

## Effect of stocking density on survival rate, growth performance, swim bladder inflation and skeletal deformity of the European sea bass (*Dicentrarchus labrax*) larvae.

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### ABSTRACT

Stocking density (STD) occupied a seniority theme in the majority of aquaculture research plans due to its impact on the interest of the farmed fish and the urgent need for future advice managing STD in marine fish hatcheries. The aim of this study was to evaluate the influence of STD (50, 75, 100 and 125 larvae l<sup>-1</sup>) on growth parameters, survival rate, swim bladder inflation (SBI) and skeletal deformity of European sea bass (*Dicentrarchus labrax*) post-larvae. Two days post-hatch (dph), *D. labrax* larvae (2.5 mm) were stocked in 4 m<sup>3</sup> tanks at different STDs. The results showed that larvae stocked at density 50 larvae l<sup>-1</sup> had significantly higher total length on 60 dph compared with the other treatments which showed no significant difference among them. Bodyweight and survival rate were significantly decreased by increasing the STD. Functional swim bladder at 21 dph was inversely correlated with STD where larvae stocked at 50 and 75 l<sup>-1</sup> had significantly higher functional swim bladder than other investigated densities. Incidence of appearance of one skeletal deformity in the pre-haemal or caudal region significantly increased with increasing STD and was inversely correlated with SBI. On the other hand, the appearance of two skeletal deformities was only recorded in larvae stocked at 125 l<sup>-1</sup>. The results of the current research showed that the best performance of *D. labrax* larvae was achieved in lower STD and that it must not exceed 75 larvae l<sup>-1</sup>.

### INTRODUCTION

European Sea bass (*Dicentrarchus labrax*) is considered one of the primarily species to be intensively cultured in the Mediterranean region due to its high commercial value. Recently, the decline in this species market price due to extra-production has forced the aquaculturists for decrement production expenses with enhancing fish quality.

Larval density in intensive larvi-culture affects larval quality because it acts as the social interactions between larvae with various ways which include: competitiveness phenomena (Schreck, 1981), cannibalism (Katavic *et al.*, 1989; Moore and Prange, 1994) and aggressiveness (Sakakura and Tsukamoto, 1999). These phenomena may have undesirable effects on the marine larvae such as impaired growth, reduced immune competence and induced abnormal deformities (Schram *et al.*, 2006; Ashley, 2007; Eid *et al.*, 2019). The effect of larval STD on growth execution was illustrated by many authors using different densities (Turnbull *et al.*, 2005; North *et al.*, 2006; Ashley, 2007; Tan *et al.*, 2018). Although many work have been published on larval STD, further research is needed because larval densities are species-specific and are affected by rearing in various water systems and also influenced with fish stage (Ellis *et al.*, 2002; Eid *et al.*, 2019).

Among the difficulties that arose during early marine larval development in commercial marine hatcheries is the failure of the swim bladder inflation (Trotter *et al.*, 2005). This obstacle is the most common circumstance observed during the early development of *D.labrax* larvae, especially if the rearing technology is poorly developed (Boglione *et al.*, 2013). Consequently, it will result in reduced growth, development of skeletal deformities, and finally increased mortality (Trotter *et al.*, 2005; Boglione *et al.*, 2013). Losses in sea bass fingerlings due to SBI problems may ranged from 5 to 10% and can reach 50% of the seed production (Woolley & Qin, 2010).

Vertebral deformities have been demonstrated in approximately all cultured marine fish species. These deformities can take the form of arches, dislocation, and shortening or flexion of skeletal elements (Boglione *et al.*, 2013). These vertebral deformities involve lordosis (V-shaped dorsal–ventral curvity); kyphosis (A-shaped dorsal–ventral curvature), and scoliosis (lateral curvature) (Boglione *et al.*, 2013). In these cases, deformities of vertebrae centra and/or arches are coupled with bending in the vertebral column that can be internally or externally examined. Internal examination of deformation is by clearing and staining of bone and cartilage or by further examination using x-ray. Severe cases of deformation can be examined externally by naked eyes. The angle initiated by axis bending formation should be of a certain extent for the identification to be done externally. Accordingly, it has to be put into consideration that many larvae may have skeletal deformities with different degrees of severity that may not appear by external examination but affect larval movement in water and feeding behavior and consequently their normal growth.

The current investigation is aiming at the evaluation of using different STDs (50, 75, 100 and 125 larvae  $l^{-1}$ ) on growth parameter measurements, survival, SBI and skeletal deformities in *D. labrax* larva, reared in large scale production from newly hatched larvae until 60 day post hatch (dph) in a continuous flow-throw water system.

## MATERIALS AND METHODS

### 1. Experimental unit

The rearing trial in this study was carried out in the Marine Governmental Hatchery of the General Authority of Fish Resources Development (GAFRD) in Alexandria, in cooperation with the Faculty of Agriculture, Alexandria University.

### 2. Experimental design

*Dicentrarchus labrax* eggs were obtained from broodstock held in captivity in GAFRD under controlled rearing conditions. Eggs were counted and incubated in 1 m<sup>3</sup> cylindroconical tanks until hatching. Newly hatched larvae were then distributed into 4 m<sup>3</sup> experimental rearing tanks in order to have final four stocking densities: 50, 75, 100 and 125 larvae l<sup>-1</sup> in triplicate treatments for 60 days. Larvae were reared using the green water technique following similar conditions used in the previous study by **El-Sayed et al. (2014)** for the same cultured species, which gave the best results in terms of survival and quality. The experimental tanks were provided by an airlift aeration system in the tank bottom to ensure a gentle homogeneity and to renew water up and down throughout the tank (**Divanach et al., 1998**). The rate of water renewal (started on 10 dph until 60 dph) were gradually increased from 5% to 40% h<sup>-1</sup>. Starting from the post larval stage 30 dph, renewal water flow-throw was 50% h<sup>-1</sup> and gradually increased to 80% h<sup>-1</sup> until the end of the experiment.

### 3. Feeding regimes

Larvae were fed live prey rotifers (*Brachionus plicatilis*), *Artemia* nauplii and *Artemia* metanauplii enriched with mixed algae according to the feeding schedule described in **El-Sayed et al. (2014)** and **El-Sayed & El-Khodary (2019)**. Exogenous feeding started from mouth opening where rotifers (5-8 ind.ml<sup>-1</sup>) were added to the tanks until 10 dph. Afterwards from 11 to 20 dph rotifers decreased gradually and *Artemia* nauplii were increased up to 1-2 ind. ml<sup>-1</sup>. Only *Artemia nauplii* were added to the larvae tanks from 21 to 25 dph then metanauplii from 26 to 30 dph. Post-larvae 30 dph were fed one day old *Artemia* metanauplii at rate of 1-2 ind. ml<sup>-1</sup> enriched with mixed algae until 45 dph. Starting from 40 dph until the end of the experiment (60 dph), artificial food gradually increased and distributed ad libitum reaching 10% of biomass tank<sup>-1</sup> day<sup>-1</sup> as described by **Hatziathanasiou et al. (2002)**.

### 4. Water quality

Water quality parameters (temperature, dissolved oxygen, salinity, pH and light intensity) were monitored continually along the experimental period. Light intensity gradually increased from 5 to 100 lux and the light phase was regulated to last 14 h (08:00 to 22:00 h). Water temperature was recorded daily by mercury thermometer and it was ranged from 16-21 °C along the experiment according to the ambient temperature. Dissolved oxygen was measured using oxygen meter and was above 6.10 mg l<sup>-1</sup>. Other water quality parameters including pH was measured every two days by

pH meter where it was  $7.7 \pm 0.7$  throughout the experiment. Water salinity was measured using temperature compensated refractometer in tanks of each treatment.

## 5. Fish performance

### 5.1. Growth Parameters

Before stocking, 10 larvae from the incubator were anaesthetised in 0.06% 2-phenoxyethanol. Their standard length was measured using an eyepiece fitted to an Olympus SZ stereomicroscope. Samples of 10 larvae were taken out of each tank on 5 dph, and larval standard length was measured and the general condition of the larvae was assessed. On 7, 14, 21, 30, 40, 50 and 60 dph, 10 larvae were siphoned from each tank prior to feed addition (except on 21 dph, 30 larvae were sampled). These larvae were anaesthetised and examined with a stereomicroscope. They were assessed on absence or presence of the swim bladder (flattened or non-flattened), feeding (retained food in the gut, an indication for poor digestion), general condition, the standard and total length, total weight, and skeletal deformity.

Mortality was estimated daily by counting all the dead larvae and post-larvae removed from the tanks' surface and bottom during cleaning. Behavioral observations (events of aggressiveness between larvae, population dispersion, attacks due to cannibalism) were made early in the morning before the first food supply and during light time before and after feeding.

Swim bladder inflation (%), survival (%), length gain (mm), weight gain (mg), weight specific growth rate (WSGR) (% day<sup>-1</sup>), length specific growth rate (LSGR) (% day<sup>-1</sup>), average daily weight gain (ADWG) (days), average daily length gain (ADLG) (days) were calculated according to the following equations:

\* **Survival (%)** = total alive larvae on 60 dph / (larvae stocked on 1 dph – (total sampled from 7 to 50 dph + live larvae removed during mortality siphoning)) \*100

\* During the **swim bladder inflation** period (approximately from 7–21 dph), the percentage of larvae with inflated swim bladders (SBI) in a sample of 30 larvae was determined as follows:

**SBI** = 100 ( $Q_{SBI} / Q_{total}$ ), Where  $Q_{SBI}$  is the number of larvae with inflated swim bladders, and  $Q_{total}$  is the total sample number ( $n = 30$ ).

\* **Weight gain (mg larva<sup>-1</sup>)**

Final weight (mg) - Initial weight (mg) (Brody, 1945)

\***Average daily weight gain (ADWG) (mg larva<sup>-1</sup> day<sup>-1</sup>)**

Final weight (mg) - Initial weight (mg) / experimental period (days) (Brody, 1945).

\***Weight specific growth rate (WSGR) (% day<sup>-1</sup>)**

$100 \times (\ln \text{ final larva weight (mg)} - \ln \text{ initial larva weight (mg)}) / \text{experimental period (days)}$  (Castell and Tiews, 1980)

Where Ln: natural logarithm.

\***Length gain (mm larva<sup>-1</sup>)**

Final mean length of larvae in mm - Initial mean length of larvae in mm

\***Average daily length gain (ADLG) (mm larva<sup>-1</sup> day<sup>-1</sup>)**

Final length - Initial length / experimental period (days)

\***Length specific growth rate (LSGR) (% day<sup>-1</sup>)**

100 × (Ln final larva length (mm)) - Ln initial larva length (mm)) / experimental period (days)

## 5.2. Examination of skeletal deformity

Larvae were examined for their vertebral column deformity. The vertebral column was divided into four distinct regions based on morphological features according to **Boglione et al., (2001)**. The first two cephalic vertebrae were characterized by only neural processes, followed by Pre-haemal vertebrae that were characterized by centra showing pleural and epipleural ribs. Afterward comes the haemal vertebrae that were characterized by neural and haemal processes (spines). The last three caudal vertebrae were characterized by longer neural and haemal processes. To identify skeletal malformations, 10 post larvae per tank were sampled on 60 dph and fixed in 10% neutral buffered formalin. These post larvae were stained with alizarin red and alcian blue for bone and cartilage on whole mounts using the method described by **Taylor & VanDyke (1985)**.

## 6. Statistical analyses

Mean values and standard deviation (mean ± SD) for each parameter was calculated. The results of the experiment were subjected to statistical analysis in a one-way analysis of variance (ANOVA), according to **Snedecor & Cochran (1981)** using the following model:

$$Y_{ij} = \mu + T_i + B_j + E_{ij}$$

Where  $Y_{ij}$  is the observation of the  $ij^{\text{th}}$  parameter mean used:

\* $\mu$  is the overall mean;

\* $T_i$  is the effect of  $i^{\text{th}}$  treatment;

\* $B_j$  is the effect of  $j^{\text{th}}$  blocks;

\*  $E_{ij}$  is the random error.

Significant differences ( $p \leq 0.01$ ) among means were tested by least significant differences (LSD) test according to (**Steel & Torrie, 1980**).

## RESULTS

### 1. Growth performance

#### 1.1. Final total length

The larval biological parameter final total length of *D.labrax* is shown in **Table (1)**. There were no significant differences ( $p < 0.05$ ) among the three fish stocking trials 75, 100, 125 larvae  $l^{-1}$  with an average  $21.50 \pm 0.21$  mg whereas rearing stocking density 50 larvae  $l^{-1}$  achieved significantly the best final total length ( $23.17 \pm 0.17$  mg) among treatments.

**Table (1):** Growth parameters of *Dicentrarchus labrax* post-larvae at 60 dph cultured at a commercial fish hatchery at different initial stocking densities 50, 75, 100 and 125 larvae  $\text{l}^{-1}$ , where  $n = 3$  replicate tanks per treatment (10 samples per tank). Values are mean  $\pm$  SD. Different letters with the same row show significant effect within various treatments (ANOVA,  $P < 0.05$ ).

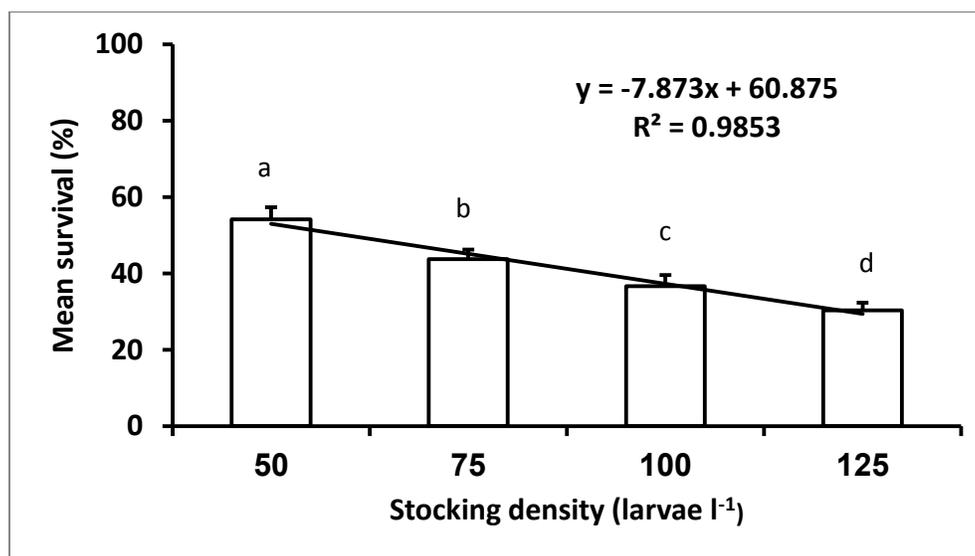
Parameters	Stocking densities (larvae $\text{l}^{-1}$ )			
	50	75	100	125
Initial length (mm)	3.0 $\pm$ 0.05	3.0 $\pm$ 0.02	3.0 $\pm$ 0.04	3.0 $\pm$ 0.01
Initial weight (mg)	0.50 $\pm$ 0.03	0.50 $\pm$ 0.01	0.50 $\pm$ 0.06	0.50 $\pm$ 0.04
Final total length (mm)	23.17 $\pm$ 0.17 <sup>a</sup>	22.33 $\pm$ 0.17 <sup>b</sup>	21.67 $\pm$ 0.17 <sup>b</sup>	20.50 $\pm$ 0.29 <sup>b</sup>
length gain(mm larvae <sup>-1</sup> )	20.17 $\pm$ 0.17 <sup>a</sup>	19.33 $\pm$ 0.21 <sup>b</sup>	18.67 $\pm$ 0.18 <sup>b</sup>	17.00 $\pm$ 0.20 <sup>b</sup>
Final total weight(mg)	75.33 $\pm$ 0.33 <sup>a</sup>	70.67 $\pm$ 0.33 <sup>b</sup>	67.83 $\pm$ 0.44 <sup>c</sup>	59.50 $\pm$ 0.29 <sup>d</sup>
weight gain(mg larvae <sup>-1</sup> )	74.83 $\pm$ 1.10	70.17 $\pm$ 2.07	67.33 $\pm$ 0.97	59.00 $\pm$ 1.98
WSGR (% day <sup>-1</sup> )	8.36 $\pm$ 0.01 <sup>a</sup>	8.25 $\pm$ 0.03 <sup>b</sup>	8.18 $\pm$ 0.02 <sup>b</sup>	7.97 $\pm$ 0.01 <sup>c</sup>
LSGR (%day <sup>-1</sup> )	3.41 $\pm$ 0.01 <sup>a</sup>	3.35 $\pm$ 0.04 <sup>b</sup>	3.30 $\pm$ 0.02 <sup>b</sup>	3.20 $\pm$ 0.05 <sup>c</sup>
ADWG (days)	1.25 $\pm$ 0.01 <sup>a</sup>	1.17 $\pm$ 0.01 <sup>b</sup>	1.12 $\pm$ 0.01 <sup>c</sup>	0.98 $\pm$ 0.00 <sup>d</sup>
ADLG (days)	0.34 $\pm$ 0.00 <sup>a</sup>	0.32 $\pm$ 0.00 <sup>b</sup>	0.31 $\pm$ 0.00 <sup>c</sup>	0.29 $\pm$ 0.00 <sup>d</sup>

### 1.2. Final total weight

The results in **Table (1)** showed that final total weight was significantly different ( $p < 0.05$ ) among treatments. The best final total weight was achieved in larvae stocked at 50 larvae  $\text{l}^{-1}$ . Results in **Table (1)** showed that the lowest stocking density of *D. labrax* larvae (50 larvae  $\text{l}^{-1}$ ) had the higher final total weight (75.33 mg  $\pm$  0.33) while the highest stocking density (125 larvae  $\text{l}^{-1}$ ) showed the lowest total weight (59.50 mg  $\pm$  0.29).

### 1.3. Survival rate

Effect of STDs on survival of *D. labrax* post-larvae at 60 dph is presented in **Fig. (1)**. Stocking density significantly ( $p < 0.05$ ) affected survival of the post-larvae at 60 dph where survival significantly decreased with increasing stocking density. There was strong inverse correlation between STDs and survival (**Fig.1**).



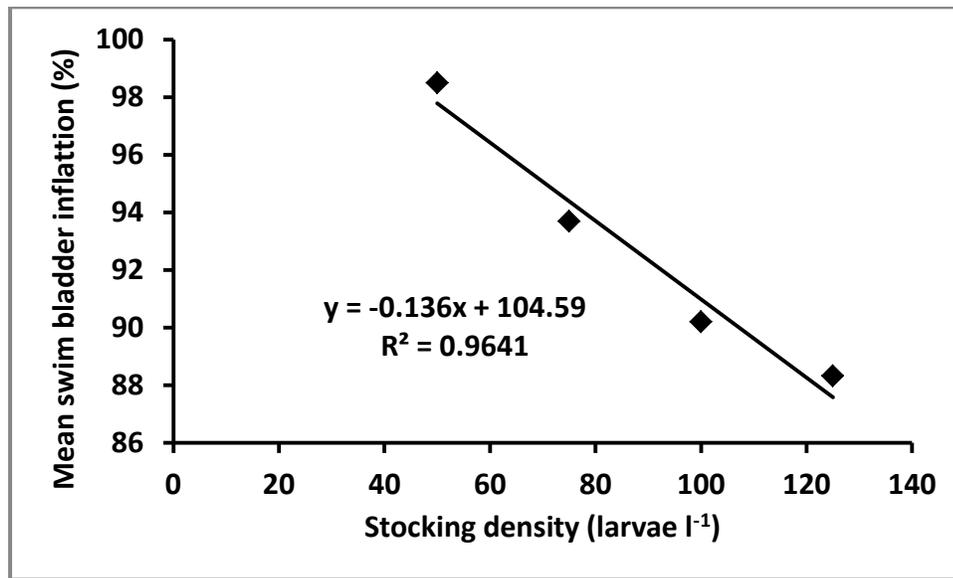
**Fig. (1):** Survival of *Dicentrarchus labrax* post-larvae at 60 dph reared in different stocking densities (50, 75, 100 and 125 larvae l<sup>-1</sup>) in a commercial fish hatchery where  $n = 3$  replicate tanks per treatment. Values are mean (%)  $\pm$  SD. Different letters show significant effect within various treatments (ANOVA,  $P < 0.05$ ).

### 2. Swim bladder inflation

The results in **Table (2)** revealed high significant ( $p < 0.05$ ) effect of increasing STDs on the formation of functional swim-bladder. The results showed that the lower larval density, the higher the percentage of SBI. Swim bladder inflation after three weeks (21 dph) were 98.50, 93.70, 90.20 and 88.33 % for larval densities 50, 75, 100 and 125 larvae l<sup>-1</sup>, respectively. There was strong inverse correlation between STDs and mean SBI at 21 dph (**Fig.2**).

**Table (2):** Effect of different stocking densities (50, 75, 100 and 125 larvae l<sup>-1</sup>) on swim bladder inflation of *Dicentrarchus labrax* larvae reared in a commercial fish hatchery, where  $n = 3$  replicate tanks per treatment (30 samples per tank). Values are mean (%)  $\pm$  SD. Different letters with the same row show significant effect within various treatments (ANOVA,  $P < 0.05$ ).

Age	Stocking density (larvae l <sup>-1</sup> )			
	50	75	100	125
<b>7 dph</b>	75.50 $\pm$ 0.58 <sup>a</sup>	69.50 $\pm$ 0.43 <sup>a</sup>	54.39 $\pm$ 0.21 <sup>b</sup>	49.22 $\pm$ 0.67 <sup>b</sup>
<b>14 dph</b>	86.33 $\pm$ 0.33 <sup>a</sup>	80.50 $\pm$ 0.35 <sup>a</sup>	76.00 $\pm$ 0.11 <sup>b</sup>	63.67 $\pm$ 0.67 <sup>c</sup>
<b>21 dph</b>	98.50 $\pm$ 0.33 <sup>a</sup>	93.70 $\pm$ 0.23 <sup>a</sup>	90.20 $\pm$ 0.13 <sup>b</sup>	88.33 $\pm$ 0.33 <sup>c</sup>

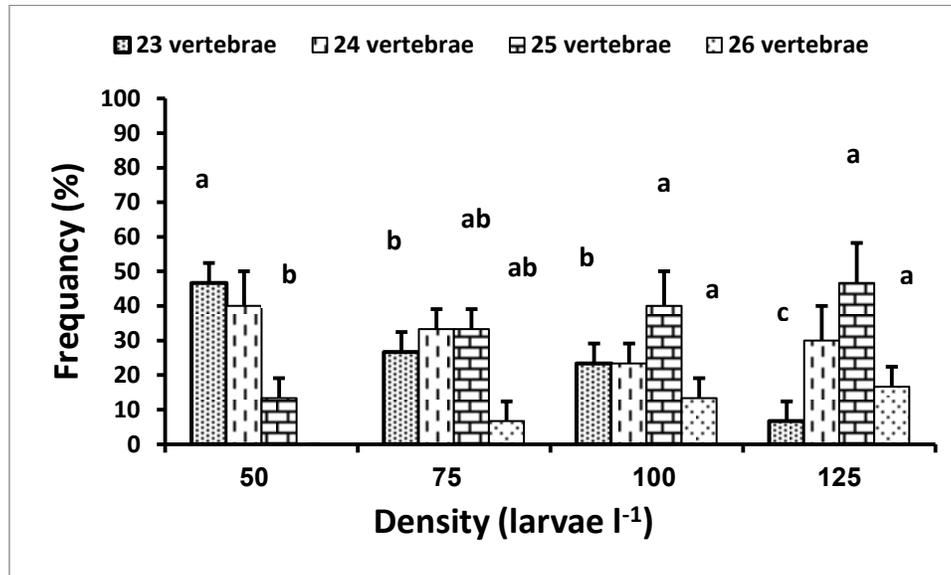


**Fig. (2).** Correlation between larval stocking densities (50, 75, 100 and 125 larvae l<sup>-1</sup>) and mean swim bladder inflation (%) on third week (21dph) of *Dicentrarchus labrax* larvae reared in a commercial fish hatchery where n = 3 replicate tanks (30 samples per tank).

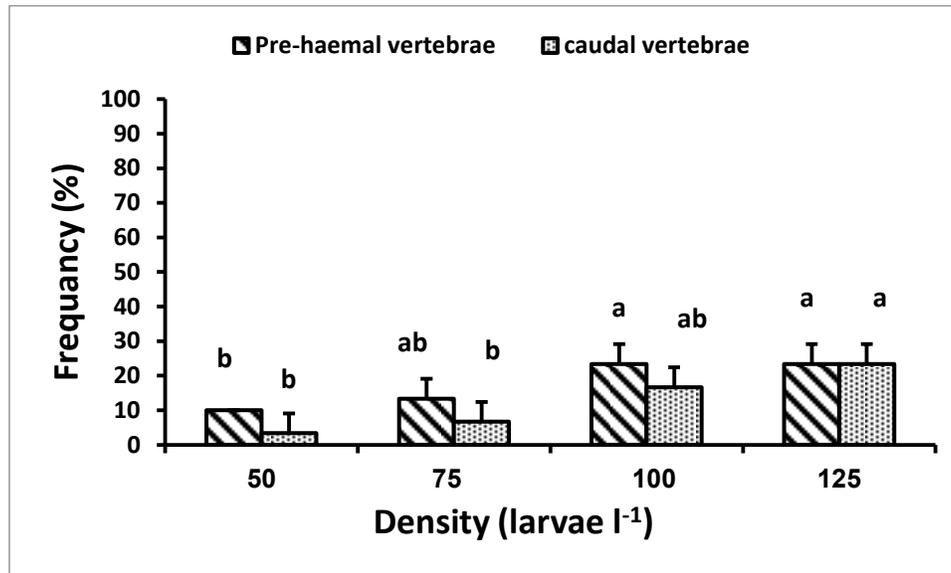
### 3. Skeletal deformity

Effect of STDs 50, 75, 100 and 125 larvae l<sup>-1</sup> on skeletal malformations of *D. labrax* post-larvae on 60 dph are presented in **figures 3** and **4**. *Dicentrarchus labrax* post-larvae on 60 dph were significantly affected ( $p < 0.05$ ) by different stocking densities in both the number of vertebrae (**Fig. 3**) and incidence of skeletal deformity (**Fig. 4**). By 60 dph, stocking densities 75, 100 and 125 larvae l<sup>-1</sup> had significantly greater number of vertebrae (25 and 26 vertebrae) than 50 larvae l<sup>-1</sup> treatment, while 23 number of vertebrae was significantly higher in the lower larval STD (50 larvae l<sup>-1</sup>) compared with the other treatments (**Fig. 3**).

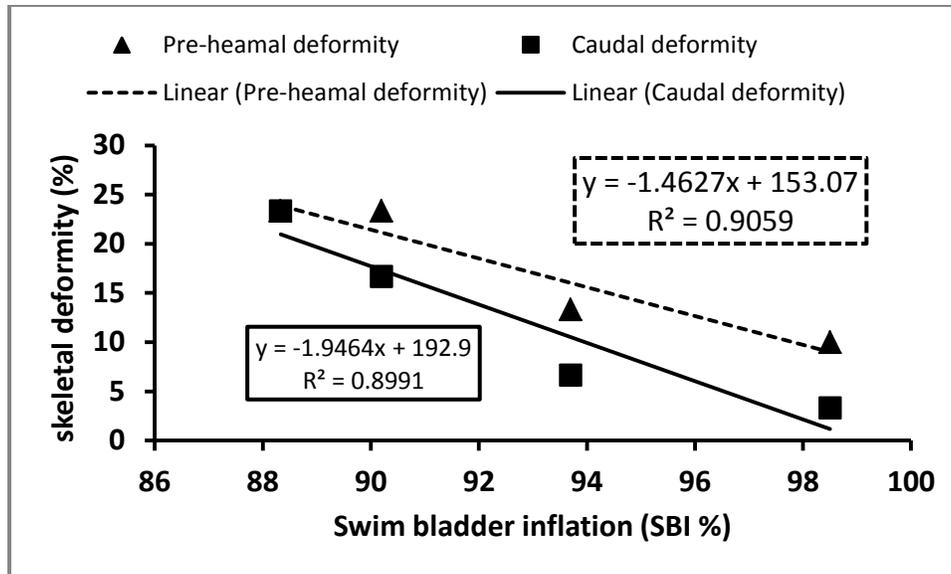
Incidence of skeletal deformity in pre-haemal region was significantly higher in larval STDs 75, 100 and 125 l<sup>-1</sup> with an average of  $20 \pm 5.77\%$  compared with  $10 \pm 0\%$  in the lower STD 50 larvae l<sup>-1</sup> (**Fig. 4**). Likewise, incidence of skeletal deformity in the caudal region was significantly higher in STDs 100 and 125 larvae l<sup>-1</sup> with an average of  $20.00 \pm 5.77\%$  compared with  $5.00 \pm 5.77\%$  in lower densities (50 and 75 larvae l<sup>-1</sup>). The incidence of appearance of more than one skeletal deformity was only observed in the treatment 125 larvae l<sup>-1</sup> with an average of  $10.00 \pm 4.00\%$ . There was inverse relationship between skeletal deformity at 60 dph and SBI at 21 dph (**Fig. 5**).



**Fig. (3).** Frequency (%) of number of vertebrae in *Dicentrarchus labrax* post-larvae at 60 dph reared in a commercial fish hatchery at different stocking densities 50, 75, 100 and 125 larvae l<sup>-1</sup> where n = 3 replicate tanks per treatment (10 samples per tank). Values are mean (%) ± SD. various letters indicate significant effect within different treatments (ANOVA, P < 0.05).



**Fig. (4).** Incidence (%) of appearance of skeletal deformity in pre-haemal and caudal regions in *Dicentrarchus labrax* post-larvae at 60 dph reared in a commercial fish hatchery at different stocking densities 50, 75, 100 and 125 larvae l<sup>-1</sup> where n = 3 replicate tanks per treatment (10 samples per tank). Values are mean (%) ± SD. various letters indicate significant effect within different treatments (ANOVA, P < 0.05).



**Fig. (5).** Correlation between incidence (%) of appearance of skeletal deformity in pre-haemal and caudal regions in *Dicentrarchus labrax* post-larvae at 60 dph reared in a commercial fish hatchery at different stocking densities 50, 75, 100 and 125 larvae l<sup>-1</sup> and percentage of swim bladder inflation at 21 dph, where n = 3 replicate tanks per treatment.

## DISCUSSION

The examined STDs in the present work did not affect the survival of newly hatched *D. labrax* larvae especially during pre-yolk sac exhaustion period (data not published). Observations of dead larvae showed that there were two durations of elevated larval mortality: the first when starting exogenous feeding and the second during SBI period mostly from 6 to 10 dph. These observed mortalities were probably not due to the initial stocking densities mainly because it was observed among all treatments. These mortalities may be attributed to the urgent need to increase energy resources as suggested by **Person Le Ruyet *et al.* (1993)** and **Hatziathanasiou *et al.* (2002)** for *D. labrax* and **Szkudlarek & Zakes (2007)** for Pikeperch larvae, *Sander lucioperca*.

No cannibalistic behaviour signs were observed on the daily larval mortality collected from the tanks. Cannibalism was recorded in *D. labrax* larvae investigated by **Hatziathanasiou *et al.* (2002)** and **El-Sayed *et al.* (2014)**. Other researchers also demonstrated the same behaviour in other marine fish larvae such as red porgy, *Pagrus pagrus*, (**Stephanou *et al.*, 1995**), marine silverside, *Odontesthes argentinensis* (**Sampaio & Phonlor, 1996**), Koi carp, *C. carpio* (**Van Damme *et al.*, 1989**) and vundu, *Heterobranchus longifilis*, (**Baras, 1999**). Cannibalism is mainly induced and increased due to a variety of reasons which include but are not limited to: larval STD, transition period to exogenous feeding such as switching from live food to another, or during weaning to an artificial diet (**Paller and Lewis 1987; Folkvord, 1991; Shawky *et al.*,**

2021). **Mohseni et al. (2000)** confirmed that morphological investigation of cannibalism in persian sturgeon, great sturgeon, and stellate sturgeon larvae was significantly higher in high stocking density trials after the onset of exogenous feeding. In the current research, no signs of cannibalistic behaviour were detected even in high larval STD (125 larvae  $l^{-1}$ ). This suggests that perhaps the continuous flow-throw water system used in the research was suitable for rearing *D. labrax* larvae.

The results of the current research showed that by 60 dph survival, growth in terms of length and weight (LSGR, WSGR, ADLG and ADWG), and swim bladder inflation were significantly affected by the STD. Survival was significantly higher in the lowest stocking density (50 larvae  $l^{-1}$ ) and minimum in the highest STD (125 larvae  $l^{-1}$ ). **Mohseni et al. (2000)** indicated that high STD can cause the area of the feeding surface to decrease and also disrupt the distribution of food among larvae. The decrease of area in the feeding surface results in the larvae crowding at a particular spot, and this leads to wounds, bodily injury, and breaking of fins (**Mokhaier, 1988**), and decline in the growth rate (**Nazari, 1996**),

The survival of *D. labrax* larvae increases with lower densities. This agrees with the findings of **Hatziathanasiou et al. (2002)** for improving sea bass aquaculture either in early or post-larval stages. The result of the current research agrees also with **Liu et al. (2019)** who reported that higher stocking densities on Lenok, *Brachymystax lenok*, resulted in lower feed efficiency and SGR compared with the lower densities. On the contrary, production of African catfish, *Clarias gariepinus*, in high density resulted in higher fish yield and annual production in extensive pond systems (**Oké and Goosen, 2019**). This is probably due to the decrement of aggressive behavior that increased in *D. labrax* larvae that were reared at high densities as reported by **Papoutsoglou et al. (1998)**.

In the current study, significant inverse correlation was found between swim bladder inflation at 21 dph and incidence of appearance of skeletal deformity at 60 dph, where incidence of appearance of skeletal deformity increases by decreasing the percentage of normally developed swim bladder. The results of the current study agrees with (**chatain, 1994**) who found that deformation in the pre-haemal region especially lordosis is correlated with the mal-formed swim bladder in *D. labrax* as well as sea bream, *Sparus aurata*, larvae. There are two kinds of swim bladders: physoclistous and physostomous swim bladders. The majority of the larval fish reared in the commercial marine hatcheries are physoclistous as adults but physostomous at the larval stages (**Boglione et al., 2013**). In physostomous swim bladders, there is a connection between the swim bladder and the oesophagus through a duct called the pneumatic duct (**Boglione et al., 2013**). During SBI phase, larvae rise to the water's surface and gasp the air needed to inflate the swim bladder and larvae who fail to do that will suffer from a mal-formed swim bladder (**Boglione et al., 2013**).

A variety of biotic and abiotic factors may be involved in stopping initial SBI during appropriate inflation time such as high STD. Failure of the larvae to develop normal swim bladder may increase the probability of incidence of skeletal deformity. This is mainly because the larvae suffering from mal-formed swim bladder have to swim vigorously with their lateral fins specifically pectoral fins to maintain balance in the water column. This excessively usage of such fins movement increases the exhaustion of pre-haemal muscles that result in mechanical overload on the newly differentiating pectoral elements. With continuing exerting effort on the pectoral fin to overcome lacking functional swim bladder, the pre-haemal vertebrae in the vertebral column can be bended (**Kranenbarg *et al.*, 2006**) or fused together (**Chatain 1994; Boglione *et al.*, 1995**) resulting in deformation in the vertebral column that could finally reach the haemal vertebrae as well (**Boglione *et al.*, 2013**). In the current study, vertebral fusions and lordosis in the haemal region and change in the normal vertebrae counts could be attributed to disorder in the differentiation of vertebral centrum (**Haga *et al.*, 2009**) that resulted from over using of pectoral fin due to mal-function swim bladder.

The results of the current research also agree with **Hattori *et al.* (2003)** and **Matsuoka (2003)** on red seabream, *Pagrus major*, and **Izquierdo & Herrero (2010)** on *P. pagrus* juveniles, who reported that combined vertebrae and decreased or increased number in vertebrae counts in the pre-haemal region was common in most of the specimens that has lordosis. **Izquierdo & Herrero (2010)** added that the skeletal deformities in *P. major* (13% lordosis) and (14% combined vertebrae) were mainly a result of STD (for example in semi-intensive had 3.9% deformity whereas the intensive had 8.8% deformity) and were not accompanied with extra-inflated or mal-functional swim bladder.

## CONCLUSION

In conclusion, the results of that study indicated that *D. labrax* larval density, especially at first feeding affected the SBI as well as the manifestation of skeletal deformity in the vertebral column at post-larval stages. In addition, newly hatched larvae STD affected larval growth in terms of weight, length and survival by 60 dph. The results of the current study recommend lower STD for *D. labrax* aquaculture that not exceed 75 larvae  $l^{-1}$  to achieve best growth results in terms of the quality of the produced larvae and reduce the cannibalism behaviour and skeletal deformities.

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