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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

ZOOPLANKTON OF KILAVUZLU DAM LAKE (KAHRAMANMARAŞ) AND THE EFFECT OF CAGE FISH FARMING ON WATER QUALITY AND ZOOPLANKTON FAUNA OF THE DAM LAKE

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Abstract:

Water quality parameters and zooplankton fauna of Kılavuzlu Dam Lake were determined. It was found that among water quality parameters, sechi depth, temperature, silica, Ca and $CaCO_3$ amounts were higher in the first (referans) station; while conductivity, dissolved oxygen, pH, chlorophyll *a*, nitrate and phosphate values were higher in second (cage) station.

A total of 57 taxa were identified in the study. Of these taxa, 33 belonged to Rotifera, 14 belonged to Cladocera and 10 belonged to Copepoda. It was found that *Keratella cochlearis*, *Daphnia longispina*, *Cyclops vicinus*, *Acanthodiaptomus denticornis* were the most common throughout the year, while *Ascomorpha ovalis*, *Dicranophorus epicharis*, *Keratella tecta*, *Notholca acuminata*, *Testudinella patina*, *T. mucronata*, *Trichotria pocillum*, *Disparalona rostrata*, *Scapholeberis kingi*, *Leydigia leydigi*, *Alona guttata*, *Eucyclops speratus*, *Paracyclops chiltoni* were the least species.

Monommata longiseta, *Trichocerca porcellus*, *Diaphanasoma birgei*, *Eurycercus lamellatus* were only found in the first station, while *Ascomorpha ovalis*, *Notholca acuminata*, *Rotaria neptunia*, *Trichotria pocillum*, *Disparalona rostrata* and *Eucyclops speratus* were only found in the second station.

Rotifera was represented with higher number of species in first station for 5 months but Copepoda was represented with higher number of species in second station for 5 months.

The abundance of groups according to months and stations revealed that Rotifera and Copepoda were abundant quantitatively in first station for 7 months; while Cladocera was abundant in first station for 8 months. On the other hand, amount of all zooplankton species were found to be more abundant in cage station in April (6605 ±4597.35 individual m^{-3}) and more abundant in first station in September (1635 ±2384.852 individual m^{-3}) ($P > 0.05$).

Key words: Zooplankton, Water quality, Fish culture, Kılavuzlu Dam

Introduction

Having rich water resources, Turkey has more than 1.5 million hectares of internal water surface area. Freshwater, salty water and brackish water resources make up of 60%, 20% and 20% of this area respectively, which mostly include stagnant water such as lakes, ponds and dam lakes. State Hydraulic Works (SHW) allowed the use of cages for fish farming in dam lakes in 2000s and increased the interest towards this sector. Several cage trout farming has been established in many dam lakes in Turkey. Discharge of organic waste (feed residue, metabolic wastes etc.) to water environment from these cages might cause pollution especially when the current is slow. Primary pollutants that are discharged to the water environment are solid materials, nitrogen and phosphor. Much of solid materials caused by feces and unconsumed feed accumulate in sediments around the farm. Although they have polluting effects in production area, their effects are not exactly known.

While the majority of aquatic organisms feed on zooplanktons throughout their lives, some of them feed on zooplankton in a certain period of their lives especially at larval stage. In this respect, there is a close relationship between the efficiency of aquatic environment and diversity and abundance of organisms. Rotifera, Cladocera and Copepoda have a character of renewal in a short time due to their short reproduction period and rapid population growth. Therefore, in addition to having a significant effect on the growth, survival and distribution of fish larvae, these species are the primary biotic factors of freshwater environments and are of great importance for freshwater ecosystem.

As the majority of zooplantonic organisms (Copepoda, Cladocera and Rotifera) filter feeding, they transform the phytoplankton to animal protein (Cirik and Gökpınar, 1993), they play a significant role in food chain. It was reported that some species are the indicators of water quality, pollution and eutrophication due to their sensitivity to environmental changes and therefore zooplankton studies on lakes have acquired significant importance (Berzins and Pejler, 1987; Mikschi, 1989; Güher and Kırgız, 1992).

Although the abundance of zooplanktonic organisms is important especially in terms of feeding of fry, this abundance is an indicator also for water quality, eutrophication and pollution levels. Abundance and composition of zooplankton are closely related with water quality parameters and increase or decrease depending on trophic levels of lakes (Canfield and Jones, 1996).

In Kılavuzlu Dam Lake, approximately 300 tons of trout are produced in cages annually. This study examines water quality and zooplankton fauna of the dam lake and analyzes the effects of fish farming on these parameters. Our findings will provide data for future studies and contribute to the follow-up of water quality and zooplanktons.

Materials and Methods

The study was carried out between March 2011 and February 2012 period in Kılavuzlu Dam Lake on Ceyhan River within the boundaries of Kahramanmaraş province. Kılavuzlu Dam Lake, constructed for irrigation and electric production in 1996-2001 period, is located at a distance of 8 km to Kahramanmaraş. It has a surface area of approximately 3.10 km^2 and a lake volume of 69.00 hm^3 at normal water level. The dam lake is at an altitude of 59.00 m from riverbed and 429 m from sea level.

The samples consisted of zooplankton and water was collected from 2 stations on monthly basis, from three depths (surface, middle and deep sections) of each station for two times. The first station (reference station) was located at the upper part of the fish farm that was not exposed to the effects of fish farming enterprise. The second station was located at the lower section of the cages (cage station) (Figure 1).

Physicochemical characteristics of the dam lake, dissolved oxygen, temperature, pH, sechi dept, chlorophyll *a*, conductivity, NO_2-N, NO_3-N, PO_4-P, silica, hardness, Ca and $CaCO_3$ were determined.

Zooplankton samples were taken from the stations with horizontal and vertical hauls by using 60 µm mesh size plankton nets on monthly basis. 5 lt of water samples were collected from every water layer (surface, middle and deep) of each station using Nansen Bottles. Plankton species were identified from the samples collected with plankton net. Zooplankton abundance, water quality parameters and chlorophyll *a* were identified from water samples.

Figure 1. Kılavuzlu Dam Lake and sampling stations

One lt of the water collected with water sampler was used for chlorophyll *a* analysis. The remaining part was filtered from a collector having a mesh size of 60 µm and zooplankton was fixed in 100 cc glass jars. Oxygen, temperature, pH and conductivity were measured directly at the field by means of digital instruments (oxygen and temperature: YSI model 52 oxygen meter; pH: YSI 600 pH meter; conductivity: YSI model 30 salinometer). YSI 9500 photometer was used to determine NO_2-N, NO_3-N, NH_4-N, PO_4-P, silica, hardness, Ca, $CaCO_3$; the method in APHA 1995 was used to determine chlorophyll *a* spectrophotometrically. Sechi depth was measured using a Secchi disk with a diameter of 20 cm.

All zooplankton samples were fixed in 4% formaldehyde. Species identifications were made using a binocular microscope according to the Works of Edmondson (1959), Dussart (1969), Kiefer (1978), Stemberger (1979), Negrea (1983), Segers (1995), De Smet (1996, 1997) and Nogrady and Segers (2002). Zooplankton count was performed using an invert microscope in a petri dish with 2 mm lines at the bottom. Filtered zooplankton was placed in a petri dish and the individuals of each species were separately counted. SPSS package software was used for statistical analyses (t test).

Results and Discussion

Narrow and long structure of Kılavuzlu Dam Lake and high water flow of Ceyhan River causes fast water flow and a high level of mixture in the dam lake. Therefore, it was found that there was no significant difference between zooplankton and water quality parameters at different depths.

The variation of water quality parameters according to stations, depth and months is presented in Figure 2. It is understood from the figure that chlorophyll *a* values decreased with depth; NO_2-N, NO_3-N and PO_4-P were high in deep sections of second station; SiO_2, Ca, $CaCO_3$, conductivity and hardness were similar at all stations and depths.

Sechi depth reached the maximum concentration of 6.8 m at first station (April) and minimum concentration of 2.2 m at second station (September), with a mean value of 4.12 ±1.03 m. Temperature varied from 9.40°C (March at second station) to 14.65°C (August at second station) with a mean value of 12.62 ±1.44°C. Mean chlorophyll *a* concentration was 8.86 ±2.81 mgL^- with a range from 4.25 mgL^- (at first station) in January to 16.13 mgL^- in June. The conductivity value varied from 247.43 µs (March at second station) to 549.13 µs (October at first station) with a mean value of 401.91 ±99.07 µs. Dissolved oxygen varied from 4.15 mgl^- (at first station) in August to a peak of 9.71 mgL^- (second station) in May with a mean value of 7.05 ±1.68 mgL^-. pH value did not vary much among the sitations. The minimum, maximum and mean pH values were 7.48 (January at second station), 8.38 (May at first station) and 7.96 ±0.22 respectively. Nitrite nitrogen reached the maximum concentration of 0.044 mgL^{-1} (June at second station) and minimum concentration of 0.002 mgl^- (July at second station), with a mean value of 0.032 mgL^{-1}. Nitrate nitrogen (1.64 ±0.46 mgL^-) varied from 0.767 mgL^- (March at first station) to 2.8 mgl^- (January at second station), and phosphate (0.93 ±0.69 mgL^-) varied from 0.127 mgL^- (October at first station) to 2.034 mgL^- (January at second station). The maximum, minimum, and mean Silica values were 4.623 mgL^- (August at first station), 0.046 mgL^- (May at second station), and 2.85 ±1.55 mgL^-, respectively. Mean $CaCO_3$ hardness was 239.84 ±108.98 with a range from 135 (at first station) in February to 460 in March (at first station). Ca (83.08 ±40.67 mgL^-) varied from 31.67 mgL^- (January at second station) to 186.67 mgL^- (March at first station), and $CaCO_3$ (78.17 ±17.5 mgL^-) varied from 51 mgL^- (May at

first station) to 122.7 mgL^{-} (February at second station) (Figure 2).

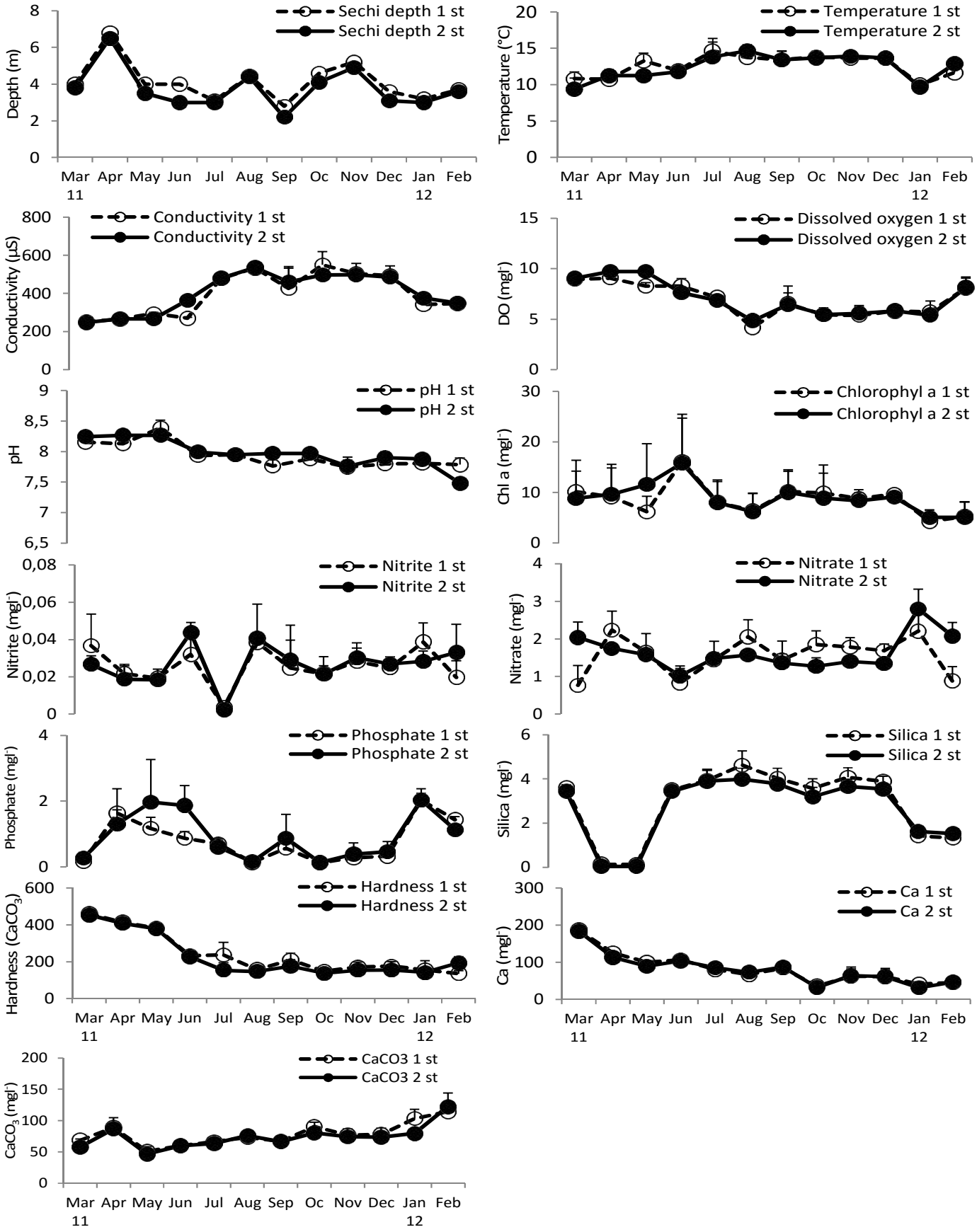

Figure 2. Monthly variations of water quality parameters at stations in the dam lake

Average annual water quality parameters at the stations were determined to display a similar distribution with each other and there was no statistically significant. It was found that sechi depth, temperature, silica, hardness, Ca and $CaCO_3$ amounts were higher at reference station; while conductivity, dissolved oxygen, pH, chlorophyll *a*, nitrate nitrogen and phosphate values were higher at cage station (Figure 3).

The zooplankton fauna of Kılavuzlu Dam Lake consists mainly of rotifers, cladocerans and copepods. A total of 57 taxa composed of 33 rotifers, 14 cladocerans and 10 copepods were identified (Table 1).

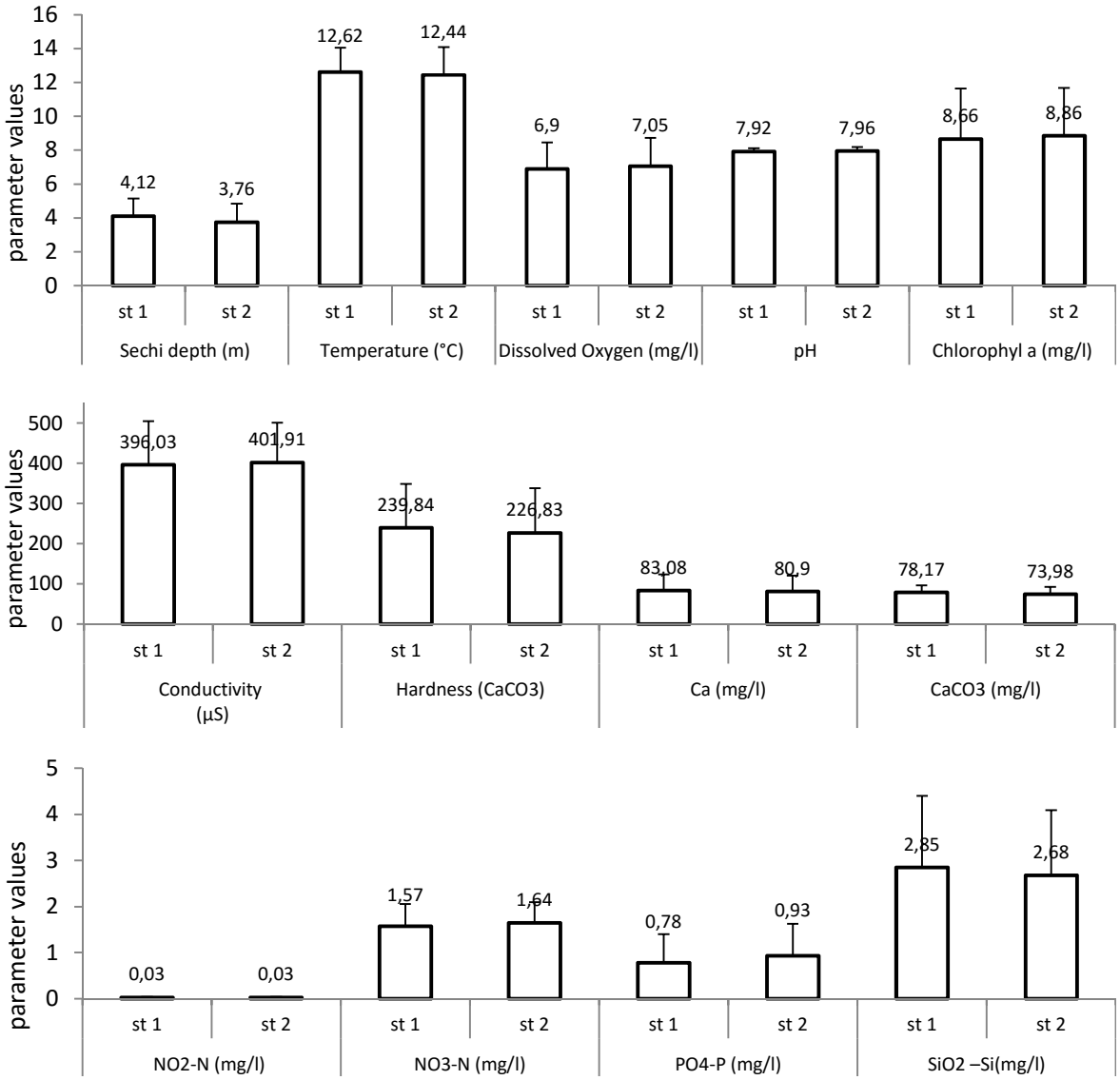

Figure 3. Change of water quality parameters at the stations

Table 1. Zooplankton of Kılavuzlu Dam Lake and monthly presence of the species

Rotifera	**M 11**		**A**		**M**		**J**		**J**		**A**		**S**		**O**		**N**		**D**		**J12**		**F**	
Stations	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Asplanchna priodonta Gosse 1850	+	+						+	+		+		+	+					+	+	+	+	+	+
Ascomorpha ovalis (Bergendal, 1892)														+										
Anuraeopsis fissa Gosse, 1851															+	+	+	+						
Cephalodella gibba (Ehrenberg, 1830)	+	+	+	+	+	+	+		+															
Collotheca pelagica (Rousselet, 1893)																			+		+			
Colurella adriatica Ehrenberg 1831					+	+	+		+							+		+						
Dicranophorus epicharis Harring and Myers, 1928																			+		+			
Euchlanis sp					+	+	+		+															
Filinia terminalis (Plate, 1886)			+	+																		+		
Kellicottia longispina (Kellicott, 1879)			+	+	+	+	+	+	+	+														
Keratella cochlearis (Gosse, 1851)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratella tecta (Gosse, 1851)															+									
Keratella quadrata (Muller, 1786)			+	+																			+	+
Lecane luna (Muller 1776)						+																	+	+
Lecane lunaris (Ehrenberg, 1832)			+	+				+		+					+	+	+	+						
Lepadella rhomboides (Gosse 1886)			+	+		+										+		+						
Lophocharis salpina (Ehrenberg, 1834)																+		+	+		+			
Monommata longiseta (Muller, 1786)											+								+		+			
Notholca squamula (Muller, 1786)		+	+	+	+	+																		
Notholca acuminata (Ehrenberg 1832)						+								+										
Polyarthra vulgaris Carlin, 1943	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pompholyx sulcata Hudson, 1885						+									+	+	+	+						
Rotaria neptunia (Ehrenberg, 1830)						+		+		+														
Rotaria sp					+	+								+										
Synchaeta stylata Wierzejski 1893	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testudinella mucronata (Gosse 1886)																		+						
Testudinella patina (Hermann, 1783)																							+	+
Trichocerca capucina (Wierzejski & Zacharias 1893)						+						+		+	+	+	+	+						
Trichocerca longiseta (Schrank 1802)					+		+	+	+	+														
Trichocerca porcellus (Gosse, 1851)	+																		+		+			
Trichocerca similis (Wierzejski, 1893)					+	+					+		+	+	+	+	+	+	+	+	+	+		
Trichocerca tenuior (Gosse, 1886)	+		+	+		+										+		+	+		+		+	+
Trichotria pocillum (Muller, 1776)																						+		
Species number of rotifers	**7**	**6**	**10**	**10**	**10**	**16**	**8**	**8**	**9**	**7**	**6**	**4**	**5**	**9**	**9**	**12**	**8**	**13**	**11**	**5**	**11**	**7**	**8**	**8**
Cladocera																								
Bosmina longirostris (Müller, 1785)	+	+	+	+	+	+	+	+	+	+		+			+	+	+	+	+		+		+	+
Ceriodaphnia pulchella Sars, 1862	+	+			+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
Daphnia longispina (Mueller, 1875)	+	+	+	+		+	+		+			+	+	+	+	+	+	+	+	+	+	+	+	+
Diaphanosoma birgei Korinek, 1981													+						+		+			
Disparalona rostrata (Koch, 1841)		+																						
Eurycercus lamellatus (Mueller, 1785)	+																		+		+			
Macrothrix laticornis (Fischer,1851)			+	+	+	+		+		+		+											+	+
Scapholeberis kingi Sars, 1903												+												
Simocephalus vetulus (Müller, 1776)						+	+	+	+	+		+	+						+	+	+	+		
Alona guttata Sars, 1862						+																		
Alona quadrangularis (Müller, 1785)	+				+	+	+	+	+	+	+									+		+		
Alona rectangula Sars, 1862			+	+	+			+		+			+	+					+	+	+	+		
Chydorus sphaericus (Muller 1776)							+	+	+	+					+	+	+	+					+	+
Leydigia leydigi (Schoedler, 1863)																					+			+
Species number of cladoceran	**5**	**4**	**4**	**4**	**5**	**7**	**6**	**7**	**6**	**7**	**1**	**6**	**5**	**3**	**4**	**4**	**4**	**4**	**7**	**5**	**8**	**5**	**5**	**5**
Copepoda																								
Cyclops vicinus Uljanin, 1875	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
Diacyclops bicuspidatus (Claus, 1857)		+					+	+	+	+						+		+	+	+	+	+		
Eucyclops speratus (Lilljeborg, 1901)						+						+												
Macrocyclops albidus (Jurine, 1820)												+	+	+		+		+	+	+	+	+		
Megacyclops latipes (Lowndes, 1927)	+													+		+		+					+	+
Microcyclops rubellus (Lilljeborg,1901)	+	+			+										+	+	+	+	+	+	+	+	+	+
Thermocyclops dybowskii (Lande, 1890)	+		+	+	+	+					+	+	+	+	+	+	+	+					+	+
Paracyclops chiltoni (Thomson, 1882)	+																							
Acanthodiaptomus denticornis (Wierzejski, 1887)	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitocra hibernica (Brady, 1880)	+		+	+	+			+		+		+			+			+						
Species number of copepods	**7**	**4**	**4**	**4**	**5**	**4**	**2**	**4**	**3**	**4**	**3**	**6**	**4**	**4**	**5**	**7**	**4**	**8**	**5**	**5**	**5**	**5**	**5**	**5**
Species number of zooplankton	**19**	**14**	**18**	**18**	**20**	**27**	**16**	**19**	**18**	**18**	**10**	**16**	**14**	**16**	**18**	**23**	**16**	**24**	**23**	**15**	**24**	**17**	**18**	**18**

Keratella cochlearis (Gosse, 1851), *Daphnia longispina* (Muller, 1875), *Cyclops vicinus* Ulyanin, 1875, *Acanthodiaptomus denticornis* (Wierzejski, 1887) were found in the lake throughout the year, followed by *Polyarthra vulgaris* Carlin, 1943, *Synchaeta stylata* Wierzejski 1893, *Bosmina longirostris* (Müller, 1776), *Ceriodaphnia pulchella* Sars, 1862 and *Thermocyclops dybowskii* (Lande, 1890). The least common species were *Ascomorpha ovalis* (Bergendal, 1892), *Dicranophorus epicharis* Harring and Myers, 1928, *Keratella tecta* (Gosse, 1851), *Notholca acuminata* (Ehrenberg 1832), *Testudinella patina* (Hermann, 1783), *T. mucronata* (Gosse 1886), *Trichotria pocillum* (Muller, 1776), *Disparalona rostrata* (Koch, 1841), *Scapholeberis kingi* (Sars, 1903), *Leydigia leydigi* (Schoedler, 1863), *Alona guttata* Sars, 1862, *Eucyclops speratus* (Lilljeborg, 1901), *Paracyclops chiltoni* (Thomson, 1883). The species that found only in one month were not included in the assessment as they are not adequately represented in the lake. It was determined that *Monommata longiseta* (Muller, 1786) (August, September, December, January), *Trichocerca porcellus* (Gosse, 1851) (December, January, March), *Diaphanasoma birgei* Korinek, 1981 (August, September, December, January), *Eurycercus lamellatus* (Mueller, 1785) (December, January, March) were found only in first station; while *Ascomorpha ovalis* (August and September), *Notholca acuminata* (May, September), *Rotaria neptunia* (Ehrenberg, 1832) (May, June, July); *Trichotria pocillum* (January, February), *Disparalona rostrata* (February, March), *Eucyclops speratus* (May, August) were found only in second station.

Comparison of number of species between the stations revealed that a higher number of Rotifera species were found in the first station for 5 months; a higher number of Rotifera species were found in the second station for 4 months and number of Rotifera species was equal in both stations for 3 months. Number of Cladocera species was found to be higher in first station for 4 months; higher in second station for 4 months and equal in both stations for 4 months. Number of Copepoda species was found to be higher in first station for 2 months; higher in second station for 5 months and equal in both stations for 5 months. Number of all zooplanktons species was found to be higher in first station for 3 months; higher in second station for 6 months and equal in both stations for 3 months (Figure 4).

Average values of all zooplankton individuals in Kılavuzlu Dam Lake according to months and stations are presented in Table 2.

An analysis of the abundance of groups according to months and stations showed that individuals of groups were more abundant in first station when compared to second station (Rotifera and Copepoda 7 months; Cladocera 8 months) (Table 2, Figure 5).

Stations and months that were found to be statistically significant are presented in Table 2 and Figure 5. It was found that Rotifera was more abundant in April, September and October in first station (6931 ±7237.71 individual m^{-3}, 2933 ±3128.89 individual m^{-3}, 2490 ±2891.39 individual m^{-3} respectively) and more abundant in July and August in second station (3490 ±6677.25 individual m^{-3}, 8629 ±3537.92 individual m^{-3} respectively). Cladocera was more abundant in August in first station (2948 ±2731.21 individual m^{-3}). Copepoda was more abundant in August and September in first station (2866 ±3381.27 individual m^{-3}, 1207 ±1360.00 individual m^{-3} respectively), and more abundant in March, May, June and July in second station (1093 ±1165.98 individual m^{-3}, 3682 ±3899.15 individual m^{-3}, 4677 ±5951.79 individual m^{-3}, 5539 ±8327.86 individual m^{-3} respectively). All zooplankton was found to be more abundant in April in second station (6605 ±4597.35 individual m^{-3}) and more abundant and statistically significant in September in first station (1635 ±2384.852 individual m^{-3}) ($P > 0.05$).

Negative effects of cage fish farming have been analyzed by various researchers and it was reported that nitrogen, phosphor and organic material load in sediment were significantly affected by these negative changes. Researches have shown that negative effects vary according to enterprise capacity, currents, change ratio and total volume of water and the technology used in fish farming (Phillips et al., 1985; Stirling and Dey, 1990; Pitta et al., 1999). The most common effects were reported to be decreased dissolved oxygen, pH values and sechi depth, and increase of suspended solid matter, nutrient, electrical conductivity and chlorophyll *a* (Rast and Holland, 1988; Weglenska et al. 1987; Beveridge 1984, Phillips et al., 1985). However, Cornel and Whoriskey (1993) reported that pH did not vary in cage and reference stations and that the enterprise did not affect pH value. In another study carried out in a rainbow trout farming enterprise,

it was found that pH and dissolved oxygen amounts did not significantly vary between the stations, while nutrient elements (N, P) (excluding nitrite nitrogen) were found to be higher in cage stations similar to the findings above (Demir et al., 2001). Similarly, other researchers reported that there was no difference between the enterprise and reference stations in terms of nitrite nitrogen and nitrate nitrogen (Stirling and Dey, 1990). Interestingly, Cornel and Whoriskey (1993) reported that in enterprises that make production below their capacity, N and P levels can be the same in the enterprise and reference stations.

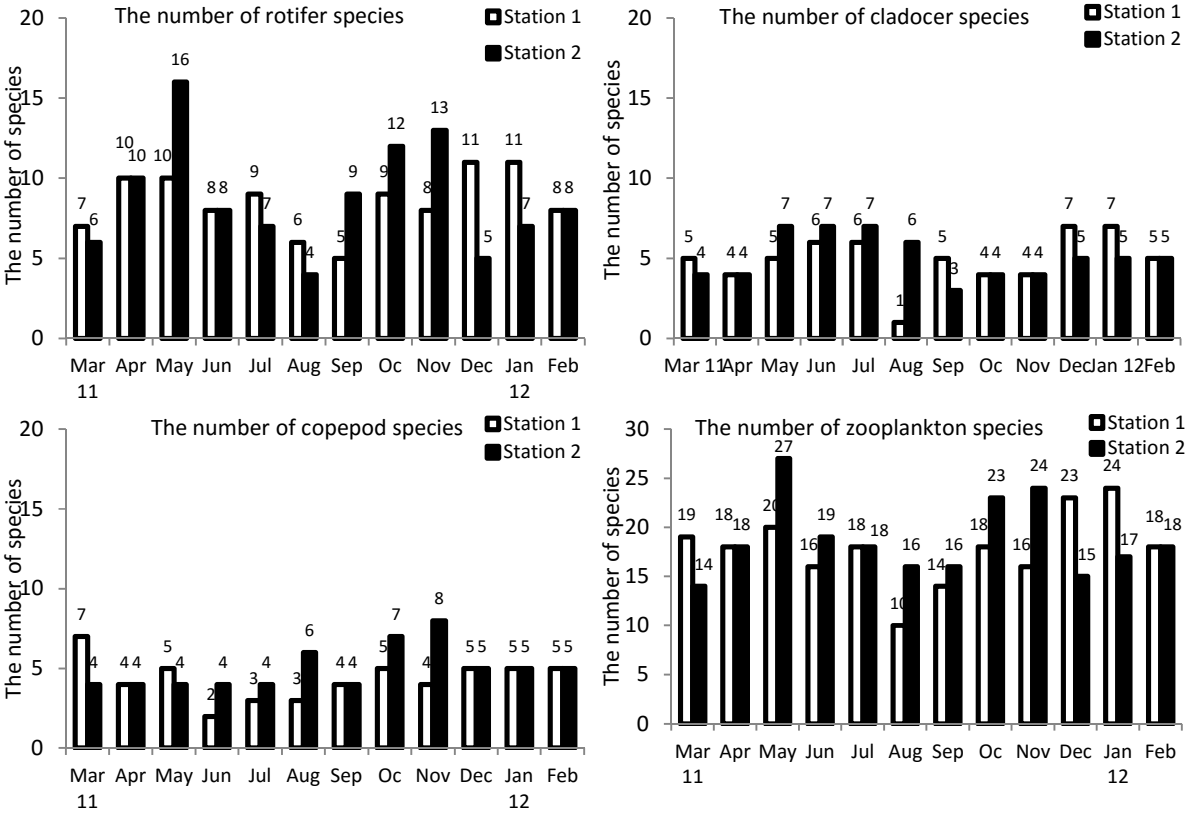

Figure 4. Monthly number of species at stations

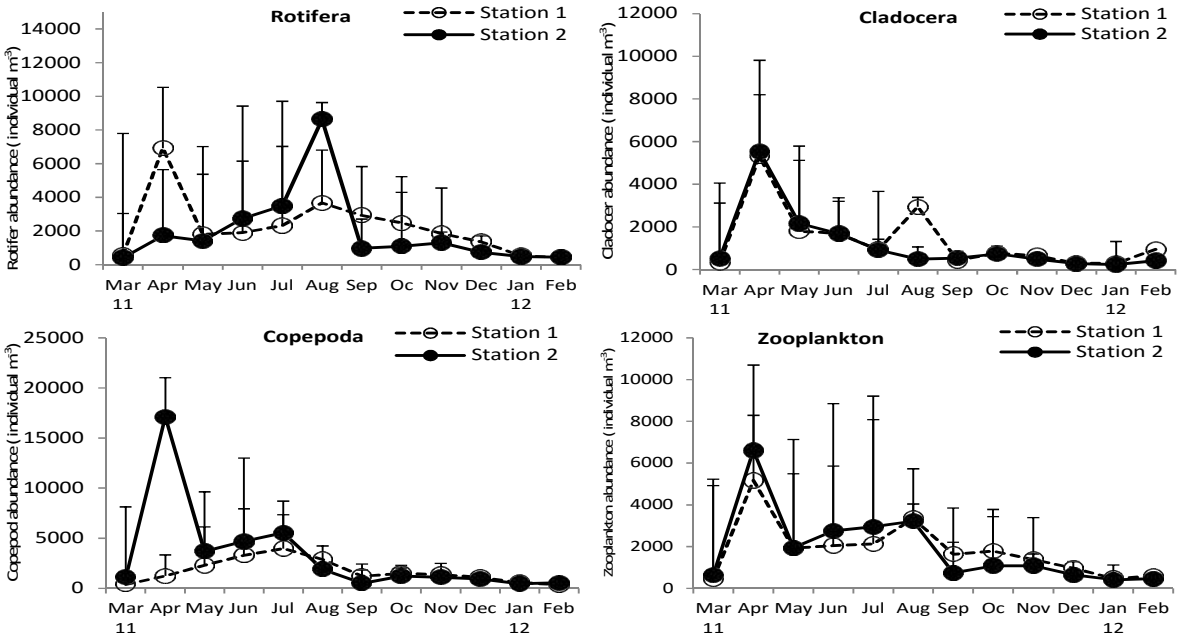

Figure 5. Monthly variation of zooplankton at the stations

Table 2. Monthly variation of the average zooplankton in stations

		Average zooplankton (individual m^{-3}), SD			
Months	**St**	**rotifer**	**cladocer**	**copepod**	**zooplankton**
Mar	1	562±269.17	335±192.53	390±152.11	448±239.7763
2011	2	414±158.50	523±532.70	**1093±1165.98***	633±741.2495
	1	**6931±7237.71***	5308±2782.13	1233±1010.97	5168±4465.77
Apr	2	1729±2622.13	5543±3533.66	17114±7050.50	**6605±4597.35***
	1	1802±3606.66	1799±2886.44	2299±2083.94	1925±3117.547
May	2	1418±3919.70	2161±4266.99	**3682±3899.15***	1946±4086.705
	1	1902±3569.12	1699±3322.31	3281±3843.58	2045±3567.666
Jun	2	2737±5593.96	1683±3624.72	**4677±5951.79***	2756±5187.113
	1	2312±4257.90	931±1496.70	3973±4666.54	2128±3808.27
Jul	2	**3490±6677.25***	915±1674.41	**5539±8327.86***	2944±6087.178
	1	3675±7393.12	**2948±2731.21***	**2866±3381.27***	3346±5952.038
Aug	2	**8629±3537.92***	495±501.42	1880±3155.77	3218±6270.778
	1	**2933±3128.89***	419±434.31	**1207±1360.00***	**1635±2384.852***
Sep	2	970±1006.22	538±565.58	502±473.66	737±824.9328
	1	**2490±2891.39***	769±314.98	1462±1212.27	1783±2213.002
Oc	2	1103±1737.93	732±309.42	1225±1324.37	1076±1467.661
	1	1848±2740.98	651±325.92	1375±814.68	1385±1995.567
Nov	2	1304±3197.90	490±247.75	1099±788.93	1079±2360.522
	1	1357±2709.73	288±84.35	1095±1130.08	975±2002.067
Dec	2	739±630.66	276±56.77	924±560.72	646±559.3482
Jan	1	542±358.73	292±88.12	572±257.78	472±304.4458
2012	2	479±219.92	238±38.00	462±225.41	411±217.7832
	1	442±225.02	951±1027.26	375±173.21	583±644.8608
Feb	2	453±172.85	417±165.06	537±112.14	467±162.3528

*bold numbers: statistically significant

Our findings are consistent with the literature. It was found that, among water quality parameters, sechi depth (4.12 ±1.03 m) was higher in reference first station (1); conductivity (401.91 ±99.07 µS), pH (7.96 ±0.22), chlorophyll *a* (8.86 ±2.81 mgl^{-}), nitrate (1.64 ± 0.46 mgl^{-}) and phosphate (0.93 ±0.69 mgl^{-}) values were higher in cage station. Similar to the literature, there was no difference between the stations in terms of nitrite nitrogen. While dissolved oxygen did not vary between the stations in our study, it was slightly higher in cage station. We believe that this can result from large water surface areas and the mixture in Dam Lake. Furthermore, silica (2.85 ±1.55 mgL^{-}), hardness (239.84 ±108.98), Ca (83.08 ±40.67 mgL^{-}) and $CaCO_3$ (78.17 ±17.5 mgL^{-}) amounts were found to be higher in first station. A review of the literature found no study on the impact of fish farming enterprises on these parameters.

Some of previous researchers reported that primary productivity increase in cage station due to the nutrients coming from feed and metabolism wastes and this increased the abundance of zooplanktonic organisms (Demir et al., 2001; Guo and Li, 2003; Köksal et al., 1997; Atay and Demir, 1998; Kirkagac and Köksal, 1999). Matsumura-Tundisi & Tundisi (2003, 2005) reported that zooplankton diversity and abundance, namely the zooplankton structure, changed in cage stations due to increased nutrients, chlorophyll-a, phytoplankton, conductivity, bacteria and other factors. In a study carried out in a tilapia farming enterprise, Santos et al., (2009) reported only small changes in zooplankton levels. Guo and Li (2003) reported that Rotifera was found in small amounts in cage station, however it was more abundant in the station that was outside of the cage; Cladocera was more abundant in the cage station and less abundant in the other station and finally Copepoda abundance was the same in the both stations.

In this study we found that Rotifera and Cladocera were more abundant first station (reference station) for 8 months, while Copepoda species were more abundant in reference station for 6

months. Similarly, total amount of Rotifera, Cladocera and Copepoda was more abundant in first station for 7 months. In parallel to Guo and Li (2003) it can be stated that fish farming enterprises have a suppressor effect on zooplankton abundance.

A total of 57 taxa consisting of 33 Rotifera, 14 Cladocera and 10 Copeoda species were identified in Kılavuzlu Dam Lake, on which no research was carried out in terms of zooplankton and water quality. A review of previous studies in Turkey revealed that 42 taxa were determined in Yenişehir Lake (Bozkurt, 2006); 38 taxa were identified in Yarseli Dam Lake (Bozkurt et al., 2004); 39 taxa were identified in Birecik Dam Lake (Bozkurt and Sagat, 2008); 17 were identified in Burdur Lake (Altındağ and Yiğit, 2002); 41 taxa were identified in Marmara Lake (Yıldız et al., 2007) by various researchers. In this respect, Kılavuzlu Dam Lake is more diverse than other reservoirs in terms of diversity of zooplankton species.

We found that *Keratella cochlearis*, *Daphnia longispina*, *Cyclops vicinus*, *Acanthodiaptomus denticornis* (Wierzejski, 1887) were cosmopolite and widely-distributed species throughout the year (Hutchinson, 1967; Ruttner-Kolisko, 1974; Margalef et al., 1976; Braioni and Gelmini, 1983; Koste and Shiel, 1986, 1987; Ramdani et al., 2001), followed by *Polyarthra vulgaris*, *Synchaeta stylata*, *Bosmina longirostris*, *Ceriodaphnia pulchella*, *Thermocyclops dybowskii*. On the other hand, the least species were found *Ascomorpha ovalis*, *Dicranophorus epicharis*, *Keratella tecta*, *Notholca acuminata*, *Testudinella patina*, *T. mucronata*, *Trichotria pocillum*, *Disparalona rostrata*, *Scapholeberis kingi*, *Leydigia leydigi*, *Alona guttata*, *Eucyclops speratus* and *Paracyclops chiltoni*. These species are known to be widely-distributed (Ruttner-Kolisko, 1974; Margalef et al., 1976).

Benthic *Collatheca pelagica* (Rousselet, 1893), *Monommata longiseta*, cosmopolite cold-water *Trichocerca porcellus*, cosmopolite *Diaphanasoma birgei*, benthic *Eurycercus lamellatus* were only found in first station; cold-water, eutrophic and mostly litoral *Notholca squamula* (Muller, 1786), alkaline, eutrophic, cosmopolite *Rotaria neptunia*, benthic *Alona guttata* and cosmopolite *Eucyclops speratus* were only found in second station. Considering the general bioecological characteristics of these species, the presence of them in the reservoir is quite normal, but the situation in the station suggested that they may be related to the fish farm.

The most dominant genus of Rotifera that was found in all stations every month was genus *Keratella,* followed by genus *Polyathra*. As genus *Keratella* is a small form with a large tolerance to the conditions of media, it was reported among the most common zooplanktonic organisms in cage fish farming in previous research (Weglenska et al., 1987, Demir et al., 2001).

Conclusion

In a study which reported that cage trout farming accelerates eutrophication, number of *Keratella*, *Polyathra* and *Bosmina* genus that are found in highly eutrophic waters was observed to increase (Weglenska et al., 1987). In our study high number of individuals of this genus shows the effects of cage system enterprises on zooplanktons.

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FULL PAPER **TAM MAKALE**

COMPARISON OF SOME FISH SORTING TOOLS FOR GRADING *Clarias gariepinus* FINGERLINGS

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Abstract:

Over 200,000 of *Clarias gariepinus* two-week old hatched fry were sorted at different ages i.e 2, 3, 4, 5 and 6 weeks using nine differet grading tools, namely Dipnet (DN), grading cage (GC), hang grading net (HGN), meshed box (MB), plastic basin (PB), ply-wood box (PWB), sorting table (ST), woven basket (WB), and wood grading panel (WGP). The result from the study indicated that sorting table (ST), grades faster in all the age of the fry, having the highest number of graded fry. In all the ages, plastic basin (PB) consistently had the lowest. The highest mortality values were observed in sorting table (ST) in all the ages of fish sorted while the lowest values were recorded in grading cage (GC). The success of the grading cage (GC) was that it grades more efficiently through the bottom unlike other sorting tools which gradel through its sides. *Clarias gariepinus* fry between the age of 2 and 4 weeks is best graded using the grading cage for high survival rate and sorting table is best used with fish from the age of 4 weeks and above.

Key words: Aquaculture, Grading tools, Size diversity, Fingerlings, *Clarias gariepinus*

Introduction

Aquaculture in Nigeria has a history of at least four decades, with spectacular growth recorded in the last few years (AIFP, 2010; Akinrotimi e*t al.,* 2011a). According to Gabriel *et al.* (2007a) the full potential of aquaculture began to be realized recently when it became obvious that the ever-increasing demand for fish cannot be met from capture fisheries alone. Therefore, reducing the widening gap between fish demand and supply and achieving the ultimate goal of self-sufficiency in fish production is the major target of aquaculture as an enterprise (Ugwumba and Ugwumba, 2003).

The steadily growing importance of fish farming has compelled improvements in the technologies necessary for securing the initial and basic requirements for productive aquaculture; namely the production of fish seed for stocking (Akinrotimi *et al*., 2011b). Fish culture today is hardly possible without the artificial propagation of fish seeds of preferred cultivable fish species (Akinrotimi et al. 2013a). The need for the production of quality fish seed for stocking the fish ponds and natural water bodies has indeed increased steadily (Dada, 2006). Artificial propagation methods constitute the major practicable means of providing enough quality seed for rearing in confined fish enclosure waters such a fish ponds, reservoirs and lakes (Conceicao *et al*., 2005; Akinrotimi *et al*., 2011c). The production of marketable fish fingerlings or juveniles into rearing environment that assures optimum and rapid growth to allow harvest in the shortest possible time. The fish farmer has to obtain adequate number of young fish to meet "his" production goals. The possibilities of obtaining fish seed in adequate numbers from natural source is rather limited. Even the spawners which, reproduces successfully in confined enclosures are propagated artificially (Madu *et al*., 2004; Gabriel *et al*., 2007b). Apart from being able to obtain quality seed, the artificial propagation technique can also be used to develop strains superior to their ancestors by the methods of selective breeding and hybridization. Depending on the perfection of the system, at least 65% of the eggs produced can be raised to viable fingerlings as against less than 1% survival rate in natural spawning. It is through this method that out of season supplies of fingerlings are achieved (Dada *et al*., 2002).

For a fish production through aquaculture to meet this projected or potential demand for fish in the country there is the need to establish a pool of fingerlings annually (Ezenwa *et al.,* 1990; Dada and Wonah, 2003). According to Ayinla (1991), there are generally two sources of fingerlings: wild collection and hatchery production. It is obvious that the supply from the wild is unreliable; hence supply of sufficient fingerlings depends on hatchery production. Fish hatchery is the bedrock upon which true and sustainable fish farming is built (Nwadukwe and Ayinla, 1993; Ugwumba *et al.,* 1998; Anyanwu *et al*., 2007).

The major problem faced by the hatcheries operators' center on the technicalities of handling fish fry on farms (Zaki *et* al., 2004). Grading of fish fingerlings is one of the most common management practices in fish farming that a serious hatcheries operator need to know in order to be able to maintain almost the same quantity of fish from fry to fingerlings stage. For example, some hatchery operators do have up to 500,000 fry at early stage, but before getting to fingerlings stage a lot of them would have been lost as a result of cannibalism which is more prevalent among the clariids (Ayinla and Nwadukwe, 2003). Hence the need for them to be sorted (graded). Sorting of fish according to FAO (2000), involves separating a mixed group of fish into different species, sexes, and sizes.

Significant size diversity within fish species requires that they be sorted. The main goal of this technique is to obtain maximum weight gain by all individuals and to increase their survival rate which result in obtaining the maximum biomass (Baarduik and Jobling 1990; Kamstra 1993, Sunde *et al.,* 1998). Sorting separates small and big fish fingerlings, thus minimizing the effect of inter individual interaction (Jobling, 1995). However, frequent sorting of the species sometimes can cause decreased growth rates as a result of stress (Baarduik and Jobling, 1990; Jamu and Ayinla, 2003).

Clarias gariepinus belong to the family clariidae and it is most popular fish for culture in Nigeria, next to the tilapia fishes (FAO, 1997; Adeogun *et al.,* 2007). They are characterized by highly variable individual growth rates. During early developmental stages this leads to intensified

cannibalism (Barki *et al.,* 2000). In order to minimize losses at this early stage the stock must be sorted frequently. According to Kestemont and Melarde (2000) sorting of fish fingerlings into various size groups normally leads to equalization of the growth rates in the reared groups. This suggests that the growth rate of particular specimen is not only determined genetically, but that the phenomena of domination and hierarchies in fish stocks might also play some roles (Melard *et al*., 1995). According to Wedemeyer (1996), young fish are under significant stress due to increased inter-individual interactions such as high stocking density, competition for food and space, that they are costantly exposed to, this leads to differential rate of growth among individuals of the same age (Carmichael, 1994) necessitating the need for sorting.

However, report on the assessment of different sorting tools for grading of *Clarias gariepinus* fingerlings, a popular culturable fish in Nigeria is essential for the sustainability of aquaculture industry, thus necessitated the need to carry out this work.

Materials and Methods

The study was carried out in a privately owned farm, located at Kpite, in Tai Local Government Area of Rivers State, Nigeria under actual farming conditions. The *Clarias gariepinus* frys used for the experiment were obtained from the hatchery unit of the farm. The fish were observed to be active and apparently healthy.

Experimental Procedure

The experimental procedure was adapted following the method described by Ezenwa *et al*. 1990, who proposed a maximum number of 1000fries per sorting tool in grading of 2 weeks old catfish, *C. gariepinus* and which can subsequently be reduced as the fries grows older. The experiment started by grading a total number of 9,000 fries (two weeks old), consisting of 1000 fries per each of the nine grading tools, this was reduced, to 7,200 fries (3 weeks old) by the second week, consisting of 800 fries per each of the nine grading tools. This was further reduced to 5,400 fries (4 weeks old) during the third week at 600 per each of the sorting tool. At week 4 however, 3,600 fries (5 weeks old) were graded at 400 per each sorting tool. Lastly 1, 800 fries (6 weeks old) were graded at week 5 at 200 per each sorting tool. In all, the experiment lasted for a period of five weeks, grading over 200,000 fry by using 9 different grading tools for each stage of fry development.

Method of Counting of Experimental Fish

In counting the number of fry, an estimation method was used. This was done by using a strainer of 2.0g, which normally contain 1,800 fry. The weight of the fry was then determined using the formula

$W_2 - W_1 = W_3$

Where

W_1= Weight of strainer

W_2= Weight of strainer fish

W_3= Weight of fish

Grading Tools

Nine different grading tools were used for this experiment, namely:

a. Plastic Basin
b. Plywood Box
c. Meshed Box
d. Woven Basket
e. Sorting table
f. Grading cage
g. Dip net
h. Hang Grading net
i. Wooden panel

The fry was collected from the rearing trough, with the aid of scoop net and put inside a strainer for estimation. After this they were then placed inside each of the grading tools. The fry was only graded once using one type of grading tools to separate the smaller ones from the bigger one and placed inside a separate rearing trough to monitor the mortality.

Evaluation of Mortality Rates

The mortality rate was recorded by carefully removing and counting the dead fry, from each of grading tool throughthe rearing trough after each grading by usiing scoop net, they were later counted. The percentage mortality was then calculated according to FAO (2005) using the formula:

$$\frac{No\ of\ mortality}{Total\ no\ of\ fish\ graded} \times \frac{100}{1}$$

Evaluation of Water Quality Parameters

During the study, the following water quality parameters were monitored: temperature, hydrogen ion concentration (pH), dissolved oxygen (DO), ammonia nitrogen and nitrite nitrogen. Temperature measurements were taken during the experimental period using mercury in glass thermometer (^{0}C). Hydrogen ion concentration (pH) was determined by the use of a pH meter (Model HI 9812, Hannah products, Portugal). Dissolved oxygen levels in the experimental tank were determined, twice at the beginning and at the end of the experiment by the Winkler method (APHA, 1985). Ammonia nitrogen and nitrite nitrogen were measured using a test kit with calorimetric chart produced by SUNPU, Biochem, Beijing, China.

Data Analysis

The data obtained from this study were collated and subjected to ANOVA (Analysis of variance), difference among mean where existed were determined by Tuckeys multiple comparison test (Zar, 1996).

Results and Discussion

Physical Observation of Fish

The experimental fish used were very active and free from disease or any external bruise, which are capable of inducing stress or mortality.

Water Quality Parameters

The results of physico chemical parameters, pH temperature, dissolved- oxygen, nitrite nitrogen, (N-N0$_2$), ammonia- nitrogen (N-NH$_3$), recorded in the course of the trial were not significantly different ($p>0.05$) in all the experimental weeks (Table 1).

Number of Fries Graded

The results of fries graded were recorded for all the ten grading tools, in all the experimental weeks. In week 2, the lowest value of graded fish (3.14 ±5.00) was observed in plywood box (PWB), while the highest (720.67 ±26.63) was recorded in sorting table (ST). In week 3, PWB, had the lowest value (390.00 ±2.00) and sorting table (ST) had the highest. In week 4, 5, and 6 plywood box (PWB) consistently had the lowest value of graded fish, while sorting table (ST) had the highest value in these weeks (Table 2).

Mortality of Fish Fries Using Different Sorting Tools

The mean values of % mortality recorded by using different tools were presented in table 4.3. The result indicated that the highest values of mortality (91.67 ±12.58, 3.12.67 ±3.51, 173.67 ±7.77, 41.00 ±4.00 and 31.33 ±1.53) were observed in week 2, 3, 4, 5 and 6 respectively. While grading cage (GC), recorded the lowest values (4.67 ±1.53, 5, 33 ±1.53, 1.00 ±0.00. 0.00 ±0.00, 0.00 ±0.00) in week 2, 3, 4, 5, and 6 respectively (Table 3).

Comparative Percentage Mortality at Different Age of Fish Using Different Sorting Tools

The percentage mortality of fish recorded by using different sorting tools are shown in (Figure 1). The results indicated that in all the sorting tools the percentage mortality tends to decrease as age of the fish increases. In Dip Net (DN) the highest value (3.26 ±0.11) was recorded at age 2, while the lowest value (0.46 ±0.11) at 6 weeks of age, for grading cage (GC) the highest value (0.80 ±0.20) was recorded at age 2, while at age 5 and 6 no mortality was recorded. In hand grading net (HGN the highest value of 1.4 ±0.20 was observed at age 2, no mortality was recorded at 6 weeks. In using meshed box (MB), the highest mortality value of 1.96 ±0.47 was recorded at age 2, while age 6 has the lowest value of 0.87 ±0.11. The highest values of 2.53 ±0.40 and 7.03 ±0.49, and the lowest values 0.90 ±0.17 and 1.16 ±0.35 were recorded in PB and PWB respectively at week 2. The highest values of 50.20 ±0.20, 1. 36 ±0.25 and 1.80 ±0.20, with the corresponding lowest values of 2.13 ±0.11, 0.60 ±0.20 and 0.53 ±0.11 recorded respectively in sorting tools (ST) Woven basket (WB), and Wooden grading panel, (WGP), In rearing of fish. Malison (2000) reported that pH, temperature and dissolved oxygen are the three most important water quality variables in fish hatchery. The differences in survival and mortality among the nine grading tools were not attributable to water quality parameters because these variables were similar in all the grading groups and were within

the acceptable limits for hatchery production (Ayinla and Nwadukwe, 1990; Haylor and Mollah, 1995).

It should be borne in mine that sorting fish according to size is a standard rearing procedure in commercial rearing system and on fish farms (Melntyre *et al.,* 1987; Popper *et al.,* 1992; Kamstra, 1993). Its aim is to simplify feeding (by applying the appropriate feed granulation size and ration) and to limit phenomena of domination and inter -individual interaction (Jobling 1995). The assumption is that separating smaller fish will protect them from domination by large fish, thus improving their growth rate and increase stock biomass (production). The results of this work indicated various degrees of effectiveness using different sorting tools as revealed in the diver's mortalities of fish recorded.

It was deduced from the results obtained in this study that the number of fish sorted, using different sorting tools varied significantly ($p<0.05$) from each other, with sorting table having the highest number, supporting the findings of Togugeni *et al.* (1997) who obtained similar results in using three different tools to sort tilapia (*Oreochromis niloticus).* This may be due to the fact that sorting table has a very flat and wide surface, which allows fry to be sorted easily. Also, the number of fry sorted using different tools in this work seemed to be increasing as the age of the fry increased. This disagreed with the findings of Melard *et al*. (1996) who observed a contrary trend in the number of larvae of perch (*Perca fluviatilis)* sorted after 44 days. This may be due to the fact that the two fishes were sorted at different ages after 44 days, the fish have grown to some extent, and cannibalism of the smaller ones by the bigger fish may have occurred thus reducing the number of the fish. According to Gabriel *et al. (*2007b), the variability of individual growth patterns especially at the early stages when growth is allometric and potentially maximal cannot be over emphasized. Therefore, huge discrepancies between individual growth patterns during this period would favour the precocious emergence of cannibal fish resulting in their lower number.

The mean mortality of baby fish observed in this work by using different sorting tools, vary significantly ($P<0.05$) from one sorting tool to another. Also, the mortality of the fish tends to decrease as the age of the fish increased. This result is in line with the findings of Koebele (1982) who observed similar results in red belly tilapia (*Tilapia Zilli)*. He postulated that sorting of fish with different tools modify the various degrees of mortality recorded. Wickins (2005) and Kamstra (1993) found that mortality of fish was mainly governed by physiological responses and not necessarily social interactions. While Purdom (2004) and Jobling (1982) found that mortality in larvae of sorted sole, *Solea solea* and plaice, *Pleuronectes platessa* depended on the efficiency of the sorting tools that were used. Conversely, Wickins (2005) observed an increase in larvae mortality as a result of defective grading tool.

Moreover, Dewandel (2002) found that the effects of four different sorting tools on the survival and mortality of Atlantic cod (*Gadus morhua)* vary from one tool to another, as observed in this study. This according to them may be as a result of social stress and increased motor activity, which ultimately leads to mortality. The various degree of mortality observed in the various sorting tools may be due to inter individual interactions as a result of the efficiency of the tools. Dill (1983) proposed the hypothesis that the highest level of inter individual interactions occurs between sorted fishes' relative to their sorting tool. In effect, this means that the survival of fish is directly proportional to the efficiency of the grading tools used.

However, the pooled data from the nine sorting tools indicated that the percentage mortality though varied according to the age of the fish, also varied in different sorting tools. Similar results were obtained with larvae of walleye (*Coho salmon)*. This support the relevance of size-sorting larvae fish, especially within a standard intensive rearing frame work, implying sorting at regular intervals as the age of the fish increases. This natural progressive sorting limits the cannibal emergence, although the variability of individual growth patterns may be substantial (Jensen 1988; Melard *et al.,* 1995).

Table 1. Physico-Chemical Parameters in Rearing Tanks During The Experiment (Mean ±SD)

	Experimental Weeks				
Parameters	**1**	**2**	**3**	**4**	**5**
pH	6.58±0.12	6.57±0.00	6.50±0.13	6.56±0.11	6.48±0.13
Temperature (^{0}C)	29.24±0.64	28.78±0.54	29.14±0.86	29.11±0.14	29.18±0.76
Dissolved oxygen (mg/l)	6.74±0.32	6.70±0.30	6.68±0.46	6.59±0.41	6.41±0.88
N-N0$_2$ (mg/l)	0.0030±0.02	0.0029±0.01	0.0028±0.01	0.0027±0.02	0.0026±0.06
NH_3 (mg/l)	0.30±0.03	0.32±0.04	0.36±0.06	0.38±0.08	0.39±0.08

Table 2. Number of Graded Fish Fries Using Different Sorting Tools.

	Tools								
AGE (WEEKS)	**DN**	**GC**	**HGN**	**MB**	**PB**	**PWB**	**ST**	**WB**	**WGP**
2	508.67±58.31^a	577.67±22.01^d	573.33±6.35^d	445.00±5.00^d	383.00±2.65^d	314.00±5.00^c	720.67±26.63^d	448.00±2.65^b	556.33±20.50^d
3	477.00±1.00^a	604.00±6.93^d	588.33±11.50^d	448.33±2.08^d	420.69±4.04^c	390.00±2.00^d	796.33±11.84^d	450.33±1.53^b	600.67±24.01^c
4	490.00±1.00^a	652.33±14.50^c	638±0.000^c	459.33±3.06^b	444.33±4.04^b	447.67±2.52^c	1013.00±52.72^c	455.00±5.00^b	616.33±7.51^c
5	492.00±1.00^a	797.00±12.12^b	698.00±16.00^b	487.33±2.65^a	474.67±3.51^d	478.67±1.53^b	1256.00±105.13^a	476.33±5.51^a	693.00±23.89^b
6	527.00±8.54^a	857.33±24.50^a	744.00±10.39^a	487.33±2.08^a	477.00±4.35^a	485.00±1.00^a	1476.33±40.99^a	482.00±2.00^a	744.00±10.69^a

Key: DN (Dip Net), GC (Grading Cage). HGN (Hang Grading Net), MB (Meshed Box), PB (Plastic Basin), PWB (Plywood Box), St (Sorting Table), WB (Woven Basket) and WGP (Wooden Grading Panel).

Means within the row carrying different superscripts are significant ($p<0.05$)

Table 3. Mortality of Fish Fries Using Different Sorting Tools

Tools									
AGE (WEEKS)	**DN**	**GC**	**HGN**	**MB**	**PB**	**PWB**	**ST**	**WB**	**WGP**
2	15.33±0.581^{c}	4.67±1.53^{e}	8.00±1.00^{d}	8.67±2.08^{d}	9.67±1.53^{d}	26.67±2.08^{a}	91.67±12.58^{a}	6.00±1.00^{e}	10.00±1.00^{d}
3	10.00±1.00^{b}	5.33±1.53^{c}	6.33±0.58^{c}	6.67±0.58^{c}	9.33±1.53^{b}	8.33±1.53^{b}	82.67±3.51^{a}	7.00±1.00^{c}	8.00±1.00^{b}
4	4.67±0.58^{b}	5.33±1.53^{c}	4.00±1.00^{b}	5.67±0.58^{c}	8.00±1.00^{b}	8.00±1.00^{b}	73.67±7.77^{a}	5.33±1.15^{b}	6.33±1.53^{b}
5	2.33±0.58^{c}	0.00±0.00^{d}	0.67±0.58^{d}	4.67±1.15^{d}	5.00±1.00^{b}	4.67±0.58^{b}	41.00±4.00^{a}	5.33±1.15^{b}	4.67±1.15^{b}
6	2.33±0.58^{c}	0.00±0.00^{d}	1.00±10.00^{d}	4.33±0.50^{b}	4.33±0.58^{b}	5.67±1.53^{b}	31.33±1.53^{a}	3.00±1.00^{c}	4.00±1.00^{b}

Key: DN (Dip Net), GC (Grading Cage). HGN (Hang Grading Net), MB (Meshed Box), PB (Plastic Basin), PWB (Plywood Box), St (Sorting Table), WB (Woven Basket) and WGP (Wooden Grading Panel).

Means within the row carrying different superscripts are significant ($p<0.05$)

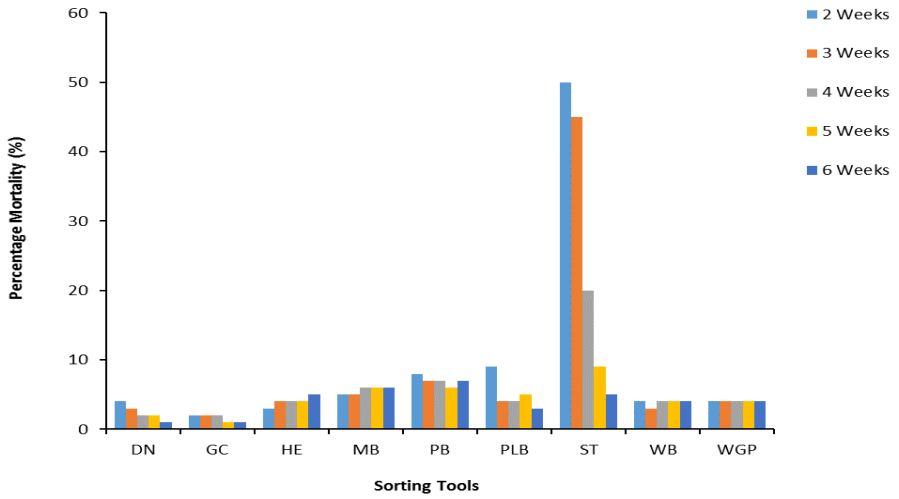

Figure 1. Comparative percentage mortality observed at different age of fish using different sorting tools

Conclusion

The results from this study indicated that the grading cage (GC) has the highest efficiency because of the very low mortality rates at all sizes and age. It is highly recommended to grade fry between 1-4 weeks old, the age regarded as the critical surviving stage of every baby fish. The sorting table (ST) has the highest capacity than other tools, as the tool has the highest mean value of graded baby fish. The highest mortality was also recorded in sorting table when compared to other tool The mortality of baby fish in various sorting tools reduced as the age of the fish increases, but the number graded increased with age. Results from the study strongly suggest that larvae of *C. gariepinus* should be sorted by using grading cage as this would proportionally increase the initial survival rate. Finally, the current experiment indicated that sorting of larvae of *C. gariepinus* using various grading tools enhance the survival, limit intra group variability and early cannibalism. This therefore helps most fish farmers especially the fish hatchery operators to achieve high survival rate of early fingerlings production.

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FULL PAPER TAM MAKALE

EFFECTS OF DIETARY ZEOLITE LEVELS ON SOME BLOOD PARAMETERS OF GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES

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Abstract:
In this study, it was aimed to investigate the effects of dietary zeolite supplementations on blood parameters of gilthead seabream (*Sparus aurata*). Zeolite was gradually included into the diets at 0%, 1%, 2%, 3% and 4% and fed to triplicated groups of fish for 10 weeks. Dietary zeolite levels did not affect red blood cell, white blood cell and hemoglobin levels of sea bream. On the other hand, serum glucose levels were linearly decreased whereas triglyceride quadratically increased with zeolite levels. There was a significant quadratic effect of dietary zeolite on serum cholesterol and alanine aminotransferase levels. Blood urea nitrogen, aspartate aminotransferase and alkaline phosphatase levels did not change in a particular trend with dietary zeolite levels, which was the case for sodium, potassium, calcium and magnesium. The results suggest that dietary zeolite inclusion up to 4% did not lead to any health impairment in gilthead sea bream when judged from blood parameters.

Keywords: *Sparus aurata*, Zeolite, Blood chemistry, Blood electrolytes, Hematology

Introduction

Zeolites, a kind of clay, are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, and have infinite three-dimensional structures (Mumpton, 1999). Former studies have showed that dietary zeolite supplementation in diets of livestock and rats was found to improve health and growth rate (Katsoulos et al., 2005; Demirel et al., 2011; Kyriakis et al., 2012; Pourliotis et al., 2012). The clays have been reported to protect the intestinal gut health and improve morphological characteristics of the mucosa (Albengres et al., 1985). As an unconventional feed additive, the clay minerals have an ability to absorb and detoxifying effect of noxious substances hence they are considered as protective against infections in warm blooded animals (Vondruskova et al., 2010).

Several clay mineral types including clinoptilolite, bentonite, modernite and sericite have been used in diets of Coho salmon, *Oncorhynchus kisutch* (Edsall and Smith, 1989), rainbow trout, *Oncorhynchus mykiss*, (Reinitz, 1984; Obradović et al., 2006; Eya et al., 2008; Ergün et al., 2008; Yiğit and Demir, 2011), European sea bass, *Dicentrarchus labrax* (Dias et al., 1998), common carp, *Cyprinus carpio* (Kanyılmaz, 2008; Khodanazary et al., 2013), tilapias, *Oreochromis niloticus and Tilapia zilli* (Hu et al., 2008; Yıldırım et al., 2009) and shrimp, *Litopenaeus schmitti* (Galindo et al., 2006). Of these aquatic species, particularly rainbow trout, shrimp and gilthead sea bream (*Sparus aurata*) (Kanyılmaz et al., 2015) have been reported to show higher growth performance when fed diets including zeolite (clinoptilolite). Earlier studies on different fish species mostly dealt with the effects of clays on growth and feed utilization. However, feeding gilthead sea bream with zeolite supplemented diets resulted in iron accumulations in the liver (Kanyılmaz et al., 2015), which could suggest that dietary zeolite can cause alterations in blood parameters. In addition, it has been reported that long term feeding with dietary zeolite in some terrestrial animals could cause physical irritations in the intestinal mucosa and subsequently affect some hematological variables as a result of their ion exchange features (Martin-Kleiner et al., 2001; Katsoulos et al., 2005; Mohri et al., 2008). Eventually, there is a serious scarcity of information about effects of dietary zeolite levels on blood parameters in fish. This study was planned to evaluate the effects of dietary clinoptilolite incorporations on blood chemical and hematological parameters of gilthead sea bream.

Materials and Methods

Zeolite and diet preparation

The zeolite material (Table 1) was procured from a commercial mining company (Gordes Zeolite, Manisa, Turkey). It was ground using a hammer mill, sieved to obtain particle size about 100 µm, washed with distilled water and then dried overnight at 105ºC. Composition of the zeolite used is presented in Table 1.

Experimental diets were prepared from a commercial sea bream diet (Çamlı Yem, İzmir, Turkey). First, the diet was ground with a hammer mill, and then zeolite was added at levels of 0, 1, 2, 3 and 4% (named as Z0, Z1, Z2, Z3 and Z4 respectively) in place of alpha cellulose (Table 2). Distilled water was added to the mixtures until a dough-like consistency, and then the resulting material was pressed through a meat mincer with a 2 mm die. The pellets were dried one night at 65°C and stored at +4°C until use.

Table 1. Chemical composition of clinoptilolite used in the experiment*

Component	g/kg
SiO_2	671
Al_2O_3	118
Fe_2O_3	14.7
MgO	11.5
CaO	21.8
Na_2O	3.8
K_2O	34.4
Moisture	124

*Statement of the supplier (Gordes Zeolite, İzmir, Turkey).

Table 2. Nutrient compositions of the experimental diets (dry matter basis).

	Z0	Z1	Z2	Z3	Z4
Dry matter (g/kg)	950	954	956	952	953
Ash (g/kg)	115	122	132	141	151
Protein (g/kg)	481	469	470	476	480
Lipid (g/kg)	174	175	171	172	172
Carbohydrate (g/kg)	190	204	206	201	196
Energy (MJ/kg)	21.6	21.6	21.5	21.6	21.6
Iron (mg/kg)	388	441	474	527	681
Aluminum (mg/kg)	137	553	891	1205	1655

Experimental design and fish rearing

This study was conducted at the Kepez Unit of Mediterranean Fisheries Research Production and Training Institute, Antalya, Turkey. The experimental system was a closed recirculation system consisting of 15 rectangular tanks (65 L), sedimentation tanks, a protein skimmer, a biological filter and an ultraviolet filter. The system was subjected to an artificial photoperiod of 12 h light (350 lux) and 12 h darkness. Daily water renewal rate was 10% and water turnover rate in the system was one hour. The culture system was also provided with continuous aeration through an air compressor. Water temperature was maintained at about 25ºC with thermostatic heaters. Fish were selected from a large population produced in the institute's marine hatchery, Beymelek, Antalya, size-graded and then transferred to the experiment unit at the Kepez Unit. Twenty-five fish were randomly allocated to each acclimatized to the experimental conditions for 2 weeks. During this period fish were fed the control at a level of 4% body weight. The number of fish in each tank was reduced to 20 at the commencement of the study. Average initial weight of fish was 9.06 ±0.04 g. Each of five treatments was tested for 10 weeks. Fish were fed carefully twice a day at 09:00 and 15:30 h near the satiation (4% first 6 weeks, 3% 7-8th weeks and 2.5% 9-10th weeks). Even if rarely observed, uneaten pellets were siphoned. Experimental fish were collectively weighed every 2 weeks after a slight anesthetization with 2-phenoxyethanol at a dose of 0.3 mL/L (Velíšek and Svobodová, 2004). Water parameters such as temperature, dissolved oxygen, pH and salinity were monitored daily with YSI 55-12 FT DO and YSI 63-12 FT pH Meter (Yellow Springs Instrument, Yellow Springs, OH, USA). Total ammonia nitrogen (TAN) and nitrite were monitored every 3 days (APHA, 1995). Water temperature, dissolved oxygen pH, Salinity, TAN and nitrite levels of the water were 24.77 ± 0.18ºC, 5.10 ±0.16 mg/L, 7.68 ±0.04, 37.35 ±0.1 ppt, 0.01 ±0.00 mg/L, 0.23 ±0.01 mg/L respectively.

Sample collection and analysis

At the end of the feeding trial, fish were starved for 24 h and five fish were randomly sampled from each tank. Following anesthetization with 2-phenoxyethanol at a dose of 0.3 mL/L (Velíšek and Svobodová, 2004), their blood was taken from the caudal vein using heparinized disposable syringes. A part of the blood samples was separated into micro tubes (Miniplast 0.6 ml, LP Italiana Spa, Milano) containing EDTA (1.26 mg/0.6 ml) as an anticoagulant agent and analyzed via a hematologic auto analyzer (MS4, Melet Scholoesing laboratories, Pontoise, Cedex–France). The remaining blood was transferred to biochemical tubes for serum analysis (BD Vacutainer SST II Advance 5 mL, Plymouth, UK) and centrifuged at 3500 rpm for 5 minutes (Elektromag M 4812 centrifuge, Istanbul, Turkey). Glucose, total cholesterol, triglyceride, blood urea ni-

trogen (BUN), alanine aminotransferase (ALT), alkaline phosphatase (Çelik et al.), aspartate aminotransferase (Ly et al.), calcium (Ca) in serum were determined using a VetTest chemistry analyzer (Model 8000, IDEXX Laboratories Inc., Westbrook, ME, USA) and magnesium (mg), sodium (Na) and potassium (K) were determined using a Roche/Hitachi chemistry analyzer (Model 911, Roche Diagnostics, Indianapolis, USA).

Data calculation and statistical analysis

Linear and quadratic effects were tested to reveal the trends resulting from the effects of various dietary zeolite levels on the observed response variables. Values were given as means ± standard errors. A statistical package JMP v.8.0 for Windows was used for the statistical analyses.

Results and Discussion

Growth and feed utilization data were given elsewhere (Kanyılmaz et al., 2015). In brief, mean final weights of Z0, Z1, Z2, Z3 and Z4 were 50.7, 52.6, 53.8, 54.5 and 52.0 g respectively. Feed conversion efficiencies of treatments with the same order were 0.83, 0.87, 0.85, 0.88 and 0.87. The study showed that nutrient ADC were generally unaffected by the diets. Overall, the zeolite supplementation made a positive contribution to the growth performance and feed utilization, and an inclusion level of 2.71% was estimated as optimum.

Dietary zeolite treatments had a significant negative linear effect on serum glucose (Linear=0.0001, Quadratic=0.798). There was a significant positive quadratic effect of dietary zeolite on serum triglyceride (Linear=0.006, Quadratic=0.018) levels. Serum ALT levels were quadratically responded to dietary zeolite levels (Linear=0.277, Quadratic=0.036). Serum AST concentrations were comparable among the treatments. There was no a remarkable trend in ALP levels in response to dietary zeolite. Cholesterol levels were quadratically effected (Linear=0.062, Quadratic=0.032), whereas there were no discernible effects of zeolite supplementations on BUN (Table 3) WBC, RBC and Hemoglobin (Hb) (Table 4), Na, K, Ca and Mg levels of gilthead sea bream (Table 5).

Table 3. Serum biochemical parameters in sea bream juveniles fed diets containing different level of zeolite (Glucose, BUN, Cholesterol, triglyceride (mg/dL), ALT, AST, ALP (IU/L).

	Z0	Z1	Z2	Z3	Z4	SEM	P values	
							Linear	Quadratic
Glucose	78.00	73.67	74.00	70.33	68.73	1.943	0.0001	0.798
BUN	22.43	24.00	22.40	23.70	23.53	1.505	0.639	0.905
Cholesterol	240.7	341.7	371.0	329.0	384.7	50.729	0.062	0.032
Triglyceride	238.3	232.0	202.7	275.0	289.5	14.069	0.006	0.018
ALT	11.00	7.67	16.00	10.00	7.00	0.699	0.277	0.036
AST	195.3	134.3	343.0	189.3	161.3	34.934	0.929	0.102
ALP	226.3	180.3	297.6	207.0	278.0	20.761	0.162	0.807

SEM = standard error of mean, BUN = blood urea nitrogen, ALT = alkaline phosphatase, AST = aspartate amino aminotransferase, ALP = alanine aminotransferase

Table 4. WBC (10^3 mm^3), RBC (billion/mm^3) and Hb (g/dL) levels in sea bream juveniles fed diets containing different level of zeolite.

	Z0	Z1	Z2	Z3	Z4	SEM	P values	
							Linear	Quadratic
WBC	224	259	187	224	228	42.412	0.939	0.991
RBC	2.42	2.73	2.45	2.41	2.61	0.277	0.955	0.758
Hb	9.07	9.43	9.10	8.70	9.17	0.786	0.797	0.956

SEM = standard error of mean, WBC = white blood cell, RBC = red blood cell, Hb = hemoglobin

Table 5. Na (mmol/dL), K (mmol/dL), Ca (mg/dL) and Mg (mg/dL) levels in sea bream juveniles fed diets containing different level of zeolite

	Z0	Z1	Z2	Z3	Z4	SEM	P values	
							Linear	Quadratic
Na	177.6	180.7	178.3	177.7	176.6	1.429	0.223	0.194
K	5.77	5.57	7.07	5.23	5.47	0.575	0.606	0.257
Ca	15.17	13.67	14.83	14.10	14.13	0.278	0.158	0.385
Mg	3.13	2.97	3.53	2.77	2.80	0.146	0.135	0.171

SEM = standard error of mean, Ca = calcium, mg = magnesium, Na = sodium, K = potassium

Blood parameters of fish are known to be affected by a number of factors such as season, water quality variables, age, sex, nutrition, health status, genetic characteristics, transportation, handling and other environmental conditions as well as those related to sampling and laboratory analysis methods (Bond, 1979; Rey Vázquez and Guerrero, 2007). In the present study, dietary zeolite supplementation significantly affected some blood parameters of gilthead sea bream. For instance, blood glucose levels varied between 68.73 and 78.00 mg/dL and were linearly reduced with increasing dietary zeolite levels. These glucose levels are within the range of reported values for gilthead sea bream (Roncarati et al., 2006; Peres et al., 2013) In agreement with the present findings, a previous study on common carp (Kanyılmaz, 2008) reported a significant decrease in blood glucose concentrations with increasing dietary zeolite contents. However, there are several other studies on various aquatic and terrestrial animals pointing out that dietary zeolite had no effect on blood glucose levels (Curtui, 2000; Yazdani and Hajilari, 2009; Ghaemnia et al., 2010; Demirel et al., 2011; Safaeikatouli et al., 2011; Peres et al., 2013). Çelik (2006) and Çelik et al. (2008) reported that when exposition of fish to heavy metals for a long time could have lowering effects on glucose levels. Although difficult to make direct connection with this, a decreasing trend in blood glucose levels could be due to increasing dietary iron and aluminum levels with zeolite in the present experiment (Table 2). Indeed, there were negative strong relationships between blood glucose and dietary iron (r^2 = -0.96) and aluminum (r^2 = -0.93) levels. Alternatively, dietary zinc, cobalt and chromium have been found to reduce blood glucose levels in fish by being involved in insulin activity (Watanabe et al., 1997; Vangen and Hemre, 2003). Compositions of these elements in the present zeolite material and their availabilities to the fish are unknown, and therefore further studies are required to clarify these points.

Dietary zeolite did not affect BUN levels in the present study. Conversely, a former study on common carp (Kanyılmaz, 2008) found dietary zeolite led to a remarkable decrease in BUN. In

terrestrial animals BUN is used as an indicator of the renal health but not in fish due to main nitrogenous excretion route being the gill not the kidney. Therefore, elevated BUN levels are suggested as an indicator of problem with nitrogen excretion by the gill (Glibert and Terlizzi, 1999). The comparable BUN levels among the treatments imply that dietary zeolite did not cause an adverse effect on nitrogen metabolism in sea bream.

In current study, cholesterol concentrations of gilthead sea bream were quadratically affected with dietary zeolite levels and varied between 240.70 and 384.70 mg/dL. This range is consistent with the findings for gilthead sea bream by Peres et al. (2013) but slightly lower than those of Roncarati et al. (2006). In contrast to our present findings, existing literature in fish point out that dietary supplemental zeolite had no effect on cholesterol levels (Kanyılmaz, 2008; Tekeşoğlu, 2010). Serum triglyceride levels showed a linear increase with dietary zeolite elevation in the present study. Previous studies report no effect of dietary zeolite on triglycerides levels of chick and fish (Curtui, 2000; Tekeşoğlu, 2010). The increase in cholesterol and triglycerides with zeolite supplementation could be partly resulted from a linear increase in lipid retention by fish fed zeolite added diets (Kanyılmaz et al., 2015). Dietary zeolite was found to have an ability of absorption of short chain fatty acids (SCFAs; butyrate, acetate and propionate) in large intestine of pigs (Ly et al., 2007). The SCFAs are fermentation products and known to reduce the synthesis of cholesterol and triacylglycerol in the liver (Ooi and Liong, 2010). Possible absorption of the SCFAs could be another reason of the cholesterol and triglyceride increasing effect of dietary zeolite, but these speculation remains to be studied.

The AST, ALT and ALP activities are associated with the tissues damages such as in the liver, gut and bile ducts (Roncarati et al., 2006; Maita, 2007; Peres et al., 2013). These variables in the present study, except ALT which had a significant quadratic contrast, were not significantly affected by the treatments, suggesting that dietary zeolite levels have no detrimental effect on fish health at least for gilthead sea bream. Similar findings were also reported by previous poultry and fish studies fed diets with varying zeolite levels (Curtui, 2000; Safaeikatouli et al., 2011; Vizcarra-Olvera et al., 2012).

Blood electrolytes are used as indictors for various physiological statuses in fish such as secondary stress response, growth and nutritional condition (Vangen and Hemre, 2003; Peres et al., 2013;). There was no significant clear trend in selected electrolytes in response to increasing dietary zeolite, being consistent with the reports from terrestrial animals (Alexopoulos et al., 2007; Demirel et al., 2011). However, Khodanazary et al. (2013) reported that dietary zeolite addition increased blood Ca and K levels, decreased Na concentrations and did not change Mg levels in common carp. Our results suggest that dietary zeolite did not alter mineral absorption and metabolism at a considerable level, but when used at levels higher than 4% there may be an adverse effect due to antagonistic relations between dietary ash levels and availabilities of certain minerals to fish such as Ca, Mg, Fe and P (Sugiura et al., 2000).

A reduction in RBC and Hb numbers below the normal range in fish is assumed to be an indicator of anemia (Houston, 1997; Maita, 2007). WBC number generally increases after deterioration of hemostasis due to an exposure to a stressful factor (Çelik, 2006). Oppositely a reduction in WBC number is also an indicator of impairment of immunity (Çelik, 2006). The Hb, WBC and RBC values obtained from the present study are within the range of literature data for gilthead sea bream (Molinero et al., 1997; Tort et al., 2002; Fazio et al., 2012). The hemogram values of sea bream were not altered by dietary zeolite levels in this study. These findings are inconsistent with those of Kanyılmaz (2008), who noted that dietary zeolite supplementation increased Hb values in common carp while similar to those of studies on fish (Eğrikılıç, 2009) and terrestrial animals (Katsoulos et al., 2005; Pourliotis et al., 2012; Yazdani and Hajilari, 2009).

Conclusion

The present findings show that dietary zeolite inclusion significantly decreased glucose whereas increased cholesterol and triglyceride levels. Other hematological and biochemical variables, except ALT levels, were not altered by the treatments. Overall the results suggest that dietary zeolite up to 4% did not affect health status of gilthead sea bream. Future studies should be focused on the effects of dietary zeolite on availabilities of minerals, gut health and microflora as well as immune parameters.

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THE USE OF GARLIC (*Allium sativum*) MEAL AS A NATURAL FEED SUPPLEMENT IN DIETS FOR EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES

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Abstract:

The incorporation of garlic meal (GM) in diets for European seabass juveniles were evaluated with a diet containing 43% protein and 17% lipid (gross energy 19kJ/g diet). Experimental diets with GM incorporation of 0, 2, 4, and 6% were fed to fish (10.60 ±0.16 g) until satiation for 60-days. Significant differences ($p<0.05$) were recorded for growth performance, with the highest rate in the 4% GM group, followed by the control group. Improved feed conversion (FCR) and protein efficiency rates (PER) were observed in the GM4 group compared to the other treatments. Nitrogen retention as a percent of intake was highest in the in GM4 group. Significantly higher values ($p<0.05$) were found for body protein and lipid, and lower values ($p<0.05$) for the hepatosomatic, viscerasomatic or mesenteric fat indexes in the GM4 group compared to the other treatments. Fish fed garlic supplemented diets showed lower saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), but higher polyunsaturated fatty acids (PUFA) compared to the control group with no garlic treatment. Results indicate that dietary GM inclusion of 4% can improve fish growth and nutrient utilization with an increase of fish muscle quality by elevated PUFA concentrations, and a reduction of total nitrogen excretion.

Keywords: Feed additives, Garlic meal, Growth performance, Nutrient utilization, Fatty acid profile

Introduction

The success in intensive aquaculture is basicly dependent on quality of feed and feeding strategies which are the most important factors influencing growth performance, feed utilization and body chemical composition of fish (Okumus and Mazlum, 2002). In intensive aquaculture, the aim is to gain maximum yields from water resources with providing artificial diets that thrives fish growth and gain maximum weight in the shortest time frame as possible (Bhosale et al., 2010). Recently, some valuable components stimulating the defense system and stress response of fish, the so-called immunostimulants, have been isolated from plants, animals or microorganisms (Sakai, 1999). The incorporation of immunostimulants in fish diets has been suggested as an effective method for the improvement of the activity of non-specific defense mechanisms increasing disease resistance (Dalmo and Seljelid, 1995) and for the control of biomass in aquaculture (Yokoyama et al., 2005). Probably known as one of the earliest medicinal plants (Farahi et al., 2010), the use of garlic in aquaculture became popular for providing protection against diseases or inducing fish feeds as a growth promoter. Garlic was used as a growth promoter in tilapia (Diab et al., 2002; Shalaby et al., 2006; Mesalhy et al., 2008; Soltan and El-Laithy, 2008; Metwally, 2009; Abdel-Hakim et al., 2010), in Asian seabass (Talpur and Ikhwanuddin, 2012), in sterlet sturgeon (Lee et al., 2014), and in Seabass fry (Saleh et al., 2015). To our knowledge, so far, no information is available on the effects of dietary garlic on growth performance, feed utilization and body chemical composition in juvenile European seabass, which is one of the main aquaculture species in the Mediterranean, where the Turkish and Greek aquaculture industries share a total production of 211.055 tons with a grand value of 1.293.082 USD for seabass together with seabream (FAO, 2012). During the last 10 years, aquaculture based fish production has doubled from around 41 million tons to over 90 million during with a market value of over 51 million USD in year 2000 and 144 million USD in 2012 (FAO, 2012). The increase of fish production worldwide triggers the demand for high quality diets improving fish growth and welfare.

The objective of this study was to evaluate the effects of dietary garlic meal as a natural feed supplement on growth performance, nutrient utilization, body biochemical composition, and nitrogen balance and muscle fatty acid profile of European seabass (*Dicentrarchus labrax*) juveniles.

Materials and Methods

Experimental fish, diets and culture conditions

European Seabass obtained from a commercial marine fish hatchery (Ida Gıda, Canakkale-Turkey) were transferred to the Marine Aquaculture Research and Development facilities of the Faculty of Marine Science and Technology at Canakkale Onsekiz Mart University. The experimental facility was a closed recirculation aquaculture system (RAS), run with mechanical and biological filtration, continuous air supply and water heaters. Water flow rate was 2.0 l/min (complete water turnover was about 2.4 times per hour). During the entire experimental period, temperature was 23.3 ±0.98ºC, dissolved oxygen 7.00 ±1.0 mgL^{-1}, salinity 24 ‰, pH 7.50 ±0.5, and Ammonia-N (NH_3-N) was 0.28 ±0.07 mgL^{-1}.

After transportation, the experimental fish were initially placed in indoor tanks (1.0 m^3) with a continuous seawater flow through system for an acclimatization period of 2 weeks. Thereafter, a total of 108 juveniles (mean initial weight of 10.60 ±0.16 g) out of the main stock were randomly distributed in to 12 identical shaped glass aquariums (9 fish per aquarium) with a water volume of 50 L, according to a triplicate design. Four different feed formulations were prepared and experimental diets were produced with garlic meal inclusion levels of 0, 2, 4 and 6%. The experimental group fed diets without garlic meal (0%) served as control. Chemical composition of the experiment diets is given in Table 1. Biochemical composition of the ingredients used in feed formulation and those for European seabass are shown in Table 2. Fish were fed until satiation twice a day (08:30 and 16:30), 6 days a week for a total period of 60 days. Satiation level was considered when sh no longer attacked food particles and refused feeding. During the trial a natural photoperiod regime was followed (N 40°04'29.98", E 26°21'35.60" - Canakkale, Turkey), and no additional light was applied.

Table 1. Feed ingredients and composition of the experimental diets

	Experimental Diets (%)				
Ingredient (g/100g)	Control	GM2	GM4	GM6	
Fish meal (FM)	57.50	57.30	57.10	56.90	
Soybean meal (SBM)	20.00	20.00	20.00	20.00	
Garlic meal (GM)	0.00	2.00	4.00	6.00	
Fish oil (FO)	10.60	10.61	10.61	10.62	
b-Corn starch	8.90	7.09	5.29	3.48	
Vit-Min Premix	3.00	3.00	3.00	3.00	
Analyzed biochemical composition (g/100g air dry basis)					
Moisture (%)	12.00	12.00	12.00	12.00	
Crude Protein (%)	43.30	42.80	43.20	43.10	
Crude Lipid (%)	16.69	17.56	17.09	16.64	
Crude Ash (%)	11.86	11.14	11.44	11.25	
Estimated nutrients					
NFE (%)	13.15	13.50	11.27	14.01	
GE (kJ/g diet)	19.05	19.33	18.86	19.13	
P/E (mg/kJ)	22.72	22.14	22.91	22.53	
PE/TE	0.54	0.52	0.54	0.53	
Amino acid composition of experimental diets (% dry matter)[a]					Seabass requirements
Arginine	3.05	3.05	3.05	3.05	1.80
Lysine	3.78	3.77	3.77	3.77	1.88
Histidine	1.26	1.26	1.27	1.27	0.63
Isoleucine	2.53	2.53	2.52	2.52	1.02
Leucine	3.93	3.93	3.94	3.94	1.68
Valine	2.70	2.70	2.70	2.70	1.13
Met+Cys	1.89	1.89	1.89	1.88	0.90
Phe+Tyr	3.98	3.98	3.98	3.98	1.02
Threonine	2.11	2.11	2.11	2.11	1.05
Triptophan	NA	NA	NA	NA	0.23
n-3 HUFA in experimental diet (%)					
Lipid in FM (%)	8.50	8.50	8.50	8.50	
Lipid from FM (%)	4.89	4.87	4.85	4.84	
Total FO in diet (%)	15.49	15.48	15.46	15.46	
n-3 HUFA in FO (%)[b]	29.76	29.76	29.76	29.76	
Total n-3 HUFA in diet (%)	4.61	4.61	4.60	4.60	
n-3 HUFA requirement (%)					0.7[c]

[a] Calculated according to values given in Table 2.
[b] Güner et al. (1998).
[c] Skalli and Robin (2004).
NFE (Nitrogen free extract) = 100 – (crude protein + crude lipid + crude ash)
GE= Gross energy, calculated according to energy fuels of 23.6 kJ/g protein, 39.5 kJ/g lipid and 17 kJ/g NFE.
P/E= mg Protein / kJ enerji ratio
PE/TE= Energy from protein to total energy ratio

Table 2. Biochemical composition of ingredients and European seabass used in the experiment

	European Seabass[a]	Fish Meal[b]	Soybean Meal[b]	Garlic Meal[c]
Analytical value (%)				
Moisture	8.0	11.0	10.0	
Crude Protein	66.0	46.3	6.5	
Crude Lipid	8.5	3.1	0.5	
Crude Ash	15.8	7.4	1.5	
Essencial amino acid (%)*				
Arginine	4.60	4.11	3.41	4.59
Lysine	4.80	5.49	3.10	4.48
Histidin	1.60	1.76	1.26	2.07
Isoleucine	2.60	3.38	2.92	2.26
Leucine	4.30	5.43	4.02	8.13
Valine	2.90	3.81	2.53	3.66
Methionine	N/A	2.16	0.72	0.78
Cystein	N/A	0.66	0.63	0.79
Met+Cys	2.30	2.82	1.35	1.57
Phenylalanine	N/A	3.03	2.45	3.89
Tyrosine	N/A	2.44	1.72	2.42
Phe+Tyr	2.60	5.47	4.17	6.31
Threonine	2.70	3.00	1.92	3.52
Tryptophan	0.60	0.82	0.68	N/A

[a] Kaushik (1998)
[b] Halver (1991)
[c] Aremu et al. (2011)
N/A = not available

Fish sampling and analytical methods

At the start of the experiment, fish samples (10 fish from an initial pool) were removed and anaesthetized at a high dose level and stored at -25 °C for subsequent analysis of fish body composition. At the end of the trial, 5 fish per tank (15 per diet) were removed following the same procedure as conducted for the initial samples and stored at -25 °C for subsequent analysis of final fish body composition. The proximate composition of the experimental diets and freeze-dried fish whole body proximate composition was determined following AOAC (2000) guidelines as follows: Moisture by weight loss after 24 h in an oven at 105 °C; crude ash by incineration in a muffle furnace at 550 °C for 24 h; crude protein (% Nx6.25) by the Kjeldahl method after acid digestion; lipids by ethyl ether extraction in a Soxhlet System. All laboratory analyses were performed in triplicate. Fatty acid was conducted using the Folch et al. (1957) method. After ethyl ether extraction of lipids in a Soxhlet System, fatty acids have been determined as ethyl esters, by Shimadzu capillary gas chromatograph equipped with flame ionization detecter (GC/FID) and cianopropyl-aryl HP-88 capillary column. For the esterization, the procedures of IUPAC (1987) were followed.

Statistical analyses

The results from the present study were analyzed by two-way analysis of variance (ANOVA) using SPSS for Windows, Version 10.0 for significant differences among treatments means. Duncan's multiple range test (Duncan, 1955) was used to compare differences among individual means. Probability values less than 0.05 were considered significant.

Results and Discussion

In the present study European seabass with initial mean weight of 10.60 ± 0.157 g were fed diets containing different levels of garlic meal for a period of 60 days. At the end of the trial, growth performance of fish showed significant ($p<0.05$) differences among dietary treatments. Best growth performance was obtained in fish fed diet with 4% garlic meal inclusion (GP4) with a final

mean weight of 25.15 ± 0.07 g, which was followed by the control diet with no garlic meal inclusion, showing a final weight of 24.27 ± 0.58 g. Relative growth rates (RGR) recorded during the study period showed a similar trend with the highest rate in the GM4 group fed 4% garlic meal diet, followed by the control group fed a diet without garlic meal inclusion, the GM2 and GM6, respectively. Spesific growth rate (SGR, %/day), which is the logarithmic expression of fish growth were similar to the RGR values. The highest SGR was recored in the GM4 group with 4% garlic meal inclusion (1.43 ± 0.03, %/day), followed by the control group (1.38 ± 0.07, %/day), GM2 (1.27 ± 0.08, %/day) and the GM6 (1.19 ± 0.03, %/day) groups, respectively (Table 3). This growth promotion effect of diets supplemented with garlic meal can be attributed to the improved feed efficiency, which is in agreement with the results in Nile tilapia (Diab et al., 2002; Shalaby et al., 2006; Mesalhy et al., 2008; Soltan and El-Laithy, 2008; Metwally, 2009; Abdel-Hakim et al., 2010), in Asian seabass (Talpur and Ikhwanuddin, 2012), in sterlet sturgeon (Lee et al., 2014), and in Seabass fry (Saleh et al., 2015), where the incorporation of different levels of garlic increased final weights and specific growth rates of fish. Soltan and El-Laithy (2008) reported that the incorporation of 1% garlic into diets improved survival rate of Nile tilapia. Similarly, Abdel-Hakim et al. (2010) found better achievements of dietary garlic on growth performance and feed utilization with low levels of garlic inclusion at 0.5 % level in tilapia. Better growth effects were found with higher incorporation levels of garlic meal in diets for Nile tilapia by Shalaby et al. (2006), who tested garlic incorporation levels from 10 g/kg to 40 g/kg diet, and recommended the incorporation of 3% dietary garlic for an increased growth, reduction of total bacteria, and improvement of fish health and welfare. Similarly, Lee et al. (2014) suggested that dietary garlic powder incorporation of about 3% could positively affect growth performance and protein retention in fingerling sterlet sturgeon. Farahi et al. (2010) used different levels (1%, 2% and 3%) of galic meal in rainbow trout diets and reported that the body protein was higher in the 3% galic group compared to the other experimental groups and that growth performance and fish health improved with the addition of galic meal in trout diets. Metwally (2009), used diets containing garlic in three different forms; natural garlic (40g/kg diet, 4%), garlic oil capsules (Strongus®, pure garlic oil capsules; 250 mg/kg diet) and garlic powder tablets (32 g/kg diet, 3.2%), and reported that the dietary addition of garlic in any form can promote growth rate, decrease mortality and increase the antioxidant activity in fish. Mabrouk (2011) tested dietary garlic and onion inclusion levels of 4 % and 6 %, respectively and a 10 % mixture of garlic and onion (4% garlic - 6% onion) in diets for Nile tilapia, and reported that the addition of 10% mixture of garlic and onion significantly increased growth performance and feed utilization rather than solitary addition. To our knowledge so far, the only one study dealed with dietary garlic inclusion in European seabass feeds is the one reported by Saleh et al. (2015), who tested garlic incorporation levels of 10, 20, and 30 g/kg diet, and recommended 3% dietary garlic for the best growth, improved fish health and welfare. However, the highest garlic incorporation level tested by Saleh et al. (2015) was 3%, so based on their report it is not possible to comment on higher levels of garlic additon in the diet for seabass. A dietary garlic incorportaiton of 4% gave better results in terms of growth performance and feed utilization in the present study. Furthermore, Saleh et al. (2015) investigated the dietary garlic incorporation for seabass fry with an initial weight of 0.4 g, while in the present study larger size of seabass with an initial weight of 10 g were used.

In general, our results are in agreement or comparable with previous findings in terms of better growth performace in fish fed diets with 4% garlic incorporation, and the results from the present study and those of earlier ones revealed that garlic incorporation in fish diets improved growth preformance, feed utilization, fish health and welfare. The discrepencies between the results of the present study and some of the previous ones regarding the effects of dietary garlic on growth performance of fish, feed utilization or body composition can be attributed to the differences in fish species or fish size, environmental conditions such as water temperature and salinity, type or level of the additives accomponying the main ingredients in diet formulation, or type of the garlic source used in the feeds, fish physiology or a combination of these factors together.

Table 3. Weight gain and feed utilization of juvenile European seabass fed diets with different garlic meal inclusion levels for a period of 60 days.

	Experimental diets			
	Control	GM2	GM4	GM6
Initial weight (g)	10.61±0.225^{a}	10.56±0.191^{a}	10.64±0.148^{a}	10.58±0.147^{a}
Final weight (g)	24.27±0.577^{c}	22.67±0.674^{b}	25.15±0.067^{c}	21.57±0.524^{a}
RGR (%)	128.9±10.07^{bc}	114.8±10.24^{ab}	136.4±3.56^{c}	103.9±3.93^{a}
SGR (%/day)	1.38 ± 0.074^{bc}	1.27 ± 0.079^{ab}	1.43 ± 0.025^{c}	1.19 ± 0.032^{a}
FI (%/day)	1.77 ± 0.101^{b}	1.67 ± 0.008^{b}	1.70 ± 0.083^{b}	1.54 ± 0.029^{a}
FCR	1.36 ± 0.074^{a}	1.38 ± 0.082^{a}	1.26 ± 0.042^{a}	1.35 ± 0.052^{a}
PER	1.75 ± 0.098^{a}	1.70 ± 0.103^{a}	1.82 ± 0.061^{a}	1.72 ± 0.067^{a}
DFI (g/fish)	0.271±0.016^{c}	0.244±0.003^{b}	0.268±0.012^{c}	0.218±0.007^{a}
DPI (g/fish)	0.130±0.008^{c}	0.119±0.001^{b}	0.133±0.006^{c}	0.107±0.003^{a}
DEI (kJ/fish)	1.28 ± 0.08^{bc}	1.18 ± 0.01^{b}	1.32 ± 0.06^{c}	1.03 ± 0.03^{a}

Values (means±standart deviation, triplicate groups) with different superscipts are statistically different at 0.05 level. (One-way ANOVA and Duncan's multiple range test, P<0.05). (W1 = initial weight, W2 = final weight, t2-t1 = feding days)
GM (Garlic meal)
RGR (relative growth rate, %) = (W2 – W1 / W1) x 100
SGR (specific growth rate, % growth/day) = ((lnW2 - lnW1) / (t2-t1)) x 100
FI (feed intake, percent of biomass per day, %/day) = (total feed offered / ((W1 + W2) / 2) / gün) × 100
FCR (feed conversion rate) = feed intake (g) / weight gain (g)
PER (protein efficiency rate) = (weight gain (g) / protein intake (g)
DFI (daily feed intake, g/fish) = (feed intake (g) / number of fish) / day
DPI (daily protein intake, g/fish) = (feed intake x crude protein in diet / 100) / day
DEI (daily energy intake, kJ/fish) = (feed intake x energy in diet / 100) / day

Interestingly, Mesalhy et al. (2008) found that the period of feeding has also affected the results. In their study, they reported that Nile tilapia fed with 10 or 20 g/kg garlic incorported diets for two months or 20 g/kg diet for one months showed significant increase in the final body weights compared to the control diet group. El-Nawawy (1991) reported that the growth promoting effect of garlic is due to the increase of glucose inflow into the tissues. It is also reported that the sulfur compounds in garlic, the active antimicrobial agents can improve the immune system, stimulating growth of the animal (El-Afify, 1997). Dietary additives which have immunostimulant effects can increase serum lysozyme activity, either because of the increase of phagocytes secreting lysozyme, or due to the increase of the amount of lysozyme synthesized per cell (Engstad et al., 1992). The lysozyme activity is mainly affected by the type of immunostimulants incorporated in the diets, and the increase of lysozyme induced by the addition of immunostimulants in diets has been reported in several fish species (Lapatra et al., 1998; Paulsen et al., 2003). Higher lysozyme has been reported in fish fed garlic supplemented diets compared to those fed diets without garlic addition (Sahu et al., 2007; Ndong and Fall, 2011). The improvements in growth performance induced by garlic inclusion to the diet may due to its antimicrobial, antioxidant, and antihypertensive characteristics (Konjufca et al., 1997; Sivam, 2001; Ibrahim et al., 2004). Block (1992) and Amagase and Milner (1993) suggested that these functions can be attributed to the bioactive components of garlic such as allin, allicin and diallylsulphides containing organosulphur compounds, particularly to thiosulfinates. Allicin in garlic promotes the performance of the intestinal flora according to Khalil et al. (2001), who indicated that the digestion is improved and the utilization of energy is enhancing, which can explain the improved growth of fish fed garlic supplemented diets.

Essential amino acid profile calculations of diet ingredients used in the present study indicate that the amino acid composition of garlic meal show quite similarities to those of the fish meal. It is well known that the incorporation of alternative feed ingredients or additives may influence the amino acid imbalance of the diet, hence linking to a reduced growth performance or decreased feed consumption. The dietary incorporation of plant sources in fish feed is mostly limited upto a

certain percent especially in carnivorous fish species, due to the lack of some essential amino acids in their composition. However, amino acid composition of garlic meal used in the present study was very similar to that of the fish meal source. Some of the essential amino acids in garlic meal were even higher then the fish meal amino acid levels, with the exception of methionine compared to fish meal. This provides important indications that garlic meal can be used in combination with other plant protein sources such as soybean meal which is considered as a strong and promising alternative protein source for fish diets, but lacking in methionine or lysine, which are the most limiting amino acids for soybean protein sources (Ergün et al., 2008a,b; Yigit et al., 2010). Results from an earlier study (Mabrouk, 2011) support this hypothesis with the work on Nile tilapia, where 50% of fish meal was replaced with soybean meal and the diet was incorporated with galic and onion meal at different levels. A diet combination of 50% fish meal and 50% soybean meal was enriched with a 10% mixture of garlic and onion, and as a result Mabrouk (2011) reported an improved growth performance and feed utilization in Nile tilapia.

Nitrogen retention as a percent of nitrogen intake was highest, while the nitrogen excretion as a percent of intake was lowest in the GM4 group compared to the other treatments (Table 4).

It is well known that the incorporation of plant feedstuffs in fish diet at an excess level may increase nitrogen excretion, lowering the retention rate of nitrogen (Burel et al., 2000; Fournier et al., 2004; Ergün et al., 2008a,b; Yigit et al., 2010). In the present study, eventhough no significance was found, the nitrogen retention as a percent of intake in the 4% garlic meal diet was higher then the control group with no garlic meal addition. When the garlic meal inclusion level increased to 6%, however, the nitrogen excretion as a percent of intake significantly decreased to a level below the control group, showing that the supplement of garlic should not exeed the 4 % level. In contrast to nitrogen retention rates, the nitrogen excretion as a percent of nitrogen intake showed a slight decline with the increase of dietary garlic meal and was recorded lowest in the 4% garlic diet, whereas again over this level the excretion rate increased to a level over the control group. Based on these tendencies of nitrogen excretion or retention rates, it might be interesting to see the long-term effect of dietary garlic meal on nitrogenous end-products.

Initial and final body moisture of experimental fish was found around 80% and did not significantly differ ($p>0.05$) among the experimental groups. Final fish body protein increased to over 46% in all treatment groups over the initial body protein of 42% at the end of the 60 days feeding trial. Highest protein content was found as 49.3 ±0.67 % in the fish fed diets with 4% garlic meal inclusion (GM4), which was followed by the GM2 (48.9 ±0.46 %) and the control diet (47.7 ±0.40 %), respectively. The lowest body protein was found in fish fed the 6% garlic meal inclusion diet (GM6) with a value of 46.9 ±0.75 %. Different then the fish body protein contents, the body lipids did not differ significantly ($p>0.05$) among diet treatments. However, compared to the initial values, body protein tended to decline, but not significantly except the GP4 group with 4 % garlic meal inclusion. Ash content in fish body showed a decline over a 60 days feeding period compared to the initial value, and these differences were recorded as significant ($p<0.05$). Nitrogen free extracts were lowest in fish fed diets with 4% garlic meal inclusion while the highest gross energy level was recorded again in the 4% garlic meal diet group and the control group. The gross energy level in the 4% garlic meal diet and the control diet group were significantly higher ($p<0.05$) then the initial values (Table 5).

The finding concerning the significant increase recorded in the body protein content of fish fed garlic meal diets at all inclusion levels, could be possibly explained by the increase in muscle free amino-acid contents that can lead to the enhanced protein synthesis. Similarly, increase in fish body protein levels were reported in rainbow trout (Gabor et al., 2010) and in seabass fry (Saleh et al., 2015), when fed on diets supplemented with 3% garlic.

Table 4. Nitrogen budget of juvenile European seabass fed diets with different levels of garlic until satiation for a period of 60 days

	Experimental diets			
	Control	GM2	GM4	GM6
N budget (mg g^{-1} production)				
N intake (NI)	91.7 ± 5.0[a]	94.4 ± 5.7[a]	88.1 ± 2.9[a]	93.3 ± 3.6[a]
Total N retention	16.7 ± 1.5[a]	17.7 ± 1.6[a]	17.4 ± 0.2[a]	16.1 ± 1.4[a]
Total N retention (%, NI)	18.2 ± 1.3[ab]	18.7 ± 0.6[ab]	19.7 ± 0.4[b]	17.2 ± 1.3[a]
Total N excretion	75.0 ± 4.3[a]	76.8 ± 4.1[a]	70.8 ± 2.7[a]	77.3 ± 3.0[a]
Total N excretion (%, NI)	81.8 ± 1.3[ab]	81.3 ± 0.6[ab]	80.3 ± 0.4[a]	82.8 ± 1.3[b]

Values with different superscripts (means±standart deviation, triplicate groups) are statistically different at 0.05 level (One-way ANOVA and Duncan's multiple range test, $p<0.05$).
GM (Garlic meal)
N intake (mg/g production) = (DPI x day / 6.25) / (W2 – W1)
N retention (mg/g production) = (total g protein remained in fish body / 6.25) / (W2–W1)
N excretion (mg/g production) = (N intake (g) – N retention in fish body (g)) / (W2 – W1)

Table 5. Body composition of juvenile European seabass fed diets with different levels of garlic until satiation for a period of 60 days.

		Experimental diets			
	Initial	Control	GM2	GM4	GM6
Moisture (%)	80.0±0.26[a]	79.9±0.74[a]	79.9±1.15[a]	80.1±1.01[a]	80.3±1.45[a]
Crude Protein (%)	42.4±1.05[a]	47.7±0.40[bc]	48.9±0.46[cd]	49.3±0.67[d]	46.9±0.75[b]
Crude Lipid (%)	25.1±2.15[a]	24.7±1.75[a]	21.7±1.64[a]	25.3±2.50[a]	23.1±3.73[a]
Crude Ash (%)	20.0±0.12[d]	14.5±0.02[b]	13.5±0.58[a]	15.9±0.28[c]	15.5±1.06[bc]
NFE (%)	12.5±2.53[ab]	13.0±2.12[ab]	15.9±0.94[b]	9.51±3.19[a]	14.5±3.89[ab]
GE (kJ)	22.1±0.48[a]	23.3±0.41[b]	22.9±0.41[ab]	23.3±0.57[b]	22.7±0.78[ab]

Values (means±standart deviation, triplicate groups) with different superscipts are statistically different at 0.05 level (One-way ANOVA and Duncan's multiple range test, P<0.05).
NFE (Nitrogen free extract) = 100 – (crude protein + crude lipid + crude ash)
GE= Gross energy, calculated according to energy fuels of 23.6 kJ/g protein, 39.5 kJ/g lipid, 17.2 kJ/g NFE.

Eventhough no significant differences (p>0.05) were observed, higher levels of total saturated fatty acids (SFA) such as Palmitoleik acid (PA, 16:1), Oleic acid (OA, 18:1n-9), Miristoleic acid (MA, 14:1) and Nervonic acid (NA, 24:1) were found in the initial fish and the control group fed garlic incorporated diets. The only exception among the SFAs was observed for Gadoleic acid (GA, 20:1n-9), which showed the lowest level (4.758 ±0.02) in the initial fish body, and presented an increase with dietary garlic meal inclusion levels with the highest value of 6.978 ±0.23 in fish fed the 4% garlic meal diet (p<0.05). Similar to the SFAs, the total monounsaturated fatty acids (MUFA) in the initial fish body samples, Linoleic acid (LA, 18:2n-6) was highest (12.77 ±0.26) in the initial fish body, while LA content in fish tissues showed a decline with the increase of dietary garlic level and the lowest value (7.99 ±3.38) was recorded for the GM4 group (p<0.05). α-Linoleic acid (α-LA, 18:3n-6) however was highest (0.152 ±0.006) in the control and lowest (0.128 ±0.004) in the GM6 group (p>0.05). Among the total MUFA, Arachidonic acid (AA, 20:4n-6) in fish body was highest in the initial fish samples, and showed a decline with the increase of dietary garlic meal in the experimental groups. Compared to the SFAs and MUFAs, the total polyunsaturated fatty acids (PUFA, n3/n6) showed a converse trend, with lower levels of PUFA in the initial fish samples or the control group and higher levels for fish fed with garlic incorporated diets. Among the PUFAs, Eicosatrienoic acid (EA, 20:3n-6) in the

final fish body samples from the GM4 treatment was significantly ($p<0.05$) higher then the values found for the initial fish, and those for the GM2 or GM6 groups, but no significant difference ($p>0.05$) was found between the 4% garlic meal and the control diet groups. Ecosapentanoic acid (EPA, 20:5 n-3) followed the same trends with EA values, with a significantly higher ($p<0.05$) value in the experimental group fed the 4% garlic diet. Docosahekzanoic acid (DHA, 22:6 n-3) in the fish muscle tissues were highest in the initial fish samples and the GM4 group, however there was no significant difference ($p>0.05$) among the experimental treatments in general for the DHA. Fatty acid composition in the muscle tissues of seabass juveniles fed experimental diets with different levels of garlic meal is given in Table 6.

The n-3 HUFA such as EPA (20:5n-3), DHA (22:5n-6) or the n-6 HUFA such as Arachidonic acid (AA, 20:4n-6) are indispansible for fish health and welfare as well as a proper growth performance. PUFA such as DHA is indispensable for breeding performance and a high growth and survival rate of larvae (Fernandez-Palacios et al., 1997). Several beneficial effects of polyunsaturated fatty acids (PUFA, n3/n6) have been reported in human health (Li et al., 2008; Buckley and Howe, 2009; Arab-Tehrany et al., 2012; Howe and Buckley, 2014; Yessoufou et al., 2015). In earlier reports, it has been observed that the incorporation of 3% fermented garlic powder in diets for laying hens increased the PUFA:SFA ratio in the egg yolk compared to laying hens fed the control diets or the diets with lower garlic incorporation (Ao et al., 2010). Similarly, Lee et al. (2012) reported that juvenile sterlet sturgeon fed diets with garlic showed lower SFA and MUFA, but higher PUFA compared to the initial fish or those fed the control diet without garlic treatment. The findings in the present study are in accordance with previous reports, indicating that dietary garlic meal may improve unsaturated fatty acid concentrations in fish body by accumulating EPA (20:5n-3) and DHA (22:5n-6) in the tissues. However, this observation found in the present study, in terms of decreasing SFAs and increasing PUFAs in European seabass with dietary garlic incorporation could not be compared with other studies, since there are no reports regarding the relation between dietary garlic and PUFA:SFA ratio in seabass, to our knowledge so far.

Hepatosomatic indexes (HSI) of the initial and the final fish samples were highest in the experimental groups fed with 2% (GM2) and 6% (GM6) garlic meal diets, while significantly ($p<0.05$) lower rates of HSI were found in the control and the 4% (GM4) garlic meal treatments. Significantly lower ($p<0.05$) viscerasomatic indexes (VSI) were also found for fish fed the control and the 4% garlic meal inclusion diet (GM4) compared to the GM2 and GM6 treatments. Similar findings were also recorded for the lipid accumulations around the internal organs, the so called mesenteric fat index (MFI), with significantly lower ($p>0.05$) values for the control and the GM4 groups compared to the GM2 and GM6 treatment groups (Table 7).

In animal nutrition studies, the hepatosomatic index (HSI) is used as an indicator for the energy reserve status of the animal. Since the liver is a target for the metabolism in the fish body, the hepatosomatic index is an effective biomarker for the detection of hazardous effects derived from environmental factors (Pait and Nelson 2003). The HSI in the present study was significantly lower in fish fed diets with 4 % garlic compared to the other garlic inclusion leves. However, the lowest HSI recorded in the 4% garlic group was not significantly different then the control group without garlic meal addition. Our results are in partial agreement with the findings of Abdel-Hakim et al. (2010) who reported that there are slight differences in HSI in fish fed the garlic meal diet however the diffeneces between the garlic treatment groups and the control group were insignificant. Shalaby et al. (2006) found that supplementing garlic meal in Nile tilapia diets at increasing levels from 1 to 4 %, did not affect the HSI in percent. In contrast, Metwally (2009) who used different forms of garlic in diets for Nile tilapia fingerlings (natural garlic 40 g/kg diet, garlic oil capsules 250 mg/kg diet, and garlic powder 32g/kg diet), reported that HSI in all experimental diets with different forms of garlic decreased significantly. Similarly, Lee et al. (2014) also presented significantly lower HSI in sturgeon fed diets containing garlic powder than that of fish group fed diets without garlic inclusion.

Table 6. Fatty acid composition (%) in muscle tissues of juvenile European seabass fed diets with different levels of garlic meal until satiation for a period of 60 days.

			Experimental diets			
		Initial	Control	GM2	GM4	GM6
ΣSFA (Total saturated fatty acid)						
16:1	PA	7.116 ± 0.66^{a}	6.994 ± 0.85^{a}	6.895 ± 0.15^{a}	6.674 ± 0.67^{a}	6.910 ± 0.02^{a}
18:1 (n-9)	OA	28.26 ± 0.44^{a}	30.65 ± 1.45^{a}	30.46 ± 0.06^{a}	30.39 ± 2.13^{a}	30.42 ± 0.11^{a}
14:1	MA	0.0388±0.003^{a}	0.0424±0.011^{a}	0.0369±0.000^{a}	0.0349±0.002^{a}	0.040±0.001^{a}
20:1 (n-9)	GA	4.758 ± 0.02^{a}	5.206 ± 1.15^{ab}	6.421 ± 0.23^{bc}	6.978 ± 0.23^{c}	6.640 ± 0.06^{c}
24:1	NA	0.0388±0.001^{b}	0.0296±0.003^{a}	0.0366±0.001^{b}	0.0369±0.000^{b}	0.036±0.001^{b}
ΣMUFA (Total mono unsaturated fatty acid)						
18:2 (n-6)	LA	12.77 ± 0.26^{b}	10.83 ± 1.03^{ab}	10.21 ± 0.13^{ab}	7.99 ± 3.38^{a}	10.34±0.20^{ab}
18:3 (n-6)	α-LA	0.149 ± 0.009^{b}	0.152 ± 0.006^{b}	0.140 ± 0.001^{ab}	0.144 ± 0.001^{b}	0.128±0.004^{a}
20:4 (n-6)	AA	0.619 ± 0.025^{b}	0.425 ± 0.054^{a}	0.439 ± 0.011^{a}	0.442 ± 0.013^{a}	0.423±0.021^{a}
ΣPUFA (Total polyunsaturated fatty acid, n3/n6)						
20:3(n-6)	EA	0.156 ± 0.004^{a}	0.158 ± 0.002^{ab}	0.155 ± 0.002^{a}	0.169 ± 0.009^{b}	0.152±0.002^{a}
20:5 (n-3)	EPA	5.127 ± 0.199^{a}	5.687 ± 0.123^{b}	5.249 ± 0.031^{a}	5.728 ± 0.152^{b}	4.967±0.099^{a}
22:6 (n-3)	DHA	12.81 ± 0.51^{a}	11.99 ± 1.31^{a}	11.89 ± 0.01^{a}	12.37 ± 0.14^{a}	11.52 ± 0.02^{a}

Values (means±standart deviation, triplicate groups) with different superscipts are statistically different at 0.05 level (One-way ANOVA and Duncan's multiple range test, P<0.05).
GM (Garlic meal), PA (Palmitoleik acid, 16:1), OA (Oleic acid, 18:1 n-9), MA (Miristoleic acid, 14:1), GA (Gadoleic acid, 20:1 n-9), NA (Nervonic acid, 24:1), LA (Linoleic acid, 18:2 n-6), α LA (α-Linoleic acid, 18:3 n-6), EA (Eicosatrienoic acid, 20:3 n-3+n-6), AA (Arachidonic acid, 20:4 n-6), EPA (Ecosapentanoic acid, 20:5 n-3), DHA (Docosahekzanoic acid, 22:6 n-3).

Table 7. Body morphological indices of juvenile European seabass fed diets with different levels of garlic meal until satiation for a period of 60 days.

	Experimental diets			
	Control	GM2	GM4	GM6
HSI	0.99±0.29^{ab}	1.46±0.41^{c}	0.93±0.25^{a}	1.27±0.26^{bc}
VSI	8.30±1.07^{a}	9.34±1.72^{ab}	8.43±1.63^{a}	10.2±1.26^{b}
MFI	1.64±0.72^{a}	4.16±1.30^{b}	1.93±0.82^{a}	3.22±1.39^{b}

Values (means±standart deviation, triplicate groups) with different superscipts are statistically different at 0.05 level (One-way ANOVA and Duncan's multiple range test, P<0.05).
HSI= Hepatosomatic index, VSI= Viscerasomatic index, MFI= Mesenteric fat index, GM= Garlic meal

Conclusion

The results obtained in the present study demonstrated that garlic meal as a natural feed additive represents alternative solutions to induce aquafeeds as a growth promoter. It might be concluded that the dietary garlic inclusion levels affect growth performance, feed utilization and body protein content of European seabass at ongrowing stage. Furthermore, a lowering effect on nitrogen excretion rate was also recorded when garlic meal was incorporated in diets for seabass at ongrowing stage. Based on the tendency of a lowered nitrogen excretion or enhanced retention rates found in the present study, it might be interesting to search the long-term effect of dietary garlic on nitrogenous end-products and fish growth. Additionally, dietary garlic meal improved unsaturated fatty acid concentrations by accumulating EPA (20:5n-3) and DHA (22:5n-6) in the tissues of seabass juveniles, as a result of lowered SFAs and MUFAs, but increased PUFAs in fish fed garlic supplemented diets. The suggested dietary garlic for seabass juveniles in the present study was 4% (40 g/kg) for a positive influence on growth performance and nutrient utilization. Further studies are encouraged to focus on the total economic cost and benefit analysis for

the use of garlic in large scale aquaculture operations.

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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

THE OCCURRENCE OF *Ligula intestinalis* IN ITS FISH HOST *Rutilus rutilus* (L.) AND THE EFFECTS OF PARASITE ON THE FISH GROWTH (BÜYÜKÇEKMECE RESERVOIR, TURKEY)

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Abstract:

Roach *Rutilus rutilus* (Linnaeus, 1758) is a very common fish species for Turkish inland waters, especially in Büyükçekmece Reservoir (İstanbul). The species is one of the most caught fish species and holds economic consumption value even though it is mostly infected by *Ligula intestinalis* (Linnaeus, 1758), a pseudophyllidean cestode causing severe pathological effects on fishes. The aim of the study was to determine the presence of *L. intestinalis* plerocercoids in its fish host *R. rutilus* and investigate the effects of the parasite on the condition of the fish. With this purpose, the fish specimens have captured from Büyükçekmece Reservoir with using gillnets having different mesh sizes (10×10 mm, 20×20 mm, 30×30 mm, 40×40 mm and 50×50 mm) from March 2009 to February 2010. The fork length and body weight of fish specimens (n=1857) were varied between 6.0–29.2 cm and 2.53–561.00 g, respectively. In total, 4.52% of specimens were infected by the plerocercoids. Infection by *L. intestinalis* was observed during summer, autumn and winter months but not spring. Parasite-host index (PSI %), prevalence (%) and mean intensity of plerocercoids for infected fishes were calculated, monthly. PSI (%) was estimated maximum in January as 18.49% while prevalence (%) was 32.31% in July and mean intensity of plerocercoids is 6.0 in October. Statistically significant differences between K values of non-infected and infected specimens among length groups and months were recorded (Mann-Whitney U Test, $p<0.05$). Results showed that, *L. intestinalis* plerocercoids seem to be affected significantly on the condition and body health of its host *R. rutilus*.

Keywords: Plerocercoid, Condition, Intensity, Parasite-host index, Prevalence

Introduction

Fish is very important protein sources as food besides their importance for the economies of countries and being an object of sportive and ornamental fishing. Besides direct losses caused by mortality, parasites may have considerable impact on growth and fish behavior, their resistance to other stressing factors, susceptibility to predation, etc.; their presence may also reduce marketability of fish (Scholz, 1999). In Turkish inland waters, especially in Marmara Region, roach *Rutilus rutilus* (L.) is very common and has an economical value (Geldiay and Balık, 2007). Its first record for Büyükçekmece Reservoir is given in 1986 (Meriç, 1986) and now it's one of the most dominant fish species of the reservoir.

Ligula intestinalis (L., 1758) is a pseudophyllidean cestode which is known to induce severe pathological effects on fish (Ergönül and Altındağ, 2005; Loot et al., 2001) and the most common infection parasite reported in Turkish inland waters (İnnal et al., 2007). Ligulids have a complex life cycle involving copepods, fishes and birds. Firstly, the coracidium larva surviving 1-2 days in the water penetrates the gut wall of a copepod and develops into a procercoid. The infected copepod is ingested by a planktivorous cyprinid fish (e.g. *R. rutilus*), and the parasite larvae develop into the plerocercoid stage in the body cavity. The definitive host is an ichtyophagous predatory bird in which *L. intestinalis* reaches sexual maturity. Parasite eggs are then released into the water with bird faeces (Loot et al., 2001; İnnal et al., 2007).

Infection with *L. intestinalis* effects cultured or free–living fish of freshwater in all over the world (Shargh et al., 2008). It has been reported from a broad range of fish families, such as Cyprinidae, Cobitidae, Salmonidae, Esocidae, Pleuronectidae or Siluridae (İnnal et al., 2007; Bouzid et al., 2008). It is known to effect especially *Alburnus escherichii, Leuciscus cephalus, Tinca tinca*, *Cyprinus carpio*, *R. rutilus* which are members of the Cyprinidae (Loot et al., 2001; Ergönül and Altındağ, 2005; İnnal et al., 2007). According to Williams and Hoole (1992), numerous ecological and pathological studies have revealed that the parasite can seriously affect the population dynamics of both wild fish and those involved in aquaculture. There is limited data on relation of the parasite and its fish host *R. rutilus* (Kennedy and Burrough, 1981; Loot et al., 2001; Oğuz et al., 2004; Carter et al., 2005). The aim of the present study is to determine the occurrence of the parasites on host and investigate the effects of parasite for condition and length–weight relationship of host fishes living in Büyükçekmece Reservoir.

Materials and Methods

The present study was carried out in Büyükçekmece Reservoir between March 2009 and February 2010. During the monthly sampling surveys, gillnets (50 m length and 2.5 m depth) having different mesh size (10×10 mm, 20×20 mm, 30×30 mm, 40×40 mm and 50×50 mm) were used for fishing. Fish specimens were measured to the nearest 0.1 cm (fork length, FL) and 0.01 g (body weight, W), and then dissected to determine the number and weight of plerocercoid larval forms of parasites occurring in the abdominal cavity. Prevalence (%) and mean intensity were calculated according to Bush et al. (1997). Parasite-host index (PSI %) was calculated according to Kesler et al. (2009). Variations of prevalence (%), mean intensity and PSI (%) among months and length groups were examined.

The length–weight relationship was calculated using the equation "$W = a*L^b$", where W is the total weight (g), L is the fork length (cm), and *a* and *b* are the equation parameters (Le Cren, 1951; Froese, 2006). Fish condition was assessed by Fulton's Condition Factor "$K=(W/L^3)*100$" (Ricker, 1975) for infected (without plerocercoids) and non–infected specimens. All statistical analyses were performed with SPSS software 16.0. Prior to statistical analysis, all data were tested for normality of distribution using the Kolmogornov–Smirnov test. Mann Whitney U test was performed to test the differences between the length, weight and condition values of infected and non-infected fish among months and length groups (Zar, 1999).

Results and Discussion

In present study, a total of 1857 *R. rutilus* specimens, captured from the Büyükçekmece Reservoir, were investigated and the results showed that 84 of the 1857 fishes (4.52%) were infected by plerocercoids. The fork length ranged from 6.5 to 25.0 cm for infected specimens while it was between 6.0 and 29.2 cm for non-infected specimens. Total weight ranged between 3.66 and 327.30 g for infected fish and 2.53 and

561.00 g for non-infected fish, respectively (Table 1).

Infection by *L. intestinalis* was observed during summer, autumn and winter months but not spring. The maximum infection rate was determined in July while it was lower during the autumn and winter (Figure 1). Parasite-host index (PSI %), prevalence (%) and mean intensity of plerocercoids for infected fishes were calculated among months (Table 2) and length groups (Table 3). PSI (%) was estimated maximum in January as 18.49% while prevalence (%) was 32.31% in July and mean intensity of plerocercoids was 6.0 in October.

The condition factors of non-infected and infected specimens (without plerocercoids) specimens are given at Table 1. Statistically significant differences between K values of non-infected and infected specimens among length groups and months were recorded (Mann-Whitney U Test, $p<0.05$). Differences between K values for non-infected and infected specimens among length groups and months are shown in Table 3 and Table 4.

The length–weight relationships were estimated and *b* values were determined as 3.383±0.001 and 3.222±0.011 for the non–infected fishes and infected fishes (without plerocercoids), respectively (Figure 2).

It was reported that the values of intensity and prevalence have changed according to seasonal conditions and fish species, increasing of the parasites especially in summer (Akmirza, 2007). Since the immune response of poikilothermic vertebrates is temperature dependent, lower water temperatures would result in a lower antibody response (Williams and Hole, 1992). Oğuz et al. (2004) were observed that the rate of infection increased steadily from winter to summer and autumn except spring. In Yenice Irrigation Pond (Turkey), the maximum density of this parasite has occurred in July. Similarly, in present study, infection wasn't observed in spring and the maximum infection rate was determined in July while it was lower during the autumn and winter.

The condition factor of infected fishes is significantly lower than that of healthy ones (Mahon, 1976). In present study, a similar result was monitored as condition factor of infected fishes is significantly lower from the non-infected specimens (Table 3 and Table 4). The parasite may have changed the food intake of the host and so, the condition as an indicator of feeding intensity of fish was effected negatively. Also, many researchers have studied some effects of this parasite for fish host such as growth, endocrine system, gonadal development and had determined that *L. intestinalis* was caused many pathogenic effect on host fish. The parasite can cause damage to the host fish specimens especially by compression and atrophy of vital organs including the gonads, liver in the coelomic cavity of the infected fish (Öztürk and Altınel, 2001; Jopling and Taylor, 2003; Oğuz et al., 2004; Carter et al., 2005; Hecker and Karbe, 2005; Dejen et al., 2006). Similarly, in present study, macroscopic investigations showed that stomachs of fish specimens were swollen and gonads were deformed. Conversely, Ergönül and Altındağ (2005) reported that the condition factors calculated for infected and non-infected tench did not exhibit a marked difference for Mogan Lake population and, Weekes and Penlington (1986) were found no significant difference in condition factors between infected and uninfected trout *Salmo gairdneri* in New Zealand.

Table 1. Descriptive statistics and estimated parameters of length-weight relationships and condition factors of infected and non-infected *R. rutilus*, Büyükçekmece Reservoir, İstanbul, Turkey (n, number of individuals; FL, fork length; W, body weight; K, condition factor; b, slope; CL, confidence limits; r^2, coefficient correlation).

	n	FL (cm)	W(g)	K	*b* (±95% CL)	♀	♂
Infected specimens	84	6.5 – 25.0	3.66 – 327.3	1.56	3.222 ±0.011	24	36
Non – infected specimens	1773	6.0 – 29.2	2.53 – 561.0	1.52	3.383 ±0.001	468	501

Table 2. Prevalence (%), mean intensity and PSI (%) among months (n*: number of infected specimens; n: number of non-infected specimens; pn: plerocercoid numbers)

Months	n*	n	Prevalence (%)	Mean Intensity	PSI (%) (min. - max.)	pn (min. - max.)
June	11	154	6.77	2.6	13.12 (4.04 - 20.91)	29 (1 - 6)
July	21	44	32.31	3.6	14.03 (3.49 - 33.98)	75 (1 - 13)
August	11	30	26.83	4.5	12.14 (4.88 - 42.21)	49 (1 - 8)
September	17	56	23.29	3.9	13.87 (4.89 - 24.39)	66 (1 - 9)
October	1	58	1.69	6.0	9.35	6
November	4	44	8.33	4.5	11.41 (4.77 - 20.17)	18 (1 - 13)
December	13	93	12.26	2.9	12.63 (5.01 - 25.32)	38 (1 - 11)
January	5	97	4.90	2.8	18.49 (12.04 - 21.07)	14 (2 - 4)
February	1	123	0.81	2.0	11.13	2

Table 3. Comparison of condition factor (K) with prevalence (%), mean intensity and PSI (%) among length groups for non-infected and infected fishes without plerocercoids (n*: number of infected specimens; n: number of non-infected specimens; pn: plerocercoid numbers; *p*: values of Mann-Whitney U Test)

Length Groups (cm)	n*	n	K		*p* (0.05)	Prevalence (%)	Mean Intensity	PSI (%) (min. - max.)	pn (min. - max.)
			Infected	Non-infected					
6.0-8.9	1	893	1.27	1.27±0.18 (0.94-2.02)	–	0.11	1	5.01	1
9.0-11.9	9	38	1.55±0.18 (1.26-1.81)	1.82±0.14 (1.51-2.24)	**0.000***	19.15	3.4	14.04 (4.99 – 25.3)	31 (1 – 7)
12.0-14.9	61	408	1.53±0.11 (1.33-1.88)	1.69±0.13 (1.22-2.24)	**0.000***	13.01	3.1	13.98 (3.49 – 42.21)	189 (1 – 9)
15.0-17.9	6	125	1.77±0.07 (1.65-1.86)	1.87±0.14 (1.57-2.21)	**0.035***	4.58	5.7	14.17 (4.04 – 28.56)	34 (2 – 13)
18.0-20.9	6	249	1.64±1.80 (1.36-1.84)	1.84±0.12 (1.58-2.24)	**0.003***	2.35	4.8	9.49 (7.85 – 12.18)	39 (2 – 11)
21.0-23.9	0	47	–	1.88±0.10 (1.72-2.18)	–	–	–	–	–
24.0-26.9	1	8	1.91	1.97±0.20 (1.72-2.27)	–	11.11	13.0	4.77	13
27.0-29.9	0	4	–	2.17±0.08 (2.12-2.30)	–	–	–	–	–

* means significant differences between groups

Table 4. The comparison of fork length (FL), weight (W) and condition factor (K) between infected and non-infected fish among months (n* the number of infected fish: n: the number of non-infected fish; *p*: values of Mann-Whitney U Test)

Months	n*	n	FL (cm)		*p* (0.05)	W (g)		*p* (0.05)	K		*p* (0.05)
			Infected	Non-infected		Infected	Non-infected		Infected	Non-infected	
June	11	154	13.7 ±2.1 (11.8 - 18.3)	14.2 ±2.5 (11.0 - 20.2)	0.909	51.59 ±27.73 (31.4 - 116.82)	59.09 ±35.01 (23.38 - 180.02)	0.927	1.66 ±0.14 (1.43 - 1.88)	1.87 ±0.13 (1.52 - 2.24)	**0.000***
July	21	44	13.2 ±1.7 (11.6 - 18.0)	14.4 ±2.6 (11.6 - 19.5)	0.087	44.10 ±21.73 (28.76 - 106.80)	59.31 ±34.88 (26.94 - 152.16)	0.126	1.82 ±0.12 (1.65 - 2.13)	1.80 ±0.15 (1.44 - 2.24)	0.715
August	11	30	12.6 ±0.8 (11.3 - 13.6)	13.7 ±2.3 (10.8 - 19.3)	0.375	36.83 ±6.62 (27.12 - 49.30)	50.15 ±32.18 (22.06 - 132.03)	0.591	1.54 ±0.14 (1.26 - 1.81)	1.78 ±0.12 (1.54 - 2.06)	**0.000***
September	17	56	13.7 ±1.8 (12.0 - 18.8)	16.1 ±3.6 (11.2 - 22.9)	**0.033***	46.56 ±26.28 (26.28 - 128.14)	85.21 ±57.46 (25.94 - 216.6)	0.070	1.54 ±0.12 (1.35 - 1.84)	1.74 ±0.13 (1.48 - 2.00)	**0.000***
October	1	58	19.5	18.3 ±3.6 (12.3 - 27.3)	–	134.53	126.20 ±72.36 (30.56 - 430.8)	–	1.66	1.82 ±0.12 (1.56 - 2.12)	–
November	4	44	15.5 ±6.3 (12.2 - 25.0)	18.3 ±4.3 (12.6 - 29.0)	0.090	105.59 ±147.81 (30.66 - 327.3)	133.99 ±111.06 (32.13 - 561.0)	0.106	1.57 ±0.25 (1.33 - 1.91)	1.80 ±0.16 (1.50 - 2.30)	0.090
December	13	93	13.5 ±3.4 (6.5 - 20.9)	15.3 ±4.7 (6.0 - 23.4)	0.114	48.41 ±38.02 (3.66 - 145.44)	80.85 ±58.28 (2.99 - 24.31)	0.124	1.47 ±0.11 (1.27 - 1.65)	1.70 ±0.18 (1.29 - 2.15)	**0.000***
January	5	97	12.9 ±0.8 (12.0 - 14.1)	16.6 ±3.4 (6.7 - 22.3)	**0.006***	35.72 ±4.39 (33.03 - 43.34)	93.38 ±52.68 (3.97 - 212.2)	**0.014***	1.44 ±0.08 (1.38 - 1.58)	1.75 ±0.17 (1.22 - 2.06)	**0.001***
February	1	123	11.9	16.3 ±3.8 (9.7 - 25.5)	–	26.42	91.84 ±69.84 (14.89 - 377.2)	–	1.43	1.75 ±0.17 (1.22 - 2.27)	–

* means significant differences between groups

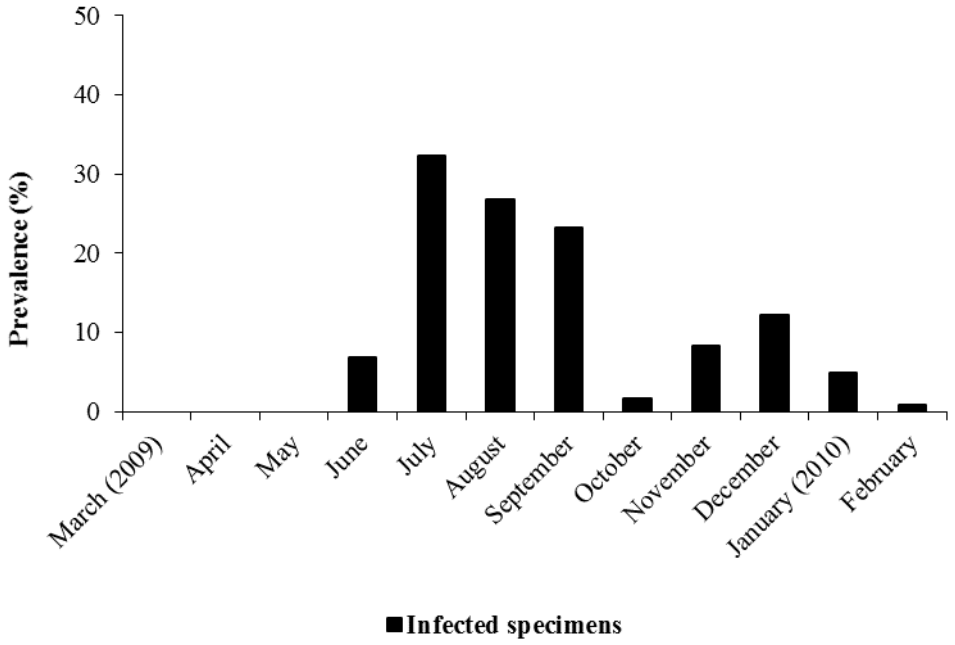

Figure 1. Monthly variations of prevalence values (%) for infected *R. rutilus* specimens, Büyükçekmece Reservoir, İstanbul, Turkey.

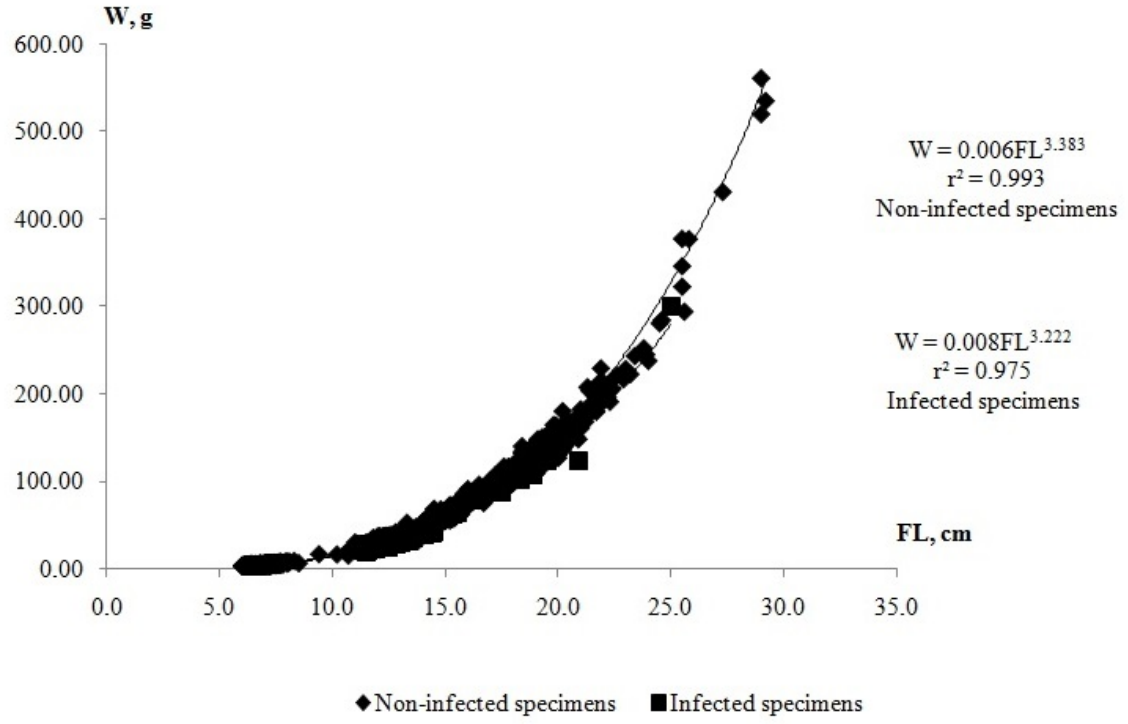

Figure 2. The length-weight relationships for non-infected and infected specimens of *R. rutilus*, Büyükçekmece Reservoir, İstanbul, Turkey.

Kelle (1978), were compared length–weight relationship between infected and non-infected specimens and were determined a reducing about 19 % for infected *Acanthobrama marmid* specimens in Devegeçidi Dam Lake. Ergönül and Altındağ (2005) were found a marked difference between the *b* value among infected and non–infected tench and were estimated value of *b* as 2.745 for infected and 3.014 for non–infected tench in Mogan Lake. But in present study, the growth was determined as positive allometrical and the *b* values were estimated with 3.222 ±0.011 and 3.383 ±0.001 for of infected and non–infected specimens, respectively.

The prevalence (%) among the length groups showed that, infection rates were high in 9.0-11.9 and 12.0-14.9 cm length groups. According to Tarkan (2006), sizes at maturation for *R. rutilus* living in Sapanca Lake (Marmara Region, Turkey) is 12.26 cm in males and 14.98 cm in females (total length). In present study, our macroscopic investigations also showed that the gonads of the roach maturated after 11.0 cm (fork length) and gonadal damages were observed in infected fish specimens. It was thought that besides the negative effects on fish growth, it may have effected on fish reproductive activity.

Conclusion

In conclusion, *L. intestinalis* plerocercoids appear to have significant effect on fish condition within certain length groups. Due to the high infection rates specifically during summer months, it is led to believe that this parasite may seriously effect fish health. After reproduction, efficient fish feeding is essential to regain general fish condition, decreased by spawning activity. According to the findings of this study, the status of *L. intestinalis* may be a threat to the host's, *R. rutilus,* presence in the lake.

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FULL PAPER TAM MAKALE

ANAESTHETIC EFFECTS OF SODIUM BICARBONATE AT DIFFERENT CONCENTRATIONS ON AFRICAN CATFISH (*Clarias gariepinus*) JUVENILES

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Abstract:

In the present study, anaesthetic effects of different sizes (5, 10, 15, 20, 25, 30, 35 and 40g) of African catfish (*Clarias gariepinus*) juveniles was investigated at different concentrations (0/control, 10, 15, 20, 25, 30, 35, 40, 45, 50 g/L) of sodium bicarbonate. Juveniles were bath immersed in each of the different concentrations of sodium bicarbonate solution for 5 minutes and recovery times in clean aerated water was established. Juveniles took between 0.67 to 5.44 minutes to reach full anaesthesia and 0.66 to 4.25 minutes to recover fully from anaesthesia. An inverse exponential relationship was observed between sodium bicarbonate concentrations and induction time, whereas exponential relationship was observed between sodium bicarbonate concentrations and recovery time. Size of *C. gariepinus* juveniles significantly affected the induction time ($P < 0.05$) with smaller juveniles getting to full anaesthesia faster than bigger juveniles at the same concentration of sodium bicarbonate. In addition, recovery time was size dependent where smaller *C. gariepinus juveniles* took significantly longer time ($P < 0.05$) to recover from anaesthesia than bigger juveniles. All juveniles reached anaesthesia and recovered in less than 5 minutes except for 30 g juveniles at 15g/L of sodium bicarbonate which was anaesthetized at 5.44 minutes.

Keywords: Immersion, Induction, Recovery, Anaesthesia

Introduction

Anaesthetics are used to minimize stress associated with handling live fish when marking, tagging, counting, injecting, transporting, weighing, artificial reproduction and surgical operations among others (Hajek et al., 2006, Altun et al., 2009, Yildiz et al., 2013, Opiyo et al., 2013). The need to handle fish without impairing their health or commercial value has led to the development of many techniques to anaesthetize fish (Yildiz et al., 2013). Different anaesthetics act with various intensity driving fish in to general anaesthesia, resulting in loss of consciousness, inhibition of reflex activity, and reduction in skeletal muscle tone (Hajek et al., 2006). Quick induction and recovery from anaesthesia is desirable in most cases (Marking & Meyer, 1985; Stoskopf, 1993). However, long recovery time is desirable while collecting fish from the wild or where fish must be handled for a longer time in the laboratory. An ideal anaesthetic should possess several attributes such as; non-toxic, inexpensive, simple to administer and result in rapid induction and calm recovery (Pawar et al., 2011). It is of paramount importance to identify the effective dose of anaesthetic for specific fish species since response to the same anaesthetic vary depending on the concentration used and the species of fish (Tytler and Hawkins, 1981; Pawar, et al., 2011). Common fish anaesthetics include clove oil, sodium bicarbonate, carbon dioxide gas, metomidate, benzocaine, tricaine, methanesulphonate (MS-222), 2-phenoxylethanol and quinaldine (Massee et al., 1995; Palić et al., 2006). Regardless of the agent, the process of anaesthesia in fish develops in a similar way and runs in a progressive pattern (McFarland, 1959).

Sodium bicarbonate (Na_2CO_3) also known as baking soda, is a white substance that gives carbon dioxide when dissolved in water (McFarland and Klontz, 1969). Its main advantages lie in its low cost, wide availability and safety to both fish and humans (Altun et al., 2009). Sodium bicarbonate has been effectively used as an anaesthetic in common carp (*Cyprinus carpio*) in both cold and warm water conditions (Booke et al., 1978, Altun et al., 2009), Rainbow trout (*Oncorhyncus mykiss*) (Keen et al., 1998) and in Nile tilapia (*Oreochromis niloticus*) (Opiyo et al., 2013).

The African catfish (Clarias gariepinus), is found in several countries throughout Africa, where it has a native distributional range as well as in Europe (the Netherlands, Germany and Belgium), Asia (Indonesia, Thailand) and South America (Brazil) (de Graaf and Janssen, 1996; Brummett, 2008). *C. gariepinus* is the second most important freshwater cultured fish (after tilapia) in Africa (Barasa et al., 2014) with the exception of Nigeria where its production far exceeds that of tilapia production and accounts for 70–80 % of the total freshwater fish production (Ponzoni and Nguyen, 2008). Successful culture of *C. gariepinus* requires artificial propagation whereby there is need of injection of the fish with gonadotropin to spawn and the juveniles are often moved from one place to another during sorting and grading at the larval growth phases (de Graaf and Janssen, 1996). These procedures may induce stress in the fish hence require use of anaesthetics to induce calming effects and reduce the mobility of the aggressive fish. *C. gariepinus* is therefore an ideal species to examine the suitability of sodium bicarbonate as an anaesthetic. The aim of the current study is to determine the efficacy and optimum concentration of sodium bicarbonate in different sizes of *C. gariepinus* which hitherto has not been reported.

Materials and Methods

C. gariepinus juveniles of average weight 10, 15, 20, 25, 30, 35 and 40 g were collected from the nursery ponds at Kenya Marine and Fisheries Research Institute, Kegati Aquaculture Research Station, Kenya. The juveniles were starved for 24 hours to allow for gut evacuation prior to experiment (Weyl et al., 1996). Average water temperature was at 23.5 ±1.2°C, pH (7.8 ±0.18), dissolved oxygen (4.5 ±1.7) mg/L, and Total Ammonia Nitrogen (0.1 ±0.1) mg/L in the experimental aquaria. To determine the optimal dosage and effect of size on anaesthetic efficacy of sodium bicarbonate, a group of 5 juveniles from each weight class were exposed to different concentrations of sodium bicarbonate by bath immersion. The experiment was carried out in 15 L glass aquaria in triplicates. The concentrations were increased by an interval of 5 based on the ability of the fish to go to stage III anaesthesia as described by Iwama et al., (1989) and Palić et al., (2006). Concentrations of sodium bicarbonate used were (0/control, 10, 15, 20, 25, 30, 35, 40, 45, 50 g/L). The juveniles were observed for three different stages of induction and recovery time (Table 1) as described by Iwama et al., (1989) and Palić et al., (2006).

Table 1. Stages of induction and recovery

Stage of induction	Description
I	Loss of equilibrium
II	Loss of gross body movements but continued opercula movement
III	Same as stage II but opercula movement ceases
Stages of recovery	**Description**
I	No body movement but opercula movement start
II	Regular opercula movements and body movements start
III	Equilibrium regained with pre-anaesthetic appearance

*Adapted from Iwama et al. (1989) and Palić et al. (2006).

After full anaesthesia, juveniles were removed from the anaesthetic solution and transferred to another aquarium with clean aerated well water for recovery. The time of induction to anesthesia and recovery were recorded and fish were maintained there for 48 hours in order to observe their behavior and possible mortality.

Differences among means were analyzed by two-way ANOVA followed by Duncan's multiple range tests. All analyses were performed with SPSS version 17.0 statistical package. Differences were considered significant at $P< 0.05$.

Results and Discussion

Induction and recovery time of *C. gariepinus* juveniles exposed to different concentrations of sodium bicarbonate are shown in table 2 and 3. In general juveniles took between 0.67 to 5.44 minutes to reach full anaesthesia in different concentrations of sodium bicarbonate (Table 2). Juveniles exposed to 0 and 10g/L concentration of sodium bicarbonate did not undergo anaesthesia. In addition, 35 and 40 g juveniles did not undergo anesthesia when exposed to 15g/L concentration of sodium bicarbonate. The recovery time for the juveniles which did not undergo anaesthesia was not recorded. Induction time decreased with increase in the concentration of sodium bicarbonate while recovery time increased with increase in the concentration of sodium bicarbonate (Figure 1 and 2). Concentration of sodium bicarbonate solution had a significant effect ($P<0.05$) on the induction time to anaesthesia with juveniles attaining full anaesthesia faster at a higher concentration than at a lower concentration. Juveniles weighing 10g took 0.67 minutes to attain anaesthesia at 50 g/L concentration of sodium bicarbonate and 3.04 minutes at 15 g/L of sodium bicarbonate. Size of *C. gariepinus* juveniles significantly affected the induction time to anaesthesia ($P<0.05$) with smaller juveniles getting to full anaesthesia faster than bigger juveniles at the same concentration of sodium bicarbonate. At a concentration of 20g/L, 10g juveniles took 2.78 minutes to attain anaesthesia while 40 g juveniles took 4.98 minutes to attain anaesthesia.

C. gariepinus juveniles generally took between 0.66 to 4.25 minutes to fully recover from the anaesthetic solution. Sodium bicarbonate concentration significantly ($P < 0.05$) affected the recovery time from anaesthesia with juveniles exposed to a higher concentration taking longer to recover than those exposed to a lower concentration (Table 3). 10 g juveniles took 4.25 minutes to recover from anaesthesia at 50g/L of sodium bicarbonate concentration and 1.66 minutes at 15g/L of sodium bicarbonate concentration. Size of juveniles also significantly ($P < 0.05$) affected the recovery time from anaesthesia with smaller juveniles taking longer time to recover than bigger juveniles at the same concentration of sodium bicarbonate. 10 g juveniles took 1.88 minutes to recover from anaesthesia while 40 g juveniles took 0.66 minutes to recover from anaesthesia when exposed to 20g/L concentration of sodium bicarbonate. No mortality was recorded in any concentration of sodium bicarbonate during the application and post recovery period.

Anaesthetics play a vital role in modern day aquaculture. It helps to reduce the stress which makes fish more susceptible to diseases due to resistance during handling (Ross and Ross, 1999). The use of sodium bicarbonate from baking powder as an anaesthetic in fish is a relatively cheap technique because of its affordability and ease of application (Opiyo et al., 2013). It is safe for human and there is no banning or restriction in its use as it leaves no tissue residues (Altun et al., 2009). Consequently, it can be used on animals entering the human food chain without any withdrawal period (Ross and Ross, 1999). The effectiveness of sodium bicarbonate depends on the weight of the fish and the concentration of administration as reported by Booke et al., (1978) and Altun et al., (2009). Similar phenomenon was realized in the present study.

Table 2. Induction time (minutes) for different sizes of *C. gariepinus* juveniles exposed to different concentrations of sodium bicarbonate

	Fish weight (g)						
Concentration (g/L)	10	15	20	25	30	35	40
0	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–
15	3.04 ± 0.01^{aA}	3.42 ± 0.00^{bA}	3.81 ± 0.05^{cA}	4.38 ± 0.01^{dA}	5.44 ± 0.01^{eA}	–	–
20	2.78 ± 0.00^{aB}	3.06 ± 0.02^{bB}	3.14 ± 0.00^{cB}	3.34 ± 0.01^{dB}	4.12 ± 0.00^{eB}	4.89 ± 0.01^{fA}	4.98 ± 0.01^{gA}
25	2.31 ± 0.01^{aC}	1.97 ± 0.01^{bC}	2.19 ± 0.01^{cC}	2.45 ± 0.00^{dC}	3.67 ± 0.00^{eC}	4.00 ± 0.00^{fB}	4.66 ± 0.00^{gB}
30	2.04 ± 0.00^{aD}	1.22 ± 0.01^{bD}	1.42 ± 0.01^{cD}	1.88 ± 0.01^{dD}	3.04 ± 0.03^{eD}	3.12 ± 0.00^{fC}	3.21 ± 0.00^{gC}
35	1.16 ± 0.03^{aE}	1.08 ± 0.08^{bE}	1.27 ± 0.06^{cE}	1.35 ± 0.01^{dE}	1.99 ± 0.00^{eE}	2.35 ± 0.01^{fD}	2.77 ± 0.00^{gD}
40	1.01 ± 0.03^{aF}	0.91 ± 0.01^{bF}	1.13 ± 0.01^{cF}	1.23 ± 0.01^{dF}	1.55 ± 0.00^{eF}	1.75 ± 0.01^{fE}	2.11 ± 0.01^{gE}
45	0.85 ± 0.01^{aG}	0.85 ± 0.01^{aG}	0.99 ± 0.01^{bG}	1.14 ± 0.01^{cG}	1.25 ± 0.01^{dG}	1.33 ± 0.00^{eF}	1.60 ± 0.00^{fF}
50	0.67 ± 0.01^{aH}	0.76 ± 0.04^{bH}	0.90 ± 0.00^{cH}	1.01 ± 0.01^{dH}	1.06 ± 0.00^{eH}	1.12 ± 0.01^{fG}	1.23 ± 0.00^{gG}

* Data are presented as Mean values ± SE of five determinations

** Means identified by different small letters in the rows or capital letters in the columns were significantly different ($P < 0.05$).

*** The sign −, means no change in the fish

Table 3. Recovery time (minutes) for different sizes of *C. gariepinus* juveniles exposed to different concentrations of sodium bicarbonate

	Fish weight (g)						
Concentration (g/L)	10	15	20	25	30	35	40
0	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–
15	1.66 ± 0.15^{aA}	1.65 ± 0.01^{bA}	1.65 ± 0.12^{cA}	1.36 ± 0.02^{dA}	0.98 ± 0.01^{eA}	–	–
20	1.88 ± 0.03^{aB}	1.62 ± 0.15^{bB}	1.70 ± 0.01^{cB}	1.55 ± 0.01^{dB}	1.37 ± 0.03^{eB}	1.02 ± 0.03^{fA}	0.66 ± 0.01^{gA}
25	2.33 ± 0.01^{aC}	2.27 ± 0.02^{bC}	2.04 ± 0.01^{cC}	1.87 ± 0.01^{dC}	1.78 ± 0.03^{eC}	1.58 ± 0.02^{fB}	1.04 ± 0.00^{gB}
30	2.72 ± 0.01^{aD}	2.62 ± 0.05^{bD}	2.17 ± 0.02^{cD}	2.46 ± 0.02^{dD}	2.50 ± 0.01^{eD}	1.90 ± 0.01^{fC}	1.61 ± 0.02^{gC}
35	3.28 ± 0.03^{aE}	3.13 ± 0.03^{bE}	2.74 ± 0.02^{cE}	3.00 ± 0.01^{dE}	2.60 ± 0.10^{eE}	2.18 ± 0.01^{fD}	2.04 ± 0.02^{gD}
40	3.62 ± 0.01^{aF}	3.47 ± 0.06^{bF}	3.24 ± 0.00^{cF}	3.13 ± 0.01^{dF}	2.94 ± 0.05^{eF}	2.40 ± 0.01^{fE}	2.28 ± 0.01^{gE}
45	3.95 ± 0.01^{aG}	3.54 ± 0.16^{bG}	3.40 ± 0.02^{cG}	3.27 ± 0.01^{dG}	3.10 ± 0.02^{eG}	2.79 ± 0.01^{fF}	2.56 ± 0.01^{gF}
50	4.25 ± 0.01^{aH}	4.21 ± 0.05^{bH}	3.93 ± 0.02^{cH}	3.45 ± 0.01^{dH}	3.25 ± 0.01^{eH}	3.05 ± 0.01^{fG}	2.71 ± 0.01^{gG}

* Data are presented as Mean values ± SE of five determinations

** Means identified by different small letters in the rows or capital letters in the columns were significantly different ($P < 0.05$).

***The sign −, means no change in the fish

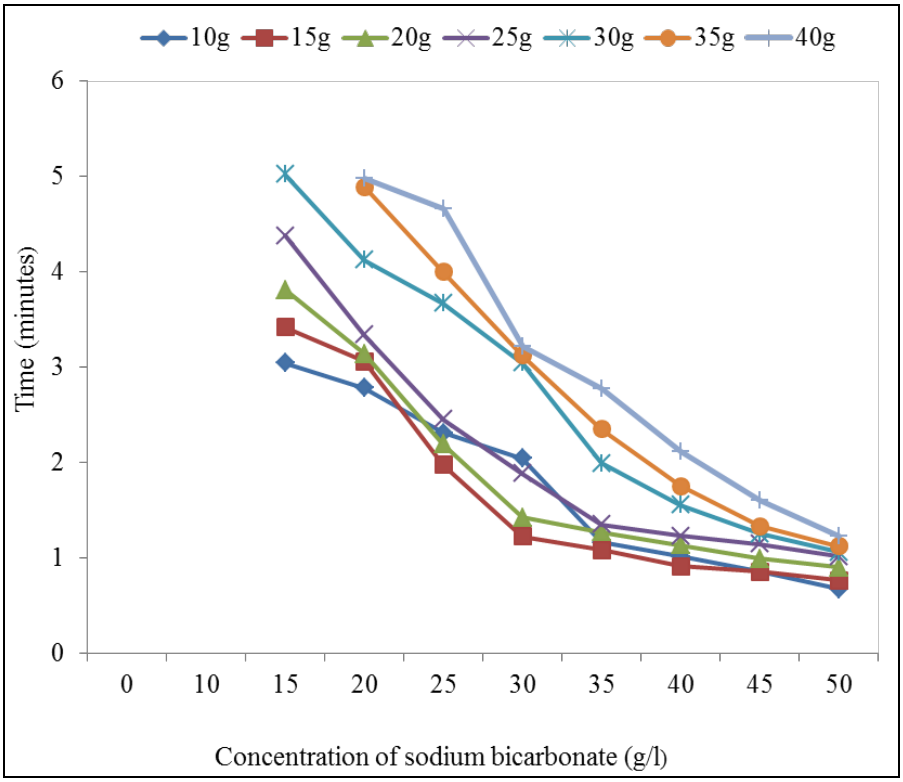

Figure 1. Induction of different sizes of *C. gariepinus* juveniles exposed to different concentrations of sodium bicarbonate.

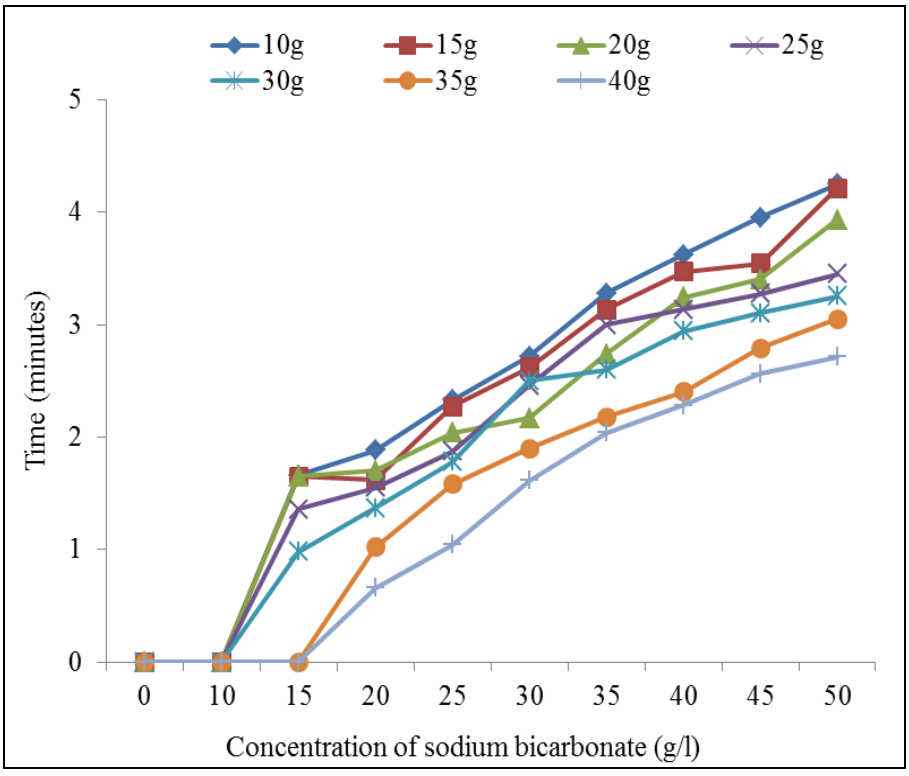

Figure 2. Recovery of different sizes of *C. gariepinus* juveniles exposed to different concentrations of sodium bicarbonate.

It took between 0.67 to 5.44 minutes for *C. gariepinus* juveniles to reach anaesthesia. Fish of the same weight reached anaesthesia faster at higher concentrations of sodium bicarbonate than at lower concentrations. This could be attributed to the fact that more anaesthesia diffused in to the body of the fish at higher concentration than those subjected to a lower concentration. This is consistent with Opiyo et al., (2013) and Altun et al., (2009) who found that higher concentration of sodium bicarbonate resulted to faster anaesthesia in Nile tilapia and Common carp respectively. In addition, smaller juveniles were also anaesthetized faster than bigger ones at the same concentration of sodium bicarbonate. This could be explained by the fact that smaller fish have a bigger surface area to volume ratio than bigger fish and therefore absorbed the anaesthetic faster. The induction time from the present study is in agreement with several authors (Marking and Meyer, 1985; Iversen, 2003; Coyle et al., 2004; Mylonas et al., 2005; Opiyo et al., 2013) who recommended a period of between 3-5 minutes for an effective induction time to anaesthesia.

The concentration of the anaesthetic solution significantly affected recovery time from anaesthesia. Juveniles exposed to higher concentration of sodium bicarbonate took longer to recover from anaesthesia than those at a lower concentration. These results are comparable with previously published work by Solomon et al. (2014) who found that when anaesthetic was in higher concentration, more of it was absorbed and accumulated in the Central Nervous System (CNS) of the fish thus suppressing the activity of the CNS to a greater degree than at lower concentrations and consequently prolonging the recovery time. King et al., (2005) also reported similar results when clove oil was used as anaesthesia in black sea bass (*Centropristis striata*). Moreover, bigger juveniles took shorter period of time to recover from anaesthesia than smaller juveniles at the same concentration. This could be linked to the fact that bigger fish have a smaller surface area to volume ratio than smaller fish and is comparable to (Yildiz et al., 2013). Similar results were recorded by Altun et al., (2009) on Common carp juveniles, Zaikov et al., (2008) on Pike and Opiyo et al., (2013) on Nile tilapia juveniles. Solomon et al., (2014) and Hseu et al., (1998) also noted that higher drug concentration increase recovery time. However, Booke et al., (1978) recorded much lower concentrations of sodium bicarbonate at 1,000 mg/L and 600 mg/L at pH 6.5 and 7.7 respectively.

Conclusion

In conclusion, 50g/L concentration of sodium bicarbonate is recommended to anaesthetize *C. gariepinus* juveniles since it led to anaesthesia in less than 2 minutes for all the sizes of fish.

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