



PRELIMINARY ASSESSMENT OF INVASIVE LIONFISH *Pterois miles* USING UNDERWATER VISUAL CENSUS METHOD IN THE NORTHEASTERN MEDITERRANEAN

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ABSTRACT

Underwater visual census (UVC) is a commonly used approach for assessing fish density and biomass. Iskenderun Bay, comprising the Samandađ coast in Turkey, is the most important introduction pathway of alien species in the northeastern Mediterranean. In this study, the density, abundance, distribution and interaction of invasive lionfish or devil firefish *Pterois miles* were assessed using the UVC method on the Samandađ coast. The total transect area monitored on the Samandađ coast was 4.500 m². Species richness, Shannon's diversity index and rate of biomass were 25, 1.81 and 73.5 m², respectively. Reduced diversity was observed with increasing depths. The sandy seafloor was a limiting factor for lionfish distribution. There was a significant positive correlation between lionfish total density and both the Shannon diversity index ($r = 0.99$, $P < 0.001$) and Shannon evenness index ($r = 0.99$, $P < 0.05$). A high number of significant positive correlations ($P < 0.05$) were detected between the occurrence of lionfish and the occurrence of both the native species and other alien species. The first stock assessment study of lionfish by the UVC method in Turkish marine waters revealed that native species are under very high pressure from lionfish species, especially Chromis, Sparids and Wrasses, indicating negative effects of lionfish on regional native biodiversity.

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INTRODUCTION

Biodiversity in the Mediterranean Sea has been considerably altered since the opening of the Suez Canal in 1869 which favored the settlement and spread of lessepsian/erythrean fish and invertebrates in the Mediterranean basin, and which appears to be accelerating (Dalyan et al., 2014; Dođdu et al., 2016; Turan et al., 2016; Gürlek et al., 2016a; Castellanos-Galindo et al., 2020; D'Amen and Azzurro, 2020; Fitori et al., 2021). Tortonese (1964) estimated there were about 30 Indo-Pacific immigrant fish species in the Mediterranean, from a total of about 550. In the Atlas of Exotic Species in the Mediterranean, Golani et al. (2002) reported 59 exotic fish species from an estimated total of 650 in the Mediterranean, which is almost 10% of the population. The checklist of Zenetos et al. (2010) comprises 106 alien fish species of Indo-Pacific origin in the Mediterranean. Turan et al. (2018) produced a checklist of 101 non-indigenous marine fish, including bony, cartilaginous and jawless species distributed along Turkish marine waters, of which 89 were bony, 11 cartilaginous and 1 jawless. In terms of the occurrence of non-indigenous fish species in Turkish marine waters, the Mediterranean coast has the highest diversity (92 species), followed by the Aegean Sea (50 species), the Marmara Sea (11 species) and the Black Sea (2 species).

Lionfish *Pterois miles* (Bennett 1828) have high invasive character and threat to the native biodiversity in the invaded areas of the eastern Atlantic, the Caribbean, the Gulf of Mexico and recently the Mediterranean Sea (Turan et al., 2014; Turan and Öztürk, 2015). Through their predation on native species, lionfish were predicted to have long-term effects on the structure and function of Mediterranean ecosystems (Turan, 2020; Turan et al., 2020). The density, biomass and distribution of lionfish must be fully understood to design and implement the most effective way of control. Thus, it is important to assess the current status of lionfish as well as other Invasive Alien Species (IAS) to contribute to management plans by identifying site-specific measures for prevention, control and management of IAS. For this reason, monitoring and providing data on IAS in relevant habitats is the first step in establishing such measures.

Underwater visual census (UVC) is an approach for assessing the density and biomass of fishes (Edgar et al., 2004; Kulbicki et al., 2012). UVCs are primarily conducted using scuba and encompass a range of methods, such as UVC strip transects (Murphy and Jenkins, 2010). At first, UVC has been developed for use in the studies of tropical coral reef fish (Brock, 1954). Nowadays, the application of UVC is widespread in temperate waters, such as the Mediterranean Sea. It has been successfully applied in marine protected areas (MPAs), as well as in other important marine habitats, such as rocky reefs, submarine caves and seagrass meadows (Tunesi et al., 2006; Bussotti and Guidetti, 2009; Soldo and Glavičić, 2020), just to

mention a few. Bias in detection across time and space could prove especially problematic for species for which accurate estimates of density and biomass are needed to inform environmental managers (Green et al., 2013).

Iskenderun Bay, comprising the Samandağ coast, is the most important introduction pathway of invasive alien species in Turkish marine waters (Nunes et al., 2014). Lionfish species *Pterois miles* is highly abundant in this area but there is no data on the abundance, density and interaction of these species in this region. Besides, in Turkish marine waters, it is important to know to control and/or decrease the abundance of these species in this region as well as other coastal waters of Turkey.

In this study, we aimed to assess lionfish *Pterois miles* density, abundance, distribution and interaction with native species using the UVC method in the Samandağ coast, northeastern Mediterranean Sea.

MATERIALS AND METHODS

Study regions

The study region, the Samandağ coast (36°00'08.0"N, 35°58'24.7"E-36°00'04.6"N, 35°58'18.7"E), *Iskenderun Bay*, the northeast Mediterranean Sea, was chosen for the monitoring based on the biodiversity importance of marine area being a spawning and feeding ground of elasmobranch species (sharks, rays and skates), hatching beach of sea turtles (*Chelonia mydas* and *Caretta caretta*), spawning and feeding ground of grouper species *Epinephelus spp.*, and breeding area of the Mediterranean monk seal *Monachus monachus*.

Transect surveys

UVC strip transect (Labrosse et al., 2002; Edgar et al., 2004; Whitfield et al., 2007; Kulbicki et al., 2012; Agudo and Salas, 2014) was used to quantify the density, abundance and distribution of lionfish in the studied areas. Transect data can reveal species zonation patterns along the line by showing where particular species occur on the line and also how many are present at any point along the line. The method consists of on-site visual counts of organisms by scuba diving along a rectangular transect (measuring tape laid on the sea bottom); in this study, the length and width of the transect were 50 m and 5 m, respectively.

A standardized underwater visual census protocol for surveying lionfish in the Samandağ coast was set according to Whitfield et al. (2007) and Murphy et al. (2010). Three depth ranges from deep to shallow - deep (21-30 m), middle (11-20 m) and shallow (5-10 m) - were monitored by two divers. For each depth category, three 5 m x 50 m replicate transects were screened by each diver, covering an area of 500 m² for each transect by two divers. Fish were recorded by genus and species, where possible, in numbers. The size of lionfish was recorded in the following

classes (to the nearest cm): 10, 11-20, 21-30, 31-40 and >40 using an underwater pointer. Lionfish smaller than 3 cm are omitted to standardize density comparisons. The observer slightly adjusted his swimming rate (15-20 min/transect), and transects with high fish densities were sampled more slowly than those with low densities. Fish counts are conducted during high neap tides, as the lower movement of the water means it is less likely for the fish to hide.

Data analysis

Density data was converted to wet weight as g/m^2 using standard L-W conversions, with published a and b values. Two main components contribute to biodiversity—species richness (S) and species evenness (E). Species richness describes the number of different species present in an area (more species = greater richness). Species evenness describes the relative abundance of different species in an area (similar abundance = more evenness).

The Shannon diversity index (H) was used to characterize species diversity in a community (Shannon and Weaver, 1949).

$$H = \sum[(p_i) \times \ln(p_i)]$$

Shannon's index accounts for both the abundance and evenness of the species present. The proportion of species relative to the total number of species (p_i) is calculated and then multiplied by the natural logarithm of this proportion ($\ln p_i$).

Species evenness refers to how close in numbers the community is. Shannon's evenness index (E_H) can be calculated by dividing H by H_{max} . Equitability assumes a value between 0 and 1 with 1 being complete evenness.

$$E = \frac{H}{H_{max}}$$

To estimate lionfish body mass, we converted individual lionfish lengths (TL in cm) to weights, using the allometric relationship:

$$W_i = aL^b$$

where W_i = weight, L = fish length, a and b are constants. The constants a and b , which were reported for lionfish species *P. miles* in *İskenderun Bay* by Dağhan and Demirhan (2020), was used in this conversion.

Biomass, which is fresh weight per surface area unit (gr/m^2), was calculated using the individual mean weights and abundances;

$$B = \frac{\sum_{i=1}^p w_i}{a}$$

where w = weight of fish (individuals) of a species and a = census area. Each data record consists of an estimated weight for each fish.

For a lionfish species, the estimate of the mean density (D) on a transect is expressed as:

$$D = \frac{\sum_{i=1}^n n_i}{a}$$

where n is the number of lionfish seen and a = census area (Whitfield et al., 2007). All statistical analyses were performed in R-Studio.

Statistical analysis

All statistical analyses were performed in R-Studio and graphics were produced using the ggplot2 package. QGIS was used for statistical geographic information (GIS) presentations. A two-factor permutation analysis of variance (PERMANOVA), based on Euclidean distance, and Analysis of Variance (ANOVA) were used to test the null hypothesis of no differences in lionfish density, biomass and other biological parameters.

RESULTS AND DISCUSSION

The total transect area monitored on the Samandağ coast was 4,500 m^2 . The species richness was 25, and Shannon diversity and evenness indices were 1.81 and 0.67, respectively. Mean biomass and mean density comprising all depths were 7.34 g/m^2 and 0.024 n/m^2 , respectively. The observed Shannon diversity index of IAS species is higher than for the Mediterranean species. Albins (2015) and Benkwitt (2015) studied lionfish *Pterois volitans* distribution in the Bahamas and reported Shannon's index as 0.826 kg/ha^{-1} and 0.322 kg/ha^{-1} , respectively, which was lower than in the present study.

Alien species, *Parupeneus forsskali*, *Diadema setosum* and *Sargocentron rubrum*, were observed as the first three species with the highest frequency species (Fig. 1). Significant differences were found in the composition of each species with depth distribution. The species are mainly located at a depth between 8 m and 15 m (Fig. 2). *P. forsskali*, *Pomadasys stridens* and *Cassiopeia nomadica* were the most abundant species at 5-10 m, 10.1-20 m and 20.1-30 m, respectively. Interestingly, all these species were IAS. At the depth of >20 m, the seafloor was sandy, which limited the species richness even for lionfish. Only the upside-down jellyfish was recorded at this depth.

Collectively for the whole depth zone (5-30 m), the mean biomass was 7.34 g/m^2 . However, there is an apparent difference among the depths of 8 m, 15 m and 25 m where biomass was found to be 8.96 g/m^2 , 13.08 g/m^2 and 0.0 g/m^2 , respectively. The increasing biomass of lionfish was increasing at 8 m and 15 m in parallel (Fig. 3). The sizes of 21-30 cm and above 41 cm showed the highest biomass at the depths of 8 m and 15 m, respectively (Fig. 3). There were no lionfish recorded at the depth of 25 m with a sandy bottom structure.

The mean density including all depths was 0.073 n/m^2 , of which 0.038 n/m^2 and 0.035 n/m^2 , and 0.0 n/m^2 for the depths 0-8 m and 8.1-15 m, and 15.1-25 m, respectively. The highest and lowest density of lionfish was observed

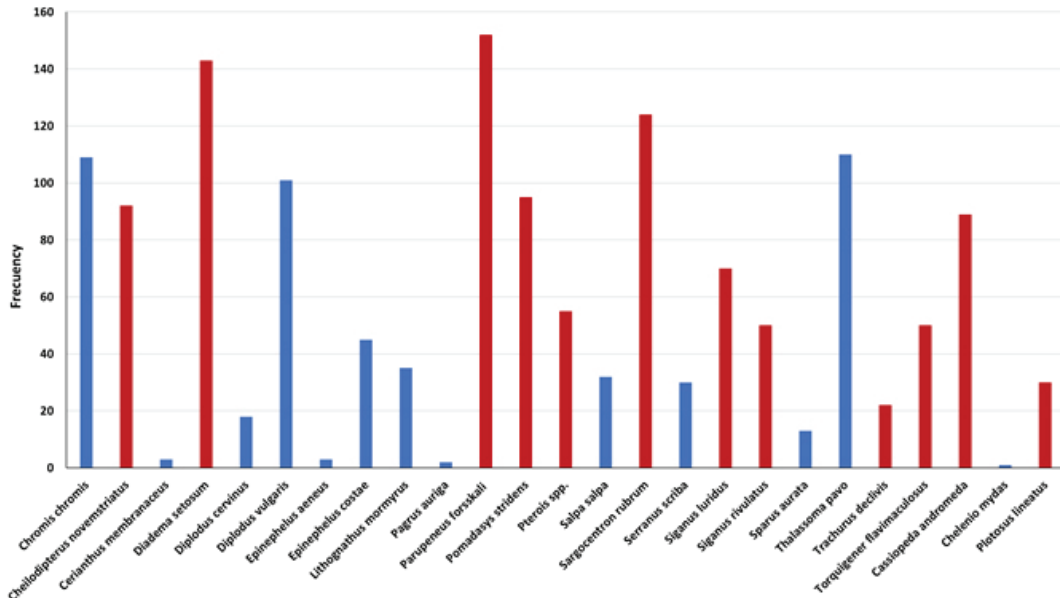


Fig. 1. Frequency of all monitored species at all study regions (Red colour: IAS, Blue colour: Mediterranean fish species)

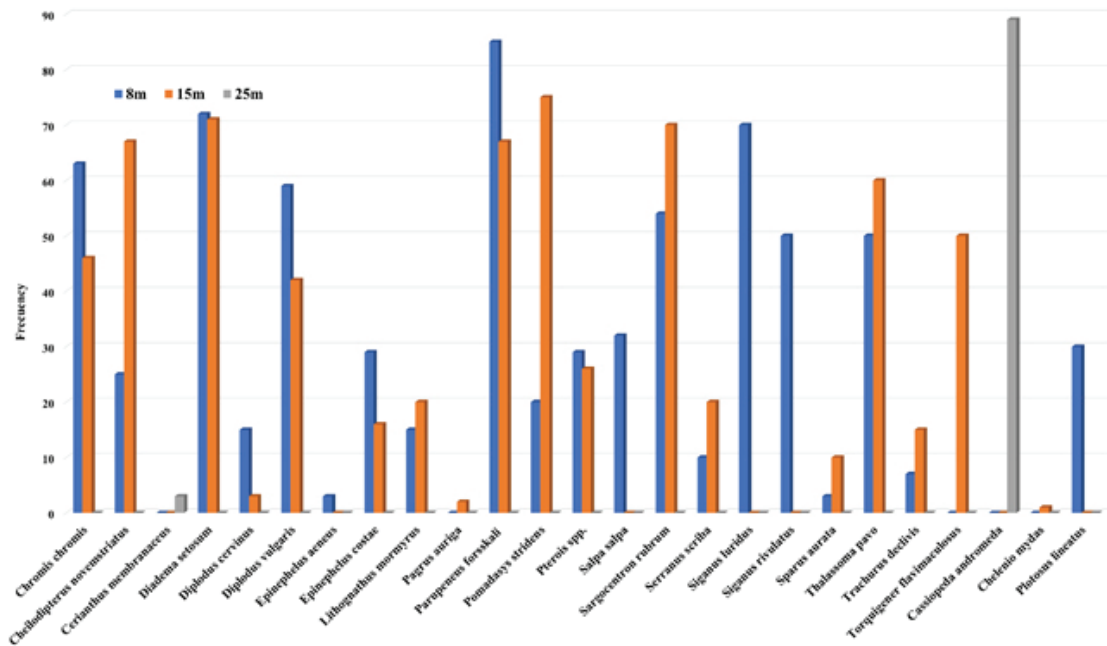


Fig. 2. The composition of monitored species with depth distribution in the study region

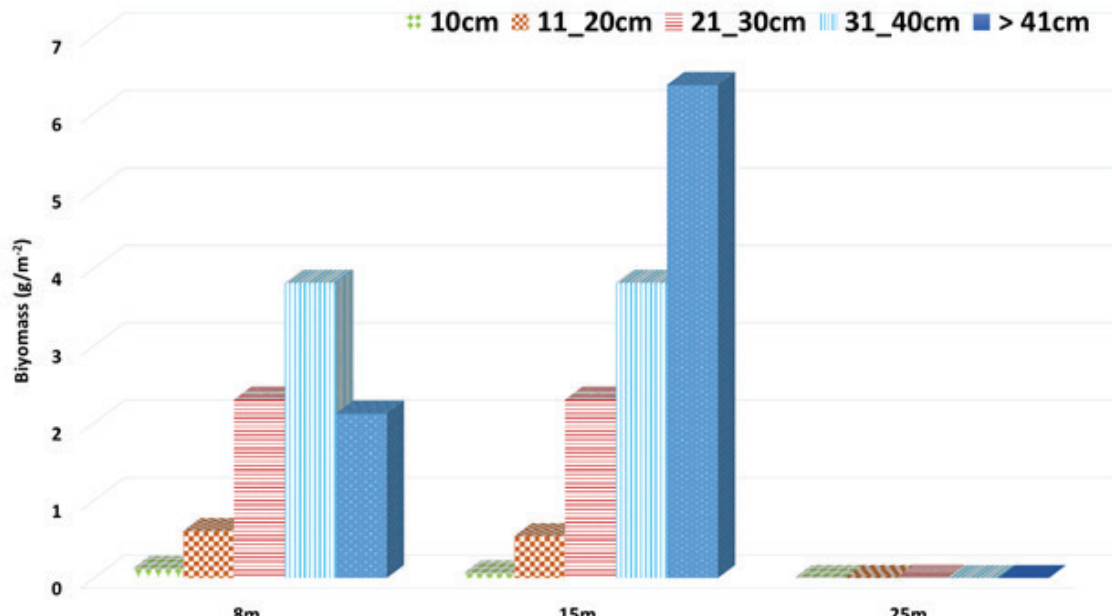


Fig. 3. Monitored biomass of lionfish (g/m^2) for each depth (8 m: 5-10 m, 15 m: 10-20 m, 25 m: 20-30 m) within the study region

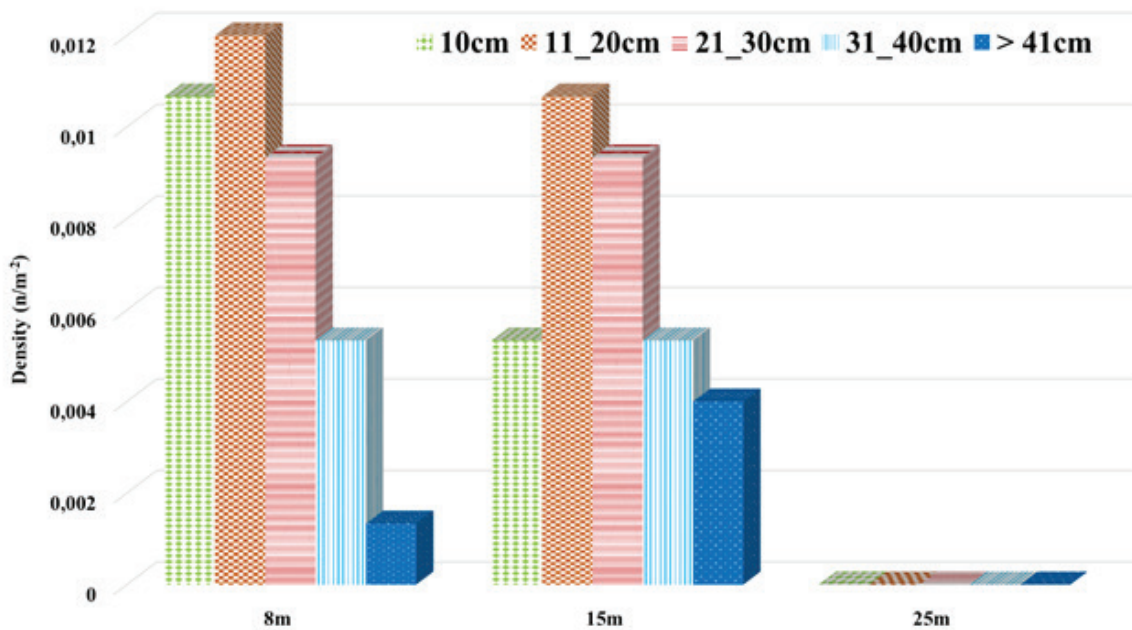


Fig. 4. The monitored density of lionfish (n/m^2) for each depth (8 m: 5-10 m, 15 m: 10-20 m, 25 m: 20-30 m) within the study region

for 11-20 cm and over 41 cm size classes both at the depths of 8 m and 15 m, respectively (Fig. 4), which is in accordance with species richness at these depths (Fig. 1). Adjeroud et al. (1998) reported the density of *P. miles* in the Mauritius coast as 3 n/ha⁻¹. Letourneur et al. (2008) reported the density of *P. miles* in the Indian Ocean as 6.2 n/ha⁻¹. Darling et al. (2011) studied lionfish in Kenya and observed a total density of *P. miles* as 25.1 n/ha⁻¹ and biomass as 1.5 n/ha⁻¹. The density and biomass in the Atlantic and Pacific Oceans mentioned above is much higher than that found in this study for the Mediterranean. This might be due to high availability of small fishes and a decrease of top predators (*Epinephelus marginatus* and *Octopus vulgaris*) as a result of overfishing in the Mediterranean Sea (Condini et al., 2017; Evliyaoğlu et al., 2020).

The proportion of lionfish *P. miles* among all 25 IAS (25 species) was 3.7 %. The sandy seafloor seems to be a limiting factor for lionfish distribution. This can be explained by two reasons. First, there is a low number of

fish species on the sandy ground which is not preferable for the lionfish that has continuous feeding habits. Secondly, lionfish also has a hiding behaviour. Therefore, they prefer habitats with reefs, caves and big rocks for feeding. There was a significant positive correlation between lionfish total density (m²) and Shannon diversity index (H) ($r = 0.99, P < 0.001$), which indicates that the number of lionfish increases with increased species diversity. Moreover, there was also a significant positive correlation between lionfish total density (m²) and Shannon evenness index ($r = 0.99, P < 0.05$), indicating that the increased abundance of each species is directly related to the abundance of lionfish.

It is important to understand the relationship between the occurrence of lionfish and other species in order to generate strategies for challenging lionfish. A high number of significant positive correlations were detected between the occurrence of lionfish, other native species and IAS (Fig. 5), indicating that lionfish put pressure on both. Our observations and another study showed that

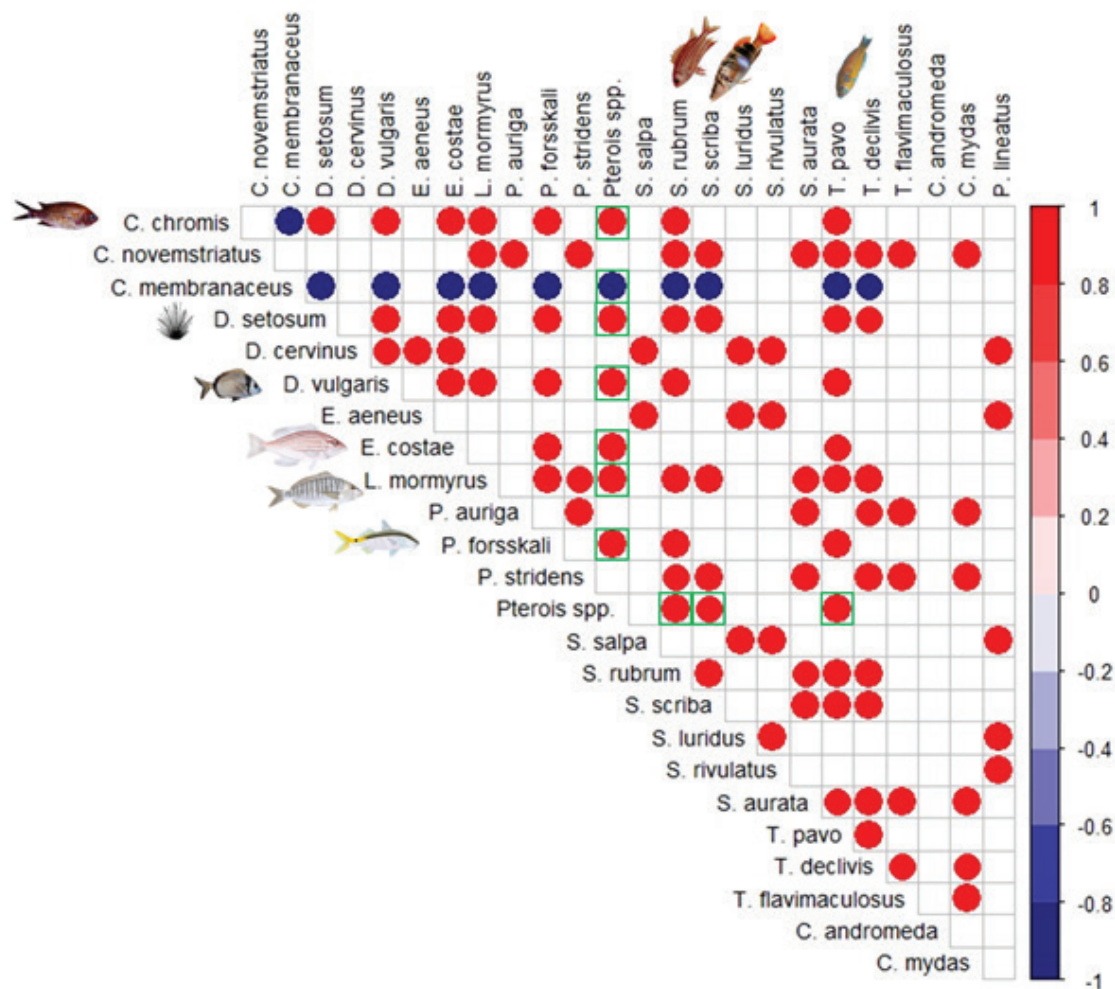


Fig. 5. Pearson correlations, showing the relationship of species occurrence. Only statistically significant ($P < 0.05$) correlations between species were redpointed with its magnitude of correlation. The significant correlations of lionfish with other species are indicated by green squares.

the diet of lionfish may consist of labrids, pomacentrids, sparids and other IAS (Zannaki et al., 2019). Especially, Sparid, Wrasse, Grouper and Chromis species seems to be threatened by lionfish. Moreover, the only significant negative correlation for the occurrence of lionfish was observed with *Cheilodipterus novemstriatus*.

In conclusion, the first stock assessment study of lionfish by the UVC method in Turkish marine waters revealed that there is a high density and biomass of lionfish in the region, which is negatively affecting regional native biodiversity as confirmed by other studies (Hixon et al., 2016; Sabido-Itzá and García-Rivas, 2019). The density and biomass of lionfish found in this study for the Mediterranean were much higher than that for the Atlantic and Pacific Oceans, which might be due to the high availability of small fishes and decrease of top predators as a result of overexploitation in the Mediterranean. The sandy seafloor seems to be a limiting factor for lionfish distribution. The lionfish abundance increases with increased species diversity and evenness. Controlling the distribution and abundance of lionfish in the region is a necessary measure for the perpetuation of native species as a management consideration.

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PRELIMINARNA PROCJENA INVAZIVNOG PRUGASTOG KOKOTA *Pterois miles* METODOM PODVODNE VIZUALIZACIJE NA SJEVEROISTOČNOM MEDITERANU

SAŽETAK

Podvodna vizualizacija (UVC) je uobičajeni pristup za procjenu gustoće ribe i biomase. Zaljev Iskenderun, kojeg okružuje obalno područje Samandağ u Turskoj, najvažniji je put unošenja stranih vrsta u sjeveroistočni Mediteran. U ovoj studiji, gustoća, brojnost, distribucija i interakcija invazivnog prugastog kokota *Pterois miles* procijenjene su UVC metodom na obali Samandağ. Ukupna promatrana površina transekta na obali Samandağ iznosila je 4.500 m². Bogatstvo vrsta, Shannonov indeks raznolikosti i stopa biomase iznosili su 25, 1,81 i 73,5 m². Uočena je smanjena raznolikost s povećanjem dubine. Pješćano morsko dno bilo je ograničavajući čimbenik za distribuciju ove vrste.

Postojala je značajna pozitivna korelacija između ukupne gustoće vrste i Shannonovog indeksa raznolikosti ($r = 0,99, P < 0,001$) i Shannonovog indeksa ravnornosti ($r = 0,99, P < 0,05$). Utvrđen je veliki broj značajnih pozitivnih korelacija ($P < 0,05$) između pojave prugastog kokota i pojave domaćih i drugih stranih vrsta. Prva studija procjene zaliha ove vrste metodom UVC u turskim morskim vodama otkrila je da su autohtone vrste pod vrlo visokim pritiskom od stranih vrsta, posebno rodova Chromis, Sparids i Wrasses, što ukazuje na negativne učinke prugastog kokota na regionalnu autohtonu biološku raznolikost.

Ključne riječi: invazivne strane vrste, prugasti kokot, monitoring, mediteranska obala Turske, podvodni vizualni popis

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