larger than the two control groups.

DAY 10. On the tenth (10) day of the experiment, all the small-fry were remeasured and the frequency exposing the small-fry in the acoustic exposed tank was reduced to 2.0 MHz but all other parameters remained the same. The acoustic exposed small-fry thrived.

DAY 14. Five of the small-fry in the small tank control group died.

DAY 16. Eighteen of the small-fry in the small tank control group had died by this time. The breeding tank group were unaffected. All remaining small-fry in all groups were measured using a centimeter ruler and the binocular microscope:

Acoustic group	7 mm long
Breeding tank control group	6 mm long
Small tank control group	5 mm long

DAY 18. All but one of the small-fry in the small tank control group had died. The control group in the breeding tank were still alive and functioning and the acoustic resonance exposed group were thriving.

DAY 19. The resonant acoustic frequencies of the growing small-fry in the acoustic tank was measured again. The acoustic field was changed to 1.55 MHz, with all other parameters remaining the same except the pulse width of each repetition was reduced to 2 microseconds. This reduction of width of pulse had a marked influence on the growth of the small-fry indicating that the 20 microseconds was at the upper end of the power range for augmentation at these frequencies.

DAY 21. The sole remaining small-fry in the small tank control group was moved into the breeding control group. This sole small-fry was noticeably smaller than the other control groups but all control small-fry were noticeably smaller than the acoustic group.

DAY 41. In the acoustic group tank, the acoustic field was changed to 0.830 MHz, having all other parameters remain constant.

DAY 65. The acoustic field exposing the small-fry in the acoustic group tank was terminated. At approximately two months old, the acoustic resonance exposed fish were approximately the same size as much older 4 month old controls from an earlier control group and much larger than their counterparts in the breeding control group.

RESULTS: There was a significant difference in level of power input or intensity between TEST A and TEST B. In TEST A, the power was continuous at 10 Volts/sec. In TEST B the power was pulsed and the acoustic field was active at the most only 0.2% of the time. Therefore, even though the power was 300 volt/sec, the overall yield was only (300 V/sec × 0.002) or 0.6 Volts/sec total power.

As the small-fry grew the acoustic resonant frequencies that induced function changes also changed due to difference in structure size and shape.

After termination of the acoustic field, the small fry were allowed to grow to maturity and breed. The fish exposed to acoustic energy at the resonant frequency matured and laid eggs significantly sooner than the control fish. No second generation effects were noted in offspring of either the acoustic exposed or control fish.

Example 10

Augmentation of Plant Growth

Testing was conducted to determine the effects of resonant acoustic energy on the germination and growth patterns of sugar snap peas. The seeds for the sugar snap peas were obtained from Lake Valley Seed Co., packed for lot 1997 lot A2B, 5717, Arapahoe, Boulder Colorado, 80303.

Initially, the resonant acoustic frequency of pea sprouts was ascertained by determining the frequency for the maximum amplitude shown on an A-scan. By varying the frequency of the audio generator, the amplitude of the pea sprout was a maximum at the resonant frequency. Seven sugar snap peas were covered half way with room temperature water in a wide-mouth glass container and left on the counter to sprout. Six days later, the sprouts were tested as follows:

The Matec Ultrasonic Inspection System, with Tb 1000 and A to D data acquisition card was used. the Tb 1000 settings were:

Gain	0-20 dB
Trigger	Internal +
Voltage	High
Rectify	None
LP filter	varied
HP filter	varied
Output level	100 %
Rep. Rate	10.000 msec
Pulse Width	2.00 usec
Frequency	0.5-20 MHz
Mode	Through transmission

A to D settings were:

Data	On
Delay	none
Range	12 usec
Signal path	RF
Volt. Range	1 V
Channel	A/AC
Trigger	External +
Threshold	1
Sample rate	100 MHz
Vid. Filtr	1.7 usec
DAC offset	1945

Transducers used in the experiment included the Matec 1.0 MHz, 2.25 MHz, 5.0 MHz and 10.0 MHz, all being 0.5 inches in diameter. These frequencies were initially chosen because calculation showed that based on the speed of sound in water (1,500 m/s) and the diameter of the sprouts (1-2 mm or 0.001-0.002 m), the resonant frequency across the diameter of the sprout should be in the low MHz range:

$$\text{velocity} = \text{frequency} \times \text{wavelength}$$

$$\text{frequency} = \text{velocity} \div \text{wavelength} = 1{,}500 \text{ m/s} \div 0.001 \text{ m} = 1.5 \text{ MHz}$$

Sprout #1 was excised from the pea halves, and was placed between two 2.25 MHz transducers, coupled with a thin coat of EKG gel. The Tb 1000 was set on scan increments of 0.005MHz, and the sprout was scanned from the lowest (50 KHz) frequency available on the system to the highest (20 MHz). Variations in amplitude were observed during this frequency sweeping process, and the low MHz region was quickly identified as the highest amplitude region. Further frequency sweeping revealed maximum amplitude at 1.7 MHz.

The same procedure was followed for test sprout #2 and #3. Test sprout #2 was still attached to half of the pea, and the resonant frequency of 1.64 MHz was detected from the entire structure, although the gain had to be increased because of the attenuation of the acoustic field by the pea half. Sprout #3 was an isolated sprout such as #1 and revealed a resonant frequency of 1.78 MHz.

The same procedure was repeated with the 1.0 MHz transducer and similar results were obtained. Thus, it was concluded that the acoustic resonant frequency for 4-5 day old sugar snap pea sprouts was 1.7 MHz ± 0.1 MHz. Having successfully identified a resonant frequency for a multicellular biological, the next step was to show disruption and/or augmentation effects from the application of an acoustic field at this frequency.

A number of germination tests were conducted using different power levels or voltages and length of exposure at the acoustic resonant frequency.

GERMINATION #1

A Matec 1.0 MHz transducer was used with the Tb 1000 system having the same settings as that described above in determining the acoustic resonant frequency except:

Frequency	1.7 MHz
Voltage	High
Rep. Rate	10 msec
Pulse Width	2 μsec
Through Mode	

Two small plastic dishes were prepared with sterile cotton balls in a single layer in the bottom of the dishes with seven sugar snap pea seeds and filled with distilled water to cover the pea seeds halfway. The pea seeds in one dish served as a control. The 1.0 MHz transducer was clamped tightly in a ring stand clamp, and the face of the transducer was lowered into the center of the dish. The acoustic field of the transducer was lowered into the center of the dish. The acoustic field was initiated on day one and interrupted several times during the next 72 hours due to frequent storms in the area. The transducer was operating approximately only 18 hours during the first 48 hours of the test.

The experiment was terminated on day five. All seven of the acoustic pea seeds sprouted, while only five of the control pea seeds sprouted. Several spots of black mold were noted in the control dish. Comparison of the root sprouts revealed that the acoustic sprouts were twice as long as the control sprouts (2.9 cm vs. 1.6 cm). Interpretation of these results was ambiguous because of the tight clamping of the transducer, the frequent and repeated interruption of the acoustic field and the contaminating mold in the control dish. Accordingly, test trays were constructed with the transducer coming up through the bottom of the tray.

GERMINATION #2

The same acoustic equipment and setup was used in this germination as that used in germination #1. The 1.0 MHz transducer was clamped loosely in a ring stand clamp, and the face of the transducer was lowered into a larger plastic dish. A second 1.0 MHz transducer, unconnected to the signal generator was lowered into a larger control dish. Interruptions were infrequent.

The study was terminated on day #7. In the control dish, 79 % had sprouted and the average root sprout length was 3.95 cm (n=81.) In the acoustic dish, only 69% had sprouted and the average root sprout length was 3.12 cm (n=80). It was concluded that this frequency at the higher power voltage output demonstrated a disruptive effect on pea sprouting and growth. GERMINATION #3

A new setup was implemented wherein the 1 MHz transducer was fitted into the bottom of two dishes which were modified by drilling a hole with rubber seals to accomodate a .5 inch diameter transducer. The transducers were placed face up through the bottom of the dish. Each dish was prepared with sterile cotton batting in a single layer in the bottom. Fifty sugar snap pea seeds were placed in the dishes and filled halfway with water. The control dish was prepared exactly as the acoustic dish but unconnected to the signal generator. The acoustic field was initiated on day #1 with the above settings used in germination #1, except that the pulse width was increased to 19.98 usec which was about 10 times the pulse width used in germination #1. It was also 10 times the power output as in experiment #2. Interruptions were infrequent.

The study was terminated on day #7. In the control dish, 82% had sprouted and the average root sprout length was similar to germination #2. In the acoustic dish, only 72% had sprouted and the average root sprout was similar to germination #2. This data confirmed that the frequency of 1.7 MHz at a high power voltage level demonstrated a disruptive effect on pea sprouting and growth.

GERMINATION #4

The same setup was used as that disclosed in germination #3 except:

Voltage Low
Rep. Rate 2 μsec
Pulse Width 0.3 μsec (this was adjusted to produce only one sonic wavelength per repetition)

The results of this germination showed that only 84% of the control dish had sprouted, while in the acoustic dish, 90% had sprouted. The average root sprout length of the acoustic peas was 24% longer than the control peas. It was concluded that this frequency and a lower power acoustic field has an augmenting effect on the pea sprouting and growth.

GERMINATION #5