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Water surface tension-related deaths in prelarval red-spotted grouper

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Abstract

Three sets of experiments were conducted on environmental changes affecting the occurrence of mass surface deaths in the prelarval stage of the red-spotted grouper, *Epinephelus akaara*. The first experiment, which was on light intensity, showed that a light source attracted the larvae intensely and they dashed toward it. The maximum number of dead fish was at the intensity of 2000 lx. The second experiment showed that an oil film on the water surface completely prevented the occurrence of mass surface deaths. The third experiment showed that the presence of a water current decreased the number of dead fish. These results suggest that water surface tension is a key environmental factor in the occurrence of surface deaths. Mucus secreted on the body surface of prelarvae functions as a glue when larvae are attracted to the water surface by light coming from above. The oil film deprives the water surface of surface tension, which seems to be the key to prevent the occurrence of mass surface deaths in the prelarval stage of groupers. The use of an oil film must be discontinued prior to swim bladder inflation.

Keywords: Mass surface death; Environmental factors; Prelarvae; *Epinephelus akaara*

1. Introduction

Groupers are challenging target fishes for aquaculture scientists because of their economic importance, especially in tropical and subtropical regions of Southeast Asia

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(Kohno et al., 1993). Even in Japan, a temperate country, they are economically important and much effort has been made to produce artificial seed (Fukuhara, 1989).

The red-spotted grouper, *Epinephelus akaara*, is a rather small-sized species mainly distributed around southwestern Japan and southern Korean Peninsula (Masuda et al., 1984). The biology of this species with respect to spawning habit and early life history (Ukawa et al., 1966), maturation and sex change (Tanaka et al., 1990), fin differentiation and squamation (Fukuhara and Fushimi, 1984), proper diet for larvae (Hamamoto et al., 1986) and development of the caudal complex (Kusaka et al., 1994) has been studied to accumulate basic information to facilitate mass seed production on a larger scale. However, complete success in artificial seed production has not been achieved.

The most serious problem for commercial mass seed production is a high mortality rate in the prelarval stage around the time of mouth opening. The prelarvae in this stage frequently die in a mass at the water surface of a rearing tank (Takaya, 1987). The causes of this unique death observed mainly a few days after hatching are a challenging issue for grouper researchers. Nevertheless, no work has dealt with it, except for a brief report on the development of mucous cells on the body surface of *E. akaara* (Kaji et al., 1995). In this study, we examined experimentally the factors affecting the occurrence of this unique death and we attempted to develop some methods to prevent it.

2. Materials and methods

We used eggs spawned naturally by rearing mature fish in heated water at Kagawa Prefectural Fisheries Experimental Station. A net was installed in the evening to collect eggs, which were sampled the next morning. After the eggs were collected, they were placed in a tank for 1 h. Only floating eggs were transferred to a 0.5 t rearing tank, where they hatched. Thai rotifer was given as food for larvae 2 days after hatching at a density of 10 rotifers/ml, and *Nannochloropsis* sp. Larvae were used in this study 2 or 3 days after hatchings.

A small acrylic case (length 13 cm × height 15 cm × width 1.5 cm) was used as an experimental tank. It was filled with seawater. Black paper was stuck on both sides of the small case to shut out light from the outside. The case was placed inside a dark box (length 32 × height 28 × width 45 cm) made of corrugated cardboard attached to the back face of the box. A hole was made in the front face of the box for the lens of a video camera.

Three kinds of experiments were designed: light, oil film and water current. Each kind of experiment consisted of light and dark experimental sets, and each was composed of trials. In each trial, 30 larvae were placed in the small case (Table 1). Seawater used for the trials was put in a bucket and its temperature was controlled by solar heat to be almost the same as that in the rearing tank. The larvae were sampled by scooping them up carefully from the rearing tank using a beaker under the dim light of the rearing room. Then, they were transferred slowly to the small experimental case using a broad-mouth pipette. The case was covered with a black curtain to avoid sunlight when transporting the larvae from the rearing room to the laboratory. The water

Table 1
Data on experiments conducted

Surface death experiment	Light intensity (lx)	Observation duration (min)	Date	Days after hatching	Number of fish used
Light	0 (control)	@60	July 19	2	@30
Experiment	500, 1000, 2000, 3000, 5000	@60	July 20	2	@30
	(experiment)	@60	July 21	2	@30
Oil film	2000 (light control),	@60	July 27	2	@30
Experiment	2000 + o.f. (light experiment)	@60	July 28	3	@30
	0 (dark control),				
	0 + o.f. (dark experiment)	@60	July 28	2	@30
		@60	July 29	3	@30
		@60	July 29	2	@30
		@60	July 30	3	@30
Water current	2000 (light control), 2000	@60	August 4	2	@30
Experiment	+ w.c. (light experiment)				
	0 (dark control), 0 + w.c.	@60	August 5	2	@30
	(dark experiment)				
		@60	August 6	2	@30
		@60	August 7	2	@30
		@60	August 8	2	@30

temperature, pH and dissolved oxygen in the rearing tank were measured just before the start of the experiments. The ranges of the water temperature during the experiments were from 26.5°C on July 20 to 29.0°C on August 5, 1995. The salinity ranged from 22.5‰ on July 19 to 31.3‰ on August 8 and its mean value was 28.0‰. The ranges of pH were from 7.31 to 8.88. Those of the dissolved oxygen were from 6.71 to 9.50 mg/l. All trials except dark ones were recorded by a video camera.

To investigate the relationships between swimming behaviour and surface death, we counted the number of water surface attachments by upward phototactic movement (dashing attachments) by the larvae every 5 min from 60 min video tapes recorded during each experimental trial.

2.1. Light experiments

We used five light intensities (500, 1000, 2000, 3000, 5000 lx) in the experimental set to examine the relationships between occurrence of surface death and luminous intensity. Fiber light illuminated the experiments from above, which kept the water temperature in the experiment constant. We also used a dark condition (0 lx) as a control. The procedures of each experiment were as follows. An experimental small tank containing 30 larvae was installed in the dark condition in the dark box. Then, it was illuminated at each light intensity for 1 h, after which the number of dead individuals at the water surface was counted.

The specified light intensity was given at the central part of the water surface. The light experiments that included one dark control and five light intensities were repeated

three times on July 19, 20 and 21, 1995, using larvae 2 days after hatching (Table 1). Total number of light experiments was 18.

The total number of attachments to the water surface by the larvae was counted for 3 days for four light intensities (1000, 2000, 3000, 5000 lx); the water surface attachment at 0 and 500 lx could not be counted because of their low light intensity.

2.2. Oil film experiments

The experiments were designed to understand the relation between surface tension of the water and occurrence of surface death. Oil of omega yeast, used to enrich the nutrition of rotifers, was used as the oil film. The omega yeast was dissolved in seawater in a 100 ml beaker and oil at its surface layer was collected using a 1 ml syringe. It was dropped onto the surface of the small experimental tank to make an oil film.

We made two experiments at 2000 lx (light control) and 2000 lx plus oil film (light experiment), at which light intensity surface death occurred most frequently (Funahashi, unpublished). Two dark experiments were made at 0 lx (dark control) and 0 lx plus oil film (dark experiment).

Each experiment was 60 min in duration. Six sets of experiments with 24 trials in total were made from July 27 to 30 at the two different light intensities using larvae at 2 or 3 days after hatching (Table 1).

Video tapes of larvae at 2 and 3 days after hatching were made on July 29 and larvae at 2 days after hatching were recorded on July 30. The tapes were analysed to count the total surface attachment number. In this case, the upward speed to the water surface was measured for 10 observable cases in the trials on larvae at 2 days after hatching for the first and last 10 min of each experiment.

The behaviour of prelarvae at the water surface was observed using a pen-like, microscopic video camera (Fig. 1). In each case, 10 larvae at 2 days after hatching were placed in a mini acrylic case (length 3 cm × height 7 cm × width 0.5 cm) and 2000 lx fiber light was applied to the water surface. Sea water was poured into the mini case to its upper limit to observe the behaviour of fish near the water surface.

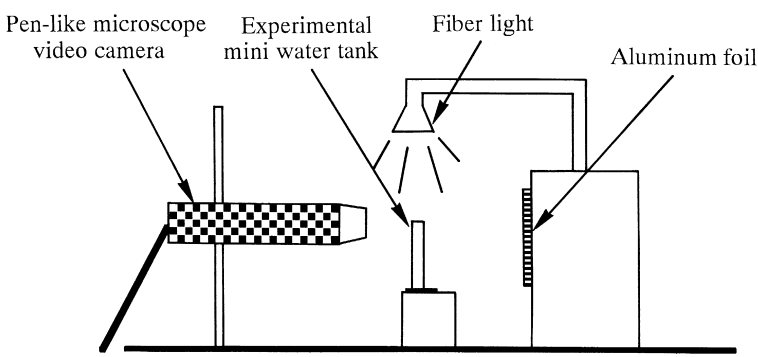

Fig. 1. Lateral view of observation device with pen-like microscope video camera.

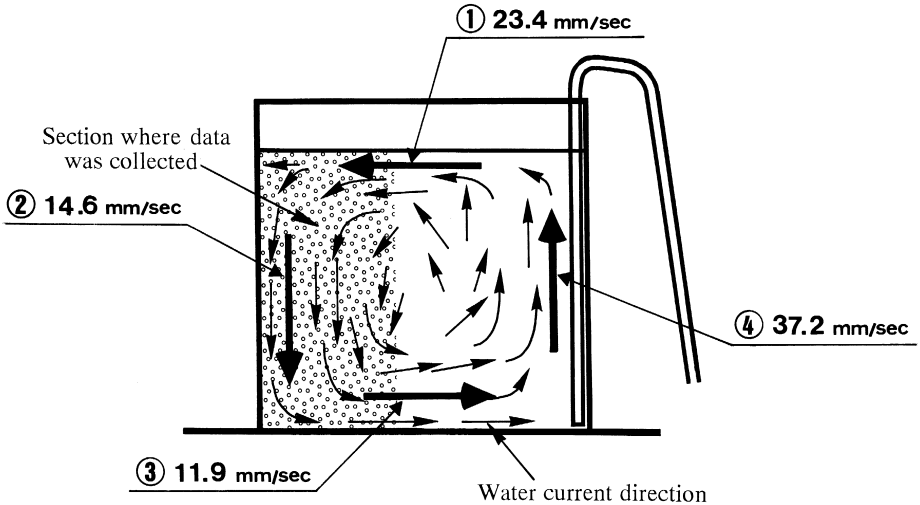

Fig. 2. Frontal view of device for water current experiment. Shadow part is a region for counting the number of individuals showing water surface attachments. Small arrows show current directions. Large arrows show places where the current speed was measured.

2.3. Water current experiments

These experiments were planned to clarify the relation between presence of the water current and occurrence of mass surface death. An aeration tube was set at the side of the small tank (Fig. 2) and a water current was generated by upward air bubbles.

We made two experiments, 2000 lx (light control) and 2000 lx plus water current (light experiment). Two dark experiments were also made at 0 lx (dark control) and 0 lx plus current (dark experiment). The experiments were made from August 4 to 8 using larvae 2 days after hatching. Other experimental procedures were the same as for the other two experiments.

Video tapes recorded from August 4 to 8 for the light control and light experiments were analysed to count the number of water surface attachments. However, considering the effect of the upward water current, the count was made only at the opposite side of the aeration tube, where no upward current was observed (Fig. 2).

The speed and course of the water current in the small tank shown in Fig. 2 were measured using dead eggs as marker particles at four sites (at right and left sides, and near water surface and bottom) for each of the 10 eggs.

3. Results

3.1. Light experiments

Surface death occurred in every experiment. We examined the mean values and standard deviation of the number of dead individuals for 60 min of the six light

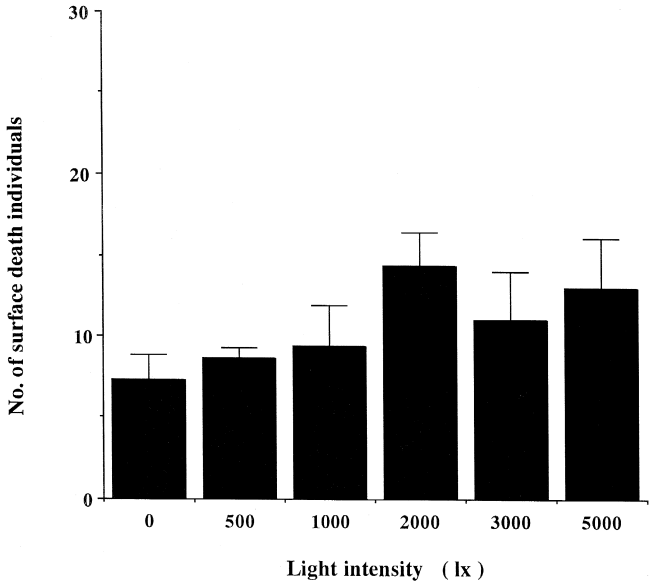

Fig. 3. Mean number and SD of surface death individuals at each light intensity.

intensities (Fig. 3). In general, lower intensity classes showed fewer dead individuals. The minimum number of dead, six, occurred in the 0 lx class and the maximum number, 16, occurred at 2000 and 5000 lx. We classified these six classes into two groups (low group: $0 + 500 + 1000$ lx, $n = 9$ and high group: $2000 + 3000 + 5000$ lx, $n = 9$) and we compared the number of dead individuals. The mean value and SD of the low group was 8.44 ± 1.74 and those of the latter high group was 12.78 ± 2.77, which showed a significant difference (Mann–Whitney's U-test, $P < 0.01$). This clearly showed that high light intensity tends to increase the number of surface death individuals.

The number of dashing attachments to the water surface under the 1000, 2000, 3000 and 5000 lx conditions for 60 min was examined. In general, its value was largest during the first 5 min and then gradually decreased with a time lapse (Fig. 4). During the first 5 min of the three trials in each light intensity, the total number of the 1000 lx was the smallest (89) and that of the 2000 lx was the largest (220). However, they might be regarded as experimental artifacts, and so the values during the first 10 min were excluded from the data. The mean value and SD at 1000 lx for the remaining 50 min was 108.67 ± 52.93, significantly smaller than values at higher light intensities (2000 lx, 273.67 ± 30.14; 3000 lx, 292.33 ± 57.86; 5000 lx, 259.33 ± 72.60) (Mann–Whitney's U-test, $P < 0.05$).

3.2. Oil film experiments

In all 12 trials without oil film, surface death individuals always appeared regardless of whether light was present, and the number at 2000 lx was always larger than that

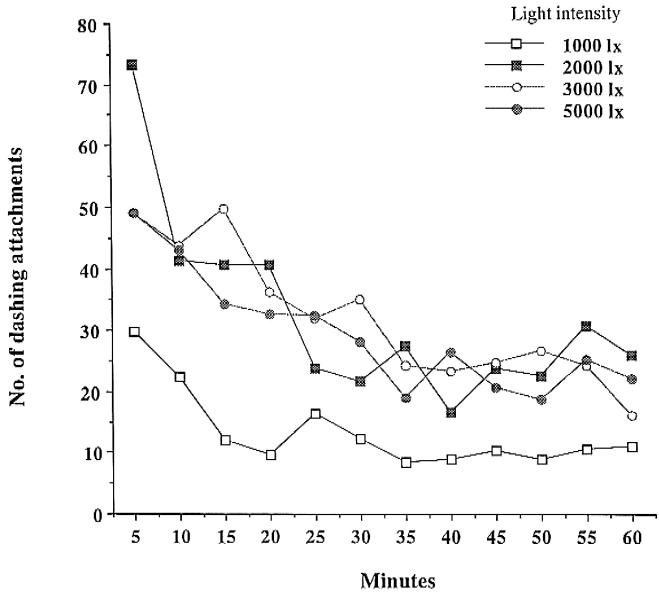

Fig. 4. Mean value of number of water surface attachments of four light intensities at every 5 min.

at 0 lx. By sharp contrast, in all experiments with oil film, we found no surface death individuals (Fig. 5).

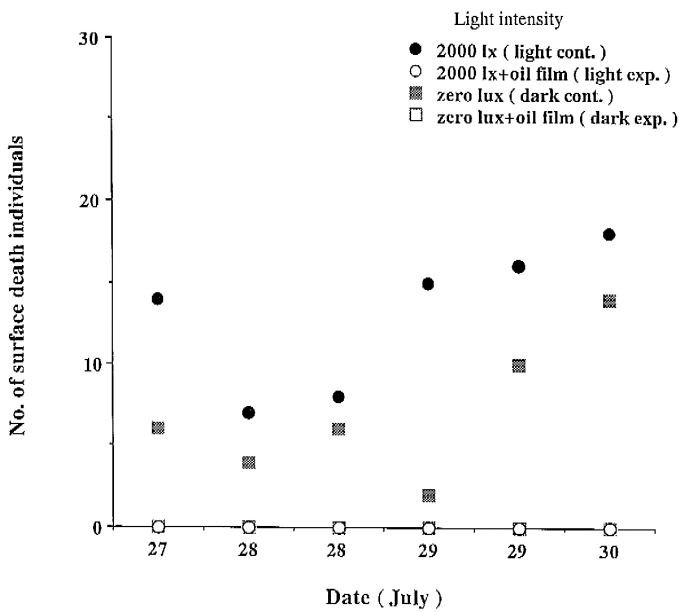

Fig. 5. Number of surface death individuals in six sets of the oil film experiment and the control.

We examined the number of dashing attachments in six trials of 2000 lx with and without oil film. No clear difference was found between the numbers with and without oil film for 60 min (Fig. 6). Statistically, we found no significant difference between the mean values of number of water surface attachments observed every 5 min (total 180 min) in experiments with (39.31 ± 12.29, $n = 36$) and without (38.58 ± 16.00, $n = 36$) oil film (Mann–Whitney's U-test, $P > 0.05$).

The behaviour of phototactic upward movement by the fish to the water surface was observed using a microscopic video camera for the control without oil film. They swam actively toward the water surface and nearly attached to it. Then, they struggled to separate from the water surface by arching the body and in many cases, they succeeded in freeing themselves. They repeated this behaviour many times. The more they repeated it, the duration attached to the surface increased gradually and the body action weakened. At the end of this repetition, they stopped at the water surface attaching to it. Although the lateral side of the body was most frequently caught by the water surface, the caudal part sometimes attached to the surface and the fish struggled at that part in a head-down position. We observed frequently individuals that separated from the surface and swam weakly. However, while swimming, their dorsal fin in many cases was attached to the surface and, after arching the body, the lateral side attached to the surface until death.

The speed of the upward swim was measured during each 10 min at the start and the end of the trial; first 10 min was 8.35 ± 7.07 mm/s (mean $\pm$ SD; $n = 10$) and the last

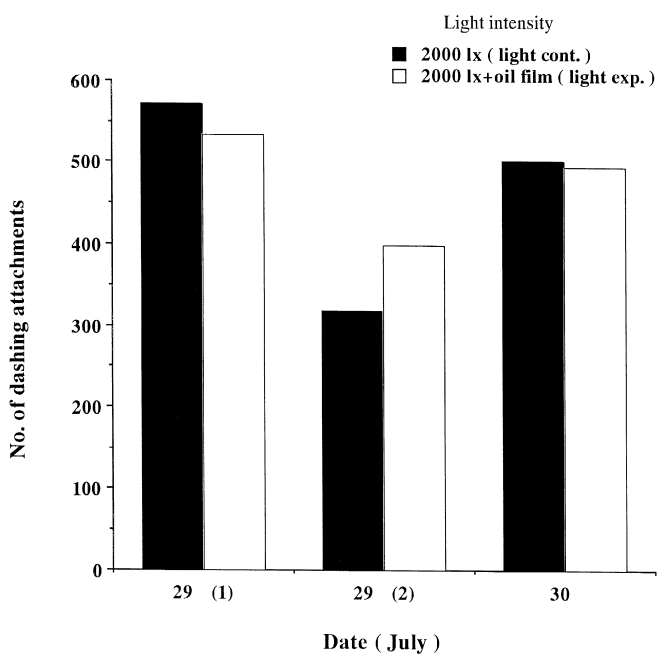

Fig. 6. Total number of water surface attachments in the oil film experiment under 2000 lx during 60 min.

10 min was 6.25 ± 2.80 mm/s ($n = 10$). No significant difference was found between them (Mann–Whitney's U-test, $P > 0.05$).

In the experiments, the oil was a layer of particles 1–2 mm thick. The boundary between the water and oil layer was uneven. Prelarvae showed the same behaviour as in the control without an oil film and they swam frequently toward the surface. However, they were repelled by the oil particles and they could not push them aside.

3.3. Water current experiments

The number of surface death individuals almost always decreased with a water current (Fig. 7). The mean value of the number of dead individuals in the light control (without current) was 11.40 ± 3.78 ($n = 5$) and that in the light experiment (with current) was 4.20 ± 2.68 ($n = 5$). A significant difference was found between them (Mann–Whitney's U-test, $P < 0.05$).

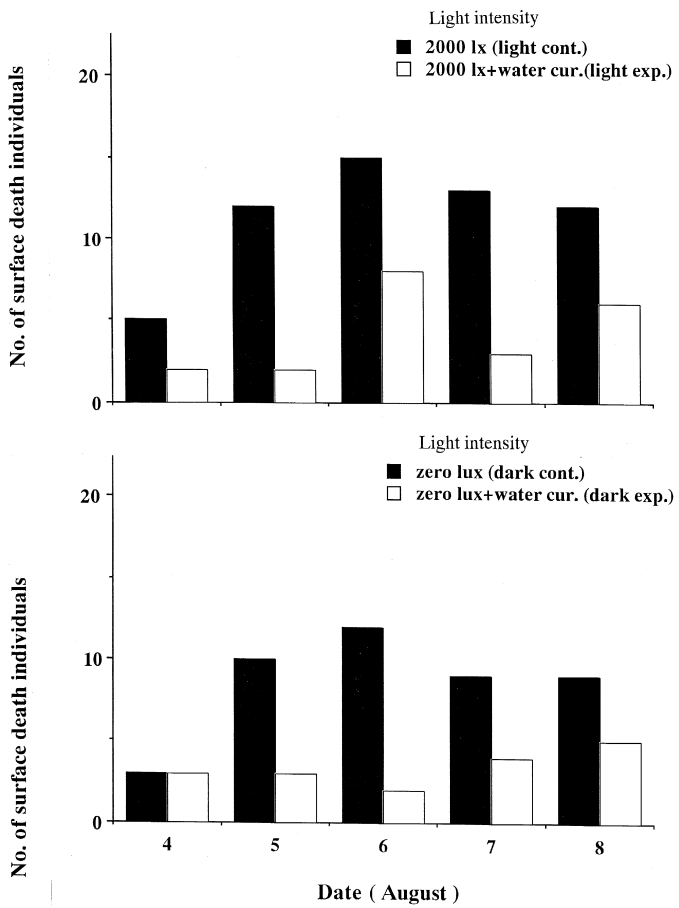

Fig. 7. Number of surface death individuals in the water current experiment and the control.

In the experiments without light (0 lx), in four of five sets, the number of surface death individuals decreased with a water current (Fig. 7). An exception was in the experimental set of August 4 in which no change in dead number occurred. The mean value and SD of the dark control was 8.60 ± 3.36 ($n = 5$) and that of the dark experiment was 3.40 ± 1.14 ($n = 5$). The former was significantly higher than the latter (Mann–Whitney's *U*-test, $P < 0.05$).

4. Discussion

The number of surface death individuals in the dark and at low light intensities was smaller than that at high light intensities, suggesting that light over 2000 lx strongly attracts prelarvae of *E. akaara*. This agrees with the fact that, in general, surface deaths frequently occur at the time of sudden positive changes in light conditions (Isshiki, personal observation). In this study, the highest value of water surface attachment just after the start of the experiments (Fig. 4) also supports this. However, surface deaths occurred even in dark conditions (Fig. 3) in this study, which suggests that the presence of light is not a key factor in the occurrence of surface deaths.

Kaji et al. (1995) studied the development of mucous cells on the body surface of these fish and concluded that larvae 2–4 days after hatching showed the highest degree of development. Thus, the larvae 2 or 3 days after hatching used in this study can be expected to have well-developed mucous cells. Considering the effect of light on larval behaviour just mentioned, mucous cell development seems to be closely related with the occurrence of mass surface deaths. When the prelarvae are given a physical stimulus, they discharge much mucous substance (Isshiki, personal observation). If one individual is caught by the water surface and is given a physical stimulus, its mucous cells function as a glue and attach the individual to other individuals, which form a mass of dead individuals at the water surface.

This study clarified that an oil film on the water surface in rearing tanks has a decisive role in preventing the occurrence of surface deaths (Fig. 5). Prelarvae cannot reach the surface because of the hindrance of the film of oil particles between the water and air, and, therefore, the mucous body is not caught by the surface tension of the water.

Although surface deaths did not occur in the presence of an oil film, prelarvae dashed frequently toward the water surface repeatedly as in the control without film. At light intensities over 2000 lx, the mean number of water surface attachments was estimated to be about 6/min during the 60 min trial in the light experiments (see Fig. 4). Because this frequency might force the fish to waste their energy, a low light intensity is favourable even with an oil film present.

The water current was suggested to assist fish caught by the water surface to free themselves from the water surface. Taking light, oil film and water current into consideration, an ideal rearing system can be produced with the following environmental conditions. The light intensity for seed production must be lower than 1000 lx at the water surface level of rearing tanks at least until 10 days after hatching. At this stage, mucous cells disappear (Kaji et al., 1995). Attention also must be paid to the spot light

on the water surface from holes in the roof and upper wall. The water current should be a speed of about 25 mm/s at the water surface for aeration. The oil film should be used until 6 days after hatching when the mucous cells tend to be reduced (Kaji et al., 1995).

The presence of an oily surface film in the rearing tank inhibited inflation by blocking access of larvae of *Sparus auratus* to the surface (Chatain and Ounais-Guschemann, 1990). In the red sea bream, *Pagrus major*, initial inflation of the swim bladder was experimentally inhibited by covering the water surface with mineral oil, which blocked the access of larvae to the surface. This frequently gave rise to the development of lordotic deformity (Kitajima et al., 1981). Since the first intake of air to the swim bladder mainly occurs on the 7th day (Kagawa Prefectural Fisheries Experimental Station, unpublished), the oil film must be removed from the water surface by that day. We think it is very interesting that the timing of the first intake of air coincides with that of the degeneration of mucous cells (see Kaji et al., 1995).

By making oil film at the water surface, Wakayam Prefectural Aquaculture Experimental Station has succeeded in mass production of the seedlings of *E. bruneus*. The number of juveniles reared to 60 days after hatching (about 40 mm TL) increased to 500,000 (a 50-fold increase) in 1998. In 1999, the survival rate at 9 days after hatching of the species is up to about 90% (Hazama, personal communication). These results show that the oil film is applicable to the successful rearing of larval grouper.

Surface death originates from a mismatch of mucous cells and surface tension of the water. The original function of mucous cells might be that of an antipredator (Kaji et al., 1995). In natural environments, prelarvae at stages with well-developed mucous cells might live in somewhat deeper and dim habitats.

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