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Prevalence of collision death in 2-year-old Pacific bluefin tuna, *Thunnus orientalis*, reared in land-based tanks for broodstock management

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ABSTRACT

The causes of Pacific bluefin tuna (PBT) mortality must be identified to increase survival rate in land-based tanks. One of the suggested causes of death is impact against the wall of a fish tank. However, little is known about the prevalence of this type of collision death in land-based tanks, except in juveniles. We investigated deaths of 2-year-old fish to estimate the prevalence of collision death in land-based tanks. Two-year-old fish were reared in land-based tanks for 182 days after transfer, and dead fish were examined by dissection and/or X-ray to detect injuries to the skin, vertebral column, and skull. The prevalence of these injuries was considerably high (91%), but no symptoms of bacterial, viral, or parasitic disease were observed. In addition, the condition factor of the dead fish was within that of surviving PBT in sea net pens, suggesting that the fish did not die from starvation. These results indicate that collision with the tank wall was the primary cause of PBT mortality in land-based tanks. Collision death occurred frequently from dusk to dawn and immediately after transfer. Thus, limited vision and the environmental change after transfer were responsible for collision death in 2-year-old PBT. Our results suggest that the collision death rate of 2-year-old PBT could be improved by increasing light intensity during night just after transfer.

Statement of relevance: Broodstock management practices of Pacific bluefin tuna need to be developed in land-based tanks to provide a stable supply of the eggs for the tuna farming industry. However, the survival rates are low in land-based tanks. Our study identified the primary cause of the mortality. We believe that this finding is a valuable first step to improve the survival rates.

1. Introduction

Commercial bluefin tuna aquaculture is increasing worldwide, particularly in Europe, Australia, Mexico, and Japan. Most bluefin tuna aquaculture businesses depend on wild-caught juveniles for seed, and negative effects of this on the management of wild tuna stocks have been reported (Kumai, 1998; Miyake et al., 2003; Masuma et al., 2008, 2011). A stable supply of artificially reared tuna must be established to sustain the tuna farming industry and preserve wild tuna stocks. Research on broodstock management and larviculture of Pacific bluefin tuna, *Thunnus orientalis* (PBT), has been conducted in Japan since 1970. In 2002, Kinki University succeeded in closing the full PBT life cycle in captivity and also achieved the full aquaculture life cycle of this species (Sawada et al., 2005).

Although nine successful PBT broodstock spawning sites have been established in sea net pens, spawning to produce larvae remains unstable (Kumai, 1998; Masuma et al., 2008, 2011). For example, the annual number of eggs collected has varied from 10,000 to 500 million at the Amami Island site (Masuma et al., 2008), where spawning has been stable compared with that at other sites. One factor suggested to affect the number of eggs based on empirical data is natural environmental variation, such as ambient temperature (Miyashita et al., 2000a; Masuma et al., 2008). Therefore, broodstock management practices need to be developed in land-based tanks where water temperature and photoperiod can be manipulated to provide a stable supply of PBT eggs (Masuma et al., 2011; Yazawa et al., 2011).

Several attempts have been made to maintain PBT in land-based tanks at research facilities and aquaria (Farwell, 2001; Yazawa et al., 2011). Although the Tokyo Sea Life Park succeeded in spontaneous spawning of PBT in a land-based tank in 1999 (Mimori et al., 2008), the survival rates were considerably lower compared with those observed in sea net pens. Mimori et al. (2008) reported that only 39.1% of PBT survived 1 year after capture, and survival decreased to 9.3% after 3 years and to 1.3% after 5 years in the land-based tank. Therefore, improving survival rates is critical for developing PBT broodstock in land-based tanks.

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It is important to identify the causes of mortality to improve survival of PBT reared in land-based tanks. One of the main causes for juvenile mortality is impact against the wall of the tank because vertebral or parasphenoid fractures have been discovered in many dead fish that had been reared in land-based tanks (Miyashita et al., 2000b; Ishibashi et al., 2013). Death by collision with the tank wall may occur frequently in land-based tanks compared with that in sea net pens because the wall is made of a solid material, such as concrete or acrylic resin. However, little is known about the prevalence of PBT collision-related death in land-based tanks, except in juveniles.

The Japanese Fisheries Research Agency (FRA) has been developing PBT broodstock management technology in land-based tanks since 2013. To estimate the prevalence of collision-related death in land-based tanks, we examined injuries to the skin, vertebral column, and skull, which would be caused by a strong physical impact, in dead 2-year-old fish (total length [*TL*], ca. 100 cm). We documented the proportion of collision-related death, the time when collision death was likely to occur, and the characteristics of the dead PBT. Based on our observations, we identified characteristics of the mortalities in land-based tanks and discuss preventive measures against collision death.

2. Materials and methods

The PBT used in this study were reared from fertilized eggs for 2 years at Amami Laboratory, Seikai National Fisheries Research Institute (SNFRI) of the FRA, Kagoshima, Japan. We transported 42, 64, and 20 individuals on 22 May, 7 June, and 20 June 2013, respectively, to two land-based tanks (diameter, 20 m; depth, 6 m; volume, 1880 m^3) at the Nagasaki headquarters of the research institute (Table 1). We measured *TL* of each fish using a tape measure and then implanted a passive integrated transponder (PIT) (Biomark, Boise, ID, USA) to identify individuals before they were released into the tanks. The *TL* of the 2-year-old fish was 99.4 ± 7.8 cm (mean ± standard deviation [SD], $n = 81$). Sixty-three fish were stocked and reared in each tank.

The land-based tanks were made of concrete, and the walls were marked with vertical lines running from top to bottom. The color of the wall and vertical line were orange and black, respectively. The lines were spaced ca. 40 cm and were ca. 10 cm wide. The purpose of the striping was to provide a visual reference for the tuna by marking the tank perimeter (Farwell, 2001; Yazawa et al., 2011; Ishibashi et al., 2013). Each tank received filtered, UV-treated seawater (560 m^3/day). The water was partially recirculated through mechanical and biological filters. Water temperature was maintained at 19.4–28.5 °C with heating and cooling systems (Fig. 1). Each tank was lit with 112 light-emitting diode (LED) arrays placed above the tank and only 16 LED arrays near the tank wall during day and night, respectively. Light intensity at the water surface near the tank wall was approximately 600 and 4 lx during the day and night, respectively. The LED arrays were controlled with a timer that turned the lights on and off sequentially (for 2 h). This allowed for subtle changes in light intensity at dawn and dusk to avoid startling fish with a sudden change in light (Farwell, 2001). In this study, daytime was called the "light period", and dawn (for 2 h), dusk (2 h), and night were collectively called the "dark period". The mean durations of the light and dark periods were 9.3 and 14.7 h, respectively. We initially fed the broodstock sand lance, *Ammodytes personatus*, followed by chub mackerel, *Scomber japonicas*, Japanese flying squid, *Todarodes pacificus*, and a formulated feed (Tunakko, Hayashikane Sangyo Co. Ltd., Shimonoseki, Japan). We measured ammonia (tank 1, 0.03 ± 0.05 mg/l; tank 2, 0.03 ± 0.04 mg/l), nitrite (tank 1, 0.01 ± 0.02 mg/l; tank 2, 0.01 ± 0.01 mg/l), and nitrate (tank 1, 0.49 ± 0.31 mg/l; tank 2, 0.40 ± 0.29 mg/l) three times per week. The rearing conditions of the two tanks were basically the same.

Table 1
Number of Pacific bluefin tuna (PBT) transported to the two land-based tanks.

Date	Number of transported PBT	
	Tank 1	Tank 2
22 May 2013	32	10
7 June 2013	31	33
20 June 2013	0	20
Total	63	63

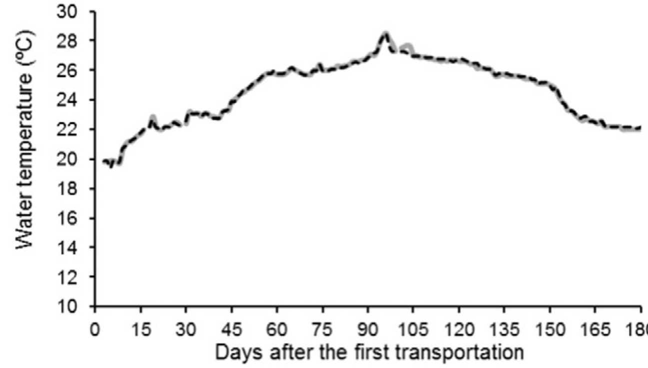

Fig. 1. Water temperatures in the two land-based tanks (*gray* and *black broken lines*).

The PBT were observed in the tanks from 22 May to 20 November 2013 (182 days), and the number of dead PBT was counted and recorded. The dead fish were sampled, and their PIT tags were checked with a scanner (Biomark 601 reader). We recorded injuries to the skin and excised the gonads to determine sex. The gonads were fixed overnight in Bouin's solution and preserved in 70% ethanol for further histological examination. Dead fish with no skin injuries were checked for injuries to the vertebral column and skull, such as the parasphenoid, by dissection and/or X-ray (CMBW-2; Softex, Tokyo, Japan). The injuries to all areas (skin, vertebral column, and skull) of the 20 dead fish were examined to reveal the frequencies of the areas injured. As skin, vertebral column, and skull injuries were caused by a strong physical impact, these injuries were collectively called "collision injuries" in this study. Mortality was relatively high 1 day after transport until day 46 and from days 84 to 152, whereas the number of mortalities decreased during days 42–83 and days 153–181 (Fig. 2). The prevalence of collision injuries during each period was calculated as follows to examine the change in collision-related injuries in dead fish during each of the four periods with different mortality rates:

$$\text{Prevalence of collision injury }(\%) = (\text{number of dead PBT with collision injuries during each period} / \text{number of all dead PBT during each period}) \times 100.$$

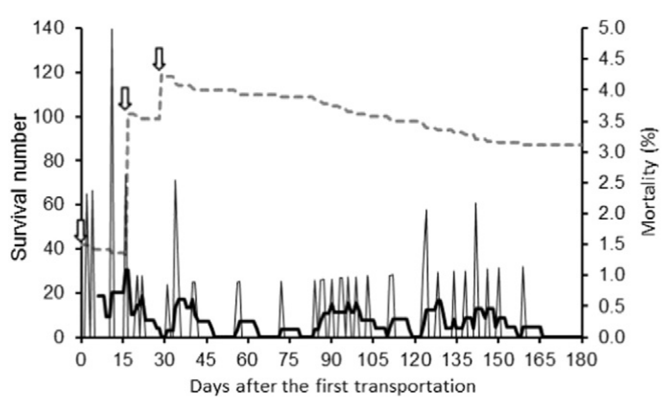

Fig. 2. Number of Pacific bluefin tuna (PBT) surviving (*bold dotted line*) and daily mortality (*thin line*) with a 7-day running average (*bold line*). *Arrows* show transportation and tank stocking of the PBT.