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Heavy Metal Levels and Human Health Risk Assessment in Some Fish Species Caught from Kuşadası Bay (Aegean Sea)

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Keywords

Metal, Fish, Kuşadası Bay, EDI-EWI, THQ-CR.

Research Article

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Abstract

The determining of Cu, Zn, Cd and Pb levels in muscle tissue and human health risk assessment of some consumable fish species (Engraulis encrasicolus, Sardinella aurita, Sphyraena sphyraena, Pagellus erythrinus, Mugil cephalus and Mullus barbatus), caught from Kuşadası Bay in 2016-2017 hunting season were aimed. Metal analysis was performed using Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS) in tissues prepared by the dry weight method. Metal level in tissue varied depending on the species, metal and season, Cu and Zn were highest in S. sphraena and E. encrasicolus in winter, while Pb was determined in M. cephalus in spring. The lowest and highest Cu, Zn and Pb levels in tissues were detected 0.80-5.82, 13.09-41.60 and 0.20-1.30 μ g g-1 ww, respectively. Cd level in all fish samples was found below the permissible level of ICP-MS. Tissue metal levels were determined within acceptable limits according to the Turkish Food Codex FAO/WHO standards. The intake rate limits and human health risk assessment based upon non-carcinogenic and cancer risk effect specified no negative impacts of investigated fish consumption.

1. Introduction

Fish have caused continuity in the human diet in the historical process, due to their high availability and easy hunting. In addition, have high protein content, low carbohydrate content, fat-soluble vitamins, minerals, unsaturated fatty acids such as EPA (Eicosa Pentaenoic Acid) and DHA (Docosa Hexaenoic Acid) ensure the continuity of consumption (Ersoy and Çelik 2009). The increase in the human population, industrial and technological development, and the agricultural applications have increased the participation of domestic, industrial and agricultural wastes in the hydrosphere, which is the main receiving environment, and caused pollution (Cicik 2003). Pollutants, including metals uptake by aquatic organisms transfered into the upper trophic level caused aquatic ecosystems to rapidly lose their food storage feature, as well as adverse effects of human and environmental health (Çiftçi et al. 2015).

Determination of metal levels in muscle tissue, which is the main consumable part of fish, is very important for both fish and public health (Türkmen et al. 2009). Metals such as Cu and Zn function at low concentrations as cofactors in various enzymes that function in metabolic events, in energy production, connective tissue development, impulse transmission. growth. development, neurotransmission and cell communication (Duran et al. 2015), while the high concentrations' cause tissue accumulation, damage, inflammation of the skin, loss of taste and smell senses, and disorders in metabolism and respiratory events (Zeitoun and Mehana 2014). The others, such as Pb and Cd, are toxic even at very low concentrations and have been found to cause disorders in the circulation, excretion, digestive system and cardiovascular system in humans (Jarup 2003).

Fish meat is often recommended by dietitians because it is healthier than other food sources in terms of both content and digestibility (Fuentes-Gandara et al.

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2018; Borowska-Korezak et al. 2021). The American Heart Association reported that consuming fish at least twice a week reduces the risk of heart disease (Zhang et al. 2008). Although the consumption of fish is important in terms of balanced nutrition and health, pollution in aquatic ecosystems poses a significant threat to both aquatic organisms and public health (Malik et al. 2014; Olmeda et al. 2013).

Kuşadası is a coastal town located in the center of the Aegean Sea, where tourism, agriculture and fishing activities take place at a high intensity. The marina due to boat and cruise traffic, and secondary residences on the coast due to cultural and religious tourism have increase the pressure on the marine ecosystem. Kuşadası Bay contains economical importance fish species. Fish and other aquatic products are widely consumed in the region as they are common elements of cuisines with different cultures.

In this study, it was aimed to determine the health risk assessments of metals (Cu, Zn, Cd and Pb) in the muscle tissues of widely consumed fish (E. encrasicolus, S. aurita, S. sphyraena, P. erythrinus, M. cephalus and M. barbatus) in Kuşadası Bay.

2. Method

2.1. Study Area

Anchovy, sardine, barracuda, red sea bream, gray mullet and red mullet species, which live in different parts of the water column, have different dietary habits and are widely consumed as food in the region, were used as material in the research. Fish samplings were made monthly, 10 of each species, between September 2016 and May 2017, and the fish were obtained from a commercial firm in Kusadasi on the first day they were caught.

Each of the fish was dissected separately, muscle tissue samples were taken and placed in polyethylene bags and stored in a deep freezer set at -20 °C until analysis. A total of 540 fish were analyzed in the study. Since the heavy metal level in fish varies depending on size and weight, fish of similar size and weight were used in the study (Table 1).

Table	1.	Length	and	weight	of the	fish	used	as materials	and th	eir habitats
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Table 1. Length and Wel	Table 1. Bength and weight of the fish used as materials and their habitats					
	n	Total length (cm)	Weight (g)	Habitat		
		$\bar{X} \pm s\bar{x}$	$\bar{X} \pm s \bar{x}$	$\bar{X} \pm s\bar{x}$		
E. encrasicolus	90	12.45±1.09	10.10±3.31	Pelajik		
S. aurita	90	13.74±1.11	20.08±2.34	Epipelajik		
S. sphyraena	90	31.87±3.98	121.50±8.58	Pelajik		
P. erythrinus	90	14.79±0.92	64.19±3.60	Bentopelajik		
M. cephalus	90	16.47±1.23	49.00±1.68	Bentopelajik		
M. barbatus	90	13.28±0.68	27.40±2.01	Demersal		

Frozen tissue samples were carried out in the laboratory of the Fisheries Faculty of Mersin University in cold chain and prepared for analysis at the end of the hunting season. Heavy metal levels in muscle tissue determined by Durmus et al. (2018). The experimental tissues were dried at 150 oC for 72 hours to reach constant weight. After the tissue dry weights were determined they were digested in nitric acid/percloric acid mixture (2/1: v/v)at 120 °C for four hours. Then digested samples transferred to polyethylene tube and their volume were completed to 10 ml with bidistiled water. The same procedures were applied to the blank and IAEA samples. Tissue metal levels calculated over dry weight were converted to wet weight values considering the moisture rate accepted for a significant part of the species and compared with previous studies (Cresson et al. 2017; Giraldo et al. 2017; Hislop et al. 1991; Payne et al. 1999). IAEA-407 obtained from fish tissue homogenates was used as reference material (IAEA, 2003). Recovery and

Table 2. Validation parameters of the analytical method

tissue homogenates for IAEA were determined as 96.90%, 98.02%, 96.15% and 95.50% for Cu, Zn, Pb and Cd, respectively. The formula of the linear regression equation was obtained from the absorbance values of the standard concentrations and this formula was used to determine the tissue concentration of each metal. The absorbance values of the standards for each metal were reread after every 10 readings to check that the device was working properly. All measurements were repeated 3 times. The validation parameters of the analytical method are shown in Table 2. SPSS v.16.0 (IBM. Corp. Armonk, NY, USA) package program was used in the analysis of the data, and homogeneity of variance was checked before starting the statistical analysis. One-way ANOVA test was applied to the data and the results found significant (P<0.05) were reanalyzed with the post-hoc test (SNK= Student Newman Keul's) to determine the difference between the groups.

correction values of control samples prepared from fish

Metals	Measured Value Concentration mg kg ⁻¹	Certificated Values %95 Confidence interval (mg kg ⁻¹)	LOD ng g ¹	LOQ ng g ⁻¹	Recovery %95	RSDr %	R ²
Cu	3.29	3.20-3.36	0.33	2.31	96.90	3.099	0.9997
Zn	67.98	66.3-67.9	2.38	7.12	98.02	2.925	0.9998
Pb	0.12	0.10-0.14	0.79	1.85	96.15	1.260	0.9993
Cd	0.189	0.185-0.193	0.79	1.92	95.50	2.195	0.9999

2.2. Risk Analysis In Terms of Human Consumption

Determining the possible risks of pollutants on human health is generally an event to determine or predict the harmful effects of toxic substances on health (Jawed and Usmani, 2016). Parameters such as Estimated Daily (EDI) and Weekly (EWI) Metal Intake, Target Hazard Ratio (THQ), Hazard Index (HI) and Cancer Risk (CR) are widely used to determine the harmful effects of heavy metals on human health (USEPA, 1989). Factors such as metal intake, exposure time, average body weight and oral reference dose affect these parameters significantly (USEPA, 1989; Harmanescu et al. 2011). In this study, EDI, EWI, THQ and CR were examined among the mentioned parameters. EDI, EWI, THO and CR values were calculated using the following mathematical formulas in case the investigated species were consumed.

EDI (µg/day/70 kg body weight) =CxFIR

Table 3. Parameters and values used in health risk analysis

EWI (μ g/week/ 70 kg body weight) = EDIx7 days C(Concentration)=Mean metal concentration in muscle tissue (μ g g-1 w.w.)

FIR (Fish Consumption Rate) = Daily fish consumption amount per capita in Turkey 20g (FAO 2005)

THQ= (EFxEDxEDI/ATxRfDxBW)x 10-3

CR = (EFxEDxEDIxCSF/ATxBW) x10-3

EF; (Frequency of exposure to a person who eats fish seven times a week, 365 days/year) (Traina et al. 2019), ED; Time of exposure (age 70 as adult), BW; Body weight is taken into account as the average weight of an adult in Turks is 70 kg. RfD; Orally, the reference dose is mg/kg/day. HORSE; mean time for non-carcinogenic substances (365 days/yearxED), CSF; Cancer Slop Factor mg/kg/day. The parameters and their values used in the risk analysis are given in Table 3.

Tuble Di	Tuble St T drameters and values used in nearth risk analysis							
Factor	Statement	Unit	Value	References				
FIR	Daily Consumption Amount	g/person/day	20	FAO 2005				
BW	Average Body Weight	kg	70	Karayakar et al. (2022)				
EF	Frequency of Exposure	day/year	365	Karayakar et al. (2022)				
ED	Influence Time	year	70	Karayakar et al. (2022)				
AT	Average Time	day	25550 (EDxEF)	Gu et al. (2017)				
RfD	Average Time	mg/kg/day	4E ⁻⁰² (Cu), 3E ⁻⁰¹ (Zn), 1E ⁻⁰³ (Cd), 4E ⁻⁰³ (Pb)	USEPA (2018)				
CSF	Cancer Slop Factor	mg/kg/day	8.5E ⁻⁰³ (Pb), 6.3E ⁻⁰⁰ (Cd)	Traina et al. (2019)				

3. Results

Metal levels determined in mussle tissues of E. encrasicolus, S. aurita, S. sphyraena, M. cephalus, P. erythrinus, M. barbatus caught from Kusadası Bay in 2016-2017 hunting season (Table 4). The Cd level in tissue could not be determined because it was below the detection limit of ICP-MS. In the autumn, winter and

spring seasons, a correlation was found between metals in terms of concentration in the muscle tissues of the species, such as Zn>Cu>Pb (Table 4). Cu ($5.82 \ \mu g \ g-1 \ ww$) was found the highest level in S. sphyraena, and Zn ($41.60 \ \mu g \ g-1 \ ww$) in E. encrasicolus in winter while the highest Pb level ($1.30 \ \mu g \ g-1 \ ww$) was detected in M. cephalus in spring (Table 4).

Table 4. Heavy metal levels (µg g-1 w.w.) determined in muscle tissues of fish

			Metals					
Saacan	n	Experimental Species	Cu	Zn	Pb	Cd		
3eas011	11	Experimental Species	$\overline{x} \pm s_x^*$	$\overline{x} \pm s_x^*$	$\overline{x} \pm s_x^*$	$\overline{x} \pm s_x^*$		
	30	E. encrasicolus	1.54±0.23 as	39.44±2.18 bs	DA cs	DA		
	30	S. aurita	1.26±0.22 as	26.06±6.05 bt	0.44±0.22 ct	DA		
	30	S. sphyraena	0.80±0.66 as	21.15±3.01 bt	0.21±0.10 ct	DA		
	30	M. cephalus	0.94±0.08 as	39.69±6.01 bs	0.28±0.10 ^{ct}	DA		
Autumn	30	P. erythrinus	0.97±0.15 as	32.64±3.99 bs	0.34±0.17 ^{ct}	DA		
	30	M. barbatus	0.96±0.22 as	41.38±2.67 bs	0.21±0.10 ct	DA		
	30	E. encrasicolus	3.79±0.90 as	41.60±8.41 bs	0.70±0.37 cs	DA		
	30	S. aurita	2.70±0.68 as	30.72±2.95 bt	0.41±0.28 cs	DA		
	30	S. sphyraena	5.82±0.58 ^{at}	13.09±1.19 bx	0.26±0.15 cs	DA		
Winton	30	M. cephalus	3.28±1.45 as	27.84±3.21 bt	0.48±0.35 cs	DA		
winter	30	P. erythrinus	1.97±0.24 ax	18.94±2.55 by	0.20±0.15 cs	DA		
	30	M. barbatus	2.89±1.31 as	26.63±4.89 ^{bt}	0.74±0.27 cs	DA		
	30	E. encrasicolus	5.28±0.85 as	41.26±3.46 bs	DA ^{cs}	DA		
	30	S. aurita	3.16±0.53 at	32.30±1.42 bt	0.46±0.23 ct	DA		
	30	S. sphyraena	2.90±0.78 at	19.96±4.50 bx	0.98±0.54 ct	DA		
	30	M. cephalus	3.44±1.40 at	25.63±2.43 bx	1.30±0.49 ct	DA		
Spring	30	P. erythrinus	3.03±1.54 at	20.11±3.84 bx	0.92±0.15 ct	DA		
	30	M. barbatus	4.41±0.93 as	37.87±2.07 bs	0.78±0.19 ct	DA		

x⁻±s_x :Arithmetic mean ± standard error, n: Number of sample

*SNK = Data shown with different letters indicate significant difference at p<0.05 level. Letters a, b, and c are used to show difference between metals and s, t, x and y among species.

The minimum and maximum Cu, Zn and Pb levels in fish muscle were determined as 0.80-5.82, 13.09-41.60 and 0.20-1.30 μ g g-1 w.w. respectively. National and international organizations such as FAO/WHO, USEPA, the European Union Commission and the National Food Codex Commission of the Ministry of Agriculture and Forestry of the Republic of Turkey have determined the

maximum acceptable limits of heavy metals in foods and limited their levels in nutrients (Table 5). In this study, it was determined that the average Cu, Zn and Pb levels in fish muscle, were rather below the acceptable upper limits determined by national and international organizations (Table 5).

Table 5. Acceptable upper limits of some heavy metals in muscle tissues of fish determined by national and international organizations.

		Metal (
Fish	Cu	7n	Dh	Cd	References
	Cu	ZII	FD	Cu	
	20	FO	1.0	0.1	Turan at al. 2000
	20	30	1.0	0.1	Turan et al. 2009
	30	40	0.5	0.5	FAO/WHO 1989
	20	50	2.0	0.2	MAFF (2000)
	20	F 0	1.0	0.2	Türlman at al. (2000)
	20	50	1.0	0.2	Turkmen et al. (2009)
	100	100	-	-	Turan et al. 2009
	60	80	-	-	Turan et al. 2009
	100	150	-	-	Turan et al. 2009
E. encrasicolus	3.53	40.76	0.23	-	
S. aurita	2.37	29.69	0.43	-	
S. sphyraena	3.17	18.06	0.48	-	
M. cephalus	2.55	26.24	0.68	-	
P.erythrinus	1.99	23.89	0.48	-	
M. barbatus	1.47	35.29	0.57	-	
	Fish E. encrasicolus S. aurita S. sphyraena M. cephalus P.erythrinus M. barbatus	Fish Cu 20 30 30 20 20 20 20 20 20 100 60 100 5. aurita 2.37 S. sphyraena 3.17 M. cephalus 2.55 P.erythrinus 1.99 M. barbatus 1.47	Fish Metal (Fish Cu Zn 20 50 30 40 20 50 20 50 20 50 20 50 20 50 20 50 20 50 100 100 60 80 100 150 <i>E. encrasicolus</i> 3.53 40.76 <i>S. aurita</i> 2.37 29.69 <i>S. sphyraena</i> 3.17 18.06 <i>M. cephalus</i> 2.55 26.24 <i>P.erythrinus</i> 1.99 23.89 <i>M. barbatus</i> 1.47 35.29	$\begin{tabular}{ c c c c c } \hline Hetal (\mu g g^{-1} w.w.) \\ \hline Fish & Cu & Zn & Pb \\ \hline Cu & Zn & Pb \\ \hline 20 & 50 & 1.0 \\ \hline 30 & 40 & 0.5 \\ 20 & 50 & 2.0 \\ \hline 20 & 50 & 1.0 \\ \hline 20 & 50 & 1.0 \\ \hline 20 & 50 & 1.0 \\ \hline 100 & 100 & - \\ \hline 60 & 80 & - \\ \hline 100 & 150 & - \\ \hline 100 & 150 & - \\ \hline E. encrasicolus & 3.53 & 40.76 & 0.23 \\ S. aurita & 2.37 & 29.69 & 0.43 \\ S. sphyraena & 3.17 & 18.06 & 0.48 \\ \hline M. cephalus & 2.55 & 26.24 & 0.68 \\ \hline P.erythrinus & 1.99 & 23.89 & 0.48 \\ \hline M. barbatus & 1.47 & 35.29 & 0.57 \\ \hline \end{tabular}$	$\begin{tabular}{ c c c c c } \hline Hetal (\mu g g^{-1} w.w.) & \hline \\ \hline Cu & Zn & Pb & Cd \\ \hline & 20 & 50 & 1.0 & 0.1 \\ \hline & 30 & 40 & 0.5 & 0.5 \\ \hline & 20 & 50 & 2.0 & 0.2 \\ \hline & 20 & 50 & 1.0 & 0.2 \\ \hline & 20 & 50 & 1.0 & 0.2 \\ \hline & 20 & 50 & 1.0 & 0.2 \\ \hline & 100 & 100 & - & - \\ \hline & 60 & 80 & - & - \\ \hline & 100 & 150 & - & - \\ \hline & 100 & 150 & - & - \\ \hline & 100 & 150 & - & - \\ \hline & & 100 & 150 & - & - \\ \hline & & & 5. aurita & 2.37 & 29.69 & 0.43 & - \\ \hline & S. sphyraena & 3.17 & 18.06 & 0.48 & - \\ \hline & M. cephalus & 2.55 & 26.24 & 0.68 & - \\ \hline & P.erythrinus & 1.99 & 23.89 & 0.48 & - \\ \hline & M. barbatus & 1.47 & 35.29 & 0.57 & - \\ \hline \end{tabular}$

Results from this study, the estimated daily and weekly heavy metal (Cu, Zn, Pb) uptake levels were rather below the tolerable levels in case of consumption of the mentioned fish species (Table 6). Therefore, the consumption of the examined species as food does not pose any risk to human health.

Table 6. Estimated weekl	y and daily met	al uptake in case of (consumption of fish s	pecies
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 te of Boundatea noomj and	a aang motar aptane m t	abe of concamption of n	on speeres	
Metal	Cu	Zn	Pb	Cd
TWI ^a	3500	7000	25	7
TWI ^b	245000	490000	1750	490
TDI ^c	35000	70000	250	70
EWI (EDI)	739.2(10.6)	5824(832)	98 (14)	-
E. encrasicolus				
EWI(EDI)	442.4(63.2)	4522(646)	644(9.2)	-
S. aurita				
EWI(EDI)	814.8(116.4)	2961(423)	137.2(19.6)	-
S. sphyraena				
EWI(EDI)	481.6(68.8)	5556.6(793.8)	182(26)	-
M. cephalus				
EWI(EDI)	424.2(60.6)	4569(652.8)	128.8(18.4)	-
P. erythrinus				
EWI(EDI)	617.4(88.2)	5824(832)	109.2(15.6)	-
M. barbatus				

a;Tolerable weekly intake (µg/week/kg/body weight)

b; Tolerable weekly intake for a 70 kg person (µg/week/70 kg body weight)

c;Tolerable daily intake ($\mu g/day/70 \text{ kg body weight}$)

EWI; Estimated Weekly Intake (µg/week/70 kg body weight)

EDI; Estimated Daily Intake (µg/day/70 kg body weight)

The mean values of the metal concentrations determined in the muscle tissues of the examined species were used to determine the THQ and CR values, and the

results showed that the THQ was below 1.0 and the CR values were within the acceptable range in terms of public health (Table 7).

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Table7. THQ and CR values according to the	mean metal levels (µg/g w.w.) in the mus	scle tissues of the examined species

Species			Metal	
species		Cu	Zn	Pb
E en angeigelue	THQ	37.8x10 ⁻³	377x10 ⁻³	50x10 ⁻³
E. encrusicolus	CR	-	-	1.7x10 ⁻⁶
C queita	THQ	22.5x10 ⁻³	307x10 ⁻³	32x10 ⁻³
S. aurita	CR	-	-	1.1x10 ⁻⁶
S. sphyraena	THQ	41.57x10 ⁻³	201x10 ⁻³	70x10 ⁻³
	CR	-	-	2.3x10 ⁻⁶
	THQ	24.57x10 ⁻³	377x10 ⁻³	92x10 ⁻³
M. cephalus	CR	-	-	3.15x10 ⁻⁶
P. erythrinus	THQ	21.64x10 ⁻³	310x10 ⁻³	65x10 ⁻³
	CR	-	-	2.23x10 ⁻⁶
	THQ	31.5x10 ⁻³	374x10 ⁻³	55x10 ⁻³
M. DUrDatus	CR	-	-	1.89x10 ⁻⁶

4. Discussion

Fish has a healthy food source for human, so the determination of heavy metal levels in fish have become the focus of attention, especially after the Minemata disaster, which occurred as a result of the consumption of methyl-mercury-containing fish in Japan (Castro-Gonzalez and Mendez-Armenta 2008). If the excretion of heavy metals from the body is negligible compared to the intake, it causes accumulation in tissues. This accumulation is tried to be regulated by activating homeostatic mechanisms. However, insufficiency of homeostatic mechanisms due to the increase in intake results in acute toxicity (Javed and Usmani 2017).

Heavy metal accumulation in fish tissues varies depending on length and weight, as well as various factors (Canpolat and Calta 2003). It was investigated whether there was a relationship between tissue metal concentration and length in *M. barbatus, Solea vulgaris* and *Diplodus annularis* in the Izmir Bay (Küçüksezgin et al. 2002). A negative correlation was found between Cd, Cr, Cu, Fe, Pb and Zn levels in liver, gill and muscle tissues and fish size in different fish species in the Northeastern Mediterranean (Canlı and Atlı 2003). So that, the same size and weight of fish were used as material in order to minimize the effect of length and weight factors on accumulation in present study.

The accumulation show an alteration also depend on the species. The muscle tissue levels of Cu, Zn, Pb and Cd in 10 different fish species sampled from the Sinop coast of the Black Sea were determined and it was found that Sprattus sprattus contained metals at higher levels than other species (Bat et al. 2012). Another research findings emphasised that, the muscle Cd, Fe, Pb, Zn, Cu, Mn, Ni, Cr, Co and Al levels in Saurida undosquamis, Sparus aurata, and M. barbatus sampled from Iskenderun Bay determined vary depending on the species (Türkmen et al. 2005). In this study, Cu, Zn and Pb tissue levels in fish caught from Kuşadası Bay differed between species, and the highest levels of Cu, Zn and Pb were detected in S. sphyraena, E. enceasicolus and M. cephalus, respectively. It is possible that this distinction between species in terms of metal levels may be due to different habitats, feeding habits and metabolisms of the species. The distinction between species in terms of tissue metal levels may be due to varies of its habitats, feeding habits and metabolisms.

Season is another factor affecting the metal accumulation in aquatic organisms. It was determined that As, Co, Cd, Cr, Cu, Fe, Mn, Ni and Zn levels in the muscle and liver tissues of *Scomber japonicus* sampled seasonally from Antalya Bay, increased in spring and decreased in autumn generally (Aktan and Tekin- Ozan 2012). In this study, Cu, Zn and Pb levels in muscle tissue changed depending on the season, while Cu and Zn were determined the highest in winter season, Pb was determined most in spring. The seasonal difference in metal levels in tissue may be due to changes in the physicochemical properties of seawater.

Fe, Cu, Cr, As, Cd and Pb levels in muscle, gill, brain and liver tissues of Pelates quadrilineatus, Upeneus moluccensis, Nemipterus randalli, Saurida lessepsianus sampled from Taşucu region (NE Mediterranean) were investigated and it was found that Cd could have not be detected in all fish tissues, except liver tissue (Karaytuğ et al. 2018). In present study, the Cd level in the muscle tissue of E. encrasicolus, S. aurira, S. sphyraena, P. erythrinus, M. cephalus and M. barbatus from Kuşadası Bay could be determined under the detection limit of ICP-MS. Cu, Zn, Pb, Cd and Fe levels in the tissues of different fish species sampled from the Yumurtalık shores of Iskenderun Bay in January, April, June and July 2002 were investigated. In the study, it was determined that the highest metal level was found in *Solea solea* sampled in June. There was a relationship between metals in terms of accumulation such as Zn>Cu>Pb>Fe>Cd was established (Çoğun et al. 2006). In this study, which was carried out in the 2016-2017 hunting season, a similar relationship was found between metals in terms of accumulation level in all of the examined species, and it is possible that this may be due to the difference in metal metabolism.

Metal levels in muscle tissue of *S. solea, M. barbatus* and *Sardina pilchardus,* sold in supermarkets in Mersin, were determined as Cu; 0.01-1.96, Zn; 1.28-45.95 and Pb; 0.02-1.37 μ g g⁻¹ ww (Korkmaz et al. 2017). Cu, Zn and Pb levels in muscle tissues of some consumable fish and crustacean species caught from the Northeast Mediterranean coast were found as 0.12-20.62, 14.77-119.01 and 4.26-6.56 μ g g w.w. respectively (Külcü et al. 2014). In another research, Cd, Cu, Zn and Pb level in muscle tissue of *E. encrasicolus* and *Spicara smaris* sampled from the Black Sea, Marmara and Aegean Sea coasts of Turkey were found to be 0.01-0.07, 0.21-8.58,

7.12-45.6 and 0.12-0.87 mg/kg w.w. respectively (Turkmen et al. 2008). In this study, Cu, Zn and Pb levels in muscle tissues of six different consumable fish species sampled from Kuşadası Bay were detected as 0.80-5.82, 13.09-41.60 and 0.20-1.30 μ g/g w.w. respectively. The results found in present study was similar to the previous research findings. It has been seen that these values are far below the acceptable limits set in foodstuffs for toxic chemicals by various national and international organizations. This indicates that the species examined in the study do not pose any health risks if consumed as food.

The tolerable daily intake of a substance is defined as the daily intake, without any risk, based on body weight. The tolerable daily intake of a metal varies depending on the amount of food consumed and the metal concentration in the food (Ikema and Egieborb 2005). According to FAO (2005), the daily fish consumption of a person weighing 70 kg in Turkey is 20 g, which corresponds to 140 g per week. The muscle tissue Cd, Cu, Pb and Zn levels of 14 different fish species sampled from the Turkish seas were determined and the estimated weekly metal uptake values as a result of their consumption were determined as 4.0-7.4, 3.2-214; 4.6-23 and 113-218 (μ g/70 kg body weight) respectively (Ates et al. 2015). If Trachurus mediterraneus, S. aurata and Pegusa lascaris offered for consumption from Karatas fishing shelter are consumed as food, the estimated daily metal intake levels for Cr, Zn, Pb, Cu, Cd and As were 1.6-5.6, 7.2-11.4, 1.4-2.2, 5.0-7.4 respectively. It has been determined that it varies between 0.1-0.6 and 6.2-9.8 μ g/70 kg body weight, and these values are well below the tolerable daily and weekly intake values (Karayakar et al. 2022). The estimated daily intake levels for Cu, Zn and Pb in consumable six different fish species sampled from Kuşadası Bay in present study that were determined between 60.6-88.2, 423-832 and 9.2-26 µg/70 kg body weight respectively. It has been determined that these values are well below the tolerable levels, so their consumption does not pose any risk in terms of health.

Systemic effects may occur when the THQ value is above 1.0. THQ means higher than the reference dose (Copat et al. 2013). In general, Cancer Risk (CR) assessment is performed by metals such as As, Cr, Cd and Pb, which are determined to be carcinogenic by the US Environmental Protection Agency (2018). The US Environmental Protection Agency has determined the average CR levels acceptable for public health as 1x10⁻⁶-1x10⁻⁴. THQ values for 16 different species of Cd, Cu, Zn, As and Pb sampled from 6 stations determined between Hatay-Samandağ and Mersin-Taşucu in the Northeastern Mediterranean was determined as 5.71 E⁻⁰⁵, 1.27 E⁻⁰², $8.74 E^{-02}$, $1.36 E^{-00}$ and $2.68 E^{-05}$ respectively (Korkmaz et al. 2019). It was determined that the THQ values for Cd, Zn, Cu and Pb in T. mediterraneus, S. aurata and P. lascaris sampled from Karataş were found as 8.5E⁻⁰³ - 1.4E⁻⁰³, 5.4E⁻⁰⁴- 3.4E⁻⁰⁴, 2.6E⁻⁰³-1.8E⁻⁰³ ve 7.8E⁻⁰³-5.0E⁻⁰³ respectively (Karayakar et al. 2022). In this study, the THO values for Cu, Zn and Pb in 6 different fish species were ascertained as 2.1E⁻⁰² - 4.1E⁻⁰², 20E⁻⁰² - 37E⁻⁰² ve 3E⁻ ⁰²-9E⁻⁰² respectively.

Since some of the heavy metals are carcinogenic, Cancer Risk (CR) values for heavy metals such as As, Cd and Pb have been determined by various organizations. According to the US Environmental Protection Agency, metals with CR values between $1x10^{-6}$ and $1x10^{-4}$ do not pose any health risks (USEPA, 2018). Korkmaz et al. (2019) reported that the CR value for Pb was determined as 2.42E⁻⁰⁵ in 16 different fish species in the Northeastern Mediterranean. In P. lascaris, T. mediterraneus and S. aurata, the CR values for Cd and Pb were stated as 9.0E-⁰⁶, 2.7E⁻⁰⁷, 5.4E⁻⁰⁵, 1.7E⁻⁰⁷ ve 1.1E⁻⁰⁵, 1.9E⁻⁰⁷ respectively (Karayakar et al. 2022). In this study, CR values could not be calculated since muscle tissue of six consumable fish species sampled from Kuşadası Bay in this study Cd levels could not be determined. However, it was determined that the CR values for Pb varied between $3.5E^{-06}$ and $1.1E^{-06}$, and it was determined that the consumption of the mentioned species did not pose any health risks.

5. Conclusion

Cu, Zn, Pb and Cd levels in muscle tissue and its seasonal changes were investigated in E. engrasicolus, S. sphyraena, S. aurita, P. erythrinus, M. cephalus and M. barbatus sampled from Kuşadası Bay. It has been revealed whether the heavy metal levels in the muscle tissues, which constitute the consumable part of the species in question, are suitable for consumption and whether they pose a risk to public health if consumed. The levels of copper, zinc and lead differed between species as well as changing depending on the seasons. While copper and zinc were determined the highest in *E*. encrasicolus is pelagic, the highest amount of lead was determined in *M. cephalus* is benthopelagic. In all examined species, a relationship was found as Zn>Cu>Pb between metals in terms of concentration in muscle tissue. It has been observed that the heavy metal levels determined in the muscle tissues of the fish are far below the acceptable levels determined by national and international organizations in terms of consumption. It has been determined that daily and weekly Cu, Zn and Pb uptake levels are lower than the tolerable daily and weekly metal uptake levels in case of consumption of the mentioned species. It was determined that the consumption of E. engrasicolus, S. sphyraena, S. aurita, P. erythrinus, M. cephalus and M. barbatus, which were caught and offered for consumption from the Kuşadası fish market, did not pose a risk.

Author contributions

The authors declare that they have contributed equally to the article.

Conflicts of interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

Research and publication ethics were complied with in the study.

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Zonation-Related Alteration in Shell Morphology of Patella caerulea (Linnaeus, 1758) Distributed in the Mersin Coastline (Mersin Bay, NE Mediterranean Sea)

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Patella caerulea, Shell morphology, Zonation, Mersin-Viranșehir.

Research Article

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Abstract

In this study, it was aimed to examine the zonation-related changes in the shell morphology of Patella caerulea (Linnaeus, 1758) belonging to the Patellidae family of the Archaeogastopoda (Thiele, 1925) order, which is distributed along the Mersin coastline. The research area consists of three regions of 4 m2 covering the supralittoral at sea level and the mediolittoral and infralittoral zones below the sea water level determined from the Mersin Viranșehir coast. A total of 80 individuals were sampled from the study area. Species identification of the samples brought to the laboratory was made by considering the morphological appearance of the radula teeth. In addition, the Shell morphology of all individuals was determined. Finally, the statistical analysis of the data was evaluated by one-way analysis of variance (ANOVA) and the relationship between the variables by calculating the Pearson linear correlation coefficient. In the study, no individuals belonging to the P. caerulea were found in the supralittoral zone of all three areas of 4 m2. It was determined that all individuals collected from the mediolittoral and infralittoral zone belonged to the P. caerulea species. Morphometrically, individuals of P. caerulea distributed in the infralittoral zone were larger than those distributed in the mediolittoral zone (p<0.01). According to the shell shape analysis findings, individuals sampled from the mediolittoral zone had a narrower, elliptical shell, with the apex closer to the center. In contrast, individuals sampled from the infralittoral zone had a wider, ovule, and the apex was asymmetrical near the anterior end. Individuals sampled from both zones did not differ in terms of conicity (p>0.01). The shell surface area and shell volume of individuals sampled from the infralittoral zone are larger than those sampled from the mediolittoral zone.

1. Introduction

Patella caerulea (Linnaeus, 1758), a native species of the Mediterranean, is a limpet distributed on the rocks in the coastal zone. It belongs to the Patellidae family of the Archaeogastopoda (Thiele, 1925) order (Sella et al. 1993; Mauro et al. 2003; Sa'-Pinto et al. 2005; Boukhicha et al. 2013). Limpets are one of the most abundant mollusks on rocky shores. These animals, which continue to live by clinging to the rocks, only move to feed. Its movement occurs during stagnant times when the water rises (Williams et al. 1999). They are firmly attached to the base, even in motion, with the secretion of pedal mucus. When disturbed, they clump or collapse, allowing the shell sub-base to make direct contact with the ground to gain resistance. This behavior prevents the animals from being dislodged or detached (Ellem et al. 2002). In this process, their clinging to the ground also reduces water loss.

Rocky coastal ecosystems are influenced by complex environmental factors due to the hard blows of the waves and drying during the low tide periods. These conditions cause stress in the biota (Cabral 2007; Ayas 2010), so they develop various adaptation mechanisms against environmental stress caused by natural changes in abiotic factors under the influence of tides (Cretella et al. 1991; Öztürk and Ergen 1999; Sá-Pinto et al. 2010; Boukhicha et al. 2013). Limpets adapt to unsuitable

Değirmenci, E., Çiftçi, N. & Ayas, D. (2023). Zonation-Related Alteration in Shell Morphology of Patella caerulea (Linnaeus, 1758) Distributed in the Mersin Coastline (Mersin Bay, NE Mediterranean Sea). Advanced Underwater Sciences, 3(1), 09-14.

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environmental conditions with changes in shell morphology. It has been reported that *P. caerulea*, which remains above the water level during the tidal period, increases the shell height in order to reduce water loss (Öztürk and Ergen 1999).

The coastal regions of the Mersin have rocky and dune ecosystems. The gulf formed by the indentation of the coastline into the land towards the north and the richness of freshwater resources causes an increase in biodiversity. The coasts are influenced by industry in the east and agricultural activities in the west. In addition, the Mersin coasts are under the influence of maritime trade activities due to the port it has and tourism activities due to the long tourism season. Furthermore, the city has an ever-increasing population due to immigration, and domestic waste is increasing in parallel.

Morphometric studies aid in identifying a species and its classification, determining biological diversity and population dynamics, and determining the effects of ecological changes on the distribution of the species. In this study, it was aimed to determine the alteration on shell morphology of *P. caerulea*, which was sampled from the mediolittoral and infralittoral zones of the Viranşehir coastline, which is under urban pressure on the Mersin coastline.

2. Method

In the study, individuals of *Patella caerulea*, one of the Archeogastropoda species distributed in the Mersin Coastline, sampled from the Mersin-Viranşehir coasts, were used as a material. Samples were collected from three areas, each covering 4 m^2 , 0.5 m above sea level and 0.5 m below sea level, 1 m vertically and 4 m horizontally in the rocky tidal zone.

Collected samples were placed in glass jars containing 4% formaldehyde and labeled. Species were identified for the samples brought to the laboratory. The morphological appearance of the radula teeth was used for species identification (Fisher-Piette and Gaillard 1959; Gaillard 1987; Öztürk and Ergen 1999). Shell morphometric properties were measured with a caliper with a precision of 0.01 mm. In determining the shell morphology of each individual, the shell length (SL), the distance between the apex and the anterior end of the shell (SAA), the distance between the apex and the posterior end of the shell (SAP), the shell width (SW), the shell width at the apex (SWA), the posterior shell length (PL), anterior shell length (AL) and shell height (SH) were determined (Figure 1).



Figure 1. Determined morphometric features of P. caerulea sampled from Mersin Viransehir coasts in the present study.

For each individual, after morphometric measurements, the conicity of the shell (SH/SL), the cone eccentricity (SAA/SAP), the base ellipticity (SW/SL), the base eccentricity (SWA/SW) ratios were determined, and their averages were compared using ANOVA. The shell surface area (SS) and shell volume (SV) were calculated using the formulas for surface area (1) and volume (2) of a parabolic cone. The shell base radius (BR) was calculated using formula (3), the base perimeter (BP) formula (4), base surface area (BS) formula (5), and the total surface area of exposure formula (6). The comparison of the mean values of the variables describing the shell shape was made with ANOVA. The relationship between the variables was evaluated by calculating the Pearson linear correlation coefficient. The least squares method was used in all regressions.

Table 1 shows the variables used in the analysis of shell shape and shape trends of Patella limpets.

$$SS = 3.6 \times BR \times \sqrt{(BR)^2 + \left(\left(\frac{4}{3}\right) \times SH\right)}$$
⁽¹⁾

$$SV = \left[\frac{\pi \times BR^2 \times SH}{2}\right] \tag{2}$$

$$BR = \frac{SL + SW}{4} \tag{3}$$

$$BP = 2\pi \times BR \tag{4}$$

$$BS = \pi \times BR^2 \tag{5}$$

$$TSA = BS + SS \tag{6}$$

Table 1. The variables used in the ana	ysis of the shell sha	pe of Patella limpets	(Cabral 2007)
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Shape parameter	Variable	Trends
Base ellipticity	SW/SL	=1 Circle <1 Ellipse/Oval/Parabole/Ovule <1, the ratio increases with decreasing ellipticity
Base eccentricity	SWA/SW	≈1 Circle / Ellipse /Oval/Parabole <1 ovule <1, the ratio increases with the transition from ovule to ellipse
Conicity	SH/SL	Increases with increasing conicity
Cone eccentricity	SAA/SAP	=1 Centred apex/Symmetrical cone <1 Apex near the anterior end <1, the ratio increases with decreasing eccentricity

The comparison of the means of the variables describing the shell shape was made with ANOVA.

3. Results

No individuals belonging to the *P. caerulea* species were found in the supralittoral zones in the sampling areas. A total of 80 individuals were sampled from the mediolittoral and infralittoral zones in all sampling areas. In the species identification made by considering the radula morphological features of 80 individuals collected from all sampling areas, it was determined that all individuals sampled belonged to the *P. caerulea* species.

It was determined that individuals distributed in the mediolittoral zone and the infralittoral zone differed in terms of the morphometric features examined (p<0.01) (Table 2). In addition, individuals in the infralittoral zone were larger than those in the mediolittoral zone (p<0.01).

Table 2. Arithmetic mean and standard errors of morphometric characteristics of *P. caerulea* sampled from Mersin Viransehir coast

Morphometric	Mediolittoral (n=41)	Infralittoral (n=39)	ANOVA
measurements	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	
(mm)			
SL	28.16 ± 7.76	30.80 ± 8.01	< 0.01
SAA	17.45 ± 4.50	19.18 ± 4.55	< 0.01
SAP	20.41 ± 5.77	23.83 ± 6.89	< 0.01
SW	22.13 ± 7.69	25.03 ± 8.10	< 0.01
SWA	19.74 ± 7.29	21.41 ± 7.61	< 0.01
PL	17.20 ± 5.47	19.70 ± 6.66	< 0.01
AL	15.13 ± 4.14	16.41 ± 4.34	< 0.01
SH	7.45 ± 2,.0	8.24 ± 2.71	<0.01

 $\bar{x} \pm s_x$: Arithmetic Mean and standard error

The base ellipticity, the base eccentricity, the conicity, and the cone eccentricity of *P. caerulea* sampled

from Mersin Viranșehir coasts were calculated, and their averages were compared using ANOVA (Table 3).

Fable 3. Shell shape analysis res	ults of <i>P. caerulea</i> sam	pled from Mersin	Viranșehir coasts
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Shape parameter	Variable	$ \begin{array}{l} \text{Mediolittoral} \\ (n=41) \\ \bar{x} \pm s_x \end{array} $	Infralittoral (n=39) $\bar{x} \pm s_x$	ANOVA
Base ellipticity	SW/SL	0.77 ± 0.07	0.80 ± 0.08	<0.01
Base eccentricity	SWA/SW	0.89 ± 0.06	0.69± 0.09	<0.01
Conicity	SH/SL	0.27 ± 0.04	0.27 ± 0.03	>0.01
Cone eccentricity	SAA/SAP	0.90 ± 0.09	0.86 ± 0.10	< 0.01

 $\bar{x} \pm s_x$: Arithmetic Mean and standard error

In the study, according to the shell shape analysis results of individuals sampled from the mediolittoral (n=41) and infralittoral (n=39) zones, the base ellipticity of the individuals distributed in the mediolittoral zone was determined as 0.77, and according to the shell shape trends classification, <1 Ellipse/Ovule/Parabole, can be defined as an ovule. On the other hand, the base ellipticity of individuals sampled from the infralittoral zone was found to be 0.80. Although, according to the shell shape trends, <1, ellipcity decreases as the ratio increases, individuals sampled from the mediolittoral zone have a narrower shell shape than individuals sampled from the infralittoral zone.

Regarding base eccentricity, individuals sampled from the mediolittoral zone were found to be 0.89. According to the shape trends, <1, a transition from an ovule to an ellipse as the ratio increases can be defined as an ellipse. Individuals sampled from the infralittoral zone were found to be 0.69. According to the shape trends, <1 can be specified ovule.

According to shell shape trends, the conicity increases as the SH/SL ratio increases. Therefore, the SH/SL ratio of individuals sampled from the mediolittoral and infralittoral zones was determined as 0.27, and there was no difference between individuals (p>0.01).

Cone eccentricity was determined as 0.90 in individuals sampled from the mediolittoral zone and 0.86 in individuals sampled from the infralittoral zone. According to the shell shape trends, individuals from both zones are suitable for grouping "<1 Apex near the anterior end". However, according to the statement "<1, the ratio increases with decreasing eccentricity", individuals sampled from the infralittoral zone were found asymmetric, while individuals sampled from mediolittoral zone were found closer to the center.

In the study, individuals sampled from the mediolittoral zone had a narrower, elliptical shell, with the apex closer to the center. In contrast, the individuals sampled from the infralittoral zone were found to be wider, with the ovule and the apex asymmetrical near the anterior end (p<0.01). Individuals sampled from both zones did not differ in terms of conicity (p>0.01).

Shell surface area (SS), shell volume (SV), and the total surface area of exposure (TSA) of *P. caerulea* sampled from mediolittoral and infralittoral zones of Mersin Viranşehir coasts were calculated (Table 4).

Table 4. The mean and standard errors of SS, SV, and TSA of *P. caerulea* sampled from Mersin Viranşehir coast

	Mediolittoral (n=41)	Infralittoral (n=39)	Pearsson
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	
SS (mm ²)	542.06 ± 78.58	775.33 ± 99.89	0.001**
SV (mm ³)	2393.76 ± 554.25	3170.96 ± 661.05	0.01*
TSA (mm ²)	1181.15 ± 169.88	667.49 ± 86.49	0.001**

 $\bar{x} \pm s_x$: Arithmetic Mean and standard error

**Correlation (Pearson) is significant at the 0.001 level (2-tailed)

* Correlation (Pearson) is significant at the 0.01 level (2-tailed)

4. Discussion

It was aimed to examine the shell morphology of the individuals of P. caerulea collected from 3 different sampling areas on the Viransehir coasts. No individual belonging to the species was found in the supralittoral zone. In a previous study, the distribution of *P. caerulea* and P. rustica in the Mersin-Viransehir coast was reported as 88.89% and 11.11%, respectively. It has been stated that *P. caerulea* is located in the upper infralittoral and mediolittoral zone, and *P.rustica* is located in the supralittoral zone (Ayas 2010). Individuals belonging to the Patella genus were collected from the supralittoral, mediolittoral and superinfralittoral zones in the Gulf of Saros (Aegean Sea), and the distribution of *P. caerulea* in the mediolittoral and upper infralittoral zones was determined (Öztürk and Ergen 1999). Our findings that P. caerulea sampled from Mersin Viransehir coasts was found in the mediolittoral, and infralittoral zones are consistent with previous research findings.

The effects of environmental factors on shell morphology have been reported in species belonging to the Patellidae family (Sá-Pinto et al., 2010). Patella *rustica* is characterized by brown spots near the top of the shell. Although these brown spots are also seen on the shell of *P. caerulea* in some regions, the shell height of P. caerulea is lower than that of P. rustica. P. caerulea and P. ulyssiponensis show wide morphological variations with overlapping shell morphology and color in various regions (Cretella et al. 1991). It was stated in a study that the shell height of *P. caerulea* sampled from the Aegean Sea, above the water level, increased, and the radula length was higher. It was noted in a previous study that individuals of P. caerulea sampled from above the waterline increased the shell height, and the radula length was higher. It is an adaptation that reduces the

water loss of patellid individuals which stay in a dry environment for a long time.

It was determined in this study that *P. caerulea* individuals sampled from the mediolittoral zone differed statistically from the individuals sampled from the infralittoral zone in terms of their morphometric characteristics (p<0.01). In the present study, individuals sampled from the infralittoral zone were larger than those sampled from the mediolittoral zone. The mean SL: 29.48, SW: 23.58 and SH: 7.85 mm were found in individuals sampled from both zones of Mersin Viranşehir coasts. The average shell length of *P. caerulea* individuals sampled from Saros Bay was reported as 31.1 mm, shell width 25.9 mm, and shell height 8.3 mm (Öztürk and Ergen 1999). The results of both studies were similar.

Morphometric features are very important in systematically defining a species, determining its biological requirements, and examining ecological effects. In P. rustica, P. ferruginea, P. caerulea and P. ulyssiponensis distributed in the Mediterranean, the shell and radula characters and the shell surface area/shell volume ratio were examined, and the results of the stress effect they encountered in the rocky ecosystem depending on their zone were compared. It has been reported that there are specific differences in shell shape, the relative size of the radula, and SSA/SV ratio among the species examined in a study (Boukhicha et al., 2013). Morphologically, the conicity of the shell was found to be the highest in P. rustica and the lowest in P. ulyssiponensis in the same study. The shell cone eccentricity was noted to show a very asymmetrical cone in *P. ferruginea* and a more centered apex in *P. rustica*. The shell base ellipticity showed that they had a narrower shell base in P. ulyssiponensis and a wider shell base in P. caerulea. It was emphasized that the relative size of the radula increased

in *P. rustica* from *P. ulyssiponensis* (Boukhicha et al. 2013).

In the present study, the shell was narrower, and elliptical, and the apex was closer to the center in the individuals sampled from the mediolittoral zone. In contrast, the individuals sampled from the infralittoral zone were found to be wider, with the ovule and the apex asymmetrical near the anterior end (p<0.01). Individuals sampled from both zones did not differ in terms of conicity (p>0.01). Shell surface area (SS), shell volume (SV) and total exposure surface area (TSA) of P. caerulea sampled from Mersin Viranşehir coasts were determined in individuals sampled from the mediolittoral and infralittoral zones. The SS is larger in individuals sampled from the infralittoral zone than those sampled from the mediolittoral zone in the present study. When individuals sampled infralittoral are compared with those sampled from the mediolittoral zone, there is a significant difference at the p<0.001 level in terms of SS. SV was higher in individuals sampled from the infralittoral zone than those sampled from the mediolittoral zone (p<0.01). On the other hand, TSA was higher in individuals sampled from the mediolittoral zone than in individuals sampled from the infralittoral zone (p<0.001). This separation in different zones can be associated with the effect of environmental factors.

It has been reported that changing environmental factors such as waves, sunlight, tidal intensity, and the geomorphological zone affect the shell morphology of the Patellidae, constituting the most critical living group of the rocky ecosystem. Therefore, the determined distinctions in the shell morphology of *P. caerulea* in this study are consistent with previous studies.

5. Conclusion

It is aimed to distinguish in terms of shell morphology in individuals of P. caerulea, one of the Patellidae species distributed along the Mersin coastline, sampled from two different tidal zones. The sampling area is under urban pressure, and the contribution of anthropogenic activities to the impact of natural changes in rocky ecosystems cannot be denied. Changes in the abiotic environment with the climate crisis also cause changes in the morphology of Archeogastropod shells. The main reason for this change is to adapt to changing environmental conditions. Environmental stress management may also be the main reason for the distinction between the mediolittoral zone and the infralittoral zone regarding shell morphology in individuals sampled from the Mersin Viranșehir coasts.

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Author contributions

The authors declare that they have contributed equally to the article.

Conflicts of interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

Research and publication ethics were complied with in the study.

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Additional record of *Priacanthus sagittarius* Starnes, 1988 from the Northeastern Mediterranean coast of Turkey

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Priacanthidae, Record, Arrow Bulleye, Mediterranean Sea, Turkey.

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Abstract

A single specimen of Priacanthus sagittarius Starnes, 1988, has been collected in the Mersin Bay (off Yeşilovacık, Turkey) at a depth of 90 m in November 2022. The presence of P. sagittarius in the Mediterranean coast of Turkey is evidently due to migration from the Red Sea through the Suez Canal. This species appears to spread rapidly and easily established in the Eastern Mediterranean Sea. Besides, this ichthyological note is important and represents the third successive record from Turkish waters in the Northeastern Mediterranean Sea. The present study will be useful in the field of fisheries scientists and, at the same time, contribute to fisheries management.

1. Introduction

The genus *Priacanthus* Oken 1817 is represented by 12 valid species worldwide (Froese and Pauly 2022). This genus is comprised of four species in the Mediterranean, namely, Atlantic bigeye *Priacanthus arenatus* Cuvier, 1829, moontail bullseye *Priacanthus hamrur* (Forsskål, 1775), elongate bulleye *Priacanthus prolixus* Starnes, 1988 and arrow bulleye *Priacanthus sagittarius* Starnes, 1988 (Quignard and Tomasini 2010; Goren et al. 2010; Gürlek et al. 2017; Ergüden et al. 2018). However, another species is known to occur in the Red Sea: paeony bulleye *Priacanthus blochii* Bleeker, 1853; it has not been recorded in the Mediterranean waters (Gürlek et al. 2021).

The arrow bulleye, *Priacanthus sagittarius* is distributed from the Indo-west Pacific to the

Mediterranean (Froese and Pauly 2022). It is a demersal marine fish species for the Mediterranean waters. This species is usually found in sheltered reefs, caves or rocky areas (Kuiter and Tonozuka 2001).

Priacanthus sagittarius in the Mediterranean coast of Turkey is evidently due to migration from the Red Sea through the Suez Canal. This species has rapidly reached to eastern Levantine coast of the Mediterranean since the first recorded from Israel in 2010 (Goren et al. 2010), and then the second record again in the Israel coast (Haifa) (Golani et al. 2011). Then several specimens were reported from Israel and Lebanon waters (Golani et al. 2021).

In recent years, *P. sagittarius* has been distributed in the Mediterranean waters, especially in Egyptian and Syrian waters (Farrag et al. 2016; Alshawy et al. 2019).

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Although *P. sagittarius* was previously recorded in the southeastern Mediterranean coast of Turkey, a misidentification of this species was probably made by Yapıcı and Hasbek (2018) based on photographic analysis. Later Ergüden et al. (2018) stated in the study that the misidentified of this species and, it was identificated as *P. hamrur* from İskenderun Bay.

In the Turkish seas, Gökoglu and Teker (2018) first recorded of *P. sagittarius* from off the Tasucu coast (Mersin). Then Gürlek et al. (2021) reported a single specimen from the Konacık beach (Iskenderun Bay).

In the present paper, we report the third occurrence of *P. sagittarius* from the N.E. Mediterranean waters of Turkey (Yesilovacık, Mersin Bay). The current record indicates a westward migration of *Pricanthus* species in the Mediterranean Sea coast of Turkey.

2. Method

On 09 July 2022, a single specimen of *P. sagittarius* was captured by a commercial trawler at a depth of 90 m off Yesilovacık (36°06'084"N, 33°37'133"E), a locality situated 30 km northwest of Silifke (Fig. 1). The *P. sagittarus* specimen was brought aboard, photographed and identified. The identification of the present specimen (Fig. 2) agrees with the description given by (Starnes 1988; Goren et al. 2010; Golani et al. 2011).



Figure 1. Map of the study area, indicating the capturing points (●) of *Priacanthus Sagittarius*



Figure 2. *Priacanthus sagittarius* from the North-Eastern Mediterranean coast of Turkey

3. Results

The Mediterranean specimen of *P. sagittarius* is described as follows: Dorsal fin ray, X+13; Anal fin ray, 13; Pectoral fin ray, 18; Caudal fin ray, 17.

The body is deep and strongly compressed, the head is large, dorsal fins are continuous. The eyes are enormous; the mouth is strongly oblique. The reoperculum margin is serrated, and the preopercular spine is short and broad. The caudal fin is emarginated (Goren et al. 2010; Golani et al. 2011).

Color (fresh specimen): The head and body are generally reddish silvery with grey mottling. The iris of the eye is pink to bright red. The dorsal, anal, and caudal fins are pink, and the margins of the dorsal, anal and caudal fins are dark and have a black mark at the pectoral fin base.

Table 1. Capture records of Priacanthus sagittari	s from Mediterranean Sea covering the period 2009-2022
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Author	N. of Samples	Record Date	Location/ Country	Gear	Depth (m)	Standard Length (mm)
Goren et al. (2010)	1	28.11.2009	Ashod, Israel	Trawl	40	114.0
Golani et al. (2011)	1	22.10.2010	Haifa Bay, Israel	Trawl	50	181.0
Farrag et al. (2016)	1	19.05.2015	Alexandria, Egypt	Trawl	35	108.0
Gökoğlu and Teker (2018)	1	27.11.2017	Tasucu, Turkey	Trawl	100	200.0
Alshawy et al. (2019)	1	15.03.2019	Banyas, Syria	Longline	100-125	154.0
Gürlek et al. (2021)	1	24.02.2021	Konacık, Turkey	Trawl	80	106.0
This study	1	09.11.2022	Yesilovacık, Mersin Bay, Turkey	Trawl	90	102.0

4. Discussion

To date, four species belonging to the genus *Pricanthus* are known from the Mediterranean. Goren et al. (2010) stated that *P. sagittarius* differs from other *Pricanthus* species, *P. arenatus* and *P. blochii*, *P. hamrur*. Although *P. sagittarius* resembles *P. blochii*, it differs from it by having pointed soft dorsal and anal fins and a black membrane between the first and second dorsal spine. At the same time, this species shows differences having the black blotch at the base of the pelvic fin and the long tenth dorsal spine, which is almost double the length of the second spine from the other three Priacanthid species.

Priacanthus sagittarus is a solitary species, usually seen alone or in small groups (Froese and Pauly 2023). It feeds on small fish, and large invertebrates (Golani et al. 2021). *P. sagittarus* is usually occur in the size range of 15 to 30 cm (Golani et al. 2021). The recorded maximum length (SL) is 35 cm for this species (Kuiter and Tonozuka, 2001). *P. sagittarus* is usually found at depths of 15-350 m (IUCN 2023). However, the single specimen reported in this study was observed at 90 m depth. This depth range is in accordance with the literature (Goldshmidt 1996).

This study gives a new location record of *P. sagittarius* from the Northeastern Mediterranean, Turkey. The finding of a third specimen in Turkey's North-eastern Mediterranean Sea coast in less than a year and a half after its previous record suggests that this species has probably been an established population in the same region. This observation indicates that the habitat of this geographic area is more efficient for its reproduction and spread. Golani (1998) reported that most alien fish species are characterized by their high mobility and high rate of reproduction in the Mediterranean Sea.

The data presented here are essential in terms of the current status of the alien species, possible population establishment, and biodiversity in the region. The historical captured record of the species in the Mediterranean Sea is documented in Table 1. Since the role of this newly established alien species in the coastal ecosystem currently constitutes a limited population, the impact of this species on local populations needs to be investigated in the next few years.

5. Conclusion

Monitoring coastal habitats and biodiversity is important, as monitoring studies increasingly contribute to the discovery of new alien fish species, especially using long-term monitoring for timely assessment and management due to marine environment changes. Thus, further research is required to reveal details about the habitat requirements for establishing new alien species in the region. Besides the present study will be useful in the field of fisheries scientists and, at the same time, contribute to fisheries management.

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Author contributions

Deniz Ergüden (DE): Investigation, data analysis, writing, sample design and methodology. Deniz Ayas (DA): Data collection, data curation and editing. Sibel Alagöz Ergüden (SAE): Investigation, validation, supervision and final editing.

Conflicts of interest

The authors declare that for this article they have no actual, potential, or perceived conflict of interest.

Statement of Research and Publication Ethics

For this type of study formal consent is not required.

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Investigation of Underwater Photogrammetry Method: Challenges and Photo Capturing Scenarios of the Method

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Abstract

Three-dimensional (3D) documentation of an underwater object and its transfer to digital platforms have gained worldwide importance in recent years. For this purpose, the photogrammetry method has been tried underwater and the term underwater photogrammetry has found its place in the literature. The most important reason for preferring the photogrammetry method is that it collects data in a shorter time compared to other methods and provides a positive contribution in terms of time and cost. However, there are both environmental and physical limitations in the underwater photographic data collection process. Various suggestions have been made to minimize these restrictions. The method, in which three-dimensional (3D) data can be produced from moving records of objects known as Structure from Motion-SfM), is also used in underwater photogrammetry. In addition, software used in other photogrammetry methods can also be used in underwater photogrammetry. But in the applications made: According to the photographs taken by other photogrammetry methods, in the photographs taken under water; It is foreseen that some corrections such as color and contrast correction, shadow removal and highlight reduction should be applied with image processing before they can be evaluated in software. Like other photogrammetry methods, underwater photogrammetry has kept pace with technological developments.

1. Introduction

Underwater photography requires special skills, experience and difficulties that require detailed work. These difficulties are being able to see living things and colors, taking shots without damaging them, using the right light and equipment according to the subject, correct timing, keeping diving safety and taking photos according to the purpose of the work. In recent years, studies have been carried out frequently in the fields of military, civil and academia with underwater photography. At the beginning of these studies, it includes many subjects such as the detection of archaeological sites, monitoring of underwater ecosystems, exploration of underwater resources, target positioning and identification. Underwater photogrammetry is the most widely used method for these subjects. Underwater photogrammetry, which is a sub-branch of photogrammetry, enables to determine the position, shape and size of objects and to extract 3D information with the help of photographs taken (Anelli et al., 2019; Baletti et al., 2015; Barnes, 1952).

biggest advantage of the underwater The photogrammetry method is the contribution it provides in terms of time and cost. Since this method can produce base data and models for many disciplines, it is frequently preferred for different purposes and directions. Especially with the developments in computer technology, the Structure from Motion (SfM) algorithm, as well as the photogrammetry technique, offered new perspectives to the operators in the use of this method. With this algorithm, the camera calibration parameters previously calculated by the operators can be calculated directly through software. The 3D geometry of the structure or object to be created with this algorithm is solved by finding the matched detail points in at least two photographs obtained by using different perspectives during the measurement. With the

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advantages provided by the SfM algorithm, changes have also emerged in photography with the photogrammetry technique. In order for the SfM algorithm to perform at an optimum level, it is necessary to take sequential photos. While sequential photography can be done easily on the ground, the situation is different under water. There are fundamental difficulties in photographing underwater photogrammetry. In this study, the main difficulties of underwater photogrammetry are mentioned and photography techniques are examined within the framework of these difficulties (Burns and Delparte, 2017; Casella et al., 2017).

2. Method

It is very important to have good planning because of sudden changing weather conditions, underwater currents, water movement and limited time underwater. In order to shoot in accordance with the photogrammetric shooting principle, an experienced and competent diver is needed in the underwater environment. Especially in photogrammetric studies performed by the diver in shallow water, problems such as camera position, camera angle, camera movement speed and image stabilization may occur, since the buoyancy of the water cannot be controlled.

Unpredictable underwater environment, unpredictable weather change creates shadow problem and reflection problem. Artificial light sources integrated into the underwater housing may be required to help with these problems (Mallet and Pelletier, 2014; Menna, 2016; Figure 1).



Figure 1. Housing and artificial light source

The blowing caused by the wind causes the sun's rays on the water surface to vibrate and thus shiny textures called caustics occur on the water floor or the objects on the bottom (Agrafiotis, 2018). Caustics are undesirable for photogrammetric applications. This is because it affects the extraction of two-dimensional content data from the photo as well as creating poor quality object texture. The intensity of the light caustics depends on the slope of the sun angle, water turbidity and depth, but after a few meters, the effect gradually decreases (Menna 2016; Kaya et al., 2019). In his study, Menna (2016) argued that it is necessary to use artificial light sources such as electronic flashes in underwater environments, especially in deep places, because both the colors can be reflected as they are and the aperture due to the limited viewing angle can be compensated. As a general rule, in waters up to 10 meters deep, diffraction effects, called caustics, can be a problem for active radial sensors (Hamal et al., 2022; Figure 2).



Figure 2. Underwater caustic

Agrafiotis et al. (2018) stated that although photogrammetric applications in deep waters are carried out at noon when higher brightness conditions are provided, strong artificial light sources are needed due to reasons such as providing the image coverage ratio in shallow waters, the sun in a low orbit, and avoiding artificial images on the sea surface. In addition to all these light-based problems, problems may arise due to the inability to control the buoyancy of the water in photogrammetric studies performed by the diver in shallow waters during the data acquisition phase (Hamal and Ulvi, 2020). Accordingly, the waves can strain the balance of the diver and the camera. All these situations can of course cause difficulties in deep waters due to the flow of water. It is not a preferred method when the distance between the camera distance and the imaged object is short enough to be less than 1 meter in studies conducted in shallow waters. Because the point where the camera is positioned cannot be marked exactly, the amount of data to be processed increases, the image scale grows, and this causes the storage capacity to be larger with the increase in data processing time. In some unusual situations there may not be enough space for the diver and the camera. To overcome this, either lenses known as fisheye or bi-media photogrammetric approaches such as water-to-air or air-to-water are applied (Hamal et al., 2021).

The loss of different wavelengths due to varying depths is a challenge in underwater photogrammetry. Water absorbs different wavelengths of light to varying degrees. The longest wavelengths with the lowest energy are absorbed first. The first absorbed is red, then orange and yellow. Colors fade underwater in the order they appear in the color spectrum. Water also absorbs light energy and scatters optical rays to create blurred images (Raoult et al., 2016; Casella et al., 2017; Vlachos et al., 2019). There are several ways to reduce the difficulties of lighting and color in underwater images. But fixing one difficulty can often create another. The photographer may have to decide between better image, color or better image quality. The exposure time of the camera can be increased to obtain better color, but this will result in poor image quality. Certain filters can be added to images to better capture certain wavelengths of light. It will require a filter and artificial lighting to better capture reds, oranges and yellows. These situations hindered underwater photogrammetric workers. However, thanks to the SfM-based software developed in recent years, it affects these situations relatively less (Bianco et al., 2015; Bryson et al., 2016; Nocerino et al., 2020).

Bodies of water are often filled with various floating particles that can obscure vision and interfere with a view. Particles suspended in water include gravel, sand, silt, clay, algae, seaweed, and others. Suspended particles under water affect field work. It is appropriate for operators to be close to objects (depending on water quality) between a minimum of 0.5 m and a maximum of 3 m. However, this makes it difficult to work on large scales. This seemingly limiting aspect requires having to produce a large amount of stereo pairs, but also ensures high accuracy and sensitivity (Maas, 2015; Cheng et al., 2020).

Various particles such as phytoplankton, organic substances, and pollution suspended in the water cause the water to become cloudy, and light is scattered in the water for these reasons. Scattering, or diffuse reflection, occurs due to the random deflection of light from its direction. Scattering; it limits the image quality, reduces the contrast and causes blurry images (Menna et al., 2016; Nocerino et al. 2021; Figure 3).



Figure 3. Blurry image underwater

Sea water is 800 times denser than air. All these dimensions vary depending on each other: as the temperature increases, the density increases, as the pressure increases, the salinity increases, and the pressure increases by 1 atm for every 10 meters of depth, in direct proportion to the depth. This corresponds to a change of 1.033 N/cm2. In this case, it affects underwater photogrammetric studies (Kaya et al., 2019).

3. Underwater Photo Shooting Scenarios

Shooting scenarios in underwater photogrammetry are very important in terms of the quality of the images to be captured and the accuracy of the resulting models. For this reason, shooting scenarios to be used in underwater photogrammetry must be created correctly.

Underwater surveying projects are quite challenging compared to the normal indoor/outdoor imaging procedure due to the fundamental challenges. Therefore, it is necessary to plan the shoot carefully before conducting a real shooting session. It is always better to take more images than necessary than to have insufficient image overlap or an incomplete dataset. It has been suggested to wait for as much calm air and bright light as possible to increase the visible distance. In addition, when shooting underwater, lighting or a fixed light source that will increase the visible distance and quality of the images obtained should be used.

It has been emphasized in the literature in underwater photography scenarios that it is necessary to shoot using "snake" or "spiral" routes (Figure 4).



Figure 4. Snake (Left) and Spiral (right) photography technique (Yakar et al., 2022).

4. Structure from Motion (SFM)

Photogrammetry is a branch of science that precisely determines the location, position and shape of an object by using photographs taken from different angles (Yiğit and Ulvi, 2020). The advancement of technology and the integration of photogrammetry and computer imaging technology have led to advances in automation of 3D model production with greater flexibility in 3D modeling work (Ulvi et al., 2020). Today, there is a variety of software available that allows us to make 3D models of surfaces from photographs taken with conventional cameras. Most of this software are based on proprietary algorithms such as Structure from Motion (SfM) (Yakar et al., 2016; Ulvi and Yiğit, 2022; Oruç, 2021). SfM; It is a photogrammetric algorithm that automatically solves the geometry of the scene, camera positions and orientation without requiring pre-definition of a target mesh with known 3D positions (Yakar et al., 2022). SfM, which is a measurement method based on computer visualization; It is an inexpensive method that has gained popularity recently because digital cameras, video cameras or smartphones with cameras are used (Döş and Yiğit, 2022; Oruç, 2021). For this reason, its use in scientific research has become very common. SfM algorithm; It has had a transformative impact on geoscience research due to its low cost, extremely fast results and easy 3D measurement capability. In the SfM algorithm, a series of overlapping picture frames is used to create 3D structures. It works by finding and matching commonalities across a series of overlapping photos.

Photogrammetric evaluation software is of great importance for 3D modeling studies. Many commercially used software are in use today. However, according to the content of the work to be done, the choice of photogrammetric software is of great importance. Each software has outstanding capabilities. Here are the most important points; The result is the quality of the product and how many steps and how long the processes will take (Yiğit and Uysal, 2021).

Structure-from-Motion (SfM); is a photogrammetric algorithm that automatically solves the geometry of the

scene, camera positions, and orientation without requiring pre-definition of a target mesh with known 3D positions. The 3-dimensional geometry of the scene to be analyzed is solved trigonometrically with the matched detail points in at least two images, by using the difference in viewpoints at the time of image recording. As a result of this process, a 3D point cloud is produced as many as the number of points that can be mapped in a local coordinate system. Software using the SfM technique is based on algorithms for automatic positioning of shared points between images to create a Sparse point cloud. The most widely used algorithm for this process is the Scale-Invariant Feature Transform (SIFT) algorithm, which works on radiometric pixel values (Senol et al., 2021).

The sparse point cloud created using SfM algorithms is relative and should be calibrated to actual dimensions. This calibration is performed using several known target marks. Another step in SfM is the creation of a dense point cloud. The algorithm used in this step is the Dense Multi View Stereo (DMVS) algorithm. At this stage, the mapped pixels and their estimated 3D positions become point clouds to produce a mesh model. Finally, images are used to give the model a photorealistic texture.

5. Human Health

Another feature of the photogrammetry technique that distinguishes it from other techniques is that it does not endanger human health. However, in underwater photogrammetry studies, this is the opposite. In particular, operators who will carry out underwater photogrammetry studies should receive professional training. Although training is received, it is recommended that no more than three dives per day be made on average due to the accumulation of nitrogen in the body, depending on the depth and duration of a diver's dive, due to human physiology (Figure 2.13). Therefore, the time that divers spend underwater is limited. Therefore, it is necessary to measure in a time that will not adversely affect human health (Kahraman et al., 2012; Kaya et al., 2019; Figure 5).



Figure 5. Nitrogen accumulation (Kahraman et al., 2012; URL-1).

6. Discussion and Conclusion

In this article, the issues that should be considered in photography scenarios and human health when applying the photogrammetry method in the underwater environment are presented. Along with the main difficulties affecting the underwater photogrammetry method, the importance of shooting scenarios in these difficulties was emphasized. When created correctly in shooting scenarios, it enables the creation of accurate and high-quality three-dimensional models. For this reason, people who will do underwater photogrammetry need to create shooting scenarios correctly.

The biggest advantage of the photogrammetry method is the contribution it provides in terms of time and cost. Since this method can produce base data and models for many disciplines, it is frequently preferred for different purposes and directions. Especially with the developments in computer technology, besides the photogrammetry technique, the Motion-Based Structural Detection (Structure from Motion/SfM) algorithm has also offered operators new perspectives in the use of this method. With this algorithm, the camera calibration parameters previously calculated by the operators can be calculated directly through software. The 3D geometry of the structure or object to be created with this algorithm is solved by finding the matched detail points in at least photographs two obtained by using different perspectives during the measurement.

The characteristics of digital technologies are accuracy, portability, low cost and rapid acquisition, each of which is important in archaeological sites, especially in with amphora loads. Underwater shipwrecks photogrammetry can be a very useful tool for archaeology. Digital techniques are essential for creating a virtual model with centimeter accuracy. It ensures that reliable 3D models are produced after the geometric accuracy is created. With these methods, one can achieve technically precise documentation. In addition, the versatility of underwater photogrammetry in terms of data acquisition is an advantage of this system. Besides the shallow conditions, it has made it difficult to use the underwater photogrammetry method in deeper areas up to about 35 m where the light conditions have become quite poor. For this reason, it is anticipated that this

technique will be useful in deeper areas by placing artificial light on the cameras. In the deep regions, the use of ROVs equipped with cameras has been used in vphotogrammetric studies in recent years. The advantage of this system is that it provides the opportunity to make measurements without the need to dive into deep areas. In addition, it saves time according to the measurement made with the help of divers. The disadvantage is; The unbalanced topographic structure of the underwater area to be photographed makes the measurement difficult. Important advice gained during underwater working experiences is related to the quality of the photos. Shadows, variation in natural light, or artificial shadowing can compromise the alignment of images, causing permanent shadows on the 3D model. Therefore, when choosing to do photogrammetric research in shallow water, in the best weather conditions, it is envisaged to avoid these problems by choosing to increase the ISO of the camera in deep water, without creating any noise in the images. In addition, before the photographs are processed in the software, it is foreseen that image enhancement processes such as color correction, contrast, removing shadows and highlight reduction should be applied to the photographs. It can be used to explore the inaccessible wrecks with a stereoscopic imaging system and to promote underwater cultural heritage information to a wide audience.

Author contributions

The article has a single author.

Conflicts of interest

The authors declare that for this article they have no actual, potential, or perceived conflict of interest.

Statement of Research and Publication Ethics

For this type of study formal consent is not required.

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