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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Artificial seagrass experiments in the Northeast Mediterranean

Kuzeydoğu Akdeniz'de yapay deniz çayırı denemeleri

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Abstract: Seagrasses provide important nursery grounds, shelter and natural habitats for juvenile fish. In this study, we evaluated if artificially created seagrass areas can play the same role as the natural seagrass (NS) habitats. The study was carried out in three different stations on the coast of Yumurtalık, Adana, selected according to the seagrass areas. Artificial seagrass (AS) was made of polypropylene ribbon and fixed on the ground in the designated areas with a depth of 0.5 m on average. Sampling was carried out with a beach seine net once a week at stations between 28 April 2016 and 11 August 2016. Sampled fish were identified to the lowest possible taxonomic level. Based on our results, the fish abundance and species richness of NS and AS habitats were not statistically different, whereas the both parameters were significantly lower in sandy (S) habitats ($p<0.001$). Moreover, the species composition of NS and AS habitats was found to be similar each other, whereas the composition was significantly different in S habitats. This study, conducted in the Northeast Mediterranean, shows that AS habitats effect the distribution of juvenile fish.

Keywords: Meadows, juvenile fish, recruitment, nursery, Levant Basin

Öz: Deniz çayırları yavru balıklar için barınak, stoğa katılma ve doğal yaşam alanları sağlar. Bu çalışmada, yapay olarak oluşturulmuş deniz çayırı alanlarının doğal deniz çayırı (NS) habitatlarıyla aynı rolü oynayıp oynayamayacağı değerlendirilmiştir. Çalışma Adana, Yumurtalık kıyısında deniz çayırı alanlarına göre seçilen üç farklı istasyonda gerçekleştirilmiştir. Yapay deniz çayırı (AS) polipropilen şeritten yapılmıştır ve belirlenen alanlarda ortalama 0,5 m derinliğe sahip zemine sabitlenmiştir. Örnekleme 28 Nisan 2016 ile 11 Ağustos 2016 tarihleri arasında istasyonlarda haftada bir ığrıp ile gerçekleştirilmiştir. Örneklenen balıklar mümkün olan en düşük taksonomik seviyede tespit edilmiştir. Sonuçlarımıza göre, NS ve AS habitatlarının balık bolluğu ve tür zenginliği istatistiksel olarak farklı değildir. Fakat her iki parametre de kumlu (S) habitatlarda daha düşük bulunmuştur ($p <0,001$). NS ve AS habitatlarının tür kompozisyonu birbirine benzerken, S habitatlarında önemli ölçüde farklı bulunmuştur. Kuzeydoğu Akdeniz'de yapılan bu çalışma, AS habitatlarının yavru balıkların dağılımını etkilediğini göstermektedir.

Anahtar kelimeler: Çayır, yavru balık, stoğa katılma, yuva, Levant Baseni

INTRODUCTION

Seagrasses are quite important for the coastal ecosystems as a source of natural habitat, shelter, oxygen and food (Gullstrom et al., 2008; Hemminga and Duarte, 2000; Tuya et al., 2014). Seagrass habitats can support high invertebrate abundance and richness and this provide important source of food for many fish species (Jenkins et al., 1997; Orth, 1992; Orth et al., 1984; Pihl et al., 2006). Moreover, they also play important role in decreasing the water movement and therefore stabilizing the sand, along with securing the quality of water and producing oxygen (Becker and Choresh, 2006). One of the most important functions of seagrasses is their role as a shelter for juvenile fish by protecting them from the other predators as they can hide in the leaves of seagrasses (Beck et al., 2001; Deegan et al., 2002; Mattila et al., 1999; Pollard, 1984; Spalding et al., 2003).

There are 66 known species of seagrasses throughout the world (Kuo and Den Hartog, 2007), five of which, *Zostera marina, Zostera noltii, Cymodocea nodosa, Posidonia oceanica* and *Halophila stipulacea* distribute in Turkish coasts (Demirci and Karakan, 2006), including the northeastern coasts of Levant Basin (Aysel et al., 2006).

The total destruction or the decline of seagrasses cause failing of its ecosystem services and resulted with adverse impacts both economically and environmentally (Orth et al., 2006; Waycott et al., 2006). Coastal structures, industrialization, marine pollution, illegal trawling along coasts are the major threats damaging the seagrass habitats (Boudouresque et al., 1994; Meinesz et al., 1991). Hence, in order to protect seagrass habitats, conservation measures have been taken over the coasts of most of Mediterranean Countries (Protocol, 1995). Additionally, habitat rehabilitation studies are in progress in disrupted areas (Irving et al., 2010). In this context, creating AS can also simulate the positive impacts of seagrasses on aquatic life. For instance Lee and Low (1991) reported that in the areas of AS, fish family increased from 12 to 14, fish species increased from 16 to 30 and created a habitat for fish. Similarly, Saad et al. (2011) identified 497 fish belonging to 17 fish families in the field AS in their study and reported that AS created an important nutrient-rich habitat for marine fish. Upston and Booth (2003) showed that there were no significant differences in species richness and diversity between AS and NS area in Botany Bay, New South Wales. Although many studies have been performed about the function of AS on fish settlement, there is no empirical evidence in the Northeast Mediterranean.

In this study, we investigate the impact of AS areas on the settlement of fish in a relatively undisturbed coastal area in Northeast Mediterranean. For this purpose, we compared the species richness, composition, and total fish abundance between the S habitats, NS and AS habitats over the course of a four-month period when NS were available.

MATERIALS AND METHODS

The study was conducted in İskenderun Bay, along the coast of Yumurtalık (35º40'50"E ile 36º50'40"N) (Figure 1). The average depth of a large part of Yumurtalık Bay is 2.8 meters, which is quite shallow (Avşar et al., 1999). The study area was built 20 meters away from the shore, on a seabed at an average depth of 0.5 meters. The site was safe from the impacts of currents, waves and wind effect and away from sites and human activities. The stations were arranged taking seagrasses into consideration as can be seen below (Table 1, Figure 1).

Table 1. Habitats at stations in the study

	Station 1 (S1)	Station 2 (S2)	Station 3 (S3)
NS	100m²	-	100m²
AS	-	25m²	100m²
S	100m²	25m²	100m²

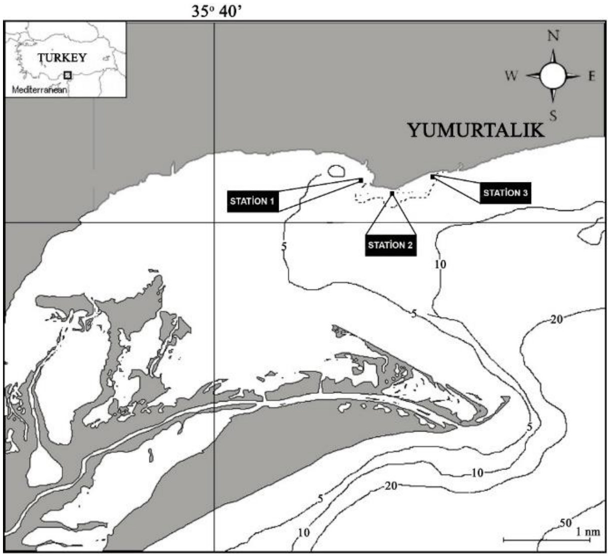

Figure 1. The locations of the stations on the map

Green thine polypropylene ribbon (d = 0.946 gr/cm³) was used in the making of AS leaf. The ribbons were attached a wire mesh fence. The fence was 1m width and 20m long and has 5 x 5 cm square. Based on the previous studies, the abundance and number of species are higher in the seagrasses the leaves of which are between 20 and 40 cm (Mattila et al., 1999). Therefore, we kept the leave length as 30 cm. Then AS model was placed into the 2nd Station (Figure 2) on 8 April 2016 and into the 3rd station on 27 May 2016. The models were fixed on the ground by using T-shaped rods.

Sampling at the stations was done weekly at the same time. Sampling in S1 and S2 started on April 28, and S3 on June 17, 2016 and ended on August 11, 2016.

Figure 2. The AS area created for Station 2

The specimens were identified at the lowest possible taxonomic level using the following references Whitehead et al. (1986), Golani et al. (2006), Turan (2007) and Froese and Pauly (2016). The number of individuals were recorded for each species. After the procedures, alive specimens were released back into the sea and the ones those were already dead were fixed in a 10% formaldehyde solution for further investigations.

The change of total abundance and total number of species based on day of sampling, habitat type and station were analyzed by using generalized additive models. The models were fitted following the protocols suggested by Zuur et al. (2009) using MASS library (Venables and Ripley, 2002) in R environment (Team, 2017). The changes of species composition were analyzed with Constrained Analyze of Principle Coordinates (CAP) using vegan and Biodiversity R libraries of R environment (Kindt and Coe, 2005; Oksanen et al., 2013)

RESULTS

A total of 29 different species of juvenile fish were detected in the samplings in NS areas. The most dominant species was found to be *Atherina boyeri* (68.92%) followed by *Siganus rivulatus* (16.46%), *Gobius niger* (6.36%) and *Diplodus sargus* (4.18%) (Figure 3.).

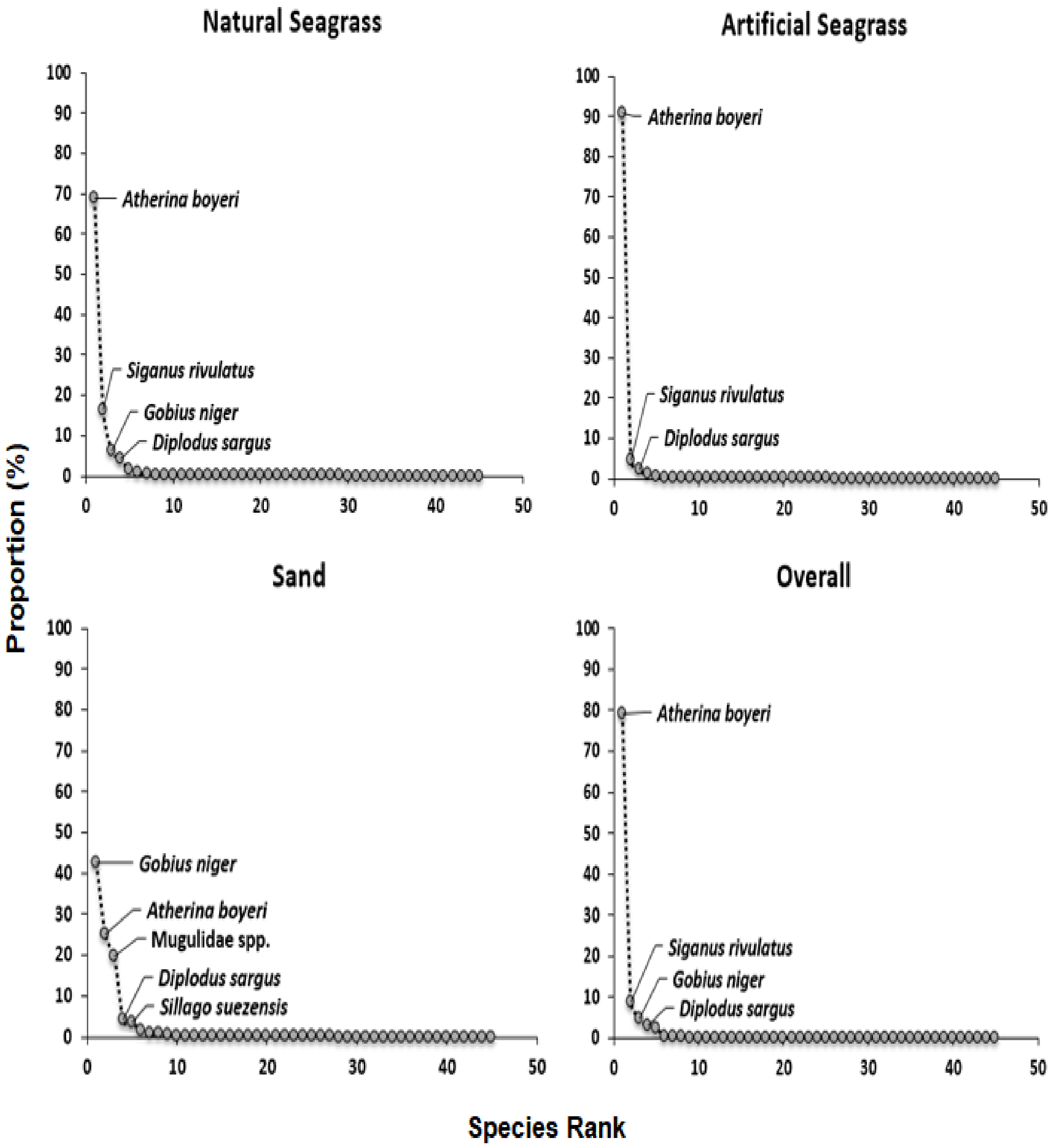

Figure 3. Rank abundance curves of NS, AS and Sand habitats, and overall study area

Results of GAMs revealed that overall average fish abundance and number of species were similar in AS and NS habitats and the both were significantly lower in S habitats (Table 2, Table 3, Figure 4, Figure 5).

Fish abundance did not reveal any significant trends along time in NS, and S habitats of S1 and S2. On the other hand, two contrasting patterns were appeared in AS. In S2, fish abundance revealed a clear increment after 80th days of experiments, whereas it monotonously decreased in S3. In stations, the number of species remained similar or decreased along the experiments in S and NS habitats, respectively. Change in AS habitat of S2 was not significant whereas GAM revealed a significantly fluctuating pattern in AS habitat of S3.

Table 2. Estimated regression parameters and approximate significance of smooth terms of negative binomial (NB) generalized additive model for total fish abundance

Abundance$_{ijk}$~NB(μ_{ijk}); ln(μ_{ijk}+1) = Intercept + H$_i$ + s(DoY$_k$: H$_i$: S$_k$) + ε_{ijk}				
Parametric coefficients:				
	Estimate	**Std. Error**	**z value**	**p value**
Intercept	**6.030**	**0.183**	**32.918**	**<0.001**
Habitat NS	0.101	0.272	0.371	0.711
Habitat S	**-1.851**	**0.234**	**-7.901**	**<0.001**
Approximate significance of smooth terms:				
	edf	Ref.df	Chi.sq	p-value
s(DoY) : S$_1$, H$_{NS}$	1.000	1.000	0.701	0.403
s(DoY) : S$_1$, H$_S$	2.642	3.282	4.775	0.211
s(DoY) : S$_2$, H$_{AS}$	**6.893**	**7.978**	**71.813**	**<0.001**
s(DoY) : S$_2$, H$_S$	3.687	4.550	9.464	0.088
s(DoY) : S$_3$, H$_{AS}$	**1.000**	**1.000**	**29.742**	**<0.001**
s(DoY) : S$_3$, H$_{NS}$	2.463	3.010	7.641	0.054
s(DoY) : S$_3$, H$_S$	**2.237**	**2.739**	**11.270**	**0.008**
R^2 (adj) = 0.798, Deviance explained = 81.6%, n = 91				

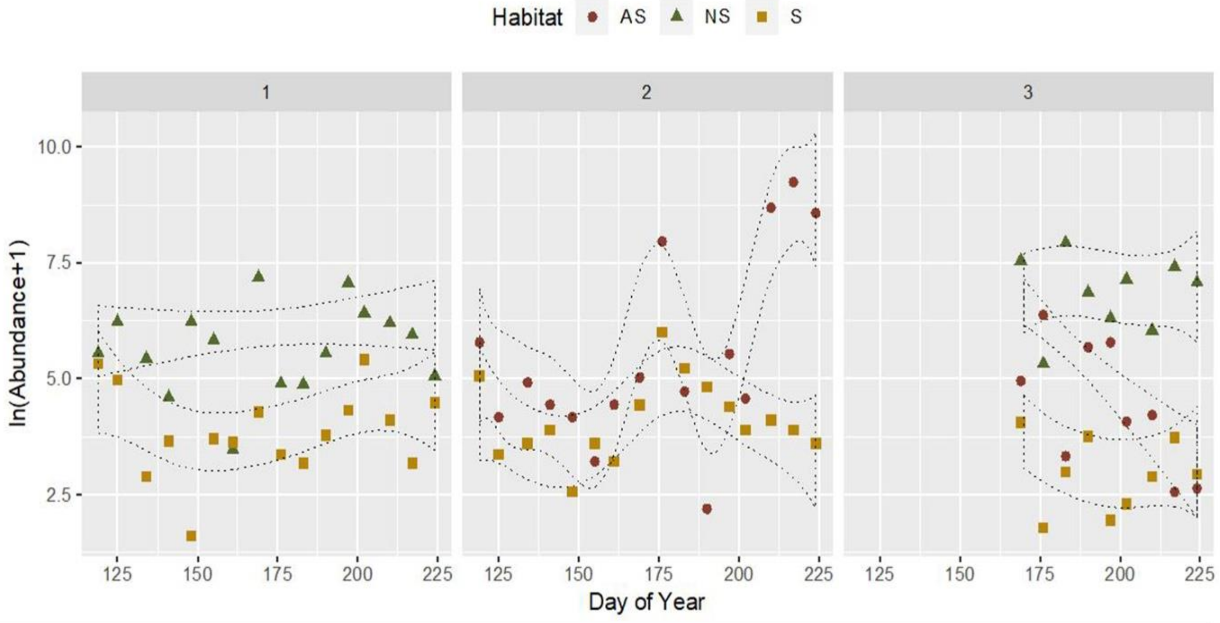

Figure 4. Fit of the negative binomial GAM in Table 2 for the total abundance

Table 3. Estimated regression parameters and approximate significance of smooth terms of quasi-Poisson (QP) generalized additive model for the richness of fish species

$Richness_{ijk} \sim QP(\mu_{ijk})$; $\ln(\mu_{ijk}+1) = Intercept + H_i + s(DoY_k : H_i : S_k) + \varepsilon_{ijk}$

Parametric coefficients:

	Estimate	Std. Error	z value	p value
Intercept	**1.443**	**0.099**	**14.599**	**<0.001**
Habitat NS	0.198	0.134	1.477	0.144
Habitat S	**-0.435**	**0.132**	**-3.308**	**0.001**

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value
s(DoY) : S_1, H_{NS}	**1.000**	**1.000**	**5.743**	**0.019**
s(DoY) : S_1, H_S	1.242	1.444	3.058	0.105
s(DoY) : S_2, H_{AS}	1.000	1.001	3.412	0.069
s(DoY) : S_2, H_S	1.000	1.000	0.163	0.687
s(DoY) : S_3, H_{AS}	**5.382**	**5.897**	**2.653**	**0.017**
s(DoY) : S_3, H_{NS}	**2.966**	**3.581**	**6.993**	**<0.001**
s(DoY) : S_3, H_S	**2.512**	**3.055**	**4.165**	**0.008**

R^2 (adj) = 0.605, Deviance explained = 61.8%, n = 91

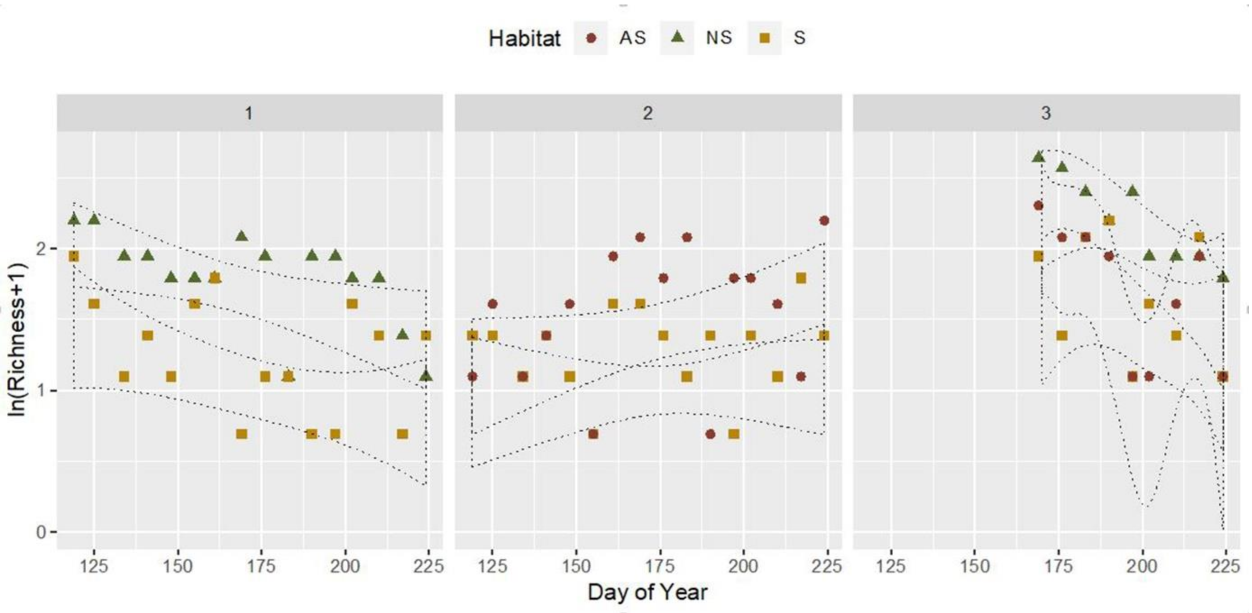

Figure 5. Fit of the quasi-Poisson GAM in Table 3 for the total richness

CAP and SimProf results revealed that the species composition was significantly changed among habitats and stations (Table 4).

Whereas artificial and NS possessed a similar composition, S habitats of all three stations were different than each other (Figure 6).

Table 4. Eigenvalues and its proportion in total and constrained inertia of first two constrained analysis of principle coordinates (CAP) axes, and the results of marginal permutation test for CAP

	CAP1	CAP2	Constrained Total	Total
Eigenvalue	0.328	0.216	0.686	0.759
Proportion in Total Inertia	0.432	0.284	0.903	1.000
Proportion in Constrained Inertia	0.478	0.314	1.000	-
Source of Variation	df	SS	F	p value
Habitat	**2**	**0.263**	**2.664**	**0.041**
Station	**2**	**0.394**	**3.988**	**0.002**
Residual	2	0.099		

n of permutations= 999

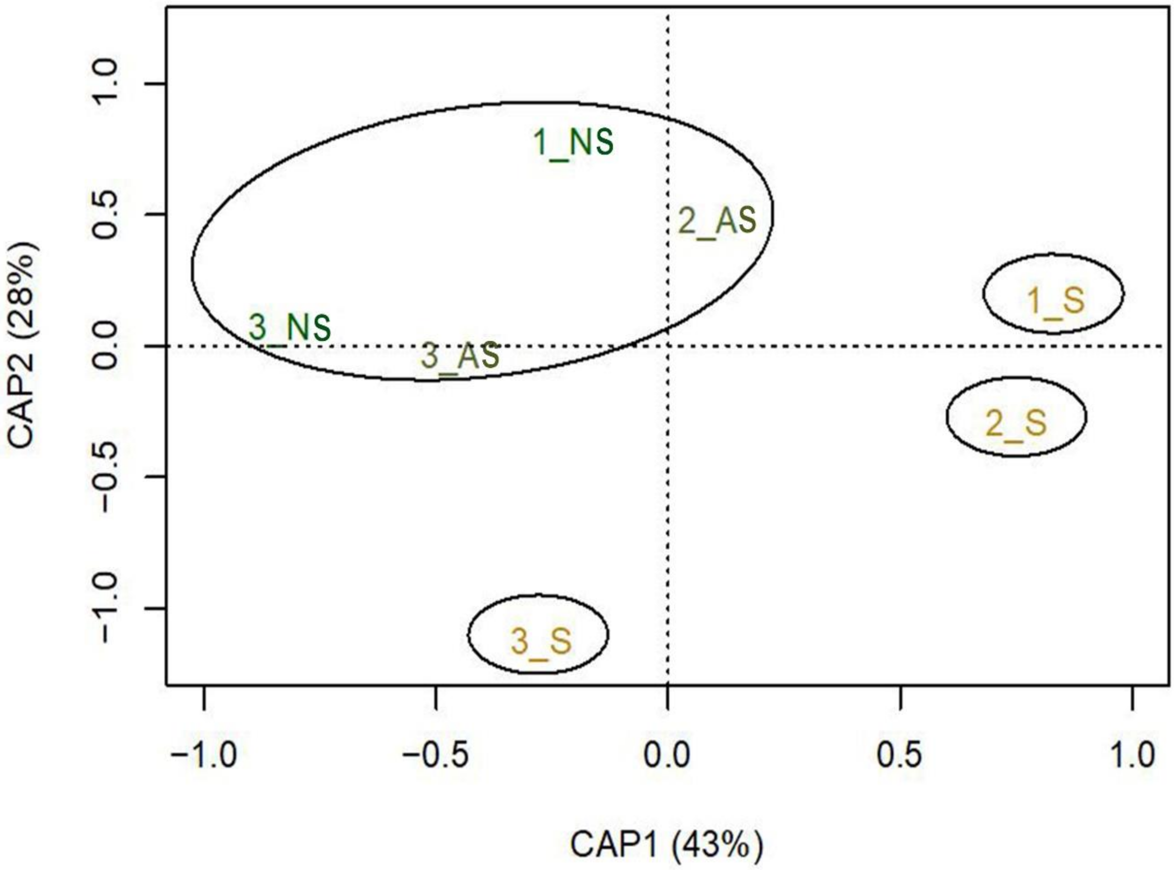

Figure 6. Results of constrained analysis of principle coordinates (CAP) ordination. Ellipses show statistically significant clusters at 95% confidence level based on a similarity profiling (SimProf) analyze

DISCUSSION AND CONCLUSION

In context of our study, a total of 45 species were detected between April and August in the Northeast Mediterranean (Table 5). In the same area also Başusta et al. (2002) reported 33 species. These numbers are not close to each other. Because of our study is in shallow water while the other study is at depths of up to 30 meters.

Table 5. Abundance of fish species (individual per 100m²) detected in context of the study (NS: Natural Seagrass, AS: Artificial Seagrass, S: Sand)

FAMILY / SPECIES	**Station I**		**Station II**		**Station III**		
	NS	**S**	**AS**	**S**	**NS**	**AS**	**S**
Atherinidae							
Atherina boyeri	2609	103	24028	460	9122	653	57
Atherinomorus lacunosus	2	1	4	4	-	-	-
Bothidae							
Arnoglossus laterna	-	-	-	-	-	-	2
Arnoglossus spp.	-	-	-	-	1	-	-
Blenniidae							
Blenniidae spp.	-	-	-	-	1	-	-
Parablennius gattorugine	-	-	-	-	1	-	-
Parablennius sanguinolentus	4	-	28	-	4	-	-
Callionymidae							
Callionymus filamentosus	-	-	-	-	-	1	2
Carangidae							
Seriola dumerili	-	-	4	4	-	-	-
Trachinotus ovatus	-	-	12	4	-	-	-
Dussumieriidae							
Dussumieria elopsoides	-	-	4	-	-	-	-
Engraulidae							
Engraulis encrasicolus	-	-	-	4	-	-	-
Fistulariidae							
Fistularia commersonii	-	-	-	-	-	-	1
Gobidae							
Gobiidae spp.	1	-	4	-	-	-	-
Gobius niger	1064	456	132	528	19	7	71
Haemulidae							
Pomadasys stridens	-	-	-	-	8	-	-
Leiognathidae							
Equulites klunzingeri	-	-	-	-	28	26	3
Monacanthidae							
Stephanolepis diaspros	-	-	-	-	3	-	-
Moronidae							
Dicentrarchus labrax	25	-	-	-	-	-	-
Mugilidae							
Mugilidae spp.	257	326	316	160	22	21	-
Mullidae							
Mullus barbatus	8	-	-	-	1	16	2
Mullus surmuletus	1	-	-	-	3	-	-
Upeneus pori	-	-	-	-	-	2	9
Serranidae							
Mycteroperca rubra	-	-	-	-	31	1	3
Serranidae spp.	-	-	-	-	-	-	1
Siganidae							
Siganus luridus	6	-	24	-	1	4	-
Siganus rivulatus	1284	-	564	12	1518	692	28
Sillaginidae							
Sillago suezensis	-	22	36	64	-	1	1
Soleidae							
Buglossidium luteum	1	2	20	-	1	3	-
Microchirus ocellatus	-	-	4	-	-	-	-
Pegusa lascaris	-	-	-	4	1	-	2
Solea solea	-	3	-	-	-	-	-
Sparidae							
Diplodus sargus	679	10	492	84	33	68	7
Lithognathus mormyrus	-	-	4	-	-	-	-
Pagellus erythrinus	3	1	-	-	-	-	-
Sparidae spp.	2	-	-	-	-	-	1
Sparus aurata	88	13	4	4	5	2	2
Sphyraenidae							
Sphyraena chrysotaenia	-	-	-	-	4	-	-
Sphyraena sphyraena	-	-	-	-	-	1	2
Syngnathidae							
Nerophis ophidion	-	-	-	-	5	-	-
Syngnathus abaster	-	-	-	-	-	1	-
Syngnathus phlegon	-	-	-	-	1	-	-
Terapontidae							
Pelates quadrilineatus	145	-	-	-	9	-	-
Trachinidae							
Echiichthys vipera	8	-	4	12	12	2	6
Trachinus araneus	-	-	-	-	-	-	3

Results of generalized additive models and similarity profiling analysis revealed that there was not statistically significant difference of the species composition, number of species and total abundance between AS and NS, whereas all three parameters were significantly different in S habitats. This situation demonstrated that the AS could simulate the NS. Similarly, Guidetti (2000) reported that seagrass areas are more preferred than sand areas. Factors such as nutrition, predators, the abundance of food come into play as reasons for fish to prefer seagrass as their habitat (Heck et al., 1997).

As larval stage, juvenile fish assemblages are also temporary associations (Miller, 2002), depends on the seasonality of spawning and settlement processes (Ak, 2004; Banbul, 2014). Accordingly, we detected the highest richness in April when the highest rate of ichthyoplankton richness and abundance were reported in the study area (Mavruk et al., 2018). In accordance with ichthyoplankton samplings performed by Mavruk et al. (2018) our richness were contentiously decreased in all stations and habitats over the periods of experiments depending on the decreasing spawning activity in the study area.

The habitats for adult and juvenile fish was ranked by Beck et al. (2001) from most to least efficient as seagrass, swamp, muddy areas, sand habitats in this order. The areas with seagrass were preferred by fish to areas without plants such as sand grounds and loams (Ferrell and Bell, 1991; Mattila et al., 1999; Pihl et al., 2006). Similarly, in our study, NS and AS have higher fish abundance values than sand habitats. The fish abundance and richness were relatively stable in NS and S habitats, as seen in the sampling. Whereas, AS revealed a fluctuating pattern in these two parameters. On the other hand, species compositions of S habitats were more variable. This may be due to the insufficient amount of sampling.

Different species preferred different kinds of habitats (Mattila et al., 1999). *Atherina boyeri*, which are carnivorous, and which represent the 79.31% of the total abundance of fish, were the predominant species in this study. Considering the fact that 31.67% of this species was observed in areas with seagrass, 66.64% in areas with AS, 1.67% in S habitats, it can be said to be a resident species of NS and AS.

High-density and low-density polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyester (PS), polyvinyl terephthalate (PET) make up 90% of the world production as the most commonly used plastics (Andrady and Neal, 2009). Therefore, it is known that most of the substances polluting the coastal and marine environments are composed of these materials (Andrady, 2011; Engler, 2012). Increasing sea pollution along with microplastics and marine debris which are discussed in many researches pose a threat to the ecosystem (Gündoğdu and Çevik, 2017; Gündoğdu et al., 2017). Nowadays, it is widely accepted that plastic pollution affects numerous marine species ranging from zooplanktons to whales (Andrady, 2011; Cole et al., 2013; Cole et al., 2011). Plastic wastes harm marine species as their gills can be clogged by plastics and they can get tangled in them and/or swallow them (Gregory, 2009; Li et al., 2016). Therefore, the use of plastic (polypropylene) as a material in AS is controversial. The general consensus is that preferring natural fibers when choosing materials is more of an eco-friendly approach and is predicted to prevent possible controversies.

Seagrass habitats have important roles in the coastal ecosystems. Therefore, they are extremely vulnerable to various stressors and declining all over the world. In this study, AS was tested as an alternative to the declining seagrass. Our study revealed that the abundance of fish is higher in the NS and AS in comparison with S habitats. This study, conducted in the Northeast Mediterranean, shows that AS habitats effect the distribution of juvenile fish.

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