



Effects of Illumination Intensity on Growth Parameters and Swim Bladder Development in Common dentex (*Dentex dentex*, L.) Larvae

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Abstract

The influences of three different light intensities were investigated on growth of common dentex (*Dentex dentex*) during larval period (0-35 day). Three different illumination levels were compared that 10 lx as group A, 30 lx described group B and 100 lx named group C in triplicates, respectively. Larvae were cultured in a recirculating aquaculture system using a green water technique. The longest total length and the heaviest weight were found as 18.43±1.9 mm and 38.48±5.1 mg in group B with 30 lx light intensity. Same parameters were 18.03±2.5 mm and 36.72±4.8 mg for group C with 100 lx light intensity; 16.23±1.7 mm and 28.53±4.2 mg for group A with 10 lx light intensity. In terms of total length and weight, there were significant differences in group A (P<0.05), however, differences were not detected between B and C groups (P>0.05). Also, swim bladder inflation rates were calculated as 88.3±2.7, 92.1±1.6 and 91.4±1.3% for groups A, B and C, respectively and did not presented significant differences among groups (P>0.05). In addition, survival rates were 8.8±3.1, 23.5±2.5 and 19.9±2.8%, respectively. While not significant differences were found between group B and C (P>0.05), but group A presented significantly difference compared to other groups (P<0.05). As a result, it is thought that illumination effected husbandry parameters were more affirmative with 30 lx light intensity in *Dentex dentex* under culture conditions.

Keywords: Illumination, larval growth, swim bladder, survival rate, common dentex, *Dentex dentex*.

Işık Yoğunluğunun Sinarit (*Dentex dentex*, L.) Larvalarında Büyüme Parametreleri ve Hava Kesesi Gelişimine Olan Etkileri

Özet

Bu çalışmada 3 farklı ışık yoğunluğunun üretim periyodu boyunca (0-35 gün) sinarit (*Dentex dentex*) larvalarının büyümesine olan etkileri incelenmiştir. 3 tekrarlı yürütülen denemede 3 farklı ışık yoğunluğu karşılaştırılmış ve sırasıyla 10 lux A grubu, 30 lux B grubu ve 100 lux C grubu olarak tanımlanmıştır. Larvalar yeşil su tekniği kullanılarak akışkanlı sistemde üretilmiştir. En yüksek total boy 18,43±1,9 mm ve 38,48±5,1 mg ağırlık değerleri olarak 30 lux uygulanan B grubunda bulunmuştur. Aynı parametreler 100 lux denenen C grubunda 18,03±2,5 mm ve 36,72±4,8 mg, 10 lux uygulanan A grubunda ise 16,23±1,7 mm ve 28,53±4,2 mg olarak ölçülmüştür. Boyca büyüme ve ağırlık artışı açısından, A grubu farklı bulunmuş (P<0,05), ancak B ve C grubu arasında önemli farklılıklar tespit edilmemiştir (P>0,05). Bunun yanında, hava kesesi gelişim oranları A, B ve C grupları için sırasıyla %88,3±2,7, 92,1±1,6 ve 91,4±1,3 olarak hesaplanmış, ancak gruplar arasında önemli bir farklılık göstermemiştir (P>0,05). Ek olarak, yaşama oranları gruplara göre sırasıyla %8,8±3,1, 23,5±2,5 ve 19,9±2,8 olarak hesaplanmıştır. B ve C grubu arasında önemli farklılık tespit edilmez iken (P>0,05) A grubu diğer deneme gruplarına göre farklı bulunmuştur (P<0,05). Sonuç olarak, kültür koşulları altında farklı ışık yoğunlukları sinarit *Dentex dentex* larvalarının büyüme parametrelerini etkilemiş, 30 lux ışık yoğunluğunda daha olumlu sonuçlar tespit edilmiştir.

Anahtar Kelimeler: Aydınlatma, larval büyüme, hava kesesi, yaşama oranı, sinarit, *Dentex dentex*.

Introduction

Common dentex (*Dentex dentex*) is a highly economic species which is distributed the Mediterranean, the Atlantic from the Bay of Biscay to Madeira and, rarely, the Black Sea (Bauchot and

Hureau, 1986; Koumoundouros *et al.*, 1999). This species is considered to promising candidate species for Mediterranean aquaculture due to a higher growth rate than gilthead sea bream *Sparus aurata*, possesses the ability to spawn spontaneously and current production technology may be applied to its culture

(Glamuzina et al., 1989; Efthimiou et al., 1994; Loir et al., 2001). During the past decade, culture of common dentex relatively increased and reached a considerable level of development in certain Mediterranean countries (Abellan, 2000; Rueda and Martinez, 2001; Loir et al., 2001). Also, common dentex has attracted wide interest therefore, more studies have been focused on its reproduction and physiology (Pavlidis et al., 2000; Saka et al., 2004), larval rearing (Glamuzina, et al., 1989; Pastor et al., 1995), nutrition (Tibaldi et al., 1996; Mourente et al., 1999), morphological and osteological ontogeny (Koumoundouros et al., 1999; 2004). It is important species for diversification of Mediterranean aquaculture industry due to faster growth rate and economical gain than the Sparid species.

More than past decades, due to over saturation of aquaculture by two species, European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*), culture of promising candidate species that would diversity to expand the market for aquacultured species. Scientific and technical interests have focused on native species and their biological cycle can be reproduced using currently available breeding techniques. Therefore, common dentex could be accepted as a credible candidate for marine culture (Abellan, 2000; Rueda and Martinez, 2001; Loir et al., 2001). Studies on this species in its natural environment are scarce, and mostly have focused on finding its distribution, determining the timing of gonadal maturation under natural conditions or describing its early life history, morphology, nutritional ontogeny, physiology and production (Santamaria et al., 2004; Koumoundouros et al., 2004; Firat et al., 2003, 2005; Pérez-Jiménez et al., 2009; Gisbert et al., 2009). Over the last decades, however, a considerable number of scientific reports have been published concerning the biology and culture techniques of common dentex in captivity (Morales-Nin and Moranta, 1997; Suzer et al., 2006; Giménez and Estevez, 2008a,b). Common dentex is normally found in the Mediterranean basin and in recent years it has been successfully produced in Greece, Italy and Spain, where its market price is similar to that of other marine farmed species.

Among the numerous abiotic factors affecting the activity of fish larvae, light and light intensity have an important crucial role under culture conditions which must be reared in a specific light ranges, depending on the developmental stage and the species (Chatain and Ounais-Guschemann, 1991; Boeuf and Le Bail, 1999). Especially, in order to enhance swim bladder inflation and promote the larval growth, illumination is the main crucial criteria under culture conditions. Besides, lack of functional swim bladder is caused to malnutrition, lack of movement, spinal deformities and delay of development (Chatain, 1986; Chatain and Ounais-Guschemann, 1991). In this study, effects of different illumination levels, which are the common light

intensities for larval rearing period in Mediterranean aquaculture on growth parameters and swim bladder inflation of common dentex (*Dentex dentex*) are studied.

Materials and Methods

Broodstock and Egg Incubation

Common dentex broodstock, 8 females (3.4 kg mean weight) and 8 males (2.6 kg mean weight), were selected from wild broodstocks and maintained in 16 m³ cylindrical tank during the natural spawning season between April and May. In order to enhance of spawning, no hormonal treatment was administered for breeders. After spawning, egg collectors on the broodstock tank were monitored and eggs were immediately taken from collectors. Following fertilization, the viable buoyant eggs were separated from the dead sinking eggs from the same batch. Eggs were incubated in 50 litre cylindrical conical incubators with 3000 eggs.L⁻¹ density in seawater at 16.0±0.5°C. Oxygen saturation was over 85%, salinity was 37 ppt and pH was 7.65±0.23. Ammonia and nitrite components were always below 0.012 mg.L⁻¹.

Larval Rearing

Larvae of common dentex were cultured in recirculating aquaculture system using a green water technique and also larval rearing parameters and protocols were carried out according to Firat et al. (2005).

On day 3, the mouth opened and also from this date until 18 DAH, larvae were fed at density of 10–15 individuals.ml⁻¹ with two strains of rotifers (*Brachionus plicatilis* but mainly with *Brachionus rotundiformis*) enriched with algae and enrichment media (DHA Protein Selco, Artemia Systems SA, Ghent, Belgium). In similar with rotifer, two grade of *Artemia* nauplii (between 9 and 17 DAH at 4–6 individuals.ml⁻¹, AF480 INVE Aquaculture) and *Artemia* metanauplii (from 15 to 35 DAH at 2–4 individuals.ml⁻¹, EG, Artemia Systems SA, Ghent, Belgium), both enriched with Protein Selco (Artemia Systems SA, Ghent, Belgium).

Experimental Design and Sampling

Experimental larval tanks were 6 m³ and also established in triplicate under three different light intensities, 10, 30 and 100 lx, named as group A, B and C, respectively. Also, in order to lighting interferences, black plastic frames were used for isolation of environment. The illumination of the experimental tanks were provided by double fluorescent lamps (type cool light, Philips, 20 W) from 1 m above of the water surface. Light intensity was measured at the water surface by a luxmeter

(Model YFE-YF1065).

In order to measure of growth rate, larvae were sampled from each group per week (30 larvae sample group⁻¹). At the end of the experiment, larval survival was determined by manually counted larvae remaining in the larval tanks. Swim bladder volume was calculated by following formulae (Ré *et al.*, 1985). $V = \frac{4}{3} \pi a^2 b$ where a is the half of maximum swim bladder length and b is the half of maximum swim bladder width.

Statistical Analysis

All measurements were conducted in triplicate. Data were presented as mean±SD. Levene test was used for determination of the variance homogeneity while Fischer's chi-square test was performed to examine of survival data. All data were compared by one-way ANOVA, whereas followed by Newman-Keul's multiple range test with a significance level of 5% and performed by SPSS 15.0 software.

Results

Larvae hatched out between 52-57 h after fertilization and hatching rate varied between 87 and 94%. Diameters of eggs and oil globules were $1004.31 \pm 17.02 \mu\text{m}$ and $232.84 \pm 5.59 \mu\text{m}$, respectively and egg parameters were not significantly different ($P > 0.05$).

In all groups, water temperature was between 16 and 20°C, as average was $18.6 \pm 0.4^\circ\text{C}$ and also mouth opening was observed at 3 DAH.

In group A with 10 lx, initial total length was $3.12 \pm 0.01 \text{ mm}$ while it was measured as $4.04 \pm 0.2 \text{ mm}$ at 7 DAH. It was $4.62 \pm 0.2 \text{ mm}$, $8.05 \pm 0.5 \text{ mm}$, $12.12 \pm 1.4 \text{ mm}$, and $16.23 \pm 1.7 \text{ mm}$ for 14, 21, 28, and 35 DAH respectively. In terms of weight development of larvae in group A, it was $0.945 \pm 0.01 \text{ mg}$ on day 7 while it was $0.603 \pm 0.01 \text{ mg}$ at 0 DAH. It was $3.04 \pm 0.8 \text{ mg}$, $15.11 \pm 1.1 \text{ mg}$, $19.18 \pm 2.1 \text{ mg}$, and $28.53 \pm 4.2 \text{ mg}$ for 14, 21, 28, and 35 DAH, respectively (Fig. 1).

In group B with 30 lx, total length development was measured as $4.07 \pm 0.1 \text{ mm}$ and $3.12 \pm 0.01 \text{ mm}$ at 0 and 7 DAH, respectively. It was $4.76 \pm 0.2 \text{ mm}$, $8.65 \pm 0.6 \text{ mm}$, $14.31 \pm 1.8 \text{ mm}$, and $18.43 \pm 1.9 \text{ mm}$ for 14, 21, 28, and 35 DAH, respectively. In terms of weight development of larvae in group B, it was $0.922 \pm 0.11 \text{ mg}$ on day 7 while it was $0.603 \pm 0.01 \text{ mg}$ at 0 DAH. It was $3.21 \pm 0.9 \text{ mg}$, $16.22 \pm 1.4 \text{ mg}$, $23.07 \pm 3.5 \text{ mg}$, and $38.48 \pm 5.1 \text{ mg}$ for 14, 21, 28, and 35 DAH, respectively (Figure 1).

In group C treated with 100 lx, total length development was estimated as $3.12 \pm 0.01 \text{ mm}$ and $3.77 \pm 0.2 \text{ mm}$ on day 0 and 7. Then, it was $4.63 \pm 0.1 \text{ mm}$, $8.11 \pm 0.8 \text{ mm}$, $13.72 \pm 1.7 \text{ mm}$, and $18.03 \pm 2.5 \text{ mm}$ for 14, 21, 28, and 35 DAH, respectively. In terms of weight development of larvae in group C, it was $0.863 \pm 0.12 \text{ mg}$ on day 7 while it was 0.603 ± 0.01

mg at the beginning of the experiment. Then it was $3.12 \pm 0.9 \text{ mg}$, $15.53 \pm 1.18 \text{ mg}$, $21.27 \pm 1.06 \text{ mg}$, and $36.72 \pm 4.8 \text{ mg}$ for 14, 21, 28, and 35 DAH, respectively (Figure 1).

Among groups, the longest larval length was found in group B treated with 30 lx. However, no significant differences were found between group B and C ($P > 0.05$), while there were significant differences between group A and other experimental groups ($P < 0.05$). Similarly, the heaviest weight was observed in group B. Although, there were no significant differences between group B and C ($P > 0.05$), significant differences were recorded between group A and other experimental groups ($P < 0.05$). Total length and weight development in groups are shown in Figure 1.

At the end of the experiment, survival rates were 8.8 ± 3.1 , 23.5 ± 2.5 and $19.9 \pm 2.8\%$ for groups A, B and C, respectively. In similar, no significant differences were noted between group B and C were ($P > 0.05$), whereas group A was analyzed significantly different than the other experimental groups ($P < 0.05$).

Initiation of swim bladder inflation percentages were observed from 5-7 DAH. The first inflation rate was determined on day 5 (Replicates A: 37.3, 39.7 and 34.6%; B: 42.2, 44.1 and 38.5%; C: 36.4, 33.8 and 29.2%) for the first time in all groups and also inflation rate exponentially increased by larval age at 6 (Replicates A: 56.1, 52.4 and 57.3%; B: 58.2, 61.4 and 54.9%; C: 54.6, 57.7 and 59.8%) and 7 DAH (Replicates A: 66.3, 62.2 and 68.7%; B: 65.4, 63.2 and 71.8%; C: 61.9, 55.1 and 63.5%). Finally, these rates reached to 88.3 ± 2.7 , 92.1 ± 1.6 and $91.4 \pm 1.3\%$ for group A, B and C, respectively at the end of the experiments. No significant differences were found among experimental groups ($P > 0.05$).

In addition, on day 7, swim bladder volume was $0.055 \pm 0.006 \text{ mm}^3$, $0.059 \pm 0.007 \text{ mm}^3$ and $0.057 \pm 0.006 \text{ mm}^3$ for groups A, B and C, respectively (Figure 2). No statistical differences were found among groups ($P > 0.05$). After this date, swim bladder volumes increased in all groups depending on larval age and size. Metamorphosis of swim bladder was observed between 15 and 20 days and about 3-5 fold increases were observed in swim bladder volumes. However, swim bladder hypertrophy was observed as 15, 11 and 18% for groups A, B and C, respectively. Increase of hypertrophic swim bladder volume was about 8-9 fold. Additionally, swim bladder metamorphosis was calculated more than these rates and also about from 14.6 to 18.4% swim bladder hypertrophy was observed between 23 and 29 DAH due to metamorphosis. At the end of the experiment, swim bladder volumes were $1.77 \pm 0.17 \text{ mm}^3$, $1.94 \pm 0.31 \text{ mm}^3$ and $1.86 \pm 0.28 \text{ mm}^3$ for groups A, B and C, respectively. The best swim bladder development was found in group B treated with 30 lx, and followed by group C treated with 100 lx and group A with 10 lx. Moreover, swim bladder inflation rates were $88.3 \pm 2.7\%$, $92.1 \pm 1.6\%$ and $91.4 \pm 1.3\%$ for

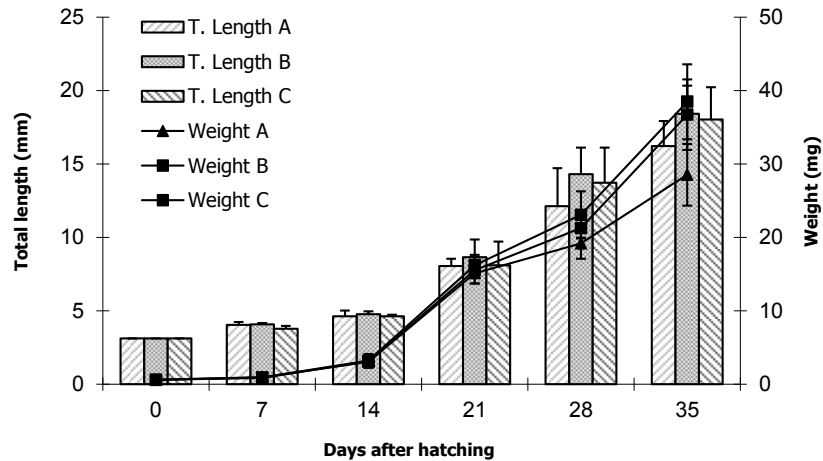


Figure 1. Growth of *Dentex dentex* larvae in experimental groups during the experiment: total length (histogram) and weight (solid line). Each mean \pm SD represents a pool of 30 larvae.

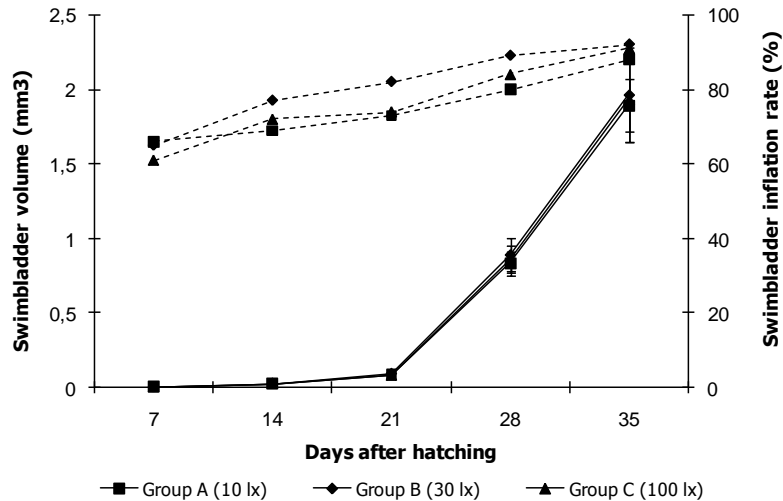


Figure 2. Swim bladder development of *Dentex dentex* larvae in experimental groups during the experiment: swim bladder volume (solid line) and swim bladder rate (dashed line). Each mean \pm SD represents a pool of 30 larvae.

group A, B and C, respectively but all experimental groups did not presented significant differences ($P > 0.05$). Swim bladder inflation and development in all groups are shown in figure 2.

Discussion

Light has an important role in the preparation of survival functions in all organisms (Boeuf and Le Bail, 1999). In aquaculture studies, light intensity and period as an abiotic factor, are very important, but have not been studied enough in culture conditions yet for many cultured fish species. These factors play a vital role in periods from incubation of eggs to forming larval pigmentation and from absorption of endogenous food reserves to formation of deformation. Many studies on larval development and

light have focused on the most cultured, gilthead sea bream *Sparus aurata*, European sea bass *Dicentrarchus labrax* and candidate species, Atlantic halibut *Hippoglossus hippoglossus* and rainbow trout *Oncorhynchus mykiss*, (Bolla et al., 1988; Saka et al., 2001; Weppe and Joassard, 1986; Firat et al., 2003). In this study, influences of three different light intensities on swim bladder development and inflation and larval survival rate of common dentex (*Dentex dentex*) during larval development (between 0-35 DAH) was studied.

Growth was satisfactory in all groups except group A treated with 10 lx. The highest total length and weight development was observed in group B with 30 lx (18.43 ± 1.9 mm and 38.48 ± 5.1 mg, respectively). For groups A and C, these parameters were 16.23 ± 1.7 mm, 28.53 ± 4.2 mg and 18.03 ± 2.5

mm, 36.72±4.8 mg, respectively.

Similar results were recorded in the other cultured Sparid species under the same experimental design. In both common pandora (*Pagellus erythrinus*) and red porgy (*Pagrus pagrus*), two recent candidate species, the highest growth were observed in larvae exposed to 30 lx illumination, while poor growth parameters were recorded in the 10 lx conditions (Suzer and Kamacı, 2004; 2005). On the other hand, Fırat et al. (2005) and Çoban et al. (2009) estimated the total length of cultured common dentex larvae at 35 DAH around 15-16 mm under 80-100 lx conditions. It is considered that the between our results and authors' results, are derived from breeders, egg and larvae quality, culture conditions, and/or larval feed (rotifera and *Artemia*). Moreover, in this study survival rates were 8.8±3.1, 23.5±2.5 and 19.9±2.8% for groups A, B and C, respectively. According to obtained results, relatively lower illumination (10 lx) resulted in poor growth parameters and survival. Critical change in growth parameters were recorded especially after 20 DAH. This could be related to lower light intensities not satisfying of biological requirement of dentex larvae during the metamorphosis stage and also relatively lower illumination levels caused to malnutrition due to dark zone in deeper side of experimental tanks. Similar pattern for survival rates were observed for both common pandora (*Pagellus erythrinus*) and red porgy (*Pagrus pagrus*) for all illumination levels in the same experimental conditions (Suzer and Kamacı, 2004; 2005). On the other hand, it was treated different photoperiod and light intensities during the larval rearing of this species, also the authors reported that the best conditions for illumination for this species were reported as 24 h L:0 h D photoperiod and at least 3.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ illumination under culture conditions (Giménez and Estevez, 2008a).

It is well reported that swim bladder is a crucial hydrostatic organ which manage the vertical distribution and changing of buoyancy of larval fishes. Besides, it is claimed that the most critical stage generally observed after finishing of yolk sac reserves during the initial inflation of swim bladder (Chatain, 1986; Chatain, 1994; Kitajima et al., 1994). Larval swim bladder becomes functional by gulping air at the water surface during the dark stage of photoperiod (Chatain, 1986; Chatain, 1994; Kitajima et al., 1994). Additionally, there are several factors effecting initial inflation and development of swim bladder such as water temperature, salinity, aeration, illumination, physico-chemical parameters of sea water, and tank shape and hydrodynamics (Chatain, 1986; Chatain, 1994; Kitajima et al., 1994). In this study, initial swim bladder inflation was observed between 5 and 7 DAH around 4.0-4.5 mm in all groups. As expected, the ratio of swim bladder to its volume increased significantly during the experiment depending on the larval age. Besides, it is reported that first swim bladder inflations was observed in 3.5-

4.0 mm total length and between 5-10 days in *Dentex dentex* (Fırat et al., 2005; Çoban et al., 2009). As is the case for other sparids, the success of swim bladder inflation depends to a large extent on the photoperiod. Some studies by Abellán et al. (2000) stated that an 18:6 (L:D) photoperiod provides the best results both in terms of swim bladder inflation and larval survival (Rueda and Martinez, 2001). Similarly, in sea bream (*Sparus aurata*) and sharpnose sea bream (*Diplodus puntazzo*) larvae, this phenomenon was determined 4.0-5.0 mm total length and between 5-9 days (Chatain, 1986; Marangos, 1995). Additionally, similar findings were recorded for both common pandora (*Pagellus erythrinus*) and red porgy (*Pagrus pagrus*) for all illumination levels in the same experimental conditions (Suzer and Kamacı, 2004; 2005).

Swim bladder hypertrophy was firstly identified as a stress syndrome in sea bass (*D. labrax*) larvae by Johnson and Katavic (1984). They observed that this syndrome could be described by failure of the larvae to inflate their swim bladder and/or hyperinflation of swim bladder due to sudden changes of abiotic factors such as temperature, salinity, aeration, illumination, and other physical-chemical parameters of culture conditions. This syndrome mostly caused vertebra deformities in fish larvae due to hyperinflation of swim bladder and higher pressure to larval notochord. In this study, several hypertrophic swim bladder were observed in all experimental groups. Similarly, a few common dentex larvae presented hyperinflation swim bladder between 25-35 DAH, which might be due to internal stress factors such as malnutrition and some organogenetic changes during the metamorphosis in this species. Besides, it is reported that lack of functional swim bladder and/or swim bladder hypertrophy could be caused to vertebral deformities in this species and marine fish larvae during early ontogeny (Chatain, 1986; Koumoundouros et al., 2004).

In conclusion, this study investigates relationships between illumination and growth parameters in *D. dentex* larvae during early ontogeny. It is thought that illumination effected husbandry parameters and also larval development, swim bladder inflation and survival rate were more affirmative with 30 lx light intensity in *Dentex dentex* culture, which is easily applicable in culture conditions.

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