

1 **Effects of Aeration Rate and Salinity Gradient on the Survival and Growth in the Early**  
2 **Life Stages of the Devil Stinger *Inimicus japonicus***

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6  
7 **Abstract:** We examined the effects of a flow field in the rearing tank exhibited by different  
8 aeration rate and salinity gradient on the larviculture of the devil stinger *Inimicus japonicus*.  
9 Two different rearing experiments using 1 *kl* rearing tanks were conducted from hatching (day  
10 0) to settlement. In the Experiment 1, fish were reared until day 21 in 5 different aeration rates  
11 (0-1200 *ml*/min). There was a significant and positive relationship between survival and  
12 aeration rate, and fish survival became stable at an aeration rate of more than 300 *ml*/min. In  
13 Experiment 2, the salinity gradient in a rearing tank was formed by pumping brackish water  
14 (22 ppt) from the surface and seawater (34 ppt) from the bottom at the same time. Control  
15 rearing tanks (34 ppt) were aerated at 300 *ml*/min and fish were reared until day 23. Growth  
16 and development was synchronized in the salinity gradient tanks with little standard deviation,  
17 although average survival at settlement in the salinity gradient tank (47.3 %) was lower than  
18 the aeration tank (68.2 %). The optimal aeration rate for devil stinger larviculture is stronger  
19 than 300 *ml*/min and introducing a salinity gradient can be a novel approach for enhancing  
20 performance of the fish.

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**Key words:** Devil stinger, Aeration rate, Salinity gradient, Larviculture

Physical environments in the rearing tanks are important factors for larviculture. In these environmental factors, water temperature and salinity are relatively easy factors to control and effects of different water temperature and salinities on larval survival have been well studied (e.g., Howell et al. 1998). On the other hand, little attention had been paid to the water flow in rearing tanks (Backhurst and Harker 1988). Recent studies on the relationships between flow field in the rearing tanks of larviculture and survival of fish larvae has revealed that the flow field in the rearing tank has the great impact on stable production for larviculture (Kolkovski et al. 2004; Ruttanapornvareesakul et al. 2007; Sakakura et al. 2007).

Devil stinger *Inimicus japonicus* is a commercially valuable demersal fish in Japan and seedling production has been conducted in many hatcheries as a candidate species for aquaculture and stock enhancement. However, rearing results fluctuate by mass mortality during larviculture, even though eggs are from the same batch and rearing is in the same temperature and tank proportions (Kadomura et al. 2007; Ruttanapornvareesakul et al. 2007). We hypothesized that this fluctuation is due to the differences in the rearing environment, such as water flow in the rearing tanks, and examined the different flow fields on the larval performance of this species. We conducted two different rearing experiments to determine the effects of different flow fields on the survival, growth and settlement of the devil stinger. First, different flow fields created by different aeration rate were investigated. Next, we examined the effects of a salinity gradient in the rearing tank on the larviculture of this species.

## Materials and Methods

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### *Materials*

Two different rearing experiments (Experiment 1 and 2) were conducted at Nagasaki Prefectural Institute of Fisheries from hatching to settlement (juvenile), and growth and survival of fish were compared. Newly hatched larvae were stocked into 1 *kl* cylindrical black tank (130 cm in diameter and 70 cm in depth). Fish were reared at the same temperature (average 25 °C) and water exchange rate (100 %/day). Feeding regime was also set to the same conditions following the previous study on the ontogenetic changes in size preference of prey in this species (Olsen et al. 2003), where L-type rotifer (Nagasaki Makishima strain) of *Brachionus plicatilis* (Fu et al. 1991) were fed from mouth opening (day 1) to 5.2 mm in standard length (SL) and *Artemia franciscana* nauplii were offered thereafter. Rotifers were enriched with HUFA enriched freshwater *Chlorella vulgaris* (Super Fresh Chlorella V12, Chlorella Industry, Fukuoka, Japan) and *Artemia* was enriched with commercial product (Super Capsule A-1, Chlorella Industry, Fukuoka, Japan). The rotifer density in each experimental tank was maintained at 10 individuals/*ml*, *Artemia* density was increased according to the fish growth (0.1-6.5 individuals/*ml*), and Super Chlorella V12 was added to the experimental tanks to adjust the density to  $5 \times 10^5$  cells/*ml* once daily. Water exchange rate of each tank was 100 %/day and dissolved oxygen was more than 4.83 mg/*l* in all experimental tanks throughout the experiment. Light condition was natural and maximum light intensity at the water surface was about 1000 lux for each experiment.

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### *Experiment 1: Effects of Aeration Rate*

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Naturally spawned eggs of devil stinger were obtained from Okayama Prefectural Institute of Fisheries and were transported to Nagasaki Prefectural Institute of Fisheries. On

69 July 12, 2005, newly hatched larvae (day 0; hatching rate, 96%) were stocked into rearing  
70 tanks at a density of 12,000 larvae/tank. A total of 10 rearing tanks were used and 5 different  
71 aeration rates (0, 50, 300, 600 and 1200 ml/min) were set with duplicates and fish were reared  
72 until day 21.

73 On day 21, survived fish were counted and survival rate was calculated for each tank.  
74 Then, settled juveniles with well developed pigmentation and transparent floating larvae were  
75 discriminated, and ratio of settled juveniles (%) was calculated for each tank. Ten settled  
76 juveniles in each tank were sampled and fixed in 5 % formalin solution and then standard  
77 lengths (SL) were measured using a digital microscope (VH6300, Keyence, Japan).  
78 Remaining fish were further reared for the stock enhancement program of the devil stinger in  
79 Nagasaki Prefecture.

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#### 81 *Experiment 2: Effects of Salinity Gradient in the Rearing Tank*

82 Naturally spawned eggs were collected at Nagasaki Prefectural Institute of Fisheries  
83 and newly hatched larvae were introduced into experimental tanks on June 26, 2006 (hatching  
84 rate, 98%). A total of 5 rearing tanks were used in this experiment. Three tanks were aerated  
85 at 300 ml/min as control group at fish density of 12,000 larvae/tank. In the other 2 tanks, a  
86 salinity gradient was formed according to Yogo (2008) by pumping brackish water (22 ppt)  
87 from the surface and seawater (34 ppt) from the bottom at the same time at a flow rate of  
88 about 350 ml/min for each salinity water (Fig. 1). Since we had realized that larvae distribute  
89 mostly in low salinity water in the preliminary trial, we introduced 6,000 larvae into a salinity  
90 gradient tank. According to the salinity in the salinity gradient tank measured by multi ware  
91 quality meter (Model 85, YSI Inc., Ohio, U.S.A.), we defined 3 layers from the free surface of  
92 water to the bottom of the rearing tank (Fig. 1); surface (0-20 cm depth, 22.0-25.0 ppt,  
93 respectively), middle (20-50 cm, 25.0-33.0 ppt) and bottom (50-70 cm, 33.0-34.0 ppt).



Fig. 1

94 Distribution of fish in each layer of the rearing tank was estimated at 2-5 days intervals. We  
95 counted larvae using the modified method of Masuda et al. (2001). We put an L-shape white  
96 board (10×10 cm) into the water at 10 cm intervals from the surface and projected the shade  
97 of the board over a water volume of approximately 1 l. We counted the number of fish in the  
98 projected water volume for 27 points and the average distribution in each layer was calculated.

99 On day 23, survival rate and ratio of settled juveniles (%) were calculated for each  
100 tank. SLs of 10 settled juveniles from each tank were measured with the same procedure as  
101 the Experiment 1. Remaining fish were further reared for the stock enhancement program of  
102 the devil stinger in Nagasaki Prefecture.

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#### 104 *Statistical Analysis*

105 In the Experiment 1, the relationship between aeration rate, and survival and growth of  
106 fish were analyzed by linear regression. In the Experiment 2, differences in survival, growth  
107 and ratio of settled juvenile between control and salinity gradient tanks were compared using  
108 Student's *t*-test. Values were considered significant at a 5% level of confidence.

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## 110 **Results**

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### 112 *Experiment 1*

113 There was a positive relationship between aeration rate and the final survival rates  
114 ( $n=10$ ,  $r=0.7477$ ,  $P<0.05$ ; Fig. 2). Tanks with higher aeration rate ( $>300$  ml/min) showed  
115 stable survival. A similar relationship was found between aeration rate and SL (range of mean  
116 SL among treatments, 8.5-9.0 mm; Fig. 2). Ratio of settled juveniles ranged 90.5-93.7 % in  
117 the aerated tanks but the ratio was relatively lower in the non-aerated tanks (85.1 %).

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Fig.2

119 *Experiment 2*

120 In the control tank, newly hatched larvae were equally distributed in the water column,  
121 and most of the fish distributed in the surface and middle layer thereafter (Fig. 3). In the  
122 salinity gradient tank, newly hatched larvae were distributed surface layer, and after mouth  
123 opening (day 1) they distributed both in the surface and middle layer (Fig. 3). On day 21,  
124 about 50 % of fish were distributed in the bottom layer as settled juveniles. Also, fish which  
125 distributed in the surface and middle layer were mostly settled juveniles and they adhered to  
126 the mesh and pipes for drainage in the tank (Fig. 1).



Fig.3

127 Average survival on day 23 in the salinity gradient tank (47.3 %) was lower than the  
128 control tank (68.2 %; *t*-test,  $P < 0.05$ ), whereas growth did not differ. Ratio of settled juvenile  
129 showed a higher trend than that of the control tank (*t*-test,  $P = 0.06$ ; Table 1).



Table.1

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131 **Discussion**

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133 We confirmed that the optimal flow field for larviculture of devil stinger is formed at  
134 over 300 *ml/min* in 1 *kl* water volume. The lower survival rate of 300 *ml/min*-aerated rearing  
135 tanks in Experiment 1 than Experiment 2 is due to the different handling for fertilized eggs.  
136 Since the eggs for Experiment 1 was transferred from another institute by a parcel delivery  
137 service, this process may affect the quality of eggs even though both batches had almost the  
138 same hatching rate. Introducing salinity gradient in the rearing tank resulted in synchronized  
139 development and growth with little standard deviation (Table 1) indicating that this is a novel  
140 approach for enhancing larval performance, although average survival at settlement in the  
141 salinity gradient tank was about 20 % lower (Table 1). Our results on survival at settlement in  
142 both aerated tanks (>300 *ml/min*) and salinity gradient tanks are fairly good compared to the

143 former studies where survival at settlement was 12.7% with 7 *kl* tanks (Kondou and Sugino  
144 1999) and 27.9-41.0% with 27 *kl* tanks (Kusaka 2007).

145 Theoretical and experimental studies (MacKenzie et al. 1994; Cury and Roy 1989;  
146 Kimura et al. 2004; Mangino and Watanabe 2006) had revealed that a particular turbulence  
147 enhances larval fish ingestion rate and survival, and a dome-shaped relationship is found  
148 between turbulence levels and survival of fish larvae. In the series of studies investigating  
149 optimal flow field created by aeration for larviculture of the seven-band grouper *Epinephelus*  
150 *septemfasciatus*, this phenomenon was also confirmed using the same rearing tank in this  
151 study (Shiotani et al. 2003, 2005; Sakakura et al. 2007). When different aeration rate (0-1000  
152 *ml/min*) was tested, the highest survival was achieved at the aeration rate of 200 *ml/min* and  
153 survival and feeding activity of larvae was extremely low both at 0 and 1000 *ml/min* for the  
154 seven-band grouper larvae (Shiotani et al. 2003) and this dome-shaped relationship was  
155 further utilized for the 100 *kl* mass-scale tank (Sakakura et al. 2007). In the larviculture of  
156 Pacific bluefin tuna *Thunnus orientalis*, mass mortality occurs when larvae sink and come  
157 into contact with the bottom of the rearing tank at the nighttime during the first 10 days after  
158 hatching (Masuma et al. 2011), and increasing in aeration rate during the night time enhances  
159 the survival both in 500 *l* scale (from 300 to 900 *ml/min*; Nakagawa et al. 2011) and in 50 *kl*  
160 scale (Tanaka et al. 2009). However, these were not the case in the devil stinger (Fig. 2) and it  
161 is noteworthy that survival rate became stable in the environment with stronger water flow  
162 (Sakakura et al. 2007), since devil stinger larvae with long pectoral fins (Kohno and Sota  
163 1998) had been believed to be fragile.

164 Ruttanapornvareesakul et al. (2007) examined the effects of rearing-tank proportions on  
165 early survival of the devil stinger using 3 different tanks with different water surface areas and  
166 the same water volume of 100 *l*. It was reported that the survival rate of devil stinger larvae  
167 was highest in the tank with the smallest free water surface, where larval movement under the

168 water surface is the shortest. Moreover, attaching the water surface significantly increased the  
169 physical and physiological stress of larvae (Ruttanapornvareesakul et al. 2010). Therefore,  
170 decreases in water surface tension by creating a smaller water surface area improve the  
171 survival rate of the devil stinger larvae. In this study, higher aeration rate resulted in higher  
172 survival with positive effects on growth indicating that active feeding was not interfered. The  
173 higher speed of the water current at the water surface, caused by strong aeration, may be  
174 strong enough to help free larvae from the water surface tension while decreasing the stress of  
175 larvae, and this can explain the positive relationship between aeration rate and survival of the  
176 devil stinger.

177         There are many studies that examined the effect of different salinities on survival in the  
178 early life stages of marine fishes (Howell et al. 1998). Some species exhibit higher survival at  
179 the lower salinity, such as orange-spotted grouper *Epinephelus coioides* (Toledo et al. 2002)  
180 and tawny puffer *Takifugu flavidus* (Zhang et al. 2010). Juvenile tiger puffer *Takifugu*  
181 *rubripes* shows higher growth rate at low salinity by enhanced food intake and food efficiency  
182 (Taga and Yamashita 2011). Midooka and Iida (2006) reported similar results in the devil  
183 stinger larvae where larvae showed higher survival when they were reared at 10-20 ppt  
184 seawater, and they concluded that less energy allocation of devil stinger for osmoregulation  
185 resulted in the higher survival than the full-strength of seawater. However, in this study,  
186 survival of the devil stinger in the salinity gradient tank was slightly lower than that of the  
187 control tank. This should be due to the differences in experimental settings between Midooka  
188 and Iida (2006) and this study. Former studies investigated the effects of salinity on fish by  
189 introducing fish to different salinity. On the other hand, we formed a salinity gradient in a  
190 rearing tank and let the fish choose where to stay. Consequently, larval distribution in the  
191 water column was different from the control tank especially during the first phase of the  
192 rearing trial where larval distribution at surface area is much higher than the control.

193 Although we introduced one-half larvae to the salinity gradient tank comparing to the control  
194 tank, aggregation at the surface layer with high density may have affected the survival of  
195 larvae in the salinity gradient tanks. Forming a salinity gradient is also quite successful in the  
196 larviculture of amphidromous fish ayu *Plecoglossus altivelis* (Yogo 2010) and Japanese  
197 flounder *Paralichthys olivaceus* at the hatchery-scale (Yogo 2005). This approach was the  
198 first trial for the devil stinger, and revealed that metamorphosis and settlement occurred  
199 synchronously during day 18 and 21 with considerable survival rate whereas the timing of  
200 settlement varied in the control tanks. Gavlik and Specker (2004) found that a decrease in  
201 rearing salinity from 30 to 20 ppt in the metamorphosing stage of summer flounder  
202 *Paralichthys dentatus* synchronized the metamorphosis and settlement. Devil stinger settle in  
203 the shallow water after the metamorphosing stage, similarly to flatfishes. Therefore, we  
204 assume that the synchronization in development of the devil stinger occurred in the salinity  
205 gradient tank considering the distribution of larvae at 22-30 ppt.

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- 297

298 通気量と塩分勾配がオニオコゼ仔稚魚の生残と成長に与える影響

299

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301

302 異なる通気量と塩分勾配がオニオコゼ仔魚飼育に与える影響を調べた。容量 1 *kl* の  
303 円形水槽で 2 つの飼育実験を孵化仔魚 (0 日齢) より着底まで実施した。実験 1 では  
304 5 つの異なる通気量 (0-1200 *ml/min*) で 21 日齢までの飼育を行った。その結果、最  
305 終日の生残と通気量の間有意な正の相関が検出され、300 *ml/min* 以上の通気量で生  
306 残が安定した。実験 2 では飼育水槽に水面から 22 ppt の汽水、底面から 34 ppt の海  
307 水をそれぞれ連続注入することで塩分勾配を形成させ、対照に 34 ppt 海水に 300  
308 *ml/min* の通気を施す水槽を設け、23 日齢まで飼育した。その結果、塩分勾配水槽で  
309 飼育した魚の成長と着底が同調したが、飼育終了時の生残 (47.3 %) は対照のそれ  
310 (68.2 %) より低い傾向があった。1 *kl* 規模でのオニオコゼの仔魚飼育には 300  
311 *ml/min* 以上の強い通気による水流が必要であることが明らかになり、さらに塩分勾  
312 配の飼育水槽への導入により種苗生産に新たな可能性を見いだした。

313

314 **Table 1.** Comparison of survival, growth and development of the devil stinger between  
315 control (aeration rate 300 ml/min) and salinity gradient (22-34 ppt) tank on 23 days after  
316 hatching

	Control	Salinity gradient
<i>n</i>	3	2
Survival (%)	68.2 ± 6.0*	47.3 ± 0.9
Standard length (mm)	8.28 ± 0.33	8.96 ± 0.13
Settled juvenile (%)	75.3 ± 10.2	93.3 ± 0.6

317 Results are mean values ± SD. An asterisk indicates significant difference between treatments  
318 (*t*-test,  $P < 0.05$ ).

319

320 **Fig. 1.** Schematic drawing of salinity gradient tank.

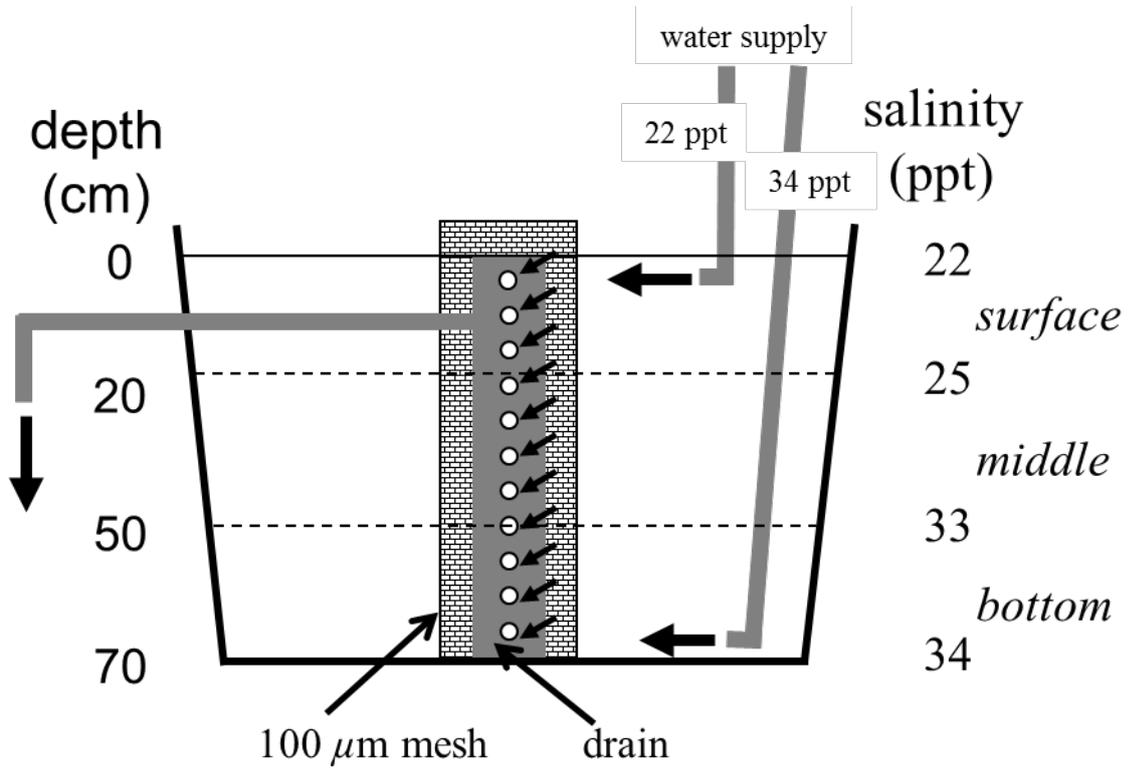
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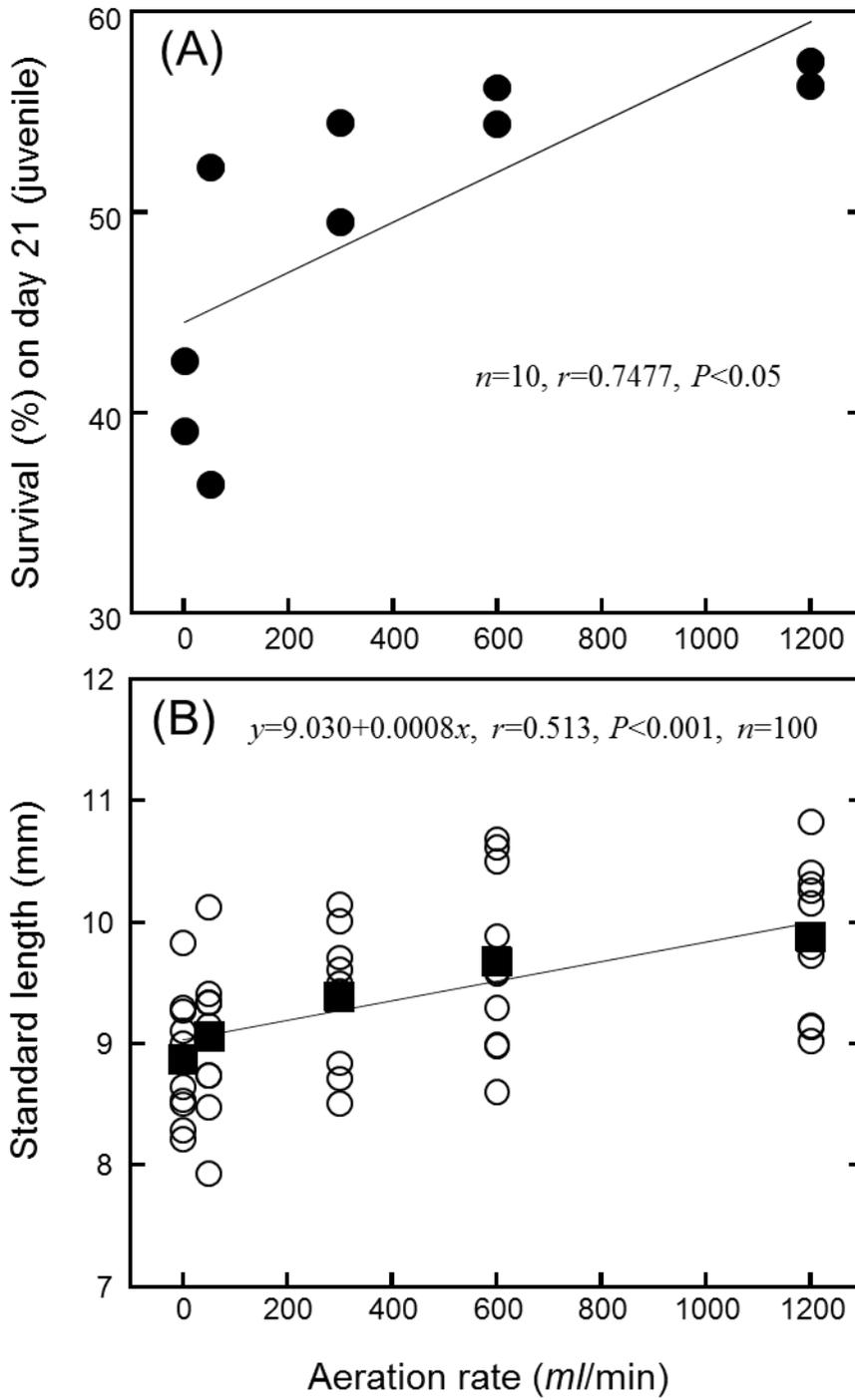
322 **Fig. 2.** Effects of aeration rate on (A) the survival and (B) growth (open circle, individual  
323 data; closed square, average) in the devil stinger reared in a 1 *kl* round tank at 21 days after  
324 hatching.

325

326 **Fig. 3.** Changes in estimated fish distribution in a 1 *kl* round tank reared at (A) aeration rate of  
327 300 *ml/min* and (B) salinity gradient. Open column denotes surface, shaded column for  
328 middle, and closed column for bottom layer of the experimental tank, respectively (see Fig. 1  
329 for the details of depth and salinity at each layer).

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