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# Effects of tank wall pattern on survival, bone injury rate, and stress response of juvenile Pacific bluefin tuna, *Thunnus orientalis*

Yasunori Ishibashi[a,*], Tatsuya Izumi[a], Michio Kurata[b], Tokihiko Okada[b]

[a] *Department of Fisheries, Faculty of Agriculture, Kinki University, Nara 631-8505, Japan*
[b] *Ohshima Station, Fish Nursery Center, Kinki University, Ohshima, Kushimoto, Wakayama 649-3633, Japan*

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ABSTRACT

Juvenile Pacific bluefin tuna (PBT), *Thunnus orientalis*, aged 30 or more days post hatching ($\geq$5 cm total length) are at high risk of mortality through contact or collision with tank walls. In this study, survival rates of PBT reared under three tank wall treatments (polka dot pattern, lattice pattern, and dark-green single color (control) were investigated. White tape was attached to the tank's surface to form either a polka dot or a lattice pattern. Fish were then reared in the tanks for 9 days. Fish survival rates in the lattice patterned and polka-dot patterned tanks were higher than that in the control tank. Bone and parasphenoid injury rates in the control group were significantly higher than those in the polka dot and lattice patterned treatment groups. The plasma cortisol content of juvenile PBT reared in the control group was higher than for fish reared in patterned tanks. These results suggest that juvenile PBT mortality from contact or collision with tank walls was reduced when wall visibility was improved by creating patterns on the tank walls.

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## 1. Introduction

Pacific bluefin tuna (PBT) (*Thunnus orientalis*) stocks have been reduced by global overfishing (Safina, 2001; Williams, 2007). Tuna cage culture (Harada et al., 1971; Kumai, 1997; Miyashita, 2002) has become more widespread worldwide (Yoshikawa and Honma, 1980), thus increasing pressure on wild fish resources because it depends on wild seed fish. Kinki University successfully developed a full-cycle culture of PBT (Sawada et al., 2005). However, the industrial mass production of PBT fingerling is constrained by mass deaths at various stages of development. The mass death of PBT fingerling can be classified into surface and sinking deaths (observed up to around 8 days post-hatching (dph), ~0.5 cm total length (TL)) (Kurata et al., in press; Takashi et al., 2006), cannibalism (observed in the larval stage from around 10 dph, $\geq$0.8 cm TL) (Ishibashi et al., in press; Sawada et al., 2005), and mass death of juveniles observed from around 30 dph ($\geq$5 cm TL) (Ishibashi et al., 2009). Mass death in the juvenile stage can be further classified into contact or collision deaths (Ishibashi, 2010), which occur at a roughly fixed rate depending on the size of the tank or sea net cage; skin injury caused by friction against the net during transport; and mass death caused by sudden environmental changes within a few days of fish being transferred from tanks to sea net cages (Ishibashi, 2010).

Contact or collision with the tank or cage is one of the primary causes of death in juvenile fish. Recently, it was reported that the high mortality by contact or collision can be prevented by means of night-time lighting of sea net cages (Ishibashi et al., 2009) and land tanks (Honryo et al., in press; Ishibashi, 2006). It has also been found that the scotopic visual threshold of cultured PBT juveniles for optomotor reactions was at least 40 fold inferior to the threshold of 4 marine teleosts, grouper (*Epinephelus septemfasciatus*), purplish amberjack (*Seriola dumerili*), ocellate puffer (*Takifugu rubripes*) and red sea bream (*Pagrus major*) (Ishibashi et al., 2009). Moreover, we reported that cultured PBT juveniles have low temporal resolution and light sensitivity compared with juvenile chub mackerel (*Scomber japonicus*) and striped jack (*Pseudocaranx dentex*) (Matsumoto et al., 2009). Ethological investigations on the relationship between light intensity and fish behavior have also been carried out (Torisawa et al., 2007a). This poor scotopic vision is one of the major causes of nighttime high mortality rates from collisions and/or contact with tank walls in cultured juvenile PBT.

Collision death of juvenile PBT is also reported to occur during the day (Honryo et al., in press) at higher light intensities. It has been reported that the caudal fin, which is the driving force that propels the fish forward, develops at the early juvenile stage, while the pectoral and ventral fins, which control swimming, remain undeveloped (Miyashita, 2002). Therefore, the high swimming speeds

*Abbreviations:* PBT, Pacific bluefin tuna; dph, days post hatching; TL, total length; BW, body weight; DO, dissolved oxygen; EIA, enzyme immunoassay; HRP, horseradish peroxidase.

* Corresponding author at: Department of Fisheries, Faculty of Agriculture, Kinki University, Nakamachi, Nara 631-8505, Japan. Tel.: +81 742 43 6305; fax: +81 742 43 1316.
*E-mail address:* isibasi@nara.kindai.ac.jp (Y. Ishibashi).

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of early juveniles (30 dph) make collision or contact deaths more likely. The eye is also considered the main sense organ of tunas (Kawamura et al., 1981). Therefore, collision or contact with tank walls might be prevented by making the wall more visible during daytime. In the present study, we attached white tape to the dark green tank wall in a polka-dot or lattice pattern and compared the survival rate, and frequency of bone injuries in PBT.

On the other hand, severe environmental stressors can induce significant stress responses in fish, including increases in cortisol and glucose levels. Significant increases in cortisol were also demonstrated in juvenile PBT reared without night-time lighting (Honryo et al., in press; Ishibashi et al., 2009). For this reason, changes in plasma cortisol and glucose levels were determined to be indicators of response to environmental stress in juvenile PBT.

## 2. Materials and methods

### 2.1. Fish

One thousand and nine hundred juvenile PBT were hatched from eggs that were spawned naturally at Kinki University Fisheries Laboratory from PBT broodstock and reared in a dark green fiber reinforced plastics circular tank (6 m internal diameter, 1.1 m depth and 30 $m^3$ volume). Juvenile PBT aged 31 dph, with a mean TL of 5.8 ± 0.4 cm, and body weight (BW) of 1.7 ± 0.2 g, were randomly distributed into 6 tanks (310 juveniles per tank) of a same background color and shape.

### 2.2. Experimental design and rearing

Three experimental groups related to wall pattern were designed in duplicate: untreated group (dark green single-color) as the control, polka-dot pattern group, and lattice pattern group (Fig. 1). The polka-dots were made out of white circular seals of 5 cm radius attached at 30 cm intervals in various directions on the tank wall. The lattice pattern was established through 30 cm intervals of vertical and horizontal lines, with the width of the white line seal maintained at 5 cm. Natural sunlight was supplied through the overhead shade sheet (about 90% of the sunlight was cut off and 10% was allowed to illuminate the tank) during the daytime (0500–1830 h). The maximum lighting intensity at the water surface at noon was about 12,400 lx. The fish were fed chopped Japanese sand lance (*Ammodytes personatus*) to satiation every 10–60 min for 9 days. Natural sea water, pumped into the front of the station and sterilized by ultraviolet rays was supplied to the tanks continuously. Half of the rearing water was exchanged 3 times a day. The water in the tanks was maintained at a temperature of 26.2 ± 2.2 °C and 99.0 ± 2.2% dissolved oxygen (DO) during the experiment.

### 2.3. Sampling and analysis methods

Fish behavior in all experimental tanks was observed and recorded during daytime. Dead fish were picked up at 0500, 1200, and 1830 h daily, and survival rates were calculated. Dead fish collected on the 7th to 9th day were further assessed for bone injuries through X-ray imaging (HB-50, HITEX Co., Ltd., Osaka, Japan, settings: 30 kV, 50 mA, 50 s).

With the aid of polyethylene hand-nets (2 mm mesh size), 6 fish were sampled from each experimental tank on the morning of the 9th day to assess stress levels. Blood samples were obtained from the caudal vessel using 2 mL heparinized syringes within 1 min of capture. The blood was centrifuged immediately and the plasma frozen in liquid nitrogen within a few seconds. The plasma samples were then preserved at −75 °C until required for the analyses of cortisol and glucose concentrations. After blood withdrawal, the sampled fish were anesthetized and killed within a few minutes using iced physiological saline.

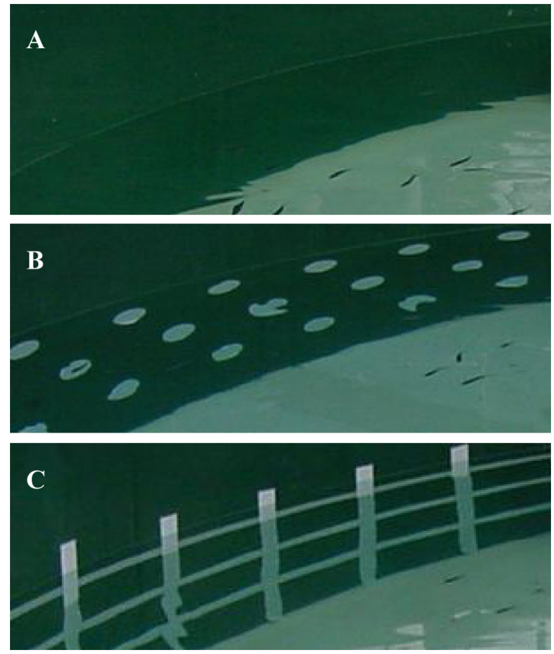


**Fig. 1.** Three types of tank walls used in the study: (A) control (dark-green single color); (B) lattice-patterned tank; (C) polka-dot patterned tank. The polka-dots were made out of white circular seals of 5-cm radius attached at 30-cm intervals in various directions on the dark-green tank wall. The lattice pattern was established through 30-cm intervals of vertical and horizontal lines, with the width of the white line seal maintained at 5 cm. Juvenile PBT aged 31 dph, with a mean body length of 5.3 ± 0.3 cm is shown in the images. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Ether-soluble matter was extracted from the plasma. Impurities were then removed using carbon tetrachloride according to Hiroi et al. (1997). Plasma cortisol levels were measured using the enzyme immunoassay (EIA) described by Asahina et al. (1995) with some modifications (Ishibashi et al., 2009). Anti-rabbit cortisol-3-carboxymethoxime bovine serum albumin (cortisol-3-CMO-BSA), IgG (FKA404E; Cosmo Bio Co. Ltd., Tokyo, Japan), goat anti-rabbit IgG (Cappel Research Reagents, ICN, Temecula, CA) as the second antibody, and cortisol-3-CMO-horseradish peroxidase (HRP) (FKA403; Cosmo Bio Co., Ltd., Tokyo, Japan) as the antigen, labeled by HRP were used for the EIA. The reacted solution was colored using the o-phenylenediamine kit EIA (Nacalai Tesque, Inc., Tokyo, Japan), and the absorbance at 450 nm was determined using a microplate reader (Model 550; Bio-Rad Laboratories, Inc., Richmond, CA, USA).

The plasma glucose concentration was measured using the glucose CII test kit (Wako Pure Chemical Industries Ltd., Osaka, Japan). Absorbance at 490 nm was determined using a microplate reader (Model 550; Bio-Rad Laboratories, Inc., Richmond, CA).

### 2.4. Statistical analysis

The survival rates across the treatment groups were compared by means of log-rank tests (Altinok, 2004) and displayed using Kaplan–Meier curves. One-way repeated measures ANOVA was used to compare all other data for the three treatments. For the cortisol concentration ($n = 12$) and parasphenoid fracture ($n = 6$) datasets, the Games–Howell post hoc test was used, as the assumption of equal variance was not met, whereas the glucose concentration ($n = 10$), bone injury ($n = 6$) and vertebra dislocation data ($n = 6$) were analyzed with the Tukey's HSD or Bonferroni test, as the transformations had equal variance. All statistical analyses

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