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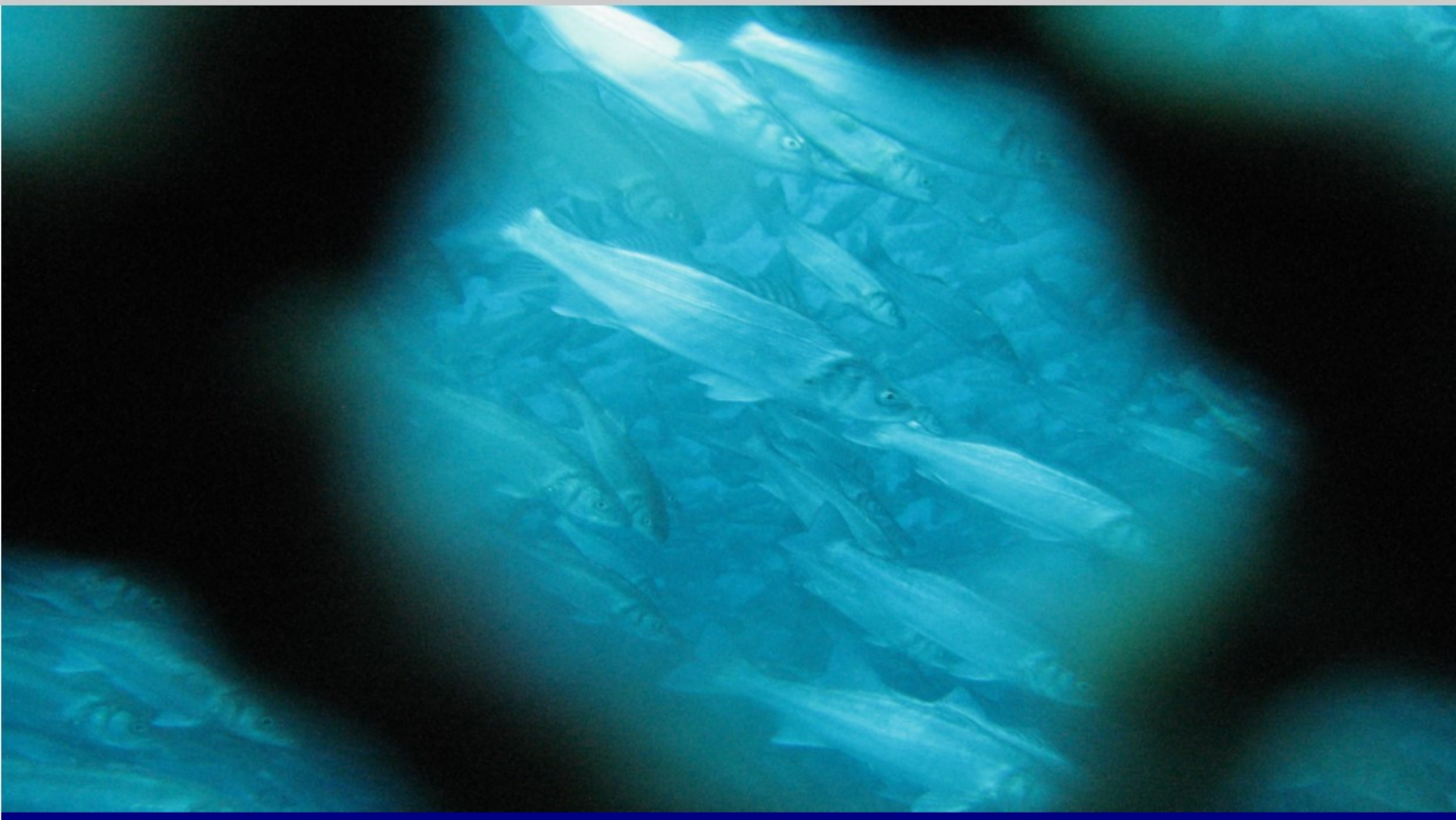

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"Journal of Aquaculture Engineering and Fisheries Research" publishes peer-reviewed articles that cover all aspects of Aquaculture and Fisheries research in the form of review articles, original articles, and short communications. Peer-reviewed (**with two blind reviewers**) open access journal published quarterly articles in **English or Turkish** language.

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Fishery Biology/Wild Fisheries/Ocean Fisheries

Biology/Taxonomy

Stock Identification/Functional Morphology

Freshwater, Brackish and Marine Environment

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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

THE ZOOPLANKTON COMMUNITY AND ITS RELATIONSHIP WITH ENVIRONMENTAL VARIABLES IN A HIGHLY POLLUTED SYSTEM, GOLDEN HORN, TURKEY

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This study is a part of master thesis of Zeynep DORAK entitled "Seasonal Changes of the Density and Composition of in Istanbul Haliç".

Abstract:

The Golden Horn Estuary (İstanbul) nutrient-rich, eutrophic and turbid at the inner part based on discharges from Alibeyköy and Kağıthane creeks. The pollution had been drastically increased by the accretion in the industry and urban development around the estuary since 1950s. The Golden Horn rehabilitation project was carried out during the 1998-2002 period. The present study was conducted following the rehabilitation process between November 2002-October 2003 at selected four stations, three at the inner part and one at the middle part of estuary. This is the first study on zooplankton community in the Golden Horn Estuary. The seasonal variation of zooplankton composition and density and 14 environmental variables (temperature, salinity, Secchi disc depth, turbidity, pH, dissolved oxygen, carbonate, bicarbonate, calcium, magnesium, total hardness, NO_2-N, NO_3-N, o-PO_4) and Chlorophyll-*a* concentration, which indicates the intensity of life in the Golden Horn Estuary have been studied, monthly. A total of 29 taxa was identified. Zooplankton composition was characterized by freshwater, estuarine and marine species. Zooplankton of Golden Horn Estuary was composed mainly by holoplanktonic organisms (53% of the total), with Copepoda as the most abundant group and a high number of taxa. Diversity were higher at all stations. A distinct relationship between zooplankton taxa composition and their environment, determined by a redundancy analysis, indicated that the measured environmental variables contributed to the variations in the zooplankton community structure to some extent. Four environmental variables and Chlorophyll-*a* explained 73.7% of the variation in the taxonomic structure. The results showed, that distribution of zooplankton was significantly associated total hardness, NO_2-N, NO_3-N, temperature and Chlorophyll-*a* ($p<0.05$).

Keywords: Golden Horn Estuary, Redundancy analysis, Water quality, Zooplankton

Introduction

Estuaries as transition areas between land and sea form aquatic ecosystems that are characterized as one of the most dynamic ecosystems by a variety of inter-related biotic and abiotic structural components and intensive chemical, physical and biological processes which influence species density and diversity (Aslan-Yılmaz et al., 2004; Telesh, 2004; Yüksek et al., 2006; Sterza and Fernandes, 2006; Sun et al., 2009).

Because of the unusual dynamic conditions experienced in estuaries zooplankton distribution is spatially and temporally heterogeneous, more so than in other aquatic ecosystems (Kibirige and Perissinotto, 2003; Sterza and Fernandes, 2006). High primary production levels make estuarine zooplankton very abundant, however some other biological factors, like environmental variables, may restrict the variety of the zooplankton species when compared to that of the marine areas.

Golden Horn lies to Sarayburnu-Tophane in northwest-southeast direction in the junction of Istanbul Bosphorus and Marmara Sea as a meander. Golden Horn formed by the invasion of sea with the junction of Alibeykoy and Kagıthane streams. The length of coasts on both sides are approximately 7.5 km. (Baştürk et al., 2001). The maximum depth of the estuary is around 15 m in mid-estuary and 4-5 m in the upper parts. The main source of the freshwater flowing into the Golden Horn is rainfall (Sur et al., 2002).

The Golden Horn Estuary has been polluted by wastewater of pharmaceutical, detergent, dye, leather industries and domestic discharges since the 1950s (Yüksek et al., 2006). The building of dam on the Alibeyköy stream weakened freshwater input. Furthermore, bridges, floating on large buoys and shipyards with large buoyant dry docks blocked circulation of upper layer and strengthen the pollution effect. Poor renewal of estuarine water and heavy nutrient load including numerous types of organic and inorganic effluents resulted in low diversity, with planktonic organisms (Taş and Okuş, 2003) at the outer part of the estuary. The inner part had only anaerobic life characterised by hydrogen sulfide formation (Doğan et al., 2001). The anthropogenic pollution at the estuary not only adversely affected the communities living in the estuary but also human life, giving a heavy odor of hydrogen sulfide and an unaesthetic appearance of this once recreational area (Aslan-Yılmaz et al., 2004).

Hence, life cycles of zooplankters are related to environmental factors. The study of the spatial and temporal variability of the Golden Horn Estuary zooplankton communities becomes important for a better understanding of the functioning of estuary ecosystems. For this reason, present study carried out throughout a year to estimate the zooplankton composition, the abundance and distribution of zoo-fauna and their relationships with environmental variables and Chlorophyll-*a* properties.

Materials and Methods

Study area and stations

The Golden Horn Estuary is in conjunction with Bosphorus and Marmara Sea covers an area of approximately $2.5x10^6$ m^2. Golden Horn is between 28º 42' and 29º 01' East Longitudes and 41º 01' and 41º 15' North latitudes.

Sampling

Three sampling stations were selected at the inner part of the Golden Horn Estuary (St.1, St.2, and St.3), determined mainly by their proximity to freshwater inputs, and one station was selected at the mid-estuary (St.4), is defined by proximity to the sea (Fig.1). Physicochemical and biological variables including Chlorophyll-*a* and zooplankton community structure and diversity were investigated monthly intervals from November 2002 to October 2003 (except February 2003). Water samples were collected vertically using 1.5 L Nansen bottle. Water salinity and pH were measured by pIONneer 65 Portable Multiparameter Instrument and light permeability with a 20 cm diameter Secchi disc onboard at each station. Turbidity was measured spectrophotometrically (Palin, 1955). Samples for nutrient analysis were pre-filtered. Nitrite was analyzed by colorimetric method (Parsons et al., 1984) and NO_2+NO_3 was detected by cadmium reduction method. o-PO_4 was detected spectrophotometrically following procedures of ADD Parsons et al. (1984). For Chlorophyll-*a* analysis, 1000 ml of seawater was filtered through GF/C filters and deep-frozen. Chlorophyll-*a* analyses were performed by acetone extraction method (Parsons et al., 1984). Dissolved oxygen (Winkler method) was measured following procedures of APHA (1989). Calcium, magnesium, total hardness, carbonate and bicarbonate were analyzed by titration methods following procedures of Boyd (1992).

Zooplankton community structure and diversity

The samples were collected with a plankton net (mesh size 55 µm), taking vertically. All zooplankton samples were immediately preserved in 4% borax-buffered formaldehyde. In the laboratory, organisms were identified to species level, when possible, and counted; all the densities are presented as number of individuals per cubic meter (ind. m^{-3}). The following references were reviewed to identify the specimens: Rose (1938), Rose and Tregouboff (1957), Boubee (1969), Mozdukhay (1969), and Pontin (1978).

Data analysis

Shannon-Wiener diversity index (*H'*) (Shannon-Weaver, 1949) were calculated for each zooplankton sample.

Spearman's correlation analysis was used to count the matrix of correlation coefficients among environmental factors completed using the SPSS 16.0 for Windows (Renner, 1970).

The mean values with a 95% confidence interval were presented in all and mean ± standard deviation described. Analysis of variance (ANOVA) was applied to the physicochemical variables and Chlorophyll-*a* in order to test differences between samples (temporal patterns/ seasonally (4 season x 4 stations x 3 replicate)) and sampling stations (spatial patterns (11 months x 4 stations x 3 replicate)). Towards equalizing the variance and normalize distribution, all data used in the ANOVA were log transformed ln(*x*+1). Where significant differences in the ANOVA were detected, a Tukey's Honestly Significant Different (HSD) test was applied to identify sources of variation. Measurements (physicochemical variables and Chlorophyll-*a)* were conducted in triplicate for each station at each month (except February 2003) during the study period.

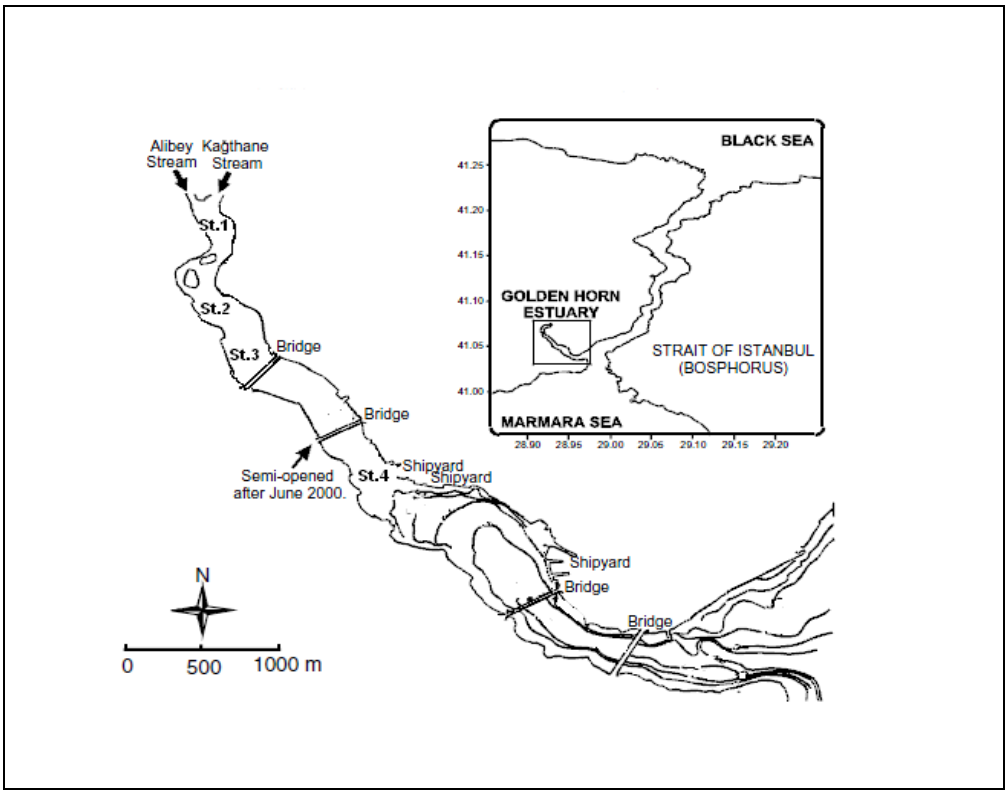

Figure 1. Golden Horn Estuary with the sampling stations.

Cluster analysis was carried out with biological data (zooplankton taxa abundances (ln(*x*+1)) using the Bray-Curtis similarity index, applying squared root data transformation). In this study, two sets of explanatory variables were built: biotic (zooplankton community and Chlorophyll-*a*) and abiotic (physicochemical factors). The abiotic matrix contained all measured physicochemical variables (including turbidity, salinity, dissolved oxygen, water temperature, pH, Secchi depth, calcium, magnesium, total hardness, bicarbonate, carbonate, nitrite, nitrate, orthophosfate). Principal Components Analysis (PCA) was used to determine the importance of environmental variables and Chlorophyll-*a*. To evaluate the association between zooplankton taxa

composition and their environment, we opted for a linear model of ordination instead of unimodal since preliminary Detrended Correspondence Analysis (DCA) showed a short gradient length on the biological data (SD=gradient length <2) (ter Braak and Šmilauer, 2002). In principle, environmental variables are displayed by their weights in an ordination diagram. In this study, there were 14 measured environmental variables and Chlorophyll-*a* (Table 1.) and 11 samples. To abstain from multicollinearity among the environmental variables, Forward Selection Method was used to reduce the number of environmental variables. The biological data in RDA were log(x+1)-transformed so as to downweight large values. To guard against interpretation of spurious axes, the statistical significance of the first and all the ordination axes was tested by Monte Carlo permutation test (999 unrestricted permutations). DCA, PCA and RDA were performed by the computer program Canoco 4.5 for Windows.

Table 1. Physicochemical characteristics of water quality and Chlorophyll-*a* concentrations in Golden Horn, during the study period.

		Station			
Variables		1	2	3	4
Turbidity (nm)	min.-max.	0.053-0.15	0.038-0.16	0.049-0.09	0.043-0.08
	average-stdev.	0.089±0.03	0.083±0.04	0.063±0.01	0.060±0.01
Salinity (‰)	min.-max.	7.45-19.15	14.6-19.15	14.4-19.55	17.1-21.45
	average-stdev.	15.94±3.29	17.21±1.67	17.35±1.71	18.59±1.39
Dissolved oxygen (mg L^{-1})	min.-max.	1.65-3.52	1.6-4.24	1.38-5.5	1.82-4.9
	average-stdev.	2.36±1.01	2.91±0.87	3.33±1.18	3.55±0.88
Water temperature (°C)	min.-max.	8.0-21.3	7.9-21.1	6.7-21.3	6.7-21.5
	average-stdev.	14.0±4.8	14.1±4.8	13.9±5.0	13.3±5.2
pH	min.-max.	7.8-10.6	7.8-10.7	7.9-10.8	8.0-10.9
	average-stdev.	8.2±0.81	8.3±0.82	8.4±0.81	8.5±0.80
Secchi-disc depth (m)	min.-max.	0.5-2.21	0.75-2.57	1.0-4.8	1.5-7.2
	average-stdev.	1.33±0.51	1.56±0.53	2.22±1.04	2.9±1.6
Calcium (mg L^{-1})	min.-max.	4.3-9.4	5.0-8.2	4.8-9.4	4.7-8.5
	average-stdev.	7.7±1.4	7.4±1.2	7.7±1.4	7.5±1.3
Magnesium (mg L^{-1})	min.-max.	5.5-9.0	4.6-9.2	4.5-11.4	4.7-10.3
	average-stdev.	7.7±1.2	8.1±1.3	8.8±1.7	8.7±1.5
Total Hardness (mg L^{-1})	min.-max.	10.5-18.3	9.8-19.2	9.8-21.5	10.2-20.1
	average-stdev.	16.2±2.5	17.0±2.6	17.4±2.8	17.5±2.7
Bicarbonate (mg L^{-1})	min.-max.	451.4-658.8	396.5-652.7	353.8-640.5	366.0-640.5
	average-stdev.	502.97±62	493.55±66.	466.37±83.7	448.63±75.5
Carbonate (mg L^{-1})	min.-max.	2.44-2.75	3.05-6.71	3.36-5.2	1.22-4.88
	average-stdev.	2.59±0.22	4.37±2.03	4.27±0.9	3.38±1.37
Nitrite (mg L^{-1})	min.-max.	0.71-3.03	0.49-3.26	0.3-2.48	0.15-3.1
	average-stdev.	1.68±0.73	1.76±0.85	1.59±0.72	1.4±1.04
Nitrate (mg L^{-1})	min.-max.	0.51-15.48	0.29-12.13	0.18-13.05	0.11-11.53
	average-stdev.	5.04±4.87	5.59±4.35	5.47±4.1	4.88±4.02
Orthophosfate (mg L^{-1})	min.-max.	0.27-5.09	0.40-4.6	0.2-5.29	0.12-1.73
	average-stdev.	1.78±1.49	1.44±1.39	1.45±1.45	0.86±0.64
Chlorophyll-*a* (mg L^{-1})	min.-max.	1.33-65.49	0.67-39.29	0.44-12.21	0.67-10.43
	average-stdev.	15.24±20.82	12.51±14.01	5.65±5.1	4.2±3.76

Results and Discussion

Environmental variables

Physicochemical characteristics of water quality and Chlorophyll-*a* concentrations are summarized in Table 1.

Regarding sampling locations, significant differences occured for turbidity (ANOVA $F_{(3,128)}$=4.777, p= 0.003, p<0,05; η=0.32 p<0.5); for dissolved oxygen (ANOVA $F_{(3,128)}$=9.878, p= 0.000, p<0,05; η=0.43 p<0.5); for Secchi disc depth $F_{(3,128)}$=16.898, p= 0.000, p<0,05; η=0.53 p>0.5); for magnesium (ANOVA $F_{(3,128)}$=4.567, p= 0.000, p<0,05; η=0.31 p<0.5); for bicarbonate (ANOVA $F_{(3,128)}$=4.184, p= 0.007, p<0,05; η=0.30 p<0.5); for orthophosphate (ANOVA $F_{(3,128)}$=3.058, p= 0.031, p<0,05; η=0.26 p<0.5); for Chl.-*a* (ANOVA $F_{(3,128)}$=6.252, p= 0.001, p<0,05; η=0.36 p<0.5);

According to Tukey's HSD post hoc test these variables showed significant differences among the stations. Highest dissolved oxygen values and Secchi disc depths occured at the mid-estuary station (Station 4), whereas the lowest values occured in the inner estuary region. Tukey's HSD post hoc test showed that turbidity, magnesium, bicarbonate, orthophosphate, and Chl.-*a* values in inner part of the estuary (first three stations), were significantly different from those collected in the middle part of the estuary (4th station), during the study period.

Significant seasonal differences occured for all physicochemical variables and Chl.-*a* (for turbidity (ANOVA $F_{(3,44)}$=4.058, p= 0.012, p<0,05; η=0.45 p<0.5); for salinity (ANOVA $F_{(3,44)}$=5.402, p= 0.003, p<0,05; η=0.52 p>0.5); for dissolved oxygen (ANOVA $F_{(3,44)}$=2.928, p= 0.044, p<0,05; η=0.42 p<0.5); for temperature (ANOVA $F_{(3,44)}$=1816.874, p= 0.000, p<0,05; η=1.00 p>0.5); for pH (ANOVA $F_{(3,44)}$=208.635, p= 0.000, p<0,05; η=0.97 p>0.5); for Secchi disc depth (ANOVA $F_{(3,44)}$=5.540, p= 0.003, p<0,05; η=0.52 p>0.5); for carbonate (ANOVA $F_{(3,44)}$=37.249, p= 0.000, p<0,05; η=0.85 p>0.5); for bicarbonate (ANOVA $F_{(3,44)}$=27.392, p= 0.000, p<0,05; η=0.81 p>0.5); for nitrite (ANOVA $F_{(3,44)}$=117.145, p= 0.000, p<0,05; η=0.94 p>0.5); for nitrate (ANOVA $F_{(3,44)}$=69.446, p= 0.000, p<0,05; η=0.91 p>0.5); for orthophosphate (ANOVA $F_{(3,44)}$=56.832, p= 0.000, p<0,05; η=0.89 p>0.5); for Chl.-*a* (ANOVA $F_{(3,44)}$=19.635, p= 0.000, p<0,05; η=0.76 p>0.5); for calcium (ANOVA $F_{(3,44)}$=202.294, p= 0.000, p<0,05; η=0.97 p>0.5); for magnesium (ANOVA $F_{(3,44)}$=3.986, p= 0.013, p<0,05; η=0.46 p<0.5) and for total hardness (ANOVA $F_{(3,44)}$=11.569, p= 0.000, p<0,05; η=0.66 p>0.5)). Tukey's HSD post hoc test showed that nutrients (NO_2-N, NO_3-N, o-PO_4) were significantly higher in autumn than the other seasons. Salinity, dissolved oxygen, Chl.-*a*, temperature, turbidity, and pH samples determined in spring, which are closely related with each other, were significantly different from those determined in other seasons. According to Tukey's HSD post hoc test Ca, Mg and total hardness spring values were significantly different from the other seasons. Bicarbonate concentrations in winter were significantly difference from other seasons, where carbonate values were significantly different in summer.

PCA was applied to 14 environmental variables and Chlorophyll *a*, first axis constituted 42.6% of the total variance. The highest positive values of the first axis were determined in Chl.-*a* (0.96) and temperature (0.81), and the highest negative value of the first axis was determined in Secchi disc depth (-0.20) (Fig. 2). Second axis constituted 22.5% of the total variance, o-PO_4 (0.42), pH (-0.03) and NO_2-N (-0.07) determined significant for the second axis (Fig. 2). The relationship of the environmental variables according to Spearman's rank correlation analysis is given in Table 2.

Zooplankton

During the study a total of 29 taxa was observed, with 22 taxa of holoplankton and 7 taxa of meroplankton (Table 3). A total of 22 taxa of holoplankton, 7 taxa for Copepoda, 4 taxa for Cladocera, 6 taxa for Rotifera, 1 taxa for Dinophyta, 2 taxa for Chordata, 1 taxa for Ctenophora, 1 taxa for Chaetognatha and 1 taxa for Protozoa, was recorded in Golden Horn Estuary (Table 3). Zooplankton community was characterized by the presence of freshwater, estuarine and marine species. Zooplankton of the Golden Horn Estuary was composed mainly by holoplanktonic organisms (75% of the total), with Copepoda as the most abundant group and a higher number of taxa. Among them, the most abundant taxa were Copepoda nauplii and *Acartia clausi* Giesbrecht, 1889 (Copepoda) (Table 4). The protozoan specimen *Favella ehrenbergii* (Claparède ve Lachmann, 1858) comprised 21.04% of the community (Table 4). Among the Cladocerans *Podon polyphemoides* (Leuckart, 1859) was the most abun-

dant (1.95%, Table 4) particularly in summer. Among the Rotifera the most abundant taxa was *Asplanchna priodonta* Gosse, 1850 (11.47%, Table 4) and was followed by *Asplanchna sieboldi* (Leydig, 1854) (2.63%, Table 4). The meroplankton comprised 25%, with 15.65 being polychaeta larvae, they were increased especially in spring (Table 4). The greatest zooplankton number was determined at 3rd station, while the lowest number was recorded at 4th station (478226 and 151066 org. m^{-3}, respectively) (Fig. 3). Zooplankton community showed also seasonal variations. The maximum zooplankton number was recorded during summer (450134 org. m^{-3}), while the lowest number was recorded during winter (59516 org. m^{-3}).

The hierarchical classification of the 29 taxa, in a rank order of abundance, based on monthly averaged data from the total period, can be summarized the above mentioned (Figure 4). Those species can depict the zooplankton community and their main assemblages in the Golden Horn Estuary.

Table 2. Correlations among the 14 environmental variables and Chl.-*a* selected for multivariate analysis of the species-environment relationships (*n*=44)

	Sal.	DO	Temp.	pH	SD	Ca^{2+}	Mg^{2+}	TH	CO_3^{2-}	HCO_3^-	NO_2-N	NO_3-N	o-PO_4	Chl.-*a*	Turb.
Sal.	1	.307*	.457**	.443**	.300*	ns	ns	.502**	ns	ns	ns	ns	-.676**	.490**	ns
DO		1	ns	.378*	.510**	.321*	ns	ns	.499**	-.301*	-.481**	ns	-.351*	ns	ns
Temp.			1	.595**	ns	ns	ns	.324*	ns	ns	-.590**	-.662**	-.587**	.721**	ns
pH				1	.451**	.313*	.411**	.562**	ns	.398**	ns	-.479**	-.411**	ns	ns
SD					1	.403**	ns	ns	ns	-.445**	ns	ns	ns	ns	ns
Ca^{2+}						1	ns	.313*	ns	ns	ns	ns	ns	ns	ns
Mg^{2+}							1	.679**	ns	-.344*	ns	ns	ns	ns	ns
TH								1	ns	ns	ns	-.390**	ns	ns	ns
CO_3^{2-}									1	-.576**	ns	-.344*	ns	ns	ns
HCO_3^-										1	ns	ns	ns	ns	ns
NO_2-N											1	.559**	.453**	-.472**	ns
NO_3-N												1	ns	-.448**	ns
o-PO_4													1	-.490**	ns
Chl.-*a*														1	-.452**
Turb.															1

*: Correlation is significant at the 0.05 level (2-tailed).
**:Correlation is significant at the 0.01 level (2-tailed).
ns: not significant.

Table 3. Zooplankton species composition in Golden Horn**.**

Holoplankton	**Holoplankton**
Copepoda	**Dinophyta**
Acartia clausi Giesbrecht, 1889	*Noctiluca scintillans* (Macartney, Kofoid et Swezy, 1921)
Microsetella norvegica (Boeck, 1865)	**Chordata**
Oncaea mediterranea Claus, 1863	larvea of *Ascida aspersa* (O.F.Mueller, 1776)
Oithona nana Giesbrecht, 1892	*Oikopleura dioica* Fol, 1872
Paracalanus parvus (Claus, 1863)	**Ctenophora**
Thermocyslos oithonoides G.O. Sars, 1863	larvea of Ctenophora
Cladocera	**Chaetognatha**
Bosmina longirostris (O.F. Müller, 1776)	*Parasagitta setosa* (Mueller, 1847)
Evadne normanni Lovén, 1836	**Protozoa**
Penilia avirostris Dana, 1849	*Favella ehrenbergi* (Claparède ve Lachmann, 1858)
Podon polyphemoides (Leuckart, 1859)	**Meroplankton**
Rotifera	Cypris
Asplanchna sieboldi (Leydig,1854)	larvea of Bivalvia
Asplanchna priodonta Gosse, 1850	larvea of Echinodermata
Keratella cochlearis (Gosse, 1851)	larvea of Gastrapoda
Gastropus stylifer Imhof, 1891	larvea of Ostracoda
Synchaeta litoralis Rousselet, 1902	larvea of Polychaeta
eggs of *Asplanchna* spp.	larvea of Zoea

Table 4. The average number of the dominant zooplankton genera (org. m^{-3}) and their frequency to the total zooplankton number in Golden Horn Estuary.

Group	Dominant taxa	No. of org. m^{-3}	% total zooplankton
Holoplankton			
Copepoda	*A.clausi*	2453	3.03
	Copepoda nauplii	19146	23.67
	Subtotal	22093	27.31
Cladocera	*P.polyphemoides*	1561	1.93
	Subtotal	1738	2.15
Rotifera	*A.priodonta*	9280	11.47
	A.sieboldi	2130	2.63
	Subtotal	14521	17.95
Dinophyta	*N.scintillans*	4567	5.65
	Subtotal	4567	5.65
Chordata	larvea of *A.aspersa*	147	0.181
	O.dioica	117	0.145
	Subtotal	264	0.33
Ctenophora	larvea of Ctenophora	470	0.58
	Subtotal	470	0.58
Chaetognatha	*S.setosa*	1	0.001
	Subtotal	1	0.001
Protozoa	*F.ehrenbergi*	17015	21.04
	Subtotal	17015	21.04
Meroplankton	larvea of Polychaeta	12661	15.65
	Subtotal	20217	25.00
	Grand total	80885	

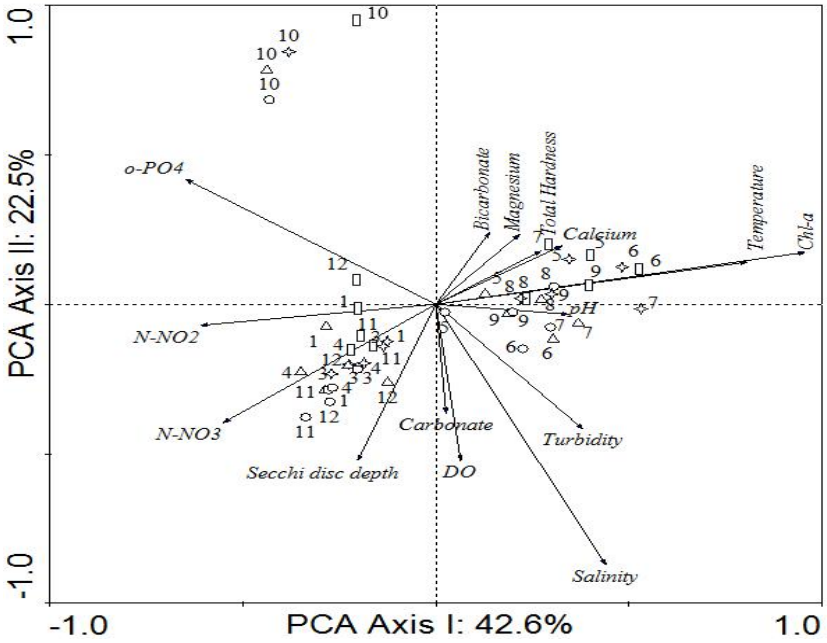

Figure 2. PCA on environmental variables. Numbers of 1–12 represented samples taken monthly. (11:November, 12:December, 1: January, 3: March, 4: April, 5: May, 6: June, 7: July, 8: August, 9: September, 10: October; rectangle: St.1, star: St.2, triangle: St.3, circle: St.4.)

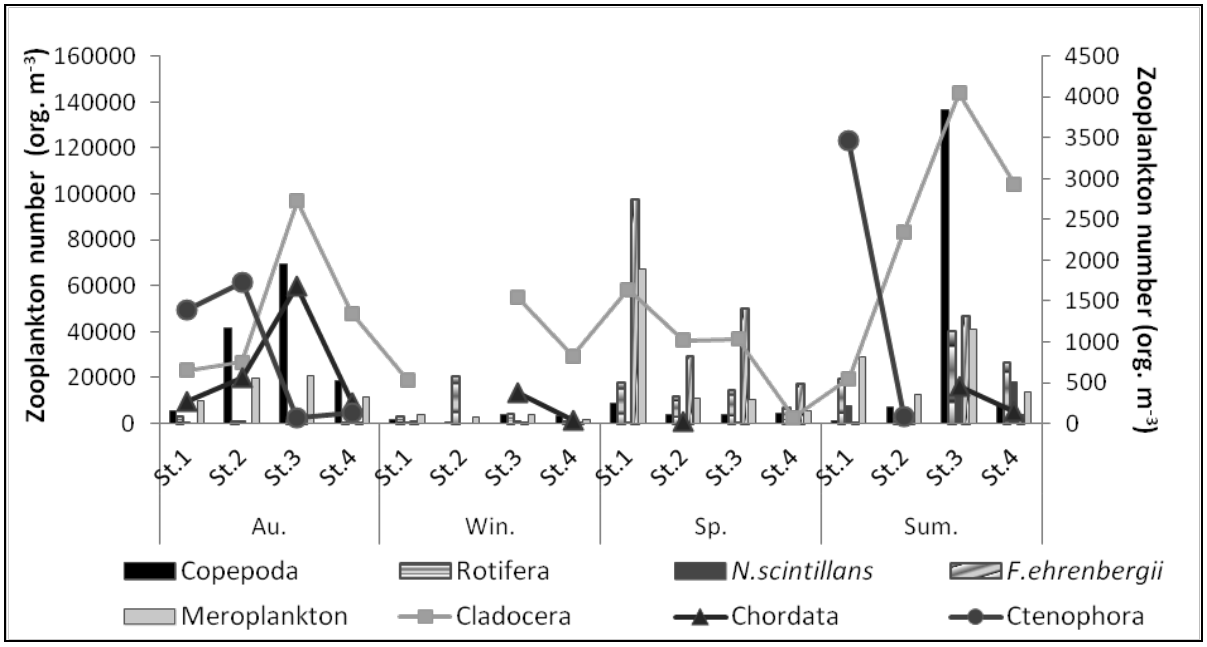

Figure 3. Distribution of numbers of zooplankton groups (org. m^{-3}) in Golden Horn Estuary during 2002/2003.

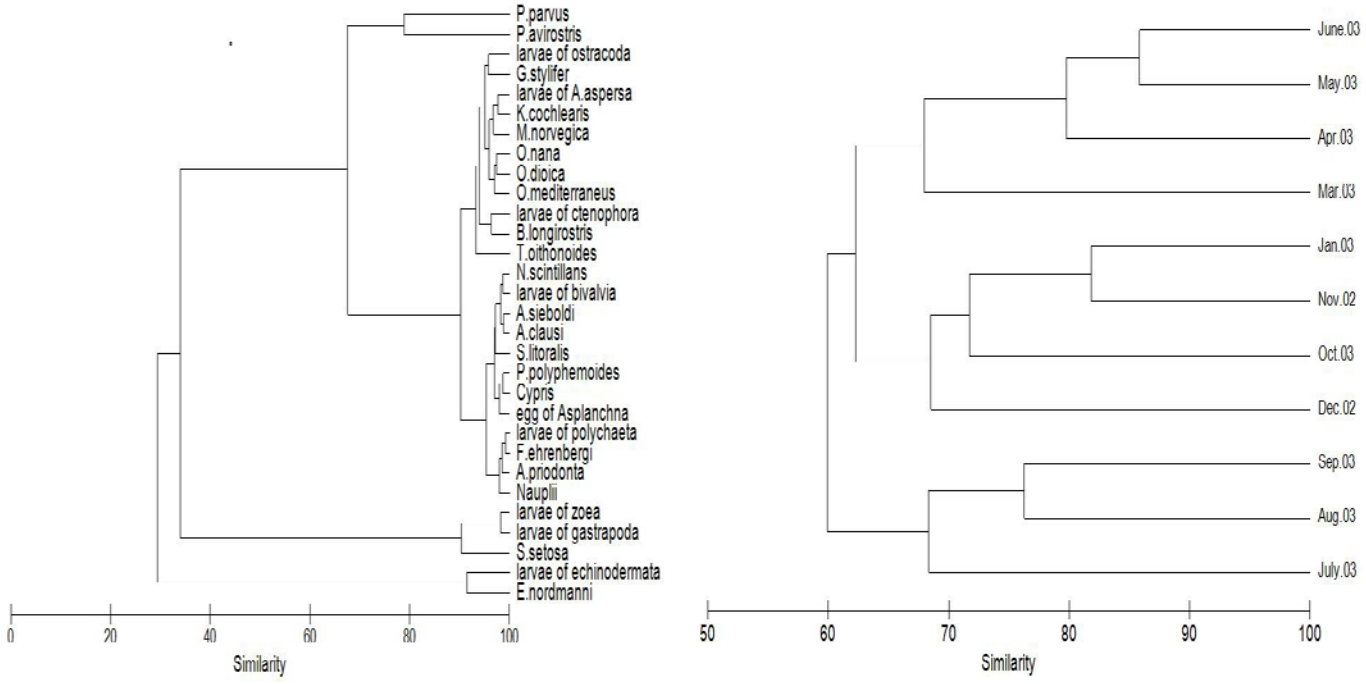

Figure 4. Dendogram using group average linking on Bray–Curtis similarity of the main species (variables) and months (samples) found from November 2002 to October.

There are three separate groups according to the dendogram. One large group is constituted by species showing a higher presence during the study period. Among these, *A. sieboldi, A. priodonto*, Cypris, *Noctiluca scintillans*, *P.polyphemoides* and Copepoda nauplii are the most abundant in summer, whereas larvae of bivalvia, *A. clausi*, eggs of *Asplanchna*, polychaeta larvae and *F. ehrenbe*rgi are more important in spring. Another large group includes the species *Gastropus stylifer, Keratella cochlearis, Microsetella norvegica, Oithona nana, Oikopleura dioica, Oncea mediterranea, Bosmina longirostris, Thermocyclops oithonoides*, larvae of ctenephora and larvae of ostracoda. Other three groups constituted by *Paracalanus parvus* and *Penilia avirostris*; *Parasagitta setosa*, larvae of zoea and larvae of gastrapoda; *Evadne nordmanni* and echinodermata larvae, respectively.

The cluster analysis of the mean monthly abundance of zooplankton taxa showed the three main assemblages, coinciding with the main annual environmental situations temporally (Figure 4).

Shannon–Wiener diversity index of the log-transformed means of zooplankton species density for the separate reaches of the estuary showed high values (2.3-4.1) during the study period for all stations. Generally, the lower reaches showed a higher zooplankton diversity index (Figure 5).

Relationships between zooplankton and their environment

With the submission of the first four synthetic gradients to RDA, the first two eigenvalues explained 26.3% of the cumulative variance of species data. Also, the species-environment correlations of axis 1 (0.900) and axis 2 (0.909) were high. The first four environmental variables explained 73.7% of the total variance in species data. The Monte Carlo permutation test was significant on the first axis (*F*-ratio = 6.034, *P*-value = 0.001) and on all axes (*F*- ratio = 3.920, *P*-value = 0.001) (Table 5).

In Figure 6a, the upper quadrant was completely confined to the distribution of zooplanktonic Crustaceans (Copepoda, Cladocera) and the lower one mainly to the distributions of two rotifers (*A. priodonta*, *A. sieboldi*). In Fig. 6b, the upper quadrant was restrict largely to the distribution of samples taken autumn and winter. Therefore, samples taken in autumn and winter were charecterized by more Crustaceans, while samples taken in spring and summer were characterized by more meroplankton, two rotifers and *N.scintillans*. According to the centroid principle and distance rule implied in RDA, in Fig. 6a NO_2-N and NO_3-N were both associated mainly with Crustaceans and two Rotifers (*A. priodonta*, *A. sieboldi*), while temperature, Chl.-*a* and total hardness mainly with meroplankton, two Rotifers and *N. scintillans.*

Table 5. Summary of the RDA analysis.

Axes		1	2
Eigenvalues		0.155	0.109
Species-environment corrlations		0.900	0.909
Cumulative percentage variance			
	of species data	15.5%	26.3%
	of species-environment relation	41.5%	70.7%
Total variance explained		73.7%	
The Monte Carlo permutation test		*F*-ratio	*P*-value
	on the first axis	6.034	0.001
	on all axes	3.920	0.001

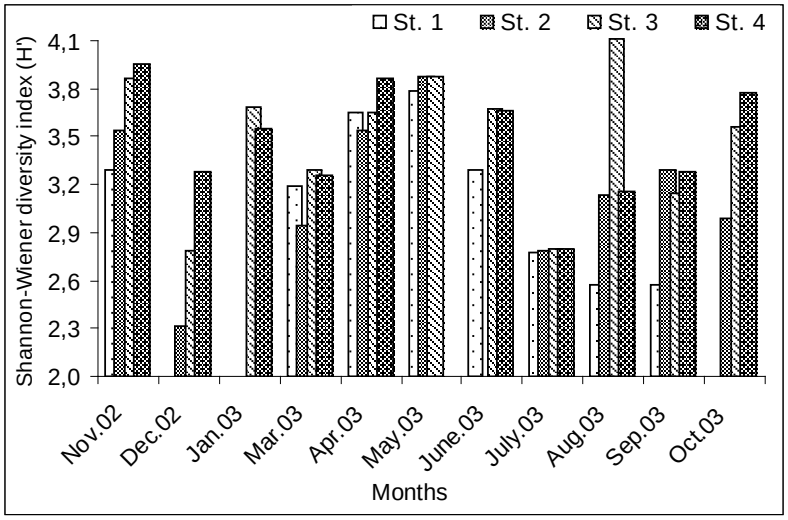

Figure 5. Shannon–Wiener diversity (*H'*) for the zooplankton community of the Golden Horn Estuary.

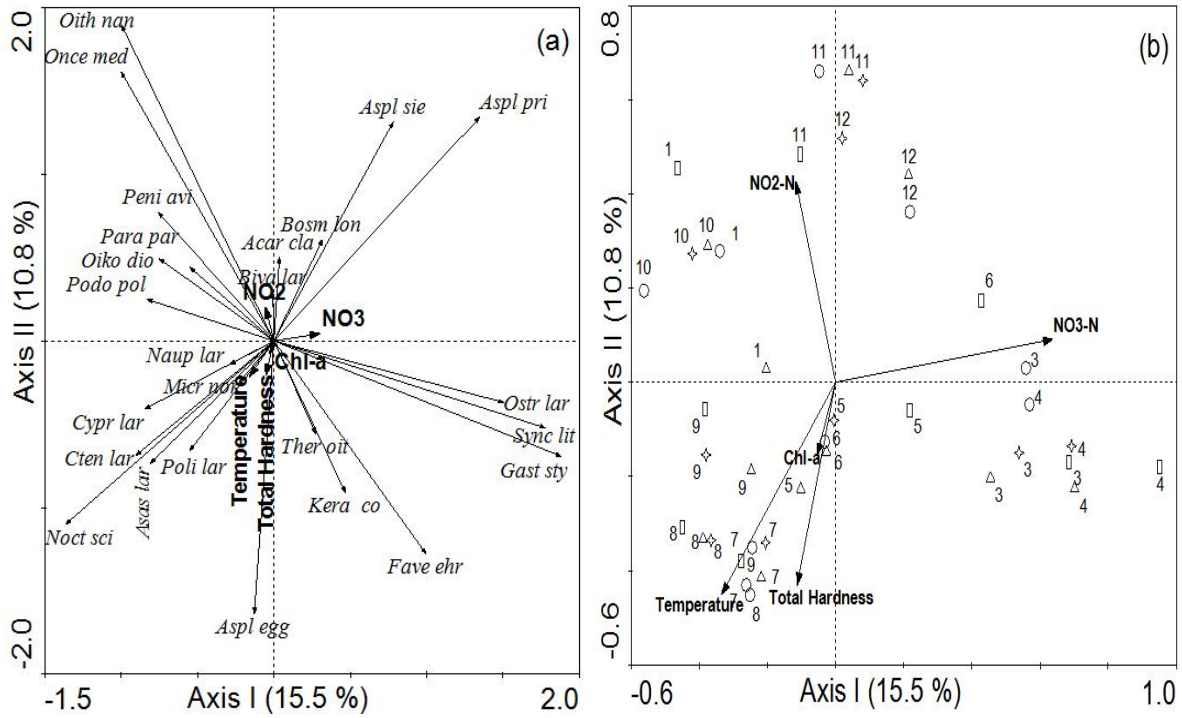

Figure 6. RDA ordination plots. (a) with species, Chl.-*a* and environmental variables. (b) with samples, Chl.-*a* and environmental variables. Numbers of 1–12 represented samples taken monthly. (11:November, 12:December, 1:January, 3:March, 4:April, 5:May, 6:June, 7:July, 8:August, 9:September, 10:October; rectangle: St.1, star: St.2, triangle: St.3, circle: St.4.)

This work characterize the variability of zooplanktonic species and their relationship with environmental variables in the inner- and middle part of the Golden Horn Estuary.

Study area characterized by a water mass with high salinity and low dissolved oxygen. Thus, low dissolved oxygen values might be related to organic matter decomposition processes, due to great input of two streams (Alibeyköy and Kağıthane) and sewages in the area. Rehabilitation workings in Golden Horn Estuary started in 1998, and the floating bridge (Valide Sultan Bridge) was opened partially. The semi-opening of the bridge enhanced of water circulation at the surface layer, relatively. The highest salinity values is the evidence of a strong input of Marmara Sea and reflecting homogenization of water mass throughout the surface waters of estuary.

Secchi disc depth increased to the sea direction, whereas turbidity decreased in this direction. These results correlates ascended depth and sea water circulation. Also, salinity values increased from the inner part to the middle part of the estuary, as a result of the impact of sea.

In the present study, the highest pH values were found in summer in general, and following heavy rains in the basin pH values dropped. The highest pH values recorded in summer can be controlled by decreasing rainfall and increasing phytoplankton production due to increasing temperature.

According to Yüksek et al. (2006), eukaryotic photosynthetic organisms dominated the plankton and succession of different species was observed in very small temporal scales following the opening of the bridge, and the heavily polluted upper layer began to show dense and frequent phytoplankton blooms. In this study, determined high dissolved oxygen concentration might depend on above-referred increasing photosynthetic activity.

Dissolved oxygen concentration and pH values ascended at the same period. There were significant positive correlations between pH-DO (Spearman's rho correlation, r=0.962, p= 0.378, p<0.05, *n*=44) and pH-temperature (Spearman's rho correlation, r=0.699, p= 0.595, p<0.01, *n*=44).

Free carbon dioxide, carbonate and bicarbonate are the three forms of carbon dioxide in melts. Bicarbonate-carbonate equation determines the alkalinity of the aquatic environments. Measured pH values can be used to determine to amount of the free CO_2, or the alkaline/asidic situation of the environment. Bicarbonate uptake is defined as indicator of algal activity (Currie and Kalff, 1984), because some plants use CO_2 in the structure of bicarbonate, when increased pH values. Because of bicarbonate concentration buffers the alteration of pH, its important variable in natural waters. According to the results, pH values and bicarbonate showed significant positive correlations (Spearman's rho correlation, r=0.398, p=0.008, p<0.01, *n*=44), similar to the literature. In the present study, the carbonate and bicarbonate values decreased from 1st Station to 4th Station. These members are more abundant in streams, and this case originate from pH balance, biological processess and the chemical structure of substratum. 4th Station is away from the impact of the creeks, so flow of the creeks, carrying the minerals in water, not attains to the middle part of the estuary.

Magnesium and calcium are the most important cations in aquatic environments, which constitute the total hardness of fresh water. Thus there was a significant correlation between total hardness-magnesium (Spearman's rho correlation, r=0.679, p= 0.000, p<0.01, *n*=44), and total hardness-calcium (Spearman's rho correlation, r=0.313, p= 0.039, p<0.05, *n*=44). On the other hand, they build up the salinity with other some cations and anions (eg. carbonate). Therefore, despite the first three stations are far from the sea, the salinity values were higher than in fresh water because of high anion and cation values. Magnesium is the important component of Chlorophyll, but according to Bohn et. al. (2004) Chlorophyll-bound Magnesium contributes a small and nutritionally insignificant part of total Magnesium intake in industrialized countries. Calcium can be used by some lime-stored plants to change to monocarbonate or shell forming animals (eg. Crutaceans).

The Golden Estuary was in eutrophic state and the main source of various nutrients were the discharges, as mentioned earlier. Also, its sediment has a fine-grain structure, and this type of sediment include high concentrations of soluble nitrogen and phosphorus (Fisher et al., 1982). Resuspension of sediments following disturbance generally causes rapid release of nutrients to the water column. For these reasons the nutrient values (NO_2, NO_3, o-PO_4) in the present study were determined in high concentrations. It was seen, that the values of nutrients were increased toward inner side of the estuary (from 4th station to 1st station), especially. Because 1st station is closer to the creeks, the quantity of pollution and sediment carried by creeks are higher in this region than the other three stations.

According to the results, the significant increase in Chl.-*a* content at the inner part of estuary may indicate an icrease in algal biomass. This was probably due to the decreased transparency and declined phosphorus levels. There were significant negative correlations between Chl.-*a* and phosphorus levels (Spearman's rho correlation, r=-0.490, p= 0.001, p<0.01, *n*=44) and Chl.-*a* and turbidity in the study (Spearman's rho correlation, r=-0.452, p= 0.002, p<0.01, *n*=44).

According to our results, some of the determined physicochemical variables (salinity, dissolved oxygen, water temperature, and pH) affect the distribution of zooplankton directly, whereas others indirectly.

Zooplankton community in Golden Horn Estuary was characterized by the presence of freshwater, estuarine and marine species. Zooplankton composition in Golden Horn Estuary is similar to other estuarine systems which holoplanktonic organisms, particularly Copepoda (Büyükateş and İnanmaz, 2010), dominated in this kind of environments. Copepoda nauplii, the most abundant taxon of Copepoda, have poor swimming ability, and therefore they can not change their habitat, until their size and swimming ability increase, after which they can then return to regions of preffered salinity (Chinnery and Williams, 2004). The other abundant Copepoda specimen *A. clausi* is known as non-migratory (Ergün, 1994). *A. clausi* population is often among the most abundant zooplankton specimen settled in many coastal and open water environments. Local populations of *Acartiidae* family may present according to highly variable temperature, salinity and available nutrient conditions (Hubareva et al., 2008). Cladocerans are not effective indicators to determine the level of pollution of estuaries (Uriarte and Villate, 2004), conversely *P.polyphemoides* (the abundant taxon of Cladocera in the present study) and *A. clausi* were reported as that they preffered highly polluted waters (Tarkan and Ergüven, 1988; Ünal et al., 2000; Benli et al., 2001). Determined abundant Rotifer species *Asplanchna* spp., noted by some authors that in general they are dominant zooplankton taxa in lotic areas, and are predatory (Bekleyen, 2001; Güher, 2003).

Also tintinnids (tintinnids represented in this study by *F.ehrenbergii*) are an important component in most marine environments (Cordeiro et al., 1997), and they can be important occasionally in estuarine environments (Dolan and Gallegos, 2001). Meroplanktonic forms, have also important components of plankton in this estuary, like the other estuary around the world (Tan et al., 2004; Ayón et al., 2008; Móderan et al., 2010).

Zooplankton community composition associates with trophic status of water body tightly, and the outcome of impacts like nutrient enrichment can be reflected in zooplankton community structure (Conde-Porcuna et al., 2002; Hietala et al., 2004). This can be illustrated by the RDA analysis in the context, which displayed a distinct relationship between zooplankton taxa composition and their environment. The first five determined variables explained 73.7% of the taxonomic structure. The zooplankton community structure responded rapidly to the environmental changes. As indicated by the RDA, temperature, NO_2-N, NO_3-N, Chl.-*a* and total hardness were significant variables that controlled species composition and temporal variations of abundance of the zooplankton assemblages.

According to the results, all the selected variables (NO_2-N, NO_3-N, Chl.-*a*, temperature and total hardness) associated with Rotifers and meroplankton, and NO_2-N, NO_3-N mainly with zooplanktonic Crustaceans.

The relation among zooplankton taxa, water temperature and high concentrations of nutrients has been detected by many authors (Park and Marshall, 2000). It is a consensus that an increase in the concentration of nutrients influences the top levels of a food web through a cascade of interactions (Anderson et al., 2002).

Rotifers are usually regarded as bio-indicators of water quality (Sládĕcek, 1983), and their abundance and population characteristics are used as effective indicators of environmental changes (Attayde and Bozelli, 1998). Rotifera species, we have identified in present study, are commonly found in eutrophic waters (Kolisko, 1974). The abundance of Rotifers decreased, while the density of zooplanktonic Crustaceans increased markedly. Increase in temperature during spring allowed the development of total Rotifers in high abundances up to 51106 ind. m^{-3}, while abundance of Crustacea was 24418 ind. m^{-3}. Inorganic nitrogen such NO_2-N and NO_3-N can help the increase of Rotifer density. According to the results NO_3-N concentration increased in spring.

Calcium is an essential structural component of crustacea carapaces (Korosi et al., 2008), and is an integral part of total hardness. Consequently has been shown to be important in influencing Crustacean assemblages in our study area.

Because zooplankton taxa are key components of aquatic ecosystems, this pattern may reflect the ability of larger Cladocera to competitively exclude smaller species when nutrients are limiting, as larger Cladocerans have lower limiting thresholds for nutrients (Brooks and Dodson, 1965). When nutrient levels are higher, competition pressures decrease and smaller individuals can proliferate. This situation could explain the relationship between nitrate and nitrite with Cladocerans. Copepoda affected indirectly by NO_2-N and NO_3-N. The presence of phytoplankton is controlled by utilizable nitrogen and phytoplank-

ton is an important diet for Copepoda (Lawrence et al., 2004).

Conclusions

The Golden Horn, in the center of Istanbul city, has been the favourite settlement area of the city in 1970s, because of its clean water, and sheltered harbors (Eyice, 1975). Therefore, it was an important fishing and recreational area, and also had touristic value and for this reason the Golden Horn is among the most important ecosystems in Turkey. In conclusion, this study presents the relationship between zooplankton distribution and the spatio-temporal patterns of environmental variables at the inner- and middle part of the Golden Horn Estuary. Longitudinal gradient has also an importance on variation of zooplankton species and physicochemical variables. According to the Water Pollution Regulation by the Ministry of Forestry and Water Affairs (Anonymous, 2004), study area was found to have IVth class of water quality according to the nutrient concentrations. However, species richness was found very high in all stations as meroplanktonic and holoplanktonic species contributes highly to the total species numbers. This study revealed the zooplankton fauna of the Golden Horn Estuary for the first time.

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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

ÇANAKKALE BÖLGESİ'NDE KUPES (*Boops boops*, L. 1758) AVCILIĞINDA KULLANILAN GALSAMA AĞLARINDA DONAM FAKTÖRÜNÜN AV VERİMİNE ETKİSİ

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Öz:

Bu çalışmada, Çanakkale kıyılarında Kasım 2011 ile Ocak 2013 tarihleri arasında ticari balıkçılıkta kullanılan 210d/3 numara ip kalınlığı olan 18 - 20 - 22 - 25 mm göz açıklığına sahip kupes ağları kullanılmıştır. Ağlar, her biri (E= 0.40 - E= 0.50 - E= 0.60) donam faktörüne sahip olacak şekilde donatılmıştır. Her ağ 105 göz yüksekliğine sahiptir. Ağların mantar yakalarında 3 numara plastik mantar ve kurşun yakalarında 50 gram kurşun kullanılmıştır. Avcılık yöntemi olarak voli yöntemi uygulanmıştır. Yapılan 15 avcılık denemesinde E=0.40 donam faktörlü 22 mm göz genişliğine sahip ağlar 174 adet kupes balığı ile en fazla avcılık yapan ağlardır. E=0.50 donam faktörlü ağlarda ise 22 mm göz genişliğine sahip ağlar 280 adet kupes balığı ile en fazla avcılık yapan ağlardır. E=0.60 donam faktörlü 25 mm göz genişliğine sahip ağlarda ise 403 adet kupes balığı ile en fazla avcılık yapan ağlar olarak göze çarpmaktadır.

Anahtar Kelimeler:

Çanakkale, Kupes (*Boops boops)*, Galsama ağı, Donam faktörü, Av verimi

Abstract:

Effect of Hanging Ratio on Catch Efficiency for Bogue (*Boops boops*, L. 1758) Gill Nets in Çanakkale Region

This study was conducted between November 2011 - January 2013, using bogue nets with 18 - 20 - 22 - 25 mm mesh size that targets bogue which used in commercial fishing commonly at Çanakkale shores were rigged in three different hanging ratios (E=0.40 - E=0.50 - E=0.60). Each net has 105 meshes height. Number 3 plastic floats were used on the floatline of nets and 50 gr leads were used on the leadline of nets. Drive-in fishery technique was used in fishery operations. At the end of 15 fishery operations, 174 bogue were caught with 20 mm mesh size, E=0.40 nets; 280 bogue were caught with 22 mm mesh size, E=0.50 nets and 403 bogue were caught with 25 mm mesh size, E=0.60 nets, as maximum.

Keywords:

Canakkale, Bogue (*Boops boops, L. 1758*), Gillnet, Hanging ratio, Catch efficiency

Giriş

Galsama ağları, düşük maliyetli ve uygulaması kolay olduğundan bütün dünyada yaygın olarak kullanılan bir av aracıdır (Hamley, 1975; Laevastu ve Favorite, 1988; Kurkilathi ve Rask, 1996). Çanakkale Boğazı'nın Ege çıkışı tür çeşitliliği açısından oldukça zengin bir yapıya sahip olmasından dolayı her türlü galsama ve fanyalı uzatma ağının bu bölgede kullanımına rastlanmaktadır. Galsama ağları dünya balıkçılığında olduğu gibi Çanakkale Bölgesinde de yüksek kullanım oranına sahiptir (Özekinci ve diğ., 2006). Galsama ağları ile avcılıkta balığın ağı görmemesi çok önemlidir. Bu yüzden galsama ağının düşük görünürlülükte olması istenir (Aydın ve diğ., 2006).

Galsama ağlarının yapısı, materyali, göz açıklığı, ip kalınlığı, rengi ve donam faktörünün av verimi üzerinde etkili olduğu bildirilmektedir (Brandt, 1984; Millner, 1985). Donam faktörü galsama ağları ile avcılıkta, ağların av verimini etkileyen en önemli faktörlerden birisidir. Donam faktörü ağın yüzdürücü ve batırıcıların bulunduğu yakalara donamı sırasında, bir birim ağın kaç birim halata donatıldığını belirtir. Ağ gözünün geometrik şekli, donam faktörü ile doğrudan ilişkilidir ve ağ gözünün şekli yakalanabilirliği etkilemektedir (Balık ve Çubuk., 1998a). Genellikle, düşük donam faktörüyle donatılan ağlar, yüksek donam faktörü olan ağlar ile karşılaştırıldıklarında aynı türün daha iri bireylerini yakalayabilmektedir. Çünkü ağların donam faktörü azaldıkça balıkların dolanarak yakalanma ihtimali artmaktadır (Karlsen ve Bjarnason., 1986). Kuzey Ege' de faaliyet gösteren balıkçılar tarafından kullanılan ağların %27' si barbun ağları, %46.4' ü marya ağları, %12.2' si tüm sezon boyunca kupes avlamada kullanılan galsama ağları, %11.8' i karides ağları ve %2.4' ü köpek balığı ağları olarak belirlenmiştir Ayaz ve diğ., (2010). Çanakkale Bölgesi'ndeki ağların geneline bakıldığında %12.2 oranla kupes ağları ile ayrıca kullanılan diğer ağlar ile de kupes balığının avcılığı yapılmaktadır.

Bu çalışmada Çanakkale kıyılarında sürdürülebilir kupes avcılığının devamının sağlanabilmesi için 18 - 20 - 22 - 25 mm göz açıklığına sahip kupes ağları ile denemeler yapılmıştır. Çalışmada, ağların donatımında uygulanan donam faktörlerinin av verimine etkisini, belirlemek hedeflenmiştir.

Materyal ve Metot

Bu çalışma, Kasım 2011 - Ocak 2013 tarihleri arasında, Gelibolu Yarımadasında ticari balıkçıların avcılık yaptıkları 2 - 15 m derinliğe sahip sahalarda gerçekleştirilmiştir. Altı farklı istasyonda, sonbahar ve ilkbahar mevsimlerinde av operasyonu yapılmıştır (Şekil 1).

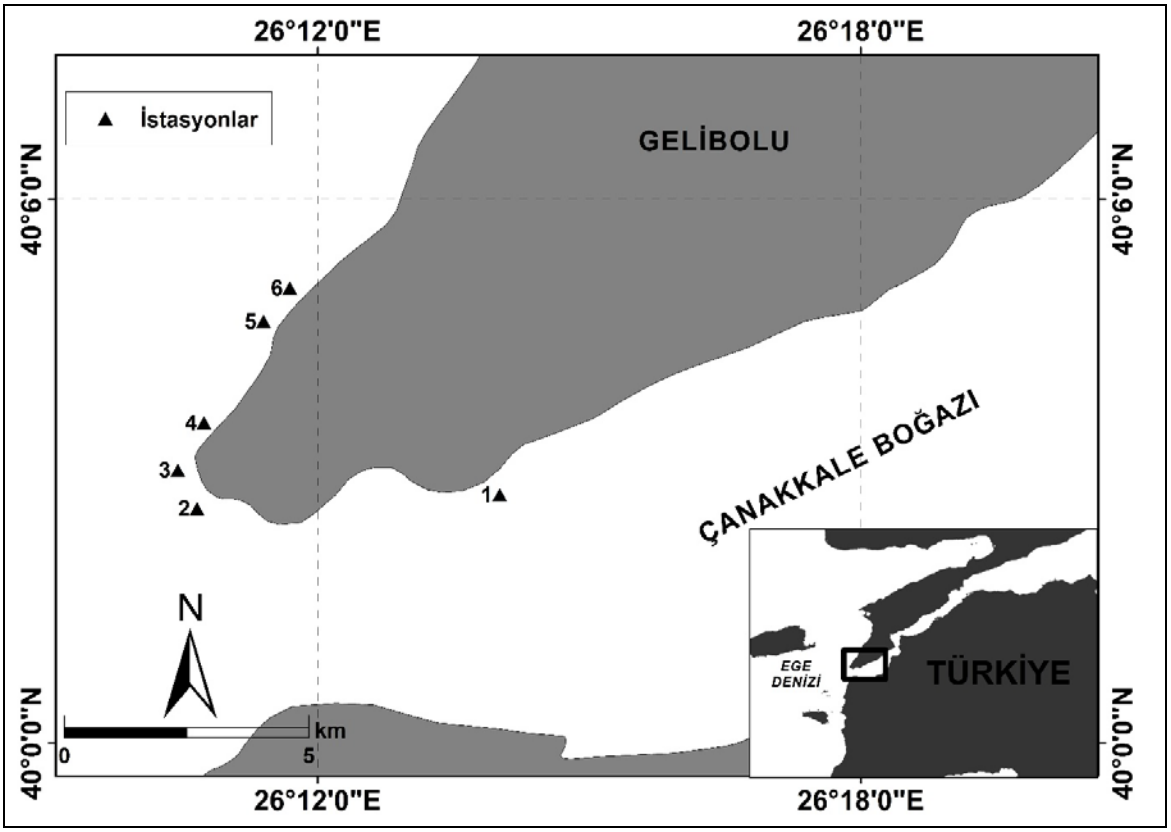
Avcılığın yapıldığı istasyonlarda en fazla av verimi bir no'lu istasyon olarak tespit edilmiştir.

Çalışmada 210d/3 numara ip kalınlığı, 36 - 40 - 44 - 50 mm göz açıklığına sahip ağlar kullanılmıştır. Ağlar, her biri E=0.40; E=0.50; E=0.60 donam faktörüne sahip olacak şekilde donatılmıştır. Her ağ 105 göz yüksekliğine sahiptir. Ağların mantar yakalarında 3 numara mantar ve kurşun yakalarında 50 gram kurşun kullanılmıştır.

Ağların her biri 30 metre uzunluğunda hazırlanmış ve farklı göz genişliğine sahiptir. Ancak aynı donam faktörüne sahip ağlar vertikal olarak birbirine küçük göz açıklığından büyük göz açıklığına gidecek şekilde dikilerek eklenmiştir. Böylece her bir donam faktöründe farklı göz genişliklerine sahip toplamda 120m uzunluğunda 3 adet ağ elde edilmiştir.

Avcılık Operasyonu

Örnekleme çalışmalarında; havanın kararmasından sonra balığın hareket yönüne paralel olarak suya bırakılan ağlara balığın ses ve ışık ile ağa doğru sürülmesi esasına dayanan voli yöntemi kullanılmıştır. Operasyonlarda ağlar donam faktörleri dikkate alınarak gruplandırılıp uçuca eklenmiştir. Her yenilenen operasyonlarda yerleri değiştirilerek tekrar denize atılmıştır. Yakalanan balıklar ağlardan temizlenip gruplandırılarak; total boyları ±1 mm hassasiyetli ölçüm tahtası ile ağırlıkları (W) ise ± 0.01g hassasiyetli dijital terazi kullanılarak ölçümleri yapılmıştır. Ağlara yakalanan balıkların maksimum ve minimum boyları tespit edilmiştir.



Şekil 1. Araştırma sahası

Figure 1. Study area

Av verimi hesaplamaları

Her bir ağın yakaladığı hedef av bireylerinin sayısı veya toplam ağırlıkları; operasyon sayısı ve ağın uzunluğuna olan orantısından yararlanılmıştır (Av verimi= Toplam Av (N) / (Operasyon sayısı x Ağ uzunluğu (m)) ve Av verimi= Toplam Av (kg) / (Operasyon sayısı x Ağ uzunluğu (m))) (Ayaz, 2010).

Bulgular ve Tartışma

Çalışma boyunca ağlarda toplam 2.048 adet (221.005 kg) kupes balığı (*B. boops*) yakalandığı tespit edilmiştir (Tablo 1).

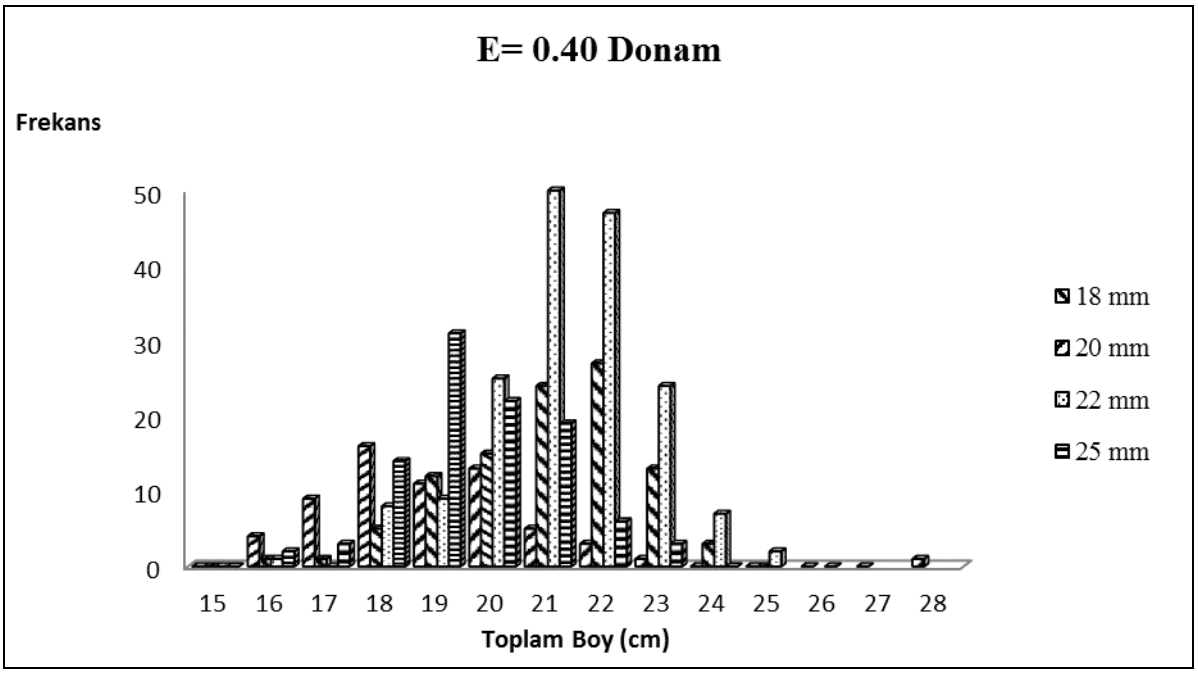
Çalışmalar sonunda elde edilen verilerde 0.4 donam faktörü ile donatılan ağlara toplam 438 adet kupes balığı yakalandığı tespit edilmiş, ağlara yakalanan balıkların total boy - frekans dağılımı çıkarılmıştır (Şekil 2). Yakalanan balıkların total boyu en büyük olanı 22 mm göz genişliğindeki ağda 28.3 cm; en küçük olanı ise 18 mm göz genişliğindeki ağda 15.9 cm olarak ölçülmüştür. Ağırlık olarak bakıldığında da 25 mm göz genişliğindeki ağda en büyük 239 g; 18 mm göz genişliğindeki ağda en küçük 40 gram olarak ölçülmüştür. E=0.40 donam faktörü ile donatılan ağlarda 22 mm göz genişliğine sahip ağlar 174 adet balık ile en fazla balığı tutan ağlar olarak göze çarpmaktadır. Ağların göz açıklıklarına göre avladıkları balıkların ortalama total boyları 18 - 20 - 22 - 25 mm göz genişliğindeki ağlarda sırasıyla; 18.55 - 20.59 - 21.88 - 23.25 cm olarak hesaplanmıştır.

Çalışmalar sonunda elde edilen verilerde 0.5 donam faktörü ile donatılan ağlara toplam 687 adet kupes balığı yakalandığı tespit edilmiş, ağlara yakalanan balıkların total boy - frekans dağılımı çıkarılmıştır (Şekil 3). Yakalanan balıkların total boyu en büyük olanı 18 mm göz genişliğindeki ağda 29.5 cm; en küçük olanı ise 25 mm göz genişliğindeki ağda 15.6 cm olarak ölçülmüştür. Ağırlık olarak bakıldığında da 22 mm göz genişliğindeki ağda en büyük 195 g; 18 mm göz genişliğindeki ağda en küçük 38 gram olarak ölçülmüştür. E=0.50 donam faktörü ile donatılan ağlarda 22 mm göz genişliğine sahip ağlar 280 adet balık ile en fazla balığı tutan ağlar olarak göze çarpmaktadır. Ağların göz açıklıklarına göre avladıkları balıkların ortalama total boyları 18 - 20 - 22 - 25 mm göz genişliğindeki ağlarda sırasıyla; 18.95 - 19.85 - 21.57 - 22.90 cm olarak hesaplanmıştır.

Tablo1. Çalışmada yakalanan balıklar ve miktarları (açıklama: ilk rakam göz genişliği, ikinci rakam ip kalınlığı, üçüncü rakam ağda uygulanan donam faktörü).Örnek; 18 3 04: 18 mm göz genişliğinde, 210d/3 numara ip, E= 0.40 donam faktörlü ağ.(105 göz: vertikal göz yüksekliği).

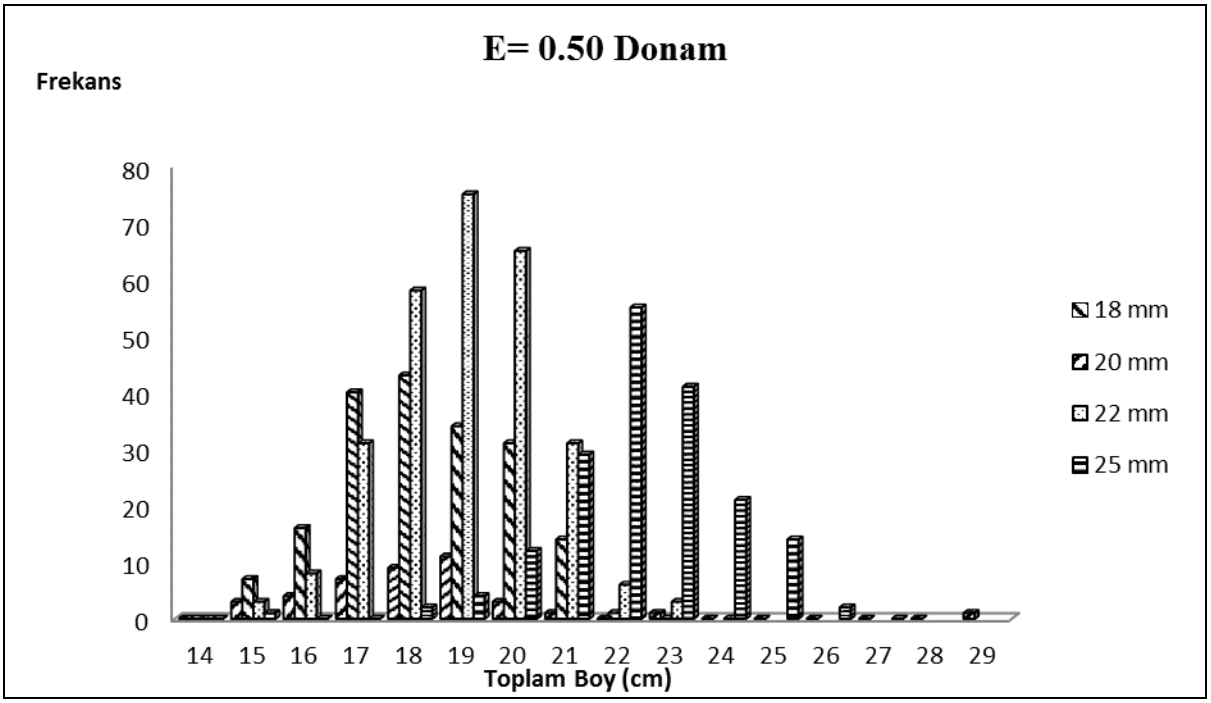
Table1. Fish caught in the study and amounts (note: fist valve is mesh size, second valve is rope thickness and third valve is hanging ratio. E.g 18 3 04: 18mm mesh size, number 210d/3 rope thickness, E=0.40 hanging ratio net. (105 meshes, vertical mesh height).

		18 mm						20 mm						22 mm						25 mm							
Türler		**0.4 Dn.**		**0.5 Dn.**		**0.6 Dn.**		**0.4 Dn.**		**0.5 Dn.**		**0.6 Dn.**		**0.4 Dn.**		**0.5 Dn.**		**0.6 Dn.**		**0.4 Dn.**		**0.5 Dn.**		**0.6 Dn.**		**Toplam**	
Türkçe Adı	**Tür İsmi**	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)	Ad (n)	Ağ (g)
Barbun	***M. barbatus***															1	55									1	55
B.iskorpit	***S.notata***																							1	38	1	38
Çipura	***S. aurata***													1	266											1	266
Ç. Hani	***S.scriba***																	1	69							1	69
Dil Balığı	***S.solea***			1	25																					1	25
Iskatari	***S. cantharus***							2	79					1	42											3	121
Isparoz	***D. annularis***							1	30	1	39	2	51	7	294	1	40	1	26	1	45	2	41			16	566
İskorpit	***S. porcus***							2	77					2	122	2	270					1	43			7	512
İstavrit	***T.mediterranus***	17	744	9	356	22	891	5	371	7	434	4	412	1	38	4	294	7	368					1	32	77	3940
İzmarit	***S.maena***	3	115	10	407	4	147	2	118	4	201			1	47	4	265	1	24							29	1324
Kalamar	***L.vulgaris***			2	618			1	180																	3	798
Karagöz	***D. vulgaris***					1	18							2	55			1	48							4	121
Kırma M.	***P. erythrinus***			1	38															1	65					2	103
Kolyoz	***S. japonicus***																	1	84							1	84
Kupes	***B. Boops***	63	4127	40	2828	46	2778	101	9558	186	15909	133	11562	174	20532	280	30793	341	32425	100	14451	181	22935	403	51103	2048	221005
Lüfer	***P.saltatrix***							2	279					2	143	6	485	1	72	2	258	10	1.102	4	447	27	2786
Mandagöz M.	***P.. bogarevo***			2	57	1	26	9	306	4	169	3	111			2	119	4	163			1	62			26	1013
Melanur	***O. melanura***			5	390	3	344	3	107	11	1.046	2	217	2	158	14	1.471			7	697	9	1.026	3	274	59	5730
Palamut	***S.sarda***															1	2.580									1	2580
Sardalye	***S. pilchardus***	6	278	1	57	2	108	1	60	1	14							30	2.386			1	69			42	2972
Sarpa	***S.sarpa***													1	274											1	274
Tekir	***M. surmuletus***																			1	114	1	300			2	414
Tirsi	***A.alosa***			3	181					3	218															6	399
Tiryaki	***U.scaber***	1	56													1	33			1	186	1	130			4	405
Turna	***S.hyalina***	1	100	2	175	2	182			5	617	1	111			1	116									12	1.301
Uskumru	***S.scombrus***																							1	116	1	116
Yabani M.	***P.acarne***	1	64					1	42	1	70	2	96	2	127	1	85	2	100			1	81			11	665



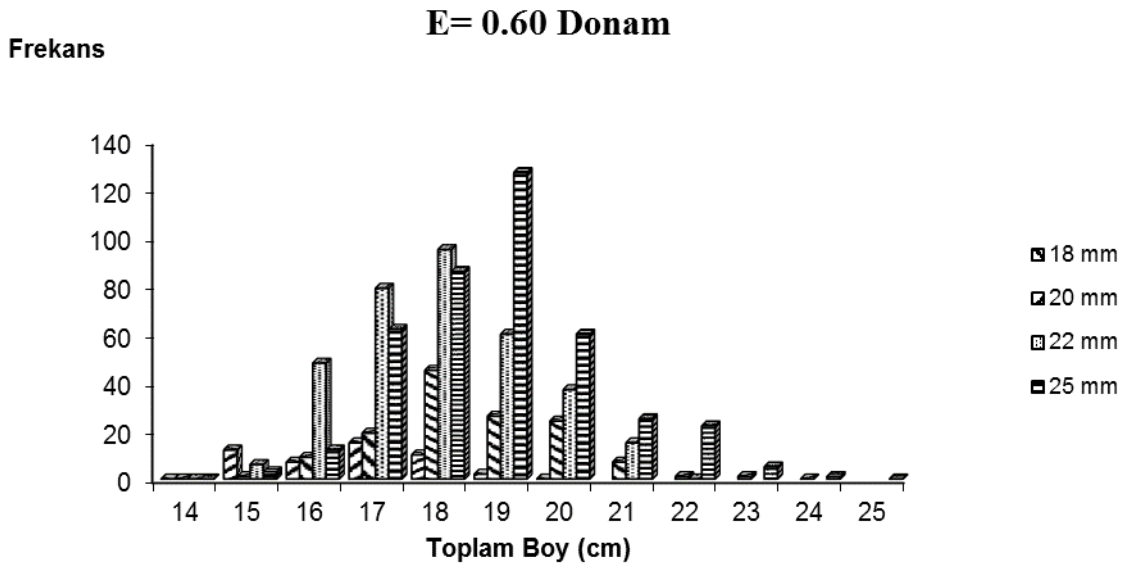
Şekil 2. E=0.40 donam faktörü uygulanan ağ göz genişliklerinin toplam boy frekans dağılımı.

Figure 2. Total lenght – frequency distribution of E=0.40 hanging ratio nets.



Şekil 3. E=0.50 donam faktörü uygulanan ağ göz genişliklerinin toplam boy frekans dağılımı.

Figure 3. Total lenght – frequency distribution of E=0.50 hanging ratio nets.



Şekil 4. E=0.60 donam faktörü uygulanan ağ göz genişliklerinin toplam boy frekans dağılımı.

Figure 4. Total lenght – frequency distribution of E=0.60 hanging ratio nets.

Tablo 2. Donam faktörlerine göre ağların birim av gücü miktarları. (Açıklama: İlk rakam ağ göz genişliği, ikinci rakam ip kalınlığı, üçüncü rakam donam faktörünü temsil etmektedir. Örnek; **18 3 04:** 18 mm göz genişliğine, 210d/3 numara ip kalığına, E= 0.40 donam faktörüne sahip ağ)

Table 2. Fish caught in the study and amounts (note: fist valve is mesh size, second valve is rope thickness and third valve is hanging ratio. E.g 18 3 04: 18mm mesh size, number 210d/3 rope thickness, E=0.40 hanging ratio net). CPUE valves of net with different hanging ratios.

		Hedef Tür		Av Verimi	
Kupez Ağları	Operasyon sayısı	Adet (N)	Ağırlık (kg)	Adet/m	Ağırlık/m
18 3 04	15	63	4.127	0.14	0.0091
18 3 05	15	41	2.828	0.0911	0.0062
18 3 06	15	46	2.778	0.1022	0.0061
20 3 04	15	101	9.558	0.2244	0.0212
20 3 05	15	186	15.909	0.4133	0.0353
20 3 06	15	133	11.562	0.2955	0.0256
22 3 04	15	174	20.532	0.3866	0.0456
22 3 05	15	280	30.793	0.6222	0.0684
22 3 06	15	341	32.425	0.7577	0.0720
25 3 04	15	100	14.451	0.2222	0.0321
25 3 05	15	181	22.936	0.4022	0.0509
25 3 06	15	403	53.107	0.8955	0.1180

Çalışmalar sonunda elde edilen verilerde 0.6 donam faktörü ile donatılan ağlara toplam 923 adet kupes balığı yakalandığı tespit edilmiş, ağlara yakalanan balıkların total boy - frekans dağılımı çıkarılmıştır (Şekil 4). Yakalanan balıkların total boyu en büyük olanı 22 mm göz genişliğindeki ağda 30.2 cm; en küçük olanı ise 18 mm göz genişliğindeki ağda 16.3 cm olarak ölçülmüştür. Ağırlık olarak bakıldığında da 25 mm göz genişliğindeki ağda en büyük 237 g; 18 mm göz genişliğindeki ağda en küçük 43 gram olarak ölçülmüştür. E=0.60 donam faktörü ile donatılan ağlarda 25 mm göz genişliğine sahip ağlar 403 adet balık ile en fazla balığı tutan ağlar olarak göze çarpmaktadır. Ağların göz açıklıklarına göre avladıkları balıkların ortalama total boyları 18 - 20 - 22 - 25 mm göz genişliğindeki ağlarda sırasıyla; 18.20 - 20.10 - 20.57 - 22.44 cm olarak hesaplanmıştır.

Hesaplamalar sonunda ağlarda en fazla balığı tutan ve en yüksek av veriminin 25 mm göz genişliği ve 0.60 donam faktörüne sahip ağda olduğu gözlenmiştir. En düşük av verimine sahip ağ ise, av adeti oranına göre 18 mm göz genişliği 0.50 donam faktörüne sahip ağ ve toplam ağırlık oranına göre 18 mm göz genişliği 0.60 donam faktörüne sahip ağ olduğu gözlenmiştir (Tablo 2).

Sonuç

Bu çalışma, sürdürülebilir kupes avcılığının devamı açısından, Çanakkale Bölgesi'nde kupes avcılığında yaygın olarak kullanılan galsama ağlarında donam faktörünün av verimine etkisini araştırmak için 18, 20, 22 ve 25 mm ağ göz genişliğine sahip ağlar 3 farklı donam faktörüyle donatılarak çalışılmıştır. Ağlarda yakalanan balık sayısına oranla yapılan av verimi hesaplamalarında; 0.6 donam faktörüne sahip 22 ve 25 mm ağ göz genişliği, 0.5 donam faktörüne sahip 20 mm ağ göz genişliği ve 0.4 donam faktörüne sahip 18 mm göz genişliğindeki ağlar ait oldukları ağ göz genişliği grubu içindeki en verimli ağlar olarak göze çarpmaktadır. Balıkların toplam ağırlık miktarına oranlanarak yapılan av verimi hesaplamalarında; 18 mm göz genişliğindeki ağlarda 0.4 donam, 20 mm göz genişliğindeki ağlarda 0.5 donam, 22 ve 25 mm göz genişliğindeki ağlarda ise 0.6 donam faktörüne sahip ağlar en verimli olarak göze çarpmaktadır. Hesaplamalarda 0.4 donam faktörü, 20, 22 ve 25 mm göz genişliğine sahip ağlar hem yakalanan balık adedi hem de toplam ağırlık oranlamasına göre en verimsiz ağlar olarak göze çarpmaktadır. 18 mm göz genişliğindeki ağlarda ise 0.5 donam faktöründeki ağ toplam yakalanan balık adedine oranla, 0.6 donam faktörüne sahip ağ ise balıkların toplam ağırlığına oranla en verimsiz ağ olarak dikkati çekmektedir. 22 ve 25 mm göz genişliğindeki ağlarda donam faktörü büyüdükçe yani ağların sudayken gerginliklerinin artmasıyla adet ve ağırlık oranlarına göre av verimliliğinin arttığı gözlenmiştir. Buna karşın, 18 mm göz genişliğindeki ağlarda 0.4 donam faktörü ile donatılan ağ yakaladığı balık sayısı fazla olmasından dolayı diğer iki donam faktörüne sahip ağlardan daha verimli çıkmıştır. 20 mm göz genişliğindeki ağlarda 0.5 donam faktörüne sahip ağa yakalanan birey sayısı ve toplam ağırlık miktarı daha fazla olmasından dolayı birim av gücü bakımından diğer iki donam faktörüne sahip ağlardan daha verimli çıktığı gözlenmiştir. Aydın ve Yüksel (2014) *Luciobarbus mystaceus* (Palas, 1814) avcılığında kulanılan farklı donam faktörlerine göre donatılmış galsama ağlarında 0.4 donam faktörüne göre donatılan ağ diğer ağlara göre daha verimli bulunmuştur. (Ayaz ve diğ., 2010) yaptıkları çalışmalarında 0.4-0.5-0.6 donam faktörü ile donatılmış galsama ağları kullanmış ve yine 0.4 donam faktörü ile en fazla isparoz (*Diplodus annularis)* balığı yakalamışlardır. (Duman ve diğ., 2006), Keban Baraj Gölü'ndeki çalışmalarında 0.50-0.60 donam faktörü ile donatılan galsama ağlarının daha verimli olduğu sonucuna ulaşmışlardır. (Nomura, 1978), galsama ağlarında en uygun donam faktörünün 0.30-0.50 arasında olduğunu bildirmiştir. Balık ve Çubuk (1998b) düşük donam faktörüyle donatılmış galsama ağlarında daha fazla sudak (*Stizostedion Lucioperca*) balığı yakalamışlardır. Bu da çalışmamızdaki 18 ve 20 mm göz genişliğindeki ağlarda, küçük donam faktörüne sahip ağların daha fazla balık yakalaması ve daha verimli çıkması ile eşleşmektedir. Bunun nedeni ise av operasyonu sırasında tesadüfi olarak balıkların ilk olarak bu ağlara sürüklenmesiyle yakalanmasından dolayı kaynaklandığı düşünülmektedir. Galsama ağları ile aktif olarak avlanılan voli yöntemiyle kupes balığının yoğun olduğu bölge ve zamanda avlanıldığı için ağların avladığı tür kompozisyonun büyük kısmını kupes balığı oluşturmaktadır. Bu sayede hedef dışı av oranı az, birim av gücü kaybı düşüktür. Son olarak ağlarda uygulanan donam faktörünün, av verimine ağların göz genişliklerine bağlı olarak suda görünürlüklerinin de etkisiyle beraber

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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

KÜLTÜR GÖKKUŞAĞI ALABALIĞI (*Oncorhynchus mykiss*)'NDAN İZOLE EDİLEN GRAM-NEGATİF PATOJENLERİN LİPOPOLİSAKKARİT PROFİLLERİ

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Öz:

Bu çalışma, Türkiye'nin farklı bölgelerindeki kültür gökkuşağı alabalıkları (*Oncorhynchus mykiss*)'ndan izole edilen patojenik Gram-negatif bakterilerin lipopolisakkarit (LPS) profillerinin belirlenmesi amacıyla yürütülmüştür. Bu amaçla, hasta balık örneklerinden izole edilen *Vibrio anguillarum*, *Aeromonas hydrophila*, *A. schubertii*, *Pseudomonas fluorescens* ve *Yersinia ruckeri* izolatlarından LPS numuneleri elde edilmiştir. LPS profilleri, Sodyum Dodesil Sülfat-Poliakrilamid Jel Elektroforezi (SDS-PAGE) ve gümüş-nitrat boyama metodu ile belirlenmiştir. Bu çalışmadan elde edilen sonuçlar, LPS analizi çalışmalarının bu bakterilerin karakterizasyonunda ve değişik coğrafik bölgelerden izole edilen suşlar arasındaki farklılıkların tespit edilmesinde kullanılabilecek faydalı araçlar olduğunu göstermiştir.

Anahtar Kelimeler:

Gökkuşağı alabalığı, *O. mykiss*, SDS-PAGE, LPS

Abstract:

Lipopolysaccharide Profiles of Gram-Negative Pathogens Recovered from Cultured Rainbow Trout (*Oncorhynchus mykiss*)

The aim of this study was to determine the lipopolysaccharide (LPS) profiles of pathogenic Gram-negative bacteria recovered from cultured rainbow trout (*Oncorhynchus mykiss*) in different regions of Turkey. For this purpose, LPS samples were obtained from 10 bacterial isolates of *Vibrio anguillarum*, *Aeromonas hydrophila*, *A. schubertii*, *Pseudomonas fluorescens* and *Yersinia ruckeri* recovered from diseased fish. LPS profiles were determined by using Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis (SDS-PAGE) and silver-nitrate staining method. Results of the study demonstrated that LPS profiling is a useful tool for characterization of these bacteria and geographic discrimination of their isolates.

Keywords:

Rainbow trout, *O. mykiss*, SDS-PAGE, LPS

Giriş

Gökkuşağı alabalığı (*Oncorhynchus mykiss*), ülkemizde 1970'li yıllardan beri başarıyla kültürü yapılan bir balık türüdür (Çelikkale vd., 1999). TUİK (2013) verilerine göre ülkemizde 2012 yılında iç sularda ve denizlerde toplam 114.569 ton/yıl gökkuşağı alabalığı üretilmiştir. Bu balığın kültüründe görülen en önemli problemlerden birisi kirlilik ve uygun olmayan yetiştiricilik koşullarına bağlı olarak gelişen bakteriyel hastalıklardır (Timur ve Timur, 2003; Roberts, 2012; Austin ve Austin, 2012).

Ülkemizin tümüne yayılan ve üretim miktarı her yıl artan gökkuşağı alabalığı kültüründe de üretim miktarını kısıtlayan bir faktör olarak Gram-negatif karakterdeki bakterilerden kaynaklanan hastalıklara sıklıkla rastlanmaktadır. Günümüze kadar gerçekleştirilen pek çok çalışma sonucunda, ülkemizde kültürü yapılan gökkuşağı alabalıklarında *Aeromonas hydrophila* (Baran vd., 1981; Diler ve Altun, 1994; Köprücü ve Sarıeyyüpoğlu, 2010), *A. schubertii* (Akaylı vd., 2011a), *A. salmonicida* (Kırkan vd., 2003), *Vibrio* (*Listonella*) *anguillarum* (Timur ve Korun, 2004; Tanrıkul, 2007; Tanrıkul ve Gültepe, 2011), *Flavobacterium columnare* (Kubilay vd., 2008), *F. psychrophylum* (Kayış vd., 2009), *Pseudomonas fluorescens* (Akaylı ve Timur, 2004), *P. putida* (Altınok vd., 2006), *P. lutoeola* (Altınok vd., 2007), *P. plecoglossicida* (Akaylı vd., 2011b) ve *Yersinia ruckeri* (Çağırgan ve Yüreklitürk, 1991; Timur ve Timur, 1991, Altun vd., 2010) gibi Gram-negatif karakterdeki patojen bakterilerin hastalıklara neden olduğu rapor edilmiştir.

Gram-negatif bakterilerdeki hücre çeperi, zorlu çevre şartlarına karşı hücreyi koruma, hücreye şeklini verme ve seçici geçirgen bir bariyer olarak görev yapmanın yanı sıra bazı enzimatik sistemleri de bünyesinde bulundurur (Lima de Faria, 1969; Koebnik vd., 2000). Gram-negatif bakterilerde hücre çift katlı bir membran ile çevrilidir ve dış membran; tamamı sitoplazmada sentezlenen fosfolipidler, lipopolisakkaritler, lipoproteinler ve integral dış membran proteinlerinden (OMP) oluşmaktadır (Bos ve Tomassen, 2004). Bakterilerin antijenik yapılarını oluşturan lipopolisakkaritlerin balıkların spesifik ve spesifik olmayan bağışıklık sisteminin gelişmesi üzerinde önemli etkileri olup bu yapıların belirlenmesi aşı geliştirme çalışmalarına temel teşkil eder (Fulop vd., 1995, Sakai, 1999). Solem vd. (1995) *A. salmonicida*'ya ait LPS'lerin Atlantik salmonu (*Salmo salar*)'nda immunostimulant olarak kullanılabileceğini belirtilirken, Acosta vd. (2004) *V. anguillarum*'a ait bu antijenlerin aynı balık türünde antikor seviyesini arttırdığını ve aşı çalışmalarında kullanılabileceği bildirilmiştir.

Bakteriyel balık patojenlerinin teşhisinde, antijenik yapılarının belirlenmesinde ve serotiplendirme çalışmalarında aglütinasyon (Sorensen ve Larsen, 1986), ELISA (Enzyme-linked Immunosorbent Assays) (Knappskog vd., 1993; Akaylı, 2001, Kubilay ve Timur, 2001), IFAT (Immune Fluorescent Antibody Technique) (Kubilay ve Timur, 2001), SDS-PAGE (Sodyum-Dodesil-Sülfat Poliakrilamit Jel Elektroforezi) (Dooley vd., 1985; Knappskog vd., 1993) ve Western-blot (Pazos vd. 1993) gibi immunokimyasal yöntemler yaygın olarak kullanılmaktadır. SDS-PAGE tekniği bakteri hücrelerinin, lipopolisakkarit yapılarının tespit edilmesinde yaygın olarak kullanılan elektroforetik yöntemlerden biridir (Laemmli, 1970; Ingram, 1993). Farklı araştırıcılar bu tekniği kullanarak *V. anguillarum* (Chart ve Trust, 1984; Knappskog vd., 1993; Boesen vd., 1999), *A. hydrophila* (Dooley vd., 1985), *P. fluorescens* (Swain vd., 2003) ve *Y. ruckeri* (Sousa vd., 2001) gibi çeşitli Gram-negatif balık patojenlerinin LPS profillerini incelemişlerdir.

Bu çalışmada da yurdumuzdaki kültür gökkuşağı alabalıklarından izole edilen Gram-negatif karakterdeki bakterilerin identifikasyonu ve bu izolatların Lipopolisakkarit (LPS) profillerinin SDS-PAGE metodu kullanılarak tespit edilmesi amaçlanmıştır.

Materyal ve Metot

Bu çalışma kapsamında Ege bölgesinde bulunan 3 adet ve Marmara bölgesinde bulunan 2 adet kültür gökkuşağı alabalığı işletmesinden örnekleme yapılmıştır. Ağırlıkları 5-130 g arasında değişen ve çeşitli hastalık belirtilerini gösteren toplam 40 adet balık örneğinden bakteriyolojik ekimler yapılmıştır. İşletmelerdeki balıkların bulunduğu havuzların su sıcaklığının Ege bölgesindeki işletmelerde ortalama 18-20 °C olduğu gözlenirken, Marmara bölgesindeki işletmelerde ise 16-18 °C arasında olduğu dikkati çekmiştir.

Bakteriyolojik inceleme

Havuzlardan canlı olarak temin edilen hasta balık örnekleri öncelikle 2-phenoxyethanol (0,1 mL/L) ile anesteziye tabi tutulduktan sonra dış ve iç klinik bulguların tespiti için otopsi işlemleri gerçekleştirilmiştir. İncelenen balık örneklerinin karaciğer, böbrek ve dalak gibi iç organlarından TSA (Triptik Soy Agar) ve BHIA (Brain Hearth Infusion Agar), *Pseudomonas* agar ve TCBS agar (Thiosulphate Citrate Bile salts Sucrose) gibi besiyerlerine bakteriyolojik ekimler yapılmıştır (Buller, 2004; Austin ve Austin, 2012; Roberts, 2012). Petri kutuları 18°C'de 4 günlük inkübasyon sonrasında primer izolasyon ile elde edilen bakteri kolonileri saflaştırılarak rutin bakteriyolojik metotlar ve API 20E hızlı teşhis kiti kullanılarak identifiye edilmiştir (Alsina vd., 1994; Austin ve Austin, 2012; Roberts, 2012). İzole edilen bakterilerin Gram boyanma özelliği, hareket testi, Oksidasyon/Fermentasyon testi, sitokrom oksidaz ve katalaz aktiviteleri ile O/129'a duyarlılık testleri ile genus bazında identifikasyon gerçekleştirildikten sonra tür düzeyinde identifikasyon için indol, MR, VP, üre, sitrat, ONPG, nitratları indirgeme, şekerlerden asit üretimi gibi testler yanı sıra *Vibrio anguillarum* Medium besiyeri, TCBS, Waltman-Shotts agar ve Pseudomonas agar besiyerlerinde üreme gibi ayırt edici biyokimyasal özellikleri incelenmiştir (Alsina vd., 1994; Buller, 2004; Kimberley ve MacNair, 2004; Altun vd.,, 2010; Austin ve Austin, 2012).

LPS örneklerinin hazırlanması ve SDS-PAGE analizi

Bu çalışmada incelenen bakteri izolatlarının identifikasyonundan sonra aynı bakterilerin hücre zarında bulunan LPS örnekleri elde edilmiştir (Hitchcock ve Brown, 1984; Santos vd. 1995). Bu amaçla, katı besiyerinde üretilen taze bakteri kültüründen elde edilen koloniler steril bir eküvyon çubuğu yardımı ile mikrosantrifüj tüpü içerisine toplanarak fosfat tamponlu tuz (PBS) solüsyonu ile yıkanmış ve 1.5 ml PBS içerisinde yoğunluğu OD_{650}'de 0.8 olacak şekilde sulandırılmıştır. *10.000 g*'de 5 dakika süre ile santrifüj edilen süspansiyonun üstte kalan sıvı kısmı uzaklaştırılmış ve pelet kısmı 50 µL'lik örnek tampon (12.5 mM Tris-HCl [pH 6,8], %2 SDS, %10 [vol/vol] gliserol, %0,002 bromfenol mavisi, %5 2-merkapto ethanol) solüsyonu ile sulandırılmıştır. Bu süspansiyona 100°C'lik sıcak su banyosunda 10 dakikalık kaynatma işleminden sonra, ortamdaki protein moleküllerinin elimine edilmesi amacıyla üzerine 10 µL proteinaz-K (2,5 mg/mL) eklenmiş ve 60°C'de 1 saat inkübe edilmiştir. Elde edilen LPS örnekleri kullanılana kadar -20°C'de saklanmıştır. SDS-PAGE analizi ise Laemmli (1970)'in metoduna göre %3'lük yükleme jeli ve %12'lik ayırma jeli kullanılarak ThermoScientific marka dikey elektroforez cihazında gerçekleştirilmiştir. Yükleme jelinde oluşturulan kuyucuklara 10 µL LPS örneği yüklenmiştir. Her bir jelde, kuyucuklardan bir tanesine de LPS örneklerinin moleküler ağırlıklarının belirlenebilmesi için protein belirteci (Fermentas PageRuler unstained protein ladder) yüklenmiştir. 80V elektrik akımında 2 saatlik elektroforetik ayrıştırmanın sonrasında LPS profillerinin görülebilir hale gelebilmesi amacıyla gümüş-nitrat boyama yöntemiyle boyanmış (Tsai ve Frasch, 1982) ve fotoğraflanmıştır.

Bulgular ve Tartışma

Klinik bulgular

Çalışmada materyal olarak kullanılan hasta balık örneklerinin dış bakı muayenesinde genel olarak deride pul kaybı, yüzgeçlerde erime, vücut yüzeyinde hemoraji ve ülserlerle seyreden bakteriyel hemorajik septisemi tablosu (Şekil 1) gözlenirken bazı balık örneklerinde ise bu gibi bulgulara ek olarak ağız bölgesinde hemorajiler ile özellikle sırt yüzgecinde erime tespit edilmiştir. Hasta balık örneklerinin iç bakı muayenesinde ise böbrekte erime, dalakta büyüme ve karaciğerde hemorajiler gibi çeşitli klinik bulgular tespit edilmiştir (Şekil 2).

Bakteriyolojik bulgular

Hasta balık örneklerinin iç organlarından yapılan bakteriyolojik ekimler sonucu elde edilen izolatlardan farklı biyokimyasal özelliklere sahip Gram-negatif karakterdeki 10 adet bakteri izolatı seçilerek çalışmaya dahil edilmiştir. Fenotipik ve biyokimyasal özellikleri Tablo 1'de gösterilen bu izolatlar *Vibrio anguillarum*, *Aeromonas hydrophila*, *Aeromonas schubertii*, *Pseudomonas fluorescens* ve *Yersinia ruckeri* olarak identifiye edilmiştir.

Tablo 1. Hasta kültür gökkuşağı balığı örneklerinden izole edilen bakterilerin (1-10) fenotipik ve biyokimyasal özellikleri

Table 1. Phenotypic and biochemical properties of bacterial isolates (1-10) recovered from diseased cultured rainbow trout samples

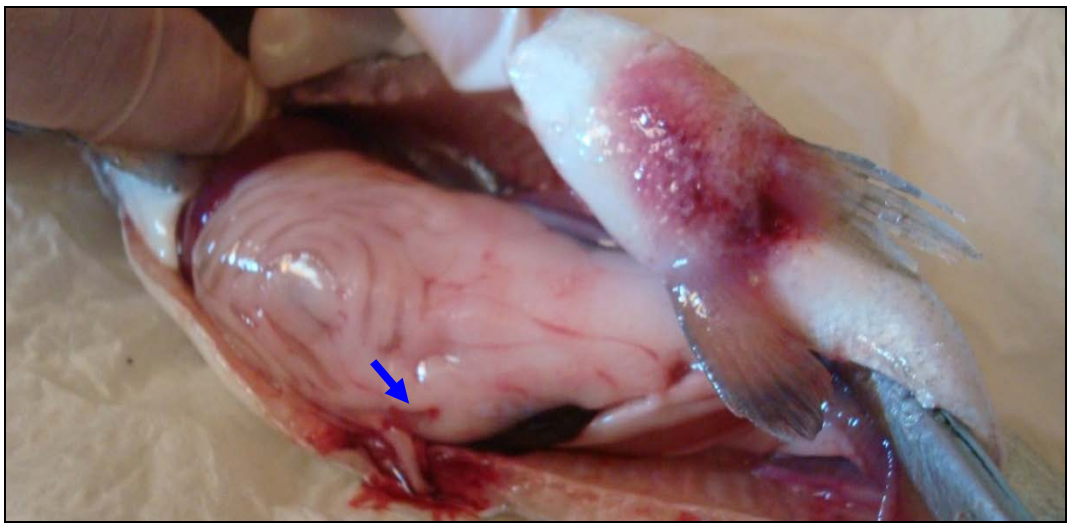
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Gram	-	-	-	-	-	-	-	-	-	-
Hareket	+	+	+	+	+	+	+	+	+	+
O/F	F	F	F	F	F	O	F	F	F	F
Sitokrom oksidaz	+	+	+	+	+	+	-	-	-	-
Katalaz	+	+	+	+	+	+	+	+	+	+
O/129 (150 µg)	H	H	D	D	D	D	D	D	D	D
İndol	-	+	-	+	-	-	-	-	-	-
MR	-	-	+	-	-	-	+	+	+	+
VP	+	+	+	+	+	Z	+	+	+	Z
Laktoz	-	-	-	-	-	-	-	-	+	-
Maltoz	+	+	+	+	+	-	+	+	+	+
Mannitol	+	+	-	+	-	-	+	+	+	+
Arabinoz	+	+	-	+	-	-	Z	-	-	-
Nitrat	+	+	+	+	-	-	+	+	+	+
Üreaz	Z	-	-	-	-	-	-	-	-	-
Amilaz	+	+	-	+	-	-	-	-	-	-
Jelatinaz	+	+	+	+	+	+	Z	+	Z	Z
ONPG	+	+	Z	Z	-	-	Z	+	+	Z
Sitrat kullanımı	-	-	-	+	-	+	-	-	+	-
Floresan pigment	-	-	-	-	-	+	-	-	-	-
Farklı besiyerlerinde üreme										
VAM	S	S	-	-	-	-	-	-	-	-
TCBS	S	S	-	-	-	-	-	-	-	-
WS	-	-	-	-	-	-	Y	Y	Y	Y
İdentifikasyon	*Vibrio anguillarum*	*Vibrio anguillarum*	*Aeromonas schubertii*	*Aeromonas hydrophila*	*Aeromonas schubertii*	*Pseudomonas fluoresens*	*Yersinia ruckeri*	*Yersinia ruckeri*	*Yersinia ruckeri*	*Yersinia ruckeri*

+: pozitif reaksiyon; -: negatif reaksiyon; K: krem; F: fermentatif; O: oksidatif;
H: hassas; D: dirençli; S: sarı; Y: yeşil; Z: zayıf reaksiyon

+: positive reaction; -: negative reaction; K: creamy coloured; F: fermentative;
O: oxidative; H: sensitive; D: resistant; S: yellow; Y: green; Z: weak reaction

Şekil 1. 130 *g* ağırlığındaki hasta balık örneğinde deride pul kaybı, yüzgeçlerde erime ve deride ülserler (okla gösterilmiştir)

Figure 1. Loss of scales, swelling of the fins and ulcers (arrowed) on the body of a 130 *g* diseased fish sample.



Şekil 2. 110 *g* ağırlığındaki hasta balık örneğinde deride kasa kadar inen ülser, hemorajik karaciğer ve dalakta büyüme (okla gösterilmiştir)

Figure 2. Ulcers expanding through the muscles on the skin, haemmorhagic liver and spleenomegaly (arrowed) in a 110 *g* diseased fish sample.

Türe özgü seçici bir besiyerlerinden VAM besiyeri, *V. anguillarum* izolatlarının; Pseudomonas agar besiyeri ise *P. fluorescens* izolatlarının identifikasyonunun doğrulanmasında kullanılmıştır. *V. anguillarum* izolatları VAM besiyeri üzerinde 24 °C'de 24 saat inkübasyon sonrasında sarı renkli koloniler oluştururken (Şekil 3a), *P. fluorescens* izolatları Pseudomonas agar besiyerinde inkübe edilip UV ışığı altında incelendiğinde floresan pigment içeren koloniler oluşturmuştur (Şekil 3b). Waltman-Shotts besiyerinde ise *Y. ruckeri* olarak identifiye edilen 4 adet izolatın yeşil renkli, etrafında sarı renkli hidroliz zonu bulunan koloniler oluşturduğu görülmüştür.

LPS analizi bulguları

Bu çalışmadaki hasta balık örneklerinden izole edilen bakterilerin LPS profilleri incelendiğinde farklı coğrafi bölgelerden izole edilen aynı türe ait bakteri izolatları arasında homojenite veya heterojenite olabileceği görülmüştür. İki farklı örnekleme bölgesinden izole edilen *V. anguillarum* izolatlarının birbirinden tamamen farklı iki LPS profili oluşturduğu ve izolatlar arasında LPS profilleri bakımından heterojenite olduğu tespit edilmiştir (Şekil 4a). Bununla beraber aynı bölgeden izole edilen iki adet *A. schubertii* izolatı ve hareketli *Aeromonas* türü olan bir adet *A. hydrophila* izolatı ise birbirlerine benzer LPS profilleri oluşturmuşlardır. Benzer şekilde *Y. ruckeri* izolatlarının LPS profilleri incelendiğinde bu izolatların sık ve çok sayıda bant oluşturduğu ve farklı bölgelerden izole edilen bu izolatlar arasında da LPS profili bakımından homojenite bulunduğu tespit edilmiştir (Şekil 4b).

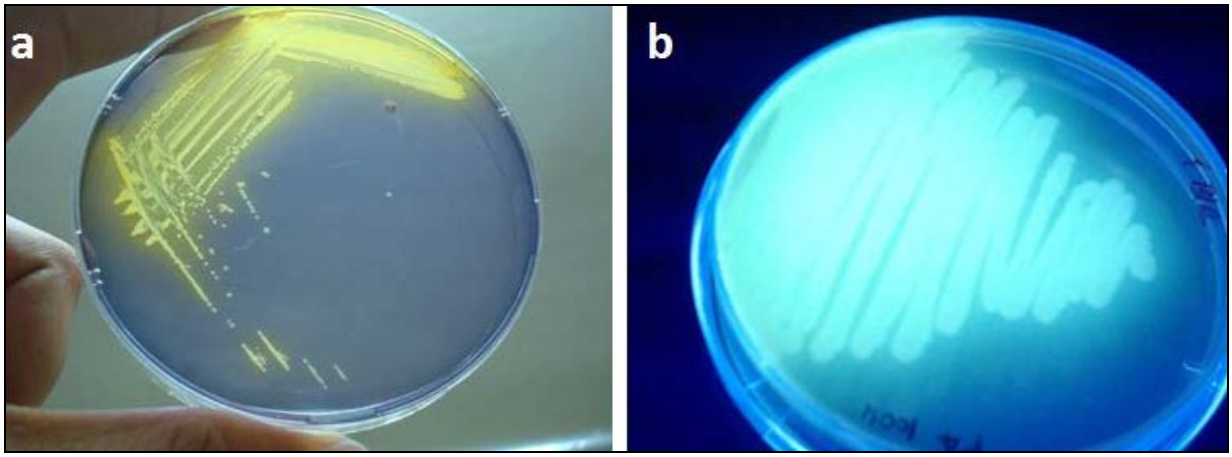
Balık hastalıklarının tedavisi amacıyla antibiyotik kullanımı konusunda gerçekleştirilen yanlış uygulamalar nedeniyle bakterilerde antibiyotik

duyarlılığı gelişmiştir ve bu da balık hastalıklarının tedavisini güçleştirmektedir (Austin ve Austin, 2012; Roberts, 2012). Bu nedenle hastalıklara karşı önleyici önlem olarak aşı geliştirme çalışmaları önem kazanmıştır. Aşı üretiminde kullanılacak suş seçimi sırasında bakterilerin identifikasyonu ve serotiplendirilmesi için genel olarak kullanılan biyokimyasal metotlar bakterilerin tür içi ayrımında yetersiz kalabilmektedir. Halbuki farklı coğrafi bölgelerden izole edilen ve aynı türe ait bakterilerin ve LPS profillerinin belirlenmesiyle daha detaylı ve güvenilir tür içi karakterizasyonları yapılmaktadır (Knappskog vd. 1993; Pazos vd., 1993; Boesen vd., 1999)

Bu çalışmada ülkemizin iki farklı coğrafik bölgesinden temin edilen ve çeşitli hastalık bulguları gösteren gökkuşağı alabalık örnekleri incelenmiş ve çeşitli bakteriler izole ve identifiye edilmiştir. Ege Bölgesi'nden temin edilen kültür gökkuşağı alabalığı örneklerinden *V. anguillarum*, *A. schubertii* ve *Y. ruckeri* izole ve identifiye edilirken, Marmara Bölgesi'ndeki işletmelerden temin edilen kültür gökkuşağı alabalığı örneklerinden ise *V. anguillarum*, *P. fluorescens* ve *A. schubertii* izole ve identifiye edilmiştir.

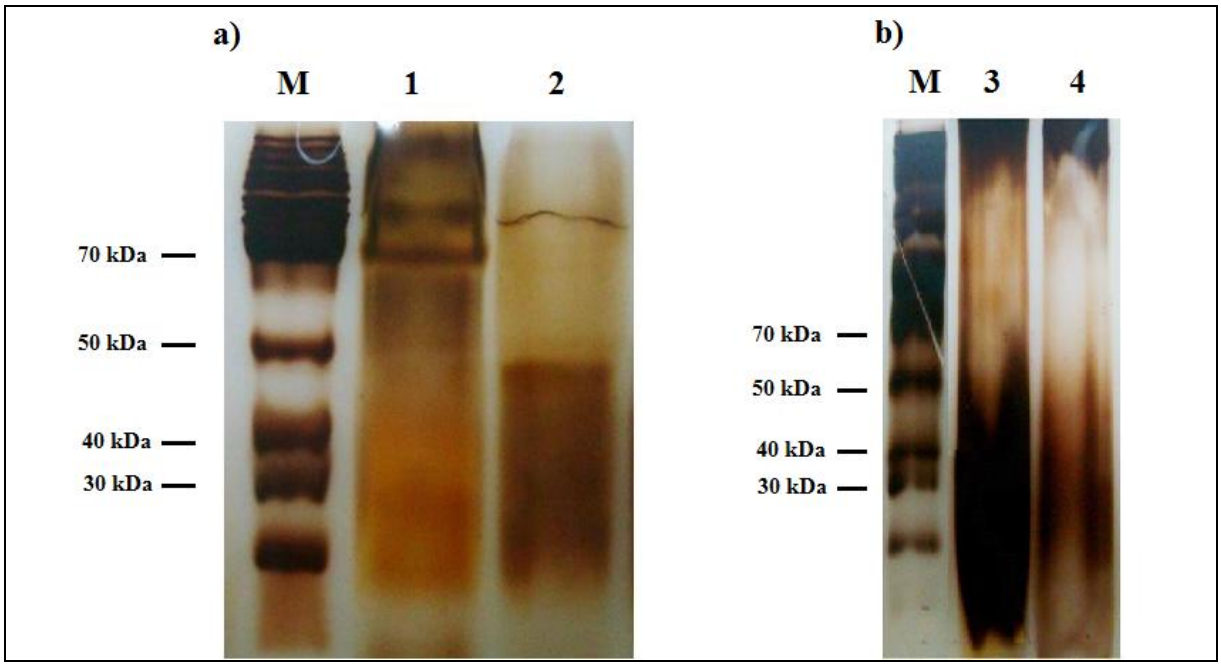
Hasta balıklarda gözlemlediğimiz ve *Vibrio, Aeromonas* ve *Pseudomonas* türü bakterilerin neden olduğu bakteriyel hemorajik septisemi vakalarında gözlenen deride pul kaybı, yüzgeçlerde erime, vücut yüzeyinde hemoraji ve ülserler gibi klinik bulgular, diğer araştırıcıların raporlarında belirtilen klinik bulgularla benzerlik göstermiştir (Austin ve Austin, 2012; Roberts, 2012). Ülkemizde balık hastalıkları konusunda çalışan diğer araştırıcıların da belirttiği gibi (Çağırgan ve Yüreklitürk, 1991; Timur ve Timur, 1991) *Y. ruckeri* ile enfekte balıklarda ise bu gibi bulgulara ek olarak ağız bölgesinde hemorajiler ile özellikle sırt yüzgecinde erime tespit edilmiştir.

Bakterilerin tür içi karakterizasyonunda ve serotiplendirilmesinde biyokimyasal testler yetersiz kalabilirken, hücre membranında bulunan protein ve lipopolisakkarit gibi yapıların SDS-PAGE analizi ile belirlenmesi sonucu daha kesin sonuçlar elde edilebilmektedir (Knappskog, 1993; Pazos vd., 1993; Pedersen vd., 1999; Boesen vd., 1999).



Şekil 3. a) VAM besiyeri üzerinde 24 saatte gelişen sarı renkli *V. anguillarum* kolonileri
b) *Pseudomonas* agar besiyeri üzerinde 24 saatte gelişen ve UV ışığı atında incelendiğinde floresan pigment üreten *P. fluorescens* kolonileri

Figure 3. a) Yellow coloured *V. anguillarum* colonies on VAM medium after 24 h incubation.
b) Fluorescent pigment producing *P. fluorescens* colonies under UV light on *Pseudomonas* Agar medium after 24 h incubation



Şekil 4. a) Farklı coğrafi bölgelerden izole edilen *V. anguillarum* suşlarının LPS profilleri (Sütun 1-2), b) Farklı coğrafi bölgelerden izole edilen *Y. ruckeri* izolatlarının LPS profilleri (3-4). M: belirteç

Figure 4. a) LPS profiles of *V. anguillarum* isolates recovered from different geographical regions (Lane 1-2), b) LPS profiles of *Y. ruckeri* isolates recovered from different geographical regions. (Lane 3-4) M: Marker

Çalışmada incelenen bakterilerden olan *V. anguillarum* izolatlarının LPS profillerinde gözlenen heterojenite durumu, daha önce salmonid balıklardan izole edilen ve aynı türe ait izolatlar arasında da tespit edilmiştir (Pazos vd., 1993; Pedersen vd., 1999; Boesen vd., 1999). Bunun yanı sıra *V. anguillarum*'un aynı serotipe ait izolatları arasında da LPS profili bakımından birtakım farklılıklar bulunduğunu bildirilmiştir (Pazos vd., 1993; Boesen vd., 199). Boesen vd. (1999) ise serotip içerisinde farklı LPS profiline sahip olan *V. anguillarum* izolatlarını, LPS profillerine göre sınıflandırmış ve bazı izolatların hem yüksek hem de düşük ağırlıklı LPS moleküllerine sahip olduğunu, bazılarının ise yalnızca düşük ağırlıklı LPS moleküllerine sahip olduğunu tespit etmiştir.

Yürütmüş olduğumuz bu çalışmadaki *Yersinia ruckeri* izolatlarının LPS profili ise Davies (1989) ve Bastardo vd. (2011) tarafından bildirildiği gibi tür içinde homojenite göstermiştir. Ancak farklı araştırıcılar *Y. ruckeri* izolatlarının LPS profilleri arasında biyotip (Tinsley vd., 2011) ve serotipe (Sousa vd., 2001) bağlı olarak ufak farklılıklar görülebileceğini bildirmişlerdir. Çalışma kapsamında hasta gökkuşağı alabalıklarından izole edilen *A. hydrophila* izolatının Dooley ve Trust (1988) tarafından; *A. schubertii* izolatlarının Kokka vd. (1992) tarafından; *P. fluorescens* izolatının ise Swain vd. (2003) tarafından bildirilen LPS profili ile benzer profiller oluşturdukları tespit edilmiştir.

Sonuç

Bu çalışmadan elde edilen veriler doğrultusunda yurdumuzun değişik bölgelerindeki kültür gökkuşağı alabalıklarından farklı patojen bakteriler izole ve identifiye edilmiştir. Farklı coğrafi bölgelerden izole edilen bakterilerin SDS-PAGE tekniği ile yapılan LPS profili analizleri incelendiğinde tür içinde heterojenite gösterdiği tespit edilmiştir. Lipopolisakkaritler, bakterilerin antijenik profillerini oluşturduklarından aşı geliştirme çalışmalarına da temel teşkil eder. Bu nedenle, kültür balıkçılığında bakteriyel hastalıkların önlenmesinde öncelikle yerel bakteriyel izolatların antijenik karakterizasyonunun yapılması ve sonrasında bu bakteriyel izolatlar kullanılarak bölgelere özgü balık aşılarının geliştirilmesi ile hastalıklara karşı daha etkin koruma sağlanabilir.

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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

SHORT COMMUNICATION KISA MAKALE

HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF *Sarotherodon melanotheron* FROM A SLUM NEIGHBOURHOOD ENVIRONMENT

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Abstract:

A total of 18 live specimens of the black jaw tilapia, *Sarotherodon melanotheron*, mean weight (82.95 ±3.27g) and mean length (13.1 ±0.35cm), were sampled bimonthly for three months from the Makoko area, a slum neighbourhood, of Lagos Lagoon, to establish the haematological and biochemical indices of the fish species which will serve as baseline for further studies and ascertain the health of the environment. The mean value and standard error of mean of some of the haematological parameters analyzed were: Hb: 89.65 ±3.63g/l, PCV: 27.47 ±0.58%, RBC: 3.05 ±0.06T/L, WBC: 10.02 ±0.40g/L, MCV: 86.87 ±1.39fl, MCH: 29.64±0.62pg, MCHC: 33.29 ±0.13g/dl, Neutrophils: 22.89 ±0.63%, Lymphocyte: 79.67 ±1.49% and Monocyte: 0.72 ±0.25%. No Eosinophils and Basophils were recorded in this study. While the mean and standard error of mean of the biochemical enzymes analyzed in IU/L, were: AST (76.84 ±4.08), ALT (23.06 ±2.12) and ALP (77.74 ±6.12). There was statistical significance ($p<0.05$) in the following haematological parameters: Hb, WBC, MCV, Neutrophils and Lymphocytes. There was statistical significance ($p<0.05$) in all the biochemical indices. It was established that the water was polluted with heavy metals, sewage and other organic pollutants such as wood wastes, and that these pollutants have altered the haematology and biochemistry of the fish species.

Keywords: Makoko, Lagos Lagoon, Pollutants, haematological, biochemical indices, *Sarotherodon melanotheron*

Introduction

Fishes are highly sought after today because of their high nutritive values. According to Ademola-Aremu *et al* (2009), fish constitute a major source of animal protein to a large number of Nigerians, particularly in the Lagos environs; they contain a high level of protein (17 - 20%) with an amino-acid profile similar to that of meat in ruminants and fowl, and when compared to beef, mutton, chicken and bush meat, fish tissues are less tough and more digestible. Today, these fishes are being threatened by pollution. Emmanuel and Ogunwenmo (2010), on their study of the Macrobenthos and the Fishes of a Tropical Estuarine Creek in Lagos, South-Western Nigeria, reported a low density of species for both macrobenthos and fish species. They attributed this decline to an unstable physically controlled environment resulting from anthropogenic induced stressors. As observed in this present study in Makoko Area of Lagos Lagoon, these anthropogenic stressors include, among others, discharges from: industrial effluents, urban wastes, agricultural runoff, untreated or partially treated sewage, oil and smoke from the engine and exhaust pipes of motorized boats and vehicles plying the third mainland bridge. Fishes are being used as biosensors to monitor the health of aquatic ecosystems, because of their sensitivity to water pollution. This point is supported by that of Summarwar and Deepali (2013) who stated that fish are relatively sensitive to changes in their surrounding environment, including an increase in pollution; and that fish health may as a result reflect, and give a good indication of the health status of the aquatic ecosystem in which the fish occurs. They further stated that the initial toxic effects of the pollution may, however, only be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance. Thus the health of an ecosystem is thus often reflected by the health of its fauna (Summarwar and Deepali, 2013). According to Nte and Akinrotimi (2011), the effects of exposure of fishes to sub lethal levels of pollutants can be measured in terms of their biochemical and physiological responses. The physical and chemical changes reflect in the blood component of the fish. Blood is therefore recognized as a potential index of fish response to water quality (Hickey, 1982). Biomarkers are measurements in body fluids, cells or tissues indicating biochemical or cellular modifications due to the presence and magnitude of toxicants, or of host response (NRC, 1987). Fish haematological and biochemical indices have been described by Nte and Akinrotimi (2011) as biomarkers used to ascertain the health status of the fish and that of the aquatic ecosystem where the fish is gotten from. Variations in blood indices in response to environmental conditions have been a major biomarker of stress in organisms. Alteration in enzymes activities of the exposed fish is one of the major biomarker indicating the level of changes consequent of pollutants in the tissues, the organs and body fluid of the fish that can be recognized and associated with established health impairment process (Akinrotimi *et al.,* 2009). However, Gabriel and Akinrotimi (2011) noted that biomarker can also be used to confirm and assess fish exposure to toxicants, providing a link between external exposure and internal structure and degree of responses to toxicant exposure observed between different individuals. The most compelling reason for using biomarkers is that they can give information on the biological effects of pollutants rather than a mere quantification of their environmental levels (Olakolu *et al.*, 2012).

The use of haematological parameters as fish health indicators was proposed by Hesser (1960) and has since been used as an index of fish health status in a number of fish species to detect physiological changes as a result of exposure to different stressful conditions such as handling, pollutants, metals, hypoxia, anaesthetics and acclimation (Duthie and Tort, 1985; Ogbulie and Okpowasili, 1999; Alwan *et al.*, 2009). Haematological indices are essential parameters for the evaluation of fish physiological status. Early diagnosis is possible when evaluating haematological data (Folmar, 1993; Golovina, 1996; Luskova, 1997). In addition, analysis of blood parameters will reveal conditions within the body of the fish long before there is any outward manifestation of disease or effects of unfavourable environmental factors such as stress (Sampath *et al*., 1993, Musa and Omoregie 1999). Thus haematological analysis of fish can be used for monitoring the aquatic ecosystem, where the fish lives, for early detection of pollution.

Enzymes are chemical substances (proteins) that help speed up a chemical reaction in the body (Ramalingam, 2011). Biochemical or liver function test also helps to determine the health status of fish as well as that of its environment. Biochemical constituents and some enzymes in the

blood are used as biomarkers as they are very sensitive, less variable and conserved between species (Owolabi, 2011). Changes in the activities of these enzymes could be an indication of tissue injury, environmental stress or diseased condition (Kori-Siakpere *et al*., 2010). An increase in enzymatic activity in the extracellular fluid or organs is a sensitive indicator of even minor cellular damage, since the levels of these enzymes within the cell exceed those in the extracellular fluid by more than three orders of magnitude (Das *et al.,* 2004). On the other hand, toxicants can also inhibit the activity or synthesis of enzymes (Jung *et al.*, 2003), resulting in decreased activities in the organs. This present study sought to establish baseline values for the haematological and biochemical parameters of *Sarotherodon melanotheron* (black jaw Tilapia) from the Makoko area – a slum neighbourhood on the Lagos Lagoon.

This present study sought to determine the impact of pollution of the Makoko Area of the Lagos Lagoon on the fish species, through the determination of the alterations in the haematology and biochemistry of the Black-jawed Tilapia (*Sarotherodon melanotheron*) caught from the area.

Materials and Methods

Study site: Makoko (Figure 1) is a slum neighbourhood community located on the eastern part of Lagos Lagoon, Nigeria. It lies approximately within latitude 6°29′46″N and longitude 3°23′16″E within the Lagos Lagoon. Majority of the people live in floating shanties built on the Lagoon and they engage in fishing as means of livelihood. Over a thousand fishing boats (paddled and motorized) could be located at a time. The water was observed to be darkly coloured, with offensive odour and human waste (faeces) was commonly observed floating on the water surface. The most discernible floras observed were the floating aquatic macrophytes; *Vossia cuspidata* and *Eichhornia crassipes* (water hyacinth).

Fish species:

Sarotherodon melanotheron (Ruppell 1852) is a cichlid that occurs commonly in West Africa and supports a major fishery in the lagoons. This status earns it the common name 'West African lagoon tilapia' (Eyeson, 1979). Pauly (1976) had earlier mentioned the species as a possible candidate for aquaculture. *S. melanotheron* is a demersal (bottom-associated) species inhabiting fresh to brackish water where it occurs. It is a tropical West African native occurring from Senegal to Zaire and southern Cameroon (Trewevas 1983, Robbins *et al.* 1991). The species is common in quiet muddy backwater habitats where aquatic vegetation is abundant (Jennings and Williams 1992). *S. melanotheron*, the blackchin (or black jaw) tilapia, is a pale (variable light blue, orange, golden yellow) cichlid whose common name refers to the dark pigmentation usually (but not always) concentrated on the underside of the head (the chin) in adult animals. Melanie pigmentation is usually also present on the posterior edge of the gill (the cleithrum) and on the tips of the soft dorsal rays. Irregular bars, spots or splotches on the body are also typical. The mouth is small and filled with up to several hundred very small teeth arranged in 3-6 rows (Trewevas 1983). In the Makoko area, the various species of Tilapia, including *S. melanotheron*, are the most landed fish species. The fishes were landed through the assistance help of the local fisher men using cast nets of various mesh sizes. The weight of specimens used in this present ranged between 47.9 – 96 g while the length ranged from 10 – 15cm.

Blood Sampling:

Bi-monthly collection of 18 live specimens of *S. melanotheron* was carried out for three months (May – July, 2013). The fishes were carefully netted and handled to minimize stress. They were put in large container (containing the lagoon water) for 2hrs prior to collection of blood samples. Collection of blood samples was done on the field to avoid stress due to transportation. Approximately 2ml of blood was collected using 2ml sterile plastic disposable syringes fitted with 0.8×38-mm hypodermic needles. The recovered blood was expressed into a vial containing dried or powdered potassium salt of ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The blood sample was rocked gently in the vial to allow thorough mixing of its contents. A further 2ml was taken with Lithium heparinised bottle and used to prepare blood films and for the determination of serum biochemical levels. The blood samples were taken in the morning time (between 08:00 and 10:00 hours) and held on ice chest until all samples were collected.

Figure 1. Map of Lagos Lagoon showing the Makoko Slum Area

Haematological and biochemical techniques

Haemoglobin (Hb) count was done with the cyanomethaemoglobin method; Packed Cell Volume (PCV) by micro haematocrit method. Red blood cell (RBC) and total white blood cell (WBC) counts were done using the Neubauer haemocytometer. Differential counts (neutrophils, monocytes and lymphocytes) were done on blood film stained with May Grumwald-Giensa stain. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the data using standard formulae.

Serum was separated from the cellular blood components by centrifugation for 5 min at 14,000 rev/min. Blood alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) were determined using a portable automated chemical analyzer following the procedure and using the reagents recommended and as described by the manufacturer (RANDOX Laboratories Ltd, UK; AST/ALT (Cat. No. Sc 2643) and ALP multi-sera level 2 (Cat. No. 1530) and level 3 (Cat. No.1532)), assay kits. All blood analyses were carried out within 48 hours of collection.

Haematological parameters and biochemical indices were analysed using one-way analysis of variance (ANOVA) at 5% level of significance. While post-hoc comparison of significance of variance result gotten from ANOVA was done using Duncan Multiple Range Test (DMRT).

Results and Discussion

Haematological parameters

Table 1 showed the mean and standard error of mean for the haematological parameters. The analysis of variance (ANOVA) for the haematological parameters showed that only Hb, WBC, MCV, NEUT, and LYMP are significant ($P<0.05$). Post-Hoc analysis using DMRT (Duncan Multiple Range Test) showed that there was significant difference ($P<0.05$) in Hb, WBC, MCV, MCH, NEUT. and LYMP.

Biochemical parameters

Table 2 showed the mean and standard error of mean for the biochemical parameters. ANOVA showed that there was significant difference ($P<0.05$) in the three biochemical parameters.

But DMRT showed that there was significant difference in: AST for weeks 1, 2, 3 and 5; ALT for weeks 1, 2, 4, 5 and 6; ALP for weeks 1, 2, 4, 5 and 6.

In this present study, the analysis of the physicochemical parameters of the water showed that the Makoko Area is polluted with heavy metals and organic pollutants – sewage and wood wastes, and oil film. These lead to low/depletion of the oxygen level. All these, in turn, cause physiological stress to the fish. This could be responsible for the low RBC and Hb and the comparable higher WBC recorded in this study. This view conforms to that of Ugwu *et al.* (2006) who reported significant changes in blood haemoglobin and neutrophil concentrations of *Heterobranchus bidorsalis* and concluded that the changes reflect the responses to the effects of stress caused by toxicants - crude oil and its fractions. According to Witeska and Kosciuk (2003), this results in cell swelling deformation and damage. According to Atamanalp and Yanık (2002) the low Hb levels may impair oxygen supply to the various tissues and result in slow metabolic rate and low energy production. Long term exposures of fish to effluents and sewages have been reported to alter haematological parameters by disrupting haematopoiesis, consequently resulting in anaemia condition (Nikinmaa and Oikari, 1992; Ellis *et al.*, 2003). Landman *et al.*, (2006) also observed changes in blood profiles of *Cyprinus carpio* and attributed it to the effect of effluents from paper mill industries.

Table 1. Mean and S.E for Haematology of *Sarotherodon melanotheron* caught at the Makoko area of the Lagos Lagoon

PARAMETER	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	GRAND MEAN
Hb(g/L)	94.57±3.35^{ab}	90.17±10.62^{ab}	84.30±7.54^{a}	83.90±4.91^{a}	111.33±6.77^{b}	73.63±3.82^{a}	89.65±3.63
PCV(%)	27.00±1.53^{a}	29.67±0.55^{a}	26.67±2.03^{a}	25.67±1.45^{a}	29.47±0.64^{a}	26.37±0.86^{a}	27.47±0.58
RBC(T/L)	3.06±0.03^{a}	3.18±0.11^{a}	3.24±0.18^{a}	2.87±0.20^{a}	3.07±0.07^{a}	2.87±0.07^{a}	3.05±0.06
WBC(g/L)	10.14±0.88^{ab}	8.96±1.43^{a}	11.93±0.41^{b}	8.29±0.65^{a}	10.77±0.45^{ab}	10.03±0.50^{ab}	10.02±0.40
MCV(fl)	91.21±1.06^{b}	89.88±1.24^{b}	78.87±2.63^{a}	89.83±1.30^{b}	90.74±1.20^{b}	80.67±3.04^{a}	86.87±1.39
MCH(pg)	32.18±0.04^{b}	30.10±0.55^{ab}	26.73±1.23^{a}	29.60±0.46^{ab}	30.67±1.0^{ab}	28.57±2.78^{ab}	29.64±0.62
MCHC(g/dL)	33.59±0.48^{a}	33.10±0.06^{a}	32.93±0.12^{a}	33.10±0.45^{a}	33.10±0.15^{a}	33.90±0.21^{a}	33.29±0.13
NEUT(%)	23.33±0.88^{ab}	20.67±1.76^{a}	23.00±1.15^{a}	26.67±0.67^{b}	23.33±0.88^{ab}	20.33±0.88^{a}	22.89±0.63
LYMP(%)	78.33±1.45^{b}	88.00±1.15^{c}	78.00±1.73^{b}	70.33±0.88^{a}	78.33±1.86^{b}	85.00±2.52^{c}	79.67±1.49
MONO(%)	0.33±0.33^{a}	0.67±0.67^{a}	0.33±0.33^{a}	1.67±0.88^{a}	0.33±0.33^{a}	1.00±1.00^{a}	0.72±0.25
EOS(%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
BAS(%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 2. Biochemical Parameters of *Sarotherodon melanotheron* caught at the Makoko area of the Lagos Lagoon

PERIODS	PARAMETER		
	AST(IU/L)	**ALT(IU/L)**	**ALP(IU/L)**
Week1	76.52 ±9.84^{ab}	22.67 ±0.67^{c}	63.48 ±5.52^{b}
Week2	74.73 ±13.63^{ab}	22.56 ±1.39^{c}	74.52 ±1.59^{bc}
Week3	94.93 ±1.85^{b}	11.14 ±2.57^{a}	45.08 ±5.12^{a}
Week4	66.11±0.77^{a}	26.33±0.38^{c}	73.62±2.43^{bc}
Week5	92.49±0.62^{b}	16.94±0.91^{b}	125.12±6.44^{d}
Week6	56.24±1.98^{a}	38.71±1.34^{d}	84.64±3.31^{c}
GRAND MEAN	76.84±4.08	23.06±2.12	77.74±6.12

NB: Mean frequencies (mean ± S.E, Standard Error) with different superscript letters in a row are significantly different in the DMRT ($p<0.05$)

In the present study, the lymphocytes, mean value 79.67 ±1.49, were observed to be numerous than any other differential cells, this is typical of most fishes (Owolabi, 2011). The abundance of neutrophils and monophils in this fish species is also typical of most fishes (Owolabi, 2011). No eosinophils and basophils were recorded in this study; this is also typical of most fish species and could be attributed to the influence of the heavy metals especially cadmium. It has been evidenced that cadmium influences the differential blood count (Gill and Eple 1993).

In this present study, there are significant differences ($P<0.05$) in the values of the serum enzymes; AST, ALT and ALP. This could be an indication that the water contains some stressors that cause damage to some organs and tissues such as the liver and the cardiovascular tissues of the fish, leading to the liberation of these enzymes into the blood. Gul *et al.* (2004) considered enzymes as sensitive biochemical indicators of toxicity in organs of fish. Blood is an active transport medium in higher animals, especially in vertebrates (Ramalingam, 2011). It constantly bathes all the organs and tissues of the body, enabling exchange of materials between the internal and external environment of these organs and tissues (Ramalingam, 2011). Therefore, a biochemical analysis of the fish blood should confirm the level of these enzymes – and this should be an indicative of the pollution status of the environment they are caught from. This view is confirmed by Parma *et al.* (2007) who stated that the enzymes AST and ALT are transaminases which are basically intercellular enzymes found in most organs of fish.

In Makoko area of Lagos Lagoon, raw sewage is commonly seen floating on the water surface.

This sewage could act as stressor and cause distress to fish. According to Ortiz *et al.* (2003), distress is one of the early symptoms of sewage poisoning in fish. Distress in fish leads to damage in cells and tissues of the organs, which, in turn, elicits the liberation of these enzymes into the blood stream; prompting their high levels in the blood as recorded in this present study. This view is supported by that of Nte *et al* (2011) who also recorded high levels of these enzymes in the organs of *S. melanotheron* exposed to different concentrations of industrial effluents. They noticed that the levels of the enzymes increased as the concentration of the effluents increased. In this present study, the levels of the enzymes are relatively constant throughout the period under survey; this could be due to continual introduction of pollutants into the Lagos lagoon all year round, causing their consistency in concentration.

The significant difference ($P<0.05$) in the ALT mean values of the *S. melanotheron* could be due to several reasons such as differences in mode of feeding, age and species. This view is supported by the report of WHO (1989) and Hunn *et al* (1993) who observed that effluents acute toxicity varies widely depending on the species, age and formulation.

Conclusion

This study has shown that the Makoko Area of Lagos Lagoon is polluted. The various pollutants are inducing serious physiological stress on the commercial feral fish species. This has resulted in alterations in the haematology and biochemical indices of these fish species, as shown by the relative high values recorded in this present study. There is, therefore, need to abate pollution of the area, to prevent decline in the commercial fish species, such as *S. melanotheron*, that serve as rich source of protein to the inhabitants of Lagos and its environs.

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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

SHORT COMMUNICATION KISA MAKALE

ABNORMALITIES IN THE WEDGE SOLE *Dicologlossa cuneata* (MOREAU, 1881) AND BLACK SEA TURBOT *Scophthalmus maeoticus* (PALLAS, 1814) FROM TURKISH SEAS

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Abstract:

Both color and morphological abnormalities on two different flatfish species [*Dicologlossa cuneata* (Moreau, 1881) and *Scophthalmus maeoticus* (Pallas, 1814)] have been recorded from Turkish seas. Abnormal flatfish species, *Dicologlossa cuneata* (Moreau, 1881), wedge sole, was sampled from two different localities in Aegean sea. First wedge sole from Izmir bay (Aegean sea) had three different colour abnormalities (ambicoloration; albinism and xanthochroism) on eyed and blind sides of their body. Other abnormal wedge sole specimen from Ekincik cove (Aegean sea) had ambicoloration on blind side of the body. Second abnormal flatfish species *Scophthalmus maeoticus* (Pallas, 1814), Black sea turbot, was caught from the Black sea coast of Istanbul and the specimen had totally ambicolored (blind side of the body was colored as like as eyed side) and morphological abnormalities (a fleshy piece-hook-above the head).

Keywords: *Dicologlossa cuneata*, *Scophthalmus maeoticus*, Wedge sole, Black sea turbot, Anomalies, Albanism, Turkish seas

Introduction

Flatfish, of the order Pleuronectiformes, has received much attention from biologists for more than a hundred years because of their complex behavior (Burton, 2002). Flatfishes have asymmetrical external pigmentation (Norman, 1934). The eyed side of the fish is colored while the blind side is completely white (Venezilos and Benetti, 1999). A variety of abnormalities have been noted among the flatfish specimens. These are morphological deformities (particularly the head and/or caudal regions) and pigment anomalies (Gartner, 1986).

There are two main pigmentation anomalies, a deficiency or absent of pigment cells on ocular side called as albinism and excess pigmentation on the blind side called as ambicoloration (Bolker and Hill, 2000). Another pigmentation anomalies is presence of yellow chromatophores (xanthophores) on the eye-side, called xanthochroism (De Veen, 1969). Color abnormalities have been reported for a variety of flatfish species (e.g. Cunningham, 1907; Hussakof, 1914; Norman, 1934; Gudger, 1936; Gudger and Firth, 1936; De Veen, 1969; Houde, 1971; Fujita, 1980; Gartner, 1986; Chaves et al., 2002; Quigley, 2003; Carnikian et al., 2006; Diaz de Astarloa et al., 2006; Macieira et al., 2006; Da Silva Junior et al., 2007; Akyol and Şen 2012).

As a result of unsuccessful eye migration, dorsal fin is blocked by anterior movement of the fin and this results in as a fleshy piece-hook-above the head. These flatfishes are called 'hooked' and sometimes it can also cause abnormal pigmentation (Norman, 1934). There were a few records for head anomalies in flatfishes (Gudger and Firth, 1936; Fujita, 1980; Gartner, 1986; Diaz de Astarloa et al., 2006; Macieira et al., 2006).

A record of any type of abnormality is important in environmental impact and background studies, and documentation of color anomalies may be particularly useful: As diffuse pigmentation may indicate a recent parasitic infestation--probably from localized sources, records of incidence of this anomaly may be useful in determining a population's residence time at a specific location (Gibson, 1972).

Here we report first record on colour anomalies in two species of wedge sole (*Dicologlasso cuneata)* and addition record on head and color anomalies of Black sea turbot (*Scophthalmus maeoticus)* in the eastern Mediterranean sea. We hope that findings obtained from the study will contribute to management of natural flatfish populations.

Materials and Methods

We found color anomalies on two the wedge sole, *Dicologlossa cuneata* (Moreau, 1881) specimen and one head and color anomalies on Black sea turbot (*Scophthalmus maeoticus)* from Turkish sea. First abnormal wedge sole specimen was caught by using trammel net (mesh size 30 mm nominal bar length and hanging ratio was 0.50%) from the Homa lagoon in Izmir bay (Aegean sea). (38º30′–38º35′N; 26º48′–26º53′E). Trammel net was used sovernight for a day in November 2008.

Second abnormal wedge sole specimen was caught by using the traditional type trawl net (mesh size 44 mm) from the Ekincik cove in SE Aegean Sea (36° 48′ 77″ N - 28° 33′ 317″E ; 36° 47′ 250″N - 28° 35′ 420″ E) (in December 2009). The other abnormal flatfish is *Scophthalmus maeoticus* (Pallas, 1814), Black sea turbot, caught by using gill net (mesh size 160 mm nominal bar length and hanging ratio was 0.33%) from Black sea coast of Istanbul region (41° 13′ 18″ N- 29° 06′ 16″ E) (in April 2011) (Figure 1.).

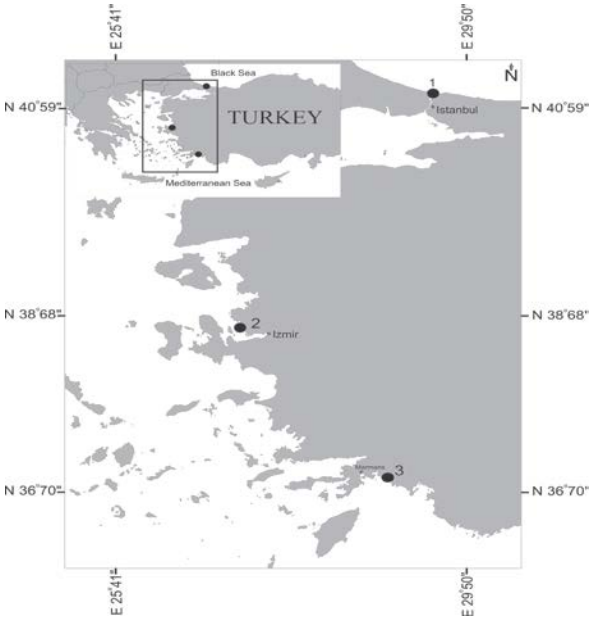

Figure 1. Sampling locations of the documented abnormal flatfish records in Turkish seas waters (1: *Scophthalmus maeoticus*; 2 and 3: *Dicologlossa cuneata*)

Results and Discussion

First color abnormal wedge sole specimen from Izmir bay was female. Total length of specimen was 18.8 cm and weighted 53.8 g. The specimen had three different types of abnormality; ambicoloration (approximately 15% coloration on the blind side). Xanthochroism (especially this characteristic was observed on two sides of the body) and albinism (ocular side of the specimen was none or less pigmented, approximately 70% of the body and also approximately 80% of the cephalic region) (Figure 2).

Second abnormal wedge sole specimen from the Ekincik cove was female. Total length of specimen was 27.0 cm and weighted 150.0 g. The specimen had ambicoloration (approximately 35% coloration on the blind side) (Figure 3).

Total length of head and color abnormal turbot was 55.5 cm and weighted 3.5 kg. The specimen was female and had totally ambicolored (whole blind side was colored as eyed side) and also had little curve (like a hook) above head region (Figure 4).

Flatfishes have typically asymetrical external pigmentation (Norman, 1934) and also the ocular surface of the fish is pigmented while the blind side is entirely white (Venizelos and Benetti, 1999). Pigmentation of teleost fishes are affected by both neural and hormonal controls (Venizelos and Benetti, 1999; Burton, 2002). Abnormalities in Pleuronectiform fish are caused by several reasons (environmental, nutritional and neurological) which are mostly caused by eye migration (Venizelos and Benetti, 1999).

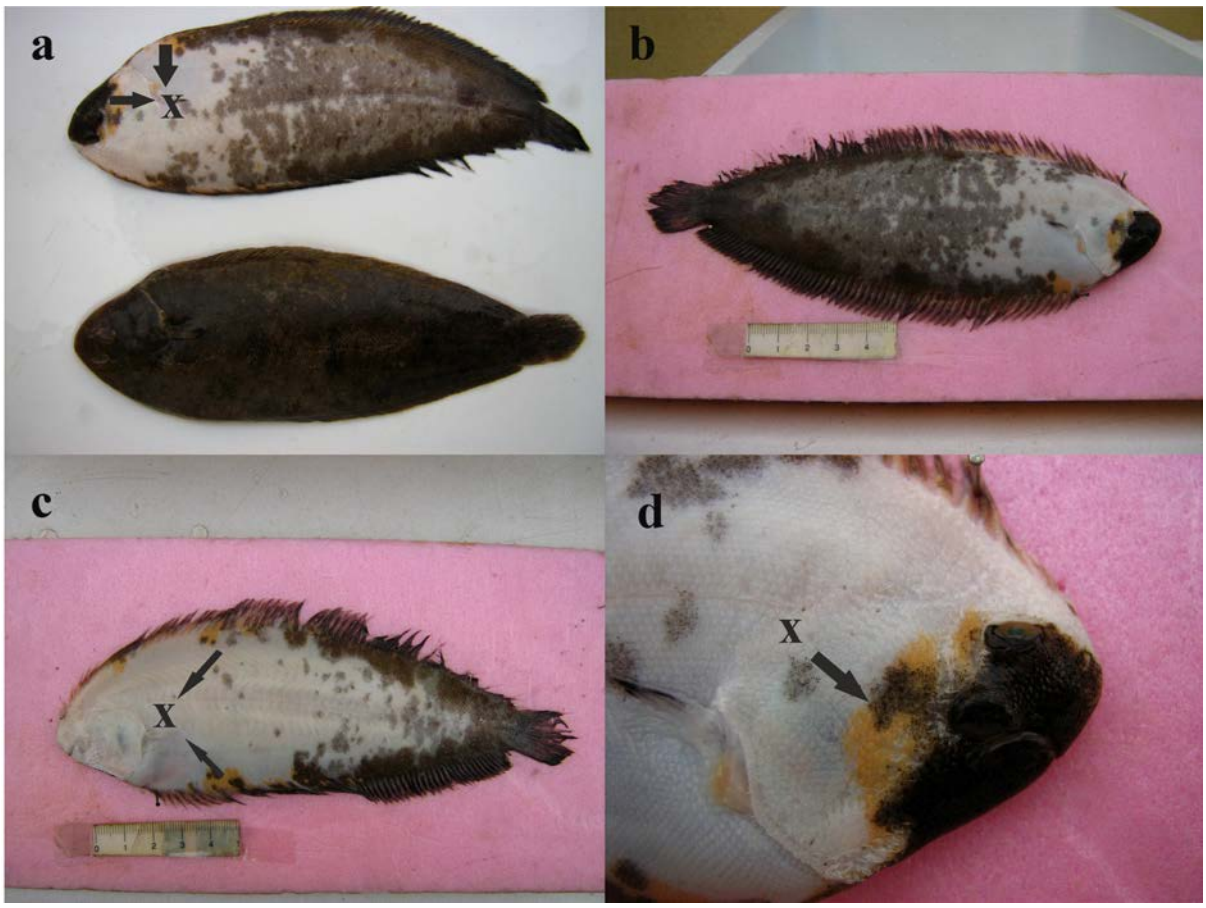

Figure 2. Partial albanism and ambicolouration in *Dicologlossa cuneata* specimen from Izmir bay (**a**: Abnormal specimen-upside and normal specimen-downside; **b**: Albanism **c**: Partial ambicolouration, on the blind side **d**: Albanism on the cephalic region **x**: refers to xanthochroism on the blind and eyed sides).

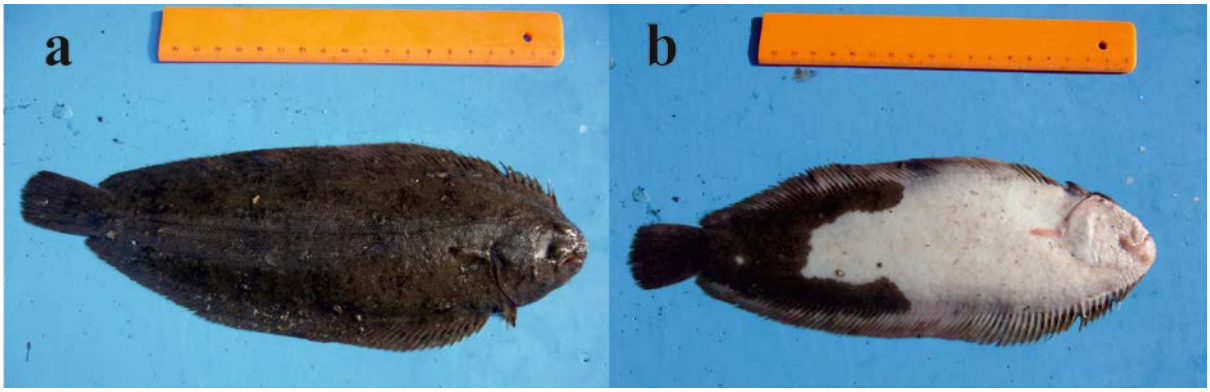

Figure 3. Partial ambicolored specimen of *Dicologlossa cuneata* from Ekincik cove (**a**: Eyed side of the body **b**: Blind side of the body).

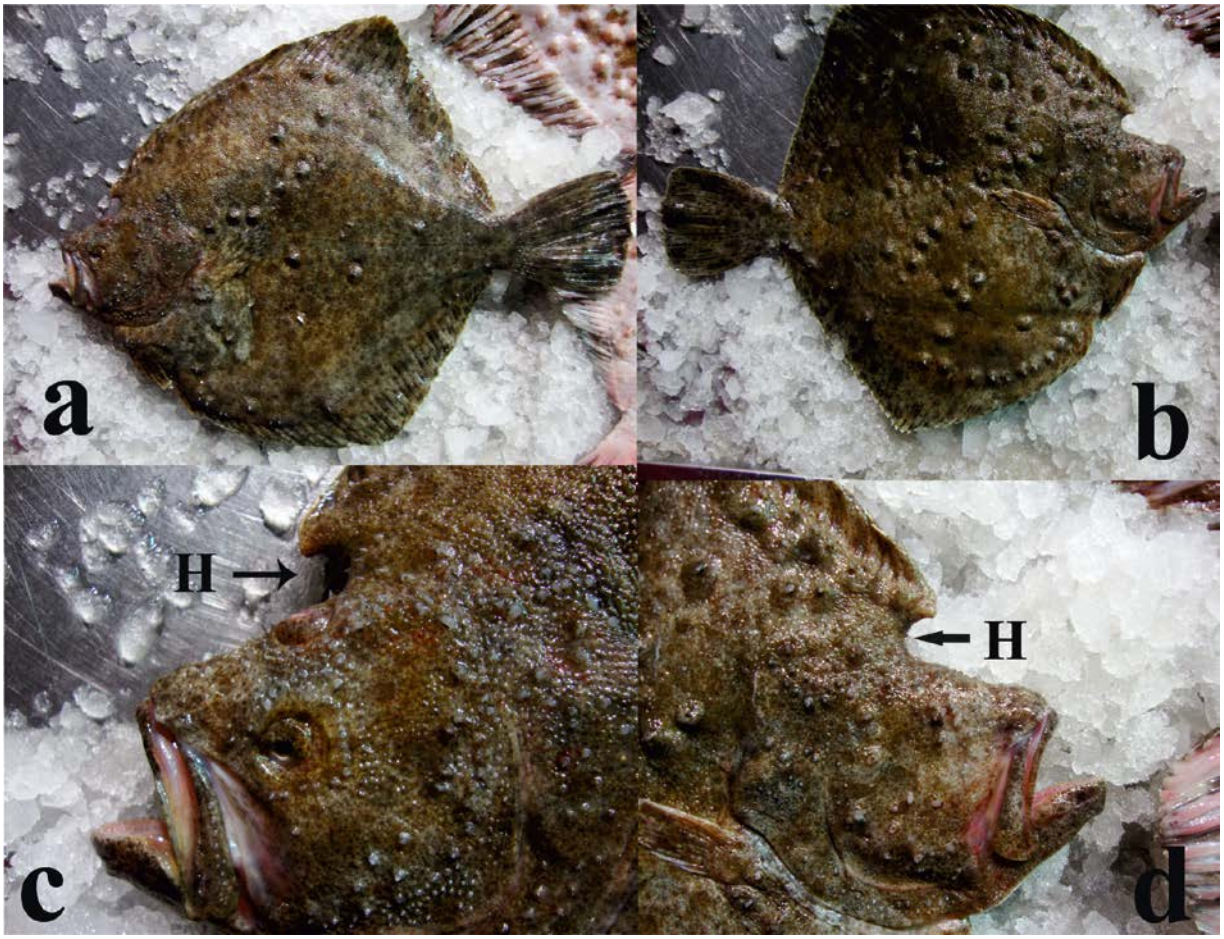

Figure 4. A totally ambicolourate (duble) specimen of *Scophthalmus maeoticus* from the Black sea (**a**: Eyed side of the specimen **b**: Blind side of the specimen **c**: Hook above the head in view of eyed side **d**: Hook above the head in view of blind side).

Pigment abnormalities are also very common in aquacultured specimens because of several reasons such as temparature, feeding protocols when they are in eye migration, etc. (Venizelos and Benetti, 1999; Bolker and Hill, 2000; Burton, 2002; Aritaki and Seikai, 2004; Tong et al., 2012). Abnormally pigmented flatfishes are very common in nature (Da Veen, 1969). For example; a study from the North Irish Seas showed that flatfish population was made out of ambicolorate European flounder (*Platichthyes flesus*, 28%), European plaice (*Pleuronectes platessa*, 8%) and common dab (*Limanda limanda*, 0.8%) individuals (Shelton and Wilon, 1973). Norman (1934) mentioned that there were several examples of abnormally pigmented flatfishes such di-

verse genera as Scophthalmus, Limanda, Platichthys, Pleuronectes and Solea.

There were some records of abnormally pigmented flatfishes in the Mediterranean Sea. First record of *Solea solea* from western Mediterranean Sea had two forms of colour abnormality (albanisim and ambicoloration) (Paris and Quingnard, 1986). The other common sole record was given from Izmir bay (Aegean Sea) which had partially ambicolorated, xanthochroistic and albanist features (Akyol and Sen, 2012). The found of abnormal colored specimen of *Dicologlossa cuneata* is the first record for wedge sole from the Mediterranean sea.

The first record of turbot (totally ambicolorate) was from Istanbul coast, which probably was *S. maeotica* (Black sea turbot) but there was not any photo or illustration (Devecian, 1915). This is additional record of *S. maeotica*, which was totally ambicolored (duble) and hooked, for the Mediterranean sea region.

Norman (1934) reported that hook above head and bony tubercules, which are developed on the blind side, are characteristic features for turbot individuals and also corresponds with our findings on the specimen from Istanbul coast.

In Norway Von Ubisch (1951) reported that, fishermen called abnormally pigment flatfish as 'Biologist Fish' (the pseudo-albinic plaice) because they were released by fisheries biologists after experiments.

Further totlly ambicolorate turbots are called "double turbot" by Turkish fisherman and French fisherman (Devecian, 1915; Paris and Quignard, 1968).

There are many reasons for color abnormalities on flatfish individuals (Norman, 1934; Venizelos and Benetti, 1999; Bolker and Hill, 2000; Aritaki and Seikai, 2004), but it still unknown (Venizelos and Benetti, 1999). Many studies have indicated that light intensity, feeding during larval stages, or neurological aspects such as hormones (i.e. endocrine system) are involved in body color patterns, while genetic factors and environmental stressors are reported as possible hypotheses to explain ambicoloration (Bolker and Hill, 2000; Tagawa and Aritaki, 2005). Moreover, environmental contamination of sediments due to anthropic and industrial activities could also contribute to the effect (Yamamoto et al., 1992).

Conclusions

So this subject needs to be studied further, and must be detailed on the subjects of frequence of occurrence in flatfish populations and genetic differences between normal and abnormal ones.

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