Application of Ultrasonic Waves to Detect Sealworms in Fish Tissue

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ABSTRACT

The potential of employing ultrasonic waves to detect sealworms embedded deep in the fish musculature was demonstrated. Images were made of sealworm-infested cod by using both the scanning laser acoustic microscopic technique, as well as the pulse-echo technique at 10 MHz. Also, attenuation of ultrasound in the frequency range from 1.0 to 12.25 MHz was studied in Atlantic cod *(Gadus morhua)*, haddock *(Melanogrammus aeglefinus)*, ocean catfish *(Anarhichas lupus)*, and the parasitic sealworm *(Phocanema decipiens)*. The difference in the attenuation of ultrasound in sealworms and in fish tissue increased as a function of frequency. The differences between ultrasonic properties (attenuation and backscatter) of fish tissue and sealworms was attributable to the high collagen content of the sealworms. The differences were sufficiently large at 10 MHz that sealworms could be detected in over 4 cm thickness of fish tissue.

INTRODUCTION

THE OCCURRENCE of larval parasitic nematodes commonly known as "sealworms" *(Phocanema decipiens)* in flesh of cod *(Gadus morhua)* and other groundfish species has become a chronic cosmetic problem for several North Atlantic fishing nations, leading to occasional consumer disapproval for fish products. In addition, some reports have indicated that there may be some cause for public health concern when sealworms are ingested in raw, marinated or undercooked form (Hafsteinsson and Rizvi, 1987). Today, the cost of removing sealworms from fish tissue is enormous. In Iceland, for example, it is estimated to be about $12 million per year (Anon., 1985), and in Canada, where the problem is probably in its worst stage ever, the annual cost related to sealworm detection and removal is estimated to be over $50 million (Anon., 1987).

The industry, through necessity, has developed various methods and techniques for sealworm detection and removal. Although techniques and methodologies vary slightly between fish processing plants, the most common practice of deworming and trimming of the fish fillet involves manual labor, using a candling table for visual detection and a knife for removal by trained personnel. The limitation of those techniques is that sealworms embedded deeper than 6 mm in the fish tissue can not be visually detected (Hafsteinsson and Rizvi, 1987). A significant proportion of the sealworms are therefore not detected. According to one Canadian study (Varga and Anderson, 1971), the average candling efficiency is about 75% under commercial operating conditions. This means that about 25% of the sealworms embedded in the fish tissue go undetected to the consumer.

To detect the sealworms while they are embedded deep inside the fish tissue, some kind of a signal has to propagate through the fillet without too much attenuation. Also, some of the physical and/or chemical properties of the sealworm have to differ enough from the same properties of the fish tissue so that the signal that goes through the worm can be differentiated from the signal that has travelled through the fish fillet only.

Light travels through a medium as an electromagnetic wave, and its propagation parameters change due to variations in the electromagnetic properties of the propagation medium. Due to the short wavelength of visible light (400 to 700 nm) and the relatively long penetration path through the fish fillet, it would scatter and be absorbed in the fish tissue. Although a difference exists in light absorption at most wavelengths between sealworms and the fish tissue and an absorption window exists in fish tissue at 800 to 900 nm and at 1100 nm (Pétursson, 1984), it has not been demonstrated that light can be employed to detect sealworms embedded deeper than just a few millimeters from the surface.

The wavelength of ultrasonic waves (150,000 nm at 10 MHz) is much longer than that of light, and the propagation parameters change due to variations in the mechanical properties of the propagation medium. The physical properties responsible for these changes are distinctly different from those governing its response to electromagnetic waves. Therefore, totally different information can be obtained about the specimen depending on whether acoustical or electromagnetic waves are employed.

The ultrasonic propagation properties that can be measured for biological materials such as sealworms and fish tissue are attenuation, velocity and impedance (Chivers, 1981). The ultrasonic velocity and the characteristic acoustic impedance embody within them both the inertial and restoring parameters of the particular material (Johnston et al., 1979). Therefore, under certain conditions, both parameters are characteristic of the material in question. However, it seems that in the last few years attenuation has attracted more attention as a useful parameter for quantitative ultrasonic tissue characterization (Linzer and Norton, 1982).

One of the most sophisticated instruments on the market today for nondestructive testing is the Scanning Laser Acoustic Microscope or SLAM (Hafsteinsson and Rizvi, 1984; Kessler and Yuhas, 1979). To be able to employ the SLAM technique to detect sealworms in fish tissue, a large difference has to exist between the attenuation of ultrasound in the fish tissue itself and the sealworms to be detected. The only way to determine it qualitatively is to form images at different frequencies, where differences in attenuation are known. Other critical factors regarding the detection operation are penetration depth and resolution. Resolution, which is dependent to a large extent on the ultrasonic wavelength (and consequently the frequency), improves at higher frequencies. However, the attenuation of ultrasonic signals has been experimentally found to increase as a function of frequency with a concomitant decrease in penetration depth. Therefore, to obtain the optimal frequency for detection of sealworms embedded in fish tissue, the attenuation of ultrasound as a function of frequency for both sealworms and fish tissue must be measured.

The SLAM technique is based on the transmission of ultrasound through the sample where it is received with a scanning laser beam. The most common ultrasonic imaging systems on the market today are, however, based on the pulse-echo principle. They are equipped with transducers mounted on an arm with freedom of movement in the plane being imaged. The same transducers serve as receivers after the signal has been reflected from an interface in the tissue being imaged. If an

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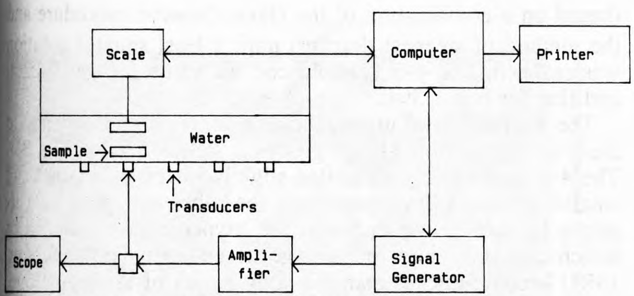

Fig. 1—Schematic diagram of radiation force system.

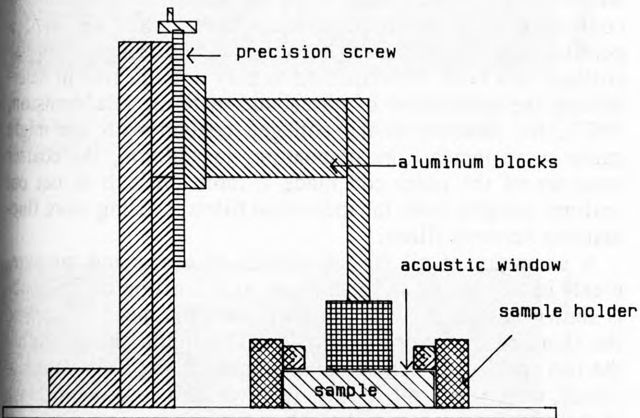

Fig. 2—Precision screw assembly for sample thickness measurements.

object in the tissue (sealworms in fish) has much higher characteristic acoustic impedance than the surrounding tissue, the object will appear brighter on the monitor than the main tissue.

The objective of this work was to study (1) the feasibility of using ultrasonic waves to detect sealworms in fish tissue, and (2) the attenuation of ultrasound as a function of frequency in sealworms and various fish tissues.

MATERIALS & METHODS

Materials

All samples were fresh and provided by the Icelandic Freezing Plants Corporation, Reykjavik, Iceland, and shipped packed in ice via air freight to Ithaca, NY.

Attenuation was measured in live sealworms as well as in sealworms which had been frozen and then thawed. In addition, attenuation was measured in five fillets from 4–5, 6–7, and 8–9 year-old cod (*Gadus morhua*), 4–5 year-old haddock (*Melanogrammus aeglefinus*), 6–7 year-old ocean catfish (*Anarhichas lupus*) and in 4–5 year-old cod that had been frozen in a walk-in freezer for 48 hr and subsequently thawed. To investigate the effect of the skin on the attenuation in nonskinned fillets, the attenuation was also measured in five samples of skin of cod.

Techniques and instruments

The radiation force technique (Goss et al., 1979; Marcus and Carstensen, 1975) was employed to measure attenuation of ultrasound. A schematic diagram of the radiation force technique employed is shown in Fig. 1. This technique was developed mainly as a primary method for measuring total output power in a phase insensitive manner. The force is a direct result of energy transport by a sound wave. Thus, a continuous wave incident on an absorbing object produces a time independent force on that object equal to, and in the direction of, the rate of change of momentum with respect to time.

The images of sealworm infested cod fillets were made with a new version of the SLAM the Sonomicroscope - System 140, at 10 and 24 MHz frequencies. To compare the pulse-echo technique with the SLAM technique an image was also made using the Diasonics Small Parts Scanner at 10 MHz frequency.

Sample preparation

Because of the high attenuation of ultrasound in air, the samples had to be submerged in an aqueous medium. To minimize the amount of tissue being dissolved, all samples were submerged in a buffer (0.05M sodium phosphate, 0.001M magnesium chloride, pH 7.1), which has been proposed as a close approximation to the ionic composition of the fluid sarcoplasmic matrix (Love et al., 1972). The samples were then placed in a 3 cm long plastic sample holder with a diameter of 7 cm and with an acoustic window on one end. Cylindrical samples 7 cm in diameter with flat ends were cut from the front section of the fillets. Skin of cod was dissected from the muscle tissue, scales were scraped off and the skin cut in circular form and packed in layers in the sample holder. Isolated, whole sealworms were randomly packed in the sample holder.

The sample holder was then placed in a vacuum (20 in, Hg) chamber for 30 min to eliminate most air bubbles, which are known to interfere with the attenuation measurements. Before measurements were conducted another acoustic window was firmly pressed on the other end of the sample holder to confine the samples within the sample holder and to provide a means for efficient thickness measurements. Sample thickness ($\pm$ 0.05 mm) was measured with the aid of a precision screw (Velmex UniSlide A2512Q1) assembly, demonstrated in Fig. 2. The water in the experimental tank had previously been degassed by boiling for 30 min.

Attenuation measurements

All measurements were controlled by an IBM PC/XT (software was written in ASYST). The computer set the signal generator (Tektronix TM 5003) to the desired value, tared the scale (Sartorius 1801) and waited 100 milliseconds (ms). The scale then sent 15 baseline values to the computer (1 value every 83 ms, total time 1.25 sec). The computer then turned the signal generator on, the signal was amplified in the RF power amplifier (EIN 2100L) and sent to the transducer (2.54 cm diameter). The signal was then received (absorbed) by a load hanging from the scale. The load was made from castable rubber (Acoustical Material Component #35075). Its density was 0.94 g/cm^3. The velocity of ultrasound in it was 1525 m/sec. The characteristic acoustic impedance was, therefore, almost the same as for water, which means that the ultrasound was absorbed by the load with insignificant reflection back to the transducer. After the signal generator was turned on, the computer waited 2905 ms to allow for scale stabilization, then obtained 15 more values from the scale. The difference between the means of the values was taken to be the radiation force value. This procedure was repeated five times for each frequency value both with and without sample in the propagation path. For each frequency point a total of 300 values were, therefore, received by the computer. Transducers with frequencies of 1.067; 2.390; 3.430; 5.720; 7.330; 8.027; 10.300; and 12.250 MHz were used for every sample.

Attenuation of ultrasound in the sample was then calculated for each frequency value according to the formula:

$$A = \ln(NS/WS)/2t \qquad (1)$$

where A (attenuation) has the unite of Nepers (Np) per cm of propagation path through the sample, t is the sample thickness in cm, NS is the mean radiation force (mg) when ultrasound does not propagate through the sample, and WS the mean force when ultrasound propagates through the sample. In the case of sealworms, the sample did not completely fill the sample holder. A dilution factor was, therefore, calculated, based on the ratio of sample volume to the volume of solution in the sample holder. To calculate attenuation per cm of tissue, the attenuation was multiplied with the dilution factor. Density of the sealworms (1.076 g/cm^3) was needed for these calculations.

RESULTS & DISCUSSION

Attenuation of ultrasound

The attenuation of ultrasound (Np/cm) as a function of frequency in five fillets from 4–5 year-old cod is shown in Fig. 3A. From the figure it is clear that the attenuation increased as a function of frequency and that there was very little difference between individual fillets. The increase in attenuation was not linear but followed a power model function:

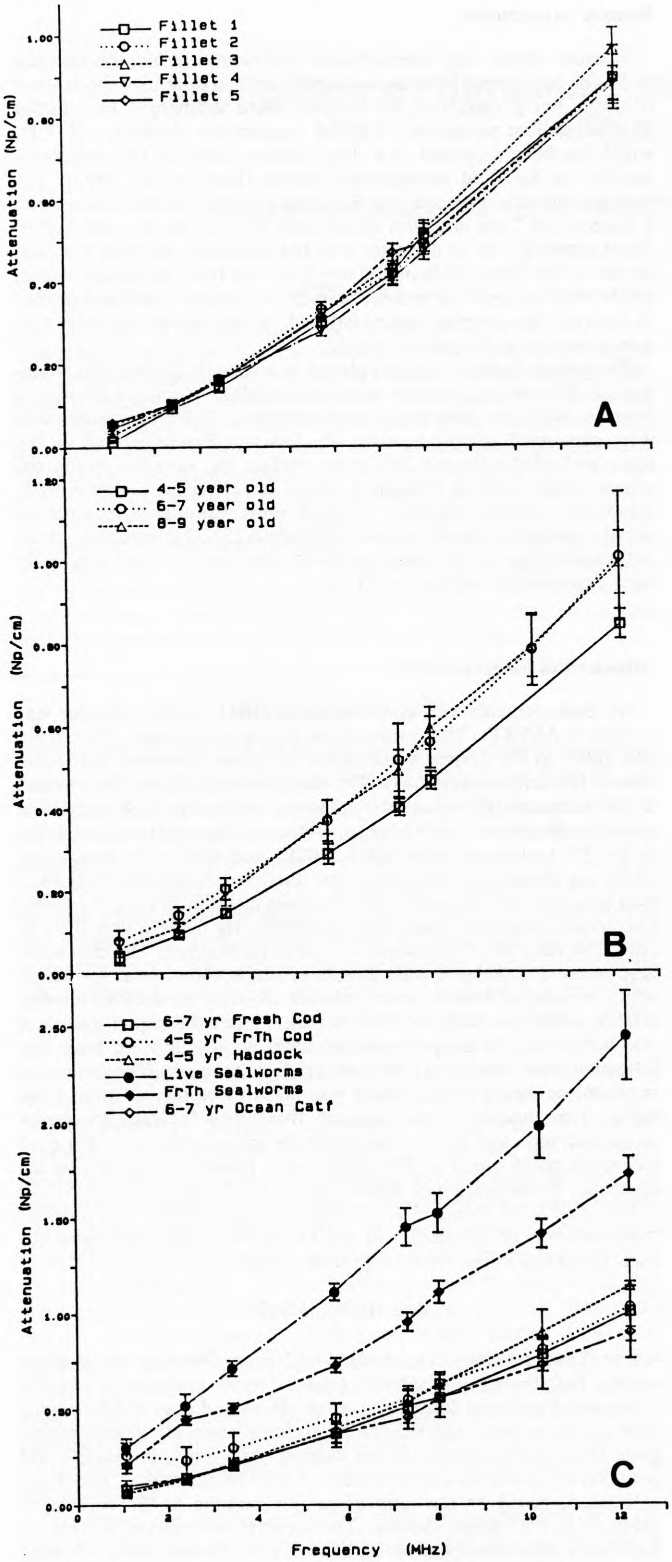

Fig. 3—(A) Attenuation of ultrasound as a function of frequency in five fillets from 4–5 year-old cod. (B) Mean values for attenuation of ultrasound as a function of frequency in three age groups of cod. (C) Mean values for attenuation of ultrasound as a function of frequency in cod, haddock, ocean catfish, and sealworms. Note that bars indicate standard deviations.

$$A(f) = af^b \qquad (2)$$

where f is the frequency in MHz. Values for a and b were obtained by fitting the mean values with repeated iterations (based on a combination of the Gauss-Newton procedure and the method of steepest descent) until a least squares solution was reached. For 4–5 year-old cod the value for a is 0.0256 and that for b is 1.394.

The attenuation of ultrasound as a function of frequency in fresh cod fillets from all age groups is summarized in Fig. 3B. The 4–5 year-old cod exhibited slightly lower attenuation and smaller standard deviations than the other age groups. This might be due to the fact that the myocommata membranes which contain a high percentage of collagen (Hafsteinsson, 1987) become thicker than the body length of the fish (Love, 1970). Also, the diameters and lengths of the individual muscle cells (taken from the same part of the fillet) increase with body length (Love, 1958), and, since the basement membrane is continuous with the myocommata (Love et al., 1969), it is possible that it also thickens according to body length. Because collagen has been demonstrated to play a major role in determining the attenuation of ultrasound in tissue (Hafsteinsson, 1987), the increase in the collagen content with age might cause some increase in attenuation. In addition, the coarser structure of the older cod made it more difficult to cut out uniform samples from the individual fillets, causing more fluctuations between fillets.

A summary of all the attenuation of ultrasound measurements in sealworms and fish tissue as a function of frequency is shown in Fig. 3C. The graphs present the mean values and the standard deviation of samples. The figure shows that all the fish species studied attenuate ultrasound by a similar magnitude, with ocean catfish slightly lower than cod, and haddock slightly higher than cod at the highest frequencies. The most important aspect of Fig. 3C is that the difference in attenuation between live sealworms and fish tissue increases as a function of frequency. Also, important was the drop in attenuation in sealworms as a consequence of the freezing-thawing cycle. At 10 MHz, for example, the attenuation in live sealworms was about 2 Np/cm, but dropped to about 1.4 Np/cm in the frozen-thawed samples. The magnitude of the attenuation in frozen-thawed cod was similar to the attenuation in fresh cod fillets.

In Table 1, the values for the power model parameters a and b are listed for all the samples. It is interesting to note the low values of b for sealworms and ocean catfish, which means that an almost linear increase in attenuation occurs with increasing frequency. Also interesting is the high value of b for the skin of cod, and the drop in the value of b as a consequence of the freezing-thawing cycle. The basic difference in chemical composition between these samples is that sealworms contain a high amount of carbohydrates, ocean catfish contains about 10% fat on a dry weight basis and the skin of cod about 85% collagen on a dry weight basis (Hafsteinsson, 1987).

The difference in attenuation between the sealworms and the cod tissue can be calculated with the aid of the power model and the values of a and b listed in Table 1. At 10 MHz the difference in attenuation between live sealworms and fresh cod was 1.34 Np/cm, and at 24 MHz it was 2.58 Np/cm. Between frozen-thawed sealworms and cod, the difference was 0.6 Np/cm at 10 MHz, and 1.26 Np/cm at 24 MHz. One layer of skin of cod (about 0.7 mm thick) attenuated the ultrasound as much as about 0.6 cm thick tissue of cod.

Table 1—Values for power model constants a and b

Sample	a	b
4-5 year-old cod	0.0256	1.394
6-7 year-old cod	0.0418	1.263
8-9 year-old cod	0.0387	1.296
Cod frozen-thawed	0.0745	1.011
4-5 year-old haddock	0.0362	1.377
6-7 year-old ocean catfish	0.0467	1.178
Live sealworms	0.2190	0.982
Sealworms frozen-thawed	0.1560	0.942
Skin of cod	0.1000	1.751

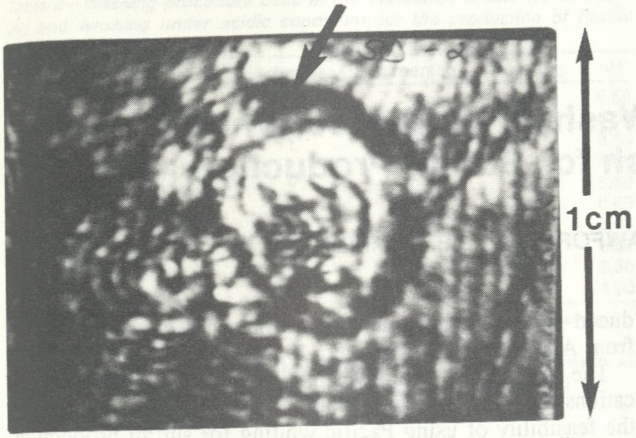

Fig. 4—An image (SLAM, 24 MHz) of a sealworm of a diameter of about 0.6 mm at the center of 3.5 cm thick cod fillet. Arrow indicates the coil.

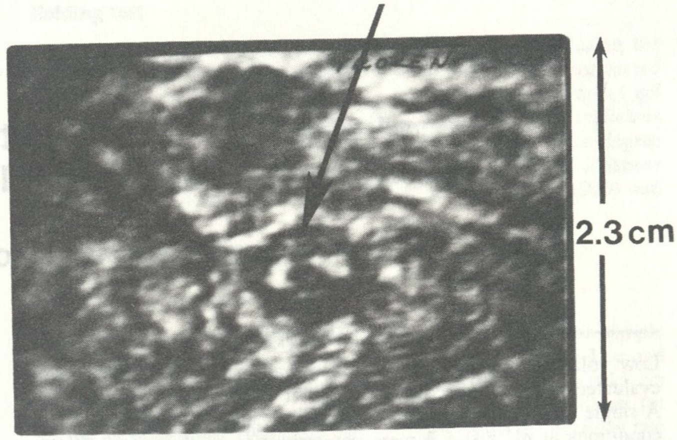

Fig. 6—An image (SLAM, 10 MHz) of a sealworm of a diameter of about 0.5 mm at the center of a 2.5 cm thick cod fillet that had been frozen and thawed. Arrow indicates the coil.

Fig. 5—An image (SLAM, 10 MHz) of a sealworm of a diameter of about 0.6 mm located at the center of a 3.5 cm thick cod fillet "sandwich" with skin on both sides. Arrow indicates the coil.

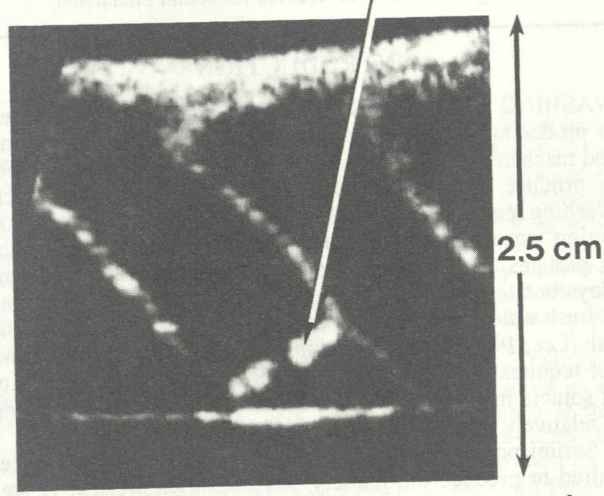

Fig. 7—An image (pulse-echo, 10 MHz) of a sealworm of a diameter of about 0.7 mm located between two myocommata membranes about 2 cm from the top surface in a 3.5 cm thick cod. Arrow indicates the sealworm.

Images

Figure 4 is an image at 24 MHz (SLAM) of a sealworm at the center of 3.5 cm thick cod fillet. The sealworm has a diameter of about 0.6 mm, is 45 mm long and forms a coil (arrow) of a diameter of 6–7 mm. Figure 5 is an image at 10 MHz (SLAM) of a sealworm of a diameter of about 0.6 mm (coil is 6–8 mm in diameter) located at the center of a 3.5 cm thick cod fillet "sandwich" with skin on both sides. The sealworms embedded deep in fish tissue can be detected with the SLAM technique (Fig. 4 and 5). In 3.5 cm thick fillets, the attenuation at 24 MHz was about 8.33 Np or 72 dB. However, at 10 MHz, the attenuation was about 2.68 Np or 23 dB in a 3.5 cm thick cod fillet. There is also enough resolution at 10 MHz for sealworm detection (Fig. 5). The difference in attenuation between live sealworms and cod tissue was sufficient (1.34 Np/cm) to form sharp images of sealworms in thick cod fillets. Figure 6 is an image at 10 MHz (SLAM) of a sealworm of a diameter of about 0.5 mm (coil is 5–7 mm in diameter) at the center of a 2.5 cm thick cod fillet that had been frozen and thawed. The difference in attenuation is only 0.6 Np/cm (Fig. 6), and the sealworm is barely seen in the 2.5 cm thick cod fillet. This was due to the sharp drop in the attenuation in sealworms when they were frozen and thawed. Figure 7 is an image at 10 MHz (pulse-echo Diasonics System) of a sealworm of diameter of about 0.7 mm (coil is 7 mm in diameter) located between two myocommata membranes about 2 cm from the top surface in a 3.5 cm thick cod.

The resolution was better in the images from the SLAM than in those obtained using the pulse-echo system, and the myocommata membranes did not interfere as much when the transmission technique was employed than when the pulse-echo technique was used. The difference in resolution was probably due to some extent to the different receivers used. The SLAM uses a scanning laser as a receiver, and the size of the focused laser spot in the SLAM determines the resolution to some degree. In the pulse-echo system, the echos are received by a group of transducers. In the SLAM, the ultrasonic wave travels just once through the sample, whereas in the pulse-echo system the wave must travel to the sealworm and back to the receiver. Due to the interference from the myocommata membranes, sealworms could not be detected with the pulse-echo system when they were too close to the membranes, and the plane the coil was in, was parallel with the membranes.

CONCLUSION

AT 10 MHz FREQUENCY there was enough difference between ultrasonic properties of sealworms and the tissue of cod, haddock and ocean catfish so that they could be detected, even

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irradiation on the color was minimal (Hunter "a" value increased with the irradiation dose from 0.9 to 2.2) with slight darkening (decreased Hunter "L" value). Since the average shelf-life for catfish is approximately 5 to 7 days, the extension of shelf-life by 13 days with irradiation would be extremely desirable.

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though they were embedded deep in the fish tissue. The sealworms could be detected in over 4 cm thick fish tissue, provided the fish was fresh. Due to the drop in attenuation of ultrasound in sealworms when they were frozen and thawed, they could not be detected in thicker than about 2.5 cm fish tissue that had been frozen and thawed. The SLAM technique was more suitable for detection of sealworms than the pulse-echo technique.

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