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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Preliminary study on otolith chemistry and otolith morphology of two demersal fish species, European hake (*Merluccius merluccius* Linnaeus, 1758) and striped red mullet (*Mullus surmuletus* Linnaeus, 1758) in the Sea of Marmara

Marmara Denizi'nde iki demersal balık türünün bakalyaro (*Merluccius merluccius* Linnaeus, 1758) ve tekir (*Mullus surmuletus* Linnaeus, 1758)'in otolit kimyası ve otolit morfolojisi üzerine ön çalışma

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Abstract: Otoliths are calcium carbonate ($CaCO_3$) accumulations. Under the influence of different ecosystems morphological and chemical composition change. In this study, economically important two demersal fish species; European hake *Merluccius merluccius* (Linnaeus, 1758) and Stripet red mullet (*Mullus surmuletus* Linnaeus, 1758) was examined. Otoliths (Sagitta) belonging to these two species have been studied both chemically and morphologicall. Morphometric measurements of otoliths (length, mm; width, mm; area, mm^2; perimeter, mm) in the sagittal of each species was made by the Leica M125 tri-ocular microscope. In the chemical analysis of otoliths, strontium (Sr), magnesium (Mg) and calcium (Ca) trace element amounts, the ratios of Sr and Mg trace elements to Ca element (Sr/Ca and Mg/Ca) were determined. For the micro-chemical analysis of otoliths ICP-MS was used. The highest magnesium (24.92±9.57 mmol/mol) and strontium (26.17±1.81 mmol/mol) element values were found in the otolith of red mullet. The difference between strontium (Sr) and magnesium (Mg) amounts for two fish species was found to be significant ($P<0.05$). In addition to it was found that the difference between them in the amount of calcium is significant ($P<0.001$). The shape indexes of otoliths are significantly different between the two fish species. Especially in terms of roundness (R_D) and aspect ratio (A_R) ($P<0.001$). The results of this study provide information about the habitats of two economic importance demersal fish species. Since such studies can give information about the habitat areas of fish species, they are important for tracking stocks, migration routes and sustainable fisheries.

Keywords: Demersal fish, otolith chemistry, otolith morphology, Sea of Marmara

Öz: Otolitler kalsiyum karbonat ($CaCO_3$) birikimleridir. Farklı ekosistemlerin etkisi altında morfolojik ve kimyasal olarak değişmektedir. Bu çalışmada, ekonomik olarak önemli iki demersal balık türü; Bakalyaro *Merluccius merluccius* (Linnaeus, 1758) ve tekir (*Mullus surmuletus* Linnaeus, 1758) balığına ait otolitler (Sagitta) hem kimyasal hem de morfolojik olarak incelenmiştir. Otolitlerin (uzunluk, mm; genişlik, mm; alan, mm^2; çevre, mm) morfometrik ölçümleri Leica M125 tri-oküler mikroskop ile yapılmıştır. Otolit yapısındaki stronsiyum (Sr), magnezyum (Mg) ve kalsiyum (Ca) iz element miktarları ile Sr ve Mg eser elementlerinin Ca elementine (Sr/Ca ve Mg/Ca) oranları belirlenmiştir. Otolitlerin mikro kimyasal analizi için ICP-MS kullanılmıştır. En yüksek magnezyum (24,92±9,57 mmol/mol) ve stronsiyum (26,17±1,81 mmol/mol) element değerleri tekir otolitinde tespit edilmiştir. İki balık türü için stronsiyum (Sr) ve magnezyum (Mg) miktarları arasındaki fark önemli bulunmuştur ($P<0,05$). Ayrıca kalsiyum miktarı açısından aralarındaki farkın oldukça önemli olduğu tespit edilmiştir ($P<0,001$). Otolitlerin şekil indeksleri iki balık türü arasında önemli ölçüde, özellikle yuvarlaklık (R_D) ve en boy oranı (A_R) açısından önemli olduğu tespit edilmiştir ($P<0,001$). Bu çalışmanın sonuçları, ekonomik önemi olan iki demersal balık türünün habitatları hakkında bilgi verdiğinden; stokları, göç yolları ve sürdürülebilir balıkçılığın takibi açısından önem arz etmektedir.

Anahtar kelimeler: Demersal balık, otolit kimyası, otolit morfolojisi, Marmara Denizi

INTRODUCTION

Otolith are stable structures in terms of metabolic activity and calcium carbonate ($CaCO_3$) deposits (Degens *et al*., 1969; Pannella, 1971; Campana, 1999). There are three pairs of otoliths in sagitta, lapillus and asteriscus in bony fishes. They undertake the task of providing balance and hearing (Popper and Coombs, 1980; Campana, 1999; Campana and Thorrold, 2001).

Otolith has been the subject of studies in many areas such as fish biology, fish ecology, fish stock detection, diet assessment, as well as age and growth (Campana, 1999;

Friedland and Reddin, 1994; Tracey *et al*. 2006; Gonzalez-Salas and Lenfant, 2007; Barrett, 1990; Martucci et al., 1993; Velando and Freire, 1999; Turan, 2006; Morat *et al*., 2014; Başusta and Khan, 2021).

Otolith constitute a source of information and data for the identifying ichthyological taxa with systematic and fleet genetic studies. Scientific studies and applications on otolith are not limited to ichthyology. It is also possible to access in-depth information about historical processes by using them in fields such as paleontology, stratigraphy, archeology and zoo-geography (Schwarzhans, 1999).

The best evidence of fish movement is observing and following their movements from one place to another. However, these data are difficult to obtain for multiple life stages (Gillanders, 2005). Although the potential importance of movement, relatively little is known about how many fish species vary within and between populations (Quinn, 1993; Gowan *et al*., 1994). Therefore, a robust and cost-effective method is needed to determine the habitat origins and movements of fish and to describe the stock structure of fish species. Otolith element composition has recently proven to be a robust natural tag for tracking population structure, species life history, habitat areas and migration routes (Campana and Neilson, 1985; Rieman *et al*., 1994; Campana *et al*., 1995; Thorrold et al., 1997; *Wells et al*., 2000; Thorrold *et al*., 2001). The structures of otolith undergo differentiation ontogenetically under the influence of different ecosystems. Their structure is three-dimensional, but not all dimensions grow equally and at the same rate. It also varies significantly among species in terms of size, shape and chemical content (Campana and Thorrold, 2001). One of the fastest growing areas of fisheries science is the use of these calcined structures (otolith) to answer ecological questions about fish movement and habitat areas. Otolith's two properties make it particularly suitable for keeping records of the environment in which the fish live (Gillanders, 2005). European hake and stripet red mullet are the most economically important demersal fish species in Turkey Seas (Yıldız and Karakulak, 2018). It mainly inhabits rocky bottoms and soft substrates and undergoes vertical movements between 5 and 100 m in depth (Froese and Pauly, 2008).

However, stocks are gradually decreasing due to overfishing, which have been decreasing recently. According to the Turkey Statistical Institute (2018-2019) 1.019 tons of *M. merluccius*, 2.914 tons *M. surmuletus* and 1269 tons *M. merluccius*, 2341 tons *M. surmuletus* were caught, respectively (TUİK, 2021).

In this study, the amount of trace elements and morphological structures of otoliths was examined and evaluated comparatively to monitor the stocks of two important demersal fish species.

MATERIAL AND METHODS

A total of 10 *M. merluccius* and 10 *M. surmuletus* were obtained from fishing vessels in the Sea of Marmara (west of "Kapıdağ Peninsula"). Otoliths were examined both morphologically and in microchemical analysis.

Otolith chemistry

Whole otoliths (right and left) were used for otolith chemistry. For each otolith to reach a constant weight, it has been subjected to drying in glass tubes for 2 h in an oven set at 120 °C. The otolith samples whose drying was completed were weighed with a sensitive scale sensitive to approximately 0.001 g and transferred to the vessels in the microwave incinerator unit. Then, 5 ml of nitric acid (HNO_3) and 2 ml of hydrogen peroxide (H_2O_2) were added to each vessel and subjected to heat-controlled microwave combustion using the SK-12 rotor in the Easy model Ethos (Rooker *et al*., 2001; Correia *et al*., 2011). After the extract reached room temperature, the final volume was diluted with 25 ml of distilled water and analyzed in solution mode on a Perkin Elmer brand Nexion 350X model Inductively Coupled Plasma Mass Spectrometer ICP-MS (Jarvis and Jarvis, 1992).

At the end of the analysis, strontium (Sr), calcium (Ca) and magnesium (Mg) trace element concentrations and elemental calcium ratios (Sr/Ca and Mg/Ca) were determined.

Otolith morphology

Firstly, the length (with a height measuring scale with 0.01 mm precision) and weight measurements (in 0.01 g precision balance) of the fish samples were examined. The sagitta otoliths were removed and soaked in 3% hydrogen peroxide (H_2O_2) and 1% nitric acid (HNO_3) for 5 min, respectively, to purify the blood, tissue and other surface contaminants. It was then kept in distilled water for 5 min to remove the acid (Rooker *et al*., 2001). There were no differences between right and left otolith of both species (*t*-test, P>0.05). Therefore, the right otolith was used for the measuring the otolith morphology of two fish species. Otolith length (OL) and otolith width (OW_i) (±0.001 mm) were determined by Leica M125. Otolith length was defined as the greatest distance between the anterior and posterior edge and otolith width was described as the greatest distance from dorsal to the ventral edge. To illustrate how measurements of otolith morphology are made *M. merluccius* otolith measurements are given as an example (Figure 1).

The raw data obtained at the end of the measurement [otolith length (OL, mm), otolith width (OW, mm), otolith area (OA, mm^2) and otolith perimeter (OP, mm)] was evaluated and the shape indexes of was calculated. The otolith shape identification process helps construct the otolith atlas and identify the species (Tuset *et al*., 2008). The formulas given in Table 1 were used to determine the shape indexes of otoliths (Tuset *et al*., 2003; Ponton, 2006).

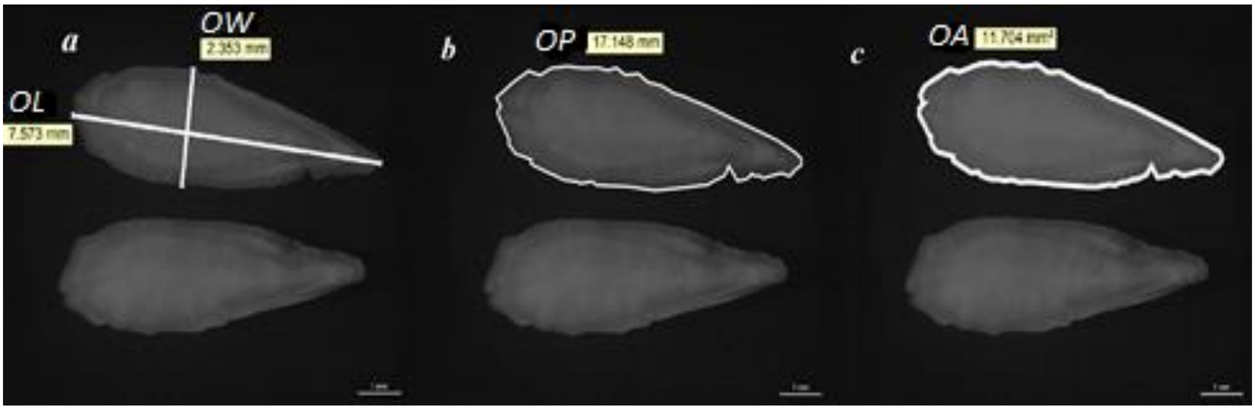

Figure 1. a: OL otolith length (mm); OW: otolith width (mm); b: OP otolith perimeter (mm); c: OA otolith area (mm^2); measurement: 1mm. Photos by H. BAL)

Table 1. Formulas to be used for the shape index otoliths of the species

Parameters	Shape indexes	Formula
OP (otolith perimeter, mm)	Form Factor (F_F)	$4.\pi.OA/(OP)^2$
OA (otolith area, mm^2)	Circularity (C_i)	$(OP)^2/OA$
OL (otolith length, mm)	Roundness (R_D)	$4.OA/\pi.(OL)^2$
OW_i (otolith witdh, mm)	Width/Length (W/L)	$OL.OW_i^{-1}$

The results of this study provide information about the habitats of two demersal fish species that have economic importance and share the same habitat. Since such studies can give information about the habitat areas of fish species, they are important for tracking stocks, migration routes and sustainable fisheries.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using the SAS package program (Version: 6.0.0) to determine the differences between two demersal fish species on otolith chemistry and morphology.

RESULTS

Both chemical and morphological analysis was performed on otolith belonging to two demersal fish species.

Otolith chemistry

Chemical trace elements (Sr, Mg and Ca) in otoliths belonging to two demersal fish species were investigated. It was determined that the highest value among the three trace elements was in otoliths belonging to *M. surmuletus* species and the average amount of strontium as 26.17±1.81 mmol/mol was determined. Similarly, it was determined that the highest amount of Mg was in *M. surmuletus* (24.92±9.57 mmol/mol). However, calcium trace element (Ca) content of *M. merluccius* was found to be 10.2±0.07 mmol/mol higher than *M. surmuletus*. The difference between strontium (Sr) and magnesium (Mg) amounts for the two fish species was found to be significant ($P<0.05$). It was found that the difference between them in the amount of calcium is significant ($P<0.001$). Data of fish size and otolith chemistry for two demersal species are given in Table 2.

Table 2. Data on otolith chemistry of two demersal fish species

Species	TL (cm) Min-max Mean±SE	W (g) Min-max Mean±SE	Sr (µmol/mg) Min-max Mean±SE	Mg (µmol/mg) Min-max Mean±SE	Ca (µmol/mg) Min-max Mean±SE	Sr/Ca (mmol/mol) Min-max Mean±SE	Mg/Ca (mmol/mol) Min-max Mean±SE
Merluccius merluccius	27.10-36.50 31.54±0.98	141.87-413.14 264.97±28.53	3.97-30.28 15.51±2.99	0.60-1.98 1.26±0.18	9.97-10.67 10.25±0.07	0.38-2.96 1.52±2.11	0.05-0.19 0.12±0.11
Mullus surmuletus	13.50-18.0 15.35±0.38	25.75-75.83 43.01±4.27	20.46-38.30 26.17±1.81	5.63-105.96 24.92±9.57	7.49-10.50 9.30±0.29	2.42-3.64 2.79±0.45	0.57-14.17 2.94±1.20
Sig.	***	***	**	***	***	**	***

TL: Total fish length (cm), W: Total fish weight (g); Sr: Strontium (mmol/mol); Mg: Magnesium (mmol/mol); Ca: Calcium (mmol/mol); SE: Standard error; Sig.: Significance, **: P<0.01, ***: P<0.001.

Otolith morphology

It was determined that the shape indexes obtained from the morphological analysis of the otoliths belonging to both fish species differ from each other. The otolith morphology of the two species was determined to be significantly different from each other. The minimum, maximum, mean and standard error of fish size and otolith measurement are given in Table 3. The raw data obtained at the end of the measurement [otolith length (OL, mm), otolith width (OW_i, mm), otolith area (OA, mm^2) and otolith perimeter (OP, mm)] was evaluated and the shape indexes of was calculated. The shape indexes differ significantly from each other. Especially in terms of roundness (R_D) and aspect ratio (A_R) (P<0.001). Data of morphological and shape indexes are given in Table 4.

Table 3. Descriptive statistics on fish size and otolith measurements

Fish size and otolith measurements	Species				
	Merluccius merluccius		*Mullus surmuletus*		
	Min-max	Mean±SE	Min-max	Mean±SE	Sig.
TL (cm)	27.10-36.50	31.54±0.98	13.50-18.0	15.35 ± 0.38	***
W (g)	141.87-413.1	248.51±2.05	25.75-75.83	43.01 ± 4.27	***
OW (mg)	75.0-159.0	107.70±7.81	4.20-10.80	6.08±0.64	***
OL (mm)	10.09-13.33	11.45 ± 0.37	2.11-3.01	2.31 ± 0.09	***
OW_i (mm)	3.96-5.82	4.65 ± 0.17	1.41-1.99	1.60±0.06	***
OA (mm^2)	28.57-52.20	37.13 ± 2.24	2.05-4.08	2.48±0.19	***
OP (mm)	22.73-30.82	25.98 ± 0.83	5.28-8.34	6.32±0.31	***

TL: Total fish length (cm), W: Total fish weight (g), OW: Otolith weight (mg), OL: Otolith length (mm), OW_i: Otolith width (mm), OA: Otolith area (mm^2), OP: Otolith perimeter (mm), SE: Standard error; Sig.: Significance, ***: P<0.001.

Table 4. Fish size and otolith shape analysis

Species	TL (cm) Min-max Mean±SE	W (g) Min-max Mean±SE	Circularity (C_i) Min-max Mean±SE	Form factor (F_F) Min-max Mean±SE	Roundness (R_D) Min-max Mean±SE	Aspect ratio (A_R) Min-max Mean±SE
Merluccius merluccius	27.10-36.50 31.54±0.98	141.87413.14 248.51±2.05	17.33-1977 18.30±0.22	0.635-0.725 0.680±0.008	0.325-0.378 0.358±0.006	2.292-2.600 2.464±0.029
Mullus surmuletus	13.50-18.0 15.35±0.38	25.75-75.83 43.01±4.27	12.87-20.05 16.33±0.83	0.626-0.976 0.789±0.042	0.547-0.642 0.592±0.009	1.322-1.620 1.445±0.030
Sig.	***	***	*	*	***	***

TL: Total fish length (cm); W: Total fish weight (g); OW: Otolith weight (mg); SE: Standard error; Sig.: Significance; *: P<0.05; ***: P<0.001.

DISCUSSION

Although the potential importance of the movement, relatively little is known about the populations of many fish species (Quinn, 1993; *Gowan et al*., 1994). Therefore, there is a need for a robust and cost-effective method to determine the habitat origins and movements of fish and to describe the stock structure of fish species. Otolith element composition has recently proven to be a powerful natural label for tracking population structure, species life histories, habitat ranges, and migration routes (Campana and Neilson, 1985; Rieman *et al*., 1994; Campana *et al*., 1995; Thorrold *et al*., 1997; *Wells et al*., 2000; Thorrold *et al*., 2001).

Although *M. merluccius* and *M. surmuletus* are demersal fish species, it has been determined that the chemical element amounts and ratios of otoliths belonging to *M. surmuletus* were higher than *M. merluccius*. Especially, when compared to the otolith size of *M. surmuletus*, Sr/Ca and Mg/Ca ratios are observed to be higher although it is much smaller than *M. merluccius*. Similarly, although *M. surmuletus* otolith is lighter (Mean±SE; 6.08±0.64 mg) than *M. merluccius* otolith (Mean±SE; 107.70±7.81 mg), it contains higher amounts of Sr and Mg trace elements (Table 2 and Table 3). But, calcium (Ca) trace element content of *M. merluccius* was found to be 10.2±0.07 mmol/mol higher than *M. surmuletus.* According to the results of scientific studies, it has been determined that salinity, temperature and conditional environmental effect the otolith chemistry (Secor *et al*., 1995; Hoff and Fuiman, 1995; Elsdon and Gillanders, 2004). It is thought that the differences between species in the inclusion of elements in our study may be partially related to environmental conditions, differences in metabolic and otolith deposition rate (Hamer and Jenkins, 2007).

This study is the first to examine the otolith chemistry and morphology of these two demersal species for Turkish waters and there is no study using this method for *M. merluccius* and *M. surmuletus* fish species in Turkey Seas. Therefore, no comparison could be made.

Only one study has been detected as research that examines both the morphological and analysis of the chemical structure of otoliths in Turkey Seas. Within the scope of the research, the horse mackerel (*Trachurus mediterraneus* Steindachner, 1868) was examined and a significant contribution was made to the separation of the stocks related to the species (Turan, 2006). Apart from this study, otolith Sr/Ca ratios were examined to determine the migration characteristics of the European eel (*Anguilla anguilla* Linnaeus, 1758) species in the Asi River (Lin *et al*., 2011). The average ratio of Sr/Ca in otoliths of *A. anguilla* taken from the Asi River was determined as 2.79×10^{-3} µm, which is approximately twice time (1.4×10^{-3} µm) the ratio of Sr/Ca in otliths of samples taken from the Garonne and Dordogne rivers (Lin *et al*., 2011). Thus, it was determined that the same fish species exist in different habitats by using otolith chemistry.

However, there are many studies examining otolith chemistry and morphology of two demersal fish species in different seas of the world (Torres *et al.,* 2000; *Lombarte et al*., 2003; Morales-Nin *et al.,* 2005; Swan *et al*., 2006; Leakey *et al.,* 2009; Mahe *et al*., 2014; Morales-Nin *et al.,* 2014; Bakkari *et al*., 2020).

In different study, it has been determined that Mg and Sr amounts in otoliths of fish samples (*M. merluccius)* taken from five different geographical locations (in north-east Atlantic and the Western Mediterranean Sea) is significantly different between the sample locations (Morales-Nin *et al*., 2005).

In another study on otolith chemistry was used to determine the relationship among populations of European hake; it was found that the ratio of Sr:Ca in the otolith core was higher than the otolith edge of the European hake. Also, the otolith edge had lower ratios for all elements except Mg (Morales-Nin *et al*., 2014). This situation shows that the habitat areas in which the juvenile stages, which are the first life stages of the fish, may differ from the adult stages. Another idiom on the otolith morphology of European hake was investigated for two different areas (North-eastern Atlantic-Cantabrian Sea and the Mediterranean Sea-Gulf of Lion). In the study, fish lengths are between 14 and 77 cm and 21-70 cm, and the average otolith area, otolith perimeter and otolith length of the samples in the North Atlantic were calculated as 80.15 mm^2, 102.01 mm, and 17.71 mm, respectively. In the Mediterranean, the average otolith area, otolith perimeter and otolith length were calculated as 102.78 mm^2, 118.8 mm, and 19.6 mm, respectively (Torres *et al.*, 2000). Another idiom conducted in the Mediterranean, trace elements of the European otoliths were examined.

All otoliths from *M. merluccius* separate areas differed not only in elemental concentrations but also in the distribution of elements among different individuals. For example, significant differences were found in the otolith concentrations between Mg, Mn and Sr among individuals in the Mediterranean region (Swan *et al*., 2006).

Although many factors such as the amount of accumulation of chemical trace elements, fish physiology, stress and genetics are effective, it may vary in proportion to environmental conditions, especially temperature and salinity (Reis Santos *et al*., 2013; Avigliano *et al*., 2014; Sarimin and Mohamed, 2014). The amount of strontium element increases positively depending on the salinity of the water (Kraus and Secor, 2004; Sturrock *et al*., 2012; Avigliano and Volpedo, 2013). However, barium is negatively affected (Miller, 2011; Avigliano *et al*., 2014). Calcium (Ca) is physiologically included in otoliths (Popper *et al*., 2005). However, magnesium increases depending on the metabolic activity and growth in the ecosystem where the fish is located (Martin and Thorrold, 2005; Sturrock *et al.,* 2015; Grammer *et al*., 2017). Also, strontium (Sr) and magnesium (Mg) are included in the otolith chemistry according to the natural abundance of trace elements carried by the fish habitat and chemical property of water (Farrell and Campana, 1996).

Additionally, environmental conditions affect the morphology of otoliths may differ between populations of the same species (Congiu et al., 2002). Determination of shape indices such as circularity and roundness by using the structural shapes and sizes of otoliths that change in a morphological sense and identification of species are secondary areas of use that help to determine the differences between stocks of the same species depending on geographical distance (Ponton, 2006; Campana and Casselman, 1993; Torres et al., 2000; Monteiro *et al*., 2005). In this study, the otolith morphology of two demersal fish species was investigated. Although samples from different regions cannot be taken and analyzed, in the present study findings are expected to form complementary data that will be a source for future scientific research on similar species. There are many studies examining the morphology of otoliths for descripting fish stocks in different parts of the world and in Seas of Turkey (Campana and Casselman, 1993; Campana *et al*., 1995; Monteiro *et al*., 2005; Özpiçak *et al*., 2017; Bal *et al.*, 2018). They made a significant contribution to the stock descriptions of the investigated species in these studies.

Since the same trace elements and the same morphological features were not examined in other studies, a comprehensive comparison could not be made with the present study.

CONCLUSION

The differences between the stocks of demersal species and their habitat areas are not yet well known. It is of great importance to define the habitats of these important fish species, which can be considered the basic stone of the marine ecosystem, the characteristics of their habitat areas and the stocks. Because; if the stocks and habitat areas of the species are not analyzed well, it is obvious that the extinction of the species will be endangered as these areas cannot be protected since stocks cannot be distinguished and defined.

However, samples could not be taken from different regions due to some limiting factors such as the costs required for laboratory analysis. To determine the differences between stocks in more detail, complementary studies are needed. It is thought that the findings obtained from this study will be a source for future research.

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