

Skeletal deformities of cultured sharpsnout seabream (*Diplodus puntazzo*) larvae during early life development

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Summary: In this study, skeletal deformities were investigated during early life stages in sharpsnout seabream, *Diplodus puntazzo*, larvae during larval development period. Whole larval period (1-42 days after hatching, DAH) was examined in three stages (preflexion, flexion and postflexion) based on notochord curve. Totally, 1210 specimens were examined during the experiment but 689 deformities were recorded in 256 larvae. Also, deformity rate was calculated as 21.2% at the end of the experiment. In terms of notochord flexion at stage-specific, no differences were found between reflexion (19.7%) and postflexion (15.8%) stages ($p>0.05$) while there was a significant difference during flexion stage (32.8%) in comparison with other groups ($p<0.05$). Correspondence analysis (CA) was performed in order to describe of skeletal deformities mainly depend on notochord flexion. According to CA analysis, it was determined that deformities were closely related with both flexion stages quantitatively and also postflexion stages qualitatively.

Keywords: *D. puntazzo*, larval development, skeletal deformities, notochord flexion.

Kültür koşullarındaki sivriburun karagöz (*Diplodus puntazzo*) larvalarında erken dönem iskelet deformasyonları

Özet: Bu çalışmada, yetiştiriciliği yapılan sivriburun karagöz (*Diplodus puntazzo*) larvalarının erken hayat döngüsü içerisinde iskelet sisteminde meydana gelen deformasyonlar incelenmiştir. Deneme boyunca larval dönem (1-42 gün) notokorda da meydana gelen değişimlere göre üç bölüme ayrılmıştır (bükülme öncesi, bükülme sırası ve bükülme sonrası). Deneme boyunca toplamda 1210 larva incelenmiş ve 256 deforme larvada 689 iskelet deformasyonu tespit edilmiştir. Deneme sonunda deformasyon oranı %21,2 hesaplanmıştır. Larval dönemdeki safhalara göre deformasyon oranları bükülme öncesi (%19,7) ile bükülme sonrası (%15,8) önemsiz bulunurken ($p>0,05$), bükülme sırası (%32,8) evresi önemlilik arz etmiştir ($p<0,05$). Notokordaya göre iskelet deformasyonları Korrespond Analiz yöntemi kullanılarak yapılmıştır. Bu analiz yöntemine göre iskelet deformasyonları safhalar arasında hem niteliksel olarak farklılıkları ortaya koymuş hem de bükülme sonrası dönemde niceliksel ayrımı belirtmiştir.

Anahtar sözcükler: *D. puntazzo*, larval gelişim, iskelet deformasyonu, notokorda bükülmesi.

Introduction

It is commonly known that morpho-anatomical abnormalities in marine fish under culture conditions usually have negative effects not only on biological performance but also on marketing image and commercial value (1, 3). Description of deformities during larval and juvenile stages could provide fish farms a tool to predict anomalies at the adult stages or could allow making changes to abiotic or biotic factors to improve the quality of specimens during growth (4,5).

Total rate of species-specific deformations in cultured marine organism are usually changed from 15 to 50% under culture conditions, however, these rates are mainly recorded as vertebral deformities between 30 and 60%. Besides, they were observed called as scoliosis,

lordosis and kyphosis, and also observed Z, V and ^ type curve of vertebra (6,8). To this date, although larval phase was evaluated until juvenile stage (2,3,9,10), recent studies were focused on skeletogenesis mainly depend on bone and cartilage developmental stages with notochordal ontogeny (11,12). However, it is well recorded that factors effecting skeletal development in larval fish are malnutrition and/or sudden changes in biotical and abiotical factors under culture conditions (8,11,12).

In this study, therefore, it is aimed that bone and cartilage deformities depend on developmental phase were investigated in terms of flexional stages (preflexion, flexion and postflexion) of the notochord during early ontogeny of *D. puntazzo* larvae.

Materials and methods

D. puntazzo broodstocks were selected from the wild breeders and also maturation and spawning occurred spontaneously. Larval rearing and feeding protocol were applied in triplicate according to Suzer et al. (13). Larval feeding regime is summarized in Figure 1. A total of 1210 larvae of *D. puntazzo* were randomly sampled from tank at 3 day intervals from hatching until 42 DAH. In order to determine deformities in bone and cartilage tissues, double staining method by alizarin red S and alcian blue was used as described by Potthoff (14), Favalaro and Mazzola (5) and Çoban et al. (11). Then, stained larvae were examined under the light microscope and photos were taken from the left side by a digital camera (Nikon Coolpix 5000, Japan) (Figure 2). Deformation analyses were classified with a letter indicating the region and a number indicating the anomaly as listed in Table 1. Also, for determination of effective period of deformities during larval stages, this period was divided into 3 stages and classified as preflexion, flexion and postflexion mainly depending on notochord flexion (15). Each stage with more than 51% of the specimens presenting a certain characteristics was used as the reference point in the description of the flexional stages.

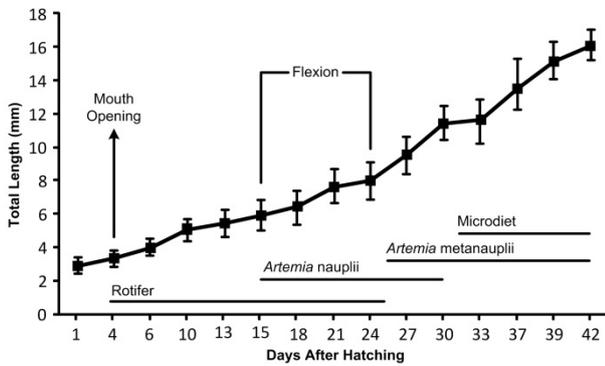


Figure 1. Total length (mean±sd) and feeding regime of *D. puntazzo* larvae.

Şekil 1. Sivriburun karagöz larvalarına ait total boy (Ort±ss) ve besleme rejimi.

Table 1. Regional deformations and their symbols in *D. puntazzo* larvae (16).

Tablo 1. *D. puntazzo* larvalarında gözlenen bölgesel deformasyonlar ve semboller (16).

Region	Abbreviations
External Skeleton	
<i>Caudal Fin</i>	A1
<i>Dorsal Fin</i>	A2
<i>Anal Fin</i>	A3
<i>Pectoral Fin</i>	A4
Appendicular Skeleton	B
Axial Skeleton (Syncranium)	C
Axial Skeleton (Vertebral Column)	
<i>Cephalic</i>	D1
<i>Pre-hemal</i>	D2
<i>Hemal</i>	D3
<i>Caudal</i>	D4
Anomalies	
Lordosis	1
Kyphosis	2
Vertebral fusion	3
Vertebral malformation	4
Malformed neural arch and/or spine	5
Malformed hemal arch and/or spine	6
Malformed ray (deformed, absent, fused, supernumerary)	7
Malformed pterygophores (deformed, absent, fused, supernumerary)	8
Malformed hypural (deformed, absent, fused, supernumerary)	9
Malformed epural (deformed, absent, fused, supernumerary)	10
Supernumerary vertebra	11
Dislocation of glossohyal	12
Malformed dentale	13
Malformed maxillary and/or pre-maxillary	14
Swim-bladder anomaly	15

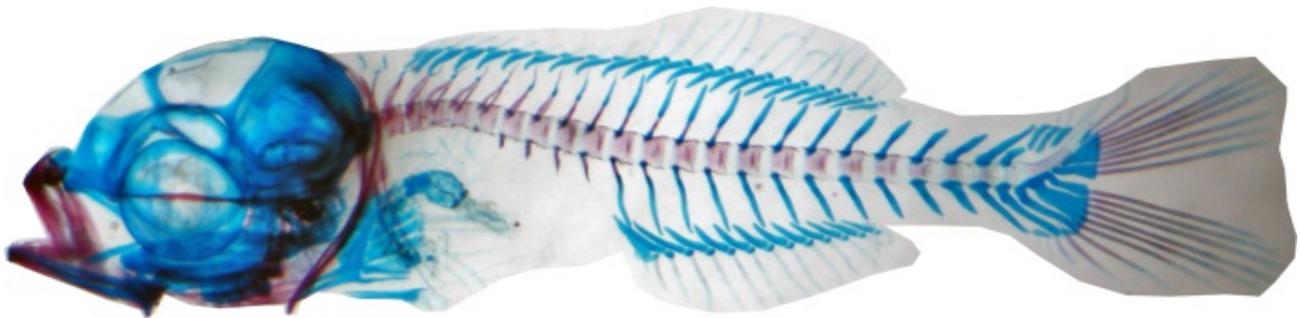


Figure 2. Double stained *D. puntazzo* larvae (7.1 mm TL).

Şekil 2. İkili boyama tekniği ile boyanmış *D. puntazzo* larvası (7,1 mm TB).

Table 2. Stage-specific growth of *D. puntazzo* larvae: Total length (TL) by means (\pm SD) and ranges.
 Tablo 2. *D. puntazzo* larval dönem-büyüme verileri. Toplam boy ortalama (\pm SS) değişimleri.

Stage	Age(DAH)	n	TL _{mean±sd}	Range (mm)
Preflexion ($\Sigma=375$)	1	81	2.91 \pm 0.11	2.73-3.18
	4	64	3.35 \pm 0.14	3.05-3.62
	6	72	3.96 \pm 0.28	3.4-5
	10	77	5.11 \pm 0.57	4.2-6.5
	13	81	5.46 \pm 0.43	4.89-7.1
Flexion ($\Sigma=296$)	15	89	5.95 \pm 0.47	5.2-7.6
	18	94	6.44 \pm 0.74	5.41-8.9
	21	113	7.61 \pm 0.66	6.44-9.5
Postflexion ($\Sigma=539$)	24	90	7.98 \pm 0.71	6.9-9.9
	27	69	9.58 \pm 0.10	7.83-10.93
	30	72	11.42 \pm 0.78	9.83-12.86
	33	98	11.63 \pm 0.95	10-13.9
	37	62	13.51 \pm 1.38	11-16.04
	39	68	15.11 \pm 1.21	12.16-17.42
	42	80	16.04 \pm 1.42	13.49-19.8

Table 3. Characterization of abnormalities and abnormal larvae regarding notochord flexion time in *D. puntazzo* larvae. Superscript * was refer to significant differences by ($p<0.05$).

Tablo 3. Notokorda bükülme zamanına göre gözlenen deformasyonların ve deforme *D. puntazzo* larvalarının özellikleri. İstatistikî önemli farklılıklar üssel * gösterilmiştir.

	Preflexion (2.91-5.95 mm TL)	Flexion (5.05-7.98 mm TL)	Postflexion (7.98-16.04 mm TL)
Total number of observed larvae (A)	375	296*	539
Total number of deformed larvae (B)	74	97*	85
Total number of observed deformation (C)	179	292*	218
Deformation rate (%), (B/A)	19.7	32.8*	15.8
The number of deformity per whole observed larvae, (C/A)	0.48	0.99*	0.40
The number of deformity per abnormal larvae, (C/B)	2.42	3.01*	2.56

In this study, deformation rate and ratio between deformed larva and total larva were found and also the number of deformations in per deformed larvae was calculated by ratio of number of total deformities to total deformed larvae (16). Deformation frequency was evaluated by duration of larval stage with individual differences of sampled larvae (16). Also, chi-square test was used in order to compare of deformations observed in three larval stages and deformation rates in different skeletal regions. Then, for determination of relations between larval stages and regional deformations in larvae, Correspondence analysis was performed.

Results

Notochord flexion was observed between 15 and 24 DAH and also larval development, total number of larvae and related anomalies according to different larval stages are demonstrated in Table 2.

During the study, 689 anomalies of 15 different types were detected in 256 specimens of 1210 larvae and deformity rate was calculated as 21.2%. In overall

results, the number of deformities per whole observed larvae and the number of deformities per abnormal larvae were estimated as 0.57 and 2.69, respectively. Characterization of abnormalities and also obtained results from different larval stages regarding notochord flexion were presented in Table 3.

Observed abnormalities according to the skeletal system: In this section, abnormalities were evaluated regarding regions of skeletal system in each flexion stage. After that, frequencies of observed deformity were equalized and compared in between flexion stages for each region.

External skeleton: The major deformity in this region was observed as malformed ray (type 7). Fin deformities which were observed in flexion and postflexion stages were 8.6% and 11.1% of overall deformities detected in larvae. Also, there were no significant differences between flexion and postflexion stages ($p>0.05$). Additionally, no deformations were recorded in the preflexion stage due to non-formation of fin rays in this stage at external skeletal system. The highest deformity

rate was observed in pectoral rays for both flexion stages (flexion 4.8%; postflexion 6.8%).

Appendicular skeleton: Several deformities were recorded in this skeletal system as malformed pterygophore (type 8), malformed hypural (type 9) and malformed epural (type 10). Although some deformities which occurred in appendicular systems that supported the fins were not observed in preflexion stage, they were detected as 10.3% for flexion and 10.6% for postflexion stages ($p>0.05$). For both flexion stages, type 8 was detected as the dominant deformity type for dorsal fin (flexion 6.8%, postflexion 6.3%). Also, saddleback syndrome was not recorded in the current study.

Axial skeleton: Some abnormalities observed in the syncranium were dislocation of glossohyal (type 12), malformed dentale (type 13) and malformed maxillary and/or pre-maxillary (type 14). The highest rate of deformity was calculated in the postflexion stage as 6.8%, and followed by preflexion (6.2%) and flexion stages (3.1%). There were no significant differences among stages ($p>0.05$).

Vertebral column: According to results, the highest rate of malformation was detected in this region compared to the other groups. A similar result was obtained in the frequency of abnormalities for this region as the highest rate. These malformations were observed as kyphosis (type 1), lordosis (type 2), vertebrae fusion and deformation (types 3 and 4), malformed neural arch and/or spine (type 5), malformed hemal arch and/or spine (type 6), and supernumerary vertebra (type 11). Deformity rates in preflexion, flexion and postflexion stage were estimated as 83.6%, 71.2% and 57.5%, respectively. Also, there were significant differences among notochord flexion stages ($p<0.05$). Swim-bladder anomalies (type 15) were detected as 10.2%, 6.8% and 13.5% for preflexion, flexion and postflexion stages, respectively. Also, no significant differences were found among notochord flexion stages ($p>0.05$).

Correspondence Analysis: In this analysis, according to overall results from table 4, differences among skeletal systems were evaluated entirely but swim-bladder anomaly was not included in the CA analysis (Figure 3). Fin deformities (especially A1-7, A2-7, A3-7, and A4-7) were located on negative CA1 due to the fact that fins were not formed in the preflexion stage and also they were presented relatively close relations with flexion and postflexion stages. Additionally, deformities observed in the appendicular system were situated on negative CA1 due to in similar with external skeleton and also exhibited similar close relations with flexion and postflexion stages. For both flexion stages (flexion and postflexion), dorsal and pectoral fin deformations and malformed pterygiophores were detected at positive CA2 and these deformities were closely related with postflexion. Moreover, anal fin deformation and

malformed epural and hypural were determined at negative CA2 and it was more effective in the flexion stage. Also, it was determined that whole caudal fin deformities were closely related with postflexion stage. Besides, deformities in syncranium were located at positive CA2 which suggested that deformities in the flexion stage were less effective than the other stages.

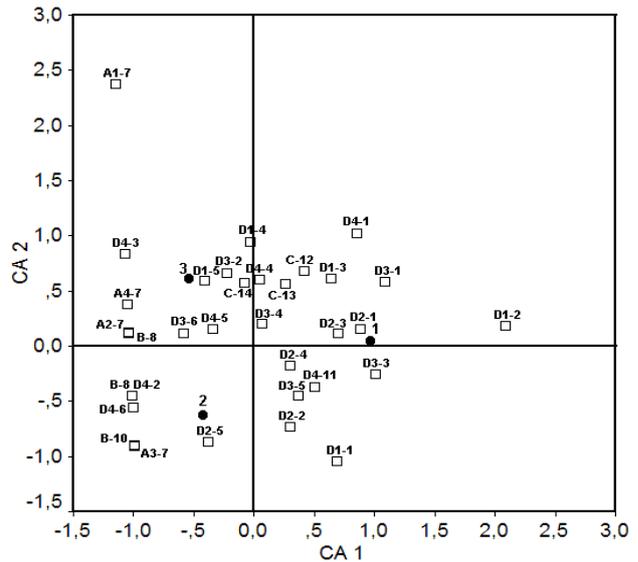


Figure 3. Correspondence analyses applied to types of abnormalities, the skeletal regions and larval period. For abnormality and region abbreviations, refer to Table 4. □ abnormalities and skeletal regions; ● larval period.

Şekil 3. Larval periyot süresince iskelet bölgeleri ve deformasyon tipine bağlı uygulanan Korrespon analizi. Deformasyon ve bölgesel kısaltmalar Tablo 4'teki gibidir. □ deformasyonlar ve iskelet bölgeleri; ● larval periyot.

Vertebral column deformities were distributed to the whole area of both axis (CA1 and CA2). Several deformities that were observed in vertebra such as lordosis, kyphosis in prehemal and caudal, vertebral fusion and malformations, malformed neural arch and/or spines in hemal region, and differences on supernumerary vertebra were located at positive CA1 and were also closely related with preflexion and postflexion stages. Other than the mentioned deformities above, vertebral column deformities were situated at negative CA1 and were entirely related with the flexion stage.

Discussion and Conclusion

This study examined skeletal anomalies and deformities in the early stages of life of sharpsnout sea bream larvae. In previous studies, skeletal deformities and meristic characteristics of sub adults (173 DAH) and adults of sharpsnout sea bream were investigated by Favalora and Mazzola (17). They reported that the number of anomalies strongly varied with age. Also, in another study, same authors pointed out that wild specimen have low malformation rate than the cultured

population of this species (17). Additionally, Boglione et al. (18) studied on skeletal abnormalities of wild and cultured *D. puntazzo* and *P. erythrinus* post-larva and juveniles. According to their results, rate of deformed fish in cultured *D. puntazzo* was 95.2%, in cultured *P. erythrinus* 72.9%, while in cultured gilthead sea bream, it varied from 98.3% to 100% (18).

Vertebral deformities were a slight deformation of the centra with a low frequency (7.7%) compared to other hatchery-reared species where larval deformities of 24% to 44% have been reported (10,19,20). Boglione et al. (16) reported that only 4% of wild caught *S. aurata* shows body deformations, in contrast to the very high values observed in hatchery-reared larvae (21). Additionally, deformity rates in wild specimens of *D. puntazzo* juveniles changed between 19.6% and 21.4% while it ranged from 88.2% to 100% for the cultured juveniles. In the current study, deformity rate was calculated as 21.5% during larval development and also the highest rate of deformity was observed as 32.8% in the flexion stage.

It is well documented that dramatical changes in abiotic factors in both intensive and extensive culture conditions could cause several deformations mainly in fins of the larvae (20). In addition to this, fin rays are more sensitive to thermal fluctuations especially during early ontogeny (8). As reported by Sfakianakis et al. (22), development of fin and fin supports initiate in notochord flexion and continue development during postflexion stages in *D. puntazzo* larvae. In this study, no deformations were recorded in external and appendicular skeletal systems during the preflexion stage, but several deformities were noted during of flexion and postflexion stages.

It is well described that malformed hypurals, epurals and pterygyphores are identified as developmental deformities and are also closely related to culture conditions such as stocking density (17). These type anomalies were reported for *D. labrax* (9), *D. dentex* (20), *S. aurata* (16), *D. puntazzo* larvae and juveniles (17). In this study, these anomalies were noted and also the rate of malformed pterygyphores was found to be relatively higher than rates of malformed epural and hypural.

Vertebral deformities, especially lordosis, kyphosis, scoliosis, are the most important skeletal malformations (7,20,21). Also, it is reported that pre-haemal kyphosis in *D. labrax* develops during the larval stage and in the range of 10–17 mm in TL (7). In the current study, the rates of lordosis and kyphosis were found to be relatively higher in the preflexion stage than both flexion and postflexion stages. Also, in preflexion stage, vertebral fusion, vertebral malformation and malformed neural and haemal arch and/or spine were relatively higher than the other stages. Sfakianakis et al. (22) indicated that

formation of neural (4.9 mm TL) and haemal arch (5.6 mm TL) is initiated during the preflexion stage in *D. puntazzo* larvae. It is possible that obtained relatively higher rates of deformations from this study could be related with differences in culture conditions and feeding protocol during the osteological development of larvae. Moreover, Koumoundouros et al. (7) reported that kyphosis was significantly associated with deformations of the branchiostegal rays, but not with lordosis or swim bladder non-inflation.

It has been found that there was negative correlation between deformities of vertebral column and larval developmental stages and that the rate of deformity concurrently decreased by larval age. There might be two reasons for this situation: firstly deformed larvae could be improved their health, secondly they could be died. In previous studies, it is clearly reported that deformities observed in this region did not recover and caused to high mortality rates (7). Concisely, it is recommended that all deformity types observed in the vertebral column should be monitored throughout each flexional stage. Also, deformities occurred in preflexion stage will be kept to continue during the other stages.

Development of haemal and neural archs on vertebra occurred between 4.5 mm and 6.4 mm TL in *D. puntazzo* larvae (22). In addition, development of digestive system and enzymatic ontogeny were completed in the same period (13). Moreover, we observed that specific activity of digestive proteases decreased between 10 and 15 DAH and could be related to some morphological and physiological changes in larvae (23). Malnutrition of larvae due to insufficient enrichment of live food by DHA/EPA and fatty acids could also cause vertebral column deformities (24). Additionally, histological analysis agreed with this phenomenon that lack of several vitamins, especially Vitamin C, minerals and various food nutrients might trigger increase of skeletal deformities during the early ontogeny (25, 26).

In the current study, the CA was useful for evaluation of characteristics of deformities in detail with stage and region. According to CA, deformities were found in relation with the flexion stage quantitatively whereas they demonstrated close relation with the postflexion stage qualitatively. In previous studies, relationships between fish origin and deformation frequency was analysed by using CA for both *D. labrax* (1) and *S. aurata* (16). Also, Ferreri et al. (27) used CA for comparison of meristic characteristics of wild and cultured *Danio rerio*. It is considered that skeletal deformities depend on notochord flexion time occurring in early larval stages could be easily analysed by CA for the monitoring and sustainability of larval quality in intensive culture.

This study investigates skeletal deformations in respect to notochord flexional stages in *D. puntazzo*

larvae during early ontogeny for the first time. Moreover, it strongly pointed out that stress originating from malnutrition of larvae by DHA/EPA, osteological development, metamorphosis and organogenesis of larvae could trigger larval deformity (23, 28). Boglione et al. (4) reported that investigation of larval quality should be crucial between 50 and 100 DAH in cultured species in commercial hatcheries. However, deformities occurred in axial skeletal system should be monitored from preflexion stage whereas deformations in external and appendicular skeletal system should be examined from flexion stage in *D. puntazzo* larvae. As a result, commercial larval producers could gain an economic profit by investigation of larval deformities during the flexion and preflexion stages. For further studies, relationships between broodstock quality and larval deformities should be clearly described, to decrease deformity rates during early larval stages, which will be useful to aquaculturists and commercial producers.

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