FOOD AND FEEDING RELATIONSHIPS BETWEEN SOME DEMERSAL NATIVE AND NON-NATIVE FISHES IN THE NORTHERN AEGEAN SEA, TURKEY

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ABSTRACT

The present study represents the feeding habits of the native (*Mullus barbatus barbatus* Linnaeus, 1758, *Mullus surmuletus* Linnaeus, 1758 and *Pagellus erythrinus* (Linnaeus, 1758)) and non-native (*Nemipterus randalli* Russell, 1986*, Upeneus moluccensis* (Bleeker, 1855) and *Upeneus pori* Ben-Tuvia & Golani, 1989) fish species collected off the Turkish Aegean coast throughout the seasonally between January to December 2017. It was determined that all species consume benthic organisms by examining a total of 672 stomach contents. The index of relative importance (IRI) revealed the most important food item according to species is: Crustacea for *N. randalli* (82.40%), *P. erythrinus* (44.15%), *M. surmuletus* (75.35%), *U. moluccensis* (98.48%) and *U. pori* (56.79%*;* Polychaeta for *M. barbatus barbatus* (56.84%). A total of 40 prey species were identified from stomach contents of native and non-native fishes via the molecular method.

KEYWORDS:
Native fish, non-native fish, Aegean Sea, Feeding habits

INTRODUCTION

Biological invasion in recent century has caused not only ecological but also economic damages [1, 2]. With the Suez Canal was completed in 1869, the geographical barrier is eliminated between the Mediterranean and the Red Sea, therefore, the Mediterranean Sea, considered as a hotspot of species richness and endemism [3], is affected dramatically by Indo-Pacific species [4]. Alien biota in the Aegean Sea includes 775 alien species of which 105 are exotic fish species consisting of more than 65 species of Indo-Pacific origin [4]. [5] stated that 512 fish species belonging to 150 families along the Turkish coasts, where 75 Lessepsian species are reported [6]. Biodiversity conservation studies in aquatic ecosystems carried out by monitoring, planning, management and inspection based on scientific infrastructure are of vital importance. This is possible by examining the biological properties of the species entering the ecosystem and their interaction with the indigenous species.

For the last two decades, the Aegean Sea has become the transition point for the organisms that expand their distribution northwardly within the Mediterranean Sea, called the Lessepsian species. Therefore, significant changes have been occurred in the ecosystem structures of the Aegean Sea due to biotic factors that occur between the Lessepsian species entering the Mediterranean and the indigenous fish species (prey-predator relationship, food competition, etc.).

Most of the studies conducted on invasive species in the Turkish Aegean coasts has been limited to the reports on the “first record” or checklist. These reports include valuable findings concerning the” detection of the non-indigenous species, however, factors such as resource partitioning, competition, ecological niche are the main processes responsible for the structuring of populations and communities [7]. If non-indigenous species prevail in food competition which can be seen among the indigenous / non-indigenous species in the similar position within the food chain, there may be changes in the abundance of the indigenous species, leading to possible negativity in the functioning of the ecosystem [8]. Therefore, the present study aims to determine the feeding relationships between some invasive species and indigenous fish species in the Aegean Sea.

MATERIALS AND METHODS

Samples were seasonally collected via trammel nets (20 mm mesh size) from the Kuşadası Bay (37.4340° N, 27.1223° E), at the depths of 40 m from January to December 2017. For all specimens, total length (TL, cm) to the nearest 0.1 cm and wet weight (W, g) to the nearest 0.01 were measured and recorded in the laboratory.

Feeding habits were determined based on the morphological identification of preserved stomach contents in 4% formalin solution. Prey in the stomach contents was identified to the lowest possible taxonomic level. Feeding state of each species was characterized using the vacuity index: %*VI*= number of empty stomachs/total number of stomachs x 100. The main food items were identified using the Index of Relative Importance (*IRI*) of [8]: $IRI = \%F \times (\%N + \%W)$ where F is the frequency of occurrence, N is the abundance of a food component, and W is the weight of a food item in a species diet [9]. The index was expressed as: $\%IRI = (IRI / \Sigma IRI) \times 100$. Prey species were sorted in decreasing order according to IRI and then cumulative %IRI was calculated. Food items were grouped into categories of preference using the method proposed by [10]. The categories were defined as follows: main important prey (MIP): $IRI \geq 30 \times (0.15 \times \Sigma\%F)$, secondary prey (SP): $30 \times (0.15 \times \Sigma\%F) > IRI > 10 \times (0.05 \times \Sigma\%F)$ occasional prey (OP): $IRI \leq 10 \times (0.05 \times \Sigma\%F)$.

Diet overlap between fish species was determined by Schoener's index (S) [11]:

$$S=100 \text{ x } [1 - 0{,}5 \ (\sum_{i=1}^{n} |P_{xi}-P_{yi}|)]$$

Where P_{xi} and P_{yi} are the IRI values of food category 'i' in the diet of species x and y; n = the number of food categories. The values that S can range from 0 to 1 according to feed utilization from completely different or the same food resources. Diet overlap percentage higher than %60 could be considered biologically significant [12].

The food diversity was evaluated using Shannon index based on the abundance of each food item:

$$H' = -\sum_{i=1}^{S} P_i \ln P_i$$

Where *P*i is the proportion (abundance) of the i*th* food (prey) category in the stomach of an individual.

In the molecular analysis of stomach contents, genomic DNA was extracted from subsample of stomach contents using DNeasy Blood & Tissue Kits (Qiagen, Germany) based on protocol of the manufacturer. mtDNA-COI gene was amplified using the following primers: LCO 1490, HCO 2198, LepF1, LepR1, FishF1, FishR2. PCR reactions were carried out in a total volume of 50 µl, containing: 0.5 U of Taq polymerase, 5 µl of 10x Taq Buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.8, 0.8% Nonidet P-40), 3 mM of $MgCl_2$, 0.2 mM of dNTPs (2.5 mM), 10 pmol of each primer and 100–200 ng of template. PCR amplifications were performed in BioRad T100™ thermal cycler with an initial denaturation of 5 min at 94°C followed by 33 cycles of 40 s at 94°C, 40 s at 51°C, 1 min at 72°C and a final extension for 10 min at 72°C.

Sequences data were subjected to blastn searches in Genbank (http://www.ncbi.nlm.nih.gov/genbank). Related species were retrieved and aligned with the obtained sequences using the Clustal-W algorithm [13] in MEGA v7.0 [14]. A Maximum Likelihood analysis in MEGA v7.0 was used to reconstruct the phylogenetic relationships among the sequences [15].

RESULTS

During the study, 238 *N. randalli*, 263 *P. erythrinus*, 79 *M. barbatus barbatus*, 36 *M. surmuletus*, 40 *U. moluccensis* and 16 *U. pori* individuals were collected. Minimum, maximum and mean values of length and weight belonging to each species were given in Table 1.

672 specimens were used for the stomach content analysis. The data of all stomach contents are presented as general feeding compositions because *M. surmuletus* in the winter season and *U. pori* in autumn and winter season could not be collected.

Diet composition. The results of the morphological identification of stomach contents are given in Table 2. As morphologically species-level identification of prey could not be performed in general due to the high digestion of the stomach content. Prey groups consist of Polychaeta, Crustacea, Mollusca, Echinodermata, Foraminifera and Teleostei, in addition, Crustacea is the dominant group for all species in terms of frequency of occurrence (Table 2). Importance of prey items classified by IRI categories of each species are given in Table 3.

TABLE 1
Maximum, minimum and mean length and weight values of collected species in the Kuşadası Bay

Species	N	Lmean ± S.D (cm) (Lmin-Lmax)	Wmean ± S.D (g) (Wmin-Wmax)
N. randalli	238	14.55±0.99 (12.3-16.3)	42.69±8.99 (22.50-63.44)
P. erythrinus	263	17.65±1.09 (15.2-19.7)	67.87±14.80 (30.98-122.70)
M. barbatus barbatus	79	14.77±3.78 (8.8-22.8)	41.50±31.92 (6.23-131.76)
M. surmuletus	36	13.75±3.22 (8.5-23.1)	29.94±26.25 (5.28-144.00)
U. moluccensis	40	14.59±2.07 (10.0-18.9)	33.68±14.87 (10.30-74.24)
U. pori	16	15.16±1.28 (13.1-17.4)	35.82±9.68 (23.19-55.23)

TABLE 2
Diet composition of native and non-native fishes in the Kuşadası Bay

Prey Items	*N. randalli*			*P. erythrinus*			*M. barbatus barbatus*		
	% N	% F	% IRI	% N	% F	% IRI	% N	% F	% IRI
POLYCHAETA	**1.52**	**2.04**	**0.04**	**15.14**	**25.79**	**12.55**	**47.10**	**74.42**	**56.84**
CRUSTACEA	**75.95**	**155.78**	**82.40**	**31.72**	**86.79**	**44.15**	**32.90**	**79.07**	**36.85**
Amphipoda	0	0	0	0	0	0	7.10	13.95	2.27
Mysida	2.78	6.80	0.27	2.42	8.18	0.68	0	0	0
Alpheus sp.	24.56	55.78	36.83	6.44	15.09	12.25	12.90	32.56	26.70
Bathynectes sp.	4.30	11.56	1.28	2.58	10.06	1.69	0	0	0
Goneplax rhomboides	12.66	27.89	11.35	9.82	19.50	17.39	0	0	0
Stomatopoda	2.78	6.80	0.27	0.81	0.93	0.09	0	0	0
Brachyura	0	0	0	6.44	19.50	7.93	6.45	18.60	3.88
Caridea	31.65	46.94	32.67	3.22	12.58	4.12	6.45	13.95	3.99
MOLLUSCA	**3.80**	**10.20**	**0.32**	**44.28**	**40.88**	**29.16**	**12.90**	**23.26**	**4.71**
Bivalvia	1.77	4.76	0.14	25.93	28.93	22.72	12.90	23.26	4.71
Gastropoda	2.03	5.44	0.18	18.36	11.95	6.44	0	0	0
ECHINODERMATA	**0.76**	**2.04**	**0.02**	**3.22**	**6.29**	**0.71**	**5.16**	**11.63**	**1.17**
FORAMINIFERA	**0**	**0**	**0**	**0.81**	**3.14**	**0.07**	**0**	**0**	**0**
TELEOSTEI	**15.19**	**39.46**	**16.95**	**4.83**	**18.87**	**13.35**	**1.94**	**4.65**	**0.43**

Prey Items	*M. surmuletus*			*U. moluccensis*			*U. pori*		
	% N	% F	% IRI	% N	% F	% IRI	% N	% F	% IRI
POLYCHAETA	**21.33**	**50.00**	**17.44**	**8.47**	**11.11**	**1.29**	**36.67**	**16.18**	**38.79**
CRUSTACEA	**64.00**	**145.00**	**75.35**	**89.83**	**166.67**	**98.49**	**50.00**	**122.22**	**56.79**
Amphipoda	18.67	50.00	21.95	20.34	37.04	13.95	23.33	55.56	23.34
Mysida	38.67	70.00	46.28	27.12	51.85	21.04	0	0	0
Alpheus sp.	0	0	0	0	0	0	6.67	22.22	3.71
Bathynectes sp.	0	0	0	0	0	0	0	0	0
Goneplax rhomboides	0	0	0	0	0	0	0	0	0
Stomatopoda	0	0	0	8.47	14.81	2.57	0	0	0
Brachyura	6.67	25.00	7.12	33.90	62.96	60.93	20.00	44.44	29.75
Caridea	0	0	0	0	0	0	0	0	0
MOLLUSCA	**4.00**	**15.00**	**0.69**	**0**	**0**	**0**	**13.33**	**33.33**	**4.42**
Bivalvia	4.00	15.00	0.69	0	0	0	13.33	33.33	4.42
Gastropoda	0	0	0	0	0	0	0	0	0
ECHINODERMATA	**4.00**	**15.00**	**1.36**	**0**	**0**	**0**	**0**	**0**	**0**
FORAMINIFERA	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
TELEOSTEI	**6.67**	**15.00**	**5.16**	**1.69**	**3.70**	**0.22**	**0**	**0**	**0**

The molecular identification results of stomach contents are shown in Table 4. Considering to results, non-native *N. randalli* was found as the widest prey consumer with 24 prey species while native *P. erythrinus* follows with 21 prey species.

Food diversity. The diet plasticity of native and non-native species was compared using Shannon's index of diversity (Figure 1). Among non-native species, *N. randalli* consumes a relatively wide range of prey items while *U. pori* has the lowest index of diversity value (<1.5). Contrary to non-native species, all of native species have generally higher diversity index values (>1.5).

Diet overlap. Schoener Index (S) results were represented in Table 5. The highest overlap was calculated between native *M. barbatus* and non-native *U. pori* (S: 76%) which feed mainly on Polychaeta and Crustacea. The most considerable diet overlapped was found between the native and non-native goatfishes (S: 70-76%). The diet overlap of the native *P. erythrinus* and non-native *N. randalli,* which two coexisting fishes, was determined as ca. 61%.

DISCUSSION AND CONCLUSIONS

Diet Composition. The morphological and molecular identification showed that stomach contents of native and non-native species derived from mostly benthic and epibentic species, such as mysids, decapods, polychaets and demersal fishes. IRI results demonstrated that the all 6 species preferred mainly crustaceans, whereas echinoderms and foraminifers were as less important preys. Previous studies on diet of *N. randalli* in the Mediterranean stated that it feeds mainly on Crustacea and Pisces [16, 17, 18]. Although *N. randalli* demonstrates relatively

TABLE 3
Preferred prey items of native and non-native fishes, based on principal indices

Species	Main Important Prey	Secondary Prey	Occasional Prey
N. randalli	IRI ≥ 942.86 *Alpheus* sp. Caridea Teleostei	IRI >104.76 *G. rhomboides*	IRI ≤ 104.76 *Bathynectes* sp. Mysida Stomatopoda Gastropoda Bivalvia Polychaeta Echinodermata
P. erythrinus	IRI ≥ 817.92 Bivalvia	IRI > 90.88 *G. rhomboides* Teleostei Polychaeta *Alpheus* sp. Brachyura Caridea Gastropoda	IRI ≤ 90.88 *Bathynectes* sp. Echinodermata Mysida Stomatopoda Foraminifera
M. barbatus barbatus	IRI ≥ 868.60 Polychaeta *Alpheus* sp.	IRI > 96.51 Bivalvia Caridea Brachyura Amphipoda	IRI ≤ 96.51 Echinodermata Teleostei
M. surmuletus	IRI ≥ 1080.00 Mysida Amphipoda Polychaeta	IRI > 120.00 Brachyura Teleostei Echinodermata	IRI ≤ 120.00 Bivalvia
U. moluccensis	IRI ≥ 816.66 Brachyura Mysida Amphipoda	IRI > 90.74 Stomatopoda Polychaeta	IRI ≤ 90.74 Teleostei
U. pori	IRI ≥ 1050.00 Polychaeta Brachyura Amphipoda	IRI > 116.67 Bivalvia *Alpheus* sp.	-

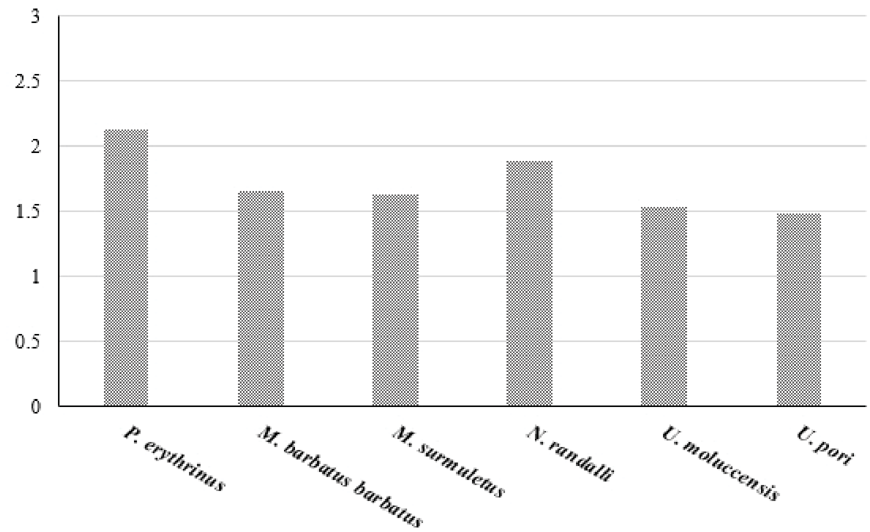

FIGURE 1
Food diversity comparison in the stomach contents of native and non-native fishes

high plasticity in feeding preference, it may have a negative effect on the Crustacea fauna (especially Natantia and Brachyura) in the Mediterranean Sea as it consumes Crustaceans highly rate (in terms of both percentage of occurrence and biomass percentage of stomach content). An examination of the molecular identification results indicated that Crustacea were represented with 12 species while Teleostei with 6 species in its diet. Pisces that encountered in the stomach contents are both native and non-native species. A prominent finding in the present study is, *N. randalli* consumes *P. erythrinus*, that competed against. This finding has previously been suggested by various researchers as a possibility [17, 18]. The diet of *P. erythrinus* in the present study show similarity of its conspecifics in the Mediterranean [18 and references therein). Nearly all of the studies about diet of *P. erythrinus* suggested that Polychaeta and Crustacea are the main important preys of common pandora. According to molecular identification, Crustacea and Mollusca were the groups that represented the most species in the diet of the common pandora. The results in terms of the main important food item of the native and non-native goatfishes in the present study and previous studies are found as compatible. *M. barbatus barbatus* feed on mainly Polychaeta and preferred Crustacea (especially *Alpheus* sp.) while *M. surmuletus* prey mainly on Mysida, Amphipoda and Polychaeta. Brachyura, Mysida, and Amphipoda are the most important food items respectively in the diet of *U. moluccensis*. *U. pori* prefers to feed on Crustacea (Caridea and Amphipoda) and Polychaeta. Consequently, Crustaceans and Polychaetes were dominant the diet of all native and non-native fishes whereas Mollusca and Teleostei were less common. Echinodermata and Foraminifera were found as occasional prey. Thus, it seems that Crustacean fauna is the most affected group by native and non-native fishes.

TABLE 4
The molecular identification results of stomach contents

	N. randalli	*P. erythrinus*	*M. barbatus barbatus*	*M. surmuletus*	*U. moluccensis*	*U. pori*
FORAMINIFERA						
Elphidium crispum		+				
POLYCHAETA						
Nereis pelagica	+	+	+			+
Micronephthys stammeri		+	+	+		
Protodorvillea kefersteini			+		+	+
Nephtys caeca		+	+	+		
CRUSTACEA						
Amphipoda						
Phtisica marina	+		+	+	+	+
Talitrus saltator		+	+	+	+	+
Mysida						
Siriella armata	+			+	+	
Paramysis helleri	+	+		+	+	
Tanaidacea						
Tanais dulongii			+		+	+
Decapoda						
Alpheus glaber	+	+	+			+
Bathynectes maravigna	+	+			+	
Goneplax rhomboides	+	+		+	+	+
Liocarchinus depurator	+		+	+	+	+
Melicertus kerathurus	+					
Parapenaeus longirostris	+					
Plesionika narval	+	+	+		+	
Processa nouveli				+		
Philocheras trispinosus	+					
Solenocera membranacea			+			+
Stomatopoda						
Erugosquilla massavensis	+				+	
Squilla mantis		+			+	
MOLLUSCA						
Bivalvia						
Donax trunculus				+	+	
Nucula sulcata	+	+	+			+
Tellina tenuis	+	+	+			
Gastropoda						
Antalis inaequicostata	+	+				
Calyptraea chinensis	+			+	+	
Cerithium vulgatum		+				
Hexaplex trunculus	+	+				
Patella caerulea		+				
ECHINODERMATA						
Echinus melo		+				
Echinaster sepositus	+			+		
Ophiothrix fragilis			+			
Ophiura ophiura		+				+
TELEOSTEI						
Bregmaceros nectabanus	+	+			+	
Callionymus lyra				+		
Lesueurigobius friesii	+	+		+		
Lesueurigobius suerii	+		+			
Pagellus erythrinus	+					
Upeneus moluccensis	+					

TABLE 5
Percentage diet overlaps between native and non-native fishes

	N. randalli	*P. erythrinus*	*M. barbatus barbatus*	*M. surmuletus*	*U. moluccensis*	*U. pori*
N. randalli	-	61%	41%	33%	52%	51%
P. erythrinus	61%	-	31%	43%	29%	28%
M. barbatus barbatus	41%	31%	-	74%	70%	76%
M. surmuletus	33%	43%	74%	-	71%	70%
U. moluccensis	52%	29%	70%	71%	-	71%
U. pori	51%	28%	76%	70%	71%	-

Resource utilization is a key factor for colonization success of invasive species. According to Shannon Index results, the diversity of food items belonging to the stomachs of the native fishes (1.63-2.13) was found higher than of that of the invasive fishes (1.48-1.53) apart from *N. randalli* (1.89). Therefore, invasive species are supposed to feed on a wide variety of food items. However, contrary to expectations, native fishes seems to display generalist predator behavior to reduce competitive effects of non-native fishes.

Diet overlap. An introduction and settlement of invasive species may alter resource partitioning, competition, ecological niche in the receiving environment and thus invasive newcomers could change the structure of populations and communities in the new environment.

The interspecific and intraspecific competition was determined in the present study, however, a competition was observed mostly between congenerics. [19] pretended to a substantial difference in resource partitioning in case of diet overlap is higher than 40%. Diet overlap values between species matches mostly were found more than 40% (Table 4). In the present study, we may suggest based on the results of IRI and Shannon Index that resource partitioning exists between congenerics. Native fishes feed on a wide variety of food items while non-native species are specialist predator except *N. randalli* in that their diet consists narrow variety of food items. Considering that relationship between resource utilization and colonization success, non-native goatfishes have been expanded their distribution range in the Aegean Sea (*U. moluccensis* was even reported in the Sea of Marmara [20]), however, they are not able to increase their abundance in invaded areas. Aforementioned non-native goatfishes were not able to observed as much as native fishes during this study too.

In conclusion, we need similar and further studies for monitoring significant and rapid changes in the marine communities structure caused by non-native species, such as in the availability of food resources and predator-prey interactions.

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