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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Determinants of economic efficiency: A case study of Rapa whelk (*Rapana venosa)* fisheries in the South Black Sea

Ekonomik etkinliğin belirlenmesi: Güney Karadeniz'deki deniz salyangozu (*Rapana venosa)* avcılığına (balıkçılığına) ilişkin bir çalışma

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Abstract: The inevitable increase in the numerical and technological capacity of fishing fleets in developed and developing countries has major implications for the management and distribution of limited natural resource capacity, as well as the ecological and socio-economic sustainability of fisheries. It is important to determine these effects to make the right decisions in fisheries management. This study aims to assess the fishing capacity, technical efficiency, scale efficiency, and capacity use in a specific subset of Rapa whelk fishers, those in Turkey's Black Sea. Economically efficient and inefficient boats were also compared in the study. The data obtained through face-to-face interviews with 452 boat owners constitute the main data of the study. Data were collected about the economic efficiency of the boats for one year. While economic efficiency ranged between 0.27 and 1, the average value was found to be 0.60. It was concluded that the age of the owner, the education period, and the length of the fishing boat positively affected economic efficiency (p<0.05). In the boats studied, technical efficiency scores were greater than economic efficiency scores. This suggests that rather than technical information, fishers require information on selecting the optimum input combination at the data cost level.

Keywords: *Rapana venosa,* economic efficiency, data envelopment analysis (DEA), Black Sea

Öz: Gelişmiş ve gelişmekte olan ülkelerde balıkçı filolarının sayısal ve teknolojik kapasitesindeki kaçınılmaz artış, sınırlı doğal kaynak kapasitesinin yönetimi ve dağıtımı ile balıkçılığın ekolojik ve sosyo-ekonomik sürdürülebilirliği üzerinde önemli etkilere sahiptir. Balıkçılık yönetiminde doğru kararların verilebilmesi için bu etkilerin belirlenmesi önemlidir. Bu çalışma, Türkiye'nin Karadeniz kıyılarında bulunan deniz salyangozu avlayan balıkçıların teknik etkinliği, ölçek etkinliğini ve kapasite kullanımını değerlendirmeyi amaçlamaktadır. Çalışmada ekonomik olarak verimli ve verimsiz tekneler de karşılaştırılmıştır. 452 tekne sahibi ile yüz yüze görüşülerek elde edilen veriler çalışmanın ana verilerini oluşturmaktadır. Teknelerin bir yıllık ekonomik etkinliğini belirlemeye ilişkin veriler toplandı. Ekonomik etkinlik 0,27 ile 1 arasında değişirken, ortalama değer 0,60 olarak bulunmuştur. Tekne sahibinin yaşı, eğitim düzeyi ve balıkçı teknesinin boyunun ekonomik verimliliği olumlu yönde etkilediği sonucuna varılmıştır. Teknelerde teknik etkinlik puanları ekonomik etkinlik puanlarından daha yüksek çıkmıştır. Bu, balıkçıların teknik bilgiden ziyade, veri maliyeti düzeyinde optimum girdi kombinasyonunu seçme konusunda bilgiye ihtiyaç duyduğunu göstermektedir.

Anahtar kelimeler: *Rapana venosa*, ekonomik etkinlik, veri zarflama analizi (VZA), Karadeniz

INTRODUCTION

One of the invasive marine gastropods, the rapa whelk, *Rapana venosa* (Valenciennes, 1846), was first seen in the Black Sea in the 1940s. Rapa whelk has been effective in colonizing the Black Sea ecosystem because of the lack of predators and competitor species (ICES, 2004). Rapa whelk with international demands to Turkey in the 1980s after the post in Bulgaria in the 1990s has also been a commercial species in Romania (STECF, 2015). Until now, Turkish fisheries mostly focused on marine fish and the Black Sea contributed a substantial proportion (80%) of the total fishery of Turkish seas (TURKSTAT, 2020). This implies that the Black Sea accounts for the majority of Turkey's marine fisheries (Dağtekin et al., 2017). Anchovy (*Engraulis encrasicolus*), sprat (*Spratus spratus*), Atlantic bonito (*Sarda sarda*), horse mackerel (*Trachurus mediterianus*), whiting (*Merlangius merlangus*), striped venus clam (*Chamelea gallina*), and rapa whelk (*Rapana venosa*) are the most important species (TURKSTAT, 2020).

Recently, rapa whelk has assumed increasing importance in small-scale fisheries as a crucial source of income, especially with the decrease in turbot, sturgeon, flounder, and other fish stocks (Dağtekin et al., 2021a). Small-scale fishers produced revenue of 5.7 million USD in 2019 (TURKSTAT, 2020). The number of workers involved in fishing, processing, and marketing of Rapa whelk is estimated to be about 5000 (Erik et al., 2020) and the processing industry contributes to local employment. Nowadays, rapa whelk is the most

important commercial species in Turkey, Romania, Ukraine, and Bulgaria because all of the catch is exported. In recent years, the volume of catch landings has started to increase (Figure 1). Rapa whelk plays a significant role in terms of socio-economic change and has also led to changes in the benthic ecosystem in the Black Sea. As a result, it is now important to establish a conventional management model for all Black Sea countries that have significant rapa whelk fisheries (STECF, 2015).

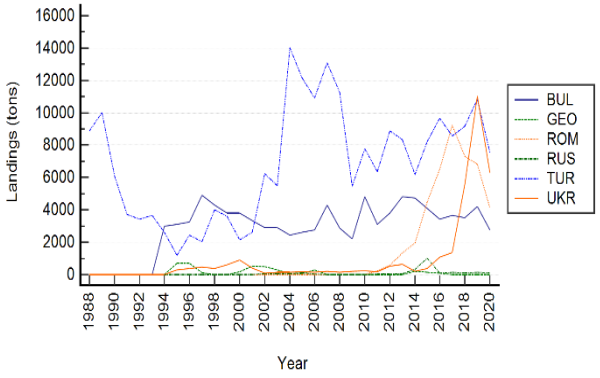

Figure 1. The volume of Rapa whelk in the Black Sea basin

Dredging and diving are two methods used for rapa whelk fishing. In Turkey, permission for rapa whelk fishing must be obtained annually from the Ministry of Agriculture and Forestry. Other fish species besides rapa whelk are also caught by boats. The number of boats licensed by the Ministry in 2018 was 793, of which 659 were dredgers and 134 were diving boats (Erik et al., 2020).

The inexorable rise of the numerical and technological capacity of fishing fleets in the developed and developing countries has brought serious problems regarding the management and share of the limited capacity of natural resources, as well as the ecological and socio-economic sustainability of fisheries (Eigaard et al. 2014; Kumar et al. 2019). This constant increase in fishing effort has resulted in decreased fish stocks decrease and sometimes, catch species population collapse. Therefore, to produce a sustainable management plan, policymakers have to first assess the extent of overcapacity in a fleet in order to regulate fishing capacity and eliminate excess capacity (Madau et al. 2009). The aim of this study was to determine the fishing capacity, technical efficiency, scale efficiency, and degree of capacity used in one sector of the Black Sea rapa whelk fishery, namely that based in Turkey. Economically efficient and inefficient enterprises were also compared.

MATERIAL AND METHODS

Data obtained from face-to-face interviews with Rapa whelk fishing boat owners were used for this study. Data were collected from 452 boat owners who agreed to interview and gave information about their boats (Figure 2). These boats operated along the coast lines of several cities, including Artvin, Rize, Trabzon, Giresun, Ordu, Samsun, Sinop, Kastamonu, Bartın, Zonguldak, Düzce, Sakarya, Kocaeli, Istanbul and Kırklareli all of which are located on Turkey's Black Sea coast.

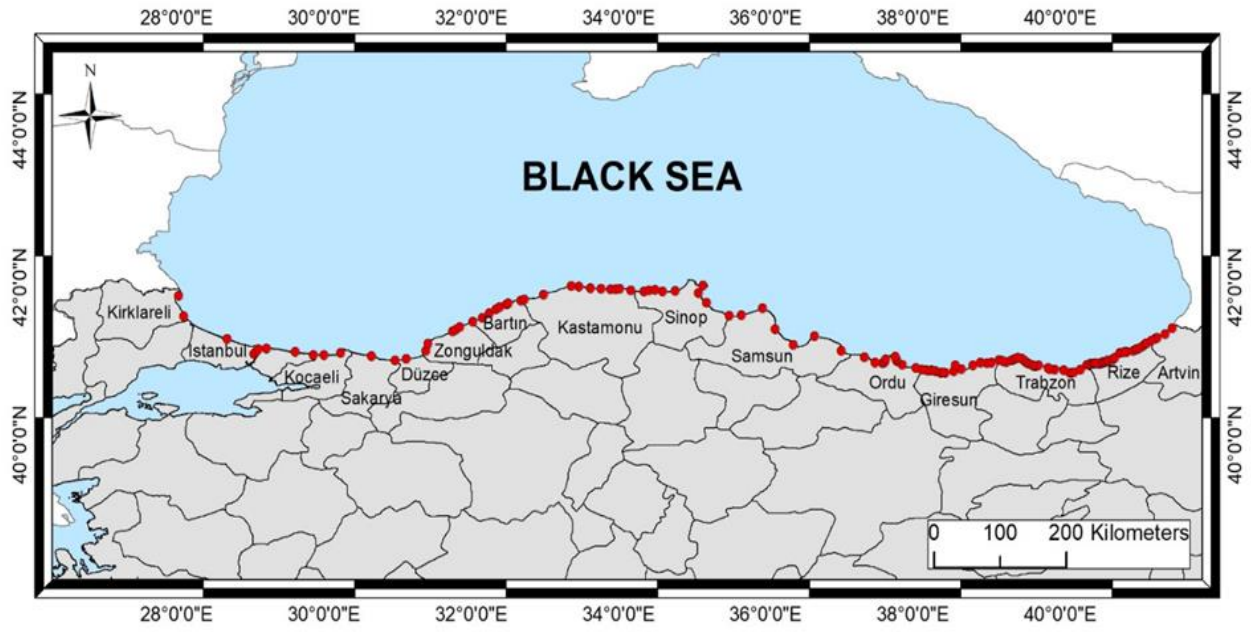

Figure 2. Location of fishing vessel ports/shelters where interviews were carried out

Data were analyzed by basic descriptive statistical methods. T-test was performed when two groups were compared while variance analysis was used when three or more groups were compared. "Two-step method" (two-stage approach) was used for efficiency analysis. This method is recommended as it does not require any prior assumption about the effect of the variables and can be used with more than one continuous or discrete variable. For this reason, this approach has been used in many efficiency analyses (Bravo-Ureta et al., 2007). In the first step of this approach, efficiency coefficients are obtained for each enterprise. In the second stage, the relationship between the variables that may affect the efficiency are assessed and efficiency is then estimated with the help of the appropriate regression model (Coelli et al., 2003).

The distribution of resources in decision-making units and differences in the current technology levels reveal economic efficiency. Economic efficiency, which is a measure of the efficiency of decision units in production, consists of two elements: technical efficiency and allocative efficiency. Technical efficiency indicates the ability to reach the maximum output with a certain amount of input. Allocation efficiency is an indicator of the ability of enterprises to distribute the inputs they use in production in proportion to their costs (Farrell, 1957). Data Envelopment Analysis (DEA), which is a non-parametric method, or stochastic frontier analysis (SFA), which is a parametric method, are widely used in efficiency analysis. However, there are three main reasons why DEA is preferred to SFA in the calculation of efficiency measurements. The first is that a special production function is not required when using DEA. Second, it is not necessary to determine in advance the type of distribution belonging to the error term, which is accepted as the measure of efficiency. The third reason is that DEA is more useful when there is more than one output (Coelli et al., 2003; Kumbhakar and Lovel, 2000).

In this study, Farrell's efficiency measures for input were preferred. Based on the suggestions of Charnes et al. (1978), each fishermen's fuel expenses, labour expenses, boat maintenance and repair expenses and Rapa whelk, and all other target species (Yi) was assumed. That is, a single output model with three inputs was created. The economic efficiency for input for each boat owner was calculated with the following linear programming model:

$$Minimum_{\lambda,xi*} \quad w_i^T x_i^*$$

$$Limitations \qquad -y_i + Y\lambda \geq 0$$

$$x_i^* - X\lambda \geq 0$$

$$\lambda \geq 0$$

where: wi is the input cost for each fisher; T is the transpose of the function; and xi * is the given input cost, wi, and the output levels; Yi is the vector showing the lowest cost input quantities calculated using the linear programming method for each fishers.

This equation shows the lowest cost for the Constant Returns Scale (CRS) conditions. Economic efficiency for each fisherman was calculated using the formula (EE)=wiTxi*/wiTxi. In this equation, EE is the ratio of the lowest cost to the observed cost for the given input costs and under CRS conditions. The allocation efficiency was calculated with the formula AE=CE/TE (Coelli et al., 2003). Since the fisheries enterprises have insufficient capital and lack information about the market, a restrictor ($N1\lambda = 1$) that provides convexity was added to the CRS model, and the model was transformed into a variable returns to scale (VRS).

Since adding this restrictor to the model prevents the calculation of scale efficiency, the minimum cost in CRS conditions was calculated by proportioning the minimum cost in the VRS conditions when calculating the scale efficiency (Banker et al., 1984). The DEAP 2.1 package program, developed by Coelli (1996), was used for estimating efficiency measurements.

Since efficiency coefficients vary between 0 and 1, and the classical least squares method predicts the coefficients to be larger than necessary, "Tobit regression" was used in this study. The Tobit model is an econometric method proposed by James Tobin that describes the relationship between a non-negative-dependent variable and an independent variable or vector. The information of the dependent variable is known as the censored sampling model, where it is found only for some observations. It is a non-parametric alternative to least squares regression (Liao, 1994). For this reason, the Tobit model is also called the censored or discrete regression model.

RESULTS AND DISCUSSION

The Rapa whelk fishing sector includes fishers and their dependent employees, brokers, and processing plants. In 2018, beam trawlers and divers spent 147 days at sea on average and landed a total of 8,675.98 tonnes of Rapa whelk by 714 boats. Rapa whelk fishing was permitted for 793 boats but not all boats with permission performed Rapa whelk fishing. In the research area an average boat had an income of 16767.1 US$ from fishing activity and to achieve this income incurred the following costs: 4593.5 US$ labour; 565.1 US$ boat maintenance; and 1534.8 US$ for fuel. The technical efficiency coefficient with variable returns to the scale varies between 0.30 and 1, and the average was found to be 0.75. This value shows that inefficient firms can reduce their inputs by 25% without a reduction in output. It was calculated that 46.02% of the enterprises have a lower value than the average technical efficiency calculated. With a constant return to the scale, the technical efficiency coefficient was found 0.72 and the scale efficiency was calculated as 0.96. Scale effectiveness shows whether the enterprises are on optimal scale. We found that 27.43% of the enterprises have a lower value than the calculated average scale efficiency value. Resource allocation efficiency was between 0.34 and 1, with an average of 0.80 for the enterprises

examined. This value indicates that boats spend 20% more than the minimum cost combination of inputs. In this area 42.26% of the enterprises have a lower value than the average resource allocation efficiency value calculated While economic efficiency ranged between 0.27 and 1, the average value was 0.60, which shows that economically inefficient enterprises would need to reduce their operating costs by 40% to reach the level of similar but economically efficient enterprises (Table 1).

Table 1. Descriptive statistics for efficiency scores

Efficiency measurements	Mean	Standard deviation	Lower	Upper
Technical efficiency	0.72	0.13	0.24	1.00
Pure technical efficiency	0.75	0.13	0.30	1.00
Scale effectiveness	0.96	0.07	0.31	1.00
Resource allocation efficiency	0.80	0.10	0.34	1.00
Economic efficiency	0.60	0.11	0.27	1.00

Scale effectiveness of present study was the same as the value found in this study (0.96). Dağtekin et al. (2021b) found that technical efficiency in pelagic trawl boats ranged from 0.413 to 0.998, with an average of 0.739. The frequency distribution of efficiency scores is given in Table 2. We found that technical efficiency scores and pure technical efficiency were mostly between 0.700-0.799. In addition, the scale effectiveness scores were mostly between 0.950-0.999 and that most enterprises were close to the appropriate scale level.

Table 2. Frequency distribution of efficiency scores

Efficiency level	Technical efficiency	Variable returns to scale	Scale effectiveness	Resource allocation efficiency	Economic efficiency
0.200-0.299	3	1	0	0	2
0.300-0.399	3	2	1	6	20
0.400-0.499	24	21	1	3	47
0.500-0.599	38	23	3	14	161
0.600-0.699	97	87	2	29	154
0.700-0.799	183	161	6	139	44
0.800-0.899	69	97	47	201	17
0.900-0.949	12	18	50	44	3
0.950-0.999	6	4	311	12	0
1.000	17	38	31	4	4
Total	452	452	452	452	452

In terms of resource allocation efficiency, the efficiency scores were predominantly between 0.800-0.899. It was striking that the obtained score values were mostly between 0.500-0.599 and 0.600-0.699, and the economic efficiency of the enterprises was low. There were 38 fully technically efficient, four fully efficient in terms of resource allocation efficiency and economic efficiency, and 31 optimal scales operating enterprises.

A sizeable proportion of the enterprises examined (46.90%) had a decreasing return to scale, while a similar proportion (46.24%) had an increasing return to scale, and 6.86% have constant returns to scale (Table 3). It was found that the income of enterprises with increasing returns to scale is considerably lower than enterprises with a constant return to scale and in turn boats with decreasing returns to scale earned 1.18 times more income than enterprises with fixed returns to scale, but the labour cost, boat maintenance costs, and fuel costs were 1.31, 1.71, and 1.11 times higher, respectively. Variance analysis results identified significant differences by return group amongst the following variables: fishing income ($F=18.444$, $p<0.001$), labour wage ($F=15.149$, $p<0.001$), boat maintenance cost ($F=10.330$, $p<0.001$) and fuel cost ($F=27.096$, $p<0.001$).

The classification of boats according to their technical efficiency is given in Table 4. Only 8.41% of the enterprises worked technically fully effectively. In addition, 0.88% of the enterprises worked efficiently, 3.98% of them worked less effectively, and the majority (86.73%) were not technically efficient.

Analyses of the calculated resource allocation efficiency scores showed that 0.88% of the enterprises were fully efficient in resource allocation, 2.65% were efficient and 9.73% were less efficient. Once again, 86.73% did not allocate resources efficiently. Thus, when the current technology level and current input costs are taken into account, most of the enterprises produce with the wrong input combination. The present study found that only 0.88% of the enterprises worked economically fully effectively, that is, they continue their production with a minimum cost input combination. While 0.66% of the enterprises were found to work less effectively, it was shown that 98.52% of them did not work economically effectively. It was also determined that the average and optimum input levels and potential improvement rates of the enterprises were not economically efficient.

Table 3. Scale effectiveness analysis results

Return to scale	Frequency	%	Fishing income (US$)	Labour cost (US$)	Boat maintenance cost (US$)	Fuel cost (US$)
Decreasing return to scale	212	46.90	22296.4^{a}	6228.5^{a}	681.0^{a}	1967.5^{a}
Increased return to the scale	209	46.24	10841.0^{b}	2911.9^{b}	472.1^{b}	1061.0^{b}
The constant return to scale	31	6.86	18907.1^{a}	4748.8^{a}	399.0^{b}	1770.0^{a}

*The averages of the groups shown with different letters are different at a 5% significance level. (1 USD:6.88 TRY)

Table 4. Classification of boats according to their technical efficiency

Efficiency status	Technical efficiency		Variable returns to scale		Scale effectiveness	
	Frequency	%	Frequency	%	Frequency	%
Fully effective (TE = 1)	17	3.76	38	8.41	31	6.86
Effective (0.95 ≤ TE ≤ 1)	6	1.33	4	0.88	311	68.81
Slightly effective (0.90 ≤ TE ≤ 0.949)	12	2.65	18	3.98	50	11.06
Ineffective (TE ≤ 0.899)	417	92.26	392	86.73	60	13.27
Total	452	100.00	452	100.00	452	100.00

Descriptive statistics of the variables used in the Tobit model are given in Table 5. Findings included the average age of boat owners was 44.48 years, the average period of education was seven years, and the average family size was 4.45 individuals. The average income of boat owners from any activity other than fishing was 301.9 US$. The average length of boats was 8.74 m, and the engine power was 108.54 HP. Nearly one fifth (19%) of owners had a second profession other than fishing. In addition, most fishers used dredge (99.12%) and only a small proportion (0.88%) caught whelk by hand diving.

Table 5. Descriptive statistics of variables used in the Tobit model

Tobit model	Average	Standard deviation	Lower	Upper
Business owner age (year)	44.48	10.99	21.00	84.00
Education period of the owner (years)	7.01	2.84	0.00	15.00
Family size (person)	4.45	1.66	1.00	11.00
Non-fishing income (US$)	301.9	1238.2	0.00	19767.4
Boat length (m)	8.74	1.76	4.70	20.00
Boat engine power (HP)	108.54	76.26	6.00	480.00
Occupation other than fishing[1]	0.00			
Way of fishing[2]	1.00			

As a measure of central tendency, the arithmetic means in the data at interval and ratio level, the median in the rank data and the model in the classified data were used.
[1] No: 0, yes: 1 is included in the model.
[2] Dredge:1, diving:2
(1 USD:6.88 TRY)

The results of the Tobit model created for determining the factors affecting economic efficiency are given in Table 6. Thepositive or negative effect of most of the variables included in the model was as expected, with the income from non-fishing and the engine power of the boat having a positive effect on economic efficiency, but the fishing style and the need for a professional other than fishing, a negative affect. However, these variables were not statistically significant (p>0.10).

Table 6. Tobit analysis results: Factors affecting economic efficiency

Variable	Coefficient	Standard error	P
Boat owner's age (years)	0.000920*	0.000519	0.0760
Business owner's education period (years)	0.004475**	0.001953	0.0219
Family size (person)	-0.006218*	0.003201	0.0521
Non-fishing income (US$)	0.0000047	0.000006	0.4641
Boat length (m)	0.008207*	0.004276	0.0550
Boat engine power (HP)	0.000009	0.000009	0.3450
Way of fishing	-0.007666	0.010300	0.4567
Professional other than fishing	-0.008652	0.014308	0.5454
Likelihood ratio	30.06660***		

*Important at the 10% probability level
**Important at the 5% probability level
***Important at the 1% probability level

The age of the owner of the boat affected the economic efficiency positively (p=0.0760). As owners got older, the economic efficiency increased. This might be expected as more experienced older people take advantage of their greater experience and knowledge. More experienced boat owners made more accurate decisions in terms of both the level of input use and application of production techniques compared to younger, less experienced operators. The boat owner's education period also positively affected economic efficiency, as the duration increased, the economic efficiency increased (p=0.0219). This situation be defined as producers with a high education level were more conscious and therefore earned more income. Family size negatively affected economic efficiency (p=0.0521). As family size increased, economic efficiency decreased. The size of the fishing boat positively affected the economic efficiency (p=0.0550). As the length of the boat increased, fishing becomes more efficient, the amount of fish caught increased, and thus larger boat size increased income and thus economic efficiency. A comparison has been made between economically fully efficient and inefficient enterprises, and the results obtained are given in Table 7.

Table 7. Socio-economic characteristics of active and ineffective boats

Variables	Effective boats	Ineffective boats
Demographic variables		
Boat owner's age (years)	48.50 (11.90)	44.44 (10.99)
Boat owner's education period (years)	14.00 (2.00)***	6.95 (2.77)***
Family size (person)	3.75 (1.50)	4.46 (1.67)
The number of fishers in the household (number)	0.75 (0.96)	0.46 (0.91)
Profession outside fishing[1]	0.00	0.00
Non-fishing income (US$)	0.00 (0.00)***	2095.85 (8554.42)***
General characteristics of boats and activity results		
Boat length (m)	10.33 (4.58)*	8.73 (1.72)*
Boat engine power (HP)	224.50 (196.29)***	107.50 (74.08)***
Boat value (US$)	26162.79 (23496.81)**	12712.35 (11020.10)**
Value of equipment (US$)	5069.04 (6415.79)	3818.65 (4418.72)
Fishing pattern[2] (median)	2.00**	1.00**
Fishing income (US$)	85356.10 (153573.80)***	16154.73 (14515.84)***
Costs		
Labour cost (US$)	25423.93 (50041.71)***	4407.50 (4502.74)***
Boat maintenance cost (US$)	1061.05 (927.96)*	560.64 (521.83)*
Fuel cost (US$)	2925.15 (3954.08)**	1522.39 (1308.53)**

As a measure of central tendency, the arithmetic means in the data at interval and ratio level, the median in the rank data and the model in the classified data were used. (1 USD: 6.88 TRY)
1 Boat with a profession other than fisheries are included in the model with a value of 1, and boats with a value of 0.
2 The type of catch is included in the model with a value of 1 dredge, and 2 with a diver.
**The difference between economically efficient and non-economically efficient enterprises is statistically significant at the 10% probability level.*
***The difference between economically efficient and non-economically efficient enterprises is statistically significant at the 5% probability level.*
****The difference between economically efficient and non-economically efficient enterprises is statistically significant at the 1% probability level.*

The average age of economically efficient boat owners and the number of people fishing together in their households tended to be slightly higher than in ineffective boats, while the family size was lower. The proportion of owners having a profession other than fishing in effective and ineffective enterprises was similar. The value of the equipment owned by effective boats also tended to be greater than for inefficient boats. However, these variables were not significant (p>0.10).

The education period of owners of effective enterprises were considerably higher than inefficient enterprises (t=-6.994, p=0.005), suggesting that education level plays an important role in efficiency. While inefficient boats earned income from sources other than fishing, efficient boats had no income other than fishing (t=5.186, p<0.001). The length of the boat (t=-1.814, p=0.070), the engine power of the boat (t=-3.083, p=0.002) and the value of the boat (t=-2.402, p=0.017) owned by effective enterprises were higher than for inefficient enterprises. This shows that higher quality tools and equipment have a positive effect on efficiency. The income of the effective enterprises from fishing activities was considerably higher than the inefficient enterprises (t=-7.197, p=0.001). Labour costs (t=-6.895, p=0.09), boat maintenance costs (t=-1.896, p=0.059) and fuel costs (t=-2.079, p=0.038) of effective enterprises were higher than ineffective enterprises. Thus, the costs of effective enterprises were higher, but their income earned from fishing was approximately fivefold higher.

An large proportion of Rapa whelk landing occurs in the Samsun shelf area. However, the length of the boats and engine power is greater than in other regions. Therefore, the unit costs increased and will have an effect on efficiency scores. Tingley et al. (2005) determined the average technical activity as 0.56, 0.76, and 0.79 according to three different fishing activity categories in England for the period 1993–2000. Esmaili, (2006) calculated the average technical efficiency for fishing activity as 0.78, in Iran. In another study

conducted in Portugal (Oliveira et al., 2010), with the same study, the technical efficiency and pure technical efficiency values for the local fleet and coastal fleet in 2005, 2006 and 2007 were calculated using a single methodology to be 0.74, 0.66, 0.58 and 0.81, 0.91, 0.79 respectively. Thean et al. (2011) found the average technical efficiency value to be 0.57 for trawlers in Malaysia. Ceyhan and Gene (2014), found that the average efficiency value was 0.667 when using trawl and purse-seine together, but 0.535 for those only using trawl in Samsun province. Kaygısız and Evren (2014) calculated the production efficiency of fishing operations in Turkey and the technical efficiency calculated value (CRS) was found to be 0.66. According to this study results, it was determined that, to become economically efficient, enterprises should make a 63.37% decrease in the labor wage, 1.39% decrease in fuel costs, and an increase of 84.01% in boat maintenance costs. Kaygısız and Evren (2014) reported that if fuel costs were reduced by 59.27%, enterprises would become fully effective. Zhaoqun et al. (2016) determined that 92.90% of fishing enterprises were not technically efficient, and only 7.10% showed technical efficiency scores above 0.90. It was calculated that 6.86% of enterprises work at the optimal scale, that is, their scale effectiveness scores were equal to 1. Besides, it was concluded that approximately 2/3 (68.81%) of the enterprises work close to the optimal scale.

According to the present study, the technical efficiency amongst fishing enterprises in Rapa whelk fishing was found to be 0.75. The technical efficiency was at a good level but was not at the optimum level. The lower pure technical efficiency scores than the scale efficiency scores indicate that the low technical efficiency was due to the ineffectiveness of the scale rather than the ineffectiveness of input use.

CONCLUSIONS

The average resource allocation efficiency was 0.80 for the enterprises examined. Considering the current technology level and input costs, this situation can be explained by production using the wrong input combination by some enterprises. These enterprises spent 20% more than the minimum cost input combination. The average economic efficiency was 0.60 in the study area. Enterprises with economic inefficiency would need to reduce their operating costs by 40% to reach the level of their peers with similar and economically efficient enterprises. Technical efficiency scores were higher than the economic efficiency scores in the enterprises examined. This indicates that producers need information on choosing the appropriate input combination at the data cost level rather than technical information. Analysis revealed the positive effect of older age and better education levels of the owner, and also of larger boat sizes. Comparative analysis revealed that efficient boats were more successful than inefficient boats in all respects. To move the efficiency measures of poorly performing enterprises towards those of their efficient peers, first, access to information resources should be facilitated with well-organized dissemination studies. More effective operation of institutions and organizations that conduct extension services and the emphasis on the technical aspect of fisheries in their studies increased the efficiency in the field of examination. It does not appear that production can be increased because Rapa whelk supplies are being utilized to their maximum capacity. As catch cannot be increased further, research in Rapa whelk fisheries should focus on identifying strategies to optimize profit.

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APPENDIX
QUESTIONNAIRE ON DETERMINING THE EFFECTIVENESS OF RAPA WHELK FISHING

A-SOCIAL PROFILS OF BOATS OWNERS

1- How many people live in the household?..... (male: age 0-14……,,
15-64 years:……., 65+ years:……. Woman:……. 0-14 years.……,, 15-64 years:……., 65+ age:…….)
2- Number of children (If there is polygamy, the total number of children from both spouses will be taken into account): ……
(Male:……, Female:…….)
3- The number of people you fish with in your household: Male:……., Female:…….
4- Age: ……..
5- Educaiton level:…….
6- Second job, if any…… and income:…….
7- What are your reasons for starting/preferring the fishing profession?
(Indicate the degree of importance numerically)
1. ( ) Because it is a family profession
2. ( ) Because I have no other profession
3. ( ) Because I couldn't find a job in another field
4. ( ) Interest/love towards the sea
5. ( ) To work after retirement (additional income)

8- Are you satisfied with fishing?	
Satisfaction level Rationale	**Reason**
a. Satisfied	( ) My main source of income ( ) Love of the sea ( ) Other (specify):……..
b. Not satisfied	( ) Low income ( ) Heavy working conditions ( ) Income instability ( ) Other (specify): ……..

B-BOATS INFORMATION

Region where the boat is licensed		
Boats	Name and Number	
	Length of overall (m)	
	Engine power (HP)	
Equipment	Communication gear	
	Radar	
	Other…..	
Fishing method	( ) Diving ( ) Dredge	

C-Rapa whelk fishing periods

	Month											
	Jan	Fab	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Fishing area												
Fishing gear												
Active fishing days												
Rapa whelk catch (kg)												
Daily operation time (hour)												
Number of fishing operaitons												
Each operation time (minute)												
Depth (m)												

D-INFORMATION ABOUT GILLNET FISHING

GILLNET FISHING PERIOD	Month											
	Jan	Fab	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Number of days												
Landings (kg)												
Target fish species												
Turbot (kg)												
Whiting (kg)												
Horse mackerel (kg)												
Atlantic bonito (kg)												
Red mullet (kg)												
Bluefish (kg)												
Other (specify)…….												
Other (specify)…….												
Other (specify)…….												
Other (specify)…….												

E- INCOME-COST STATUS OF BOATS

COSTS	VALUE (TURKISH LIRA)
Crew	
Maintenince	
Fuel costs	
Technical device purchase	
Gears	
Commercial costs	
Other …..	
Other…….	
Value of boats	
Value of equipments	

INCOME	VALUE (TURKISH LIRA)
Rapa whelk	
Turbot	
Whiting	
Horse mackerel	
Atlantic bonito	
Red mullet	
Bluefish	
Other……..	
Other……	
Other…..	
Other……..	

NON FISHING INCOME OF THE BOAT OWNER	VALUE (TURKISH LIRA)
Retirement	
Farming	
Small business	
Other	
Name and surname of fishers	
Tel	

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Ege dalyanlarında kullanılan av araçlarının çeşitliliği

Diversity of used fishing gears in the Aegean lagoons

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Öz: Bu çalışmada, Ege Denizi boyunca lagün balıkçılığında kullanılan bazı av araçlarının teknik özelliklerini ve hedef türlerini belirledik. 2014-2016 yılları arasında beş dalyanda (Enez, Homa, Karina, Akköy ve Güllük) örneklemeler yapılmıştır. Balık ağlarının teknik planları MS-Visio programı tarafından ağlar için ölçekli, ancak paragatlar için ölçeksiz çizilmiştir. Dalyanlarda toplam 8 adet fanyalı ve 2 adet solungaç olmak üzere toplam 10 adet uzatma ağı ve 3 tip paragat belirlenmiştir. Dalyanda incelenen tüm av araçları için hedef türler, 8 aile ve 15 türden oluşmaktadır. İncelenen dalyanlarda yaygın olarak *Dicentrarchus labrax, Chelon auratus, Chelon labrosus, Chelon ramada, Chelon saliens, Mugil cephalus, Sparus aurata* türleri görülmüş, *Callinectes sapidus, Platichthys flesus* türleri sadece Enez dalyanında ve *Cyprinus carpio* türü ise sadece Güllük dalyanında tespit edilmiştir.

Anahtar kelimeler: Lagün, av araçları, hedef türler, Ege Denizi

Abstract: In this study, we determined the technical characteristics and target species of some fishing gears, used in lagoon fisheries along the Aegean Sea. The samplings were carried out in five lagoons (Enez, Homa, Karina, Akköy and Güllük) between 2014 and 2016. Technical plans of the fishing gears were drawn by MS-Visio programme with scale for gillnets, but no scales for longlines. A total of 10 type set nets of which 8 trammel nets and 2 gillnets, and 3 type longlines were determined. Target species comprise 8 families and 15 species for all fishing gear examined in the lagoon. *Dicentrarchus labrax, Chelon auratus, Chelon labrosus, Chelon ramada, Chelon saliens, Mugil cephalus* and *Sparus aurata* were commonly seen in the studied lagoons. *Callinectes sapidus* and *Platichthys flesus* were determined only in the Enez lagoon and *Cyprinus carpio* was determined only in the Güllük lagoon.

Keywords: Lagoon, fishing gears, target species, Aegean Sea

GİRİŞ

Kıyı lagünleri, kara ve deniz arasındaki sınırda bulunan önemli ekosistemler olup, genellikle en yüksek biyolojik üretkenliğe sahip deniz habitatları arasındadır (Day vd., 1989). Bunlar çok sayıda balık türü için uygun yaşam alanları sağlar ve deniz balıklarının erken evreleri için de korunma alanı işlevi görürler (Elliott vd., 2007). Bu sistemler omurgasızlardan balıklara ve kuşlara kadar çok çeşitli türler için besin kaynakları, yuvalanma alanları, avcılardan korunma ve yavruların büyümesi ve hayatta kalması için uygun ortamlar sağlar (Kjerfve, 1994).

Bolluk ve tür zenginliği açısından Mugilidae ve Sparidae familyaları lagün sistemi balık toplulukları arasında en önemli ailelerdir (Elliott vd., 2007). Akdeniz bölgesinde yer alan irili ufaklı 400'den fazla kıyı lagününün alanı yaklaşık 640.000 hektardır (Aalto vd., 2015; Cataudella vd., 2015). Geçmişte, Türkiye'de 72 adet dalyan bulunmaktaydı ve bu dalyanların %40'ı Ege kıyılarında olup, toplam yüzey alanı %54'lük payla 38.000 hektardı. Tüm dalyanlardan elde edilen su ürünleri üretiminin %63'ü de bu Ege dalyanlarından sağlanmaktaydı (Elbek vd., 2003). Ancak lagüner alanların bir ömrü vardır ve meteorolojik şartların etkisiyle oluşan, denizlerdeki fırtınalar sonucu dalgaların yükselmesi veya yoğun yağış sonrası dalyanları besleyen derelerden dalyanlara doğru oluşabilecek seller, dalyan kıyılarında sıklıkla bozulmalar oluşturur; ayrıca düzenli derinleştirme yapılmazsa, sığlaşarak zamanla yok olurlar (Akyol, 1999).

Ege Denizi kıyılarında günümüzde faal durumda sadece 6 dalyan (Enez, Homa, Karina, Akköy, Güllük ve Köyceğiz) kalmıştır. Lagünlerde temel avcılık "dalyancılık" olarak adlandırılan ve çit kuzuluklardan balıkların hasat edilmesi şeklinde yapılmaktadır. Dalyanlarda kefal, levrek, çipura ve dil balıkları gibi türlerin avcılığı için ayrıca uzatma ağları, paragat ve pinterler de yaygın olarak kullanılmaktadır (Tosunoğlu vd., 2017). Bu av araçlarının çeşitliliği ve kullanımı yöreden yöreye değişmekte, bazı türlere karşı özel av araçları da geliştirilebilmektedir.

Bu çalışma, Ege Denizi kıyılarında bulunan beş dalyanda (Enez, Homa, Karina, Akköy, Güllük) kullanılan av araçlarının çeşitliliğini ve avcılık metotlarının güncel tespitlerini yapmayı amaçlamaktadır.

MATERYAL VE YÖNTEM

Bu çalışma, Haziran 2014 – Aralık 2016 yılları arasında Ege Denizi kıyılarında bulunan, kuzeyden güneye Enez, Homa, Karina, Akköy ve Güllük dalyanlarında gerçekleştirilmiştir (Şekil 1). Dalyanların kooperatif başkanları, müdürleri ve dalyan balıkçılarıyla yüz yüze anketler yapılmış, yine dalyan rıhtımlarında hem balık, hem de av araçlarının tespiti yapılarak teknik özellikleri yerinde gözlemlemeyle ilgili formlara kaydedilmiştir. Bu av araçlarının teknik özellikleri FAO standartlarına göre tanımlanmıştır. Av araçlarının teknik çizimleri uzatma ağları için ölçekli, paragatlar için ölçeksiz olarak MS Visio 10.0 programı yardımıyla yapılmıştır

Şekil 1. Ege Denizi'nde örneklenen dalyanlar (Google Earth)
Figure. 1. Lagoons sampled in the Aegean Sea (Google Earth)

BULGULAR

Enez, Karina ve Güllük dalyanları kooperatifler tarafından işletilirken, Homa dalyanı araştırma ve uygulama dalyanı olarak Ege Üniversitesi Su Ürünleri Fakültesi tarafından kullanılmaktadır ve Akköy dalyanı ise bir özel firma tarafından işletilmektedir.

Kullanılan Av Araçları

Kuzuluk Sistemleri

Örneklenen dalyanlarda kuzuluk (tuzak) sistemlerinin şeması Şekil 2'de verilmiştir.

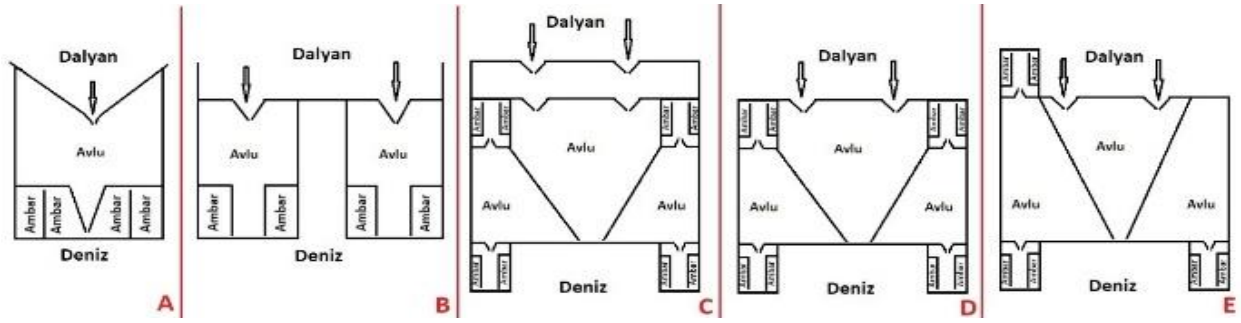
Yöre halkı Enez dalyanı kısımlarından Işık gölüne "Üzmene", Dalyan gölüne ise "Peso" ismi vermektedir. Enez dalyanında biri Üzmene bölümünde, diğer ikisi Peso bölümünde olmak üzere toplam 3 adet kuzuluk bulunmaktadır. Kuzuluklar Haziran-Aralık ayları arasında kurulmaktadır. Her kuzulukta bir adet avlu ve 4 adet ambar bulunmaktadır. Kuzuluk sisteminin yapımında doğal malzeme olarak kargı çit ve çam kazıklar kullanılmıştır. Avlu kısmına geçiş yapan balıklar daha sonra ilerleyerek ambar kısmına geçiş yapmakta ve buradan kepçe ile hasat edilmektedir (Şekil 2A).

Homa dalyanında bir adet kuzuluk bulunmaktadır. Kuzuluk Haziran-Kasım ayları arasında kurulmaktadır. Kuzulukta 2 adet avlu ve 2 adet ambar bulunmaktadır. Kuzuluk sisteminin yapımında tonozlar ve dış kısmı oluşturan çerçeve sıcak galvaniz kaplı demir ızgara ile avlu ve ambar iç kısımlarında kısmında doğal malzeme olarak kargı çit kullanılmıştır (Şekil 2B).

Karina dalyanında toplam 6 adet kuzuluk bulunmaktadır. Kuzuluklar Haziran-Ocak ayları arasında kurulmaktadır. Kuzulukta 3 adet avlu ve 8 adet ambar bulunmaktadır. Kuzuluk sisteminin yapımında doğal malzeme olarak kargı çit ve çam kazıklar kullanılmıştır (Şekil 2C).

Akköy dalyanında toplam 5 adet kuzuluk bulunmaktadır. Bunlar Karina dalyanı kuzuluklarına benzer yapıdadır. Kuzuluklar Haziran-Ocak ayları arasında kurulmaktadır. Kuzulukta 3 adet avlu ve 8 adet ambar bulunmaktadır. Kuzuluk sisteminin yapımında doğal malzeme olarak kargı çit ve çam kazıklar kullanılmıştır (Şekil 2D).

Güllük dalyanında 2 adet kuzuluk bulunmaktadır. Kuzuluklar Haziran-Şubat ayları arasında kurulmaktadır. Kuzulukta 3 adet avlu ve 6 adet ambar bulunmaktadır. Kuzuluk sisteminin yapımında doğal malzeme olarak kargı çit ve çam kazıklar kullanılmıştır (Şekil 2E). Tüm kuzuluklarda çit aralıkları Su Ürünleri Tebliğine uygun olarak 3 cm genişlikte ayarlanmıştır.

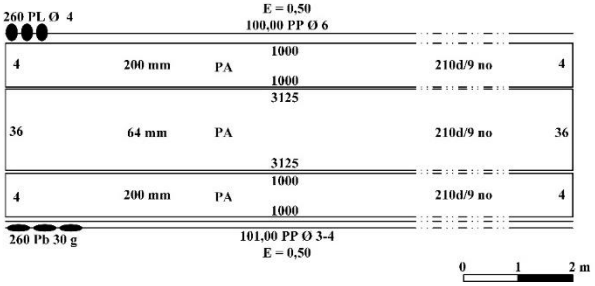


Şekil 2. Dalyan kuzuluk şeması (A) Enez, (B) Homa, (C) Karina, (D) Akköy, (E) Güllük
Figure 2. Lagoon trap scheme (A) Enez, (B) Homa, (C) Karina, (D) Akköy, (E) Güllük

Uzatma Ağları

Fanyalı kefal ağı -I (Enez)

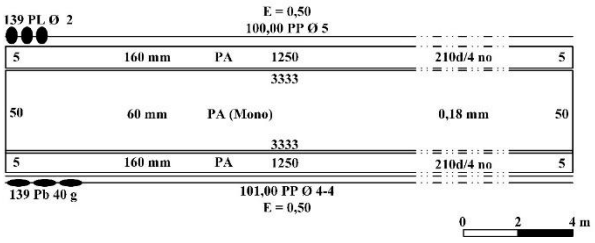
Multifilament polyamid (PA), 64 mm tam göz boyu (TGB)'nda, 36 göz yüksekliğindedir ve 210d/9 no ip kalınlığındaki tor ağın boyu 100 m'dir. Ağın her iki tarafında bulunan 4 göz yüksekliğinde fanyalar 200 mm TGB'unda ve 210d/9 no ip kalınlığındadır. E=0,5 oranında donam uygulanmaktadır. Yaka halatlarında, üst yakada 6 mm ile alt yakada 3–4 mm kalınlıkta (biri koşma halatı) PP materyal kullanılmaktadır. Halatlarda 4 numara plastik mantarlar ile 30 g'lık bakla kurşun kullanılmaktadır (Şekil 3).



Şekil 3. Fanyalı kefal ağı -I (Enez)
Figure. 3. Trammel net for grey mullets –I (Enez)

Fanyalı kefal ağı-II (Enez)

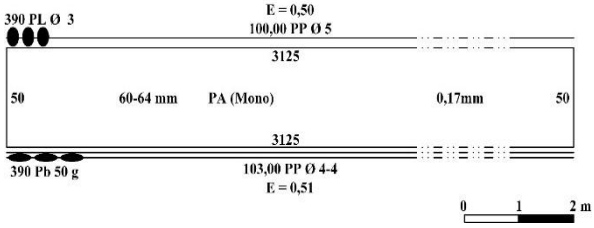
Monofilament PA, 60 mm TGB'nda, 50 göz yüksekliğindedir ve 0,18 mm ip kalınlığındaki tor ağın boyu 100 m'dir. Ağın her iki tarafında bulunan 5 göz yüksekliğinde fanyalar 160 mm TGB'unda ve 210d/4 no ip kalınlığındadır. E=0,5 oranında donam uygulanmaktadır. Yaka halatlarında, üst yakada 5 mm ile alt yakada 4–4 mm kalınlıkta (biri koşma halatı) PP materyal kullanılmaktadır. Halatlarda 2 numara plastik mantarlar ile 40 g'lık bakla kurşun kullanılmaktadır (Şekil 4).



Şekil 4. Fanyalı kefal ağı -II (Enez)
Figure. 4. Trammel net for grey mullets –II (Enez)

Sade kefal uzatma ağı (Enez)

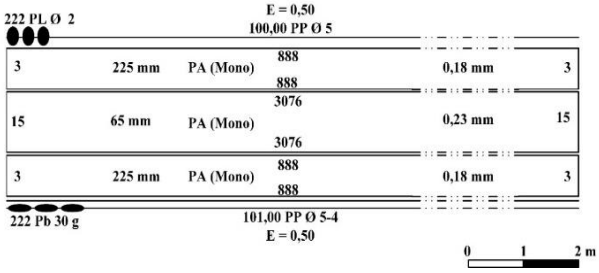
Monofilament PA, 60-64 mm TGB'nda, 50 göz yüksekliğindedir ve 0,17 mm ip kalınlığındaki tor ağın boyu 100 m'dir. E=0,5 oranında donam uygulanmaktadır. Yaka halatlarında, üst yakada 5 mm ile alt yakada 4–4 mm kalınlıkta (biri koşma halatı) PP materyal kullanılmaktadır. Halatlarda 3 numara plastik mantarlar ile 50 g'lık bakla kurşun kullanılmaktadır (Şekil 5).



Şekil 5. Sade kefal ağı (Enez)
Figure. 5. Gillnet for grey mullets (Enez)

Fanyalı levrek uzatma ağı (Enez)

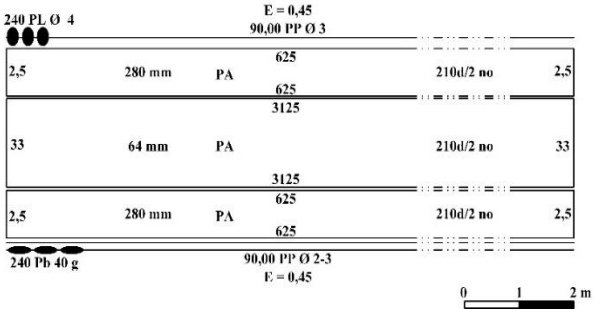
Monofilament, 65 mm TGB'nda, 15 göz yüksekliğindedir ve 0,23 mm ip kalınlığındaki tor ağın boyu 100 m'dir. Ağın her iki tarafında bulunan 3 göz yüksekliğinde fanyalar 225 mm TGB'unda ve 0,18 mm ip kalınlığındadır. E=0,5 oranında donam uygulanmaktadır. Yaka halatlarında, üst yakada 5 mm ile alt yakada 5–4 mm kalınlıkta (biri koşma halatı) PP materyal kullanılmaktadır. Halatlarda 2 numara plastik mantarlar ile 30 g'lık bakla kurşun kullanılmaktadır (Şekil 6).



Şekil 6. Fanyalı levrek ağı (Enez)
Figure. 6. Trammel net for seabass (Enez)

Fanyalı uzatma ağı–I (Homa)

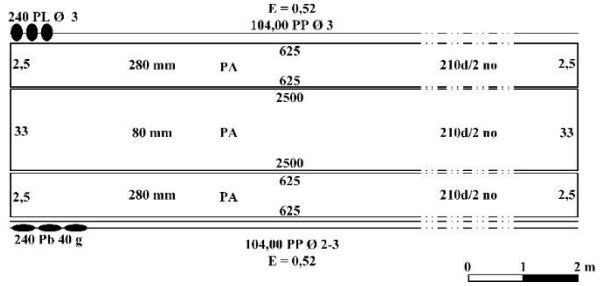
Multifilament, 64 veya 68 mm TGB'nda, 33 göz yüksekliğindedir ve 210d/2 no ip kalınlığındaki tor ağın boyu 90 m'dir. Ağın her iki tarafında bulunan 2,5 göz yüksekliğinde fanyalar 280 mm TGB'unda ve 210d/2 no ip kalınlığındadır. E=0,45 oranında donam uygulanmaktadır. Yaka halatlarında, üst yakada 3 mm ile alt yakada 2-3 mm kalınlıkta (biri koşma halatı) PP materyal kullanılmaktadır. Halatlarda 4 numara plastik mantarlar ile 40 g'lık bakla kurşun kullanılmaktadır (Şekil 7).



Şekil 7. Fanyalı uzatma ağı-I (Homa)
Figure. 7. Trammel net-I (Homa)

Fanyalı uzatma ağı-II (Homa/Karina)

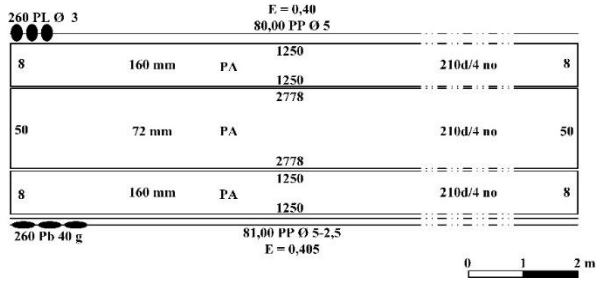
Multifilament, 80 mm TGB'nda, 33 göz yüksekliğindedir ve 210d/2 no ip kalınlığındaki tor ağın boyu 96 m'dir. Ağın her iki tarafında bulunan 2,5 göz yüksekliğinde fanyalar 280 mm TGB'unda ve 210d/2 no ip kalınlığındadır. E=0,48 oranında donam uygulanmaktadır. Yaka halatlarında, üst yakada 3 mm ile alt yakada 2-3 mm kalınlıkta (biri koşma halatı) PP materyal kullanılmaktadır. Halatlarda 3 numara plastik mantarlar ile 40 g'lık bakla kurşun kullanılmaktadır (Şekil 8).



Şekil 8. Fanyalı uzatma ağı-II (Homa)
Figure. 8. Trammel net-II (Homa)

Fanyalı uzatma ağı (Karina)

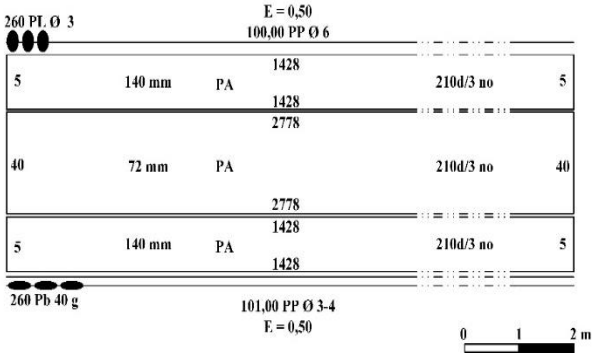
Multifilament, 72 mm TGB'nda, 50 göz yüksekliğindedir ve 210d/4 no ip kalınlığındaki tor ağın boyu 80 m'dir. Ağın her iki tarafında bulunan 8 göz yüksekliğinde fanyalar 160 mm TGB'unda ve 210d/4 no ip kalınlığındadır. E=0,40 oranında donam uygulanmaktadır. Yaka halatlarında, üst yakada 5 mm ile alt yakada 5-2,5 mm kalınlıkta (biri koşma halatı) PP materyal kullanılmaktadır. Halatlarda 3 numara plastik mantarlar ile 40 g'lık bakla kurşun kullanılmaktadır (Şekil 9).



Şekil 9. Fanyalı uzatma ağı-I (Karina)
Figure. 9. Trammel net-I (Karina)

Fanyalı uzatma ağı-I (Akköy)

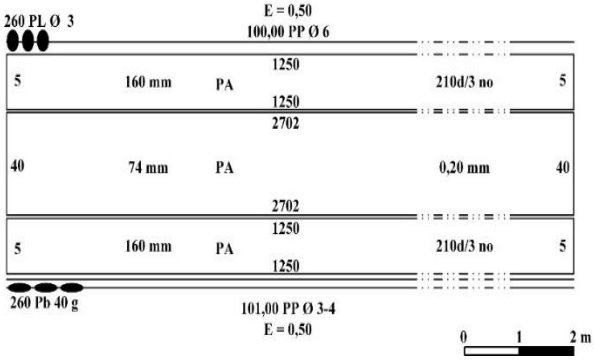
Multifilament, 72-74 mm TGB'nda, 40 göz yüksekliğindedir ve 210d/3 no ip kalınlığındaki tor ağın boyu 100 m'dir. Ağın her iki tarafında bulunan 5 göz yüksekliğinde fanyalar 140-160 mm TGB'unda ve 210d/3 no ip kalınlığındadır. E=0,50 oranında donam uygulanmaktadır. Yaka halatlarında, üst yakada 6 mm ile alt yakada 3-4 mm kalınlıkta (biri koşma halatı) PP materyal kullanılmaktadır. Halatlarda 3 numara plastik mantarlar ile 40 g'lık bakla kurşun kullanılmaktadır (Şekil 10).



Şekil 10. Fanyalı uzatma ağı-I (Akköy)
Figure. 10. Trammel net-I (Akköy)

Fanyalı uzatma ağı-II (Akköy)

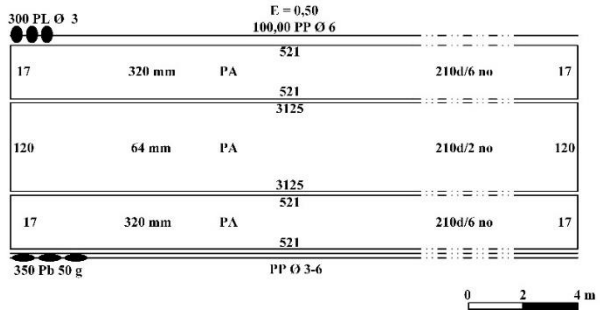
Monofilament, 74 mm TGB'nda, 40 göz yüksekliğindedir ve 0,20 mm ip kalınlığındaki tor ağın boyu 100 m'dir. Ağın her iki tarafında bulunan 5 göz yüksekliğinde fanyalar 160 mm TGB'unda ve 210d/3 no ip kalınlığındadır. E=0,50 oranında donam uygulanmaktadır. Yaka halatlarında, üst yakada 6 mm ile alt yakada 3-4 mm kalınlıkta (biri koşma halatı) PP materyal kullanılmaktadır. Halatlarda 3 numara plastik mantarlar ile 40 g'lık bakla kurşun kullanılmaktadır (Şekil 11).



Şekil 11. Fanyalı uzatma ağı-II (Akköy)
Figure. 11. Trammel net-II (Akköy)

Fanyalı uzatma ağı (Güllük)

Dalyanda tek tip uzatma ağı kullanılmaktadır. Bu ağ, PA materyalden yapılmış, 64 mm TGB'nda, 120 göz yüksekliğindedir ve 210d/2 no ip kalınlığındaki tor ağın boyu 100 m'dir. Ağın her iki tarafında bulunan 17 göz yüksekliğinde fanyalar 320 mm TGB'unda ve 210d/6 no ip kalınlığındadır. E=0,5 oranında donam uygulanmaktadır. Yaka halatlarında, üst yakada 6 mm ile alt yakada 3–6 mm kalınlıkta (biri koşma halatı) PP materyal kullanılmaktadır. Halatlarda 3 numara plastik mantarlar ile 50 g'lık bakla kurşun kullanılmaktadır (Şekil 12).

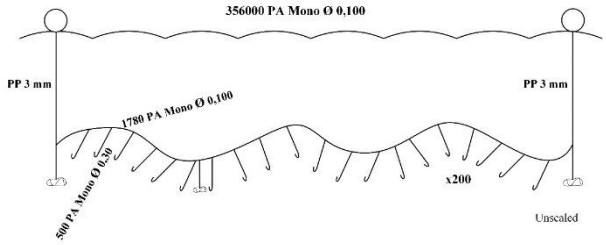


Şekil 12. Fanyalı uzatma ağı (Güllük)
Figure. 12. Trammel net (Güllük)

Paragatlar

Yılanbalığı paragatı (Enez)

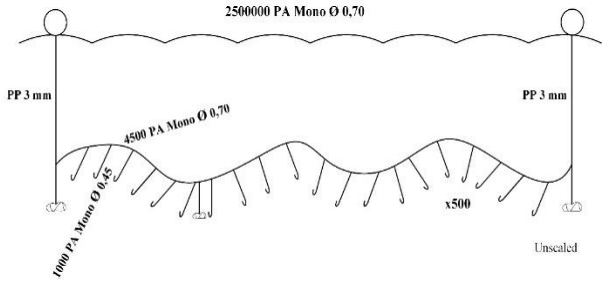
İğneler düz ve beyaz renklidir. Paragatın bedeni PA misina olup 0,70 mm çapındadır. 0,30 mm kalınlığında kösteklerin boyu 0,5 m'dir. İki köstek arası mesafe 1,78 m dir (Şekil 13). İğneler, sardalya- hamsi, mürekkepbalığı parçaları ve madya ile yemlenmektedir.



Şekil 13. Yılanbalığı paragatı (Enez)
Figure. 13. Longline for eel (Enez)

Yılanbalığı paragatı (Akköy/Karina)

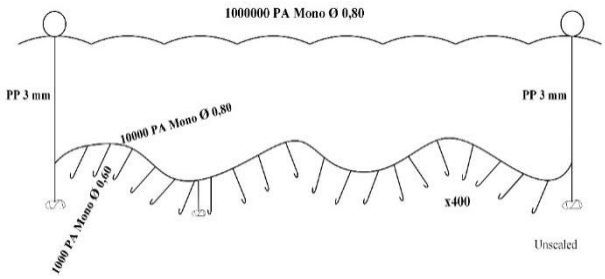
İğneler düz ve beyaz renklidir. Paragatın bedeni PA misina olup 0,70 mm çapındadır. 0,70 mm kalınlığında kösteklerin boyu 0,45 m'dir. İki köstek arası mesafe 4,5 m dir (Şekil 14). İğneler, sardalya- hamsi, mürekkepbalığı parçaları ve madya ile yemlenmektedir.



Şekil 14. Yılanbalığı paragatı (Akköy/Karina)
Figure. 14. Longline for eel (Akköy/Karina)

Levrek paragatı (Akköy/Karina)

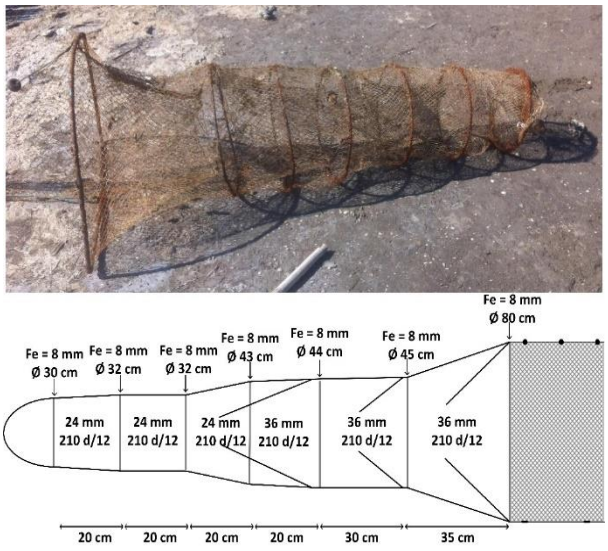
İğneler düz ve beyaz renklidir. Paragatın bedeni PA misina olup 0,80 mm çapındadır. 0,50-0,60 mm kalınlığında kösteklerin boyu 1 m'dir. İki köstek arası mesafe Akköy dalyanında 3 m, Karina dalyanında 10 m dir (Şekil 15). İğneler, sardalya- hamsi, mürekkepbalığı parçaları ve madya ile yemlenmektedir.



Şekil 15. Levrek paragatı (Akköy/Karina)
Figure. 15. Longline for seabass (Akköy/Karina)

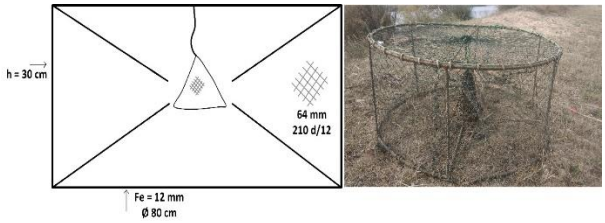
Pinter ve sepetler

Dalyanların tamamında yılanbalığı avcılığı için pinterler kullanılmaktadır. Yılanbalığı avcılığında yağmurlar ile dalyanı besleyen tatlı su girişlerindeki debi artışı önemli rol oynamaktadır. Bu nedenle pinter avcılığı yağmurların başladığı dönem olan Ekim'den itibaren Mart ayına kadar sürmektedir. Bu dönemde dalyan içerisine genellikle 600-700 adet pinter bırakılarak, gün aşırı veya her gün pinterler ellenerek avcılık gerçekleştirilir. Pinterler ağızları 80 cm'den 30 cm genişliğe doğru daralarak devam eden 7 çemberden ve ilk 3 çember içerisinde bulunan boğazlardan oluşmaktadır (Şekil 16).



Şekil 16. Pinter (Akköy)
Figure. 16. Fyke net (Akköy)

Diğer dalyanlardan farklı olarak Enez dalyanında 'davul' adı verilen bir sepet ise bölgede mavi yengeç (*Callinectes sapidus* Rathbun, 1896) avcılığında kullanılmaktadır. Davul tuzakların çapı 80 cm ve yüksekliği 30 cm olacak şekilde 64 mm ağ gözü ile donatılmışlardır. Sepetlerin karşılıklı olarak 2 adet giriş boğazları bulunmaktadır. Orta kısımda bulunan yem torbasına balık artıkları konulmak suretiyle bahar ve yaz aylarında yoğun olarak kurulmaktadır (Şekil 17).



Şekil 17. Davul sepet (Enez)
Figure. 17. Pot (Enez)

Dalyanlarda avlanan ekonomik türler

Enez dalyanında avcılığı yapılan 7 familyaya ait 14 tür, Homa dalyanında 3 familyaya ait 7 tür, Karina dalyanında 5 familyaya ait 9 tür, Akköy dalyanında 5 familyaya ait 12 tür ve Güllük dalyanında 5 familyaya ait 9 tür tespit edilmiştir (Tablo 1).

Tablo 1. Avlanan türlerin dalyanlara göre dağılımı
Table 1. Distribution of captured fish species at lagoons

Tür	Enez	Homa	Karina	Akköy	Güllük
Anguilla anguilla Linnaeus, 1758) (Yılan balığı)	+		+	+	+
Callinectes sapidus (Rathbun, 1896) (Mavi yengeç)	+				
Cyprinus carpio (Linnaeus, 1758) (Sazan)					+
Dicentrarchus labrax (Linnaeus, 1758) (Levrek)	+	+	+	+	+
Chelon auratus (Risso, 1827) (Altınbaş kefal)	+	+	+	+	+
Chelon labrosus (Risso, 1827) (Mavraki)	+	+	+	+	+
Chelon ramada (Risso, 1827) (İnce dudaklı kefal)	+	+	+	+	+
Chelon saliens (Risso, 1810) (Kastroz)	+	+	+	+	+
Mugil cephalus (Linnaeus, 1758) (Has kefal)	+	+	+	+	+
Solea solea (Linnaeus, 1758) (Dil balığı)	+		+	+	
Diplodus sargus (Linnaeus, 1758) (Sargoz)	+			+	
Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817) (Karagöz)	+			+	
Lithognathus mormyrus (Linnaeus, 1758) (Mırmır)	+			+	
Sparus aurata (Linnaeus, 1758) (Çipura)	+	+	+	+	+
Platichthys flesus (Linnaeus, 1758) (Pisi balığı)	+				

TARTIŞMA VE SONUÇ

Bu araştırmada, Ege bölgesinde aktif halde bulunan 5 dalyandan Enez dalyanı, Homa dalyanı, Karina dalyanı, Akköy dalyanı ve Güllük dalyanında kullanılan av araçlarının ve hedef türlerin çeşitliliği ortaya konmuştur. Dalyanlarda aktif olarak balıkçılık faaliyetinde bulunan balıkçıların çoğunluğu aynı zamanda bölgelerinde deniz balıkçılığı faaliyetlerinde de bulunmaktadır. Dünyada da lagünlerde balık avcılığında birden fazla türün avcılığının hedeflendiği ve bunun gerçekleşmesi için çeşitli av araçlarından kuzuluk, pinter, sepet vb. tuzaklar, uzatma ağları (fanyalı veya sade), olta ve paragat takımlarının kullanıldığı bilinmektedir (Cataudella vd., 2015).

Dalyanlarda kullanılan av araçları denizlerde küçük ölçekli balıkçılıkta kullanılan av araçları ile benzerlik göstermektedir. Tüm dalyanlarda genellikle fanyalı kefal uzatma ağları değişik versiyonlarıyla ortak av aracı olarak kullanılmaktadır. Yılanbalığı paragatı üç dalyanda (Enez, Karina ve Akköy) kullanılırken, levrek paragatı sadece iki dalyanda (Akköy ve Karina) gözlenmiştir. Monofilament misina uzatma ağı ise sadece Enez ve Akköy dalyanlarına kullanılan av aracı olarak belirlenmiştir. Kaykaç ve Tosunoğlu (2015), Karina ve Akköy dalyanındaki pasif av araçları içerisinde pinter, paragat ve uzatma ağlarının yapısını ortaya koymuşlardır. Bu çalışmaya benzer olarak, levrek için paragat takımı kullanıldığından ve aynı zamanda yılanbalığı pinter avcılığından bahsetmişlerdir. Bu çalışmada, diğer dalyanlardan farklı olarak Enez dalyanında 'davul' adı verilen bir sepet tuzağın mavi yengeç (*Callinectes sapidus*) avı için kullanıldığı ilk kez ortaya konmuştur.

İncelenen dalyanlardaki aktif kullanılan kuzuluk sayısı ve bazı kısımlarında yapım materyali bölgelere göre farklılık göstermiştir. Kuzulukla avcılık sistemi hedef türlerin sıcak dönemlerde dalyana giriş yapması ve su soğuması ile denize geçiş yapması prensibine dayanmaktadır ve incelenen dalyanlardaki kuzulukların açık ve kapalı olduğu aylar dalyanların bulunduğu bölge sıcaklığına göre farklılık göstermektedir.

Uzatma ağları açısından dalyanları karşılaştırdığımızda, bütün dalyanlarda fanyalı uzatma ağlarının kefal ve levrek avı için kullanıldığı görülmektedir. Çalışmada sadece Enez ve Akköy dalyanlarında monofilament (misina ağ) sade uzatma ağının kullanıldığı tespit edilmiştir. Bu durum çalışmanın yapıldığı dönemdeki 3/1 numaralı Ticari Amaçlı Su Ürünleri Avcılığını Düzenleyen Tebliğ'e (Anonim, 2012) uymamaktadır. Balıkçılarla yapılan görüşmelerde balıkçılar bu durumun farkında oldukları; fakat bazı dönemlerde yosun oluşumunun çok olduğundan ve yosunlu zeminde misina ağlar ile avcılık sonrasında ağların daha rahat temizlendiği gerçeğinden hareketle balıkçıların bu ağları kullandıkları düşünülmektedir. Monofilament ağ kullanımı, en son yayımlanan 5/1 numaralı Ticari Amaçlı Su Ürünleri Avcılığını Düzenleyen Tebliğ ile bu ağların yasak kapsamı sadece deniz avcılığı olarak sınırlandırılmış ve lagünlerdeki avcılık serbest bırakılmıştır

(Anonim, 2020). Tosunoğlu vd. (2017) Homa dalyanında monofilament misina ağlarının yasaklandığı yıl olan 2011 yılına kadar yoğun olarak kullanıldığından, ancak sonrasında kargılı ağ ve pinterlerin yanı sıra dalyanda 32, 36 ve 40 mm fanyalı kefal uzatma ağlarının Ekim-Aralık ayları arasında yoğun kullanıldığından bahsetmişlerdir. Fakat bu çalışmada kargılı ağ kullanımına rastlanmamıştır.

Enez, Akköy ve Karina dalyanlarında paragat avcılığı da yapıldığı kaydedilmiştir. Paragat avcılığı donamı dalyanlara göre bazı farklılıklar gösterse de hedef türlerin yılanbalığı ve levrek olduğu tespit edilmiştir. Burada Akköy ve Karina dalyanları birbirlerine komşu dalyanlar olarak az farklı benzer paragatlar kullandıkları belirlenmiştir. Paragat yemi olarak hepsinin hamsi veya sardalya, mürekkepbalığı parçaları ile madya kullandıkları anlaşılmıştır. Aynı zamanda yılanbalığı avcılığında tüm dalyanlarda pinter kullanımı da söz konusudur.

Çalışmada incelenen dalyanlar içerisinde ekonomik değere sahip toplam 8 familyaya ait 15 tür (*A. anguilla, D. labrax, C. auratus, C. labrosus, C. ramada, C. saliens, M. cephalus, S. solea, D. sargus, D. vulgaris, L. mormyrus, S. aurata, P. flesus, C. carpio* ve *C. sapidus*) tespit edilmiştir. Bu türlerin en büyük çeşitliliğinin 14 türle Enez dalyanında olduğu görülmüştür. Ayrıca, Akyol ve Ceyhan (2010), Enez Dalyanı'nda 1997-2007 yılları arasında bu türlerin yanı sıra Tablo 1'de yer almayan *Pomatomus saltatrix*, *Mullus barbatus*, *Umbrina cirrosa*, *Sarpa salpa* ve *Penaeus kerathurus* gibi türlerin de az da olsa dalyandan çıktığını belirlemişlerdir. *C. carpio* ise Enez dalyanında tuzluluğun iyice azaldığı yağmurlu dönemlerde kısmen ortaya çıkan hedef dışı bir tatlı su balığıdır.

Dalyanlarda balıkçılık, iyi denetlendiği sürece hem dalyanın tercihen bir kooperatifçe aktif kullanılması yoluyla sahiplenilmesi, hem de bulunduğu yöreye sağlayacağı kaliteli ve ucuz balık gıdası için faydalı bir faaliyet olarak değerlendirilebilir. Burada hassas bir ekosistemde avcılık yapıldığı göz önüne alınarak, balıkçı sayısının ve av aracı normlarının iyi tespit edilmesi ve seçici takımlarla av yapılması gerektiği unutulmamalıdır. Lagünler ve sulak alanlar, kuşları ve balıklarıyla bulundukları coğrafyayı zenginleştiren, önemli sahalardır. Bu lagüner sahaları ve geleneksel dalyan balıkçığını sürdürülebilir kılmak ve gelecek nesillere aktarabilmek için yerel yönetimler, bakanlık, kooperatifler, sivil toplum kuruluşları ile üniversitelerin iş birliği sürekli kılınmalıdır.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Occupational health and safety in large-scale fishing vessels registered in Aegean ports

Ege limanlarına kayıtlı büyük-ölçekli balıkçı teknelerinde iş sağlığı ve güvenliği

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Abstract: This study aimed to determine the occupational safety knowledge level of the fishers and the factors affecting it in large-scale (trawl-purse seine) fishing vessels registered in Aegean ports. In the survey study, conducted with the participation of 295 person from both types of fishing, 40% (118) of the participants consisted of fishers working in trawlers and 60% (177) in purse-seiners. The knowledge level of 51.5% of the fishers was insufficient under score 7, while 48.5% of them were found to be sufficient with score 8 or more. It was determined that 40% of the fishers had occupational accidents, and hastiness with 15.5%, carelessness with 14.9% and fatigue with 5.8% followed the first place among the causes of accidents. It has been determined that the accidents occurred not only during the operation, but also at the port operations and during the cruise. As a result, it is considered that awareness and training activities to increase the knowledge level of fishers on occupational health and safety will be the most effective method in reducing the possibility of accidents and getting occupational diseases.

Keywords: Trawl, purse seine, accident, safety

Öz: Bu çalışma, Ege limanlarına kayıtlı büyük ölçekli (trol-gırgır) balıkçı gemilerinde, çalışanların iş güvenliği bilgi düzeylerini ve bunları etkileyen faktörleri belirlemeyi amaçlamıştır. Her iki balıkçılık tipinden toplam 295 kişinin katılımı ile gerçekleştirilen anketin, %40'ını (118) trol teknesinde, %60'ını da (177) gırgır teknesinde çalışan balıkçılar oluşturmuştur. Balıkçıların %51,5'inin bilgi düzeyi 7 puanın altında yetersiz kalırken, %48,5'i ise 8 puan ve üzerinde yeterli bulunmuştur. Balıkçıların %40'ının iş kazası geçirdiği tespit edilmiş olup kaza nedenleri arasında ilk sırayı %15,5 ile acelecilik, %14,9 ile dikkatsizlik ve %5,8 ile yorgunluk takip etmiştir. Kazaların, sadece operasyon sırasında değil, limanda ve seyir sırasında da yaşandığı tespit edilmiştir. Sonuç olarak balıkçıların iş sağlığı ve güvenliği konularında bilgi düzeylerini artırmaya yönelik farkındalık ve eğitim çalışmalarının, kaza yapma ve hasta olma olasılıklarını azaltmada en etkili yöntem olacağı değerlendirilmektedir.

Anahtar kelimeler: Trol, gırgır, kaza, güvenlik

INTRODUCTION

Commercial fishing is one of the oldest occupation and regarded as the most dangerous job due to its workplace and working conditions in the world (Msed, 2010). Furthermore, fishing turns out to be an occupational group with high disease and accident rates. There are many risks in the activities performed in fishing vessels. Every year more than 24 000 fishermen lose their lives and about 24 million fishermen are estimated to be injured in this sector worldwide (Håvold, 2010). The injury and mortality rates caused by fishing in Australia, America and many European countries are substantially higher than the average rates. Jin and Thunberg (2005) reported that 16 out of every 10 000 fishers died in various accidents at sea in America in 1996 and that this rate was 16 times greater than the deaths experienced at the fire and police departments. According to a study carried out in England between 1976 and 1995, some 454 (74%) of the 616 fishermen who lost their lives died as a result of the accident they got involved in while working and 394 of them (87%) drowned. When compared with the other sectors, the rate of fishermen's getting involved in an accident resulting in death is 52.4 times greater (Roberts and Roberts, 2005). The dangerousness of fishing may be ascribed to many factors like the fact that people work for quite long hours under the hardest conditions of sleeplessness, the cold, and wetness. A safe and healthy working environment may be provided as a result of fishermen's knowledge of the existing risks and the fulfillment of sector-related legal obligations.

Although fishing is known to have high disease and mortality rates, there is rather little research on the effect of working conditions on the health of fishers particularly in developing countries (Matheson et al., 2001). Jin and Thunberg (2005) determined the common factors concerning the accidents in fishing vessels as the poor condition of the

fishing vessel or its equipment, inadequate emergency training, inadequacy in using the water survival equipment and the attention deficit about the vessel balance. In their study on this subject, Wang et al. (2005) concluded that a high rate (20.7%) of the accidents experienced in fishing vessels was caused by the crew and that, apart from this, the inadequacy of the number of crew members, their fatigue, the lack of crew management, poor boat design, hard working conditions and inappropriate arrangements substantially increased the accident rate.

Turkey has a total of 14 092 fishing vessels; 370 of them are purse seines and 790 of them are trawlers according to 2019 fishing records (TUİK, 2020). A total of 28 717 fishermen serve on these vessels, 3 649 in trawlers and 7 549 in purse seiners. A total of 5 995 fishers work in 63 trawler and 55 purse seiner in the Aegean Sea (TUİK, 2020). According to Social Security Institution of Turkey, the number of occupational (fishing and aquaculture) accidents in 2017 and 2020 are 273 and 372, respectively (SGK, 2021). From the reference, the number of occupational accidents in the fishing activities in Turkey has gradually increased over the years. Studies on the on occupational health and safety related to fisheries in Turkey are very limited. For this reason, this study aims to determine knowledge level of the fishers working in large-scale fishing vessels regarding occupational safety and factors influencing it.

MATERIALS AND METHODS

The numbers of trawler (63) and purse seiner (55) vessels operating in the Aegean Sea with a length greater than 12 meters were obtained from the Izmir Provincial Directorate of Agriculture. This study was carried out with 33 trawlers and 32 purse seiners and their employees (295 fishers).

A questionnaire was prepared by making literature review and taking in consideration International Convention on Standards of Training, Certification and Watchkeeping for Seafarers (STCW) prepared by the International Maritime Organization IMO, Fishing Boats Sea Survey Checklist of the Ministry of Transport, Maritime Affairs and Communications, Seafarers Regulations and Regulation on the Safety of Fishing Vessels published by this ministry and Regulation on Health and Safety Precautions in the Work Performed on Fishing Vessels published by Ministry of Labor and Social Security, and the Occupational Health and Safety Law No. 6331.

The questionnaire consisted of 35 questions in total. The first 15 questions were related to socio-demographic variables. In the survey, there are 6 questions that measure the level of knowledge about maritime safety and 10 questions about occupational accidents and work safety.

The scoring of the questions related to the level of knowledge is given in Table 1. According to the average score of the participants according to the knowledge level scores; those who scored “average and below” had “insufficient knowledge”, those who scored above the average were evaluated as “sufficient knowledge”.

This study covered the large-scale fishing vessels, purse seiners and trawlers registered in the Aegean Sea ports. Sample selection was carried out by simple random sampling method (Kadilar and Cingi, 2006). The total number of fishers working on trawler and purse seine vessels was firstly estimated, and then it was calculated to conduct a sub sampling group a total of 1250 person sampling volume (Calculated as Prev 50%, Pattern Effect 1, Error 5%, Confidence Interval 95%). A total of 295 person participated in the survey. Of the person who participated in the survey, 118 were trawl and 177 purse seine fishermen. Some of the fishermen participating in the study were captains and personnel of the vessels.

The independent variables in the study were age, experience, whether or not he has a second profession, marital status, educational status, reason for choosing fishing, thinking of quitting fishing, wanting his children to do this job, position, type of boat he works, daily working time, income, income satisfaction, social security and document he has identified as the type. Dependent variables were determined as the level of knowledge about safety at sea and measured as "Occupational Health and Safety Score", which was formed from the answers to the questions.

The survey data were evaluated using the SPSS 18 package program. Chi-square test was used to find differences between the groups according to $p<0.05$.

Table 1. Questions regarding maritime safety knowledge and score table

Questions	Answers	Score
Which role scale does your ship have?	Fire, Abandon ship Man over board	3
Should it be done frequently until abandon ship or fire drills?	Once a month	1
In what situations is an emergency alert issued on the vessel?	Fire, Abandon ship Man over board	3
How many fire extinguishing equipment should be on the vessel?	At least 2 trawlers At least 3 purse seiners	1
What is the protective equipment that must be used in terms of safety when going on deck in bad weather conditions and at night?	Life jacket	1
Can you tell me a feature that should be on life buoys on the vessel?	Reflective tape, Smoke, Light	3
Total Score		12

RESULTS

According to socio-demographic data, the mean age and age group of the fishers were found 44.6 ± 9.93 years and 25-69 years. The average experience of the fishermen was 26.6 ± 10.7 years. About ¾ of the fishermen were married and 58.3% of them graduated from elementary school, while only 2% of them had higher education. It was determined that 53.6% of them performed fishing as it was their fathers' occupation. 21% of them were skippers, whereas 79% of them were crew. 84.1% of them stated that they worked more than 13 hours per day. 72.9% of them stated that they earned their income for a share. It was found that 22% of them had a Master certificate that 77.6% of them had a Fisherman-class deck crew certificate, and that 0.3% of them had a Deck-class seaman certificate.

It was determined that, 62.4% of the fishermen smoked and 47.1% consumed alcohol. In the findings, 99.7% of the fishermen stated that they wore gloves while working on the boat. 96.3% of them stated that they wore a hard hat in addition to wearing gloves. In our study, it was found that the types of accidents were distributed as follows: 16.7% cut injuries and 8.5% fall injuries (Table 2).

In the study, it was determined that the accidents were caused by different reasons. While 69% of the accidents (118 fishers) occurred during fishing, accidents experienced during navigation and port operations were 18% and 13%, respectively.

Table 2. Distribution of the types of accidents

Type of Accident	Number	Percent (%)
Cut	40	16.7
Fall	25	8.5
Hitting	11	3.7
Striking	7	2.4
Slipping and falling	5	1.7
The falling of the material	5	1.7
Hitting and striking	5	1.7
Hand and arm injuries	3	1.0
The falling of the equipment	3	1.0
The falling of a man into the sea	2	0.7
Getting stuck	1	0.3
The falling of an object	1	0.3
The falling of an eyebolt	1	0.3
Total	118	40.0

The busy work schedule (hastiness) was the primary reason for the accidents on boats. Lack of experience was more frequently seen among those crew members who have just started working on the boat (Figure 1).

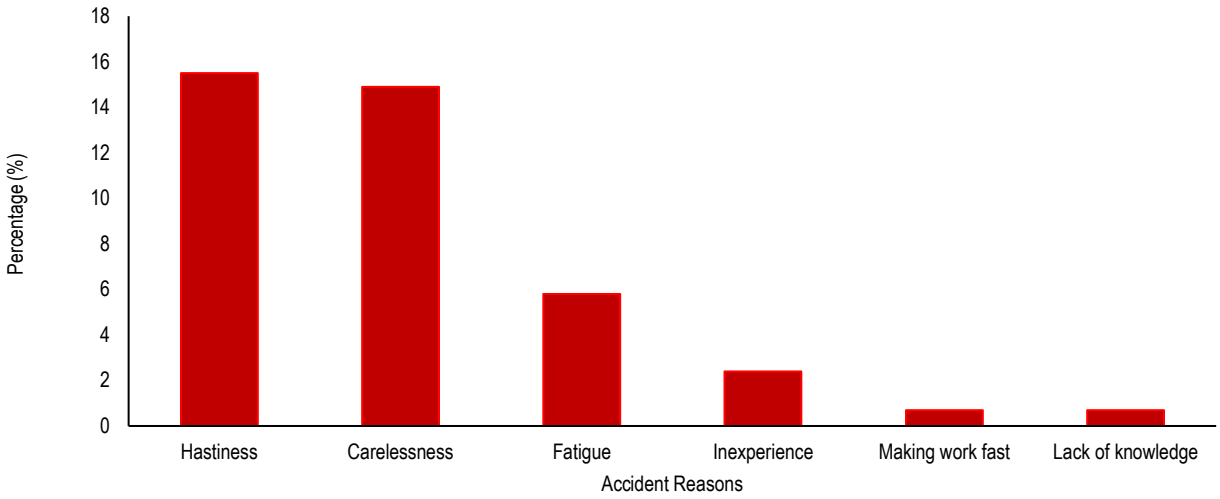

Figure 1. The percentage of the accidents according to the occurrence reasons

In the assessments of safety knowledge level of the fishers, 51.5% was found insufficient with scores 7 and below (inadequate), while 48.5% reached sufficient level with scores 8 and above (adequate). In the statistical evaluation made between the factors affecting the knowledge levels of the fishermen and the other variables, it was seen that some variables were statistically significant (Table 3).

In addition a statistical evaluation was made to determine the factors affecting the occupational accidents. The variables of marital status, reason for choosing fishing, thinking of quitting fishing, boat type, working time, income, and social security were included in the analysis. The results were given in Table 4.

Table 3. The factors affecting the knowledge levels of the fishermen (p<0.05)

Factors	*p value*
Age	0.622
Experience	0.328
Non-fishing occupation	0.580
Marital status	0.132
Education	**0.048**
Reason for choosing fishing	0.484
Thinking of quitting fishing	**0.072**
Children's doing this job	0.900
Type of boat	**0.000**
Duty	**0.000**
Working period	**0.000**
Income	**0.058**
Income satisfaction	0.908
Social security	**0.000**
Certificate held	**0.000**
How safe is fishing	**0.013**

Table 4. The factors affecting the occupational accidents (p<0.05)

Factors	*p value*
Age	0.717
Experience	0.298
Non-fishing occupation	0.333
Marital status	**0.026**
Education	0.306
Reason for choosing fishing	**0.005**
Thinking of quitting fishing	**0.020**
Children's doing this job	0.059
Type of boat	**0.000**
Duty	0.129
Working period	**0.001**
Income	**0.032**
Income satisfaction	0.543
Social security	**0.018**
Certificate held	0.152
How safe is fishing	0.392

It was statistically determined that there was no significant difference between knowledge levels and occupational accidents (Table 5).

Table 5. Distribution of the occupational accidents by knowledge level group.

	Experiencing of an occupational accident					
	Yes		No		Total	
Knowledgelevel	Number	%	Number	%	Number	%
Adequate	62	21	81	27.5	143	48.5
Inadequate	56	19	96	32.5	152	51.5
Total	118	40	177	60	295	100

DISCUSSION AND CONCLUSION

The occupational health and maritime safety knowledge level of more than half of fishermen participating in the study was insufficient. McGuinness et al. (2013) conducted a study investigating deaths in the Norwegian fishing fleet in 1990-2011. He found that fishermen had severely limited knowledge of health and safety practiced within the common coastal industry. It has been determined that the participants, who are sufficient in terms of their knowledge level about health and occupational safety at sea, have 1-20 years of experience. However, it was found that there was no significant relationship between work experience and knowledge levels.

In the study, we asked the participants about occupational accidents. It was stated that 40% of them had work accidents, 60% had no accident. In a study Håvold (2010) carried out in Norway concerning the safety culture in fishing vessels, it was stated that the primary accidents with injuries most experienced between 1999 and 2008 by Norwegian fishermen were falls on the vessel. In the study Piniella et al. (2008) carried out on the safe working conditions of Andalusian fishermen, they stated that 35% of the most frequently experienced injuries were caused by cuts. They further stated that of the other accidents, 15% were due to the striking of an object, 12% were accidents resulting from the moving equipment, and 8% were due to falls from high levels, followed by hitting and striking. In his study on the English commercial fishing, Roberts (2010) stated that 86% of the fatal fishing accidents experienced in England between 1996 and 2005 resulted from drowning. However, in our study, it was determined that the most common accidents experienced by fishers in Turkey were injuries caused by cuts, followed by fall and crash-impact accidents, respectively.

As stated by the fishers in this study that hand injuries constituted a high risk among the accidents experienced in the ports. McGuinness et al. (2013) investigated the deaths in the Norwegian fishing fleet, they stated that 20% of the most important mortality rates were comprised of the disasters of drowning in the port. They stated that the most common headings among all groups in a period of 22 years were drowning in the port (20%) and getting stuck/striking (10%). In their study for the classification and coding of the commercial fishing injuries in Denmark according to the work processes, Jensen et al. (2005) stated that the injuries concerning the preparation and repair of the equipment included in the work processes while working in the port made up about half of all injuries. They indicated the work processes as the repair of nets, installation of the otter boards used, working with gear, and the repair of gear. In another study carried out in Denmark, it was stated that the commercial fishermen generally fell from the wharf or that there was a risk of being damaged after falling from a lower level and that, in some cases, they could even drown within the port. It was explained that these tragedies could have been connected with the fact that the fishermen went to foreign ports and returned to the

vessel at nights (Jensen et al., 2005). In the study of Thomas et al. (2001) investigated the fatal and nonfatal workplace injuries of Alaskan commercial fishermen, they stated that fall injuries were the most common ways of injury, that twenty-four of these injuries had not taken place on a vessel, and that most of them had occurred as a result of falls from the port. Moreover, in our study, it was determined that foot and head injuries could also occur in the ports and that they made up half of these injuries.

Fishermen generally stated that they had an accident while fishing. It was found that the accidents had been experienced during the operation and that no fatal injury had occurred. According to Roberts (2004), most of the fatal accidents at sea happen while leaving or collecting the fishing gear during the operation. In the classification of the commercial fishing injuries in Denmark according to the work processes, the injuries occurring while pulling the gear and equipment at sea made up half of the injuries caused by the repair of the gear and the collection of the nets in Denmark trawl fishing. The work processes of collecting the nets include such practices as the casting and removal of the nets as well as the throwing and removal of the otter boards (Jensen et al., 2005). These practices were determined to have caused injuries in various areas of the body. The highest rate among them belongs to the accidents in which the whole body was injured. It was established that the overwhelming majority of the occupational accidents had developed during fishing and that these accidents had damaged the whole body. It was stated that the majority of the occupational accidents experienced on the fishing vessels had been experienced during the operation from the studies.

The accidents experienced during navigation were determined depending on the statements by the fishermen. Accidents during navigation could damage any part of the fisherman's body, moreover, the feet and heads were the most frequently injured limbs. The accidents had been experienced not only during the operation but also in the port operations. Almost all fatal occupational injuries in the US commercial fishing industry was occurred during navigation or as a result of docking (NIOSH, 2010). Thomas et al. (2001) investigated the fatal and nonfatal workplace injuries of Alaskan commercial fishermen, they determined that at least 90% of the injuries was occurred on vessel, 5% on land and generally at the pier, and that the scenes of the other 5%.

Roberts (2010) stated that fatal accidents resulted from fishing under bad weather conditions. Furthermore, he stated that the accidents experienced under bad weather conditions depended on the fishing performed without having adequate consciousness of safety.

Wang et al. (2005) analyzed the accidents on fishing boats, they stated that a high percentage (20.7%) of the accidents experienced had been caused by the negligence or carelessness of the employees. They further stated that employees' competence, fatigue, poor management of the vessel, hard working conditions, poor design and inappropriate arrangements made great contributions to such accidents. In our study, hastiness ranks first with 15.5% among the reasons for accidents, followed by carelessness with 14.9% and fatigue with 5.8%. Hastiness appeared the most important of the reasons for accidents.

In the study Piniella et al. (2008) carried out on the safe working conditions of Andalusian fishermen, they stated that the accidents experienced were found significant only in terms of age, work experience, and service period. Such factors as marital status, type of boat, working period, and income status were found statistically significant in our study. Additionally, in our study, it is seen that such factors as educational status, type of boat, duty, working period, and income status among the factors affecting the knowledge levels were statistically significant. They do not feel themselves in danger in terms of the job. This reveals that fishermen's knowledge levels about maritime safety were inadequate. They must use the necessary equipment and gear while performing their duties. It is of importance that they have adequate occupational knowledge. It should be known that even the minor mistake made while working might lead to serious injuries and even deaths.

Fishing sector cannot benefit from occupational health and safety services sufficiently. It was seen that this was due to a lack of education and knowledge. It has been determined that training is the first and most effective method on occupational safety and worker health, and even on safety at sea. It is important to reduce occupational accidents in the fishing sector and to increase the corresponding labor productivity. Creating occupational safety awareness among fishers is also important. Raising awareness of fishermen about their activities and providing safer working conditions will contribute to fisheries management.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Diversity of the EPT complex (Ephemeroptera, Plecoptera and Trichoptera) in the Western and Eastern Ghats (South India) caused by the variations of landscape elements and mesohabitats

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Abstract: The present study was carried out in 27 streams of the southern part of the Western Ghats and the Eastern Ghats from January to December 2018. The outcomes show that there is an increase in the percentage of Ephemeroptera (71%) in the first order and there was a negligible fall in the abundance of the fourth-ordered stream. LSE results show a gradual increase of Plecoptera and a gradual decrease of Trichoptera and no such variations in Ephemeroptera. Variations in mesohabitat richness were highest in the run (38.38%) and it was lowest in silt (0.5%). To compare the taxa composition in Western and Eastern Ghats taxa, five different types of river basins were classified. The species richness and abundance were higher in Tampiraparani East flowing and Vamanapuram River basin communities (Western Ghats) than in the Eastern Ghats and this shows mega diversity of Western Ghats compared to the Eastern Ghats. The results of both cluster and ordination analysis also strongly support the discrimination between Western and Eastern Ghats diversity and distribution of EPT taxa.

Keywords: Western Ghats, Eastern Ghats, LSE, PCA, DCA, ordination

INTRODUCTION

The larvae of Ephemeroptera, Plecoptera, and Trichoptera are important members of the freshwater ecosystem due to nutrient cycling (Ross and Wallace, 1983), coarse organic particulate breakdown and they form the diet of many fishes and other aquatic vertebrates (Wiggins, 1996; Pflieger, 1997). Moreover, members of these orders generally act as bioindicators of good water quality (Rosenberg and Resh, 1993; Wiggins, 1996), although an exception exists. The altitudinal/ latitudinal gradient which have a direct and indirect influence on spatial distribution and community structure of organisms (Sivaramakrishnan and Venkataraman, 1990). The anthropogenic factors or stressors (Dinakaran and Anbalagan, 2007), riparian landuse (Subramanian et al., 2005; Chakona et al., 2009), and habitat heterogeneity also determine the diversity and distribution of EPT organisms.

Studies of aquatic insects give more knowledge about the species-habitat relationship and interpretation of water quality (Azrina et al., 2005). Pollution becomes a major concern nowadays; it causes adverse effects to aquatic ecosystem health. Aquatic insects play a vital role in the functioning of the stream ecosystem. EPT along with other benthic macroinvertebrates find a wide range of suitable substrates in headwater streams. EPT diversity increases with habitat diversity. Given the lack of information concerning EPT biodiversity of some unexplored River basins in Eastern and Western Ghats, especially those in the unprotected areas, and in the context of the high potential for these organisms to be important and diverse biotic components of the stream ecology.

This study is aimed to characterize the diversity of mayflies, stoneflies, and caddisflies taxa occurrences in terms of geographic distributional patterns, species-genera/ habitat preferences, and distinctive faunal elements. This EPT faunistical survey is essentially descriptive in scope and attempts to investigate patterns of EPT richness and assemblage structure and to test their correlation to physico-chemical parameters associated with stream altitudinal/latitudinal environmental gradients.

MATERIAL AND METHODS

Study area

The present study was carried out in 27 streams of the southern part of the Western Ghats and the Eastern Ghats

(Figure 1) from January to December 2018 and they were listed in Table 1. Each sampling site was selected after assessing the habitat heterogeneity, canopy cover, and riparian taxa. Each stream was sampled during three seasons namely summer (February, March, April, May), south-west monsoon (June, July, August, September), and north-east monsoon (October, November, December, January).

Measuring water quality parameters

The physico-chemical parameters of stream water like dissolved oxygen, pH, conductivity, hardness, alkalinity, calcium, magnesium, sodium, iron, and chloride were analyzed in all the 27 sites using the guidelines of APHA, (2005). Water temperature was measured with a thermometer. Water velocity was measured by a flow meter.

Macroinvertebrate collection and identification

EPT complex was sampled by using a 1m wide Kick-net (Burton and Sivaramakrishnan,1993) with a mesh size of about 1mm. Limited opportunistic collections (hand picking) were also made. The organisms were carefully picked from the net and were preserved in 80% ethyl alcohol. All specimens from each of the 27 streams were sorted and identified with the help of a field guide by Sivaramakrishnan et al. (1998) and other taxonomic literature.

Analysis of data

Alpha and beta diversity

Alpha and beta diversity were used to measure the generic diversity within and between latitudinal and altitudinal zones. Simpson's index and Shannon-Wiener index were used to calculate alpha diversity whereas Jaccard's index was used to measure the beta diversity. The data analysis was done with the help of the PAST software to measure the various diversity indices (Hammer et al. 2001).

Cluster analysis

Insect assemblages were analyzed with relative abundance data, cluster analysis employing both Q (the relationship between regions based on the description of taxa) and R (measures the relationship between descriptions based on regions) modes were performed using the unweighted group method of arithmetic averages (UPGMA). To test that the data contains clusters, the matrix of the original data to produce a cophenetic correlation value. Values of $r>0.9$, $0.8<0.9$, $0.7<0.8$, and $r<0.7$ indicate very good, poor, and very poor fits (Unmack, 2001). Spearman similarity matrix is a widely used clustering procedure used to group stations.

Principal component analysis and detrended correspondence analysis

Principal Component Analysis (PCA) is employed to evaluate the relationship between the abundance of taxa and the environmental variables of 27 study sites. Sites having unique physico-chemical features were being clustered together, whereas the ones having extreme conditions were plotted very far. Detrended Correspondence Analysis (DCA) is used to compare the Western and Eastern Ghats species richness and abundance. PCA and DCA were analyzed using PAST software (Hammer et al., 2001).

Description of mesohabitats

Based on flow, depth, and substrate mesohabitat has been evolved following the method of Vadas and Orth (1998) which was characterized by the EPT and habitat associations in temperate streams (Ferro and Sites, 2007). Seven mesohabitats were identified in the study.

The landscapes in study localities were classified into six Land Scape Element (LSE) types. The landscapes were assigned to specific LSE types (Nagendra and Gadgil 1998; Ghate et al., 1998) in the Western Ghats. LSE types of 27 stations are given in Table 2.

Table 1. Name, abbreviation and stream order of 27 study sites

No	Sites	Abbreviation	Stream order
1	Kumbakkarai	Kumb	Third
2	Sothuparai stream	Soth	Fourth
3	Suruli	Suru	First
4	Kurangani falls	Kura	Second
5	Gadana Nathi	Gada	Third
6	Iluppaiar	Ilup	Second
7	Ramanadi	Rama	Second
8	Chittar	Chit	Third
9	Ayyanar falls	Ayya	Second
10	Karuppar	Karu	First
11	Mundar	Mund	Third
12	Mothiramalai	Moth	Second
13	Kumbar	Kumr	Second
14	Illanguruparai	Illa	Second
15	Kalikesam River	Kali	Third
16	Kaippillai thodu-Kallar	Kaip	Third
17	Golden valley-Kallar	Gold	Second
18	Kallar	Kall	Third
19	Aranakuzhi-Kallar	Aran	Second
20	Panivadi-Kallar	Pani	Second
21	Meenmutti	Meen	Third
22	Downstream-Kallar	Down	Fourth
23	Odamundurai odai-Karanthamalai	Odam	Second
24	Ayyan odai-Karanthamalai	Ayyn	First
25	Sirumalai	Siru	Third
26	Bison vally-Alagarmalai	Biso	First
27	Periaaruvi-Alagarmalai	Peri	Second

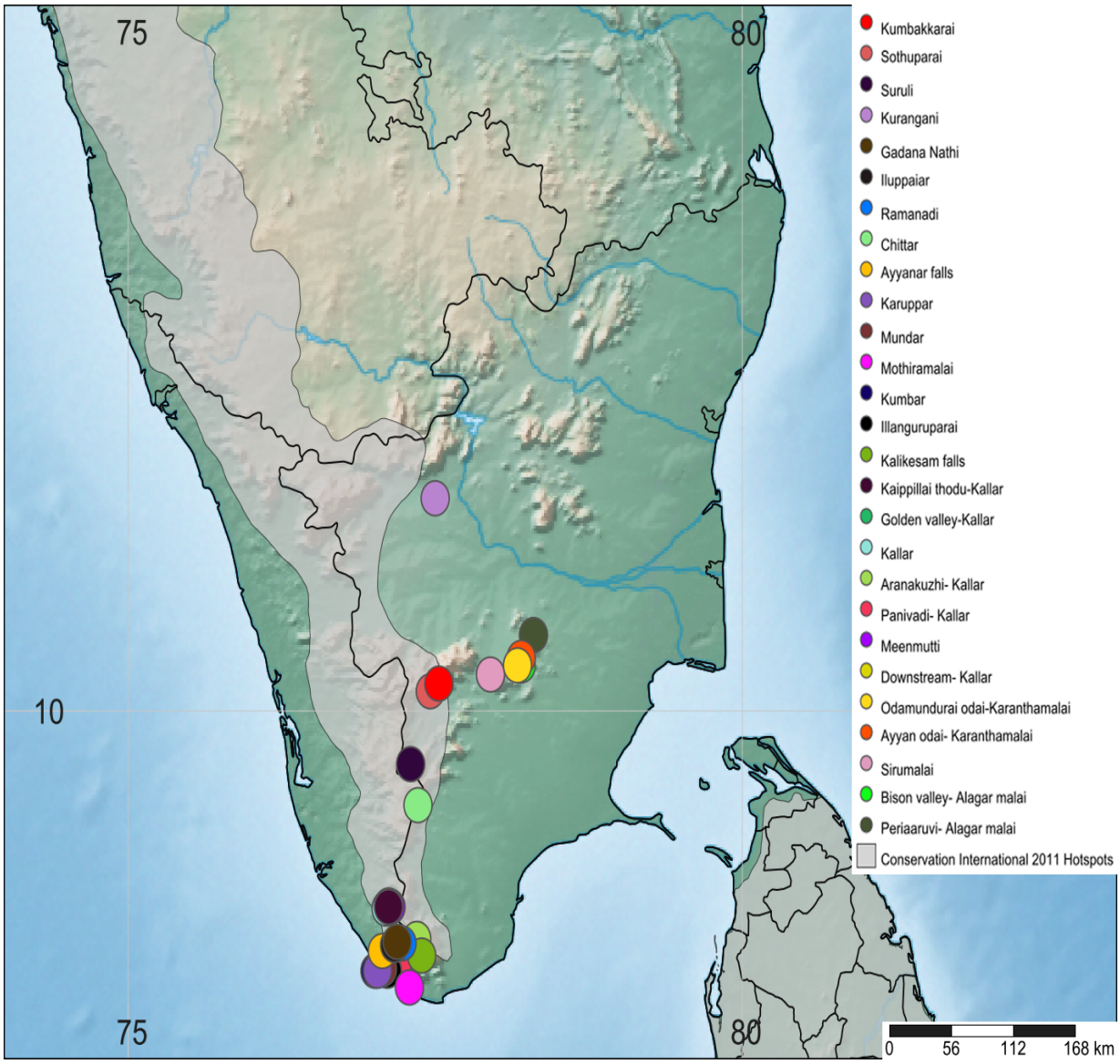

Figure 1. Map showing 27 study sites

Table 2. Categorization of study sites in terms of LSE

Evergreen	Semi evergreen	Scrub	ARE	Forestry plantation	Dry deciduous
Suruli	Kumbakkarai	Ramanathi	Mundar	Panivadi	Odamundurai odai
Gadana	Illangurupparai		Mothiramalai	Chittar	Ayyan odai
Illuppaiar	Kumbar		Kurangani	Ayyanar falls	Sirumalai
Karuppar	Kalikesam			Bisonvalley	
Kaipillai thodu	Goldenvalley			Periaaruvi	
KTC	Aranakuzhi				
Downstream	Meenmutti				
	Sothuparai				

RESULTS AND DISCUSSION

Overall, 4,216 specimens were collected and studied. The result shows that 21 genera of Ephemeroptera belonging to 6 families, two species of genus *Neoperla* belonging to one family, and 19 genera of Trichoptera were belonging to 12 families were identified in the Western Ghats. In the Eastern Ghats, 10 genera of Ephemeroptera belonging to 6 families, one species of genus *Neoperla* belonging to one family, and 11 genera of Trichoptera belonging to 9 families were identified.

The abundance of EPT changes with stream order. Species diversity was generally high and pollution intolerant organisms were present at most stations throughout the study period, indicating the high quality of the water in the streams. There is an increase in the percentage (71%) of Ephemeroptera in the first order and there was a negligible fall in the abundance of the fourth-order stream. The Plecoptera shows a gradual decrease from first to fourth-order. Most stonefly nymphs are related to cool, lotic waters while those of mayfly nymphs have a broader ecological range with a preference for warmer lotic water (Wiggins and Mackay, 1978).

Of the 27 stations, five streams belong to the first order; nine streams belong to the second order; eleven streams belong to the third order, and two streams belong to the fourth order.

The percentage of Ephemeroptera shows no major deviation in any of the LSE types (Table 3). The percentage of Plecoptera shows a gradual increase from 3.8 – 10.9. This increase of predatory Plecoptera which are in dry deciduous LSE types may be due to the presence of more mayflies and caddisflies. The gradual decrease in the percentage of Trichoptera from 29% to 19% may be attributed to their feeding habits (shredders). Nair et al. (1989) stated that shredders were the dominant organisms in the headwaters of the Neyyar River in Southern India. Even in primary rainforest streams in New Guinea, shredders (a majority of Trichoptera in the present study) do not exceed 2% of the benthic populations (Dudgeon, 1994). Likewise, shredders are not more abundant in forested streams in Nepal. The under-representation of shredders which is typical of Hong Kong streams is a general feature of typical Asian Rivers. A possible explanation for this phenomenon is trophic flexibility and hence functional feeding group misclassification i.e. the same taxon acting as a shredder or collector of fine organic material under different circumstances (Dudgeon, 1999).

Table 3. EPT(%) in land scape element types

LSE types	% of E*	% of P**	% of T***
Evergreen	67.1	3.8	29.1
Semievergreen	66.9	5	28.1
Scrub	78.6	2.8	18.6
ARE	72.7	3.3	24
Forest plantation	70.6	6.8	22.6
Dry deciduous	70	10.9	19.1

*E – Ephemeroptera, **P – Plecoptera, ***T – Trichoptera

The EPT taxa present in different mesohabitats are listed in Table 4. Of the seven mesohabitats, richness was the highest in the run (38.38%) followed by riffle (24.2%) and bank (23.97%). The richness was the lowest in silt (0.5%) and the pool (1.1%). Leaf pack substrate types may influence species distributions; however, velocity and complex hydraulic characters also may be important (Sites and Willing, 1991; Llyod and Sites, 2000). The Riffle is dominated by the family Heptageniidae (Ephemeroptera) and Plecoptera. These forms require high velocity and turbulence of a riffle which increases aeration and provides an area where these forms can exploit the current and gather food with minimum energy expenditure (Cummins and Merrit, 1996).

Species richness and abundance values were higher in Tampiraparani (East flowing) River and Vamanapuram River sites and lower in Southern Eastern Ghats sites. Nine environmental variables were selected and the variance was explained a total variance of about 65.8%. The present variances explained by the first three axes were 35.49, 17.20, and 13.11 respectively. The PCA loading above >0.65 was considered significant and variables that showed higher loadings were Alkalinity, Fe, conductivity followed by hardness in the first component (Table 5). Mg showed in the second component and Ca was significant in the third component. Mg was higher in Bison valley and less in Ayyan Odai, Kaipillai thodu, Kallar, and Karuppar. Conductivity is very less compared to all the study sites and it was higher in Kumbakkarai, Kumbar, and Ayyanar falls (Figure 2).

Table 4. EPT complexes present in different mesohabitats in 27 sites

Silt/Mud	Bank	No flow organic	Pool	Leaf pack	Run	Riffle
Ephemera nadinae	*Centroptella similis*	*Caenis* sp.	*Polycentropus* sp.	*Afronurus* sp.	*Tenuibaetis frequentus*	*Afronurus kumbakkaraiensis*
	Choroterpes (Euthraulus) sp.	*Wormaldia* sp.		*Neoperla* sp.	*Baetis ordinatus*	*Epeorus petersi*
	Edmundsula lotica	*Macrostemum* sp.		*Anisocentropus* sp.	*Labiobaetis germinatus*	*Thalerosphyrus flowersi*
	Isca sp.	*Polymorphanisus* sp.		*Leptocerus* sp.	*Acentrella vera*	*Indialis badia*
	Nathanella indica	*Lepidostoma* sp.			*Rhyacophila* sp.	*Notophlebia jobi*
					Diplectrona sp.	*Petersula courtallensis*
					Hydropsyche sp.	*Thraulus gopalani*
					Potamyia sp.	*Dudgeodes bharathidasani*
					Goerodes sp.	*Teloganodes* sp.
					Oecetis sp.	*Stenopsyche kodaikanalensis*
					Setodes sp.	*Adicella* sp.
					Helicopsyche sp.	*Neoperla biseriata*
					Ecnomus sp.	
					Gumaga sp.	

Table 5. PCA loadings for the environmental variables in the first three components

	Environmental variables	First component	Second component	Third component
1	Water temperature	-0.613	-0.192	0.246
2	Conductivity	**-0.687***	0.011	0.372
3	Hardness	**-0.652***	0.121	0.33
4	Alkalinity	**-0.794***	0.241	0.148
5	Ca	0.154	-0.576	**0.669***
6	Mg	-0.185	**-0.859***	-0.371
7	Na	-0.63	-0.508	-0.278
8	Fe	**-0.722***	0.078	-0.35
9	Cl	0.551	-0.319	0.251

* >0.65 Significant

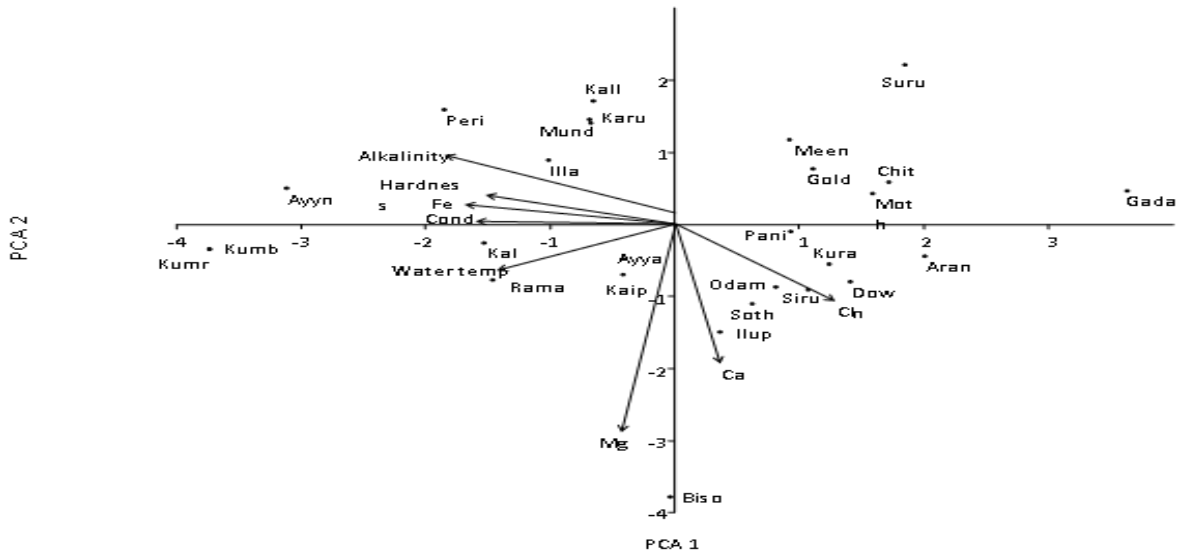

Figure 2. Principal component analysis for the 27 study sites

The similarity matrix and UPGMA dendrogram (Figure 3) quantify the degree of similarity in the EPT complex among 27 stations. Stations were grouped into a hierarchical framework that is useful for proposing a community classification.

The dendrogram shows hat two highly similar (0.97) stations in the Vamanapuram River basin namely Golden valley and Kallar which represent a distinct community showing low similarity (0.45) to stations constituting the Southern Eastern Ghats River basin.

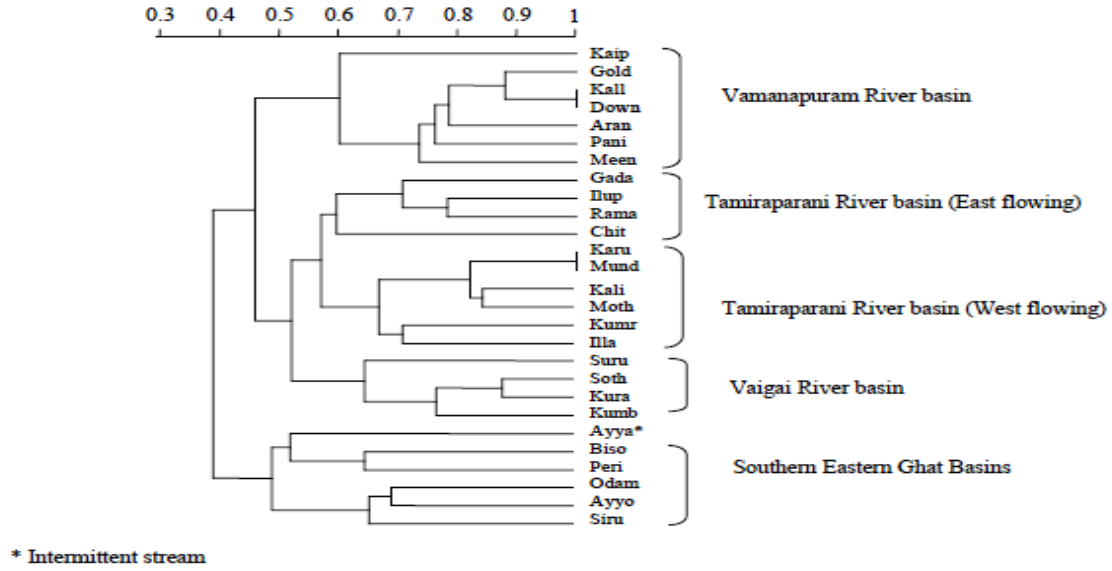

Figure 3. Cluster analysis of the sampling sites using Spearman's rank order correlation coefficient as measure of similarity based on the abundance of EPT complex

Based on the interpretations of the DCA analysis, five distinct communities are recognized namely Vamanapuram River basin, Tamiraparani East flowing, Tamiraparani West flowing, Vaigai River basin, and Southern Eastern Ghats River basin (Figure 4). The sampling sites namely Ayyanar falls is excluded as it is an intermittent stream.

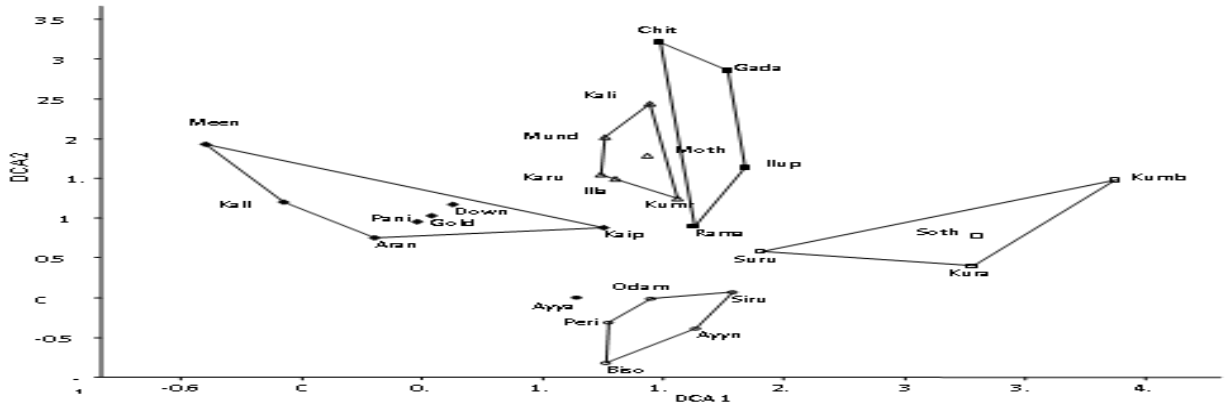

Figure 4. DCA for the 27 study sites based on the EPT complex distribution.

Tamiraparani east flowing basin (closed square), Vaigai River basin (open square), Vamanapuram River basin (closed diamond) and Tamiraparani west flowing (open triangle) Of the five distinct communities recognized, species richness and species abundance value are greater in Tampiraparani East flowing and Vamanapuram River basin (Figure 5). These values are least in southern Eastern Ghats River basins. The Western Ghats is a recognized 'hot spot' for mega diversity (Myers et al., 2000), species richness and species abundance values are higher in streams located in it than in the southern Eastern Ghats which is not as high as the Western Ghats or not even a rain forest. Streams are not canopied and the

water flow is less when compared to the Western Ghats. These reasons may be attributed to the less biodiversity of southern Eastern Ghats Rivers. In all the study sites of southern Eastern Ghats Rivers, the mayfly species *Epeorus* sp. is conspicuously absent, since this is a rhithrogenic form and requires shade and less temperature.

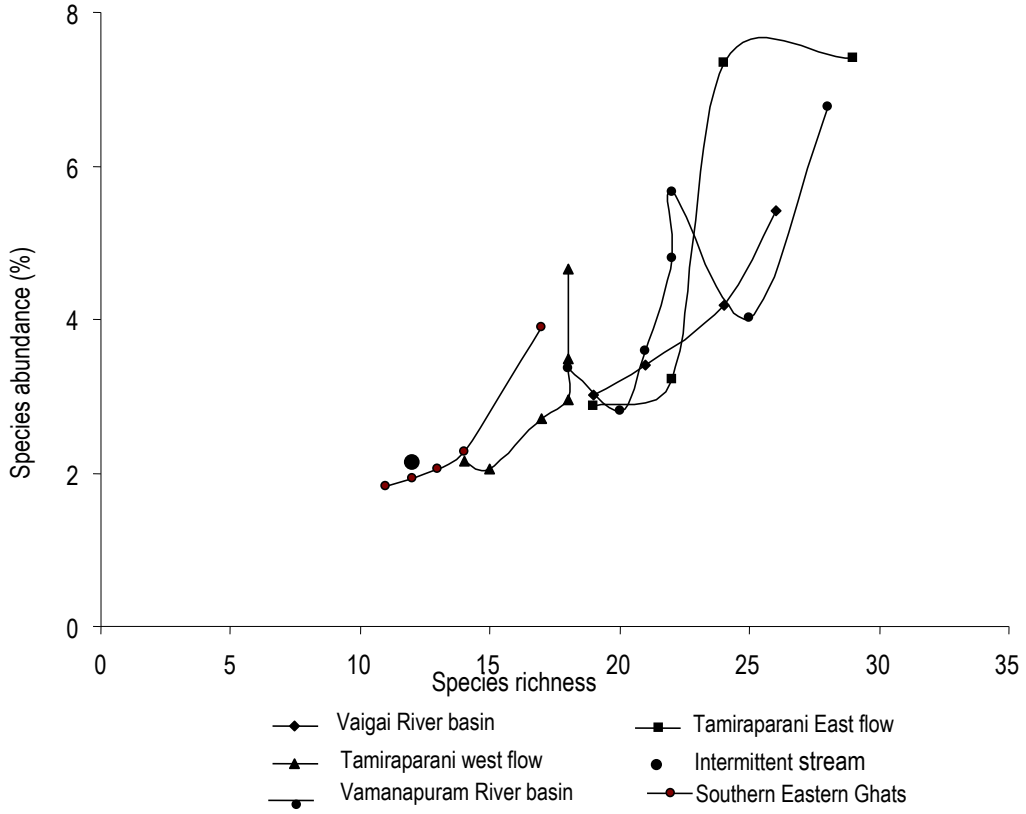

Figure 5. Species richness and abundance in 27 sites

The DCA ordination of all 27 stations, from the five different Rivers of Western and Eastern Ghats, is shown in Figure 4. The analysis resulted in the station being ordinated along four axes with values of 0.2636, 0.1319, 0.07904, and 0.04588 respectively. It does not appear in general axis 1 that is related to the geographic gradient (different mountain ranges of Western and Eastern Ghats) and axis 2 is related to the abundance of EPT complex among the 27 stations. This assessment is in general agreement with the cluster analysis wherein, basal grouping corresponds with stations of Rivers in Western and Eastern Ghats.

CONCLUSIONS

The results of the present study reveal that Ephemeroptera tends to increase in the first-order stream and a negligible fall in the abundance of the fourth-order stream. LSE results show a gradual increase and decrease of Plecoptera and Trichoptera respectively. Mesohabitat results show, these EPT taxa prefer to run (38.38%) compared to silt (0.5%). Based on DCA results of both Western and Eastern Ghats taxa, species richness and abundance were higher in the Western Ghats compared to the Eastern Ghats and the results again prove the mega diversity of Western Ghats.

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DOI: 10.12714/egejfas.39.1.05

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Assessing of fluctuating asymmetry in otolith of the *Alburnus* spp. from Anatolian lotic and lentic systems

Anadolu lotik ve lentik sistemlerindeki *Alburnus* spp. otolitlerinde dalgalı asimetrinin değerlendirilmesi

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Abstract: Fluctuating asymmetry can cause developmental disorders in fish, and particularly high levels of asymmetry adversely affect fish life. In this study, the fluctuating asymmetry levels of the otoliths of four *Alburnus* species (*A. chalcoides* (Güldenstädt, 1772), *A. tarichi* (Güldenstädt, 1814), *A. escherichii* Steindachner, 1897, and *A. mossulensis* Heckel, 1843) found in Turkish inland waters were investigated. A total of 160 fish were collected in the Turkish inland waters. The fluctuating asymmetry level was calculated for the width and length characters of the asteriscus. *Alburnus* species, which were examined in the present study, were divided into four total length classes and fluctuating asymmetry of otolith was evaluated according to both the total length classes and species. There was significant difference between the right and left asteriscus otolith measurements for *A. escherichii* ($P<0.05$). Besides, there were no significant differences between females and males' otoliths measurements of the species ($P>0.05$). The highest and lowest asymmetry levels in the otolith length and otolith width were calculated in the *A. escherichii* among the *Alburnus* species. However, the highest and lowest asymmetry levels in otolith length and otolith width were calculated in the *A. mossulensis* among the total length groups of the *Alburnus* species. In this study, it was determined that the fluctuating asymmetry level varies according to different fish species and habitats.

Keywords: *Alburnus*, asteriscus, asymmetry, freshwater, otolith

Öz: Dalgalı asimetri, balıklarda gelişimsel bozukluklara neden olabilir ve özellikle yüksek düzeyde asimetri balık yaşamını olumsuz etkiler. Bu çalışmada Türkiye iç sularında bulunan dört *Alburnus* türünün (*A. chalcoides* (Güldenstädt, 1772), *A. tarichi* (Güldenstädt, 1814), *A. escherichii* Steindachner, 1897 ve *A. mossulensis* Heckel, 1843) otolitlerinin dalgalı asimetri düzeyleri araştırılmıştır. Türkiye iç sularından toplam 160 adet balık örneklenmiştir. Asteriskus otolitlerinin genişlik ve boy karakterleri için dalgalı asimetri düzeyi hesaplanmıştır. Bu çalışmada incelenen *Alburnus* türleri dört boy sınıfına ayrılmış ve otolitin dalgalı asimetrisi hem total boy sınıflarına hem de türlere göre değerlendirilmiştir. Sağ ve sol asteriskus otolit ölçümleri arasında *A. escherichii* için önemli farklılıklar bulunmuştur ($P<0.05$). Ayrıca, türlerin dişi ve erkek bireylerinin otolit ölçümleri arasında önemli bir farklılık yoktur ($P>0.05$). *Albumus* türlerinden otolit boyu ve otolit genişliğindeki en yüksek ve en düşük asimetri düzeyleri *A. escherichii* türünde hesaplanmıştır. Ancak *Alburnus* türlerinin total boy grupları arasında otolit uzunluğu ve otolit genişliğindeki en yüksek ve en düşük asimetri düzeyleri *A. mossulensis* türünde hesaplanmıştır. Bu çalışmada dalgalı asimetri düzeyinin farklı balık türlerine ve habitatlara göre değişiklik gösterdiği tespit edilmiştir.

Anahtar kelimeler: *Alburnus*, asteriskus, asimetri, tatlı su, otolit

INTRODUCTION

In fish species, otoliths are constituted by three pairs of calcium carbonate structures such as asteriscus, lapillus, and sagitta which are commonly used in many studies because of their species-specific features, morphological diversity, and chemical compositions (Campana, 1999; Tuset et al., 2008; Bostancı et al., 2015; Pavlov, 2019). The otoliths are bony structures that play an active role in the vital functions of fish, such as balance and sound. Therefore, asymmetric otoliths can negatively affect the balance and sensory sensitivity of the fish (Lychakov and Rebane, 2005; Gagliano et al., 2008). In many studies, otolith asymmetry has been used as an indicator to test the similarities and differences between fish populations and even has been used to test different environmental effects such as temperature (Miller, 2011), salinity (Elsdon and Gillanders, 2002), and pollution (Hardersen, 2000), in the relevant populations. When the literature is examined, it is determined that fish otoliths are used extensively in age determination studies, but few studies have observed the fluctuating asymmetry in otoliths (Jawad et al., 2012; Yedier et al., 2018; Abdulsamad et al., 2020).

The inconsistency between the left and right features of an organism due to the different development of the bilateral characteristics is called asymmetry (Leary and Allendrof, 1989; Yedier et al., 2018). Fluctuating asymmetry (FA) is expressed as random deviations from the perfect bilateral

symmetry observed in many animal groups and reflects variable growth during the development of organisms (Fey and Hare, 2008). Besides, FA is thought to reflect the environmental pressures and genetic that the organism experiences throughout its development. For instance, environmental factors that cause developmental disorders, have been reported to cause deviations from the symmetrical character of bilateral organisms (Fey and Hare, 2008). It was stated that asymmetry observed in the otoliths is a valuable index for body and health conditions during the early development and growth of fish (Gagliano and McCormick, 2004; Allenbach, 2011).

The genus *Alburnus* (Bleaks) belongs to the Leuciscidae family, one of the largest families of teleost and is represented by 48 species from Europe to the southern regions of Southwest Asia (Freyhof and Kottelat, 2007; Çiçek et al., 2015). The genus is an excellent example for endemism and high diversity in the western Palaearctic freshwater fishes in Turkey with 26 species, of which 17 are endemic (Gülle et al., 2017; Frose and Pauly, 2020). A comprehensive evaluation of FA in otolith length and width characteristics of four *Alburnus* species in Turkish waters was not investigated. Therefore, the main objective of the present study was to provide some valuable information about the otolith asymmetry of *A.chalcoides* (Güldenstädt, 1772), *A. tarichi* (Güldenstädt, 1814), *A. escherichii* Steindachner, 1897, and *A. mossulensis* Heckel, 1843 in Turkish inland waters.

MATERIALS AND METHODS

All fish samples were obtained from Turkish inland waters (*A.chalcoides* from Cevizdere Stream - 41°05'10.1"N, 37°19'28.6"E, *A. escherichii* from Seydisuyu Stream - 39°24'51.9"N, 31°07'29.7"E, *A. mossulensis* from Munzur River - 39°05'23.2"N, 39°32'31.6"E and *A. tarichi* from Lake Van - 38°32'02.9"N, 43°17'16.7"E) using different sized (7-10 cm) fishing nets and also from local fishermen. *A. tarichi* is an endemic species for Lake Van Basin, *A. escherichii* from Anatolia, and *A. mossulensis* from Tigris–Euphrates Basin. Total length of the samples was measured to the nearest ± 1 mm. The gender of fish samples was determined and their left and right asteriscus otoliths were removed. The cleaned and undamaged otoliths were examined. The otolith width (OW, mm) and length (OL, mm) of the left and right otoliths were taken by the same research scientist to exclude any unwanted error and were repeated three times. The right and left otoliths were photographed using a microscope with a computer-connected camera system (Leica S8APO) (Figure 1). Otolith width and length were measured by ImageJ software (Ver. 1.50b).

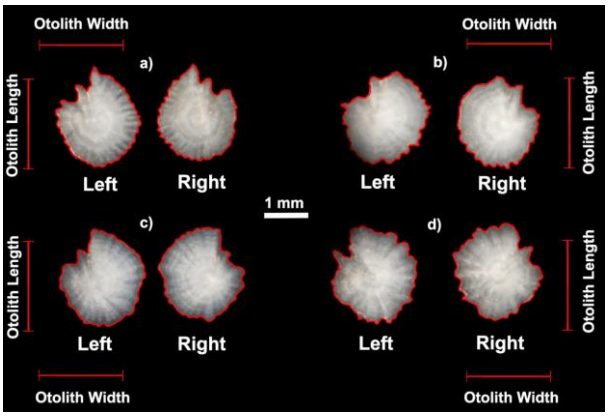

Figure 1. The otolith length and width of asteriscus pairs for a) *A. chalcoides*, b) *A. escherichii*, c) *A. mossulensis*, and d) *A. tarichi* from the Anatolian freshwaters

Kolmogorov-Smirnov and Levene's tests were used for testing whether the variables have normal distribution and homogeneity of variances, respectively. While t-test was applied to determine differences between females and males' otolith measurements, paired t-test was used to determine differences between right and left otoliths measurements (Bostancı et al., 2015). In the present study, otolith length and width were used to calculate the Fluctuating Asymmetry (FA) in the *Alburnus* species from Turkish waters. The statistical analysis included calculating the squared coefficient of asymmetry variation (CV^2_a) for otolith length and width, according to Valentine et al. (1973): $CV^2_a = (S_{r-l}*100/X_{r+l})^2$, where X_{r+l} is the mean of the character, which is calculated by adding the absolute scores for both sides and dividing by the sample size and S_{r-l} is the standard deviation of signed differences. ANOVA test was used to compare CV^2_a values in the otoliths between the different total length classes. The relationships between total length classes and fluctuating asymmetry values in otolith length and width were calculated with linear regression model (y=ax+b). Minitab 17.0 statistical program was used for all statistical analyses.

RESULTS

A total of 160 specimens (n=40 for each species) were collected from Turkish inland waters in 2021. The samples were divided into four total length classes, with the differences between the total length classes being equal and each class containing at least one individual. Total length classes are 100-120 mm, 121-140 mm, 141-160 mm, and 161-180 mm for *A. chalcoides* in the Cevizdere Stream (Turkey), 80-100 mm, 101-120 mm, 121-140 mm, and 141-160 mm for *A. escherichii* in the Seydisuyu Stream (Turkey), 110-130 mm, 131-150 mm, 151-170 mm and 171-190 mm for *A. mossulensis* in the Munzur River (Turkey) and 170-190 mm, 191-210 mm, 211-230 mm and 231-250 mm for *A. tarichi* in the Lake Van (Turkey) (Table 1).

All variables were passed the Kolmogorov-Smirnov test of normality ($P>0.05$) and Levene's test of homogeneity ($P>0.05$). The otolith measurements of both sexes were

pooled in the study because there were no significant differences between female and male otoliths measurements of the species (t-test, $P>0.05$). There were no statistical differences between left and right otolith measurements of *A. chalcoides*, *A. mossulensis,* and *A. tarichi* (paired t-test, $P>0.05$); however, statistically differences were found between left and right otoliths of *A. escherichii* (paired t-test, $P<0.05$).

Table 1. Squared coefficient of asymmetry and otolith characters mean, min-max values by total length class of *A. chalcoides*, *A. escherichii*, *A. mossulensis*, *A. tarichi*

	Character	Total length class (mm)	N	CV_a^2	Character mean	Character Min-Max	% of individuals with asymmetry
A. chalcoides Cevizdere Stream	Otolith Length	100-120	7	10.922	1.352	1.246-1.450	85.71
		121-140	9	15.320	1.752	1.573-1.981	88.89
		141-160	17	16.192	2.272	1.955-2.582	88.24
		161-180	7	21.642	2.588	2.254-2.980	100.00
	Otolith Width	100-120	7	7.755	1.335	1.252-1.527	85.71
		121-140	9	9.829	1.678	1.522-1.895	77.78
		141-160	17	13.981	2.055	1.852-2.326	82.35
		161-180	7	26.011	2.310	2.054-2.597	100.00
A. escherichii Seydisuyu Stream	Otolith Length	80-100	11	13.118	1.558	1.438-1.724	90.91
		101-120	11	20.800	1.827	1.587-1.997	90.91
		121-140	14	23.418	1.919	1.720-2.193	78.57
		141-160	4	35.024	2.105	1.838-2.258	100.00
	Otolith Width	80-100	11	10.974	1.447	1.229-1.644	90.91
		101-120	11	29.682	1.632	1.381-1.929	81.82
		121-140	14	30.835	1.797	1.472-2.109	85.71
		141-160	4	39.614	1.952	1.758-2.223	100.00
A. mossulensis Munzur River	Otolith Length	110-130	5	5.783	1.944	1.775-2.096	80.00
		131-150	15	14.487	2.131	1.935-2.392	93.33
		151-170	14	18.447	2.353	2.096-2.613	92.86
		171-190	6	59.103	2.535	2.281-2.898	100.00
	Otolith Width	110-130	5	5.841	1.735	1.578-1.846	80.00
		131-150	15	14.622	1.907	1.719-2.149	86.67
		151-170	14	21.949	2.115	1.826-2.396	85.71
		171-190	6	77.367	2.403	2.104-2.711	100.00
A. tarichi Lake Van	Otolith Length	170-190	11	17.130	2.498	2.226-2.697	81.82
		191-210	15	21.551	2.693	2.511-2.983	86.67
		211-230	10	25.344	2.752	2.515-3.106	80.00
		231-250	4	33.438	2.904	2.582-3.253	100.00
	Otolith Width	170-190	11	18.344	2.330	2.112-2.511	90.91
		191-210	15	20.330	2.450	2.183-2.825	93.33
		211-230	10	22.459	2.570	2.240-2.960	90.00
		231-250	4	26.492	2.829	2.711-3.079	100.00

In the current study, results exhibited that the level of FA for the two otolith traits at its lowest values in the fish species ranging in total length between 100–120, 80–100, 110–130 and 170–190 for *A. chalcoides, A. escherichii, A. mossulensis,* and *A. tarichi,* respectively. The highest FA levels for the two otolith traits were determined in total length groups such as 161-180 for *A. chalcoides*, 141-160 for *A. escherichii*, 171-190 for *A. mossulensis,* and 231-250 for *A. tarichi*, respectively. In all of the four *Alburnus* species examined in our study, the percentage of individuals showing

asymmetric characteristics in both otolith length and otolith width is in the largest total length class (Table 1). Besides, the FA values of total length classes for each species were significantly different (ANOVA, $P<0.05$).

In the current study, it was determined that fluctuating asymmetry values in both otolith length and otolith width tend to increase with the total length of the four *Alburnus* species and there was a linear relationship between them for all bleaks. The highest and lowest relationships between total length classes and fluctuating asymmetry values in otolith length and width were determined in *A. tarichi* (r^2= 0.968 and 0.967) and *A. mossulensis* (r^2= 0.798 and 0.787), respectively.

Similarly, the highest and lowest correlations in total length classes and fluctuating asymmetry values in otolith width were determined in *A. tarichi* and *A. mossulensis*, respectively.

Fluctuating asymmetry results of the otolith width and length of the four *Alburnus* species from Turkish inland waters are presented in Table 2. FA level of the otolith width was greater than otolith length for *A. escherichii*, *A. mossulensis,* and *A. tarichi,* but it was not the case with *A. chalcoides* (Table 2). The percentage of individuals showing asymmetry in otolith length characteristics was highest for *A. mossulensis*, while the percentage of otolith width was calculated as the highest in *A. tarichi*. In addition, the percentage of individuals showing asymmetry in these two otolith characters in *A. escherichii* was equal to each other (Table 2).

Table 2. Squared coefficient of fluctuating asymmetry values and otolith characters means with minimum and maximum values for four bleaks from Turkish inland waters

	Character	Total Length (mm)	n	CV_a^2	Mean	Min-Max	% of individuals with asymmetry
Alburnus chalcoides	Otolith Length	100-180	40	17.540	2.049	1.246-2.980	90.00
Cevizdere Stream	Otolith Width	100-180	40	15.914	1.889	1.252-2.597	85.00
Alburnus escherichii	Otolith Length	80-160	40	23.358	1.813	1.438-2.258	87.50
Seydisuyu Stream	Otolith Width	80-160	40	27.857	1.671	1.229-2.223	87.50
Alburnus mossulensis	Otolith Length	110-190	40	22.455	2.246	1.775-2.898	92.50
Munzur River	Otolith Width	110-190	40	26.962	2.033	1.578-2.711	87.50
Alburnus tarichi	Otolith Length	170-250	40	21.744	2.675	2.226-3.253	85.00
Lake Van	Otolith Width	170-250	40	22.946	2.485	2.112-3.079	92.50

DISCUSSION

In the literature, it was observed that most otolith asymmetry studies are conducted extensively on marine species (Jawad at al., 2012; Kontaş at al., 2018; Yedier et al., 2018; Abdulsamad et al., 2020), while few studies were carried out in inland fishes (Green and Lochmann, 2005; Estes et al., 2006; Green and Lochmann, 2006). The lack of data regarding the natural asymmetry of the fish is present in the Turkish inland waters as in many parts of the world. Therefore, it is difficult to decide whether the asymmetry values obtained in the present study are higher or lower than normal. However, in the previous study, it was reported that asymmetry has many negative effects on fish species such as hearing problems (Lychakov and Rebane, 2005), abnormal swimming activity (Helling et al., 2003), and adaptations problems in the habitat (Jorgensen and Fiksen, 2010).

This is the first study to determine the fluctuating asymmetry in otolith of *Alburnus* spp. in Turkish inland waters. The FA values of the otolith length in the compared *Alburnus* species were ranged from 10.922 to 21.642 for the *A. chalcoides*, 13.118 to 35.024 for the *A. escherichii,* 5.783 to 59.103 for the *A. mossulensis* and 17.130 to 33.438 for the *A. tarichi.* On the other hand, the lowest and highest fluctuating asymmetry values in the otolith width were varied such as 7.755 and 26.011 in *A. chalcoides* from the Cevizdere Stream, 10.974 and 39.614 in *A. escherichii* from the Seydisuyu Stream, 5.841 and 77.367 in *A. mossulensis* from the Munzur River and 18.344 and 26.492 in *A. tarichi* from the Lake Van. The highest asymmetry value differs among otolith characters in fish species. For example, some researchers were determined the highest asymmetry value in the otolith length (Al- Mamry et al., 2011; El-Regal et al., 2016), while others were indicated in the otolith width (Sadighzadeh et al., 2011; Jawad, 2012; Jawad et al., 2012; Abdulsamad et al., 2020). Similarly, some differences also were identified with the highest asymmetry value of otolith characters for four *Alburnus* species from the inland waters of Turkey. Although the highest asymmetry value was found in the otolith length in *A. chalcoides* species, it was determined in otolith width in the *A. escherichii, A. mossulensis* and *A. tarichi*. The highest and lowest asymmetry values in the otolith length and otolith width were found in the *A. escherichii* among the *Alburnus* species. However, the highest and lowest asymmetry in otolith length and otolith width were calculated in the *A. mossulensis* species among the total length groups of *Alburnus* species.

When these four *Alburnus* species are compared, it is concluded that the fish from the Seydisuyu Stream and Munzur River are more stressed than from Cevizdere Stream and Lake Van.

In order to evaluate the fluctuation asymmetry values in both otolith length and otolith width of the four *Alburnus* species, there is no study conducted with the same species in different regions or different species in the same regions. Therefore, fluctuating asymmetry values of the otolith characters of *Alburnus* spp. examined in the study were compared among themselves. In many studies, a relationship between otolith asymmetry and total length was examined and it was stated that asymmetry increases with total length (Jawad, 2001; Al-Mamry et al., 2011). In the current study, it was determined that the value of otolith asymmetry was positively correlated with the fish length for four *Alburnus* species from Turkish inland waters. Similar results are available in several studies in different habitats (Jawad et al., 2012; Mabrouk et al., 2014; Abdulsamad et al., 2020). The increase in asymmetry levels depending on the total length may be the result of longer exposure to fish under environmental conditions depending on the age and fish size (Thiam, 2004). In many studies, it has been reported which fluctuating asymmetry in the morphometric character of the organism, is negatively correlated with the animal fitness (Martin and Lopez, 2001; Jawad et al., 2012). Pollutants such as heavy metals, pesticides, DDT, and detergents in water can accumulate in the fish body and can then be transferred to humans through the food chain (Ongley, 1996; Afshan et al., 2014). Determining the environmental stress of heavy metals and other pollutants in aquatic ecosystems is very important for both humans and fish. For this, it should be well known to collect detailed information about habitat conditions and animal welfare of the fish species. In such cases, habitats should be protected for populations by measuring the effects of these stresses in fish species before they are irreversibly affected (Lens et al., 2002). Therefore, fluctuating asymmetry is one of the methods applied in determining the quality and health of individuals and populations, since it is both easy to apply and not destructive (Møller and Thornhill, 1998; Lens et al., 2002). In addition, it was stated in previous studies that developmental instability was determined using fluctuating asymmetry values and this asymmetry could be an indicator of environmental stress (Parsons, 1990; Alados et al., 1993). Furthermore, based on previous research in this field, it is likely that there is a relationship between asymmetry in fish species and environmental factors (Yedier et al., 2018). The environmental problems in aquatic habitats are also present in the sampling stations of the current study, as in many inland waters of Turkey. Cevizdere Stream (*A. chalcoides*), which is surrounded by hazelnut lands, is located in the Ordu province (Turkey) and it is one of the main drinking water resources of the Ordu district of Ünye (Anonymous, 2018). In the literature, there seems to be no study to determine water pollution in this stream, although there are some quarries and cement plants as the obvious pollutants around the stream. Seydisuyu Stream Basin (*A. escherichii*), which has important agricultural lands and borate deposits on its basin, is located in Eskişehir province (Turkey) (Atıcı et al., 2018). The pollution of Seydisuyu Stream has been expressed in many studies and the main reasons for the pollution of the stream system are the discharge of agricultural pesticides, urban sewage water from settlements, and mineral mining activities (Çiçek et al., 2013; Köse et al., 2014; Tokatlı et al., 2014). It is reported that these pollutants can be an important limiting factor in aquatic life in the region unless a precaution is taken for the pollution in Seydisuyu Stream (Köse et al., 2014). Munzur River (*A. mossulensis*), which is an important water source, is located in Tunceli province (Turkey). Unfortunately, it is stated that domestic wastewater and agricultural activities cause pollution by causing changes in some parameters of the water quality of Munzur River (Yıldırım et al., 2011a; Ural et al., 2012). It was reported that this pollution in the Munzur River causes oxidative stress in fish and this stress adversely affects the life of the fish (Yıldırım et al., 2011b; Lushchak, 2016). Lake Van (*A. tarichi*), which is the largest soda lake, is located in Turkey's eastern Anatolian region and the primary water sources feeding the lake are rains and streams (Poyraz and Mutlu, 2017). It has been reported that Lake Van is under the influence of many pollutants such as quarries, mining activities, non-mining mineralization areas, geothermal discharges in the basin, natural radioactive contaminations, and domestic wastes (Öğün et al., 2005; Çiftçi et al., 2008).

Since fish are very sensitive to changes in their habitats (Bassem, 2020), they are one of the model organisms used in the assessment of environmental and ecological factors in aquatic ecosystems (Ali et al., 2008). While the asymmetry caused by environmental stress has direct or indirect effects on fish species, such as developmental disorders, it may cause problems in the short or long term. For this reason, fish populations should be checked at regular intervals and the increase or decrease in asymmetry should be monitored.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Determinations of the effects of cyfluthrin on the hemocytes parameters of freshwater mussel (*Unio delicatus*)

Tatlı su midyesinin (*Unio delicatus*) hemosit parametreleri üzerine Cyfluthrinin etkilerinin belirlenmesi

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Abstract: The overuse of pesticides has been increasing since the 20th century. Depending on this use, non-target organisms are also affected apart from target organisms. Cyfluthrin is a synthetic pyrethroid pesticide used in agriculture, domestic and veterinary medicine against insects. It may also affect non-target aquatic organisms as a result of mixing with aquatic ecosystems. This study was aimed to investigate the effect of cyfluthrin on freshwater mussels, one of the aquatic invertebrate species, with hemocyte parameters. Total hemolymph counts, hemolymph cell morphology, and differential hemocyte counts were performed from hemolymph taken from mussels exposed to different cyfluthrin doses exposures for 24 and 48 hours. Compared to the control group, the total hemocyte counts of the experimental groups were found to increase in 24h and decrease in 48h significantly ($p<0.05$). In the examination of hemocyte morphologies, granular, semi granular, and hyalinocyte cells were observed. Similar values of differential hemocyte counts were found both 24 and 48h exposure times. As a result, in aquatic toxicology studies, besides total hemocyte count, analysis of hemocyte morphologies and differential hemocyte counts are found to be good biomarkers.

Keywords: Cyfluthrin, hemolymph, total hemocyte counts, hemocyte morphology, differential hemocyte counts

Öz: Pestisitlerin aşırı kullanımı 20. yüzyıldan beri artan bir şekilde devam etmektedir. Bu kullanıma bağlı olarak hedef organizmalar dışında hedef olmayan organizmalar da etkilenmektedir. Cyfluthrin tarımda, evsel alanda ve veteriner sağlığında böceklere karşı kullanılan bir sentetik piretroit pestisittir. Sucul ekosistemlere karışması sonucunda hedef dışı sucul organizmalar üzerinde de etki gösterebilir. Bu çalışmada, sucul omurgasız türlerinden biri olan tatlı su midyelerinde cyfluthrinin etkisi hemosit parametreleri ile incelenmesi amaçlanmıştır. 24 ve 48 saat süre ile farklı cyfluthrin maruziyetine bırakılan midyelerden alınan hemolemften total hemosit sayısı, hemolemf hücre morfolojisi ve diferansiyel hemosit sayımı yapılmıştır. Kontrol grubuna göre, deney gruplarının total hemosit sayıları önemli ölçüde değişkenlik gösterdiği bulunmuştur ($p<0.05$). Hemosit morfolojilerinin incelenmesinde ise granüllü, yarı-granüllü ve hiyalinosit hücreleri gözlenmiştir. Ardından yapılan diferansiyel hemosit sayımı ile 24 ve 48 saatlik maruziyet sonucunda benzer değerler bulunmuştur. Sonuç olarak, sucul toksikoloji çalışmalarında total hemosit sayısının yanı sıra hemosit morfolojilerinin incelenmesi ve diferansiyel hemosit sayıları da iyi bir biyobelirteçtir.

Anahtar kelimeler: Cyfluthrin, hemolemf, toplam hemosit sayısı, hemosit morfolojisi, diferansiyal hemosit sayısı

INTRODUCTION

Pyrethroids, synthetic insecticides, are made of pyrethrins that are naturally produced from the flowers of *Chrysanthemum cinerariaefolium* (Soderlund et al., 2002). The first compound of the pyrethroids is allethrin that was produced in 1949 and commercialized in 1952. Then, tetramethrin, resmethrin, phenothrin, permethrin, fenvalerate, deltamethrin, cypermethrin were synthesized and marketed in the 1970s. During the 1980s, bifenthrin, cyfluthrin, and other second-generation pyrethroids were made and started to use for against corps, public health, and household application (Palmquist et al., 2012). Even though the natural pyrethrums consisting of pyrethrins, cinerins and jasmolins were used against insects in the 1800s (Palmquist et al., 2012), pyrethroids have been widely applied in agricultural and household areas since the 1970s (Soderlund et al., 2002).

The mechanism of pyrethroids is the disruption of the peripheral nervous system of insects. Their main effects on sodium-voltage channels and hence they cause paralysis. According to their mechanism of action, pyrethroids are divided into two groups: Type 1 and Type 2 (Palmquist et al., 2012; Williams et al,. 2018). Exposure to the first causes restlessness, overstimulation and body shake whereas the last causes hyperactivity, incoordination, and writhing. Among pyrethroids compounds, Type 1 consists of allethrin, bifenthrin, d-phenothrin, permethrin, resmethrin, and tetramethrin while Type 2 includes many of the second generation pyrethroids such as cyhalothrin, cypermethrin, cyfluthrin, and deltamethrin (Palmquist et al., 2012).

Cyfluthrin (Cas Number: 68359-37-5; $C_{22}H_{18}Cl_2FNO_3$) is widely used for various pests in agriculture, household, veterinary, and food industry. After hypersensitiveness of the

insects, it causes convulsion and death. It affects either on depolarization of sodium-voltage channel or calcium transport mechanism on the cell membrane (Verma et al., 2021).

Cyfluthrin is detected in different parts of aquatic environments. Duavi et al. (2021) found that cyfluthrin concentration in water and sediment samples of the Ceara River (CE, Brazil) were from 15.60 to 178.35 ng/L and from 5.75 to 55.45 ng/g, respectively. Cryder et al. (2021) found that the mean concentration of cyfluthrin ranged from 13.8 to 455 ng/L in the water, from 0.263 to 52.4 ng/g in the sediment, and from non-detected to 3.18 ng/g in the plant samples of Prado Wetlands (California, USA). Lizotte et al. (2021) found that cyfluthrin was detected 16-18 µg/kg in sediment samples from Mississippi Stream Bayous. They also found that *Hyelalla azteca* were exposed to collected sediment for 28 days and the residues of cyfluthrin in its tissue were found between 179 and 1003 µg/kg. In addition to being detected in the aquatic ecosystem, it has also been found to have toxic effects on aquatic organisms such as green algae (Sáenz et al., 2012), mussels (Serdar, 2021), and fish (Selvi et al., 2008; Sepici-Dinçel et al., 2009; Farag et al., 2021) in laboratory experiments.

Aquatic invertebrates have cellular and humoral defence mechanism against to pathogens, pollutants (Ciacci et al., 2009). The blood tissue of invertebrates is known as hemolymph that is an important bioindicator parameter of the species health status (Günal et al., 2018). Hemocytes, blood cells, are responsible for cellular defence mechanism via phagocytosis, release of lysosomal enzymes and etc (Ciacci et al. 2009).

Aquatic invertebrates have been affected by alterations of abiotic parameters including salinity (Nunes et al., 2021; Pérez–Velasco et al., 2021), temperature (Matoo et al. 2021; Wu et al., 2021), pollutants (Baussant et al., 2009; Tresnakova et al., 2020; Günal et al., 2021) and by biotic factors such as bacteria (Canesi et al., 2002; Ciacci et al. 2009), invasive species (Berber et al., 2018) in the aquatic environment. Therefore, their immune system may be also affected by these factors.

Freshwater mussels have been effectively used for biomonitoring studies of aquatic systems. Due to their filter-feeding characteristics, they reflect the environment where they inhabit (Negishi et al., 2013; Lundquist et al., 2019). Therefore, they are bioindicator organisms many studies including biomonitoring (Wagner and Boman 2004), biodiversity (Bolotov et al., 2020), and aquatic toxicology (Gillis et al., 2004; Machado et al., 2014; Yurdakok-Dikmen et al., 2018; Tresnakova et al., 2020; Arslan et al., 2021a, 2021b).

The freshwater mussel organism in the current study is *Unio delicatus* Lea, 1863. It is one of the most common mussel species in the southwest to east Anatolia in the large river basins. Due to the distribution areas, it is an indicator species in biomonitoring, ecotoxicological, and phylogenetic studies (Lopes-Lima et al., 2021). The current study was aimed to investigate immunological response of non-target organisms, freshwater mussels (*Unio delicatus*) via the total hemocyte counts, differential hemocyte counts, and hemocyte morphology of exposure different concentrations of cyfluthrin.

MATERIALS AND METHODS

The freshwater mussels *Unio delicatus* were obtained from fishermen from Gölbaşı Lake in Adıyaman (Turkey) situated in the southern of Anatolia. It has rich biodiversity and also located in the migration routes of birds between Africa and Europe (Alkan Uçkun, 2018). The species were taken to the laboratory in the aerated water. The mussels were brought to the laboratory and were placed in 15 L aquariums. Before starting the experiments, the mussels were adapted to the laboratory conditions for 2 weeks. The dechlorinated municipal tap water was used in the aquariums and the temperature was kept constant with thermostat heaters. The water parameters are as follows: the mean temperature as 21.4±1.21°C, the mean dissolved oxygen as 5.46±0.17 mg/L, the mean conductivity as 223.15±0.48 mS/cm, the mean pH as 7.70±0.03. During the adaptation period, they were fed by *Chlorella* sp. The aquariums water were changed every two day.

Cyfluthrin was prepared via solvent (dimehtylsulfoxide, DMSO) in stock concentration as 10.05 mg/L. Then, it was diluted with water 1.05, 0.105, and 0.0105 mg/L.

In the experiment, five aquariums consisting 10 L water in 15 L aquariums were used. Ten mussels were randomly placed in the aquarium (n=50). The mean weight, mean length, mean thickness, and mean height of mussels were 31.59±4.8 g, 4.87±0.43 cm, 1.18±0.30, and 1.88±0.17 cm, respectively. Three of the aquariums were the experiment groups (1.05, 0.105, and 0.0105 mg/L cyfluthrin, CF) and two of them were the control groups (control and solvent control (DMSO)). The acute toxicity test was applied for 24 and 48 hours. The cyfluthrin concentrations in the exposed groups were analysed according to AOAC Official Method 2007.01 by LC-MS/MS (AOAC 2007). The results were within acceptable limits of nominal concentrations. At the end of each exposure period, 5 mussels were taken from the aquariums and hemolymph samples (about 1 mL) were taken by stimulating the adductor muscle with the help of a 2.5 mL sterile syringe.

Total hemocyte counts (THCs) were made using hemocytometer according to Yavuzcan and Benli (2004). After taking hemolyph, some of the hemolymph samples (around 200 µL) was mixed with 4% formaldehyde fixative solution (1:1) and then the mixed hemolymph samples were counted in Thoma counting chambers. Every hemolymph specimen was prepared and counted three times.

Among morphological analysis of hemocytes, hemocyte types and differential hemocyte counts were detected. The hemolymph samples were stained Giemsa dye according to

Silva et al., (2002). After the hemocyte types were analyzed under the light microscope, the differential hemocyte counts were carried out to count four different parts of the slides. Lysosomal observations were made Neutral Red staining according to Matozzo and Marin (2010).

The results were evaluated by using a non-parametric statistical method (Kruskal Wallis) via the GraphPad Prism 5 program. The percentage of differential hemocyte counts was calculated via the Microsoft Excel 13 program. The cells diameters were determined by using the ImageJ program.

RESULTS AND DISCUSSION

The blood parameters of invertebrates are very important to detect the health status of the species as well as the environment where they inhabit. Exposure to environmental pollutants, adverse effects including abnormal offsprings (Saidov and Kosevic 2021), deformation in their morphology (Banumathi et al., 2017) were detected. In the short- and long- term exposure, the blood parameters such as total hemocytes counts, differential hemocyte counts are used for the immunological investigations of the species (Matazzo and Marin 2010; Günal et al., 2018; Ayhan et al., 2021). Due to exposure to pollutants, the immune system of species were effected, and hence their total hemocyte counts increase (Katalay et al., 2019).

The health assessment of aquatic invertebrates generally occurred lethal tissue collection from species for alterations histopathological (Costa et al., 2013; Larguinho et al., 2014; Yee-Duarte et al., 2018; Noleto et al., 2021) and enzymatic (Baussant et al., 2009; Larguinho et al., 2014; Noleto et al., 2021) analysis. These methods have resulted in the impacts on the habitat status of aquatic invertebrates. In some cases, the investigated species may be threatened/endangered due to the decrease in the number of specimens in the habitat. Thus, rapid techniques and the use of a small number of animals are required to study the health status of aquatic organisms (Gustafson et al., 2005). Therefore, in this study, the number of individuals was used to examine the hemolymph parameters, which is one of the physical parameters of the mussels, to be low.

In the present study, the acute effects of cyfluthrin on the freshwater mussels were detected via hemocyte parameters. Differential hemocyte parameters were examined after the determination of the total hemocyte counts used in many studies in which pollutant research was carried out. By examining the cells morphologically, it was determined whether the granules in the hemocytes were lysosomes.

The values of THCs of the freshwater mussels exposed to cyfluthrin for 24h and 48h were given in Figure 1. At 24-hour exposure, hemocyte counts significantly increased compared to control groups ($p<0.05$). In the high concentration applied (1.05 mg/L), an increase of THCs occurs almost twice of the control group, while a decrease in the number of THCs occurs when the dose concentration is decreased. During the 48-hour exposure period, the THCs values observed at a high rate in the control group led to a decrease in half at the lowest concentration of CF (0.0106 mg/L). A decrease was observed in the exposure groups from high dose to low dose at 48 hours of exposure ($p<0.05$). However, the difference between exposure times did not affect on THCs ($p>0.05$). On the other hand, the alterations in THCs in this study were agreed with other studies exposed to different pollutants on aquatic invertebrates (Günal et al., 2018; Katalay et al., 2019; Tresnakova et al., 2020; Ayhan et al., 2021).

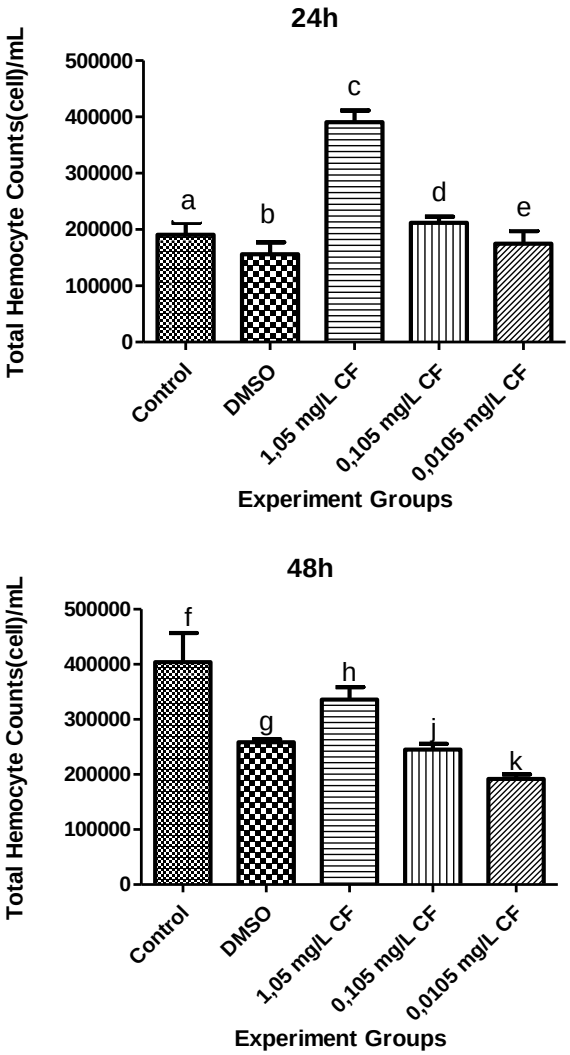

Figure 1. The values of THCs (±SEM) of *Unio delicatus* exposed to cyfluthrin for 24 and 48h and control groups. (Different letters shows the significant difference, $p<0.05$)

In the morphological examination of hemocyte types by light microscopy, three types of hemocytes were detected: granulocytes (Figure 2.A), semigranulocytes (Figure 2.B), and hyalinocytes (agranulocytes, Figure 2.C). The first has great cytoplasmic granules in the cells, the second has lots of cytoplasmic granules in the cells, and the third has no

cytoplasmic granules. The granules in the cytoplasm of granulocytes and semigranulocytes were lysosomes and they were shown in Figure 2.D. The obtained hemocyte type results in the current study are similar to Matozzo and Marin (2010).

The mean diameters of granulocytes, semigranulocytes, and hyalinocytes in the control group were 15.97±1.42 µm, 10.48±0.87 µm, and 11.04±0.16 µm, respectively. Matozzo and Marin (2010) found that granulocytes (11.94 µm), semigranulocytes (12.38 µm), and hyalinocytes (7.88 µm) in the hemocyte cells of the crab *Carnicus aestuarii*. Bolognesi and Fenech (2012) observed that granular (8-12 µm) and agranular (3-4 µm) hemocytes in the hemolymph of the Mediterranean mussel *Mytilus galloprovincialis.* According to the results obtained from the control group in this study, the difference in hemocyte distribution and size from Matozzo and Marin (2010) and Bolognesi and Fenech (2012) may be due to the difference between species and the marine/freshwater species.

On the other hand, the mean diameters of granulocytes, semigranulocytes, and hyalinocytes in the exposed groups were 19.91±0.96 µm, 23.68±0.31 µm, and 27.30±1.20 µm, respectively. The diameter of the hemocytes in the control groups were smaller than the exposed groups. This may be related to phagocytosis of the contaminant by hemocyte cells.

In the exposure of Cyfluthrin for 24h and 48h, all the groups had semigranulocytes, granulocytes and hyalinocytes (Figure 3). The highest and lowest values of the cells obtained from the experiment groups are same in the 24h and 48h exposure time. For instance, the highest semigranulocytes were 80.24% in the 0.0105 mg/L CF group where the lowest were in the control group (23.73%) in the 24h exposure duration. Likewise, the highest semigranulocytes were 71.42% in the 0.0105 mg/L CF group where the lowest were in the control (31.42%) in the 48h. The highest granulocytes were 58.21% in the 0.105 mg/L CF group where the lowest were in the 0.0105 mg/L CF group (20%). The highest hyalinocytes were 16.9% in the control group where the lowest were in the 1.05 mg/L CF group (5.79%). Besides, the highest granulocytes were 57.89% in the 0.105 mg/L CF group where the lowest were in the 0.0105 mg/L CF group (19.05%). The highest hyalinocytes were 11.43% in the control group where the lowest were in 4.35% the 1.05 mg/L CF group.

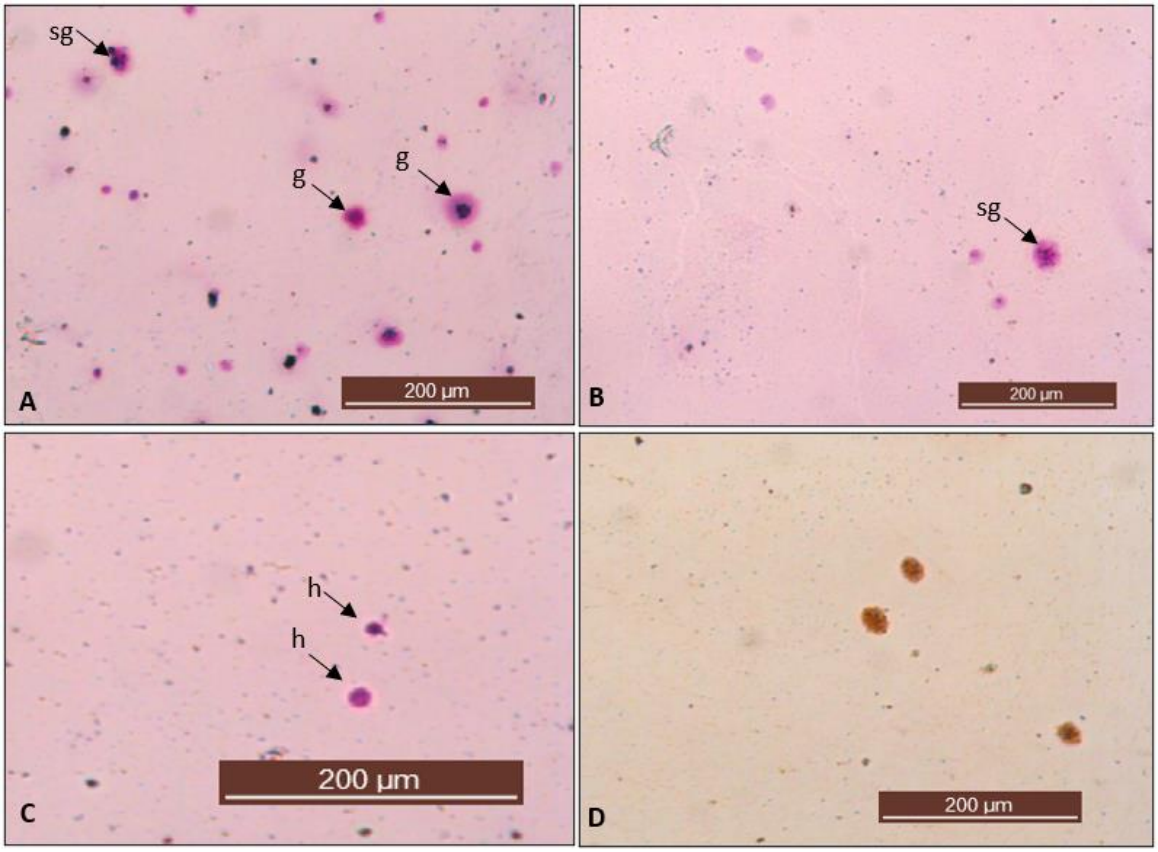

Figure 2. *Unio delicatus* hemocytes stained with Giemsa's dye (A-C; g: granulocytes, sg: semigranulocyte and h: hyalinocyte) and Neutral Red dye showing lysosomes in the cell (D).

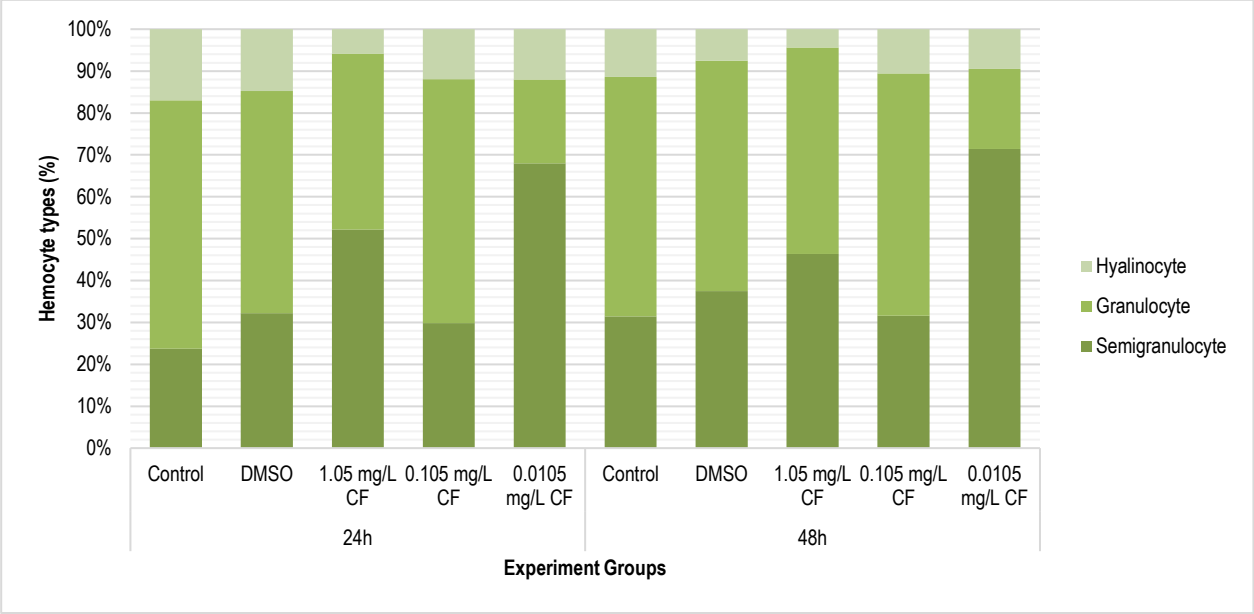

Figure 3. The percentage of hemocyte types in the experiment groups for 24h and 48h

CONCLUSION

It has been found appropriate to use changes in the total hemocyte counts (THCs), which is one of the important physiological markers, in many studies investigating the effects of environmental pollutants in the aquatic toxicology. In this study, the decrease in the THCs due to cyfluthrin exposure indicates that the THCs parameter is a good indicator. The hemocyte cell types and differential hemocyte cell counts (DHCs) in hemolymph tissue were also investigated in the current study. The fact that hemocyte cells with granules, semigranules and agranules are of the same type as the cells in other studies in the literature. When the DHCs results between the experimental groups were examined, it was found that the cell counts were high or low within the same groups depending on the exposure times. Thus, it has been shown that differential hemocyte counts can be used in addition to the total hemocyte counts parameter in investigating the effects of environmental pollutants on aquatic invertebrates.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Tüketime hazır halde satışa sunulan işlenmiş midye ürünlerinin mikrobiyal kaliteleri

The microbial qualities of ready-to-eat sold processed mussel products

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Öz: Bu çalışmada İzmir'in Bornova ilçesinin farklı semtlerinden farklı mevsimlerde toplam 180 adet midye tava ve 180 adet midye dolma örneği satın alınarak incelenmiştir. Örneklerin hiçbirinde *Vibrio vulnificus* ve *Vibrio cholerae* bakterilerine rastlanmazken, yaz aylarında incelenen 45 midye dolma örneğinin 11 tanesinde (%24,4), sonbahar aylarında incelenen 45 örneğin 3 tanesinde (%6,7) *Vibrio parahaemolyticus* tespit edilmiştir. Midye dolma örneklerinde ise *V. parahaemolyticus*'a kış ve ilkbahar aylarında rastlanılmazken, yaz ve sonbahar aylarında rastlandığı tespit edilmiştir. Analiz edilen toplam 360 işlenmiş midye örneğinin 14'ünde (%3,8) *Salmonella spp.*, 11'inde (%3,1) *Listeria monocytogenes* tespit edilmiştir. İncelenen midye tava örneklerinin hiçbirinde patojen bakteri tespit edilmemiştir. Sonuç olarak, midye dolmaların yaz ve sonbahar aylarında patojen bakteri içerdiğinin bulgulanmasından dolayı bu ürünlerin tüketiminin halk sağlığı açısından riskli olduğu belirlenmiştir. Bu nedenle midye dolmaların hammadde aşamasından itibaren işlendiği ve satışının yapıldığı yerlerde denetimlerin özellikle yaz ve sonbahar aylarında sıklaştırılması gerekmektedir.

Anahtar kelimeler: Tüketime hazır midye ürünleri, işlenmiş midye, midye tava, midye dolma, mikrobiyal kalite

Abstract: In this study, the total of 180 fried and 180 stuffed mussel samples from the different districts of Bornova in different seasons were sold and examined. While none of the specimens of *Vibrio vulnificus* and *Vibrio cholerae* were found, *Vibrio parahaemolyticus* was detected in 11 (24.4%) of 45 mussel stuffed samples examined in summer and 3 (6.7%) of 45 samples examined in autumn. In the stuffed mussel samples, *V. parahaemolyticus* was not found in winter and spring, while it was found in summer and autumn. *Salmonella spp.* was found in 14 (3.8%) of the 360 processed mussel samples and *Listeria monocytogenes* in 11 (3.1%). No pathogenic bacteria were detected in any of the fried mussel samples examined. As a result, it is always necessary to keep in mind that stuffed mussel can be risk for human health since it was found that it contained pathogenic bacteria, especially in the summer and in the autumn months. For this reason, it has become necessary to tighten the inspections especially in the summer and autumn months, where mussels are processed and sold.

Keywords: Ready-to-eat mussel products, processed mussel, fried mussel, stuffed mussel, microbiological quality

GİRİŞ

Ülkemizde yaygın olarak tüketilen midyenin Akdeniz midyesi (*Mytilus galloprovincialis,* Lamarck, 1819) olduğu ve kara midye olarakta adlandırıldığı bildirilmektedir (Ayvaz, 2018). *Mytilus galloprovincialis* Mytilidae ailesinin ülkemiz kara sularında karaya çıkarılan, işlenerek insanlara servis edilen ve ekonomik olarak değerlendirilen önemli bir türdür. Bu türün İzmir'den Karadeniz sularına kadar geniş bir alanda toplandığı belirtilmektedir (Balcıoğlu ve Gönülal, 2017). Türkiye İstatistik Kurumu (TÜİK), 2018 yılı su ürünleri verilerine göre 603,8 ton kara midyenin karaya çıkarıldığı ve insanlar tarafından tüketildiği bildirilmiştir (TUİK, 2018). Kabuklu su ürünlerinin lezzetli ve insanlar tarafından sevilerek tüketilen bir gıda maddesi olduğu belirtilmektedir. Midyenin hem pişmiş hem taze olarak tüketilebilen yüksek besin değerine sahip ve suyu uzaklaştırılmış ağırlığının %60'ının proteinden oluşan bir kabuklu su ürünü olduğu bildirilmektedir (Ayvaz, 2018). Ancak midyeler deniz suyunu süzerek beslenme faaliyetlerini gerçekleştirdiği için çeşitli patojen mikroorganizmayı içerebilmektedirler. Bu nedenle karaya çıkarıldıkları bölgedeki mikroorganizma yoğunluğununda dolaylı olarak hasat edilen midyelerin mikrobiyolojik kalitesini etkilediği ve midyelerin kalitelerininde mikrobiyolojik testlerle belirlenebileceği bildirilmektedir (Şirin, 2012). Dünyada midyeler genelde çiğ veya minimal işlem görmüş hali ile buharda pişirilmiş olarak insanlar tarafından tüketilen su ürünlerinden olsa da ülkemizde midyeler pişirilmeden yenilmemektedir (Başçınar, 2009).

Deniz kabuklularının vücutlarında ve kabuklarında çoğalma özelliği gösterebilen *Vibrio cholerae, Vibrio parahaemolyticus*, ve *Vibrio vulnificus* önemli *Vibrio* türleridir. Dolayısı ile insanlara bu kaynaklardan meydana gelen bulaşmanın başlıca nedenlerinin deniz kabuklularının tüketilmesi veya toplanması esnasındaki temas ile yakın ilgili olduğu vurgulanmaktadır (Aydın ve Soyutemiz, 2002). Taze ve işlenmiş su ürünlerinde patojen bakterilerin varlığı konusunda (Kılınç, 2001; Kocatepe vd., 2013; Kılınç ve

Beken, 2016; Novoslavskij vd., 2016; Zhang vd., 2016; Kılınç, 2019; Choi vd., 2019; Kılınç, 2020), midye dolmaların mikrobiyolojik kalitesi (Durgun, 2013; Kök vd., 2015; Kılınç vd., 2018; Öztürk ve Gündüz, 2018; Güngörür ve Bostan, 2019) konusunda çok sayıda çalışma yapılmış olmasına karşın, midye tavaların mikrobiyolojik kalitelerinin belirlenmesi konusunda yapılmış sınırlı sayıda çalışmaya (Bindu vd., 2002; Hampikyan vd., 2008) rastlanılmıştır.

Türkiye'de midyeler tüketime hazır halde midye tava ve midye dolma şeklinde satışa sunulmaktadır. Midye dolma midye eti, pilav ve baharatlar kullanılarak, midye tava ise midye etinin un ile kaplanarak derin yağda kızartılmasıyla hazırlanan ürünlerdir. Bu amaçla çalışmada tüketime hazır halde satışa sunulan işlenmiş midye tava ve midye dolma örneklerinin İzmir'in farklı semtlerinde satışı yapılan yerlerden temin edilmiştir. Ayrıca tüketime hazır haldeki midye ürünlerinin mevsime bağlı olarak mikrobiyolojik kalitelerinin incelenmesi yanısıra halk sağlığı açısından risk oluşumunun incelenmeside hedeflenmiştir.

MATERYAL VE METOT

Materyal

İşlenmiş midye çeşidi olarak çalışmada Türkiye'de tüketim şekli en fazla olan midye tava ve midye dolma örnekleri tercih edilmiştir. Midye tava ve midye dolma örnekleri İzmir'in Bornova (Bornova, Merkez), Çamdibi, Altındağ, Mersinli ve Yeşilova) olmak üzere 5 farklı semtte satış yapılan yerlerden temin edilmiştir. Örneklerin mevsime bağlı olarak (İlkbahar, Yaz, Sonbahar, Kış) mikrobiyal değişimleri ve patojen bakteri içerikleri araştırılmıştır. Midye tava ve midye dolma örnekleri aylık olarak her ay 3 defa olmak üzere 1 yıl boyunca örneklenmiştir. Çalışmada her mevsim için 45 midye tava ve 45 midye dolma örneği olmak üzere toplamda 1 yıl boyunca 180 adet midye tava ve 180 adet midye dolma örneği analiz edilmiştir.

Mikrobiyolojik analizler

Dilusyon hazırlanması

10 gram tüketime hazır halde satışa sunulan midye tava ve midye dolma örnekleri alınarak içerisinde 90 ml peptonlu su (LABM, LAB204) ile seyreltme işlemi yapılmıştır. Bu seyreltme pedallı blender torbasına konularak cihazda (IUL, Barcelona, Spain) 2,5 dakika homojenize edilmiştir. Hazırlanan dilüsyondan diğer desimal dilüsyonlar peptonlu su kullanılarak (TSE, 2001) metoduna göre hazırlanmıştır.

Toplam mezofilik ve psikrofilik aerob bakteri sayımları

Toplam mezofil ve psikrofil aerobik bakteri sayımları yayma plak yöntemine göre (TSE, 2013) metodu kullanılarak yapılmıştır. Seri dilüsyonları yapılan örnekler her dilüsyondan 0,5 ml olacak şekilde içerisinde Plate Count Agar (Liofilchem, 10032) hazır besiyeri bulunan petrilere ekilerek drigalski yayma çubuğu ile yayılmıştır. Petriler 30°C deki inkübatörde 72 saat aerobik ortamda inkübe edilmiştir. İnkübasyon sonunda petrilerde üreyen koloniler sayılarak dilüsyon faktörünün tersi ile çarpılıp, inokülasyon miktarına bölünerek örneklerin toplam mezofilik aerobik bakteri sayıları hesaplanmıştır. Psikrofil aerobik bakteri sayımı için ise yine her dilüsyondan 0,5 ml olacak şekilde içersinde Plate Count Agar (Liofilchem, 10032) hazır besiyeri bulunan petrilere ekilerek drigalski yayma çubuğu ile yayılmıştır. Petriler bu kez psikrofil bakteri sayımları için buzdolabı sıcaklığında 4-8°C de 7 gün aerobik ortamda inkübasyona bırakılmıştır. İnkübasyon sonunda petrilerde üreyen kolonilerin sayımı yapıldıktan sonra koloni sayısının dilüsyon faktörünün tersi ile çarpılıp, inokülasyon miktarına bölünmesi ile işlenmiş midye örneklerinin psikrofil aerobik bakteri sayıları hesaplanmıştır (TSE, 2013).

Toplam koliform bakteri sayımı

Koliform bakteri sayımı için seri dilüsyonları yapılan örnekler En muhtemel Sayı Yöntemine göre Lauryl Sulphate Tryptose Broth (LST) (Liofilchem, 24453) besiyerine 1 ml olacak şekilde ekimleri yapılmıştır. Daha sonra tüpler 37±1°C'deki inkübatörde 48 saat aerobik ortamda inbübe edilmiştir. Gaz oluşturan tüplerden paralel olarak steril öze yardımı ile Brilliant Green Bile Broth 2% (Liofilchem, 24102) besiyerine ekimleri yapılmıştır. Brilliant Green Bile Broth 2% besiyerleri 37±1°C'de 48 saat aerobik ortamda inkübe edilmiştir. İnkübasyon sonunda durham tüplerinde gaz oluşumu görülmüş olan tüpler (+) olarak, gaz oluşumu görülmemiş tüpler (-), olarak değerlendirilmiştir. Koliform bakteri sonuçları EMS tablosu kullanılarak hesaplanmıştır (TSE, 2015).

Fekal koliform bakteri sayımı

Fekal koliform bakteri sayımı için, inkübasyon sonunda gaz oluşturan koliform saptanan pozitif tüplerden steril öze yardımı ile EC Broth (Liofilchem, 24122) besiyerine ekimler yapılmıştır. EC Broth besiyerlerine ekim yapılan tüpler 44±1°C'deki inkübatörde 24 saat aerobik ortamda inkübe edilmiştir. İnkübasyon sonunda durham tüplerinde gaz oluşumu görülmüş olan tüpler (+) olarak, gaz oluşumu görülmemiş olan tüpler (-) olarak değerlendirilmiştir. İşlenmiş midye örneklerinin fekal koliform bakteri sayıları EMS tablosu kullanılarak hesaplanmıştır (TSE, 2015).

E. coli sayımı

E. coli sayımı için seri dilüsyonları yapılan örnekler her dilüsyondan 0,5 ml olacak şekilde içersinde TBX (Liofilchem, 10522) Agar hazır besiyeri bulunan petrilere ekilerek drigalski yayma çubuğu kullanılarak yayılmıştır. Ekim yapılan petriler 44±1°C'deki inkübatörde 24 saat aerobik ortamda inkübasyon sonrasında petrilerde üreyen mavi-yeşil kolonilerin sayımı gerçekleştirilmiştir. İşlenmiş midye örneklerindeki *E. coli* sayısı, koloni sayısının dilüsyon faktörünün tersi ile çarpılıp, inokülasyon miktarına bölünmesiyle hesaplanmıştır (TSE, 2015).

Koagulaz pozitif *Stafilakok* sayımı

Koagulaz pozitif *Stafilakok* sayımı için seri dilüsyonları yapılan işlenmiş midye örneklerden her dilüsyondan 0,5 ml

olacak şekilde içersinde Tavşan Fibrinojenli Baird Parker Agar (Baird Parker Agar Base + RPF Supplement, Liofilchem, 420010) hazır besiyeri bulunan petrilere ekimi gerçekleştirilmiştir. Ekim yapılan petriler üzerindeki inokulum drigalski yayma çubuğu kullanılarak yayılmıştır. İnoküle edilen petriler 37 ± 1 °C'deki inkübatörde 48 saat aerobik ortamda inkübe edilmiştir. İnkübasyon sonunda petrilerde üreyen lesitinaz pozitif zonlu koloniler sayılarak, koloni sayısının dilüsyon faktörünün tersi ile çarpılıp, inokülasyon miktarına bölünmesi ile örneklerdeki koagülaz pozitif *Stafilakok* sayısı hesaplanmıştır (TSE, 2006).

***Bacillus cereus* sayımı**

Bacillus cereus bakteri sayımı sadece midye dolma örneklerinin pirinç içeriğinden gerçekleştirilmiştir. *Bacillus cereus* sayımı için seri dilüsyonları yapılan örneklerden her dilüsyondan 0,5 ml olacak şekilde içersinde Chromatic™ *Bacillus cereus* agar (Liofilchem, 11628) hazır besiyeri bulunan petrilere yayma plak yöntemine göre ekim yapılarak drigalski yayma çubuğu kullanılarak yayma işlemi gerçekleştirilmiştir. Ekim yapılan petriler 30 ± 1 °C'deki inkübatörde 18-24 saat aerobik ortamda inkübe edilmiştir. İnkübasyon sonucunda petrilerde üreyen mavi-yeşil opak zonlu koloniler sayılarak, koloni sayısının dilüsyon faktörünün tersi ile çarpılıp, inokülasyon miktarına bölünerek *B. cereus* sayısı hesaplanmıştır (TSE, 2009).

Patojen bakterilerin analizi

***Salmonella spp.* analizi**

Salmonella analizi için 25 g tüketime hazır halde satışa sunulan işlenmiş midye örnekleri içerisinde 225 ml peptonlu su (LABM, LAB204) bulunan sıvı besiyeri içerisine tartılarak konulmuştur. Muller Kauffmann Tetrathionate Novobiocin Broth (MKTTn) (Liofilchem, 20072) besiyerinde 37±1°C'de 18-24 saat, Rappaport Vassiliadis Soy (RSV) Broth (Liofilchem, 26400) besiyerinde 41,5±1°C'de 18-24 saat aerobik ortamda inkübe edilmiştir. İnkübasyon sonunda Xylose Lysine Deoxycholate (XLD) Agar (LABM, LAB032) ve Brilliant Green Agar (LABM, LAB034) besiyerlerine ekimler yapılarak 37±1°C'deki inkübatörde 18-24 saat inkübasyona bırakılmıştır. İnkübasyondan sonrasında besiyerinde etrafı parlak kırmızı zon ile çevrili pembe-kırmızı renkli koloniler *Salmonella* şüpheli olarak değerlendirilmiştir. Daha sonra spesifik koloniler Nutrient Agar (Liofilchem, 10044) besiyeri kullanılarak saflaştırılmıştır. Besiyerinden izolasyonu gerçekleştirilen kültürlerin identifikasyonu amacıyla biyokimyasal testler uygulanmıştır. Bu amaçla steril öze yardımı ile besiyerlere ekimler yapılmıştır. Yapılan ekimler sonrasında ekim yapılan petriler 37±1C°'deki inkübatörde 24 saat inkübe edilmiştir. İnkübasyon sonrası Lysine Iron Agar (Liofilchem, 30098) besiyerinde rengin değişmemesi (menekşe rengi) ve siyahlaşma olması tespit edilmiştir. Metil Red-Voges Proskuer Broth (Liofilchem, 24149) besiyerinde pembe rengin oluşması saptanmıştır. Triple Sugar Iron agar (Liofilchem, 30100) besiyerinin dip kısmının sarı, yatık kısmının kırmızı ve siyahlaşma olması gözlemlenmiştir. Simmon's Sitrat Agar (Liofilchem, 412030) besiyerlerinde renk değişikliği olması saptanmıştır. Üre-İndol Besiyerinde (Liofilchem, 403060) ise Kovacs ayıracı ilave edildiğinde menekşe renginde halka oluşmaması ve test tüpünde kırmızı renk oluşmaması durumunda örnekler *Salmonella spp.* yönünden pozitif olarak değerlendirilmiştir (TSE, 2005).

***Listeria monocytogenes* analizi**

Listeria monocytogenes analizi (TSE, 1997) metodu kullanılarak yapılmıştır. Analiz için 25 g işlenmiş midye örnekleri 225 ml yarım konsantrasyonda inhibitör içeren Fraser Broth (Oxoid, CM0895) besiyerinde homojenize edilmiştir. Daha sonra 30±1°C'de inkübatörde 24 saat inkübe edilmiştir. Ön zenginleştirme kültüründen doğrudan Palcam Agar (Oxoid, CM0877) ve/veya Ottoviani-Agosti Agar (Liofilchem, 10620) selektif besiyerlerine sürme yapılarak ekim gerçekleştirilmiştir. İnkübasyon sonrasında yine Palcam Agar ve/veya Ottoviani-Agosti Agar besiyerlerine sürme yapılarak besiyerleri 37°C' de 48 saat inkübe edilmiştir. İnkübasyon sonunda petrilerde oluşan tipik kolonilere biyokimyasal doğrulama testleri uygulanmıştır. Her bir petride üreyen yaklaşık 1 mm çaplı mavi-yeşil renkli zonlu olan tipik kolonilerden 2-4 adet seçilerek biyokimyasal ve diğer testlerin yapılması için Tryptic Soy Agar-Yeast Extract (Liofilchem, 610241) besiyerine ekim yapılmıştır. Daha sonra petriler 30°C'de 24 saat inkubasyona tabi tutulmuştur. Sonrasında petrilerde kolonilere sırası ile belirtilen testler (Gram boyama, katalaz (%3'lük H_2O_2 ile), oksidaz (Merck, 113300) ve 21-26°C'de Sulphate Indole Motility Medium (Liofilchem, 30095)'te hareketlilik testleri) uygulanmıştır. Gram pozitif, oksidaz negatif, katalaz pozitif, kısa çubuk formlu ve SIM mediumda oksijenli kısımda çoğalıp şemsiye görünümü oluşturan kolonilerinin identifikasyonu amacıyla %7'lik defibrine koyun kanı ile hazırlanmış kanlı agarda ß-hemoliz ve CAMP testleri ile Mannitol, L-ramnoz, D-ksiloz, fermentasyon ve nitrat redüksiyon testleri yapılarak izolatların identifikasyonu gerçekleştirilmiştir. Kolonilerden CAMP testte *S. aureus* ile sinerjik etki göstererek hemoliz veren, kanlı agarda β-hemoliz oluşturan, şeker testlerinden D-ksiloz negatif, mannitol negatif, nitrat redüksiyon testi negatif ve L-ramnoz pozitif olan örnekler *L. monocytogenes* olarak tanımlanmıştır. (TSE, 1997).

Örneklerin *Vibrio spp.* için Real-time PZR analizleri

Örneklerin *Vibrio* türleri yönünden Real-Time PZR ile analizleri Dupot Q7 BAX sistem analiz kiti kullanılarak (Dupont, BAX Part D12863877) yöntemine göre yapılmıştır. (Anonymus, 2016). Bu metot ön zenginleştirme, DNA ekstraksiyonu ve cihaza aplikasyon aşamalarını içermektedir. 25 gram işlenmiş midye örnekleri 225 ml Alkali Pepton besiyeri (Liofilchem, 451404) içerisine konulup pedallı blender (IUL, Barcelona, Spain) cihazında 2,5 dakika homojenize edilmiştir. Homojenize edilen örnekler 35-37°C'de 18-24 saat inkübe edilmiştir. Daha sonra örneklerden 500 µl ependorf tüpüne alınarak DNA ekstraksiyonu için kullanılmıştır.

Ekstraksiyon için Lysis buffer içersine 150 µl proteaz eklenip votekste (İka, Almanya) karıştırıldıktan sonra cluster tüplerine 200 µl lizis buffer ilave edilmiştir. Daha sonrasında cluster tüpleri içine 500 µl ependorf tüpünden alınan 30 µl örnek eklendikten sonra karışım önce 37°C'de 20 dakika, daha sonra 95°C'de 10 dakika ısı bloklarında inkübe edilmiştir. Real-Time PZR ekstraksiyon işlemleri tamamlandıktan sonra cihaza aplikasyon işlemleri için derin dondurucudan soğutma blokları üzerine PZR reaksiyon tüpleri konulmuştur. Elde edilen ekstraklardan liyofilize halde Real-Time PZR reaktifi olan PZR reaksiyon tüplerine 50 µl DNA ekstraktı konularak tüpler kitle birlikte gelen optik kapakları ile kapatılmıştır. Analiz için hazırlanan Dupont BAX Q7 cihazına reaktif tüpleri yerleştirilerek çalıştırılmıştır. *Vibrio* türleri için sonucu pozitif çıkan örneklerin ön zenginleştirmelerinden Thiosulfate citrate bile salts sucrose agar (TCBS agar) (Himedia, M189) besiyerine ekimler yapılmış ve 37°C'de 18-24 saat aerobik koşullarda inkübasyona bırakılmıştır. Petrilerde üreyen 2–3 mm çapında, mavi-yeşil renkli ortası koyu kolonilerden flagellar hareketli, oksidaz pozitif, gram negatif kıvrık çomak şeklinde olanlardan %1,5 NaCl içeren Tryptic Soy (TSA) agara subkültürleri yapılmıştır. Üreyen koloniler ön zenginleştirme yapılmadan tekrar PZR işlemine tabi tutularak doğrulama işlemleri yapılmıştır (Anonymus, 2016).

İstatistiksel analiz

Çalışmada işlenmiş midye tava ve midye dolma örneklerinin mikrobiyolojik verileri İstatistik programı olarak MiniTAB 17. sürüm kullanılarak istatistiksel olarak değerlendirilmiştir. Bu programla One-Way ANOVA analizi ve Tukey gruplandırma testleri (Aktaş vd., 2016)'ya göre yapılmıştır. İşlenmiş midyelerde mikroorganizma türlerine rastlanma oranları (pozitif oranları) arasındaki farkın önemli olup olmadığı Z testi ile (Montgomer ve Runger, 2003)'e göre kontrol edilmiş ve ilgili tablolarda anlamlılık (p-değeri<0,001) düzeyleri ile birlikte verilmiştir.

BULGULAR

İşlenmiş midyelerin *Vibrio spp.*, analiz sonuçları Tablo 1'de verilmiştir.

Tablo 1. İşlenmiş midyelerin *Vibrio* türleri analiz sonuçları
Table 1. The analysis results of the species of *Vibrio* in processed mussels

Örnekler	Örnek Sayısı	*V. vulnificus*			*V. cholerae*			*V. parahaemolyticus*			p değeri
		Pozitif	Negatif	%	Pozitif	Negatif	%	Pozitif	Negatif	%	
Midye tava	180	0	180	0	0	180	0	0	180	0	
Midye dolma	180	0	180	0	0	180	0	14	166	7,8	p<0,001
Toplam	360	0	360	0	0	360	0	14	346	7,8	

Çalışma sonuçlarına göre işlenmiş midye tava ve midye dolma örneklerinin hiçbirinde *V. vulnificus* ve *V. cholerae* bakterilerine rastlanılmamıştır. Buna karşın, *V. parahaemolyticus* türü sadece midye dolma örneklerinde tespit edilmiştir. Midye dolma örneklerinin 14 tanesinde (%7,8) *V. parahaemolyticus*'a rastlanılmıştır. Midye tava ile midye dolma örneklerinde *V. parahaemolyticus* görülme oranları arasındaki farkın önemli olduğu belirlenmiştir (p-değeri<0,001). İşlenmiş midyelerdeki *Vibrio* türlerinin mevsimlere göre analiz sonuçları Tablo 2'de verilmiştir.

Tablo 2. İşlenmiş midyelerdeki *Vibrio* türlerinin mevsimlere göre analiz sonuçları
Table 2. The analysis results of the species of *Vibrio* according to the seasons

Mevsimler	Örnek Sayısı	Midye tava			Midye dolma			p değeri
		Pozitif	Negatif	%	Pozitif	Negatif	%	
Kış	45	0	45	0	0	45	0	
İlkbahar	45	0	45	0	0	45	0	
Yaz	45	0	45	0	11	34	24,4	p<0,001
Sonbahar	45	0	45	0	3	42	6,7	p=0,037
Toplam	180	0	180	0	14	166	7,8	

Analiz sonuçlarına göre yaz aylarında incelenen 45 midye dolma örneğinin 11 tanesinde (%24,4), sonbahar aylarında incelenen 45 örneğin 3 tanesinde (%6,7) *V. parahaemolyticus* tespit edilmiştir. Mevsimsel açıdan bakıldığında midye dolma örneklerinde *V. parahaemolyticus*'a kış ve ilkbahar aylarında rastlanılmazken, yaz ve sonbahar aylarında rastlanıldığı tespit edilmiştir. Yaz aylarında midye tava ile midye dolma örneklerinde *V. parahaemolyticus* görülme oranları arasındaki farkın önemli olduğu belirlenmiştir (p-değeri<0,001). Sonbahar aylarında da pozitif oranlar arasındaki farklar yine anlamlı olarak saptanmıştır (p-değeri=0,037). Midye dolma örneklerinin farklı semtlere göre *V. parahaemolyticus* analiz sonuçları Tablo 3'te gösterilmiştir.

Tablo 3. Midye dolmaların farklı semtlere göre *V. parahaemolyticus* analiz sonuçları
Table 3. The analysis results of *V. parahaemolyticus* in stuffed mussels according to the different districts

Semtler	Örnek Sayısı	*V. parahaemolyticus*		
		Pozitif	Negatif	% Pozitiflik
Bornova	36	3	33	8,33
Çamdibi	36	3	33	8,33
Mersinli	36	2	34	5,56
Altındağ	36	3	33	8,33
Yeşilova	36	3	33	8,33
Toplam	180	14	166	7,78

Semtler arasında *V. parahaemolyticus* varlığı açısından önemli bir fark bulunmamaktadır. Diğer bir ifadeyle, *V. parahaemolyticus* tespit edilemeyen semt bulunmamaktadır. Bornova'dan toplanan 36'şar örneğin 3 tanesinde (%8,33) Çamdibi'nden toplanan 36'şar örneğin 3 tanesinde (%8,33), Altındağ'dan toplanan 36'şar örneğin 3 tanesinde (%8,33) ve Yeşilova'dan toplanan 36 örneğin 3 tanesinde (%8,33), Mersinli'den toplanan 36 örneğin 2 tanesinde (%5,56) *V. parahaemolyticus* tespit edilmiştir.

Diğer patojen bakteri analizleri sonuçları

İşlenmiş midyelerin diğer patojen bakteri analizi sonuçları Tablo 4'te verilmiştir.

Tablo 4. İşlenmiş midyelerin diğer patojen bakteri analizi sonuçları
Table 4. The analysis results of other pathogenic bacteria in processed mussels

Örnekler	Örnek Sayısı	*Salmonella spp.*				*Listeria monocytogenes*			
		Pozitif	Negatif	%	p değeri	Pozitif	Negatif	%	p değeri
Midye	180	0	180	0		0	180	0	
Midye	180	14	166	7,8	p<0,001	11	169	6,1	p<0,001
Toplam	360	14	346	3,8		11	349	3,1	

Analiz edilen toplam 360 işlenmiş midye örneğinin 14'ünde (%3,8) *Salmonella spp.*, 11'inde (%3,1) *Listeria monocytogenes* tespit edilmiştir. İncelenen midye tava örneklerinin hiçbirinde *Salmonella spp.* ve *Listeria monocytogenes* tespit edilmemiştir. Analiz edilen 180 midye dolma örneğinin 14'ünde (%7,8) *Salmonella spp.* ve 11'inde (%6,1) *Listeria monocytogenes* tespit edilmiştir. Midye tava ve midye dolma örneklerinde *Salmonella spp.* ve *Listeria monocytogenes* türlerine rastlanma oranları arasındaki fark istatistiksel açıdan anlamlı olarak bulunmuştur (p-değeri<0,001). İşlenmiş midyelerin *Salmonella* türlerinin mevsimlere göre sonuçları Tablo 5'te gösterilmiştir.

Tablo 5. İşlenmiş midyelerin *Salmonella spp.* analizlerinin mevsimlere göre sonuçları
Table 5. The analysis results of *Salmonella spp.* in processed mussels according to the seasons

Mevsimler	Örnek Sayısı	Midye tava			Midye dolma			p değeri
		Pozitif	Negatif	%	Pozitif	Negatif	%	
Kış	45	0	45	0	0	45	0	
İlkbahar	45	0	45	0	0	45	0	
Yaz	45	0	45	0	14	31	31,1	p<0,001
Sonbahar	45	0	45	0	0	45	0	
Toplam	180	0	180	0	14	166	7,8	

Midye tava örneklerinde hiçbir mevsimde *Salmonella* türlerine rastlanılmazken, midye dolma örneklerinde sadece yaz aylarında *Salmonella* türlerine rastlanılmıştır. Kış, ilkbahar ve sonbahar aylarında satın alınan midye dolma örneklerinde *Salmonella* türleri bulunmamasına karşın, yaz aylarında alınan 45 örneğin 14'ünde (%31,1) *Salmonella* türleri tespit edilmiştir. Yaz aylarında midye tava ile midye dolma örneklerinde *Salmonella* türleri bulunma oranları arasındaki fark istatistiksel olarak anlamlı bulunmuştur (p-değeri<0,001). Örneklerin *Listeria monocytogenes* analizlerinin mevsimlere göre sonuçları Tablo 6'da gösterilmiştir.

Tablo 6. İşlenmiş midyelerin *Listeria monocytogenes* analizlerinin mevsimlere göre sonuçları
Table 6. The analysis results of. *Listeria monocytogenes* in processed mussels according to the seasons

Mevsimler	Örnek Sayısı	Midye tava			Midye dolma			p değeri
		Pozitif	Negatif	%	Pozitif	Negatif	%	
Kış	45	0	45	0	0	45	0	
İlkbahar	45	0	45	0	0	45	0	
Yaz	45	0	45	0	9	36	20,0	p<0,001
Sonbahar	45	0	45	0	2	43	4,4	p=0,074
Toplam	180	0	180	0	11	169	6,1	

Kış, ilkbahar, yaz ve sonbahar aylarında satın alınarak incelenen midye tava örneklerinin hiçbirinde *Listeria monocytogenes'*e rastlanılmamasına rağmen, yaz ve sonbahar aylarında satın alınarak incelenen midye dolma örneklerinde *Listeria monocytogenes'*e rastlanılmıştır. Kış ve ilkbahar aylarında satın alınan midye dolma örneklerinde *Listeria monocytogenes* bulunmamasına karşın, yaz aylarında satın alınarak incelenen 45 örneğin 9'unda (%20), sonbahar aylarında satın alınarak incelenen 45 örneğin 2'sinde (%4,4) *Listeria monocytogenes* tespit edilmiştir. Yaz aylarında midye tava ve midye dolma örneklerinde *Listeria monocytogenes* görülme oranları arasındaki fark önemli iken (p-değeri<0.001) sonbahar aylarındaki oranlar arasındaki farklılığın istatistiksel olarak anlamlı olmadığı (p-değeri=0,074) belirlenmiştir.

İndikatör mikroorganizma analizleri sonuçları

İşlenmiş midyelerin indikatör mikroorganizma analizleri sonuçları Tablo 7'de verilmiştir.

Tablo 7. İşlenmiş midyelerin indikatör mikroorganizma analizleri sonuçları
Table 7. The analysis results of indicator microorganisms in processed mussels

Analizler	Midye tava	Midye dolma
TMBS log(kob/g)	2,45±0,07	5,30±1,09
TPBS log(kob/g)	2,21±0,09	3,39±0,27
TKBS MPN/g	<3	2,12±0,44
FKBS MPN/g	<3	1,64± 0,36
EC log(kob/g)	-	1,24±0,36
KPS log(kob/g)	<1	2,41±0,20
BCS log(kob/g)	-	2,04±0,52

n=180; X ort. (ortalama değer) ±SS (standart sapma). TMBS: Toplam mezofilik aerobik bakteri sayısı, TPBS: Toplam psikrofilik bakteri sayısı, TKBS: Toplam koliform bakteri sayısı, FKBS: Fekal koliform bakteri sayısı, EC: *Escherichia coli*, KPS: Koagulaz pozitif *Stafilokok*, BCS: *Bacillus cereus*

Midye tava örneklerinin TMBS 2,45±0,07 log (kob/g) düzeyinde ve TPBS ise 2,21±0,09 log (kob/g) düzeyinde bulunmuştur. Midye tava örneklerinde TKBS, FKBS, EC, KPS bakterilerine ise rastlanılmamıştır. Buna karşın, midye dolma örneklerinin ise TMBS, TPBS, TKBS, FKBS, EC, KPS ve BCS sırasıyla 5,30±1,09 log (kob/g), 3,39±0,27 log (kob/g), 2,12±0,44 MPN/g, 1,64±0,36 MPN/g, 1,24±0,36 log (kob/g), 2,41±0,20 log (kob/g) ve 2,04±0,52 log (kob/g) olarak saptanmıştır. Midye tava örneklerinin mevsimlere göre indikatör mikroorganizma analiz sonuçları Tablo 8' de verilmiştir.

Tablo 8. Midye tava örneklerinin mevsimlere göre indikatör mikroorganizma analiz sonuçları
Table 8. The analysis results of indicator microorganisms in fried mussels according to the seasons

Mevsimler	Örnek Sayısı	TMBS log(kob/g)		TPBS log(kob/g)	
		Ortalama	Tukey	Ortalama	Tukey
Kış	45	2,46	A	2,40	C
İlkbahar	45	2,45	A	2,46	AB
Yaz	45	2,43	A	2,49	A
Sonbahar	45	2,43	A	2,44	B

TMBS: Toplam mezofilik aerobik bakteri sayısı, TPBS: Toplam psikrofilik bakteri sayısı

Midye tava örneklerinin yapılan TMBS analiz sonuçları mevsimler yönünden incelendiğinde analiz sonuçları ile mevsimler arasında istatistiksel olarak fark (p>0,05) bulunmamaktadır. Midye tava örneklerinin TPBS analizindeki sonuçları istatistiksel olarak farklılıklar (p<0,05) gösterse de sonuçların birbirine oldukça yakın olduğu açıkça görülmektedir. Midye dolma örneklerinin mevsimlere göre indikatör mikroorganizma analiz sonuçları Tablo 9'da verilmiştir. Midye dolma örneklerinin analiz sonuçları mevsimler yönünden incelendiğinde yapılan analiz sonuçları ile mevsimler arasında belirgin şekilde değişimler olduğu tespit edilmiştir. Midye dolma örneklerinin TMBS analiz sonuçları en çok yaz aylarında yükselmekte (p<0,05), kış ve ilkbahar aylarında ise istatistiksel olarak (p>0,05) benzerlik göstermektedir. Midye dolma örneklerinin TPBS sonuçları ise aksi yönde değişim göstermiş, en yüksek düzeye kış ve ilkbahar mevsimlerinde ulaşmış (p>0,05) ve kış, yaz ve sonbahar mevsimleri arasında istatistiksel olarak birbirinden farklılık (p<0,05) gözlenmiştir.

Tablo 9. Midye dolma mevsimlere göre indikatör mikroorganizma analiz sonuçları
Table 9. The analysis results of. indicator microorganisms in stuffed mussels according to the seasons

Mevsimler	Örnek Sayısı	TMBS log(kob/g)		TPBS log(kob/g)		TKBS log(kob/g)		FKBS log(kob/g)		EC log(kob/g)		KPS log(kob/g)		BCS log(kob/g)	
		Ort.	Tukey	Ort.	Tukey	Ort.	Tukey	Ort.	Tukey	Ort.	Tukey	Ort.	Tukey	Ort.	Tukey
Kış	45	4,39	C	3,61	A	1,76	B	1,34	C	1,00	C	2,23	D	1,35	D
İlkbahar	45	4,56	C	3,64	A	1,77	B	1,35	C	1,01	C	2,31	C	1,77	C
Yaz	45	6,54	A	3,08	C	2,46	A	2,04	A	1,60	A	2,63	A	2,57	A
Sonbahar	45	5,73	B	3,23	B	2,49	A	1,83	B	1,35	B	2,43	B	2,45	B

Ort: Ortalama değer, TMBS: Toplam mezofilik aerobik bakteri sayısı, TPBS: Toplam psikrofilik bakteri sayısı, TKBS: Toplam koliform bakteri sayısı, FKBS: Fekal koliform bakteri sayısı, EC: *Escherichia coli*, KPS: Koagulaz pozitif *Stafilokok,* BCS: *Bacillus cereus*

Midye dolmaların TKBS analiz sonuçlarına göre, koliform sayısı en yüksek düzeye yaz ve sonbahar mevsiminde ulaşmış olup bu mevsimlerde istatistiksel olarak fark (p>0.05) gözlenmezken, kış-ilkbahar ile yaz-sonbahar mevsimleri arasında istatistiksel olarak birbirinden farklılık (p<0.05) gözlenmiştir. FKBS ve EC analizleri mevsimsel olarak birbirine paralel ilerlemiş en yüksek düzeye ise yaz aylarında (p<0.05) ulaşmıştır. Midye dolma örneklerinin KPS ve BCS analiz sonuçları arasında mevsimler arasında istatistiksel olarak fark saptanmış (p<0.05) ve her iki grup bakteride de en yüksek sayıya (p<0.05) yaz aylarında rastlanmıştır.

TARTIŞMA

Gıdalarda TMBS gıdanın işleme emniyeti bakımından önemlidir. Gıdalarda en fazla bozulmaya TMB grubu mikroorganizmalar neden olduklarından bunların örneklerdeki miktarı raf ömründe ve üretimin hijyen koşullarının belirlenmesinde önemli bir kriter oluşturdukları belirtilmektedir (Ayhan, 2019). Tıpkı diğer gıda ürünlerinin mikrobiyolojik özelliklerinin incelendiği çalışmalarda olduğu gibi midye ve midye ürünlerinin TMBS araştırıldığı birçok çalışma yapılmıştır. 20 midye dolma örneğinin mikrobiyolojik açıdan incelendiği bir çalışmada midye dolmaların TMBS 2,5 x 10^4 kob/g olarak tespit edilmiştir (Öner, 1997). Ergönül vd., (2014) tarafından yapılan çalışmada İzmir'in değişik ilçelerinden, 25 satıcıdan toplam 100 adet midye dolma örnekleri satın alınarak mikrobiyolojik kaliteleri incelenmiştir. Yapılan bu çalışmada midye dolmaların TMBS'nın 1 ile 4,67 log(kob/g) arasında değişim gösterdikleri bildirilmiştir (Ergönül vd., 2014). Aksu vd., (2017) tarafından yapılan diğer bir çalışmada ise, 10 adet midye dolma örneğinin TMBS'nın 1,51 x 10^5 kob/g olarak belirlendiği belirtilmiştir (Aksu vd., 2017). Öztürk ve Gündüz (2018) tarafından yapılan başka bir çalışmada inceledikleri midye dolmaların mikrobiyal kalitelerinin düşük olduğu ve TAMB sayısının 2,8-6,82; KBS'ın <2,00-5,43 ve *Staphylococcus aureus* bakteri sayısının <2,00-5,04 log kob/g arasında değişim gösterdiğini tespit etmişlerdir. Yapılan diğer çalışmada İzmir'in üç farklı bölgesinde kış mevsiminde

satışa sunulan ve marketlerden rastgele örnekleme yöntemiyle alınıp incelenen midye dolma örneklerinin TMBS'nın (<10 kob/g - 4,2x10^5 kob/g) aralığında değişim gösterdiği bildirilmiştir (Kılınç vd., 2018). İstanbul'un farklı ilçelerinden temin edilen toplam 50 adet midye dolma örneğinin TMBS'ın sokakta satılan 25 örnekte 2,20-7,23 log(kob/g) arasında ve kapalı mekânlarda satılan 25 örnekte ise 2,11-5,34 log(kob/g) değerleri arasında değişim gösterdiği belirtilmiştir (Güngörür ve Bostan, 2019). Yukarıda belirtilen çalışmalarda (Öner, 1997; Ergönül vd., 2014; Aksu vd., 2017; Kılınç vd., 2018; Öztürk ve Gündüz, 2018; Güngörür ve Bostan, 2019) midye dolmaların TMBS'nın satış yerlerine ve mevsimlere göre değişiklik göstermesi yönünde elde edilen bulgular yapılan çalışma ile paralellik göstermektedir. ICMSF (1986)'ya göre; tüketime hazır haldeki ürünler için belirtilen mikrobiyolojik açıdan tüketilebilirlik üst limit değer TMBS için 1,0x10^6 kob/g olarak bildirilmiştir. Yapılan çalışmada; sonbahar mevsiminde satın alınarak incelenen midye dolma örnekleri mikrobiyolojik olarak belirtilen bu limit değere yakın saptanırken, yaz mevsiminde mikrobiyolojik açıdan tüketilebilir limitlerin üzerinde saptanmıştır. Buna karşın, kış ve ilkbahar mevsimlerinde ise tüketilebilir oldukları bulgulanmıştır.

Satın alınarak incelenen midye tava örneklerinin ise TMBS açısından her mevsim tüketilebilir olduğu saptanmıştır. Güngörür ve Bostan (2019) tarafından yapılan çalışmada İstanbul'un farklı ilçelerindeki sokakta satılanlardan ve kapalı (restoran, büfe) müesseselerde satışa sunulan toplam 50 midye dolma örneğinin incelendiği çalışmada patojen *Salmonella* türlerine rastlanmadığı bildirilmesine karşın, yapılan çok sayıda çalışmada da (Bingöl vd. 2008**;** Ateş vd., 2011; Durgun, 2013; Kök vd., 2015, Güngörür ve Bostan 2019) midye dolmaların patojen bakteri türlerini içerdiği vurgulanmaktadır. Yapılan çalışmada midye dolma örneklerinin sonbahar ve yaz aylarında *L. monocytogenes* ve *V. parahaemolyticus* gibi patojen bakterileri içermesi yönünde elde edilen bu sonuçlar yukarıdaki çalışmalarla uyum göstermektedir. *E. coli*'nin gıda hijyeni kontrollerinde hijyen indikatörü olarak kullanıldığı belirtilmektedir (Halkman, 2013). Bu grup bakteriler fekal kontaminasyonunda göstergesi olduğundan tüketime hazır halde satışa sunulan işlenmiş midye dolma örneklerinde bu bakteri grubunun varlığı üretim yeri ve satış yeri hijyeninin uygun olmadığını açıkça göstermektedir. Yapılan çalışmada midye tava örneklerinde *E. coli* ve diğer patojen bakterilere hiçbir mevsimde rastlanılmamasına rağmen, incelenen midye dolma örneklerinde tüm mevsimlerde *E. coli*'ye ve diğer patojen bakterilere rastlanılması hijyen kurallarına dikkat edilmediğine işaret etmektedir.

İnsanların el, kulak, burun ve yüzlerinde bulunan stafilokokların tüketime hazır gıdalarda saptanması insan kaynaklı bulaşma olduğunu göstermektedir (Halkman, 2013). Midye dolmalarda koagulaz pozitif stafilokok varlığı birçok araştırmacı tarafından saptanmıştır (Ateş vd., 2011; Durgun, 2013; Kök vd., 2015; Güngörür ve Bostan, 2019). Yapılan çalışmada midye dolmalarda koliform, fekal koliform, *E. coli*, *Staphylococus aureus, Bacillus cereus* saptanması yönündeki bulgular yapılan diğer çalışmalarda (Öner 1997; Ateş vd. 2011; Kök vd. 2015; Öztürk ve Gündüz, 2018) midye dolmalarda patojen bakteri bulunması veya mikrobiyal kalitenin düşük olduğunun belirlenmesi yönünde elde edilen bulgularla paralellik göstermektedir.

Çalışmamızda satın alınarak incelenen iki farklı şekilde işlenmiş midye dolma ve midye tava örnekleri mikrobiyal açıdan karşılaştırıldığında; midye dolma örneklerinin mikrobiyal yükünün fazla ve patojen bakterileri içermesinin midyeden, dolma içeriğine giren diğer malzemelerden (pirinç ve baharatlar) veya personel kontaminasyonu gibi faktörlerden kaynaklandığı düşünülmektedir. Midye tava örneklerinde ise patojen bakteri saptanmamasının sebebinin örneklerin derin kızgın yağda kızartma işleminin patojen bakteriler üzerinde yapmış olduğu öldürücü etkisinden kaynaklandığı tahmin edilmektedir. Ayrıca midye tava örnekleri midye dolma örnekleri ile kıyaslandığında; midye tava örneklerinin işlendikten sonra ve satışa sunuş aşamalarında da hijyen ve sanitasyon kurallarının uygulandığını ve patojen bakteriler ile ürünlerin kontamine olmadığını açıkça göstermektedir. Satışa sunulan midye dolma örneklerinin ise işlendikten sonra satışa sunuş aşamalarına kadar ki süreçtede kontaminasyona maruz kalmış olabileceğini düşündürmektedir.

Midye ve midyeden üretilen gıda ürünlerinin insanlar için çok tehlike arzeden patojen bakterileri taşıyıp taşımadıkları mevsimlere göre büyük ölçüde değişkenlik göstermektedir. Bu durum yukarıda belirtilen çalışmalarda da açıkça görülmektedir. Yapılan kimi çalışmalarda işlenmiş midye dolma ürünlerinde yüksek oranda bakteri sayısı ve patojen bakteri varlığı tespit edilirken, yapılan kimi araştırmalarda bu ürünlerde patojen bakterilere rastlanılmaması alınan örneklerin mevsimsel zamanı ile ilişkili olduğunu göstermektedir.

Mevsimsel analizlerdeki bulgular riskin daha çok yaz ve sonbahar mevsimlerinde oluştuğunu işaret etmektedir. Burada deniz suyunun ısınması midyelerin hasat alanlarındaki bakteri miktarlarının artmasına neden olabilmektedir. Bu riski gidermek için bu mevsimlerde toplanan midyelerin depurasyon tesislerinde işlem görmesi ve sertifikalı ham madde kullanılması (tesis çıkışlı) önerilebilir. Ayrıca işlenmiş midye üretiminin yapıldığı yerlerde hijyen ve sanitasyon uygulamalarına dikkat edilmediğinde bu ürünler halk sağlığı açısından büyük risk oluşumuna neden olabilmektedir. Bu nedenle gerek işleme öncesinde gerekse işleme esnasında ve sonrasında işlenmiş ürünlerde ve üretim yerlerinde hijyen ve sanitasyon kurallarının uygulanmasına dikkat edilmeli bu konuda gereken önlemler alınmalıdır.

SONUÇ

Çalışma sonucunda analiz edilen midye dolma örneklerinde özellikle yaz mevsiminde *V. parahaemolyticus*,

L. monocytogenes ve *Salmonella spp*. gibi patojen bakterilerin saptanması bunun yanısıra sonbahar mevsiminde de *V. parahaemolyticus* ve *L. monocytogenes* gibi patojen bakterilere rastlanılmıştır. Bu durum midye dolmaların özellikle yaz ve sonbahar mevsimlerinde hammaddeden gelen bir sıkıntı olabileceğini belki de hijyen ve sanitasyon kurallarına uygun olarak işlenmediğini ve satışa sunulmadığını göstermektetir. Çalışmada incelenen midye dolmalarda patojen bakteri varlığının saptanması bu ürünlerin uygun şekilde ısıl işleme tabi tutulmadığını veya midyelerin kirli alandan temiz alana doğru akışı olmayan sağlıksız ortamlarda üretiminin yapıldığını göstermektedir. Bunun yanısıra üretimden satışa kadar ki aşamalarda da hijyen ve sanitasyon kurallarının uygulanmasında eksiklikler olduğu ve ürünlerin rekontaminasyonunu işaret etmektedir.

Bu nedenle elde edilen sonuçlara göre midyelerin işleme tekniklerinin başarılı olan yönlerini (hijyen ve sanitasyon kurallarına uygun işlenmiş midyelerin üretimi) öne çıkarma yönünden ileriki çalışmalara devam edilerek halkımızın sağlıklı gıdaya ulaşması için alınan önlemlerin arttırılacağı öngörülmektedir. Özellikle bakteriyel yükü fazla olan zamanlarda bu ürünlerin çocuklar ve bağışıklık sistemi zayıf olan bireylerde hastalık yapabileceği göz önünde bulundurularak üretim ve satış yerlerinin denetlenmesi noktasındaki denetimlerin özellikle yaz ve sonbahar mevsimlerinde sıklaştırılması gerekliliği ortaya çıkmıştır. Burada hammaddenin menşei ve kontrollerinin baştan denetlenmesinin her mevsim güvenilebilir hammadde temininde etkili olabileceği düşünülmektedir. Ayrıca yetkili otorite tarafından menşei durumuna (Hasat ortamı su kriterlerine göre A, B, C sınıfı sular AB mevzuatı) göre depurasyon tesisi çıkışlı hammadde kullanım zorunluluğu da getirilebilir. Halk sağlığı açısından önemli olan denizden sofraya olan yolculukta iyi üretim uygulamaları, hijyen sanitasyon gibi kavramların uygulanmasının sağlanması ile tüketicideki güven daha da artacaktır.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effects of ceramic balls on trout welfare during their live transfer conditions

Canlı balık transferi süresince kullanılan seramik topların alabalık refahı üzerine etkisi

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Abstract: The effects of ceramic balls on live transfer conditions of rainbow trout (*Oncorhynchus mykiss,* (Walbaum,1792)) were determined by examining ammonia, pH, dissolved oxygen, and temperature of water and gill histology of fish. Salt addition and various pH levels of water were also studied for comparison. The study was designed two different pH levels (high 7.98±0.2 and low 6.01±0.1), salt (6g/L), and ceramic ball (10g/l) addition and fish stock density to be 62.3 kg/m^3. The trial tanks were mounted on a vehicle to represent actual transport conditions. Fish were sampled every hour for gill histology. As a results, the lowest ammonia value was determined as 1.98 mg/L in the ceramic ball. The highest ammonia value in the high pH group was 2.83 mg/l. When the gill tissues of the fish were examined, the significant differences observed were oedema and epithelial lifting in the control and high pH groups. Hyperplasia, epithelial lifting, and multiple deformations were observed in all the experimental groups except the ceramic ball group. This study showed that based on the histological results of the gills and the stability of the pH levels of the water and the effect on the reduction of the ammonia value of the water, that the ceramic balls are particularly useful for the transport of live fish.

Keywords: Trout, ceramic balls, pH, ammonia, gill histology

Öz: Seramik topların gökkuşağı alabalığının (*Oncorhynchus mykiss*, (Walbaum, 1792)) canlı transfer koşulları üzerindeki etkileri, balıkların solungaç histolojisi ve taşıma suyunun amonyak, pH, çözünmüş oksijen ve su sıcaklığı değerleri incelenerek belirlenmiştir. Seramik topların karşılaştırılması için tuz ilavesi yapılmış grup ve farklı pH seviyeleri hazırlanmış taşıma grupları oluşturulmuştur. Çalışma iki farklı pH seviyesi (yüksek 7.98±0.2 ve düşük 6.01±0.1), tuz (6g/L) ve seramik top (10g/l) ilavesi ve balık stok yoğunluğu 62.3 kg/m^3 olacak şekilde tasarlanmıştır. Deneme tankları, gerçek nakliye koşullarını temsil etmek için bir araca monte edildi. Solungaç histolojisi için balıklardan her saat başı numune alındı. Sonuç olarak seramik bilyede en düşük amonyak değeri 1,98 mg/L olarak belirlendi. Yüksek pH grubunda en yüksek amonyak değeri 2,83 mg/l idi. Balıkların solungaç dokuları incelendiğinde, kontrol ve yüksek pH gruplarında ödem ve epitel dokuda ayrılma olduğu gözlendi. Seramik top grubu dışındaki tüm deney gruplarında hiperplazi, epitel dokuda ayrılma ve çoklu deformasyonlar gözlendi. Bu çalışma, solungaçların histolojik sonuçlarına ve suyun pH seviyelerinin stabilitesine ve suyun amonyak değerinin azalmasına olan etkisine dayanarak, seramik bilyelerin özellikle canlı balıkların taşınması için yararlı olduğunu göstermiştir.

Anahtar kelimeler: Alabalık, seramik top, pH, amonyak, solungaç histolojisi

INTRODUCTION

There are several stages in aquaculture production. Many practices such as brood stock fish care, egg fertilization, grading, disease treatment, and hygiene applications are carried out in fish farms (Kayis, 2019). An important stage during these practices is the transportation of live fish to different systems. Transporting fish to other systems in different life stages has become very common and applicable in a wide geography. Today, live fish can be transported even between different countries. Systems and transportation procedures that allow the transfer of ornamental fish from the Far Eastern countries to countries in other regions of the world are widespread.

In this sense, practices such as starvation of fish before the transfer, the addition of oxygen and antibiotics in water, fish density, and different anaesthetic additions (tetracycline and nitrofurazone) help fish to be healthy during these transfers (Belema et al., 2017).

Rainbow trout (*Oncorhynchus mykiss*) has an important place among the fish used for human consumption. Therefore, it is one of the most intensively produced species. During the production process, trout are frequently subjected to live transfer. In live trout transfer, water temperature values are required to be maintained at 8–10 °C, and dissolved oxygen value not to fall below 5–6 mg/L. Similarly, it is stated that the pH value should be around 7–8. Water conditions, fish size and weight, and transportation time determine the stocking density of trout transfer. An average stocking density of 100 kg/m^3 is acceptable (Shabani et al., 2016). At this stage, the addition of certain substances to the transport water has been suggested in the literature. Zeolite and ceramic materials that act as ammonia retainers and pH regulators are good examples. In recent years, the addition of ammonia retainers such as zeolite has come to the fore to prevent the accumulation of ammonia and reduce ammonia toxicity during transportation. In addition, natural ceramic balls or rings with ammonia retainers and pH regulators with various effects on bacteria have become useful for fish welfare, especially in the aquarium industry. These products are the subject of studies; especially for the improvement of wastewater, values related to pH, ammonia excretion, and temperature have been investigated (Sajuni et al., 2010). In addition, it has been reported that ceramic products are used as a suitable attachment material for bacteria and contribute to bacterial growth. Contrarily, it has also been reported that ceramic balls are used to inhibit the growth of pathogenic *E. coli* bacteria (Ni et al., 2007).

A few indicators determine the sustainability of ecological balance or the welfare of living organisms in aquatic ecosystems. These reagents can be biotic or abiotic. For example, heavy metals, antibiotics, and nitrogenous compounds are considered to be abiotic markers of pollution in aquatic ecosystems (Barlas et al., 2005; Topal et al., 2012). Biotic markers are used for some bacteria and many aquatic organisms. Gills are the leading tissues that can be used as markers and reveal the environmental effects of fish diseases and welfare (Strzyżewska-Worotyńska et al., 2017). Since gills are the centres of respiration and excretion of many metabolic wastes that can be toxic (Abrahamson et al., 2008), gills can develop symptoms in adverse conditions earlier than other tissues (Sorour, 2001). Because, gills play an important role on the uptake of dissolved substances (suspended solids, heavy metals, nitrogenous compounds, pesticides and other pollutants) from the water. Therefore, gills are the most used bioindicator organs in the determination of water pollution (Birungi et al., 2007).

In the present study, we aim to determine changes in water during the live transfer of rainbow trout by examining water quality parameters and gill histology.

MATERIAL AND METHODS

In this study, the effects of two different pH levels, salt addition, and ceramic ball addition to water for live transport of rainbow trout (18.7±0.8 g) were tested. For this purpose, five different groups were designed, namely, control (C), low pH (LpH), high pH (HpH), salt (S), and ceramic balls (CB). Low pH was named for the value lower than neutral pH, and high pH was named as the value higher than neutral pH. The contents of the ceramic balls are given in Table 1. 0.1 N NaOH was used to increase the pH value of the water for the HpH group (pH 7.98), and 0.1 N HCl was used to decrease for the LpH (pH 6.01) group. The amounts and properties of the substances used in the experimental groups are given in Table 2. The pH value of the ceramic ball group was initially recorded as 7.91 due to the addition of the ceramic ball. The experimental fish were observed on a fish farm located in the Eastern Black Sea Region of Turkey. In the study, the fish were transported at a density of 62,3 kg/m^3.

Table 1. The contents of the ceramics ball

Content	(%)
Al_2O_3	>99
Fe_2O_3	<0.1
Na_2O	<1.0
SiO_2	<0.2
MgO	~0.11
CaO	<1.0
Max. temperature resistance	>1500°C
Water absorption	<1.0
Specific gravity	3.50-3.75 (g/cm^3)

Table 2. The amount and properties of the substances used in the experimental groups, and fish weight and some water quality parameters. Control (C), High pH (HpH), Low (pH), Salt (S), ceramic ball (CB)

Parameters and Properties	Experimental Groups				
	C	H (pH)	L (pH)	S	CB
Temperature (ºC)	11.1±0.2	11.5±0.4	11.4±0.2	11.2±0.1	11.1±0.3
pH	7.10±0.3	7.98±0.2	6.01±0.1	6.71±0.2	7.91±0.4
Dissolved oxygen (mg/l)	14.1±0.1	14.2±0.2	13.9±0.2	14.0±0.3	14.2±0.1
Fish weight (g)(total)	187±2.1	187±2.3	186±2.1	186±2.2	188±2.4
*Ceramic ball (g/l)	-	-	-	-	10
Salt (NaCl) (g/l)	-	-	-	6	-
Initial Ammonia (mg/l)	0.043±0.008	0.034±0.008	0.034±0.122	0.033±0.011	0.037±0.004

In order to carry out the experiment, the fish were placed in plastic containers (each of 5 L capacity) with a black surface to prevent light transmission and minimize stress. HACH DR 3900 model device and HACH kits were used to measure the ammonia content of the water. Temperature, dissolved oxygen, and pH values were measured with the HACH LANGE HQ40d portable multiprop. The experiment was conducted in two sets. Ten fish were placed in each group containing three liters of water. Thus, a total of 100 rainbow trout were used. The study was initiated by moving the vehicle on which the tanks were mounted. At the end of the one-hour movement period, ammonia, pH, and temperature values of the water were recorded for each group. In addition, samples were taken from each group every hour for gill histology that were fixed in neutral buffered formalin (10%). The trial was terminated at the end of the fourth hour. This time has been determined by considering the average time spent in fish transfer in the region.

The live fish were anesthetized with benzocaine, and transferred as a whole to neutral formalin. One day later, the fish were transferred to 50% ethyl alcohol. The gills of the fish were removed, passed through alcohol series and xylose, and kept in paraffin at 65°C for 12 h, after, which they were embedded with paraffin. Tissues were sectioned in 0.5 µm thickness with a microtome device (Leica RM2125 RTS), kept at 65°C overnight, then passed through xylose and alcohol series, and stained with haematoxylin and eosin. Stained sections covered with rapid mounting medium (Entellan, Merck 107961) and were inspected under a light microscope (Leica DMi1) and photographed (Luna, 1968). Histopathological changes were graded as follows; none (-), low (+), middle (++) and extreme (+++).

Data were statistically analyzed using the SPSS 25 Software program for Windows. Statistical significance was determined at p=0.05. Prior to statistical analysis, the normality of data was tested. All the data were analyzed using one way analysis of variance (ANOVA). Significant differences between groups were determined by Tukey Post Hoc test.

The study above has been approved by the Local Ethics Commitee of Recep Tayyip Erdogan University (Decision no:2016/31).

RESULTS

As an important detail, there were no deaths in any group during the trial. It was observed that the initial ammonia content of the experimental water was 0.037 mg/l. At the end of the experiment, the lowest ammonia value was in the CB group at 1.98 mg/l. The highest ammonia value was measured in the HpH group at 2.83 mg/l. At the end of the 4th hour, CB group had a lower ammonia value than the control group, while ammonia values in the HpH and S group were higher than the control group ($p<0.05$). There was no statistical difference between the LpH group and the control ($p>0.05$) (Figure 1).

Although the pH value of the CB group was 7.98 at the beginning of the experiment, it remained at a constant value of 6.4 in the subsequent measurements. This standard value measurement was not observed in the other groups. In the HpH group, the suddenly falling pH value tended to increase during the study. Similarly, in the salt group, the pH value was in an up and down course. In the control group, a result was obtained in continuous descent. Although no significant difference was observed in the low pH group, a continuously increasing trend was noted (Figure 1).

When the gill tissue samples of each group were examined histologically, the most observed histopathological finding was hyperplasia. This pathology was mostly detected in the salt, HpH, and LpH groups. Histological changes occurring in the gills are given in Table 3.

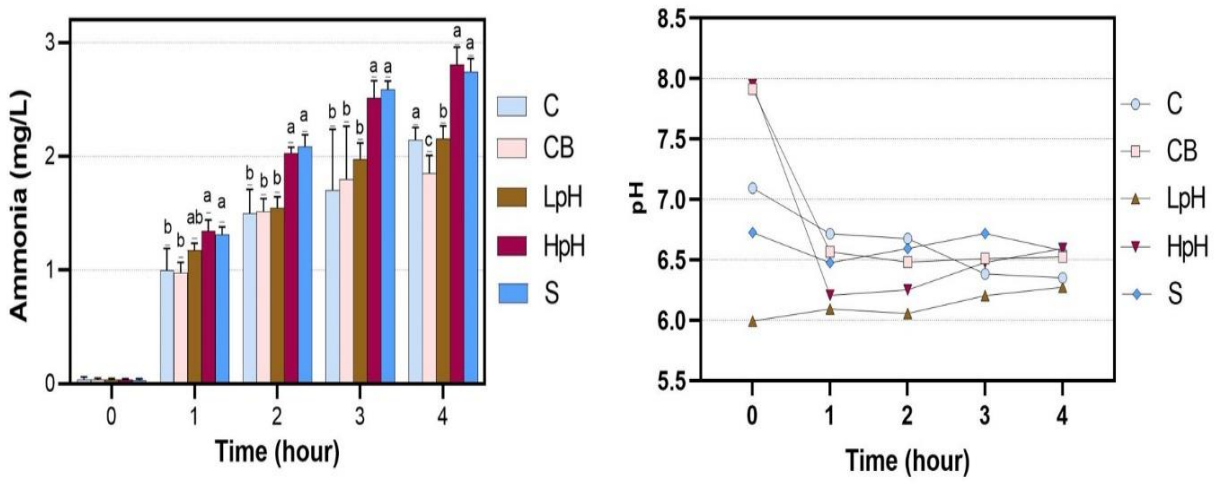

Figure 1. Ammonia and pH values of the water during the experiment. Control (C), High pH (HpH), Low (pH), Ceramic ball (CB)

Table 3. Histological changes occurred at the end of the experiment. control (initial FC), control (end of the trial LC), High pH (HpH), Low (pH), ceramic ball (CB)

Histopathological change	FC	LC	CB	H (pH)	L (pH)	S
Hyperplasia	-	+	+	+++	+	++
Hypertrophy	-	-	-	+	-	-
Epithelial lifting	-	++	-	+++	-	+
Lamellar fusion	-	-	+	+	-	-
Deformation	-	-	-	++	+	+
Oedema	-	++	-	-	-	-

none (-), low (+), middle (++) and extreme (+++)

Another important pathology of the gills was epithelial lifting in the HpH and control groups. In addition, a few deformations of the gill structure in the control group were observed. However, the most distinctive feature observed was oedema; this pathology was observed only in the control group (Figure 2, 3).

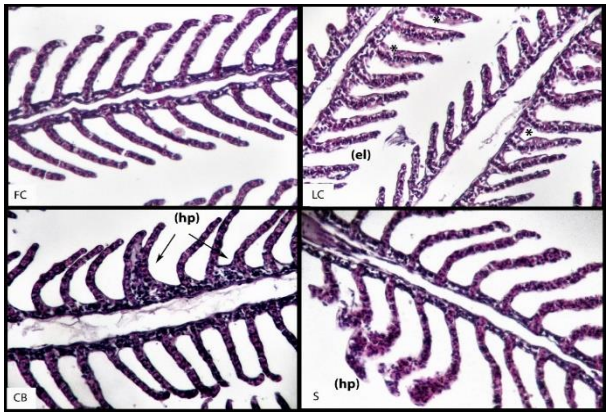

Figure 2. Histopathologic changes on gills. (el) epithelial lifting, (hp) hyperplasia, (•) oedema. First control (FC), last control (LC), Salt (S), ceramic ball (CB)

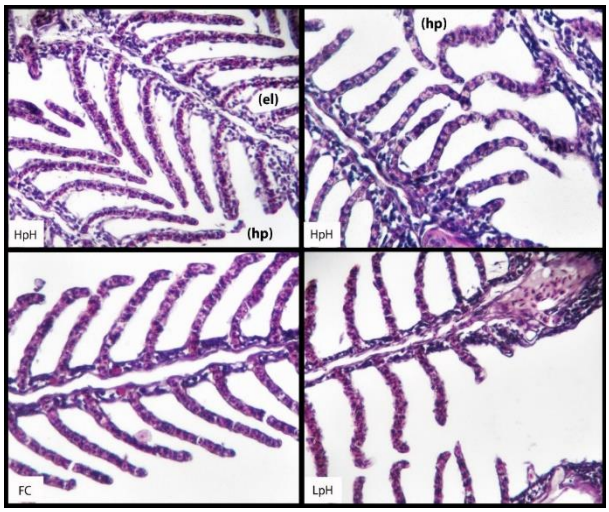

Figure 3. Histopathologic changes on gills. (el) epithelial lifting, (hp) hyperplasia. First control (FC), high pH (HpH), low (LpH)

DISCUSSION

Fish remove most of their metabolic waste as ammonia from the gills. The high amount of ammonia in water causes toxicity in fish (Randall and Tsui, 2002). For trout, the highest ammonia value in water is 0.02 mg/l (Vosyliene and Kazlauskiene, 2004). One of the important factors causing stress and toxicity during the transfer of fish is the presence of nitrogenous compounds that can show high accumulation (Kurtoğlu et al., 2021). In this study, ammonia values were calculated for the different groups and matched with gill histological results. According to the results obtained at the end of the trial, the highest ammonia content was recorded in the HpH group at 2.83 mg/l. The lowest value was observed at 1.98 mg/l in the CB group. In the control group, this value was 2.13 mg/l. These results may be due to the ammonia-binding and ion-exchange-enabled properties of the ceramic balls (Bhakta and Munekage, 2009). The gill histological equivalents of these values were observed as diffuse oedema and epithelial lifting in the control group, epithelial lifting and hyperplasia in the HpH group, and hyperplasia in the salt and LpH groups. It cannot be stated that these pathologies were only related to ammonia values. It should also be noted that high pH levels are an important factor affecting ammonia toxicity. As the fish transport time increases, the CO_2 in the water increases and causes to decrease the pH of the water towards acidity. The pH levels in the water around 7-9 are considered optimum for nitrification bacteria and fish. pH can cause rapid changes in fish stress levels, but buffers can be used to stabilize the pH of the water during fish transport (Berka, 1986).

The presence of nitrification bacteria is very important in recirculating aquaculture system. Because these bacteria are affected by sudden pH changes (Allison, and Prosser, 1993). The results show that ceramic balls positively affect the survival of nitrification bacteria because they keep the pH of the water in balance. In this study, the fact that the ammonia values in the CB group were lower than the other groups explains this situation. In this sense, in the experimental CB group, the presence of the lowest ammonia value and the fact that the pH level did not undergo a sudden change and remained constant at 6.5 revealed a healthier histological structure for the gills in this group.

Salt is a common chemical used in the treatment of parasitic, mycotic and bacterial diseases of fish and for the prevention of nitrite toxicity in static aquatic systems. (Schelke et al. 2011; Williams and Eddy, 1986). In addition, the use of salt (NaCl at the rate of 5 g/l) in the live transfer of fish is a recommended practice to balance osmoregulation and reduce stress (Tacchi et al., 2015). It has even been stated that salt application in the form of salt addition to transport water has the effect of suppressing pathogenic bacterial load. However, Amend et al., (1982) reported that the addition of salt during fish transportation had no effect on increasing fish welfare. In this study, adding salt at the rate of 6 g/l to transport water did not suppress the ammonia value in the salt group more than in the other groups. It was also determined that salt application

was not very effective in keeping the pH values constant and eliminating pathologies on the gills.

The usage of ceramic balls has widely increased in recent years. In addition to creating an attachment surface for beneficial bacteria and inhibiting some harmful bacteria, it has also been used to improve water quality in ensuring fish welfare (Ni et al., 2007; Sajuni et al., 2010). In order to increase the effectiveness of ceramic balls in improving water quality, the use of porous form has been studied. In the study, it was determined that porous ceramic balls were 10 times more effective than normal (Di et al., 2013). In another study, in which the effect of ceramic balls on the hardness of water was studied, it was also stated that these balls were effective on the water hardness (Kawarada and Pironneau, 2019). Although there are many similar studies on water quality and microbial load, our study plays a leading role as a research between fish welfare and ceramic ball. In this study, ceramic balls with a large amount of aluminium oxide (Al_2O_3) were used. In addition, these ceramic materials containing Fe, Na, Si, Mg, and Ca have been shown to reduce ammonia accumulation in water compared to that of other groups. In addition, the ability of this substance to buffer the pH value of water has drawn much attention. This feature can be recommended as a very useful application in ensuring fish welfare, especially for live transport. The data obtained at the end of this study and the gill histology findings revealed that the use of ceramic products can be particularly useful for live fish transport.

CONCLUSION

During the transportation of live trout, the most important challenge is that the ammonia value of the transport water gets much higher than the optimum level. The practices that will keep this value constant or at lower levels are very important for live fish transport. The fact that the addition of ceramic ball (B type) to trout transport water can keep the ammonia value of the water at a lower level than that of the other groups emerged as an important result. Fish welfare in the live transfer can be achieved by generalizing this practice. High pH level in fish farming has a significant effect on ammonia toxicity. Therefore, it is desirable to keep the pH levels stable, especially below 7. In this study, it has been observed that ceramic balls can keep the pH level close to 7 and provide stability. In this respect, this application can also be applied in other growth conditions (especially in closed circuit systems). In addition, important results were obtained in this study during gill histological studies. It has been determined that ceramic ball application ensures that the gill structure, which is especially important for fish, is not damaged as much as possible during transportation. In this study, fish transportation was carried out for 4 hours, the effects of longer transportation should be investigated. In addition, histological changes in the gills may be related to enzyme activity and stress hormones in fish. Accordingly, different materials that will increase fish welfare in fish transfer should be researched.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Investigation of harmful algae in İzmir Bay for the 30 years

İzmir Körfezi'ndeki zararlı alglerin 30 yıllık incelenmesi

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Abstract: Marine pollution, which has been seen for the recent years in cities with a coast that industrializes day by day, was also seen in İzmir, the third most populous city of Turkey. After the destruction of Ragıp Paşa jetty and especially the completion of the Grand Canal Project, the improvement in the gulf is increasing day by day. In İzmir Bay, which is one of the most fertile areas of the Aegean Sea, excessive algae growth is occasionally observed. In this study, 360 quantitative and 1080 qualitative samples were obtained during the monitoring projects carried out jointly by İzmir Metropolitan Municipality, Dokuz Eylül University Marine Sciences and Technology Institute and Ege University Faculty of Fisheries between 1990-2016 were examined. The distribution of the 2 dominant major classes Dinophyceae and Bacillariophyceae were investigated. In addition to the species that produce toxins such as DSP, ASP and PSP into the marine environment, species that do not contain any toxins but cause adverse conditions in the environment by mass-formation were investigated.

Keywords: Harmful species, toxic, dinoflagellate, diatom, DSP, ASP, PSP

Öz: Son yıllarda endüstrileşme ve artan sanayi faaliyetleri nedeniyle denize kıyısı olan şehirlerde görülen deniz kirliliği, Türkiye'nin üçüncü en kalabalık kenti olan İzmir'de de görülmekteydi. Ege Denizinin en verimli alanlarından birisi olan "İzmir Körfezi'nde başlatılan büyük kanal projesi ve yıkılan Ragıp Paşa Dalyanı sayesinde körfez akıntısında düzelme ve kirlilikte azalma olmasına rağmen; dönem dönem gözlenen alg patlamaları, denizdeki canlıları olduğu kadar insan sağlığını da tehdit etmektedir. Bu çalışmada 1990-2016 yılları arasında İzmir Büyük Şehir Belediyesi, Dokuz Eylül Üniversitesi Deniz Bilimleri ve Teknolojisi Enstitüsü ve Ege Üniversitesi Su Ürünleri Fakültesinin ortaklaşa yürüttüğü izleme projeleri sırasında elde edilen 360 kantitatif ve 1080 kalitatif örnek incelenerek; hem körfezde geçen süre boyunca gözlenen zararlı alg patlamaları hem de baskın 2 büyük sınıfı olan Dinophyceae ve Bacillariophyceae türlerinin dağılımları incelenmiştir. DSP, ASP ve PSP gibi toksinler üreten türler yanında, herhangi bir toksin içermeyen fakat aşırı üreyerek ortamda olumsuz koşulları oluşturan türler incelenmiştir.

Anahtar kelimeler: Zararlı türler, toksik, dinoflagellat, diyatom, DSP, ASP, PSP

INTRODUCTION

Over the last 30 years, excessive algal blooms have been increasingly reported around the world, both negatively affecting interactions between species and reducing the populations of other aquatic organisms. Sustainability of the ecosystem affects important economic areas such as tourism and seafood farming, as well as human health (Shumway, 1990; Landsberg, 2002; Wells et al., 2015, Lin et al., 1994). Massive blooms are particularly affect filter-feeding bivalves (oysters, mussels, scallops, clams), crustaceans, and finfish. When the overgrowth, which we call bloom, the color of sea changes, this event named according to the color of the pigment contained in the species that reproduces and the density of the species (red-tide, green-tide, brown-tide, etc.) (Hallegraeff, 1995; Lindahl, 1998). Only 2% of the approximately 5000 phytoplankton species are harmful. However, the effect of these mass blooming species, whether toxic or not, is significant on the ecosystem (Landsberg, 2002). Excessive algal blooms, which are discussed in this study, are called Harmful Algal Blooms (HABs), which have a significant impact on the food chain and human health in the seas by releasing various toxins into the environment, especially Dinoflagellates (Hallegraeff et al., 2004). Dinoflagellates are also excellent indicators of the environment, especially water temperature, salinity and eutrophication in the seas (Graham, 1942; Wood, 1954).

The study by German researcher Wilhelm Nümann, who was in our country for a short time between 1954-1955 and conducted research, in 1955 is the first study on excessive algae blooms in Izmir Bay (1955). Various newspapers of that period attributed the mass fish deaths observed in İzmir Bay to 'excessive chlorine gas increase in seawater, gas plant wastes, oil spilled from ships and tectonic movements on the seabed'. He stated that the causative organisms, especially dinoflagellates, in the environment were responsible for fish deaths. The amount of oxygen detected as 9.3 mg/liter on the surface was measured as 2.8 mg/liter in the inner bay bottom water taken from 10 meters. The water samples taken in August were sent to the Humboldt University in Berlin. The samples were analyzed by Prof. Dr. Alfred Heilbronn and observed many cells abundantly belonging to the genus *Gymnodinium*. According to Heilbronn, in the shallow regions, where water mixing are constantly observed, the oxygen in

the water reaches a normal level despite the mixing, only after the aforementioned dinoflagellate species starts to decrease in the environment. According to the results of the research, the fish died first by poisoning massively on the surface because of the dinoflagellate toxin, and then the still-living ones drowned due to the decomposition of the dead dinoflagellates at the bottom and the oxygen depletion caused by the oxidation of organic materials (Nümann, 1955).

The study conducted by Yurga (1992) in the areas selected from muddy sea coasts that are polluted by industrial and domestic wastewater in the Inner Bay of İzmir, showed that while diatoms and dinoflagellates were never found in samples taken from heavily polluted areas throughout the year, some tintinnids were adapted to live in these polluted areas. In the study, it was observed that tintinnids were found only in the surface of muddy seawater in the inner gulf coasts, where there is no oxygen and there is intense pollution, and no harmful algae growth has been reported.

Non-native species that transported to the bay by ballast waters of ship and currents (Oligotrichea class (*Leptotintinnus nordqvisti* Brandt, 1906 and *Rhizodomus tagatzi* Strelkow & Wiketis, 1950 (=*Tintinnopsis corniger*)) did not show any excessive reproduction, they were rarely observed and reported seasonally. The same two alien species, Balkıs and Koray's study in 2014 were detected and reported in the Marmara and Aegean Seas, these species were rarely encountered throughout the year, and any excessive reproduction was not recorded (Balkıs and Koray (2014).

The amount of harmful and poisonous algae species that show excessive reproduction in the phytoplankton groups distributed in the seas of our country is only about 10%, and it is known that only 5% of these affect the ecosystem, especially mass fish deaths, by synthesizing neuro-toxins Koray (2001), Bargu et al., (2002), Koray (2002a), Koray (2002b), Koray and Cihangir (2002), Koray and Çolak-Sabancı (2003), Çolak-Sabancı and Koray (2005), Koray and Çolak-Sabancı, (2007).

MATERIAL AND METHODS

To determine the qualitative distributions of the phytoplanktonic organism groups that form the first food chain in the İzmir Bay, between 1990 and 2016, in Outer Bay (Station 1), Middle (Station 2), and Inner Bay (Station 3) (Figure 1), samples were collected with a standard plankton net with 55 µm mesh size seasonally from stations 1, 2 and 3, with the horizontal method at speed 2 mils for 10 minutes and fixed with formaldehyde at a resulting concentration of 4%. 5-liter samples collected from selected stations as surface (0.5 meters), middle (5.0 meters), and bottom water (10.0-20.0 meters) were fixed with acidic Lugol to be used in quantitative studies. The samplings between 2017-2020 were made during the excessive algal blooms in the bay.

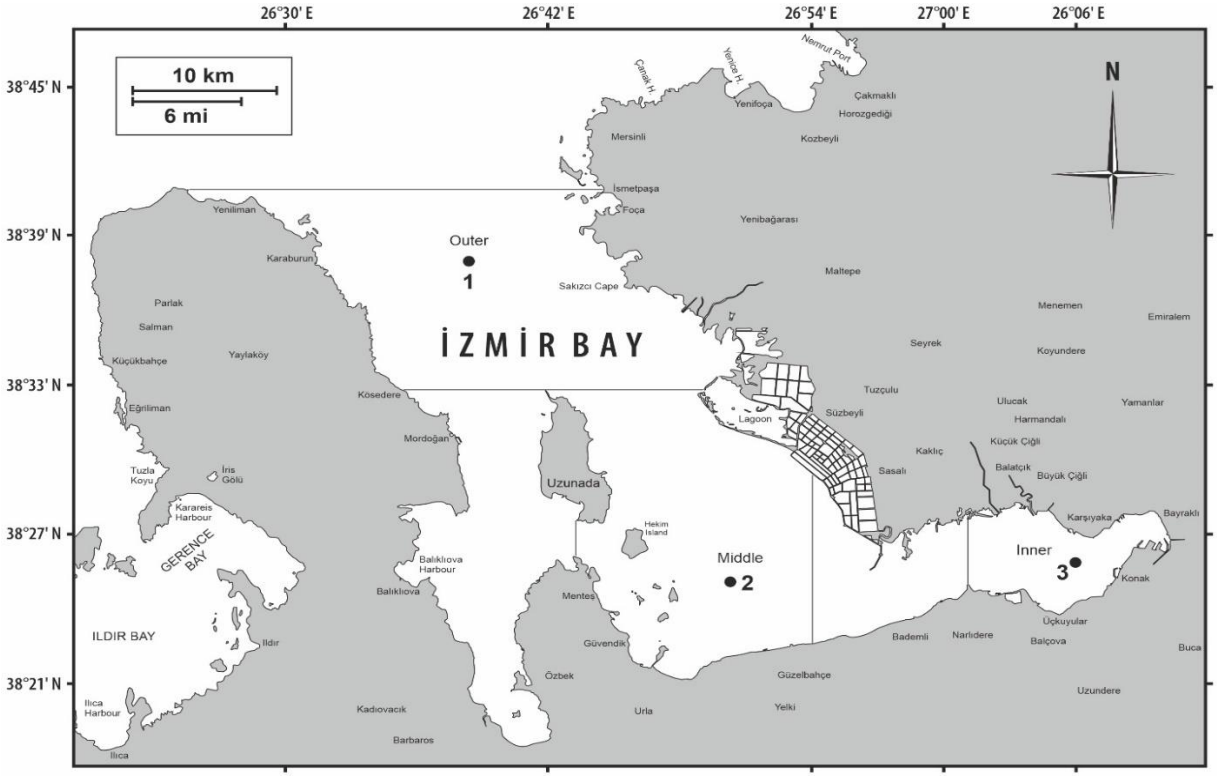

Figure 1. Sampling stations in İzmir Bay

Seawater temperature, pH, salinity, and oxygen values were measured in situ and recorded seasonally. Brought quantitative samples bottles were transferred to 250 cc measuring glass cylinders in the laboratory by removing excess seawater after 1 week of sedimentation and kept for the second sedimentation for another week. After removing excess seawater, the remaining samples were transferred to standard glass tubes and labeled and the concentrations of the species belonging to the planktonic organism groups per liter were counted and determined using the one-drop method, phase-contrast Olympus BX-50 and Olympus CX-31 research microscopes at 15x20 and 14x40 magnifications for 3 times for each sample. To determine the amount of the species per liter, the cell and individuals count results were calculated separately for each sample and converted into cell/liter and individual/liter. In each sampling period, quantitative samples were taken from 3 depths and a plankton net was used for each station. 12 samples obtained per expedition is 48 per year and 1440 samples in 20 years. In qualitative studies, 360 tubes and 1080 bottles of quantitative samples were examined. Seasonal quantitative plankton data were regularly transferred to spreadsheet software in each sampling period and the seasonal and annual distribution of the research stations's class, family, and species was obtained. The seasonal qualitative and quantitative data obtained as a result of the examination of the samples were transferred to a spreadsheet software periodically, the frequency of species and the concentrations per liter of the bay, which changed depending on the years, were obtained eventually. Class, family, and species distributions in the content of 120 seasonal qualitative lists in spreadsheet software covering the years 1990-2020 were converted into annual lists jointly with the calculation formula. The data in the lists were gathered together to be used in this study. Toxic species detected using standardized methods (Hallegraef, 2003) by UNESCO-IOC/HAB bureau were marked seasonally in spreadsheet software and their distribution over 30 years was determined qualitatively and quantitatively. In the determination of the species belonging to the diatom and dinoflagellate classes, Anderson et al., (1995), Balech (1988), Cupp (1943), Delgado and Fortuno (1991), Dodge (1982), Hasle and Syvertsen (1997), Hendey (1964), Koray *et. al.,* (2007), Lebour (1930), Marshall (1969a), Rampi and Bernhard (1978, 1980), Ricard (1987), Steidinger and Williams (1970), Steidinger and Tangen (1997), Sournia (1968, 1976, 1986), Taylor (1976), Tomas (1997), Tregouboff and Rose (1957), and Wood(1954); for Ciliophora species; Boltovskoy (1981), Isamu (1982), Lee, J.B. and Kim (2010), Margalef (1963), Marshall (1969b) and Pierce and Turner (1993); for HAB species, the works of Anderson et al., (1995) and Landsberg (2002) were used for the identification of the diatom and dinoflagellates. The names of the authors of the designated species and the current status of the scientific names of the species were periodically checked and rearranged on the websites of AlgaeBase. Samples were used to determine the distribution of phytoplankton groups have been collected using "The Physical, Chemical, Biological, and Microbiological Effects and Microbiological Effects of the DBTE-180, DBTE-199 Grand Canal Projects on the Marine Environment of the İzmir Bay", jointly organized by Dokuz Eylul University, Institute of Marine Sciences and Technology and İzmir Metropolitan Municipality between 1990-2016. During the seasonal research, expeditions were carried out by the R/V. K. Piri Reis. Ege University Faculty of Fisheries, Department of Hydrobiology carried out the preservation, storage, and evaluation of the collected all samples.

RESULTS AND DISCUSSION

Considering the distribution of the species belonging to the large groups in the bay over a 30-year period, it was determined that the dominant class in the bay was Bacillariophyceae with 42.8%, followed by Dinophyceae with 38.6% (Figure 2).

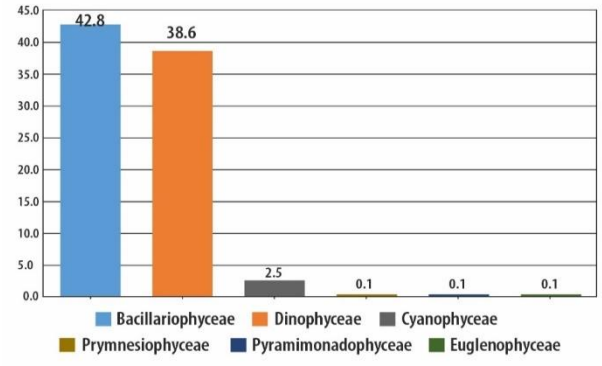

Figure 2. Percentage distribution of major classes in the bay

It was seen that the distribution of the frequency of occurrence of planktonic HAB classes in Izmir Bay, the most dominant class was the dinoflagellate (Dinophyceae) class (14.8%). Percentage distributions of 6 orders belonging to the class Dinophysales 5.9%; Gonyaulacales 3.0%; Prorocentrales 2.6%; Gymnodiniales 1.3%; Noctilucales are 0.3% and Peridiniales 1.6%. The percentage distributions of the detected Cyanophyceae, Prymnesiophyceae, Pyramimonadophyceae and Euglenophyceae classes were determined as 0.3% (Figure 3).

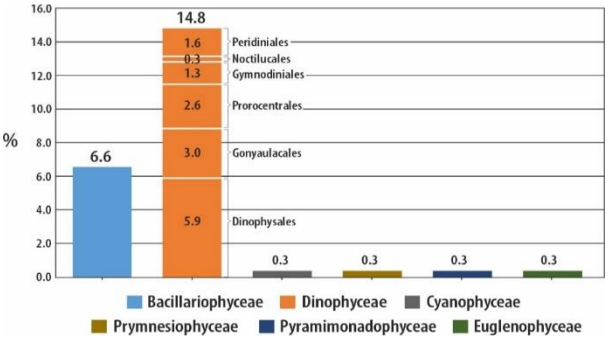

Figure 3. Percentage distribution of major HAB classes in the bay

It has been observed that species that release toxins such as DSP, PSP, ASP occasionally cause blooms in the bay. *Alexandrium minutum* Halim 1960, *Alexandrium tamarense* (Lebour) Balech 1995, *Gymnodinium catenatum* H. W. Graham 1943, *Lingulodinium polyedra* (F. Stein) J. D. Dodge 1989, *Prorocentrum lima* (Ehrenberg) F. Stein, 1878 and *Prorocentrum minimum* (Pavillard) J. Schiller 1933 species that cause PSP (Paralytic shellfish poisoning or PST (Paralytic shellfish toxins) in the bay. The secretions of these species contain Saxitoxin, Neosaxitoxin and Gonyatoxin. DSP (Diarrhetic Shellfish Poisoning) species containing Okadaic acid and Dinophysis toxin: *Dinophysis acuminata* Claparède & Lachmann, *Dinophysis acuta* Ehrenberg, *Dinophysis caudata* Saville-Kent, 1881, *Dinophysis fortii* Pavillard, *Dinophysis mitra* (F. Schütt) T.H. Abé, *Dinophysis rotundata* Claparède & Lachmann, *Dinophysis sacculus* F. Stein, *Dinophysis tripos* Gourret, *Gonyaulax grindleyi* Reinecke, 1967 and *Prorocentrum cassubicum* (Woloszynska) Dodge, 1975. The two species that cause ASP (Amnesic Shellfish Poisoning) by releasing domoic acid into the marine environment are *Pseudo-nitzschia delicatissima* (Cleve) Heiden, 1928 and *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle, 1993 species from the diatom class. It is a type of *Gymnodinium breve* dinoflagellate that causes NSP (Neurotoxic Shellfish Poisoning by releasing Brevetoxin into the environment.

Species of poisoning by AZP (Azaspiracid shellfish poisoning), (CFP) Ciguatera fish poisoning and VSP (Venerupin shellfish poisoning) toxins seen in other seas were not encountered in the bay during this period. Although it has been reported in other studies that dinoflagellate species *P. minimum* causes VSP, there was no evidence of an overgrowth of this species observed throughout the year in the bay. As a result of combining the species lists obtained from the studies in the bay, when the frequencies of the species detected in the 30-year period are examined, it is seen that the dominant class of the bay, which reproduces excessively, is Dinophyceae with 45 species (14.8%). 5 species (1.6%) producing PSP belonging to this class; It was observed that there were 11 species (3.6%) producing DSP and 3 (1.0%) producing ASP. In 67 species, 48 of them, which reproduce excessively from time to time and do not release any neurotoxin to the environment, were detected in the bay, and these species were found at seasonally varying rates (Table 1).

Table 1. Distribution of classes of toxin-producing and non-toxin-producing species

	CLASSES							TOXIC						
	CY	**DI**	**PR**	**BA**	**EU**	**PM**	**OL**	**PP**	**DP**	**AP**	**NP**	**NT**	**Mu**	**Color and type**
Achnanthes brevipes C. Agardh, 1824				•								•		
Alexandrium minutum Halim		•						•						Reddish brown
Alexandrium tamarense (Lebour) Balech 1995		•						•						
Anabaena variabilis Kützing ex Bornet & Flahault	•											•		Reddish brown, HT
Ceratium furca (Ehrenberg) Claparède & Lachmann		•										•		Orange, HAO, AO
Ceratium fusus var. *fusus* (Ehrenberg) Dujardin, 1841		•										•		Orange, HAO, AO
Ceratium tripos var. *atlanticum* (Ostenfeld) Paulsen, 1908		•										•		
Chaetoceros danicus Cleve, 1889				•								•		
Chaetoceros densus (Cleve) Cleve, 1899				•								•		
Coscinodiscus granii Gough, 1905				•								•		
Cyclophora tenuis Castracane, 1878				•								•		Greenish brown, HAO, AO
Cylindrotheca closterium (Ehrenberg) Reimann & J.C. Lewin, 1964				•								•	•	
Dinophysis acuminata Claparède & Lachmann		•							•					Pale brown
Dinophysis acuta Ehrenberg		•							•					Orange
Dinophysis caudata Saville-Kent, 1881		•							•					Orange
Dinophysis fortii Pavillard		•							•					Orange
Dinophysis infundibulum J. Schiller		•										•		Orange
Dinophysis mitra (F. Schütt) T.H. Abé		•							•					

Table 1. Continued

	CLASSES							TOXIC						
	CY	**DI**	**PR**	**BA**	**EU**	**PM**	**OL**	**PP**	**DP**	**AP**	**NP**	**NT**	**Mu**	**Color and type**
Dinophysis odiosa (Pavillard) Tai & Skogsberg		●										●		Orange
Dinophysis ovata Claparéde & Lachmann, 1859		●										●		
Dinophysis ovum (F. Schütt) T.H. Abé		●							●					
Dinophysis rotundata Claparède & Lachmann		●							●					
Dinophysis rudgei (G. Murray & F.G. Whitting) T.H. Abé		●										●		Orange
Dinophysis sacculus F. Stein		●							●					
Dinophysis tripos Gourret		●							●					Orange
Diplopsalis lenticula Bergh		●										●		Orange
Emiliania huxleyi (Lohmann) W.W. Hay & H.P. Mohler			●									●		
Eutreptiella gymnastica Throndsen, 1969					●							●		Milky
Gonyaulax grindleyi Reinecke, 1967		●						●						Green Anoxia, hyperoxia
Gonyaulax polygramma Stein, 1883		●										●		Orange
Gonyaulax spinifera (Claparède & Lachmann) Diesing, 1866		●										●		
Gymnodinium breve C.C. Davis 1948		●									●			Reddish brown
Gymnodinium catenatum H.W. Graham 1943		●						●						
Gymnodinium simplex (Lohmann) Kofoid & Swezy		●										●		
Gyrodinium spirale (Bergh) Kofoid & Swezy 1921		●										●		Orange
Heterocapsa triquetra (Ehrenberg) F. Stein		●										●		
Lingulodinium polyedra (F. Stein) J.D. Dodge		●						●						Reddish brown
Mesodinium rubrum (Lohmann, 1908)							●					●		
Noctiluca scintillans (Macartney) Kofoid & Swezy		●										●	●	Pink, NH3
Ornithocercus magnificus F. Stein, 1883		●										●		
Ornithocercus quadratus Schütt, 1900		●										●		
Ornithocercus steinii Schütt, 1900		●										●		
Oxytoxum scolopax F. Stein		●										●		Pale orange
Phaeodactylum tricornutum Bohlin				●								●		Pale brown
Phalacroma mitra		●		●								●		
Phalacroma rotundatum (Claparéde & Lachmann) Kofoid & J.R. Michener 1911		●		●					●					
Prorocentrum aporum (Schiller) Dodge, 1975		●										●		
Prorocentrum balticum (Lohmann) Loeblich, 1970		●										●		
Prorocentrum cassubicum (Woloszynska) Dodge, 1975		●										●		Pale orange, HAO, AO
Prorocentrum dentatum Stein, 1883		●										●		Orange, HAO, AO
Prorocentrum lima (Ehrenberg) F. Stein, 1878		●							●					Orange

Table 1. Continued

	CLASSES							TOXIC						
	CY	DI	PR	BA	EU	PM	OL	PP	DP	AP	NP	NT	Mu	Color and type
Prorocentrum micans Ehrenberg, 1834		•										•		Orange, HAO, AO
Prorocentrum minimum (Pavillard) J. Schiller		•										•		Pale brown
Prorocentrum triestinum J. Schiller, 1918		•										•		Orange, HAO, AO
Protoperidinium longipes Balech, 1974		•										•		Orange
Protoperidinium steinii (Jörgensen, 1899) Balech, 1974		•										•		Orange
Pseudo-nitzschia delicatissima (Cleve) Heiden, 1928				•						•				Milky
Pseudo-nitzschia pseudodelicatissima (Hasle) Hasle, 1993				•						•				Milky
Pseudo-nitzschia pungens (Grunow ex Cleve) G.R. Hasle, 1993				•						•				Pale green
Pseudosolenia calcar-avis (Schultze) B.G. Sundström, 1986				•								•		
Pyramimonas propulsa Moestrup & Hill, 1991						•						•		Green
Rhizosolenia setigera Brightwell, 1858				•								•		
Scrippsiella trochoidea (Stein) Loeblich III, 1976				•								•		Brown
Skeletonema costatum (Greville) Cleve, 1873				•								•	•	Greenish orange, HO, AO
Thalassiosira allenii H. Takano 1965				•								•		Pale Green, HAO, AO
Thalassiosira angustelineata (A.W.F. Schmidt) G. Fryxell & Hasle				•								•		
Thalassiosira rotula Meunier				•								•		PUA, Greenish orange, HO, AO

(CY: Cyanobacteria, DI: Dinophyceae, PR: Prymnesiophyceae, BA: BacillariophyceaE, EU: Euglenophceae, PM: Pyramimonadophyceae, OL: Oligotrichea, PP: Paralytic Shellfish Poison, DP: Diarrhetic Shellfish Poison, AP: Amnesic Shellfish Poison, NT: Non toxic blooms, Mu: Mucilage secretion, HO: Hyperoxia, AO: Anoxia, HT: Hepatotoxic, NH3: Ammoniac, PUA: Polyunsaturated short chain aldehydes.

When we review the history of algal blooms in the bay, it is seen that the Cyanophyceae, Dinophyceae and Bacillariophyceae classes are responsible for. Cyanophyceae class started to decrease gradually after the Grand Canal Project came into operation after 2000, and it was determined in 1994 as the most abundant throughout the period. This group was observed at all stations in 1994, increased depending on the depth at station 1, 6000 cells l^{-1} at 15 meters, 17000 cells l^{-1} at 15 meters at station 2 and all levels at station 3 in the inner bay. It was determined as 60000 cells l^{-1} in the water column in Spring. In the summer of 1994, it was found at a concentration of 6500 cells l^{-1} at station 6, 8000 cells l^{-1} at station 2, increasing depending on the depth at station 3, and 80000 cells l^{-1} "on the surface, station 3. In the autumn of 1994, this class was the highest in the outer bay surface water at a concentration of 40000 cells l^{-1} at station 6, 50000 cells l^{-1} at station 2, and 400000 cells l^{-1} in the entire water column at station 3 reached high density.

Pseudo-nitzschia pungens (Grunow ex Cleve) G.R. Hasle, 1993 and *Ceratium fusus* var. *seta* (Ehrenberg) Sournia was observed in all stations in March 2000, *P. pungens* was detected at a concentration of 8370 cells l^{-1} at 5 meters in the inner bay, and *Ceratium fusus* var. *seta* (Ehrenberg) Sournia at a concentration of 8056 cells l^{-1} at 5 meters.

In May 2006, *L. polyedra* bloomed at a concentration of 4630 cells l^{-1} on the surface in the middle bay, making the color of the sea brownish-red. *C. furca var. furca* was detected at a concentration of 8624 cells l^{-1} per liter at station 3 in July 2008. In February 2009*, R. setigera* was detected at all depths by blooming massively in the entire bay, most abundantly at station 2, at a concentration of 53280 cells l^{-1} at 5 meters, and changed the color of the sea in coastal areas in Güzelbahçe and İnciraltı. *G. spinifera* species, which bloomed excessively in Urla, Güzelbahçe and Foça in September 2010, turned the seawater in these regions brownish-red. *P. pungens* changed the color of the sea to pale green in February 2011 and was observed at all stations, especially at a concentration of 7611 cells l^{-1} at 5 meters at station 2, and 7437 cells l^{-1} at 10 meters in the inner bay. The same species

was detected in January 2020 and again at all stations, with the most abundant concentration of 6556 cells l^{-1} at 5 meters at station 2. In November 2018, green water color was observed on the sea surface on the shores of Karşıyaka, and it was determined that the species showing excessive proliferation was diatom *T. allenii* at a concentration of 6228 cells l^{-1} at 5 meters at station 2. *P. triestinum* dinoflagellate species was detected in March 2014 at a concentration of 67203 cells l^{-1} on the surface, 28296 cells l^{-1} at 5 meters and 81351 cells l^{-1} at 10 meters by causing excessive proliferation in the inner bay and turned the color of seawater orange. The same species was intensely observed in the inner bay 3 months later in June, with a concentration of 67203 cells l^{-1} on the surface and 28296 cells l^{-1} at 5 meters. The diatom species *L. minimus*, belonging to the class Coscinodiscophyceae, which causes mass fish kills, was detected only at station 3 in March 2014 at all depths, changing the color of the sea from light brownish to dark green at 106111 cells l^{-1} concentration on the surface. It was detected at a concentration of 24759 cells l^{-1} and 45981 cells l^{-1} at 10 meters. In April 2018, *G. spinifera* showed excessive bloom and was detected at a concentration of 9760 cells l^{-1} at station 2 and 10880 cells l^{-1} at station 3, and turned the color of the sea in red color on the Konak and Narlıdere shores.

A species known to cause anoxic and hypoxic conditions in water with excessive growth, *P. micans* made a mass blooming in February 2020 and changed the color of the sea to orange, 8772 cells l^{-1} at 5 meters of station 2 and 9360 cells l^{-1} at 5 meters of station 3. It was determined most intensely at the concentration of 10030 cells l^{-1}.

The massively blooming classes are mostly diatom and dinoflagellate and cases where ciliates blooms are rarely encountered in the bay. *H. subulata*, a tintinnid from the Oligotrichea class, was observed in all stations in July 2010, it was most intensely detected at station 24 at a concentration of 49648 individuals/l^{-1}, and the same species was detected in a very small amount in winter. It is known that the genus *Helicostomella* is widely distributed in neritic waters (Pierce and Turner, 1993).

When the phytoplanktonic organisms included in the mucilage formation, the environmental disaster that started to be seen in the Adriatic (Rinaldi and Vollenweider, 1995) and the Tyrrhenian Bay in 1991 (Innamorati et al., 1998) in the summer of 1988 is examined, the important species detected are *Dinophysis caudata* Saville-Kent, 1881; *Ceratium fusus* var. *fusus* (Ehrenberg) Dujardin, 1841 from the Dinophyceae class; *Ceratium tripos* var. *atlanticum* (Ostenfeld) Paulsen, 1908; *Prorocentrum micans* Ehrenberg, 1834; *Coscinodiscus radiatus* Ehrenberg, 1840; *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C.Lewin, 1964; *Dactyliosolen fragilissimus* (Bergon) Hasle, 1996; *Gonyaulax fragilis* (Schütt) Kofoid, 1911; *Grammatophora marina* (Lyngbye) Kützing, 1844; *Leptocylindrus danicus* Cleve, 1889; *Licmophora abbreviata* C. Agardh, 1831; *Nitzschia longissima* (Brébisson) Ralfs, 1861; *Pleurosigma elongatum* W. Smith; *P. delicatissima*, *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle, 1993 and *Pseudo-nitzschia pungens* (Grunow ex Cleve) G.R. Hasle, 1993, *Pseudosolenia calcar-avis* (Schultze) B.G.Sundström, 1986; *Rhizosolenia setigera* Brightwell, 1858; *Skeletonema costatum* (Greville) Cleve, 1873; *Striatella unipunctata* (Lyngbye) C. Agardh, 1832; *Synedra undulata* (J.W. Bailey) Gregory, 1857; *Thalassiosira pseudonana* Hasle & Heimdal, 1970; *Thalassiosira rotula* Meunier; *Thalassiothrix frauenfeldii* (Grunow) Grunow from Bacillariophyceae class and *Eutreptiella gymnastica* Throndsen, 1969 from the class Euglenophyceae have been reported by the above researchers. The first observation of mucilage formation in the Turkish Seas was made by Aktan in the fall of 2007 in the Marmara Sea, and the *G. fragilis* seen in the Tyrrhenian Sea and Adriatic was also reported for the first time in Marmara. Aktan detected *G. fragilis* at a density of $3.9x10^6$ l^{-1} in the study. (Aktan et al., 2008). With the exception of the dinoflagellate *Gonyaulax fragilis* (Schütt) Kofoid 1911, which has the ability to produce mucilage (Pistocchi et al., 2005), all of the species found in the mucilage structure are also present in İzmir Bay, according to the species lists of all periods in our study. Although diatom species *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin, 1964; *Skeletonema costatum* (Greville) Cleve, 1873 and dinoflagellate speices *Noctiluca scintillans* (Macartney) Kofoid & Swezy which are capable of producing mucilage substances, reproduce from time to time in the bay, it has not been observed that they cause any mucilage formation other than changing the color of the sea.

As a result of the observations in İzmir Bay, considering the species belonging to all classes in the Bay, the rate of harmless species that do not reproduce excessively is 77.6%, the rate of blooming species whether toxic or not is 22.4%, the rate of non-toxic species that worsen the marine environment by excessive reproduction is 16.1%. and the rate of both toxic and overproducing species was determined as 6.3% (Figure 4).

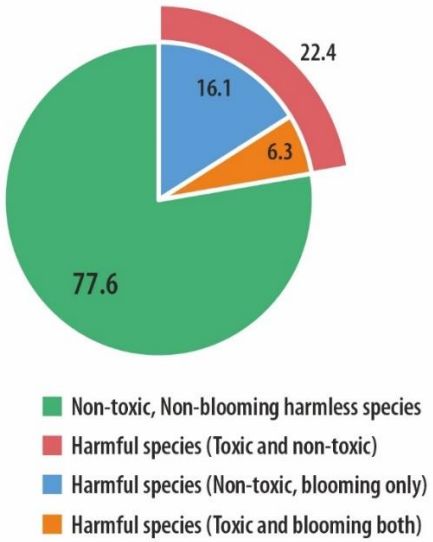

Figure 4. Percentage of HAB species and Non-toxic, Non-blooming harmless species

When 67 planktonic organism species that cause harmful algae overgrowth are examined in the bay (Table 1), the ratio of the species in the bay that affect the other organisms by releasing DSP, PSP, ASP and NSP toxins into the sea is given in Figure 5. Among the 67 species with harmful increase the most producing common toxin is DSP with 16.4% (Figure 5).

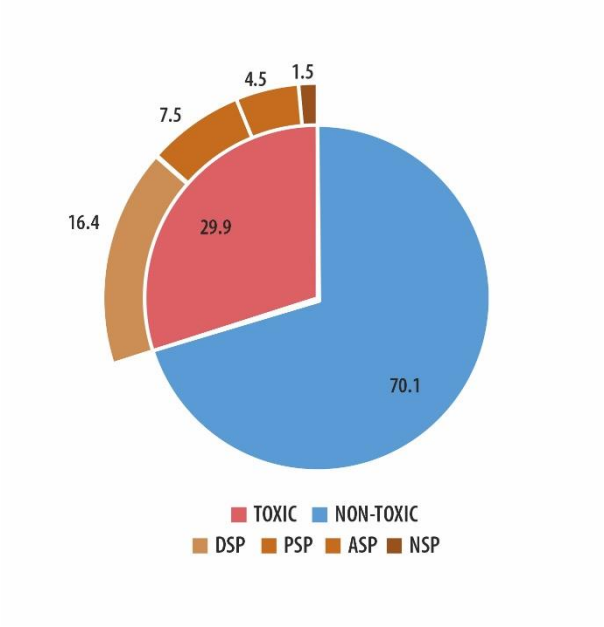

Figure 5. Percentage of algal bloom forming species and toxin producers

Successful commissioning of the İzmir Grand Canal Project has made an substantial improvement in diversity. The seasonal excessive algal blooms in the Izmir Bay, which has an extremely dynamic structure, should be continuously monitored.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Extraction of protein from fresh rainbow trout (*Onchorhynchus mykiss*) viscera and smoked trout trimmings using commercial enzymes

Ticari enzimler kullanılarak taze alabalık (*Onchorhynchus mykiss)* iç organlarından ve tütsülenmiş alabalık kırpıntılarından protein ekstraksiyonu

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Abstract: In the current investigation, fresh trout viscera and smoked trout trimmings were enzymatically extracted using papain, alcalase, protamex, and flavourzyme. Protein extraction was performed at different concentrations (0.5%, 1%, 1.5% and 2%) and times (30 minute, 1 hour and 4 hours). The moisture, crude protein, lipid and crude ash contents of trout viscera used as raw materials, in the study, were respectively found as 60.26±0.78%, 12.18±0.21%, 31.18±0.36% and 1.33±0.07%, while these values in smoked trout trimmings were determined as 54.53±0.93%, 18.39±0.13%, 17.71 ± 1.06% and 8.50±0.13%, respectively. Following the conclusion of the study, protein content (g protein/100 g waste) and Protein Recovery Rate (PRR, %) in liquid protein hydrolysate extracted from trout viscera and smoked trout trimmings were found to be significantly affected by enzyme type, enzyme concentration, and extraction time. The results showed that the flavourzyme, followed by the protamex, produced the highest protein content (g protein/100 g waste) and PRR (%) in the liquid protein hydrolysate extracted from trout viscera. Furthermore, the protamex, followed by the flavourzyme, was shown to have the highest protein content (g protein /100 g waste) and PRR (%) in the liquid protein hydrolysate extracted from smoked trout trimmings.

Keywords: Trout, viscera, protein extraction, smoked trimmings, commercial enzymes

Öz: Bu çalışmada, alabalık iç organları ve tütsülenmiş alabalık kırpıntıları papain, alkalaz, protameks ve flavourzyme kullanılarak enzimatik olarak ekstrakte edilmiştir. Protein ekstraksiyonu, farklı konsantrasyonlarda (%0,5, %1, %1,5 ve %2) ve sürede (30 dakika, 1 saat ve 4 saat) gerçekleştirilmiştir. Çalışmada, hammadde olarak kullanılan alabalık iç organlarının nem, ham protein, lipid ve ham kül içerikleri sırasıyla %60,26±0,78, %12,18±0,21, %31,18±0,36 ve %1,33±0,07 olarak bulunurken, bu değerler tütsülenmiş alabalık kırpıntılarında sırasıyla %54,53±0,93, %18,39±0,13, %17,71±1,06 ve %8,50±0,13 olarak belirlenmiştir. Çalışmanın sonuçlanmasının ardından, alabalık iç organlarından ve tütsülenmiş alabalık kırpıntılarından ekstrakte edilen sıvı protein hidrolizatlarının protein içeriği (g protein / 100 g atık) ve Protein Geri Kazanım Oranlarının (PRR, %), enzim tipi, enzim konsantrasyonu ve ekstraksiyon süresinden önemli ölçüde etkilendiği bulunmuştur. Sonuçlar, alabalık iç organlarından ekstarkte edilen sıvı protein hidrolizatlarda en yüksek protein içeriği (g protein/100 g atık) ve PRR' nin (%) flavourzyme ile elde edildiğini bunu protameksin takip ettiğini göstermiştir. Ayrıca, tütsülenmiş alabalık kırpıntılarından ekstakte edilen sıvı protein hidrolizatlarında, protameks, ardından flavourzyme ile en yüksek protein içeriğine (g protein / 100 g atık) ve PRR'ye (%) sahip olduğu gösterilmiştir.

Anahtar kelimeler: Alabalık, iç organlar, protein ekstraksiyonu, tütsülenmiş kırıntı, ticari enzimler

INTRODUCTION

Rainbow trout **(***Onchorhynchus mykiss***)**, which is originated in North America and is the most commonly grown variety of trout in our country, is marketed fresh in our nation as well as smoked and exported abroad. According to TEPGE (Republıc of Turkey Mınıstry of Agrıculture and Forestry Agrıcultural Economic and Policy Development Institute), the production of rainbow trout in Turkey is estimated to reach 113,678 tons (TEBGE, 2020). The majority of the trout produced in our country are shipped fresh or smoked in processing factories. In industries that process smoking of trout have two types of waste which are produced. These include visceral waste that happens during the cleaning of the fish prior to the smoked process, as well as smoked flesh trimmings, which consists of head, skin, bone, and flesh parts, and is created during the processing of smoked fillets after they have been smoked. The heads (15.3% of total weight), bones (6.9% of total weight), tails (2.3% of total weight) and intestines (8.8% of total weight) of the trout were removed from the fish before it was smoked as a fillet. Thus, around 33% of the body of the fish was considered waste to be discarded in the procedure (Kotzamanis et al., 2001). Smoked trout trimmings are also used to make lower-value items such as fish meal and fish feed after they have been smoked. Its portion is around 3–5 % depending on numerous processing

conditions such as the size of the fish and the kind of smoker (Tolasa et al., 2012). Most of the fish wastes in Turkey are used to make fish meal with low biological and economic value. The remainder is dumped into the environment as a pollutant without being assessed. These sources, on the other hand, may be transformed into protein products that are nutritionally valuable, functional, easily digested, and have a high economic value. Studies conducted that have previously been performed on fish waste have revealed a large amount of protein. Zamora-Sillero et al. (2018) states that the protein content in fish waste might be 10-20% (w/w) of the total protein in fish.

Adding enzymes to 'waste' material from fish, along with other procedures like filtering and centrifugation, was discovered many years ago to be an efficient technique to separate and recover the proteins contained in the waste (Kristinsson, 2007). The proteins in fish processing waste can be separated from other compounds to which they are associated with hydrolysis by using proteases. Several different authors have studied and described the enzymatic proteolysis and solubilization of proteins from a variety of different fish materials (Aspmo et al., 2005; Vieira et al., 1995). Pigott and Tucker (1990) define fish protein hydrolysate as a liquid product prepared from fish with the addition of proteolytic enzymes to accelerate hydrolysis under controlled conditions, resulting in a protein mixture. Proteins extracted from fish muscle by using enzyme have been found to contain a variety of bioactive peptides with nutritional and functional properties (Benjakul and Morrisey, 1997; Theodore et al., 2008). Furthermore, a wide range of bioactivities, including antihypertensive, antithrombotic, immune-modulatory, and antioxidant characteristics, have been found in peptides generated from the enzymatic breakdown of proteins (Dong et al., 2005; Fitzgerald et al., 2005; Ghaly et al., 2013; Liaset and Espe, 2008; Underland et al., 2009).

Researchers have shown that by applying regulated enzymatic hydrolysis, a wide variety of high-quality protein components may be generated from low-value waste materials (Quaglia et al., 1987; Aspmo et al., 2005). The enzyme utilized in hydrolysis has a significant impact on the content and properties of the extracted proteins, as well as the amino acid sequence of the peptides generated. The functional characteristics of the generated extracted proteins, which are referred to as hydrolysate, are significantly influenced by protease species and protein substrate. Temperature, hydrolysis time, and enzyme concentration all have an impact on the speed and specificity of protein hydrolysate production. The quality of the recovered protein is most strongly influenced by the duration of the hydrolysis process. All parameters that can influence the structure of the product such as pH, hydrolysis duration, enzyme-substrate level and temperature, can influence enzymatic hydrolysis (Utomo et al., 2014; Ananey-Obiri et al., 2019).

There has been a lot of studies on the use of commercial proteases to convert fish processing waste and inadequate or low-value fish into protein hydrolysates (Quaglia and Orban, 1987; Uhlig, H., 1998; Wu et al., 2015). The choice of enzyme is critical in the extraction of proteins from fish byproducts and waste (Ramakrishnan et al., 2013). Alcalase, -chymotrypsin, Neutrate, papain, pepsin, trypsin, pancreatin, flavourzyme, bromelain, pronase E, protamex, orientase, thermolysin, validase, protease amano and protease N are some of the most often used proteolytic enzymes. Among the commercial enzymes employed as proteolytic agents, effective investigations have been achieved by using plant proteases such as papain, bromelain, and ficin, as well as bacterial prostheses such as alcalase, neutrate, protease N, and protamex (Ananey-Obiri et al., 2019).

Using various proteases, successful investigations have been carried out on extracting fish protein hydrolysate from fish internal organs, which are discarded by fish processing companies (Batista et al., 2010; Chalamaiah et al., 2012; Siddik et al., 2021). Many enzymes are employed in the commercial production of fish protein hydrolysate. The amount of protein obtained from these enzymes has been determined on the waste profile and the surrounding circumstances. In this investigation, trout viscera and smoked trout trimmings were extracted at varied rates (0.5%, 1%, 1.5%, and 2%) and times (30 minutes, 1 hour, and 4 hours) by utilizing commercial enzymes (protamex, flavourzyme, alcalase and papain), and extracted protein content (g protein/100 g waste) and Protein Recovery Rate (PRR, %) were measured in liquid protein hydrolysate.

MATERIAL AND METHODS

Material

Fresh trout viscera and smoked trout trimmings were received from the processing factory of of Kılıç Holding Bafa Su Ürünleri A.Ş., which processes fresh and smoked trout in Maraş, Turkey. The enzymes needed for protein hydrolysate production, including alcalase, protameks, and flavourzyme, were provided by Sigma-Aldrich, and papain enzymes, which were supplied by Novozymes A/S (Bagsvaerd, Denmark).

Methods

Enzymatic extraction of protein

The enzymatic extraction of protein from trout viscera and smoked trout trimmings was produced by the enzymatic method according to Ramakrishnan et al. (2013) and He et al. (2013) with slight modifications. The wastes were vacuum-packed into 5-kilogram packages, placed in ice-filled foam boxes, and transported to the Protein Research Laboratory at the Faculty of Fisheries, Department of Fishing and Processing Technologies, where they were held at -18°C until protein hydrolysate was produced. After being thawed at room temperature, frozen wastes were minced by using a meat grinder. The wastes were then heated for 20 minutes at 90°C to assure that endogenous enzymes were inactivated (Nasri et al., 2013). Each sample of minced viscera and smoked trout trimmings, whose enzymes were inactivated,

was cooled and homogenized by adding distilled water at a ratio of 1:1. The optimal hydrolysis temperatures, pH values, inactivation times, and temperatures of four different enzymes (papain, alcalase, protamex, and flavourzyme) used in the production of fish protein hydrolysate (FPH) were conducted in accordance with the manufacturer's recommendations. With the use of 2 N NaOH, the pH values at which these enzymes displayed optimal activity were adjusted to 8.0 for alkaline protease, 7.0 for protamex and flavourzyme, and 7.0 for papain. The hydrolysis temperature was set in accordance with the manufacturer's recommendations, with alcalase at 55°C, flavourzyme and protamex at 50°C, and papain at 40°C being selected as the best temperatures for each enzyme. During the hydrolysis stage, papain, alcalase, protamex, and flavourzyme were evaluated at three different hydrolysis times of 30 minutes, 1 hour, and 4 hours, as well as four different enzyme concentrations of 0.5%, 1%, 1.5%, and 2%. The inactivation time and temperature of each enzyme were applied according to the recommendation of the company from which the enzymes were obtanied, in order to terminate the hydrolysis in the samples that were applied for a certain time, temperature and enzyme-substrate concentration. Accordingly, it was applied for alcalase at 85°C for 10 minutes, for flavourzyme at 90°C for 5 minutes, for protamex at 85°C for 10 minutes and for papain for 30 minutes at 70°C and then 15 min cooled. The cooled extracted solution was then centrifuged at 3600 rpm for 20 minutes to separate into phases.

Figure 1 shows the flow chart to the production of extracted fish protein hydrolysate. The formation of four different phases was achieved in all as an oil phase in the top phase, a light oil phase in the second phase, a protein phase in the third phase, and an insoluble material phase in the fourth phase. The protein content in the hydrolyzing solution were collected from the liquid proteins in step 3 for the production of hydrolyzed fish protein extract (Figure 2).

Figure 1. The production of hydrolyzed fish protein extract flow chart

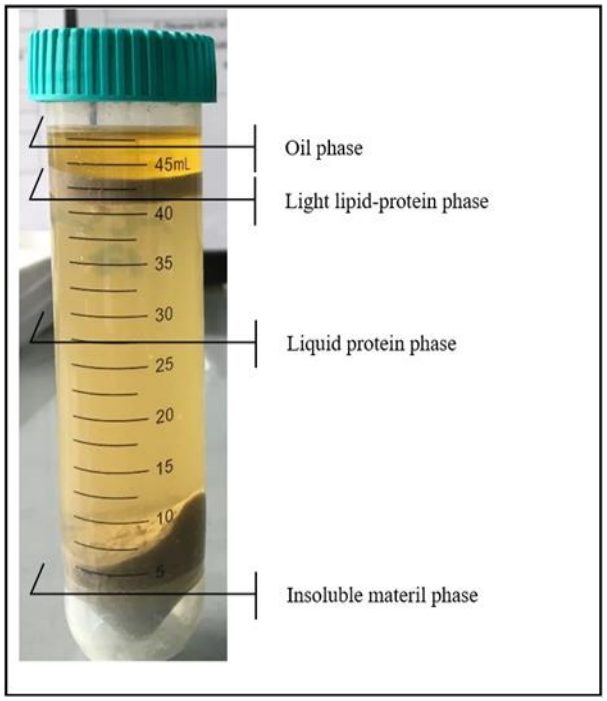

Figure 2. Fractions of soluble hydrolyzed fish protein extract derived from trout viscera and smoked trout trimmings

Chemical analysis

The proximate composition of the trout viscera and the smoked trout trimmings were determined in triplicate by using the following methods: lipid content by Bligh and Dyer (1959), moisture content by AOAC (1998), total crude protein by Kjeldahl technique (AOAC 981.10, 1998) and ash content by AOAC (1998). The quantity of protein in the extracted solution samples was measured by using the Lowry method (1951), using bovine serum albumin as a standard protein. Absorbance was measured at 660nm in a UV/vis spectrophotometer. Protein Recovery Rate (%) estimated by Ovissipour et al. (2009) using the formula below:

$$\text{Protein Recovery Rate (\%)} = \frac{\text{[the content of protein present in the hydrolysate]}}{\text{[the initial content of protein present in the extracted mixture]}} \times 100$$

Statistical analysis

Using the SPSS (SPSS 16.0 Inc. Chicago, IL) package program, one-way analysis of variance (ANOVA) and General Linear Model (GLM) was used in data obtained in the study, and Duncan multi-way analysis of variance was used to assess the differences between the means at the 0.05 significant level.

RESULTS AND DISCUSSION

Proximate composition of trout viscera and smoked trout trimmings

Crude protein, lipid, crude ash and moisture contents of trout viscera and smoked trout trimmings used in the production of protein hydrolysates are given in Figure 3.

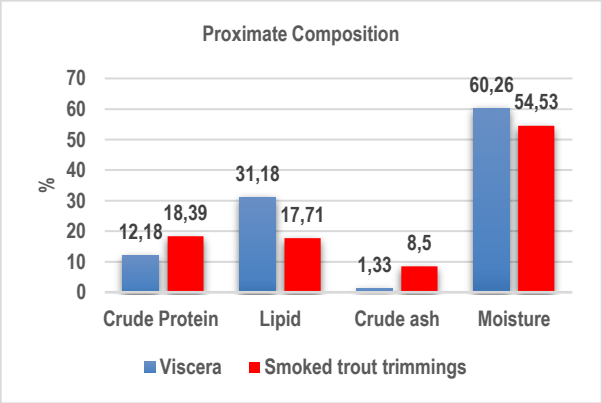

Figure 3. The Proximate composition of trout viscera and smoked trout trimmings

In the proximate composition analysis, trout viscera included 60.26±0.78% moisture, 12.18±0.21% crude protein, 31.18±0.36% lipid, and 1.33±0.07% ash, while smoked trout trimmings contained 54.53±0.93% moisture, 18.39±0.13% crude protein, 17.71±1.06% lipid, and 8.50±0.28% crude ash.

Taheri et al. (2013) observed that trout viscera had moisture content of 71.65%, fat content of 13%, protein content of 15% and ash content of 2.73%. Dong et al. (1993) found that minced salmon viscera contained 78.7% moisture, 12.1% protein, 18.1% lipid and 7.1% crude ash. Kotzamanis et al. (2001) determined the average crude protein, fat content, ash content and moisture of trout waste (head, tail, bone, and intestines) to be 14.5%, 11.1%, 3.3%, and 70.1%, respectively. In a previous study, Tokur (2007) reported that the moisture, crude protein, lipid, and crude ash content of smoked trout were 61.14%, 26.53%, 6.4%, and 1.71%, respectively, which was in contrast to our findings. In the study, written by Tosun and Özden (2014), the researchers found that the protein, fat, moisture, and ash contents of hot-smoked rainbow trout were 22.06%, 7.02%, 66.70%, and 3.50%, respectively. Those findings were in contrast to the data provided in this study, which included fish viscera and smoked trout trimmings. The proximate composition of fish as well as viscera varies according to the fish species, sex, age, nutritional status, season and health (Villamil et al., 2017; Korkmaz and Tokur, 2019). Lipid and protein content of cultured fish have been observed to depend mostly on the activity of fish muscle and feed (Thammapat et al., 2010). The current research findings, when compared to previous study findings for proximate compositions, are predicted to be dependent on feeding and moisture content of used materials (Kotzamanis et al., 2001; Kołakowska et al., 2006). After the

smoking process, previous studies showed that the proximate composition of the fish might alter based on a variety of parameters such as the brine concentration and time applied, the smoked temperature and duration, and the kind of fish (Bjørnevik et al., 2018; Fuentes et al., 2010; Jittinandana et al., 2002; Tosun and Özden, 2014). This might explain why differences in proximate composition were observed.

Papain

Table 1 shows the protein content (g protein/100 g waste) and Protein Recovery Rates (%) in the liquid protein hydrolysate extracted from trout viscera and smoked trout trimmings using four different rates of papain during three different time periods.

Table 1. Protein content (g protein/100 g waste) and Protein Recovery Rates (PRR %) in liquid protein hydrolysate derived from trout viscera and smoked trout trimmings using papain[1,2]

		Enzyme concentrations (%)							
	Extraction Time	0.5	1	1.5	2	0.5	1	1.5	2
		Viscera				Smoked trout trimmings			
Protein Content	30 min.	5.93^{a1} (0.01)	6.23^{a4} (0.01)	6.18^{a3} (0.01)	6.12^{a2} (0.02)	5.59^{a1} (0.01)	5.60^{a1} (0.01)	5.85^{a2} (0.01)	5.92^{a3} (0.01)
	1 h	6.15^{b1} (0.01)	6.33^{b4} (0.01)	6.31^{b3} (0.01)	6.18^{b2} (0.01)	5.87^{b1} (0.01)	6.33^{b2} (0.01)	6.36^{c3} (0.01)	6.40^{c4} (0.01)
	4 h	6.13^{b1} (0.01)	6.37^{c3} (0.01)	6.39^{c4} (0.01)	6.29^{c2} (0.01)	6.01^{c2} (0.01)	6.24^{c4} (0.01)	5.99^{b1} (0.01)	6.14^{b3} (0.00)
PRR (%)	30 min.	48.71^{a1} (0.06)	51.12^{a4} (0.08)	50.70^{a3} (0.04)	50.23^{a2} (0.14)	30.41^{a1} (0.06)	30.47^{a1} (0.03)	31.80^{a2} (0.06)	32.18^{a3} (0.06)
	1 h	50.46^{c1} (0.07)	51.98^{b4} (0.10)	51.81^{b3} (0.09)	50.76^{b2} (0.06)	31.92^{b1} (0.03)	34.42^{c2} (0.08)	34.56^{c3} (0.06)	34.81^{c4} (0.08)
	4 h	50.34^{b1} (0.06)	52.28^{c3} (0.11)	52.44^{c4} (0.12)	51.64^{c2} (0.11)	32.65^{c2} (0.05)	33.94^{b4} (0.04)	32.58^{b1} (0.07)	33.38^{b3} (0.02)

[1]Parentheses indicate the standard deviation

[2] Different letters in the same column and numbers in the same row indicate differences at a significance level of 0.05 ($p<0.05$)

In the extracted of trout viscera with papain enzyme, there was a particularly notable increase in the content of protein with respect to extraction time in all papain concentrations studied, except for 0.5% which shown an increase after 1 hour of extraction and no significant differences between the time intervals ($p<0.05$) of 1 hour and 4 hour extraction. The highest protein content after 30 minutes, 1 hour and 4 hours of extraction were determined to be 1% enzyme concentration with 6.23g protein/100g waste, 1% enzyme rate with 6.33g protein/100g waste and 1.5% enzyme rate with 6.39g protein/100g waste, respectively ($p<0.05$). As result of 4 hour extraction, the PRR (%) of trout viscera treated with 0.5%, 1%, 1.5% and 2% papain contcentrations significantly increased from 48.71% to 50.34%, from 51.12% to 52.28%, from 50.70% to 52.44% and from 50.23% to 51.64%, respectively ($p<0.05$). Under consideration of all extraction periods and papain concentrations, it was shown that 1 and 4 hour of extraction, as well as 1% and 1.5% papain concentrations, resulted in increased protein content and PRR (%) in liquid protein hydrolysate extracted from trout viscera ($p<0.05$).

After extracted of smoked trout trimmings for 30 minutes, 1 hour, and 4 hours, the highest protein content was found in samples extracted with 2% papain concentration with 5.92g of waste, 2% papain concentration with 6.40g of waste, and 1% papain concentration with 6.24g of waste, respectively ($p<0.05$) (Table 1). When the enzyme concentration was raised from 0.5% to 2%, the PPR (%) for liquid protein hydrolysate from smoked trout trimmings increased from 30.41% to 32.18% after 30 minutes of extraction, from 31.91% to 34.81% after 1 hour of extraction, and from 32.65% to 33.38% after 4 hours of extraction ($p<0.05$).Using smoked trout trimmings treated with papain, the results revealed that all extraction periods had a statistically significant impact on protein content and PRR (%), with 1 hour having the highest protein content and PRR (%) achievable with the use of 1%, 1.5%, and 2% ($p<0.05$).

Papain (EC 3.4.22.2), a plant cysteine protease endolytic enzyme, is obtained by cutting the skin of an unripe papaya (*Carica papaya* L.) and then collecting and drying the latex that flows from the cut (Mombaya, 2012; Hoyle and Merritt, 1994; Shahidi et al., 1995). According to Uhlig (1998), papain has wide proteolytic activity against proteins, short chain peptides, amino acid esters, and amide linkages and has been commonly used in the food and pharmaceutical industries. Utoma et al. (2014) used papain enzyme to hydrolyze catfish fillet wastes for 6, 12, 24, 36, and 48 hours and found that the content of protein in the liquid protein hydrolysate increased significantly with time. Similarly, it was found in this study that as the extraction time increased, so did the content of protein in the liquid hydrolysate solution extracted from viscera and smoked trout trimmings. Fan et al (2018) discovered that the hydrolysis duration in the hydrolysis of trout bone proteins caused an increase in the hydrolysate grade, which was similar to the values found in this study. Adler-Nissen (1986) stated that the enzyme

substrate ratio has an important influence on peptide bonding of the protein substrate as well as on the pH and temperature. According to Noman et al. (2018), the enzyme/substrate ratio over 3% (w/w) in the hydrolysis of Chinese sturgeon (*Acipenser sinensis*) by using papain had no effect on the degree of hydrolysis. They noted that their findings were most likely due to enzyme aggregation, which causes an increase in substrate diffusion inhibition, resulting in reaction rate saturation. Similar findings from this study were also disclosed in that, when the impact of the ratio was studied on the protein contents of liquid protein hydrolysate derived from trout viscera, it was shown that higher papain concentration greater than 1.5 % resulted in reduced protein contents in all periods. This impact, on the other hand, was not observed in smoked trout trimmings. This finding indicates that not only the type of enzyme, enzyme concentrations, and duration of the hydrolysis, but also the type of waste used, have a significant impact on protein content and recovery during enzymatic extraction.

Alcalase

Table 2 shows the protein content (g protein/100 g waste) and Protein Recovery Rates (%) in the liquid protein hydrolysate extracted from trout viscera and smoked trout trimmings using four different rates of alcalase during three different extration periods.

Table 2. Protein content (g protein/100 g waste) and Protein Recovery Rates (%) in the liquid protein hydrolysate from viscera and smoked trout trimmings utilizing alcalase[1,2]

		Enzyme concentrations (%)							
	Extraction Time	**0.5**	**1**	**1.5**	**2**	**0.5**	**1**	**1.5**	**2**
		Viscera				**Smoked trout trimmings**			
Protein Content	**30 min.**	6.22^{b2} (0.02)	6.21^{b2} (0.02)	6.15^{a1} (0.02)	6.47^{b3} (0.01)	5.95^{b1} (0.01)	6.13^{a2} (0.02)	6.09^{a2} (0.06)	6.11^{b2} (0.07)
	1 h	6.14^{a1} (0.01)	6.15^{a1} (0.02)	6.14^{a1} (0.02)	6.18^{a1} (0.06)	6.16^{c2} (0.01)	6.20^{b2} (0.06)	6.25^{c3} (0.01)	5.92^{a1} (0.04)
	4 h	6.14^{a1} (0.07)	6.15^{a12} (0.01)	6.15^{a12} (0.01)	6.16^{a2} (0.01)	5.38^{a1} (0.07)	6.15^{a2} (0.01)	6.19^{b2} (0.01)	6.19^{c2} (0.00)
PRR (%)	**30 min.**	51.10^{b2} (0.13)	50.97^{b2} (0.17)	50.46^{a1} (0.13)	53.13^{b3} (0.11)	32.37^{b1} (0.07)	33.31^{a2} (0.13)	33.11^{a2} (0.32)	33.24^{b2} (0.38)
	1 h	50.40^{a1} (0.06)	50.53^{a1} (0.08)	50.39^{a1} (0.05)	50.73^{a1} (0.50)	33.49^{c2} (0.04)	34.01^{c3} (0.46)	34.95^{c4} (0.10)	32.17^{a1} (0.22)
	4 h	50.39^{a1} (0.05)	50.51^{a2} (0.09)	50.48^{a12} (0.05)	50.56^{a2} (0.09)	$29.^{24a1}$ (0.36)	33.46^{b3} (0.05)	34.02^{b4} (0.06)	33.65^{c2} (0.02)

[1]Parentheses indicate the standard deviation

[2] Different letters in the same column and numbers in the same row indicate differences at a significance level of 0.05 ($p < 0.05$)

The highest protein content in liquid protein hydrolysate from trout viscera was obtained after 30 minutes of extraction at all alcase concentrations, and it then significantly decreased ($p<0.05$), except for samples treated with 1.5 % alcalase, which had no significant influence on the protein content throughout the extraction period ($p>0.05$). By increasing the alcalase concentration from 0.5 to 2%, the PRR/%) of liquid protein hydrolyzate generated from trout viscera increased after 30 minutes and 4 hours of extraction. However, no significant change in PRR (%) was seen after 1 hour of extraction at all concentarions. In the extraction of trout viscera with alcalase, the results revealed that the highest PRR (%) and protein content were obtained after 30 minutes of extraction and at 2% alcalase concentration ($p<0.05$).

The highest protein content and PRR (%) in protein liquid protein hydrolysate derived from the smoked trout trimmings treated with the alcalase was found after 1 hour extraction and 1.5% alcalase concentration. Findings from the current study reveal that alcalase extraction of smoking trout trimmings had a significant impact on protein content and PRR (%) depending on extraction time and alcalase concentrations ($p<0.05$). (Table 2).

Many researchers have found Alcalase® 2.4L, an alkaline enzyme produced from *Bacillus licheniformis* and developed by Novozymes for the detergent industry, to be one of the most effective enzymes for solubilizing proteins among the numerous proteases tested (Diniz and Martin, 1997; Aspmo et al., 2005). Because of its high degree of hydrolysis (DH), which can be attained in a relatively short time compared to moderately neutral or acidic enzymes, Alcalase® 2.4L (*Bacillus licheniformis*) is frequently preferred for fish extraction (Lee, 2007).

Protein Recovery Rate (%) refers to the percentage of total proteins which are soluble in the raw material, as well as the percentage of protein from the extracted materials in the protein phase and nitrogen recovery reflects the yield of proteins that can be recovered during the extraction process (Benjakul and Morrisey,1997). Benjakul and Morrisey (1997) revealed that enzyme concentration, reaction time, and waste/buffer ratio all have a significant effect on extraction and nitrogen recovery (NR) in protein hydrolysates produced from pacific whiting solid wastes using alcalase. The same researchers found that the enzyme concentration between 0 and 34 AU/kg significantly increased nitrogen recovery (NR), but when the enzyme concentration was higher (57 AU/kg),

there was no significant effect on NR. In this study, it was revealed that, in addition to extraction duration and enzyme concentrations, the materials used had a significant impact on the content of protein and PRR (%) after alcalase extraction. Shahidi et al. (1995) used alcalase, neutralase, and papain to extract protein hydrolysate from capelin (*Mallotus villosus*). The extraction solution was also subjected to autolytic hydrolysis. The results showed that the protein recoveries using commercial enzymes achieved 22,9%, 51,6% and 70% compared with the efficiency of autolytic hydrolysis. Similarly, they also observed that while considerable soluble protein was generated at the beginning of the hydrolysate, adding more enzymes throughout the stationary phase of hydrolysis had little influence on the dissolution of the hydrolysate. The presence of a high concentration of soluble peptide in the reaction mixes appears to limit the rate of hydrolysis. Ovissipour et al. (2009) produced protein hydrolysate from the viscera of Persian sturgeon (*Acipenser persicus*) using commercially available Alcalase, and they observed that protein recovery ranged from 34.97% to 61.96% depending on the hydrolysis progressed (30-205 min) protein source. It was reported by them that enzyme absorption onto insoluble protein particles is rapid, cleaving the polypeptide chains that are only weakly linked to the surface of the particles. The more compacted the core proteins are, the longer it takes for them to be hydrolyzed to be broken down (Klomklao and Benjakul, 2017).

Protamex

Protein content (g protein/100 g waste) and Protein Recovery Rates (%) in liquid protein hydrolysate extracted from viscera and smoked trout trimmings using protamex is shown in Table 3.

Table 3. Protein content (g protein/100 g waste) and Protein Recovery Rates (%) in liquid protein hydrolysate exctracted from viscera and smoked trout trimming using protamex [1,2]

		Enzyme concentrations (%)							
	Extraction Time	0.5	1	1.5	2	0.5	1	1.5	2
		Viscera				Smoked trout trimmings			
Protein Content	30 min.	6.51^{a1} (0.01)	6.54^{a2} (0.02)	6.62^{a3} (0.02)	6.70^{b4} (0.02)	6.54^{a1} (0.03)	6.69^{b3} (0.03)	6.72^{b3} (0.02)	6.58^{a2} (0.02)
	1 h	6.65^{b1} (0.02)	6.75^{c2} (0.01)	6.76^{c2} (0.01)	6.74^{b2} (0.01)	6.61^{b2} (0.04)	6.24^{a1} (0.34)	6.72^{b2} (0.01)	6.67^{b2} (0.02)
	4 h	6.65^{b12} (0.07)	6.62^{b12} (0.02)	6.70^{b2} (0.02)	6.37^{a1} (0.35)	6.75^{c2} (0.06)	6.65^{b1} (0.05)	6.64^{a1} (0.01	6.64^{ab1} 0.07)
PRR (%)	30 min.	53.47^{a1} (0.11)	53.69^{a2} (0.13)	54.32^{a3} (0.15)	55.04^{b4} (0.15)	35.57^{a1} (0.15)	36.38^{b3} (0.15)	36.56^{b3} (0.10)	35.79^{a2} (0.11)
	1 h	54.62^{b1} (0.13)	55.39^{c23} (0.08)	55.49^{c3} (0.11)	55.31^{b2} (0.10)	35.94^{a2} (0.20)	33.91^{a1} (1.87)	36.55^{b2} (0.06)	36.25^{b2} (0.12)
	4 h	54.61^{b12} (0.54)	54.31^{b12} (0.15)	55.03^{b2} (0.20)	52.34^{a1} (2.87)	36.72^{b2} (0.35)	36.19^{b1} (0.29)	36.08^{a1} (0.08)	36.12^{ab1} (0.39)

[1]Parentheses indicate the standard deviation
[2] Different letters in the same column and numbers in the same row indicate differences at a significance level of 0.05 ($p < 0.05$)

The content of protein and PRR (%) in the liquid protein hydrolysate of trout viscera extracted with protamex for 30 min. increased significantly as the enzyme concentration increased ($p<0.05$) and the highest protein content and PRR (%) were found in samples treated with 2% protamex ($p<0.05$). However, after 1 hour of extraction, the same effectiveness was not observed, with this increase seen only in increasing enzyme concentration from 0.5% to 1% ($p<0.05$). Furthermore, the enzyme concentration did not have a significant effect on the increase of protein content and PRR (%) after 4 hours extraction.

Protein content and PRR (%) in liquid protein hydrolysate produced from smoked trout trimmings treated with protamex for 30 minutes was highest at 1% and 1.5% enzyme concentrations; however, after the 4 hour extraction, the highest protein content was found at 0.5 % enzyme concentration. The increase in enzyme concentration in 1 and 4 hour extraction did not substantially contribute to the increase in protein content and PRR (%).

Protamex is a protease complex for *Bacillus* designed for food protein degradation. It has been demonstrated to exhibit non-bitter protein hydrolysates, unlike other endoproteases (Lee, 2007). Soufi-Kechaou et al. (2012) investigated the effect of extraction time on protein recovery rate in hydrolysates produced from cuttlefish (*Sepia officinalis*) viscera. Researchers have found that the rate of soluble nitrogen increases during hydrolysis. They noted that this was an indication that the proteins were solubilized under the influence of commercial enzymes used during hydrolysis and move from the substrate to the soluble phase. In the study, the total content of nitrogen in the soluble fraction increased rapidly in the first two hours and then the content of protein in the substrate decreased while reaching a stationary phase for the remainder of the hydrolysis reaction. The researchers obtained the maximum yield for protamex after approximately 120 minutes of extraction reaction. In this study, the highest content of protein was obtained in 1 hour extraction of visceral waste and 4 hour extraction for smoked trout trimmings. This shows that the material used in protein recovery is effective.

Molla and Hovannisyan (2011) used protamex to optimize the enzymatic hydrolysis of beluga (*Huso huso*) visceral waste proteins. They observed that increasing the temperature, time, and enzyme activity in the hydrolysis of beluga protein resulted in an increase in hydrolysis rate up to a certain point, but thereafter hydrolysis rate considerably decreases. They indicated that a decrease in hydrolysis rate with increasing enzyme activity levels, temperatures, and time might be attributed to a decrease in enzyme activity.

Flavourzyme

Table 4 shows the protein content (g protein/100 g waste) and Protein Recovery Rates (%) in the liquid protein hydrolysate extracted from trout viscera and smoked trout trimmings using four different rates of flavourzyme during three different time periods.

Increasing the enzyme concentration at all times in the extraction of trout viscera with the flavourzyme did not have a significant effect on the increase in the content of protein and PRR (%) in the liquid protein hydrolysate (Table 1). However, considering all extraction time for all enzyme concentarions, extraction times of 30 minutes and 4 hours resulted in the highest protein content and PRR (%) ($p<0.05$).

In the smoked trout trimmings, the highest protein content and PRR (%) in the liquid protein hydrolysate was recovered at 0.5% enzyme concentrations for 30 minutes, followed by 2% enzyme concentration for 4-hour extraction ($p<0.05$).

Table 4. Protein content (g protein/100 g waste) and Protein Recovery Rates (%) in the liquid protein hydrolysate extracted from viscera and smoked trout trimmings utilizing flavourzyme [1,2]

		Enzyme concentrations (%)							
	Extraction Time	0.5	1	1.5	2	0.5	1	1.5	2
		Viscera				Smoked trout trimmings			
Protein Content	30 min.	7.02^{b12} (0.00)	7.03^{c2} (0.02)	7.00^{b12} (0.01)	6.99^{c1} (0.01)	7.01^{c4} (0.01)	6.69^{b2} (0.02)	6.64^{b1} (0.03)	6.88^{b3} (0.02)
	1 h	6.99^{b3} (0.02)	6.51^{a1} (0.01)	6.94^{a2} (0.02)	6.97^{b3} (0.01)	6.00^{a1} (0.00)	5.99^{a1} (0.02)	6.00^{a1} (0.01)	6.03^{a2} (0.01)
	4 h	6.94^{a2} (0.02)	6.98^{b1} (0.01)	6.98^{ab2} (0.05)	6.90^{a2} (0.02)	6.05^{b1} (0.03)	6.31^{a1} (0.39)	6.90^{c2} (0.04)	6.96^{c2} (0.01)
PRR (%)	30 min.	57.62^{c23} (0.10)	57.68^{c3} (0.14)	57.45^{b12} (0.11)	57.43^{c1} (0.10)	38.10^{c4} (0.06)	36.39^{b2} (0.10)	36.12^{b1} (0.18)	37.41^{b3} (0.11)
	1 h	57.40^{a3} (0.19)	53.44^{a1} (0.09)	56.97^{a2} (0.17)	57.21^{b3} (0.11)	32.60^{a1} (0.03)	32.60^{a1} (0.10)	32.65^{a1} (0.05)	32.77^{a2} (0.05)
	4 h	57.33^{b2} (0.27)	57.33^{b2} (0.11)	57.32^{ab2} (0.39)	56.62^{a1} (0.11)	32.89^{b1} (0.18)	34.29^{a1} (2.14)	37.54^{c2} (0.20)	37.84^{c2} (0.06)

[1]Parentheses indicate the standard deviation

[2] Different letters in the same column and numbers in the same row indicate differences at a significance level of 0.05 ($p < 0.05$)

Flavourzyme is generated by a strain of *Aspergillus oryzae* and also is composed of a number of enzymes, including endoproteases and exopeptidases, each with varied activity and optimal pH values. Exopeptidase activities cause the removal of terminal amino acids that can cause bitter taste (Lee, 2007). It has been noted that hydrolysis of proteases such as flavourzyme, which can degrade bitter peptides (from Novozymes), contributes to eliminating the problem of bitter hydrolysates (Guerard, 2007). In the study of Nemati et al. (2012) with wastes belonging to shad *(Alosa caspia*) species, the protein recoveries of using flavourzyme throughout 1 hour increased significantly with time and their protein recovery obtained by flavourzyme during 60 minutes was 47.66%. Although it was shown in this investigation that visceral and smoked trout trimmings did not produce a significant increase in time on protein recovery in hydrolysates obtained with flavourzyme, protein recovery was assessed to be greater in trout viscera than that observed by Nemati et al (2012). Their also found that major peptide cleavage happened within the first 15 minutes of hydrolysis. Mohr (1980) stated that the proteins in the sarcoplasmic fraction may denature and precipitate during heating to the hydrolysis temperature, and the denatured proteins would be highly resistant to enzymatic degradation. Moreover, it was noted that there was no statistically significant difference found between the yield of proteins following enzymatic hydrolysis and hydrophobic interactions between peptides or self-assembly of larger peptides, meaning that precipitation would likely occur, reducing the yield of proteins (Mutilangi et al., 1996).

The flavourzyme, followed by protamex, produced the highest protein content and PRR (%) in liquid protein hydrolysate extracted from trout viscera when all times and rates were taken into consideration ($p<0.05$). Protamex, on the other hand, was found to be the enzyme responsible for the highest protein content and PRR (%) in smoked trout trimmings, followed by flavourzyme ($p<0.05$). Additionally, it was demonstrated that the effects of papain and alcalases were not comparable ($p>0.05$).

CONCLUSION

Based on the findings of the investigation, it was found that the content of and the protein recovery rate protein in the liquid protein hydrolysate were significantly affected by the type of waste, the amount of enzyme utilized, and the extraction time.

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SHORT COMMUNICATION KISA BİLDİRİ

Occurrence of the silky shark, *Carcharhinus falciformis* (Bibron, 1839) (Carcharhiniformes: Carcharhinidae), from Iskenderun Bay, northeast Levant Sea

İpeksi köpekbalığı, *Carcharhinus falciformis* (Bibron, 1839)'in (Carcharhiniformes: Carcharhinidae), İskenderun Körfezi sularında (Kuzeydoğu Levant Denizi) görülmesi

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Abstract: An immature male specimen of *Carcharhinus falciformis* (Bibron, 1839) was caught by a commercial long-liner off Samandağ coast (Iskenderun Bay, northeastern Levant Sea, Turkey), on 2nd of November, 2021. This record is the fourth observation of this species in Turkey. Monitoring the coastal occurrences of the silky shark throughout its distribution range is critical, where the species is considered as very rare.

Keywords: Record, Carcharhinidae, distribution, Turkey

Öz: *Carcharhinus falciformis*'in (Bibron, 1839) erişkin olmayan bir erkek örneği, 2 Kasım 2021'de Samandağ sahili açıklarında (İskenderun Körfezi, kuzeydoğu Levant Denizi, Türkiye) ticari bir paraketa teknesi ile yakalandı. Bu kayıt, türün Türkiye'deki dördüncü gözlemidir. Türün çok nadir görüldüğü yerlerde, ipeksi köpekbalığının dağılım aralığı boyunca kıyı oluşumlarını izlemek çok önemlidir.

Anahtar kelimeler: Kayıt, Carcharhinidae, dağılım, Türkiye

INTRODUCTION

Carcharhinidae is one of the largest and most important families of sharks, with many common and wide-ranging species, represented by 12 genera and 57 species (Ebert and Stehmann, 2013). In the Mediterranean Sea, the family is represented by 4 genera and 11 species, one of which is *Carcharhinus falciformis* (Bibron, 1839) (Serena et al., 2020).

The silky shark, *C. falciformis*, is a large and fairly slender shark, occurring in oceanic and coastal waters, from the surface down to at least 500 m (Ebert and Stehmann, 2013). It is a circumtropical shark and it's distribution range covers both western and eastern Atlantic, Indo-Pacific region and extends to the Mediterranean Sea (Bonfil and Abdallah, 2004; Ebert and Stehmann, 2013; Serena et al., 2020). Although, it was recorded in several localities in eastern Mediterranean waters (Azab et al., 2019; Kabasakal and Bilecenoğlu, 2020) and western in the Ligurian Sea (Garibaldi and Orsi-Relini, 2012), its occurrence status in the region is considered as very rare (Bariche, 2012).

Following its first record in Turkish Mediterranean waters by Kabasakal and Bilecenoğlu (2020), several sightings of *C. falciformis* in northeastern Levant Sea were reported (personal observation by the first author); however, this encounters could have not been confirmed due to the lack of preserved specimens or reliable photographs documenting the capture of a silky shark.

In the present paper, authors report the regional occurrence of a specimen of *C. falciformis* in the southern part of Iskenderun, and provide supporting information for a better understanding of the distribution and status of the species in the mentioned region.

MATERIAL AND METHODS

The examined male specimen of *C. falciformis* was caught by a commercial long-liner, at a depth of 30 m, nearly 10 km off Samandağ coast (Figure 1), on 2nd of November, 2021. Species identification follows Serena (2005) and Ebert and Stehmann (2013), and taxonomic nomenclature follows Serena et al. (2020). Total length of the specimen was measured according to Serena (2005), where the total length is the distance between the tip of the snout and to the tip of the upper lobe of the caudal fin, depressed to body axis. Morphometric measurements were measured to the nearest centimetre, according to procedure proposed by Ebert and Stehmann (2013). Preserved specimen is being kept in the ichthyological collections of Iskenderun Technical University Marine Sciences and Technology Faculty, with the following registration number: (MSM-PIS-2021-2; Figure 2).

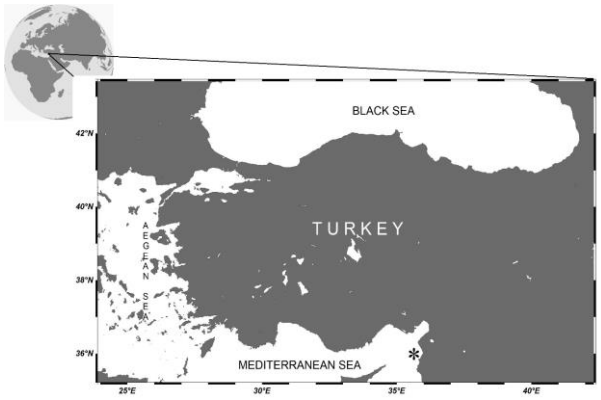

Figure 1. Map shows the approximate locality (*) of capture of the present silky shark in northeastern Levant Sea

RESULTS

Total length (TL) of the present specimen of *C. falciformis* (Figure 2) was 103 cm and the weight was 7638 g. Some morphometric measurements are presented in Table 1, which are all in accordance with previous descriptions of Ebert and Stehmann (2013).

Coloration of the examined specimen was dark grey above and white below, with narrow dark blotches on tips of pectoral fins, and upper and lower caudal lobes (Figure 2). The observed descriptive characteristics were in agreement with those described by Ebert and Stehmann (2013). Thus, the present specimen was identified as *C. falciformis*.

Figure 2. (A) Side view of the silky shark, *Carcharhinus falciformis*; (B) arrow denotes the interdorsal ridge; (C) arrow denotes the free rear tip of second dorsal fin; and (D) uncalcified and soft claspers of the specimen

Table 1. Morphometric measurements of the examined specimen of *C. falciformis*

Measurement	cm	% of TL
Total length (TL)	103	100
Standard length	75	72
Head length	23	22.3
Mouth length	10	9.7
Snout length	3.5	3.4
Eye length	1.6	1.55
Eye height	1	0.9
Pre-branchial length	21	20.38
Pre-orbital length	7.5	7.2
Pre-D1 fin length	35	34
Pre-D2 fin length	85	82.5
Pre-pectoral fin length	24	23.3
Pre-D1 to anal fin length	51	49.5
Pre-D2 to anal fin length	86	83.4
D1 fin length	13	12.6
D1 fin height	9.5	9.2
D2 fin length	7	6.8
D2 fin height	1.4	1.35
D2 fin free rear tip length	4	3.8
Pectoral fin length	15.5	15
Ventral fin length	8	7,7
Anal fin length	7,5	7,2
Caudal upper lobe length	29,5	28,6
Caudal lower lobe length	13	12,6
Clasper length	3	2,9

DISCUSSION

Kabasakal and Bilecenoğlu (2020) recorded 3 specimens of *C. falciformis* off Turkish Mediterranean coasts, 1 in the Gulf of Antalya and 2 specimens off the eastern coast of Gulf of Mersin. We are presenting hereby the fourth record of the species from Turkey, which supports the existence of an established population in the region. According to Ebert and Stehmann (2013), males of *C. falciformis* mature at about 210 and 220 cm. Claspers of the examined silky shark were uncalcified, soft and shorter than the pelvic fins (Figure 2D). Thus, the present specimen was an immature male.

Besides the silky shark, several other Carcharhinid species have been previously encountered through the northern Levant coasts of Turkey (Ayas et al., 2019, 2020; Ergüden et al., 2020; Kabasakal and Bilecenoğlu, 2020; Kabasakal et al., 2021).

Based on the recent IUCN Red List assessment (Rigby et al., 2017), conservation status of *C. falciformis* is considered 'Vulnerable' globally, with a decreasing population trend. Therefore, monitoring the coastal occurrences of the silky shark throughout its distribution range is critical, where the species is considered as 'very rare' (Bariche, 2012; Azab et al., 2019).

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SHORT COMMUNICATION **KISA BİLDİRİ**

The northernmost dispersal record of the lionfish, *Pterois miles* (Bennett, 1828) for the Aegean Sea

Aslan balığı, Pterois miles (Bennett, 1828)' in Ege Denizi için en kuzey dağılış kaydı

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Abstract: *Pterois miles* (Bennett, 1828) is an aquatic invader and disruptive predator for marine ecosystems. A single lionfish was photographed and sampled in March 2021 during a scientific survey at 36 m depth in Karaburun/İzmir Bay. In this study presents an update on the lionfish's northward progression in the Aegean Sea.

Keywords: Invasive species, *Pterois miles*, lionfish, Mediterranean Sea, Aegean Sea, İzmir Bay

Öz: *Pterois miles* (Bennett, 1828) sucul istilacı tür olup deniz ekosistemleri için yıkıcı bir predatördür. 2021 yılının Mart ayında, Karaburun/İzmir Körfezi'nde gerçekleştirilen bilimsel araştırma esnasında 36 m derinlikte tek bir Aslan balığı fotoğraflanmış ve örneklenmiştir. Bu çalışmada Aslan balığının Ege Denizi'ndeki kuzey yönlü ilerleyişi için bir güncelleme sunulmaktadır.

Anahtar kelimeler: İstilacı tür, *Pterois miles*, aslan balığı, Akdeniz, Ege Denizi, İzmir Körfezi

INTRODUCTION

Pterois miles (Bennett, 1828) is regarded as one of the most invasive marine fish species which affects native fish assemblages and human health negatively in invaded ecosystems (Sutherland et al., 2010). Lionfish possess several ecological traits such as high tolerance to a wide range of environmental conditions (Whitfield et al., 2007); rapid growth rate (Johnson and Swenarton, 2016); capability of spawning throughout the year, and high fecundity (Savva et al., 2020) and opportunistic feeding habits (Eddy et al., 2016). This species is currently considered as established in all the Eastern Mediterranean (Savva et al., 2020). Although the lionfish was firstly reported from Israel by Golani and Sonin in 1992, the invasion of *P. miles* in the Mediterranean began in Lebanon in 2012 (Bariche et al., 2013). The twenty-year period between the reports of lionfish was commented by the researchers as the first specimen entered the Suez Canal like many marine organisms (Zenetos et al., 2012); released from captivity (Golani et al., 2002) or it has not been established a population in the Mediterranean (Bariche et al., 2013). In the intervening years, *P. miles* rapidly spread throughout most sectors of the Mediterranean Sea (e.g. Levantine Sea, southern and central Aegean Sea, Ionian Sea, southern Adriatic Sea, Tunisia, and Italy) (Kletou et al., 2016; Azzurro et al., 2017; Giovos et al., 2018; Dimitriadis et al., 2020; Vavasis et al., 2020; Di Martino and Stancanelli, 2021).

Concerning the Turkish coasts of the Aegean Sea, *P. miles* was firstly reported at Dalyan in 2015 by Turan and Öztürk and has since, the lionfish has expanded towards the northeast Aegean Sea, being reported at Datça (Bilge et al., 2016), Didim (Yapıcı, 2018), Bodrum and Teos (Ulman et al., 2020) and Kokar (Özgül, 2020).

This scientific report presents information about the northern progression of *Pterois miles* in the Aegean Sea.

MATERIAL AND METHODS

A single specimen of *P. miles* was collected from İzmir Bay (Karaburun), Aegean Sea (38.65 N°-26.52 E°) on 18 March 2021, at a depth of 36 m (Figure 1). The water temperature was 15.0 °C. Photographs and underwater videos of *P. miles*, when it was detected in the propeller gap of the 9 Eylül shipwreck was taken (Figure 2).

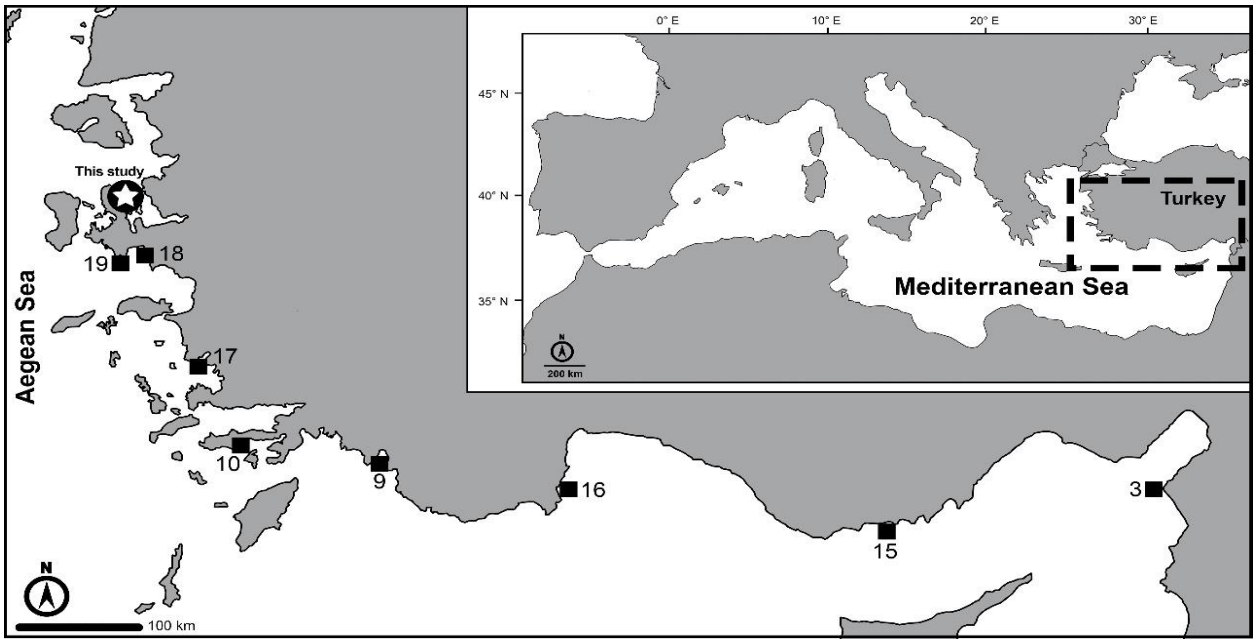

Figure 1. (★) *Pterois miles* in İzmir Bay (Aegean Sea) (■) Previous records of *P. miles* in Turkish coasts. The numbers refer to the references listed in Table 1

Table 1. Scientific reports of *P. miles* from the Levantine Sea to the Aegean Sea (Report localities from Turkish coasts were shown in Figure 1 with numbers indicated in the table. Updated from Özgül (2020)

No	Location	Coordinates	Length Range (TL mm)	Habitat	Depth (m)	Observation Method	Reference
1	Herzliya, Israel	-	328	-	35	Trawl	Golani and Sonin, (1992)
2	Al Minie, Lebanon	34.29N 35.54E	209	Coralligenous	30	Gill net	Bariche et al., (2013)
3	İskenderun, Turkey	36.17N 35.46E	276	Rocky bottom	25	-	Turan et al., (2014)
4	Rhodes, Greece	36.38N 28.24E	-	Rocky bottom	7	Diving	Crocetta et al., (2015)
5	Rhodes, Greece	35.91N 27.85E	-	Shipwreck	-	Diving	Crocetta et al., (2015)
6	Rhodes, Greece	36.45N 28.21E	-	Rocky bottom	2	Diving	Crocetta et al., (2015)
7	Ormidia, Cyprus	-	170	-	10	Gill net	Iglesias and Frotte, (2015)
8	Karpas, Cyprus	-	373	Rocky bottom	40	Gill net	Oray et al., (2015)
9	Dalyan, Turkey	-	-	Sandy bottom	11	Diving	Turan and Öztürk, (2015)
10	Datça, Turkey	36.69N 27.68E	-	-	10	Gill net	Bilge et al., (2016)
11	NE Crete, Greece	35.20N 26.30E	250	Rocky bottom	33	Gill net	Daillianis et al., (2016)
12	SE Crete, Greece	35.01N 25.96E	100	Rocky bottom	12-37	-	Daillianis et al., (2016)
13	Karpathos Island, Greece	35.55N 27.20E	100	Rocky bottom	17	Diving	Mytilineou et al., (2016)
14	Karpathos Island, Greece	35.50N 27.22E	200	Rocky bottom	16	Diving	Mytilineou et al., (2016)
15	Mersin, Turkey	36.08N-33.40E	250	-	100-110	Trawl	Yağlıoğlu and Ayas (2016)
16	Kemer, Turkey	-	85-293	Rocky Bottom	10-15	Spearfishing	Özgür-Özbek et al., (2017)
17	Didim-Aydın, Turkey	37.20N 27.14E	-	Rocky bottom	18	Diving	Yapıcı, (2018)
18	Bodrum and Teos, Turkey		100	Rocky bottom	10	Diving	Ulman et al., (2020)
19	Kokar Bay, Turkey	38.13N 26.61E	144	Rocky bottom	15	Spearfishing	Özgül, 2020
20	İzmir Bay, Turkey	38.65N 26.52E	309	Sandy bottom	36	Diving	This study

Figure 2. An underwater photograph of *P. miles* sampled in this study

The specimen was identified according to Golani and Sonin (1992) and Bariche et al. (2013). Morphometric measurements were taken using a digital caliper to the nearest 0.1 mm. Total length (TL, mm), standard length (SL, mm), and total weight (TW, g) were measured. The specimen was fixed in 4% formaldehyde solution and deposited in the fish collection of the Sea Museum of Izmir Kâtip Çelebi University, Turkey (IKC PIS 1262).

RESULTS

The specimen was 309 mm in total length (TL), 208 mm in standard length (SL) and 402 g in total weight. Morphometric measurements and meristic counts of the *P. miles* specimen were as follows: dorsal fin rays XIII+10; anal fin III+6; pectoral fin rays 14; pelvic fin rays I, 5; caudal fin rays 14, gill rakers 14. Body depth 36.9; head length 32.7 of % SL snout length 39.7; eye diameter 18.5 and interorbital width 20.1 % of head length (HL). Pelvic longest fin ray 38.2 and pectoral longest ray 63.4 % of SL (Figure 3).

Figure 3. Adult specimen of *P. miles* from the İzmir Bay, Turkey

After the diagnostic characters were determined, dissection of the lionfish was performed. It was observed that the sex of the lionfish was male, stomach and gut were empty.

DISCUSSION

The Eastern Mediterranean is threatened by highly invasive fish species (Bonanno and Orlando-Bonaca 2019). One of these invasive species is *P. miles*, which is raising serious concerns for the potential impacts on the local marine ecosystems and native species (Charles and Dukes 2008).

The northernmost occurrence of *P. miles* in the Mediterranean Sea was reported by Di Martino and Stancanelli (2021) at three locations in the Adriatic Sea. In the Aegean Sea, previously *P. miles* reported by Özgül (2020) from Kokar Bay as the northernmost location. This scientific record represents the most northward range expansion of the *P. miles* in the Aegean Sea reported up today.

In this study, *Pterois miles* was sampled at 15 °C at the propeller gap of the shipwreck. According to Kimball et al. (2004) lionfish were stationary and fed infrequently at 15 °C and also Özgür-Özbek et al., (2017) stated that *P. miles* continued feeding at 14.9 °C. However, the specimen of this study had a completely empty stomach and gut. As stated in Balazy et al., (2019) shipwrecks can act as a stepping stones in bio-invasion and some non-indigenous species even thrive better on the added structures (Airoldi et al., 2015).

The Mediterranean Sea is the world's most invaded marine region (Edelist et al., 2013). Following the invasion process with scientific studies and monitoring activities are globally important to minimize the negative effects of *P. miles* on marine habitats.

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