic methods developed for estimating sound speed show good consistency, and together with low-frequency acoustic resonance (Wilson *et al.*, 2010), offer sound options for future research into the material properties of macroalgae and seagrass that may otherwise be difficult to measure. Density contrast was 1.04 and 1.20 for stipe and blade tissue, respectively. When corrected for a temperature of 18 °C sound speed for *E. radiata* blade tissue was between 1568 m/s and 1572 m/s, depending on the method used, with sound speed contrasts of 1.035 and 1.033 for blended and stacked blades, respectively. Stipe tissue showed lower sound speed, with an average of 1536 m/s and contrast of 1.135. These results support the development of acoustic propagation modeling to be used in future management of temperate kelp forests.

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