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Research Article

The Effects of Different Light Intensities on Early Life Development of Sharpsnout Seabream (*Diplodus puntazzo*, Cetti, 1777) Larvae

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Abstract: The effect of different levels of illumination in the early life development stage of sharpsnout seabream (*Diplodus puntazzo*) larvae was examined. It was determined that illumination affected the relationships between total length and digestive tube length, total length and oil globule volume, and total length and yolk sac volume. The difference in the development of total length of the larvae kept in the dark and in other lighting conditions (30-450 lx) was significant ($P < 0.05$); however, it was determined that digestive tube development, yolk sac utilisation, and oil globule absorption differences of the larvae were not significant ($P > 0.05$). As for the covariance test, while the relationship between total length and oil globule volume was not significantly ($P > 0.05$) different between Group A (dark) and Group B (30 lx), it was significantly ($P < 0.05$) different between Group A (dark) and Group C (450 lx), and Group B (30 lx) and Group C (450 lx). Additionally, the relationship between total length and yolk sac volume was significantly different ($P < 0.05$) between Group A (dark) and Group B (30 lx), Group A (dark) and Group C (450 lx), and Group B (30 lx) and Group C (450 lx) ($P < 0.05$). However, the relationship between total length and digestive tube length was not significantly different ($P > 0.05$) between Group A (dark) and Group B (30 lx), Group A (dark) and Group C (450 lx), or Group B (30 lx) and Group C (450 lx). Survival rate was not significantly different among the 3 experimental groups ($P > 0.05$).

Key Words: Sharpsnout seabream, *Diplodus puntazzo*, light intensity, survival rate

Farklı ışık yoğunluklarının Sivriburun Karagöz (*Diplodus puntazzo*, Cetti, 1777) Larvalarının Erken Dönem Gelişimleri Üzerine Etkileri

Özet: Bu çalışmada farklı yoğunluktaki ışıklandırmanın sivriburun karagöz (*Diplodus puntazzo*) larvalarının erken yaşam gelişim evrelerine etkisi incelenmiştir. Işıklandırmanın; total boy ve sindirim tübü uzunluğu, total boy ve yağ damlası hacmi ve total boy ve vitellus kesesi arasındaki ilişkiyi etkilediği bulunmuştur. Larvaların total boy gelişimindeki farklılık karanlıkta ve diğer ışıklandırma koşullarında (30-450 lx) önemli bulunmuştur ($P < 0,05$). Bununla birlikte larvaların sindirim tübü gelişiminin, vitellus kesesi tüketilmesinin ve yağ damlası emiliminin önemli olmadığına karar verilmiştir ($P > 0,05$). Dağılım testine göre total boy ve yağ damlası hacminin ilişkisi grup A (karanlık) ve grup B (30 lx) arasında önemli değilken ($P > 0,05$), grup A (karanlık) ve grup C (450 lx) arasında ve grup B (30 lx) ve grup C (450 lx) arasında önemlidir ($P < 0,05$). Bunların yanında, total boy ve vitellus kesesi hacminin ilişkisi grup A (karanlık) ve grup B (30 lx), grup A (karanlık) ve grup C (450 lx) ve grup B (30 lx) ve grup C (450 lx) arasında önemli bulunmuştur ($P < 0.05$). Bununla birlikte total boy ve sindirim tübü uzunluğu ilişkisi grup A (karanlık) ve grup B (30 lx) arasında, grup A (karanlık) ve grup C (450 lx) arasında ve grup B (30 lx) ve grup C (450 lx) arasında önemli bulunmuştur ($P > 0,05$). Deneme gruplarında (A, B ve C) yaşama oranlarında önemli derecede farklılık bulunmamıştır ($P > 0,05$).

Anahtar Sözcükler: Sivriburun karagöz, *Diplodus puntazzo*, ışık yoğunluğu, yaşama oranı

Introduction

Sharpsnout seabream (*Diplodus puntazzo*) is a commercially important Sparidae, which is a rocky bottom dweller. The distribution of the species includes the Mediterranean and Black Seas, as well as the Atlantic Ocean. The spawning period of sharpsnout seabream is September and October (1). *D. puntazzo* is one of the most valuable alternative fish species for many Mediterranean finfish farmers, and for this reason its cultivation techniques are of great interest to many scientists. Of particular interest are its reproduction and physiology (2,3), embryonic and yolk sac larval development (4-7), larval rearing (8-11), morphology (12-14), and nutrition and growth (15-17), under cultivation conditions.

Among the numerous abiotic factors that regulate fish larvae activity, light plays a major role in aquaculture (18). Light has a powerful influence on behaviour and

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development in teleost larvae, which must be reared in a specific light range, depending on the developmental stage and the species (19). In addition, light also has a great influence on pigmentation. When there is insufficient light, serious developmental abnormalities appear in larvae (20). Moreover, absorption of the yolk sac and development of the digestive tract and its associated organs are all affected by illumination (21).

In the present study, the influence of different illumination levels on total length development, digestive tube length, yolk sac volume, and oil globule volume were investigated in *D. puntazzo* larvae. Additionally, survival rates were examined. This study investigated the effects of light intensities on survival and growth rates of larvae in the lecithotrophic stage, during the absorption of endogenous food reserves.

Materials and Methods

Sharpsnout seabream broodstock, 20 females (1.2 ± 0.2 kg mean body weight) and 20 males (0.9 ± 0.1 kg mean weight), were selected from wild breeders and stocked in a 10 m^3 tank with a seawater flow of 1.5 m^3 per h. The fish were hand-fed once a day to satiation at noon, with approximately equal amounts (by weight) of a moist-pellet broodfish diet, cuttlefish (*Sepia officialis*), and shrimp (*Palaemon elegans*). The fish were subjected to the natural photoperiod of natural rearing seasons (16 h of light and 8 h of dark), and the water temperature varied throughout the experimental period between 23 and 24 ºC. No hormonal treatment was applied to the breeders and spawning occurred spontaneously. Eggs that spawned were immediately collected with a net (mesh size: 500 µm). Following fertilisation, viable buoyant eggs were separated from the dead sinking eggs. The volumetric method was used to determine stock density of the eggs. Eggs were incubated in 10-l incubators at an initial density of 2000 eggs/l, with a gentle flow of 23.5 ± 0.2 ºC seawater. Oxygen saturation was over 85%, salinity was 38 ppt, and pH was approximately 7.6. Ammonia and nitrite components were always < 0.011 mg/l.

After hatching, larvae were stocked at a density of 100/l in 15-l cylindrical-conical tanks. The colour of the tanks was dark grey. The water flow rate was adjusted to replace 5% of the total volume in the tanks per hour. Aeration was supplied at 40 ml/min. Temperature and salinity were kept at 23.5 ºC and 37-38 ppt, respectively. Three different experimental groups each consisting of 3 tanks were established. In these groups, only the illumination factor was changed; the other conditions of the experiment were kept consistent. Group A received no illumination. Group B received 30 lx and Group C received 450, both for 25 h/day, from a yellow light source with a rheostat placed 1 m above the surface of the tanks. Light intensity was measured with a luxmeter on the tank surface and each experiment was performed 3 times for each group.

The length of the larvae (from the tip of the lower jaw to the posterior margin of the caudal fin), digestive tube length (from the tip of the lower jaw to the end of the anus), the length and width of the yolk sac, and the diameter of the oil globule (the length of the 2 axes of the spheroid yolk sac (L, major axis; H, minor axis)), as well as the diameter (d) of the spherical oil globule of 10 individuals from each tank were measured every 8 h. The first measurement following hatching was made prior to exposure of the larvae to the different levels of illumination. Therefore, initial values for all the experimental specimens are the same. The total body length, digestive tube length, the length of the 2 axes of the spheroid yolk sac, as well as the diameter (d) of the spherical oil globule were measured using a microscope with an ocular micrometer lens and read to the nearest 0.01 mm. Observations and measurements were made on anaesthetised (phenoxy-2 ethanol, 0.5 ml/l) specimens. Curled larvae were not used for measurements. Yolk sac (V_{ys}) and oil globule (V_{og}) volumes were calculated using the formulae produced by Blaxter and Hempel (22) and Cetta and Capuzzo (23) as follows:

$$V_{ys} = 4/3\pi*(L/2)*(H/2)^2$$

$$V_{og} = 4/3\pi*(d/2)^3$$

The experiment was terminated when the larvae were first observed to open their mouths. At the end of the experiment, survival rates were determined by counting the number of survivors. Survival rate differences between the groups were calculated by chi-square test. Differences in total length, digestive tube length, yolk sac volume, and oil globule development between the groups were detected by ANOVA. Regression analyses of the relationship of total length-digestive tube length, total length-yolk sac volume, and total length-oil globule volume were performed for each group, and the degree to which they were significant was calculated using ANCOVA.

Results

During the experiments, the average temperature and the salinity values in the tanks were 23.46 ± 0.0242 ºC and 37.87 ± 0.031 ppt, respectively. These values were not significantly different between the tanks ($P > 0.05$). Table 1 shows the oil globule volume, yolk sac volume, digestive tube length, growth in total length, and survival rates of sharpsnout seabream larvae in Groups A, B, and C at the beginning and end of the experiment. Mouth opening onset was 46 h for Group A, and 45 h for Groups B and C.

Analyses of the results showed negative allometry in all groups for the relationship between total length and oil globule volume. This relationship was $y = -0.0024x + 0.0095$ ($r = 0.79$; $n = 210$) for Group A, $y = -0.0025x + 0.0098$ ($r = 0.79$; $n = 210$) for Group B, and $y = -0.0029x + 0.0104$ ($r = 0.83$; $n = 210$) for Group C. Covariance analysis found that the relationships among all groups were not significant ($P > 0.05$) (Table 2). Results of the covariance test showed that the relationship between Group A and Group B was not significant ($P > 0.05$); however, between Group A and Group C and between Group B and Group C, it was significant ($P < 0.05$). The results of the analyses are given in Table 3.

Statistical analysis of the results showed negative allometry for the relationship between total length and yolk sac volume. The value of y was $y = -0.0879x + 0.3054$ ($r = 0.87$; $n = 210$) for Group A, $y = -0.0967x + 0.3161$ ($r = 0.91$; $n = 210$) for Group B, and $y = -0.0976x + 0.3241$ ($r = 0.88$; $n = 210$) for Group C. Covariance analysis found that the relationships among all groups were not significant ($P > 0.05$) (Table 2). Results of the covariance test showed that the relationship between Group A and Group B, Group A and Group C and Group B and Group C was significant ($P < 0.05$) (Table 3).

Additionally, analyses of the results showed a positive allometry for the relationship between total length and digestive tube length in all groups. These relationships were $y = 198.97x + 288.49$ ($r = 0.87$; $n = 210$) for Group A, $y = 195.95x + 295.5$ ($r = 0.91$; $n = 210$) for Group B, and $y = 194.62x + 298.52$ ($r = 0.89$; $n = 210$) for Group C. Covariance analysis revealed that the relationships among all groups were not significant ($P > 0.05$) (Table 2). Results of the covariance test showed that the relationship between Group A and B, Group A and Group C and Group B and group C was not significant ($P > 0.05$) (Table 3).

The developmental differences of total length in the dark and light conditions (30-450 lx) were significant ($P < 0.05$); however, it was determined that digestive tube development, yolk sac utilisation, and oil globule absorption differences were not significant ($P > 0.05$).

The average survival rates at the end of the experiment were 84% ± 1.21%, 79.4% ± 1.01%, and 78.1% ± 0.68% in Groups A, B, and C, respectively. The survival rate was higher in the dark condition (Group A), but the difference was statistically insignificant ($P > 0.05$).

Table 1. Mean initial and final oil globule volume, yolk sac volume, digestive tube length, total length (± SD), and survival rate of *D. puntazzo* larvae.

		Group A	Group B	Group C
Oil Globule volume (mm^3)	Initial		0.00458 ± 0.000774	
	Final	0.00082 ± 0.000155	0.00076 ± 0.000144	0.00074 ± 0.000125
Yolk sac volume (mm^3)	Initial		0.1260 ± 0.017214	
	Final	0.0047 ± 0.000628	0.0051 ± 0.000877	0.0089 ± 0.0001549
Digestive tube length (mm)	Initial		0.662 ± 0.025	
	Final	0.949 ± 0.064082	0.926 ± 0.004079	0.898 ± 0.04349
Total length (mm)	Initial		1.91 ± 0.0703	
	Final	3.32 ± 0.01447	3.20 ± 0.160	3.07 ± 0.087
Survival rate (%)		84 ± 1.21	79.4 ± 1.01	78.1 ± 0.68

Table 2. Covariance analysis of results of *D. puntazzo* larvae grown for 45-46 h without illumination (Group A), in 30 lx illumination (Group B), and 450 lx illumination (Group C).

		Source of Variation	df	SS	MS	Fs
Total length-Oil globule	Group A	Adjusted means (among ai's)	2	1.68555E-08	8.4278E-09	0.013
		Error (deviations from a common slope)	206	0.000130983	6.3584E-07	
	Group B	Adjusted means (among ai's)	2	6.53303E-07	3.2665E-07	0.497
		Error (deviations from a common slope)	206	0.000135122	6.5593E-07	
	Group C	Adjusted means (among ai's)	2	1.7154-E-07	8.577E-08	0.142
		Error (deviations from a common slope)	206	0.000123743	6.0069E-07	
Total length-Yolk sac volume	Group A	Adjusted means (among ai's)	2	0.000458054	0.00022903	0.486
		Error (deviations from a common slope)	206	0.096995208	0.00047085	
	Group B	Adjusted means (among ai's)	2	0.001606709	0.00080335	2.382
		Error (deviations from a common slope)	206	0.06945505	0.00033716	
	Group C	Adjusted means (among ai's)	2	0.001503339	0.00075167	1.846
		Error (deviations from a common slope)	206	0.083854388	0.00040706	
Total length-Digestive tube length	Group A	Adjusted means (among ai's)	2	54.09043248	27.0452162	0.011
		Error (deviations from a common slope)	206	502524.5524	2439.43957	
	Group B	Adjusted means (among ai's)	2	226.9593855	113.479693	0.080
		Error (deviations from a common slope)	206	291984.505	1417.40051	
	Group C	Adjusted means (among ai's)	2	130.6785876	65.3392938	0.043
		Error (deviations from a common slope)	206	306662.399	1488.65242	

Table 3. Covariance analysis of the relationships between results for *D. puntazzo* larvae grown for 45-46 h without illumination (Group A), in 30 lx illumination (Group B), and 450 lx illumination (Group C) (*statistically different).

		Source of Variation	df	SS	MS	Fs
Total length-Oil globule	Relation A-B	Adjusted means (among ai's)	5	5.99339E-06	1.1987E-06	1.855
		Error (deviations from a common slope)	416	0.000266838	6.4610E-07	
	Relation A-C	Adjusted means (among ai's)	5	3.44609E-05	6.8922E-06	10.943*
		Error (deviations from a common slope)	416	0.000260103	6.2979E-07	
	Relation B-C	Adjusted means (among ai's)	5	1.37783E-05	2.7557E-06	4.3606*
		Error (deviations from a common slope)	416	0.00026099	6.3194E-07	
Total length-Yolk sac volume	Relation A-B	Adjusted means (among ai's)	5	0.022777065	0.00455541	11.193*
		Error (deviations from a common slope)	416	0.16807591	0.00040696	
	Relation A-C	Adjusted means (among ai's)	5	0.008451601	0.00169032	3.820*
		Error (deviations from a common slope)	416	0.182708381	0.00044239	
	Relation B-C	Adjusted means (among ai's)	5	0.006471003	0.0012942	3.486*
		Error (deviations from a common slope)	416	0.153324697	0.00037125	
Total length-Digestive tube length	Relation A-B	Adjusted means (among ai's)	5	489.816174	97.963234	0.051
		Error (deviations from a common slope)	416	794706.945	1924.2298	
	Relation A-C	Adjusted means (among ai's)	5	578.784259	115.75685	0.059
		Error (deviations from a common slope)	416	809554.321	1960.1799	
	Relation B-C	Adjusted means (among ai's)	5	401.389409	80.277881	0.055
		Error (deviations from a common slope)	416	598676.093	1449.5789	

Discussion

Light is one of the most important abiotic factors, playing a major role in providing life continuity for all organisms. Light is also indispensable for body pigmentation, an important phenomenon involved in early development and growth; however, light can create stress for larvae and may result in reduced growth or high mortality (19). Yolk conversion efficiency is dependent upon environmental factors, including light and composition of the yolk (24). In the present study, the effects of different illumination conditions on absorption of endogenous food reserves were examined.

In *D. puntazzo*, newly hatched larvae have a massive yolk sac containing the vitelline reserves and the lipid globule. It has been related to transformation of a greater part of the total energy obtained from absorption into metabolic energy in illuminated conditions. The yolk reserves of fish contain glycogen, proteins, lipoproteins, lysosomal enzymes, and other enzymes related to protein, carbohydrate, and lipid metabolism. The nutrition of yolk sac larvae is supported by the yolk reserves (25) and ontogeny depends exclusively on the abiotic parameters (26,27). The vital importance of successful feeding onset is certified by the developmental priority of the organs that support this function as well as by the nutritional support of the early larvae by the lipid globule. The consumption rate is negatively correlated with the nutritional value of the available food. Lipid consumption in the digestive tube starts on the same day the mouth first opens (28). It has been observed in sharpsnout seabream larvae in the lecithotrophic phase that illumination has an effect on the speed of consumption of the yolk sac. It has also been suggested that illumination has an effect on larval use of energy obtained from the absorption of the yolk sac. This fact supports the hypothesis that length development occurs faster in a dark environment. It is known that larvae in dark conditions could be moved spasmodically by water flow; however, this movement can also be observed more frequently in larvae under illumination.

It was found that light conditions increase the consumption of the oil globule and considerably affect the consumption of the oil globule and the development of the digestive tube. The speed of oil globule absorption in light conditions has indicated an increase in lipid absorption from the surface of the digestive tube. These results suggest that the larvae in light conditions (30-450 lx) had a better ability to digest.

Results of total length development in this study showed similarities to the findings of Boglione et al. (7); however, they were different from those of Faranda et al. (6). Moreover, yolk sac volume and oil globule diameters were larger. It is possible that, in addition to different natural conditions such as temperature, food, and geographic location, feeding of broodstock with food high in HUFA, such as squid, during the spawning season could also affect yolk sac volume and oil globule diameter. Total length development was higher in the tanks kept in the dark. In our study, the positive effects on development of spending the early larval stage in a dark environment have been proven. A similar result was found for gilthead seabream (*Sparus aurata)* by Saka et al. (29) and common dentex (*Dentex dentex)* larvae by Fırat et al. (30). The need for metabolic energy increases in light conditions as a result of convulsive movements, which leads to fast absorption by larvae. In contrast, there is a difference in the total length-oil globule relationship between sharpsnout sea bream larvae and common dentex, which are reared in dark and light conditions (30). However, both species show similar results in the total length-yolk sac volume relationship, and light affects the absorption of yolk reserves. In addition, under light and dark conditions, there was no significant difference in the total length-digestive tube length relationship. This result indicated that light did not play a major role in the differences between the relationship of total length and digestive tube length in these species. On the other hand, differences in the relationship between total length development and oil globule absorption in 2 species suggest that sharpsnout sea bream larvae might be more sensitive to light. Similarly, significant differences were found in total length-oil globule volume and total length-yolk sac volume relationships in gilthead sea bream larvae by Saka et al. (29).

The best survival rates were found in dark conditions, which were similar to previous studies with *D. dentex* and *S. aurata* (29,30). In experiments in which illumination (15-20 lx) was applied, light has not negatively affected survival rates (18). The survival rate in tanks with 450 lx intensity was the lowest; however, it was higher in the tanks with 30 lx illumination; the best survival rate results were achieved in dark conditions. In the tanks with no illumination, total length was also the greatest. This has been related to the absence of stress caused by light and the use of endogenous food reserves for

development rather than movement. In these experimental conditions, significant positive effects of a dark environment in the early larval stage have been proven.

In conclusion, in culture conditions, larvae could utilise the endogen food reserves during the early developmental period. Additionally, the main factor affecting the success of initial feeding of Sparidae larvae is a smaller mouth width than other marine fish larvae. Moreover, providing live food and/or feeding of larvae with these organisms could be interrupted; therefore, it is suggested that the spontaneous absorption of endogen food reserves during the lecithotrophic phase is a vital factor for larval rearing. Furthermore, in starvation and/or interrupted feeding conditions, larvae could utilise a reserve and this situation is required for survival and for further physiological development.

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