FEEDING INCIDENCE, GROWTH AND SURVIVAL RATE IN THE EARLY STAGE OF THE RED-SPOTTED GROUPER, *Epinephelus akaara*, IN RELATION TO TANK COLOR

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ABSTRACT

The red-spotted grouper, *Epinephelus akaara*, is one of marine fish species targeted for mariculture in Japan. The artificial mass seedling production of these species has been largely successful. However, the survival is still unstable. The effect of tank colors on the feeding incidence, feeding proportion, growth and survival rates in the early stage of the red-spotted grouper were examined. The results showed the feeding incidence, feeding proportion, growth, and survival rates at different tank colors were highly significantly different (P<0.001). Post hoc multiple comparisons based on Tukey's test showed significant differences (P<0.05) regarding feeding incidence between yellow (70.45%) compared to white (55.83%), black (48.42%), green (41.67%), blue (35.17%), and red (32.50%). The highest feeding proportion (number of rotifer in the stomach/larvae) was found at yellow (5.62), followed by white (2.47), black (1.97), green (1.92), blue (1.71), and red (1.28). The specific growth rate showed significant differences (P<0.05) were found at yellow (2.14%) and white (1.84%) compared to black (1.46%), green (1.20%), blue (1.15%), and red (1.13%). The survival rate at yellow color (1.22%) was the highest, followed by white (1.09%), black (0.79%), green (0.57%), blue (0.38%), and red (0.37%). Yellow was suitable as tank wall color for rearing of red-spotted grouper larvae.

KEYWORDS: **tank color, growth, survival, larvae, grouper**

INTRODUCTION

Understanding physical and biological factors that act on larval development is essential to create protocols that maximize survival and growth encountered under culture conditions (Downing & Litvak, 1999). Light has been shown to be important ecological factor for fish, influencing development from egg to sexually mature adult (Saunders *et al.*, 1989; Suquet *et al.*, 1992; Mangor-Jensen & Waiwood, 1995). Light regimes are easily manipulated, whether intentionally or unintentionally, by selection of light source, distance of light source from water surface, photoperiod, light spectrum, depth of tank, water color, and tank color (Gulbrandsen *et al.*, 1996; Naas *et al.*, 1996; Downing & Litvak, 1999; Downing & Litvak, 2001; Setiadi *et al.*, 2003). Consequently, care should be taken to ensure that environmental conditions in the aquaculture hatchery are not detrimental to larval vision (Downing & Litvak, 1999).

Conditions that maximize contras between prey and environment should facilitate detection and capture of food by larvae, particularly during critical switch from endogenous to exogenous feeding (Downing & Litvak, 1999). Foraging success is depending on a series of events involving encounter, attack, and capture of prey (WanzenbÖck & Schiemer, 1989). Manipulation of light intensity has been shown to influence growth, survival, and feeding success of many larval teleosts (Brizt & Pienaar, 1992). However, color and reflectance of tank walls may also influence contrast between prey and background, resulting in different prey capture.

Since groupers are generally large and good tasting fishes (Katayama, 1984), they have

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become challenging subjects for aquaculture scientists (Kohno *et al.*, 1993). The red-spotted grouper, *Epinephelus akaara*, is a rather small-sized species mainly distributed around southwestern Japan and the southern Korean Peninsula (Masuda *et al.*, 1984). Seedling production of *E. akaara* has been studied from the middle of the 1960s (Ukawa *et al.*, 1966). However, complete success in artificial seedling production has not been achieved. The most serious problem involved in commercial mass seedling production is the high mortality rate in the prelarval stage around the time of mouth opening. Several studies on this species to support the 'critical period' had been reported. These studies have focused on larval and early stage development (Mito *et al.*, 1967), fin differentiation and squamation (Fukuhara & Fushimi, 1988), development of mouth parts and feeding (Kayano, 1988), the effects of water temperature on embryonic development (Kayano & Oda, 1991), development of the caudal skeleton (Kusaka *et al.*, 1994), the effects of oil film, light intensity, and water current in the early stage (Yamaoka *et al.*, 2000), the early development of dorsal and pelvic fins (Kusaka *et al.*, 2001), and the effects of light intensity and water color in the early stage (Setiadi *et al.*, 2003). However, research focusing on the tank color as a background for larval rearing of the red-spotted grouper has not yet been reported.

The objective of present study was to determine the influence of tank color on feeding incidence, feeding proportion, growth, and survival in the early stage of red-spotted grouper. This information is important for grouper culture, especially in an intensive culture system.

MATERIALS AND METHODS

The experiment was conducted in July 2002 at the Tamano Station of the Japan Sea-Farming Association, Okayama Prefecture, Japan. Eggs were obtained from natural spawning broodstock, which were reared in a circular concrete tank with a water volume of 50 m^3. Fertilized eggs (floating ones) were collected from a collector using a scoop and put into a bucket. Thereafter eggs were transferred into a hatching tank filled with seawater to 500 L. On the following day, newly hatched larvae (D0) were taken using a beaker plastic with a water volume of 1 L and stocked into 100 L seawater in a circular polycarbonate transparent tank. Eighteen of circular polycarbonate transparent tanks were used. Each tank was stocked with 40 larvae/L. To make the tank color, plastic cutting sheet colors with index number i.e., yellow (841), white (840), black (839), green (857), blue (8502), and red (823) colors were used. The plastic cutting sheets colors were attached at the inside walls of the experimental unit tanks. The light intensity was adjusted to 2,000 lx using florescent tube 40 W each. Each treatment was consisted of three replicates. For measurements of total length, feeding incidence, feeding proportion, and growth, 30 larvae were sampled from 3 to 6 days after hatching. All of the larvae were anaesthetized using MS222. Observation on feeding incidence and feeding proportion, larval stomach contents were expressed under the pressure of a cover slip, and number of rotifers consumed was counted under microscope. The SS-type rotifers were fed to larvae from 3 to 6 days after hatching at a density of 5 rotifers/mL. Phytoplankton (*Nannochloropsis* sp.) as a food for rotifer were added into the experimental unit tanks at a density of 5—10 x 10^5 cells/mL. To make an oil film at the water surface, 0.1 mL feed oil (E 100 Riken) was dropped in each tank.

Statistical analysis one-way ANOVA was used to compare data (feeding incidence, feeding proportion, growth and survival) among the treatments. If there was significant differences among the treatments, post hoc multiple comparisons test using Tukey's test was performed in order to know which treatment was different from each other. All statistical data analysis was performed using JMP statistical program version 3.2.6, SAS Institute Inc., USA (1989).

For each color treatment specific growth rate (SGR) over the experimental period was determined (Ricker, 1979; Kamler, 1992):

$$SGR = (\ln w_2 - \ln w_1)/(t_2 - t_1) \times 100\%,$$

where w_1 is total length (*mm*) at time t_1 and w_2 is total length (*mm*) at time t_2.

The SGR was calculated from day 0—6.

RESULTS AND DISCUSSION

The result of feeding incidence at different tank colors is shown in Figure 1.

The result of Figure 1 showed the feeding incidence at yellow color (70.45%) was the highest, followed by white (55.83%), black

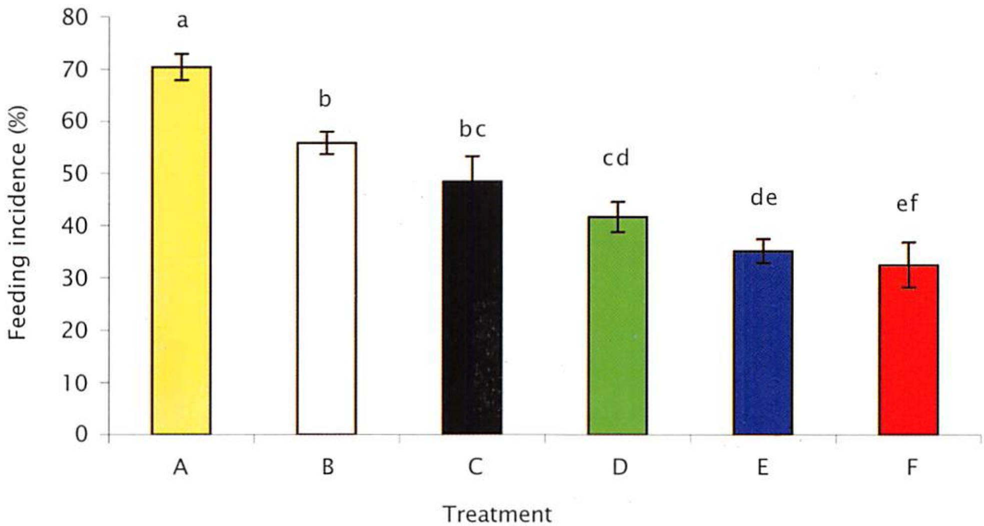

Figure 1. Feeding incidence at different tank colors: (A) yellow; (B) white; (C) black; (D) green; (E) blue; (F) red. Bars with similar letters are not significantly different (P>0.05)

(48.42%), green (41.67%), blue (35.17%), and red (32.50%) colors. Based on statistical analysis (one-way ANOVA), the feeding incidence at different tank colors revealed significant differences (P<0.001) among the treatments. According to post hoc multiple comparisons (Figure 1), in the feeding incidence between yellow versus white, black, green, blue, and red revealed significant differences (P<0.05). Significant differences (P<0.05) were also found between white versus yellow, green, blue, and red, black versus yellow, blue, and red, and green versus yellow, white, and red. No significant differences (P>0.05) in the feeding incidence were found between white versus black, black versus green, green versus blue, and blue versus red.

The highest feeding incidence found at yellow color may be caused by background. This study suggests that the yellow color has made a good contrast between prey and background. On the other hand, the yellow color may be resulted in different between reflection and dispersion of light illumination in the tank compared to the other colors. Thus at yellow color, prey are easily to recognized by larvae compared to the other colors. White, black, green, blue, and red colors have resulted in impaired recognition of prey, due to poor light dispersion and contrast between prey and background. Naas *et al.* (1996) stated that different tank colors have resulting different reflection and dispersion of light illumination that affect larvae in capturing prey.

Many aquaculturists recommend used to black tank color for larvae rearing because the larvae were not concentrated along the wall and resulting in less damage due to abrasion on their body (Howell, 1976; Gilham & Baker, 1985; Naas *et al.*, 1996). In contrast, white color was the best compared to black color had also been observed (Downing & Litvak; 1999). The result of this study showed discrepancy with black and white tank colors that had been reported (Howell, 1976; Gilham & Baker, 1985; Naas *et al.*, 1996; Downing & Litvak; 1999). The present study showed yellow color was the best for feeding incidence of red-spotted grouper larvae. According to larval behavior observed had indicated the larvae were distribute in the middle layer and not to accumulate along the wall. Otherwise, larvae reared at the other colors had indicated most of the larvae were concentrated along the wall. Therefore, the red-spotted grouper cannot be equated to the other marine fishes that could be reared in the black or white tank colors as mentioned above.

The result of feeding proportion at different tank colors is shown in Figure 2.

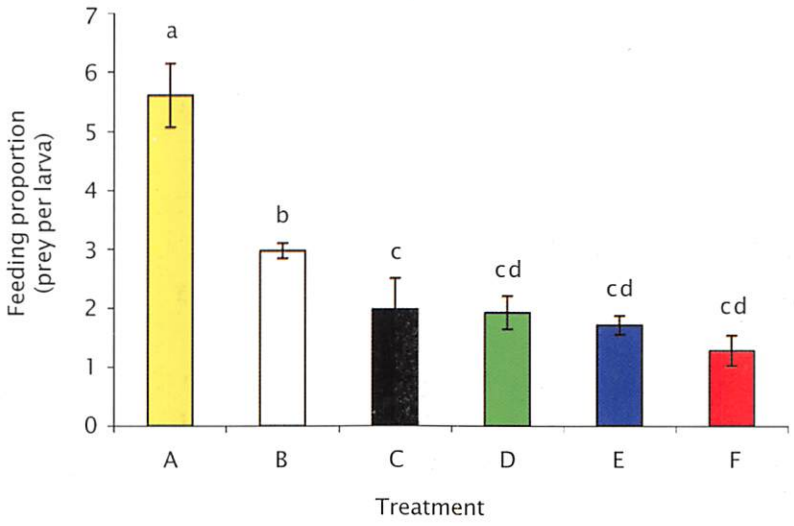

Figure 2. Feeding proportion at different tank colors: (A) yellow; (B) white; (C) black; (D) green; (E) blue; (F) red. Bars with similar letters are not significantly different (P> 0.05)

Figure 2 showed the feeding proportion at yellow color (5.62) had indicated the highest, followed by white (2.97), black (1.97), green (1.92), blue (1.71), and red (1.28) colors. One-way ANOVA in the feeding proportion at different tank colors revealed significant differences (P<0.0001) among the treatments. Post hoc multiple comparisons (Tukey's test) in the feeding proportion between yellow versus white, black, green, blue, and red showed significant differences (P<0.05). White versus yellow, black, green, blue, and red were also significant differences (P<0.05). No significant differences (P>0.05) in feeding proportion were found between black versus green, black versus blue, black versus red, green versus blue, green versus red, and blue versus red.

Larvae reared at yellow color showed success in capturing prey, while white, black, green, blue, and red colors were poor. Downing & Litvak (1999) reported that tank color may influence contrast between prey and background, resulting in different capture rates. However, the contrast between prey and background only cannot be considered as a reason for capturing success in term of feeding incidence (Figure 1) and feeding proportion (Figure 2). This study suspects that an interrelationship seems to exist between tank color and the eye retina (visual pigment) of the larvae. Most teleost larvae are visual feeders and some of theoretical works have indicated the importance of ontogeny and irradiance on larval visual range (Blaxter, 1986; Miller *et al.*, 1988; Aksnes & Utne, 1997). The Epinepheline possesses visual pigment (cone) type (A_1) with a wave length of maximum absorption (l_{max}) of 491 to 501 *nm* (Ali & Wagner, 1975). It has been reported that the range of yellow-greenish to yellow is 500—580 nm (McFarland, 1986). Therefore, Epinepheline's eye pigment is most sensitive to yellow, which suggests the reason why the feeding incidence and feeding proportion of the red-spotted grouper were the highest at yellow color than the other colors.

The result of specific growth rate (SGR) at different tank colors is shown in Figure 3.

The result of figure 3 showed the SGR at yellow color (2.14%) was the highest, followed by white (1.84%), black (1.46%), green (1.20%), blue (1.15%), and red (1.13%) colors. One-way ANOVA in the SGR at different tank colors revealed significant differences (P<0.0001) among the treatments. Post hoc multiple comparisons (Tukey's test) in the SGR at yellow and white compared to black, green, blue, and red showed significant differences (P<0.05). No significant differences (P>0.05) in SGR were found between yellow versus white, black versus green, black versus blue, black versus

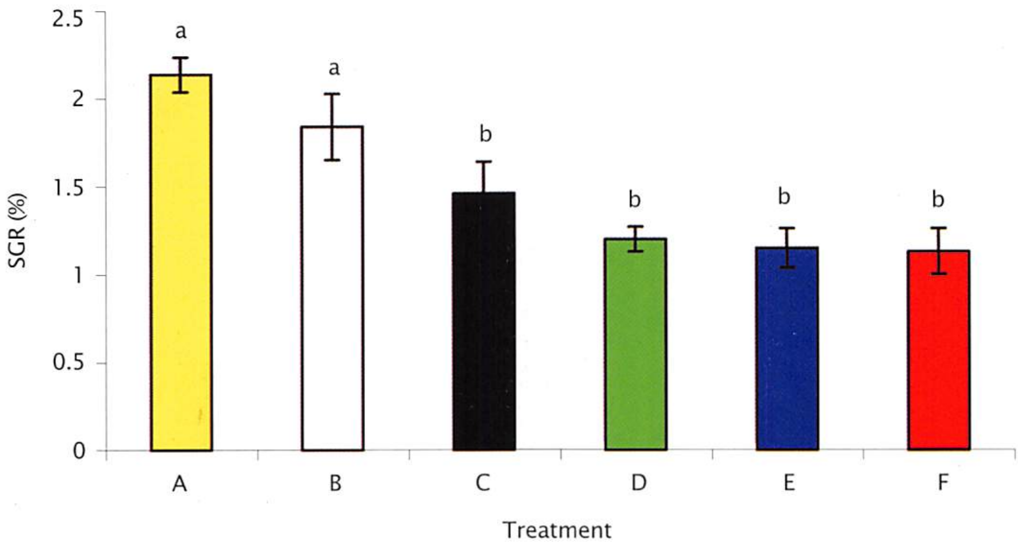

Figure 3. Specific growth rate at different tank colors: (A) yellow; (B) white; (C) black; (D) green; (E) blue; (F) red. Bars with similar letters are not significantly different (P> 0.05)

red, green versus blue, green versus red, and blue versus red.

Food is essential for larval growth. The lack of food consumption by larvae is the main problem for grouper, especially in the early stage or in the critical period (endogenous to exogenous). This study showed the yellow color had indicated the best in the SGR due to the larvae were sufficient to capture of prey. Otherwise, black, green, blue, and red colors had indicated the larvae were very poor, resulting in low SGR. This study suspects that sufficient to capture of prey by larvae may be correlated with nutrition demand for larvae that reflect in the improvement for their growth. Downing & Litvak (1999) stated that inability to detect and capture of prey by larvae might have resulting inhibition for their growth due to lack of nutrition. Successful initiation of feeding behavior during the switch from endogenous to exogenous nutrition is very important for larval growth (Downing & Litvak, 2001). KjØrsvik *et al.* (1991) reported that 3 to 5 days after hatching is the time for start feeding and its point-of-no-return in cod (*Gadus morhua* L.) larvae. The first feeding of the red-spotted grouper, *E. akaara*, was at 3 days after hatching, where the yolk-sac was completely absorbed (Setiadi *et al.*, 2003). In tiger grouper (*Epinephelus fuscoguttatus*) and humpback grouper (*Cromileptes altivelis*) larvae, the yolk-sac would completely being absorbed in 3 days after hatching had also been observed (Kohno *et al.*, 1993; Slamet *et al.*, 1996; Sugama *et al.*, 2001). This study suggests that from 3 to 6 days after hatching plays a role in critical period for the red-spotted grouper and also the other groupers.

The result of survival rate at different tank colors is shown in Figure 4.

Figure 4 showed the survival rate at yellow color (1.25%) was the highest, followed by white (1.08%), black (0.78%), green (0.57%), blue (0.39%), and red (0.37%) colors. One-way ANOVA in the survival rate at different tank colors revealed significant differences (P<0.0001) among the treatments. Post hoc multiple comparisons (Tukey's test) in the survival rate at yellow and white versus black, green, blue, and red showed significant differences (P<0.05). No significant differences (P>0.05) in the survival rate were found between yellow versus white, black versus green, black versus blue, black versus red, green versus blue, green versus red, and blue versus red.

The survival of larvae is related to success in the first feeding. Based on feeding incidence (Figure 1) and feeding proportion (Figure 2) showed yellow and white colors were much

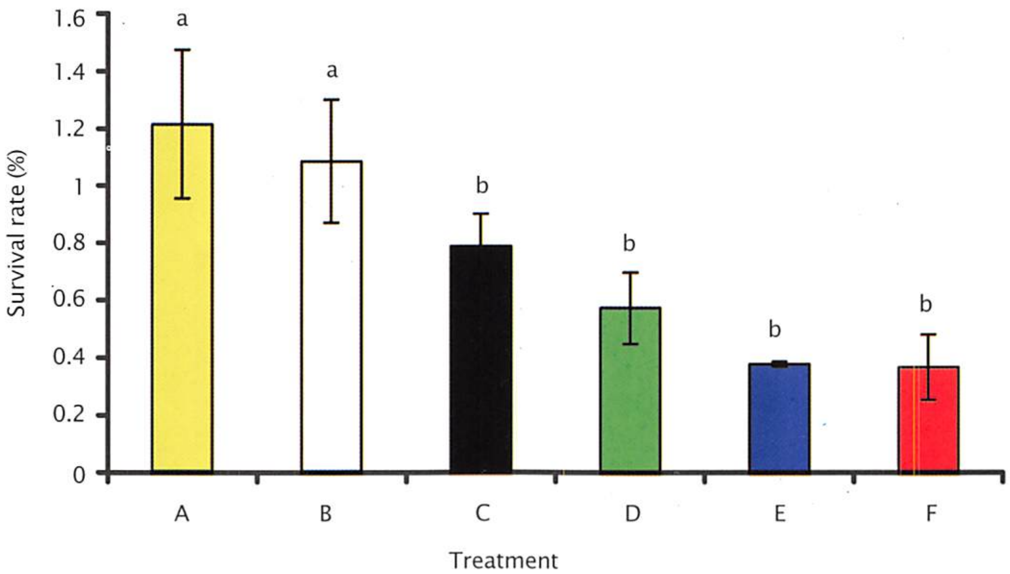

Figure 4. Survival rate at different tank colors: (A) yellow; (B) white; (C) black; (D) green; (E) blue; (F) red. Bars with similar letters are not significantly different (P>0.05)

higher compared to the other colors in both of feeding incidence and feeding proportion. Therefore, yellow and white colors indicated much higher compared to the other colors for survival rates. According to the stomach of larvae examined at black, green, blue and red colors indicated most of their stomach did not present rotifers. This study assumes that larvae did not feed rotifers might died on the following day during the larval rearing period, due to lack of nutrition. Thus, these conditions may have resulted in low survival rates. Downing & Litvak (1999) reported that larvae were probably unable to detect and capture of prey resulted in the greater mortalities observed due to starvation.

Although many aquaculturists recommended that black and white tank colors were the best for marine fishes culture to obtained maximize growth and survival (Howell, 1976; Gilham & Baker, 1985; Naas *et al.*, 1996; Downing & Litvak, 1999). However, those recommends cannot be applied for larval rearing of the red-spotted grouper, because the result of the present study showed yellow color was suitable as a background for feeding incidence, feeding proportion, growth and survival. To decide which background color is suitable for fish culture that should initially be conditioned based on visual pigment before start to culture. There has been known that one species possesses visual pigment different from another.

CONCLUSIONS

Yellow for tank background is suitable to maximize feeding incidence, feeding proportion, growth, and survival of red-spotted grouper larvae.

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