Aquat. Living Resour. 24, 403–410 (2011)

DOI: 10.1051/alr/2011150
www.alr-journal.org

Aquatic Living Resources

Enhancement of survival rate of Pacific bluefin tuna (*Thunnus orientalis*) larvae by aeration control in rearing tank

Yoshizumi Nakagawa[1,2], Michio Kurata[1], Yoshifumi Sawada[1], Wataru Sakamoto[1,a] and Shigeru Miyashita[1]

[1] Fisheries Laboratory, Kinki University, 3153 Shirahama, 649-2211 Wakayama, Japan
[2] Department of Aquatic Bioscience, Faculty of Bio-industry, Tokyo University of Agriculture, 099-2493 Hokkaido, Japan

Received 25 April 2011; Accepted 14 October 2011

Abstract – High levels of larval mortality are a significant barrier to the artificial mass production of Pacific bluefin tuna (*Thunnus orientalis*). Mortality may occur when larvae sink and come into contact with the bottom of the rearing tank during the first 10 days after hatching. We evaluated the effect of flow control by aeration on the survival of *T. orientalis* larvae. These larvae were held in 500-L tanks in which the aeration rate was varied during the night. Larval survival increased with air supply. We documented the cross-sectional flow pattern and gravitational sinking velocities of larvae to assess the correlation between survival and circulation patterns in the tank. The sinking velocity of *T. orientalis* larvae at night increased with larval body density, which varied with swimbladder volume. Larvae with uninflated swimbladders sank significantly faster than larvae with inflated swimbladders. Both water circulation speed and survival increased at higher aeration rates. Our results suggest that aeration rates >900 ml min^{-1} may increase larval survival by counteracting sinking.

Key words: Gas bladder inflation / Swimbladder / Flow field / Survival rate / Sinking velocity / Aeration / *Thunnus orientalis*

1 Introduction

Pacific bluefin tuna (PBT, *Thunnus orientalis*) are the basis of economically important commercial fisheries throughout the world. PBT are highly valued for both their taste and health benefits. Efforts to artificially culture this species are, therefore, expanding rapidly to meet an increasing demand. At present, all tuna aquaculture, including PBT, is reliant on natural seed resources. In 2008, the International Commission for the Conservation of Atlantic Tunas (ICCAT) decided to reduce the total allowed catch of Atlantic bluefin tuna (*Thunnus thynnus*) from 28 500 tons in 2008 to 18 500 tons in 2011. As the catch of wild-caught tuna declines, demand will increase for a complete full-cycle tuna aquaculture system to eliminate the need for wild-caught broodstock fish.

The Fisheries Laboratory of Kinki University (FLKU) succeeded in developing a full-cycle aquaculture system for PBT in 2002 (Sawada et al. 2005) and techniques for seedling production continue to be developed. FLKU is now able to produce PBT seedlings on an annual basis. In 2009, more than 200 000 juveniles >5 cm in length were produced and transferred from land facilities to net cages in the sea (unpublished data). However, a number of problems still impede reliable mass production of PBT seedlings for commercial aquaculture.

The success of PBT seedling production is currently dependent on three high-mortality stages (Miyashita 2002; Sawada et al. 2005): (1) initial mortality until 10 days after hatching (DAH); (2) cannibalism from 10 to ~30 DAH; and (3) collision with tank walls or net cages from 30 DAH onwards. The survival rate up until 10 DAH (10%) for PBT is much lower than that for red seabream (*Pagrus major*, 70–80%), for which larval rearing techniques are well established in Japan (Miyashita 2002; Chen et al. 2004; Tomoda et al. 2004). Initial mortality is thus a major factor in determining the success of PBT production.

The early survival of marine fish larvae is affected by egg quality, water quality, light conditions, and food quality and density. It has also been reported that the survival of PBT larvae is affected by rotifer size (Sawada et al. 2000) and prey type (Nakagawa et al. 2007). Sakamoto et al. (2005) identified two patterns of mortality for larvae up to 10 DAH: surfacing death and sinking death. Surfacing death occurs when larvae are brought to the surface layer by aeration or phototaxis and are

[a] Corresponding author: sakamoto@kindaisuiken.jp

subsequently trapped by surface tension. Surfacing death can be prevented by spreading a thin film of oil on the water surface, which reduces surface tension (Yamaoka et al. 2000).

Sinking death, which has been identified as a more important problem during the initial peak in mortality for PBT (Miyashita 2006), occurs when PBT larvae sink and touch the tank bottom during the night (Sakamoto et al. 2005; Tanaka et al. 2009). This phenomenon is affected by several factors. Larval body density increases as larvae grow and larval sinking velocity increases with body density. After first feeding, larvae swim in the mid-water to feed during the day, but swimming activity decreases at night (Takashi et al. 2006). Swimbladder inflation plays an important role in controlling PBT larval body density and hence their buoyancy. Both PBT larval body density and swimbladder volume are lower during the day and greater at night (Takashi et al. 2006). Although inflation of the swimbladder decreases density and hence sinking velocity, larvae gradually sink to the tank bottom at night (Takashi et al. 2006). Larvae may be killed by direct contact with the tank bottom, infection by pathogenic bacteria concentrated near the bottom of the tank (Miyashita 2006), or by reduced oxygen levels in this zone (Kayaba 2003). As a result, techniques to maintain larval suspension at night may decrease larval mortality.

At FLKU, the air supply to PBT larval rearing tanks is increased at night. Bubbles from the aerators drag water upwards and increase water circulation. The flow pattern is typically characterized by an inner segment of relatively low flow and an outer segment of higher flow, often shaped like a doughnut. Larvae remain suspended in the water column segments where larval sinking velocity is balanced by upward flow velocity.

Although increased air supply likely increases larval suspension, higher air input and water flow during the night may also decrease larval feeding during the day and cause skeletal deformities or injuries as a result of collisions with air bubbles (Tucker 1998). However, there is no empirical method of determining the ideal air supply rate, and control depends on staff experience. An understanding of the effects of air supply on water circulation and larval downward velocity would allow more accurate control of water movement in larval rearing tanks.

Our goal was to improve larval survival by evaluating the effects of air supply on water circulation. Larvae were reared in small tanks (500 L) in which the air supply rate was varied at night. Circulation patterns were determined by acoustic measurements and compared with larval sinking velocity to determine the relationship between larval survival and water circulation.

2 Materials and methods

2.1 Larval rearing

Fertilized eggs were obtained from broodstock at Amami Experiment Station, the Fisheries Laboratory of Kinki University, and transported by air cargo to the Shirahama Center, Kinki University Fish Nursery Center. We conducted two trials in which PBT larvae were reared in essentially the same conditions, except for the number of eggs per tank (Table 1). The first trial was conducted between 29 July and 9 August, 2007 (trial 1) and the second between 24 June and 4 July, 2010 (trial 2). The broodfish differed in age between the two trials but were from the same strain (8-year-old F1 during trial 1 and 4-year-old F2 during trial 2). Between 5 000 and 6 000 fertilized eggs were introduced into each of nine 500-L cylindrical polycarbonate tanks (diameter 100 cm, height 62 cm). Egg diameters were 1.01 ± 0.01 mm in trial 1 and 1.01 ± 0.02 mm in trial 2. Hatching rates were 92.3% in trial 1 and 99.0% in trial 2.

Table 1. Rearing conditions for larviculture of Pacific bluefin tuna.

Conditions	Trial 1	Trial 2
Number of eggs introduced per tank	6 000	5 000
Photoperiod	07:00–19:00	
Luminance (lx)	500	
Water exchange ratio (% day^{-1})	day number after hatching × 10% of tank volume	
Density of *Nannochloropsis oculata* (cells ml^{-1})	0.75 million	
Density of *Brachionus plicatilis* sp. complex (ind. ml^{-1})	5–7	
Water temperature (°C)	24.0–24.9	25.6–26.9
Dissolved oxygen (mg L^{-1})	6.1–8.2	5.3–6.6
pH	7.96–8.28	7.97–8.31
Salinity	32.8–33.9	33.0–33.5

Water was replenished daily at 10% of tank volume × days after hatching. Seawater was filtered through a high-speed fibre filter (≤2 μm) system (Unitica Co. Ltd., Osaka, Japan) and sterilized with a UV lamp.

The photoperiod was 12 h light (07:00–19:00) via fluorescent lights with 500-lx luminance during the rearing period. PBT larvae were fed S-type rotifers (*Brachionus plicatilis* sp. complex) at 10:00 and 14:00 and condensed *Nannochloropsis oculata* (Marine Fresh, Marine Bio Co., Ltd., Yatsushiro, Japan) from 2 DAH onwards. The *N. oculata* were added to the rearing tank before 07:00 to maintain a density of 0.75 million cells ml^{-1}.

The rotifers were enriched with commercial supplements to enhance n-3 highly unsaturated fatty acids (n-3 HUFA) (Marine Gloss and Marine Gloss EX, Nisshin Marine Tech Co., Ltd., Yokohama, Japan), and taurine and vitamin content (Aquaplus ET, Marubeni Nisshin Feed Co., Ltd., Tokyo, Japan) before feeding them to the larvae. Rotifers were supplied at a sufficient rate to maintain a density of 5–7 rotifers ml^{-1}.

In both trials, 0.1 ml of feed oil (Ueda Oils And Fats MFG, Co., Ltd., Kobe, Japan) was added to the tank from just after hatching to 3 DAH to prevent surfacing death. To allow the larvae to inflate their swimbladders, the oil film on the surface was removed each night between 16:00–17:00 from 3 days to 10 days (DAH), using paper during trial 1 and a handcrafted skimmer during trial 2.

Air was supplied through a circular ceramic aerator (32 mm diameter) placed 25 mm above the bottom in the centre of each tank. All tanks were supplied with air at 300 ml min^{-1} during the day between 07:00 and 19:00, using a rotameter (Model RK1250, Kojima Instruments Inc., Kyoto, Japan). After first feeding (2 DAH), air supply was changed to 0, 300, or 900 ml min^{-1} at night ($N = 3$ tanks/flow rate). During the rearing period, temperature (°C), dissolved oxygen (mg L^{-1}), pH and salinity (practical salinity unit: psu) were measured with a stick thermometer and a multiple water quality sensor (556 MPS, YSI Inc., Yellow Springs, OH, USA) at 16:00 every day. The water conditions during larval rearing are shown in Table 1. At the end of each trial, and before turning on the light, the flow rate in all tanks was set to 900 ml min^{-1}, except for the tanks that were set at 0 ml min^{-1}. We then collected 20–30 live larvae from the surface water of each tank using a 3-L beaker and observed their swimbladders under a microscope. After turning on the light, the total number of live PBT larvae in each tank was counted and survival rate was calculated. Because there were only low numbers of larvae in the tanks set at 0 ml min^{-1}, we collected larvae for swimbladder observation at the time of counting the total larval number in the tank. Then, we calculated the percentage of larvae with an inflated swimbladder.

Table 2. Survival rates and the percentage of larvae with an inflated swimbladder (ISB) (%, average value ± standard deviation) for *Thunnus orientalis* at 10 days after hatching in 2 trials, $n = 3$ tanks at each air supply. Survival rate and ISB values with different superscripts are significantly different within each row (Scheffé's test, $p < 0.05$).

Trial	Air supply at night time		
	0 (ml min^{-1})	300 (ml min^{-1})	900 (ml min^{-1})
Trial 1			
Survival (%)	0.4 ± 0.1^{a}	18.2 ± 13.1^{a}	48.6 ± 4.2^{b}
ISB (%)	0^{a}	5.0 ± 0.0^{b}	6.7 ± 2.9^{b}
Trial 2			
Survival (%)	1.8 ± 1.0^{a}	26.6 ± 15.7^{ab}	43.2 ± 4.5^{b}
ISB (%)	5.6 ± 6.9	54.4 ± 23.4	21.0 ± 22.2

2.2 Larval sinking velocity

In trial 2, we measured the sinking velocity of larvae during the night (20:00–22:00) between 1 and 9 DAH. Thirty larvae were selected at random from a rearing tank in which the air was supplied at 900 ml min^{-1}. The larvae were anesthetized with 200 ppm eugenol (FA-100, Mitsubishi Tanabe Pharma Co., Ltd., Osaka, Japan), their swimbladders were examined under a microscope, then each fish was dropped gently into a 2-L cylinder filled with water from the rearing tank. This sinking velocity measurement was conducted in an air conditioned room (26 °C) which was equal to the larval rearing temperature. We assumed that terminal velocity was achieved 5 cm below the surface, and therefore measured the time for each larva to sink from 5 to 15 cm depth.

2.3 Flow field measurements

We measured the flow fields in a 500-L rearing tank at two air supply rates (300 and 900 ml min^{-1}) with an acoustic Doppler flow meter (velocimeter, Vectorino, Nortec AS, Rud, Norway) between 3 and 14 September, 2008. The velocimeter was fixed to a movable trestle that allowed the sensor to take horizontal and vertical measurements. Measurements were taken in three planes: horizontal (x-, y-) and vertical (z-) axes, at 137 points at 25- and 50-mm intervals. We added fossil shell powder (Fish Green, Green Culture Co., Ltd., Takaoka, Japan) to the test tank to improve sonic reflection. The flow rate at each point in both experiments was measured at 25 Hz for 3 min. Preliminary data suggested that the velocity reached a steady state within 2 to 3 min. For the analysis, we therefore only analyzed the data over the 1 min period between 2 and 3 min. Because air bubbles, physical structures, and the surface/air interface may interfere with measurements, all measurement points were >5 cm from the tank wall and >5 cm below the surface.

2.4 Estimated danger zone

The risk of larval sinking death is highest when the larval sinking velocity (V_l) is higher than the upward water velocity (V_z) during the night. We defined the "Estimated danger zone" (EDZ) as the area within the cross-section of the rearing tank where, based on flow readings, the upward flow velocity was less than the larval sinking velocity ($V_l > V_z$). The EDZ between 3–9 DAH was calculated daily from flow field measurements and mean larval sinking speed. The velocity at each sample point was compared with daily mean larval sinking speed between 3 and 9 DAH. The EDZ was plotted as a cross-sectional spatial percentage of the vertical section of the tanks with flow rates of 300 and 900 ml min^{-1}.

2.5 Statistical analysis

We tested for differences in survival or swimbladder inflation ratio at different air supply rates using one-way ANOVA followed by Scheffe's test. Data were assumed to be normally distributed. The effect of swimbladder inflation on sinking velocity was evaluated using a t-test. All analyses were performed in StatView (SAS Institute, Cary, NC, USA) and $p < 0.05$ was considered to represent a significant difference.

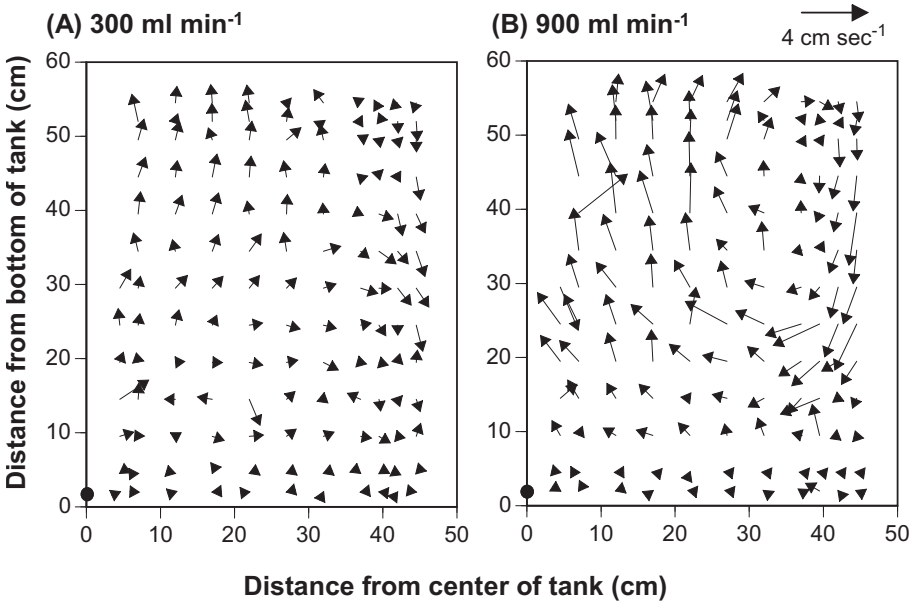

Fig. 1. Half of a horizontal, cross sectional view of mean flow velocity in *Thunnus orientalis* rearing tanks at air flow rates of (A) 300 and (B) 900 ml min^{-1}. The arrows indicate mean flow velocity (length) and direction for a point in the rearing tank. The black circle indicates the location of the aerator at the bottom centre of the tank. The arrow at the top of the figure indicates the speed scale.

3 Results

3.1 Survival rate

In both trials, survival rates were higher at higher flow rates (ANOVA, trial 1, $df = 2, 6$, $F = 28.3$, $p < 0.05$; trial 2, $df = 2, 6$, $F = 14.7$, $p < 0.05$; Table 2). Larval survival was highest in the tanks with a nighttime air supply rate of 900 ml min^{-1} (trial 1, 48.6 ± 4.2%; trial 2, 43.2 ± 4.5%) (Table 2). In both trials, more larvae developed a fully inflated swimbladder by the final day of rearing when held at higher flower rates (ANOVA, trial 1, $df = 2, 6$, $F = 13.0$ $p < 0.05$; trial 2, $df = 2, 6$, $F = 5.2$, $p < 0.05$; Table 2). The swimbladder inflation rates of trial 1 were higher than those of trial 2.

3.2 Flow field

We observed water circulation in the larval tank at 300 and 900 ml min^{-1} (Fig. 1). Vertical flows were generated as bubbles from the aerator at the centre of the tank bottom rose, dragging water upwards until the bubbles burst at the surface. At the surface, the flow direction changed from vertical to horizontal (Fig. 1). Water flowed horizontally across the surface to the side of the tank, and then flowed down the sidewall. Upon reaching the bottom, the flow moved towards the centre of the tank, completing the circulatory pattern. Circulation velocity increased and the thickness of the boundary layer between the turbulent zone and the bottom of the tank decreased at higher air supply (Fig. 1). At 300 ml min^{-1}, the boundary layer was 30–40 cm above the bottom, whereas at 900 ml min^{-1} the boundary layer was 20–30 cm above the bottom.

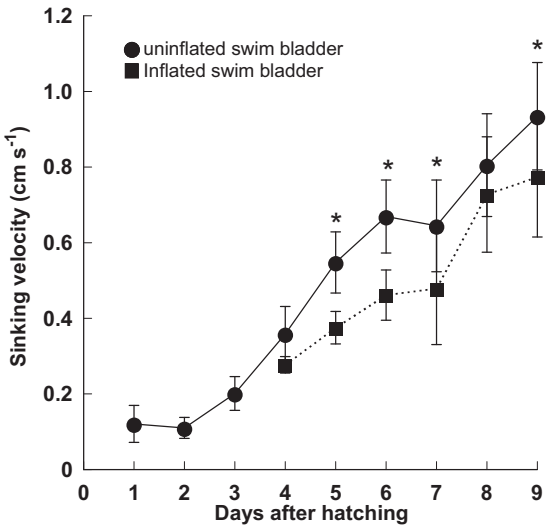

Fig. 2. Average sinking velocities (cm s^{-1}) of *Thunnus orientalis* larvae with inflated swimbladders (squares) or uninflated swimbladders (circles) during trial 2 between 1-9 days after hatching. Vertical bars indicate standard deviations. Asterisks indicate significant differences between inflated and uninflated swimbladder larvae according to age.

3.3 Larval sinking velocity

The sinking velocity of larvae increased as the larvae grew (from 0.12 ± 0.05 cm s^{-1} 1 DAH to 0.20 ± 0.04 cm s^{-1} 3 DAH) (Fig. 2). At 4 DAH, some larvae were found to have inflated swimbladders; their mean sinking velocity was 0.28 ± 0.02 cm s^{-1}, whereas the larvae with uninflated swimbladders had a mean sinking velocity of 0.36 ± 0.07 cm s^{-1} (Fig. 2).

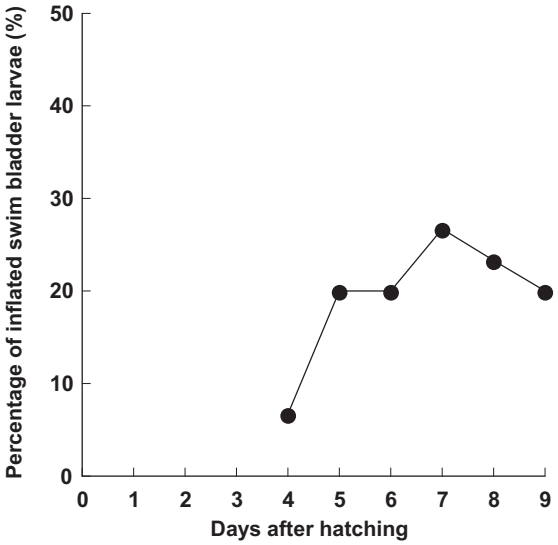

Fig. 3. Percentage of *Thunnus orientalis* larvae with inflated swimbladders during larval sinking velocity measurements between 4–9 days (DAH). Larvae were first observed with an inflated swimbladder 4 days after hatching.

Approximately 6.7% of larvae completed swimbladder inflation at 4 DAH (Fig. 3). From 5 DAH, the sinking velocities of larvae with uninflated swimbladders were significantly higher than those of larvae with inflated swimbladders. At 9 DAH, the sinking velocity was 0.77 ± 0.16 cm s^{-1} for larvae with inflated swimbladders and 0.93 ± 0.14 cm s^{-1} for larvae with uninflated swimbladders (Fig. 2), with the former accounting for 20% of the total number of larvae (Fig. 3).

3.4 Estimated danger zone

At all air supply rates, the area of the EDZ increased with larval growth (Figs. 4, 5), and the EDZ of larvae with inflated swimbladders tended to be smaller than that of larvae with uninflated swimbladders (Fig. 6). For all DAH, the EDZ at 300 ml min^{-1} air supply were much larger than those at 900 ml min^{-1} (Fig. 6). The depth of the EDZ above the tank bottom on 9 DAH was 30–40 cm from the tank bottom at 300 ml min^{-1} and 10–20 cm from the tank bottom at 900 ml min^{-1} (Figs. 4, 5). There was a significant difference in larval survival between 0 and 300 or 900 ml min^{-1} in trial 1, but not in trial 2 (Scheffe's test, $p < 0.05$).

4 Discussion

We demonstrated that the rate of air supply at night plays a role in preventing sinking death among PBT larvae. Larval survival in both trials increased with increasing air supply during the night (Table 2), although there were a few statistical discrepancies between the trials. These discrepancies may have been caused by differences in the quality of eggs or by small variations in environmental conditions, such as water temperature and dissolved oxygen concentration (Table 1). Despite these differences, we conclude that both experiments demonstrated a clear relationship between PBT larval survival and characteristics of the flow field in the rearing tank.

These results are also consistent with larval survival reported for other aquaculture fish species. Kayaba (2003) reported that aeration reduced mortality in barfin flounder (*Verasper moseri*) larvae at early-stage settlement. Similarly, larval sinking and survival in seven-band grouper (*Epinephelus septemfasciatus*) is related to aerator distribution and air supply (Shiotani et al. 2003), and to the pattern of circulation in the rearing tank (Sakakura et al. 2006). Teruya et al. (2009) quantified the flow field that prevented the sinking of amberjack larvae, and Tanaka et al. (2009) demonstrated that the number of sinking PBT larvae was decreased by vertical turbulent mixing of the rearing water through aeration.

We surmise that higher circulation rates might retain larvae within the water column, thus reducing mortality. This may be a result of the balance between downward larval sinking and upward flow velocities. If this hypothesis is valid, it may be possible to describe typical larval movement based on flow velocity in the tank and larval sinking speed in the following way. In the early stages of development, fish larvae are generally unable to maintain a single position within a water column under normal rearing conditions. Swimming ability increases as larvae grow and PBT larvae swim actively during the day (Kawamura 2003). However, larvae are less active at night and, consequently, sink (Sakamoto 2005; Takashi et al. 2006). As larvae develop and inflate their swimbladders, larval body density decreases (Takashi et al. 2006), and survival rate increases.

Larvae appear to be less active at night during early developmental stages. As a result, it may be difficult for larvae that sink into the EDZ at night to re-enter the upper layers. Because increased air supply rates reduce the depth of the EDZ, the risk of larvae sinking into the EDZ might also decrease. Further, higher air supply rates produce stronger horizontal flow, which may move larvae towards the centre of the tank and, thus, back into circulation.

The body density of PBT larvae increases after hatching and varies over a diel cycle, decreasing at night and increasing during the day (Takashi et al. 2006). Our results are consistent with those of Tanaka et al. (2009), who suggested that larval density and PBT larval sinking depend on the development of the swimbladder. It follows that PBT larvae will sink faster if their swimbladders are not inflated and be at greater risk of sinking death.

There was a significant difference in larval survival between 300 and 900 ml min^{-1} in trial 1, but not in trial 2 (Scheffe's test, $p < 0.05$). The higher percentage of larvae having an inflated swimbladder in trial 2 perhaps contributed to higher larval survival under lower flow conditions (300 ml min^{-1}) (Table 2). The differences in the ratio of inflated: uninflated swimbladders may be explained by differences in the method of surface skimming: paper was used in trial 1 and a handcrafted skimmer in trial 2. The higher percentage of larvae having an inflated

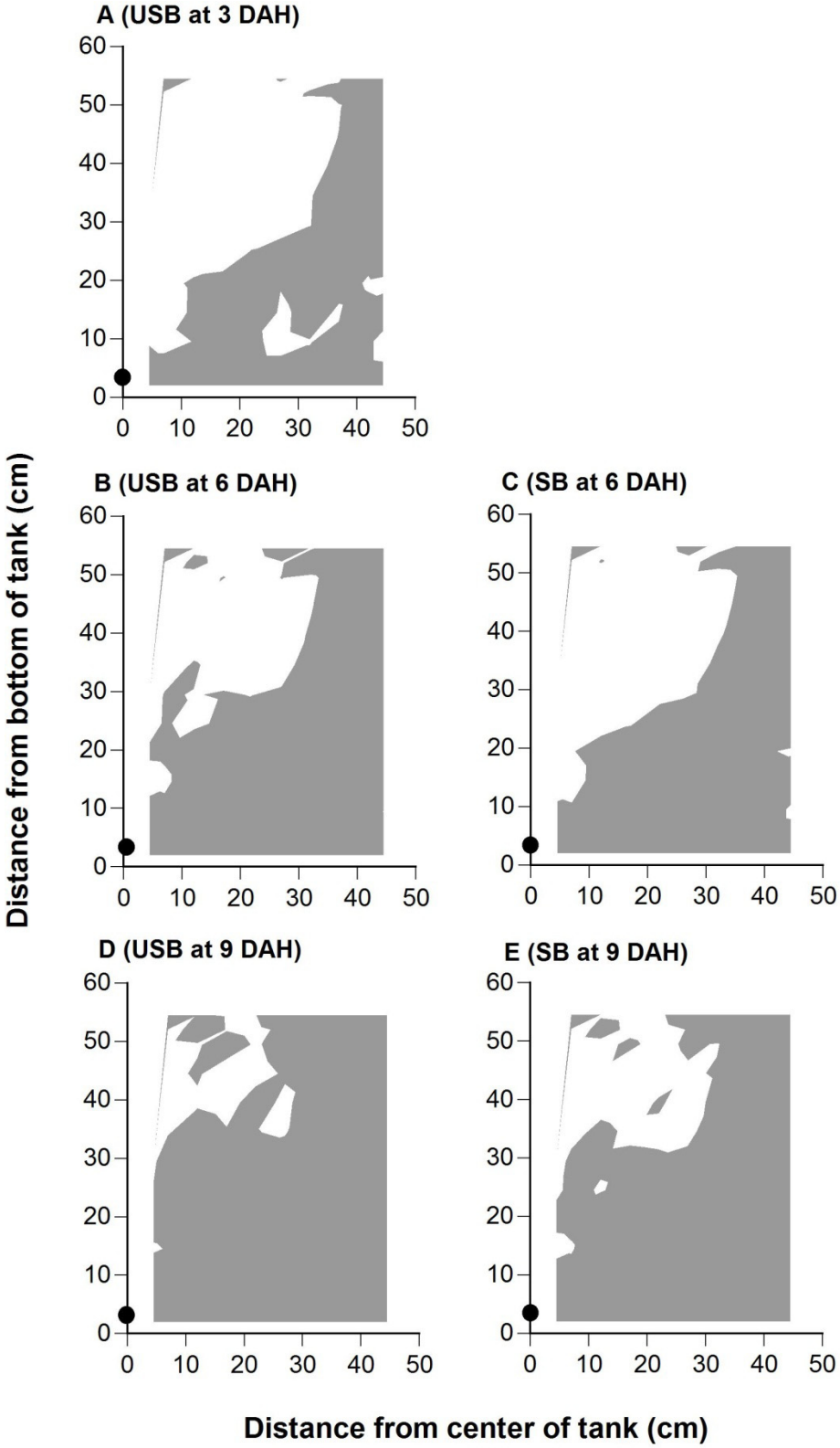

Fig. 4. Half of a horizontal, cross sectional view of the estimated danger zone (EDZ) for each day at an air supply of 300 ml min^{-1}. A: *Thunnus orientalis* larvae with uninflated swimbladders (USB) at 3 days (DAH); B: USB at 6 DAH; C, larvae with inflated swimbladders (SB) at 6 DAH; D, USB at 9 DAH; E, SB at 9 DAH. Grey areas indicate the EDZ, where larval sinking velocity on each DAH was higher than water upward flow velocity. The black circle at the bottom centre indicates the location of the aerator.

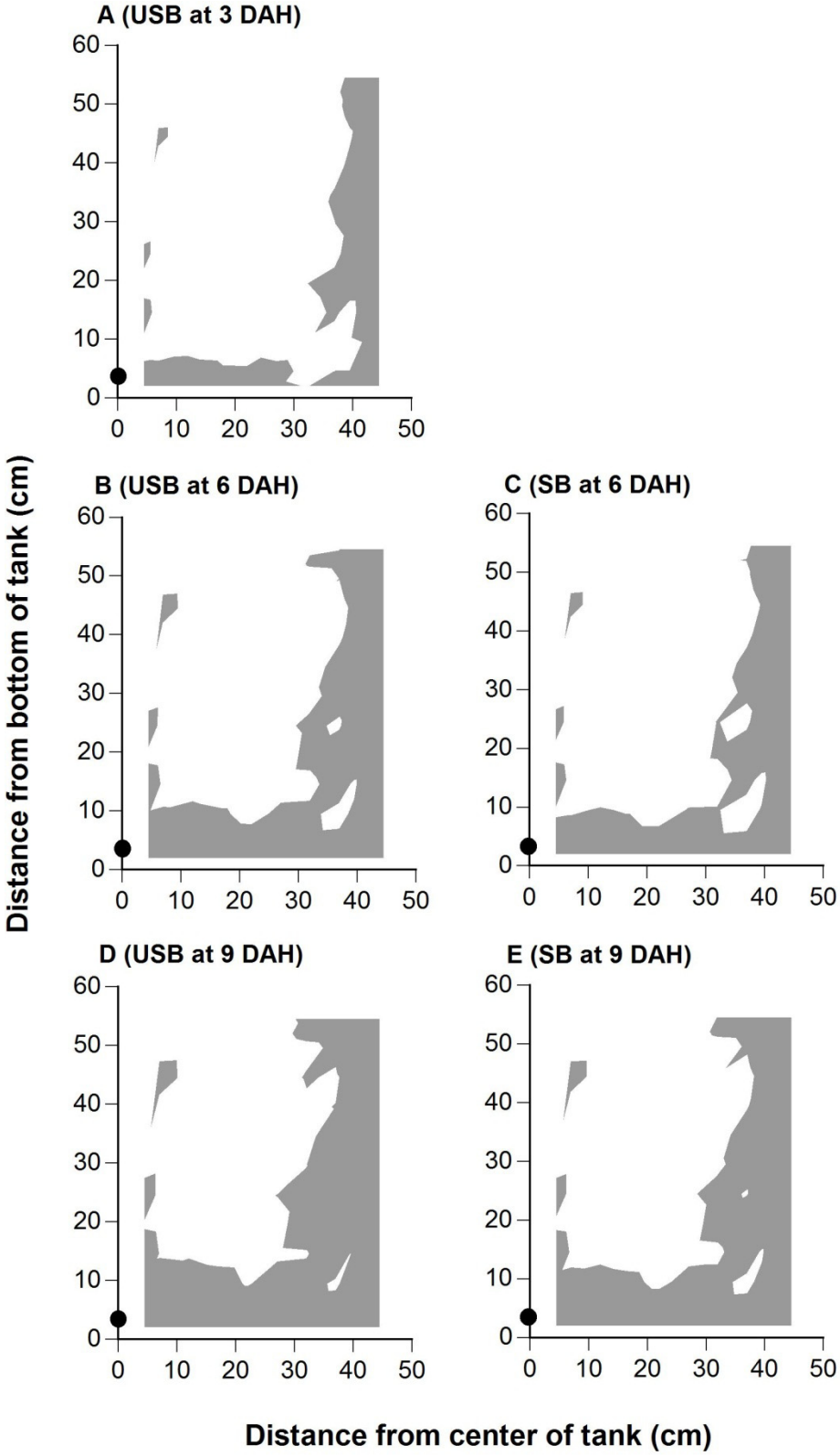

Fig. 5. Half of a horizontal, cross sectional view of the estimated danger zone (EDZ) at 3, 6, and 9 days (DAH) at an air supply of 900 ml min^{-1}. A: *Thunnus orientalis* larvae with uninflated swimbladders (USB) at 3 DAH; B, USB at 6 DAH; C, larvae with inflated swimbladders (SB) at 6 DAH; D, USB at 9 DAH; E, SB at 9 DAH. Grey areas indicate the EDZ, where larval sinking velocity at each DAH was higher than water upward flow velocity. The black circle at the bottom centre indicates an aerator.

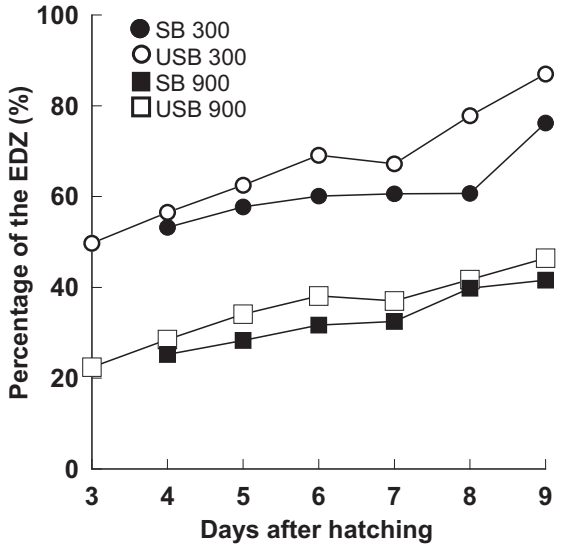

Fig. 6. Percentage of the estimated danger zone, EDZ relative to the area of the vertical tank section under different night-time air supply conditions: *Thunnus orientalis* larvae with inflated (SB 300, close circle) and uninflated swimbladders (USB 300, open circle) at 300 ml min^{-1}; larvae with inflated (SB 900, closed square) and uninflated swimbladders (USB 900, open square) at 900 ml min^{-1}.

swimbladder perhaps contributed to higher larval survival under lower flow conditions (300 ml min^{-1}).

Although other factors are likely to affect larval survival, it is clear that increasing the air supply at night increases circulation, reduces the size of the estimated danger zone , and enhances PBT larval survival. Our results provide insight into the relationship between PBT larval survival and flow field characteristics, as well as techniques to prevent larval sinking death in mass production tanks. Future studies should examine the relationship between inflation of the swimbladder and larval sinking velocity, and means of controlling air in larger-scale tanks for use in mass production of Pacific bluefin tuna seedlings.

Acknowledgements. The present study was partly supported by the 21st century Center of Excellence (COE) program (Center of Aquaculture Science and Technology for Bluefin Tuna and Other Cultivated Fish), the global COE program (International Education and Research Center for Aquaculture Science of Bluefin Tuna and Other Cultured Fish) of the Ministry of Education, Culture, Science, Sports and Technology of Japan, and by the Research and Development Projects for Application in Promoting New Policy of Agriculture Forestry and Fisheries (1905) of the Ministry of Agriculture, Forestry and Fisheries of Japan. The authors also thank A. Shibahara and S. Fukui for assistance with PBT larval rearing. This manuscript was improved by constructive comments from two anonymous referees.

References

Chen J.E., Takeuchi T., Takahashi T., Tomoda T., Koiso M., Kuwada H., 2004, Effect of rotifers enriched with taurine on growth and survival activity of red sea bream *Pagrus major* larvae. Nippon Suisan Gakkaishi 70, 542–547.

Kayaba T., Sugimoto T., Matsuda T., Mass mortality associated with sudden sinking of larval barfin flounder, *Verasper moseri*. Suisanzosyoku 51, 443–450.

Kawamura G., Masuma S., Tezuka N., Koiso M., Jinbo T., Namba K., 2003, Morphogenesis of sense organs in the bluefin tuna *Thunnus orientalis*. In: Browman H.I., Skiftesvik A.B. (Eds.). The Big Fish Bang. Proc. 26th Annual Larval Fish Conference, Bergen, Institute of Marine Research, pp. 123–135.

Miyashita S., 2002, Studies on the seedling production of the Pacific bluefin tuna, *Thunnus thynnus orientalis*. Bull. Fish. Lab. Kinki Univ. 8, 1–173.

Miyashita S., 2006, Surfacing and bottoming death in seedling production. Nippon Suisan Gakkaishi 72, 947–948.

Nakagawa Y., Eguchi M., Miyashita S., 2007, Pacific bluefin tuna, *Thunnus orientalis*, larvae utilize energy and nutrients of microbial loop. Aquaculture 267, 83–93.

Sakakura Y., Shiotani S., Chuda H., Hagiwara A., 2006, Improvement of the survival in the seven-band grouper *Epinephelus septemfasciatus* larvae by optimizing aeration and water inlet in the mass-scale rearing tank. Fish. Sci. 72, 939–947.

Sakamoto W., Okamoto K., Uehabu T., Kato K., Murata O., 2005, Specific gravity change of bluefin tuna larvae. Nippon Suisan Gakkaishi 71, 80–82.

Sawada Y., Miyashita M., Aoyama M., Kurata M., Mukai Y., Okada T., Murata O., Kumai H., 2000, Rotifer size selectivity and optimal feeding density of bluefin tuna, *Thunnus orientalis*, larvae. Suisanzosyoku 48, 169–177.

Sawada Y., Okada T., Miyashita S., Murata O., Kumai H., 2005, Completion of the Pacific bluefin tuna, *Thunnus orientalis*, life cycle under aquaculture conditions. Aquac. Res. 36, 413–421.

Shiotani S., Akazawa A., Sakakura Y., Chuda H., Arakawa T., Hagiwara A., 2003, Measurements of flow field in rearing tank of marine fish larvae: a case study of the seven band grouper *Epinephelus septemfasciatus*. Fish. Engineer. 39, 205–212.

Takashi T., Kohno H., Sakamoto W., Miyashita W., Murata O., Sawada Y., 2006, Diel and ontogenetic body density change in Pacific bluefin tuna, *Thunnus orientalis* (Temminck and Schegel), larvae. Aquac. Res. 37, 1172–1179.

Tanaka Y., Kumon K., Nishi A., Eba T., Nikaido H., Shiozawa S., 2009, Status of the sinking of hatchery-reared larval Pacific bluefin tuna on the bottom of the mass culture tank with different aeration design. Aquac. Sci. 57, 587–593.

Teruya K., Hamasaki K., Hashimoto H., Katayama T., Hirata Y., Tsuruoka K., Hayashi T., Mushiake K., 2009, Ontogenetic changes of body density and vertical distribution in rearing tanks in greater amberjack *Seriola dumerili* larvae. Nippon Suisan Gakkaishi 75, 54–63.

Tomoda T., Koiso M., Kuwada H., Chern J.N., Takeuchi T., 2004, Dietary value of marine rotifer *Brachionus plicatilis* in different population growth stages for larval red seabream *Pagrus major*. Nippon Suisan Gakkaishi 70, 573–582.

Tucker J.W., 1998, The rearing environment. In: Tucker J.S. (Ed.). Marine Fish Culture. Kluwer Academic Publishers, London, pp. 49–148.

Yamaoka K., Nanbu T., Miyagawa T., Isshiki T., Kusaka A., 2000, Water surface tension-related deaths in prelarval red-spotted grouper. Aquaculture 189, 165–176.