



Morphological Development and Allometric Growth of Sharpsnout Seabream (*Diplodus puntazzo*) Larvae

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Abstract

In this study, morphological development and allometric growth were investigated in the sharpsnout seabream, *Diplodus puntazzo*, during larval development until the end of the weaning on day 42. Average total length (TL) of newly hatched larvae was 2.91±0.11 mm and it was 3.35±0.13 mm TL at the onset of feeding at 4 days after hatching (DAH). Initial swimbladder inflation occurred at 10 DAH (5.11±0.45 mm TL) and post-inflation became more elongate at 15 DAH (5.95±0.43 mm TL). The notochord flexion occurred between 5.95±0.43 mm TL at 15 DAH and 7.98±0.72 mm TL on 24 DAH. At the end of weaning, larvae were 16.03±1.74 mm TL at 42 DAH. The majority of all allometric changes from inflection point were integrated with the larval and the metamorphosis stage. Inflections in body proportion changes occurred mainly at 5.12, 5.95 and 7.98 mm TL, corresponding to initial swimbladder inflation and flexion period of notochord, respectively.

Keywords: Sharpsnout seabream, *Diplodus puntazzo*, larval rearing, allometry, ontogeny.

Sivriburun Karagöz (*Diplodus puntazzo*) Larvalarında Morfolojik Gelişim ve Allometrik Büyüme

Özet

Bu çalışmada, sivriburun karagöz (*Diplodus puntazzo*) larvalarında morfolojik gelişim ve allometrik büyüme siumurtadan çıkıştan itibaren 42. güne kadar incelenmiştir. Yumurtadan yeni çıkmış larvada toplam boy (TB) 2,91±0,11 mm olmuş ve yumurtadan çıktıktan sonraki 4. günde 3,35±0,13 mm TB'da dışarıdan canlı yem ile besleme başlamıştır. Hava kesesi ilk şişmesi yumurtadan çıktıktan sonraki 10. günde 5,11±0,45 mm TB'da gözlenmiş olup, ikinci şişme 15. günde 5,95±0,43 mm TB'da meydana gelmiştir. Notokordanın küçük bir açıyla bükülmeye başlaması 15 ile 24. günlerde 5,95±0,43 mm TB ile 7,98±0,72 mm TB arasında meydana gelmiştir. Denemenin sonunda 42. günde larva 16,03±1,74 mm TB'da olmuştur. Kırılma noktaları ile ortaya çıkan tüm allometrik değişimler larval dönem ve metamorfoz gelişim safhaları ile ilişkilendirildi. Vücut bölgelerinin toplam boya oranına bağlı ortaya çıkan kırılma noktaları daha çok 5,12, 5,95 ve 7,98 mm TB'da meydana gelirken bu değerlerde hava kesesi şişmesi ve notokorda bükülmesi gerçekleşmektedir.

Anahtar Kelimeler: Sivriburun Karagöz, *Diplodus puntazzo*, larval yetiştiricilik, allometri, ontogeni.

Introduction

Sharpsnout seabream, *Diplodus puntazzo*, is one of the most valuable alternative fish species for the Mediterranean aquaculture industry (Boglione *et al.*, 2003a; Papandroulakis *et al.*, 2004; Suzer *et al.*, 2007), and for this reason its cultivation techniques are of great interest. Due to its increasing importance as a new aquaculture species in the Mediterranean, many scientists are interested in its reproduction and physiology (Micale *et al.*, 1996; Hernandez *et al.*, 2003; Pajuela *et al.*, 2008), skeletal anomalies

(Favaloro and Mazzola, 2000; 2003; 2006; Boglione *et al.*, 2003b;), morphology (Loy *et al.*, 2000; Favaloro and Mazzola, 2000, 2003), nutrition and growth (Hernandez *et al.*, 2001; Atienza *et al.*, 2004; Suzer *et al.*, 2007). However, literature about its early life history is scarce or limited to embryonic and larvae development in rearing conditions (Boglione *et al.*, 2003a; Korkut *et al.*, 2006).

Developmental processes of fish are not yet completed at hatching and morphogenesis is very intense during the early life developmental process (Gisbert, 1999). During the process of larval

development and organogenesis marine finfish larvae undergo major developmental changes in their body and behavior for transition to juvenile stage (Koumoundouros *et al.*, 1999; Firat *et al.*, 2006). It is well known that major morphological changes and their developmental components in fish larvae reflect the close relationships between form and function (Fuiman, 1983; Kendall *et al.*, 1984; Suzer *et al.*, 2007). Increasing our knowledge of the larval morphology and ontogenic developmental process of the larvae of new candidate species for aquaculture will promote the development of optimal rearing protocols and greatly improve effective production of high quality juveniles and adults (Fukuhara, 1991; Koumoundouros *et al.*, 1999; Çoban *et al.*, 2009a; b; Russo *et al.*, 2007, 2009).

The objective of this study was to define the development of the early life stages in the sharpnose seabream, *Diplodus puntazzo*, larvae under intensive culture conditions, from mouth opening until the end of weaning at 42 days after hatching (DAH).

Materials and Methods

Ten females (1.6 kg mean body weight) and ten male (2.4 kg mean body weight) sharpnose seabream broodstocks were selected from wild breeders and stocked in a 12 m³ tank with a seawater supply of 35 L.min⁻¹. Frozen cuttlefish (*Sepia officinalis*) and leander squilla (*Palaemon elegans*) were provided daily as the primary food source. The fish were subjected to natural photoperiod (38° 92' N; 27° 05' E) and the water temperatures varied between 20–22°C throughout the spawning period. Eggs spawned were immediately collected in recirculator and viable buoyant eggs were separated from the dead sinking eggs. Eggs were incubated in 500 L incubators at an initial density of 2,500 eggs L⁻¹ with a gentle flow of seawater at 20.0±0.5°C. Oxygen saturation was over 85%, salinity was 37 ppt and pH was around 7.65. Ammonia and nitrite components were <0.012 mgL⁻¹ during the study.

After hatching, larvae were reared in a cylindrical dark grey tank (15 m³), at an average density of 100 ind L⁻¹. Larval rearing was carried out in a closed sea water system with UV filters. Water temperature, dissolved oxygen, salinity, pH, ammonia and nitrite levels were monitored daily. Water temperature increased from 20.0°C to 24.0°C. During the larval period oxygen, salinity and pH were maintained at >85%, 37‰ and 7.6, respectively. Ammonia and nitrite were kept constant always below 0.01 mg L⁻¹. Water in the tank was static during the first 3 days of the rearing period. From 4 DAH to 12 DAH, the tank water was partially replaced (5-7% daily) by draining through a 200 µm mesh. Water exchange rate was increased gradually with the age of the larvae. Light intensity was set at 30 lx between 3 and 10 DAH, 50 lx between 10 and 20 DAH and 150 lx until the end of the larval rearing. Daily

photoperiod was set at 24 h light until the end of algal addition and then 16 h light and 8 h dark until the end of the experiment.

D. puntazzo larvae (when the mouth opened) were fed with rotifers (70%, *Brachionus rotundiformis* and 30% *Brachionus plicatilis*), cultured with algae and enriched with DHA (Protein Selco, Artemia Systems SA, Gent, Belgium) from 4 DAH to 25 DAH at a density of 10-15 rotifers ml⁻¹. From 4 DAH to 30 DAH, green-water composed of *Nannochloropsis* sp., *Chlorella* sp. and *Isochrysis* sp. at a density of 3-4x10⁵ cells ml⁻¹ was added daily. From 15 DAH to 30 DAH, they were fed *Artemia* nauplii (AF 480, INVE Aquaculture, Gent, Belgium) at 4-6 individuals ml⁻¹ and from 25 DAH until the end of the experiment, *Artemia* metanauplii at 2-4 individuals ml⁻¹ (EG, Artemia Systems SA), both enriched with Protein Selco (Artemia Systems SA). Extruded micro diet (Proton, INVE Aquaculture) was used from 32 DAH until 42 DAH as 4-10% of biomass per day (Figure 1).

Morphological observations and body measurements were conducted on samples of minimum 30 specimens taken randomly every 3 days from 1 to 42 DAH (end of the weaning). Anaesthetized specimens (ethyleneglycol-monophenylether, Merck, 0.2-0.5 ml L⁻¹) were photographed from their left side using a stereoscopic microscope (Novex, Zoom Stereo Microscopes, Holland). Morphometric characters were measured by using TpsDig (version 1.37) software with 0.01mm on the photographs (Table 1). Eighteen body parts were measured from these images (Table 1). Curled larvae were not measured in the present study.

The alterations of body shape were identified by studying of the morphometric ratios (R) of all the characters (Y) to TL: R=Y/TL (Koumoundouros *et al.*, 1999). The developmental stages were identified between TL values (Fukuhara 1988; Yoshimatsu *et al.*, 1992; Koumoundouros *et al.*, 1999). The allometric equation on TL (Y=aTL^b, Fuiman 1983) was calculated severally for each stage of development by linear regression analysis (after logarithmic transformation of all the variables) (Koumoundouros *et al.*, 1999). T-test was also applied to test any differences in the slopes from unity (Sokal and Rohlf, 1981).

Results

Growth of *D. puntazzo* larvae followed an exponential curve during the study and their growth is described by equation $y=3.1004e^{0.0406x}$, ($r^2 = 0.96$, $n = 795$), where y is total length in millimeters and x is days after hatching (Figure 1).

The changes in larval stages of reared *D. puntazzo* until 42 DAH are shown in Figure 2. The mean TL of newly hatched larvae was 2.91±0.11 mm with 2.78±0.1 mm notochord length (NL). Newly hatched larvae (1 DAH) were transparent with closed

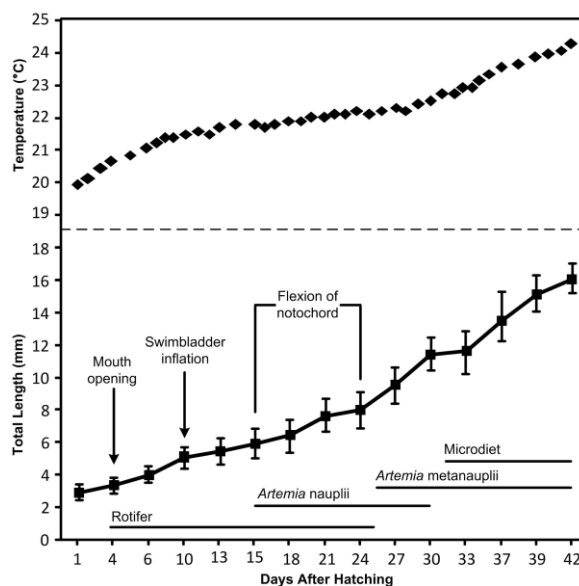


Figure 1. Temperature, feeding regime and growth in TL of *D. puntazzo* from 1 to 42 DAH.

Table 1. Abbreviations and description of morphometric characters measured during larval development

Character	Abbreviations	Description on Larvae
Head Depth (A)	HD-A	Posterior to eye
Head Depth (B)	HD-B	Posterior to gill cover
Body Depth	BD	Posterior to the anus
Eye Diameter	ED	Parallel to the longitudinal axis of the body
Head Length	HL	From tip of snout to the margin of gill cover
Ventral Margin of Cleithrum	VMC	From tip of snout to ventral margin of the cleithrum
Notochord Length	NL	From tip of snout to posterior margin of the notochord
Pre-Anal Length	PreAL	From tip of snout to the anus
Pre-Orbital Length	PreOL	From tip of snout to anterior margin of the eye
Total Length	TL	From tip of snout to tip of the caudal fin
Standard Length	SL	From tip of snout to base of caudal fin rays
Fork Length	FL	From tip of snout to the fork of the tail
Caudal Peduncle Depth	CPD	Minimum depth of the caudal peduncle
Pre-Anal Fin Length	PreAFL	From tip of snout to anterior margin of the anal fin base
Pre-Pelvic Fin Length	PrePFL	From tip of snout to the base of the pelvic fin
Pre-Dorsal Fin Length	PreDFL	From tip of snout to anterior margin of the dorsal fin base
Post-Anal Fin Length	PostAFL	From tip of snout to posterior margin of the anal fin base
Post-Dorsal Fin Length	PostDFL	From tip of snout to posterior margin of the dorsal fin base

mouth and anus (Figure 2A). The trunk of the larva was surrounded by the primordial fin. On this day, larval pigmentation consisted of punctuated and stellate melanophores which were located on posteriorly to the eye, cephalic region, around the oil globule, between myomeres 6 to 7 in pre-hemal region and between myomeres 12 to 13 and 19 to 20 in hemal region. At the onset of feeding on 4 DAH, larvae were 3.35 ± 0.13 mm TL and 3.28 ± 0.15 mm NL and were observed with a functional mouth, opened anus, developed stomach, completely consumed vitelline reserve and with no oil globule (Figure 2B).

At 4 DAH, punctuate and stellate melanophores were scattered around the eye, dorsal part of cephalic region, between myomeres 6 to 7 in pre-hemal region and between myomeres 12 to 13 and 19 to 20 in

hemal region. In *D. puntazzo*, initial swim bladder began to inflate at 5.11 ± 0.45 mm TL and 4.67 ± 0.43 mm NL at 10 DAH (80%; n=41). Fins were present the pectorals and the primordial marginal fin fold (Figure 2C). Xanthophores were clearly observed on the trunk and also around the eyes. Notochord flexion started at 5.95 ± 0.43 mm TL at 15 DAH. Punctuate and stellate melanophores were visible on digestive tract, swimbladder and dorsal part of the head (Figure 2D). Xanthophores were present from tip of snout to anus. Post-inflation the swim bladder became more elongate (88%; n=102) and occupied a greater length of the body cavity. At 15 DAH, the dorsal, anal, caudal, and pectoral fin shapes were present. The completion of notochord flexion was characterized by the antero-posteriorly formation of the caudal fin rays,



Figure 2. Ontogenic development of *D. puntazzo*: A, 1 DAH at 2.88 mm TL; B, 4 DAH at 3.36 mm TL; C, 10 DAH at 5.12 mm TL; D, 15 DAH at 5.98 mm TL; E, 24 DAH at 7.96 mm TL; F, 30 DAH at 11.43 mm TL; G, 37 DAH at 13.62 mm TL; H, 42 DAH at 16.08 mm TL.

presented at 7.98 ± 0.72 mm TL on 24 DAH. Melanophores and xanthophores were visible on the head, cephalic and pre-hemal region (Figure 2E). The dorsal, anal, caudal, and pectoral fin shapes began to develop on 24 DAH. *D. puntazzo* larvae were 11.42 ± 0.86 mm TL at 30 DAH. Punctuate and puncto-stellate melanophores and xanthophores shown on the head, cephalic and pre-hemal region (Figure 2F). At 37 DAH, melanophores and xanthophores increased on trunk, mainly on notochord, dorsal and anal fins at 13.50 ± 1.54 mm TL. In addition, vertical lateral bands were characterized between dorsal and anal fins (Fig. 2-G). Larvae were 16.03 ± 1.74 mm TL on 42 DAH, which is the end of the study. Punctuate and small stellate melanophores were visible all over the trunk, mainly in the head and around the fins except caudal fin (Figure 2H).

The allometric growth patterns of 18 body parts (including TL) were measured in 1075 *D. puntazzo* larvae (Figure 3). Larval morphometric ratios (R) were not permanent during the study except PrePFL, PreDFL, PostAFL and PostDFL. PrePFL, PreDFL and PostAFL showed isometry while PostDFL showed negative allometry during the larval development. Alterations of R in graphs showed high in ranges of TL. The allometric equations of the morphometric characters are shown in Table 2.

The majority of the morphometric characters became distinct at initial swimbladder inflation on 10 DAH at 5.12 mm TL. Also, flexion of notochord was observed on 15 DAH at 5.95 mm TL and completion of the flexion on 24 DAH at 7.98 mm TL. In 17 of 38 respective regression equations, allometry coefficients were positive allometry, while 10 equations showed

negative allometry. Twelve coefficients of regression equations showed isometry, which were mainly observed after notochord flexion (5.95 mm TL) in HD-A (5.96-22.07 mm TL), HD-B (3.40-5.95 mm TL), HL (7.99-22.07 mm TL), SL (10.05-22.07 mm TL), CPD (10.05-22.07 mm TL), PreAL (5.96-22.07 mm TL), PreOL (5.96-22.07 mm TL), PrePFL (6.96-22.07 mm TL), PreAFL (11.00-22.07 mm TL), PreDFL (6.28-22.07 mm TL) and PostDFL (5.89-22.07 mm TL) while only NL was before notochord flexion (2.73-5.95 mm TL).

Discussion

Feeding of larvae during the autotrophic phase is provided by absorption of yolk sac and oil globule (Watanabe *et al.*, 1995; Saka *et al.*, 2001). The yolk reserves of larvae include glycogen, proteins, lysosomal enzymes, and other enzymes related to protein, carbohydrate, and lipid metabolism (Korkut *et al.*, 2006). Absorption of endogenous food reserves, or more accurately the transition from endogenous to exogenous nutrition, is also a critical developmental and ecological transition for fish larvae (Kamler, 2008). The efficiency of yolk conversion decreases during the yolk-sac stage, since this could be more nutrients in the yolk is used for repair and energetic purposes as increase in the larval development. Besides, the major distinction of the autotrophic phase in Sparidae is anatomically and functionally undeveloped digestive tract resulting in inability to feed (Koumoundouros *et al.*, 1999; Russo *et al.*, 2007). Larval nutritional requirement is connected to digestion of proteins and digestive physiology.

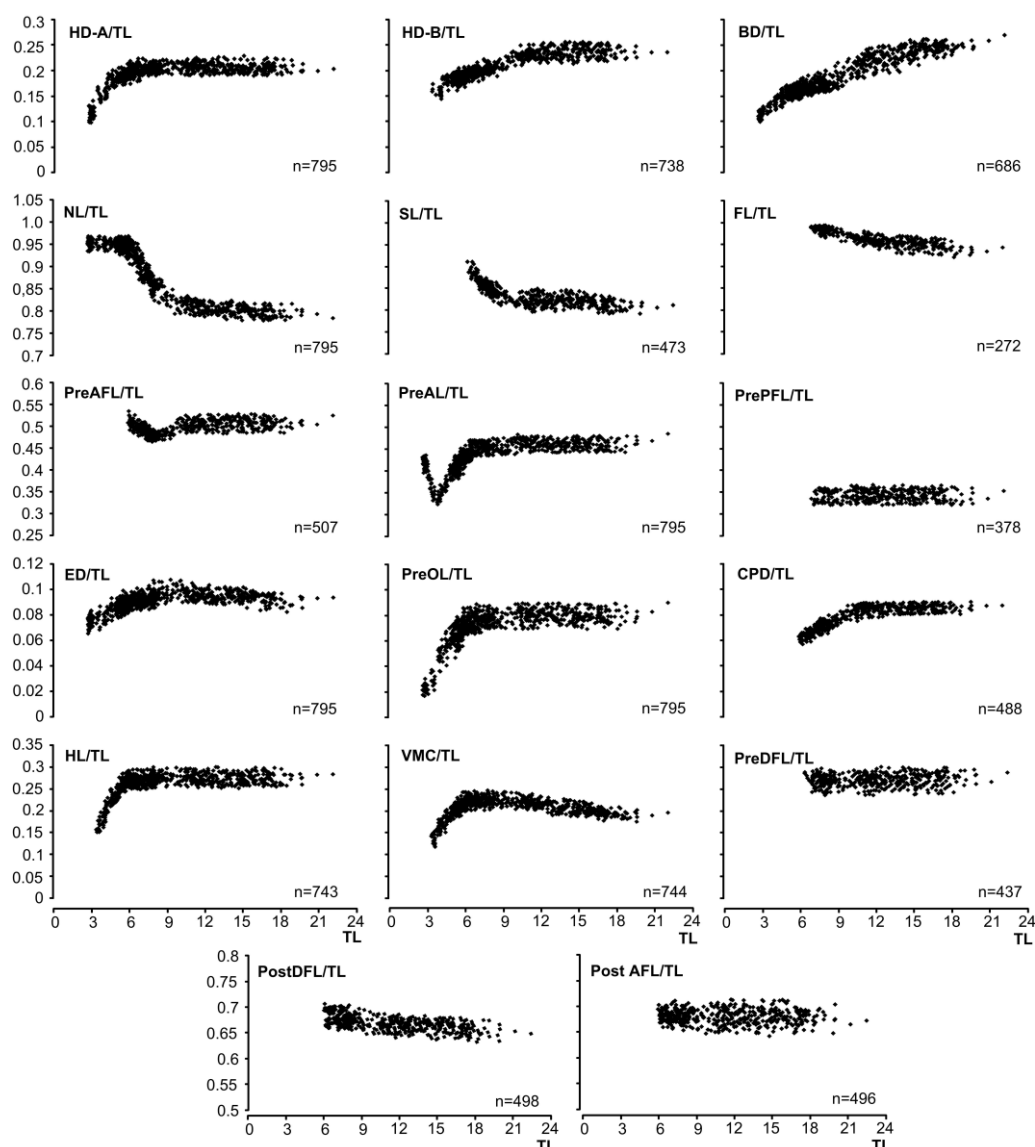


Figure 3. Development of the morphometric ratios in relation to TL. Morphometric abbreviations are listed in Table 1.

Furthermore, it is well known that competence at searching and assimilating food prior to depletion of the endogenous energy source of the yolk-sac is crucial for the survival of marine fish larvae (Kjørsvik *et al.*, 2004; Sveinsdóttir *et al.*, 2006; Kamler, 2008). The important role of different temperatures in yolk utilization results in different ontogenic developmental process of morphological properties and behavioral patterns in the cultured species (Fukuhara, 1990). Moreover, the initial feeding of larvae is important for survival and improving the organogenesis. Also the most crucial developmental stages during the early ontogeny of larval fish are hatching, and yolk absorption (Firat *et al.*, 2005). As described in other cultured species, in this study, newly hatched larvae of *D. puntazzo* have intense endogenous food reserves (yolk sac and oil globule). However, absorption of yolk sac of *D. puntazzo* was completed at 3.74 ± 0.13 mm TL on 4 DAH while most of the oil globule remained. In Sparidae, yolk

absorption is usually completed in 4 DAH at 3.93 ± 0.09 mm TL (Mihelakakis *et al.*, 2001) and 5 DAH at 3.5-4.2 mm TL in *P. pagrus* (Stephanou *et al.*, 1995), 3.2 mm TL in *Pagrus major* (Kitajima, 1978), 6 DAH at 3.55 mm TL in *Dentex dentex* (Firat *et al.*, 2003), and 3 DAH at 3.9-4.1 mm TL in *Sparus aurata* (Saka *et al.*, 2001; Russo *et al.*, 2007). It is well recorded that, larvae of physoclistous fishes can inflate the swimbladder for establishment of hydrostatic regulation during the onset of exogenous feeding (Ronnestad *et al.*, 1994; Firat *et al.*, 2005). It is known that the inflation and having a functional swimbladder is crucial in larval development, for controlling buoyancy and swimming activity and also larvae capture prey more efficiently (Bjelland and Skiftesvik, 2006). In this study, initial swimbladder inflation in *D. puntazzo* was first seen at 5.11 ± 0.45 mm TL on 10 DAH, but only in 80% of the larvae sampled ($n=41$). This rate increased to 98% at 15 DAH and post inflation of swimbladder (80%)

Table 2. Parameters of the allometry equations ($Y = a.TL^b$) of the morphometric characters studied (Y) against TL (b allometry coefficient; a constant of the allometry equation; r coefficient of determination; n number of measured individuals; ti test of isometry) (Morphometric abbreviations are listed in Table 1).

Y	TL range	b	Log a	r	n	ti
HD-A	2.73-5.12	1.802	-1.594	0.981	124	+
	5.13-5.95	1.328	-1.341	0.961	130	+
	5.96-22.07	0.995	-1.008	0.994	541	*
HD-B	3.40-5.95	0.997	-1.092	0.966	198	*
	5.96-11.00	1.230	-0.741	0.977	292	+
	11.01-22.07	1.171	-0.879	0.987	248	+
BD	2.73-5.12	1.324	-0.984	0.986	124	+
	5.13-11.00	1.209	-0.769	0.971	424	+
	11.00-22.07	1.381	-0.923	0.954	248	+
ED	2.73-7.98	1.213	-0.759	0.976	444	+
	7.99-22.07	0.933	-0.484	0.960	352	-
HL	3.40-5.95	1.408	-0.819	0.966	204	+
	5.96-7.98	1.162	-0.646	0.908	188	+
	7.99-22.07	0.989	-0.456	0.981	351	*
VMC	3.470-5.11	1.227	-0.899	0.961	73	+
	5.12-5.95	1.382	-0.745	0.908	130	+
	5.96-22.07	0.933	-0.411	0.951	541	-
NL	2.73-5.95	1.008	-0.029	0.883	255	*
	5.96-7.98	0.685	0.288	0.960	188	-
	7.99-22.07	0.937	0.149	0.865	352	-
SL	5.95-10.00	1.183	-0.659	0.937	183	-
	10.05-22.07	0.987	-0.073	0.926	290	*
FL	6.87-13.69	0.943	0.033	0.957	255	-
	13.69-22.07	0.967	0.041	0.931	147	-
CPD	5.95-10.00	1.293	-1.347	0.970	198	+
	10.05-22.07	1.018	-1.080	0.981	290	*
PreAL	2.73-5.12	0.676	0.281	0.916	124	-
	5.13-5.95	1.718	-1.391	0.930	132	+
	5.96-22.07	1.011	-0.789	0.965	540	*
PreOL	2.73-5.95	1.475	-1.381	0.981	254	+
	5.95-22.07	1.043	-0.841	0.967	541	*
PrePFL	6.96-22.07	1.047	0.453	0.958	378	*
PreAFL	5.89-7.98	0.941	-0.223	0.925	155	-
	7.98-11.00	1.201	-0.436	0.884	104	+
	11.00-22.07	0.989	-0.258	0.875	248	*
PreDFL	6.28-22.07	1.066	0.574	0.954	437	*
PostAFL	5.89-22.07	0.990	-0.063	0.932	496	*
PostDFL	5.98-22.07	0.934	-0.019	0.946	498	-

+, positive allometry; -, negative allometry; *, isometry

occurred on this day. It is recorded that in *P. pagrus* larvae, the swimbladder started to inflate and to be functional in larvae about 5-7 DAH (Mihelakakis *et al.*, 2001). Also, it is reported that inflation of swimbladder occurs at 3.5-4 mm TL between 5-10 DAH in *P. major*, 4-5 mm TL during the period from 5 to 9 DAH in *S. aurata* (Chatain, 1986) and 5-9 DAH *Puntazzo puntazzo* (Marangos, 1995). In *Latris lineta*, the initial inflation of swimbladder lasted 4 days (9-12 DAH) at 5.7-6.1 mm TL (Trotter *et al.*, 2005). Additionally, start of notochord flexion Sparids mainly depends on the cultured species, size of newly hatched larvae, and culture conditions (especially rearing temperature) (Çoban, 2009b). It is well known that water temperature is the most important environmental factor affecting larval

development and metamorphosis (Walsh *et al.*, 1991; Sfakianakis *et al.*, 2004; Saka *et al.*, 2008). Also, the development of caudal complex begins with the formation of the hyparalia, which is closely related to the flexion of the notochord (Koumoundouros *et al.*, 1999; Fırat *et al.*, 2006). In the present study, notochord flexion of *D. puntazzo* started at 5.95 ± 0.43 mm TL. It is reported that the beginning of the notochord flexion of *P. pagrus* occurred at 4.40 mm standard length (Machinandiarena *et al.*, 2003). In addition to this, notochord flexion was observed at 5.4 mm TL in *Pagellus erythrinus* (Sfakianakis *et al.*, 2004), 7.1 mm TL in *S. aurata* (Koumoundouros *et al.*, 1997), 5.4-6.4 mm TL in *D. puntazzo* (Sfakianakis *et al.*, 2005) and 6.4 mm TL in *D. sargus* (Koumoundouros *et al.*, 2001).

It is commonly known that growth in fish is often combining with mainly changes husbandry parameters (Katsanevakis *et al.*, 2006). Use of the allometric equation is the most common method for the analysis of the relative growth during early ontogeny in fish. Moreover, morphometric ratios are useful to determine intraspecific variations both culture methods and species of larvae and juveniles (Fukuhara, 1983, Suda *et al.*, 1987; Mihelakakis *et al.*, 2001; Koumoundouros *et al.*, 1999). These ratios could be used as a complementary criterion for evaluation of quality and control of cultured larvae (Wyatt, 1972; Yin and Blaxter, 1986; Koumoundouros *et al.*, 1995). In the present study, proportional changes with growth, the main inflection points the proportions of *D. puntazzo* occurred at 5.12, 5.95 and 7.98 mm TL. At 5.12 mm TL, *D. puntazzo* larvae inflated initial swimbladder which is necessary for the subsequent growth and survival of the cultured physoclistous larvae (Battaglione, 1995) and success to inflate swimbladder can result in high larval growth (Battaglione and Talbot, 1990, 1992). Also, notochord flexion started at 5.95 mm TL and the commencement of the upward bending of the notochord occurred at 7.98 mm TL. According to Kouttoui *et al.* (2006), the inflection points in shape ontogeny of *D. puntazzo* were present at 6.2 and 11.4 mm TL. Sfakianakis *et al.* (2005) reported that osteological development is mainly observed with the formation of cartilaginous elements of the vertebral column, caudal and pectoral fins and notochord flexion at 5.4-6.4 mm TL in *D. puntazzo*. As described by Tachihara and Kawaguchi (2003), proportional changes of *Plecoglossus altivelis ryukyuensis* appeared at the end of the prelarval stage, notochord flexion and completion of squamation. Also, Mihelakakis *et al.* (2001) reported that inflection point in body proportion changes in *P. pagrus* occurred at 4 mm and 7 or 9 mm TL, corresponding to morphological transitions to the postlarval stage and juvenile stage. Besides, Koumoundouros *et al.* (1999) pointed that the morphometric development of *D. dentex* was characterized by the transition of the sharp allometric growth of the early larval stages (mainly of from 3.6 to 6.7 mm TL). In addition, many morphological characters showed a relatively fast growth in early larvae and the larval inflexion point was found at 7 mm TL, which corresponds closely to the inflexion point in head length and head width in *Clarias gariepinus* and *Cyprinus carpio* (Van Snik *et al.*, 1997). Allometric growth during early developmental stages is mainly associated with muscle and bone characteristics.

This is the first study to investigate major morphological developmental process and morphometric modifications and development of feeding, sensorial, pigmentation, fin, and respiratory systems in larvae of *D. puntazzo* during the early life stages. These types of studies are needed to optimize

larval culture conditions (i.e. light intensity, photoperiod, stocking density, salinity etc.) in order to improve the reliability of the present protocols for larval rearing of *D. puntazzo*, which will contribute to increasing diversity of cultured species in both aquaculture and fisheries industries.

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