Flow field control in marine fish larviculture tanks: lessons from groupers and bluefin tuna in Japan

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Abstract

Flow field in a larviculture tank is often based on the empirical assumptions by fish culturists and science-based information had been very limited. Therefore, we studied and reviewed research on the flow field dynamics in larviculture tanks of groupers and Pacific bluefin tuna, and compared them with results from our own and other studies. For radial symmetry tanks (cylindrical or octagonal), aeration by one aerator at the bottom of the

tank forms vertical circulation. We quantified and visualized this flow at different aeration rates in a 1 kL tank and compared survival rates of seven-band grouper *Epinephelus septemfasciatus* and devil stinger *Inimicus japonicus*. The highest survival with the lowest surface tension-related death (STRD) was achieved when vertical flow velocity above an aerator was 8 cm/sec (200 mL/min) for larvae of *E. septemfasciatus*, while better survival was observed at more than 8 cm/sec in *I. japonicus*. Larviculture tank proportions also influence both flow field and performance of fish larvae. When tanks were set with the same water volume (100 L) and aeration rate (50 mL/min) but different aspect ratios (*AR*: water depth/tank radius), survival of larvae for the above two species in a tank with *AR* greater than 2.0 was found to be significantly higher with lower STRD. Flow field in a vertical cross-section of a tank changed from a single-pair vortex system to two-pair vortex systems as *AR* changed from 1.0 to 2.0. However, sinking syndrome, which causes high mortality by sinking of larvae to the tank bottom during darkness in some marine fishes (i.e., Pacific bluefin tuna *Thunnus orientalis*), cannot be prevented with the above conventional flow field management. Two flow field control methods have been proposed in order to prevent sinking syndrome. One is increasing the aeration rate at darkness for vertical mixing of the rearing water. The other is a 'water pump system', where a water pump was put in a small net cage with fine mesh connected to a drain at the center of the tank, and water from the water pump was discharged via a cross-shape pipe on the tank bottom. Both were found to be effective to prevent sinking syndrome. Flow fields in the conical-cylindrical tanks and the rectangular tanks, which are used widely and have many different proportions, have not been well quantified yet. We have expanded our approach investigating the flow patterns in rectangular tanks by three dimensional two-phase aerated flow simulations, and found that velocity fields were different from those of cylindrical tanks.

Key words

grouper; Pacific bluefin tuna; aeration; surface tension-related death; sinking syndrome

1. Introduction

Physical environment of larviculture tanks is an important aspect for larviculture. Many fish culturists claim that management of the flow field and the distribution of fish larvae, live feeds (i.e. rotifers and *Artemia*) and artificial pellets in the rearing tanks are important for the success of marine fish larviculture. Flow field in a larviculture tank is commonly generated by aerators, and is very important to prevent stratification, to insure oxygenation and to disperse live feeds and artificial diets (Sakakura, 2017; Backhurst and Harker, 1988). However, the bubbles by aerators will hit and/or injure larvae, and strong aeration may keep larvae from feeding and waste energy for orientation in the water column which is lethal to larvae (Tucker, 1998). Thus, flow in a rearing tank is assumed to have great impact on marine fish larvae and to provide a basis for tank design for larviculture (Harboe et al., 1998; Kolkovski et al., 2004a). Tank volume (Theilacker, 1980; Estudillo et al., 1998), tank shape (Ruttanapornvareesakul et al., 2007; Moody et al., 1992; Moore et al., 1994), both tank volume and shape (Cook et al., 2015), aeration rates (Sakakura et al., 2007; 2014), and water inlet and outlet (Oca and Masalò, 2013) are known to affect water circulation and larval performance such as survival and growth. However, these considerations are mainly based on the empirical assumptions by fish culturists and science-based information had been very limited. Therefore, we studied the flow field in the rearing tanks in order to investigate the optimal flow for some marine fish larviculture. This paper reviews our recent findings on flow field control by integration of rearing trials of some marine fish larvae (mainly groupers and Pacific bluefin tuna) in Japan and hydrodynamics in larviculture tanks.

Both aeration rate and flow rate (water inlet/outlet) are important factors for the flow field in rearing tanks. Some studies which quantified flow field in the fish culture tanks (Klapsis and Burley, 1984; Burley and Klapsis, 1985; Oca and Masalo., 2013; Gorle et al., 2018) used high flow rates to create circular flow in the tanks with different shapes, assuming the grow-out and broodstock culture. However, in case of larviculture, water exchange rates by water inlet/outlet are set to very low levels (from static condition to 100 %/day; Shields, 2001) compared to the juvenile culture (> 100 %; Takebe et al., 2011) and grow-out (>1200 %; Oca and Masalo, 2013). When we compared the flow velocity created by aeration, water inlet and a combination of these during larviculture, it was revealed that flow velocity by water inlet (water exchange rate < 100 %/day) was negligible both in small scale tanks (<1 kL water volume) and in large scale tanks (100 kL water volume; Shiotani et al., 2003; Sakakura et al., 2006). Thus, in this paper, we mainly focus on the flow field created by aeration (aeration rate) rather than by flow rate.

We conducted larviculture trials with different aeration rates and tank shapes to compare larval survival and growth, studying two crucial biological phenomena in the early phase of marine fish larviculture, namely “surface tension-related death” (STRD) and “sinking syndrome”. In STRD, mucus secreted on the body surface of larvae functions as a glue when larvae are attracted or carried to the water surface causing high mortality (Yamaoka et al. 2000). STRD is known to occur in the larviculture of groupers (Sakakura et al., 2007) and Pacific bluefin tuna *Thunnus orientalis* (Masuma et al., 2011) shortly after hatching. Therefore, reduction of STRD is essential for effective larviculture of these species. We used cylindrical tanks in order to determine the optimal flow field and aeration methods for reducing STRD by hydrodynamic approach that quantify and visualize flow field in rearing tanks. Recently, sinking syndrome was found to be more problematic than STRD in larviculture of groupers (Hirata et al., 2009; Takebe et al., 2011) and Pacific bluefin tuna (Tanaka et al., 2009). Sinking syndrome causes high mortality (Masuma et al., 2011; Tanaka et al., 2009), and occurs when larvae settle on the tank bottom during darkness. The larval body density of Pacific bluefin tuna is higher than that of the sea water density, and larval swimming activity is low (Sakamoto et al., 2005; Takashi et al. 2006). It was realized that sinking syndrome cannot be prevented with conventional flow field management and several methods were proposed. We also expanded our approach and have started measuring the flow field in the rectangular tanks with different aspect ratios.

2. Flow field in the radial symmetry tanks

2.1 Management of flow field to prevent STRD by aeration rate

We conducted a series of larviculture experiments using seven-band grouper *Epinephelus septemfasciatus* (Sakakura et al., 2007) and devil stinger *Inimicus japonicus* (Sakakura et al., 2014) clarifying the optimal flow in rearing tanks, and distribution of rotifers and fish larvae. These experiments used flat-bottomed cylindrical tanks with one aerator (air stone) at the center of the bottom of the tank and different aeration rates (Fig.1a). In these studies, we chose commercially available cylindrical tanks which are commonly used in the Japanese hatcheries (SPS-1000 or SPE-1000, Tanaka Sanjiro Co., Ltd., Fukuoka, Japan). The tank was cylindrical with a 154 cm diameter at the top and a height of 82 cm, and water volume was 1 kL, and water exchange rate was set at 100% per day. The air stone was spherical and ceramic-made which is commercially available (C-1B, Tanaka Sanjiro Co., Ltd.). Air bubble size from aerators affects not only the bubble surface area for gas exchange (Kolkovski et al., 2004b), but also the larval survival (Ellis et al., 1997). The average bubble size from the air stone in this review was 1-2 mm in diameter (Sumida et al., 2013), and we assume that the bubble size is comparable to the other larviculture studies ranging from 0.15 mm (Pavlidis et al., 2000) to 5 mm (Olivotto et al., 2008). Distribution of rotifers (*Brachionus plicatilis* sp. complex) and fish larvae in the water column was observed, and survival rates of fish larvae 10-21 days after hatching were compared. We also measured flow field in these tanks with different aeration rates.

Fish larvae formed dense patchiness beneath the free water surface, however, rotifers distributed evenly in the water column when aeration was provided. The highest survival and the lowest STRD were achieved when aeration rate was 200 mL/min for larvae of *E. septemfasciatus* (Fig.2a; Shiotani et al., 2003). Theoretical and experimental studies (MacKenzie et al., 1994; Cury and Roy, 1989; Kimura et al., 2004; Mangino and Watanabe, 2006) had revealed that a particular turbulence enhances prey consumption rate and larval survival, and a dome-shaped relationship is found between turbulence levels (aeration rates) and survival of fish larvae. Our result of *E. septemfasciatus* matches these former findings, indicating that there is an optimal aeration rate for larviculture. However, this was not the case in the devil stinger (Sakakura et al. 2014) and a significant and positive relationship between aeration rate and larval survival was detected ($n=10$, $r=0.7477$, $p<0.05$), and fish survival became stable at an aeration rate of greater than 300 mL/min (Fig. 2b). It is noteworthy that strong aeration resulted in higher survival of devil stinger larvae, because this species with long pectoral fins (Kohno and Sota, 1998) had been believed to be fragile. Thus, we propose that the optimal flow field for larviculture created by aeration is species specific and should be carefully examined in each target species.

We found that water exchange did not significantly affect the flow field in the tank and also dissolved oxygen levels were the same among the different aeration rates (0-1200 mL/min). We used an acoustic Doppler velocity meter (NVD Field, Nortek, Sandvika, Norway) and measured velocity distribution of flow in a vertical section on a radius of the tank. The number of grid points for measurements was 9×24 in the horizontal directions, and the grid spacing was at 1-10 cm. The mean velocities of three dimensional components of flow in the rearing tank were obtained from sampling data. When aeration was provided in the tank, the stationary flow in the rearing tank was vertical regardless the aeration rates and the horizontal circulation was almost negligible (Fig.1b). The optimal flow field for *E. septemfasciatus* larviculture was at the aeration rate of 200 mL/min, where vertical flow velocity above an aerator was 8 cm/sec and the maximum flow velocity at free water surface was 6 cm/sec for larvae of *E. septemfasciatus*.

The above approach was informative because we quantified the optimal flow field and presented actual values of velocity and/or aeration rates for *E. septemfasciatus* larvae. It was also informative for managing flow field for larviculture of other species. Thus, we expanded this approach to the mass-scale culture and investigated the optimal flow field for *E. septemfasciatus* larviculture (Sakakura et al., 2006). The larviculture tank in the Nagasaki Prefectural Institute of Fisheries, Nagasaki, Japan, was used, which size is a diameter of 8.0 m, a depth of 1.87 m and a volume of 100 kL. When several aerators were installed in these large-scale tanks, which was common in many hatcheries using such mass-scale rearing tanks for larviculture, early survival of *E. septemfasciatus* larvae fluctuated and average survival was less than 30% at 10 days after hatching. We hypothesized that setting the multiple aerators in a mass-scale rearing tank produces several upwelling zones in the tank which may cause physical damage to larvae more frequently. Additionally we hypothesized that the optimum flow field for larviculture of the seven-band grouper in the mass-scale rearing tank can be the same level as 1 kL tank where vertical upwelling was at about 8 cm/sec. With these hypotheses, we set an aerator at the center of the rearing tank, surrounding the cylindrical drain (1.2 m in diameter) to generate the flow field so that the upwelling is gathered in the center of the tank and kept at about 8 cm/sec (630 mL/min aeration rate). Then STRD reduced significantly and the survival rate at 10 days after hatching in the new aeration method (61.5 ± 5.1 %, n=7) was about 3 times higher than the former methods (21.2 ± 13.7 %, n=6).

However, measuring flow in the larval tanks is time consuming. In addition, since the optimum flow field varies not only with fish species, but with developmental stage of fish and tank shape, the measurement of flow for each case is impractical in terms of time and cost. In order to streamline these flow field measurements, we developed a computation model for estimating the flow field in cylindrical tanks (Shiotani et al., 2005; Sumida et al., 2013). The flow in a rearing tank was calculated two-dimensionally based on experimental results of actual measurements. The simplified method of calculation was satisfactory for determining the stationary flow and velocity in the rearing tank; the method compared favorably to the results obtained in the actual experiments. Therefore, this computation model enables us to estimate the flow field in various scales of rearing tanks and utilize the estimated flow for designing the rearing conditions for larviculture.

2.2 Effect of aspect ratio of the tank on STRD

The proportion of a rearing tank influences both the flow field and performance of marine fish larvae (Cook et al., 2015; Ruttanapornvareesakul et al., 2007; 2010). We introduced 2 marine fish species larvae, seven-band grouper and devil stinger, into commercially available cylindrical tanks with 3 different proportions (SPS-200, SPS-100 and SLP-100, Tanaka Sanjiro Co., Ltd.). These tanks were set with the same water volume (100 L) and aeration rate (50 mL/min), but with different aspect ratios (*AR*: water depth/tank radius), where greater *AR* indicates that a tank has lower water surface with greater water depth. Those *AR* values were respectively 0.74, 1.36, and 3.29. The highest survival and the lowest STRD of larvae in both species were achieved in a tank having an *AR* 3.29 and significantly low survival with higher STRD in tanks with lower *AR*s were observed (Fig.3). Fish in tanks with a high *AR* (3.29) showed less physiological stress as determined by enzyme activities (Ruttanapornvareesakul et al., 2010). Ruttanapornvareesakul et al. (2007) speculated that the chances of larvae being captured by the surface tension were reduced because the speed of larval movement over the water surface is fast, and as a result the number of STRD was reduced. However, they only observed larvae which had been carried as far as the water surface by the water flow, and they assumed that the vertical circulation in the tank of *AR*=3.29 is similar to those cylindrical tanks of 1 kL (*AR*=0.53) and 100 kL (*AR*=0.50).

Recently, we quantified and visualized the flow field in cylindrical tanks with different *AR*s (Sumida et al. 2013). A transparent circular tank with diameter=390 mm and height=590 mm was used in the experiments. An air stone was set at the center of the tank bottom and aeration rate was set at 10, 25, or 50 mL/min. Then, water depth was varied according to the value of the projected cross-section *AR*s. The AR was varied as 0.5, 1.0, and 2.0, respectively. In order to accurately discriminate the detailed flow field, we applied Particle Image Velocimetry (PIV) by a combination of the dye streakline method and the suspension method. The dye streakline method is a method where a dye solution is injected into the flow as a tracer so that the streaklines depicted by the tracer can be observed. A sodium fluorescein solution was used as the dye. The suspension method was one in which a microscopic solid-state tracer was injected directly into the flow field and after being suspended in the water, the flow pattern was obtained by following the tracer. The tracer consisted of aluminum powder with an average particle diameter of 40 μm and specific gravity of 2.7. The light source used for the visualization was a slide projector. A slit light source of width 20 mm was set up by inserting a film with a slit cut in it into the light source section. To reduce the light incident in the tank, a 10 mm slit was fashioned with black paper stuck onto the tank at the position where the light was incident. Visual images were collected at a position perpendicular to the light path using a digital camera. Moving images were obtained with a video recorder. Fig.4 shows photographs visualizing the flow patterns over the whole area in the tank for a fixed aeration rate = 50 mL/min, and *AR* = 0.5, 1.0, and 2.0 respectively. The vortex structure can be seen that large vortex structures exist inside the tank, one pair symmetrically arranged left and right (*AR*=0.5 and 1.0). However, two pairs are seen in *AR*=2.0, one pair in the upper region and another in the lower region. Flow field in a vertical cross-section of a cylindrical tank changed from a single-pair vortex system, which is generally accepted for the flow pattern in a rearing tank with one air stone, to two-pair vortex systems as the value of *AR* changed from 1.0 to 2.0. Further, aeration had a weak effect on the changes in the vortex pair system.

These findings added new insights about the effects of *AR* on the survival of some larvae. Larvae in high *AR* (>2.0) tanks were transported into either of the two pairs of vortex structures as shown in Fig.4 and then the chance to attach free water surface may be reduced comparing to a tank with an *AR* less than 2.0. Thus, these two-pair vortex systems were formed in the tank with *AR*=3.29 (Ruttanapornvareesakul et al., 2007) and led the results of the highest survival with the lowest STRD. The cylindrical tank with *AR*=3.29 (SLP-100) was originally produced for the culture of microalgae to illuminate the tank effectively by fluorescent lights from side. However, this tank was proven to be effective for small-scale marine fish larviculture, and is convenient in terms of space saving for the laboratory. This tank is now used for small-scale laboratory studies for nutritional study of live feeds (Wullur et al., 2011; Kim et al., 2014; Hagiwara et al., 2016) and for developmental engineering (Takeuchi et al., 2009; 2017; Yazawa et al., 2016) in marine fish larvae.

2.3 Management of flow field to prevent sinking syndrome

In addition to a marked number of STRD of larvae, 'sinking syndrome' has become problematic. Larvae stop swimming during darkness and then sink to the bottom of the tank and die. Sinking syndrome has been reported in many fish species: amberjack *Seriola dumerili* (Shiozawa et al., 2003; Teruya et al., 2009), barfin flounder *Verasper moseri* (Kayaba et al., 2003), Pacific bluefin tuna (PBT) *Thunnus orientalis* (Tanaka et al., 2009; Masuma et al., 2011), seven-band grouper *Epinephelus septemfasciatus*, kelp grouper *E. bruneus* (Hirata et al., 2009), and leopard coral grouper *Plectropomus* *leopardus* (Takebe et al., 2011). Recently, it was confirmed that the initial mortality of PBT is mainly due to sinking syndrome rather than to STRD (Tanaka et al., 2009). The sinking of PBT larvae to the tank bottom occurs during darkness (Masuma, 2011; Tanaka et al., 2009) as same as the other reported species, presumably because the larval body density of PBT is higher than that of the sea water density, and larval swimming activity is low during the night (Sakamoto et al., 2005; Takashi et al. 2006). Ina et al. (2014) found that larvae of PBT which failed swim bladder inflation were more frequently observed at the bottom layer of larviculture tank, and these larvae with un-inflated swim bladders are hard to maintain the buoyancy from sinking to the tank bottom during darkness. Several methods are proposed to prevent the larval death by sinking syndrome: 1) continuous illumination (Teruya et al., 2008), 2) increasing the aeration rate during darkness (Nakagawa et al., 2011), 3) “water pump system” (Masuma et al., 2011), and 4) combinations of continuous illumination and water pump system (Takebe et al., 2011).

Continuous illumination is common practice in larviculture of marine fishes, and is known to enhance survival and growth of larvae (Villamizar et al., 2011; Cook et al., 2015). Continuous illumination was also reported to decrease the mortality by sinking syndrome in the seven-band grouper (Teruya et al., 2009), yellow fin tuna *Thunnus albacores* (Partridge et al., 2011) and PBT (Kurata et al., 2017). Kumon et al. (2018) compared larval survival and growth of PBT between cycling photoperiod (14h light:10h darkness) and continuous illumination at 2000 lux on the water surface. They used 500 L cylindrical tanks (diameter 100 cm, height 62 cm, *AR*=1.24; SPS-500, Tanaka Sanjiro Co., Ltd.) with one aerator at the center of the bottom of the tank at the aeration rate of 100 mL/min. Larval survival of PBT was 9-times higher under continuous illumination (15.5 ± 7.6 %, n=3) than cycling photoperiod (1.8 ± 0.6 %; Two-way ANOVA, n=3, photoperiod: df=1, F=4.94, p<0.05, age: df=4, F=72.64, p<0.0001), where cumulative numbers of dead larvae by sinking syndrome was lower in continuous illumination. Further, larvae at continuous illumination was significantly larger (4.8±0.3 mm in total length) than those at cycling photoperiod (4.5±0.3 mm). Thus, continuous illumination is effective for preventing sinking syndrome in PBT larvae. However, applying continuous illumination for long duration requires careful consideration, because fast gut transit in continuously feeding larvae may cause essential but slowly digestible compounds to be lost in the feces, while in larvae on a cycling-photoperiod feeding regime, a longer transit time increases the absorption of nutrients that are critical for development (Rønnestad et al., 2013).

The idea of increasing the aeration rate during darkness is that larvae remain suspended in the water column segments where larval sinking velocity is balanced by upward flow velocity for vertical mixing of the rearing water with increasing aeration rate during darkness (Nakagawa et al., 2011). It is simple method, however, higher air input and water flow during darkness may cause physical stress to larvae. Therefore, Nakagawa et al. (2011) determined optimal aeration rate during darkness. They used 500 L cylindrical tanks (SPS-500), where one air stone was set at the bottom center of each tank. They set the aeration rate at 300 mL/min in the daylight and changed aeration rate from 0 to 900 mL/min in the nighttime, and compared the survival of PBT larvae at day 10. The flow field created by aeration was similar to that of Sakakura et al. (2007; Fig.1b) with a single-pair vortex system. The highest survival was achieved at aeration rate of 300 mL/min in daytime and 900 mL/min in nighttime, indicating that increasing the aeration rate during darkness significantly reduce the risk of sinking syndrome. Similar positive effect was found in the kelp grouper *Epinephelus bruneus* (Fui et al., 2014) using the same condition as Nakagawa et al. (2011). When aeration rate was increased at 900 mL/min in the darkness, kelp grouper larvae showed higher survival with low sinking syndrome than those at 0 and 300 mL/min at darkness. However, this approach has not been determined in the mass-culture tanks with higher water volume.

The water pump system produces a water current at the bottom of the rearing tank (Masuma et al., 2011). We introduced the water pump system for the mass-culture of leopard coral grouper *Plectropomus leopardus* (Fig.5; Takebe et al., 2011). We used 60 kL octagonal tank (water volume 50 kL, base area 25 m^2), where a water pump (CSL-100, Terada Pump Mfg Co., Ltd, Nara, Japan) was put in a rectangular net cage connected to drain (50×50×150 cm, 0.252 mm mesh opening) at the center of the tank. Rearing sea water from the water pump was discharged via a cross-shape PVC pipe (diameter 13 mm) on the tank bottom, which has 2 mm diameter holes at intervals of 10 cm. The angle of water flow direction from the holes was adjusted to 0º against the tank bottom. The water flow rate of the pump was 1.5-2.2 kL/h. A counter-clockwise and upward current was produced. In addition to the water pump system, two rectangular aerators (5×5×17 cm) were set next to the cage outside of drain in order to prevent larvae from aggregating into the cage mesh. Further, because of the difficulty for setting the air stone at the center of the tank for vertical circulation of rearing water, four aerators (diameter 13 mm, length 500 mm) were set at the four corners of the tank bottom. The aeration rate was 500 mL/min for each aerator. We conducted larviculture trials using this water pump system and compared the survivals from the former rearing method with several aerators in the rearing tank. The larvae in the water pump system showed significantly higher survival and numbers of produced juveniles/tank was 113±32 thousands (20.3±8.7 % in survival rate, n=3), while former method only produced 2 thousands juveniles/tank in average

(0.6 % in survival). It clearly indicates that water pump system can significantly reduce the early mortality of leopard coral grouper larvae as well as PBT (Masuma et al., 2011; Tanaka et al., 2009). In this water pump system, the counter-clock wise water current is formed at the bottom of the rearing tank, and it is assumed that the upwelling current prevented the larvae from sinking. Although this system greatly improved the survival rate of larvae in *P. leopardus* mass-culture, flow field in the water pump system could not be measured with conventional surveying instruments and clarifying the flow field and larval distribution in a tank equipped with a water pump system will be the future research topic.

2.4. Management of flow field in cylindrical tanks with conical bottoms

Many hatcheries also utilize cylindrical tanks with conical bottoms (e.g., Kolkovski et al., 2004a,b; Lika et al., 2015; Moorhead, 2015). Since the water volume and vertex angle of the conical tanks vary among the tanks in hatcheries, we found difficulty in generalizing flow field in these conical tanks. It is generally accepted that cylindrical tanks with conical bottoms are superior to those with flat-bottomed tanks in regard to the uniform circulation of water and self-cleaning (with center drain), however, the literatures which quantified flow field in the conical tanks are scarce. Backhurst and Harker (1988) compared the aeration rates for suspending particles (1.3 mm in diameter and 1.024 g/cm^3 in specific gravity) in the water column between a cylindrical tank with flat bottom and a tank with conical bottom. They revealed that the minimum aeration rate suspending particles in a cylindrical tank with conical bottom (520 mL/min/volume of tank (L)) is lower than that of a tank with flat bottom (1410 mL/min/volume of tank (L)).

Vertical circulation can be easily formed by aerator or upwelling water inlet. Harboe et al (1998) used a conical tank and set one air stone at the center of the bottom of the tank for larviculture of Atlantic halibut *Hippoglossus hippoglossu*s. They noted bubbles from air stone created circular flow and distribution the larvae, but did not quantify the flow field. Lika et al. (2015) measured flow fields in the conical tanks with three different water volumes (40, 500, and 2000 L). They created vertical circulation by the combination of upwelling water inlet from the bottom of the tank (100%/h of water exchange rate) and aeration at 30 mL/min (40 L tank), 180 mL/min (500 L tank) and 250 mL/min (2000 L tank), respectively. Average water velocity at the surface layer (0.148 mm/sec) and the bottom layer (0.661 mm/sec) in 2000 L tank were 10 times higher than those in 40 L tanks, and 500 L tank showed intermediate velocity. The velocities in the 500 and 2000 L tanks by Lika et al. (2015) is one digit lower than those by Shiotani et al. (2003) where they used 1000 L cylindrical tanks with flat bottoms (Fig. 1). This may suggest that the conical tanks provide more gentle circulation flow than flat bottomed tanks as indicated by Backhurst and Harker (1988). Comparison of flow field and larviculture performance between cylindrical tanks with flat bottoms and tanks with conical bottoms at the same scale will be necessary for future research.

A tank with similar bottom structure as the conical bottom is the Kreisel tank which is like a horizontal cylinder. This apparatus was developed for zooplankton culture (Greve., 1968) and has been modified for larviculture of marine invertebrates such as phyllosoma larvae (Kittaka, 1997; Matsuda and Takenouchi, 2005; Goldstein and Nelson, 2011). Vertical circulation of water is formed along the tank wall by aeration or water inlet and high water velocity around the tank wall prevents larvae from attaching to both tank wall and water surface. This feature is ideal for fragile organisms for keeping them in the center of the water column. Therefore, this apparatus is also used for larviculture at experimental scale for ornamental marine fishes (Moorehead, 2015) and Japanese eel *Anguilla japonica* (Okamura et al., 2009), which are known to be fragile. Blanco et al. (2014) compared the survival and growth of long-snouted seahorse *Hippocampus guttulatus* between rectangular and Kreisel tanks with the same water volume. They found that fish in Kreisel tanks had better survival and growth. We propose that Kreisel tanks will be applicable for the experimental larviculture of groupers and tunas to prevent sinking syndrome.

3. Flow field in the rectangular tanks

Rectangular tanks including raceway tanks are commonly used in larviculture facilities. In general, rectangular tanks are easier to handle and clean than cylindrical tanks. However, low velocities and poor mixing of water in rectangular tanks lead to the creation of stagnate areas, causing the accumulation of biosolids on the tank bottom (Oca and Masalò, 2007). Attempts have been made to create circular flow field in the rectangular tanks (Burley and Klapsis, 1985; Cripps and Poxton, 1993; Oca et al., 2004; Oca and Masalò, 2007; Duarte et al., 2011). These studies investigated the optimal flow field for grow-out culture in the rectangular tanks by comparing the different flow rates, water volumes, water depths, and designs of water inlet/outlet. These studies reported that higher flow rate, shallower depth and arrangement of water inlet/outlet are effective for water circulation in the rectangular tanks. Backhurst and Harker (1988) examined the effects of both tank shapes and air stone types on suspending particles (1.3 mm in diameter and 1.024 g/cm^3 in specific gravity) in the water column. For the minimum aeration rate suspending particles in a rectangular tank with flat bottom (60×29×25 cm), a long air stone (15 L/min) is lower than that of a cylindrical air stone (30 L/min).

However, high flow and shallower depth of water are not realistic to marine fish larviculture, where we handle fragile fish larvae. Therefore, we assumed the flow field in the rectangular tanks of larviculture where flow rate is very low or static and water circulation is mainly created by aeration. Since there are many different shapes in rectangular tanks with volume and *AR*s, it is not realistic to measure every case. Thus, we have started investigating the flow patterns in rectangular tanks by three dimensional two-phase bubbling flow simulations (Takakuwa et al., 2018). We prepared a rectangular tank with square bottom (288×288 mm), and set 3 different *AR*s (0.5, 1.0, 2.0) by changing the water volume (Fig.6). One air stone was set and air was diffused at 432 mL/min. Given the experimental conditions, PIV was applied to measure the bubble velocity at the air stone by tracing air bubbles, which was used as the initial setting of the simulations. Then, we calculated the velocity fields in the tanks by three dimensional two-phase bubbly flow simulations (Sumida et al., 2013) using a dispersed flow model.

We visualized flow field calculated by the model at 3 different vertical sections of the tank (Fig.7a-c); cross-section 1) the midsection of the tank including air stone, cross-section 2) a section at quarter from the tank wall to the other side of the tank wall, and cross-section 3) at the tank wall, respectively. Fig.8 shows the 2-dimensional flow velocity distribution at the different vertical sections as indicated in Fig.7. At the cross-section 1, flow velocity accelerates with the bubble flow above the air stone, and the area with high velocity increased as *AR* increases. This is coincident with the case of cylindrical tanks (Sumida et al., 2013). Also we observed a single-pair vortex system similar to the cylindrical tanks at *AR*=0.5 and 1.0. However, we could not observe the 2-

pair vortex system at *AR*=2.0 which was found in the cylindrical tanks. At the cross-section 2 and 3, the flow fields were more complicated and were different from the case of cylindrical tank. At the cross-section 2 of *AR*=0.5, four vortices were lined in the horizontal direction, while its vortex structure at the cross-section 1 was similar to that observed in *AR*=1.0 and 2.0. In the cross-section 3 (tank wall) of *AR*=0.5, downward flow from the water surface to the bottom was observed, and there were small eddies at the corners of tank bottom. In case of *AR*=1.0 and 2.0, downward flow from the water surface to the bottom formed flows from the tank wall to the center of the bottom wall, and this flow created small eddies around the boundary of downward flow. These small eddies and slow velocity layers around the bottom of the tank may be specific flow field in the rectangular tanks. These small eddies and slow velocity layers found in the corners of rectangular tanks may affect the distribution of larvae and live feeds, and the water quality.

4. Summary and perspectives

Table 1 summarized the optimal aeration and flow requirements for larviculture of marine fishes in this review. It is clear that the optimal flow is different by fish species even in the same volume and proportion (*AR*), and the flow requirement is different whether STRD and/or sinking syndrome occur in the larvae of the target species. We also found that the structure and velocity were different between cylindrical and rectangular tanks. In the rectangular tanks, there were so-called ‘dead spots’ (Kolkovski et al., 2014b) which are the stagnate areas, and these dead spots may have negative effects on larviculture. We need to conduct larviculture experiments investigating the survival and growth of fish using the rectangular tanks with different *AR*s, air stone types and aeration rates to seek the optimal flow field. We also assume that the flow field will vary not only with *AR*s but also with proportion of tank bottom (square to oblong). Modelling approaches as we applied in this review (Shiotani et al., 2005; Sumida et al., 2013; Takakuwa et al., 2018) will be effective to estimate the optimal flow field in larviculture.

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Figurs

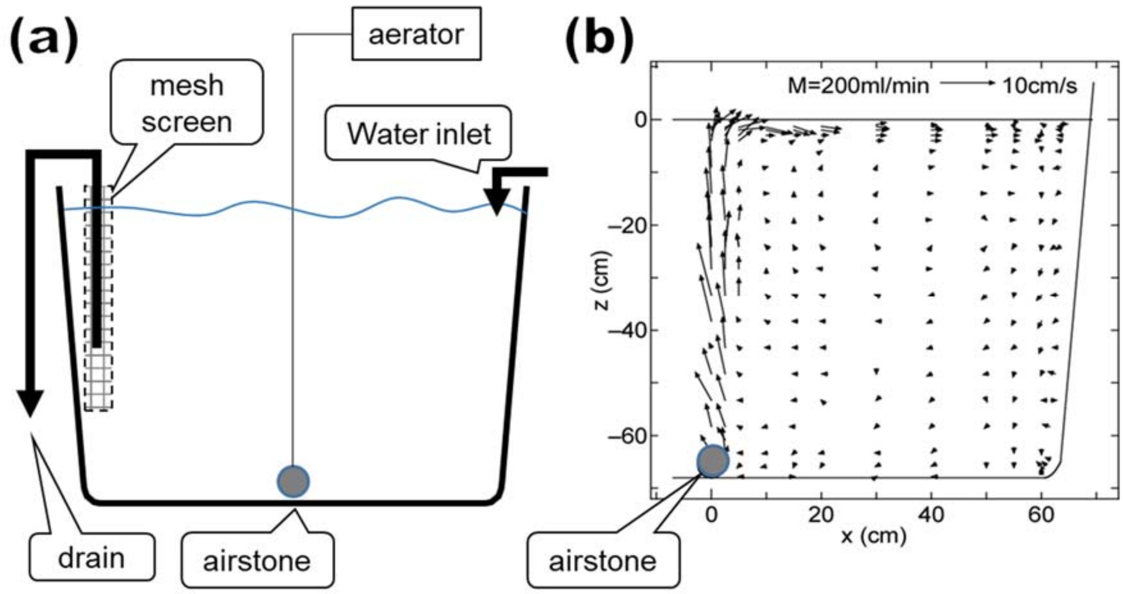

Fig. 1. (a) a 1 kL cylindrical tank used for the larval rearing experiments (1320 mm in diameter), and (b) flow velocity distribution in this tank at 200 mL/min aeration rate (redrawn from Sakakura et al., 2007). A horizontal half section of the 1 kL tank is shown, and a circle at the left side bottom indicates an air stone.

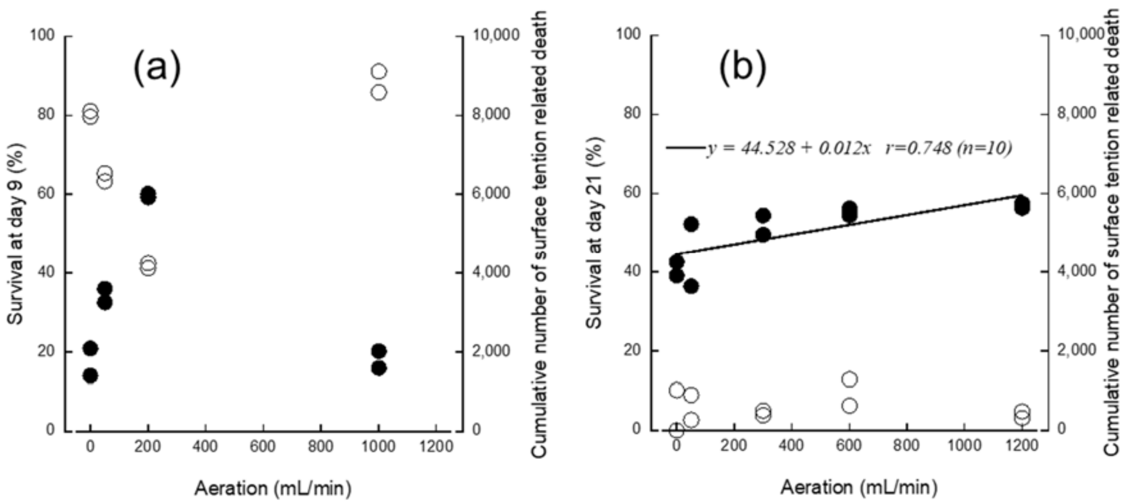

Fig. 2. Effects of aeration rate on the survival (closed circle, n=10) and cumulative number of surface tension related death (open circle, n=10) in the 1 kL cylindrical tanks for (a) the seven-band grouper larvae at 9 days after hatching reared (redrawn

from Sakakura et al., 2007) and (b) the devil stinger at 21 days after hatching (redrawn from Sakakura et al., 2014). Significant positive correlation was detected between aeration rate and survival of the devil stinger larvae (r=0.748, n=10)

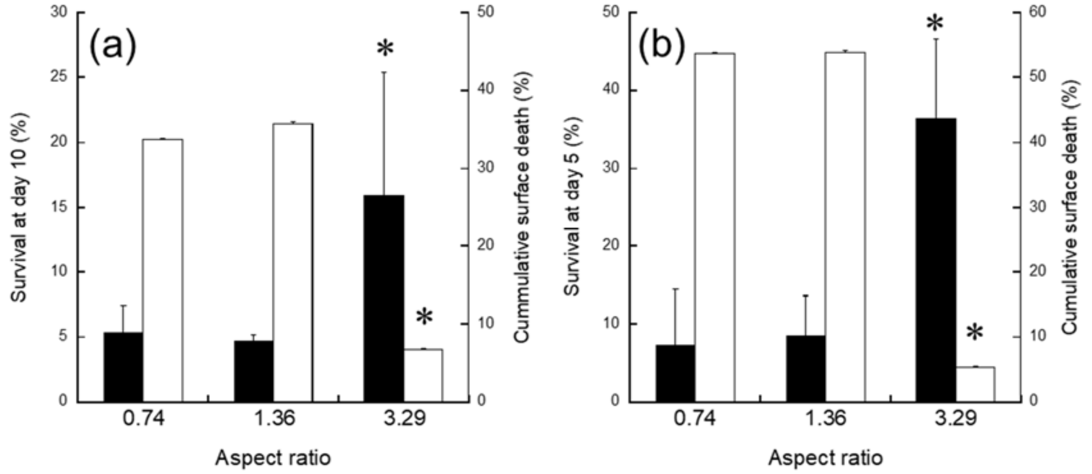

Fig. 3. Effects of aspect ratio (*AR*: the ratio of water depth to tank radius) of cylindrical tanks on the survival (closed column) and cumulative surface tension related death (open column) in (a) the seven-band grouper larvae at 10 days after hatching reared in the 100 L cylindrical tank and (b) the devil stinger at 5 days after hatching (redrawn from Ruttanapornvareesakul et al., 2007). Each column and bar indicate average and standard deviations, and an asterisk denotes significant difference among *AR*s (Tukey-Kramer post hoc test, p<0.05, n=3).

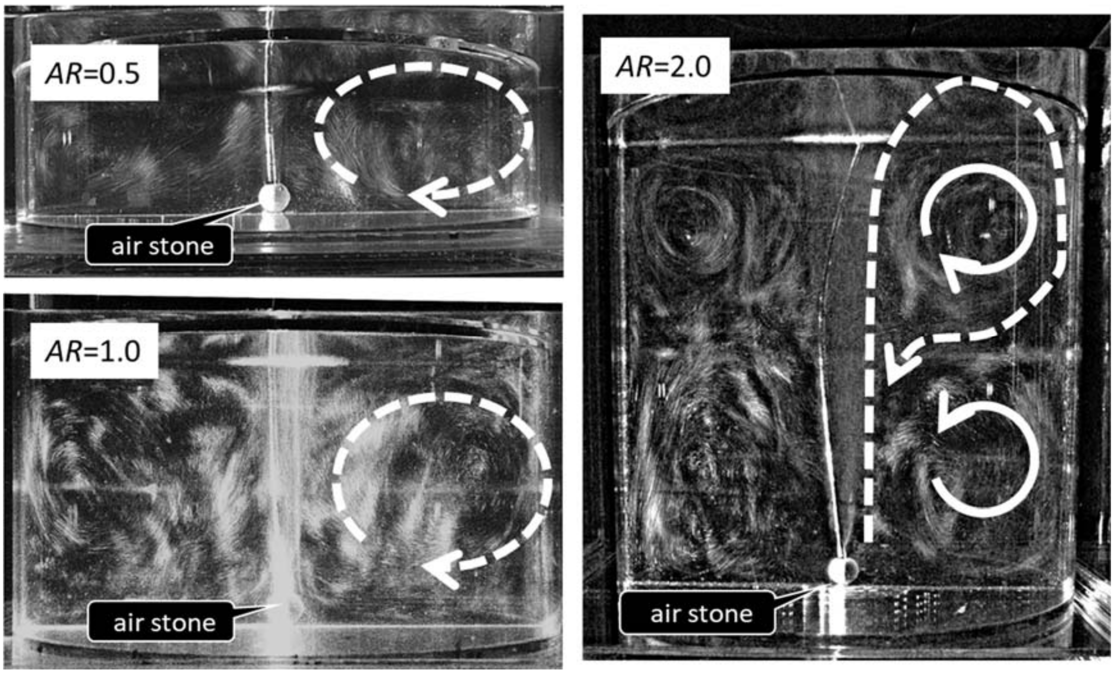

Fig. 4. Flow visualization of overall flow patterns in vertical cross-section of cylindrical tank at *AR*=0.5, 1.0 (left) and at *AR*=2.0 (right). A dotted line in *AR*=0.5 and 1.0 indicates a single-pair vortex system, and dotted lines in *AR*=2.0 indicate a 2-pair vortex system and solid lines indicate eddies formed by the 2-pair vortex system (redrawn from Sumida et al., 2013).

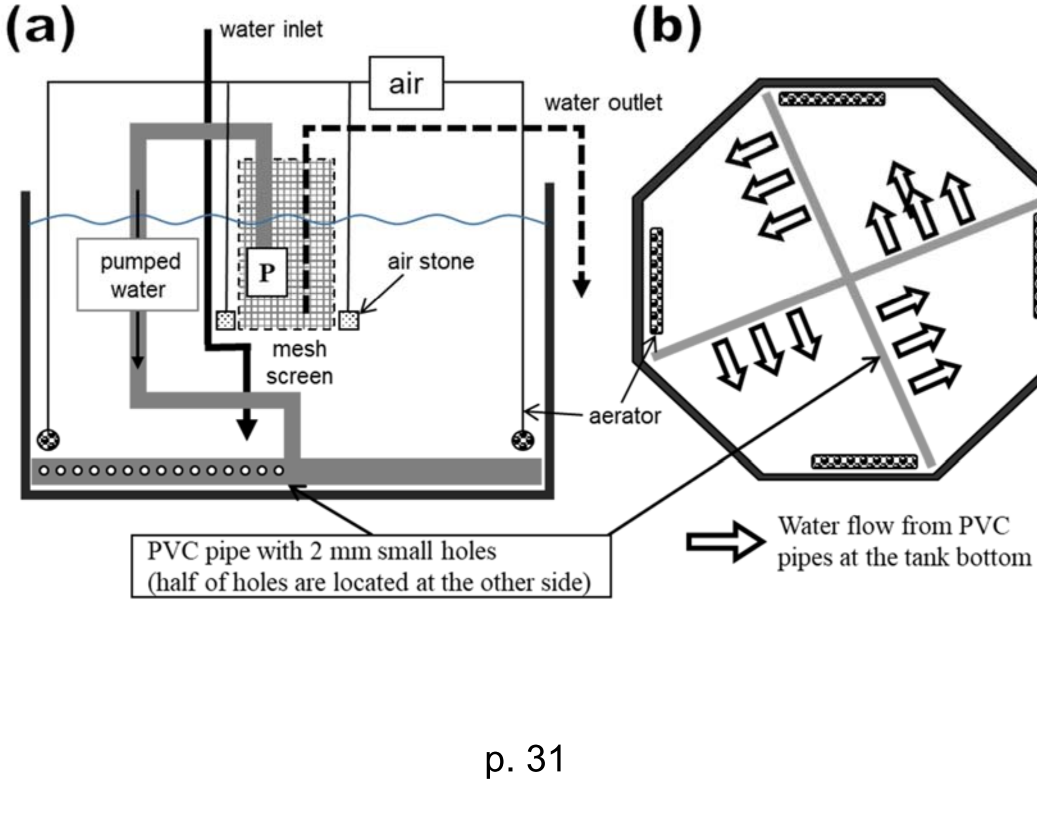

Fig. 5. Schematic drawings of settings of the "water pump system" in the 60 kL octagonal rearing tank for mass-culture of leopard coral grouper in Yaeyama Station, Japan Fisheries Research and Education Agency: (a) lateral view of the rearing tank and (b) horizontal view of the bottom of the tank equipped with water pump (P) in order to create horizontal flows on the bottom of the tank (redrawn from Takebe et al., 2011).

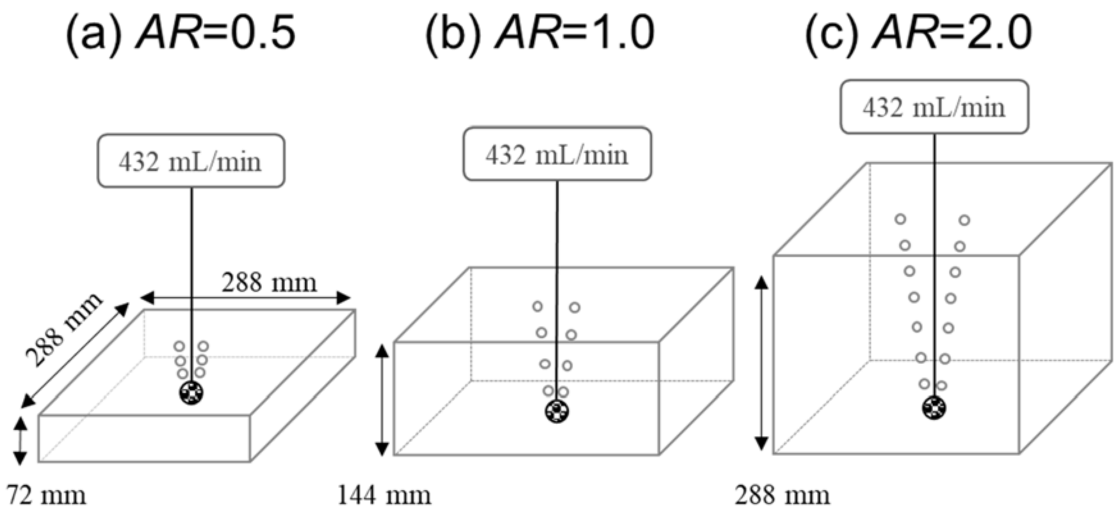

Fig. 6. Schematic drawings of the rectangular tanks with different *AR*s examined by Takakuwa et al. (2018).

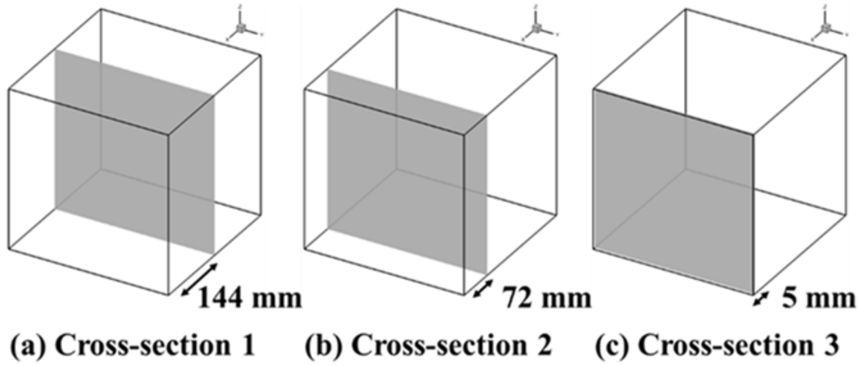

Fig. 7. Definition of vertical cross-sections for visualization of flows in a rectangular tank (Takakuwa et al., 2018).

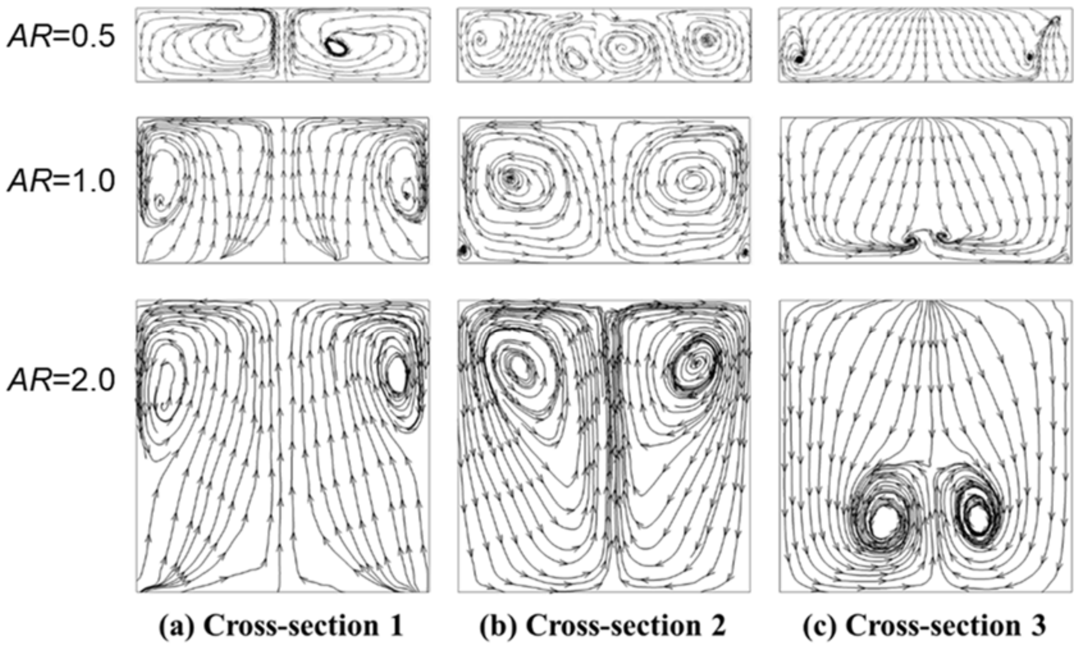

Fig. 8. Schematic drawings of flow patterns in the rectangular tank at three vertical cross-sections by three dimensional two-phase bubbly flow simulations (Takakuwa et al., 2018).

Table 1. Optimal flow for larviculture in marine fishes studied in this review

Fish species	Tank				Flow		Note
	Shape	Bottom	Aspect ratio	Volume (l)	aerator	aeration rate (ml/min)	
Seven-band grouper (*Epinephelus septemfasciatus*)	Cylindrical	Flat	3.29	100	Spherical air stone	50	
	Cylindrical	Flat	0.53	1,000	Spherical air stone	200	
	Cylindrical	Flat	0.50	100,000	Tube aerator* surrounding drain	630	* FAL, Unihose Co., Ltd., Japan
Devil stinger (*Inimicus japonicus*)	Cylindrical	Flat	3.29	100	Spherical air stone	50	
	Cylindrical	Flat	0.53	1,000	Spherical air stone	300-1200	
Leopard coral grouper (*Plectropomus leopardus*)	Octagonal	Flat	0.67	50,000	Tube aerators* at 4 corners of bottom	500	A counter-clockwise and upward current was produced by "water pump method"
Pacific bluefin tuna (*Thunnus orientalis*)	Cylindrical	Flat	1.24	500	Spherical air stone	100	Continuous illumination
	Cylindrical	Flat	1.24	500	Spherical air stone	Daytime: 300 Nighttime: 900	