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Effect of environmental fluctuations on mortality of juvenile Pacific bluefin tuna, *Thunnus orientalis*, in closed life-cycle aquaculture

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A B S T R A C T

Although full-cycle aquaculture of Pacific bluefin tuna, *Thunnus orientalis*, has been established by Kinki University, Japan, most juvenile die after transfer to net cages offshore due to the stress associated with fluctuations in environmental conditions. We examined the effect of environmental conditions on mortality of juvenile using a generalized liner model (GLM). Juveniles were reared for more than 300 days in a net cage and number of dead fish was examined over time and in relation to water temperature, salinity, moon phase, and water transparency. Mortalities soon after transferring the fish from the indoor rearing tank to the net cage occurred independently of environmental factors and were considered to be due to the handling stress and lack of acclimation. In subsequent periods, fluctuations in water temperature were the most critical factor affecting juvenile survival. Our results showed that optimal water temperatures for juvenile bluefin tuna aquaculture are considered to range between 15 and 25 °C. Salinity and lunar irradiance (half moon to full moon) fluctuations had less marked effects on juvenile survival. Juveniles in net cages cannot move to avoid those unfavorable environmental fluctuations and this inability to locate areas of optimal temperature may complicate their body temperature maintenance because the capacity for endothermy in juveniles is undeveloped. We propose that sites selected for culturing juvenile Pacific bluefin tuna have water temperatures ranging from 15 to 25 °C with constant temperatures that avoid large fluctuations.

1. Introduction

Commercial aquaculture of bluefin tuna is increasing in all over the world, particularly in Europe, Australia, Mexico and Japan. Since most of these operations rely on wild-caught juveniles for seedlings or wild-caught adults, a decrease in natural stocks is a concern due to the high fishing pressure in the ocean. There is thus a need to develop and optimize full-cycle aquaculture of bluefin tuna, as such a method does not rely on wild-caught juveniles. In 2002, the Fisheries Laboratory at Kinki University (FLKU) succeeded in the life-cycle of the Pacific bluefin tuna, *Thunnus orientalis* (Sawada et al., 2005). Despite the success of subsequent rearing trials, numerous issues need to be resolved before bluefin tuna can be achieved on a commercial-scale. The most significant of these challenges is how to decrease larval and juvenile mortality. While recent improvements have decreased the high mortalities previously associated with the larval stage, problems affecting the juvenile stage are still severe (Ishibashi et al., 2009).

In full-cycle aquaculture, bluefin tuna juveniles are transferred from indoor rearing tanks to offshore net cages after reaching 5 cm in total length (juvenile stage, approximately 30 days after hatching). It is at this stage in the production cycle that particularly high mortalities are routinely reported (Sawada et al., 2005), with fluctuations in environmental conditions at the aquaculture site and nutrition problems considered to be the most serious. In addition, the stress associated with the handling of juveniles when they are transferred to the net cage also contributes to high mortalities (Ishibashi et al., 2009). In the first 1 to 2 weeks in the net cage, a major cause of juvenile mortality is due to collisions with the sides of the net at dusk and dawn; the juveniles do not appear to register that the net cage differs in size compared to the indoor tank, and in the approximately 2 weeks that it takes to acclimatize to the net environment, they frequently collide with the sides of the net. Even after this initial period of acclimatization, accidental deaths due to fluctuations in the environment are frequently encountered (Sawada et al., 2005). Indeed, this sensitivity of bluefin tuna juveniles to fluctuations in environmental conditions is characteristic feature of the juvenile stage (Block and Stevens, 2001; Brill, 1994). In addition, the lights of fishing boats near the aquaculture site may increase mortality considerably as the fish panic and swim into the sides of the net cages (Ishibashi et al., 2009; Masuma et al., 2001; Miyashita et al., 2000; Sawada et al., 2005).

Interestingly, few investigations have been conducted on the relationship between fluctuations in oceanic environmental conditions and the mortality of juvenile bluefin tuna reared in nets offshore. We therefore quantitatively investigated the relationship between daily mortality in juvenile bluefin tuna reared for 300 days in net cages and four environmental factors: water temperature, salinity,

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moon phase, and water transparency. The resulting data were applied to a generalized liner model (GLM) to clarify the effect of environmental factors on bluefin tuna mortality.

2. Materials and methods

2.1. Culturing of juvenile bluefin tuna

Full-cycled culturing Pacific bluefin tuna, *Thunnus orientalis* eggs were obtained from the Amami Experimental Branch of the Fisheries Laboratory at Kinki University and transported by air to the Uragami Experimental Branch where they were reared for 33 days after hatching on 15 July 2009. A total 3320 juveniles were then transferred to the Shirahama Branch on 18 August 2009 where they were fed S-type rotifers (*Brachionus plicatilis* sp. complex, Shizuoka strain), *Artemia franciscana* nauplii, and newly hatched parrotfish Lethrinus *nebulosus* larvae before being transferred to the offshore net cages. We divided and stocked 1245 juveniles into two offshore net cages (12 m × 12 m × 4 m depth) in Tanabe Bay in Wakayama Prefecture, Japan, on 31 August 2009. The number of juveniles in each group (Groups A and B) was 625 in Group A and 620 in Group B, with the average size and weight of each juvenile being 10.0 cm of total length and 10.5 g, respectively (Table 1). For the first 42 days, the fish in both groups were fed artificial tuna pellets according to their mouth size (Marubeni Nisshin Feed Co., Ltd., Tokyo). Thereafter, Group B fish was fed Pacific sand lance, *Ammodytes personatus*, while Group A was fed artificial tuna pellets until the end of the experiment. The night time mortalities of juveniles in the net cage are degreased using an artificial lighting during night time (Ishibashi et al., 2009). Light by artificial LED light (1 m of length, 10 cm of wide, 1wt LED) during night time were exposed from 30 cm above water surface in each net cage from dawn to dusk in time with season during experiments.

2.2. Data collection, statistical pre-treatment and modeling

After collecting the dead fish from each group at 11:00, the body length and weight of each fish was measured and the mortality recorded. In addition, the water temperature below 3 m, salinity in the surface layer, and water transparency were recorded adjacent to the net cage. To clarify the change in the lunar irradiance, the phase of the moon (0–30) was converted to a numerical value of 0 to 15 (from new moon to a full moon), and 15 to 0 for full moon to a crescent. Water transparency was determined using a Secchi disk.

In the area around the aquaculture nets, daily and seasonal fluctuations in water temperature were recorded; these fluctuations included long-term, seasonal fluctuations and short-term fluctuations arising from semi- and diurnal changes in tides. Statistical pre-treatment was performed on the water temperature data to estimate the inflection points and to clarify seasonal trends in water temperature and its effect on mortality. The best-fitting second-degree polynomial equation was applied to find inflection points of the water temperature fluctuation $F(T_{w)}$ corresponding to mortality before analysis:

$$F(T_w) = 0.0005\,(\text{date}-150.5000)^2 + 0.0175 \cdot \text{date} + 19.2088\,\left(R^2 = 0.8949, P < 0.001\right)$$

where *date* is the number of days that have elapsed after the tuna were transferred to the net cages offshore. The first stage (S1) of the analysis was taken as the initial 2-week period after the fish were transferred, when mortality was consistently >10 fish deaths per day (1 to 14 September 2009). The second stage (S2) was taken as the period after the initial 2 weeks to the day when the average water temperature exceeded 20 °C (15 September 2009 to 20 November 2009). The third stage (S3) was taken as the time when water temperature dropped below 20 °C to the lowest temperature recorded (21 November 2009 to 17 February 2010). The fourth stage (S4) was taken as the period from 18 February 2010 to the end of the experiment on 27 June 2010. Daily temperature fluctuations were related to mortalities in each period.

Since tuna mortality usually manifested approximately one day after the fish actually died, total daily mortality in both groups was compared with fluctuations in environmental parameters occurring the previous day. We fitted our dataset to a generalized liner model (GLM) to identify the independent variable affecting the daily mortality of juvenile bluefin tuna in each stage. The model was constructed using the statistical analysis program R (version 2.10.1; R Development Core Team, 2009). Four independent environmental variables were considered for inclusion in the model: temperature (T_w), salinity, water transparency, and phase of the moon. The phase of the moon was included to determine the effect, if any, of background irradiance on tuna behavior at night. We applied a negative binomial generalized linear model to estimate the parameters fitted to the *glm.nb* function in R.

$$\log(\text{DailyMortality}) = a_0 + a_1\text{Temperature} + a_2\text{Salinity} + a_3\text{Transperancy} + a_4\text{Moon}$$

where a_i ($i = 0$ to 4) is the regression coefficient; if a_i is high then the correlation is strong. The coefficient can have a positive or negative value.

Result

3.1. Seasonal changes in survival rate

Time series data of the survival rate of each group, T_w, salinity, transparency, phase of the moon and daily mortality are shown in Fig. 1. The mortality in each phase is shown in Table 2. In the S1 stage, mortality was 218 individuals for the 2 weeks after transferring of the juveniles to the net cage offshore. Mortality then decreased over the S2 period (15 to 20 November 2009). Highest mortality was recorded in the mid-S3 period when a rapid decrease was observed in T_w, and total mortality in this period was 152 (Group A: 57, Group B: 95). Especially, from 1 to 7 February. A marked

Table 1
Summary of experimental conditions for the culture of Pacific bluefin tuna, *Thunnus orientalis*.

Group	Date of hatching	Date of transfer to net cage	Duration of experiment (days)	Number of fish	Number of fish remaining at 300 days	300 days Survival rate (%)	Size at transfer to net cages		Type of food*
							Total length (cm)	Body weight (g)	
A	27 Jun. 2009	31 Aug. 2009	300	625	104	16.6	10.0	10.5	Pellet
B				620	22	3.5			Sand lance
Total				1245	126	10.1			

*Fish in both net cages were fed the artificial tuna pellets for 42 days. After 42 days, the fish in Group B were fed Pacific sand lance, *Ammodytes personatus*, while the fish in Group A were kept on the artificial tuna pellet diet.