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**Title:**

Larval rearing without aeration: A case study of the seven-band grouper, *Epinephelus septemfasciatus*, using a wave maker

**Running title:**

larval rearing without aeration

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通気を使用しない仔魚飼育：造波装置によるマハタ仔魚の飼育事例

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マハタ種苗生産の初期減耗を軽減する飼育技法開発の基礎知見を得るために，通気による従来の飼育方法（容量 1 kL，φ130 ㎝，水深 70 ㎝：通気量 200 mL/分）と，直径 5 ㎝の球を水面で上下させて（1 Hz）水面に波を発生させる造波装置を用いた飼育方法で仔魚の生残を比較した。21 日間の飼育実験での造波装置の生残率は 55.5%（n=1）で，対照区のそれ（11.6±14.3%，n=3）よりも顕著に高い値を示した。造波装置による水槽垂直断面の流れを計測したところ，波が水深とともに減衰していくのが確かめられた。

## Introduction

Generally the flow field in a rearing tank for seedling production is generated by aerators to prevent stratification, to insure oxygenation, and to disperse live and artificial foods. [1] On the other hand, the bubbles hitting larvae might stun or injure them, and strong aeration might keep larvae from feeding, make them struggle and waste energy which is lethal to the larvae.[2] Howell *et al.* [3] pointed out that oscillating horizontal paddles or vertical plungers can be used for creating the water field in a rearing tank instead of aeration. There are studies on the effect of turbulence using the vertical plungers on the survival and feeding activity of fish larvae. [4,5] However, so little attention has been paid to compare the effect of the flow field generated by aeration and other flow generating methods in rearing tanks on larval survival and growth. Therefore, we compared the larval survival and growth between two different generating methods for flow field in the larval rearing tank; one is aeration and the other is a wave maker which creates a constant wave on the free water surface in the rearing tank.

The seven-band grouper *Epinephelus septemfasciatus* was chosen as an experimental fish in this study. Although *E. septemfasciatus* is expected as a new candidate species for aquaculture and stock enhancement in Japan, physical stress, such as strong flow and light intensity, causes the water surface tension-related death, [6] which is critical in the early phase (from hatch to abound 10 days after hatching) of the seedling production of this species. [7,8] Oil film forming is commonly used in order to prevent the surface tension-related death in the seven-band grouper larviculture.[7-9] However, surface films will not only reduces gaseous exchange across the air-water interface, but also prevent the larvae swallowing the air that is essential to normal swim bladder inflation. [3,10] We hypothesized that continuous movement of the free water surface by creating small wave will reduce the surface tension-related death of the seven-band grouper larvae, and conducted larval rearing trial for 3 weeks in order to compare early survival and growth in this species reared by the wave maker and aeration.

## Materials and Methods

Four 1 $m^3$ cylindrical black-polyethylene tanks (140 cm in diameter, 68 cm in depth) were used as experimental tanks. Three tanks were set as the same conditions as the former studies [8,9] for the control group, where the flow in the rearing tank was generated with a spherical aerator with a diameter of 5 cm placed at the bottom center of the rearing tank. [8] Another experimental tank was equipped with the "wave maker", which can generate constant wave on the free water surface by a vertically oscillating rubber ball (5 cm in diameter) with a stroke of 2 cm at the center of the surface (Fig. 1).

Fertilized eggs of *E. septemfasciatus* were obtained by artificial insemination followed by the hormonal treatment [11] of a female brood stock (8.1 kg body weight) in the Nagasaki Prefectural Fisheries Experimental Station on 20 May 2004. They were transferred into each experimental tank at a density of 10 eggs/L, and were kept at aeration rate of 1000 mL/min until hatching (day 0). Immediately after hatching (90 % hatching rate), the aeration rate of the control group was set at 200 mL/min following Shiotani *et al.* [8] in which the optimal aeration rate (200 mL/min) and the flow field in the rearing tank for the seven-band grouper larvae had been revealed. For the wave maker tank, aeration was stopped at hatching of larvae and oscillating period was set at 1 Hz creating a 1 mm wave height. Surface film was formed from hatching by addition of oil at 0.2 mL/m$^2$ water surface (Riken Feed Oil Omega, Riken Vitamin Co. Ltd., Tokyo, Japan) for the control group once daily, while oil was not added to the wave maker tank throughout the experiment. Water temperature was kept at 25 °C, water exchange rate was 100 %/day (dissolved oxygen; 5.0-6.0 mg/L), and light condition was natural throughout the experimental period. Feeding regime was designed according to Tanaka *et al.* [9]; S-type (Thai strain) of *Brachionus plicatilis* complex was fed from day 4 (mouth opening of larvae) to day 13, and L-type (Nagasaki Makishima Strain) was fed from day 14 to 21. Rotifers were cultured with HUFA enriched *Chlorella* (Super *Chlorella* V12, *Chlorella* Industry Co. Ltd., Fukuoka, Japan). Rotifer density in each experimental tank was maintained at 10 rotifers/mL and Super *Chlorella* V12 was added to the experimental tanks at a density of $5\times10^5$ cells/mL once daily.

We collected samples on day 10 from the water column from surface to the bottom of the tank using a long polyvinyl chloride pipe (5 cm in diameter) about 5 times in the night (dark condition) and counted the fish density following estimation of live fish numbers in the rearing tank. On day 21, all fish in the experimental tank were counted and were preserved in 5 % formalin solution. Standard lengths of 20 fish from each tank were measured by a digital microscope (VH6300, Keyence Co. Ltd., Osaka, Japan).

We used an acoustic doppler velocity meter (NVD Field, Nortek, Sandvika, Norway) and measured velocity distribution of flow in a vertical section on a radius of the wave maker tank. The number of grid points for measurements was 9×24 in the ($r$, $z$) directions, and the grid spacing was at 1-10 cm. The mean velocities of three components ($u$, $v$, $w$) of flow in the rearing tank were obtained from sampling data. The time for sampling the flow was 0.1 sec, and the sample continued for 50 sec. This measurement design was comparable of the former study [8] that measured the velocity distribution of the control group.

**Results and Discussion**

The survival of the seven-band grouper larvae in the wave maker tank (65.3 % for day 10, 55.5 % for day 21) showed about 3 times higher than the average survival of the control group (20.5 ± 20.4 % on day 10, 11.5 ± 14.3 % on day 21; Fig. 2) which is comparable as the former study with the same rearing condition (22 %). [9] Also the survival rate of the wave maker tank was remarkably high comparing with other rearing experiments of *E. septemfasciatus* using 1 $m^3$ tank (0.06-56.9 % on day 10). [7] No surface tension-related death was observed both in the control and the wave maker tanks during the experimental period, while the oil film was not formed in the wave maker tank. Patchiness of larvae was formed under the water surface near the sidewall of the control tank and larval movement was observed according to the vertical circulation of water mass formed by aeration. On the other hand, most of larvae in the wave maker tank were observed within 10 cm from the water surface and formed more dense patchiness than control group. Standard length of fish in the wave maker tank on day 21 (n=20, 4.1 ± 0.2 mm) was smaller than that of control tank (n=60, 4.9 ± 0.5 mm; *t*-test, $t$=7.031, $p$<0.0001), indicating that there was a negative density-effect on larval growth in the wave maker tank due to the high survival and dense patchiness.

The flow velocity distribution in the wave maker tank showed that the weak undulation (about 1 mm/sec) was formed in the entire water column (Fig. 3). The maximum flow in the wave maker tank was about 1 cm/sec beneath the oscillator, and this is one eighth lower than the maximum upwelling flow in the control tank. [8] We presume that this weak undulation around the free water surface prevented the surface tension-related death of larvae and also reduced physical damage by water movement. Synthesizing these findings, it is noteworthy that the seven-band grouper larvae can be reared without both aeration and surface film showing quite high survival after 3 weeks rearing, even though we had only 1 replicate for the wave maker tank.

One of the specific features in grouper larvae is characterized by the dense mucus cell distribution in the body surface. [12] Physical stress, such as strong flow and light intensity, causes the excess secretion of mucus and leads to the water surface tension-related death. [6] However, surface films will prevent the swim bladder inflation and can cause malformation of juveniles. [3,10] Although we did not check the swim bladder inflation or malformations of larvae in this study, oil film formation must be carefully considered for the quality of seedlings. Recently, it is reported that controlling water viscosity adding proteins into the rearing water reduces surface tension-related death of Japanese flounder *Paralichthys olivaceus* [13] and striped bonito *Sarda orientalis* [14] larvae. However, in the mass-scale rearing tank, addition of these materials into the rearing water is costly and has risk for reducing water quality. Therefore, we conclude that the wave maker is a possible solution for increasing the survival and quality of the seven-band grouper larvae in the process of larviculture.

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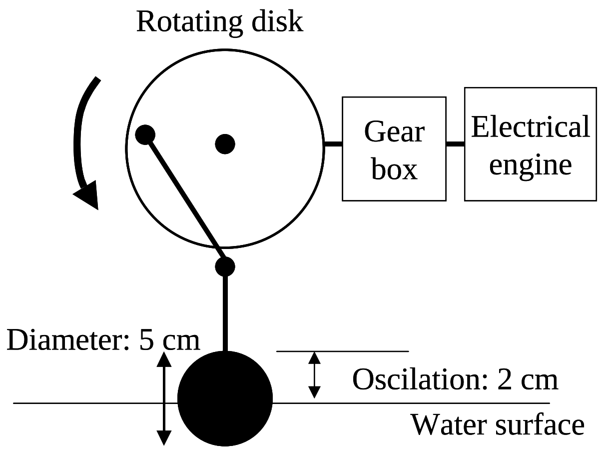


Fig. 1 Schematic illustration of the wave maker, flow field generating apparatus.

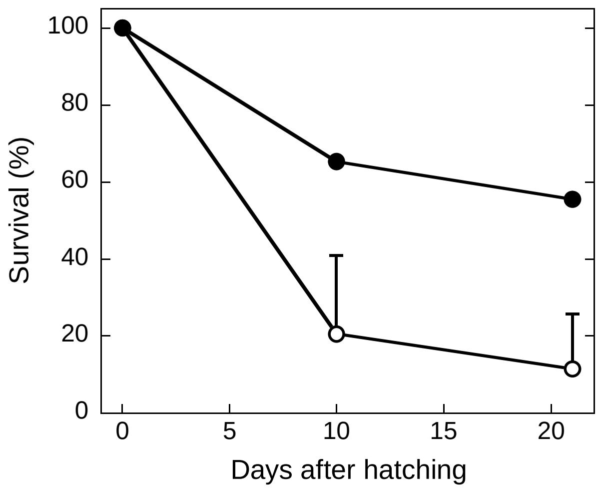


Fig. 2 Changes in percent survival of *E. septemfasciatus* larvae reared in 1 $m^3$ tank with aeration at 200/mL aeration rate (open circle, n=3) and wave maker (closed circle, n=1). Vertical bars indicate standard deviations.

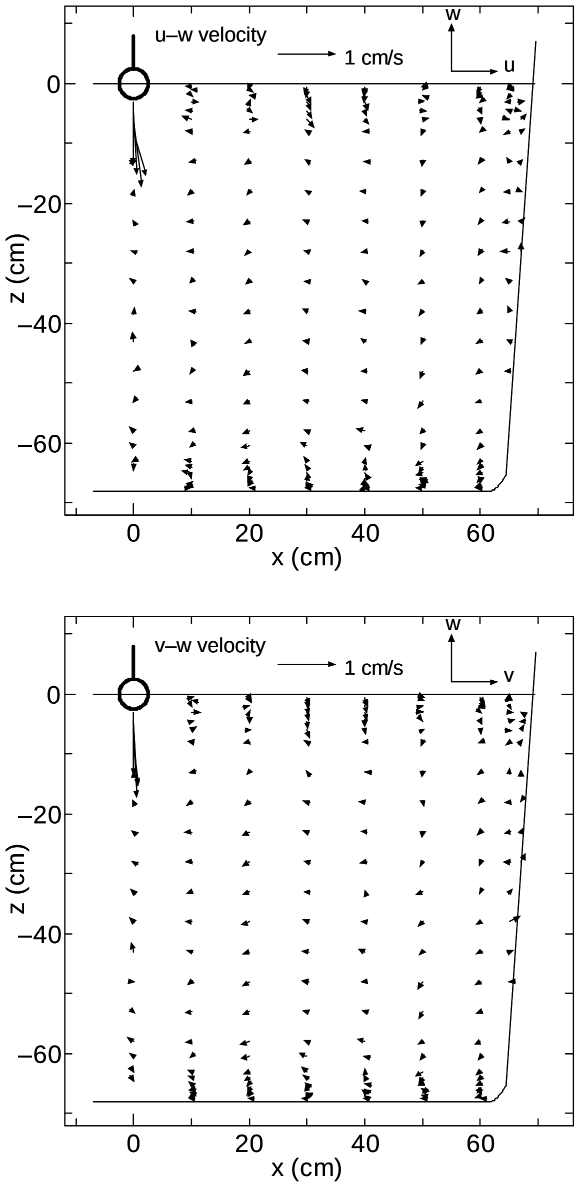


Fig. 3 Flow velocity distribution at 1 Hz oscillation period and 0.1 mm wave length created by the wave maker in the 1 $m^3$ tank. Upper figure; velocity distribution (*u*-*w*) and bottom figure; velocity distribution (*v*-*w*).