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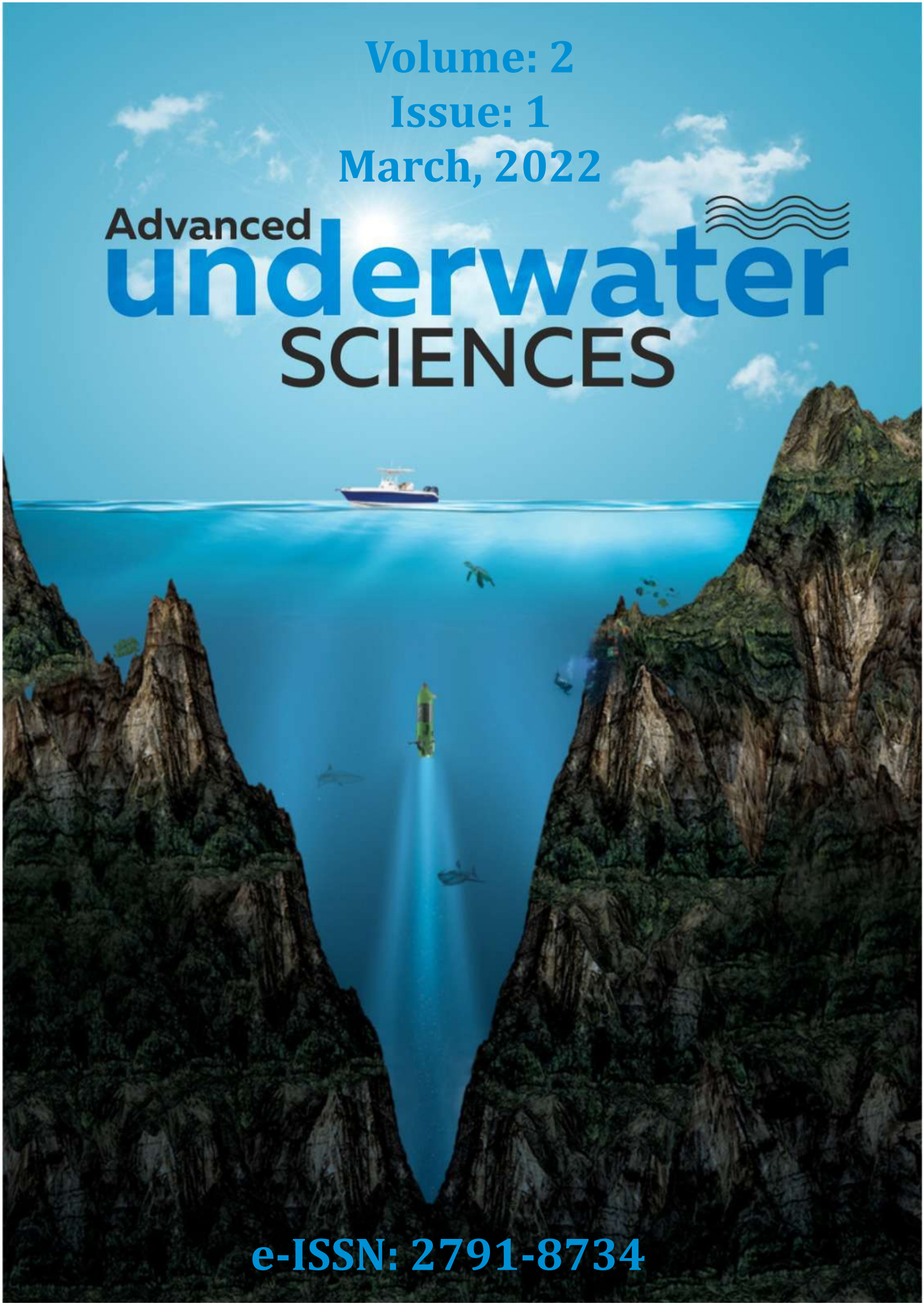

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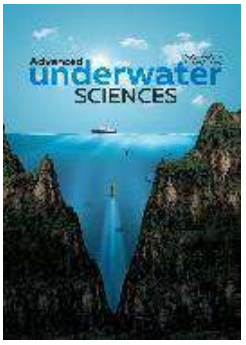

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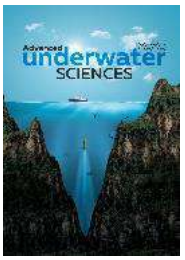

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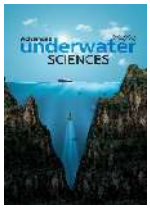

Morphometric comparison of different populations of *Nemipterus Randalli* Russell 1986 distributed in the Mediterranean coasts of Turkey

İrfan Ay [1], Nuray Çiftçi [*2], Deniz Ayas [3]

[1] *Mersin University, Vocational School of Technical Sciences, Mersin, Turkey*
[2] *Mersin University, Faculty of Fisheries, Department of Basic Sciences of Fisheries, Mersin, Turkey*
[3] *Mersin University, Faculty of Fisheries, Department of Processing Technology, Mersin, Turkey*

Keywords
Biometry,
Morphometry,
Nemipterus randalli,
Lessepsiyan Species,
Biomorph.

ABSTRACT

It was aimed to compare the populations of *N. randalli* sampled from Antalya, Anamur, Silifke, Mersin, and Iskenderun by the biometric method. BioMorph program was used in the research. Principal Components Analysis (PCA), Intercluster Correlation Analysis (ICA), and Univariate Analysis of Variance (UAV) were used in the multivariate analyzes of the individuals. The comparison of the data was made with SPSS and Excel package programs. A statistical difference was found between the populations in terms of morphometric characteristics ($p<0.05$). There was no distinction between populations in terms of meristic characters. Fin rays of the samples (D: X+9-10; A:III+7-8; PEL:I+5; PEC:16-17) and the number of lateral line scales (44/47) were determined. While positive strong correlations were determined in Antalya, Anamur, Silifke, and Iskenderun populations in terms of morphometric characters ($p<0.001$). Positive and weak correlations were found between preorbital length, dorsal-fin base, anal fin base, eye diameter, and total length in the Mersin population ($p<0.05$). Length-weight relationships of different populations of *N. randalli* were determined (b=2.25-3.0; r2=0.90-0.99). It was determined that the species showed isometric growth in Anamur and Silifke populations and negative allometric growth in Antalya, Mersin, and Iskenderun populations. The condition factor values of the species were calculated as 1.21 in Antalya, 1.29 in Anamur, 1.36 in Silifke, 1.32 in Mersin, and 1.28 in Iskenderun. The distinction between populations may vary depending on fish size, the female-male ratio in the population, habitat conditions, sampling time, sampling method, nutritional status of individuals.

1. INTRODUCTION

Morphometric characteristics of the individual are used in biological research such as species identification, growth parameter, and population dynamics for ages (Slice, 2007). Traditional morphometric applications were enhanced by modern morphometric analysis in the mid-twentieth century, combining the quantitative description of morphometric characters with statistical analyzes showing shape variations within and between groups. In this application, the set of quantitative variables such as the individual's length, width, and height are determined by multivariate statistical analyzes (Adams et al., 2004) and most of the variables are created by the development of Cartesian coordinate analysis methods of anatomical points (Slice, 2007). In modern morphometric studies using biometric methods, morphological diversity analyzes can be made according to the biological characteristics of the individual. These analyzes allow the systematic classification of the organism and the determination of the founder effect within a particular species, family, or population (Hockaday et al., 2000).

The Northeast Mediterranean was influenced by the migration of aquatic organisms originating from the Indian Ocean and the Red Sea with the opening of the Suez Canal in 1869, which connects the Mediterranean Sea to the Red Sea for the purpose of international

[*] **Corresponding Author**
(irfanay@mersin.edu.tr) ORCID ID 0000-0002-8260-2050
[*](nciftci@mersin.edu.tr) ORCID ID 0000-0002-2925-0332
(ayasdeniz@mersin.edu.tr) ORCID ID 0000-0001-6762-6284

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maritime trade (Por, 1978; Golani, 1998; Galil, 2000). The establishment of populations by migratory non-native species in the Eastern Mediterranean has brought a dynamic structure to the ecosystem and biodiversity has changed significantly. Opportunistic invasive species, which are among these species, are common to the food and habitat of native species, as well as causing adverse effects on ecological balance and fisheries as some species as predators (Zibrowius, 1994). The similar ecological character of the Northeast Mediterranean with the Red Sea increases the migration of the species. The Mediterranean coasts of Turkey have high nutrient content due to the abundant freshwater inflow, especially Mersin, Iskenderun, and Antalya Bays, due to the wide continental shelf, constitute important spawning areas. It has made the coast of our country the main migration route of migratory species (Golani, 1999).

There are also economically important and consumable species among the Lessepsian fish migrations. *N. randalli*, a demersal fish species belonging to the Nemipteridae family of the order Perciformes, has been recorded as one of the economically important species populating the coasts of Turkey. It is known that the species lives on sandy and muddy surfaces between 22-450 m depths of tropical waters and generally feed on crustaceans, mollusks, and small fish. Records of *N. randalli* from different regions have been reported in the Northeastern Mediterranean Sea. The continuity of its spread and the fact that each new population is formed from the group that left the previous population can cause variations between populations.

The feeding habits (Gürlek et al. 2010), age and growth relationships (Ergüden et al. 2010; Innal et al. 2015; Demirci et al. 2020), length-weight relationships (Erguden et al. 2010), the relationship between length and otolith size (Uyan et al. 2019), reproductive characteristics (Demirci et al. 2020 and Innal et al. 2015; Yazici et al. 2020) of the species from the Northeast Mediterranean. However, there are limited studies on morphometric characters.

In this study, it was aimed to analyze the possible differentiation between populations of *N. randalli* sampled from Iskenderun, Mersin, Silifke, Anamur, and Antalya on the Mediterranean coasts of Turkey, using the modern morphometric method.

2. MATERIAL AND METHOD

N. randalli was examined as material in this study. The economic importance and the origin and contribution to fisheries of *N. randalli* were taken into account in the selection of materials. Fish were purchased dead from trawler fishing boats from Antalya, Anamur, Silifke, Mersin, and Iskenderun. The migration route has been taken into account in the selection of the stations where the fish are sampled.

Fifty samples were taken from each station determined in the research and brought to Mersin University Fisheries Faculty Processing Technology Laboratory with a portable icebox. After determining the weight and total length of the samples brought to the laboratory, their photographs were taken by placing a millimetric ruler next to them on a white background. Photographs of each individual were uploaded to the BioMorph program and 14 different morphometric points were marked on them. With the help of these points, total length (TL), fork length (FL), head length (HL), eye diameter (ED), preorbital length (POL), body width (BW), dorsal fin base (DFB), predorsal length (PDL), anal fin base (AFB), preanal length (PAL), pectoral fin length (PFL), and caudal peduncle height (CPH) were measured (Figure 1).

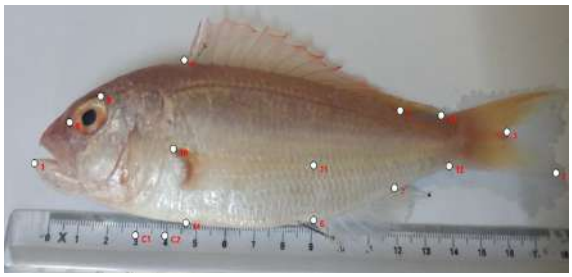

Figure 1. Points determined for morphometric measurements in the BioMorph program (C1 and C2 points were used for calibration)

The calibration value was taken as 1 cm (C1-C2; Figure 3.3.1) and the length between these points was measured using the BioMorph program. A linear ($y = ax + b$) regression model was used to determine the equations describing the relationships between morphometric characters and total length and head length. In calculated equations, 'y' is the dependent variable (morphometric character), 'x' is the independent variable (total length), and 'a' and 'b' are constants. To describe these relationships, the model with the highest coefficient of determination (r2) was chosen.

The allometric growth equation $W(i)=a \times (L(i))b$ was used to examine the length-weight relationship of *N. randalli* sampled from five different populations (Ricker, 1975). Here, W(i): the total weight of each fish (g), L(i): the total length of each fish (cm), a and b are the relationship parameters, and a: a constant related to growth (where the line cuts the weight axis). point), b: is a constant (slope of the line) that represents growth and is calculated with the formula $\ln[W(i)] = \ln a + b \times \ln[L(i)]$.

Fulton's condition factor was used to compare the conditions of individuals belonging to *N. randalli* sampled from a different population, and the formula CF=W/L^3 x100 was used to calculate the condition factor (Froese, 2006). Here, CF is the condition factor, W is the bodyweight of the fish (g), L is the total length of the fish (cm).

The meristic characters of the species were determined separately for each individual and compared. The characters used in meristic measurements were D (Dorsal fin ray number), Pel (Pelvic fin ray number), Pec (Pectoral fin ray number), A (Anal fin ray number), Lateral line scale number was determined.

Principal Components Analysis (ABA), Intercluster Correlation Analysis (CCI), and Univariate Analysis of Variance (VA) were used in the multivariate analyzes of the individuals measured. Related calculations were made with SPSS and Excel package programs.

3. RESULTS

A statistical difference was found between the populations in terms of morphometric measurements determined in the study (p<0.05) (Table 1). It was determined that Antalya and Iskenderun populations were similar in terms of some morphometric characters. It was determined that the head length, eye diameter, predorsal length, and anal fin base had the highest values in the Mersin population while the other morphometric characters had the highest value in the Silifke population.

There is no distinction between populations in terms of some meristic characters of individuals sampled from determined stations. Fin ray numbers of the samples used in study D: X+9-10; A:III+7-8; Pel:I+5; Pec:16-17, the number of lateral line scales was determined as 44/47.

The results of the analysis of variance applied to the values obtained by taking the % of the total length ratio within each population of the determined morphometric characters were determined (Table 2).

In the study, some morphometric characters of *N. randalli* were determined to have a positive linear correlation in Antalya, Anamur, Silifke, Mersin, and Iskenderun populations (p<0.001), however, it was determined that the correlation between preorbital length, dorsal-fin base, anal fin base, eye diameter, and total length showed a weak positive correlation in Mersin population.

The length-weight relationship of different populations was determined and the range of the r2 value was determined as 0.90-0.99 among the populations (p<0.001). The b value of the populations was found in the range of 2.25-3.0. Negative allometric growth was observed in Antalya, Mersin, and Iskenderun populations, and isometric growth in Anamur and Silifke populations (Table 3). The length-weight relationship graph of the studied population of *N. randalli* is shown in Figure 2.

Table 1. Average of some morphometric characters determined in individuals belonging to different populations of *N. randalli* sampled from the Turkish coasts

Morphometric Characters (mm)	Antalya $\bar{x} \pm SE$	Anamur $\bar{x} \pm SE$	Silifke $\bar{x} \pm SE$	Mersin $\bar{x} \pm SE$	Iskenderun $\bar{x} \pm SE$
TL	172.96±3.63 ^{a}	183.26±6.17 ^{a}	212.56±6.76 ^{c}	196.81±3.44 ^{b}	173.08±2.88 ^{a}
FL	155.32±3.45 ^{a}	163.84±5.29 ^{a}	197.71±6.00 ^{c}	178.07±3.29 ^{b}	156.65±2.53 ^{a}
HL	41.38±1.08 ^{a}	41.87±1.49 ^{a}	46.37±1.39 ^{a}	46.93±4.12^{a}	42.40±0.88 ^{a}
ED	12.85±0.45 ^{a}	13.51±0.42 ^{ab}	14.39±0.44 ^{ab}	17.24±3.00 ^{b}	11.75±0.24 ^{a}
BW	45.55±1.28 ^{a}	48.19±1.78 ^{ab}	58.02±2.27 ^{c}	50.86±0.98 ^{b}	46.93±1.42 ^{ab}
DFB	68.75±1.86 ^{a}	74.14±2.73 ^{a}	89.71±3.08 ^{b}	74.17±6.52 ^{a}	68.27±5.25 ^{a}
PDL	54.09±1.46 ^{a}	54.36±1.53 ^{a}	63.82±1.81 ^{ab}	72.12±6.12 ^{b}	58.47±5.05 ^{a}
PFL	45.31±1.69 ^{a}	50.04±1.73 ^{ab}	56.32±2.26 ^{b}	48.70±4.13 ^{a}	47.87±1.14 ^{a}
AFB	25.15±0.69 ^{a}	28.09±1.08 ^{a}	34.71±2.14 ^{ab}	40.02±6.98 ^{b}	31.38±4.49 ^{ab}
PAL	92.58±2.01 ^{ab}	95.01±3.28 ^{ab}	111.14±3.46 ^{c}	100.02±6.18 ^{b}	86.29±3.85 ^{a}
CPH	13.98±0.26 ^{a}	14.53±0.41 ^{a}	16.99±0.50 ^{c}	15.70±0.33 ^{b}	14.20±0.36 ^{a}

$\bar{x} \pm SE$: Arithmetic mean ± standard error; The letters a,b,c indicate the statistical separation between the data. Statistical discrimination is at the p<0.05.

Table 2. Statistical differentiation (%) of *N. randalli* sampled from the Turkish coasts among different populations in terms of some morphometric characters (FL: Fork Length, TL: Total Length, HL:Head Length, ED: Eye Diameter, BH: Body Height, DFB: Dorsal Fin Base, PDL: Predorsal Length, PFL: Pectoral Fin Length, AFB: Anal Fin Base, PAL: Preanal Length, CPH: Caudal Peduncle Height).

Populasyon	FL/TL	BH/TL	ED/HL	BH/TL	DFB/TL	PDL/TL	PFL/TL	AFB/TL	PAL/TL	CPH/TL
	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$
Antalya	89.79± 0.51^{a}	23.96± 0.53^{bc}	31.07± 0.74^{c}	26.31± 0.35^{a}	39.70± 0.38^{a}	31.29± 0.58^{ab}	26.18± 0.77^{a}	14.53± 0.17^{a}	53.53± 0.37^{ab}	8.10± 0.13^{a}
Anamur	89,44± 0,45^{a}	22.92± 0.63^{ab}	32.49± 1.24^{c}	26.29± 0.38^{a}	40.43± 0.37^{bc}	29.76± 0.57^{a}	27.38± 0.80^{a}	15.32± 0.27^{ab}	51.85± 0.48^{a}	7.94± 0.07^{a}
Silifke	90.68± 0.33^{a}	21.96± 0.77^{a}	30.98± 0.60^{c}	27.60± 1.30^{a}	42.27± 1.05^{c}	30.10± 0.58^{a}	26.44± 0.46^{a}	16.20± 0.70^{b}	52.34± 0.63^{a}	7.99± 0.07^{a}
Mersin	90.47± 0.29^{a}	25.88± 0.76^{c}	28.06± 0.60^{b}	25.90± 0.62^{a}	42.26± 0.90^{c}	32.10± 0.56^{b}	24.57± 1.93^{a}	15.27± 0.32^{ab}	55.01± 1.06^{b}	7.98± 0.08^{a}
Iskenderun	90.52± 0.44^{a}	24.53± 0.53^{bc}	27.73± 0.41^{a}	27.11± 0.66^{a}	42.37± 1.73^{c}	30.91± 0.38^{ab}	27.68± 0.60^{a}	15.66± 0.30^{ab}	52.82± 0.73^{a}	8.20± 0.15^{a}

Duncan; The letters a, b, c indicate the statistical separation between stations for each group. Statistical discrimination is at the p<0.05. x̄±SE:Arithmetic mean ± Standard error

Table 3. Length-weight relationship of different populations of *N. randalli* sampled from the Turkish coast

		Total Lengthn (mm)	Weight (g)	$W=a \times TL^b$			
Populations	**N**	**Min-max**	**Min-max**	**a**	**b**	**95% CI (b)**	**r^2**
Antalya	50	156-190	43.06-78.00	0.0240	2.75	2.70-2.79	0.90
Anamur	50	150-210	46.53-111.5	0.0126	3.00	2.93-3.06	0.96
Silifke	50	190-235	93.44-173.62	0.0140	3.00	2.16-3.84	0.97
Mersin	50	179-215	80.60-119.21	0.0007	2.25	2.20-2.30	0.98
Iskenderun	50	153-189	58.43-82.30	0.0004	2.34	2.32-2.36	0.99

N: number of samples, a: intercept, b: slope, r2: coefficient of determination, CI: Confidence Interval

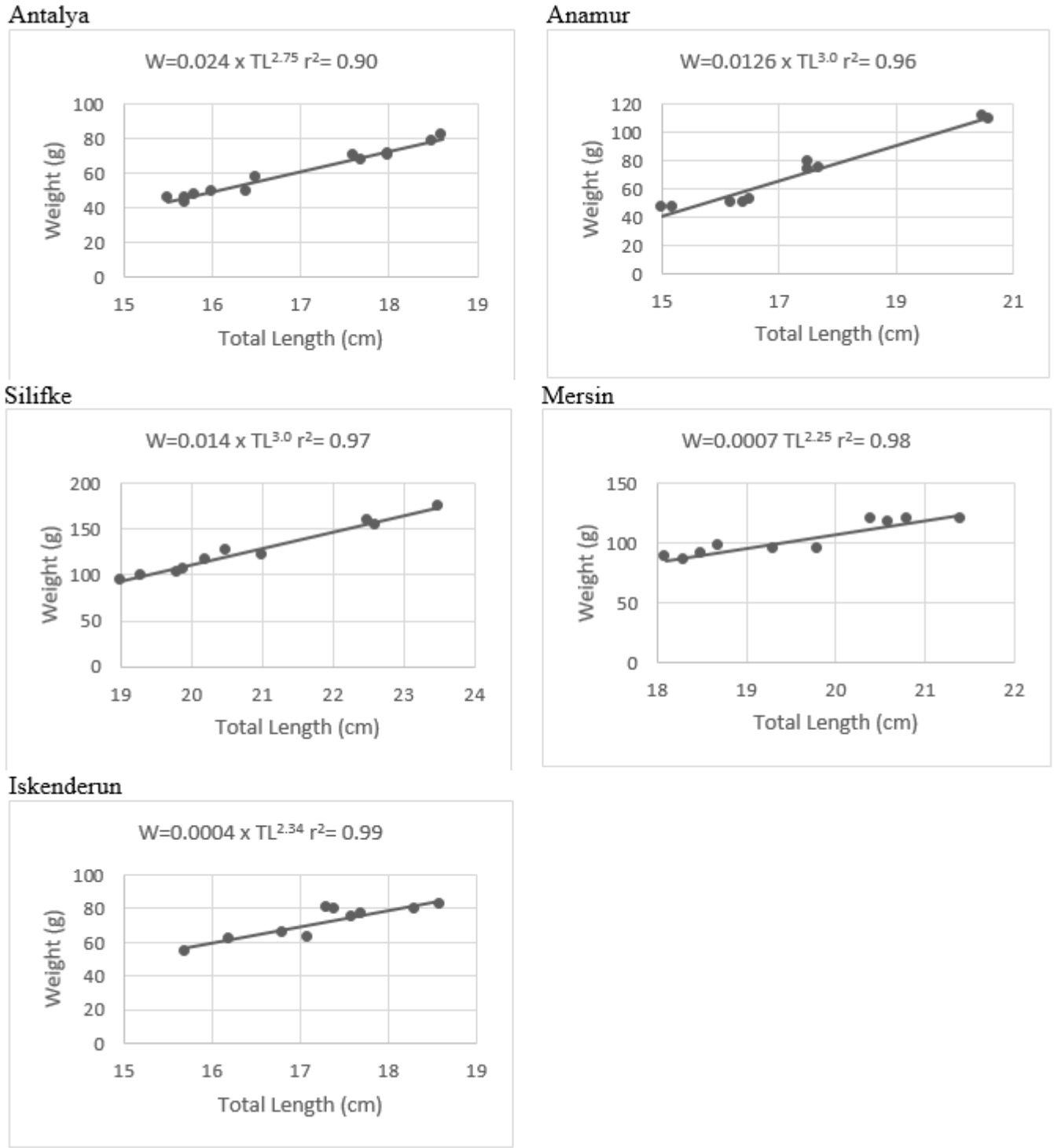

Figure 2. Length-weight relationship graph of the studied population of *N. randalli*

The mean of condition factor values of *N. randalli* was calculated as 1.21 in the Antalya population, 1.29 in the Anamur population, 1.36 in the Silifke population, 1.32 in the Mersin population, and 1.28 in the Iskenderun population.

4. DISCUSSION

Some morphometric characters of different populations of *N. randalli* were compared with previous studies. The maximum total length of *N. randalli* known in a previous study was reported as 25.0 cm (Gürlek et al., 2010). The highest total length among the populations examined in this study was found 23.5 cm from Silifke. It is consistent with the highest total length reported by Ali et al. (2013) from Syria (Table 4).

Since morphometric characters may vary depending on individual differences, environmental factors, age, and sex, the total length ratio of morphometric characters can be determined and the comparison of proportional values allows to obtain reliable results. The total length ratio of some morphometric characters of *N. randalli* was determined and compared with the different populations studied present and with previous studies (Table 5).

No distinction was found between the populations of *N. randalli* in terms of some meristic characters. Fin ray numbers of the samples examined in the study was D: X+9-10; A:III+7-8; Pel:I+5; Pec:16-17, the number of lateral line scales was determined as 44/47, which is consistent with previous studies (Lelli et al., 2008; Ali et al., 2013; Akyol & Aydın, 2016).

The positive linear correlation was found in some morphometric measurements in different populations of *N. randalli* except for preorbital length, dorsal-fin base, anal fin base, eye diameter, and total length were shown weak positive correlations in the Mersin population ($p<0.001$). Yazici et al. (2020) stated that some morphometric characters of *N. randalli* sampled from the Iskenderun Bay had positive regressions with total length, and the relationship between head length and total length showed the highest correlation among the morphometric characters examined. These research findings are consistent with previous research findings.

The length-weight relationships of *N. randalli* from Antalya, Mersin, and Iskenderun were shown negative allometric growth while from Anamur and Silifke were found isometric growth. Silifke has a rich nutritional content due to the nutrient salts carried by the Göksu River. This may have allowed the Silifke population to show isometric growth. Anamur population showed a similar growth relationship with the Silifke population. This may be due to the fact that Anamur and Silifke, unlike other study areas, are less affected by anthropogenic factors and thus provide suitable habitat and food. On the other hand, it can be thought that these two populations may have similar characteristics in terms of founder effect. The comparison of the determined length-weight relationships of different populations of the species with previous studies is presented in Table 6.

Table 4. Comparison of some morphometric characters of *N. randalli* sampled from different populations of Turkish coasts with previous studies (mm).

	Antalya	Anamur	Silifke	Mersin	Iskenderun	Bilecenoğlu and Russell, 2008 Iskenderun Bay	Ali et al., 2013 Syria	Gülşahin and Kara, 2013 Gökova Bay	Aydın and Akyol, 2016 Izmir Bay
	min-max								
TL	190.2-156.0	150.1-210.0	190.1-235.0	179.4-215.6	153.2-189.0	73.8-102.9	151-233	179-225	183
FL	136.3-185.1	135.3-200.0	170.1-232.1	159.9-192.8	143.2-168.0	-	-	164-208	157
HL	37.1-49.9	35.3-55.1	40.3-53.8	40.8-60.2	37.0-47.8	23.5-33.1	40-58	46.6-61.5	43
ED	10.4-17.3	11.5-15.6	11.4-16.4	12.2-15.6	10.5-13.3	7.4-8.9	12-15	13.6-18.7	13
BH	37.5-59.1	42.0-59.5	50.2-72.2	44.6-59.7	42.5-51.0	24.2-35.4	37-58	22.5-65.4	47
DFB	58.7-83.7	57.1-92.0	76.6-110.3	67.9-90.0	63.5-79.3	36.3-51.7	60-97	-	-
PDL	44.7-68.2	46.0-65.7	53.9-74.1	55.2-71.9	39.2-67.7	24.3-32.6	41-61	-	42
PFL	33.9-59.9	39.0-60.2	51.3-65.0	38.7-60.2	41.9-52.1	19.1-27.0	40-64	17-50.2	-
AFB	21.7-30.6	21.5-34.2	23.4-50.7	27.1-34.6	24.4-31.1	14.1-19.1	22-37	-	-
PAL	78.5-105	77.8-114.7	97.1-131.3	98.5-121.9	80.4-98.4	45.5-61.8	78-122	-	90
CPH	16.6-12.2	12.1-17.6	15.1-20.3	13.6-17.1	12.7-16.0	8.5-11.3	17-26	8.15-21.8	-

Table 5. The comparison of the total length ratio (%) of some morphometric characters among different populations of *N. randalli* sampled from the Turkish coasts with previous studies

	Present study					Russell, [11]	Aydın and Akyol, [30]
	Antalya	Anamur	Silifke	Mersin	Iskenderun		
FL/TL	89.79	89.44	90.68	90.47	90.52	87.4	85.8
HL/TL	23.96	22.92	21.96	25.88	24.53	23.7	23.5
ED/HL	31.07	32.49	30.98	28.06	27.73	27.5	30.2
BH/TL	26.31	26.29	27.60	25.90	27.11	26.5	25.7
PDL/TL	31.29	29.76	30.10	32.10	30.91	24.8	23.0
PFL/TL	26.18	27.38	26.44	24.57	27.68	25.0	-
PAL/TL	53.53	51.85	52.34	55.01	52.82	48.9	49.2

Table 6. The comparison of the length-weight relationships of different populations of *N. randalli* with previous studies.

			$W=a \times TL^b$		
Populations		**Total Length (mm)**	**a**	**b**	**r^2**
Antalya	Present study	156-190	0.0240	2.75	0.90
Anamur	Present study	150-210	0.0126	3.00	0.96
Silifke	Present study	190-235	0.0140	3.00	0.97
Mersin	Present study	179-215	0.0007	2.25	0.98
Iskenderun	Present study	153-189	0.0004	2.34	0.99
Indian Coast	Murty, (1982)	-	0.0223	2.88	-
Iskenderun Bay	Erguden et al. (2010)	48-215	0.0011	3.06	0.98
Israeli Coast	Edelist, (2014)	-	0.0101	3.08	0.97
Gulf of Oman	Al-Kiyumi et al., (2014)	-	0.0135	3.06	0.94
Antalya Bay	Ozvarol, (2014)	95-220	0.0120	2.97	0.93
Antalya Bay	Innal et al., (2015)	60-240	0.0105	3.04	0.98
Pakistani Coast	Kalhoro et al., (2017)	-	0.035	2.74	0.97
Tamil NaduPortonovo	Bandana et al., (2017)	130-259	0.0309	2.67	0.81
Gökova Bay	Ateş et al., (2017)	-	0.0201	2.98	0.96
Gökova Bay	Uyan et al., (2019)	108-219	0.0171	2.92	0.96
Iskenderun Bay	Demirci et al., (2020)	77-210	0.0106	3.09	0.97
Antalya Bay	Özen and Çetinkaya, (2020)	-	0.0173	2.85	-

The b value of *N. randalli* has been reported in previous studies ranged from 2.86 to 3.09 (Murty, 1982; Turan, 1999; Ergüden et al., 2010; Gürlek et al., 2010; Al-Kiyumi et al., 2014; Edelist, 2014; Ozvarol et al., 2014; Innal et al., 2015; Ateş et al., 2017; Kalhoro et al., 2017; Bandana et al., 2017; Uyan et al., 2019; Demirci et al., 2020; Özen et al., 2020; Yazıcı et al., 2020), in the present study it was found to be between 2.25 and 3.0. The reason why the species shows different growth characteristics may vary according to the sampling region, sampling tool, sampling time, sampling frequency, nutrient abundance, and other environmental factors.

The length-weight relationship of *N. randalli* in Antalya Bay was determined as W=0.08xTL3.1365 (r2=0.97) in females and W= 0.0079xTL3.1498 (r2=0.98) in males and positive allometric growth has been reported (Innal et al., 2015). Özvarol (2014) reported that the length-weight relationship of *N. randalli* in Antalya Bay was determined as W= 0.0120xTL2.9750 (r2=0.93) and the species showed negative allometric growth. In another study from Antalya Bay, the length-weight relationship of the species was reported as W=0.0173×TL2.8584 (Özen & Çetinkaya, 2020). In the present study, the length-weight relationship of *N. randalli* from Antalya was determined as W=0.0240xTL2.75 (r2=0.90), and it was determined that the species showed negative allometric growth that is consistent with Özvarol (2014) and Özen & Çetinkaya (2020).

The growth parameters of the species previously reported from Anamur, Silifke, and Mersin could not be reached. In this study, the length-weight relationship were found as from Anamur W=0.0126xTB3.00 (r2=0.96), from Silifke W=0.0140xTB3.0 (r2=0.97) and from Mersin W=0.0007xTB2.25 (r2=0.98). It was determined that the species showed isometric growth in Anamur and Silifke while negative allometric growth in Mersin and the findings are the first recorded growth records for the species from the Anamur, Silifke, and Mersin regions.

The length-weight relationship of the Iskenderun was determined as W= 0.0004xTB2.34 (r2=0.99) in present study. The previous research findings from Iskenderun Bay notified the growth relationships of *N. randalli* as W= 0.013xTB2,687 (r2=0.978) (Ergüden et al., 2009), W= 0.0011xTB3.061 (r2=0.982) (Gürlek et al., 2010), W= 0.00106xTB3.09 (r2 =0.97) (Demirci et al., 2020). Negative allometric growth was found in present study from Iskenderun and the result was consistent with Erguden et al (2010).

Other studies reporting the growth characteristics of the species on the Turkish coast are presented from the Gulf of Gökova (Ateş et al., 2017; Uyan et al., 2019). Ateş et al., (2017) found the length-weight relationship of *N. randalli* in Gökova Bay as W=0.0201xL2.98(r2=0.96), Uyan et al., (2019) found W=0.0171xL2.92 (r2=0.92). Both studies reported showing negative allometric growth of *N. randalli*.

In the Gulf of Oman, the length-weight relationship of *N. randalli* was determined as W=0.0109xL3.1569 in female individuals and W=0.0066xL3.3247 in male individuals, and it was reported that the species showed positive allometric growth. Edelist (2013) where the species showed positive allometric growth (b=3.08) on the coast of Israel, Edelist (2014), however, it showed negative allometric growth from the coast of Pakistan (Kalhoro et al., 2017) and the coast of East Indian Portonova (Swagat et al., 2017) has been reported.

In this study, it was determined that the species has isometric growth in Anamur and Silifke populations, and negative allometric growth in Antalya, Mersin, and Iskenderun populations, and the b value in Mersin and Iskenderun populations is outside the range of 2.5-3.5 commonly reported for bony fish (Teleost) by Froese (2006). The distinction between the populations examined in this study can be explained by changing environmental factors, as well as intra-species and inter-species competition for food and habitat. Among the sampling areas selected in the study, Iskenderun, Mersin, and Antalya regions are more affected by anthropogenic factors. This can cause changes in growth characteristics due to stress on populations.

The distinction between populations studied in present and previous studies may vary depending on fish size, the female-male ratio in the population, habitat conditions, sampling time and sampling method, and nutritional status of individuals (Innal et al., 2015).

The slope b value varied greatly between populations of *N. randalli*. It has been reported that the b value of Nemipterus species in the world varies between 2.63 and 3.28, and it has been stated that the separation between populations may depend on the season, geographical conditions such as the sampling region, as well as conditions such as limited food availability, disease and parasite loads (Bagenal & Tesch, 1978).

The condition factor values of *N. randalli* sampled from the Turkish coasts were between 1.92 and 3.77 in the present study. It has been reported that the range of condition factor values determined in a study conducted in Antalya Bay is 1.089 and 1.346 (Innal et al., 2015). It was emphasized that the condition factor of the species was high during the spawning season and these values decreased after the spawning took place. According to 44, performing the sampling during the spawning period of the species may explain the high condition factor due to gonad development.

There was a distinction between the populations in terms of the parameters examined in the present study. The distinction may be a result of the founder effect as well as ecological factors. This can be determined by genetic studies to be conducted with *N. randalli*.

5. CONCLUSION

It was reported that *N. randalli* is increasing along the northeastern Mediterranean coast, especially in the Iskenderun Bay, where fishing activities are important. It is estimated that the growth in the population of the species may place prey pressure on the economically important local species (Demirci et al., 2020). It is thought that the determination and management of successful species such as *N. randalli* among the alien species that joined the Eastern Mediterranean through tropical migration, and the relationship between local species and other migrating alien species will affect ecological change as well as fishing activities (Fox & Copp, 2014).

Each new population that the species establishes on the migration route may cause loss of genetic diversity and genetic variations due to its founding effect. Genetic diversity allows the species to adapt to the new environment. Its success in increasing new populations established in different habitats shows that *N. randalli* has genetic diversity. The weakening of gene variations of the species during adaptation to new habitats, and the success of adaptation may cause them to need anatomical changes. Increasing genetic diversity within a population will allow for increased variation selected from the most compatible alleles. Genetic variation also indicates mutation. Genetic drift that occurs in this way is one of the basic mechanisms of the evolutionary process and is important for the evolutionary development process of the species. In this study, the morphometric differences between different populations of *N. randalli* can be explained by the founder effect.

The distinction between the populations of the species may also be influenced by various factors such as ecological factors, developmental stage, prey-predator relationship, amount of food, the male-female ratio in the population, fishing pressure, and hunting method. Studies have shown that when the growth parameters of *N. randalli* are examined, it shows rapid growth and is less affected by prey pressure than species with similar growth characteristics (Demirci et al., 2020).

Finally, the finding of more than one record of *N. randalli* living in warm and salty waters in the Aegean Sea suggests that it can easily adapt to colder and lower salinity waters and establish populations. These data also confirm the hypothesis that tropical origin species migrated westward (Zenetos et al.,2008).

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Author contributions

The authors contributed equally.

Conflict of interest

The author declare that no conflict of interest pertaining to the publication of this manuscript.

Statement of Research and Publication Ethics

For this study is ethical approval not required.

REFERENCES

Adams, DC., Rohlf, FJ., & Slice, DE. (2004). Geometric morphometrics: ten years of progress following the'revolution'. *ItalianJournal of Zoology*, 71, 5-16.

Akyol, O. & Aydın, İ. (2016). A newrecord of Lagocephalus guentheri (Tetraodontiformes: Tetraodontidae) from the north-eastern Aegean Sea. *Zoology in the Middle East*, 62(3), 271-273.

Al-Kiyumi, F., Mehanna, S. & Al-Bulush, N. (2014). Growth, mortality and yieldperrecruit of the Randall'sthread finbream Nemipterus randalli (Russell, 1986) from the Arabian Sea off Oman. *Thalassas: An International Journal of Marine Sciences*, 30(1), 67-73.

Ali, M., Saad, A., Reynaud, C. & Capape, C. (2013). First records of Randall'sthreadfin bream Nemipterus randalli (Osteichthyes: Nemipteridae) off Syriancoast (Eastern Mediterranean). *Annales, Series Historia Naturalis*, 23, 119-124.

Ateş, C., Cerim, H. & Celik, M. (2017). Length-weight relationships of commercial in digenous and Lessepsian fishes in Gökova Bay, Turkey. *Cahiers de Biologie Marine*, 58(1), 43-47.

Bagenal, TB. & Tesch, F. (1978). Age and Growth. In: Methods for assessment of fish production in fresh waters. T. B. Bagenal (Ed.) Blackwell Scientific Publications, London. IBHHandbook, 3,101-136.

Bandana, DG., Saravna Kumar, A., Thangaradjou, T., Nagarja Kumar, M., Srinivasa Kumar, T. & Swagat, G .(2017). Length-weight relationship for 15 commercially ımportant fish species of Portonovo Coast, Tamil Nadu, South East of India. *Int JOceanogr Aquac*, 1(3), 000116.

Demirci, S., Demirci, A. & Şimşek, E. (2020). The validation of different growth models of randall's thread fin bream, Nemipterus randalli (Russell, 1986), in Northeastern Mediterranean Sea. *Pakistan Journal of Zoology*, 52(3), 1113- 1119.

Edelist, D. (2014). New length–weight relationships and Lmax values for fishes from the South eastern Mediterranean Sea. Journal of Applied Ichthyology, 30(3), 521-526.

Erguden, D., Turan, C. & Gurlek, M. (2009). Short Communication Weight–length relationships for 20 Lessepsian fish species caught by bottom trawl on the coast of Iskenderun Bay (NE Mediterranean Sea, Turkey). *Journal of Applied Ichthyology*, 25,133-135.

Erguden, D., Turan, C., Gurlek, M., Yaglioglu, D. & Gungor, M. (2010). Age and growth of the Randall's thread fin bream Nemipterus randalli (Russell, 1986), a recent Lessepsian migrant in Iskenderun Bay, northe astern Mediterranean. *Journal of Applied Ichthyology*, 26, 441-444.

Froese, R. (2006). Cubelaw, condition factor and weight–length relationships: history, meta-analysis and recommendations. *Journal of Applied Ichthyology*, 22, 241-253.

Galil, BS. (2000). A sea undersiege – alien species in the Mediterranean. Biological Invasions, 2, 177–186.

Golani, D. (1998). Distribution of Lessepsian migrant fish in the Mediterranean. *Italian Journal of Zoology*, 65, 95–99.

Golani, D. (1999). The Gulf of Suez Ichthyofauna-Assembl age Pool for Lessepsian Migration in to the Mediterranean. *Israel Journal of Zoology*, 45,79-90.

Gurlek, M., Erguden, S., Yaglioglu, D., Turan, F., Demirhan, S., Gurlek, M., Özbalcılar, B. & Özcan, T. (2010). Feeding Habits of Indo-Pacific Species Nemipterus randalli Russel, 1986 (Nemipteridae) in Iskenderun Bay, Eastern Mediterranean Sea. *Rapp Commint MerMédit*, 39, 539.

Hockaday, S., Beddow, TA., Stone, M., Hancock, P. & Ross, LG. (2000). Using truss networks toestimate the biomass of Oreochromis niloticus, and toinvestig age shape characteristics. *Journal of Fish Biology*, 57, 981-1000.

Innal, D., Aksu, M., Akdoganbulut, D., Kisin, B., Unal, MC., Oztop, M., Dogangil, B. & Pek, E. (2015). Age and growth of Nemipterus randalli from Antalya Gulf, Turkey. *International Journal of Fisheries and Aquatic Studies*, 2, 299–303.

Kalhoro, MA., Tang, DL., Ye, HJ., Morozov, E., Liu, Q., Memonand, KH. & Kalhoro, MT. (2017). Population dynamics of Randall's threadfin bream Nemipterus randalli from Pakistani waters. *Northern Arabian Sea. Indian J. Geo Mar Sci*, 46(3), 551-561.

Lelli, S., Collaca, F., Carpentieri, P. & Russell, BC. (2008). The tread fin bream Nemipterus randalli (Perciformes: Nemipteridae) in the eastern Mediterranean Sea. *Journal of Fish Biology*, 73, 740–745.

Murty, VS. (1982). Observations on some aspects of biology of threadfin bream Nemipterus mesoprion (Bleeker) from Kakinada. *Indian J.Fish*, 28, 199-207

Ozvarol, Y. (2014). Length–weight relation ships of 14 fish species from the Gulf of Antalya (North eastern Mediterranean Sea,Turkey). *Turkish Journal of Zoology*, 38, 342-346.

Özen, MR. & Çetinkaya, O. (2020). Population composition, growth and fisheries of Nemipterus randalli Russell, 1986 in Antalya Gulf, Mediterranean Sea, Turkey. *Acta Aquatica Turcica*, 16(3), 330-337.

Por, FD. (1978). Lessepsian Migration – the Influx of Red Sea Biota in to the Mediterranean Seaby Way of the Suez Canal. Berlin: Springer.

Ricker, WA. (1975). Computation and interpretation of biological statistics of fish populations. *Journal of the Fisheries Research Board of Canada*, 191, 382.

Slice, DE. (2007). Geometrics morphometrics. *Annual Review of Anthropology*, 36, 261–281.

Turan, C. (1999). A note on the examination of morphometric differentiation among fish populations: The truss system. *Turkish Journal of Zoology*, 23, 259-264.

Uyan, U., Filiz, H., Tarkan, AS., Çelik, M. & Top, N. (2019). Age and growth of Nemipterus randalli in the Southern Aegean Sea, Turkey. *J. Black Sea/Mediterranean Environment*, 25(2), 140-149.

Yazıcı, R., Yazıcıoğlu, O. & Akkan, T. (2020). Morphometric Relationships of Nemipterus randalli (Russell, 1986) from Iskenderun Bay. *J. Anatolian Env. and Anim. Sciences*, 5(4), 772-776.

Zibrowius, H. (1994). Introduced Invertebrates: Examples of Successand Nuisance in the European Atlantic and the Mediterranean. In: Introduced Species in European Coastal Waters. Boudouresque CF, Briand F, Nolan C. (Eds), *Ecosystems Research Report*, 8, 44-50.

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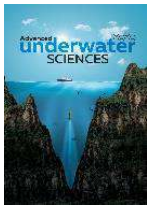

Histopathological changes in the gill tissue by some ectoparasites detected in poecilia reticulata (peters,1859)

Cafer Erkin Koyuncu [*1], **Kenan Engin** [1]

[1] *Mersin University, Faculty of Fisheries, Mersin, Turkey*

Keywords
Poecilia reticulate,
Ecto parasites,
Edema,
Hyperplasia,
Hyperplastic areas.

ABSTRACT

This research was carried out to reveal the histopathological changes in the gill tissues of ecto parasites found in the Poecilia reticulata species belonging to the Poecilidae family in an aquarium fish farming enterprise in Mersin. The investigation revealed that five parasites were present in the fish classified as Chilodonella hexasticha, Ichtyobodo necator, Trichodina sp. Gyrodactylus bullatarudis and Dactylogyrus extensus. In the histopathological examination of gill tissue samples prepared from parasitic fish, it was determined that the parasites were located between the gill lamellae and edema, hyperplasia, and hyperplastic areas developed in the epithelium.

1. INTRODUCTION

It is estimated that the aquarium fish industry has a market of around 900 million dollars worldwide (Evans and Lester, 2001). In recent years, great developments have been observed in aquarium fish production in Turkey. There have been significant increases in the number of goldfish producing farms and aquarists selling them. In almost every city in Turkey, there are many businesses selling aquarium fish, amateur and a small number of professional aquarium fish breeders (Alpbaz 1984: 163-193).

It is known that there are about 1000 ornamental fish species trading into the aquarium sector globally. Most of them are freshwater species. Poecilidae family that lives in freshwater has an important place among aquarium fish trading in the world (Yanar, 1998). Poecilia reticulate (Peters,1859), which belongs to the family Poecilidae, is a species originating from South America and is widely produced all over the world and has an important place among aquarium fish (Bassler, 1996:1- 267; Riehl & Baensch, 1996: 1-991; Evans, vd. 2001: 51-55).

Recently, aquarium fish are cultured mainly in Southeast Asia and exported from there to many parts of the world. In many countries where aquarium fish are imported, appropriate quarantine methods are neglected, and as a result, imported fish die due to parasitic infections during their transport or just after completing their transport. Great economic losses occur when they are included in the natural species (Şahin, 2004: 1-127).

If the stock density of fish in aquariums is high and the water quality is not suitable, a parasite that can enter the aquarium multiplies in a short time and cause high mortality rates (Al.pbaz, 1984:163-193). Problems caused by ectoparasites have an important place among aquarium fish diseases (Schperclaus, 1992: 1-707; Cengizler, 2000:1-136). Flagellates, ciliates and amoebae from protozoa parasites, trematodes, crustaceans, annelids and mollusks from metazoan parasites have been reported as ectoparasites in fish. The general effects of these parasites have been identified as irritation of the epithelial surfaces by increasing mucus production, the destruction of mucus cells, gill damage and respiratory distress due to the number of parasites (Southgate, 1993:1-447). In histopathological examinations, edema in the lamella, hypertrophy, hyperplasia, degeneration and necrosis in epithelial cells were determined according to the degree of irritation.

This investigation aimed at determining the ectoparasites encountered in the Poecilia reticulate (Peters,1859) species and the histopathological changes caused by these parasites in the gill tissues of the fish in an aquarium fish farming enterprise located in Mersin.

* **Corresponding Author**

*(ekoyuncu@mersin.edu.tr) ORCID ID 0000-0002-5264-7055
(kengin@mersin.edu.tr) ORCID ID 0000-0002-4335-7515

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2. MATERIAL and METHOD

Guppy fish samples used in this study were obtained from an enterprise engaged in aquarium fish farming in Mersin. During the research, a total of 120 fish of different ages and sizes, with an average of 10 fish per month, were examined for ectoparasites.

The fish to be examined were brought to Mersin University Faculty of Fisheries Diseases Laboratory, and the total length and weight of the fish were recorded before starting the parasite examination. In addition, the water temperature, pH and oxygen determinations were measured with the Orbego-Hellige brand water parameter meter by visiting the facility every month.

Protozoa living in fish as ecto parasites left the fish shortly after the fish died, so the fish were studied live. The gills of the individual fish used in the study were examined under a stereo microscope fallowing an examination macroscopically. The gill leaves, numbered 1, 2, 3, 4 from the outside to the inside, were cut with fine scissors in the order of operation and taken on a slide. The scraping preparations taken from the gills were diluted with ambient water and examined under a binocular microscope with a coverslip covered.

The parasites detected in the prepared preparations were fixed in 70% ethanol and kept for a while, and the parasites in the unit area were counted and recorded. Then the parasites were made into permanent preparations for storage. In the identification of Trichodina specimens, the gills, which were placed on a slide and dried in air, were detected using the Klein's silver impression method (Lom & Dykova 1992:1-26). Ichtyobodo necator specimens were stained with heamatoxylin for the determination of the types of parasites according to Thomas&Wellborn 1967:399-412, Lom 1970:153-177, Hoffman 1979: 153-157, Özer 1995: 441-454, Dove, vd.1998: 1755-1764., Martins vd.2012: 281-286 .

Histopathological sections were taken from the gills of infected fish and these sections were stained according to hematoxylin-eosin (H&E) staining methods and examined histopathologically (Ferguson, 1989: 1-260, Takashima &Hibiya, 1995: 1-195).

3. RESULTS

Parasites determined in this investigation were; Chilodonella hexasticha Kiernik, 1909 (Protozoa: Chlamydodontidae: Chilodonella), Ichtyobodo necator Henneguya, 1884 (Protozoa: Bodonidae: Ichtyobodo) Trichodina sp. Ehrenberg 1831 (Protoazoa: Urceolariidae: Trichodina), Gyrodactylus bullatarudis Turnbull, 1956 (Monogenea:Gyrodactlidae:Gyrodactylus), Dactylogyrus extensus Mueller and Van Cleave, 1932 (Monogenea:Dactylogyridae): Dactylogyrus.

In clinical examination and autopsy findings, It was observed that fish had difficulty in breathing trying to breathe on the water surface and the gills were covered with a dense mucus layer having a dark color and bleeding appearance compared to that of normal fish. Tears and ruptures were also spotted in the gill filaments, causing the edges of the gill filaments grayish. The operculum of fish was found open during the examinations.

In the histopathological examination of the gills; It was determined that the parasites settled between the gill lamellae (Figure 1, 2, 3) and hyperplastic areas developed in the lamellae (Figure 4). Edema and hyperplasia of the gill epithelium were also detected (Figure 5).

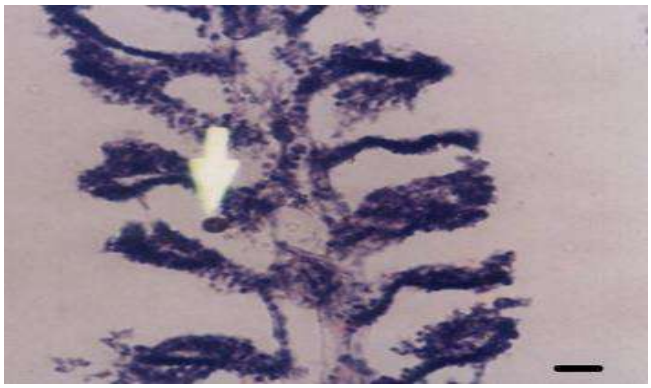

Figure 1. The image of Trichodina sp. on gill filaments (H&E). (Scale bar=50µm)

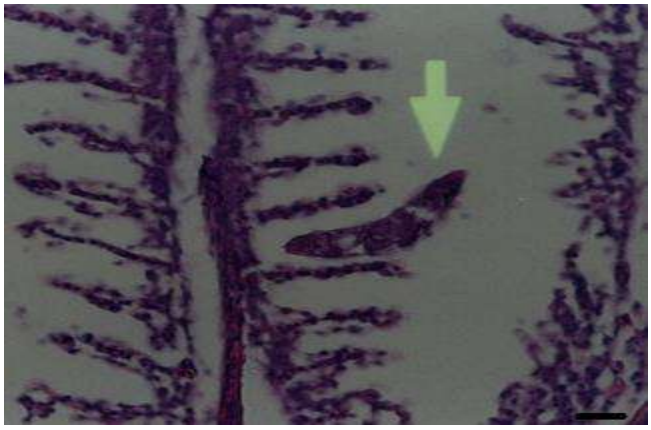

Figure2. The image of D.extensus on gill filaments(H&E). (Scale bar =100µm.)

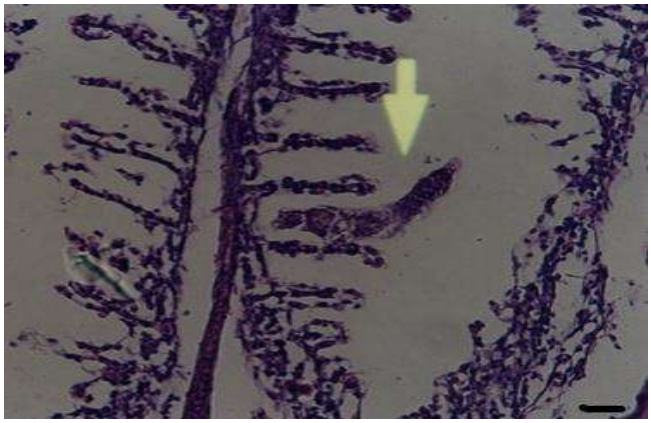

Figure 3. The image of G.bullatarudis on gill filaments(H&E).(Scale bar=100µm.)

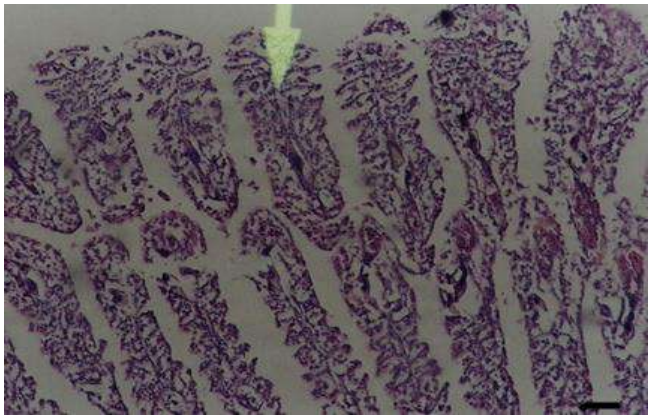

Figure 4. Hyperplastic areas in the gills (H&E) (Scale bar =100µm.)

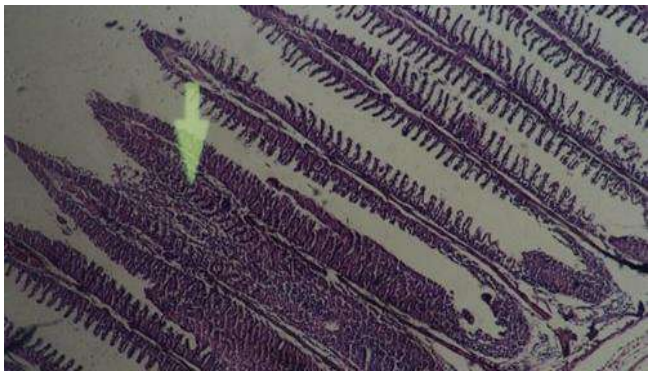

Figure 5. Hyperplasia of the gill epithelium(H&E).(Scale bar=100µm.)

4. DISCUSSION and CONCLUSION

The increase in parasitic diseases in aquarium fish farming has become an important issue that has reduced production, especially in recent years. Parasites, directly or indirectly, damage fish at different rates and cause intense mortality rates. Parasites cause damage to the gill tissue with their attachment organs such as hooks, claws and suckers, and prepare a suitable environment for the entry and reproduction of microbial disease agents. It is necessary to know well the biological characteristics of this infestation mechanism by way of understanding the cycle, the histopathological damage that the parasite causes and the selection of attachment area of the parasite in fish. (Kabata 1985: 1-318; Grabda 1991:1-306; Hibiya, 1992: 1-145).

This research was able to identify five species of parasites as Chilodonella hexasticha, Ichtyobodo necator, Trichodina sp., Gyrodactylus bullatarudis and Dactylogyrus extensus. In addition, the clinical appearance and autopsy findings of these parasite species were also investigated.

Miyazaki et al. (1986) reported that Ichtyobodo necator generally causes swelling in the gill filaments and causes increased mucus secretion in fish. Histopathological changes caused by Ichtyobodo species as hyperplasia of epithelial cells in inter lamellar regions, fusion of gill filaments and intense fusion of lamellae. In this study, on the histopathological examination of the gills of fish, it was determined that I. necator parasites settled between the gill lamellae and hyperplasia developed in the gill flaments.

Studies by the authors have shown that fish infected with the parasite C. hexasticha caused proliferation of the epithelial cells to settle between the secondary lamellae, which tends to clump together and dissolve. They also cause an increase in mucus and chloride cells. Hyperplastic epithelium may undergo dystrophic changes with enlargement of capillaries, edema, petechiae and hemorrhages (Paperna & Van As, 1983: 441-459, Shariff, 1984: 69-75, Langdon vd., 1985: 409-413). In this study, it was determined that the parasites settled between the gill lamellae and hyperplastic areas developed in the gills. In addition, edema and hyperplasia were detected in the gill epithelium.

Kayis et al. (2013) Trichodina sp. was found in the gills of severum (Heros efascatus) and both in the gills and skin of the yellow princess in Turkey. Kerek and Özdemir (2016) also detected Trichodina sp. in goldfish and in guppies. Özer, (1995) reported that Trichodina sp. caused gray bluish discoloration due to excessive mucus secretion and peeled epithelial tissue, especially in heavy infestations. Trichodina species cause epithelial hyperplasia between gill lamellae, and may also cause epithelial shedding in gill filaments (Lom & Holdar, 1977: 193-210). As a result of the clinical examination of this study, trichodina sp was found in gill of guppy fish. Also autopsy findings it was determined that the gills of the fish had a dark, bleeding appearance compared to normal, ruptures in the gill filaments, and graying at the edges.

Especially in heavy infestations of Gyrodactylus species; It has been reported that the gills are covered with a mucus mixed with dead tissue (Roberts, 1978, Kabata, 1985, Dove, et al.1998, Cone et al. 2011). Dactylogyrus species, on the other hand, cause intense mucus secretion, bruises and ulcers due to strong irritations in the gill filaments (Ribelin&Migaki 1975: 117-143, Daghigh Roohi et al., 2019: 1-6.). Dactylogyrus and Gyrodactylus species also cause deformation in gill lamellae by hyperplasia of epithelial cells and mucus cells in the gills (Wootten 2012: 415-446, Kerek & Özdemir 2016: 1-50, Durgun & Özdemir 2021: 145-157). In this study, It was determined that the parasites settled between the gill lamellae of the fish and hyperplasia developed in the gills.

In this study, the guppy, the most preferred aquarium species in Mersin province was examined for its ectoparasites using microscopic and histopathological methods and the pathological changes caused by them were revealed.

Author contributions

The authors contributed equally.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence this paper.

Statement of Research and Publication Ethics

The authors declare that this study complies with Research and Publication Ethics.

REFERENCES

Alpbaz, A. (1984). Akvaryum Tekniği ve Balıkları. Acargil Matbaası, İzmir, 163-193.

Bassler, G. (1996). Bil datlas Der Fishkrankheiten in Sübwasser aquarium Weltbil Verlag Gmblt, Augsburg, 1- 267.

Cengizler, İ. (2000). Balık Hastalıkları Ders Kitabı. Çukurova Üniv. *Su Ürünleri Yayınları*, Yayın No:7, Adana,1-136.,

Cone, DK. & Odense, PH. (2011). Pathology of five species of Gyrodactylus Nordmann, 1832 (Monogenea). *Canadian Journal of Zoology*, 62(6):1084-1088.

Daghigh Roohi, J., Dalimi Asl, A., Pourkazemi, & Shamsi, S. (2019). Occurrence of Dactylogyrid and Gyrodactylid Monogenea on common carp, Cyprinus carpio, in the Southern Caspian Sea Basin. *Parasitology International*, 73, 1-6.

Dove, D. M. & Ernst, I. (1998). Concurrent Invanders-Four Exotic Species of Monogenea Now Established an Exotic Freshwater Fishes in Austaria, *Journal of Parasitogy*, 28, 1755-1764.

Durgun, B. & Özdemir, Ö. (2021). Akvaryum Balıklarından Vatoz Balığı (Hypostomus sp) ve Tetra Balıklarında (Characidae sp) Patolojik ve Parazitolojik İncelemeler. *Manas Journal of Agriculture Veterinary and Life Sciences*, 11(2), 145-157.

Evans, BB. & Lester, RJG. (2001). Parasites of Ornamental Fish Imported into Australia. *Eafp Bul*, 21(2), 51-55.

Ferguson, HW. (1989). Systemik Pathology of fish. *Iowa State University Press*, 1-260, USA

Grabda, J. (1991). Marine Fish Parasitology: An Outline PWN-Polish Scientific Puplisher, Warszazawa, 306.

Hibiya, T. (1992). An Atlas of Fish Histology Normal and Pathological Features. Kodansha Ltd., Tokyo, 1-145.

Hoffma, GL. (1979). Chilodonella hexasticha (Kiernik,1909) (Protozoa, Ciliata) From North Fish. *Journal of Fish Diseases*, 2, 153-157.

Kabata, Z. (1985). Parasites and Diseases of Fish Cultured in The Tropics. *Taylor and Francis. Philedelphia, Pennsylvania,* 1-318.

Kayış, Ş. Balta, F. Serezli, R. & Er, A. (2013). Parasites on different ornamental fish specıes in Turkey. *J. Fisheries Sciences*, 7, 114-120.

Kerek, G. & Özdemir, Ö. (2016). Konya bölgesinde yetiştirilien akvaryum balıklarından Lepistes Poecilia reticulate) ve Japon balıklarında (Carassius auratus) patolojik ve parazitolojik incelemeler. SÜ Sağ.Bil. Enst. Yüksek lisans tezi, 1-50, Konya.

Langdon, JS., Gudsovs, N., Humphrey, JD. & Saxon, EC. (1985). Death In Australian Freshwater F Fishes associated With Chilodonella hexasticha Infection. *Australian Veterinary Journal*, 62: 409-413.

Lom, J. (1970). Observations On Trichodinid Ciliates from Freshwater Fishes. *Arshiv FürProtistenkunde* 112,153-177.

Lom, J. & Dykova, I. (1992). Protozoan Parasites of Fishes., Developments in Aquaculture and Fisheries Science, 26.

Lom, J. & Haldar, DP. (1977). Ciliates of Generea Trichodinella, Tripartiella and Paratrichodina (Peritricha, Mobilina) Invading Fish Gills. *Folia Parasitologica*, 24, 193-210.

Martins, M., Marchiori, N., Roumbedakis, K. & Lami, F. (2012). Trichodina nobilis Chen, 1963 and Trichodina reticulata Hirschmann et Partsch, 1955 from ornamental freshwater fishes in Brazil. *Brazilian journal of biology*, 72(2), 281-286.

Miyazaki, TRogers, WA & Plumb, JA. (1986). Histopathological Studies on Parasitic Protozoan Diseases of The Channel Catfish in The United States. Bulletein of The American Fisheries, Mien University, 13: 1-9.

Özer, A. &Erdem, O. (1998). Ectoparasitic Protozoa Fauna Of The Common Carp (Cyprinus carpio L. 1758) Caught In The Sinop Region Of Turkey, Journal Of Natural Histology, 32(3), 441-454.

Paperna, I. & Van, As. JG. (1983). The Pathology of Chilodonella hexasticha (Kiernik) Infection in Cichlid Fishes. Journal Of Fish Biology, 23, 441-459.

Riehl, R. & Baensch, HA. (1996). Aqarium Atlas, Publishers of Natural History And Pet Books, Germany, 1-991.

Ribelin, EW. & Migaki, G. (1975). The Patology of Fishes. The University of Wiscosin Press,117-143

Roberts, RJ. (1978). Fish Pathology. Bailliere Tindall A Division of Cassel Ltd. Printed in Great brition at the Universty Press.Aberdeen, London, 1- 318.

Shariff, M. (1984). Occurrence Of Chilodonella hexasticha (Kiernik, 1909) (Protozoa, Ciliata) On Big Head Carp Aristichys Nobilis (Richardson) In Malaysia. *Tropical Biomedicine*, 1, 69-75.

Şahin, G. (2004). İthal Edilen Altın Balıkların (Carassius auratus auratus) Ektoparazitolojik Olarak Incelenmesi. Yüksek LisansTezi, Ankara Üniversitesi Fen BilimleriEnstitüsü Su Ürünleri Ana Bilim Dalı, Ankara. 1-127.

Schaperclaus, W. (1992). Fish Diseseas. Third Edition, Akadamie Verlag, Berlig, 1-707.

Southgate, W. (1993). Aquaculture For Veterinarians (Ed. Lydia Brown). Pergamon Pres. Ltd. Oxford. 1-447.

Takashima, F. & Hibiya, T. (1995). An Atlas of fish Histology Normal and Pathologica Features. second Edition, Kodansa Ltd., Tokyo,1-195.

Thomas, L. & Wellborn, JR. (1967). Trichodina (Ciliata:Urceolariidae) of Freshwater Fishes Of southeastern United States, *J.Protozool*., 14 (3), 399-412.

Tonguthai, K. (1997). Control Of Freshwater Fish Parasites: A Southeast Asia Perspective, İnternational Journal for Parasitology, 27(10), 1185-1191.

Yanar, M. (1998). Akvaryum Balıkları Yetiştiriciliği Ders Notu, Çukurova Üniv. Su Ürünleri Yayınları, 1-58.

Woo, P. T. K. (1995). Fish Diseases and Disorders Protozoan and Metazoan Infections. 1, 1-446.

Wootten, R. (2012). The Parasitology of Teleosts In: Fish Patholog.4th Edition. Ed: Roberts Rj. London Wiley-Blackwell, Pp. 292 – 338.

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A rare record of European finless eel *Apterichthus caecus* (Linnaeus, 1758) (Family: Ophichthidae) from the Eastern Mediterranean

Deniz Ayas [*1], Deniz Ergüden [2]

[1]*Mersin University, Fisheries Faculty, Fishing* and Seafood *Processing Technology Department, Mersin, Turkey*
[2]*I*skenderun *Technical University, Marine Sciences and Technology Faculty, Marine Sciences Department, Iskenderun, Hatay, Turkey*

Keywords
Record,
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Mersin Bay,
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Turkey.

ABSTRACT

The European finless eel *Apterichthus caecus* was recorded from the Mersin Bay. One specimen was caught by a scoop net at a depth of 42 m on 20 June 2021 in the Taşucu coast, Turkey. *A. caecus* is extremely rare in the eastern part of the Mediterranean Sea. However, this report is the fourth record from Turkish marine waters and the first record in the Mersin Bay (Northeastern Mediterranean, Turkey).

1. INTRODUCTION

The genus *Apterichtus* Duméril 1805 is represented with 21 valid species worldwide. This genus comprises two species in the Mediterranean, namely Slender finless eel *Apterichthus anguiformis* (Peters, 1877) and European finless eel *Apterichthus caecus* (Linnaeus, 1758) (Froese & Pauly, 2021).

A. caecus was known from the western and eastern basins of the Mediterranean and the west coast of Africa (McCosker & Hibino, 2015). Golani (1996) reported a single specimen from the coast of Israel. Fricke et al. (2007) reported the occurrence of this species from Antalya as the first record from Turkish waters based on the collection of a single specimen.

Although the occurrence of *A. caecus* has been reported from Turkish marine waters in the Mediterranean Sea in previous years (Fricke et al., 2007; Gökoğlu et al., 2009; Ergüden et al., 2017), this species is extremely rare in the eastern part of the Mediterranean Sea. However, until now *A. caecus* has not been reported with certainty from the Bay of Mersin. Thus, in the present paper, we report the first records of *A. caecus* from Mersin Bay, eastern Mediterranean Turkey.

2. METHOD

One specimen of *A. caecus* (Fig. 1 and Fig. 2). was collected by a scoop net at Mersin Bay, Taşucu coast of Turkey at a depth of 42 m on 20 June 2021 (Coordinates: 36.12500°N 33.49230°E), (Fig. 3). This specimen was preserved in 4% formaldehyde and deposited in the Museum of the Marine Life, Mersin University, (catalog number: MEUFC-21-11-133). Morphometric measurements of the specimens were made to the nearest 0.01 mm using a digital caliper. All measurements, counts, morphological descriptions, and colors agree with the descriptions given by McCosker & Hibino (2015).

Figure 1. Dorsal view of *A. caecus* from Mersin Bay

[*] **Corresponding Author**

[*](ayasdeniz@mersin.edu.tr) ORCID ID 0000-0001-6762-6284
(deniz.erguden@iste.edu.tr) ORCID ID 0000-0002-7682-6867

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Figure 2. Head of *A. caecus*

Figure 3. Sampling area

3. RESULTS

Body very elongated, almost round in cross-section. The posterior tip stiffened and pointed. Anterior nostril tubular. Posterior nostril with a distinct nasal fold located just in front of the upper lip. Gill opening ventral with delicate flap. Head length (HL) 6.3% of total length (TL), body depth 1.2% of TL. Pointed snout 15.3% of HL, small eye covered by a membrane 7.1% of HL, interorbital length 8.9% of HL.

Color (fresh): dorsal surface beige, mouth black, ventral surface creamy-white, caudal tip a white. The historical captured record of the species in the Mediterranean Sea was documented in Table 1. Besides, this current specimen is also a fifth successive record from Mediterranean marine waters.,

Table 1. Records of *A. caecus* in the Mediterranean Sea in 1996-2021

Author	Year	Location	Country	Depth (m)	Sampling	TL (cm)	W (g)
Golani (1996)	1994	Levantine coast, Eastern Mediterranean	Israel	-	-	24.2	-
Fricke et al. (2007)	Oct. 2005	Kaş, Antalya Bay	Turkey	1	Surface	29.1	-
Gökoğlu et al. (2009)	Feb. 2007	Antalya Bay	Turkey	8	Scuba diving (Hand net)	32.2	4.36
Ergüden et al. (2017)	March 2015	Cevlik coast, Iskenderun Bay	Turkey	20	Trammel net	32.4	4.96
This study	June 2021	Tasucu coast, Mersin Bay	Turkey	42	Scuba diving (Scoop net)	44.6	53.54

4. DISCUSSION

European finless eel *A. caecus* is found in demersal, continental self, usually in the sand between 0 m and 85 m (Leiby, 1990) and can reach up to 60 cm in TL (Bauchot, 1987). It commonly feeds on small fishes and benthic invertebrates.

A. caecus has been confused with *A. anguiformis* during the last two centuries (Blache and Bauchot, 1972; Leib, 1990). Later, Blache and Bauchot (1972) was re-analyzed the references for this species and revealed that these two species are different from each other. According to Blache & Bauchot (1972) *A. caecus* is distinguished from the other species, *A. anguiformis*, by having 135-138 vertebrae and a preanal distance of 40.5-42.3% of the total length. However, *A. anguiformis* has a higher vertebral count (150-157 vertebrae) than *A. caecus*.

In the present study, our measured specimen was 44.60 cm in TL and 53.55 g in TW. Thus, this specimen was found longer and higher than the previous record that has been reported for all Mediterranean samples.

5. CONCLUSION

Consequently, *A. caecus* is rare in the eastern Mediterranean Sea. To date, *A. caecus* has never been recorded in Mersin Bay. This study reveals the fourth record from Turkish Mediterranean waters and the first record from the northeastern Mediterranean coast of Turkey.

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Author contributions

The authors contributed equally to the article.

Conflicts of interest

The authors declare that they have no conflict of interest.

Statement of Research and Publication Ethics

For this type of study formal consent is not required.

REFERENCES

Bauchot, M. L. (1987). Poissons osseux. pp. 891-1421. In W. Fischer, M.L. Bauchot and M. Schneider (eds.) Fiches FAO d'identification pour les besoins de la pêche. (rev. 1). Méditerranée et mer Noire. Zone de pêche 37. Vol. II. Commission des Communautés Européennes and FAO, Rome.

Blache, J. & Bauchot, M. L. (1972). Contribution à la connaissance des poissons Anguilliformes de la côte occidentale d'Afrique. 13e note: les genres Verma, Apterichthus, Ichthyapus, Hemerorhinus, Caecula, Dalophis avec la description de deux genres nouveaux (Fam. des Ophichthidae). *Bulletin de l'Institut Fondamental d'Afrique Noire Sciences Naturelles*, Series A, 34, 692-773.

Ergüden, D., Doğdu, S. A., Uyan, A., Gurlek, M. & Turan, C. (2017). New record of the European Finless Eel Apterichtus caecus from Iskenderun Bay, eastern Mediterranean Sea. *Zoology in the Middle East*, 63(4), 374-376.

Froese, R. & Pauly, D. (2021). FishBase. World Wide Web electronic publication. www.fishbase.org, version (08/2021).

Fricke, R., Bilecenoğlu, M. & Sari, H. M. (2007). Annotated checklist of fishes and lamprey species (Gnathostomata and Petomyzontomorphi) of Turkey, including a Red List of threatened and declining species. Stuttgarter Beiträge zur Naturkunde, Series A, 706, 1-169.

Golani, D. (1996). The marine ichthyofauna of the eastern Levant-history, inventory and characterization. *Israel Journal of Zoology*, 42, 15–55.

Gökoğlu, M., Güven, O., Balcı, B. A., Çolak, H. & Golani, D. (2009). First records of Nemichthys scolopaceus and Nemipterus randalli and second record of Apterichtus caecus from Antalya Bay, southern Turkey. *Marine Biodiversity Records*, e29, 1–3.

Leiby, M. (1990). Ophichthidae. pp. 176-192. In J.C. Quero, J.C. Hureau, C. Karrer, A. Post and L. Saldanha (eds.) Check-list of the fishes of the eastern tropical Atlantic (CLOFETA). Vol. 1. JNICT, Lisbon; SEI, Paris; and UNESCO, Paris.

McCosker, J. E. & Hibino, Y. (2015). A review of the finless snake eels of the genus Apterichtus (Anguilliformes: Ophichthidae), with the description of five new species. *Zootaxa*, 3941(1), 49-78.

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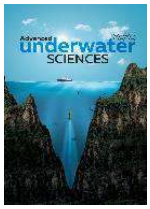

Rare occurrence of *Ariosoma balearicum* (Delaroche, 1809) from the Eastern Mediterranean

Deniz ERGÜDEN*[1], Servet Ahmet Doğdu[2], Necdet Uyğur[2]

[1]Iskenderun *Technical University, Marine Sciences and Technology Faculty, Marine Sciences Department, Iskenderun, Hatay, Turkey*
[2]*Vocational School of Maritime, University of Iskenderun Technical, TR 31220 Iskenderun, Hatay, Turkey*

Keywords
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Iskenderun Bay,
Turkish coast.

ABSTRACT

The Bandtooth conger *Ariosoma balearicum* was recorded from the Iskenderun Bay. One specimen was caught by a trammel net at a depth of 32 m on 13 February 2018 in the Konacık/Arsuz coast, Turkey. Another specimen was photographed at a depth of 10 m during a SCUBA diving expedition in Keldag/Cevlik (Iskenderun Bay) on 20 June 2018. *A. balearicum* is extremely rare in the eastern part of the Mediterranean Sea. This paper reporting two specimens confirms the occurrence of the species in Iskenderun Bay (Southern Mediterranean coast of Turkey) in addition to the present report is the first observation for this location in the Mediterranean coast of Turkey.

1. INTRODUCTION

The family Congridae is represented in the Mediterranean Sea by four genera as *Arisoma, Conger, Gnathophis,* and *Rhynchoconger* (Golani et al., 2006). The genus *Arisoma* Swainson 1838 is represented a single species in the Mediterranean, namely Bandtooth conger *A. balearicum* (Delaroche, 1809). *A. balearicum* is also known as the Baleares conger, the Balearic conger, or the Conger eel (IUCN, 2022).

The bandtooth conger *A. balearicum* is found from the Northwest Atlantic, western Atlantic, and eastern Atlantic including the Mediterranean (Froese and Pauly, 2022). Although Varezic et al. (2013) first reported of leptocephali of *A. balearicum* in the Adriatic Sea. This species is more common in its southern part, it never occurs in the extreme northern part of the Adriatic coast (Froese & Pauly, 2022).

To date, the underwater observation record and caught specimen of *A. balaericum* has not been reported from the Eastern Mediterranean waters of Turkey. Although the occurrence of *A. balearicum* has been reported in checklist from Turkish marine waters in the Mediterranean Sea in previous years (Aksiray, 1987; Fricke et al., 2007). Previous studies have neither given any specific location nor any detailed information about the fish.

A. balearicum is extremely rare in the eastern part of the Mediterranean Sea. However, until now *A. balearicum* has not been reported with certainty from the Iskenderun Bay (Southern Mediterranean coast of Turkey). This report, hence, is very important information since it is the first confirmed occurrence of *A. balearicum* and with detailed information about its presence from Iskenderun Bay, Eastern Mediterranean Turkey.

* **Corresponding Author**

*(derguden75@gmail.com) ORCID ID 0000-0002-7682-6867
(servet.dogdu@iste.edu.tr) ORCID ID 0000-0003-2939-5838
(necdet.uygur@iste.edu.tr) ORCID ID 0000-0002-4460-1735

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2. METHOD

The first specimen of *A. balaericum* was captured in Konacık (Arsuz) coast (Coordinate: 36°21′N, 35°48′E), Iskenderun Bay on 13 February 2018. This specimen was caught on rocky bottoms, partially covered with algae and seagrass, by a trammel net of 22 mm mesh size (Fig. 1). The second specimen of *A. balaericum* was photographed at a depth of 10 m during a SCUBA diving expedition in Keldag/Cevlik (Iskenderun Bay) on 20 June 2018 (Fig. 2).

Morphometric measurements of the specimen were made to the nearest 0.1 mm using a digital caliper and weighed to the nearest gram (g). All measurements, counts, morphological descriptions, and colors agree with the descriptions given by Bauchot (1987). The specimen is deposited in the Museum of the Faculty of Marine Sciences and Technology, Iskenderun Technical University with catalog number MSM-PIS/2018-5.

Figure 1. Dorsal view of A. balaericum from Konacık coast (Iskenderun Bay)

Figure 2. Underwater observation of A. balaericum in Cevlik, Iskenderun Bay (Photo: Necdet Uyğur)

3. RESULTS

The body is stout. The snout is round, projecting a little, its length almost equal to a large eye. The front nostril is tubular, forward-pointing near the tip of the snout. The rear nostril is a small oval hole in front of and a little below the middle of the eye. Dorsal fin begins over gill opening. Dorsal and anal fins are continuous with the tail fin. The tail fin is reduced and stiff. No scales on the body.

Head length (HL) 7.3% of total length (TL), body depth 5.0% of TL, Eye diameter 24.3% of HL, interorbital distance 30.1% of HL. The morphometric data of the specimen was given in Table 1.

Color (fresh): The body is brownish with the lower half of the flank with silvery to golden reflections. There is an orange crescent on top of the eye. The pectoral fin is red with margins of vertical fins black.

Table 1. Morphometric measurements of Arisoma balaericum from the Iskenderun Bay

Measurement	Value (mm)
Total length	114
Head length	8.33
Head/total length	13.63
Pre-orbital distance	2.54
Eye diameter	2.03
Max body depth	5.72
Body depth/total length	0.044
Depth at anal origin	5.14
Pre-dorsal distance	4.374
Pre-dorsal/total length	0.039
Pre-anal distance	17.21
Pre-anal/total length	0.132
Pectoral fin length	3.50
Weight	97.0

4. DISCUSSION

The bandtooth conger *A. balaericum* is found common on sandy bottoms on the continental shelf, usually in the sand between 1 m and 732 m (Smith, 1990), most frequently between 20-100 m and can reach up to 35 cm in TL (Sanches, 1991). This species is a nocturnal species that spends its days buried in sand in the Mediterranean. It feeds on invertabres and small fishes (Bauchot & Saldanha, 1986; Golani et al., 2006).

Although Sanches (1991) claimed that the species is usually found at 10-200 m depths. The first specimen was caught at a depth of 32 m from Konacık (Arsuz) by a trammel net (mesh size 22 mm). The second specimen reported in this study was observed at 10 m depth in its natural habitat in Keldag (Çevlik) on sandy bottoms, during a SCUBA diving expedition (Fig. 2). This depths range is in accordance with the literature (Sanches, 1991).

In the present study, our measured specimen was 11.40 cm in TL and 97.0 g in TW. Although *A. balaericum* is commonly reported as 20-30 cm in total length. The present length record for this specimen was found smaller and lower than the previous record for Mediterranean samples report (Grassi, 1993; Morey et al., 2003). The historical captured record of the species in the Mediterranean Sea was documented in Table 2.

5. CONCLUSION

A. balearicum is non-commercial fish species that typically inhabit bottoms with burrowed sand and mud in coastal areas. *A. balearicum* is rare in the eastern part of the Mediterranean Sea. It is considered as "Least Concern" in the Global Red List Categories and Criteria, (Carpenter et al., 2015). However, this species is assessed as "Data Deficient" in Turkey (Fricke et al., 2007). Thus, we propose that further studies are needed in this region to monitor this species in the eastern Mediterranean, Turkey.

Table 2. Records of *A. balaericum* in the Mediterranean Sea in 1913-2018

Author (s)	Year (s)	Location	Depth	Sampling	Total Length(cm)
Grassi (1913)	1913	Mediterranean Sea, Italy	-	-	21.2
Morey et al. (2003)	April 2000-June 2001	Balearic Islands and Iberian Peninsula, Spain	8-35	Trammel net	16.7-28.4
This study	Feb. 2018	Arsuz coast, Iskenderun Bay, Turkey	32	Trammel net	11.4

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Author contributions

Deniz Ergüden (DE): Investigation, data analysis, writing, sample design and methodology. Servet Ahmet Doğdu (SAD): Data collection, data curation and editing. Necdet Uyğur (NE): Scuba diving expedition and underwater photo

Conflicts of interest

The authors declare that they have no conflict of interest.

Statement of Research and Publication Ethics

For this type of study formal consent is not required.

REFERENCES

Bauchot, M. L. (1987). Poissons osseux. pp. 891-1421. In W. Fischer, M.L. Bauchot and M. Schneider (eds.) Fiches FAO d'identification pour les besoins de la pêche. (rev. 1). Méditerranée et mer Noire. Zone de pêche 37. Vol. II. Commission des Communautés Européennes and FAO, Rome.

Bauchot, M. L. & Saldanha, L. (1986). Congridae (including Heterocongridae). pp. 567-574. In P.J.P. Whitehead, M.L. Bauchot, J.C. Hureau, J. Nielsen and E. Tortonese (eds.) Fishes of the north-eastern Atlantic and the Mediterranean. Volume 2. UNESCO, Paris.

Carpenter, K. E. (2015). Ariosoma balearicum. The IUCN Red List of Threatened Species 2015: e.T194968A2369313. https://dx.doi.org/10.2305/IUCN.UK.2015.2.RLTS. T194968A2369313.en. Accessed on 15 January 2022.

Froese, R. & Pauly, D. (Editors) (2022). FishBase. World Wide Web electronic publication. www.fishbase.org, version (01/2022).

Fricke, R., Bilecenoğlu, M. & Sari, H. M. (2007). Annotated checklist of fishes and lamprey species (Gnathostomata and Petomyzontomorphi) of Turkey, including a Red List of threatened and declining species. Stuttgarter Beiträge zur Naturkunde, Series A, 706, 1-169.

Golani, D., Öztürk, B. & Başusta, N. (2006). Fishes of the Eastern Mediterranean. Turkish Marine Research Foundation (TUDAV), Istanbul, Turkey.

Grassi, B. (1913). Contributo alla conoscenza delle uova e delle larve dei Murenoidi. Mem. R. Talassogr Com. Italy, 45, 1-32.

IUC. (2022). The IUCN Red List of Threatened Species. Version 2021-3. https://www.iucnredlist.org

Morey, G., Moranta, J., Massutí, E., Grau, A., Linde, M., Riera, F. & Morales-Nin, B. (2003). Weight-length relationships of littoral to lower slope fishes from the western Mediterranean. Fisheries Research, 62(1), 89-96.

Sanches, J. G. (1991). Catálogo dos principais peixes marinhos da República de Guiné-Bissau. *Publ. Avuls. Inst. Nac. Invest. Pescas* 16, 429.

Smith, D. G. (1990). Congridae. pp. 156-167. In J.C. Quero, J.C. Hureau, C. Karrer, A. Post and L. Saldanha (eds.) Check-list of the fishes of the eastern tropical Atlantic (CLOFETA). Vol. 1. JNICT, Lisbon; SEI, Paris; and UNESCO, Paris.

Varezić, D. B., Tutman, P., Matić-Skoko, S., Pallaoro, A., Tičina, V. & Dulčić, J.. (2013). First record of leptocephali of bandtooth conger Ariosoma balearicum (Pisces: Congridae) in the Adriatic Sea. *Journal of Applied Ichthyology,* 29, 663-665.

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Tetrodotoxin (TTX) extraction methods applied for the Silver Cheeked Toadfish (*Lagocephalus sceleratus* (Gmelin, 1789))

Hasan Deniz Akbora [*1], **Deniz ayas** [2]

[1] *Eastern Mediterranean University, Faculty of Arts and Sciences, Famagusta, North Cyprus*
[2] *Mersin University, Faculty of Fisheries, Department of Processing Technology, Mersin, Turkey*

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Pufferfish,
Neurotoxin,
Aquatoxicology,
Technique,
Reliable.

ABSTRACT

Silver cheeked toadfish is one of the most popular invasive species that originated from the Red Sea. They can inflate themselves inside or outside the sea with water, making them hard to swallow by predators. This fish also has a neurotoxin named Tetrodotoxin in various body tissues and makes them non-edible for human consumption. There are many death reports in the Red Sea and the Mediterranean Sea because of the consumption of this fish by humans. In this review, we have summarized the techniques used to detect the amount of Tetrodotoxin in the Silver Cheeked Toadfish. The main aim of this study is to give researchers a fast source of TTX extraction procedures.

1. INTRODUCTION

Lagocephalus sceleratus which is also known as the Silver Cheeked Toadfish is a species of fish belonging to the family Tetraodontidae. This bony fish species are originated from Indo-Pacific and can reach up to a total length of 110 cm (Boustany et al. 2015; Yaglioglu et al. 2011; Akbora et al. 2020). In case of danger, they collect air or water in their bodies, grow in volume, and avoid being swallowed by predators (Golani et al., 2006).

What is Tetrodotoxin (TTX)?

It is a non-protein neurotoxic molecule (see fig. 1) found in some terrestrial and marine animals. It prevents nerve impulses by blocking voltage-gated sodium channels (Nzoughet et al. 2013).

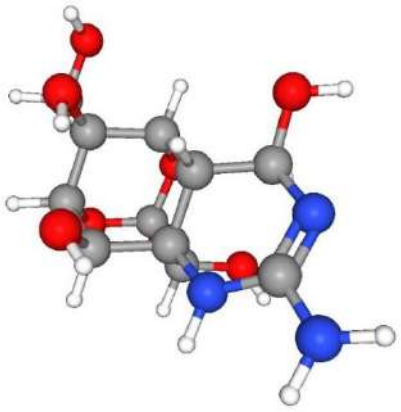

Figure 1. 3D structure of Tetrodotoxin molecule (https://pubchem.ncbi.nlm.nih.gov/compound/6324668#section=3D-Conformer).

* **Corresponding Author**
*(hdakbora@hotmail.com) ORCID ID 0000-0002-6647-866X
(ayasdeniz@mersin.edu.tr) ORCID ID 0000-0001-6762-6284

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Some terrestrial animals like Colostethus inguinalis (Cope, 1868) (Common Rocket Frog), has TTX on their body (Daly et al., 1994). In addition, most of the fish species belonging to the Tetraodontidae family can harbor TTX in their body. Another popular marine animal having TTX in its body is Greater Blue-Ringed Octopus Hapalochlaena lunulata (Quoy & Gaimard, 1832). They can transfer TTX from their salivary glands. There are some human toxification issues reported from Japan (Asakawa et al., 2019).

Analysis Techniques

- Extraction: According to Evans (1969), the tetrodotoxin molecule is quite soluble in dilute acetic acid; besides, It is soluble in small amounts in water, ether, and ethanol. In addition, when exposed to strong acids or bases, its molecular structure deteriorates. For these reasons, diluted acetic acid is used for extraction in all methods, regardless of the analysis method. After extraction, to stabilize the molecule or, in methods needs to use enzymes, pH adjustment can also be made by researchers.

- Biological Analysis Methods: ELISA (Enzyme-Linked Immunosorbent Assay) and MBA (mouse bioassay) techniques are biological techniques commonly used in tetrodotoxin analysis. Since enzymes are used in the ELISA technique and mice are used in the MBA technique, these techniques are classified as biological.

- Chemical Analysis Methods: IR (infrared spectroscopy), NMR (nuclear magnetic resonance), GC-MS (Gas Chromatography-Mass Spectrometry), LC-FLD (Liquid chromatography fluorescence detector) and LC-MS (Liquid Chromatography-Mass Spectrometry) techniques are reliable for tetrodotoxin analysis, and used by many researchers (Bane et al.2014).

Techniques used for tetrodotoxin (TTX) analysis in *L. sceleratus*

Although there are many techniques that can be used for tetrodotoxin analysis, for *L. sceleratus*; MBA (Kosker et al.2016; Katikou et al. 2009), ELISA (Akbora et al. 2020), GC-MS (Man et al.2010) and LC-MS (Rodriguez et al. 2012; Azman et al. 2014; Kosker et al. 2016) techniques were used.

- MBA- Mouse Bioassay: Kosker et al. (2016) analyzed the amount of TTX using the MBA method and LC-MS / MS methods in their study on *L. sceleratus*. If the procedure is explained with the help of the steps in this study;

Extraction: 10 grams of tissues to be analyzed are weighed and placed in 25 ml of 0.1% CH_3COOH solution and homogenized for 10 minutes at 2400 rpm. The mixture is kept in a 100ºC hot water bath for 10 minutes and kept until it comes back to room temperature. The cooled mixtures are filtered with the help of 110 mm filter paper and the residues remaining on the paper are washed with 0.1% acetic acid. All the filtered and washed solutions are combined and filled with 0.1% acetic acid to make up to 50 ml. Each 1 ml of the prepared solution corresponds to 0.2 g of tissue. Mice allowed to be used in biochemical experiments are listed in Labome (2019). Kosker et al. (2016) used the Swiss Webster Albino mouse in their research. From the mixture obtained, 1 ml per tissue is taken and injected into 3 different mice intraperitoneally. With the help of a stopwatch, the time between the time of injection and the time of death is recorded. TTX levels are calculated in units of MU (mouse unit) with the help of the table given in Kawabata, (1978).

- ELISA: Enzyme-Linked Immunosorbent Assay (ELISA) method is basically based on the immunological working principle based on the antibody-antigen relationship. It is a technique that can be used to determine the presence of antigen, antibody, protein, peptide, or hormone present in a sample. The most common modification of the ELISA technique, which has many different modifications, used in TTX analysis is competitive ELISA methods (Akbora 2018).

Extraction: TTX extraction is similar in all techniques due to the chemical properties of the molecule. Basically, when using the ELISA method, the extraction steps are as follows:

Disintegrating the tissue with the help of a homogenizer in 0.1% acetic acid and keeping the mixture in a boiling hot water bath. Making the pH adjustment determined in the ELISA Kit procedure. Centrifugation is followed by the removal of the supernatant.

Competitive ELISA modifications used in TTX analysis:

Indirect Competitive ELISA (ic ELISA)

In this modification of competitive ELISA, the TTX molecule in the sample is competing with a pre-coated antigen to bind with the primary antibody.

Basically, when you add your extracted TTX solution into a microtiter tube and add primary antibody; TTX molecules inside the sample solution and precoated TTX molecules inside the tube start to compete. If the TTX molecules inside the sample are too much, they will bind a lot of antibodies. If there is not too much TTX in the sample, precoated TTX molecules will attract primary antibodies. After the washing step, only antibodies bind to precoated TTX molecules will remain inside the tube. The next step is the addition of enzyme-linked secondary antibody which is also known as a detection antibody. Detection antibodies are specific for primary antibodies. So, during incubation detection antibodies binds to primary antibodies. After washing, only the detection

antibodies bind to primary antibodies will remain inside the tube. The last step is the addition of enzyme-substrate. There will be a reaction between enzyme and substrate and a color change observed. The color change is a measurable product to detect the amount of TTX inside the original sample (See fig.2).

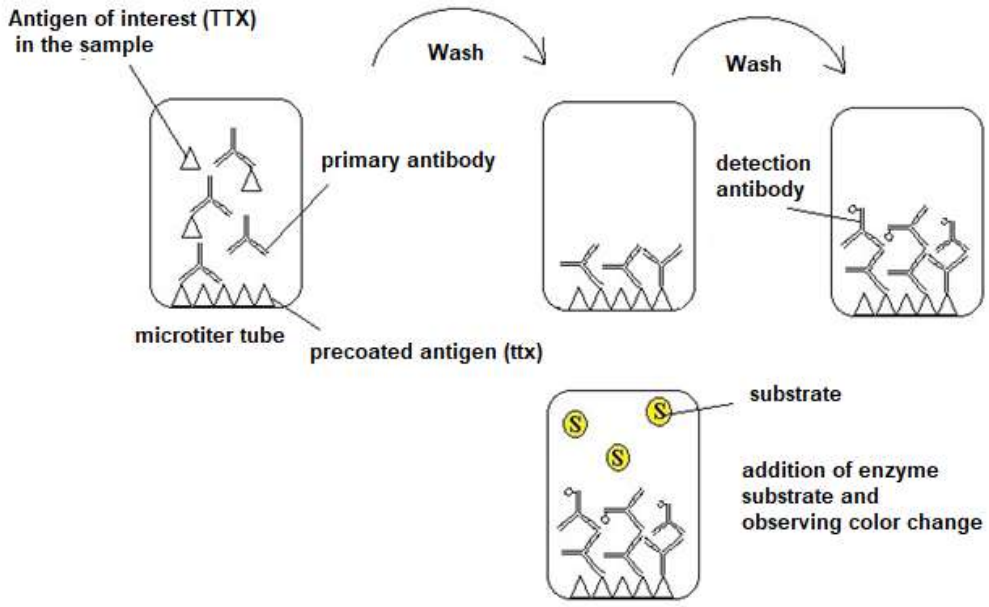

Figure 2. Schematisation of the Indirect Competitive ELISA method (This figure has been created according to Zhao et al. (2006)).

Direct Competitve ELISA (dc ELISA)

This modification of ELISA is quite similar with the ic ELISA. In this method, there is no primary antibody. TTX molecules inside the sample and precoated TTX molecules are competing to bind the detection antibody. After the washing step, and adding enzyme-substrate, there will be a color change which is the measurable product of the dc ELISA (See fig.3).

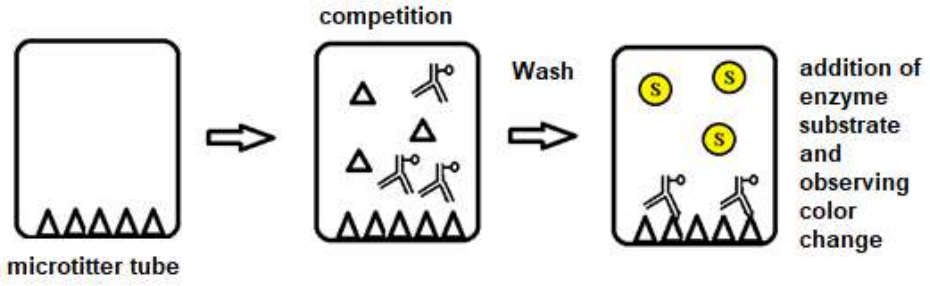

Figure 3. Schematisation of the Direct Competitive ELISA method (This figure has been created according to Zhao et al. (2006)).

In both competitive ELISA modifications, the color change and the amount of TTX inside the sample are inversely proportional. So, if the color change in a tube is higher than the other one, the amount of TTX inside the sample is less.

- GC-MS (Gas Chromatography-Mass Spectrometry): Because the TTX molecule is not volatile, this technique involves a different step compared to other techniques. This is the step of making the molecule volatile. In a study conducted in Malaysia, TTX amounts in muscle tissue were analyzed using the GC-MS method (Man et al., 2010). Again in Malaysia, another researcher analyzed TTX amounts with the LC-MS method and obtained results approximately 17.5 times higher than the GS-MS method (Azman et al., 2014). Bane et al. (2014) stated in their review that the GS-MS method is not a reliable technique in terms of TTX analysis and that it is a waste of time and money. For this reason, the details of the GC-MS technique were not discussed in our review, only it was mentioned that this technique has also been studied before.

- LC-MS (Liquid Chromatography-Mass Spectrometry): This technique has been accepted as the most widely used and most reliable method for researching TTX and its derivatives worldwide.

Extraction: As with all other techniques, the first few steps of TTX extraction from tissues are similar. Tissues

are homogenized in 0.1% acetic acid solution. Homogenized tissues are mixed with the help of a vortex and an ultrasonic mixer. These steps are repeated 2 times, and the final products are combined and centrifuged. The supernatants obtained are rounded to the desired volume by adding acetic acid. 1 ml of the final mixture is taken and cleaned using a solid-phase extraction cartridge (SPE), which was previously filled with methanol and distilled water. The cleaned sample is diluted by adding 100% methanol until reaching the desired volume. Each sample is concentrated by heating until dry and resuspended in 1 ml of methanol. 100 µl are taken from the samples, passed through 0.45 µm filters, and made ready for analysis. Extracts are given one by one to the LC-MS system and analyzed according to their mass/charge ratio (Rodriguez et al. 2012; Azman et al. 2014; Kosker et al. 2016).

2. DISCUSSION

Many techniques are used worldwide for TTX analysis in L. sceleratus. Techniques may differ according to the technical equipment infrastructure and budget of the researchers. For example, an LC-MS/MS system can be installed with a budget of around $145000, and the ELISA system with a budget of $1000-10000 (depending on the brand and features). Researchers with limited budgets can use techniques suitable for their budgets. Considering the toxin's chemical structure, it has been observed that the gas chromatography method is not reliable.

Ethical approval is required for the technique with the mouse. The use of experimental animals is prohibited in some countries if analysis can be made with alternative methods.

Although the ELISA method can be used as an alternative technique for TTX analysis; problems that may occur during the blocking of ELISA cuvettes cause incorrect antibody binding, and misleading color changes can be seen. In addition, plates containing ELISA wells may lose their properties when they are kept for a long time and may cause faulty color change.

LC-MS / MS method is the most common and reliable method used for TTX analysis. The only disadvantage of the LC-MS / MS method is that it is quite expensive to set up. Both methods (ELISA and LC-MS) can give proper results under suitable conditions. In selecting the analysis method and equipment, it is important to choose the device that will work in the longest term by considering the future research fields. It is important to establish a laboratory infrastructure accordingly.

Author contributions

The authors contributed equally to the article.

Conflicts of interest

The authors declare that they have no conflict of interest.

Statement of Research and Publication Ethics

For this type of study formal consent is not required.

REFERENCES

Akbora, HD., Kunter, İ., Erçetin, T., Elagöz, AM. & Çiçek, BA. (2020). Determination of tetrodotoxin (TTX) levels in various tissues of the silver cheeked puffer fish (*Lagocephalus sceleratus* (Gmelin, 1789)) in Northern Cyprus Sea (Eastern Mediterranean). *Toxicon*, 175, 1-6.

Asakawa, M., Matsumoto, T., Umezaki, K., Kaneko, K., Yu, X., Gomez-Delan, G., Tomano, S., Noguchi, T. & Ohtsuka, S. (2019). Toxicity and Toxin Composition of the Greater Blue-Ringed Octopus Hapalochlaena lunulata from Ishigaki Island, Okinawa Prefecture, Japan. *Toxins*, 11(5), 245.

Azman, AMN., Samsur, M. & Othman, M. (2014). Distribution of tetrodotoxin among tissues of pufferfish from Sabah and Sarawak waters. *Sains Malaysiana*, 43(7), 1003-1011.

Bane, V., Lehane, M., Dikshit, M., O'Riordan, A. & Furey, A. (2014). Tetrodotoxin: Chemistry, toxicity, source, distribution and detection. *Toxins*, 6(2), 693-755.

Boustany, L., El Indary, S. & Nader, M. (2015). Biological characteristics of the Lessepsian pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) off Lebanon. *Cah. Biol. Mar*, 56, 137-142.

Daly, JW., Gusovsky, F., Myers, CW., Yotsu-Yamashita, M. & Yasumoto, T. (1994). First occurrence of tetrodotoxin in a dendrobatid frog (Colostethus inguinalis), with further reports for the bufonid genus Atelopus. *Toxicon*, 32(3), 279-285.

Evans, MH. (1969). Mechanism of saxitoxin and tetrodotoxin poisoning. British Medical Bulletin, 25(3), 263-267

Golani, D., Öztürk, B. & Başusta, N. (2006). Fishes of the Eastern Mediterranean–Turkish Marine Research Foundation (Publication No. 24). Istanbul, Turkey.

Akbora, HD. (2018) Kuzey Kıbrıs Denizlerinde yaşayan benekli balon balığı (*Lagocephalus sceleratus* (Gmelin 1789))'nın çeşitli dokularında bulunan tetrodotoksin (TTX) düzeylerinin belirlenmesi (Master Thesis, Ankara Üniversitesi, 16-19.

Katikou, P., Georgantelis, D., Sinouris, N., Petsi, A. & Fotaras, T. (2009) First report on toxicity assessment of the Lessepsian migrant pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) from European waters (Aegean Sea, Greece). *Toxicon*, 54(1), 50-55.

Kawabata, T. (1978). Tetrodotoxin. In Veterinary Sanitation Division, Environmental Health Bureau, Ministry of Health and Welfare (Ed.), Food hygiene examination manual (shokuhin eisei kensa shishin) (pp. 223–241). Tokyo: Japan Food Hygiene Association.

Kosker, AR., Özogul, F., Durmus, M., Ucar, Y., Ayas, D., Regenstein, JM. & Özogul, Y. (2016). Tetrodotoxin levels in pufferfish (*Lagocephalus sceleratus*) caught in the Northeastern Mediterranean Sea. *Food Chemistry*, 210, 332-337.

Labome. (2019) Laboratory Mice and Rats. [ONLINE] Available at: https://www.labome.com/method/Laboratory-Mice-and-Rats.html. [Accessed 27 May 2021].

Man, CN., Noor, NM., Harn, GL., Lajis, R. & Mohamad, S. (2010). Screening of tetrodotoxin in puffers using gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1217(47), 7455-7459.

Nzoughet, JK., Campbell, K., Barnes, P., Cooper, KM., Chevallier, OP. & Elliott, CT. (2013). Comparison of sample preparation methods, validation of an UPLC–MS/MS procedure for the quantification of tetrodotoxin present in marine gastropods and analysis of pufferfish. *Food Chemistry*, 136(3-4), 1584-1589.

PubChem. (2019). Maculotoxin | C11H17N3O8 - PubChem. [ONLINE] Available at: https://pubchem.ncbi.nlm.nih.gov/compound/6324668#section=3D-Conformer. [Accessed 27 May 2021].

Rodríguez, P., Alfonso, A., Otero, P., Katikou, P., Georgantelis, D. & Botana, LM. (2012). Liquid chromatography–mass spectrometry method to detect Tetrodotoxin and Its analogues in the puffer fish *Lagocephalus sceleratus* (Gmelin, 1789) from European waters. *Food Chemistry*, 132(2), 1103-1111.

Yaglioglu, D., Turan, C., Erguden, D. & Gurlek, M. (2011). Range expansion of silverstripe blaasop, *Lagocephalus sceleratus* (Gmelin, 1789), to the northeastern Mediterranean Sea. *Biharean Biologist*, 5(2), 159-161.

Zhao, J., Li, G., Yi, GX., Wang, BM., Deng, AX., Nan, TG., Li, Z-H. & Li, QX. (2006). Comparison between conventional indirect competitive enzyme-linked immunosorbent assay (icELISA) and simplified icELISA for small molecules. *Analytica Chimica Acta*, 571(1), 79-85.