

Effects of Different Tank Types on Growth and Survival of European Sea Bass (*Dicentrarchus labrax*, L. 1758) Larvae

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Özet: Farklı tank tiplerinin levrek (*Dicentrarchus labrax* L., 1758) larvasının büyüme ve yaşama oranları üzerine etkileri. Bu çalışmada, farklı tank tiplerinin levrek larvası yaşama, gelişim ve büyüme oranları üzerindeki etkileri araştırılmıştır. Bunun için, yaygın olarak kullanılan farklı şekil, hacim, derinlik ve yüzey alanına sahip 3 farklı tipte (sırasıyla A, B ve C) larva tankı kullanılmıştır. Her tank tipi için iki tekrar gerçekleştirilmiş olup, her bir tanka 100 g/m³ oranında yumurta stoklanmıştır. Yumurtadan çıkıştan sonra, larval yetiştiricilik aynı tanklarda intensif akışkanlı su yöntemi ile 60. güne kadar sürdürülmüştür. Ayrıca 60. günde, hava keseli ve hava kesesiz tüm larvalar sayılmış ve her bir tanktan rasgele seçilen 100 larvanın yaş ağırlığı ve total boyu ölçülmüştür. C tanklarındaki larvaların yaşama oranı hem A hem de B tanklarındaki larvaların yaşama oranından daha yüksek olmuştur (p<0.05). Yaşama oranlarına benzer şekilde, C tanklarındaki larvaların hava kesesi geliştirme oranı hem A hem de B tanklarındakinden daha yüksektir (p<0.05). Bununla birlikte, üç tank tipindeki hava keseli larva oranları arasında ise önemli bir fark görülmemiştir (p>0.05). En düşük ortalama yaş ağırlık değeri C tanklarında kaydedilirken, en yüksek ortalama yaş ağırlık değeri A tanklarında elde edilmiştir. Fakat hem A hem de B tanklarında ortalama total boy değerleri C tanklarında kaydedilen değerden daha yüksek bulunmuştur (p<0.05). Bu çalışma intensif akışkanlı su yetiştiricilik tekniğinde kullanılan tank tipinin levrek larvasının hava kesesi geliştirme ve yaşama oranları üzerinde önemli etkileri olduğunu göstermiştir. Karşılaştırılan tank tipleri arasında, silindirik-konik C tipi tanklar, özellikle ilk 45 günlük larval dönem için en uygun fiziksel ortamı sağlamıştır. Ancak, larvanın bu tip tanklarda 45 günden daha uzun süre tutulması büyümenin yavaşlamasına neden olabilmektedir. Kırk beş gün sonunda larvanın A veya B tipi tanklara nakledilmesi ile daha yüksek büyüme ve yaşama oranları elde edilebilir. Çünkü daha geniş yüzey alanına ve hacme sahip olan bu tip tanklar larvanın yapay yeme alıştırılması için daha uygun bir ortam sağladıkları gibi yapay yem ile besleme sırasında karşılaşılan su kalitesinde sorunlarının hafifletilmesine yardımcı olabilirler.

Anahtar Kelimeler: Levrek, *Dicentrarchus labrax*, tank tipi, yaşama oranı, hava kesesi gelişimi, larval büyüme.

Abstract: In this study, we investigated that the effects of different tank types on survival, development and growth of European sea bass, *Dicentrarchus labrax*, larvae. Therefore, we used three different types (A, B and C) of common hatchery tanks having different shapes, volumes, depths and surface areas. Each tank type had two replicates and each tank was stocked with 100 g eggs/m³. After eggs hatched, larvae were reared in the same tank for 60 days. On 60 days-post-hatching, all larvae with or without a functional swim bladder were counted and wet weights and total lengths of 100 randomly selected larvae from each tank were measured. Survival of larvae in C tanks was higher (p<0.05) than that of larvae both in A and B tanks. Similar to survival ratios, swim bladder development ratios of larvae were higher (p<0.05) in C tanks than that of larvae both in A and B tanks. Nevertheless, the ratio of larvae with functional swim bladder did not show any differences (p>0.05) among the three tank types. The highest mean wet weight was observed in A tanks while the larvae in C tanks had the lowest mean wet weight. Mean total length of larvae was higher (p<0.05) both in A and B tanks than that of larvae in C tanks. The results of this study showed that tank type have profound effects on swim bladder development and survival of sea bass larvae. Among the three different tank types, cylinder-conic C tanks provided the best physical environment for the first 45 days of larval period. Nevertheless, keeping larvae longer than 45 days in these tanks caused slower growth. Higher growth rates can be obtained by transferring larvae to A or B types of tanks after 45 days because these tanks with larger surface areas and volumes are more suitable for feed training and can alleviate the water quality problems encountered during feeding with artificial diets.

Key Words: European sea bass, *Dicentrarchus labrax*, tank type, survival rate, swim bladder development, larval growth.

Introduction

As in intensive culture of any fish species, the most crucial step in sea bass culture is the production of high quality juveniles in large quantities. A healthy organogenesis and successful production of high quality juveniles requires an appropriate combination of both biotical and abiotical factors. Many physical-chemical factors such as salinity (Johnson and Katavic, 1984, 1986; Dendrinis and Thorpe, 1985; Freddi, 1985; Barnabe and Guissi, 1993; Firat, 1995; Tandler, 1995;

Firat *et al.*, 1999; Saka *et al.*, 1999), temperature (Bertolini *et al.*, 1991; Bronzi, 1993; Koumoundouros *et al.*, 2001), gas saturation of the rearing water (Colt, 1986; Firat *et al.*, 1998), light intensity (Weppe and Jossard, 1986; Coves, 1987; Barnabe, 1990; Chatain, 1992; Chatain, 1997) and tank coloration (Naas *et al.*, 1996; Freddi, 1985; Chatain, 1997; Petersen, 1998) have been known to directly affect a healthy larval development.

The major causes of mortality in sea bass larvae culture are mostly related to initial inflation and later developments of

swim bladder. For successful initial inflation of swim bladder, larvae have to reach the water surface to gulp air. Hence, the oily film layer impeding the access of larvae to water surface must be cleaned carefully for successful initial inflation of swim bladder (Chatain, 1989; Chatain and Quanis-Guschmann, 1990; Firat, 1995; Firat et al., 1999). After initial inflation, posterior enlargement of swim bladder continues by means of gas transfer (Demir, 1992). The gas contained within the swim bladder is composed mainly of O₂ but other gases such as N₂ and CO₂ in much lower proportions are also present. At abnormal environmental conditions, as the acidity and the partial pressures of O₂, CO₂ and N₂ in the blood serum of larvae increase, the entry of these gasses to swim bladder by diffusion through retina-mirabilia increases and over inflate the bladder (Demir, 1992; Jobling, 1995) causing the problem called hypertrophy syndrome (Chatain, 1989; Chatain and Corrao, 1992; Firat et al., 1999). To avoid this problem, hatcheries commonly use degassing devices (Colt, 1986).

In nature, swim bladder of larvae is more inflated at night than that of larvae in daytime. However, no such volumetric differences was observed for the swim bladder of larvae reared under controlled conditions and this situation is attributed to limited depth of the rearing tanks (Rosa and Dinis, 1985). A study on *Mugil cephalus* larvae also shows a positive relationship between the tank volume and larval survival (Harrel et al., 1998). Therefore, another important factor affecting the normal development of swim bladder could be the depth of the rearing tanks. Besides this, highly specific techniques and protocols have been developed to rear sea bass larvae (Coves et al., 1991; Chatain, 1997; Firat et al., 1998; Buke, 2002). All these techniques require certain changes in rearing parameters at specific phases of larval period to promote a healthy development. However, all larvae in a rearing tank should not be expected to adapt these changes in a short time. Providing an extra space in terms of volume or area in the rearing tanks might help larvae to make such adaptations gradually. Hence, this study was conducted to investigate the effects of different tank types with different shapes, volumes, surface areas and depths on survival and development of sea bass larvae.

Materials and Methods

This research was conducted at the hatchery of Bodrum Fisheries Research Institute in Güvercinlik, Bodrum, Muğla in between January and February 2000.

Three widely used hatchery tank types having different shapes, volumes, surface areas and depths were tested to evaluate the effects of different tank types on survival, development and growth of sea bass larvae. The experiment was carried out in 6 tanks with each tank type having two replicates.

All tanks received either sea and/or well water from a flow through system. Both sources of water were filtered through 40-60 µm sand and 10 µm cartridge filters, UV sterilized and degassed before reaching the tanks. Salinity of

full strength sea water was 40‰, but it was decreased to 25-26‰ on between 4 and 17 days post hatching (dph). Afterwards, it was gradually increased back to 40‰ by 30 dph.

To avoid tangential currents and mechanical manipulation to larvae, water inlets connected to degassing columns were positioned at the periphery of the tanks, approximately 20 cm below the water surface. At the beginning of the larval period, water discharged only from the surface. As the larval development progressed, both surface and bottom water discharges were used. Since the intensive clear water larval rearing techniques was applied, the initial water renewal rate was adjusted to 10% of the tank volumes per hour. Later, it was increased to 15%, 20%, 25%, 40%, and 50% per hour on the 11, 15, 20, 30, and 35 dph, respectively. Dissolved oxygen concentrations were measured twice a day and maintained between 5 to 7 mg / l by adding pure oxygen into the incoming water when necessary. Additionally, tanks were aerated through only coarse bubbles on from 4 to 17 dph, and commonly wooden air diffusers giving small bubbles were used later on.

Lighting for the tanks was provided through 60-150 watt tungsten lamps placed 0.75 m above the water surface. Based on the surface area, sufficient lighting was provided with 4 lamps in A tanks, with 2 lamps in B tanks and with 1 lamp in C tanks. Photoperiod application was initiated on 8 dph as 8 h light (L): 16 h dark (D) periods. On 15 dph, it was changed to 16 h L: 8 h D periods. Light intensity used on 8 dph was 20 lux and increased to 500 lux on 18 dph. In the case of swim bladder hypertrophy, light intensity was increased to 900-1500 lux to lead the larvae downward so that swim bladder can reduce to its' normal size before giving any permanent damage to larvae. Additionally, photoperiod was kept at 24 h light until a normal distribution of larval population in the tank was observed.

The study was conducted at ambient water temperatures. During the study period, sea water temperatures ranged between 14.8 to 17.5°C (Figure 1).

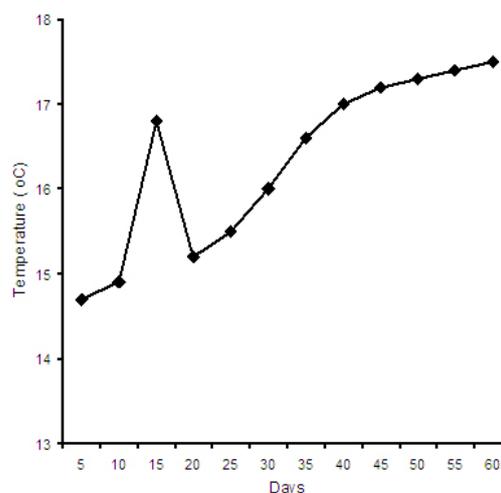


Figure 1. Temperatures during the 60 day larval rearing period.

Feeding was started on 8 dph with *Artemia* nauplii (A_0). Larvae were fed A_0 *Artemia* until 25 dph, enriched *Artemia* metanauplii (A_1) on from 21 to 59 dph and microparticulate diets of 100-200 μm on between 28 and 45 dph, 200-400 μm on from 40 to 57 dph and 300-500 μm on between 45 and 60 dph (Figure 2).

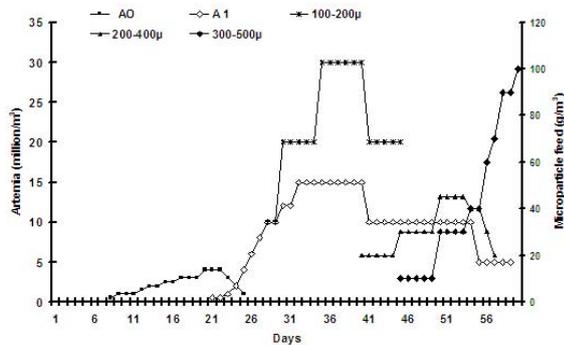


Figure 2. Larval feeding protocol.

Since the death of larvae with un-inflated or over-inflated swim bladder is one of the major problems observed in intensive culture of sea bass, the ratio of larvae with functional swim bladder was taken as the criteria for a normal larval development. At the end of the experiment period (60 dph), larvae without swim bladder were separated using the sleeping method (Chatain and Corrao, 1992). For this, larvae were placed into plastic buckets containing high salinity (up to 60‰) water with 70 ppm MS-222 as an anesthetic. Since larvae with swim bladder floated in high salinity water, they were easily separated from the larvae without swim bladder, which stayed at the bottom of the buckets. After counting the number of larvae with swim bladder, the below values were calculated for the comparisons of tank type effects:

Survival ratio: the number of live larvae at 60 dph x 100 / the number of larvae at the beginning,

Swim bladder development ratio: the number of larvae with functional swim bladder at 60 dph x 100 / the number of larvae at the beginning,

The ratio of larvae with functional swim bladder: the number of larvae with functional swim at 60 dph x 100 / the number of live larvae at 60 dph.

To determine the effect of tank types on larval growth, 100 larvae from each tank was randomly sampled on 60 dph. Their wet weights to the nearest 0.01mg and total lengths to the nearest 1 mm were measured.

Statistical analysis was carried out by SAS software (version 8.0; SAS Institute Inc., Cary, NC, USA). Before the analysis, swim bladder development ratio, survival ratio and the ratio of larvae with functional swim bladder were arcsine ($x^{1/2}$) transformed and wet weights and total lengths of larvae were $-1/y$ transformed to normalize the data distribution and/or to homogenize variances. Then, the data were analyzed using one-way ANOVA. When ANOVA indicated a significant tank type effect at $\alpha=0.05$ significance level, Duncan's multiple range test was used to separate the means of tank types.

Results

Through out the experimental period, all dead larvae collected during the surface cleaning and bottom siphoning were examined in order to determine the possible cause of death. These examinations showed that the dead larvae were either weak individuals or the ones with over-inflated swim bladder. Hypertrophy syndrome was observed particularly in A and B tanks between 9 and 12 dph, at the initial stage of swim bladder inflation, and later between 20 and 25 dph. It was also observed that some larvae floating on the water surface due to hyper-inflated swim bladder died in a short time because they could not swim or take feed while the others with over-inflated swim bladder were able to went deeper (mostly by the mid of water column) under the effect of high light intensity and could keep feeding. When the presence of feed in the guts of larvae was examined, however, an important difference between the normal and affected larvae was observed. The digestive tract of normal larvae was completely full while the larvae with over-inflated swim bladder have much lesser feed in their gut (Figure 3).

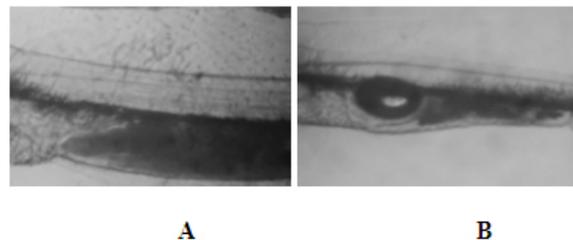


Figure 3. Digestive tract and swim bladder of 12 days-old sea bass larvae. A. larvae with normal swim bladder and full digestive tract; B. larvae with over-inflated swim bladder and unfilled digestive tract

On contrary to A and B tanks, hypertrophy syndrome did not cause relatively much trouble in C tanks. Although hypertrophy syndrome caused some deaths in one of the C tanks, this was at much lower rate than the other tank types. Some deaths also occurred in the same C tank on between 50-60 dph when feeding rate with microparticulate feed increased. The cause of these deaths, however, was probably deteriorating water quality at bottom of the tank because this time majority of the death larvae consisted of individuals with normally developed swim bladder.

Survival ratios of larvae by 60 dph showed significant differences ($p<0.05$) among the three tank types (Table 1). Survival of larvae in C tanks was higher than that of larvae both in A and B tanks, but there was no significant differences in survival of larvae between these two tank types.

Similar to survival ratios, swim bladder development ratios of larvae were significantly higher ($p<0.05$) in C tanks than that of larvae both in A and B tanks and there was no significant difference between A and B tanks (Table 1). The ratio of larvae with functional swim bladder however, did not show any significant differences ($p<0.05$) among the three tank types (Table 1).

When the within group variations in survival and swim

bladder development ratios were examined, the highest differences were observed in C tanks (Table 1). These high variations between the two C tanks can be explained by the fact that deaths both due to hypertrophy syndrome and deterioration of water quality at the beginning of feeding with artificial diets occurred in only one of the C tanks.

Table 1. The effects of different tank types on survival and swim bladder development ratios (% , ±SD) of sea bass larvae.

Tank groups	Survival ratio	Swim bladder development ratio	The ratio of larvae with functional swim bladder
A	16.1 (2.2) b	14.8 (1.5) b	92.6 (3.1) a
B	20.0 (1.4) b	18.5 (0.1) b	93.0 (7.1) a
C	35.7 (2.5) a	33.5 (5.0) a	93.6 (7.6) a

According to Duncan's multiple range test, mean percentages within the same column followed by different lower case letters are significantly different ($p < 0.05$).

Growth rate of larvae showed significant differences ($p < 0.05$) among the tank types (Table 2). The highest mean wet weights were observed in A tanks while the larvae in C tanks had the lowest mean wet weights. Mean wet weight of larvae in B tanks was in between that of larvae in the other two tank types, but no significant differences can be detected ($p > 0.05$). However, mean total length of larvae on 60 dph was significantly higher ($p < 0.05$) both in the A and B tanks than that of larvae in C tanks (Table 2).

When the within group variations in mean wet weights and mean total lengths were examined, the lowest variation was observed between the C tanks (Table 2). This indicates that growth of larvae in the C tanks was more uniform than that of larvae in the other two tank types.

Table 2. The effects of different tank types on growth of sea bass larvae.

Tank groups	N / tank	Mean wet weight (mg, ±SD)	Mean total length (cm, ±SD)
A	100	63.3 (18.3) a	2.1 (0.2) a
B	100	55.3 (14.5) ab	2.0 (0.2) a
C	100	40.1 (10.6) b	1.8 (0.1) b

According to Duncan's multiple range test, means within the same column followed by different lower case letters are significantly different ($p < 0.05$).

Discussion

Problems encountered in initial inflation and later developments of swim bladder are the major causes of deaths in sea bass larvae culture. For successful initial inflation of swim bladder, larvae have to reach the water surface to swallow air. Hence, a proper method for cleaning of oily film

layer which prevent larvae from reaching to water surface carries crucial importance (Chatain, 1989; Chatain and Quanis-Guschemann, 1990; Firat, 1995). After initial inflation, posterior enlargement of swim bladder continues by means of gas transfer (Demir, 1992). Since over-inflation of swim bladder can cause deaths as much as unsuccessful initial inflation, concentrations of dissolved gases in the rearing water must be controlled carefully. Beside these, depth of the rearing tanks can be another important factor for normal development of swim bladder because its' volume inversely changes with depth according to Boyle-Marriott law. Hence, the amount of gases within swim bladder and the volume of the bladder changes with the changing outside pressure (Demir, 1992; Malcolm, 1995; Fıret et al., 1998). While larvae in nature have a chance of adjusting the volume of their swim bladder by swimming up or down, having such a chance for larvae in hatcheries depends on the depth of their rearing tanks (Rosa and Dinis, 1985). In this study, deaths due to hypertrophy syndrome occurred both in A and B tanks and one replicate of the C tanks in between 9 to 12 and 20 to 25 dph. Yet, hypertrophy syndrome lasted longer and affected a higher number of fish in A and B tanks than the C tank and no hypertrophy was observed in the second C tank. Using the negative photo-taxis characteristic of sea bass larvae, hypertrophic larvae floating on or close to water surface were forced to swim down by increasing the light intensity and photoperiod. With the help of this light treatment, partially hypertrophic larvae were saved, but highly hypertrophic larvae stayed floating on the water surface and died in short time. Additionally, we obtained better results with this treatment in the C tank than the other two tank types. The C tanks were deeper than the other tank types. Hence, larvae in C tanks had more vertical space to benefit from the reverse relationship between gas pressures and depth in order to adjust the size of their swim bladder.

Decreasing salinity at the beginning of the larval period reduces the osmotic and ionic regulation loads of larvae (Malcolm, 1995; Tandler, 1995) as well as it provides the sedimentation and vertical distribution of larvae within the rearing tanks (Freddi, 1995; Firat, 1995). Because of the reduced density and buoyancy of water at lower salinities, larvae without developmental problems had to use their swim bladder in order to maintain the same position in low salinity waters (Broomage and Shepherd, 1988). As a result, low salinity treatment helps a healthy and more functional development of swim bladder. It also prevents further increases in swim bladder size of larvae with over-inflated swim bladder by keeping them below the water surface. In this study, larvae in C tanks showed a more uniform distribution in a deeper water column compared the other two types of tanks. Hence, swim bladder development ratio and the number healthy larvae with functional swim bladder were higher in the deeper C tanks than the shallower A and B tanks.

When surface cleaning of larvae tanks was done properly, the success of initial swim bladder inflation can be

expected to be 80% or higher (Chatain, 1990; Firat, 1995). In this study, the ratio of larvae with functional swim bladder on 60 dph (92.6 to 93.6%) did not differ among the three tank types. This also indicates no differences in the ratio of larvae without functional swim bladder on 60 dph among the tank types. Concurrently, the similar ratio of larvae without functional swim bladder in all tank types shows that initial swim bladder inflation success of larvae should be similar among the tanks. Hence, the results of our study show that surface cleaning, which carries crucial importance for successful initial inflation of swim bladder, can be done properly in tanks with different surface areas. Nevertheless, various environmental conditions of different tank types should have affected the later development of swim bladder differently. The high intensity and severity of hypertrophy syndrome in A and B tanks and the better success of light treatment in the C tank were the consequences of different physical conditions among the tank types. With the death of highly hypertrophic larvae in A and B tanks, higher survival ratios were obtained in C tanks.

Chatain (1997) reported that, with the present culture methods, survival ratio of sea bass larvae at 45 dph could range from 35 to 65%, but higher survival ratios can be obtained when a healthy development of larvae was supported with appropriate physical environmental conditions and feeding strategies. In this study, the highest survival ratio by 60 dph ($35.7 \pm 2.5\%$) was obtained in C tanks. Similar to Hatzithanasiou *et al.* (2002), we observed some deaths due to no apparent reason in all tank types during the first 10 days of the culture period. After that, all dead larvae consisted of either weak individuals without a swim bladder or larvae with over-inflated swim bladder. Hypertrophy syndrome occurred in all three tank types. However, it affected a larger number of larvae in A and B tanks than C tanks. Similar to survival ratios of larvae, swim bladder development ratios of larvae was higher in C tanks than the other two tank types. This similarity between the survival ratios and swim bladder development ratios of the tank types once more indicates that normal development of swim bladder is the determining factor for the survival of sea bass larvae in culture tanks.

Studies on growth showed that sea bass larvae could attain 30-45 mg mean wet weight and 16.3-19.9 mm mean total length by 45 dph (Coves *et al.*, 1991; Firat 1995; Firat *et al.*, 1998). In our study, larvae reached a similar size (40.1 ± 10.6 mg and 18 ± 0.1 mm) by 60 dph in C tanks. The slower growth of larvae in our study could be attributed to lower water temperatures, higher stocking density or differences in feeding regimes. Yet, larvae in A and B tanks showed higher growth rates (63.3 ± 18.3 mg and 55.3 ± 14.5 mg, 21 ± 0.2 mm and 20 ± 0.2 mm, respectively) than larvae in C tanks. Since both the volumes of these tanks were larger than the C tanks and the survival ratios of larvae were lower in these tanks, the slower growth of larvae in C tanks could mainly be attributed to higher stocking rates. Buke (1997) and Hatzithanasiou *et al.* (2002) also reported that density affected the growth of sea bass larvae at this stage and growth increases at low stocking

rates. Another explanation for the better growth of larvae could be that these tanks might have provided better physical conditions for larval growth, particularly during the gradual passage from live food to artificial diets between 45 and 60 dph. Since these tanks, especially A tanks, have the typical characteristics of large surface area and volume of larval adaptation tanks used at this sensitive stage.

As well as fast growth, uniform growth of larvae is an important criterion in determination of the best culture conditions. Size distribution of larvae in C tanks was more uniform than that of larvae in A and B tanks. It can be concluded that the high stocking rate although slowed the growth of larvae in C tanks, it provided more uniform growth. However, the higher size variation of larvae in A and B tanks can be explained by the presence of high numbers of partially hypertrophic larvae with slowed growth. These larvae established stressed and crowded schools closer to water surface while normally developing larvae evenly distributed within the rearing tanks. Since they can not compete with normal larvae for food during this period, their growth probably slowed down and this might have caused the observed high size variations in A and B tanks.

Low salinities provide a homogenous distribution of both live food and larvae in the rearing tanks (Moretti *et al.*, 1999). This allows much easier access of larvae to food and promotes more uniform growth. Although we applied the same salinity level in all tanks, we were not able to provide a uniform distribution of live food, particularly Artemia, in A and B tanks because of the severe hypertrophy syndrome occurred in these tanks. The high light intensity treatment we used against the hypertrophy syndrome caused positive photo-taxis showing Artemia to accumulate in the upper water column of these tanks while their distribution were more homogenous in C tanks. Therefore, the non-homogenous distribution of Artemia might be another reason contributing the highly variable size distributions of larvae in A and B tanks.

In conclusion, the results of our study showed that the tank type used in intensive clear water rearing method have profound effects on swim bladder development and survival ratios of sea bass larvae. Among three different tank types compared in the study, 2 m³ cylinder-conical tanks with 1.67 m depth and 1.5 m radius provided the best physical environment, especially for the first 45 days of larval period. However, keeping larvae longer than 45 days in this type of tanks can cause slow growth. Higher growth rates can be obtained by transferring larvae to A or B types of tanks after 45 days. Transferring larvae to A or B types of tanks which are more suitable for feed training with their larger surface areas will also alleviate the water quality problems encountered during feeding with artificial feeds and will increase the survival rates.

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