Preliminary Observations on the Development of Aggressive Behavior in Pacific Bluefin Tuna, *Thunnus orientalis*

Francisco de la Serna Sabate¹, Yoshitaka Sakakura²,*, Takayuki Takebe³, Hideki Nikaido³, Naouki Matsumoto¹, Satoshi Shiozawa³, Atsushi Hagiwara¹ and Shukei Masuma⁴

Abstract: We investigated the optimal conditions for behavioral observation of Pacific bluefin tuna *Thunnus orientalis* and observed behavioral development with special reference to aggressive behavior. Different colored tanks and light intensities were tested against survival in 10-h period. While low survivals (0 – 10%) were observed in dark colored containers and high light intensity (>900 lx), clear tanks and medium light intensity (600 lx) had high survival (60 – 80%). With latter conditions, preliminary observations on the behavioral development of swimming behavior (swimming speed) and aggressive behavior (chase frequency) were performed at different developmental stages; mouth opening (2 days after hatching, DAH), pre-flexion (3 DAH), flexion (13 DAH), post-flexion (18 DAH) and juvenile (19 DAH) using different batches. Mouth opening was at 2 DAH (standard length, *SL* mean ± SD, 2.9 ± 0.1 mm) and juveniles first appeared at 19 DAH (*SL* 6.3 mm). Frequency of chase behavior in relation to feeding was observed for 36 DAH juveniles. Swimming speed maintained constant values from hatch until the flexion stage (3.7 ± 3.9 – 9.5 ± 14.3 mm/s). Aggressive fish first appeared on 16 DAH (*SL* 5.4 ± 0.9 mm, flexion stage) and frequency of chase behavior did not change until the juvenile stage. Chase behavior was more frequent when juveniles were starved.

Key words: *Thunnus orientalis*; Aggressive behavior; Swimming behavior; Ontogeny

Pacific bluefin tuna, *Thunnus orientalis*, is a commercially important species that has become target species for aquaculture in Japan (Nakagawa et al. 2007). The Japanese Fisheries Agency, Kinki University, and National Center for Stock Enhancement (formerly Japan Sea-Farming Association) started a project to develop rearing techniques for bluefin tuna larvae for future enhancement trials (Kaji et al 1996; Miyashita 2002). Rearing beyond post-flexion was first successful in 1979 (Harada 1980) and bluefin tuna was successfully cultured through its complete life cycle in 2002 (Sawada et al. 2005). However, mass production of bluefin tuna larvae still fluctuates due to several unsolved problems, such as initial mortality up to 10 days after hatching (60 – 90% mortality; Kumai 1998; Miyashita 2002), aggressive behavior and frequent collision with the rearing tank after the juvenile stage (Miyashita 2002). Miyashita (2002) pointed out that aggressive behavior, including cannibalism, during the juvenile stage as one of the major causes of mortality.

Many studies have reported the early development of this species such as morphological development (Miyashita et al. 2001; Sawada 2006), morphogenesis of sense organs (Kawamura et al. 2003), and feed selectivity on...
rotifers (Sawada et al. 2000). However, no study has been performed on the behavioral development of this species. In this study, we aimed to establish the optimal experimental conditions to monitor behavioral development and obtain preliminary data of swimming speed and aggressive behavior of Pacific bluefin tuna.

**Materials and Methods**

**Rearing conditions**

Naturally spawned eggs from 3 years old broodstocks in a floating net cage at the Amami Fisheries Experimental Station were collected by plankton net between June and August of 2007. Eggs were maintained in 500-l tanks and newly hatched larvae were transferred into 50-kl rearing tanks. Three batches of eggs were selected for this experiment and reared in the same conditions: batch 1, hatched on 12-June 2007; batch 2, hatched on 28-June 2007; and batch 3, hatched on 13-July 2007. Seawater was filled from the bottom of the rearing tanks at a daily rate of 150% to prevent bottom death (Tezuka et al. 2005). Water temperature ranged from 26°C to 29°C and light condition was natural.

Fish were fed with S-type rotifers (*Brachionus plicatilis* sp. complex, Shizuoka strain) or L-type rotifer (*B. plicatilis* sp. complex, Kindai strain) from mouth opening (2–3 days after hatching, DAH) to 22 DAH, nutritionally enriched (Sujiko Nyukayu, Nisshin Marine Tech Co., Yokohama, Japan) *Artemia franciscana* nauplii between 17 DAH and 24 DAH, newly hatched parrotfish, *Lethrinus nebulosus*, larvae between 17 DAH and 29 DAH, and minced sand eel, *Ammodytes personatus*, between 22 DAH and 35 DAH.

**Investigation of optimal conditions for behavioral observations**

In order to assess the optimal conditions to perform behavioral observations of Pacific bluefin tuna, two experiments were carried out to determine the optimal light intensity and color of observation container.

For the light intensity experiment, five 14 DAH larvae from the first batch were distributed at 5:30 A.M. into three 1-l crystal beaker (11 cm diameter) and each container was set at the different light conditions, either high (> 900 lx), medium (50–350 lx) and low (< 50 lx). Survived fish were counted every two h from 6:00 to 19:00 in each beaker. Meanwhile, light intensity at which larvae formed patchiness at the rearing tank was measured.

To determine the optimal container color for behavioral observation, survival of 9 DAH larvae from batch 2 was compared when transferred from the rearing tank into either one of six different containers: transparent glass beaker; and five different colored plastic containers (green, white, gray, blue and black). Five larvae were transferred into each container at 8:30. Remaining fish were counted every two h from 9:00 until 19:00.

**Behavioral observations**

In order to quantify the behavioral observations the following indexes were defined: swimming speed (in mm/s; Fukuhara 1986) and relative swimming speed (in standard lengths/s, *SL*/s); chasing behavior. We defined chase as an index of aggressive behavior, because we could clearly observe that the fish showed aggression towards another fish. To measure swimming speed and relative swimming speed, video recordings of the fish behavior from the second (0, 11 and 16 DAH) and third batch (13 and 15 DAH) and all hatched parrotfish, *Lethrinus nebulosus*, larvae between 17 DAH and 29 DAH, and miniced sand eel, *Ammodytes personatus*, between 22 DAH and 35 DAH.
DIPP Motion Pro Version 2.0 (DITECT Co., Tokyo, Japan).

Chase behavior was directly observed for fish from the second (2, 16, 17 and 19 DAH) and third batch (12 and 14 DAH) in observation containers. According to fish size either 1-l crystal beaker (11 cm diameter), or 5-l white plastic (26 cm diameter) was used under medium light conditions (600 lx). Four replicates were set for each observation. Five fish were randomly sampled from the rearing tank and introduced into an observation container and behavior was observed for 10 minutes every 2 h, from 6:00 to 19:00. On each observation the chase frequency was counted. SL was measured and developmental stage of each fish was determined according to Miyashita et al. (2001). Frequencies of chase behavior were pooled by hour and age and mean value (per minute per fish) was obtained for each time and age group, respectively. Additionally, twenty minutes observations for the rearing tank were made daily.

Direct observations on 36-DAH juveniles of the first batch before, during and after feeding were performed and chases in the rearing tank were counted. The behavior of twenty-two randomly selected juveniles was observed at each observation period. Observations were made four times from 7:30 to 10:30. After the observations, 10 juveniles were randomly sampled from the rearing tank, anesthetized with MS222 and preserved in 10% formalin to measure SL.

Statistics

To determine if there were differences in standard length, swimming speed or relative swimming speed between developmental stages of the same batch, t-test or one-way ANOVA was performed. In case significant difference was detected by ANOVA (p < 0.05), Tukey-Kramer test was used. To assess differences in chase behavior frequency among hours Kruskal-Wallis test was performed, and if no significant differences were detected, data were pooled and mean values were calculated for each sampling day. To determine if there were differences in frequency of chase behavior between developmental stages or starving conditions of fish in the same batch, U-test or Kruskal-Wallis test was performed. In case significant difference was detected (p < 0.05) Dunn’s nonparametric multiple comparison test was performed.

Results

Growth and development

Mouth opening was on 2 DAH (SL, mean ± SD, 2.9 ± 0.1 mm, Fig. 1). Flexion stage larvae appeared from 13 DAH (SL 4.7 mm), and juveniles first appeared at 19 DAH (SL 6.3 mm).

Behavioral observations conditions

In the light condition experiment, survival of larvae was highest in medium (50 – 350 lx, Fig. 2) or low light (< 50 lx). In the rearing tank, fish formed patchiness beneath water surface at around 650 – 750 lx.

For the tank color, white and transparent tanks showed the best survivals (80% at the end of the experiment, Fig. 2). The lowest survival was observed in the black tank, where fish tended to swim very actively towards the bottom and push the snout against the walls.

Behavioral development

Swimming speed at hatch was 3.0 ± 3.9 mm/s

![Figure 1](image-url)
(mean ± SD; Fig. 3) and did not change until the flexion stage (9.5 ± 14.3 mm/s; ANOVA, df=1–2, \(F=0.785–2.108, p=0.14–0.39\)).

Relative swimming speed showed a similar pattern to swimming speed (Fig. 3), stable from hatch (1.2 ± 1.6 SL/s; ANOVA, df=1–2, \(F=0.968–3.396, p=0.052–0.337\)) until the juvenile stage (2.0 ± 2.5 SL/s).

There was no effect of hour of the day on the expression of chase behavior (ANOVA, df=6, \(F=1.000, p=0.4512\)). Then data were pooled and mean values were calculated for each sampling day. Chase behavior was first observed in the observation containers for 16 DAH fish (SL 5.4 ± 0.9 mm, flexion stage; Fig. 4). Afterwards, frequency of chase behavior did not change until the juvenile stage (ANOVA, df=5, \(F=0.945, p=0.4787\)).
Chase behavior was more frequent before the first feed time in the morning than in the rest of the day in the rearing tank (Kruskal-Wallis test, $p < 0.05$, Fig. 5).

**Discussion**

Fish growth and development in our experiment are comparable to those reported in similar conditions of temperature (Kaji et al., 1996). Kawamura et al. (2003) and Miyashita et al. (2001) described a better Pacific bluefin tuna growth than in the present experiment, presumably due to differences in temperature ($27.3^\circ \text{C} - 29.0^\circ \text{C}$) and feed, respectively.

Light can be a limiting factor for fish growth in aquaculture depending on turbidity and depth, and different responses in different species and different developmental stages are reported (Boeuf and Le Bail 1989). Too intense light condition might be stressful or even lethal (Han et al. 2005). Eurasian perch, *Perca fluviatilis*, (Tamazouzt et al. 2000) and juvenile Chinese longsnout catfish, *Leiocassis longirostris*, (Han et al. 2005) showed better survival at medium lights ($250$ and $74 - 312$ lx, respectively) than at high light intensities ($800$ and $432$ lx, respectively). Although in a much shorter time scale, Pacific bluefin tuna showed a higher survival at low and medium intensities, while larvae died in less than three h at the high light intensity environment. While at high light intensity conditions, Pacific bluefin tuna larvae tended to swim very actively towards the bottom of the tanks presumably as a sign of stress. This behavior may have caused the larvae to suffer injuries on their snout and jaw, which could lead to the high mortality observed in these tanks, as no aggressive behavior was observed during the experiment. On the other hand, when in low or medium light intensity conditions larvae tended to swim smoothly in the entire water column. Although further research is needed to assess the effect of light intensity on survival and growth, we strongly advise a light intensity of around $600$ lx for the observation of Pacific bluefin tuna behavior at their early life stages.

Pacific bluefin tuna had a better survival after transfer to containers with light colors (white and transparent), under the same light intensity conditions. Survival and growth of Eurasian perch larvae were higher in the tanks with light color walls (white or light gray) for 15 days from hatch (Tamazouzt et al. 2000). However, our results are different from those on walleye, *Stizostedion vitreum*, (Corraza and Nickum 1981) and on striped bass, *Morone saxatilis*, (Martin-Robichaud and Peterson 1998) for early rearing (until aprox. two weeks post hatching). Tanks with dark walls improved early larval rearing of these 2 species by improving the prey perception and consumption (rotifers, *Artemia nauplii*). Black-walled tanks are also preferable for marine fish larviculture (Naas et al. 1996). In case of Eurasian perch, light walls seem to induce a strong contrast between food particles and tank wall and, consequently, improved food perception (Tamazouzt et al. 2000). Moreover, in our experiment larvae tended to swim actively towards the bottom when put into dark colored containers, possibly provoking injuries on the snout and jaw of the larvae, while they swam evenly in the entire water column in the light colored tanks. In our experiment with Pacific bluefin tuna the rapid
effect on survival observed in the dark-walled containers (60% mortality 2 h after transfer into black or green colored tanks) and the absence of aggressive behavior suggests a stress and/or injuries effect on survival instead of a feeding success effect. Accordingly, we strongly suggest the use of light colored or clear observation containers to monitor the behavior of Pacific bluefin tuna.

Swimming speed of Pacific bluefin tuna larvae tended to increase with developmental stages. Swimming speed of Pacific bluefin tuna larvae was similar to that of the comparable body size of chub mackerel, *Scomber japonicus*, larvae, (Nakayama et al. 2007), and was slightly faster than those of comparable size larvae of other non-migratory species, such as Japanese flounder *Paralichthys olivaceus* (Fukuhara 1986) and red drum *Sciaenops ocellatus* (Smith and Fuiman 2004). Also, juvenile relative swimming speed was comparable to that of other highly migratory species juveniles, such as yellowtail, *Seriola quinqueradiata* (Sakakura et al., 1998). This seems to be reasonable since Pacific bluefin tuna migrates long distances throughout its life history (Kitagawa et al. 2002; Takii et al. 2007).

Chase behavior was first observed in flexion stage of Pacific bluefin tuna, earlier than yellowtail (Sakakura and Tsukamoto 1998, 1999) and Japanese flounder (Sakakura and Tsukamoto 2002) in which aggressive behavior onsets from the juvenile stage. Chase frequency of Pacific bluefin tuna was much lower than yellowtail (around 0.03 chases/min/fish; Sakakura and Tsukamoto 1999) and Japanese flounder (0.05 chases/min/fish; Sakakura and Tsukamoto 2002). Chase behavior was expressed equally towards individuals of smaller or comparable size of aggressive individual and was expressed only during starvation. Therefore, we speculate that chase behavior of Pacific bluefin tuna is directly related to feeding behavior, namely cannibalistic behavior, which is different from social hierarchy like aggressive behavior of yellowtail (Sakakura and Tsukamoto 1998, 1999) and Japanese flounder (Sakakura and Tsukamoto 2002).

### Acknowledgements

The Ministry of Education, Culture, Sports, Science and Technology of Japan is gratefully acknowledged for the scholarship awarded to the first author. This study was financially supported by the Research Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries (1905), Ministry of Agriculture, Forestry and Fisheries, Japan.

### References


Martin-Robichaud, D. J. and R. H. Peterson (1998) Effects of light intensity, tank colour and photoperiod on swimbladder inflation success in larval striped bass,


クロマグロの攻撃行動発達の予備的観察

Sabate DLSF・阪倉良孝・武部孝行・二階堂英城
松本尚之・塩澤 聡・萩原篤志・井間 主計

クロマグロの行動観察のための至適条件を検討し、攻撃行動に着目した行動の発達を調べた。異なる水槽の色および照度に鰤を置いたところ、暗い色の水槽と高照度（900 lx 以上）では生殖が低下（0-10%）、透明な水槽で中程度の照度（600 lx）で生殖が高かった（60-80%）。後者の条件で、異なる孵化日と発育段階の魚；すなわち開口、脊索帯曲前期、脊索帯曲期、脊索帯曲後期、稚魚、を用いて遊泳および攻撃行動の発達を観察した。また、36日齢の稚魚で、給餌と攻撃行動の関係を調べた。開口は4日齢（体長 2.9 ± 0.1 mm）で、稚魚は19日齢（体長 6.3 mm）に初めて出現した。遊泳速度は脊索帯曲期まで変化しなかった（3.7 ± 3.9 - 9.5 ± 14.3 mm/s）。攻撃行動は16日齢（体長 5.4 ± 0.9 mm、脊索帯曲後期）より観察され、その頻度は稚魚期にも変化しなかった。攻撃行動は稚魚が空腹状態のときに観察された。