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Early Morphological Development and Allometric Growth Patterns in Hatchery-Reared Red Porgy (*Pagrus pagrus*)

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Abstract

Morphological development and allometric growth were investigated in the red porgy, *Pagrus pagrus*, during larval development until the end of weaning at 46 days after hatching (DAH). Total length (TL) of newly hatched larvae was 2.77±0.09 mm (mean±SE). Onset of exogenous feeding occurred at 4 DAH (3.74±0.13 mm TL). Initial swimbladder inflation occured at 10 DAH (4.22±0.18 mm TL) and post-inflation became more elongate at 16 DAH (4.95±0.26 mm TL). Notochord flexion occurred between 22 DAH (5.56±0.43 mm TL) and 34 DAH (8.22±0.63 mm TL). At the end of the weaning at 46 DAH, TL of larvae was 15.81±1.36 mm. According to individual measurements of a total of 1026 larvae of 18 body parameters for allometric growth, in 19 of 35 respective regression equations, the allometry coefficients were positive, while coefficients of 7 equations showed negative allometry. The majority of all allometric changes from inflection point were expressed during the larval stage and were integrated with the metamorphosis stage. Inflections in body proportion changes occurred mainly at TL values of 3.74, 4.22 and 5.56 mm, coinciding with mouth opening, initial swimbladder inflation and flexion of notochord, respectively.

Keywords: Red porgy, *Pagrus pagrus*, early development, allometry

Kültürü Yapılan Fangrilerde (*Pagrus pagrus*) Erken Dönem Morfolojik Gelişim ve Allometrik Büyüme Modelleri

Özet

Bu çalışmada, kültürü yapılan fangrilerde *Pagrus pagrus* larval dönem boyunca toz yem girişinin sona erdiği 46. güne kadar erken dönem morfolojik gelişim ve allometrik büyüme modelleri incelenmiştir. Yumurtadan yeni çıkan larvalarda total boy (TB) 2,77±0,09 mm (ort±SH) olarak ölçülmüştür. İlk hava kesesi şişmesi 10. günde (4,22±0,18 mm TB) meydana gelmiş ve kesede uzama şişme sonrası 16. günde (4,95±0,26 mm TB) tespit edilmiştir. Notokorda bükülmesi 22. gün (5,56±0,43 mm TB) ile 34. günler (8,22±0,63 mm TB) arasında izlenmiş ve toz yem giriş periyodunun sonu olan 46. günde larvalarda total boy 15,81±1,36 mm ulaşmıştır. Allometrik büyüme için toplam 1026 larvada 18 vücıt parametresi incelenmiş, 35 adet regresyon eşitliğinden 19 adedinde allometri katsayısı pozitif tespit edilirken 7 adet eşitlikte ise negatif allometri belirlenmiştir. Regresyon grafiklerindeki kırılma noktalarındaki allometrik değişimlerin büyük çoğunluğu larval dönemde meydana gelmiş ve metamorfoz dönemi ile ilişkili bulunmuştur. Vücut bölümlerine bağlı olarak regresyon grafiklerindeki kırılmalar 3,74, 4,22 ve 5,56 mm TB değerlerinde olmuş, sırasıyla ağız açılımı, ilk hava kesesi şişmesi ve notokorda bükülmesi ile eş zamanlı olarak tespit edilmiştir.

Anahtar Kelimeler: Fangri, *Pagrus pagrus*, erken dönem gelişim, allometri.

Introduction

Efficient production of high quality fry is one of the most important requirements for aquaculture expansion and to obtain independence from wild populations to produce healthy and high quality fish stocks (Lahnsteiner and Patarnello 2003). The red porgy, *Pagrus pagrus*, is one of the most attractive candidate species for diversification of aquaculture (Mihelakakis *et al.* 2001; Conides and Glamuzina, 2001; Suzer *et al.* 2007). It is a species intensively farmed in the Mediterranean (Basurco and Abellan 1999) and has been targeted because of its fast growth under intensive farming conditions (Divanach *et al.* 1993; Stephanou *et al.* 1995; Kentouri *et al.* 1995).

As described by Koumoundouros *et al.* (1999), ontogeny is a complex process of growth and differentiation during which larvae undergo extreme changes in body shape, morphology, metabolism and behavior in order to transform into juveniles. The change of body shape, which results from the growth of its components at different relative rates (allometry), reflects the close relationship between ontogeny of morphology and functions (Fuiman 1983; Kendall *et al.* 1984; Koumoundouros *et al.* 1999; Çoban *et al.*, 2009b). At hatching, most functional systems of fish are incomplete and morphogenesis and differentiation are very intense during early life stages of development (Gisbert 1999). Consequently, important quantitative morphometric changes take place and are responsible for a progressive transformation of recently hatched specimens from a larval body shape to a juvenile or adult form in a relatively short time, suggesting that growth functionally optimized for survival is a common feature among teleost fish larvae (Osse *et al.* 1995).

In the last decade, numerous studies have been carried out on red porgy reproduction and physiology (Kokokiris *et al.* 1999; Hood and Johnson 2000), larval rearing (Hernandez-Cruz *et al.* 1999; Roo *et al.*, 1999; Machinandiarena *et al.* 2003), feeding (Papandroulakis *et al.* 2004), osteological development (Çoban *et al.*, 2009a) and digestive enzyme activities (Darias *et al.* 2006; Suzer *et al.* 2007). In contrast, literature regarding the early life history of larval red porgy reared in intensive culture conditions is scarce, either fragmentary or related to the internal morphology of a few individuals caught in the wild (Alarcon and Alvarez, 1999), morphological and histological features (Diaz et al, 2013) and/or limited to yolk-sac larvae and larval development under captivity (Mihelakakis *et al.* 2001). Therefore, the objective of this study was to describe the ontogeny of the early stages of development of red porgy larvae with focus on the age and size in the morphological and functional development under intensive culture conditions using ambient temperature regime.

Materials and Methods

Broodstock and Egg Incubation

Red porgy broodstocks were collected from the wild and stocked in a 20 m^3 tank with a seawater supply of 35 $L.min^{-1}$. Frozen cuttlefish (*Sepia officinalis*) and leander squilla (*Palaemon elegans*) were provided as the primary food source twice daily to apparent satiation. The fish were subjected to natural photoperiod in rearing seasons.Water temperatures varied throughout the experimental period between 17.5ºand 19.0º C. Eggs spawned were immediately collected following fertilization, and the viable buoyant eggs were separated from the dead sinking eggs. Eggs were incubated in 50 L incubators at an initial density of 1500 $eggs.L^{-1}$ with a gentle flow of seawater of 18.0±0.5 °C. Oxygen saturation was 85%, salinity was 37 ppt and pH was approximately 7.65. Ammonia and nitrite were <0.012 $mg.L^{-1}$.

Larval Rearing

Biotical and abiotical parameters and rearing procedures during larval rearing of red porgy was based on Marangos (1995). After hatching, the larvae were reared in cylindrical tank (15 m^3), at an average density of 100 $ind.L^{-1}$. The color of the tank was dark-gray. To improve water quality, seawater was recirculated through a sand filter, a biofilter, UV system, a protein skimmer, and a battery of 4 screening cartridges down to 1 μm, before being pumped into the larvae tank. Physico-chemical parameters of seawater during the experiment are summarised in Table 1.

Newly hatched larvae were fed from day 4 (when the mouth opened) to day 15 with rotifers (70%, *Brachionus rotundiformis* and 30%, *Brachionus plicatilis*) cultured with algae and enriched (DHA Protein Selco, Artemia Systems SA, Gent, Belgium) at a density of 10-15 rotifers/mL. In addition, the tank was maintained with green-water composed of *Nannochloropsis* sp., *Chlorella* sp. and *Isochrysis* sp. at a density of 3-4 x 10^5 $cells.mL^{-1}$. From day 8 to day 20, they were fed *Artemia* nauplii (AF 480, INVE Aquaculture, Gent, Belgium) at 4-6 $individuals.mL^{-1}$ and from day 15 until the end of the experiment, *Artemia* metanauplii at 2-4 $individuals.mL^{-1}$ (EG, Artemia Systems SA), enriched with Protein Selco (Artemia Systems SA). Extruded micro diet (Proton, INVE Aquaculture) was used from 32 DAH until 46 DAH as 3-8% of biomass per day.

Morphological Observations and Measurements

Morphological observations and body measurements were conducted on subsamples of minimum 30 specimens per sample taken randomly at 3-day intervals from 1 to 46 DAH. Photographs (left side of each larva) were taken on anaesthetized (ethyleneglycol-monophenylether, Merck, 0.2-0.5 $ml.L^{-1}$) specimens using a stereoscopic microscope. Morphometric characters were measured by using TpsDig (version 1.37) software with 0.01 mm on the photographs. Eighteen body parts were measured from these images (Table 2). Curled larvae were not measured in the present study. Measured morphometric results are given as mean±SE.

The alterations of body shapes such as morphometric ratios (R) of all the characters (Y) to TL (i.e. R=Y/TL) during the process of development and also the different developmental stages were identified according to Fukuhara (1988), Yoshimatsu *et al.* (1992) and Koumoundouros *et al.* (1999). The alteration of R values as TL increases is identical to

Table 1. Physico-chemical parameters of seawater during the experiment

Temperature (°C)	18-24 (depending on age and size)
Salinity (‰)	37.2-37.8
Ammonia and nitrite ($mg.L^{-1}$)	<0.01
Flow rate (%)	5-6<
Light intensity (lx)	30-100 (depending on age and size)
Photoperiod (h)	24 h L (by algal addition), 16:8 L:D
Dissolved oxygen (mg/lt)	6.6-8.8
pH	7.2-8.4

Table 2. Abbreviations and description of morphometric characters measured during larval development

Chracter	Abbreviations	Description on Larvae
Head Depth (A)	HD-A	Posterior to eye
Head Depth (A)	HD-B	Posterior to gill cover
Body Depth	BD	Posterior to the anus
Eye Diameter	ED	Paralel 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
Muscle Depth	MD	The depth of the dorsal myotome, posterior to the skull
Occipital Spine	OS	
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

the allometric growth of the body. The allometric equation of every character on TL ($Y=aTL^b$, Fuiman 1983) was estimated separately for each stage of development by linear regression analysis (after logarithmic transformation of all the variables) according to Koumoundouros *et al.* (1999). Also, a Student *t*-test was applied to test any differences between stages (Sokal and Rohlf, 1981).

Results

Larval Growth

During the experimental period, speed of growth was slow from 1 to 22 DAH ($y=3.139e^{0.027x}$, R^2=0.896, n= 566) and accelerated exponentially ($y=1.830e^{0.046x}$, R^2=0.967, n= 460) between 22 and 46 DAH, once the notochord flexion occurred (Figure 1). Y is total length (TL) in millimeters and x is days after hatching.

Morphological Development

The changes in TL of reared *P. pagrus* larvae are shown in Figure 2. The mean TL of newly hatched larvae was 2.77±0.09 mm with 2.69±0.12 mm notochord length (NL). Hatched larvae were transparent with closed mouth and anus (Figure 2-A). On this day (1 DAH), larval pigmentation consisted of punctuate and stellate melanophores which were located on dorsal part of the head, pre-hemal region and between 16 and 20 vertebra in hemal region. At the onset of exogenous feeding on 4 DAH, larvae measured 3.74±0.13 mm TL and 3.61±0.08 mm NL and they were identified with a functional mouth, opened anus, developed stomach, completely consumed vitelline reserve but not oil globule (Figure 2-B).

On 4 DAH, punctuate and stellate melanophores were scattered on the anterior and posterior of the eye, dorsal side of the digestive tract, anus, and also between 16 and 20 vertebra in hemal region. In *P. pagrus*, initial swim bladder began to inflate at 4.22±0.18 mm TL and 4.04±0.20 mm NL on 10 DAH (41%; n=61) and also fins were present at the pectorals and the primordial marginal fin fold (Figure 2-C). On 16 DAH, larvae were 4.95±0.26 mm TL and 4.81±0.36 mm NL (Figure 2-D). Notochord flexion started on 22 DAH at 5.56±0.43 mm TL (Figure 2-E). On 28 DAH, the dorsal, anal, caudal, and pectoral fin shapes were present and the pelvic fin rays began to develop at 6.24±0.56 mm TL (Figure 2-F). The complement of the notochord flexion was characterized by the antero-posterior orientation of the caudal rays, present at 8.22±0.63 mm TL on 34

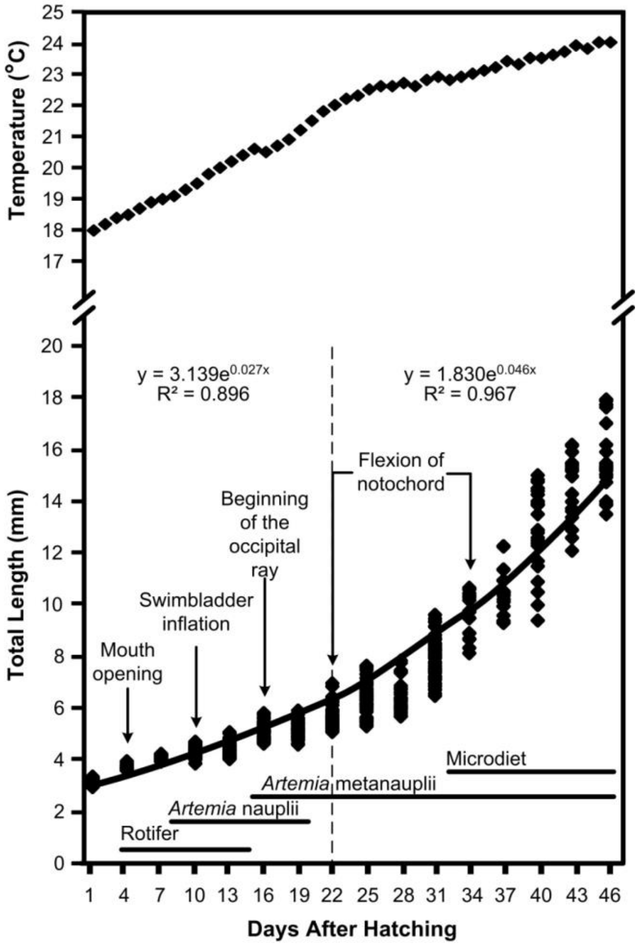

Figure 1. Feeding regime and growth in TL of *P. pagrus* from 1 to 46 DAH.

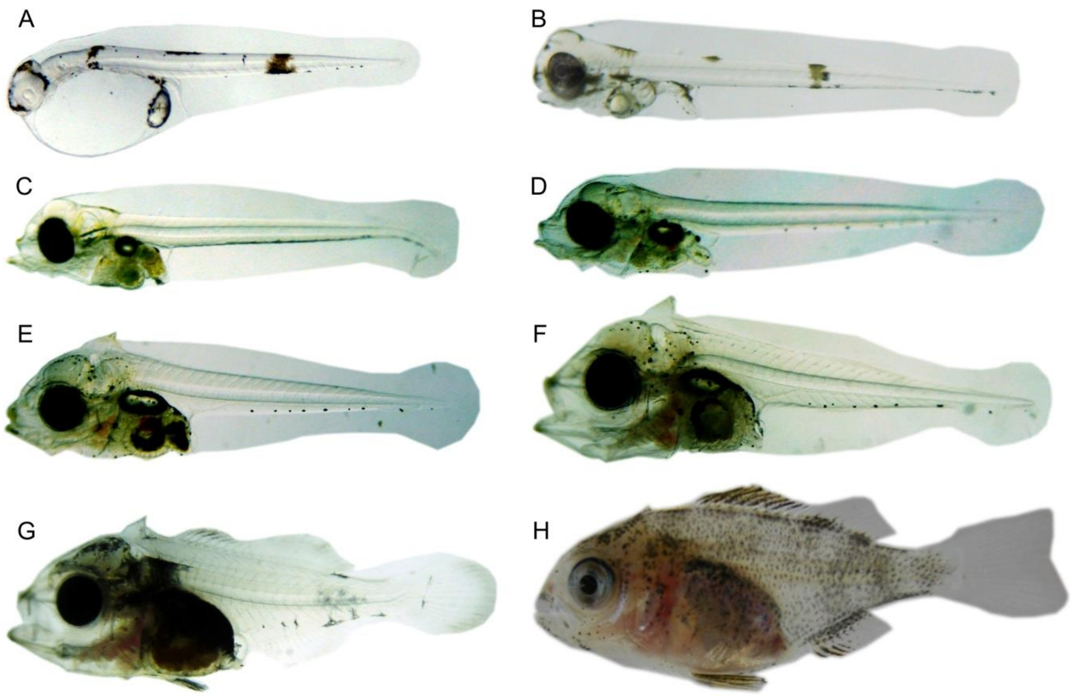

Figure 2. Ontogenic development of *P. pagrus*: A, 1 DAH at 2.81 mm TL; B, 4 DAH at 3.76 mm TL; C, 10 DAH at 4.20 mm TL; D, 16 DAH at 4.98 mm TL; E, 22 DAH at 5.55 mm TL; F, 28 DAH at 6.22 mm TL; G, 34 DAH at 8.25 mm TL; H, 46 DAH at 15.78 mm TL.

DAH (Figure 2-G). At the end of the study larvae were 15.81±1.36 mm TL on 46 DAH, punctuate and small stellate melanophores were visible all over the trunk, mainly in the head and around the fins except the caudal fin (Figure 2-H).

Allometric Growth

The allometric growth patterns of 18 body parts (including TL) measured in 1026 specimens of *P. pagrus* larvae are presented in Figure 3. Morphometric ratios (R) of larvae were not constant during the study except NL, OS, PrePFL, PreAFL, PreDFL, PostAFL and PostDFL. In contrast, only NL showed negative allometry during the larval development. These inflection points of TL were differentiated between measured characters. The allometric equations of the morphometric characters were studied in comparison to TL as shown in Table 3.

The majority of the morphometric characters became distinct at opening of mouth on 4 DAH at 3.74±0.13 mm TL. Also, initial swimbladder inflation was observed on 10 DAH at 4.22±0.18 mm TL and flexion of notochord was detected on 22 DAH at 5.56±0.43 mm TL. Additionally, BD, PreAL and ED showed negative allometry between 2.86 and 3.74 mm TL, which was the yolk sac period. In 19 of 35 respective regression equations, the allometry coefficients were positive allometry, while coefficients of 7 equations showed negative allometry. Finally, remaining nine coefficients of regression equations showed isometry, which were observed mainly between 5.57 and 17.85 mm TL in HD-A, ED, HL, VMC, PrePFL, PreAFL and PreDFL.

Discussion

Different temperatures play important roles in yolk utilization, as well as, result in different development sequences of morphological characters and behavioral patterns within the same species

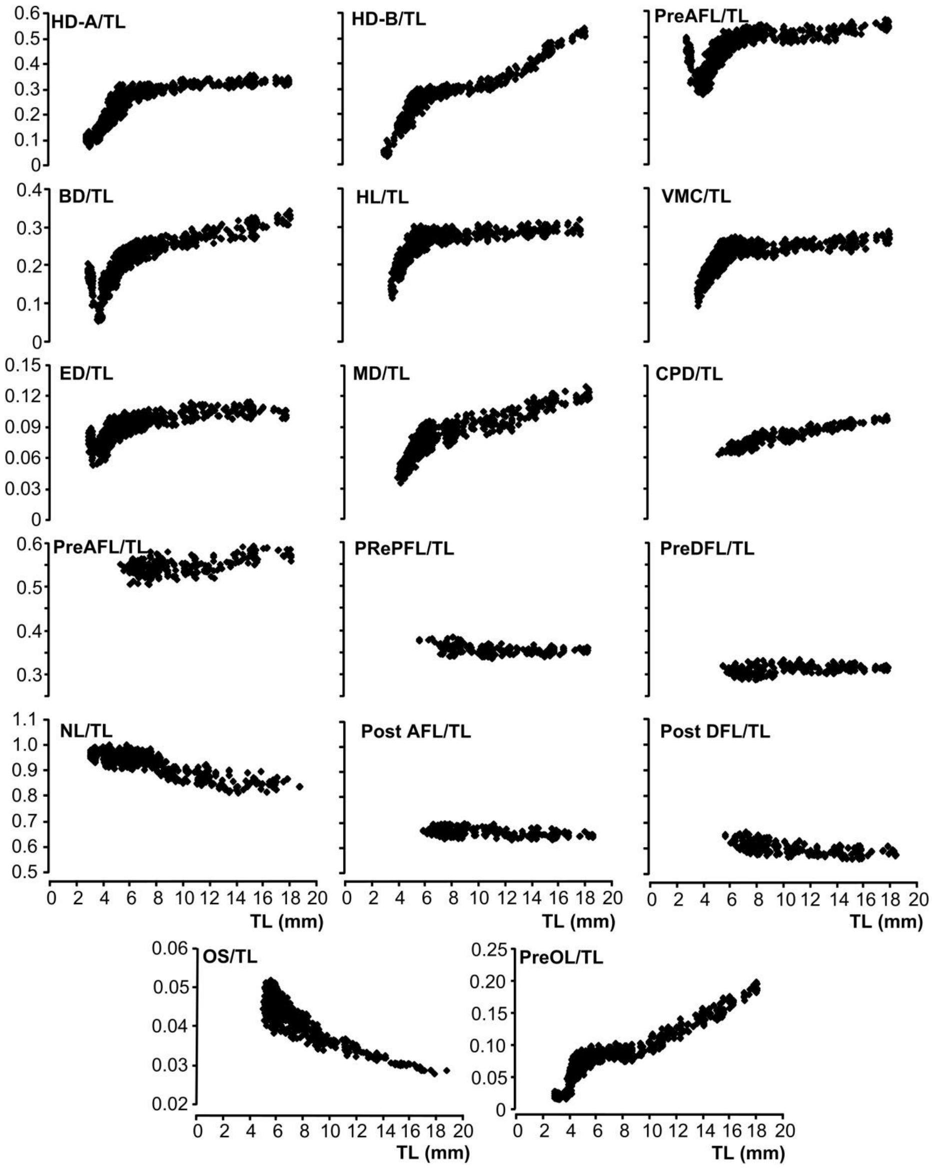

Figure 3. Development of the morphometric ratio in relation to TL. Morphometric abbreviations are listed in Table 1.

Table 3. 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; SE_b standard error of b; *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	SE_b	r	n	ti
HD-A	2.86-3.74	1.5207	-1.3694	0.0126	0.9115	155	+
	3.75-5.56	1.3447	-0.9829	0.0201	0.9385	487	+
	5.57-17.85	1.0954	-0.8356	0.0094	0.9689	378	*
HD-B	2.86-4.22	1.3802	-0.9159	0.0215	0.9758	365	+
	4.23-10.60	1.1628	-0.8074	0.0209	0.9329	444	+
	10.61-17.85	1.2198	-0.7309	0.0218	0.9831	145	+
BD	2.86-3.74	0.7470	-0.6636	0.0070	0.8126	161	-
	3.75-6.21	1.6619	-1.1385	0.1691	0.9206	515	+
	6.22-17.85	1.1701	-0.9798	0.1299	0.9780	345	+
ED	2.86-3.74	0.7155	-0.3892	0.0078	0.9472	242	-
	3.75-5.56	1.1922	-0.7388	0.1159	0.9205	262	+
	5.57-17.85	1.0134	-0.6029	0.0920	0.9794	517	*
HL	3.54-4.22	1.4249	-0.9042	0.0159	0.9110	72	+
	4.23-5.56	1.3247	-1.3776	0.0349	0.9392	406	+
	5.57-17.85	1.0340	-0.3231	0.0875	0.9738	395	*
VMC	3.5-5.56	1.5758	-1.3952	0.1424	0.9353	385	+
	5.57-17.85	1.0767	-0.5492	0.0985	0.9561	489	*
NL	2.86-16.93	0.8993	-0.9092	0.0120	0.7801	984	-
PreAL	2.86-3.74	0.6673	0.2597	0.0076	0.8817	156	-
	3.75-6.20	1.6046	-1.3317	0.1441	0.9460	517	+
	6.20-17.85	1.1909	-0.7316	0.1168	0.9504	348	+
PreOL	2.86-3.74	0.9818	-0.4882	0.0733	0.9259	160	*
	3.75-5.56	1.6830	-1.1986	0.1502	0.8381	314	+
	5.57-7.95	1.0174	-0.6218	0.0701	0.9399	308	*
	7.95-17.85	1.2170	-0.7949	0.1329	0.9878	237	+
MD	3.77-5.56	1.3602	-0.3321	0.0330	0.9614	410	+
	5.57-17.85	1.1369	-1.0391	0.1003	0.9843	379	+
OS	4.5-16.89	0.8169	-0.7852	0.0198	0.9710	485	-
CPD	5.34-8.57	1.3702	-1.4904	0.0299	0.9689	113	+
	8.57-17.85	1.1784	-1.1636	0.1210	0.9895	203	+
PrePFL	5.56-17.85	0.9895	-0.2515	0.0756	0.9755	274	*
PreAFL	5.34-17.85	1.0831	-0.3622	0.0949	0.9696	326	*
PreDFL	5.56-17.85	1.0411	-0.5289	0.0868	0.9766	311	*
PostAFL	5.56-17.85	0.9419	-0.1850	0.0642	0.8951	317	-
PostDFL	5.56-17.85	0.9263	-0.1747	0.0635	0.8510	304	-

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

(Fukuhara 1990). In this study, absorption of yolk sac of *P. pagrus* was complete at 3.74±0.13 mm TL on 4 DAH but most of the oil globule remained. Other studies also reported that yolk absorption was complete in 3.93±0.09 mm TL at 4 DAH (Mihelakakis *et al.* 2001) and 2.5-3.2 mm TL at 5 DAH in *P. pagrus* larvae (Stephanou *et al.* 1995). The differences might result from changes in rearing temperatures. In other Sparid species, this absorption occurred at 3.2 mm TL in *Pagrus major* (Kitajima 1978), 3.22 mm TL at 5 DAHin *Dentex dentex* (Fırat *et al.* 2003), and 2.6-2.8 mm TL at 3 DAH in *Sparus aurata* (Saka *et al.* 2001). Generally, the presence and functioning of swimbladder are crucially important in fish larvae which can occur at and/or follow the onset of exogenous feeding, as they control buoyancy and make swimming activity and prey capture more energy efficient (Ronnestad *et al.* 1994; Fırat *et al.* 2005; Bjelland and Skiftesvik 2006). In this study, initial swimbladder inflation was first observed at 4.22±0.18 mm TL on 10 DAH, but only in 41% of the sampled larvae. This increased to 96% at 16 DAH and post inflation of swimbladder (80%) occurred on this day. In this species, Mihelakakis *et al.* (2001) reported that inflation of swimbladder began to inflate and be functional between 5 and 7 DAH. Also, inflation of swimbladder occurred at 3.5-4 mm TL during the 5-10 DAH in *P. major* (Kitajima 1978), 4-5 mm TL during the period from 5 to 9 DAH for *S. aurata* (Chatain 1986) and 5-9 DAH for *Diplodus puntazzo* larvae (Marangos 1995). The start of notochord flexion in Sparids depend on the species, size of newly hatched larvae, and culture conditions (especially rearing temperature)(Koumoundouros *et al.* 1999; Sfakianakis *et al.* 2004; Fırat *et al.* 2005). In the present study, notochord flexion of *P. pagrus*appeared at 5.56±0.43 mm TL. In contrast, it was 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), 6.5 mm TL and 7.1 mm TL in *S. aurata* (Çoban *et al.*, 2008b; Koumoundouros *et al.* 1997), 5.6 mm TL in *P. pagrus*

(Çoban *et al.* 2008a) and 6.4 mm TL in *D. sargus* larvae (Koumoundouros *et al.* 2001).

It is known that growth in fish is often accompanied with changes in proportion as well as in size, the phenomenon of relative or allometric growth (Katsanevakis *et al.* 2007). Moreover, morphometric ratios are useful indicators for intraspecific variations between rearing methods and cultivated populations of larvae and juvenile (Fukuhara 1983; Suda *et al.* 1987; Mihelakakis *et al.* 2001). These ratios may be used as supplementary criteria for quality assessment and control of cultured larvae (Wyatt 1972; Yin and Blaxter 1986; Koumoundouros *et al.* 1995). In the present study, main proportional changes and inflection points in the proportions of *P. pagrus* occurred at 3.74, 4.22 and 5.56 mm TL with growth. Besides, *P. pagrus* larvae inflated initial swimbladder at the second turning point (4.22 mm TL) and also the commencement of the upward bending of the notochord occurred at third turning point (5.56 mm TL). 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. Koumoundouros *et al.* (1999) pointed out that the morphometric development of *D. dentex* was basically characterized by the transition of the sharp allometric growth of the early larval stages (mainly of 3.6 to 6.7 mm TL) to the lighter allometric or isometric of the following stages.

In conclusion, the present study desribes important morphological developments and morphometric modifications which occurred in *P. pagrus* larvae during early life stages under gradually increasing temperature profile. The data are hepful for management using similar regimes. Further studies with sufficient replicates are needed at several constant tempratures to provide a baseline data set for comparison with other studies.

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