Effect of tank shape on survival and growth of Pacific bluefin tuna *Thunnus orientalis* larvae

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Abstract

We examined the effect of rearing tank shape on survival and growth of Pacific bluefin tuna *Thunnus orientalis* larvae. Cylindrical (1.7×10^3 cm^2 water surface area; 30 cm deep) and rectangular (1.8×10^3 cm^2 water surface area; 28 cm deep) tanks (n=3 each) were filled with 50 liters of seawater. One air stone with a 100 ml/min aeration rate was set at the bottom center of each tank. Light intensity at the water surface was 2000 lux with a photoperiod of 24L:0D. Larvae were introduced into each tank at a rate of 10 individuals/l at 2 days post-hatching (dph). Rotifers were fed at 10 individuals/ml and their distribution in tanks was measured. Survival of larvae in cylindrical tanks (CT; 52.7±5.1%) at 8 dph was higher than that in rectangular tanks (RT; 0.8±0.7%, *p*<0.01). Meanwhile, larvae growth was not significantly different between tank shapes either in body length (CT: 4.23±0.26 mm; RT: 4.09±0.20 mm) or dry weights (CT: 95.1±17.6 μg; RT: 67.7±10.9 μg). The swimbladder inflation rate of larvae also did not differ significantly between tank shapes (CT: 16.5±14.5%; RT: 56.9±3.47%). Rotifer distribution was higher at tank bottom in both shapes (*p*<0.05). Two-phase bubbly flow simulations in the tanks revealed that the low-flow area was larger in the RT. The low-flow area at tank bottom varied by tank shape, occurring at the edge of the tank wall on the bottom in the CT, and from the center of the tank (air stone) to the tank wall in the RT. These low-flow areas at tank bottom coincided with areas of higher rotifer distribution, which may be a cause of sinking syndrome in fish larvae. Our results indicate that small-scale (50-l) PBT larviculture experiments can be conducted using a CT with the present aeration system, and that an RT requires an improved aerator in place of the single air stone.

Keywords: Pacific bluefin tuna; rearing tank shapes; sinking syndrome; survival; flow

44   field

1. Introduction

Pacific bluefin tuna (PBT) *Thunnus orientalis* is a commercially important fish in Japan, Korea, Taiwan and the United States (Craig et al., 2017). Aquaculture for PBT utilizes wild-caught juveniles as seedlings (Ottolenghi, 2008), and overfishing of PBT juveniles has led to a decline of the PBT population in the wild (Craig et al., 2017). Recently, the full life cycle of PBT was successfully completed under aquaculture conditions for the increasing demands of PBT seedlings (Sawada et al., 2005). However, high mortality occurred during the first 10 days post-hatching (dph), followed by cannibalism during the late larval and juvenile stages, and high mortality occurred by collision with tank or net walls during the juvenile stage (Sawada et al., 2005). The high mortality during the first 10 days is an obstacle that must be solved for mass production of *Thunnus* species to gain a stable large-scale supply of seedlings (Woolley et al., 2013; Nakagawa et al., 2011; Sawada et al., 2005).

The sinking syndrome is a main cause of high mortality during the early larval stages in PBT larviculture (Masuma et al., 2011). This occurs when larvae sink to the bottom during dark periods because larval swimming activity is low at night, and their body density is higher than that of seawater (Nakagawa et al., 2011; Tanaka et al., 2009; Takashi et al., 2006). Sinking syndrome occurs in many marine fish larvae, including the striped trumpeter *Latris lineata* (Trotter et al., 2005), greater amberjack *Seriola dumerili* (Teruya et al., 2009), yellowtail kingfish *S. lalandi* (Woolley and Qin, 2013), leopard coral grouper *Plectropomus leopardus* (Takebe et al., 2011), kelp grouper *Epinephelus bruneus* (Ching et al., 2014) and tiger grouper *E. fuscoguttatus* (Ching et al., 2016). Sinking syndrome can be reduced by increasing the aeration rate at night (Tanaka et al., 2018; Nakagawa et al., 2011) and/or creating conditions in which the larvae are suspended

within the water column of the rearing tank (Kurata et al., 2017; Ching et al., 2016, 2014; Takebe et al., 2011; Tanaka et al., 2009) and by continuous illumination (Kumon et al., 2018; Kurata et al., 2017).

In PBT larviculture, water temperature (Tanaka et al., 2018), aeration rates (Tanaka et al., 2018; Kurata et al., 2017; Nakagawa et al., 2011) and light conditions (Kurata et al., 2017) have been studied as physical environmental factors that affect sinking syndrome in PBT larvae. However, information on the effects of tank shape, which affect the early survival of marine fish larvae (Ruttanapornvareesakul et al., 2007) are lacking. In the present study, we hypothesized that tank shape may affect the survival and growth of marine fish larvae. To examine this possibility, we conducted larviculture experiments in small 50-l tanks of different shapes. We chose a cylindrical tank (CT) with axisymmetrical flow field patterns (Sumida et al. 2013), and a rectangular tank (RT) with three-dimensional (3-D) complicated flow field patterns (Takakuwa et al., 2018), to investigate the effect of tank shape on survival and growth of PBT larvae. We also examined the distribution of rotifers to estimate the flow field in tanks, and visualized the flow fields by simulation.

2. Materials and Methods

Three blue plastic CT (46 cm in diameter) and blue acrylic RT (60 cm × 30 cm × 35 cm depth) with a 50-l working volume were used in this study. The aspect ratio (liquid depth/internal radius of the tank) of the CT was 1.3. We assumed that the aspect ratio of the RT was also 1.3, since the water surface area and depth of the RT (1.8×10^3 cm^2 × 28 cm) were almost equal to those of the CT (1.7×10^3 cm^2 × 30 cm). Tanks were filled with

92 32 parts per thousand (ppt) artificial seawater (Marine Art Hi, Tomita Pharmaceutical,

93 Japan), and placed in a 25 ℃ temperature-controlled room of the Aquaculture Biology

94 Laboratory, Nagasaki University, Japan. A spherical aerator (5 cm in diameter; 100

95 ml/min aeration rate) was placed at the bottom center of each tank to generate water flow.

96 Light intensity at the water surface was 2000 lux with a photoperiod of 24L:0D to

97 decrease the sinking syndrome that occurs in PBT larvae during dark periods (Nakagawa

98 et al., 2011; Tanaka et al., 2009; Takashi et al., 2006).

99   We followed the PBT larval rearing procedure described by Tanaka et al. (2018), who

100 successfully reared PBT larvae for 7 days dph in 200-l tanks under static conditions (no

101 water exchange). Heavy mortality has been observed within 10 dph in the PBT mass

102 culture process due to the sinking syndrome, and the percentage of sinking PBT larvae

103 on the tank bottom has been shown to peak at around 5 dph (Tanaka et al. 2009). We thus

104 decided on a rearing period of 8 dph. Fertilized eggs of PBT were obtained from Seikai

105 National Fisheries Research Institute, and were transported to the Aquaculture Biology

106 Laboratory, Nagasaki University, Japan, on 30 June 2018. Eggs were first transferred into

107 a 100-l polycarbonate tank and larvae were kept until 2 dph in the same tank at 25 ℃ and

108 32 ppt. Larvae were distributed into each experimental tank at 10 individuals/l on 2 dph,

109 and reared until 8 dph under static conditions. Super Chlorella V12 (Chlorella Industry

110 Co., Fukuoka, Japan) was added to the experimental tanks as green water, and the density

111 was adjusted to 5×10^5 cells/ml once daily. Rotifers *Brachionus plicatilis* enriched with

112 Super Chlorella V12 were fed to larvae at 10 individuals/ml when the mouth opened (2

113 dph). Water samples to assess rotifer density were collected from 9 stations (3 ml for

114 each) in a vertical cross-section of CTs (Fig. 1(a)) and 27 stations in the quarter segments

115 of RTs (Fig. 1 (b)) using a pipet. Since the rotifer numbers in the experimental tanks

116   increased during the experimental period, we standardized the rotifer distribution in the

117   tanks each sampling day using the following equations:

118                           $deviation\ value\ at\ station\ x\ on\ day\ i =$

119          $\dfrac{rotifer\ density\ at\ station\ x - mean\ rotifer\ density\ on\ day\ i}{standard\ deviation\ of\ rotifer\ density\ on\ day\ i}$.

120   On 8 dph, all surviving larvae in the experimental tanks were counted to calculate

121   the survival rate. Then, about 30 fish in each tank were anaesthetized with 200 ppm of

122   MS222 (Tricaine; Sigma-Aldrich) and observed under a dissecting microscope with

123   transmitted light to see whether the swimbladder was inflated by checking air bubbles in

124   the bladder. Larvae were then fixed with 5% formalin solution. Formalin-preserved fish

125   were individually measured for morphometric characteristics by a digital microscope

126   (VH-6300; Keyence, Osaka, Japan), and then dried at 60 °C for 24 h for measurement of

127   the dry body weight by an ultra-micro balance (UMX2; Mettler Toledo, Columbus, OH,

128   USA).

129   Two-phase bubbly flow simulations were performed in the experimental tanks using

130   a dispersed flow model that was developed by Takakuwa et al. (2018). Its governing

131   equations are composed of the conservation laws of mass and momentum of liquid (water)

132   and gas (air bubble) phases, in which the effects of pressure gradient, drag and lift forces

133   acting on bubbles, gravitational acceleration and flow viscosity are taken into account. A

134   simplified marker and cell (SMAC) method was used to solve the governing equations.

135   For the liquid phase, the free surface was assumed to be flat, and a no-slip boundary

136   condition was used. On the other hand, an outflow condition was given for the gas phase

137   at the free surface. As boundary conditions on the wall surface of tanks, a no-slip

138   condition was given for the liquid phase, while a slip condition was given for the gas

139   phase. An air inlet (square with a side length of 22 mm; aeration rate, 100 ml/min) was

140   set at the center of the bottom surface. The diameter of a bubble was set to 2.0 mm. Flow

141   simulations were performed for 450 s, and averaged flow fields for the last 150 s are

142   discussed in this research.

143   Physical environmental parameters during the experiments were as follows: water

144   temperature 24.3–24.8 ℃; salinity 32.1–32.2 ppt; dissolved oxygen 6.2 mg/l; pH 7.94–

145   7.96; and ammonia (NH_3-N) 0.18–0.19 mg/l.

146   2.1 Statistical analysis

147   Differences in the survival, growth and swimbladder inflation rates of larvae between

148   tanks were determined using either Student's *t*-test or Mann-Whitney *U*-test after

149   Shapiro-Wilk normality test. The rotifer distribution in tanks was standardized by the

150   deviation value on day *i*, and determined by two-way ANOVA followed by Tukey HSD

151   test. All analyses used R 3.4.1 software, and a 5% level of confidence was considered a

152   significant difference.

153

154   **3. Results**

155

156   *3.1. Survival, growth and swimbladder inflation of larvae*

157   The hatching rate of fish eggs was 100%. The survival rate of PBT larvae at 8 dph in CTs

158   (52.7±5.1%) was significantly higher than in RTs (0.8±0.7%, *p*<0.01, *t*-test; Table 1).

159   Neither standard length nor dry weight of larvae was significantly different between tank

160   shapes (Table 1). Other morphological parameters of larvae at 8 dph were also not

161   significantly different: total length (CT, 4.23±0.26 mm; RT, 4.09±0.20 mm; *p*=0.9111, *t*-

162   test, n=3), body depth/standard length (0.18±0.02 mm; 0.17±0.02 mm; *p*=0.8526, *t*-test,

8

163   n=3), head length/standard length (0.23±0.01 mm; 0.24±0.02 mm; p=0.0765, Mann-

164   Whitney U-test, n=3) and eye diameter/standard length (0.11±0.01 mm; 0.11±0.01 mm;

165   p=0.6667, Mann-Whitney U-test, n=3). The swimbladder inflation rate of larvae was not

166   significantly different between CT and RT (Table 1).

167

168   *3.2 Flow field in the experimental tank*

169   3-D visualizations of streamlines in the rearing tanks are shown in Fig. 2. In the case

170   of the CT, upward flows by the effect of air bubbles generated from the air stone radiated

171   outward in the vicinity of the water surface, and then downward flows were observed

172   along the sidewalls of the tank. A single-pair vortex system could be observed at arbitrary

173   central sections. In the case of the RT, the central upward flows radiated outward in the

174   same manner as the CT. Due to the effect of rectangular corners and the non-axisymmetric

175   shape of the tank, however, a more complicated flow field was generated. Figure 3

176   compares cumulative area distributions of the tanks with respect to the flow velocity

177   magnitude obtained from the flow simulation results. Low-velocity regions in the RT

178   were larger than in the CT regardless of the increasing flow velocity. In Fig. 4, streamlines

179   at the bottom regions (where water depth is more than 25 cm) are visualized, colored by

180   the flow velocity magnitude. In the CT, although low-velocity regions were observed

181   along the outside edge, most streamlines eventually moved toward the center of the tank,

182   which reverted to the upward flow from the air stone. In the RT, the flow velocity

183   magnitude at most bottom regions was larger than the CT, and the flow structures were

184   more complicated. Streamlines from both short sides of the rectangle collided in the

185   vicinity of the center of the long sides, creating a vortex at the upper side of Fig. 4(b), and

186   a large low-velocity area at the lower side of the figure.

187

3.2 Rotifer distribution

Rotifer density increased during the experimental period, reaching 54.7 individuals/ml in CT and 52.2 individuals/ml in RT on 8 dph (n=6). Rotifer distribution expressed as an average of deviation values was significantly higher at the edge of the tank wall on the bottom in the CT, and from the air stone to the tank wall on the bottom in the RT ($p<0.05$, Tukey HSD test, n=6; Fig. 5).

194

4. Discussion

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4.1 Survival, growth and swimbladder inflation of PBT larvae

The present study examined whether the shape of small-scale larval-rearing tanks affects the survival and growth of PBT larvae, together with the rotifer distribution in tanks. The survival rate of PBT larvae in CTs at 8 dph was about 50-fold higher than that in RTs. Usually, better survival and growth of larvae occurs in large rearing tanks rather than in smaller systems (Houde, 1972). However, the survival rate in this study (52.7%) in the 50-l CT under a 24-h photoperiod was higher than that in the 500-l CT of a previous study at 7 dph under natural photoperiod with strong aeration during darkness (about 35%; Tanaka et al., 2018), whose protocol with static conditions was followed here. Survival rates in mass-scale tanks at similar ages were also lower than this study: 19.3% in a 50 m^3 octagonal tank with a water pump system under a natural photoperiod at 8 dph (Tanaka et al., 2009), and 20.3% in 30 m^3 circular tanks with 1.7 l/min aeration rates in daytime and stronger aeration rates (3.0 l/min) during the dark period at 8 dph under a natural

210   photoperiod (Kurata et al., 2014).

211   Moreover, the survival rate in the present study was comparable to that at 10 dph in 1

212   m^3 cylindrical fiberglass tanks (22.2–42.3%) with 130 ml/min daytime aeration rates, and

213   1.2 l/min dark-period aeration rates under natural and artificial fluorescent lighting

214   (Kurata et al., 2012), as well as those at 10 dph in 500-l cylindrical polycarbonate tanks

215   (43.2–48.6%) with 300 ml/min daytime aeration rates and 900 ml/min dark-period

216   aeration rates at 12L:12D (Nakagawa et al., 2011). Therefore, we propose that rearing

217   experiments of PBT larvae can be conducted in small-scale tanks in which the rearing

218   environment, such as water temperature and illumination, can be easily manipulated.

219   We did not find significant differences in growth between different rearing tank

220   shapes. Similar findings were reported in the seven-band grouper *Epinephelus*

221   *septemfasciatus* and devil stinger *Inimicus japonicus*, for which the rearing tank shapes

222   affected larval survival but not growth (Ruttanapornvareesakul et al., 2007). The growth

223   of PBT larvae at 8 dph in the present study (about 4.1 mm TL) appeared to be smaller

224   than that of larvae of the same age in previous studies (5.8–6.1 mm: Kurata et al., 2012,

225   2014). Growth measurements were conducted with formalin-fixed specimens in the

226   present study and fresh specimens in Kurata et al. (2012, 2014). If we assume 10%

227   shrinkage in the case of formalin-fixed larvae (Hay, 1982) to the previous studies (Kurata

228   et al., 2012, 2014), body size of our sample (4.1 mm) was still smaller than that of the

229   previous studies (5.2–5.5 mm). The inferior growth of PBT larvae in this study may have

230   been due to the smaller tank volume; thus, we should consider the larval density and water

231   exchange when applying small-scale tanks for PBT larviculture experiments.

232   Sinking syndrome of PBT larvae occurs because larvae with higher body density

233   than seawater sink to the bottom of the tank during dark periods due to low swimming

234 activity (Tanaka et al., 2009; Takashi et al., 2006). Swimbladder inflation failure is one of

235 the causes of sinking syndrome (Kurata et al., 2017, 2015, 2012; Ina et al., 2014; Woolley

236 and Qin, 2013), as the swimbladder controls the buoyancy of fish by reducing body

237 density relative to that of surrounding water (Taylor et al., 2010; Phleger, 1998). However,

238 Takashi et al. (2006) found that the body density of PBT larvae was higher than seawater

239 density during dark periods even in swimbladder-inflated larvae. In the present study, the

240 swimbladder inflation rate varied among tanks but was not significantly different between

241 tank shapes. We presume that swimbladder inflation failure may not be the main cause of

242 sinking syndrome. These results may be in agreement with the present finding that the

243 survival rate in the CT was higher than that in the RT despite the same swimbladder

244 inflation rates between tank shapes. We could not conclusively determine whether

245 swimbladder inflation is directly connected to the cause of sinking syndrome; additional

246 studies with more detailed observations of the diel movement of PBT larvae in the rearing

247 tank will be needed.

248

249 *4.2 Flow field in the cylindrical and rectangular tanks*

250 Unfavorable flow in rearing tanks can cause mass mortality of marine fish larvae

251 (Sakakura et al., 2019; Shiotani et al., 2003; Yamaoka et al., 2000; Backhurst and Harker,

252 1988). Flow field structures in tanks differ by tank size and shape (Sakakura et al., 2019;

253 Moore and Prange, 1994). CTs have an axisymmetrical flow field pattern (Sumida et al.,

254 2013), and the flow field provides a uniform current environment and facilitates the

255 elimination of biosolids from the tank bottom (Masaló and Oca, 2016; Oca and Masaló,

256 2013; Timmons et al., 1998). In the case of RTs, 3-D complicated flow fields and low-

257 flow areas were observed in this study, confirming the findings of a previous study

258　(Takakuwa et al., 2018). Low-velocity areas were larger in the RT, as shown in Fig. 3.

259　Moreover, these low-flow areas and the eddy at the tank bottom coincided with the areas

260　where rotifers were distributed at high density. Thus, the low-flow areas in RTs may be

261　larger at the bottom than in CTs, leading to the sinking syndrome of PBT larvae because

262　low velocities and poor mixing of water in rectangular tanks lead to the creation of

263　stagnate areas, causing the accumulation of biosolids on the tank bottom (Oca and Masalò,

264　2007). These low-flow areas may have negative effects on larviculture (Sakakura et al.,

265　2019).

266　　　　　The advantage of numerical modeling of the flow field in a larviculture tank is

267　that we can visualize the field in the rearing tank without a flow meter or intensive labor,

268　and that one established model can be expanded to similarly shaped tanks with different

269　water volumes. Thus, the flow field in similar tank shapes can be easily estimated and the

270　model can help in designing the number and location of aerators and water inlets for the

271　larviculture tank. Our results also demonstrated that comparison of rotifer densities at

272　various sites in a rearing tank could approximately predict the low-flow areas that will

273　cause the sinking syndrome of marine fish larvae.

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275　　　　　In conclusion, the flow field in different larval rearing tank shapes affected the

276　survival of PBT larvae in our experiments. The present study demonstrated that flow field

277　patterns in small-scale CTs (50-l) at *AR*=1.3 are more feasible for the survival of PBT

278　larviculture experiments than those in RTs, and that improvement of aerators beyond a

279　single air stone to decrease low-flow areas at the tank bottom should be developed for

280　RTs.

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Figure captions

Fig. 1. Sites for rotifer distribution in (a) a cylindrical tank, and (b) a rectangular tank. One air stone was set at the center of the tank bottom.

Fig. 2. Three-dimensional streamlines predicted in this study in (a) a cylindrical tank (ø 46 cm × 29 cm depth), and (b) a rectangular tank (60×30×28 cm depth) with an aerator at the center of the bottom with 100 ml/min aeration.

Fig. 3. Cumulative area (%) of the velocity magnitude (< x mm/s) between the tanks applied in this study. For instance, the water mass having a velocity between 0–10 mm/s occupied 40% and 65% of total water volume in the CT and RT in this study, respectively.

Fig. 4. Visualization of streamlines at bottom regions of (a) a cylindrical tank (ø 46 cm), and (b) a rectangular tank (60×30 cm) in this study. An air stone was located at the center of each graph.

Fig. 5. Rotifer distribution (a) at half-cross section in the CT, and (b) in the quadrisection in the RT. Values are average of deviation values during the culture period (n=6, a>b>c>d, Tukey HSD, $p<0.05$).

1   **Table. 1.** Survival, growth and swimbladder inflation rates of *T. orientalis* larvae at 8 dph

2   in tanks of different shapes

Tank	n	Survival (%) *	Standard length (mm)	Dry weight (μg)	Swimbladder inflation (%)
Cylindrical	3	52.7 ± 5.1	4.06±0.25	95.1±17.6	16.5 ± 14.5 (n=30)
Rectangular	3	0.8 ± 0.7	3.91±0.20	67.7±10.9	56.9 ± 37.4 (n=1-8)

3   Results are mean values ± SD. The asterisk indicates a significant difference by *t*-test (p

4   < 0.01).

(a)

St.1 St.2 St.3

St.4 St.5 St.6

Air stone

St.7 St.8 St.9

23 cm

(b)

St.1 St.10 St.19
St.2 St.11 St.20
St.3 St.12 St.21

St.4 St.13 St.22
St.5 St.14 St.23
St.6 St.15 St.24

Air stone

St.7 St.16 St.25
St.8 St.17 St.26
St.9 St.18 St.27

30 cm

15 cm

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

(a)

-0.3^b -0.4^b -0.5^b

-0.2^b 0.1^b -0.1^b

Air stone

0.4^{ab} 0.1^b 0.9^a

23 cm

(b)

-0.3^{bcd} -0.4^{bcd} 0.3^{abcd}

-0.7^d -0.5^{bcd} -0.3^{abcd}

-0.4^{abcd} -0.5^{bcd} -0.3^{abcd}

-0.2^{abcd} 0.5^{abc} 0.4^{abcd}

-0.2^{abcd} 0.3^{abcd} -0.4^{abcd}

0.0^{abcd} 0.1^{abcd} -0.4^{abcd}

Air stone 0.9^a 0.2^{abcd} -0.4^{abcd}

0.6^{abc} 0.2^{abcd} -0.1^{abcd}

1.3^a 0.4^{abcd} -0.2^{abcd}

30 cm 15cm

Fig. 5.

(a)

(b)