Inhibitory Effect of Ultrasonic Waves on the Larval Settlement of the Barnacle, *Balanus amphitrite* in the Laboratory

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Abstract: The effects of ultrasonic waves for survival and settlement of the barnacle, *Balanus amphitrite*, were investigated under laboratory condition. Among the three frequencies (19.5, 28, and 50 kHz) of ultrasonic waves tested, 19.5 kHz was found to be the most effective in decreasing the survival rate of the barnacle nauplii. A 50% reduction in cyprid settlement was achieved by a total irradiation of about 140 kPa·sec, defined as the product of acoustic pressure (kPa) and irradiation time (sec), whereas some 4,300 kPa·s was necessary to reduce to 50% the survival rate of nauplii or cyprids.

Fouling organisms, such as barnacles, mussels, bryozoans and algae, are responsible for many economical problems, including increase in fluid frictional resistance and corrosion of ship's hulls, cooling water systems for power plants, and aquaculture facilities. Although antifouling agents, for example, organotin are available, their use is limited owing to their toxicity to fish, shellfish and other organisms. Hence, more environmentally friendly antifouling systems are required to combat this problem.

The use of irradiation of ultrasonic wave is found to be an effective antifouling method. Earlier laboratory studies on the mortality effects on the larvae of fouling animals, such as barnacles and mussels, demonstrated the effectiveness of ultrasonic wave irradiation at frequencies of several tens of kHz (MORI *et al.*, 1969; SUZUKI and KONNO, 1970). However, there are no reports available on its effects on barnacle settlement. Hence, investigations were undertaken to study: (1) the effect of ultrasonic wave on the survival of laboratory reared *Balanus amphitrite* nauplii, at three frequencies (19.5, 28.0, and 50.0

kHz); (2) more detailed effects on both nauplii and cyprids at 19.5 kHz; and (3) the effect on larval settlement at 19.5 kHz.

Materials and Methods

Irradiation instruments

An acrylic acid resin tank (30 cm × 25 cm × 120 cm high, with the seawater level at 100 cm) was used for the exposure experiments (Fig. 1). An ultrasonic transducer (total area 195 cm²) was set at the bottom. A sonicpressure sensor (Kaijou Denki Co.) was also immersed in the water column. Water-filled, 10 ml capped glass tubes, each containing twenty laboratory-reared *B. amphitrite* nauplii (II–VI stage) or cyprids, were set in the tank at various distances (1–100 cm) from the transducer.

Larval culture

Nauplii, obtained from adult barnacles cultured with *Artemia salina*, were fed with the diatom *Chaetoceros gracilis* at 25°C. Larval density was lower than two individuals/ml. More than 70% of the reared nauplii reached the cyprid stage in five days. Newly-transformed cyprids were collected and used on the following day for settlement assay.

Key words: ultrasonic waves, settlement inhibition, *Balanus amphitrite*

Fig. 1. Diagrammatic view of the radiation tank.

Survival assay

The effects on the survival of nauplii were investigated initially at three frequencies (19.5, 28.0, and 50.0 kHz) for diverse exposure times (5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 sec), at constant power (240 W, 1.3 W/cm^2) and distance (1 cm) from the transducer. For each experiment, three to ten glass tubes containing twenty larvae were used.

The effects of 19.5 kHz, the most effective frequency in killing the nauplii, on both nauplii and cyprids survival were investigated in detail to understand its impact of total irradiation ($\text{kPa} \cdot \text{s}$) on the survival rate of the larvae. Total irradiation is defined as the product of the acoustic pressure (kPa) and the irradiation time (sec). Each test tube containing twenty nauplii or cyprids was irradiated with 19.5 kHz ultrasonic wave for various times ranging from 5–120 sec and distances between test tubes and the transducer (1–100 cm), at constant power (1.3 W/cm^2). Acoustic pressures on each test tube were detected by a sonic pressure sensor. Five to 32 capped glass tubes were used in each experiment.

Settlement assay

After treatment with 19.5 kHz ultrasonic wave irradiation, the settlement activity of *B. amphitrite* cyprids was examined by settlement assay (KITAMURA and NAKASHIMA, 1993). The cyprids were placed into a 3 l capacity, plankton-net (opening: $96 \mu\text{m}$) cage, which was set up in a 15-l glass-tank containing 10 l of filtered seawater. Water circulation was maintained by a pump directing seawater to each

Fig. 2. Changes in the survival rate of *Balanus amphitrite* nauplii, exposed to ultrasonic waves at three frequencies, 19.5 (○), 28 (△), and 50 kHz (□) at 1.3 W/cm^2 power, distance 1–2 cm. Error bars denote standard deviation.

corner of the cage. For each experiment, 12 ceramics plates ($76 \times 26 \text{ mm}$) were provided as substrata for settlement, of which six plates were pretreated (each plate coated with 1 ml of crude extract of adult *B. amphitrite* equivalent to about 10 g wet-weight) and allowed to air-dry. The remaining plates were not treated. After 24 h at 25°C , the number of individuals permanently settled on each plate was counted.

Settlement assays, each of two hundred cyprids (total of ten capped glass tubes), were subjected to total irradiations of 0 (control), 95, 125, 150, and 300 $\text{kPa} \cdot \text{s}$. Exposure to 19.5 kHz ultrasonic wave irradiation was at a distance of 100 cm, under powers of $0.05\text{--}1.3 \text{ W/cm}^2$ (Table 1). Settlement assays were run with up to three replicates.

Results

Survival assay

The relationship between exposure time and survival rate of *B. amphitrite* nauplii at 19.5, 28, and 50 kHz (Fig. 2) showed the 19.5 kHz ultrasonic wave to be the most effective in decreasing the survival rate. A gradual decrease in survival rates occurred under 28 kHz irradiation, whereas the 50 kHz ultrasonic wave did not clearly reduce the survival rate of nauplii with increasing exposure time. The 19.5 kHz

Fig. 3. Changes in the survival rate of *Balanus amphitrite* nauplii; (○), cyprids (●) against total irradiation (kPa·s). Error bars denote standard deviation.

Table 1. Number of settled barnacles after subjected to 19.5 kHz at five levels of total irradiation (distance from transducer 100 cm)

Acoustic pressure (kPa)	Irradiation time (sec)	Total irradiation* ¹ (kPa × sec)	Number of settled barnacles			Number of replicates
			12 plates (total)	(Treated plates)	(Untreated plates)	
0* ²	0	0	62 ± 22* ³	52 ± 16	10 ± 9.6	3
19	5	95	53 ± 1.4	36 ± 9.9	17 ± 8.5	2
25	5	125	51 ± 13	42 ± 8.1	9 ± 5.5	3
30	5	150	24 ± 13	22 ± 12	2 ± 1.0	3
30	10	300	18	16	2	1

*¹ Total irradiation = (acoustic pressure) × (irradiation time).

*² Control.

*³ Standard deviation.

wave produced a linear decrease in the survival rate between 5 and 90 sec, with irradiation for 60 sec reducing the survival rate to 50%.

The relationships between total irradiation and the survival rate of nauplii and cyprids at 19.5 kHz with diverse exposure times (5–120 sec) and distances (1–100 cm) are shown in Fig. 3, the survival rates of both gradually decreasing with increasing total irradiation. Between 100 to 3,000 kPa·s, the survival rates of cyprids were lower than those of nauplii, but no clear differences in survival rates were apparent in the range 5,000 to 8,000 kPa·s. The levels of total irradiation which resulted in 50% survival rates of both nauplii and cyprids were almost the same, their

values being calculated (method of DOUDOROFF *et al.*, 1951) as 4,400 and 4,200 kPa·s, respectively.

Settlement assay

The mean number of settled barnacles on 12 ceramics plates was 62 in the control assays (without irradiation). The numbers on the pretreated and untreated plates being 52 and 10, respectively (Table 1). Exposure to the 19.5 kHz ultrasonic wave progressively reduced the number of settled barnacles on the 12 plates, with increasing total irradiation from 95 to 300 kPa·s. At irradiation levels of 95 and 125 kPa·s, the mean barnacle numbers on the 12 plates remained high, being 53 and 51, respectively, but decreased to less than 50% of the control number,

Fig. 4. Relationship between total irradiation (kPa·s) of the 19.5 kHz ultrasonic wave and settlement percentage of *Balanus amphitrite* cyprids (see text for explanation). Error bars and parentheses denote standard deviation and number of experiments, respectively.

under 150 and 300 kPa·s. The settlement numbers on treated plates remained higher than those on untreated plates throughout the experiments (Table 1). Almost all the cyprids exposed to ultrasonic waves swam normally, as did the control specimens.

The relationship between total irradiation and percentage of settled barnacles (Fig. 4), which was calculated on the basis of the barnacle numbers on the 12 control plates representing 100% settlement, indicated that the settlement percentage decreased sharply from 82% at 125 kPa·s to 39% at 150 kPa·s. From the figure, irradiation of 140 kPa·s achieved 50% inhibition on larval settlement.

Discussion

The results of the initial survival experiment using ultrasonic waves at three frequencies indicated that the 19.5 kHz ultrasonic wave was the most effective in decreasing the survival rate of *B. amphitrite* nauplii (Fig. 2). The 28 kHz wave, however, was also effective, but to a lesser extent, and the 50 kHz wave not at all. The results obtained in the present investigation agreed with those of other studies (MORI *et*

al., 1969; SUZUKI and KONNO, 1970).

It appeared that the lethal or destructive effect of ultrasonic waves on larvae was related to the total irradiation (kPa·s). Although the survival rates of cyprids were lower than those of nauplii within the total irradiation range of 100 to 3,000 kPa·s, there was no difference in the survival rates from 5,000 to 8,000 kPa·s. The reason for the differing effects of the ultrasonic waves is not clear, but this may be related to the different shape of the two larval forms. The total irradiation levels which resulted in 50% survival rates of both nauplii and cyprids were almost the same, being about 4,300 kPa·s.

The experimental inhibition of larval settlement by the 19.5 kHz wave indicated that a total irradiation of 300 kPa·s reduced barnacle numbers to about 30% compared to the control, whereas 125 kPa·s reduced numbers to only about 80% (Fig. 4). In order to reduce the settlement of cyprids by half, total irradiation of about 140 kPa·s is necessary. The barnacle numbers on treated and untreated plates (Table 1) indicated that cyprids subjected to ultrasonic waves retained the ability to select the pretreated plates, in the same manner as the control specimens. Throughout the experiments, the great majority of the exposed cyprids swam normally, evidence that the 19.5 kHz ultrasonic wave injured neither the organs related to settlement, nor those necessary for swimming. Although the settlement abilities of the cyprid larvae were affected, microscopic examination of individual specimens failed to show the details.

On the present study, a total irradiation of about 4,300 kPa·s was required to reduce the survival rate of larvae to 50%, however, a considerably lower level of 140 kPa·s also achieved 50% inhibition of larval settlement. The use of ultrasonic waves is considered to have the disadvantage of high energy consumption (FISCHER *et al.*, 1984), but it is clear that much lower energy levels can be used to inhibit barnacle settlement under natural conditions. Consequently, the use of ultrasonic waves, at low energy levels as an antifouling tool, may well resolve the existing environmental problems of antifouling.

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