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Assessment of temperature effects on body shape in Nile tilapia, *Oreochromis niloticus* juveniles using the truss network system

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ABSTRACT

Among the environmental factors, water temperature has a significant effect on many aspects of fish life. This study was carried out to examine effects of water temperature on body shape of Nile tilapia, *Oreochromis niloticus* during the early stages of life using truss network system. A total of 150 newly-born fry were reared in three temperature treatments (22, 28 and 34°C) for 40 days. At the end of experiment, the left side of the specimens was photographed. To achieve the body shape data, 13 landmark-points were digitized using tpsDig2 software. The extracted data were justified with generalized procrustes analysis, then the differences of body shape among groups were investigated using the principal component analysis (PCA), canonical variate analysis (CVA) and cluster analysis (CA). There were significant differences in body shape of the treatments. The results of CVA and CA indicated that one of the important factors in the aquatic habitats is water temperature that influences the body shape of the Nile tilapia during the first stages (larval and juvenile stages) of growth.

Keywords: Fish, Temperature, Tilapia, *Oreochromis niloticus*, Truss distance.

Article type: Research Article.

INTRODUCTION

Environmental factors such as chemical, physical and biological factors can affect biological functions and survival rate of fishes directly through their action, or indirectly via environmentally affected body shape variation (Georgakopoulou *et al*. 2007). The structural and functional morphology of fish, in addition to geomorphological factors like bed morphology and channel slope, is affected by environmental characteristics like velocity, depth and temperature of water (Mahon 1984; Rajput *et al*. 2013). Temperature is known as one of the most important environmental factors that strongly influences the body shape (Sfakianakis *et al*. 2011; Nasrolah-Pourmoghadam & Eagderi 2013; Eagderi *et al*. 2015; Rowiński *et al*. 2015), meristic characters (Murray & Beacham 1988; Sfakianakis *et al*. 2011), fertilization and hatching rates, egg and yolk-sac larval development (Polo *et al*. 1991; Bolla & Holmefjord 1988; Klimogianni *et al*. 2004; Barón-Aguilar *et al*. 2013; Thépot & Jerry 2015), skeletal ontogeny (Pavlov & Moksness 1997; Campinho *et al*. 2004; Georgakopoulou *et al*. 2010), Morpho-anatomical abnormalities (Sfakianakis *et al*. 2004; Roo *et al*. 2010) and feed efficiency ratio (Imsland *et al*. 1996) in fishes. One of the most important mechanisms in phenotypic adaptation is phenotypic plasticity. It is defined as the ability of a genotype to create several forms in response to environmental conditions (Georgakopoulou *et al*. 2007; Zamani-Faradonbe *et al*., 2014, 2015). Studies have also been reported the effects of temperature on fish meristic characters. Taning (1952) reported that *Salmo trutta trutta* had a more number of vertebrae at a brief low temperature pulse. Hempel & Blaxter (1961) also reported a similar effect of brief temperature pulse on the number of vertebrae in herring fish, *Clupea harengus*. Georgakopoulou *et al*. (2007) showed that in sea bass, *Dicentrarchus labrax* the body shape at lower temperature (15 °C) tended to be more slender than at higher temperature (20 °C), the fish which had experienced 15°C treatment had more pectoral and dorsal lepidotrichia,

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while in 20°C treatment, the fish had more dorsal spines and caudal dermatotrichia, but the caudal lepidotrichia, number of anal and pelvic fin rays and vertebral number were unaffected by the temperature and there were no significant differences between the two temperature groups. Sfakianakis *et al*. (2011) reported that in zebra fish (*Danio rerio*) juveniles, the number of anal, and dorsal fin rays and vertebrae in 22 °C group were significantly higher than those in 25, 28 and 31°C groups, while the number of pelvic and pectoral fin rays were significantly lower in 31°C group. In the upper caudal dermatotrichia, intermediate temperatures (25 and 28 °C) presented higher count when compared to the two extreme temperature groups (22 and 31 °C), such that, in 22 °C group the number of the lower caudal dermatotrichia was lower. Also, in the upper and lower caudal lepidotrichia, the four temperature groups had no significant differences.

Morphometric and meristic characters are the two types of morphologic characters that have been most frequently employed to identify stocks of fishes. The truss network system is one of those tools that can be successfully used to investigate fish stocks within a species that, in a long term, allows better and direct comparison of morphological evolution of stocks, while using the same set of measurements (Turan 1999). Nile tilapia (*Oreochromis niloticus*) is one of the most important and valuable species in fresh-water aquaculture (FAO 2004). Moreover, this species has features such as high fecundity rate, year-round breeding capability (2–3 week spawning cycle) and short generation period (half a year), so this species is an excellent model species in studying evolutionary developmental biology (Fujimura & Okada 2007). The purpose of this study is investigating the impacts of rearing temperature on body shape variations in Nile tilapia, *O. niloticus* juvenile using truss network system.

MATERIALS AND METHODS

Sampling

After hatching eggs, the freely-swimming larvae were transferred into three 65-L aquaria with 22, 28 and 34 ºC and were kept until the end of the study. Temperature treatments included two tolerable threshold temperatures including 22 and 34ºC and optimum temperature (28 ºC as the control, 50 larvae each; according to Bardach *et al*. 1972). Three different groups of eggs were used to repeat the experiment. At late stages of yolk-sac absorption, commercial feed was used to feed free-swimming larvae. 40-day-old fish (40 days after hatch) were collected from the treatment tanks with a scoop and anesthetized in 1% clove oil solution. The left side of body was photographed with a digital Cannon camera (12 megapixel resolutions), then 13 landmark-points were selected and digitized using tpsDig2 software (Fig. 1) to extract appropriate information about body shape.

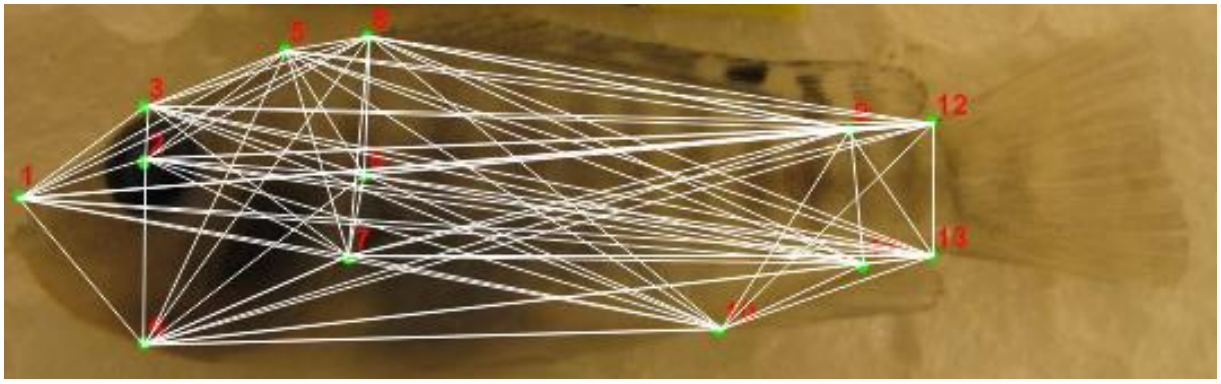

Fig. 1. Defined landmark points to extract the body shape data of *Oreochromis niloticus*: (1) anterior point of the snout tip on the upper jaw, (2) center of the eye, (3) dorsal and (4) ventral edge of the head perpendicular to the center of the eye, (5) end of the head, (6) posterior end of the opercular edge, (7) dorsal origin of pectoral fin (8) anterior and (9) posterior end of the dorsal fin base, (10) anterior and (11) posterior ends of the anal fin base, (12) posterodorsal end of caudal peduncle at its connection to caudal fin, (13) posteroventral end of caudal peduncle at its connection to caudal fin.

Data collection and analysis

Measurements (78 distances between 13 landmark points) were performed by collecting X-Y coordinate information for appropriate body shape traits using a three-step process described by Turan (1999). For having a basic shape of the body of each fish, a box truss was created by connecting these landmarks (Cadrin & Friedland 1999). For further analyzes, the X-Y coordinate data were converted to linear distances (Turan 1999; Anvarifar

et al. 2011) and then these linear distances were analyzed using generalized procrustes analysis (GPA) to remove non-shape information such as scale, direction and position. Finally, all measurements were transferred to a spreadsheet file in SPSS Ver. 19. Analysis of variance (ANOVA) was performed to evaluate the significance of the differences between the three treatments and only traits with significant differences ($p < 0.05$) were used in subsequent analyses. To determine which body shape characters are more effective in differentiating treatments, the participation of characters to principal components (PC) were examined. To examine the suitability of the data for PCA, Bartlett's test of sphericity and Kaiser–Meyer–Olkin (KMO) measure were used. For comparison of treatments, PCA, CVA and CA were used according to AnvariFar *et al*. (2011), Samaee *et al*. (2006, 2009) and Veasey *et al*. (2001). Statistical analyzes were conducted for morphological information in tpsDig2 (Ver. 2.04; Rohlf 2005), PAST (Ver. 2.17b; Hammer 2012) and SPSS (Ver. 19).

RESULTS

Amongst the 78 characters, 71 were significantly different ($p < 0.05$) and were used further for multivariate analysis (Table 1). In this study, the value of KMO for overall matrix was 0.605 and the Bartlett's test of sphericity was significant ($p < 0.01$). The results (KMO and Bartlett's) suggested that the data is appropriate to proceed to factor analysis procedure. Principal component analysis of the 71 morphometric measurement with an eigenvalue > 1 were included, explaining 94.19% of the variance and others were discarded. PCA of 71 morphometric measurement showed that PC1 and PC2 accounts for 23.55% and 17.78% of the variation, respectively (Table 2) and the most significant of morphometric measurements on PC1 were the distances 2-12, 3-12 and on PC2 were the distances 1-2, 1-4, 1-6 and 1-12 (Table 3). Visual examination of plotted PC1 and PC2 scores for each sample (Fig. 2) revealed that the three treatments are distinct from each other and there is little overlap between the 28 and 34 ºC treatments. For the DFA, the averages probability of correct classification (PCC) was 89.73% for morphometric measurements in truss network system. Since the Wilks' Lambda test proved the existence of differences among treatments, morphometric measurements of groups were compared using discriminant function analysis and this test revealed that in both system, two functions are highly significant ($p < 0.01$) and Lambda values were 0.064 and 0.502 for first and second functions, respectively (Table 4). The high values for successful grouping for the 22 ºC (100.0%), 28 ºC (84.3%) and 34 ºC (84.9%) treatments indicate correct classification of individuals into their original populations with respect to morphometric characters. Percentage of specimens classified in each group and cross validation are shown in Table 5. The result of CVA and grouping of three treatments in the two canonical variables for each treatments are shown in Fig. 3. The CVA scatter plot shows that studied treatments occupy different areas. Cluster analysis based on Euclidean distances among groups of centroids was used to draw the dendrogram plot, three treatments classified in two groups; 28 ºC and 34 ºC treatments were classified in one group and 22 ºC was grouped in one (Fig. 4).

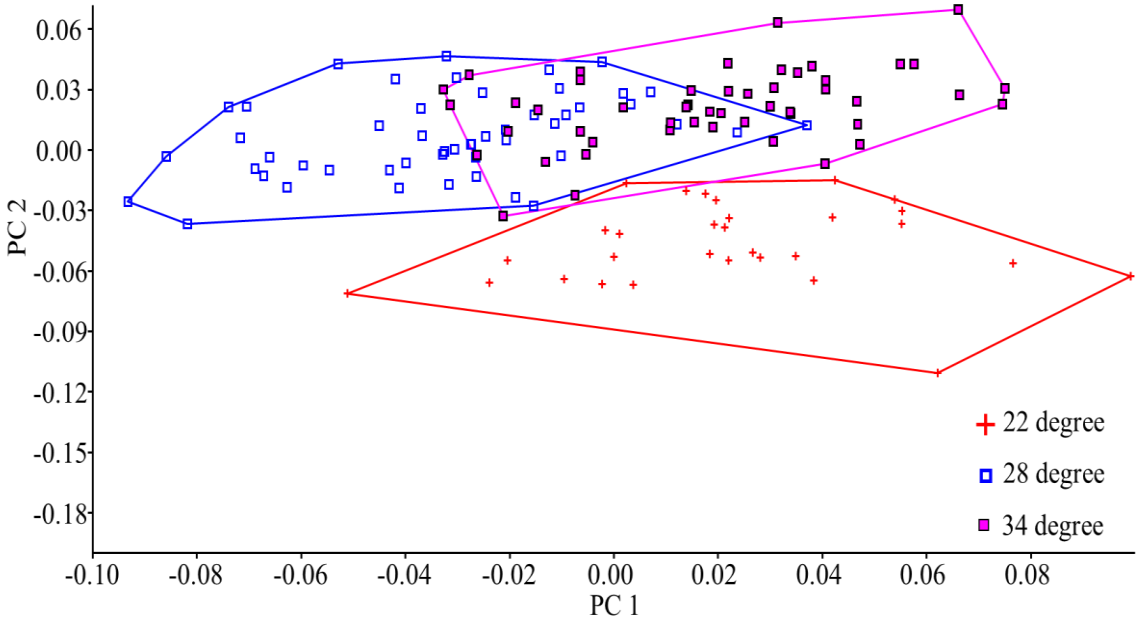

Fig. 2. Plot of the factor scores for PC1 and PC2 for *Oreochromis niloticus* specimens from the three treatments.

Table 1. The results of ANOVA among samples of *Oreochromis niloticus* from three treatments.

measurement	*F* value	*p* value	measurement	*F* value	*p* value	measurement	*F* value	*p* value
1-2	17.480	0.000	3-7	17.024	0.000	6-9	29.274	0.000
1-3	44.439	0.000	3-8	9.828	0.000	6-10	32.990	0.000
1-4	13.153	0.000	3-9	11.666	0.000	6-11	24.346	0.000
1-5	4.661	0.011	3-10	36.809	0.000	6-12	41.302	0.000
1-6	33.980	0.000	3-11	7.647	0.001	6-13	14.496	0.000
1-7	50.467	0.000	3-12	24.113	0.000	7-8	35.481	0.000
1-8	26.884	0.000	3-13	1.092	0.338	7-9	46.997	0.000
1-9	7.718	0.001	4-5	10.998	0.000	7-10	22.510	0.000
1-10	26.224	0.000	4-6	18.999	0.000	7-11	26.211	0.000
1-11	7.439	0.001	4-7	56.275	0.000	7-12	31.625	0.000
1-12	28.751	0.000	4-8	4.342	0.015	7-13	18.158	0.000
1-13	21.433	0.000	4-9	19.366	0.000	8-9	4.889	0.009
2-3	26.665	0.000	4-10	5.171	0.007	8-10	95.573	0.000
2-4	4.642	0.011	4-11	5.977	0.003	8-11	34.998	0.000
2-5	15.093	0.000	4-12	29.255	0.000	8-12	4.723	0.010
2-6	9.363	0.000	4-13	8.908	0.000	8-13	19.173	0.000
2-7	26.707	0.000	5-6	18.078	0.000	9-10	1.833	0.164
2-8	7.735	0.001	5-7	11.914	0.000	9-11	7.927	0.001
2-9	15.222	0.000	5-8	0.603	0.549	9-12	12.599	0.000
2-10	39.250	0.000	5-9	0.634	0.532	9-13	3.404	0.036
2-11	9.499	0.000	5-10	84.689	0.000	10-11	13.092	0.000
2-12	26.973	0.000	5-11	23.473	0.000	10-11	26.301	0.000
2-13	18.858	0.000	5-12	0.029	0.971	10-13	60.694	0.000
3-4	13.636	0.000	5-13	21.222	0.000	11-12	2.940	0.056
3-5	10.595	0.000	6-7	14.943	0.000	11-13	23.373	0.000
3-6	19.573	0.000	6-8	0.415	0.661	12-13	31.663	0.000

Table 2. Eigenvalues, percentage of variance and percentage of cumulative variance for the 11 principal components of *Oreochromis niloticus* samples from the three treatments.

Component	Eigenvalues	% of Variance	Cumulative (%)
1	16.72	23.55	23.55
2	12.63	17.78	41.33
3	8.88	12.51	53.84
4	6.77	9.54	63.38
5	5.93	8.35	71.73
6	4.79	6.75	78.48
7	3.53	4.98	83.46
8	2.81	3.96	87.42
9	1.87	2.63	90.05
10	1.57	2.21	92.26
11	1.37	1.93	94.19

Table 3. Results of factors extraction in PCA after Varimax normalized rotation.

measurements	1	2	3	4	5	6	7	8	9	10	11
1-2		-0.833									
1-3	0.601		-0.484								
1-4		-0.850									
1-5			-0.419						0.482		
1-6		-0.966									
1-7					-0.442			0.458			
1-8		-0.784				-0.499					
1-9					-0.887						
1-10	-0.590	-0.706									
1-11				0.877							
1-12		-0.907									
1-13	-0.483		0.772								
2-3							0.504				0.557
2-4			0.485				0.695				
2-5									0.845		
2-6		-0.503								-0.562	
2-7								0.884			
2-8		0.608			0.604						
2-9	-0.442	0.458		-0.589							
2-10	-0.643			-0.548							
2-11		0.462									
2-12	0.905										
2-13		-0.565					0.403				
3-4		-0.556				-0.429					0.412
3-5									0.796		
3-6		-0.639								-0.479	
3-7								0.888			
3-8		0.653			0.535						
3-9			0.484	-0.503							
3-10	-0.625			-0.560	0.413						
3-11						0.456					
3-12	0.909										
4-5	-0.409		-0.505			-0.655					
4-6		-0.489	-0.653								
4-7	0.402				-0.468			0.424			
4-8						-0.857					
4-9					-0.749						
4-10	-0.585		-0.657								
4-11				0.933							
4-12	0.506		-0.768								
4-13			0.791								
5-6							-0.840				
5-7										0.707	
5-10	-0.654										
5-11				0.923							
5-13			0.927								

Continue Table 3.

6-7						-0.804		
6-9		0.822						
6-10	-0.529			-0.512	0.442	0.403		
6-11		0.791						
6-12	0.948							
6-13		0.602				-0.436		0.406
7-8						-0.418	-0.732	
7-9		0.746		-0.408				
7-10	-0.504			-0.522		0.462		
7-11		0.785						
7-12	0.946							
7-13		0.660					-0.489	
8-9					-0.827			
8-10	-0.539						-0.534	
8-11				0.856				
8-12	0.403					0.405	-0.620	
8-13			0.938					
9-11						0.723		
9-12						0.727		
9-13			0.856					
10-11				0.847				
10-12	0.898							
10-13	0.780			0.424				
11-13	0.810							
12-13	-0.891							

Table 4. Wilks' Lambda and significant scores for *Oreochromis niloticus* specimens from the three treatments.

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1	0.064	349.758	16	0.000
2	0.502	87.952	7	0.000

Table 5. Percentage of specimens classified in each group and after cross validation for *Oreochromis niloticus* specimens from the three treatments.

		Treatment	Predicted Group Membership			Total
			22 ºC	28 ºC	34 ºC	
Original	Count	22 ºC	30	0	0	30
		28 ºC	0	46	5	51
		34 ºC	0	7	46	53
	%	22 ºC	100.0	0	0	100.0
		28 ºC	0	90.2	9.8	100.0
		34 ºC	0	13.2	86.8	100.0
Cross-validated	Count	22 ºC	30	0	0	30
		28 ºC	0	43	8	51
		34 ºC	0	8	45	53
	%	22 ºC	100.0	0	0	100.0
		28 ºC	0	84.3	15.7	100.0
		34 ºC	0	15.1	84.9	100.0

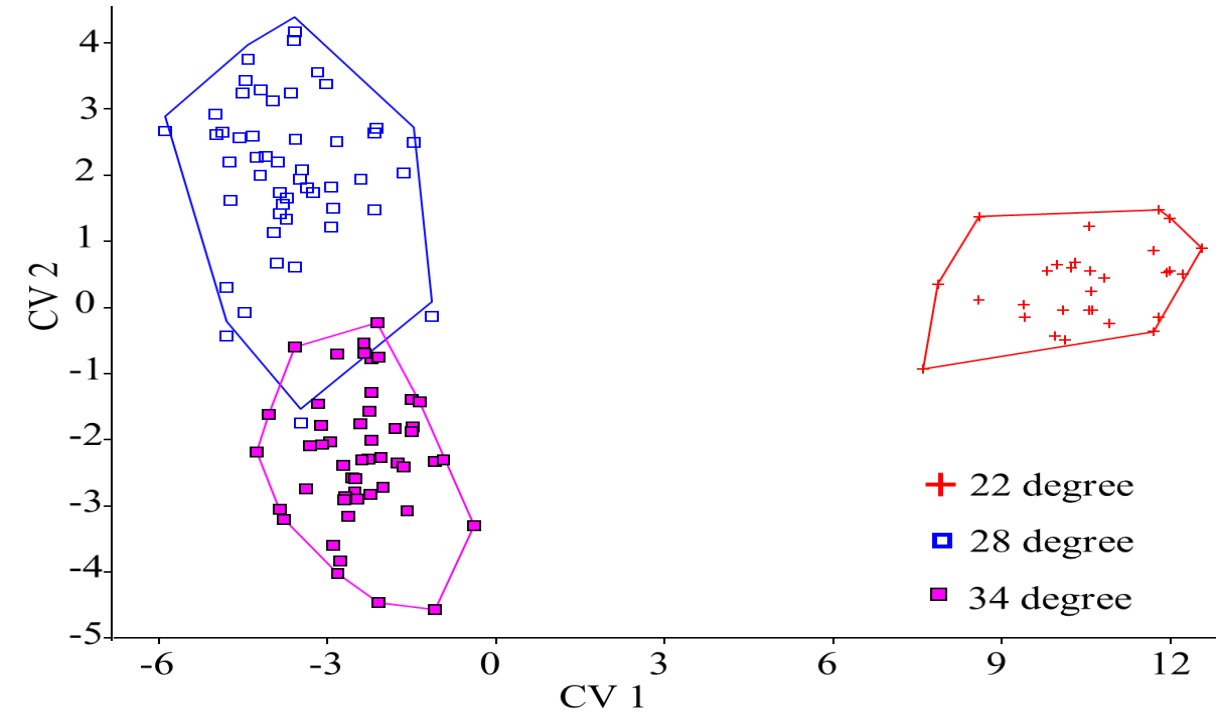

Fig. 3. Results of canonical variable analysis (CVA) showing the relative position of *Oreochromis niloticus* specimens from the three treatments.

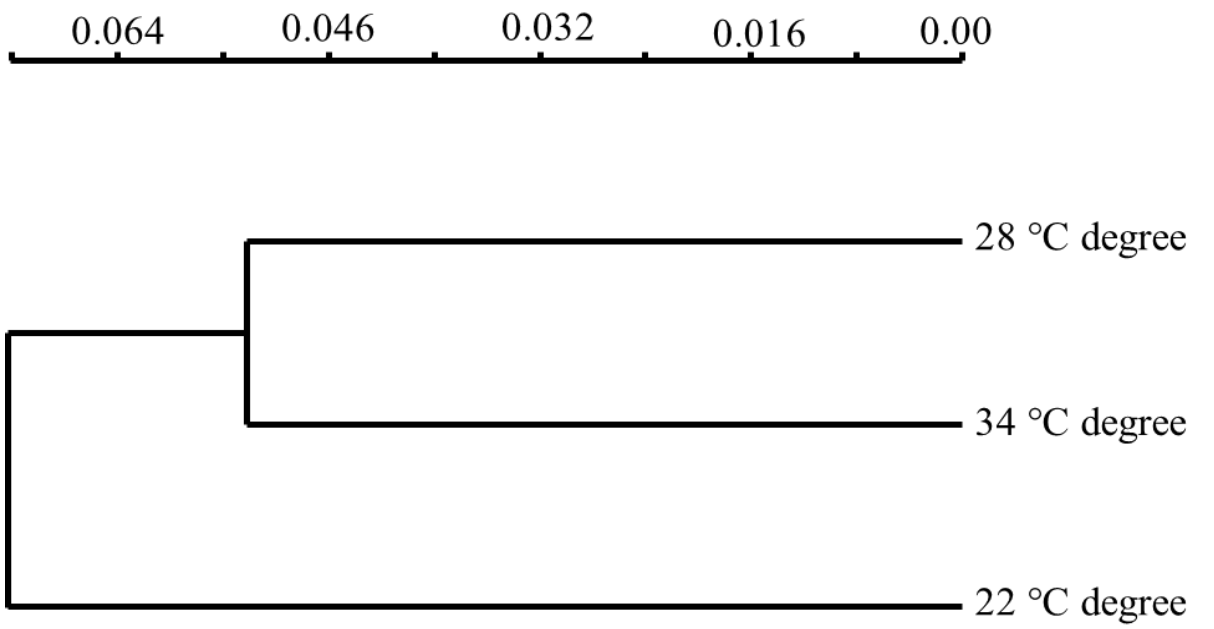

Fig. 4. Dendrogram derived from cluster analyses on the basis of Euclidean distance for *Oreochromis niloticus* specimens from the three treatments.

DISCUSSION AND CONCLUSION

The environmental factors that fishes experience has a significant influence on their phenotype throughout the entire life cycle. The effects of water temperature on morphology (meristic and morphometric characters) during embryonic and larval stages have been studied in some fish species including European sea bass; *Dicentrarchus labrax* (Georgakopoulou *et al*. 2007), zebrafish, *Danio rerio* (Sfakianakis *et al*. 2011), swordtail, *Xiphophorus hellerii* (Eagderi *et al*. 2015) and Angel fish, *Pterophyllum scalare* (Nasrolah-Pourmoghadam & Eagderi 2013). In this study, the effects of water temperature on the phenotype throughout early life stages in larvae and juveniles

of Nile tilapia were observed and results indicate that the water temperature during the larval period significantly affects the body shape of the fish (71 distances of 78 distances). Guill *et al*. (2003) suggested that the phenotypic characters of fish reflect the genetic characteristics of the population as well as the characteristics of the environment that fishes experienced in the entire life history, especially in early stages of their life. Eagderi *et al*. (2015) examined the effects of two different water temperatures (17 and 26 °C) on body shape in early developmental stages of *X. hellerii* and showed that water temperature is an important environmental parameter impacting the body shape. Their study showed that the specimen experienced higher temperature had shorter tail length, deeper body depth, and deeper head at the level of the operculum and caudal peduncle, so they had significant difference in body shape between the two treatments. Our results showed that body shape of *O. niloticus* larvae in three temperature treatments were significantly differed, especially in 22 ºC. The colder water can lead to creation of a slim body that could be a response to low temperature effects on physiological rates of the individuals due to changes in muscle and bone growth patterns (Wimberger 1992) and changes in kinematic viscosity of the water in which fish move in (Fuiman & Batty 1997; Johnson *et al*. 1998; Hunt von Herbing 2002). So, it is possible that different habitat conditions in which fish live and swim, require different manoeuvrability responses that can lead to the distinctive morphological variance, during the developmental period (Sfakianakis *et al*. 2011; Wimberger 1992). In addition, Campinho *et al*. (2004) showed that the temperature caused changes in the appearance patterns of the cartilaginous and bony structures of the *O. mossambicus*, which means that the epigenetic mechanisms favor sequence heterochrony. They also reported that the development timing of those skeletal structures that are functionally important for swimming, breathing and feeding like the centra, the cleithrum, the branchial arches and Meckel's cartilage was well conserved at all temperatures, so they suggested that these skeletal structures can be appropriate index of teleost developmental stages. Morphological plasticity can be an explanation to the variance in fish body shape that fish present in different habitat, and this is caused by temperature effects on the number and the diameter of 'fast' and 'slow' muscle fibers (Johnston 2006; Koumoundouros *et al*. 2009) and muscle development with possible further impacts on external morphology (Sfakianakis *et al*. 2011). In the goldfish *Carassius auratus* (L.) low culture temperatures also have a detrimental effect on viability and cause a range of developmental abnormalities (Wiegand *et al*. 1989). In *Perca fluviatilis*, body depth and caudal peduncle size were the most pronounced differences in different habitat temperature, so low temperatures of water in a habitat causes deeper bodies than higher temperatures in this kind of fish (Rowiński *et al*. 2015). The most important mechanism for responding to environmental conditions is the flexibility in the body shape. The results of the study show that temperature plays an important role during the early stages of the development of Nile tilapia larvae and by making changes in body shape, and maybe, it will provide biological requirements to increase survival rates and reduce the negative effects of related pressures.

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