Influence of UV-Irradiation on the Nauplius Larvae of the Barnacle *Chthamalus* sp.

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The purpose of this study is to investigate the influence of UV-irradiation on the nauplius larvae of the barnacle and to use this as the basis for researching the possibility of UV-irradiation as a new method for anti-macrofouling control. The nauplius larvae in the petri dishes were exposed to UV-irradiation (λ max = 253.7nm), then the larval states and behaviour were observed. UV-intensities were 3.0 mW·cm⁻²-7.0 mW·cm⁻² and the exposure time was 15 sec-10 min. The water in the petri dishes was changed intermittently but the larvae were not fed. The results were as follows:

1) UV-irradiation may have a delayed lethal effect and metamorphosis-inhibitory effect on the nauplius larvae.
2) The lethal effect of the sum of the dosages of UV on the larvae may nearly equate each other despite differences in each UV-intensity.
3) Within ca. 72hr, 100% of UV-irradiated larvae were dead with the dosage of at least 572 mW·sec·cm⁻² and were incapable of swimming with the dosage of at least 158 mW·sec·cm⁻².
4) UV-irradiated larvae could not exuviate with the dosage of at least 246 mW·sec·cm⁻² and could only exuviate with the dosage of 45 mW·sec·cm⁻².
5) After UV-irradiation even the "actively swimming" larvae may have suffered some sort of physiological damage.

1 INTRODUCTION

Our industrial use of sea water as a coolant brings the problem of marine biofouling to the forefront. Especially in power stations, a wide range of organisms colonizes the surface of the cooling water system and causes several problems.

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Key words : UV-irradiation, the nauplius larvae of the barnacle, the mortality rate, the delayed lethal effect, swimming activity, the ecydysis-metamorphosis.
In many papers, the sterilization effect of UV \(^{13,22}\), the induction of mutation by UV \(^{37,41}\), and the influence of UV on the early development of some animals \(^{5,6}\) (fishes, frogs and sea-urchins) have been discussed. It is also known that the physiological state of the living body is seriously affected by the irradiation of UV around 254 nm. The influence of UV-irradiation on the barnacles' larvae however has yet to be investigated in detail.

The purpose of this study is to investigate the influence of UV-irradiation on the nauplius larvae of barnacles, and to use this as the basis for researching the possibility of UV-irradiation as a new method for anti-macrofouling control.

2 EXPERIMENTAL

The flow chart of experiments is shown in Fig. 1.

2.1 Materials

The larvae were collected before use with a NXX13 plankton net in waters adjacent to the Sekiheki seashore, part of the shoreline of Himeji, from April to June, 1987. The zooplankton with the nauplius larvae were selected from the collected sample by their phototaxis. Then only nauplius larvae of Chthamalus sp. were selected using a pipet and transferred to petri dishes that were filled with filtered sea water. The growth stage of nauplius larvae is classified into 6 stages. The samples used in this paper are mostly at the stage II or III \(^{6,9}\) as shown in Fig. 2. Only very active larvae were selected and applied to the UV-irradiation experiment. UV-irradiation were started within 1 to 12 hr after the larvae were collected. For the observation of appendages' beating, samples at the stage VI was used, since their appendages were larger than those at other stages.

2.2 Apparatus for UV-irradiation

Apparatus for UV-irradiation was self-made and is shown in Fig. 3. UV-lamps were 10W (Toshiba Co., Tokyo), 15W (Matsushita Electric Co., Tokyo) and 30W (Chiyoda Kohan Co., Tokyo).

The wavelength of the UV of these lamps was around 254 nm. The intensities of UV

- Collecting zooplankton with a NXX13 net.
- Carrying them rapidly to our laboratory, with just a little cool and delicate aeration.
- Transferring only active nauplius larvae of Chthamalus sp. to the petri dishes with the filtered sea water.
- Measuring the water temperatures.
- UV-irradiation Non-irradiation (control)
- Measuring the water temperatures. (A observing the larvae).
- Replacing the water. Observing & counting the larvae. Measuring the water temperatures.

Fig. 1 The flow chart of experiments

Fig. 2 A micrograph of a nauplius larva stage II or III of Chthamalus sp.

a: appendages

Fig. 3 Apparatus for UV-irradiation

1: 10W UV lamp
2: 15W UV lamp
3: 30W UV lamp
were measured by UV-radiometer (type UVR-254 TOPCON Co., Tokyo). The intensities were adjusted by changing the distance from UV-lamps to the objects and the number of UV-lamps. The change in UV-intensity with time at a fixed distance was examined before larval exposure.

As shown in Fig. 4, UV-intensity stabilized approximately 2 min after the switch was turned on. Therefore, UV-intensity obtained was measured at least 2 min after switching on. The larvae were then exposed to UV-irradiation.

2.3 The method of UV-irradiation and observation

The conditions of UV-irradiation are shown in Table 1.

Each experiment was performed in still water. The water in the petri dishes was changed after the UV-irradiation and also after every 2 to 12 hours to avoid any type of water pollution caused by the UV-irradiation or by the metabolism of larvae. The larvae were not fed after UV-irradiation.

The water temperatures in the petri dishes were 16 to 19 °C similar to that of the larvae collecting field. Each observation was continued for 70 to 84 hr.

Control and UV-irradiated larvae were observed beneath a stereoscopic and a light microscope.

The state of the larvae was classified into 5 ranks: actively swimming, barely swimming, incapable of swimming, spasmodic, and motionless (dead). The definition of each rank is shown in Fig. 5. The percentages of each rank were obtained. So the mortality rate, the process to larval death, and the percentage of the swimming larvae were examined.

The speed and the continuity of the beating of the appendages in larval swimming was measured. One, the former, the time required for larval appendages to beat 10 strokes in swimming was measured three or more times using a stopwatch and their average was defined as the beating speed. On the latter, the larvae were traced for about 3 min. and the periods of beating and ceasing were measured with a stopwatch, respectively.

Therefore, the continuity of the beating of larval appendages (while larvae were swimming) was examined.

The control and the UV-irradiated larvae were illuminated with spotlight from one side to observe the phototactic behaviour of the larvae. The number of the larval exuviae in the petri dishes was counted to examine the state of ecdysis-metamorphosis of the larvae. The surface and eyes of dead larvae were observed under a light microscope.

![Fig. 4 The change in UV-intensity after lighting UV-lamps. Room temperature: 19.5°C](image)

<table>
<thead>
<tr>
<th>Table 1: The conditions of UV-irradiation</th>
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<tbody>
<tr>
<td>UV-intensity</td>
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<tr>
<td>Exposure time</td>
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<tr>
<td>Water temperature</td>
</tr>
<tr>
<td>Test receptacle</td>
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<tr>
<td>Type of water</td>
</tr>
<tr>
<td>Quantity of water</td>
</tr>
<tr>
<td>Conditions of water</td>
</tr>
<tr>
<td>Number of larvae</td>
</tr>
<tr>
<td>Feeding conditions</td>
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</tbody>
</table>
Ranks | State of the larvae under observation
--- | ---
Actively Swimming | Swimming by continuous beating of appendages
Barely Swimming | Swimming slowly with intermittent movement of appendages and transient sinking
Incapable Swimming | Long continuous beating of appendages of the sunken supine larva limited to forward swimming movement, incapable of swimming
Spasmodic | Sunken, supine, only spasmodic movement of appendages
Motionless | No movement, nor response to stimulus of light and to vibrating the water or to touch
Judged to be dead

Fig. 5 Definition of the ranks of the larval state

3 RESULTS AND DISCUSSION

3.1 The changes of the mortality rate of UV-irradiated larvae over a period of time

(1) Fixed exposure time and varied UV-intensity:

The exposure time was fixed at 1 min and the intensities of UV were varied as follows: 4.1, 5.6 and 7.0 mW·cm⁻². This observation was continued for 84 hr after UV-irradiation.

The changes of the mortality rate of the larvae over a period of time are shown in Fig. 6.

As the UV-intensity decreased, the delayed death occurred. In control, the mortality rate of the larvae was only 5 to 14%. On the other hand, the mortality rate of UV-irradiated larvae increased according to the post UV-irradiation time. With UV-intensity of 4.1 mW·cm⁻², the mortality rate of the larvae was 96.8 to 98.6% at 84 hr after UV-irradiation. With UV-intensity of 5.6 mW·cm⁻², it was 98.6 to 100%, and there 100% death at UV-intensity of 7.0 mW·cm⁻².

(2) Fixed UV-intensity and varied exposure time:

The UV-intensity was fixed at 3.0 mW·cm⁻² and 5.6 mW·cm⁻². The length of exposure time was varied: 0.25, 0.5, 1, 2, 3, 4, 5 and 6 min. Additionally a 10 min exposure was made at 3.0 mW·cm⁻². Observation was continued for ca. 72 hr because the mortality rate of the control larvae tend to increase after 84 hr in the previous experiment. The changes of the mortality rate of the larvae over a period of time are shown in Fig. 7 and 8, and the mortality rate after 71 to 75 hr are summarized in Table 2.

Fig. 6 Relationship between the mortality rate of the larvae and post-UV-irradiation time using variable UV-intensities and fixed exposure time, 1 min
- ○: 7.0 mW·cm⁻² maximum
- △: 5.6 mW·cm⁻²
- ●: 4.1 mW·cm⁻² minimum
- □: control (0 mW·cm⁻²)
In control, the mortality rate of the larvae remained at only 0 to 1.1%. On the other hand, with UV-irradiation, the longer the exposure time was, the more early death and the less delayed death there were. The shorter the exposure time was, the less early death and the more delayed death there were. As compared with the 99-100% survival rate of control larvae, Table 2 shows, 100% of UV-irradiated larvae were dead within 71 to 75 hr when using an intensity of 5.6 mW·cm⁻² for a minimum of 2 min and also with 30 mW·cm⁻² for a minimum of 4 min. In other words, with dosage of at least 672 mW·sec·cm⁻², 100% of UV-irradiated larvae were dead within ca. 72 hr.

These results suggest that on the nauplius larvae UV-irradiation has a certain delayed lethal effect which progresses with time even if the water is changed intermittently after UV-irradiation.

![Graph](image-url)

**Figure 7** Relationship between the mortality rate of the larvae and post-UV-irradiation time using variable exposure times and a fixed UV-intensity, 5.0 mW/d

**Table 2** The mortality rate of UV-irradiated larvae after 71 to 75 hr

<table>
<thead>
<tr>
<th>UV-intensity (mW/cm²)</th>
<th>Exposure (min)</th>
<th>Dosage (mW·sec·cm⁻²)</th>
<th>Mortality Rate of the Larvae after 71 to 75 hr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1800</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>720</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>540</td>
<td>98.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>360</td>
<td>96.1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>180</td>
<td>75.0</td>
<td></td>
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<tr>
<td>0.5</td>
<td>90</td>
<td>52.8</td>
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<tr>
<td>0.25</td>
<td>45</td>
<td>33.0</td>
<td></td>
</tr>
<tr>
<td>0(control)</td>
<td>0</td>
<td>0-0.8</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2016</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>672</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>5.6</td>
<td>336</td>
<td>99.1</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>168</td>
<td>93.1</td>
<td></td>
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<tr>
<td>0.25</td>
<td>84</td>
<td>32.6</td>
<td></td>
</tr>
<tr>
<td>0(control)</td>
<td>0</td>
<td>0-0.5-11</td>
<td></td>
</tr>
</tbody>
</table>
3.2 A comparison of the lethal effect between the intensities of 3.0 mW·cm⁻² and 5.6 mW·cm⁻² where both dosages are almost equal. The dosage of UV-irradiation is expressed by the following formula.

\[ \text{Dosage of UV} = \text{the intensity of UV} \times \text{the exposure time} \]

It is known that the effect on the bacteria of the same dosage of UV-irradiation is equal even if each UV-intensity is different (in the limited condition). A comparison of the effect on nauplius larvae between the intensities of 3.0 mW·cm⁻² and 5.6 mW·cm⁻² was made where both dosages were almost equal. The results of the comparison of the mortality rate between these two intensities are demonstrated in Fig. 9.

The results suggest that the effects on nauplius larvae (including the delayed effect), under the condition of the equivalent sum dosage of UV-irradiation, are almost equal even with different intensity.

3.3 The dosage of UV-irradiation resulting in loss of swimming activity in the larvae (including the delayed effect)

Figure 10 shows the changes of the percentage of the swimming larvae over a period of time.

In control, the most larvae were able to swim for 71 hr, while 100% of the larvae with the dosage of 168 or 336 mW·sec·cm⁻² were unable to swim after 45 to 70 hr.

These results suggest that even in low UV dosages the swimming larvae suffer some sort of physiological damages.

3.4 The changes in speed and continuity of the beating of the larval appendages resulting from UV-irradiation

In the dosage of 672 mW·sec·cm⁻², it was observed how the speed and the continuity of beating appendages in larval swimming changed after UV-irradiation and also after the water replacement. Here, actively swimming larva (stage VI) that had been UV-irradiated was traced. The results are shown in Fig. 11.

Before UV-irradiation, the larvae swam almost rhythmically with its appendages beating continuously. The mean speed of beating appendages was 6.5 times·sec⁻¹. Every 6 to 42 sec the speed of appendages beating slowed down slightly.

However, immediately after UV-irradiation, the larval swimming pattern lost its rhythm and the beating became a little intermittent. The mean speed of the beating of the
appendages was 4.8 times·sec\(^{-1}\) and the beating stopped for less than about 1 sec in every 3 to 20 sec.

After the water was changed, the swimming of the larva remained rhythmless, intermittent, and not recoverable. The mean speed of the beating was 4.8 times·sec\(^{-1}\) and the beating stopped for less than 1 to 11 sec in every 2 to 18 sec. The mean speed and the continuity of the beating were not changed before or after the water replacement.

These results suggest that after irradiation even the "actively swimming" larvae suffer practically irreparable physiological damage despite water replacement. The progress of the damage to the UV-irradiated larvae may lead to their death or emaciation.

3.5 The phototactic behaviour of UV-irradiated larvae

In control, the larvae headed in a straight line for the source of light. After UV-irradiation (dosages of 246 to 420 mW·sec·cm\(^{-2}\)), firstly some of larvae swam in a circular motion, but then eventually most of the larvae showed phototaxis. Further, even some of the larvae unable to swim moved with their dorsal side down toward the light source.

These results suggest that the larvae able to move forward after UV-irradiation do not suffer damage in all photoreceptive functions.

3.6 The state of ecdysis-metamorphosis of UV-irradiated larvae

Figure 12 shows the larval exuvia. The number of the larval exuviae in the petri dishes is summarized in Table 3.

In control, the number of the larval exuviae in each petri dish was varied from 4 to 20 plus within 77 hr. However, with dosages of 45 and 180 mW·sec·cm\(^{-2}\) of UV, there were only 1 to 3. And, with dosages of 246 to 2016 mW·sec·cm\(^{-2}\) of UV, there were no larval exuviae.

These results suggest that ecdysis-metamorphosis of the larvae tend to be inhibited with dosage of 45 mW·sec·cm\(^{-2}\) and becomes totally impossible with dosage of at least 246 mW·sec·cm\(^{-2}\). However, the number of ex-
uviae of control larvae was low due to the larvae being left unfed during this experiments. Therefore a further experiment on these larvae that are fed is necessary.

3.7 Observation of the body surface and the eye of UV-irradiated larvae

The larvae killed by higher dosage (2016 mW·sec·cm⁻²) of UV are shown in Fig. 13.

Further, the killed larvae were observed individually beneath a light microscope for abnormalities to their body surfaces and eyes.

As the result, no abnormalities were observed. However further observation at histological level may be necessary.

3.8 The changes of water temperatures before and after UV-irradiation, and after water replacements

The changes of water temperatures before and after UV-irradiation, and after water replacements were examined.

With UV-intensity of 3.0 mW·cm⁻² and the exposure-time of 10 min (for a total dosage of 1800 mW·sec·cm⁻²), the water temperature increased from 19.0 to 22.0°C. With UV-intensity of 5.6 mW·cm⁻² and the exposure-time of 6 min (for a total dosage of 2016 mW·sec·cm⁻²), the water temperature increased from 18.0 to 21.8°C. This increase in the water temperature may be due to the heat of UV-lamps. After the water was changed, the water returned to its approximately original temperature. That is, the changes of water temperature in this experiment were within only 3.8°C.

It may be considered that the nauplius larvae of Chthamalus sp. suffer less damage from water temperature changes and more from the direct influence of UV-ray (253.7nm).

4 CONCLUSION

The influence of UV-irradiation on the nauplius larvae of the barnacle Chthamalus sp. was investigated. The larvae in the petri dishes filled with sea water were exposed to UV-irradiation (253.7nm), then the larval state and behaviour were observed for 70 to 84 hr. UV-intensities were 3.0 to 7.0 mW·cm⁻² and the exposure time was 15 sec to 10 min. The water in the petri dishes was changed but the larvae were not fed. The results were as follows.

1) UV-irradiation may have a delayed lethal effect on the larvae.

2) The lethal effect of the same dosage of UV seems almost equal on the larvae even if each individual UV-intensity is different.

3) At dosage of at least 672 mW·sec·cm⁻², 100% of UV-irradiated larvae were dead within about 72 hr, and at dosage of at least 168 mW·sec·cm⁻², they lost their swimming ability within about 72 hr. Besides, at the dosage of 84 mW·sec·cm⁻², about 93% of the UV-irradiated larvae were unable to swim after 72 hr.

4) The ecdysis-metamorphosis of UV-irradiated larvae become impossible at the dosage of at least 246 mW·sec·cm⁻² and tend to be inhibited at dosage of 45 mW·sec·cm⁻².

5) After UV-irradiation, even the "actively swimming" larvae have suffered some sort of physiological damage.

These results can be the basis for the research on the possibility of UV-irradiation as a new method for antiaircraft control.

In further work, the nauplius larvae must be fed and reared so that the delayed lethal effect and the metamorphosis-inhibitory effect of UV-irradiation can be investigated. Moreover, the influence of UV-irradiation on the adherancy of the cypris larvae must be investigated.

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References