Suitable Photoperiod and Light Intensity of Laboratory-reared Juvenile Pacific Bluefin Tuna *Thunnus orientalis*

Yasunori Ishibashi¹,*, Tomoki Honr YO¹,², Shigeru Miyashita³ and Seiji Oda³

Abstract: Effect of photoperiod on survival rate of juvenile Pacific bluefin tuna (PBT) was investigated. The 24L (24 hours lighting) significantly improved the survival rate compared with the 12LD (12 hours lighting and 12 hours darkness) and 24D (24 hours darkness) tanks. Next, juvenile PBT were reared under 3 different 24 hours light intensities. Fish survival rates in the 1500 lx and 150 lx tanks were higher than in the 15 lx tank. These results suggest that a combination of 24 hours lighting and 150 lx or more light intensity would be effective for reducing the mortality of cultured juvenile PBT.

Key words: Pacific bluefin tuna; Fingerling production; Night-time lighting

The cage culture of the Pacific bluefin tuna (PBT), in which juvenile fish are captured in the wild and raised in enclosures, was first carried out in 1970 (Harada et al. 1971; Kumai 1997). However, the production of seedlings is laborious (Sawada et al. 2005) and it is still difficult to mass produce the required numbers. Problems encountered in mass production includes floating and sinking deaths of larvae, cannibalism of larvae by juveniles, and mass death by collision with tank walls (Miyashita 2002). In particular, the mass death of the juvenile PBT is of primary concern because they are difficult to produce.

The mass death of the PBT juveniles is caused by a number of factors such as collision (Miyashita 2002) or contact with the walls of land tank or sea net cage among others (Ishibashi 2012). High mortality is sometimes seen during net handling and transportation. The mass deaths also occur on a large scale in several days after the transportation of juveniles (Ishibashi et al. 2009). Other factors that are responsible for the mass mortality includes change of environment (Tsuda et al. 2012; Higuchi et al. 2013), outbreak of infectious diseases such as iridovirus (Sawada et al. 2005) and blood fluke (Shirakashi et al. 2012), loss of appetite, accidental ingestion of inorganic matter and others.

It was reported that the high mortality that occurs several days after transportation can be prevented by providing night-time lighting in sea net cages (Ishibashi et al. 2009). This is because the eye is considered the main sensory organ of tunas (Kawamura et al. 1981) and cultured PBT juvenile have low scotopic vision (Torisawa et al. 2007; Ishibashi et al. 2009; Matsumoto et al. 2009). However, it is necessary to examine the relationship between the photo environment and mass deaths in detail because daily mortality occurs at high frequency not only during night but also at daytime (Ishibashi 2012). In this study, effects of photoperiod (experiment I) and light intensity (experiment II) on the survival rate were investigated using laboratory juvenile PBT reared in small tanks.

The juvenile PBT used in both experiments were obtained from Kinki University Fish Nursery Center, Shirahama Station in 2003. All fish were fed daily with commercially available formulated diets for yellow tail (Otohime, Marubeni Shiryo Co., Ltd., Tokyo, Japan) at the opportune time in 20 kl concrete tank. To investigate the cause of mass deaths that occur at a constant daily rate during rearing, effects of photoperiod on survival rate were examined in Experiment I. A total of 120 juvenile fish (32 dph, TL 4.8 ± 0.6 cm, body weight 1.2 ± 0.4 g) were transferred using a small tank (200 l) from the 20 kl concrete tank to 3 × 3 kl blue fiber reinforced plastics tanks (40 fish per a tank).

The experimental design includes three tanks (n = 1), where the first tank received 12 hours of light and 12 hours of darkness (12LD), the second tank was illuminated for 24 hours (24L), and the third tank was in darkness for 24 hours (24D). Tanks were surrounded with shading sheets and a 14W fluorescent light (Compact fluorescent lamp, Panasonic Corporation, Osaka, Japan) was installed in the center.

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of the 24L and the 12LD tanks. In the tanks that received lighting the maximum light intensity at the water surface was 128.0 ± 5.3 lx. The lighting intensity under the un-lit phase in 24D and 12LD tanks was 0.2 ± 0.1 lx. Fish were moved to each experimental tank during daytime on the first day, and lighting in the 24L and the 12LD tanks were turned on before the movement. In the 12LD tank light was provided for 30 minutes at 0600 h and 1800 h using small lamp of about 10 lx to prevent possible mass death due to a rapid change in the photo environment. In the 24D tank, the natural light intensity gradually decreased at twilight on the first day of the experiment and then the darkness was kept for the whole experimental period. The fish were fed with formulated diet up to satiation five times a day at three-hour intervals. Sea water, sterilized using an ultraviolet sterilizer (Chiyoda Kohan Co., Ltd., Tokyo, Japan), was continuously supplied to the tanks. The median drainage pipe at the tank’s bottom is connected with the vertical drainpipe outside the tank, and the water level of tank is maintained by the overflow of the rearing water at vertical drainpipe. Half of the rearing water was gradually decreased by extracting the upper half of the vertical drainpipe and then placing the pipe back to its original place. These water exchange operation was performed twice a day. Dead fish were collected and counted on a daily basis. The survival rate was monitored over a period of seven days. The water temperature, pH, dissolved oxygen level, and salinity during rearing are shown in Table 1. There was no difference in these parameters across all rearing tanks. All of the data are expressed as mean ± SD. The survival data were plotted using Kaplan-Meier curves (Altinok 2004), and the survival of the different groups in experiment I were compared using the log-rank test. Kaplan-Meier analyses were performed using the Statistical Package for the Social Sciences (SPSS) program for Windows (version 15.0J).

In Experiment I, the survival rates of PBT juveniles under different photoperiods are shown in Fig. 1. In the 24D group, mass deaths occurred 2 days after the experiment started and only 11.3% of the juveniles remained by the third day. The number of dead fish in 12LD group also increased as the number of experimental days increased such that by the fourth day, a low survival rate of about 24.5% was observed.

In contrast, the survival rate of the 24L group was significantly higher than that of the 24D and 12LD groups (P<0.05, n=40).

To determine the appropriate luminance for the 24-hour lighting condition, the effect of three light intensities on survival rate of juvenile PBT in small

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Water temperature (°C)</th>
<th>pH</th>
<th>Dissolved oxygen (mg/l)</th>
<th>Salinity (psu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15lx</td>
<td>24.6 ± 0.5</td>
<td>8.1 ± 0.0</td>
<td>6.7 ± 0.1</td>
<td>34.8 ± 0.1</td>
</tr>
<tr>
<td>150lx</td>
<td>24.5 ± 0.5</td>
<td>8.1 ± 0.0</td>
<td>6.6 ± 0.1</td>
<td>34.8 ± 0.1</td>
</tr>
<tr>
<td>1500lx</td>
<td>24.5 ± 0.5</td>
<td>8.1 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>34.8 ± 0.1</td>
</tr>
</tbody>
</table>
tanks was examined in Experiment II. A total of 120 juvenile fish (40 dph, TL 6.7 ± 0.7 cm, body weight 3.1 ± 0.9 g), which were from the same cohort as those used in experiment I, were transferred to 3 × 3 kl FRP tanks (40 fish per a tank). Three tanks (n = 1) were exposed to the 24L conditions described in Experiment I above and the maximum lighting intensities at the water surface of each tank were set at 15 lx (13.7 ± 1.7 lx), 150 lx (146.4 ± 13.7 lx) and 1500 lx (1507 ± 101 lx), respectively. The same facilities and diet as in experiment I were used in this experiment. Dead fish were collected and counted on a daily basis. The survival rate was monitored over a period of seven days. The water temperature, pH, dissolved oxygen level, and salinity during rearing are shown in Table 2. There was no difference in these parameters across all rearing tanks. Statistical analyses were performed using the same methods and software in Experiment I above.

In Experiment II, the survival rates of PBT juveniles under different light intensities are shown in Fig. 2. On day 7, the survival rates in the medium (150 lx) and high (1500 lx) light intensity groups (41.8% and 56.0%, respectively) were significantly (P < 0.0001) higher than that in low (15 lx) light intensity group (4.1%). However, there was no significant difference (P > 0.05) in survival rates of juvenile PBT between 150 and 1500 lx light intensities. There was also no significant difference (P > 0.05) in the total length (TL) of juvenile PBT between 150 lx (8.0 ± 0.5 cm TL) and 1500 lx (8.2 ± 0.7 cm TL) light intensities.

Our results showed that most juvenile PBT died within 2–3 days when reared under 24 hours of darkness. Mass deaths still occurred when juveniles were reared under the 12L-D tank. On the contrary, providing the juveniles with 24-hour lighting resulted in superior survival rates. However, even under 24-hour lighting survival rate was extremely low at low light intensity (15 lx). The relationship between light intensity and survival rate during the 7-day periods in experiment I and II was calculated by mean of Broken-line regression analysis (Zeitoun et al. 1976) and the optimal light intensity is shown in Fig. 3. This analysis shows that the survival rate increased in direct proportion to an increase in light intensity from 0 to 150 lx (r = 0.860) after which a plateau was reached at about 150 lx and beyond (r = 0.048). The point of intersection of both approximation lines was 142 lx. Thus it was concluded that a lighting intensity of about 150 lx or more for 24 hours is suitable for successful rearing of PBT juveniles. These results also indicate that a 3 kl tank can be successfully used for juvenile PBT rearing experiments for short periods.

A number of previous studies provide evidence that continuous lighting promotes the growth of fish (Simensen et al. 2000; Trippel and Neil 2003; Biswas et al. 2005). However, most juvenile fish do not die within a few days under dark conditions in the same manner as cultured PBT juvenile. The reason why PBT juveniles suffer mass mortalities by contact, collision, feed intake reduction and other factors is revealed by this experiment. By means of the optomotor reaction, we found that poor scotopic vision was one of the major causes of mortalities in juvenile PBT (Ishibashi et al.)

![Fig. 2. Cumulative survival (Kaplan-Meier plot) of juvenile Thunnus Orientalis reared under different light intensities with 24 hours lighting for seven days. Different lowercase letters of the low light intensity (●, 15 lx), medium light intensity (▲, 150 lx) and high light intensity (□, 1500 lx) groups represent significantly different (Kaplan-Meier analysis, log-rank test; P < 0.0001, n = 40).](image)

![Fig. 3. Relationship between light intensity under 24 hours lighting and 7-day survival rate in juvenile Thunnus Orientalis. ○, Data from Experiment 1; ●, Data from Experiment 2. The arrow indicates the optimal light intensity derived with the broken-line regression.](image)
2009), in addition to school behavior (Torisawa et al. 2007) after this experiment in 2003. Moreover, the PBT juvenile has low visual temporal resolution compared with the chub mackerel, and low light sensitivity when compared with the striped jack. It has been suggested that the low level of scotopic vision is based on both low temporal resolution and low light sensitivity (Matsumoto et al. 2009). Since the mass deaths that occur immediately after transfer to sea net cages (Ishibashi et al. 2009) and the daily mortality in this experiment were reduced by continuous lighting, it is clear that one of the main reasons for these mortalities is poor scotopic vision in cultured PBT juveniles.

With the aid of retinomotor response experiments, (Masuma et al. 2001) concluded that the light intensity at which the transition from scotopic to photopic vision takes place in juvenile Pacific bluefin tuna was 7.52 lx. However, the survival rate of the juvenile PBT reared under a light intensity of 15 lx in this study was significantly lower than at 150 lx or more tanks. This may mean that even though retinomotor response occurs under the low light intensity (7.52 lx), visual function of PBT juvenile still is suboptimal. This may point to the reduced sensitivity of not only their rod cells but also cone cells (Ishibashi 2012). The interesting characteristic of cultured PBT juvenile revealed in our study is that the optimal light intensity for survival rate is high at around 150 lx or more. However, the turbidity differs depending on the culture sea area. This light intensity should be used as a reference for futures studies in which cultured and wild PBT and other tunas are cultured in industrial scale, such in sea net cages. Even under 24-hour lighting at 150 lx or more, survival rate for the seven-day period was around 60% and the dead fish had skin lesions. Thus, there is still need to develop additional strategies to reduce mass death.

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