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# Fungal and Bacterial Co-Infection of Sea Bass (*Dicentrarchus labrax*, Linnaeus 1758) in a Recirculating Aquaculture System: *Saprolegnia parasitica* and *Aeromonas hydrophila*

Ezgi Dinçtürk<sup>1</sup> , Tefvik Tansel Tanrıku<sup>1</sup> , Saniye Türk Çulha<sup>2</sup> 

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## ABSTRACT

*Aeromonas hydrophila* causes symptoms of hemorrhagic septicemia in acute cases and can cause death in freshwater fish, whereas *Saprolegnia parasitica* is the cause of ulcers complicated by fungal mycelia located on skin that has lost its scales for various reasons. Both pathogens co-infect recirculating aquaculture systems at the İzmir Katip Çelebi University Fisheries Research and Training Center. Clinical, bacteriological, parasitological, and mycological studies were carried out on 25 fish samples during the infestation. Sabouraud glucose agar and malt extract agar were used to isolate the fungus, and the bacterial isolates were streaked on tryptic soy agar (Oxoid) with 5% defibrinated sheep blood. Gray-white cotton-like patches, erosions on the skin, and hemorrhaging were detected on the infected fish samples. The analytical profile index test and molecular identification showed that the bacterial agent was *A. hydrophila* and a fungal examination and amplification by polymerase chain reaction confirmed that the mycotic agent was *S. parasitica*.

**Keywords:** *Aeromonas hydrophila*, *Dicentrarchus labrax*, *Saprolegnia parasitica*

## INTRODUCTION

*Aeromonas hydrophila* is an opportunistic microorganism that is widely distributed in water, soil and food (Laith and Najiah, 2013). It is a Gram-negative motile bacterium with aerobic and facultative anaerobic, oxidase-positive characteristics and can be found in aquatic environments and gastrointestinal tracts of healthy fish (Rey et al., 2009; Laith and Najiah, 2013). It is known as Bacterial Hemorrhagic Septicemia, Aeromonad Septicemia and Red Pest (Roberts and Shepherd, 2001) the major symptoms of which are skin ulcers, hemorrhaging and necrosis of visceral organs (Huizinga et al., 1979; Austin and Austin, 2007; Cipriano et al., 2001). *A. hydrophila* has a great impact on fish farms causing outbreaks with a mortality rate of 80-100% in a short period of time (Lukistyowati, 2012; Kusdarwati et al., 2017). To date the pathogen has been reported in various fish species such as mirror carp and gold fish (Timur, 1986), rainbow trout (Diler and Al-

tun, 1995), guppy (Timur et al., 2003) and xiphophorus (Akayli and Zeybek, 2005) caught in Turkey.

Saprolegniasis is a mycotic freshwater disease that especially, affects fish and eggs (Gaikowski et al., 2003). It usually starts with cotton wool-like patches on the head and dorsal fin region then spreads all over the body as focal patches (Abdel-Aziz et al., 2002; Bangyakkum et al., 2003; Osman et al., 2008; Roberts, 2012). Erosion, poor water quality, overcrowding, handling, malnutrition, spawning or bacterial and parasitic infections may be the cause of this mycotic disease and it can lead to mortality (Noga, 1993; Pickering, 1994; Hussien et al., 2010). In Turkey, Saprolegniasis has, generally, been reported in freshwater fish species, especially in rainbow trout eggs (Diler, 1992).

Co-infections are described as infections caused by two or more genetically different pathogens and each pathogen has pathogenic

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effects that cause harm to the host when other pathogens are present (Cox, 2001; Bakaletz, 2004; Kotob et al., 2016). Interactions between different pathogens can have either synergistic or antagonistic effects on fish. *Aeromonas hydrophila* and *Saprolegnia parasitica* were detected in sea bass (*Dicentrarchus labrax*) with high mortalities and clinical signs of both pathogens in this study.

## MATERIAL AND METHOD

Infected sea bass (*D.labrax*) with fungal infections and hemorrhages on different parts of the body were found in the university pond at İzmir Katip Çelebi University Fisheries Research and Training Center. Fish weighing approximately 40 gr were examined during the infestation and mortalities with water parameters were observed. 25 of the samples were subjected to clinical, microbiological, parasitological and pathological examination. An external and internal examination was carried out on the skin, abdomen, fins, gills, kidneys, intestines, liver and spleen. Bacterial examinations were conducted according to Austin and Austin (2007). Bacterial isolates were streaked on Tryptic Soy Agar (TSA, Oxoid; Merck, Germany) and Tryptic Soy Agar supplemented with 5% defibrinated sheep blood (BTSA) from the anterior of the kidneys, spleen and liver of the fish.

Fungal samples were provided from the patches on the body surfaces and streaked on Sabouraud Glucose Agar (SGA, Oxoid; Merck, Germany) and Malt Extract Agar (MEA, Oxoid; Merck, Germany). The bacterial samples were incubated at 21°C for 2 days and fungal samples were incubated at 21°C for 3-4 days. Native and colored samples were examined with a light microscope (BX53; Olympus, Japan) after incubation. Colorization of the fungus samples was conducted according to Arda (2006).

A scanning electron microscopic examination was carried out at İzmir Katip Çelebi University's Central Research Laboratory and for preparation, samples were sputtered with gold by QUORUM Q150 RES (Quorum Technologies, UK) and examined with a scanning electron microscope (Carl Zeiss 300VP, Germany).



**Figure 1.** Hemorrhagic septicemia and fungal growth on sea bass (*D.labrax*)

*Aeromonas hydrophila* strains were passaged onto TSA and BTSA three times in order to purify the colonies after primer isolation. They were then cultivated onto TSA for biochemical tests. Gram staining and an oxidase test were carried out according to standard procedures. The motility of the bacteria was detected by the hanging-drop technique. For further information, biochemical tests were carried out with API 20E (BioMerieux S.A., France) (Tannikul et al., 2004).

Molecular identification of the bacteria and fungus were conducted. The 16SrRNA gene sequence was polymerase chain reaction (PCR) amplified in order to ensure that strains were *A.hydrophila*. Strains were obtained from the samples that were isolated from infected *D.labrax* during the outbreak. A EurX GeneMATRIX Tissue Bacteria DNA Isolation Kit (EURx, Poland) was used for DNA isolation. Then with a Thermo Scientific Nanodrop 2000 (ThermoFisher Scientific, USA), the density and quality of the isolates were determined. 27F and 1492R primers were used for PCR amplifications. Band screening of the PCR products was observed in the gel electrophoresis. Amplified products of template DNA were sent to the Macrogen direct sequencing service (Macrogen, Holland) for sequence determination. Sequences were then checked with the BLASTN 2.6.1. database. The Same procedure was used for fungus samples. DNA isolation was conducted using a EurX Tissue Bacteria DNA isolation kit. For PCR amplification, ITS1 and ITS4 primers were used and DNA samples were sent to Macrogen direct sequencing service (Macrogen, Holland) and sequences were checked with the BLASTN 2.6.1. database.

The antibiotic susceptibility of *A. hydrophila* strains was determined by the Kirby-Bauer disk diffusion method onto Mueller-Hinton medium (Hudzicki, 2009).

The water parameters such as temperature, salinity, oxygen and pH parameters were determined as 10°C, 3.93‰, 10.07 mg/L and 7.7, respectively. The suspended solid matter was analyzed with gravimetric methods (Stirling, 1985). Analyses of ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, phosphate phosphorus, silica and chlorophyll-a were performed using a Spectrophotometer (DR 6000 Hach LANGE; Germany) (Strickl and Parsons, 1972; Parsons et. al., 1984; Stirling, 1985). Alkalinity, Ca and Mg were analyzed according to Egemen and Sunlu, (2003).

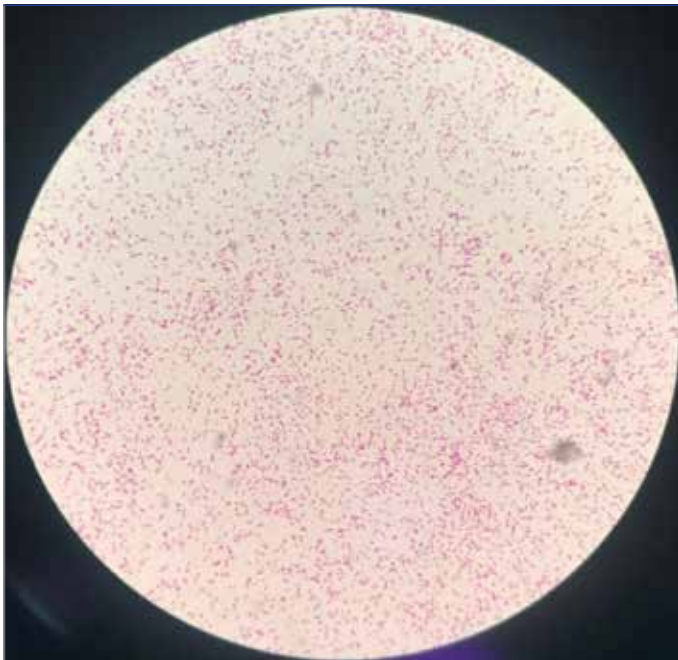
## RESULT AND DISCUSSION

Stunned swimming, loss of balance and appetite were observed during the outbreak. Grey-white cotton like patches, erosion on skin and hemorrhages were shown on the infected fish samples. Mycelial growth and hemorrhages were observed on the fins and body surface (Figure 1).

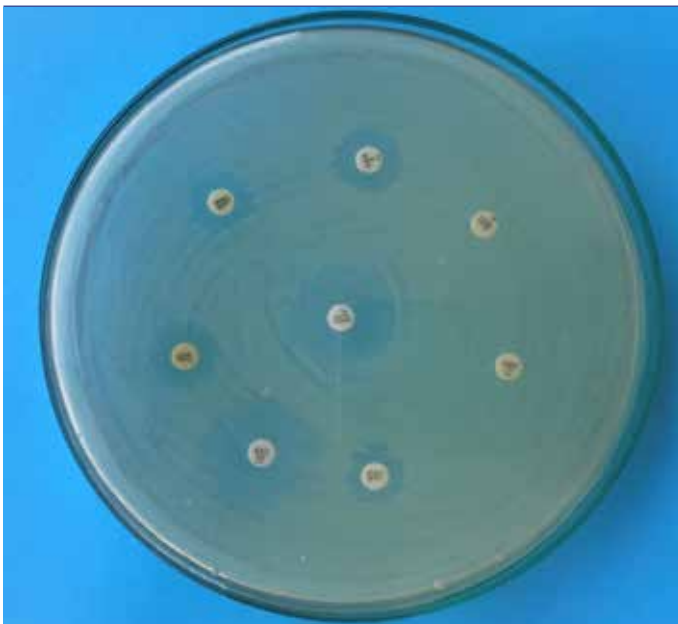
*Aeromonas hydrophila* was isolated from the internal organs of sea bass with bacteriological studies from all the fish samples. Gram staining of the bacteria was shown in Figure 2. API test and molecular identification were also conducted (Figure 2, Table 1) with similar results.

The PCR amplification of the *A. hydrophila* gene sequence was registered in the BLASTN 2.6.1 database. It resulted in 100% nucleotide identity between the current isolate and *A. hydrophila* (accession number MF445123.1).

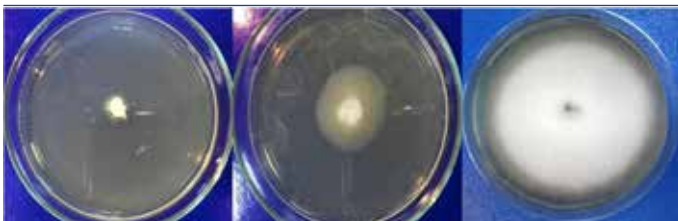
The antibiotic susceptibility test results are shown in Figure 3 and Table 2.



**Figure 2.** Gram staining of *A. hydrophila*



**Figure 3.** Antimicrobial susceptibility of *A. hydrophila*

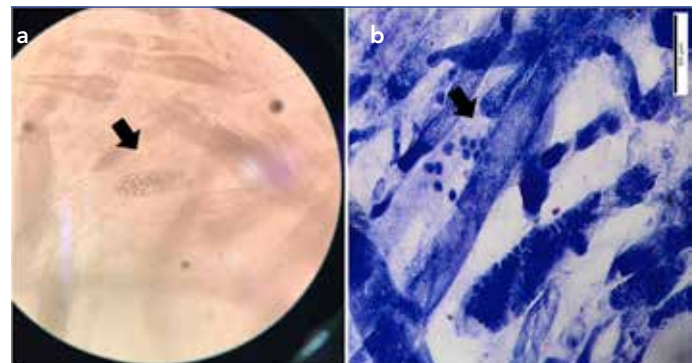


**Figure 4.** Fungal growth on SGA from skin samples of sea bass

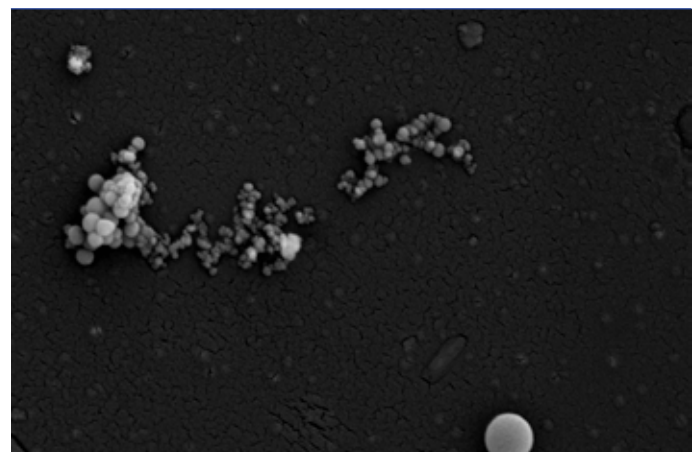
Fungal growth was detected on SGA and MEA within 4 days of the samples being taken from mycelial growths on body surfaces (Figure 4). Long and branched hyphae with cysts were detected with a native and colored examination (Figure 5). Fungal zoospore was displayed from scanning electron microscopy overviews (Figure 6).

The PCR amplification of the 18S rRNA gene sequence was registered in the BLASTN 2.6.1 database. The FASTA homology showed 100% nucleotide identity between the current isolate and *Saprolegnia parasitica* (accession number AM228725.1).

*Aeromonas hydrophila* is a part of the normal intestinal microflora of healthy fish and stress is often a contributing factor (Yu et al., 2004). It is an opportunistic pathogen (Arda et al., 2002) and effected by environmental factors such as oxygen, temperature, pH and stocking density (Klein and Wu, 1974). Like *A. hydrophila*, *Saprolegnia spp.* was reported from the natural environment of fish and, with suitable conditions, it is possible to be present as a secondary infection. Fish which are more vulnerable to physical conditions tend to be infected by Saprolegniosis (Neish and Huges, 1980; Diler, 1992). Saprolegniosis is considered as a secondary infection by Bruno et al. (2011) with immunosuppression, bacterial infections, poor husbandry and parasite infestations. The effect of this pathogen is reduced osmoregulation, which



**Figure 5. a, b.** (a) Zoosporangiums of *S. parasitica* (x40) (b) Giemsa staining view of zoosporangiums



**Figure 6.** The zoospores of *S. parasitica*. SEM. Bar. 200 µm scale

**Table 1.** API 20E results of isolated bacteria confirmed that the isolate was *Aeromonas hydrophila*

ONPG	+	GLU	+
ADH	+	MAN	+
LDC	-	INO	-
ODC	-	SOR	+
CIT	-	RHA	-
H2S	+	SAC	+
URE	-	MEL	-
TDA	-	AMY	+
IND	+	ARA	+
VP	+	OX	+
GEL	+		

**Table 2.** The antibiotic susceptibility test results of *A. hydrophila*

Antibiotic ( $\mu\text{g disc}^{-1}$ )	
Ampicillin (10)	R
Enrofloxacin (5)	I
Florfenicol (30)	I
Oxytetracycline (30)	I
Amoxycillin (10)	R
Doxycycline (30)	I
Sulphamethoxazole/Trimethoprim (25)	R
Flumequine (30)	I
R: Resistant, I: Intermediate, S: Sensitive	

**Table 3.** Water parameters during the infestation

Temperature ( $^{\circ}\text{C}$ )	10	$\text{NO}_3^- \text{-N}$ (mg/L)	0.101
Salinity ( $\text{‰}$ )	3.93	$\text{NO}_3^-$ (mg/L)	0.461
Oxygen (mg/L)	10.07	$\text{PO}_4^{3-} \text{-P}$ (mg/L)	4.16
pH	7.7	Chlorophyll-a ( $\mu\text{g/L}$ )	1.59
$\text{NH}_4^+ \text{-N}$ (mg/L)	0.136	Ca (mg/L)	280.54
$\text{NH}_4^+$ (mg/L)	0.175	Mg (mg/L)	272.23
$\text{NO}_2^- \text{-N}$ (mg/L)	0.0115	Alkalinity ( $\text{CaCO}_3$ mg/L)	338
$\text{NO}_2^-$ (mg/L)	0.0385	Suspended solids	2.25
$\text{SiO}_2$ (mg/L)	6.45		

may even lead to death (Pickering and Willoughby, 1988). Kubilay et al. (2008) reported *Saprolegnia spp.* in rainbow trout fry infected with *Flavobacterium columnare* as a secondary opportunistic infection.

*Aeromonas hydrophila* has been reported in *Salmo gairdneri* (Peters et al., 1988), *Carassius auratus* (Citarasu et al., 2011), *Oreochromis niloticus* (Ibrahim et al., 2008), *Clarias batrachus*

(Angka, 1990), *Cyprinus carpio* (Citarasu et al., 2011) with skin lesions and hemorrhagic septicemia over the body, tail and fin regions (Sarder et al., 2016). It is generally considered to be a secondary invader (Rogers, 1971) like *Saprolegniosis*. Clinical symptoms of both pathogens were detected on *D.labrax* in this study. Similarly, hemorrhagic septicemia, tail or fin rot, changes on the body surface, fins, gills and tail because of fungus infection were reported in catfish (*Clarias gariepinus*) with the same clinical symptoms as this study (Kusdarwati et al., 2017) caused by *A. hydrophila* and *Saprolegnia spp.*

The water parameters during the outbreak are shown in Table 3. Temperature, salinity, oxygen and pH parameters were determined as  $10^{\circ}\text{C}$ , 3.93‰, 10.07 mg/L and 7.7, respectively. Suspended solid was measured as 2.25 mg/L while the Ministry of Food, Agriculture and Livestock of the Turkish Republic determined maximum 2 mg/L for sea bass farming.

*Aeromonas hydrophila* infections are mostly found in warm waters but under stress conditions, it may cause infections down to  $5^{\circ}\text{C}$ . With a sudden increase of turbidity and a decrease in water temperature to  $10^{\circ}\text{C}$ , *Saprolegniosis* and *A. hydrophila* were observed together in this case.

The antibiotic susceptibility tests were conducted from isolated *A. hydrophila* strains but after applying 200 mL/tonne formol bath for 30 minutes everyday and revision of physical conditions, it was no longer necessary to apply antibiotics to infected fish.

The problems in the filtration unit of İzmir Katip Çelebi University Fisheries Research and Training Center caused water pollution and mortalities were observed, particularly in tanks which had high stocking density. The mortality rate was calculated to be 18% during that period. The sudden change of water quality and intensive fish stock may have caused this agent to show itself as hemorrhagic septicemia in this study. The primer pathogen could not be determined but after fixing the filtration system and formol bath, the infections were successfully treated. This result suggested that *S. parasitica* could be the dominant pathogen in this case.

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# Relationship between Sagittal Otolith Size and Fish Size in *Engraulis encrasicolus* and *Sardina pilchardus* (Osteichthyes: Clupeiformes) in the Southern Aegean Sea, Turkey

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## ABSTRACT

The objective of this study was to determine the regressions between otolith size (length and height), otolith weight vs. fish length, and weight of European anchovy *Engraulis encrasicolus* (Linnaeus, 1758) (n=360) and European pilchard *Sardina pilchardus* (Walbaum, 1792) (n=360), living off Güllük Bay, Turkey. Fish were caught using a purse seine between January and March 2014 in the southern Aegean Sea. No differences were found between the size and weight of the left and right otoliths. Equations were used to reconstruct the original dimensions of prey from the size of hard structures found in food samples of piscivorous predators living in or in the vicinity of the aquatic habitat. A linear regression model was used to determine the relationship between fish length and otolith size, whereas an exponential regression model was used to describe the relationships between lengths and weights of otoliths and fish for both species. All regressions yielded high coefficients of determination ( $r^2$ ) of 0.78–0.93 for *E. encrasicolus* and 0.80–0.95 for *S. pilchardus*. We conclude that otolith length and otolith weight are good indicators of the length and weight of the two species.

**Keywords:** European anchovy, European pilchard, Güllük Bay, otolith morphometry

## INTRODUCTION

Otoliths are small opaque structures composed of calcium carbonate in an organic matrix and they also have vestibular and sound detection function in fishes other than lampreys, sharks, and rays (Campana, 2004). Otoliths also have a distinctive shape, which varies widely among fish families, yet can be highly species-specific (Maisey, 1987). Although they are composed of protein and calcium carbonate crystals, they are situated in the skull and therefore protected from digestion. Thus, several identification guides and keys have been published for South Africa by Smale et al. (1995), for the northeast Atlantic Ocean by Härkönen (1986), the Bering Sea by Morrow (1976), the northwest Atlantic Ocean by Campana (2004), the western Mediterranean, north and central eastern Atlantic by Tuset et al. (2008), and fossil fishes by Nolf (1985).

Otoliths can be used in diet studies of piscivorous animals, providing the whole fish is consumed or at least if the head is not discarded to such an extent that the results of the study are heavily biased (Härkönen, 1986). During feeding studies, the identification and quantification of this prey is often a difficult task: in most cases specimens are already partially or totally digested and the hard remains in the stomach, intestines, and faeces are the only diagnostic features that can be considered (Battaglia et al., 2010). Otoliths are somewhat resistant to digestion and may be used as an important tool for prey classification in several feeding studies (Pierce and Boyle, 1991; Pierce et al., 1991; Granadeiro and Silva, 2000; Battaglia et al., 2010). Furthermore, the relationship between fish length and otolith size and weight has been used with several fish species to draw conclusions on the body size and biomass of prey species (Frost and Lowry, 1980; Al-Mamry

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et al., 2010). Thus, marine biologists frequently depend on the morphology and size of conserved otoliths to examine the species and size composition of the diet of piscivore animals (Campana, 2004).

In the present study, the relationships between fish size and otolith size were studied in two marine species in the southern Aegean Sea: the European anchovy *Engraulis encrasicolus* (Linnaeus, 1758) and the European pilchard *Sardina pilchardus* (Walbaum, 1792). *E. encrasicolus* is a pelagic-neritic fish species forming large schools and it is a unique member of the Engraulidae family distributed in the Black Sea, the Sea of Marmara, the Aegean and the Mediterranean and estuaries of adjacent watersheds in Turkish waters (Fricke et al., 2007). The conservation status of this species was reported as vulnerable (VU) and the threats were FIT (a species that is commercially exploited as a target species) and FIB (a species that is not regularly commercially exploited, but frequently caught as bycatch in fisheries) in Turkey (Fricke et al., 2007). *Sardina pilchardus* is a pelagic-neritic species forming schools and it is a member of the Clupeidae family distributed in the Black Sea, the Sea of Marmara, the Aegean and the Mediterranean including adjacent estuaries and lower reaches of watersheds in Turkey (Fricke et al., 2007). The conservation status of this species was stated as near threatened in Turkey (NT) and the threats on the species were reported as FIT and as FIB by Fricke et al. (2007). Both keystone species, which have medium priority for conservation action, are sensitive to human activities (Fricke et al., 2007) and they are also economically one of the most important fish species for Turkish waters. In 2012, the total marine fish catch was 315636.5 tones in Turkey; of the catch 163981.9 tones were *E. encrasicolus* (51.95% of the total catch) and 28248 tones were *S. pilchardus* (8.95% of the total catch). A total of 34784.1 tones, *E. encrasicolus* (11141.4 tones, 32.03 % of total catch) and *S. pilchardus* (9973.5 tones, 28.67 % of total catch) have the highest ratio of catch of marine fishes for the Aegean Sea, where the present study was conducted (TUIK, 2013). Başçınar & Atılğan (2016), Zengin et al. (2015a, b) studied otolith morphometry and shape analysis of *E. encrasicolus* in the Black Sea and the Sea of Marmara. The aim of this study was to provide new data on the morphology and the relations between the otolith size and the fish size for the researchers studying the stomach contents and trophic interactions among marine animals in the Aegean Sea.

## MATERIAL AND METHOD

The fish were collected in monthly intervals during the period of January-March 2014 off Güllük Bay (southern Aegean Sea) (Figure 1) using commercial purse seine boats. The total length (TL) of the fish was measured to the nearest mm. Fish weight (W) was determined to the nearest 0.01 g on a digital balance. Sagittae (Figure 2) (total of 360 individuals, i.e., 720 otoliths per species) were removed with forceps through a cut in the cranium. Otoliths were then cleaned with 10% NaOH solution and dried, and the left and right otoliths were considered separately. Each sagitta was placed with the sulcus acusticus oriented upwards and otolith length (OL) was measured in mm through an eye-piece micrometer under a stereo zoom microscope (Olympus SZX-16). It was defined as the longest dimension between the rostrum and postrostrum axis (nomenclature of Smale et al., 1995; Tuset et al., 2008) through the focus of the otolith (Al-Mamry et al., 2010). Otolith height (OH) was measured in mm as the longest dimension between the ventral and dorsal surfaces of each sagitta. The image was taken of the internal side (medial or proximal) of the otolith as this side presents the sulcus acusticus (Tuset et al., 2008). Otolith weight (OW) was determined in mg. The paired t-test was applied to examine any dissimilarities between sagittae. When there is no significant difference ( $p < 0.05$ ), the  $H_0$  hypothesis ( $b_{right} = b_{left}$ ) was used. A single regression was used for each parameter (OL, OW, and OH). Linear regression equations ( $y = ax + b$ ) and exponential regression equations ( $y = ax^b$ ) were fitted to determine which equations (TL-OL, TL-OH, TL-OW, W-OL, W-OH, W-OW, OW-OL, OH-OL and OW-OH) are best describing various relations between otolith and fish size (Tarkan et al., 2007). The highest  $r^2$  scores were used to determine which type of regression model (Linear or exponential) was used between the parameters. Moreover, some otolith shape indices were calculated: aspect ratio (OH/OL:%) and OL/TL:% (Tuset et al., 2008) for both species to compare with other studies on the same species.

## RESULT AND DISCUSSION

The sagittal otoliths of 360 *Engraulis encrasicolus* and 360 *Sardina pilchardus* specimens were examined. Table 1 shows the descriptive statistics regarding length and weight of both species and their sagittal otoliths (with otolith width): In *E. encrasicolus*

**Table 1.** Descriptive statistics of length and weight data of specimens and their otoliths obtained from the Southern Aegean Sea; Values given are the mean  $\pm$  standard deviation (SD) and range in brackets

Species	N	Fish		Otolith		
		TL Mean ( $\pm$ SD) Min.-Max.	Weight Mean ( $\pm$ SD) Min.-Max.	Length Mean ( $\pm$ SD) Min.-Max.	Height Mean ( $\pm$ SD) Min.-Max.	Weight Mean ( $\pm$ SD) Min.-Max.
<i>E. encrasicolus</i>	360	109.07 $\pm$ 22.96 [57-150]	8.61 $\pm$ 5.26 [1.21-21.21]	2.68 $\pm$ 0.41 [1.7-3.4]	1.17 $\pm$ 0.17 [0.8-1.5]	8 $\pm$ 4 [1-21]
<i>S. pilchardus</i>	360	126.70 $\pm$ 25.39 [67-177]	17.44 $\pm$ 9.25 [2.29-42.67]	2.39 $\pm$ 0.49 [1.2-3.4]	1.22 $\pm$ 0.18 [0.7-1.5]	17 $\pm$ 9 [2-37]

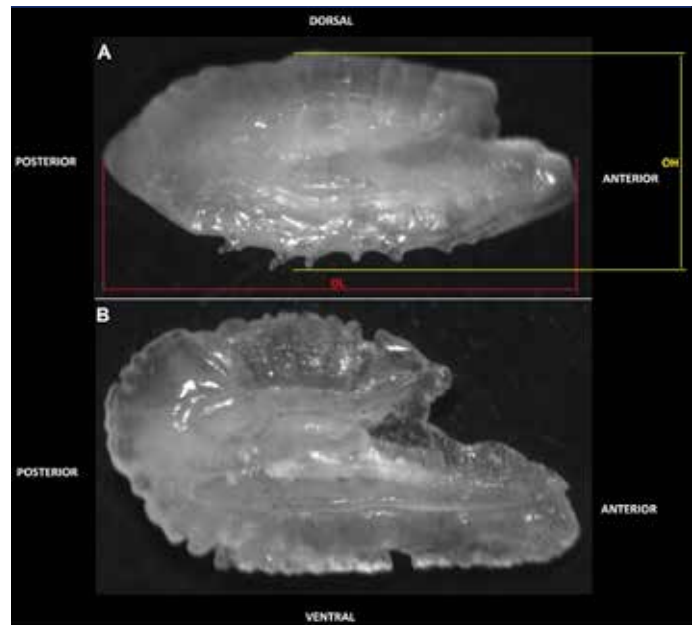
N: sample size; TL: Total Length; Min.: Minimum; Max.: Maximum  
All lengths in mm, fish weight in g, otolith weight in  $10^4$  g

**Table 2.** Intercept values (a), regression slope (b) and coefficients of determination ( $r^2$ ) for linear (L) and exponential (E) relationships between otolith morphometric parameters, fish length and weight of *Engraulis encrasicolus* and *Sardina pilchardus*

	Relationship	Regression	a	b	$r^2$	Significance
<b><i>Engraulis encrasicolus</i></b>						
Fish Length	TL vs. OL	L	55.427	- 39.467	0.93	$p < 0.05$
	TL vs. OH	L	137.28	- 51.382	0.86	$p < 0.05$
	TL vs. OW	E	1587.4	0.3714	0.83	$p < 0.05$
Fish Weight	W vs. OL	E	0.1033	4.3187	0.86	$p < 0.05$
	W vs. OH	E	3.4932	4.6922	0.78	$p < 0.05$
	W vs. OW	L	12647	- 15877	0.85	$p < 0.05$
Otolith	OW vs. OL	E	2E-05	3.6356	0.84	$p < 0.05$
	OH vs. OL	L	0.3987	0.1002	0.80	$p < 0.05$
	OW vs. OH	E	0.0004	3.9387	0.81	$p < 0.05$
<b><i>Sardina pilchardus</i></b>						
Fish Length	TL vs. OL	L	52.028	2.2924	0.95	$p < 0.05$
	TL vs. OH	L	140.2	- 44.851	0.84	$p < 0.05$
	TL vs. OW	E	880.69	0.2962	0.83	$p < 0.05$
Fish Weight	W vs. OL	E	1.1935	2.9498	0.82	$p < 0.05$
	W vs. OH	E	6.6862	4.0985	0.80	$p < 0.05$
	W vs. OW	L	9880	1.1075	0.84	$p < 0.05$
Otolith	OW vs. OL	E	8E-05	3.2972	0.87	$p < 0.05$
	OH vs. OL	L	0.3455	0.3972	0.82	$p < 0.05$
	OW vs. OH	E	0.0006	4.5842	0.80	$p < 0.05$

**Figure 1.** Map of the study area

lus, the mean total length was 109.07 mm (57–150 mm), and the length of otoliths ranged from 1.7 to 3.4 mm, height from 0.8 to 1.5 mm, and weight from 0.0001 to 0.0021 g; in *S. pilchardus*, the mean total length was 126.70 mm (67–177 mm), and the length of the otoliths 1.2–3.4 mm, their height 0.7–1.5 mm, and their weight 0.0002–0.0037 g. Statistically no significant difference was detected (Student's t-test for paired comparisons,  $p > 0.05$ ) between otolith pairs. So, measurements of left sagittae were used for detecting fish and otolith size relations. The relations between fish and otolith measurements are given in Table 2. All

**Figure 2. a, b.** Left sagittal otoliths of (a) *Engraulis encrasicolus* (TL=150 mm, OL=3.4 mm), (b) *Sardina pilchardus* (T=150 mm, OL=2.8 mm) from proximal side. OL=Otolith length, OH=Otolith height



of them yielded a high coefficient of determination ( $r^2$ ) between 0.78 and 0.93 for *E. encrasicolus* and between 0.80 and 0.95 for *S. pilchardus* (Table 2). % (OH/OL) and % (OL/TL) ratios were calculated with the ranges of 39.6-48.1 and 2.2-3.1 for *E. encrasicolus* and 42.0-61.6 and 1.8-2.0 for *S. pilchardus*, respectively.

Both species are of great economic importance. Furthermore, they are significant for the trophic level in the marine environment because they are consumed by several piscivorous fishes: *E. encrasicolus* are consumed by other *E. encrasicolus* (Valdés et al., 1987), *Seriola dumerili* (Matallanas et al., 1995), *Trachurus mediterraneus* (Santic et al., 2003), *Huso huso* (Berg, 1962), *Alosa fallax* (Assis et al., 1992), *Coryphaena hippurus* (Palko et al., 1982), *Etmopterus spinax* (Macpherson, 1979), *Merluccius merluccius* (Cabral and Murta, 2002), *Ophichthus rufus* (Casadevall et al., 1994), *Scomber scombrus* (Cabral and Murta, 2002), *Thunnus thynnus* (Sanz Brau, 1990), *Galeus melastomus* (Macpherson, 1979), *Saurida undosquamis* (Golani, 1993), *Uranoscopus scaber* (Sanz, 1985), *Xiphias gladius* (Cavaliere, 1963), *Ciliata mustela* (Costa, 1988), *Oblada melanura* (Pallaoro et al., 2004) and *Elops lacerta* (Hie Dare, 1980). For *E. encrasicolus*, our % ratio relationships between fish (57–150 mm TL,  $n=360$ ) and sagitta sizes were calculated as % (OL/TL)= 2.2-3.1 and % (OH/OL)= 39.6-48.1 for the Southern Aegean Sea; Tuset et al. (2008) reported these ratios as % (OL/TL)= 2.1-2.5 and % (OH/OL)= 42.5-46.3 for three specimens (134, 155 and 177 mm TL) from the Western Mediterranean Sea and the Atlantic Ocean. In the present study, OL/TL ratios were found to be similar to those of Tuset et al. (2008). Başçınar and Atılğan (2016) calculated otolith length and width (height) ratios ( $A_R$ , please see for the Method to Başçınar and Atılğan, 2016) of *E. encrasicolus* ( $n=54$ ) for the Black Sea coast of Ukraine, Rize and Samsun as: 1.69-2.18, 1.82-2.23 and 1.76-2.26. They calculated the equations between otolith height and length:  $y=0.3224x+0.7619$  ( $r^2=0.39$ ) in Ukraine,  $y=0.41x+0.31$  ( $r^2=69$ ) in Rize and  $y=0.3296x+0.5985$  ( $r^2=56$ ), with the linear regression model. According to their results, otoliths in Ukraine are different from the other two regions. Zengin et al. (2015a) were used otolith shape analyses and calculated dimensions of *E. encrasicolus* in the Black Sea ( $n=137$ ) and Marmara Sea ( $n=126$ ). According to their data, they could not find any difference between localities and between the left and right otoliths from the same locality. In addition, they offered that, otolith length was the best index for estimating fish length (please, see Zengin et al. (2015a) for the detailed data). Although Zengin et al. (2015a) noticed that the linear regression model was preferred for the examination of the relationship between fish length and otolith characteristics in most of the studies, in the present study, the exponential regression model gave higher  $r^2$  scores than the linear regression model, especially, for the length-weight relations. Therefore, an exponential regression model was used to explain relations between length-weight variables. Zengin et al. (2015b) also investigated some morphometric and otolith features of *E. encrasicolus* caught in the Black Sea and the Marmara Sea. Their results showed that there are statistical differences between the Black Sea and the Marmara Sea for otolith length and otolith weight.

*Sardina pilchardus* are eaten by *Seriola dumerili* (Matallanas et al., 1995), *Seriola rivoliana* (Barreiros et al., 2003), *Trachurus*

*mediterraneus* (Santic et al., 2003), *Trachurus trachurus* (Cabral and Murta, 2002), *Alosa fallax* (Assis et al., 1992), *Coryphaena hippurus* (Massutí et al., 1998), *Merluccius merluccius* (Cabral and Murta, 2002), *Sarda sarda* (Yoshida, 1980), *Scomber scombrus* (Kyrtatos, 1992), *Thunnus thynnus* (Sanz Brau, 1990), *Lepidorhombus whiffiagonis* (Morte et al., 1999), *Serranus cabrilla* (Labropoulou and Eleftheriou, 1997), *Serranus hepatus* (Labropoulou and Eleftheriou, 1997), *Synodus saurus* (Soares et al., 2003), *Chelidonichthys lucernus* (Morte et al., 1997), *Uranoscopus scaber* (Sanz, 1985), *Xiphias gladius* (Cavaliere, 1963), *Zeus faber* (Silva, 1999), *Oblada melanura* (Pallaoro et al., 2004), *Trisopterus luscus* (Costa, 1988), and *Dicentrarchus labrax* (Costa, 1988). Tuset et al. (2008) reported a % ratio relationship between the length of *S. pilchardus* (138, 175 and 214 mm TL,  $n=3$ ) and sagitta sizes as OL/TL=1.8-2.0 and OH/OL=45.5-48.7; in the present study (67–177 mm TL,  $n=360$ ) these ratios were calculated as OL/TL=1.8-2.0 and OH/OL=42.0-61.6. In the present study, OL/TL ratios were found to be higher than those of Tuset et al. (2008), but again this may be related to the small sample size of Tuset et al. (2008).

OH/TL ratios have larger ranges in the present study than those of Tuset et al. (2008) for both species. The largest specimen examined by Tuset et al. (2008) was larger than the specimens in this study for both species. However, in the present study, the number of specimens examined was higher than those of Tuset (2008). Tuset et al. (2008) described the sagittal otoliths of both species: having an elliptic shape and a funnel like ostium which is longer than the cauda. The cauda is tubular, straight, ending far from the posterior margin in both species. The Sulcus acusticus is heterosulcoid in *E. encrasicolus* but pseudo-archaeulcoid in *S. pilchardus*. The anterior region is peaked in both species, but the rostrum is short, broad, and pointed while the antirostrum is short, broad, peaked or poorly defined in the larger otoliths of *E. encrasicolus*. *S. pilchardus* has a broad, long and pointed rostrum; and it has a larger antirostrum with otolith growth.

Many otolith atlases, such as Härkönen (1986), Smale et al. (1995) or Tuset et al. (2008), were prepared for large geographic areas. Even though they include many fish species, but with fewer sample sizes. This paper supplies information about the TL–OL, TL–OH, TL–OW, W–OL, W–OH, W–OW, OW–OL, OH–OL and OW–OH relationships for *E. encrasicolus* and *S. pilchardus* in the Southern Aegean Sea. According to the data of the present study, otolith length is the best index for estimating fish length ( $r^2>0.93$ ) for both species.

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## Akuaponik Yetiştiricilik Sisteminde Farklı Bitkilerin Besin Dinamiği

### Nutrient Dynamics of Different Plants in an Aquaponics Aquaculture System

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#### ÖZ

Bu çalışmada modern ve sürdürülebilir üretim metotlarından olan akuaponik sisteminde farklı dönemlerde yetiştirilen bitkilerin su kalitesindeki performansı değerlendirilmiştir. Kurulan akuaponik üretim sisteminde koi, *Cyprinus carpio* var. Koi, balıklarının ve farklı bitkilerin (yapraklı bitki olarak; marul, *Lactuca sativa* var. *Crispa*, ve meyveli bitki olarak; çilek, *Fragaria* sp.) büyüme performansı, makro besinlerin döngüsü ve bitkilerin su kalitesi parametrelerine etkileri izlenmiştir. Balık ve bitki üretim üniteleri ve filtreleme ünitelerinden oluşan akuaponik üretim sistemde yapılan denemelerde makro besinlerin sistemden giderilme oranları marul uygulamasında fosfat için %42,95 ve nitrat için %55,45 ve çilek uygulamasında da fosfat için %35,49 ve nitrat için %46,36 olarak bulunmuştur. Çalışma sonucundan bulgulara göre marul ile yapılan denemede, genel olarak makro besinlerin ortamdaki giderilmesi çilek ile yapılan denemelere göre daha yüksek olduğu görülmüştür. Bu çalışmada elde edilen makro besinlerin giderilme oranları, yetiştiricilikte artan çözünmüş besinlerin etkili bir şekilde azaldığını göstermiş bu üretim sisteminin çevre-dostu ve sürdürülebilir bir metot olduğunu doğrulamıştır.

**Anahtar Kelimeler:** Akuaponik, entegre akuakültür sistemi, sürdürülebilir akuakültür, balık, bitki

#### ABSTRACT

This study evaluates the performance of plants grown at different times in the aquaponics system—a modern and sustainable production method—on water quality. In the established aquaponics system, this study monitored the growth performance of koi fish, *Cyprinus carpio* var. koi, and different plants (lettuce, *Lactuca sativa*, var. *Crispa* as a leafy plant and strawberry, *Fragaria* sp. as a fruit plant), pertaining to macro nutrient cycling and effects of plants on water quality parameters. Experiments in an aquaponics system comprising a biological filter and plant unit found that the removal rates of macro nutrients from the system were as follows: 1) 42.95% for phosphate and 55.45% for nitrate in lettuce and 2) 35.49% for phosphate and 46.36% for nitrate in strawberries. It was observed that, in general, the rate of removing macro nutrients was higher in the lettuce experiment than in the strawberries one. The elimination rates of macro nutrients in this study show that increasing dissolved nutrients in an aquarium is effectively reduced, which confirms that this production system is an environmentally-friendly and sustainable method.

**Keywords:** Aquaponics, integrated aquaculture system, sustainable aquaculture, fish, plant

#### GİRİŞ

Dünyada ve ülkemizde balık üretimi daha çok geleneksel havuz ve kafes sistemlerinde yapılmakta olup, üretimi yapılabilen balık türleri konusunda da sınırlıdır. Son çeyrek yüzyılda teknolojik sistemler olarak kapalı devre akuakültür sistemleri devreye alınmakta ve endüstriyel

birçok tür, neredeyse her ortamda üretilmektedir. Bu tür sistemlerde sıcaklık, tuzluluk ve su kalitesi parametreleri kontrol edilebildiği için tür ve coğrafik bölge seçiminde sınır tanımamaktadır. İstenilen türe göre ayarlanan sistem, istenilen bölgeye de yerleştirilebilir bu şekilde geleneksel üretim sistemlerinde en bü-

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yük problemi oluşturan pazara ulaşım da giderilmiş olmaktadır. Kapalı devre sistemleri üretim için gerekli arazi ve su ihtiyacını büyük oranda azaltırlar. Bu tür sistemlerde su kalitesi filtrasyon sistemleri ile düzenlenir ve sistemde su döngüsü pompalar aracılığı ile sağlanır (Losordo ve ark., 1992). Teknoloji ile bütünleşen bu sistemler elbette maliyet yüksektir, ancak kapalı devre sistemler suyun az ve değerli olduğu bölgelerde üretim yapmak için idealdir ve ticari değeri yüksek balık türleri üretilerek sabit giderler ve işletme giderleri kısa sürede amorti edilebilir.

Günümüzde yetiştiricilik şartları su kalitesinde bazı standartlara uyma gerekliliği nedeni ile ağırlaşmış olup üretim tesisleri su kaynaklarının kullanımında bazı düzenlemeler gereği ekonomik ve sosyal açıdan baskı altında işlem yapmaktadırlar (Summerfelt, 1998). Bunun sonucunda da yeni teknolojik gelişmelerin ihtiyacı doğmakta olup üretimden kaynaklanacak atıkların en alt seviye ye indirilmesi için gelişmelere ihtiyaç duyulmaktadır. Kapalı devre sistemleri bu tür taleplere cevap verebilmesine rağmen, atık üretimi halen devam etmekte olup akuakültürden oluşacak atık sorunu çevresel olarak daha kabul edilebilir düzeyde çözümlenmelidir (Rakocy, 1997). Ayrıca artık günümüzde tekli-akuakültür (mono-aquaculture) uygulamalarından ziyade bu çevresel atıkların değerlendirilmesi amacı ile entegre çoklu-besinsel (multi-trophic) akuakültür teknikleri geliştirilmiştir. Bu sayede çevresel, ekonomik ve toplumsal faydanın arttığı fark edilmiştir. Bu sistemlerde temel prensip, çevresel etkiyi azaltma amacı ile doğal ekolojik işlemler oluşturarak, besin yüklemesini kullanarak ve tür etkileşiminde faydalanarak farklı ticari ürünler elde etmektir (Chopin ve ark., 2012).

Artık akuakültür teknikleri günümüzde ekolojik bir yaklaşıma dikkat çekmekte, enerji tasarrufu ile birlikte yenilenebilir enerjiyi en üst seviyeye çıkarmakta, besinler arası geri dönüşümü sağlamakta, farklı türler yetiştirmekte ve diğer üretimi yapılabilen çeşitli ürünlerle entegre olabilmektedir. Bu entegrasyon kapalı devre akuakültür ile topraksız tarım tekniğinin birleşimi ile sağlanır ve akuaponik (aquaponics) olarak anılır (Rakocy, 1987). Balık ve bitki üretimi birleştirildiği zaman, üretim su kullanımına oranla en üst seviyeye çıkmaktadır (Rakocy ve ark., 1993). Akuaponik dünyada besin tedarik edilesinde ve küresel sorunların (su kıtlığı, gıda güvenliği, kentleşme, enerji kullanımının azaltılması ve gıda nakliyesi) üstesinden gelinmesinde önemli rol oynayan bir sistemdir (COST, 2013). Akuaponik sistemleri tipik olarak en az su kullanımını ile en fazla ürün elde etmek için tasarlanmıştır. Böylece ticari bir kazanç elde edilirken sürdürülebilir bir balıkçılık ve sebze üretimi bir arada sağlanmış olmaktadır (Adler ve ark., 2000). Ayrıca gübreleme ve enerji kullanımı oransal olarak diğer zirai üretimlere göre daha azdır. Bundan başka ürünler daha küçük alanlarda da ve şehir merkezine yakın yerlerde yetiştirilebilir.

Balık-bitki entegre yetiştiricilik sistemi akuaponik, akuakültür ile topraksız tarımın teknolojik olarak simbiyotik anlamda birleştirilmesi olarak da tanımlanır (Rakocy, 1989). Bu sistem ekolojik anlamda geri dönüşümlü olarak çalışan ve akuakültür ile oluşan suda çözünmüş haldeki besinlerin değerlendirilip başka bir ürün (bitki) tarafından kullanılmasını sağlayan bir mühendislik harikasıdır. Sistem işletim karlılığını da ekstra ürün üreterek, aynı alanı kullanarak ve su kullanımını düşürerek arttırmaktadır. Balıklar tarafından sisteme bırakılan çözünmüş besinler, ikinci bir ürün (bitki)

tarafından kullanılarak çevresel etki en aza indirilmektedir. Yapay ve kapalı bir ekosistem olarak tasarlanan bu sistem üç önemli biyolojik bölümün (balık, bitki ve bakteri) entegrasyonu sayesinde balık üretmekte, çözünmüş besinler ile bitki olarak ikinci bir ürün sağlamakta ve her ikisinin kombinasyonu ile de kullanılan suyu geri dönüşümlü bir halde sisteme kazandırmaktadır.

Ayrıca balık-bitki entegre yetiştiricilik sisteminin uygulamadaki avantajları şu şekilde de özetlenebilir;

- Suyun ve enerji kaynaklarının nadir bulunduğu az gelişmiş veya gelişmemiş ülkelerde, bölgelere göre güneş enerjisinden de yararlanılarak yapılan bu yoğun balık ve bitki üretim sistemi bölgesel besin üretimine katkıda bulunur (Quiellere ve ark., 1993).
- Bu sistem balık yetiştiriciliğinden doğan negatif çevresel etkiyi azaltır (Rakocy, 1989; Rakocy ve ark., 1992).
- Bu tür bir sistem su kaynağının mesafesi ve toprak yapısı dikkate alınmadan her hangi bir yerde kurulup (Ör: kentsel alan) ve hemen hemen bütün iklim şartlarında işletilir (Clarkson ve Lane, 1991). Özellikle arid (kurak) bölgelerde uygulanması mümkündür (Rakocy, 2007).
- Geleneksel tarım sistemlerinde kullanılan sulama masrafının ve teknikleri gerekmekte, ayrıca su tüketim miktarı diğer yetiştiricilik koşullarına göre oldukça düşüktür.
- Balıklar tarafından sisteme bırakılan çözünmüş besinler sayesinde bitkiler gübre kazanmakta ve ek gübreleme gerekmemektedir.
- Bütün yıl boyunca istenilen zamanda yetiştiricilik yapılabilmektedir.
- Ekolojik ve sürdürülebilir tarıma, organik yetiştiricilik şartlarına uygundur.
- Sistem ve teknolojisi gelişmekte olan ülkeler için düzenlenebilir ve lokal olarak gıda güvenliği sağlayabilir.
- Endüstriyel ölçekte rekabetçi bir sistem ile uygun maliyetli, sağlıklı ve sürekli gıda sağlayabilir.

Son yıllarda da daha bilimsel olarak incelenmeye başlanmış çeşitli sistemler tasarlanmış, farklı türlerde balık ve bitkiler üzerinde farklı araştırmalar yapılmıştır (Rakocy ve Hargreaves, 1993; Seawright ve ark., 1998). 1990 yıllarından sonra sistem başka araştırmacılar tarafından da biraz daha geliştirilerek günümüze kadar farklı tasarım ve uygulamalar ile gelişmiştir (Mathieu ve Wang, 1995; Faucette, 1997). Sistem 2000'li yıllardan itibaren yeni tekniklerin ilavesi ile uygulanabilir seviyelere gelmiştir (Diver, 2006). NASA bile 1998 yılında uzaya balık (tilapia) yollamış, gelecekte uzaydaki atıkların değerlendirilmesi, topraksız tarımla protein ihtiyacının giderilmesi konusunda çalışmalar başlatmıştır (Falls ve Hudson, 1999).

Akuaponik sistemi içinde yetiştirilen balık türleri genellikle kapalı devre yetiştiricilik sistemine ve çevre toleransı yüksek balık türleri (Ör: tilapia, sazan, koi, sudak ve levrek) seçilir. Diğer entegre ürün bitkiler içinde bu sistemdeki performansı, bitkinin büyüme evrelerine ve besin gereksinimine bağlı olarak sebze (Ör: domates, ıspanak, marul, çilek, salatalık, biber) ve ekonomik değere sahip tıbbi veya aromatik bitkiler (Ör: nane, fesleğen), hayvansal üretimde yem olarak kullanılabilen sucul bitkiler (Ör: su teresi, su marulu) ve sucul ortamda yaşayan fitoremediasyon özelliğine sahip



yabani bitkiler (Ör: *Lythrum salicaria* L., Sun ve ark. (2017)) kullanılabilir.

Bu çalışmada amaç koi, balıkları içeren akuaponik sistemde yeşil yapraklı bitki olarak marulun ve meyveli bitki olarak da çileğin üretimini sağlamak ve üretim periyodu boyunca bu kapalı entegre sistemde mikro ve makro besinlerin dengesini belirlemek, balıklar tarafından üretilen çözünmüş besinler ile bitkiler tarafından uzaklaştırılan besin miktarını tespit etmek, su kalitesi parametreleri ile balık-bitki büyüme verilerini gözlemlemektir.

## MATERYAL VE METOT

Koi balıkları, *Cyprinus carpio* var. *koi*, renkli görünüşleri açısından özellikle havuz ve akvaryum sektöründe talebi olan bir süs balığıdır. Akuaponik sistemlerinde çevre toleransı yüksek olduğundan tercih edilen bir türdür. Sistemde yapraklı ve meyveli olarak iki farklı tür bitkisel ürün elde edilmesi planlanmış ve yeşil yapraklı sebzelerden kıvırcık yağlı marul, *Lactuca sativa* var. *crispa*, salata olarak tüketimi oldukça yaygın olup düşük fosfor ve potasyum içerikli ortamlara dayanıklı olması ile meyveli bitkilerden de kültür çileği, *Fragaria* sp., topraksız tarıma uygunluğu açısından tercih edilmiştir.

Deneme de kullanılan akuaponik sistem, balık ve çökeltme tankı (900 ve 100 l), biyolojik filtre (600 l) ve bitki tavaları (900 l) ile toplam 2500 l su kapasitesi olacak şekilde kurulmuştur. Ortama ağırlıkları  $15,7 \pm 0,6$  g olan koi (*Cyprinus carpio*) balıkları ticari koi üreticisinden (Balıkselsu, Çekmeköy, İstanbul) satın alınarak sisteme konmadan önce 5 mg/l  $KMnO_4$  çözeltisinde dezenfekte edilmiş ve balık üretim ünitesine her bir uygulama öncesi 5 kg/m<sup>3</sup> olacak şekilde stoklanmıştır. Bilimsel araştırma protokolü ilgili mevzuat ve yükümlülükler göz önüne alınarak Abant İzzet Baysal Üniversitesi Etik Ku-

rul Karar No:2014/12 ile onaylanmıştır. Balıklar günlük olarak ağırlıklarının %1,5 kadar %45-50 protein ve %17-20 yağ içeren alabalık yemleri (Abalioğlu Yem A.Ş., Manisa) ile beslenmişlerdir. Zaman ayarlı floresan (soğuk beyaz) ışıklandırma kullanılarak 16 saat aydınlık 8 saat karanlık uygulanmıştır. Deney ortam sıcaklığı 22-23°C, su sıcaklığı da 24°C'de tutulmuştur. Tatlı su kaynağı olarak şebeke suyu ön filtreden geçirilip ardından dinlendirilip sisteme verilmiştir. Çökeltme tankı haftalık olarak katı atıklardan kısmi temizlenmiş ve dinlendirilmiş şebeke suyu ile yenilenmiştir.

Bitkiler sisteme eklenmeden önce 5 kg/m<sup>3</sup> olarak koi stoklanmış balık üretim tankları için bir ay boyunca sistemde TAN ve  $NO_3$  dengesi takip edilip 600 litrelik biyofiltre ünitesinde bakteri oluşumu ile nitrifikasyon dengesi sağlanmıştır. Ayrıca bu işlemin devamında sistemde her bir üretim periyodu başlangıcında başlangıç olarak konsantrasyonlarını 2,1 mg/l Fe, 147 mg/l K, 0,14 mg/l Zn, 0,04 mg/l Cu, 20,3 mg/l Mg ve 0,02 mg/l Mo sağlayacak şekilde ve ölçüm sonrası haftalık olarak FeEDDHA,  $KNO_3$ ,  $ZnCl_2$ ,  $CuCl_2$ ,  $MgSO_4$  ve  $Na_2MoO_4$  ilavesi yapılmıştır (Seawright ve ark., 1998). İki haftalık periyodlar halinde ilaveler tekrarlanmıştır. Ayrıca kullanılan alabalık yem formülasyonunda mikro besinlerden 58-60 mg/kg Fe ( $FeSO_4$  olarak), 5-7 mg/kg Cu ( $Cu_2O$ ), 100-150 mg/g Zn ( $ZnO$ ) ve makro besinlerden Ca %2,21-2,48, toplam fosfor %1,54-1,67 olarak bulunduğu üretici tarafından belirtilmiştir.

Her biri 300 l olan 1 m<sup>2</sup> yüzey ve 30 cm derinliğe alanına sahip 3 adet bitki üretim tavaları yüzer sal (floating raft) sistemi olarak tasarlanmıştır. Tanklar bitkilerin büyümelerini ve köklenmelerini sağlayan 5 cm kalınlıkta köpük kaplama (styrofoam) ile kaplanmış ve su üstünde yüzey alanınca yüzmeleri sağlanmıştır. Daha sonra bu kaplamalar bitkilerin dikim yapıldığı delikli saksılar için uygun ebat ve sayı da delinmiştir. Bitki üretimi 8'er haftalık üretim boyunca ilk uygulama da marul sistemin yenilenmesi ardından da

**Tablo 1.** Marul ve çilek üretim periyodu boyunca su kalitesi parametreleri. İki uygulama içinde aynı harfe sahip olan satırlar arası ortamlar istatistiksel olarak farklı değildir ( $p > 0,05$ )

**Table 1.** Water quality parameters during the lettuce and strawberry production period. Inter-line spaces with the same letter in two applications are not statistically different ( $p > 0,05$ )

Bitki türü	Parametre	Su Kalitesi								Ort.	± SH
		Haftalar									
		1	2	3	4	5	6	7	8		
Marul	Sıcaklık (°C)	23,8	23,5	23,9	23,7	23,8	23,8	24,0	23,9	23,8 <sup>a</sup>	0,05
	DO (mg/l)	6,6	6,5	6,6	6,3	6,4	6,6	6,7	6,4	6,5 <sup>a</sup>	0,04
	TAN (mg/l)	1,23	1,35	1,42	1,29	1,04	1,25	1,32	1,52	1,3 <sup>a</sup>	0,05
	$NO_2$ (mg/l)	0,41	0,22	0,16	0,12	0,18	0,15	0,26	0,31	0,2 <sup>a</sup>	0,04
	pH	7,89	7,93	7,98	7,84	7,86	7,94	7,55	7,8	7,8 <sup>a</sup>	0,02
	Alkalinite (mg/l)	140	130	150	140	130	130	140	150	138,8 <sup>a</sup>	2,90
Çilek	Sıcaklık (°C)	24,1	23,8	23,9	24,3	24,0	23,9	23,8	24,2	24,0 <sup>a</sup>	0,06
	DO (mg/l)	5,9	6,4	6,3	6,6	6,0	6,1	6,7	6,2	6,3 <sup>a</sup>	0,09
	TAN (mg/l)	1,17	1,22	1,25	1,13	1,41	1,36	1,61	1,73	1,4 <sup>a</sup>	0,04
	$NO_2$ (mg/l)	0,29	0,53	0,74	0,65	0,96	0,73	0,41	0,58	0,6 <sup>b</sup>	0,08
	pH	7,22	7,66	7,42	7,77	7,35	7,17	7,55	7,41	7,4 <sup>a</sup>	0,08
	Alkalinite (mg/l)	130	140	140	150	140	130	150	140	140,0 <sup>a</sup>	2,67

**Tablo 2.** Marul ve çilek üretim periyodu boyunca makro besin değerleri ve değişim oranları. İki uygulama içinde aynı harfe sahip olan satırlar arası ortamlar istatistiksel olarak farklı değildir ( $p>0,05$ )

**Table 2.** Macro nutrients and rates of change during the lettuce and strawberry production period. Inter-line spaces with the same letter in two applications are not statistically different ( $p>0,05$ )

Bitki türü	Makrobesinler (mg/l)	Sisteme Giren Su								Sistemden Çıkan Su								*Giderilme Verimliliği (%)				
		Haftalar								Haftalar												
		1	2	3	4	5	6	7	8	Ort.	± SH	1	2	3	4	5	6		7	8	Ort.	± SH
Marul	PO <sub>4</sub>	2,58	2,88	2,25	2,53	2,41	2,05	2,15	2,22	2,38 <sup>a</sup>	0,10	2,25	1,86	1,32	1,24	1,39	1,01	0,92	0,89	1,36 <sup>a</sup>	0,16	42,95 <sup>a</sup>
	NO <sub>3</sub>	22,76	23,82	22,48	25,56	24,62	23,42	25,15	23,54	23,92 <sup>a</sup>	0,41	12,06	10,1	11,78	12,21	10,91	10,33	8,33	9,52	10,66 <sup>a</sup>	0,32	55,45 <sup>a</sup>
Çilek	PO <sub>4</sub>	1,78	1,95	2,05	2,15	1,98	1,82	2,12	2,35	2,03 <sup>b</sup>	0,05	1,42	1,35	1,48	1,32	1,14	0,98	1,64	1,12	1,31 <sup>a</sup>	0,07	35,49 <sup>b</sup>
	NO <sub>3</sub>	24,42	25,56	24,78	26,63	25,89	26,10	25,45	24,89	25,47 <sup>b</sup>	0,29	15,75	14,19	10,56	12,23	14,16	15,45	15,5	11,42	13,66 <sup>b</sup>	0,70	46,36 <sup>b</sup>

\*Giderilme (ortadan kaldırılma) Verimliliği (%) = [(Giren su - Çıkan su) / (Giren su)] x 100

ikinci uygulama olarak çilek planlanmıştır. Marul yetiştirme uygulamasında ilk olarak marul tohumları sisteme konacak saksılarda bulunan sünger medyumlar içinde ilk birkaç günü karanlık daha sonraki üç hafta boyunca aydınlık bitki kültürü laboratuvarında çimlendirilmiş yaklaşık 3-4 cm boyuna ulaşınca 3 m<sup>2</sup>lik bitki üretim alanına 24 adet/m<sup>2</sup> olacak şekilde yerleştirilmiştir. Marullar yüzey sal ortamına alındıktan 8 hafta sonra hasat edilmiş, yaprak uzunlukları ve ağırlıkları kayıt edilmiştir. Hasat sonu sistem temizlenmiş, yeni grup balık satın alınmış ve stok miktarı aynı oranlarda yenilenerek tekrar düzenlenmiş, TAN ve NO<sub>2</sub> dengesi sonrası ikinci bitki üretim uygulaması olan çilek üretimine hazırlanmıştır. Çilek uygulaması için de fideler hazır satın alınmış yaprak adedi eşit olacak şekilde bitki üretim alanına 24 adet/m<sup>2</sup> olarak yerleştirilmiştir.

Üretim periyodları süresince haftalık olarak pH seviyesi kontrol edilmiş (Orion pH metre, Boston, ABD) ve pH'nın 7'nin ve alkalinitenin (Merck test kiti, Darmstadt, Almanya) de 100 mg/l'nin üstünde tutulması KOH ve sönmüş kireç ilavesi ile sağlanmıştır. PO<sub>4</sub> ve NO<sub>3</sub> gibi makro besinler ve su kalitesi parametrelerinden TAN ve NO<sub>2</sub> ile Fe, K, Zn, Cu, Mg ve Mo gibi mikro besinler her haftanın aynı günü örnek üzerinden Hach Lange DR 1900 (Düsseldorf, Almanya) spektrofotometreye ait olan hazır kitler ile ölçülmüştür. Makro besinler için örnekler 'Sistemden Çıkan Su' olarak çökeltme tankından ve 'Sisteme Giren Su' olarak da akuaponik ünitesinde çıkan ve sisteme dönen sudan alınmıştır.

Elde edilen rakamsal verilerin istatistiksel analizi MedCalc (versiyon 15.8; Ostend, Belçika) istatistik programı kullanılarak yapılmış ve ortalamalar arasındaki farklılıkların %5 düzeyinde önemlilik durumu t-testi ile karşılaştırılmıştır.

## BULGULAR VE TARTIŞMA

Marul ve çilek uygulaması periyodunca su kalitesi parametreleri değişimi koi balıklarına uygun aralıklarda görülmüş, kültüre uygun seviyelerde seyretmiş ve uygulamalar arasında NO<sub>2</sub> hariç fark gözlemlenmemiştir ( $p>0,05$ ) (Tablo 1). Çözünmüş oksijen sisteme hava motorları ile sağlandığından seviyesinde herhangi bir değişim olmamıştır. Ölçülen ortalama toplam amonyak (NH<sub>3</sub>+NH<sub>4</sub>) su sıcaklığı ve pH değerinden iyonize olmamış serbest NH<sub>3</sub>'e çevrildiğinde marul için 0,04 mg/l ve çilek için 0,01 mg/l olduğu gözlemlenmiştir. Serbest NH<sub>3</sub> miktarları kapalı devre sistemler için tavsiye edilen değerlerde (<1 mg/l) kalmıştır (Nijhof ve Bovendeur, 1990; Van Rijn ve Rivera, 1990). Uygulamalar arasında sadece NO<sub>2</sub> konsantrasyonları arasında fark görülmüş, marul için 0,2 mg/l ve çilek için de 0,6 mg/l olarak ölçülmüştür. NO<sub>2</sub> için genellikle 0,5 mg/l altı olması istenir ancak sazan balıkları bu değerlere oldukça toleranslıdır (Williams ve Eddy, 1986).

Yüksek nitrat sucul ortamlarda bitkiler için en çok tercih edilen besinlerden olup, balıklara toksik açıdan zararı oldukça azdır (Rakocy ve ark., 2007). Özellikle koi'lere genel olarak olumsuzluk teşkil etmemektedir. Ebeling ve ark.'larının (1993) yetiştiricilik sistemleri içinde nitrat konsantrasyonlarının 25 mg/l aştığı durumlarda bile balıklarda bir zarar oluşturmadığını bildirmiştir. Licamele (2009), marul-tilapia akuaponik sisteminde nitrat seviyesini 50 mg/l olarak rapor etmiştir. Bu çalışma da akuaponik sistemi içinde ortalama nitrat değerleri sisteme giren ve çıkan su

**Tablo 3.** Marul ve çilek üretim periyodu boyunca mikro besin değerleri)

**Table 3.** Micro nutrients during the lettuce and strawberry production period

Bitki türü	Mikrobesinler (mg/l)	Başlangıç	Haftalar								Ort.	± SH
			1	2	3	4	5	6	7	8		
Marul	Fe	2,10	2,00	2,20	2,10	2,30	2,00	1,90	2,20	2,10	2,10	0,05
	K	20,00	18,30	21,60	22,80	19,60	21,50	20,40	22,90	22,10	21,15	0,57
	Zn	0,14	0,22	0,30	0,35	0,37	0,48	0,55	0,62	0,58	0,43	0,04
	Cu	0,04	0,05	0,08	0,11	0,09	0,12	0,11	0,09	0,10	0,09	0,01
	Mg	20,30	25,80	29,20	31,20	30,30	32,60	33,50	28,10	28,90	29,95	0,97
	Mo	0,02	0,05	0,03	0,05	0,07	0,04	0,05	0,08	0,06	0,05	0,01
Çilek	Fe	3,11	2,80	2,86	2,56	2,89	2,44	2,36	2,78	2,55	2,66	0,08
	K	18,90	20,11	20,42	21,20	20,60	21,11	21,52	21,63	20,03	20,83	0,19
	Zn	0,26	0,18	0,36	0,28	0,48	0,55	0,85	0,79	0,74	0,53	0,08
	Cu	0,10	0,08	0,09	0,13	0,75	0,55	0,61	0,41	0,30	0,37	0,11
	Mg	18,62	20,80	25,46	27,45	28,56	30,78	29,15	28,34	29,23	27,47	1,25
	Mo	0,04	0,06	0,08	0,06	0,05	0,04	0,08	0,07	0,09	0,07	0,01

**Tablo 4.** Marul ve çilek üretim periyodu boyunca balık büyüme parametreleri. İki uygulama içinde aynı harfe sahip olan kolonlar arası ortamlar istatistiksel olarak farklı değildir ( $p>0,05$ )

**Table 4.** Parameters of fish growth during the production period of lettuce and strawberries. Averages between two columns having the same letter in two applications are not statistically different ( $p>0,05$ )

	Marul	Çilek
Balık büyüme parametreleri	5 kg/m <sup>3</sup>	5 kg/m <sup>3</sup>
İlk ağırlık (g)	15,32±0,06 <sup>a</sup>	16,03±0,28 <sup>a</sup>
İlk uzunluk (cm)	7,81±0,03 <sup>a</sup>	7,34±0,11 <sup>a</sup>
Son ağırlık (g)	21,42±0,13 <sup>a</sup>	20,91±0,22 <sup>a</sup>
Son uzunluk (cm)	13,24±0,07 <sup>a</sup>	14,52±0,43 <sup>a</sup>
Ağırlık kazancı (%)	39,82±0,02 <sup>a</sup>	30,44±0,13 <sup>b</sup>
Spesifik büyüme oranı (%g/gün)	0,56±0,01 <sup>a</sup>	0,44±0,08 <sup>b</sup>
Yem değerlendirme oranı	2,6±0,01 <sup>a</sup>	2,9±0,22 <sup>b</sup>
Ağırlık Kazancı (%) = [ (son ağırlık-başlangıç ağırlığı) / (başlangıç ağırlığı) ] x 100		
Spesifik Büyüme Oranı (%g / gün) = [ Ln(son ağırlık) - Ln(başlangıç ağırlığı) ] / (gün) x 100		
Yem Değerlendirme Oranı = (Tüketilen yem miktarı) / (Ağırlık Kazancı)		

da marul uygulamasında 23,92 ve 10,66 mg/l ve çilek uygulamasında da 25,47 ve 13,66 mg/l olarak gözlemlenmiştir. Marul ve çilek uygulamasındaki nitrat değerleri sisteme giren ve çıkan su da istatistiksel olarak karşılaştırıldığında farklı olduğu bulunmuştur (Tablo 2). Akuaponik uygulamaları içinde diğer önemli bir makro besin olan fosfat dengesi de bu çalışmada normal seviyelerde seyretmiştir. Lennard ve Leonard (2006) morina balıkları için kurduğu akuaponik sisteminde marullarla yaptığı uygulamada fosfat oranını 3,47 mg/l olarak ve Petrea ve ark.nın (2009) gökkuşağı

**Tablo 5.** Marul ve çilek üretim periyodu boyunca bitki büyüme parametreleri

**Table 5.** Plant growth parameters during the lettuce and strawberry production period

	Marul
Bitki büyüme parametreleri	24 adet/m <sup>2</sup>
İlk uzunluk (cm)	2,6±0,1
Son uzunluk (cm)	24,3±1,3
Hasat (kg/m <sup>2</sup> )	1,8±0,2
	Çilek
	24 adet/m <sup>2</sup>
İlk yaprak sayısı	6±1
Son yaprak sayısı	28±2
Meyve hasatı (kg/m <sup>2</sup> )	0,6±0,2

alabalığı-ıspanak uygulamasında 3,7 mg/l olarak bildirmiştir. Bu çalışmada da giren ve çıkan suda marul için 2,38-1,36 mg/l ve çilek için 2,03-1,31 mg/l olarak bulunmuştur (Tablo 2). Uygulamalar arasında sisteme giren su içerisinde farklı fakat çıkan su da farklı olmadığı görülmüştür (Tablo 2).

Sekiz haftalık üretim periyodunca nitrat ve fosfat besinlerinin giderilme (ortadan kaldırılma) oranlarının marul ve çilek uygulamaları arasında farklı olduğu gözlemlenmiştir (Tablo 2). Marul uygulaması ile elde edilen giderilme oranlarının fosfat için %42,95 ve nitrat için %55,45 iken çilek uygulamasında fosfat için %35,49 ve nitrat için %46,36 olarak daha yüksek olduğu hesaplanmıştır. Marul uygulamasında makro besinlerin daha yüksek seviyelerde giderilme oranları görülmesinin nedeni marulun çileğe göre yeşil yaprak alan ve sayısının daha fazla olması ve kök yapısının daha sık ve geniş alana yayılmış olarak gösterilebilir. Lennard ve Leonard'nın (2006) fosfat giderilme oranını %36,3 buna karşın

nitrat giderilme oranını %93,2 olarak bulmuştur. Yine Hussain ve ark.'nın (2014) ıspanak uygulamasında, giderilme oranlarını nitrat için %80, 75 ve 58 ve fosfat için %53, 49 ve 39 olarak üç farklı koi stok oranında (1,4; 2,1; 2,8 kg/m<sup>3</sup>) gözlemlemiştir.

Akuaponik sistemleri içinde en yüksek bitki üretimi yapabilmek için temel elementleri içeren uygun mikro besinlerin düzenli olarak sisteme eklenmesi gerekmektedir. Seawright ve ark.'nın (1998) önerdiği başlangıç miktarlarına marul ve çilek üretim dönemleri başında uyum sağlanmış üretim periyodu boyunca seviyeleri takip edilip eklenmiştir (Tablo 3). Bu nedenle mikro besinler açısından her hangi bir olumsuzluğa rastlanılmamıştır.

Günlük olarak ağırlıklarının %1,5 kadar beslenen balıkların altmış günlük üretim periyodu sonunda yapılan hasadın da, koi balıklarında ağırlık kazancı, spesifik büyüme oranı ve yem değerlendirme oranı uygulamalar arasında istatistiksel olarak farklı bulunmuş olup, en iyi büyüme ve yem değerlendirme marul uygulamasında gerçekleşmiştir (Tablo 4). Sırası ile ağırlık kazancı, günlük büyüme oranı ve yem değerlendirme oranı marul uygulamasında %39,82, %0,56 ve 2,6 çilek de ise %34,44, %0,44 ve 2,9 olarak hesaplanmıştır. Bir üretim sisteminde balık stok yoğunluğu büyümeye etkili hassas bir değişken olup üretimi etkileyen en önemli faktördür. Ayrıca akuaponik sistemlerde de uygun stok yoğunluğu belirlenmesi gerekli temel faktörlerin başında gelir. Bu nedenle benzer çalışmalar ile ilgili karşılaştırma yapmak uygun olmamaktadır. Örneğin; Shelton ve ark.'nın (1981) yaptığı bir çalışmada stok yoğunluğunun artması ile ot sazanlarında büyüme parametrelerinin azaldığı, Licamele'in (2009) çalışmasında Nil tilapyası ile yapılan marul üretiminde artan stok yoğunluğu ile yem değerlendirme katsayısının arttığı rapor edilmiştir. Yine Vijayan ve Leatherland'in (1988) alabalıklarla ve Imanpoor ve ark.'nın (2009) adi sazanlarla çalışmalarında stok oranına bağlı büyüme ve yem değerlendirmenin azaldığı gözlemlenmiştir. Bu çalışmaya benzer olarak ıspanak-koi akuaponik sisteminde stok oranına (1,4; 2,1; 2,8 kg/m<sup>3</sup>) bağlı olarak ağırlık kazancı %66, 35 ve 28 ve yem değerlendirme oranı 1,95; 3,9 ve 4,7 olarak bulunmuştur (Hussain ve ark., 2014).

Yüzer sal şeklinde köpük kaplama üzerine yerleştirilerek yapılan bitki üretim alanında yetiştirilen marul ve çileklere ait büyüme verileri Tablo 5'te özetlenmiştir. Buna göre sekiz haftalık üretim periyodu sonunda marullar tüketim boyuna ulaşmış ve çilekler de meyve vermeye başlamışlardır. Sisteme düzenli olarak mikrobesin takviyesi yapıldığından ve değerleri de haftalık olarak tespit edildiğinden her hangi bir olumsuzluğa rastlanılmamıştır. Yapraklı bitkiler için en iyi büyümenin sağlandığı yüzer sal metodunun akuaponik sistemler içinde en fazla tavsiye edilen olduğu belirtilmiştir (Lennard ve Leonard, 2006).

Bu çalışmanın sonunda; modern ve sürdürülebilir üretim metodlarından olan topraksız tarım tekniğinin ve kapalı devre sisteminin entegrasyonu olan akuaponik sisteminde balık ve bitkilerin büyümesini optimize eden yapay ve kontrollü bir çevre sağlanmış ve besin döngüsü değerlendirilmiştir. Üretim boyunca su kalitesinin ve makro besinlerin uzaklaştırılmasının etkin bir şekilde sağlanması koi-yapraklı bitki (marul) veya meyveli bitki (çilek) uygulanabilirliğini göstermiştir. Ancak makro besinlerin uzaklaştırılmasında marul uygulamasının çileğe göre daha etkili olduğu gözlemlenmiştir. Bu çalışma ile akuaponik uygulaması üretim sistemleri için

de ortamdaki artan besin maddelerinin başka bir ürüne çevirerek gidermesi ve uygun su kalitesi sağlanması açısından en fazla uygulanması gerekli sürdürülebilir teknik olarak önerilir.

## TEŞEKKÜR

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## Güneydoğu Karadeniz’de *Noctiluca scintillans* (Macartney) Kofoid & Swezy Dinamiği ve Mesozooplanktona Katkısı

### *Dynamics of Noctiluca scintillans* (Macartney) Kofoid & Swezy and its Contribution to Mesozooplankton in the Southeastern Black Sea

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#### ÖZ

Son yarım yüzyılda Karadeniz’de zooplankton komunitelerinde önemli değişimler gözlenmiş, bazı türler hemen hemen kaybolurken, bazı türler artış göstermiştir. Bu türlerden biri olan heterotrofik dinoflagellat *Noctiluca scintillans*, dünya denizlerinde yaygın olarak dağılım göstermektedir. Büyük boyutu (>200 µm) ve fagotrofik beslenme davranışı nedeniyle mesozooplankton içinde değerlendirilen *Noctiluca scintillans* 1970’lerden sonra Karadeniz’de mesozooplanktonun baskın katılımcılarından biri olmuştur. Bu çalışma ile Mayıs 2015-Nisan 2016 tarihleri arasında Güneydoğu Karadeniz yüzey sularında *Noctiluca scintillans* popülasyon yapısı ve mesozooplanktona olan katkısı araştırılmıştır. Yüzey sularında *Noctiluca scintillans* hücre bolluk değerleri en düşük yaz ayları, en yüksek Şubat-Mayıs periyodu olmak üzere 0-23357 hücre.m<sup>-3</sup> arasında değişmiş ve nehir ağzından açığa doğru artış eğilimi sergilemiştir. Örnekleme süresince *Noctiluca scintillans* hücre çapı 352-629 µm ve hacmi 2.28x10<sup>7</sup>-1.3x10<sup>8</sup> µm<sup>3</sup> arasında değişmiştir. Toplam mesozooplankton bolluğu 10-24020 birey.m<sup>-3</sup> arasında değişim göstermiştir. Örnekleme istasyonları arasında *Noctiluca scintillans* ve mesozooplankton bolluğu bakımından istatistiksel olarak önemli farklılık bulunamamıştır (p>0,05). *Noctiluca scintillans* toplam mesozooplanktona en düşük katkıyı yaz aylarında yaparken, Şubat-Mayıs periyodunda toplam mesozooplanktona katkısı nehir ağzında %79±4, kıyı sularında ise %98±2 olarak belirlenmiştir. Çalışma, bölgede *Noctiluca scintillans*’ın zooplanktonun hala önemli bir katılımcısı olduğunu doğrulamaktadır.

**Anahtar Kelimeler:** *Noctiluca scintillans*, heterotrofik dinoflagellat, mesozooplankton, Karadeniz

#### ABSTRACT

During the last half century, significant changes have been observed in the zooplankton communities in the Black Sea. While some species have almost disappeared, some other species have increased in number. *Noctiluca scintillans* is a red-tide forming heterotrophic dinoflagellate, which is widely distributed in the world’s oceans. Due to its large cell size (>200 µm) and phagotrophic feeding behavior, *Noctiluca scintillans* is assessed within mesozooplankton communities. After the 1970s, *Noctiluca scintillans* became dominant in the mesozooplankton community in the Black Sea. Between May 2015 and April 2016, the population characteristics of *Noctiluca scintillans* and its contribution to mesozooplankton communities were investigated in surface waters in the southeastern Black Sea. The abundance of *Noctiluca scintillans* in surface waters ranged between 0–23357 cells/m<sup>-3</sup> with the lowest number found in the summer and the highest number found from February to May. Abundance tended to increase from the river mouth toward open waters. During the study, the cell diameter and volume of *Noctiluca scintillans* varied between 352–629 µm and 2.28 x 10<sup>7</sup>–1.3x10<sup>8</sup> µm<sup>3</sup>, respectively. The total mesozooplankton abundance ranged between 10–24020 individuals/m<sup>-3</sup>. There are no statistically significant differences in *Noctiluca scintillans* and mesozooplankton abundance between sampling stations (p>0.05). The contribution of *Noctiluca scintillans* to the mesozooplankton population was the lowest during summer, but from February to May, *Noctiluca scintillans* comprised 79%±4% and 98%±2% of the total mesozooplankton population in the river mouth and coastal waters, respectively. These results confirm that *Noctiluca scintillans* is still an important contributor of the mesozooplankton community in the southeastern Black Sea.

**Keywords:** *Noctiluca scintillans*, heterotrophic dinoflagellate, mesozooplankton, Black Sea

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## GİRİŐ

Karadeniz ekosisteminde ötrofikasyon, aşırı avlanma, kirlilik, nütrient rejiminde deęişimler, istilacı türlerin giriő ve iklimsel deęişimin etkisiyle son yarım yüzyılda deęişimler gözlenmektedir (Zaitsev, 1992; Beőiktepe ve ark., 1999; Kideys, 2002; Daskalov, 2002; Oęuz ve Gilbert, 2007; Oęuz ve ark., 2012). Bu deęişimler Karadeniz'de denizel besin zincirinin temelini oluőturan fitoplankton ve zooplankton komunitelerini ciddi Őekilde etkilemiőtir. 1970'lerden sonra zooplankton bolluęu, biyoması ve komünite yapısında çok önemli deęişimler gözlenmiőtir, bazı zooplankton türleri ortadan kaybolurken, bazı türler de ortamda hâkimiyet saęlamaya baőlamıőtir (Shiganova ve ark., 2008).

Bu türlerden biri olan heterotrofik dinoflagellat *Noctiluca scintillans* (Macartney) Kofoid & Swezy ılıman ve tropikal bölgelerde yaygın olarak daęılım göstermektedir (Elbrachter ve Qi, 1998; Harrison ve ark., 2011). Hızlı üreme özellikleri ve beslenme davranıőı ile *N. scintillans*'ın miktarı kısa bir süre içinde hızla artarak, red-tide oluőturabilmekte ve plankton biyoması üzerinde baskı kurabilmektedir (Tiselius ve Kiørboe, 1998). Büyük boyutlu oluőu (>200 µm) ve fagotrofik beslenme davranıőı dolayısı ile mesozooplankton içerisinde deęerlendirilmektedir. 1970'lerden sonra Karadeniz ekosisteminde meydana gelen deęişimlerle birlikte *N. scintillans* sayıca yaklaşık 8-10 kat artış göstererek zooplankton içinde önemli bir yere gelmiőtir (Shiganova ve ark., 2008). Karadeniz'in kuzey ve kuzeybatı kıta sahanlığında *N. scintillans* miktarındaki artış ötrofikasyon ile iliŐkendirilmiőtir (Shiganova ve ark., 2008). *N. scintillans* Karadeniz'de genellikle Mayıs-Haziran periyodunda artış göstermekte ve çevresel koőullardaki deęişikliklerden ötürü, yaz aylarında Karadeniz'in üst karıőım tabakasında neredeyse ortadan kaybolmaktadır (Mikaelyan ve ark., 2014). Kuzeybatı Karadeniz'den Ekim-Kasım periyodunda da bolluk deęerlerinde artışlar kaydedilmiőtir (Velikova ve Mihneva, 2005). Karadeniz'in güney kıyılarında ise Nisan-Mayıs aylarında *N. scintillans* bolluęundaki artışlar rapor edilirken, Temmuz-Aęustos veya Aralık aylarında da artıőı gözlenmiőtir (Üstün, 2005). Güneydoęu Karadeniz'de yapılan çalıőmalarda *N. scintillans*'ın su kolonunda daęılımı rapor edilmiőtir (Feyzioęlu ve Sivri, 2003; Özdemir ve Ak, 2012; Yıldız ve Feyzioęlu, 2014). Güneydoęu Karadeniz'den 2011 yılı Nisan ayı sonunda ilk kez *N. scintillans*'ın neden olduęu red-tide vakası rapor edilmiőtir (Kopuz ve ark., 2014).

Karadeniz ekosisteminde gerçekteően dramatik deęişimler süresince artış göstererek mesozooplanktonun baskın katılımcılarından biri haline gelen ve pelajik besin zincirinin önemli bileőenlerinden biri olan *N.scintillans*'ın izlenmesi önem arz etmektedir. Bu çalıőma ile Mayıs 2015-Nisan 2016 tarihleri arasında balıkçılık bakımından önemli bir alanı temsil eden Güneydoęu Karadeniz yüzey sularında *N.scintillans*'a ait hücre bolluęu (hücre.m<sup>3</sup>), çapı (µm) ve hacminin (µm<sup>3</sup>) aylık olarak deęiőimi araŐtırılmıőtir. Ayrıca *N.scintillans*'ın mesozooplanktona katkısı belirlenmiőtir ve çevre ile etkileőimi deęerlendirilmiőtir.

## MATERYAL VE METOT

Örnekleme dar bir kıta sahanlığı ile karakterize olan Güneydoęu Karadeniz'de, nehir aęzı (40° 55'14" N; 40°11'31" E) ve kıyı (41°

00'01" N; 40°10'33" E) (5 deniz mili) sularında seçilen iki istasyonda Mayıs 2015-Nisan 2016 tarihleri arasında gerçekteőirilmiőtir (Őekil 1). Deniz suyuna ait sıcaklık, tuzluluk, iletkenlik parametreleri Sea bird SBE-19 Plus CTD prop kullanılarak, çözünmüő oksijen konsantrasyonu ise CTD prop üzerinde yer alan SBE 63-DO sensörü kullanılarak optik olarak yerinde ölçülmüőtür. In-situ floresan ölçümleri CTD üzerinde bulunan WETLAB flourometre kullanılarak gerçekteőirilmiőtir.

Zooplankton örneklere su yüzeyinden 09:00-15:00 saatleri arasında 200 µm göz açıklığına ve 70 cm aęiz açıklığına sahip WP2 net ile 2 knot hızda 5 dk süre ile alınmıőtir. Kepçede dışarıdan nazıkçe yıkanarak tüm örneğin kolektörde toplanması saęlanmış ve örneklere %4'lük formaldehit ile fikse edilmiőtir. Laboratuvarında örneklere Stempel pipet ile alt örnekleme yapılarak Bogorov sayım kamerasına aktarılmıőtir ve ZEISS Stemi 508 stereo mikroskop altında ana taksonomik gruplara ayrılmıőtir (Copepoda, Chaetognatha, Appendicularia, Cladocera, Scyphozoa, Ctenophora, *Noctiluca*). *N. scintillans* hücre çapının belirlenmesi için 10-50 hücrenin fotoęrafları çekilmiőtir ve ölçümler (µm) resim yazılımı aracılığı ile gerçekteőirilmiőtir. Hücrenin küresel olduęu varsayımı yapılarak hacim hesaplanmıőtir (Hillebrand ve ark., 1999). Ana taksonomik gruplara ait hücre bollukları birey.m<sup>-3</sup> ve *N.scintillans*'a ait hücre bolluęu hücre.m<sup>-3</sup> olarak hesaplanmıőtir. İstatistiksel analizlere baőlamadan önce verilere logaritmik dönüőüm yapılarak normal daęılım gösterip göstermedikleri test edilmiőtir. *N.scintillans* ile fiziksel ve biyolojik veriler arasındaki iliŐkinin anlaşılabilmesi için parametrik olmayan Spearman Rank-Order korelasyonu gerçekteőirilmiőtir. İstasyonlar arasında *N.scintillans* ve mesozooplankton bolluęu bakımından farklılık olup olmadıęı one-way ANOVA ile test edilmiőtir.

## BULGULAR VE TARTIŐMA

### Hidrografi

Örnekleme istasyonları, 2001 yılından bu yana birçok araŐtırmaya konu olmuőtir ve hidrografik koőulları iyi bilinmektedir (Aęırbaő, 2010; Kopuz, 2012; Aęırbaő ve ark., 2015). Çalıőma bölgesinde yüzey suyu sıcaklığı en düşük (9,7 °C) Őubat ve en yüksek (28,2°C) Aęustos ayında kaydedilmiőtir (Őekil 2). Nehir aęzı sularında tuzluluk yüzeyde %15,82-18,05, kıyı sularında ise %17-18 arasında deęiően tuzluluk deęerleri (Őekil 2), derinliğe baęlı artış göstermiőtir. Çalıőma bölgesi tuzluluk ve sıcaklık bakımından bölgede yürütülmüő önceki çalıőmalarla uyum içerisinde (Aęırbaő, 2010; Kopuz, 2012). Çözünmüő oksijen konsantrasyonu nehir aęzı istasyonu yüzey sularında 7,43 (Ocak)-8,57 mg.l<sup>-1</sup> (Temmuz), kıyı istasyonunda ise 7,06 (Őubat)-8,77 mg.l<sup>-1</sup> (Ocak) arasında deęiőim göstermiőtir. Örnekleme süresince yüzey sularında en yüksek klorofil-a deęerleri nehir aęzı istasyonunda tespit edilmiőtir ve kıyıda açığa doęru azalma eğilimi göstermiőtir. Nehir aęzı istasyonunda yüzey klorofil-a deęerleri 0,50 (Mayıs)-3,90 µg.l<sup>-1</sup> (Ekim) ve kıyı istasyonunda ise 0,28 (Haziran)-3,43 µg.l<sup>-1</sup> (Ekim) arasında deęiőim göstermiőtir (Őekil 2). Güneydoęu Karadeniz'de 2009-2010 periyodunda yüzey suyu klorofil-a deęerleri kıyısal bölgede ortalama 1,97 µg l<sup>-1</sup> olarak tespit etmiőtir (Aęırbaő, 2010). Aynı bölgede Ekim 2010- Aralık 2011 tarihleri arasında yapılan bir başka çalıőmada ise yüzey klorofil-a deęerleri 0,17-2,21 µg l<sup>-1</sup> arasında rapor edilmiőtir (Kopuz, 2012). Bu çalıőma esnasında ölçülen yüzey klorofil-a deęerleri Aęırbaő (2010) tarafından rapor

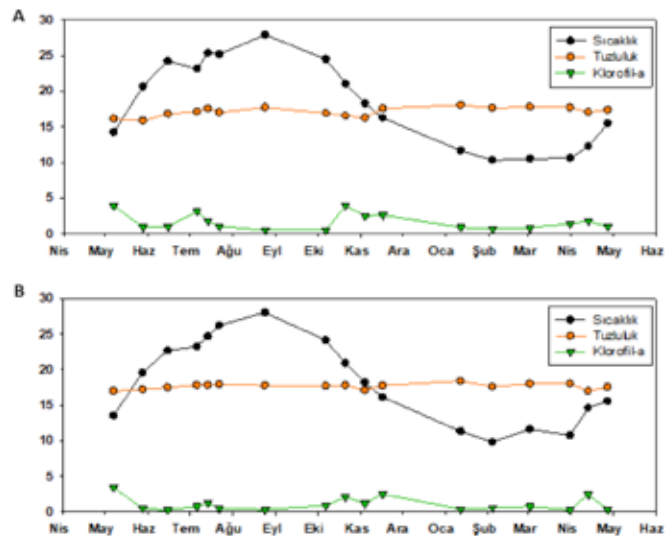
edilen deęerlerle benzerlik sergilerken, Kopuz (2012) tarafından rapor edilen deęerlere gre yksek bulunmuŐtur. Son yıllarda Gneydoęu Karadeniz'de klorofil-a'nın iklimsel deęiŐime baęlı olarak dalgalanmalar gsterdięi bildirilmiŐtir (AęırbaŐ ve ark., 2015). Karadeniz kıyasal blgesi nehir girdileri ve zellikle siklonik sırt akıntısı ve bununla iliŐkili meso-lçekli girdaplar, filament ve cephelerin etkisiyle olduka dinamik bir yapı sergilemektedir. Her ne kadar klorofil-a fitoplankton biyomasının bir gstergesi olarak kullanılsa da, klorofil-a konsantrasyonu ıŐık, besin elementi konsantrasyonu ve tr kompozisyonu gibi faktrlerin etkisi ile deęiŐim gsterebilmektedir.

### *Noctiluca scintillans* poplasyon yapısı

Gneydoęu Karadeniz'de alıŐma sresince yzey sularında *N.scintillans* hcre bolluk deęerleri en dŐuk yaz ayları, en yksek Őubat-Mayıs periyodu olmak zere 0-23357 hcre.m<sup>-3</sup>

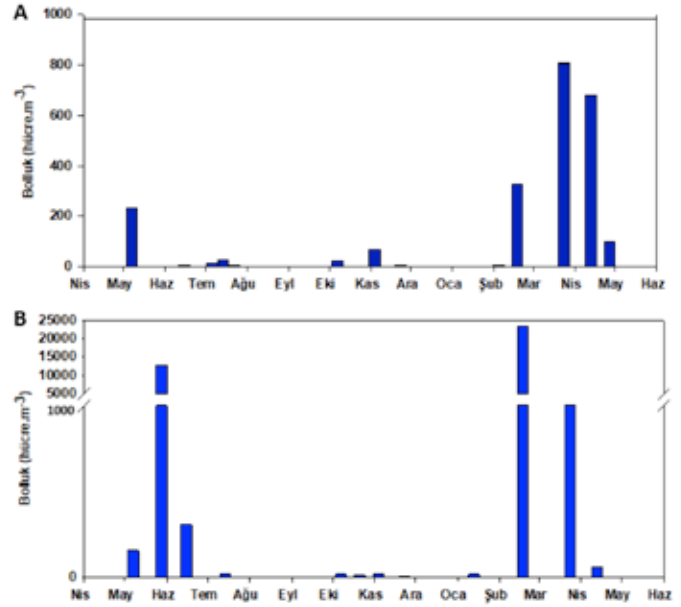


**Őekil 1.** alıŐma blgesi ve rneklemeye istasyonları  
**Figure 1.** Study area and sampling stations in the Black Sea

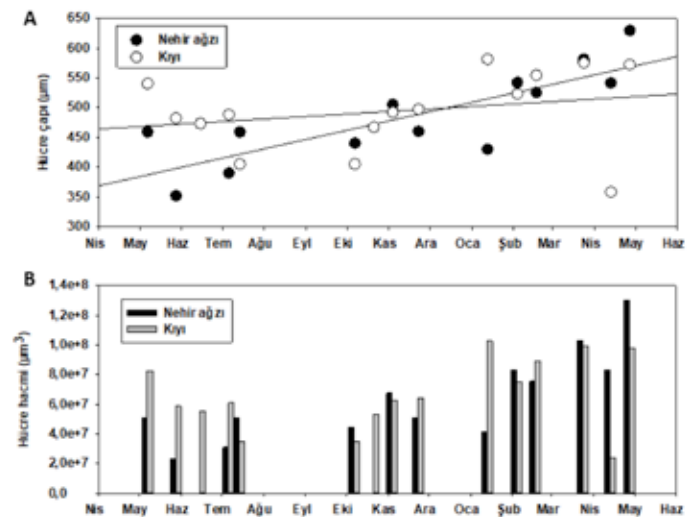


**Őekil 2. a, b.** rneklemeye istasyonlarına ait yzey suyu sıcaklık (°C), tuzluluk (‰) ve klorofil-a (µg.l<sup>-1</sup>) deęerleri (A: nehir aęzı, B: kıyı)  
**Figure 2. a, b.** Sea surface temperature (°C) (black line), salinity (‰) (orange line) and chlorophyll-a (green line) at the sampling stations (A: river mouth, B: coastal)

arasında deęiŐmiŐtir (Őekil 3). rneklemeye istasyonları arasında *N.scintillans*'ın bolluęu bakımından istatistiksel olarak nemli bir farklılık bulunmamıŐtır (ANOVA, p>0,05). Dnya genelinde olduęu gibi (Harrison, 2011) Karadeniz'de de *N.scintillans* hcre

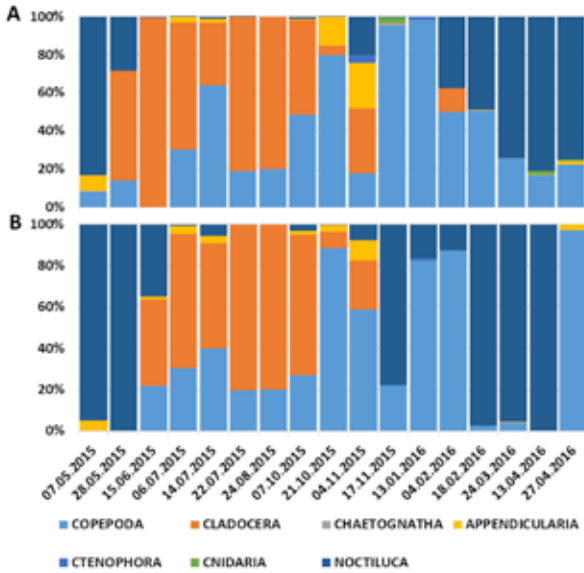


**Őekil 3. a, b.** *Noctiluca scintillans*'ın rneklemeye istasyonları yzey sularında daęılımı (A: nehir aęzı, B: kıyı)  
**Figure 3. a, b.** Surface distribution of *Noctiluca scintillans* at the sampling stations (A: river mouth, B: coastal)



**Őekil 4. a, b.** alıŐma sresince rneklemeye istasyonlarında *Noctiluca scintillans* hcre apı (A) ve hacminin (B) deęiŐimi  
**Figure 4. a, b.** Changes on cell diameter (A) and volume (B) of *Noctiluca scintillans* at the sampling stations during study





Şekil 5. a, b. *Noctiluca scintillans*'ın örnekleme istasyonları yüzey sularında mesozooplanktona katkısı (%) (A: nehir ağızı, B: kıyı)

Figure 5. a, b. Contribution of *Noctiluca scintillans* to mesozooplankton at the sampling stations (A: river mouth, B: coastal)

sayısındaki artışın ilkbahar aylarında gerçekleştiği bilinmektedir (Nikishina ve ark., 2011; Özdemir ve Ak, 2012; Mikaelyan ve ark., 2014; Kopuz ve ark., 2014). Bulgaristan kıyılarında *N.scintillans* popülasyonunun Nisan ayında artmaya başlayıp Haziran ve Temmuz aylarında en yüksek değerlere ulaştığı (yaklaşık 10000 hücre.m<sup>-3</sup>) rapor edilmiştir (Shiganova ve ark., 2008). Kuzeydoğu-merkez Karadeniz ve Kuzey Adriyatik Denizi'nde *N.scintillans* popülasyonların karşılaştırıldığı çalışmada, 2008-2012 yılları arasında en yüksek hücre bolluğu Mayıs'ta ve Haziran'ın ilk yarısında tespit edilmiş ve 1x10<sup>3</sup>-25x10<sup>3</sup> hücre.m<sup>-3</sup> arasında değişmiştir (Mikaelyan ve ark., 2014). Güneydoğu Karadeniz'de Ekim 2007 ile Eylül 2008 tarihleri arasında yürütülen bir çalışmada *N.scintillans* hücre bolluğu 5-3753 hücre.m<sup>-3</sup> arasında değişmiş ve en yüksek bolluk değerleri Mayıs ayında rapor edilmiştir (Özdemir ve Ak, 2012). Çalışma süresince her iki örnekleme istasyonunda Şubat ayı ile birlikte bolluk değerlerinde ciddi artışlar görülmüş ve nehir ağızı istasyonunda en yüksek bolluk değerleri (806 hücre.m<sup>-3</sup>) Mart 2016'da, kıyı istasyonunda ise Mayıs 2015 (12776 hücre.m<sup>-3</sup>) ve Şubat 2016'da (23357 hücre.m<sup>-3</sup>) tespit edilmiştir. Hücre bolluk değerleri ve en yüksek değerlere ulaştığı dönem Karadeniz'de yapılan çalışmalarla uyum içerisindedir (Nikishina ve ark., 2011; Özdemir ve Ak, 2012; Mikaelyan ve ark., 2014). *N. scintillans*'ın su sıcaklığının 25 °C üzerine çıktığı dönemlerde büyüme hızının ciddi şekilde düşmesi dolayısıyla çok düşük bolluk değerleri sergilediği rapor edilmiştir (Tada ve ark., 2004). Mikaelyan ve ark., (2014) tarafından yapılan çalışmada da beslenme ve çevresel koşullardaki değişikliklere bağlı olarak yaz aylarında *N. scintillans*'ın Karadeniz'de üst karışım tabakasından neredeyse kaybolduğu bildirilmiştir. Benzer şekilde bu çalışmada da en düşük hücre yoğunluğu (0-29 hücre.m<sup>-3</sup>) su sıcaklığının 25 °C

üzerine çıktığı yaz aylarında tespit edilmiştir. Dünya genelinde *N.scintillans*'ın neden olduğu red-tide vakaları sıklıkla rapor edilmektedir (Harrison ve ark., 2011). Güneydoğu Karadeniz'den *N.scintillans*'ın neden olduğu red-tide ilk kez 2011'de bildirilmiş ve hücre sayısının 1x10<sup>6</sup> hücre.l<sup>-1</sup> ulaştığı rapor edilmiştir (Kopuz ve ark., 2014). Bu çalışma esnasında *N. scintillans*'ın oluşturduğu bir red-tide vakasına rastlanılmamıştır.

Örnekleme süresince yüzey sularında *N.scintillans* hücre çapı nehir ağızı istasyonunda 352-629 µm (ortalama 486±78 µm), kıyı istasyonunda ise 405-581 µm (ortalama 494±67 µm) arasında değişim göstermiştir (Şekil 4a). Karadeniz'de uzun yıllara dayalı değerlendirme sonucunda *N.scintillans*'a ait hücre çapı 401-600 µm arasında rapor edilirken (Mikaelyan ve ark., 2014), Karadeniz'den ilk kez rapor edilen red-tide vakasında 425-809 µm arasında tespit edilmiştir (Kopuz ve ark., 2014). Büyük boyutlu hücrelerin red-tide öncesinde ve esnasında bölünmek üzere olan hücreler olduğu bildirilmiştir (Kopuz ve ark., 2014). Genel olarak değerlendirildiğinde Mayıs 2015-Nisan 2016 periyodunda bölgede *N.scintillans* 500 µm'den küçük hücreler (ortalama 490±72 µm) ile temsil edilmiştir. Bulunan değerler Karadeniz'in kuzey sahillerinden Mikaelyan ve ark. (2014) tarafından rapor edilen değerlere benzer olup, red-tide esnasında bulunan değerlere göre düşük bulunmuştur (Kopuz ve ark., 2014). Küçük boyutlu hücreler (340-450 µm) iyi beslenen ve hızlı büyüyen hücreleri temsil ederken, büyük boyutlu hücrelerin (450-1200 µm) yetersiz koşullar altındaki durumu temsil ettiği rapor edilmiştir (Murray ve Suthers, 1999; Dela-Cruz ve ark., 2008). Bu çalışma süresince bulunan değerlerin bölgede sağlıklı büyüyen bir *N. scintillans* popülasyonunun göstergesi olduğu düşünülmektedir. *N.scintillans*'ın hücrelerinin küresel olduğu varsayılarak çap kullanılarak hesaplanan hücre hacmi değerleri ise nehir ağızı istasyonunda 2.28x10<sup>7</sup>-1.3x10<sup>8</sup> µm<sup>3</sup> (ortalama 6.4x10<sup>7</sup>±3x10<sup>7</sup> µm<sup>3</sup>), kıyı istasyonunda ise istasyonunda 2.4x10<sup>7</sup>-1.03x10<sup>8</sup> µm<sup>3</sup> (ortalama 6.6x10<sup>7</sup>±2.5x10<sup>7</sup> µm<sup>3</sup>) arasında değişim göstermiştir (Şekil 4b).

Çalışma esnasında *N. scintillans* bolluğu sıcaklık ve tuzluluk ile istatistiksel olarak önemli bir ilişki sergilememiştir. *N. scintillans* artışında potansiyel besin artışının büyük etkisi olduğu (Harrison ve ark., 2011), özellikle diatom blomları esnasında *N. scintillans*'ın sayıca artış gösterdiği bilinmektedir (Dela-Cruz vd., 2008; Kopuz ve ark., 2014). Yapılan bir çok çalışmada *N. scintillans* ile fitoplankton biyomasının bir göstergesi olarak kabul edilen klorofil-a arasında istatistiksel olarak önemli bir ilişki bulunurken (Kopuz ve ark., 2014; Tsai ve ark., 2018), diğer bir çok çalışmada ise *N. scintillans* bolluğu ile klorofil-a arasında istatistiksel olarak önemli bir ilişki bulunmamıştır (Huang ve Qi, 1997; Tada ve ark., 2004; Özdemir ve ark., 2017). Bu çalışma esnasında da örnekleme istasyonlarında *N.scintillans* ile klorofil-a arasında istatistiksel olarak önemli bir ilişki bulunamamıştır. *N.scintillans* bakteriden, fitoplankton, protozoa, kopepod ve nauplileri, metazoa yumurta ve larvalarına kadar geniş bir av aralığı üzerinden beslenebilmektedir (Elbrachter ve Qi, 1998; Nikishina ve ark., 2011). Bu çalışmada *N.scintillans* ile klorofil-a arasındaki ilişki yıl genelinde değerlendirildiğinden, *N.scintillans* bolluğunun yalnızca kısa süreli fitoplankton artışlarına bağlı olarak değil, ortamda mevcut diğer av kompozisyonuna bağlı olarak da değiştiği düşünülmektedir.

### **Noctiluca scintillans'ın mesozooplanktona katılımı**

Çalışma bölgesi yüzey sularında mesozooplankton 7'si Copepoda, 3'ü Cladocera, 1'i Chaetognatha, 1'i Appendicularia, 1'i Ctenophora, 1'i Cnidaria ve 1'i Dinoflagellata'ya (*N. scintillans*) ait toplam 15 tür tarafından temsil edilmiştir. Toplam mesozooplankton bolluğu (*Noctiluca* dahil) nehir ağızı yüzey sularında 10-6021 birey.m<sup>-3</sup>, kıyı sularında ise 12-24020 birey.m<sup>-3</sup> arasında değişim göstermiştir. *N.scintillans*'ın mesozooplanktona katkısı nehir ağızı yüzey sularında %0-83, kıyı sularında ise %0-99 arasında değişmiştir (Şekil 5). *N.scintillans* toplam mesozooplanktona her iki istasyonda da en düşük katkısı (<%3) yaz aylarında yaparken, aynı dönemde Cladocera baskın grup olmuştur (>%65). *N. scintillans*'ın toplam mesozooplanktona en yüksek katkısı nehir ağızı istasyonunda Mayıs 2015 (%83) ve Nisan 2016'da (%81), kıyı istasyonunda ise Mayıs 2015 (%99) ve Şubat-Nisan 2016 (%96) periyodunda gözlenmiştir (Şekil 5).

Karadeniz komisyonunun raporunda, *N.scintillans*'ın Karadeniz kıyılarında özellikle 1970'lerden sonra artış göstererek mesozooplanktonun en önemli katılımcılarından biri olduğu bildirilmiştir (Shiganova ve ark., 2008). Bulgaristan kıyılarında ötrofikasyon periyodu boyunca (1970-1990) *N.scintillans* zooplankton komünitelerinde baskın grup olarak rapor edilmiştir. Bu dönemde *N.scintillans* toplam zooplanktona katılımı 1970 başlarında %35-42, 1970 ortaları ve 1980 sonlarına kadar olan dönemde %90'dan fazla olmuştur. Türkiye kıyılarında 1999-2005 yıllarında Bulgaristan kıyılarına oranla daha küçük değerlerde katkı yaparak zooplanktonun %70'ini oluşturmuştur. Romanya kıyılarında 2002 yılında *N.scintillans*'ın toplam zooplanktona %98 oranla katılım gösterdiği bildirilmiştir (Shiganova ve ark., 2008). Özdemir ve Ak (2012) tarafından Güneydoğu Karadeniz'de yürütülen çalışmada *N.scintillans*'ın hücre bolluğunun en yüksek olduğu Mayıs ayında toplam zooplanktona katkısının %90'nın üzerinde olduğu bildirilmiştir. Güneydoğu Karadeniz'de yürütülen bu çalışmada da Karadeniz'de yapılan çalışmalarla uyum içerisinde *N.scintillans* mesozooplanktonun önemli bir katılımcısı olmuştur ve mesozooplanktona en yüksek katkısı (%99), Özdemir ve Ak (2012) tarafından rapor edildiği şekilde sayıca en yüksek olduğu dönem olan Mayıs ayında gerçekleştirmiştir.

### **DEĞERLENDİRME**

Güneydoğu Karadeniz'de *N.scintillans*'ın popülasyon yapısı Karadeniz'de daha önce yapılmış çalışmalarla uyum içerisinde. Beklendiği üzere yüzey sularında ilkbahar periyodunda artış göstererek zooplanktonun önemli bir katılımcısı olmuş, ancak güçlü termal tabakalaşma periyodunda yüzey sularında neredeyse bulunamamıştır. Karadeniz'de mesozooplankton içinde bu denli baskın olan *N.scintillans*, Deniz Stratejisi Çerçeve Direktifi'nin "İyi Çevresel Durum" göstergelerinden 'Besin Ağı' amaçları kapsamında Karadeniz pelajik ekosisteminde indikatör bir tür olarak göz önünde bulundurulmalıdır. Karadeniz pelajik besin zincirinde gerçekten besinsel kör uç olup olmadığı ve besin zincirindeki rolünün anlaşılması gerçekçi karbon döngüsü yaklaşımları için önem arz etmektedir.

### **TEŞEKKÜR**

Bu çalışma TÜBİTAK 114Y232 nolu "Güneydoğu Karadeniz Planktonik Besin Zincirinde Mikrozooplanktonun Rolü" başlıklı proje tarafından desteklenmiştir. Deniz çalışmalarındaki yardımlarından

ötürü Doç. Dr. Ertuğrul AĞIRBAŞ, Yrd. Doç. Dr. İlknur YILDIZ, Yrd. Doç. Dr. Rahşan Evren MAZLUM, Fatma Başak ESENİSOY ŞAHİN, Rıza USTA, Mustafa BAKIRCI ve Yusuf ÖZDEN'e, harita için Dr. Andre VALENTE'e teşekkür ederiz. Ayrıca R/V SURAT ARASTIRMA, R/V DENAR and R/V KARADENİZ ARAŞTIRMA gemi adamları ve kaptanlarına çalışma esnasındaki desteklerinden ötürü teşekkür ederiz.

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## Predicting the Size Selectivity Based on the Striped Red Mullet Morphology (*Mullus surmuletus*) in Bottom Trawl Fisheries

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### ABSTRACT

The striped red mullet (*Mullus surmuletus*) is a commercially important demersal species caught by mixed trawl fishing in the Mediterranean Sea. Although it is not among the target species of bottom trawl operations, the striped red mullet is an important species due to its high commercial value in trawl catch composition. The primary target is to catch adult individuals while allowing the immature and juvenile fish to escape for sustainable fisheries using selective fishing gears. Various selectivity studies regarding trawl codends aimed at improving selectivity have been carried out in the past two decades. However, the selectivity sea trials generally require a great deal of work, time, and labor on the deck and are also expensive. Therefore, instead of experimental-based selectivity studies, simulation-based studies (i.e., the FISHSELECT methodology) were recently started to predict the size selectivity of species in bottom trawl fisheries. In this study, sampled individuals of the striped red mullet were used in morphological measurements, fall-through experiments, and simulation phases. Diamond mesh sizes of 40, 44, and 50 mm and a square mesh size of 40 mm of bottom trawl codends were simulated, and the L50 values were calculated as 9.87, 10.75, 12.19, and 12.3 cm for the aforementioned mesh sizes, respectively. Design guides were then created for various mesh sizes and opening angles. The study results were compared with those of previous studies on the red mullet (*M. barbatus*) that were conducted using the FISHSELECT methodology, and the selectivity results of these two species were found to be similar.

**Keywords:** FISHSELECT, striped red mullet, *Mullus surmuletus*, selectivity, bottom trawl

### INTRODUCTION

Certain rules and guidelines have been established for the scientific use of resources in fisheries management. One of these is to prevent juveniles from being caught in fishing operations by means of selectivity and to ensure that fish populations are used in a sustainable manner. Using the morphological characteristics of fish, predicting the basic selectivity characteristics of fishing nets and adapting this to Mediterranean fisheries is one of the main objectives of such methods.

It has been reported that 30% of the species caught in the Mediterranean and Black Sea are demersal species (FAO, 2011). Among these demersal species the striped red mullet (*Mullus surmuletus*) is one of the most commercially important species. According to the latest data, a

total of 3,047 tons of fish were caught in the seas off Turkey (TÜİK, 2016). 12,014 tons of striped red mullet were caught worldwide according to latest reports (FAO, 2014). Generally, gill net, trammel net and bottom trawling methods are used in the fishing of the striped red mullet.

Conventional selectivity studies are usually carried out by analyzes of experimental data obtained from sea trials. However, these studies are very expensive and require manpower and time. To avoid such problems, less experimental data is used in the theoretical selectivity prediction studies which have started to be used extensively in recent years. The relationship between the morphological characteristics of captured species and mesh openings can be examined in terms of selectivity through these studies.

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İzmir Bay and the vicinity were selected as the study area. In this region, there is intensive fishing of the striped red mullet. Generally, gill and trammel nets are using as fishing methods but bottom trawling is also used as a method although, in this case, the red mullet is not among the primary target species. It also shows morphologically significant similarities with the red mullet which indicates that the comparative selectivity properties of these two species can be studied. Thus, problems that can be encountered in fisheries management could be solved much more quickly and scientifically.

To briefly mention the similarities between the striped red mullet and the red mullet, the striped red mullet has a longer snout structure than the red mullet. In addition, the red mullet has a more perpendicular head shape than the striped red mullet. The red mullet also has a purple-maroon color while the striped red mullet has a red stained body color. The striped red mullet has a clear red line on the lateral side starting from the back of the eye, above which there are three yellow lines extending up to the caudal keel.

In conventional selectivity studies, the main method of evaluating selectivity is based on obtaining experimental data with sea expeditions and analyzing this data statistically. But during trawl hauling, difficulties and unpredictable conditions can arise such as streams, weather, equipment, crew etc. If we take these variable factors into consideration, many high cost and time-consuming expeditions at sea are required to get reliable results. Scientists have introduced many methods to solve these problems. The FISHSELECT methodology, which simulates and predicts codend selectivity based on fish morphology (Herrmann et al., 2009), could be one of the solutions to such issues in sustainable fisheries management.

This method has already been applied in a number of studies to estimate the selective properties of codends of towed fishing gears for larger species of some round fish (Herrmann et al., 2009, 2012; Krag et al., 2011; Sistiaga et al., 2011), flatfish (Herrmann et al., 2013), nephrops (Frandsen et al., 2010) and krill (Krag et al., 2014). This method was also used on the red mullet as a Mediterranean species (Tokaç et al., 2016). The results of these studies show that there is a similarity between the estimated and actual results when compared with actual marine selectivity studies. This is the first theoretical basis selectivity study on the striped red mullet, which has an economically important place in bottom trawl catch composition.

**Table 1.** Striped red mullet (*Mullus surmuletus*) experimental selectivity data for diamond mesh and square mesh codends. n denotes the number of open meshes in codend circumference

Nominal/ Measured Mesh Size	L50 (cm)	SR (cm)	n	References
40 mm Square	12.2	2.1	-	Ordines et. al., 2006
40 mm Diamond	9.8	2.9	51	Ateş et. al., 2010
40 mm Square	13.2	3.2	28	Ateş et. al., 2010

## MATERIAL AND METHOD

### Study Area and Experimental Selectivity Data

In this study, the striped red mullet was used as a material. The striped red mullet is a demersal species belonging to the Actinopterygii class, Perciformes order, Mullidae family. The striped red mullet has a relatively high commercial value among other species caught by bottom trawl fishing in the Mediterranean Sea. Although there were not enough selectivity studies on trawl fisheries of this species, published historical study results are listed below with retention rates (L50) and selection range (SR) (Table 1).

The FISHSELECT method was used in this study. This method has three different phases, in chronological order; sea trials, laboratory phase, simulation analyzes.

Individual samples were collected from İzmir Bay and bought fresh and with no deformities from fish auctions in the İzmir region. A total of 65 fish from the smallest to the largest possible size of the species were obtained and used in this study. Species are sampled according to length-frequency data about the striped red mullet population in İzmir Bay (İlhan et al., 2009). Specimens were brought to the laboratory before body deformation such as dehydration and rigor mortis occurred.

### Measuring Fish Morphology

Fresh samples were transferred directly to the laboratory for length and weight measurements. A weight scale (TypeBD6000: Mettler, USA) of 0.01 gr precision was used during this process. Measured weights were recorded in grams. To take size measurements, a specially designed PVC ruler was used.

A morphometer was used for body cross section measurements, which are required for the simulation study. Cross sections (CS) are coded as CS1 and CS2 (Figure 1). CS1, a spiny structure located in the middle of the opercula which is located at the widest bony part of the head, and CS2, the foremost point of the spiny dorsal which is located at the point of the maximum transverse perimeter, fin were used for the red mullet cross section measurements in Tokaç et al., 2016.

These cross sections were determined using a morphometer and were scanned by a flatbed scanner and these measurements were marked and digitized by computer using FISHSELECT software (Figure 2).

### Fall-through Experiments

A new set of mesh templates comprised of 478 different mesh shapes, were made specifically to carry out fall-through experiments for small fish species. Mesh templates were made from 4 mm thick solid polyethylene material (Figure 3). Each fish was held by the tail and dropped into the mesh templates upside-down. The template plates were held horizontally and each fish turned optimally to fall through the mesh. The only force that influenced the fish was gravity as they faced the mesh templates (Herrmann et al., 2009). At the end of this experiment, it was decided whether or not fish passed through plates.

### Simulation of Mesh Penetration and Selection of a Penetration Model

After length and weight data obtained in the laboratory were converted into a list called a fish list, a mesh list was created which includes 478 different mesh models representing mesh sizes from 20 to 245 mm. The shapes include diamonds (252 meshes), hexagons (98 meshes) and rectangles (128 meshes).



Figure 1. Cross section positions

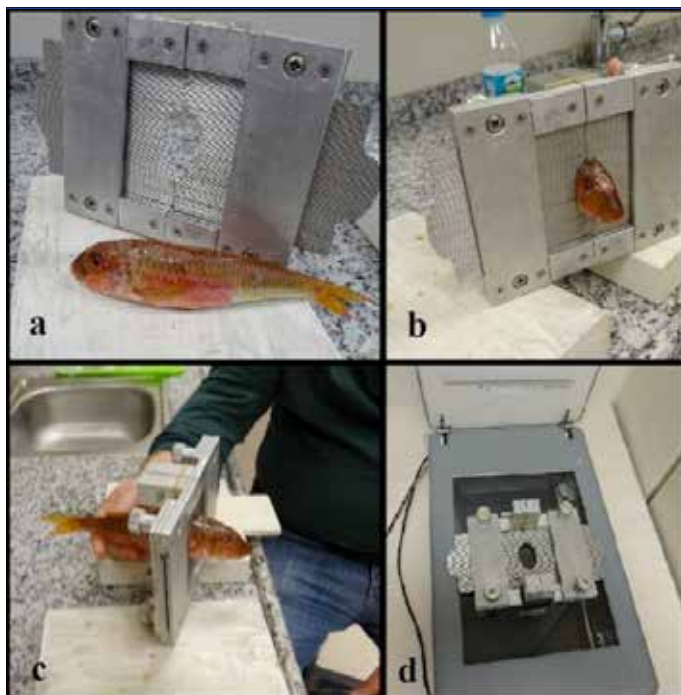


Figure 2. a-d. (a) Morphometer and sample, (b) CS1 measurement, (c) CS2 measurement, (d) Flatbed scanner



Figure 3. Fall-through experimental setup

By using the cross-sectional body models available in the program, the closest matching model for each digitised cross section was determined as the most appropriate model for the relevant cross section. At this stage, 3 cross-section models: ellipse, flexellipse and flexdrope shapes were chosen for analyzes. These cross-sections were chosen for their potential to determine a striped red mullet's ability to pass through meshes of different configuration and size size and shape.

After the determination of the cross-sectional shapes, the compression ratio was estimated. In order to make the closest estimate of the results obtained in laboratory studies, the tissue stiffness differences in the dorsal, lateral and abdominal regions were estimated and the compression rates were calculated. Compression models obtained for cross-sections were compared with fall-through experiment results and simulated by FISHSELECT. The simulation process varies depending on the processing performance of the computer, however, in this study, it took approximately two weeks. The most appropriate model was then selected for the best resultant compression model type.

A virtual population of 5,000 individuals was created for use in predict size selectivity regarding length-weight distributions of 65 specimens morphological data. The selected best compression model was tested along with the mesh size and virtual population, and the selectivity parameters for mesh sizes were calculated. These were determined as the 50% retention rate (L50) selectivity parameter of the species. All these results were transformed into the design guide with the help of R statistic program. These isobar graphics show all retention rate possibilities and help us to estimate theoretical selectivity of target species.

## RESULT AND DISCUSSION

A total of 65 striped red mullet individuals were sampled and ID numbers for each fish were created (Figure 4). Sampled species were measured and found to be between 87-234 mm and 4-192 g in length and weight, respectively. This data was recorded in the FISHSELECT software for the study.

### Fish Shape and Cross Section Analyzes

The striped red mullet has a fusiform body shape. According to this shape, three similar forms were chosen; ellipse, flexellipse1 and flexdrope. Cross sections have been imaged from a flatbed scanner and those images pinpointed from their edges one by one, each image is marked with at least 120 dots to enable the shape of the most suitable model to be analyzed (Figure 5).

To determine the most suitable shapes for CS1 and CS2 separately, the mean AIC (Akaike, 1974) and  $R^2$  values were calculated for each striped red mullet individually for each of the three models for both CS1 and CS2. The highest  $R^2$  and the lowest AIC values were considered as parameters. According to this analysis, the most suitable body shape has been found as the flexellipse1 model (Table 2).

### Fall-through Results and Penetration Model

Fall-through pass results were recorded as yes or no for the 65 samples for each of the 478 meshes with varying sizes of open areas and a total of 31,070 trials were completed. Based on the results of these fall-through trials, we selected a penetration model

(compression model) to use for simulating size selection of the striped red mullet.

This process determines the best model to be used in selectivity simulations by entering the estimated compression rates for the most appropriate model obtained from analyzes of fall-through experiments and cross sections. The maximum compressibility estimated for this model was 20% lateral, 6% dorsal and 30% ventral. A combination of different compression rates determined for each side resulted in  $6 \times 4 \times 6 = 144$  compression models (Table 3).

Virtual simulation was carried out by selecting the best 5 models obtained from the result of the fall-through experiments. The simulation was then carried out based on the degree of agree-

ment value (DA-value) for the different models compared with these fall-through experiments result.

S: The number of successful test results compared with the model's free pass result

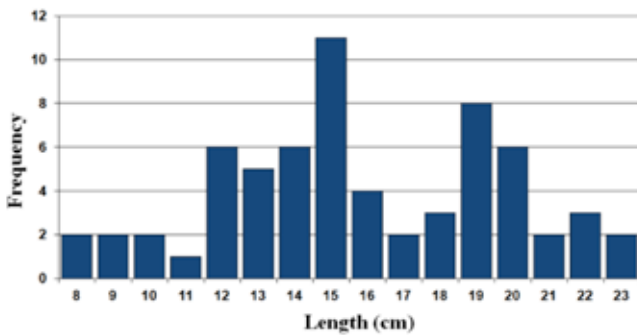
D: The number of unsuccessful test results compared with the model's free pass result

$$DA = \frac{S}{S + D}$$

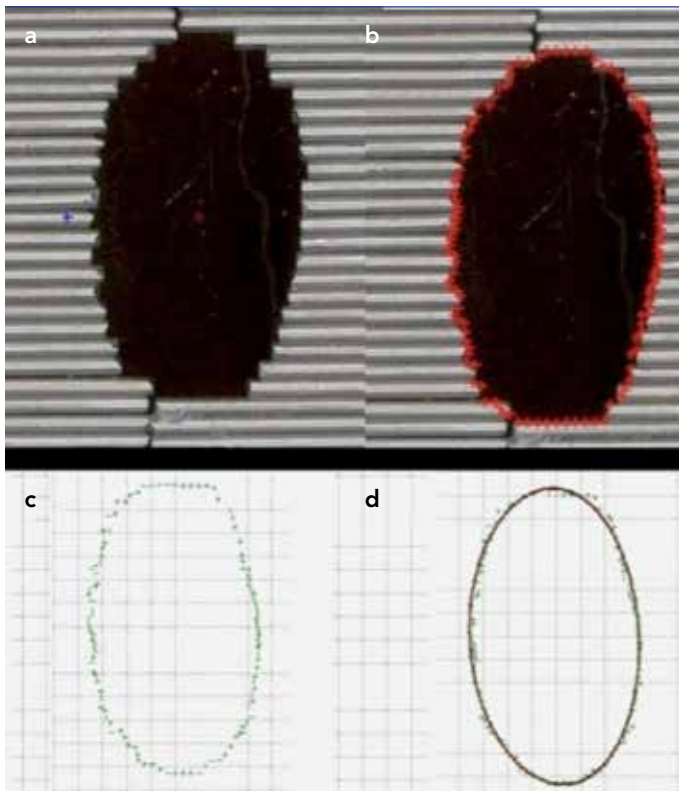
The model with the highest value of 0.95 above the acceptance grade was chosen as the best model for both cross sections (CS1 and CS2) and was selected for the compression model (Table 4).

A virtual population of 5,000 individuals was created based on the length-weight data of the 65 samples. The recalculation of the compression values to  $\pm 0.01$  resulted in a test with  $3 \times 3 \times 3 = 27$  possible combinations of compression, and a virtual simulation has been run. As a result, the highest DA value model was found to be as the best compression model (Table 5).

When the compression models were examined, it was seen that the model giving the highest result belongs to the CS2 coded section with a rate of 97.19%. Estimated compressions were found as 4% from lateral, dorsal 2%, ventral 18% at the end of the simulation.



**Figure 4.** Length/frequency histogram graphic of sampled 65 species



**Figure 5. a-d.** (a) Scanned raw image (b) Edge marking (c) Digitalised CS (d) CS model selection

**Table 2.** Mean  $R^2$  and AIC values for different shape descriptions. The highest  $R^2$  and lowest AIC values for each cross section are in bold

		<i>M. surmuletus</i>		
		Ellipse	Flexdrope	Flexellipse1
CS1	AIC	149.552164	147.857345	<b>146.766025</b>
	$R^2$	0.966616	0.967569	<b>0.967817</b>
CS2	AIC	183.129680	179.886555	<b>178.338775</b>
	$R^2$	0.960203	0.961754	<b>0.962417</b>

**Table 3.** Estimated compression rates matrix

Comp0	Comp90	Comp270
1.00	1.00	1.00
0.96	0.98	0.94
0.92	0.96	0.88
0.88	0.94	0.82
0.84		0.76
0.80		0.70
Total Compression Model Number: $6 \times 4 \times 6 = 144$		



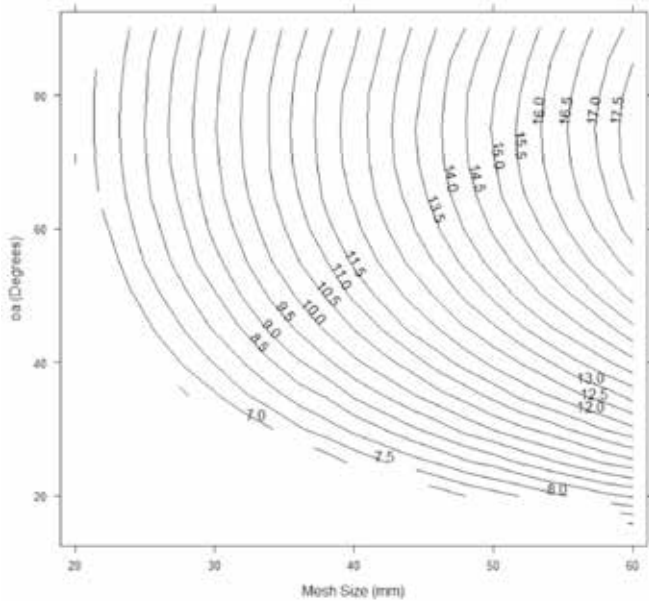
### Design Guides

The size selection predicted by FISHSELECT can be applied to produce design guides in the form of iso-curves for L50 values dependent on mesh size and mesh openness (Tokaç et al., 2016). With the help of the R statistics program, design guides have been created. Diamond and square mesh types have been designed in two guides (Figure 6, 7). In addition, it has been plotted the FISHSELECT predicted L50 values for 40° opening angle (OA). This specific OA value was reported by Tokaç et al., 2016 as the typical value for meshes observed during trawling from underwater recording.

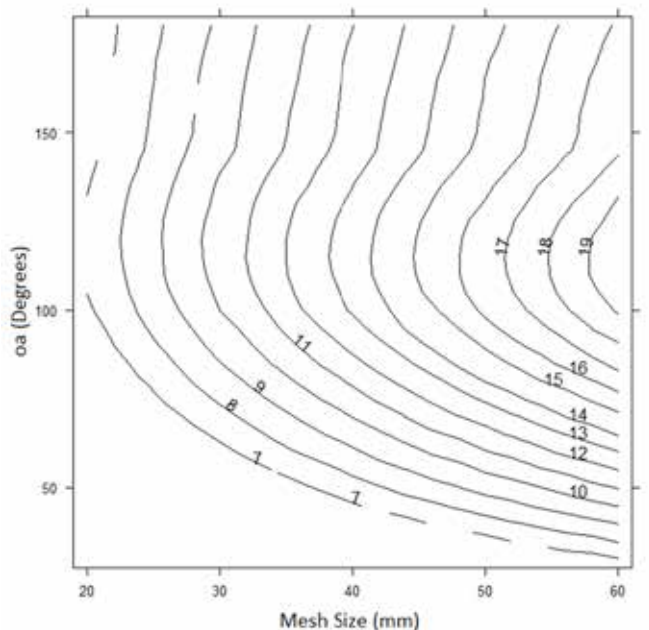
Diamond mesh codend L50 values for 40 mm, 44 mm, 50 mm mesh sizes are predicted as 9.87 cm, 10.75 cm, 12.19 cm striped red mullet. Square mesh codend L50 retention rates are predicted as 12.38 cm mean value between 70° and 120° OA which are estimated OA values during bottom trawl towing (Table 6).

Diamond mesh codend with 40 mm, 44 mm and 50 mm, and 40 mm square mesh codend were used in the study in accordance with the European Union's (1995/2006) mandatory regulation and the recommendations of the General Fisheries Commission for the Mediterranean on mesh sizes.

When the results obtained were examined, it was concluded that it is difficult to make a precise and clear comparative analysis due to incomplete and inadequate previous studies. However, if we compare the results obtained in the controlled computer environment with the actual selectivity studies, it can be seen that



**Figure 6.** Design guide for diamond mesh



**Figure 7.** Design guide for square mesh

**Table 4.** Best 5 models that given highest results for both cross sections. Best resultant model is marked in bold

	CS 1					
	comp0	comp90	comp270	S	D	DA
1	1.00	1.00	1.00	29835	1235	0.96025
2	1.00	0.98	1.00	29779	1291	0.95844
3	1.00	0.96	1.00	29723	1347	0.95664
4	1.00	1.00	0.94	29663	1407	0.95471
5	1.00	0.94	1.00	29658	1412	0.95455
	CS 2					
1	0.96	0.98	0.82	30715	895	0.97119
2	0.96	1.00	0.82	30165	905	0.97087
3	0.96	1.00	0.76	30158	912	0.97064
4	0.96	0.96	0.82	30156	914	0.97058
5	0.96	0.98	0.76	30152	918	0.97045

**Table 5.** Compression results end of precise simulation gives the best model

	CS 2					
	comp0	comp90	comp270	S	D	DA
Best Compression Model	0.96	0.98	0.82	30197	873	0.97190

**Table 6.** Important L50 values from this study

Mesh Opening Angle	Mesh Size	Mesh Shape	L <sub>50</sub>
40°	40mm	Diamond	9.87 cm
40°	44mm	Diamond	10.75 cm
40°	50mm	Diamond	12.19 cm
70°	40mm	Square	11.21 cm
120°	40mm	Square	13.56 cm



the results are close to the previous study results. In addition, if the selectivity study is compared to that of numerous previous studies of the red mullet, it shows that the selectivity parameters between the two species are very similar.

In the previous FISHSELECT study (Tokaç et al., 2016) the red mullet had L50 value in 40 mm and 50 mm diamond mesh size predicted as 11.0 cm and 13.5 cm respectively. When the comparison result was reviewed both species showed similar results, which is thought to be due to the low morphological differences between the two species. Due to the fact that the red mullet has a more perpendicular head structure than the striped red mullet, result L50 values are thought to be lower for the striped red mullet.

As can be seen from the design guide (Figure 6), 44 mm diamond mesh codend was found to be the most appropriate for the striped red mullet when we compare this result with its minimum landing size (MLS) 11 cm.

Based on the results of the 31,070 fall-through trials, a compression model has been selected to use for simulating size selection of striped red mullet. This model consists of CS2 and resulted in a DA-value of 97.19%. The compressibility for this model at CS2 was 4% lateral, 2% dorsal and 18% ventral. These compression rates can be related to the fact that the dorsal area has a bony structure because of the spine and skull, towards the lateral area tissue starts to lose stiffness and at the ventral area the abdominal tissue is soft.

## CONCLUSION

The prediction of the size selection for trawl codends in different mesh size and shapes can be easily done using the design guides for guidance and, hence, can be a useful tool for sustainable fisheries. In this study, the FISHSELECT methodology was used with only one species to obtain cross section data defining the striped red mullet's ability to penetrate different mesh configuration and sizes. This methodology can also be used to predict the selectivity for other species relatively easily and quickly at a low cost.

Like other FISHSELECT studies, this study shows that selective studies conducted with laboratory and computer experiments give reliable results. It is suggested that further studies on similar selectivity methods should be conducted on the same species or different species and should be compared and interpreted with these and previous studies.

One of the most important problems is the pressure of overfishing of the fish stocks with by-catch. Useful technical measures should be found, such as improving codend selectivity for sustainable fisheries. The FISHSELECT methodology is of crucial importance in order to provide solutions by predicting the size selectivity for different species versus different mesh size and mesh configuration in the mixed bottom trawl Mediterranean fishery.

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## Sensorial Evaluation of Fish Croquettes Produced from Different Seafood

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### ABSTRACT

This study evaluated the sensorial characteristics of fish croquettes produced from different seafood such as deep-water rose shrimp (*Parapenaeus longirostris*), sardine (*Sardina pilchardus*), and rainbow trout (*Oncorhynchus mykiss*). First, a sensorial appreciation test and a quantitative descriptive analysis (QDA) were conducted by 12 expert panelists. Then, a consumer appreciation test was carried out with 100 consumers aged between 20 and 55 years. The appreciation tests showed that all the croquettes were appreciated by both the panelists and the consumers. The shrimp croquette was found to be the overall favorite in terms of flavor, odor, and general appreciation parameters among the groups. The QDA results revealed that the shrimp and sardine croquettes maintained their characteristic flavor and odor, whereas those of the rainbow trout croquettes were preserved less compared with other croquettes. These results indicate that sardine croquettes can be recommended to consumers who enjoy consuming seafood with a strong fish aroma. Trout and shrimp croquettes can be recommended to some consumers who wish to consume seafood but do not necessarily like the taste of fish or shellfish.

**Keywords:** Deep-water rose shrimp, sardine, rainbow trout, quantitative descriptive analysis, croquette, deep-fried.

### INTRODUCTION

Fish and seafood are important sources of protein which provide 40% of the world's protein intake (Diaz and Hu, 2009) in addition to essential micronutrients which have various health benefits (Budtz-jorgensen et al., 2013). Moreover, they are rich in amino acids (Özden and Erkan, 2008) and unsaturated fatty acids especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Krzynowek and Murphy, 1987). Thus, the global fish supply obtained from both aquaculture (Kobayashi et al., 2015) and captured fish (Rickertsen et al., 2016) is increasing day by day. This increase of seafood consumption is likely to lead to improved health (Lund, 2013). Along with this trend, people want to consume new aquatic species or new seafood products (European Commission, 2017) which can be processed in various ways

such as fresh, frozen, dried, marinated and coated products (croquettes) (Tveteras et al., 2012). Among these processing technologies, the coating technique is one of the leading methods of producing new seafood products. Coating technology can be used to produce food products using non-economic fish or by-catch fish species (Yean, 1998) as well as aquatic species with a high economic value like shrimp and scallop (Çaklı, 2007). Fish croquettes are a type of small breaded food produced by mixing minced fish meat and various ingredients (Barros Fuchs et al., 2013) and are usually consumed after being deep-fried (Stastny et al., 2014). The deep-frying is one of the methods of frying most favored by consumers (Pokorny, 1987) and adds desirable flavor and textural properties to food (Holownia et al., 2000). Therefore, the evaluation of sensorial properties of fish croquettes is important.

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One of the most important aims of the food industry is understanding consumer's senses and acceptance of foods (Mani-Lopez et al., 2018). There is an increase in the number of consumers who will not compromise on flavor and appearance, in addition to food quality. In the competitive food production sector, not only is it imperative to ensure products pass the required food safety checks but it is also vital that products obtain the approval of consumers. In view of this, consumer attitudes are becoming increasingly important both in terms of nutritional and sensorial aspects. The aim of this study is to provide an insight into the preferences of consumers due to sensorial characteristics of croquettes produced from different seafood sources, each having a distinct and diverse aroma, which are easily accessible and address different budgets. Thus, the sensorial characteristics of fish croquettes obtained from deep-water rose shrimp (*Parapenaeus longirostris*), sardine (*Sardina pilchardus*) and rainbow trout (*Oncorhynchus mykiss*) were evaluated in this paper.

## MATERIAL AND METHOD

### Main materials

In this study, deep-water rose shrimp (*Parapenaeus longirostris*), sardine (*Sardina pilchardus*) and rainbow trout (*Oncorhynchus mykiss*) were used for croquette production. Deep-water rose shrimp and sardines caught in the Northern Aegean Sea were purchased from Çanakkale fish market. The length of all individuals were above the minimum legal size in compliance with Turkish fishing legislation. Rainbow trout were obtained from a freshwater aquaculture facility operated in Mount Ida Çanakkale, Turkey. In the production of the croquettes, 15 kg of each species were used.

### Supplemental Materials

The frying oil preferred was sunflower oil and main ingredients like wheat flour, corn flour, bread crumbs, wheat starch and ingredients such as dried coconut, dried onion, dried garlic, cilantro, black pepper, white pepper and salt that were used in the production of the croquettes were obtained from the local marketplace.

### Croquette Production

Croquettes were produced in previous studies carried out by Çankırılıgıl and Berik (2017a, 2017b, 2017c). According to these studies; croquette formulations consisted of 75.50% meat, 9.70% wheat flour, 1.31% corn flour, 9.70% breadcrumbs, 0.68% wheat starch, 1.78% dried coconut, 0.43% dried onion, 0.20% dried garlic, 0.13% cilantro, 0.28% black pepper, 0.23% white pepper and 1.00% salt. Initially, shrimp, pilchard and trout were eviscerated to remove internal organs and bones. Afterwards, the meat was pre-cooked at 150°C for 5 minutes in an oven and then minced with a grinder. The aforementioned ingredients were then added to the minced meat and mixed together until they become a homogeneous croquette paste. To form the croquette, this paste was shaped by hand, sliced evenly and coated with both a liquid soda-based material consist of 70% egg white, 12% carbonate, 8.50% breadcrumbs, 5.0% wheat starch, 1.20% salt and a dry coating material containing breadcrumbs, wheat flour and corn flour. The croquettes were deep-fried with sunflower oil at 180°C for 2 minutes which is the most preferred cooking method for

croquettes (Kilincceker and Hepsag, 2011). In the final stage of croquette production; 10.40, 10.51 and 10.94 g croquettes were produced from 15 kg of shrimp, sardine and trout, respectively. The loss in weight caused by the removal of undesirable parts such as the head, fins, bones, skin or shells from each species, was recovered in a ratio of 25% by adding the ingredients above.

### Sensorial Analyses

A sensorial analysis was carried out using the fried croquettes. In the analyses, 50 gram of fried croquettes from each group was evaluated by both panelists and consumers. The panelist group consists of expert panelists (6 men and 6 women) between the ages of 25 and 45. In order to determine the consumer group, volunteers from students and personnel of Çanakkale Onsekiz Mart University were listed with their name, age and gender. 100 consumers were selected from the list above to create an equally distributed and homogeneous group in terms of gender and age groups. Thus, the consumer group was formed with 50 men and 50 women between the ages of 18 and 55. Once the groups were selected, the sensory appreciation test, consumer sensory analysis and quantitative descriptive analysis were conducted on the fried croquettes.

### Sensory Appreciation Test

The Sensory appreciation test was modified from Mason and Nottingham (2002) and conducted on deep-fried croquettes by 12 expert panelists. The parameters of flavor odor, texture, appearance and general appreciation were evaluated by the panelists using a point scale ranging from 1 to 9. The descriptive terms and sensory appreciation test form are shown in Table 1 and Figure 1, respectively.

### Consumer Sensory Analysis

With the aim of assessing the market potential of the fish croquettes, a quantitative sensory test was carried out with the participation of 100 consumers (Mason and Nottingham, 2002). Flavor, odor and general appreciation parameters were evaluated by the consumer test group using a short and easy-to-understand form with five-point hedonic appreciation categories. The data obtained was expressed as mean values. The consumer sensory analysis test form is shown in the Figure 2.

### Quantitative Descriptive Analysis

In the quantitative descriptive analysis (QDA), the differences between the deep-fried croquettes were described by the same 12 expert panelists who carried out sensory appreciation test. The QDA was modified from Mason and Nottingham (2002) and the descriptive terms used for the fish croquettes were modified from Hayes (2011) and Shaviklo et al. (2010). The descriptive parameters of fish flavor, fish odor, saltiness, spiciness, color, hardness, oiliness and frying were evaluated by the panelists using a point scale ranging from 1 to 9. Results obtained were expressed in spider diagrams drawn in Excel (Office 2010; Microsoft, U.S.A.). The descriptive terms and QDA test form are shown in Table 1 and Figure 3, respectively.

### Statistical Analyses

Differences between the mean values were evaluated with data obtained from the 12 expert panelists who had participated in the sensory appreciation test and quantitative descriptive analysis.

**Table 1.** Descriptive terms used for fish croquettes modified from Hayes (2011) and Shaviklo et al. (2010)

Descriptor	Definition
<i>Sensory appreciation test</i>	
Flavor	General flavor of deep-fried croquettes
Odor	General odor of deep-fried croquettes
Texture	Structure of croquette felt with fingers or teeth
Appearance	Color and general look of deep-fried croquettes
General appreciation	General acceptance considering all parameters
<i>Quantitative descriptive analysis</i>	
Fish flavor	Flavor of selected species like shrimp, sardine or trout
Fish odor	Odor of selected species like shrimp, sardine or trout
Saltiness	Density of salt
Spiciness	Aromatics associated with spices or other ingredients
Color	Outside color of croquettes from light to dark
Hardness	Force required to compress the sample in first bite
Oiliness	Absorbed oil associated with frying oil
Frying	Frying rate of croquettes

	0	1	2	3	4	5	6	7	8	9	Point scale	
Flavor											Like extremely	9
Odor											Like very much	8
Texture											Like moderately	7
Appearance											Like slightly	6
General approval											Neither like nor dislike	5
											Dislike slightly	4
Name:											Dislike moderately	3
Date:											Dislike very much	2
											Dislike extremely	1

**Figure 1.** Evaluation form of sensory appreciation test

Parameters	1	2	3	4	5	6	7	8	9	Point scale	
Fish flavor										Extreme	9
Fish odor										Very strong	8
Saltiness										Strong	7
Spiciness										Above-moderate	6
Color										Moderate	5
Hardness										Below-moderate	4
Oiliness										Slightly	3
Frying										Very slightly	2
Name:										Absent	1
Date:											

**Figure 3.** Evaluation form of quantitative descriptive analysis (QDA)

Name:	Date:	Flavor	Odor	General

**Figure 2.** Evaluation form of consumer sensory analysis

However, the mean values of the consumer sensory analysis with a total of 100 participants were evaluated, separately according to age and gender groups, with the aim of evaluating possible differences between age or gender. Participants were divided into 7 categories representing the age ranges of 18-25, 25-30, 30-35, 35-40, 40-45, 45-50 and 50-55. SPSS Statistics package program version 21.0 (SPSS 21; IBM, U.S.A.) was used in all statistical analyses. The results were expressed as mean±standard

error. Differences between the mean values were evaluated with Tukey's multiple comparison test using a one-way variance analysis (ANOVA) after the normality and homogeneity of the data were tested with Anderson-Darling and Levene tests, respectively. The level of significance was set as 0.05.

## RESULT AND DISCUSSION

According to the results of the sensory appreciation test all deep-fried croquettes were liked by consumer groups. The shrimp croquettes were determined as the most preferred group with 8.44±0.30 points by the panelists. Moreover, the highest flavor and odor values were found to be 8.33±0.41 and 8.11±0.45, respectively in shrimp croquettes. The panelists stated that additions like onion, garlic and pepper into the croquette paste improved the flavor and odor of the products, particularly in shrimp croquettes. Thus, shrimp croquettes received the highest flavor and odor values. Several studies have shown that shrimp species are both nutritional and delicious (Nasiri et al., 2012) and they are



an aquatic food source in high demand by consumers (Fan et al., 2017; Khan et al., 2013). In addition, Yanar and Fenercioğlu (1999) stated that the addition of onion and garlic affected the flavor of coated products, positively. Texture is one of the most important attributes that indicates quality in fish and fish products (Cheng et al., 2014). Fried fish croquettes have a crisp texture on the outside and a soft texture on the inside, similar to other battered and fried foods. This characteristic texture is formed with a combination of high temperature, oil uptake and water loss due to deep-frying (Soto-Jover et al., 2016). In this research, the texture characteristics of all fish groups were liked by the panelists and the highest texture value was specified in sardine croquettes as  $8.33 \pm 0.41$ . The appearance value was detected as highest in trout croquettes as  $8.56 \pm 0.42$ . The color of trout meat was found to be lighter than other materials. Fish with white meat such as whiting, Alaskan Pollock etc. are preferred mostly in production of croquettes and surimi products (Boran and Köse, 2007). It is thought that trout croquettes have got the highest appearance points because of their similarity to fish croquettes sold commercially in the market. The results of the sensorial appreciation test are shown in Table 2.

According to the consumer sensory analysis, shrimp croquettes were found to be the most popular in terms of both flavor and odor parameters, followed by trout and then sardine croquettes. When the irresolute consumers are not distributed, 70% of consumers liked shrimp croquettes, 62% of them liked sardine croquettes and 63% of them liked trout croquettes. Among consumers 19%, 23% and 19% of all did not like shrimp, sardine and trout croquettes, respectively. A significant difference was not detected for either sex or age groups statistically. The results of the consumer sensory analysis are shown in Table 3.

**Table 2.** Results of sensory appreciation test

Parameters	Shrimp	Sardine	Trout
Flavor	$8.33 \pm 0.41^a$	$7.56 \pm 0.42^c$	$8.00 \pm 0.41^b$
Odor	$8.11 \pm 0.45^a$	$7.22 \pm 0.56^b$	$8.00 \pm 0.29^a$
Texture	$7.89 \pm 0.45^b$	$8.33 \pm 0.41^a$	$7.44 \pm 0.51^c$
Appearance	$7.56 \pm 0.42^b$	$7.44 \pm 0.51^b$	$8.56 \pm 0.42^a$
General approval	$8.44 \pm 0.30^a$	$7.22 \pm 0.39^c$	$7.89 \pm 0.35^b$

Values are expressed as mean  $\pm$ SD, mean values in rows with different superscripts were significantly different ( $p \leq 0.05$ )

The aim of the quantitative descriptive analysis was to evaluate sensorial differences between products. Çaklı (2007) stated that fish and other seafood can reach out to larger masses by enhancing the flavor, odor and texture, as is done in croquette production technology. Therefore, the differences between croquettes in terms of sensorial quality owing to unique aromas of raw materials and the description of final products are important. In our research, the parameters of spiciness, saltiness and frying were found to be the same, statistically, in all croquettes because of the implementation of exact formulation to each group ( $p \geq 0.05$ ). The highest flavor and odor values of the species used in the croquette production were specified in sardine croquettes whereas the lowest values were determined in shrimp croquettes ( $p \leq 0.05$ ). Coated products have specific flavor components which come from the breading, frying oil and raw materials used in production (Albert et al., 2012). Deep-fat frying can produce desirable or undesirable flavor compounds that can affect final products (Choe and Min, 2007). It is clearly shown that deep-frying affects croquettes positively with medial frying rate as well as non-dominant aromas of spices or salt. The darker outer-color was specified in sardine croquettes due to the color of sardine meat and followed by shrimp and trout croquettes, respectively ( $p \leq 0.05$ ). The color parameter showed distinct differences between croquettes due to the specific meat color of each of the species used in the production of the croquettes. The inner color of sardine croquettes was found to be darker compared to that of shrimp and trout croquettes. In addition, the unique rosy color of shrimp meat was preserved in croquettes. Oiliness was found to be highest in sardine croquettes ( $P \leq 0.05$ ) whereas no differences were found statistically between the shrimp and trout croquettes ( $p \geq 0.05$ ). Several sardine species are rich in terms of crude fat and unsaturated fatty acids (Homayooni et al., 2014; Sánchez et al., 2013; Senapati et al., 2017). Thus, this can explain why the oiliness of sardine croquettes was deemed the highest. One of the results of deep-frying is gaining a distinctive structure of food (Stevenson et al., 1984). Even if just by a slight amount, hardness was found to be higher in sardine croquettes than the others statistically ( $p \leq 0.05$ ). As a result of deep-frying, food gains a crispy exterior crust and moist center by virtue of water evaporation and oil migration between the frying oil and the food (Nieto Salvador, 2014). According to Albert et al. (2012) this contrasting texture is an important and desirable feature for consumers. The results of the quantitative descriptive analysis are shown in Table 4 and Figure 4, respectively. According to the opinions of the panelists,

**Table 3.** Results of consumer sensory analysis

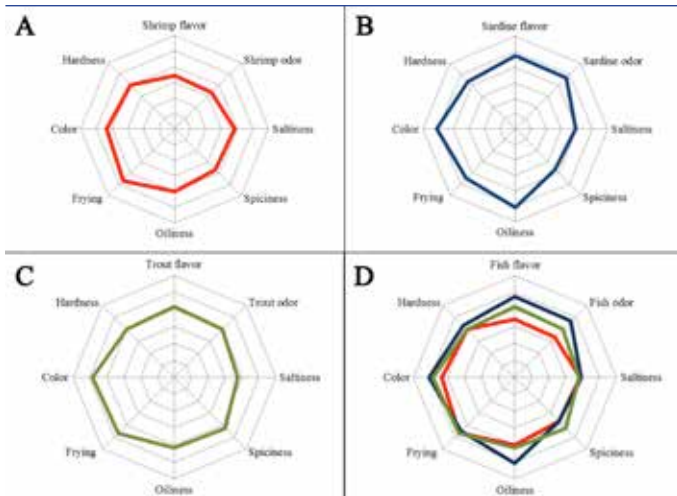
Parameters	Shrimp			Sardine			Trout		
	F	O	G	F	O	G	F	O	G
Like very much	41%	35%	39%	27%	19%	23%	32%	24%	29%
Like moderately	24%	28%	31%	35%	28%	32%	33%	36%	34%
Neither like nor dislike	16%	19%	17%	16%	28%	22%	14%	27%	18%
Dislike moderately	11%	10%	9%	12%	16%	14%	9%	7%	8%
Dislike very much	8%	7%	4%	10%	9%	9%	12%	6%	11%

\*F: flavor, O: odor, G: general

**Table 4.** Results of quantitative descriptive analysis (QDA)

Parameters	Shrimp	Sardine	Rainbow trout
Fish flavor	4.41±0.21 <sup>c</sup>	5.75±0.42 <sup>a</sup>	5.17±0.33 <sup>b</sup>
Fish odor	4.33±0.42 <sup>c</sup>	5.67±0.28 <sup>a</sup>	5.00±0.58 <sup>b</sup>
Saltiness	4.83±0.17 <sup>a</sup>	4.91±0.20 <sup>a</sup>	4.83±0.23 <sup>a</sup>
Spiciness	4.66±0.21 <sup>a</sup>	4.67±0.31 <sup>a</sup>	5.25±0.42 <sup>a</sup>
Color	5.33±0.27 <sup>c</sup>	6.08±0.54 <sup>a</sup>	5.83±0.45 <sup>b</sup>
Hardness	5.00±0.16 <sup>b</sup>	5.33±0.24 <sup>a</sup>	5.00±0.31 <sup>b</sup>
Oiliness	5.00±0.17 <sup>b</sup>	6.08±0.23 <sup>a</sup>	5.17±0.33 <sup>b</sup>
Frying	5.66±0.42 <sup>a</sup>	5.50±0.56 <sup>a</sup>	5.67±0.25 <sup>a</sup>

Values are expressed as mean ±SD, mean values in rows with different superscripts were significantly different (p<0.05)



**Figure 4. a-d.** Quantitative Sensory Analysis (QAD) results of croquettes; (a) shrimp croquettes, (b) sardine croquettes, (c) trout croquettes, (d) combined results of all croquettes

sardine croquettes preserved the characteristics of the raw material such as fish flavor, odor, color and oiliness used in croquette production more than others. Shrimp and trout croquettes can be recommended to consumers who need to consume seafood but do not particularly like the taste of fish or shellfish. However, sardine croquettes, which have dominant fish aroma, can be recommended as a new fishery products to consumers who enjoy eating seafood. Moreover, according to the European Commission (2017), 65% of European consumers want to try new fish and fishery products.

## CONCLUSION

In conclusion, fish croquettes produced from different aquatic species such as deep-water rose shrimp, sardine and rainbow trout were evaluated in terms of their sensorial quality. According to the results, all products were evaluated by both panelists and consumers. Shrimp croquettes received the highest approval by both consumers and panelists with their exquisite flavor. A number of different seafood products have been cho-

sen as raw material. It is necessary to use sustainable and easily accessible food sources such as fish species from healthy stocks, aquaculture species, discard species of commercial fishing operations or edible waste of seafood processing facilities for substantial croquette production. The results of the present comparative study would be beneficial for the seafood production sector.

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## Kahverengi Alabalık (*Salmo trutta fario*) Hematoloji Parametreleri Üzerine Anyonik Yüzey Aktif Maddelerin Etkileri

### *Effects of Anionic Surfactant Ingredients on Hematological Index of the Brown Trout (Salmo trutta fario)*

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#### ÖZ

Bu çalışmanın amacı, sucul canlılar için sodyum dodesil sülfatın (SDS) toksisitesini belirlemektir. Kahverengi alabalıklarda (*Salmo trutta fario*) SDS'nin etkileri hematolojik indekslerle araştırılmıştır. Bu amaçla balıklar 21 gün süreyle kontrol (0 mg/l), düşük doz (1,5 mg/l) ve yüksek doz (2,25 mg/l) SDS'ye maruz bırakılmıştır. Uygulama süresi sonunda kontrol ve deneme gruplarında hematolojik indeksler [eritrosit sayısı (RBC), lökosit sayısı (WBC), hemoglobin değeri (Hb), hematokrit oranı (Hct), trombosit sayısı (PLT), eritrosit çökme oranı (ESR), eritrosit başına düşen hemoglobin sayısı (MCHC), eritrosit miktarı hemoglobin (MCH) ve ortalama eritrosit hacmi (MCV)] araştırılmıştır. Araştırma sonuçları, yüksek dozda SDS konsantrasyonuna maruz bırakılan grubun RBC, WBC, PLT, Hb, ESR, MCV, MCH ve MCHC indeks değerlerinin düşük doz ve kontrol gruplarına göre önemli derecede arttığını ( $p<0,05$ ) göstermiştir. Düşük konsantrasyona maruz bırakılan grupta ise, Hct değeri diğer uygulama gruplarına göre daha yüksek bulunmuştur ( $p<0,05$ ).

**Anahtar Kelimeler:** Hematoloji, balık, deterjan, kirlilik

#### ABSTRACT

The aim of this study was to determine the toxicity of sodium dodecyl sulfate (SDS) to aquatic organisms. The effects of SDS were investigated using the hematological index of the brown trout (*Salmo trutta fario*). Fish were exposed to control (0 mg/l), low dose (1.5 mg/l) and high dose (2.25 mg/l) of SDS over a 21-day period. At the end of the treatment period, the control and the treatment groups were investigated for the hematological index [total erythrocyte count (RBC), total leukocyte count (WBC), hemoglobin (Hb), hematocrit (Hct), total platelet count (PLT), erythrocyte sedimentation rate (ESR), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), and mean cell volume (MCV)]. The results showed a significant increase in RBC, WBC, PLT, Hb, ESR, MCV, MCH, and MCHC values of the group exposed to high SDS concentrations compared to those in the low-dose treatment and control ( $p<0.05$ ) groups. At low SDS concentrations, the Hct value was significantly higher than that in the other treatment groups ( $p<0.05$ ).

**Keywords:** Hematology, fish, detergent, pollution

#### GİRİŞ

Deterjanlar; yüzey aktif bileşikler sınıfına giren ve temizlik amacı ile kullanılan kimyasal maddelerdir. Kullanımları giderek artan bu bileşikler anyonik, katyonik ve noniyonik olarak gruplandırılarak sadece evlerde değil aynı zamanda tekstil, kozmetik, medikal, metal, boya, deri, kâğıt ve lastik sanayilerinde kullanılmaktadır. Deterjan artıkları hemen hemen tüm dünyada yaygın bir

biçimde çevre kirliliği sorunlarına dahil edilmelerinden dolayı büyük ilgi toplamaktadır (Yeğin ve Uçar, 2017; Esenbuğa ve ark., 2017). Özellikle günümüzde, evsel ve endüstriyel atıklardan sucul ortama gelen deterjanlar oldukça önemli olup boşaltıldıkları alıcı ortamlarda bir takım olumsuzluklara (biyolojik ayrışma sonucu oksijen tüketimi, köpük oluşturma, sudaki canlılar üzerine olumsuz etkileri, içme sularına etkileri ve ötrofikasyon) sebep olmaktadır (Mineraci ve

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ark., 2009). Balık, akuatik ortamda neredeyse her yerde bulunur ve ekosistemde önemli bir rol oynamaktadır (Gaber and El-Kasheif, 2013). Bu nedenle, balıktaki hematolojik ve biyokimyasal parametrelerin analizi hayvan sağlığı ve ekolojik koşulların değerlendirilmesine katkı sağlamaktadır (Pimpão et al., 2007). Hematolojik parametrelerin (eritrosit sayısı (RBC), lökosit sayısı (WBC), hemoglobin değeri (Hb), hematokrit oranı (Hct), trombosit sayısı (PLT), eritrosit çökme oranı (ESR), ortalama eritrosit hacmi (MCV), eritrosit başına düşen ortalama hemoglobin (MCH) ve eritrosit başına düşen ortalama hemoglobin konsantrasyonunun (MCHC)) incelenmesiyle her türlü sosyal, fizyolojik ve çevresel faktörün (sosyal hiyerarşi, hastalık, beslenme yetersizliği, toksik madde, su kalitesindeki değişimler, sıcaklık, fotoperyot, yoğunluk, tuzluluk, pH, oksijen, ağır metaller, pestisitler, deterjanlar gibi) balık sağlığı ve fiziksel durumuna olan etkisi belirlenebilmektedir (Yeğin ve Uçar, 2017; Parlak, 2016). Hayvanlarda kan parametrelerinin değerlendirilmesi önemli bir marker ve yaygın bir yöntemdir. Bu teknik ile hayvanın fizyolojik durumu ve bulunduğu ortam şartlarının belirlenmesinde güvenilir kararlar verebilmek mümkün olmaktadır (Yeğin ve Uçar, 2017).

Bu çalışma, kullanımı giderek artan deterjanların akuatik canlılardan olan balıklar üzerinde oluşturduğu etkilerin belirlenmesi, kahverengi alabalık (*Salmo trutta fario*) kan parametrelerinin kullanılarak toksisitesinin değerlendirilmesi amacıyla planlanmış ve yürütülmüştür.

## MATERYAL VE METOT

Denemede Atatürk Üniversitesi Su Ürünleri Fakültesi İç Su Balıkları Uygulama ve Araştırma Merkezinden temin edilen 30 adet, iki yaşlı, 165±25 g ortalama ağırlığa sahip kahverengi alabalıklar (*Salmo trutta fario*) kullanılmıştır. Balıklar 1 m çap ve 1 m derinliğe sahip, su tahliyesi eçik boru sistemiyle yapılan fiberglass tanklarda tutularak, sodyum dodesil sülfatın (SDS) kontrol (0 mg/L), düşük doz (1,5 mg/L) ve yüksek dozlarına (2,25 mg/L) 21 gün süreyle maruz bırakılmışlardır. Sodyum dodesil sülfat ticari bir firmadan (Sigma) temin edilmiştir. Araştırma süresince yapılan analizler Atatürk Üniversitesi Su Ürünleri Fakültesi İç Su Balıkları Uygulama ve Araştırma Merkezi, Akvaryum Balıkları Uygulama ve Araştırma Merkezinde bulunan Toksikoloji Deneme Ünitesi ve Su Ürünleri Fakültesi Laboratuvarlarında

yapılmıştır. Araştırma başlangıcında Atatürk Üniversitesi Hayvan Deneyleri Yerel Etik Kuruludan (HADYEK) çalışmasının yürütülmesinin etik kurallarına uygun olduğuna dair Etik Kurul Onayı alınmıştır.

Balıkların kaudal venalarından girilmek suretiyle alınan kan örnekleri kullanılarak yapılan hemoglobin tayininde Cyanmethemoglobin yöntemi, hematokrit tayininde mikrohematokrit metodu, eritrosit, lökosit ve trombosit seviyelerinin tespitinde ise Dacie's solüsyonu ile boyama yapılarak thoma lamı üzerinden mikroskopta belirlenen alanlarda sayımlar yapılmıştır. Bu sayım yöntemi ile elde edilen veriler aşağıda verilen formüllerde kullanılarak diğer indeks (ortalama eritrosit hacmi (MCV), eritrosit miktarı hemoglobin (MCH) ve eritrosit başına düşen hemoglobin sayısı (MCHC)) değerleri hesaplanmıştır (Blaxhall and Daisley, 1973; Atamanalp, 2000; Uçar, 2010; Alak ve ark., 2012).

$$MCV(fl) = Hct (\%) * 10/RBC (\text{million}/\text{mm}^3)$$

$$MCH(pg) = Hgb (\text{gm}/\text{dL}) * 10/RBC (\text{million}/\text{mm}^3)$$

$$MCHC (\%) = Hgb (\text{gm}/\text{dL}) * 100/Hct (\%)$$

Araştırmadan elde edilen veriler SPSS (Statistical Package for the Social Sciences) paket programı kullanılarak varyans analizine tabi tutulmuş ve ortalamalara alfa 0,05 seviyesinde Duncan testi uygulanmıştır (Alak ve ark., 2012; Javed et al., 2016).

## BULGULAR VE TARTIŞMA

Hematoloji indeksleri açısından gruplar arası fark istatistiki olarak önemli bulunmuştur ( $p < 0,05$ ). Çalışılan parametreler açısından artış ve azalışlar kaydedilmekle birlikte deterjan uygulanan gruplarda hemoglobin değeri (Hb), eritrosit çökme oranı (ESR) ve eritrosit miktarı hemoglobin (MCH) arasında istatistiki olarak fark gözlemlenmemiştir (Tablo 1). Yüksek konsantrasyon uygulamasına maruz kalan grubun eritrosit sayısı (RBC), lökosit sayısı (WBC), trombosit sayısı (PLT), hemoglobin değeri (Hb), eritrosit çökme oranı (ESR), eritrosit başına düşen hemoglobin sayısı (MCHC), eritrosit miktarı hemoglobin (MCH) ve ortalama eritrosit hacmi (MCV) indeksleri düşük doz uygulaması ve kontrole kıyasla olduk-

**Tablo 1.** Farklı dozlardaki SDS'nin *Salmo trutta fario* hematolojik indekslerine etkisi

**Table 1.** Effect of different concentration of SDS on hematological index of *Salmo trutta fario*

Parametre/Grup	Kontrol	1,5 mg/Lt SDS	2,25 mg/Lt SDS
RBC ( $10^6/\text{mm}^3$ )	0,81±0,18 <sup>b</sup>	0,81±0,20 <sup>b</sup>	0,90±0,33 <sup>a</sup>
WBC ( $10^4/\text{mm}^3$ )	2,60±0,16 <sup>b</sup>	0,95±0,71 <sup>c</sup>	4,52±3,33 <sup>a</sup>
PLT ( $10^4/\text{mm}^3$ )	1,65±0,42 <sup>b</sup>	0,70±0,25 <sup>c</sup>	4,16±6,47 <sup>a</sup>
Hb (g/dl)	6,35±0,12 <sup>b</sup>	11,65±4,11 <sup>a</sup>	12,00±4,78 <sup>a</sup>
ESR (mm/h)	2,46±0,34 <sup>a</sup>	0,63±0,74 <sup>b</sup>	0,66±0,52 <sup>b</sup>
Htc (%)	25,25±1,5 <sup>b</sup>	33,43±24,71 <sup>a</sup>	24,50±18,39 <sup>b</sup>
MCV ( $\mu\text{m}^3$ )	321,29±69,84 <sup>c</sup>	591,07±50,40 <sup>a</sup>	414,26±144,63 <sup>b</sup>
MCH (pg)	99,78±14,63 <sup>b</sup>	148,30±64,71 <sup>a</sup>	146,30±64,24 <sup>a</sup>
MCHC (g/100ml)	31,30±0,06 <sup>b</sup>	24,73±8,84 <sup>c</sup>	39,69±28,99 <sup>a</sup>

Aynı satırda aynı harfle (a, b) gösterilen ortalamalar arasında fark yoktur ( $p < 0,05$ )  
Lowercase superscripts (a, b) indicate significant differences among same line within each experimental treatment group

ça yüksek belirlenmiştir ( $p < 0,05$ ). Düşük konsantrasyonlarında ise hematokrit oranı (Hct) diğer uygulama gruplarına oranla yüksek belirlenmiştir ( $p < 0,05$ ).

Sucul organizmaların strese tepki olarak verdiği primer yanıt fizyolojik etkisiyle, zincirleme bir şekilde sekonder yanıtlar oluşur. Sekonder yanıtlar ise histolojik, histopatolojik, biyokimyasal ve hematolojik parametrelerde meydana gelen değişikliklerle belirlenebilmektedir. Balıklarda stres sonrasında homeostaziyi sağlamak amacıyla, hematolojik, hormonal ve enerji metabolizmasını düzenleyen bazı fizyolojik değişiklikler açığa çıkmaktadır (Kayhan, 2009). Balıklarda strese bağlı fizyolojik değişikliklerin belirlenmesinde hematolojik parametreler, bir stres faktöründen kaynaklanan fizyolojik ve biyokimyasal değişikliklerin ölçülmesinde gösterge olarak yaygın bir şekilde kullanılmaktadır (Stoskopf, 1993; Cataldi et al., 1998; Adeyemo et al., 2003).

Yüksek eritrosit sayısı (RBC) ve hemoglobin değeri (Hb), hipoksi veya anoksi için ortak yanıtlardır. Yapılan bu çalışmada, deterjan uygulaması sonrası hemoglobin değerleri kontrol grubuna göre daha yüksek bulunmuş ve bu artışın kırmızı kan hücrelerindeki artışla ilişkili olduğu sonucuna varılmıştır. Bu durumda balık kanın oksijen taşıma kapasitesini arttırmak için RBC ve Hb'yi arttırarak bu stresi elemine etmeye çalışmıştır (Hedayati and Tarkhani, 2014). Eritrosit sayısı kan oksijen taşıma kapasitesini ve eritropoietik dokuların fonksiyonlarını belirlemek için önemli bir parametredir (Witeska, 2005). Eritrosit sayısındaki ani artışlarda, stres ve kan dolaşımına yeni eritrosit salınmasına bağlı olarak katekolamin kaynaklı dalak kontraksiyonlarının etkili olabileceği düşünülmektedir. Benzer şekilde eritrosit sayısındaki artış ya mukusla kaplanan solungaçtan kaynaklanan hipoksik koşulların bir sonucu olarak ya da eritrosit oluşumu üzerine deterjanların uyarıcı etkisi ile solungaç yapısında deformasyonlar ve dokuların oksijen ihtiyacının artmasına neden olabilmektedir. Hematopoietik dokularda kirlenici konsantrasyonu ve maruz kalma süresinin, balıkların eritrosit hücrelerinde değişikliğe neden olduğu bilinmektedir (Yeğin ve Uçar, 2017). Eritrosit sayısına bağlı olan ESR değerinin artış veya azalışı balıkta fizyolojik işlev bozukluğunu göstermektedir (Jagtap and Mali, 2012).

Doğan ve Can (2011), hematolojik parametrelerin farklı çevresel faktörlere ve kimyasallara karşı farklı duyarlılık seviyeleri gösterdiğini ve balıkların kirlenici kaynaklı stres ve hematolojik parametrelere oldukça duyarlı olduğunu belirtmiştir. Bu çalışmadan elde edilen sonuçlara göre lökosit değerleri için, deterjan uygulamasının farklı konsantrasyonlarından kaynaklanan değişiklikler bulunmaktadır. Lökosit hücrelerinin sayısı (WBC) fizyolojik ve çevresel faktörlerden etkilenmekte ve kirleniciye maruz kalan balıklarda görülen WBC ve diferansiyel lökosit sayısındaki değişim, kirlenicilerin immüno-modülasyonunu göstermektedir. Sucul organizmalarda ksenobiyotiklerin immünsupresyon etkiye sahip olduğu bilinmektedir (Heyedati and Tarkani, 2014). Balıklarda WBC'lerin immünolojik fonksiyon ve sayıları strese karşı koruyucu bir yanıt olarak artar. Benzer olarak solungaç hasarlarında da WBC düzeylerinin arttığı bilinmektedir (Saravanan et al., 2011). Araştırma bulgularımızda yüksek düzeydeki WBC değerleri hematopoietik uyarımı tetiklemiştir (Ullah et al., 2018).

Çalışmada, ortalama eritrosit hacmi (MCV)'inde gözlemlenen artışın, eritrositlerin şişmesine bağlı olarak makrositer bir sonuç oldu-

ğu ve anemiyi işaret ettiği gözlemlenmiştir. MCV'de ki artış, aynı zamanda hipoksik bir artış sonucu RBC'lerin şişmesinden veya strese maruz kalan balıklarda bozulmuş su dengesi (ozmotik stres) veya makrositer anemide kaynaklanmaktadır; bu durumda kandaki oksijene olan afiniteyi artırmaktadır (Harikrishnan et al., 2009). Bu çalışmada, eritrosit başına düşen hemoglobin sayısı (MCHC), çalışma süresi boyunca deterjan uygulanan balıklarda önemli ölçüde artmıştır, MCHC seviyesindeki bu değişimde Hb'deki artışın etkili olduğu düşünülmektedir (Saravanan et al., 2011). Bu çalışmada, deterjan uygulanmasının kırmızı hücrelerin büzülmesine neden olduğu (artmış MCHC) ve eritrosit miktarı hemoglobin(MCH) değerinin anlamlı bir şekilde arttığını göstermiştir ve yüksek MCHC ve MCH değerlerinin daha az hemoglobin içeriği olan büyük boyutlu RBC varlığını gösterdiği bildirilmiştir (Alwan et al., 2009; Kumar ve Banerjee, 2016). Çalışmamızda, deterjana maruz kalan balıkların MCV ve MCH seviyelerindeki artışta anemik bir durumun etkili olduğunu düşünmekteyiz. Benzer şekilde, gökkuşağı alabalığında yapılan bazı çalışmalarda yüksek MCV seviyesinin aneminin makrositer tipine bağlı olarak geliştiği kaydedilmiştir (Jayaprakash ve Shettu, 2013; Kumar ve Banerjee, 2016). Çalışma sonuçlarımıza paralel sonuçlar farklı kirlenicilerin farklı balık türlerinin hematolojik indeksi üzerindeki etkilerinin araştırıldığı çalışmalarda benzerlik göstermektedir (Sinha ve ark.2000; Devi ve Banerjee 2007; Ramesh and Saravanan, 2008; Alwan et al., 2009; Jahanbakhshi et al., 2015; Murussi et al., 2015; Southamani et al., 2015).

Balıklarda stres reaksiyonu, ozmotik dengesizliğe ve iyonik değişiklik düzenleyici sistemlerde etkili olup kan pH'sında düşüş, eritrosit hacminde artış ve bunun sonucunda da hematokrit yüzdesinde artışa neden olur (Saravanan et al., 2011). Elde edilen hematokrit bulguları bu durumu destekler nitelikte ve kontrole oranla yüksektir. Kumar et al., (1999) kimyasalların, enerji metabolizması ve hematolojik özelliklerde özel etkilerinin olduğunu ve balığın genel fizyolojik profilini etkilediğini ifade etmişlerdir. Kontrol grubu ile karşılaştırıldığında, trombosit sayısı yüksek doz deterjan uygulanan gruplarda artmıştır. Stres koşulları altında balıkların kan pıhtılaşma sistemi daha aktif hale gelir ve bu nedenle trombosit sayısı önemli ölçüde artabilir (Casillas and Smith, 1977). Trombositlerin en tanınmış fizyolojik rolü hemostaz sürecinde kan pıhtılaşmasını başlatmaktır (Engelmann, 2012). Balıklarda trombosit hücreleri koruma duvarları oluşturur, fagositik özelliklere sahip olur ve savunma mekanizmasına katılır. Bu hücreler, doğuştan edinilmiş immünite ile bağışıklık fonksiyonlarını da içeren intraselüler ve hücre dışı molekülleri ifade etme bağlantısını temsil eder (Yeğin ve Uçar, 2017). Stres koşullarında, kan koagülasyon sistemi daha aktif hale gelir ve bu nedenle trombosit sayısında artışa neden olabilir. Balık üzerinde trombositopeninin olumsuz bir etkisi olabilir, çünkü bu hücreler yalnızca kan koagülasyonundan sorumlu olmayıp aynı zamanda yüzeysel yaralar ve kan akışının da kontrolünde rol oynamaktadır (Campbell, 2007).

## SONUÇ

Bu çalışmada, uygulanan deterjan konsantrasyonları kahverengi alabalıklarda hematolojik değişimlere neden olmuştur. Bu çalışmanın sonuçları, sodium dodecyl sulfate varlığının sudaki çok düşük konsantrasyonlarının bile, suda yaşayan organizmalar üzerinde zararlı olumsuz etkilere neden olduğu sonucuna varılmıştır. Söz konusu kirleniciler için hematolojik indekslerin farklı zaman aralıkları ve farklı sucul canlılarla araştırılmasının yararlı olacağı düşünülmektedir.

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## Türkiye'ye İthal Edilen İki Tatlısu Akvaryum Balığı Türünde Saptanan Digenetik *Centrocestus Sp. Metaserkeri* (Trematoda: Heterophyidae): Hastalık Profili ve Risk

### *Digenetic Centrocestus Sp. Metacercariae (Trematoda: Heterophyidae) Were Detected on Two Freshwater Ornamental Fish Species Imported Into Turkey: Disease Profile and Risk*

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#### ÖZ

Akvaryum balıklarının ticareti ile ülkeler arası patojen transferi, günümüzde en önemli patojen yayılım risklerinden birisidir. Akvaryum balığı ticareti ile Türkiye'ye giren parazit tehlikesini irdelemek üzere Türkiye'ye ithal edilen 50 altın balık (*Carassius auratus auratus*) ve 10 dişi kılıçkuyruk balığı (*Xiphophorus hellerii*) Aralık 2015 ve Ekim 2016 tarihleri arasında paraziter olarak incelenmiştir. *Centrocestus sp.* metaserkerleri incelenen balıkların sadece solungaçlarında bulunmuştur. Altın balıklarda *Centrocestus sp.* metaserkerlerinin enfeksiyon oranı %34, dişi kılıçkuyruk balıklarında %30'dur. Ortalama yoğunlukları ise sırasıyla  $10,58 \pm 2,38$  ve  $14,3 \pm 13,3$ 'dür. Bu, dişi kılıç kuyruk (*X. hellerii*) balıklarında *Centrocestus sp.* metaserkeri enfeksiyonunun ilk raporudur. Bu çerçevede akvaryum balığı ithali yoluyla Türkiye'ye patojen girişinin mevcut olduğu açıktır ve kendi sucul türlerimizi koruyabilmemiz için gerekli önlemler acilen alınmalıdır.

**Anahtar Kelimeler:** Parazit, metaserker, ithal, akvaryum balığı, *Centrocestus sp.*

#### ABSTRACT

Ornamental fish trade is one of the reasons for pathogen transfer among different countries. To underline the threat of exported parasite risks, 50 specimens of goldfish (*Carassius auratus auratus*) and 10 specimens of the female swordtail (*Xiphophorus hellerii*) imported into Turkey between December 2015 and October 2016 were examined for the presence of parasites. *Metacercariae* of encysted *Centrocestus sp.* were found only on the gills of the examined fish. The prevalence rates of *Centrocestus sp. metacercariae* were 34% on goldfish and 30% on the female swordtail. The mean intensities of infection were  $10.58 \pm 2.38$  and  $14.3 \pm 13.3$ , respectively. To our knowledge, this is the first report of infection with *metacercariae* of *Centrocestus sp.* in the female swordtail (*X. hellerii*). These results clearly emphasize that pathogen entrance into Turkey occurs through ornamental fish trade and urgent measures must be implemented to conserve our own aquatic species.

**Keywords:** Parasite, metacercariae, import, ornamental fish, *Centrocestus sp.*

#### GİRİŞ

Uluslararası akvaryum balığı ticareti tüm dünyada önemli bir sektördür ve ihracatçı ülkelerin başında Güneydoğu Asya ülkeleri gelmektedir (Evans ve Lester, 2001; Türkmen ve Alpaz, 2001; Kim ve ark., 2002; Whittington ve Chong, 2007). Parazitlerin çoğu enfekte balıkların ticari olarak taşınmaları ile yayılmakta ve bu yolla dünyanın farklı ve yeni bölgelerine giriş yapmaktadır (Velez-Hernández ve ark., 1998; Evans ve Lester, 2001). Enfekte balıkların ülkelerarası

ticaretiyle taşınan parazitler de bu ülkelerin faunası için potansiyel olarak risk oluşturmaktadır (Velez-Hernández ve ark., 1998; Evans ve Lester, 2001). Son yıllarda yapılan çalışmalar, ithal edilen balıklarla çok sayıda ülkelerarası patojen hareketinin olduğunu ve ekolojik riskler kapsamında egzotik organizmaların ithali ile bölgelerarası patojen transferinin giderek daha da önem kazandığını göstermektedir (Mood ve ark., 2010; Pinto ve Melo, 2012; Mehrdana ve ark., 2014; Krailas ve ark., 2016; Yousif ve ark., 2016). Akvaryum balıklarının da içinde bulundu-

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ğu sucul organizmaların taşınımı ile gerçekleşen patojen transferinin sürekli olarak takip edilmesi gerekmektedir.

Özellikle *Centrocestus* sp. nin de dahil olduğu Heterophyidae ailesine ait intestinal zoonotik digenetik trematodların metaserker evresindekilerin akvaryum balığı ticareti ile Meksika, İtalya, İsrail, Sri Lanka, Avustralya, Venezuela, Çin, Hawaii, Hindistan, Japonya Filipinler, Çekoslovakya, Tayvan, Mısır, Hırvatistan, Danimarka ve İran'a taşındığı bildirilmektedir (Farstey, 1986; Saad, 1994; Paperna, 1996; Diaz ve ark., 1998; Velez-Hernández ve ark., 1998; Scholz ve Salgado-Moldonado, 2000; Evans ve Lester, 2001; Crespo ve Crespo, 2003; Thilakarathne ve ark., 2003; Gjurčević ve ark., 2007; Mehrdana ve ark., 2014).

*Centrocestus* sp. Heterophyidae familyasına ait digenetik trematodlardandır; birinci ara konakçısı salyangozlar (*Melanooides tuberculatus*), ikinci ara konakçısı tatlı su balıklarının pek çok türü ve son konakçısı da piscivor kuşlar ve memelilerdir. Heterophyid kurtlardan *Centrocestus* sp.'nin de içine girdiği Süper familya: Opisthorchioidea'e ait serkeriler balıkların solungaçlarına ya da iç organlarına yerleşirler. Yumurtaları kabukludur. Heterophyid kurtların tüm üyeleri insanlarda zoonoz oluşturmaktadırlar. Araştırmacılar, *Centrocestus formosanus* metaserkerlerinin balıklarda solungaçlara kan taşıyan damarlara yakın yerleştiklerini tespit ettiklerini bildirerek; metaserkerleri solungaç kıkırdağının tabanında ve solungaç kemerinin kas dokusu içinde bulduklarını belirtmişlerdir (Farstey, 1986; Madhavi, 1986; Madhavi ve Rukmini, 1991; Paperna, 1996; Velez-Hernández ve ark., 1998; Srisawangwong ve ark., 1997).

Heterophyid metaserkerlerinin, sub-tropik ve tropik havuzlarda büyütülen balıklarda şiddetli solungaç hasarı ve solunum toleransında azalmaya neden olduğu, solungaç dokusunda hafif hiperplasi, solungaç lamellerinde epithelial hiperplasi, solungaç hiperemisi ve konjesyon meydana getirdiği saptanmıştır (Nowak ve ark., 2000; Velez-Hernández ve ark., 1998).

*Centrocestus* mataserkerlerinin balığın solungaçlarına yerleşmesi su akışı ile olmaktadır. Bu da metaserkerlerin yayılışının ne kadar hızlı olabileceğinin bir göstergesidir (Farstey, 1986).

Heterophyidae familyasına ait *Centrocestus armatus* (Kore), *C. caninus* (Tayvan), *C. cuspidatus* (Mısır ve Tayvan), *C. formosanus* (Çin ve Japonya), *C. kurokawai* (Japonya), *C. longus* (Tayvan)'un insanlarda bulunan intestinal bir trematod olduğu kalbe, beyine ve omuriliğe yerleşebildiği ve daha çok çiğ balık yeme alışkanlığı olan Uzak Doğu ülkelerinde görüldüğü kaydedilmiştir (Maleewong ve ark., 2003).

Bu çalışma, uluslararası akvaryum balığı ticareti ile ülkemize giriş yapan parazitlerin saptanması ve ülkemiz faunası için taşıdıkları riskin yeniden değerlendirilmesi amacıyla yapılmıştır.

## MATERYAL VE METOT

Bu çalışmada Ülkemize Singapur'dan ithal edilen altın balık (*Carassius auratus auratus*) (n:50,3,4-7,5cm), ve dişi kılıç kuyruk (*Xiphophorus hellerii*) (n:10, 3,7-5,3cm) balıkları klinik muayenelerinin ardından, anestezik madde olarak kullanılan yüksek dozdaki karanfil yağı ile bayıltıldıktan sonra dekapitasyonla öldürülmüş, ekto

ve endo parazitler yönünden muayeneleri yapılmıştır. Araştırma, Ankara Üniversitesi Hayvan Deneyleeri Yerel Etik Kurulu'nun 2015-12-136 sayılı izni ve hayvan deneyleeri için belirlenen etik değerlere uygun olarak gerçekleştirilmiştir.

Metaserkerlerin tespit edildiği solungaç yaprakları dıştan içe doğru 1,2,3,4 olarak numaralandırılmış ince bir makasla kesilip bir lam üzerine alınarak fizyolojik tuzlu su ile sulandırılmış ve üzerine bir lamel kapatılmış olarak binoküler mikroskopta (Nikon E100, Tokyo, Japan) incelenmiştir.

Parazitlerin enfeksiyon oranı ve ortalama yoğunluk değerleri Bush ve ark., (1997)'ye göre hesaplanmıştır.

## BULGULAR VE TARTIŞMA

*Centrocestus* sp. metaserkerlerinin sırasıyla enfeksiyon oranı (%) ve ortalama yoğunlukları (OY±SH) altın balıklarda %34, 10,58±2,38, dişi kılıç kuyruk balıklarında ise %30, 14,33±13,33 olarak belirlenmiştir (Tablo 1). *Centrocestus* sp. metaserkerlerinin tespit edildiği bazı balıkların solungaçlarında *Dactylogyrus* sp. (enfeksiyon oranı %14) saptanmıştır. *Centrocestus* sp. ile yoğun enfekte balıklarda uyusukluk ve düzensiz yüzme görülmüştür.

Metaserkerlerin tanımlanmasında morfolojik özellikleri dikkate alınmış; kist içerisinde, oval şekilli, koyu renkli granüllü, *Centrocestus* cinsinin karakteristik özelliği olan oral sakırın ışınlı ve x şeklinde bir boşaltım torbası olduğu gözlemlenmiştir (Scholz ve Salgado-Moldonado, 2000; Evans ve Lester, 2001; Scholz ve ark., 2001; Yıldız, 2005; Mehrdana ve ark., 2014; Yousif ve ark., 2016).

Son yıllarda yapılan çalışmalara bakıldığında; Gjurčević ve ark., (2007) Hırvatistan'a ithal edilen altın balıklarda *Centrocestus formosanus* metaserkerlerinin enfeksiyon oranını %40, Mood ve ark., (2010) İran'a ithal edilen altın balıklarda %25 olarak tespit etmiştir. Mehrdana ve ark., (2014) ise Danimarka'ya ithal edilen *Xiphophorus maculatus*'da *Centrocestus* spp. metaserkerlerinin enfeksiyon oranını %100 olarak bulmuşlardır.

Yıldız (2005), Türkiye'ye ithal edilen altın balıklarda *C. formosanus* metaserkerlerinin enfeksiyon oranını %37,5 olarak bulmuştur. 2005 yılından günümüze kadar geçen bu süre içerisinde *Centrocestus* sp. metaserkerlerinin ülkemize girişinin %34 enfeksiyon oranı ile devam ettiği bu çalışma ile ortaya konmaktadır. Çalışmamızda *Centrocestus* sp. metaserkerlerinin dişi kılıçkuyruk balıklarında saptanması Türkiye'deki ilk bulgulardan biri olmuştur.

**Tablo 1.** İncelenen balıktaki *Centrocestus* sp. enfeksiyon oranı (%) ve ortalama yoğunluk (Ortalama Yoğunluk±Standart Hata)

**Table 1.** Prevalance rate and mean intensity of *Centrocestus* sp. in examined fish

Balığın Türü	Enfeksiyon Oranı (%)	Ortalama Yoğunluk (OY±SH)
<i>Carassius auratus auratus</i>	34	10,58±2,38
<i>Xiphophorus hellerii</i>	30	14,33±13,33

Mehrdana ve ark., (2014) tropikal orijinli olan *Centrocestus* spp.'nin larval evrelerinin gelişiminde optimal sıcaklık değerlerinin 15-25°C arasında değiştiğini, yaz döneminde bu sıcaklığın avrupa sularına uygun olduğunu ancak küresel ısınma ile birlikte sonbahar ve ilkbahar aylarında da parazitin hayatta kalma olasılığının artacağını bildirmektedir.

Bu parazitlerin balıkların refahı, çevre ve insan sağlığı açısından olumsuz etkileri olduğu, endemik olmayan bölgelere girmelerinin engellenmesi gerektiği de Mehrdana ve ark., (2014) tarafından belirtilmektedir.

Türkiye'ye ithal edilen çeşitli akvaryum balıkları üzerinde yapılan çalışmalar ile birlikte bu çalışmanın sonucunda saptanan *Centrocestus* sp. metaserkerlerinin zoonoz oluşturan digenetik bir trematod olması, balıkları metaserkerlerinin geliştirdiği bir ara konakçı olarak kullanması ve zaman içerisinde diğer balık popülasyonları üzerinde de yayılım gösterebilmesi; canlı süs balıklarının ithalatinde, üründen numune alınıp analiz yapılmaması, ancak ürünle gelen sağlık sertifikasındaki bilgilerin değerlendirilmesi gibi nedenlerle ülkemize ithal edilen akvaryum balıkları ile taşınan parazitlerin kapsamlı bir şekilde incelenmesi gerektiğini bir kez daha ortaya koymaktadır.

## SONUÇ

Egzotik akvaryum balıklarının ithali ile taşınan yerli olmayan parazit türleri, yerli balık popülasyonları ve su ürünleri endüstrisi için de bir risk oluşturmaktadır. Yeni parazitlerin girişi ile yerli balık türlerinin yabancı parazitlere karşı uygun savunma mekanizması geliştiremeyeceği, parazitlerin yerli türlerin üzerinde baskı yaratarak harap edici bir etki gösterebilecekleri belirtilmektedir (Mouton ve ark., 2001).

Uluslararası ticaretteki risklerin analizi ve gerekli önlemlerin alınabilmesi için rutin hastalık kontrollerinin yapılması önem taşımaktadır (Adel ve ark., 2015).

Akvaryum balığı ithalatı ile taşınan parazitlerin ve taşıdıkları risklerin çeşitli platformlarda sunulması, bilgilendirme yapılması ile ulusal bir çözüme ulaştırılmasının hedeflenmesi gerekmektedir.

*Centrocestus* türlerinin ara konak seçiciliğinin düşük olması sebebi ile şimdiye kadar pek çok balık türünde metaserkerlerinin saptandığı bildirilmiştir (Scholz ve Salgado-Moldanado, 2000; Ortega ve ark., 2009). Metaserkerlerin yayılımının su akışı ile olduğu düşünüldüğünde, *Centrocestus* metaserkerlerinin ülkemiz sularına karışarak ülkemiz balık faunası üzerindeki enfestasyon ihtimali önemli bir risktir. Masraflı olması sebebiyle gözardı edilen hastalık denetimlerinin ve düzenli karantina uygulamalarının yapılabilmesi için etkili yönetim stratejileri geliştirebilmek, ülkemiz balık faunasının korunması yönünde önemli bir adım olacaktır.

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