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Aquatic Sciences and Engineering aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of aquatic sciences. The journal publishes original research and review articles that are prepared in accordance with the ethical guidelines.

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Research Article

Ultrasonic Pre-Treatment and Vacuum Effect on the Drying of *Cancer Pagurus* Meat

Zehra Özden Özyalçın¹ 💿, Azmi Seyhun Kıpçak¹ 💿

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ABSTRACT

This study investigates the impacts of vacuum and ultrasonic pre-treatment on drying kinetics, mathematical modeling, and color changes of *Cancer pagurus* (brown crab). The Midilli & Kucuk model provided the best match for all the drying techniques examined. Using a variety of drying techniques such as oven drying (OD), vacuum oven drying (VOD), ultrasonic pre-treated oven drying (US-OD), and ultrasonic pre-treated vacuum oven drying (US-VOD), effective moisture diffusivities (D_{eff}) and activation energies (E_a) were calculated. According to the findings of the study, vacuum and ultrasonic pretreatment shortened drying times, which is crucial for improving drying effectiveness and lowering costs. On the other hand, the OD method had the lowest D_{eff} values (0.397-0.673 × 10⁻¹⁰ m²/s), indicating that it was the least efficient method for removing moisture. The study also found that the highest E_a value was observed in the US-VOD method (44.60 kJ/mol) and the lowest in the OD method (25.69 kJ/mol). Interestingly, the US-VOD method also had the lowest ΔE values (17.09 - 20.33), indicating that both US pre-treatment and vacuum application were successful in preserving the color of the brown crab.

Keywords: Crab, oven, seafood, ultrasonic pre-treatment, vacuum oven

INTRODUCTION

Owing to their high production costs and short shelf life, meat and meat products are in need of high-cost storage and transfer, such as a cold chain. These products, with high nutritional values and water activities, are prone to microbiological and biochemical degradation. Today, many different methods are used to prevent meat and its derivatives from degradation, to extend their shelf life, to ensure microbial safety, and to reduce transfer costs (Ozbay & Saricoban, 2015a, b). As an alternative to meat products, seafood stands out with its distinct tastes and high nutritional values. For products which have unit prices higher than many meat products conservation processes are very desirable and cost-effective.

The edible crab or brown crabs, scientifically known as *Cancer pagurus*, belongs to the *Can*-

cridae family of large crabs and is found in the eastern Atlantic and Mediterranean regions. The length of the species is about 6-15 cm. The edible crab lives in shallow and rocky areas and is carnivorous (Tonk & Rozemeijer, 2019). *Cancer pagurus* is one of the most economically important crab species in terms of commercial value. These ten-legged shellfish are the most caught crab species in Western Europe, and the world annual production is over 1.25 million tons (FAO, 2020).

Drying is the predominant preservation method in the food industry, relying on the evaporation of water from food products through the application of thermal energy. However, high temperatures involved in this process can adversely impact the visual quality of food products due to enzymatic browning, leading to

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quality deterioration (Mothibe, Zhang, Nsor-atindana, & Wang, 2011). To overcome such challenges, innovative drying methods are continuously being developed with a focus on minimizing quality losses that consumers associate with processed products while maximizing drying efficiency to minimize processing costs. Additional applications are used to meet these expectations, especially in thermally sensitive foods such as seafood. The most common side applications used in drying are vacuum and ultrasound pre-treatment.

In traditional drying methods, fishery products are generally sundried (Ozbay & Sarıcoban, 2015a, b). As this method takes a long time and relies on weather conditions, it is not a very efficient one. Besides that, oven drying operates on a similar principle, but using a constant temperature commonly used in food drying applications.

Supporting drying with vacuum and ultrasound pre-treatment increases the drying rate and quality parameters of the dried product. The vacuum process provides a protective effect from oxidation, especially for oxygen-sensitive products (Punathil & Basak, 2016). Ultrasound is a method based on creating micro-channels in the structure of the product with sound waves at frequencies between 20 kHz and 1 MHz (Soria & Villamiel, 2010). Sufficient ultrasound power reduces the surface tension on the product surface, disrupting tissue continuity, and causing the pores to expand. This increases the mass transfer rate (Nowacka, Wiktor, Śledź, Jurek, & Witrowa-Rajchert, 2012). The decrease in the drying time with the application of vacuum and the increase in the porosity with ultrasound enable the texture, color, and taste changes of the dried product to decrease (Punathil & Basak, 2016).

Consumption amounts of dried foods is rising day by day, and therefore, the product range is diversifying. Especially, dry meat derivatives are very popular products due to their high protein content and easy accessibility. Among the diversified dried meat products, seafood also finds its place and diversifies the range of products. Examples can be given for drying-related studies of meat and seafood. Kipcak & Ismail (2021) studied microwave drying of various meat products, including fish, chicken, and beef, and Kumar, Tarafdar, Kumar, & Badgujar (2019) studied convective drying of chicken breast slices. Compared to other meat products, investigations on seafood are less common. Some examples of studies on drying seafood include Shamsuddeen, Cha, Kim, & Kim (2021), who studied hybrid heat pump vacuum drying of oysters, Nguyen, Ngo, & Le, (2019) who investigated convective hot air drying of shrimps, and Kouhila et al. (2020) who studied the effects of convective and solar drying of Mediterranean mussels. Kipcak, Doymaz & Derun, (2019) examined infrared drying of blue mussel and drying kinetics, Kipcak (2017) studied microwave drying of blue mussels, Duan, Jiang, Wang, Yu, & Wang, (2011) studied hot air-microwave drying of tilapia fish fillets.

In addition to these studies, studies on vacuum and ultrasound pre-treatment, which reduce drying time and are effective in preserving visual quality, are often performed on fruit and vegetable products. Examples that can be given as; Tao et al. (2021) studied airborne and contact ultrasound effect on air drying of blackberry, Li, Wang, Wu, Wan, & Yang, (2020) studied ultrasound-assisted vacuum drying of hawthorn fruit juices, and Bozkir, Ergün, Serdar, Metin, & Baysal, (2019) examined ultrasound assisted drying of persimmon fruit. Nowacka et al. (2012) studied apple drying with ultrasound pre-treatment, da Silva et al. (2019) investigated ultrasound and vacuum effect on drying nectarine, and Wang, Ye, Wang, & Raghavan, (2019) studied drying of kiwifruit slices with ultrasound pre-treatment.

Among the numerous drying studies performed with oven drying, sea creatures have rarely been studied. Therefore, this study aims to explore the impact of temperature on the drying rate of a well-known shellfish, *Cancer pagurus*. The drying process was carried out at 60, 70, and 80°C using both an oven dryer and a vacuum oven dryer, and the effect of ultrasonic pre-treatment was also examined. Additionally, effective moisture diffusivity, activation energy, and color changes were calculated to gain a better understanding of how the drying process affected the product.

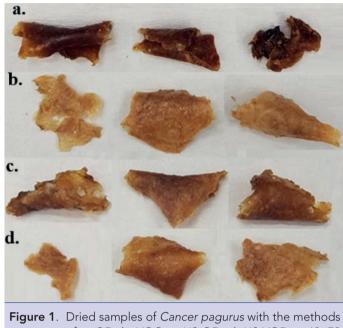
MATERIALS AND METHODS

Samples and equipment

Cancer pagurus was purchased from a regional market in July 2019 and stored in a freezer at -18°C. Prior to experimentation, the frozen crab was defrosted at a temperature of +4°C, and its hard shell was broken with the aid of a hammer to extract all the edible meat. The extracted crab meat was weighed to approximately 5 ± 0.1 g for each drying process and subsequently pelletized to dimensions of 2.4 \pm 0.2 cm in length, 2.4 \pm 0.2 cm in width, and 2 \pm 0.1 mm in thickness. The impact of pretreatment on drying efficiency was investigated using both pretreated and untreated samples. Moisture content was determined through the use of an oven (KH-45; Kenton, Guangzhou, China) at a temperature of 105°C for 3 hours. Ultrasonic pretreatment (US) was administered using an Isolab Ultrasonic Bath at an ultrasonic power of 180 W (Isolab, Escau, Germany). Drying experiments were conducted using a Nüve EV-018 (Nüve, Ankara, Turkey) at temperatures of 60, 70, and 80°C. Vacuum assistance was provided by a KNF N022AN.18 (KNF, Freiburg, Germany) operating at a frequency of 40 kHz and 100 W. This equipment was employed to examine the effect of temperature on the drying rate of Cancer pagurus meat.

Experimental method

Cancer pagurus pellets of 5±0.1 g were placed onto watch glasses. Drying parameters were selected as 60, 70, and 80°C. During the drying processes for each method, the samples for each temperature were weighed at 15-minute intervals. In the first stage, OD was performed with the aforementioned parameters. In the second stage, VOD was applied with the same drying parameters. The pressure inside the oven was kept constant at 0.32 atm during the drying process using a vacuum pump. During the drying process, the samples were weighed at 15-minute intervals to determine the rate of moisture loss. The experimental procedures were divided into four stages. In the first stage, oven drying (OD) was performed at the selected temperatures. In the second stage, vacuum oven drying (VOD) was applied with the same drying parameters. In the third stage, ultrasonic pre-treatment was applied before the drying process, and in the fourth and final stage, the same procedure was applied with vacuum assistance (US-VOD).



of a. OD, b. VOC, c. US-OD, d. US-VOD at 60, 70 and 80 °C, respectively.

Drying processes ended when the humidity of the samples dropped below 10%. After all the drying processes were completed, the samples shown in Figure 1 were placed in polyethylene bags and placed in a desiccator to protect them from moisture.

Mathematical modeling of drying curves

The drying process is governed by Fick's second law, which describes the diffusion of water from the interior to the surface of the sample during drying. To model the drying rate of *Cancer pagurus*, moisture content (*M*), moisture ratio (*MR*), and drying rate (*DR*) were calculated using equations (1), (2), and (3) developed by Kipcak & Ismail (2018), Kipcak, Doymaz, & Derun (2019), and Sevim, Derun, Tugrul, Doymaz, & Kipcak, (2019):

$$M = \frac{m_w}{m_d} \tag{1}$$

Here, M represents the moisture content in kgW/kgDM, $m_{\rm w}$ represents the water content in kg, and $m_{\rm d}$ represents the dry matter content in kg.

$$DR = \frac{M_{t+dt} - M_t}{dt} \tag{2}$$

DR represents the drying rate in kgW/kgDM \times min, t is the drying time in minutes, and M_{t+dt} is the moisture content at time t+dt in kgW/kgDM.

$$MR = \frac{M_t - M_e}{M_0 - M_e} \tag{3}$$

MR represents the moisture ratio, which is dimensionless. M_r , M_o , and M_e and represent the moisture content at any time, initial, and equilibrium states, respectively. In most cases, the moisture content at the equilibrium state is negligible compared to the initial and any other moment during the drying process, so it can be neglected in the calculations (Doymaz, Kipcak, & Piskin, 2015; Kipcak, 2017). The calculated values of M, MR, and DR were used to draw drying rate curves for modeling the drying process of *Cancer pagurus*.

Regression analysis of the data to be obtained as a result of drying seafood was done with the help of Statistica 10.0 (StatSoft Tulsa, USA) program. Levenberg-Marquardt algorithm was used for the parameter estimation in nonlinear regression in the modeling of the data, and it was examined in the 14 most widely used models taken from the studies of Doymaz, Kipcak, & Piskin, 2015; Silva et al., 2014).

In determining the suitability of the models for all models used, the value of the regression coefficient (R^2) was taken into consideration first. Besides R^2 , the lowest root mean square error (*RMSE*) and reduced Chi-square (χ^2) values were evaluated. Coefficients are calculated using Eq. (4), (5), and (6) (Tunckal & Doymaz, 2020).

$$R^{2} \equiv 1 - \frac{\sum_{i=1}^{N} \left(MR_{exp,i} - MR_{pre,i}\right)^{2}}{\sum_{i=1}^{N} \left(MR_{exp,i} - \left(\frac{1}{n}\right)MR_{exp,i}\right)^{2}}$$
(4)

$$RMSE = \left(\frac{1}{N}\sum_{i=1}^{N} \left(MR_{exp,i} - MR_{pre,i}\right)^2\right)^{\frac{1}{2}}$$
(5)

$$\chi^{2} = \frac{\sum_{i=1}^{N} (MR_{exp,i} - MR_{pre,i})^{2}}{N-z}$$
(6)

The moisture ratios, $MR_{exp,i}$ and $MR_{pre,i}$ denote the calculated and predicted values of moisture content, respectively. The parameter N signifies the total number of experiments conducted, while z refers to the constant values incorporated in the models (Kipcak, 2017; Tunckal & Doymaz, 2020).

Effective moisture diffusivity

Removal of moisture in the structure during the drying process in foodstuffs takes place in a constant or falling rate period and features a complex mass transport mechanism. Fick's second diffusion equation is commonly used in determining the effective moisture diffusion coefficient in foodstuffs as given in Eq. (7).

While solving this equation, assumptions in which the shrinkage was neglected, the diffusion coefficient was accepted as constant, and the mass transfer occurred symmetrically with respect to the center only by diffusion were made. The applicable unsteady state condition equation of Fick's second law thin layer is given in Eq. (7) (Kipcak & Doymaz, 2020; Doymaz et al., 2015):

$$MR = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n+1)^2} \exp\left(-\frac{(2n+1)^2 \pi^2 D_{eff} \times t}{4L^2}\right)$$
(7)

The moisture ratio is denoted by *MR*, where n is a positive integer, *t* represents the time in seconds, $D_{\rm eff}$ represents the effective moisture diffusivity in square meters per second, and *L* denotes the half-thickness of the sample in meters. However, when the drying time is long, n is considered as 1 (Kipcak & Doymaz, 2020). To derive the exponential equation, the natural logarithm of MR is plotted against *t* using Eq. (8), which is obtained by taking the naturel logarithm of Eq. (7). The slope of the ln(*MR*) versus *t* graph allows for the calculation of $D_{\rm eff}$.

$$\ln(MR) = \ln\left(\frac{8}{\pi^2}\right) - \left(\pi^2 \frac{D_{eff} \times t}{4L^2}\right) \tag{8}$$

Activation energy

The activation energy plays a significant role in the drying process of food products. It is the energy required to separate water molecules from the product, which makes it a crucial factor in the thermodynamics of drying. A lower activation energy implies a faster drying process. To determine the relationship between the drying process's Eq. (9)-derived $D_{\rm eff}$ and temperature change (Kara & Doymaz, 2015). The $D_{\rm eff}$ in m²/s, the pre-exponential factor (D₀) in m²/s, the activation energy (E_a) in kJ/mol, the universal gas constant (R) in kJ/mol K, and the drying temperature (T) in Celsius are all taken into account in this equation (Kipcak & Doymaz, 2020).

$$D_{eff} = D_o exp\left(-\frac{E_a}{R(T+273.15)}\right) \tag{9}$$

The natural logarithm of Eq. (9) is applied to calculate the $E_{a'}$ resulting in Eq. (10). The activation energy can then be determined by calculating the slope of the graph of D_{eff} versus 1/T, as given in Eq. (11). It is worth noting that E_a is an important parameter in the drying process since a lower activation energy implies a faster drying rate, making it a crucial factor to consider in food processing (Tunckal & Doymaz, 2020).

$$\ln(D_{eff}) = \ln(D_0) - \left(\frac{E_a}{R}\right) \left(\frac{1}{T}\right)$$
(10)

$$slope = -\frac{E_a}{R} \tag{11}$$

Color analysis

The color of food products is an essential feature that conveys information about the product to consumers. In fact, consumers often expect treated products to have consistent color with their pre-processing color. One of the widely used methods for analyzing color is the Hunter color analysis, which involves measuring the lightness-darkness value L^* , redness-greenness value a^* , and yellowness-blueness value b^* . To measure the color parameters of the samples, a hand-held PCE-CSM 1 model colorimeter from PCE Instruments UK Ltd. was used. Samples were measured at least five times before and after each method to ensure accuracy. To calculate the total color changes (ΔE), Eq. (12) was used. The accurate measurement and analysis of color parameters are crucial to ensure that the final product meets the consumers' expectations (Tunckal & Doymaz, 2020).

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2}$$
(12)

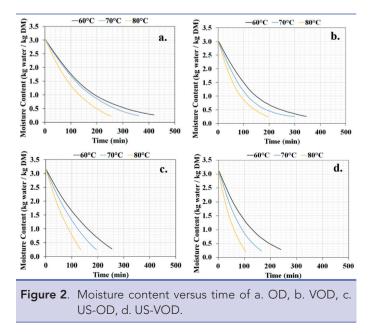
where "0" represents the fresh sample.

RESULTS AND DISCUSSION

Drying curves

Initial moisture content was calculated as 75.27% on a wet basis, 3.0431 kg water/kg dry matter (kgW/kgDM) for unpretreated samples and as 75.99% on a wet basis, 3.1651 kgW/kgDM for US pre-treated samples. The moisture content of the sample was reduced to 0.2704, 0.2400, and 0.2189 kgW/kgDM for 60, 70, and 80 °C, respectively, and the drying process took 420, 360, and 255 minutes using the OD method. On the other hand, for the VOD method, the moisture content was reduced to 0.2573, 0.2268, and 0.1997 kgW/kgDM for 60, 70, and 80 °C, respectively, and the dryings took only 345, 285, and 210 minutes. These results indicate that the vacuum effect significantly reduces the drying time compared to the standard OD method, and can be considered a more efficient method for drying food products.

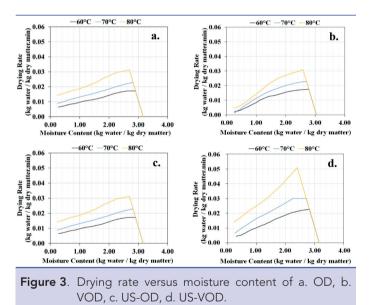
Moving on to the next method, US-OD, the initial moisture content was higher at 3.1651 kgW/kgDM, but it was still reduced to 0.2773, 0.2406, 0.2397 kgW/kgDM for 60, 70, 80°C, respectively, with drying times of 255, 195, and 135 minutes. The fourth method, US-VOD, reduced the moisture content even further to 0.2294, 0.1817, 0.1048 kgW/kgDM for 60, 70, 80°C, respectively, with drying times of 240, 180, and 120 minutes. These results are also presented in Figure 2 and demonstrate that the US pre-treat-



ment method, similar to the vacuum effect, can significantly reduce the drying time when compared to the OD method. This highlights the potential of US-OD and US-VOD as effective and efficient alternatives for drying food products.

Drying rate curves

During the drying process, as the water content in the sample decreases, the structure of the material shrinks, and the number of pores decreases, as explained in Sarpong et al. (2019). As a result, the resistance to water loss increases, making it more difficult for water to escape, which leads to a falling rate period in the drying process (Doymaz et al., 2015; Kipcak, 2017). This phenomenon was also observed in the drying of Cancer pagurus, as shown in Figure 3. The drying rates obtained in the OD process were between 0.0141 - 0.0014, 0.0158 - 0.0022 and 0.0202 - 0.0041 kgW/kgDM \times min for 60, 70 and 80 °C, respectively. Meanwhile, in vacuum oven drying (VOD), the drying rates were observed between 0.0175 - 0.0019, 0.0229 - 0.0026 and 0.0307 - 0.0037 kgW/kgDM \times min for 60, 70 and 80 °C, respectively. The drying rates were found to increase with increasing temperature, as higher temperature results in faster heat and mass transfer, and hence faster water loss. Furthermore, the results indicated that vacuum application effectively accelerates the drying process.



Modeling and regression analyses results

Experimentally obtained moisture ratios and drying times were tested in 14 different mathematical models. In order to find the model that best fits with experimental data, R^2 , value is expected to be close to 1, while the lowest χ^2 and *RMSE* values are desired. All data placed on the 45° line and the most suitable models were listed in Table 1 and Table 2 with values $R^2 > 0.9998$.

The most compatible model for all drying methods was observed with Alibas model. For OD, the coefficients were found as 0.999967, 0.999970, 0.999995 R^2 ; 0.000003, 0.000003, 0.000001 χ^2 and 0.001541, 0.001498, 0.000622 *RMSE* for 60, 70 and 80 °C, respectively. For VOD, the coefficients were found as 0.999831, 0.999956, 0.999977 R^2 ; 0.000013, 0.000004, 0.000003 χ^2 and

0.003301, 0.001822, 0.001354 *RMSE* for 60, 70 and 80 °C, respectively. For US-OD, the coefficients were found as 0.999985, 0.999996, 0.999986 R^2 ; 0.000002, 0.000001, 0.000003 χ^2 and 0.001117, 0.000583, 0.001179 *RMSE* for 60, 70 and 80 °C, respectively. For US-VOD, the coefficients were found as 0.999996, 0.999987, 0.999996 R^2 ; 0, 0.000002, 0.000001 χ^2 and 0.000567, 0.001085, 0.000634 *RMSE* for 60, 70 and 80°C, respectively.

Effective moisture diffusivity results

The calculation of effective moisture diffusivity values was based on the slope of the ln(MR) versus drying time graphs, and the results were obtained for temperatures of 60, 70, and 80 °C. The first two methods, OD and VOD, resulted in effective moisture diffusivities of 3.97×10^{-11} , 4.78×10^{-11} , and 6.73×10^{-11} m²/s, and $4.90\times10^{\text{-}11},\,6.12\times10^{\text{-}11}$, and $8.67\times10^{\text{-}11}$ m²/s, respectively. It was observed that the temperature increased, and low-pressure medium resulted in a slight increase in the effective moisture diffusion values. The third and fourth methods, US-OD and US-VOD, resulted in effective moisture diffusivities of 6.08 imes 10⁻¹¹, 8.31 imes $10^{\text{-}11}$, and $1.20\times10^{\text{-}10}$ m²/s, and $7.2\times10^{\text{-}11}$, $1.05\times10^{\text{-}10}$, and $1.75\times10^{\text{-}10}$ 10⁻¹⁰ m²/s, respectively. These results indicated that ultrasonic pre-treatment was a factor that increased the effective moisture diffusivities values. Upon evaluating all the results, it was seen that the US-VOD method yielded the best diffusion values among the methods. Furthermore, it was determined that all the calculated values were within the range of 10⁻⁸ to 10⁻¹² m²/s specified in the literature for diffusion coefficients of biological materials (Ayriksa et al., 2022). Overall, the study provides valuable insights into the effective moisture diffusivity values for various temperatures and models, highlighting the importance of ultrasonic pre-treatment in enhancing the diffusion process.

Activation energy results

The effect of temperature on diffusivity is defined by the Arrhenius equation, and the activation energy is obtained from the slope of the $ln(D_{eff})$ plot against 1/T by multiplying by universal gas constant *R* (8.314 J/mol × K).

In the first method of OD, the slope of the graph was obtained as 3090 K, hence, the activation energy of drying was calculated as 25.69 kJ/mol. In the next methods, the slopes were obtained as 3287, 3994.5, and 5364.5 K, hence the activation energies as 27.33, 33.21, and 44.60 kJ/mol for VOD, US-OD, and US-VOD stages, respectively. When the results were examined, it is seen that vacuum and US pre-treatment have an increasing effect on the activation energy.

Color analysis results

When the *Cancer pagurus* color parameters given in Table 4 were compared, from the highest to lowest, *L** values were obtained in US-VOD, VOD, US-OD, and OD, respectively. Accordingly, it is seen that vacuum application and subsequent US pre-treatment are quite effective in preventing discoloration due to the shortening of drying times.

As the drying time increased, a^* redness-greenness value increased inversely to L^* values. The highest a^* value was obtained in the OD, US-OD, VOD, and US-VOD methods, respectively. In addition, b^* blueness-yellowness values were obtained from the highest to the

Table 1.	OD and VOD methods coe	fficients and statistical	data.		
Method	Model	Parameter	60°C	70°C	80°C
		а	0.826245	1.125136	0.750317
		k	0.003378	0.003859	0.006218
		n	1.141137	1.067582	1.099287
OD	Alibas	b	-0.000269	0.000175	-0.000891
OD	Alibas	g	0.171153	-0.126821	0.249788
		R^2	0.999967	0.999970	0.999995
		χ²	0.000003	0.000003	0.000001
		RMSE	0.001541	0.001498	0.000622
		а	1.000793	0.997232	1.001945
		k	0.003768	0.003837	0.006250
		n	1.085134	1.088471	1.044227
OD	Midilli & Kucuk	b	0.000048	-0.000056	-0.000225
		R^2	0.999928	0.999966	0.999966
		χ ²	0.000010	0.000003	0.000003
		RMSE	0.002291	0.001608	0.001621
		а	-0.047378	-1.142330	-3.667693
		k	0.045039	0.002930	0.014252
		g	0.005961	0.004220	0.012623
OD	Verma	R^2	0.999921	0.999677	0.999378
		χ ²	0.000006	0.000028	0.000059
		RMSE	0.002403	0.004969	0.007023
		а	0.907010	0.978223	0.786354
		k	0.007020	0.005348	0.008596
		n	1.004697	1.136896	1.142108
NOD	A 1:1	b	-0.000060	0.000113	-0.000774
VOD	Alibas	9	0.096707	0.019303	0.212804
		R^2	0.999831	0.999956	0.999977
		χ ²	0.000013	0.000004	0.000003
		RMSE	0.003301	0.001822	0.001354
		а	1.006291	0.998019	1.002602
		k	0.007431	0.005441	0.009861
		n	0.971580	1.128988	1.052590
VOD	Midilli & Kucuk	b	0.000101	0.000166	0.000021
		R^2	0.999797	0.999955	0.999824
		χ ²	0.000135	0.000004	0.000019
		RMSE	0.010851	0.001853	0.003795
		а	0.906495	0.000074	-0.034320
		b	-0.007166	0.019274	-2.639500
		С	0.097983	1.021629	1.034320
VOD	Two-term	d	-0.000791	-0.009614	-0.012820
		R^2	0.999831	0.999071	0.999867
		χ ²	0.000012	0.000083	0.000013
		RMSE	0.003297	0.008444	0.003297

lowest in US-VOD, VOD, US-OD, and OD with L^* values and in contrast to a^* values. When Table 4 is examined, it is seen that increasing drying temperatures reduce the change in color values. This is because the increasing temperature reduces the drying time. In addition, it was stated in the literature that the temperature increase increased the L^* and b^* values and decreased a^* values for all drying methods, and it was found to be compatible with this study (lsik, Ozdemir, & Doymaz, 2019). Total change of color ΔE values was obtained as 22.67, 21.84, 21.18 in OD; as 21.57, 19.23, 18.00 in VOD; as 22.30, 21.63, 19.85 in US-OD and as 20.33, 17.69, 17.09 in US-VOD at 60, 70 and 80 °C, respectively. When the ΔE values given in Table 4 were evaluated, as expected, the US-VOD was the least color change, and the OD was the method with the highest color change. It can be said that the drying-thermal exposure time plays an im-

Table 2.	US-OD and US-VOD methods	s coefficients and statisti	ical data.		
Method	Model	Parameter	60°C	70°C	80°C
		а	0.819830	1.576497	0.556739
		k	0.005351	0.005091	0.011153
		n	1.036487	0.992529	1.067161
	Alibas	b	-0.000972	0.000233	-0.003223
US-OD	Alibas	g	0.180970	-0.576187	0.443556
		g R²	0.999985	0.999996	0.999986
		χ^2	0.00002	0.000001	0.000003
		RMSE	0.001117	0.000583	0.001179
		а	1.359024	1.439650	1.519793
		k	0.004374	0.005260	0.006919
US-OD	Le gerithmie	С	-0.357436	-0.439931	-0.520653
03-00	Logarithmic	R^2	0.999975	0.999995	0.999934
		χ^2	0.000002	0.000000	0.000009
		RMSE	0.001437	0.000630	0.002515
		а	1.001334	0.999696	1.001047
		k	0.005029	0.006301	0.009206
		n	1.017187	1.017572	0.999035
US-OD	Midilli & Kucuk	b	-0.000614	-0.000942	-0.001585
		R^2	0.999983	0.999992	0.999968
		χ^2	0.000002	0.000001	0.000010
		RMSE	0.001191	0.000858	0.002515
		а	1.006000	1.003000	1.002100
		k,	-159.3560	-125.9880	-91.13980
US-Oven	Peleg	$rac{k_2}{R^2}$	-0.462000	-0.431000	-0.405200
03-0ven	releg		0.999938	0.999954	0.999968
		χ^2	0.000006	0.000005	0.000004
		RMSE	0.002242	0.001995	0.001761
		а	1.037983	1.138559	1.537330
		k	0.006203	0.007587	0.014648
		n	1.060824	1.073670	0.928680
		b	0.000022	0.000207	0.000912
US-VOD	Alibas		-0.037979	-0.138069	-0.537275
		g P²			
		R^2	0.999996	0.999987	0.999996
		χ^2	0.000000	0.000002	0.000001
		RMSE	0.000567	0.001085	0.000634

Table 4.	Color parameters for each m	ethod.			
Method	T (°C)	L*	a*	b*	ΔE
Fresh	-	35.93	-6.56	-7.13	-
	60	18.15±0.20	10.86±0.25	4.95±0.67	22.67
OD	70	18.99 ±0.61	9.81±0.20	6.17±0.31	21.84
	80	19.36±0.05	8.50±0.18	7.23±0.17	21.18
	60	20.61±0.58	9.50±0.22	8.15±0.23	21.57
VOD	70	23.12±0.40	6.76±0.27	9.52±0.07	19.23
	80	25.23±1.04	5.48±0.11	10.25±0.01	18.00
	60	19.08±0.18	10.46±0.28	6.31±0.12	22.30
US-OD	70	20.57±0.56	9.70±0.74	7.93±0.42	21.63
	80	22.15±0.15	7.65±0.65	8.79±0.79	19.85
	60	22.84±0.40	8.38±0.04	9.36±0.02	20.33
US-VOD	70	27.59±0.97	5.92±0.21	10.49±0.14	17.69
	80	30.36±0.66	4.92±0.81	11.20±0.47	17.09

portant role in the change of color values, and the color change increases in direct proportion to the exposure time. This situation is also clearly seen in Figure 1.

CONCLUSION

The study aimed to investigate the drying kinetics and color analysis of Cancer pagurus using various drying methods, including OD, VOD, US-OD, and US-VOD at different drying temperatures. In addition, mathematical modeling of drying curves was conducted to determine the best fit model. The results showed that vacuum and US pre-treatment significantly reduced the drying times compared to the traditional OD method. Specifically, vacuum reduced the drying times from 420 - 255 min to 345 - 210 min in the OD method, while US pre-treatment reduced the drying times to 255 - 135 min for the OD method and to 240 - 120 min for the VOD method. The Alibas model provided the best fit for the drying curve data with R^2 values greater than 0.9999. Moreover, US pre-treatment and vacuum increased the D_{aff} and E_{a} values, which were found to be 0.367 - 0.673 \times 10⁻¹⁰ m²/s, 0.490 - 0.867 × 10⁻¹⁰ m²/s, 0.608 - 1.20 × 10⁻¹⁰ m²/s, and 0.721 - 1.75 × 10⁻¹⁰ m²/s for the OD, VOD, US-OD, and US-VOD methods, respectively. The *E*₂ values were calculated as 25.69, 27.33, 33.21, and 44.60 kJ/mol for the OD, VOD, US-OD, and US-VOD methods, respectively. It was observed that US pre-treatment and vacuum decreased the ΔE values, which ranged from 22.67 - 21.18, 21.57 - 18.00, 22.30-19.85, and 20.33 - 17.09 for the OD, VOD, US-OD, and US-VOD methods, respectively. As a result, vacuum and US pretreatments significantly reduced drying times and increased D_{eff} and $E_{a'}$, which are critical factors in final product quality. The use of pre-treatment or drying aiding technologies, such as vacuum and ultrasound, has a substantial influence on improving drying performance and the guality of the end product, according to the study's findings. The observed decrease in color change values further supports this assertion.

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Research Article

Effects of Total Suspended Solids at Different Levels on the Eggs and Larvae of Endemic Fish, Tarek (*Alburnus tarichi* Güldenstädt, 1814) in the Karasu River (Van, Turkey)

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ABSTRACT

The Karasu River (Van, Turkey) is one of the most important sources of intensive reproduction for Tarek (Alburnus tarichi Güldenstädt, 1814) in the Van Lake basin. However, sand pits and sand extraction activities in the Karasu River threaten the spawning areas of A. tarichi. This study aimed to investigate how increased sediment concentration in the Karasu River affects the eggs and larvae of A. tarichi during the reproductive period. The study in the laboratory was designed by measuring total suspended solids and turbidity associated with the river over two years for five different experimental groups (corresponding to 0, 10, 50, 100, and 600 mg L⁻¹, and associated groups: control group, Group 1, Group 2, Group 3, and Group 4, respectively). The percentage of hatched out larvae was statistically different between the groups (p<0.05). The highest percentages of hatched out larvae were found in the control group (73.98±5.89%) and Group 1 (68.05±2.96%). The highest survival rates in larvae were determined in the control group (53.35±5.62%) and Group 1 (44.98±4.41%). These results demonstrate that A. tarichi is sensitive to suspended solid concentrations that are very common in the natural environment. In conclusion, sand pits and sand extraction activities must be stopped completely during the reproductive period of A. tarichi between May and July and strict controls should be put in place during this time. These measures will provide an important contribution to ensuring the continuity of this species.

Keywords: Destruction zones, sand extraction activities, sand pits, sediment effects, turbidity

INTRODUCTION

Alburnus tarchi (Güldenstädt, 1814) is endemic to the Van Lake Basin (Elp et al., 2014, 2016; Sen et al., 2015). Considering the biological characteristics of this species, *A. tarichi* migrates to the rivers from Van Lake for the purpose of reproduction and shows mass distribution in the rivers between May 15 and June 15 (Cetinkaya et al., 2014). Moreover, tons of A. tarichi are caught from Van Lake every year during the fisheries season and offered for human consumption (TUIK, 2020).

Sediment transport, suspension and settlement play important roles in water quality and aquat-

ic lives. Because of their influence on density, light penetration, and nutrient availability, total suspended solids effects eutrophication processes, biological and chemical reaction rates in the water column (Sutherland & Meyer, 2007; Jin & Ji, 2013).

The Karasu River is one of the most important sources of intensive breeding for *A. tarichi.* However, it has been reported that this density is reducing each passing year (Elp et al., 2006). In the first half of 2000s, there was over 90% reproductive success compared to today. In the Van Lake basin, sand pits and sand extraction activities in

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the Karasu River, threaten the reproductive areas of *A. tarichi*. Due to sand extraction activities, the silt forms a sediment that slowly settles at the river bed and then passes into the form of total suspended solids in the water. During this sedimentation process, silt accumulates on the eggs and hatching of the eggs is prevented (Atici et al., 2018).

It was seen that sand has been extracted from the river beds of Hosap, Ilica, Karmuc and Zilan as well as the Karasu River. Also the structure of the river bed had been damaged by sand extraction and produced observable turbidity in the river. Sand washing activities in sand pits also affects the water turbidity. On the other hand, while the sand extraction activities are stopped due to snow in winter, they are reactivated with the arrival of spring and the warming of the weather. These activities take place in the spring during the breeding migration of *A. tarichi* in the Karasu River (Sen et al., 2015). Because of sand extraction from the Karasu River, mass fish mortality was observed in 2003 to 2004, especially in the months of May to June (Elp et al., 2006).

Studies on sand pits and sand extraction effects have shown that these activites are very harmful for the ecosystem. There are many sand pits in Karasu River and increasing sand extraction activities in spawning aeras threaten the amount of *A. tarichi* catching in Van Lake (Atici et al., 2018).

On the other hand, while there are many studies that report on bioecology, life history, environmental conditions, morpholgy, systematic characters, population structure, growth, condition, reproduction biology, feding, stock assessment, fisheries management and histologic studies on *A.tarichi*, there are no data about the effects of suspended solid concentrations on the population of *A. tarichi*. This study was performed to determine how increased suspended sediment concentration in the Karasu River affects the eggs and larvae of the *A. tarichi* during the reproductive period.

MATERIALS AND METHODS

Migration area

Van Lake (pH 9.81, 22‰ total salinity), is the largest soda-lake on Earth, present an extreme living environment to which comparatively few orgasims have adapted. The only commercial fish species known to occur in Van Lake is the anadromus cyprinid *A. tarichi* (Danulat & Sekcuk, 1992). Since the freshwater environment is needed for the eggs to hatch, they must migrate to the Karasu River (pH 8.36, 0.31‰ total salinity). Karasu River, which sources from the Turkey - Iran border, is poured into Van Lake from the Citoren Reeds (38°34′51.54″N - 43°13′21.42″E) to the north of the Van center (Figure 1). There are Sarımehmet Dam and Satibey Regulator built in 1991 for irrigation purposes on the river (Cetinkaya et al., 1994).

Experimental design

This study was carried out during the reproductive period of the *A. tarichi* between May 2016 and August 2016. During the experimental period, temperature, oxygen and pH parameters were measured with a multimeter (HACH model DR-5000, Loveland, USA). The total suspended solids were analyzed using a spectrophotometer (HACH model HQ-40d, Loveland, USA) and turbidity was measured in nephelometric turbidity unit (NTU) with a turbidy meter (HACH model 2100-Q, Loveland, USA).

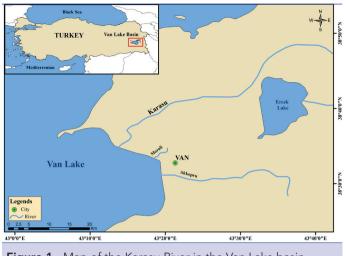


Figure 1. Map of the Karasu River in the Van Lake basin.

Sediment suspension apparatus were modified from Sutherland (2005, 2006) and Sutherland and Meyer (2007). The all experiments were carried out in 10 experimental units, each composed of a 30 liter glass aquarium ($50 \times 20 \times 30$ cm) and a motor-driven paddle which moves slowly ($\sim 3 - 5$ mm sec⁻¹) back and forth along the bottom of the aquarium (Figure 2). The paddle was traveled along the bottom, sediment that has settled, was resuspended. To aid in resuspension, air lines have been attached to each paddle allowing it to function as a slow moving air diffuser.

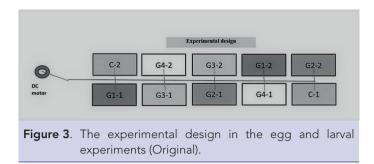


Figure 2. Sediment suspension apparatus used in the experiments: drive mechanism, drive shaft cross coupling, and arm (Original).

The study in the laboratory was designed by measuring total suspended solids and turbidity associated with the river over two years (Atici et al., 2018) for five different experimental groups (corresponding to 0, 10, 50, 100, and 600 mg L⁻¹, and associated groups: C group, G1, G2, G3, and G4, respectively). Suspended sediment treatments were set up with 2 replicates of 5 inorganic sediment concentrations (Figure 3). Natural sediment used in trials was collected from Karasu River.

Egg trials

The mature female and male *A. tarichi* were taken from the Karasu River in May 2016. The eggs and sperms were stripped from the spawner artifically and fertilized by the dry method under labora-



tory conditions. Fertilized eggs ranged from 329-344 were placed in the incubator of trial aquariums (i.e. 5 treatments × 2 tanks per treatment level × 3 trials × 329-344 egg / hatchery). For each egg experiment, a total of 10 incubators ($20 \times 14.3 \times 4.5$ cm) were used. The incubators were placed at 10 cm above the bottom of trial aquariums. Egg experiments lasted 7 days and the average temperature, dissolved oxygen, and pH were found to be: 22.1 ± 0.04 °C, 8.43 ± 0.00 mg L⁻¹ and 8.41 ± 0.00 , respectively.

Sediment accumulation at hatchery were measured to the nearest 0.01 mm using a digital caliper. The percentage values of hatched out eggs (HE), dead larvae (DL) and live larvae (LL) were calculated using the equations 1, 2, and 3, respectively.

HE (%) = the number of dead larvae (%) + the number of live larvae (%) (1)

DL (%) = [the number of dead larvae \div the total values of fertilized eggs]×100 (2)

LL (%) = [the number of live larvae \div the total values of fertilized eggs]×100 (3)

Larvae trials

The larval experiments were carried out with larvae hatched from eggs of A. tarichi in the laboratory. The fertilized eggs were incubated in at 18 \pm 1 °C incubators for approximately 4 days. On the 5th day after hatching, larvae were fed with commercial feed (45% crude protein, 4% crude fat and 3% crude fiber) twice a day and the water in the tanks was changed daily. After acclimation, the larvae were exposed to sediment treatments for 14 days. The same procedures used in the first larval experiment were also performed for the second larval trial. Two 14-day trials were conducted using 1-1.5 month old post larvae. In each of two trials, 300 lab-reared A. tarichi were randomly chosen from holding tanks and placed in the experimental aquariums (i.e., 30 fish per tank \times 2 tanks per treatment level \times 5 treatments \times 2 trials = 600 fish). Before each trial began, the fish were allowed to acclimate to the apparatus for 48 hours. The mean temperature, dissolved oxygen, and pH were found as 23.0 ± 0.01 $^{\circ}$ C, 8.43 ± 0.00 mg L⁻¹ and 8.40 ± 0.00, respectively.

The initial weight and final weight of the larvae were measured on scales to a precision of 0.0001 g. The number of dead larvae (DL) was determined by equation 4. The survival rate (SR) was calculated using the equation 5 (Pechsiri & Yakupitiyage, 2005).

The number of DL= the total number of larvae -the number of live larvae (4)

SR (%)= [number of live fish \div initial number of fish]×100 (5)

Statistical analysis

A statistical software package, SPSS 21.0, was used to statistically evaluate the data obtained at the end of the experiment. A one-way ANOVA analysis was applied and the difference between the averages was assessed by the Duncan Multiple Comparison Test at a significance level of 0.05. The results are expressed as the mean \pm standard deviations (SD). Pearson's rank correlation was used to establish relationships between parameters (Uzgoren, 2012).

RESULTS AND DISCUSSION

The total suspended solids (TSS) in water are known to have an effect on the reproduction, growth and nutrition of fish such as salmon, trout, carp, bass, herring, anchovy, and killifish (New-combe & Jensen, 1996; Barton, 2002). Sand pits and sand extraction activities in the water column can result in increased TSS levels. However, although the source and the effect of TSS are known, these activities need to be expressed numerically. In order to sustain the Karasu River as a breeding environment for *A. tarichi*, there is a need for these studies.

The average sediment accumulations in the egg experiments were recorded as 0.00 ± 0.00 cm (control group), 0.31 ± 0.07 cm (G1), 0.55 ± 0.12 cm (G2), 1.35 ± 0.12 cm (G3) and 1.70 ± 0.17 cm (G4) at the end of the 7th day (Table 1). The percentages of hatched eggs (HE) were highest in the control group (73.98 ± 14.43%) and in G1 (68.05 \pm 7.25%), while the rate of hatched eggs in G2 (44.62 \pm 21.85%), G3 (8.14 \pm 5.09%) and G4 (2.87 \pm 2.50%) decreased with sediment accumulation. A negative correlation was found between TSS and the number of hatched eggs, and there was a positive relationship with the sediment accumulation (Table 2). These results show that the rate of hatching decreases with an increasing amount of TSS. The duration and degree of exposure to TSS are important factors to consider in determining effects on aquatic organisms (Berry et al., 2003). In another study, salmon (Oncorhynchus keta) eggs were exposed to 97 mg L⁻¹ of TSS for 117 days resulting in a mortality rate of 77% (Langer, 1980), while rainbow trout (Oncorhynchus mykiss) eggs were exposed to 57 mg L⁻¹ of TSS for 62 days, resulting in a mortality rate of 47% (Slaney et al., 1977). In addition, it was observed that egg development was slowed and hatching was delayed in striped bass (Morone saxutilis) and white perch (Morone americana) exposed to 800 mg L⁻¹ TSS for 24 hours (Morgan et al., 1983). Sutherland (2005) reported whitetail shiner (Cyprinella galactura) eggs in the experimental group exposed to 500 mg L⁻¹ TSS could not be opened. In our study, hatching percentages were found to be very low in G2, G3 and G4. It was determined that this situation was related with the amount of sedimentation on eggs at the high TSS levels (100-600 mg L⁻¹) for 7 days.

In other studies, mean TSS were reported as 212.5 mg L⁻¹ at Karasu River (Atici et al., 2018), 11.8 mg L⁻¹ at Catakdibi (Aydın, 2018), 18.5 mg L⁻¹ at Donerdere, 6.5 mg L⁻¹ at Yumruklu, 110 mg L⁻¹ at Dolutas, and 44 mg L⁻¹ at Degirmigol Ponds (Atici, 2020) in Van Lake basin. In the measurements made using sediment trays to determine sediment accumulation in the Karasu River has accu-

Aquat Sci Eng 2023; 38(3): 145-150 Atici, Elp and Sen. Effects of Total Suspended Solids at Different Levels on the Eggs and Larvae of Endemic Fish, Tarek (Alburnus tarichi...

Table 1.

The total number of fertilized eggs (FE), the percentage values of hatched out eggs (HE), dead larvae (DL) and live larvae (LL) and the values of sediment accumulation (SA) in three trials (Mean ± SD).

	Groups							
	С	G1	G2	G3	G4			
FE	344.67±10.51	329.17±17.51	329.83±12.15	331.83±10.61	331.00±13.64			
HE (%)	73.98±14.43°	68.05±7.25ª	44.62±21.85 ^b	8.14±5.09°	2.87±2.50°			
DL (%)	12.38±7.25 ^b	16.51±6.81 ^b	17.94±5.32 ^b	3.42±2.06ª	0.76±0.51ª			
LL (%)	61.61±18.44°	51.54±13.01°	26.68±23.47 ^b	4.72±6.00°	2.11±2.65ª			
SA (cm)	0.00±0.00ª	0.31±0.07 ^b	0.55±0.12 [°]	1.35±0.12 ^d	1.70±0.17°			

In the same column, the differences between the values indicated by the same letters are statistically insignificant (p>0.05) and the differences between the different letters are significant (p<0.05).

mulated an average of 3.23 ± 0.35 cm sediment at the end of the 3rd day (Atici et al., 2018). The present study, the highest sediment accumulations in the egg experiments were recorded as 1.35 ± 0.12 cm in G3 and 1.70 ± 0.17 cm in G4 at the end of the 7th day. Due to the excess amount of sediment carried in the study conducted in Karasu River, approximately twice as much sediment was accumulated in the half time of the current study.

It has been observed that increased sedimentation was effective in reduced the success of hatching (Figure 4).

At the beginning of the experiment, there were 30 larvae in all groups (Table 3), but at the end of the experiment there was a statistically significant difference between the dead larva groups (p<0.05). The highest numbers of dead larvae were in G2 (26.0 ± 2.0), G3 (28.0 ± 2.0) and G4 (29.0 ± 2.0), while the lowest values were in the control group (14.0 ± 4.0) and G1 (17.0 ± 4.0).

The survival rates in the groups showed statistically significant differences (p 0.05) (Table 3). The survival rate was the highest in the control group (53.35 ± 11.24%) followed by G1 (44.98 ± 8.82%). The survival rate was comparatively less in the G2 (13.33 \pm 2.74%), G3 (7.50 \pm 6.34%) and G4 (2.50 \pm 3.20%) groups. The larval survival rate was reported as 94.3% at 25 mg L⁻¹ TSS in Arctic grayling (*Thy*mallus arcticus), 58% at 500 mg L⁻¹ TSS in striped sea bass (Morone saxatilis), and 82% and 64% at 100 and 500 mg L⁻¹ TSS respectively, in American shad (Alosa sapidissima) (Auld & Schubel, 1978; Berry et al., 2003). In increasing amounts of sediment, mucus and sediment accumulates in the gills of fish, the gill epithelium thickens, respiratory function is reduced and the fish epidermis is affected by mechanical damage (Goldes, 1998). The highest larval survival rate for A. tarichi was determined to be in the control group and G1, which had the lowest level of TSS. Our study is consistent with other studies, which show that as the amount of TSS increases, the survival rate of the larvae decreases (Table 3).

There was a statistical difference between the final live weights of the larvae in the groups (p 0.05). The final live weights were measured as 0.50 g (control group), 0.37 g (G1), 0.16 g (G2), 0.13 g (G3), and 0.06 g (G4), respectively.

A negative correlation was found between the increase in the amount of TSS and the survival rate. However, there was a positive relationship between TSS and the number of dead larvae (Table 4).

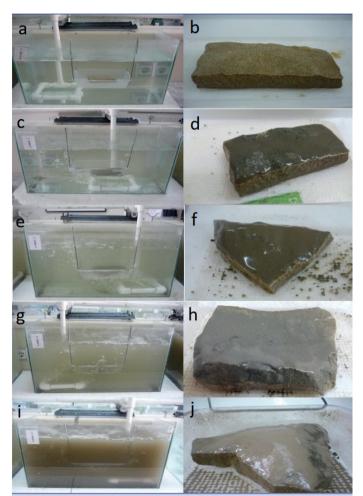


Figure 4. Sediment accumulation in the egg experiments: a. experiment of control group, b. sediment accumulation in control group, c. experiment of G1, d. sediment accumulation in G1, e. experiment of G2, f. sediment accumulation in G2, g. experiment of G3, h. sediment accumulation in G3, i. experiment of G4, j. sediment accumulation in G4 (Original).

Table 2. The correlation results in egg experiments.										
Egg Experiment	TSS	Turbidity	LL	DL	HE	SA				
TSS	1	0.999**	-0.564**	-0.660**	-0.654**	0.800**				
Turbidity	0.999**	1	-0.561**	-0.660**	-0.651**	0.797**				
*: p<0.05 significant, **: p<0.01 significant.										

Table 3.The total number of larvae (TNL), the number of dead larvae (DL), the initial weight (IW), the final live weight (FW)
and the survival rate (SR) in the two larval experiments (Mean ± SD).

Groups									
	С	G1	G2	G3	G4				
TNL	30.0	30.0	30.0	30.0	30.0				
DL	14.0 ± 4.0^{a}	17.0 ± 4.0^{a}	26.0 ± 2.0^{b}	28.0 ± 2.0^{b}	29.0 ± 2.0^{b}				
IW (g)	0.31 ± 0.06	0.30 ± 0.04	0.29 ± 0.08	0.28 ± 0.08	0.26 ± 0.04				
FW (g)	$0.50 \pm 0.10^{\circ}$	0.37 ± 0.06^{b}	$0.16 \pm 0.06^{\circ}$	$0.13 \pm 0.08^{\circ}$	$0.06 \pm 0.02^{\circ}$				
SR (%)	53.35 ± 11.24 ^b	44.98 ± 8.82^{b}	13.33 ± 2.74°	$7.50 \pm 6.34^{\circ}$	2.50 ± 3.20ª				

In the same column, the differences between the values indicated by the same letters are statistically insignificant (p>0.05) and the differences between the different letters are significant (p<0.05).

Table 4. The correlation results in larvae experiments.	
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	TSS	Turbidity	DL	SR			
TSS	1	1.000**	0.580*	-0.580*			
Turbidity	1.000**	1	0.582*	-0.582*			
*: p<0.05 significant, **: p<0.01 significant,							

CONCLUSION

The results obtained in this study generally emphasized the effects of TSS concentrations on *A. tarichi* eggs and larvae. Studies on sand pits and sand extraction effects have shown that these activities are very harmful for the ecosystem. While there are many studies that report TSS concentrations effects for aquatic organisms, there are no data on *A. tarichi*. *A. tarichi* migrates to Karasu River lays 8500 - 9000 sticky eggs in shallow and vegetative areas (Elp, 1996) (Figure 5a). However, there are many sand pits in Karasu River and increasing sand extraction activities in spawning aeras threaten to *A. tarichi* (Figure 5b). So, this study has demonstrated under laboratory conditions how increased TSS may directly affect *A. tarichi*.

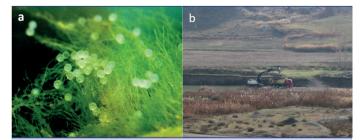


Figure 5. a. sticky eggs on vegetative structure, b. sand extraction process in Karasu River (Original).

In our study, TSS concentrations ranged from 100 to 600 mg L⁻¹ were determined to have a damaging effect on the eggs and the larval survival rates were significantly lower at the these high treatment levels. These results demonstrate that *A. tarichi* is sensitive to suspended solid concentrations that are very common in the natural environment. It is necessary to take various measures to sand extraction and the sand pits activities. Sand extraction activities in the reproductive regions should be prohibited during the reproductive period of *A. tarichi* between May and July and sand pits should be consciously planned when selecting their locations.

Conflict of interest: The authors declare no conflict of interest.

Ethics committee approval: Final report of the research project detailed above was approved by Van Yuzuncu Yil University Animal and Research Local Ethics Committee in the session held on 25.05.2017 (decision number 2017/05).

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Research Article

Brood Rearing and Dose Optimisation for Induced Breeding of Raikor, *Cirrhinus reba* (Hamilton, 1822)

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ABSTRACT

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An experiment on brood rearing and induced breeding of the near threatened fish species Raikor, *Cirrhinus reba* using the pituitary gland (PG), was conducted from March to August 2020 at the Floodplain Sub-station of Bangladesh Fisheries Research Institute, Santahar, Bogura. Broods were collected and reared in the ponds of the hatchery complex. The total length (cm), body weight (g), gonad weight (g), and gonado-somatic index (%) of this species were measured during the rearing period. To standardize the breeding technique, a total of 90 brood fish of *C. reba* were treated with different doses of PG, specifically, 2.0, 4.0, 6.0 mg/kg body weight for females and 1.0, 2.0, 3.0 mg/kg body weight for males in different treatments, namely T_1 , T_2 , and T_3 respectively. A significant difference (p<0.05) was observed in fecundity, ovulation (%), and the fertilization rate (%) among the treatments. Based on the results, T_2 (4.0 mg/kg body weight for females, 2.0 mg/kg body weight for males) produced the most favorable results. The current observations could be applied to *C. reba* stimulated breeding for the advancement of hatchery formation. More research on the nursing, nurturing, and culture of the near- threatened *C. reba* at varied densities and feedings is necessary for their conservation and restoration.

Keywords: Induced breeding, embryology, gonado-somatic index, C. reba

INTRODUCTION

Bangladesh, a self-sufficient country that ranks third in inland open-water capture production, fifth in world aquaculture production, and fourth in world tilapia production (FAO, 2022), is supplying a total output of 4.62 million metric tons per year (DoF, 2022). The Fisheries sector contributes up to 60% of animal protein consumption in Bangladesh (Sufian et al., 2017; DoF, 2022; Mustafi et al., 2022) and fish is the primary source of protein for most people (Haque et al., 2015).

The floodplain contains both persistent and temporary wetlands which are both highly important and vulnerable to various hydrological changes and threats (Maria et al., 2016; Mondal & Pal, 2017). Bangladesh has an extensive number of unique watercourses, including rivers, lakes, floodplains, estuaries, canals, *beels*, *haors*, and *baors* (IUCN Bangladesh, 2015; Suravi et al., 2017; Pandit et al., 2020) which occupies about 80% of the country's land area (Brammer, 1990) and is home to 65% of Bangladesh's inhabitants (di Baldassarre et al., 2014). Most of the rural populations in the floodplains rely on natural water sources for their livelihoods and agricultural activities (Pandit et al., 2022).

Among the 260 freshwater fish species (Rahman, 2005; DoF, 2022), about 64 and 27 freshwater species are under threatened and near-threatened conditions, respectively (IUCN Bangladesh, 2015). *Cirrhinus reba* (Hamilton, 1822) is a highly nutritious, near-threatened fish which is rich in protein, fat, vitamins, and minerals (Bogard et al., 2015) and is dispersed in India, Bangladesh, Pakistan, Nepal, Myanmar, and Thailand (Bhuiyan, 1964; Jayaram, 1981; Davis & West, 1993; Rahman, 2005; Talwar & Jhingran, 1991; Bogard et al., 2015). This delicious and popular fish (Reba carp) is locally known as Reba, Raik, Bata, Aikhor, Raikor, and Tatkini. In the past, Raikor was found in large quantities in natural water bodies in Bangladesh, but this fish species is also currently under threat, due to both human activities and ecological factors such as habitat destruction and loss, construction of flood protection embankments and roads, overfishing, water pollution, climate change, and the introduction of invasive alien species (Galib et al., 2009, 2013; Imteazzaman & Galib, 2013; Mohsin et al., 2009; Mia et al., 2017; Islam et al., 2019; Pandit et al., 2021; Talukder et al., 2021; Das et al., 2022a; Mia et al., 2022; Kamal et al., 2022; Kunda et al., 2022). In these circumstances, Bangladesh is working to develop artificial breeding, fry production, and farming management technologies through research to save this fish from extinction.

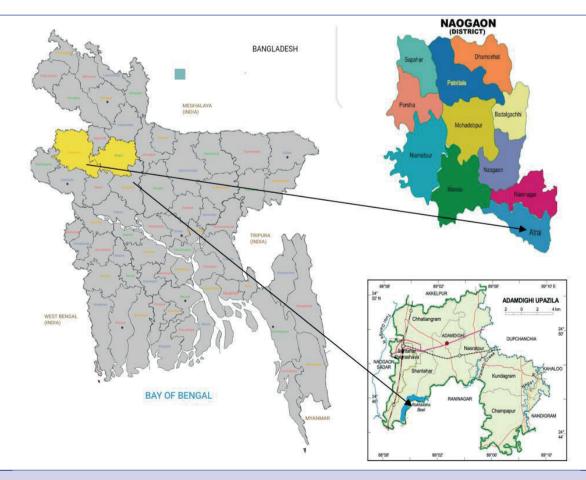
Production of *C. reba* from natural water bodies is decreasing day by day (Gupta & Banerjee, 2016). Artificial breeding can increase the number of fingerlings and fry, and the development of culture technology can offer cultivation of these fish in shallow waterways (Haque et al., 2023). This species has a high market

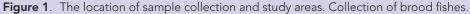
price in the commercial market (Sultana et al., 2018), is locally available, is cultivable in small ponds, matures in 1 year, is easier for artificial breeding, has a prolonged breeding period, is delicious to eat, and is environmentally friendly. Numerous explorations on the biology of this species are available, such as on feeding (Naik et al., 2015, Lashari et al., 2010), life-history character (Hossain et al., 2013), and induced spawning (Sarkar et al., 2004). Lashari et al. (2007) sampled the gonado-somatic index (GSI) and reproductive success of C. reba in fishponds in the area of Jacobabad, Sindh, Pakistan. Although induced breeding techniques of this species have been attempted in some cases, the technique has not yet been standardized. The recording of reproductive capacity is a vital part of the fisheries realm because it has a direct impact on fish manufacturing (Mian et al., 2017; Shafi et al., 2012). The current study aims to understand C. reba's brood-rearing process, determine the appropriate pituitary gland (PG) hormone dosage for induced breeding, know the fecundity and nursing of fry, and evaluate water quality factors.

MATERIALS AND METHOD

Experimental site

From March to August 2020, the study was conducted at the Bangladesh Fisheries Research Institute's floodplain sub-station at Santahar, Bogura (Figure 1).





The Raikor species is commonly found thriving in various aquatic environments such as rivers, canals, ponds, *beels*, and floodplains across Bangladesh, including the Ratargul swamp forest (Kunda et al., 2022), Kawadighi *Haor* (Kamal et al., 2022), Bookbhara *Baor* (Mohsin et al., 2009), Chalan *Beel* (Galib et al., 2009), Surma River (Mia et al., 2022), Choto Jamuna River (Galib et al., 2013), Juri River (Islam et al., 2019), Shari-Goyain River (Talukder et al., 2021; Das et al., 2022a), Dhanu River (Pandit et al., 2021), and Halti *Beel* (Imteazzaman & Galib, 2013). Full-grown, robust, and pathogen-free fish were collected from Bogura's Raktadaho *Beel* and Naogaon's Little Jamuna River.

Pond preparation and brood nurturing

Prior to the start of the research, the ponds, which were 1.5m deep, were cleared of vegetation, dried, limed (250kg/ha), and manured with cow dung at a rate of 500kg/ha. Liming was done 4-5 times per year during pond preparation and during the culture period at 0.40-1.20kg per decimal. Fertilization was done prior to fish stocking and as needed during the culture period. Urea and TSP (Tripple Super Phosphate), as well as MP (Murate of Potash) and DAP (Di-ammonium Phosphate), were used as fertilizers at the study site. Underground water was used to meet the mandatory water depth of the fishpond.

Feeding

Following stocking, the fish were fed 10% of their body weight once daily in the morning in the form of dough balls. Supplemental feed included 60% rice bran, 30% mustard oil cake, and 10% flour.

Selection of broods for breeding

Healthy and disease-free Reba broods can be recognized by their external sexual characteristics. Male fish have tough, sandy scales on the flanks, neck area, and anterior dorsal side, whereas females have smooth scales. Males are longer and have larger pectoral fins than females. Males are distinguished by their flat abdomens and long, protruding genital papillae, whereas females have soft, oozing abdomens.

The brood fish conditioning and PG extract preparation

Broods were measured and placed in separate cisterns with continuous water flow for 8–9 hours to condition. A freshly prepared extract of widely viable wet pituitary glands was used to induce ovulation. The specified volume of PG was carefully measured using an electronic balance, and the PG was prepared for administration to the broods.

Experimental design

During the study period, nine fishponds were assigned for three treatments, each with three replicates, in a Completely Randomized Design (CRD) (Table 1).

Reba's gonads were accumulated between March and August of 2020. During the experiment, 50 samples were collected to calculate the GSI. The ovary was removed completely, weighed, and stored in 10% buffered formalin.

Histology of gonads

Throughout the experiment, both types of gonads were collected from the brood fish. Fish were dissected and arranged in a

Table 1.	Desig	Designing the experiment.								
nents	f fish	ation	atio	PG c (mg/						
Treatments	No. of	Replicati	Sex ra	М	F					
T1	10	3	Male: Fe-	1	2					
T2	10	3	male=1:1	2	4					
Т3	10	3		3	6					
Gonado-som	Gonado-somatic index (GSI)									

balanced buffered formalin solution. Specimens were prepared in a scaled alcohol and xylene installation before being encapsulated in paraffin columns in plastic cassettes. The conventional hematoxylin and eosin staining methodology described by Van-Dyk & Pieterse (2008) was used. Portions of 3-5 microns were cut using a servomotor microtome machine (Leica RM 2125), and photographs of the besmirched phases were picked with a composite photomicroscope.

Fecundity

The Von Vayer method was used to calculate fecundity. The gonads were picked out with scissors and the exterior connective tissues of the ovaries were removed. Blotting paper was used to absorb any excess moisture, and the ovaries were weighed with an electronic balance (Model FX- 300). Then, 10 mg from each gonad was precisely extracted. The overall egg count was determined by multiplying the overall average number of eggs counted by the total weight of the ovary.

Injecting PG extract into the experimental fish

To inject PG extract into the experimental fish, each Reba was carefully placed on a soft fabric piece. A graded 1-ml syringe was filled with the required amount of PG solution, which was injected intramuscularly under the fish's pectoral fin. A specific amount of the PG extract was injected into brood fishes according to the experimental protocol (Table 1).

Ovulation, fertilization, and embryonic development

Broods were separated into different aquifers based on intervention, and the breeding attitude was considered. During the rutting season, an ephemeral fountain was kept running smoothly to disperse the eggs and keep them from sinking to the bottom, as well as to keep the eggs aerated. The amount of hormone transfused affected the ovulation time. Both male and female sperm and eggs were forced to release and were fertilized 7-9 hours after injection. The fertilized embryo was then collected and incubated in a separate plastic container. Fertilized eggs swell, harden, and become sticky when they are exposed to water. Throughout the time of incubation, dead eggs were lifted from the water tanks every two hours. After the completion of the hatching process, the hatchlings were counted and recorded.

Tending of Reba fry

The ponds were drained and left to dry to eliminate rapacious and weed fishes. After drying and weed removal, 1kg/decimal lime was applied to the pond bottom. One day after liming, the ponds were

refilled with water to a depth of 1.0m and 2kg/decimal flour, 1.5kg/ decimal mustard cake, 50gm/decimal yeast, and 250gm/decimal molasses were incorporated into the ponds three to four days before releasing the fry. Sumithion was used to control pest expansion. The spawn of the fish was not fed for the first day after release to allow them to adapt quickly to their changed surroundings. After three days, flour and hard-boiled eggs were added. Mustard oil cakes were used for 10–15 days, then again for 28–30 days.

Hydrological parameter monitoring

The temperature, transparency, dissolved oxygen (DO), pH, and ammonia were evaluated at monthly intervals in the morning. The water temperature was measured using a Celsius thermometer. To achieve transparency, a Secchi disc was used. The dissolved oxygen was measured using a portable DO meter (YSI digital DO meter). The pH of the pond water was measured using a pH meter (Digital pH meter). Ammonia levels were determined using an ammonia test kit.

Evaluation of growth parameters

The total length, body weight, and mortality were all tracked at regular intervals, and growth metrics were analyzed using the following equations:

(Mollah et al., 2008). where Wb = the total body weight (kg) of all fishes to be injected and Pt = the dose in mg of PG to be injected per kg body weight under a particular treatment.

The volume of extract (ml) = Wt (PG weight (mg)) \times 1.0 (extract volume (ml) to be injected per kg body weight of fish) (Mollah et al., 2008).

The Gonado-somatic Index (GSI%) = (Weight of gonad/Weight of fish) * 100

Fecundity = (Number of eggs in the fraction×Total weight of ovary) / weight of the fraction

Fertilization = (Number of fertilized eggs/Total number of egg counted)*100

Hatchability = ((Number of hatchlings (two days old)/(Total number of fertilized egg))*100

Survival rate = Number of fries harvested/Number of fries stocked)*100

Ovulation (%) = (Number of fish ovulated/Total number of fish injected)*100

Observation of embryonic development

An optical micrometer, a stage micrometer, and a computer-linked microscope were used to examine the embryonic iterations of fertilized eggs. Collected specimens were fleetingly tainted with methyl orange and safranin to allow for a concise examination under an electronic microscope. This study outlined ten distinct embryonic developmental phases.

Statistical evaluation

All data were analyzed using one-way ANOVA (Analysis of Variance) followed by DMRT in SPSS (Statistical Package for Social

Science) version 25.0. A one-way ANOVA was used to compare mean differences. The hypotheses of normal distribution and variance similarity were verified prior to conducting the analysis.

RESULTS AND DISCUSSION

The temperature (°C), pH, dissolved oxygen (mg/l), transparency (cm), and NH₂ (mg/l) were measured every month in the brooder pond and found to be within a suitable range (Table 2). The thermal range of water in three treatments was found to range between 28.00 and 31.50°C during the experimental period and was found to coincide with Ali et al. (2017), which is within the optimum range (26.06-31.97°C) for fish culture (Boyd, 1982). The fact that there were no clouds in the sky and a strong sun in July may have contributed to the highest temperature of 31.50°C that was recorded in T_1 in July and the lowest water temperature (29.0°C) ever recorded in T₁ in March. The most favorable range of pH needs to be between 6.5-9.0 for fishponds and aquatic life (Swingle, 1969); a value below 5.5 turns the water too acidic for fish (Makori et al., 2017), where our findings (6.9-7.6) are above the acidic criteria. Similar water quality has been maintained by Rahman et al. (2021) and Araf et al. (2021) during the captive breeding of freshwater fish.

The pH readings from this investigation were consistent with the findings of the authors mentioned above. In July, T₂ had the greatest DO level (6.82mg/l), whereas in August, it had the lowest dissolved oxygen content (5.29mg/l). The suitable dissolved O₂ level for fishponds should be greater than 5mg/l (Bhatnagar and Šingh, 2010); Oxygen below 3mg/l is considered detrimental to fish progression (Ross et al., 2001). To support fish life in freshwaters, 5.0mg/l of dissolved oxygen is necessary, according to Chapman and Kimstach (1996). According to Mallasen et al. (2012), oxygen concentrations below 4.0mg/l are necessary for tropical fish growth. As a result, the experiment's DO values support the conclusions of the authors mentioned above. The ideal transparency coverage for fish farming is 15-40cm (Boyd, 1982). The current study's findings were consistent with those of Begum et al. (2017). The safe limit of ammonia concentration was not specified, but it was higher than the value of 0.012 mg/l generally cited by fish culturists, as mentioned by Meade, 1985. The current report's ammonia levels stayed within an acceptable range (>0.012mg/l).

Both histological and morphological changes were considered when identifying brood fishes. C. reba ovarian development was studied to determine the structure and timeline of germ cell growth and maturation stages. The yolk granule stage in the histological process indicates the ripening condition of brood fish. During July, the ovary had the late yolk granule stage (LYGS) and the testes had spermatozoa (SZ) and spermatids (ST) (Figure 2). To assess the maturity of fish gonads, morphological changes in the brood fish were observed. Fish length, gonad weight, and gonado-somatic index were measured monthly for the majority of the experiment. The mean values of these parameters varied significantly (p<0.05) between months, as shown in Figure 3. During the study period, there was a positive relationship between total length and body weight. The GSI values of female C. reba changed from 3.32±0.15 to 12.2±1.30% and in the male it was 1.68±0.02 to 3.4±0.03 with the change of months (Figure 3).

Hydrological parameters (mean±SD) were assembled throughout the study period under various treatments from

	March to A	ugust 2020.				
Months	Treatments	Temperature (°C)	рН	Dissolved oxygen (mg/l)	Transparency (cm)	NH ₃ (mg/l)
	T ₁	29.0±2.15	7.6±.6	6.11±0.37	36.33±4.22	0.54±0.11
March	T ₂	30.12±0.9	7.1±0.5	6.55±0.40	35.9±2.58	0.49±0.09
	T_3^{-}	30.2±1.2	7.2±0.47	5.9±0.11	37.51±0.44	0.50±0.09
	T ₁	30.99±1.2	7.3±0.8	5.8±0.31	36.33±4.22	0.48±0.11
April	T ₂	29.23±0.7	7.6±0.4	6.4±0.31	35.6±2.5	0.49±0.12
	T ₃	30.22±1.2	7.2±0.14	5.9±0.55	34.66±5.1	0.51±0.11
	T ₁	31.00±2.3	7.2±0.41	6.2±0.11	36.33±2.01	0.50±0.09
May	T ₂	29.88±2.1	6.9±0.42	5.81±014	33.99±4.22	0.54±0.12
	T ₃	30.44±1.8	7.3±0.32	6.24±0.35	34.88±4.3	0.49±0.10
	T ₁	30.19±1.1	7.1±0.81	6.64±0.41	36.66±3.33	0.50±0.10
June	T ₂	31.00±1.0	6.9±0.28	5.64±0.55	36.33±3.18	0.48±0.07
	T ₃	30.09±1.0	7.3±0.47	5.51±0.19	35.5±5.5	0.49±0.08
	T ₁	31.50±1.9	6.8±0.61	6.65±0.54	33.5±2.22	0.51±0.11
July	T ₂	29.91±1.7	7.5±0.21	6.82±0.71	36.33±2.89	0.50±0.12
	T ₃	30.33±1.9	7.2±0.35	6.3±0.48	35.5±4.9	0.54±0.12
	T ₁	29.47±1.0	7.2±0.87	5.6±1.00	36.88±3.22	0.49±0.10
August	T ₂	31.14±1.9	6.9±0.17	5.29±0.87	34.5±2.8	0.50±0.13
	T ₃	31.21±1.0	7.6±0.87	5.91±1.0	34.8±4.45	0.47±0.18

The average GSI of the fish is supposed to augment with the fish's ripeness, and it deters after spawning. The lowest GSI was found during the quiescent stage, but in females, it was found that the GSI was steadily augmented from March to May. Then it was hastily improved in June. The peak GSI was recorded in July for both females and males, indicating that July is the apex breeding season of *C. reba* (Figure 3). These values started to plummet in August, both in males and females. Male gonad weights ranged from 1.68 to 5.16g, with the largest weight being discovered in July and the smallest weight being discovered in March. The reproductive progress of the fish was studied by observing the GSI values as they started going up with fish progression, peaking during maximal ripening, and rapidly dropping after that when the species had been spent (le Cren, 1951).

Table 2.

The highest female GSI was $12.2\pm1.30\%$ in July, while the lowest was $3.32\pm0.15\%$ in March. During the study period, a rising trend in weight and GSI (%) was seen monthly with a significant difference (p<0.05). As a result, the gonad weight increased along with the GSI (%) of *C. reba*, reaching a high in July. Jewel et al. (2019), Akther & Akther (2011), Mathialagan and Sivakumar (2012), and Lashari et al. (2007) all came to more or less the same conclusions.

The fecundity was calculated using the Von Vayer method. Significantly different fecundities were estimated at 21,119.44 \pm 1,731, 37,805.44 \pm 1,509, and 29,468.22 \pm 1,784 in T₁, T₂, and T₃, respectively. The fecundity of *C. reba* was found to vary significantly (*p*<0.05) between regimens, and it was highest in T₂, followed by T₃ and T₁ (Table 3). The fecundity range during the research period was within the range of 20,722 to 211,200 eggs, according to Lashari et al. (2007), who previously studied the same species of fish in aquaculture farms in Pakistan.

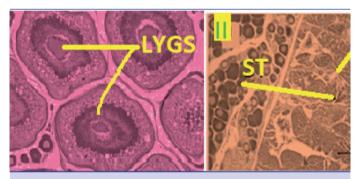
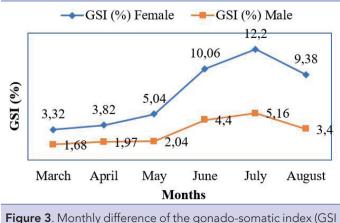
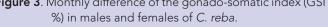


Figure 2. Histological section of *C. reba* showing (i) the ovary with the late yolk granule stage (LYGS) and (ii) the testes with spermatozoa (SZ) and spermatids (ST) stained with hematoxylin and eosin.





From the Baigul Reservoir (U.P.), India, Khan (1986) reported greater fertility of *C. reba* at 22,356 to 437,400 eggs. Jewel et al. (2019) also discovered fecundity (11,542.10 to 53,602.28) with comparable outcomes. The discrepancies between the current findings and those of certain research articles result from elements that have an influence on fish productiveness, such as fish habitat and diet, as well as characteristics like fish size, age, and condition.

For three treatments, separate doses of the hormone were injected into broods. After injecting the proper dose of PG, 7-9 hours are needed for the latency period. The ovulation rate was 68.22 ± 2.11 , 75.25 ± 2.43 , and $71.12\pm1.69\%$ in T₁, T₂, and T₃, respectively (Table 3). The significantly highest (p<0.05) ovulation rate was found in T₂ ($75.25\pm2.43\%$) and the lowest was found in T₁ ($68.22\pm2.11\%$).

It might be because the medication efficiently triggered eggs in the ovary to ovulate. Average fertilization rates (%) of *C. reba* were 60.25±3.05%, 88.88±5.17%, and 75.71±7.27 in T₁, T₂, and T₃, respectively, during the study period, and significantly (p<0.05) it was higher in T₂ followed by T₃ and T₁ where 2mg/kg for the male and 4mg/kg for the female of the PG hormone was used on *C. reba*. This was like the discovery made by Sarkar et al. (2004), who did captive breeding of *C. reba* using synthetic estrogen ovaprim and found the high side fertilization rate which ranged from 90 to 95%.

The hatching rate (%) of *C. reba* measured during the study period in T₁, T₂, and T₃ were 87.5±2.25, 88.23±3.27 and 86.67±4.15%, respectively (Table 3). The hatching rate (%) of *C. reba* was higher (p<0.05) in T₂ followed by T₁ and T₃. The survival rates of *C. reba* from hatchlings to fry in T₁, T₂, and T₃ were 75.57±2.27, 76.45±2.37, and 76.57±3.04%, respectively (Table 3). In this study, it was found

that the survival rate of C. reba did not vary significantly (p>0.05) among the treatments. Significantly higher ovulation (%) was recorded from T₂ (75.25±2.43) followed by T₃ (68.22±2.11) and T₁ (71.12±1.69). The latency period in the present study was found to be 7-9 hours, whereas the latency period of Heteropneustes fossilis was 22-25 hours (Kohli & Goswami, 1987), that of Clarias gariepinus was 16-20 hours (Munshi & Hughes, 1991), and 30 hours for C. stiriatus (Marimuthu et al., 2001). The higher hatching rate (%) of C. reba was in T_2 (88.23±3.27) followed by T_1 (87.5±2.25) and T₂ (86.67±4.15). Das et al. (2022b) reported 61-86% hatching rate in Nandus nandus using a dose of PG hormone of 2mg/kg for males and 4mg/kg for females. Our findings comply with the above result. No significant (p>0.05) difference in the endurance rate of C. reba among the treatments was recorded. Sarkar et al. (2004) found that in the survival rates of C. reba, hatching started 10-12 hours after injection. The present findings of the survival rate of C. reba from hatchlings to fry were comparable to the findings of the above authors.

The embryonic developmental stages of this fish were observed (Figure 4). Reba's fertilized eggs (Figure 4b) demonstrate meroblastic cleavage within 30–35 min after fertilization (Figure 4c). By administering Gn-RH in *Mystus cavasius*, Ali et al. (2021) observed the first cleavage to a 32-cell formation in 00:35–2:20h, whereas the new research ascertained the first cleavage achieved a 32-cell formation in 00:30–3:20h. At 190–210 minutes after fertilization, the morula phase was acquired (Figure 4c). At that point, the blastoderm had dispersed well over the protein and the embryo was distinguishable. Ali et al. (2021) discovered the very same phase in *M. cavasius*, but much earlier, at 2:20h after fertilization. The same progression was discovered by Nesa et al. (2017) by administering PG extract to *M. cavasius*. The Blastula phase was found in the present experiment (3:50-4:20h after fertilization) (Figure 4d), but Ali et al. (2021) found 3:30-4:00h after

Table 3. Details of the induced breeding of *C. reba* through the use of different doses of PG.

	Mean brood weight (g)		d (hr)	(hr)					
Treatments	Male	Female	Latency period (hr)	Hatching time (hr)	Fecundity (%)	Ovulation (%)	Fertilization (%)	Hatching (%)	Survival (%)
T ₁	102±10.2	118±14.6	7	15	21119.44 ±1731°	68.22±2.11°	60.25±3.05°	87.50 ±2.25	75.57±2.27
T ₂	105±6.8	115±11.4	8	16	37805.44 ±1509ª	75.25±2.43ª	88.88±5.17ª	88.23±3.27	76.45±2.37
T ₃	103±5.8	116±12.2	9	17	29468.22 ±1784 ^b	71.12±1.69 ^b	75.71±7.27 ^b	86.67±4.15	76.57±3.04

Mean values in a single row containing the same superscript letters do not differ significantly (p<0.05).

fertilization in *M. cavasius*. The C-shaped gastrula part (Figure 4e) starts at 6:0-6:40h post-fertilization, whereas Ali et al. (2021) recorded it at 5:0–5:30h after fertilization. The winding motion of the embryo was seen in current research as it unwinds from being encircled over the yolk realm. The tail had already become separate, but the neck remained attached to the yolk sac.

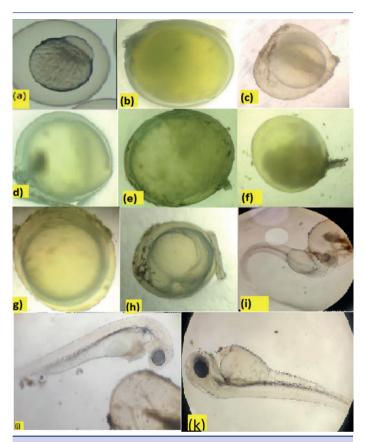


Figure 4. Embryonic developmental stages of *C. reba*: (a) unfertilized egg, (b) fertilized egg, (c) morula stage, (d) Blastula stage, (e) Gastrula stage, (f) Late gastrula stage, (g) Yolk plug stage, (h) Organogenesis, (i) Stage before hatching, (j) Hatching stage, and (k) Hatchling.

The rhythm of the tail is rapid just prior to starting the larval stage and beats around 50-60 times per minute. The larvae began hatching between 10:00 a.m. and 12:00 p.m. The small larvae were easily recognized by their distinct heads, trunks, and rear regions (Figure 3k). Hatching time in *M. cavasius* was 24–25h (Ali et al., 2021); in *Rita rita* hatching was started after 22h of incubation and completed after 30h of incubation at 35°C (Mollah et al., 2011); and in the *C. batrachus* hatching time varied between 23-26 hours at a temperature range of 31–37.5°C (Das, 2002).

CONCLUSION

The current findings could be applied to induce the breeding of C. *reba* to advance hatchery reproduction. This insight may be useful for sustainable strategic planning and maintenance of C.

reba, which could have an important impact on mitigating the overall nourishment of Bangladesh's villagers. More exploration on nursing, nurturing, and culture of this near-threatened fish at various densities or feeding levels are needed at both on-station and on-farm stages in Bangladesh and throughout this territory to save these fish from extinction or to preserve and restore them.

Conflict of interest: The researchers acknowledge that they have no conflicts of interest.

Ethics committee approval: All procedures used in experiments involving humans and animals (fish) were following the ethical standards of the "Hajee Mohammad Danesh Science and Technology University, Dinajpur" Ethical Committee. All survey participants provided informed consent.

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Research Article

Morphological Evolution of the Binahaan River, Palo, Leyte, 6501, Philippines

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ABSTRACT

Using Landsat satellite images, this study evaluated the morphological evolution of the Binahaan River, Palo from 2000-2020. It focuses on quantifying the morphological changes in terms of river planform parameters namely channel width, channel length, channel area, and sinuosity, as well as erosion and accretion rates. Multi-temporal satellite images were collected and analyzed to recognize the quinquennial and seasonal alterations. With the quantification of the spatial extent and rates of bank-line alterations by means of remote sensing, the purpose is to better understand the erosion and accretion processes of the Binahaan River from the perspective of its planform parameters. Throughout the study period, the river experienced significant morphological changes in planform; however, no consistent trend was found due to the dynamic nature of the river. The seasonal and quinquennial erosion and accretion rates of the river were also analyzed. It was found that the right bank line was more susceptible to erosion and accretion. Seasonally, the river was more prone to sediment accumulation which led to the narrowing of channel widths and decreasing channel area. In the guinguennial comparison during the dry seasons, the river was mostly dominated by erosion, while during wet seasons, the river was more prone to accretion. In terms of change in the area, whether by loss or gain of land, the river consistently displayed increasing change from 2000 to 2020. The results of this study could be utilized to develop an integrated strategy for its management and restore the fundamental processes that shape and maintain the river.

Keywords: River, remote sensing, morphological changes

INTRODUCTION

River morphology and channelization are attributed to sediment flow, deposit characteristics, river discharge patterns, and erosion-accretion processes occurring in spatial and temporal scales. In addition to natural interferences, human constructions such as the building of bank protection structures, channel width widening, bridges, artificial cutoffs, dams, and land use adjustments also modify the physical configuration and fluvial processes of these river systems (Credit Valley Conservation, 2012; Dar, Mir, & Romshoo, 2019). The unpredictable character of seasonal rain during monsoons in the Philippines encourages diverse behavior in river flow, which can alter channel characteristics and result in floods, changes in drainage patterns, and sediment deposition (Asio & Cagasan, 2014). These morphological alterations may aggravate erosion, accretion, and channel instability, inducing environmental problems such as flooding, debris flows, and water quality degradation (Baki & Gan, 2012; Hossain et al., 2013). Consequently, these directly influence the riverine and riparian coenoses because these provide the setting for the ecological processes and the habitats of

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the flora and fauna of the river (Elosegi, Díez, & Mutz, 2010; Gran et al., 2015; Michal & Gran, 2014).

The Binahaan River is one of the biggest rivers in the province of Leyte, Philippines. It supplies the Leyte Metropolitan Water District (LMWD) with potable water for the city and several towns of the province (Figure 1). However, due to varying natural occurrences such as moderate to heavy flooding, exacerbated by human activities and urban development, it is perceptible that the primeval condition of the river and topography has been altered.



Figure 1. Binahaan River study area taken from Google Map images.

As watersheds within the Philippines are characteristically prone to flooding, debris flows, liquefaction, storm surge, and soil erosion, the Binahaan River is likewise predictably prone to these naturally-occurring geological alterations. In fact, for the past eight years, the Department of Environment and Natural Resources (DENR) discussed significant laws concerning environmental protection and preservation of the river. Further, according to the Philippine Information Agency (2011), there has been extensive destruction of the Binahaan Watershed mainly due to illegal or unmonitored activities being conducted in the past years.

As mentioned, the Binahaan River in the Philippines is an important source of water, but there has been little research done on its features. Obbus et al. (2021) aimed to develop a model to classify the river's geomorphic typologies and identified 12 types based on parameters such as sinuosity, entrenchment ratio, and channel material. They found that Typology B5c was dominant and sand was the most common channel material. More importantly, sand mining has disturbed the river and reduced the recovery potential of the river. The identified typologies of the Binahaan River will be significant components for future research focusing on biodiversity and its relationship with the river's geomorphology. Other than sand mining, channel interventions, such as riparian land use, bank line infrastructures, and sand extraction areas for construction purposes, were also present. Locals were also found to fish and mine in the river based on ocular surveillance.

Hence, to mitigate the risk of flooding and debris flows due to the mentioned natural and anthropogenic activities and contrib-

ute to improving the ecological conditions of the river, it is necessary to analyze the seasonal morphological evolution and the sediment transport dynamics and river flow of the Binahaan River (Department of Environment and Natural Resource, n.d.). In this regard, this present study assessed the seasonal and quinquennial morphological changes of the Binahaan River.

Data from assessing the morphological changes of the Binahaan River using satellite images provided important information on the river's geomorphology. Using Landsat satellite images, this study assessed and quantified the morphological changes of the Binahaan River throughout the years 2000 -2020. Seasonal data was acquired through the recommendation of the Philippine Atmospheric, Geophysical and Astronomical Services Administration (Retrieved May 20, 2023). Using temperature and rainfall as bases, the wet season was defined from June to November while the dry season was defined from December to May. However, the unavailability of higher-resolution images, as well as the sporadic capture of imagery in the area of interest and cloud cover reduced optical data and limited the potential outcomes of the study.

By assessing the morphological evolution of the Binahaan River in Palo, the study described the probable impacts on the river and its geomorphology, proving to be helpful in developing an integrated strategy to enhance the management, if not restore, the fundamental processes that shape and maintain the river. This could additionally contribute to drafting policies for its maintenance and flood management, without compromising the socio-economic aspect of this river system, assisting concerned government bodies in planning for effective and sustainable flood risk and land use management, river restoration projects, and agricultural land management practices.

MATERIAL AND METHODS

Satellite data collection

Multitemporal Landsat Collection 2 Level 1 image products from Landsat 7 and 8 were collected through USGS Earth Explorer and were used as data sources. Landsat images with a spatial resolution of $30 \times 30 \text{ m}^2$ were collected from 2000 - 2020. These images were geometrically matched and projected onto the same map coordinates (Table 1).

Satellite image processing

Satellite images were processed and analyzed using the QGIS software. These images were subjected to two correction procedures before the final image processing. The radiometric correction involved the conversion of the measured multispectral brightness values to top-of-atmosphere (TOA) reflectance units to eliminate discrepancies between images as a result of sensor differences, Earth-sun distance, and solar zenith angle caused by the different scene dates, overpass time, and latitude distances (Table 2). The images were subjected to pansharpening and merging with high-resolution panchromatic images. A Modified Normalized Difference Water Index (MNDWI) was calculated through Eq. 1:

Table 1.List of satellite data used in the study.

Satellite Data	Acquisition Date	Spatial Res- olution (m)	Band Number
Landsat-ETM	May 26, 2000	30	2, 5, 8
	December 04, 2000		
	April 19, 2004		
	November 13, 2004		
	April 07, 2011		
	October 16, 2011		
Landsat-OLI	May 28, 2015	30	3, 6, 8
	November 20, 2015		
	May 25, 2020		
	November 17, 2020		

Table 2.	Satellite data specifications for MNDWI calculation.			
Landsat	Green	MIR	Panchro- matic	Final Spatial Resolution (m)
Landsat 7	Band 2	Band 5	Band 8	15
Landsat 8	Band 3	Band 6	Band 8	15

$$MNDWI = \frac{Green - MR (Mid Infrared)}{Green - MIR (Mid Infrared)}$$
(1)

Satellite data analysis

Delineating the bank lines in the processed satellite images is a principal step in the assessment of the collective impact of geomorphic processes and anthropogenic activities on the morphological evolution of the river. A bank line is defined as the feature that separates the outer margin of a river channel from the floodplain (Hossain et al., 2013). Four river planform parameters were selected in accordance with the topographic and geographical characteristics of the river – channel width, area, length, and sinuosity.

Spatial analysis and parameter value calculation were conducted, wherein the channel area was the instantaneous water surface area of the river, the channel width was calculated as a range, the left bank length was defined as the left side along the river flow direction, the right bank length was defined as the right side along the river flow direction, and the sinuosity index was calculated through Eq. 2:

Sinuosity Index
$$= \frac{cL}{sL}$$
 (2)

wherein CL = Channel length and SL = Straight line distance between start and endpoints (García, 2015).

Policy assessment and recommendation

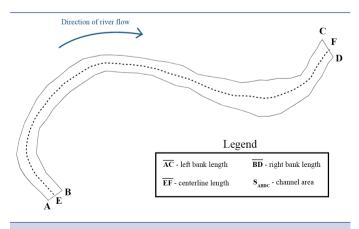
Current policies of the Local Government Unit were reviewed and assessed to determine the current state of the environmental protection and management of the river. Policy recommendations based on the outcome of the study will be drafted at a later date. For a comprehensive assessment of the morphological evolution of the Binahaan River, Palo, seasonal changes within a year, as well as quinquennial changes, were evaluated. The results of the morphological changes of the river are discussed below.

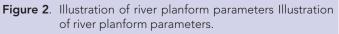
RESULTS AND DISCUSSION

Ideally, the best approach to accurately detect the morphological changes of a river body is by analyzing multiple satellite data over a consistent time interval. However, sporadic capture of imagery of the area of interest as well as cloud cover, which impedes optical data, restricted the availability of satellite images appropriate for the study. Gathering a continuous time series of data with a consistent interval proved to be unachievable. As such, the researchers employed satellite images from neighboring years on periods with no available data. In this study, Landsat satellite images from 2000, 2004, 2011, 2015, and 2020 were acquired. The 30 imes30 m² spatial resolution of Landsat data limited the extent of the precision of the monitoring of morphological changes. Even with the application of higher-resolution panchromatic images, assessments of changes in erosion and accretion along the river banks were approximate and subject to an order of accuracy comparable to the spatial resolution of the satellite images.

River planform parameters

Changes in the river channel of the Binahaan River, Palo were estimated in terms of channel width, channel length, channel area, and sinuosity (Figure 2).





Channel width

Based on the results found in Table 1, the channel width of the river ranged from 19.348 m to 135.633 m, both found in the wet seasons of 2004 and 2015. Typically, the area within the first to third meander from the river source measured the smallest width (Figure 3), while the area near the river mouth measured the greatest width (Figure 4). This phenomenon is reasonable for several factors, such as the increase in the volume of water as well as the velocity downstream, changes in the landscape, channel patterns, and human activities. All these contribute to the changes in the river width as these lead to erosion and accretion or lateral movement in both banks (Mohammadi et al., 2008).

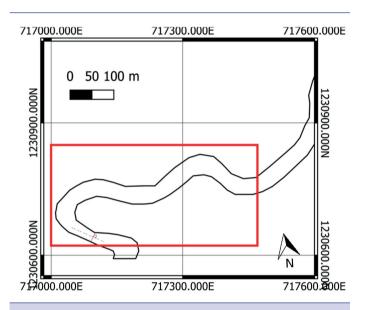
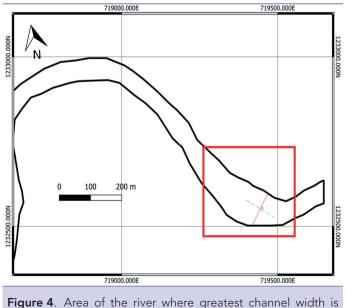


Figure 3. Area of the river where least channel width is usually observed.



usually observed.

There was no uniform pattern in the changes in maximum and minimum widths between the two seasons (Figure 5). In 2000, the maximum width increased while the minimum width decreased from the dry to the wet season of the year, so the range difference between the two increased by about 24 m. This change was identified to be the greatest throughout the study period. Such an event was similar for 2004 and 2015 but with a slight increase and decrease in the maximum and minimum width within the seasons of the said years. However, an opposite pattern was seen in the year 2011 between its dry and wet seasons when the maximum width decreased while the minimum width increased. This resulted in the lowering of the width range between seasons with a range difference of 13 m. From the dry to wet seasons of the year 2020, both the maximum and the minimum widths decreased. As for the quinquennial change, the average maximum and minimum width alteration were graphed in Figure 6. Only a minimal change was detected, except from 2000 to 2004 when a more noticeable increase in the maximum channel width could be recognized. On the other hand, the average minimum width showed an increasing pattern of data starting from the year 2000 to 2015, but a decreasing one from 2015 to 2020. On average, the maximum channel width, minimum channel width, and width range resulted in 126.613 m, 24.341 m, and 102.272 m, respectively.

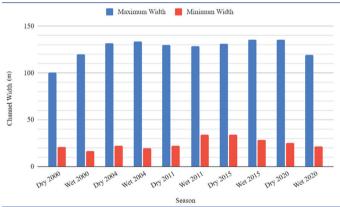
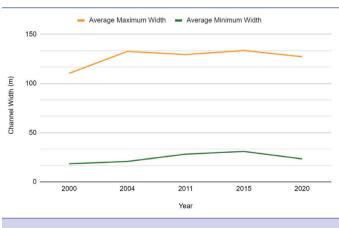
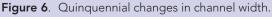


Figure 5. Seasonal changes in channel width.





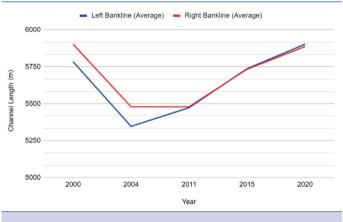
Channel length

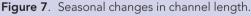
Minimal changes were observed between the dry and wet seasons for both the left and the right bank lines of the river (Figure 7) apart from 2004 which measured length differences of about 369 m and 230 m, respectively. Within a year, 2011 had the least change of length in the left bank line with a measure of approximately 11 m, as well as in the right banking with a length difference of about 30 m.

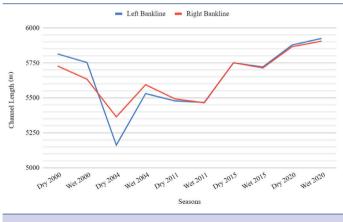
Starting from 2011 to 2020, an increasing pattern can be recognized for the left and right bank lines of the river as represented by Figure 8. It is different, however, from 2000 to 2004 as there was a decrease in length for both of the bank lines. Generally, the average channel length of the left and right bank lines measured higher in 2020 than in 2000 with a calculated difference of about 118 m and 206 m, respectively. Moreover, there was only a slight change in channel length for the different sides of the river during the time period of the study.

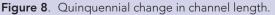
Channel area

In the years 2004, 2015, and 2020, the channel area was greater during the dry season than the wet season with a difference of $6,450.662 \text{ m}^2$, $43,597.662 \text{ m}^2$, and $19,997.125 \text{ m}^2$, respectively (Table 3). On the contrary, in the years 2000 and 2011, the values of



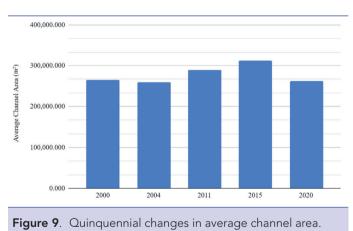






Year	Casaan	Channel Width (m)		
rear	Season	Maximum	Minimum	
2000	Dry	100.564	20.736	
2000	Wet	120.055	16.174	
2004	Dry	131.513	22.323	
2004	Wet	133.784	19.348	
2011	Dry	130.109	22.275	
2011	Wet	128.653	22.275	
2015	Dry	131.395	33.759	
2015	Wet	135.633	28.131	
2020	Dry	135.219	25.373	
2020	Wet	119.204	21.320	

the channel area were greater during the rainy season than in the dry season. The wet seasons of 2000 and 2011 had a channel area greater by 28,220.716 m² and 20,917.073 m². Overall, there was no consistent trend between the channel areas of the processed river segments with regard to seasonality. The area of the river segment approximated 265,511.301 m² by 2000 but slightly decreased to 259,355.595 m² by 2004. As seen in Figure 9, the average channel area continued to increase throughout 2011 and was successively maximized by 2015, particularly during the dry season, with a value of 311,691.882 m². Afterwards, the channel area fell substantially during the year 2020 with around 48,907.667 m² less in value.



Sinuosity

The sinuosity of the river is regarded as a measure deviation of a river from its ideal path movement. However, as a straight-line path is nearly impossible to achieve due to several geomorphic factors, the sinuosity index was used to highlight irregularities in the channel path instead. In Table 4, the sinuosity indices of the river are enumerated. In accordance with the river sinuosity classification, the Binahaan River, Palo was characterized as a sinuous river with an average sinuosity of 1.778 in the span of two decades (Table 5). The right bank consistently measured greater sinuosity than the left bank, suggesting that the right bank is more susceptible to erosion and accretion. Furthermore, the sinuosity of the centerline of the river follows the sinuosity of the left

Table 3.Changes in channel width.

Table 4.	Changes in channel area.		
Year	Channel A	Channel Area (m²)	
Tear	Dry	Wet	
2000	251,400.943	279,621.659	
2004	262,580.926	256,130.264	
2011	278,783.067	299,700.140	
2015	333,490.768	289,892.996	
2020	272, 782.778	252, 785.653	

Table 5.Changes in sinuosity.

Year Season		Bank lines		
rear	Season	Left	Right	Centerline
2000	Dry	1.785	1.822	1.784
2000	Wet	1.767	1.780	1.751
2004	Dry	1.593	1.711	1.637
2004	Wet	1.711	1.777	1.720
2011	Dry	1.721	1.789	1.739
2011	Wet	1.716	1.789	1.732
2015	Dry	1.807	1.886	1.823
2015	Wet	1.798	1.868	1.843
2020	Dry	1.841	1.917	1.863
2020	Wet	1.869	1.928	1.885

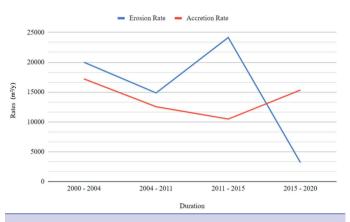
bank line more closely than the right bank. No consistent pattern in sinuosity was recognized between the dry and wet seasons of the same year. However, average quinquennial changes revealed a more consistent trend. A decreasing sinuosity index was observed only in the period between 2000 and 2004 because of the straightening of the channel path that led to the loss of a meandering section of the river between the two time periods. Apart from that, the general trend showed that the sinuosity gradually increased, with an increase of 0.195 in the centerline sinuosity between 2004 and 2020 which implies the presence of shifting of the channel and bank lines. The increase in sinuosity of a river is related to an increase in bank erosion as it causes a decrease in streamflow velocity (Hossain et al., 2013).

Erosion and accretion Seasonal erosion and accretion

The largest eroded bank area was recorded in the year 2000, while the smallest was recorded in 2015. On the other hand, the year 2015 recorded the largest accreted area, while the year 2011 recorded the smallest accreted area. On average, the river seasonally eroded 40,905.634 m² and accreted 45,300.617 m² for a seasonal net gain of 4,394.983 m² of land. With this, it can be inferred that the river is more prone to accretion, which may lead to the narrowing of the channel width and a decrease in the channel area from dry to wet seasons, as seen in Figure 10.

Quinquennial erosion and accretion during dry seasons

The river consistently lost land from 2000 to 2015. To be specific, the river lost 11,179.920 m² from 2000 to 2004, 16,202.162 m² from 2004 to 2011, and 54,707.774 m² from 2011 to 2015 (Table 6).



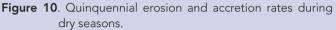


Table 6.	Quinquennial eroded and accreted areas during dry seasons.			
Veer		Area (m²)		
Year		Eroded	Accreted	
2000 - 2004		79,990.204	68,810.284	
2004 - 2011		103,975.412	87,773.25	
2011 - 2015		96,604.862	41,897.088	
2015 - 2020		15,994.3958	76,702.442	

This continuous erosion contributed to the widening of the channel width and an increase in the channel area. However, during the recent time period from 2015 to 2020, the Binahaan river exhibited decreasing erosion and increasing accretion with a net gain of 60,708.047 m². In terms of change in the area whether by loss or gain of land, the river consistently displayed increasing change from 2000 to 2020. As observed in Figure 11, the river was mostly dominated by erosion throughout the period covered by the study with an estimated average annual erosion rate of 15, 550.319 m² and an average annual accretion rate of 13, 889.092 m² for an average annual net loss of 1, 661.227 m². The river recorded the fastest rate of erosion of 24,151.216 m²/yr. between 2011 and 2015 while the slowest rate of erosion of 3,198.879 m²/yr. between 2015 and 2020. Meanwhile, the river recorded the fastest rate of accretion of 17,202.571 m²/yr. between 2000 and 2004 and the slowest rate of accretion of 10,474.272 m²/ yr. between 2011 and 2015.

Quinquennial erosion and accretion during wet seasons

In the three consecutive time intervals, the river exhibited an alternating pattern between land gain and land loss with a net gain of 23, 491.496 m² from 2000 to 2004, a net loss of 43,569.847 m² from 2004 to 2011, and a net gain of 13,300.136 m² from 2011 to 2015. It was only from 2011 to 2020 that the river followed a consistent trend of land gain while the period from 2015 to 2020 followed the previous interval's trend with a net gain of 33,615.345 m². Overall, it can be observed in Figure 12 that only during the period between 2004 to 2011 did the river have a greater erosion rate than accretion while the others exhibited an inverse phe-

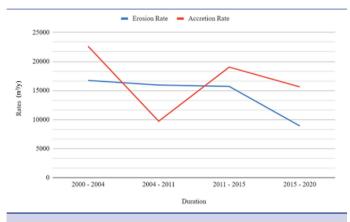
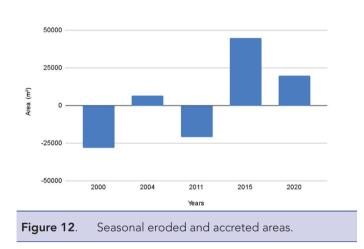


Figure 11. Quinquennial erosion and accretion rates during dry seasons.



nomenon, indicating that the river is more prone to accretion with an estimated average annual erosion rate of 14,321.949 m² and average annual accretion of 16,746.077 m² for an annual net gain of 2,424.128 m². The river recorded the fastest rate of erosion of 16,737.000 m²/yr. between 2000 and 2004 while the slowest rate of erosion of 8,906.666 m²/yr. between 2015 and 2020. Meanwhile, the river recorded the fastest rate of accretion of 22,609.874 m²/yr. between 2000 and 2004 and the slowest rate of accretion of 9,713.865 m²/yr. between 2004 and 2011 (Table 7).

Impact of natural phenomena

In a study by Marteleira (2019) about the impacts of climate change on some rivers, the Binahaan River was found to have an insufficient flow, making it one of the water bodies to be categorized as not a resilient water source and river basin based on the hydrological model QSWAT. With that, it could be said that such a river system is prone to deformation and change.

As stated on the Modified Corona's Climate Classification, the Binahaan River, Palo is characterized by fair rainfall distribution for the entire year and is also categorized under tropical wet climate having significant rainfall even amidst dry seasons. As high amounts of water flow in streams or rivers, such water bodies experience flooding. According to the College of Forestry and Natural Resources, the Binahaan River, Palo, is susceptible to flood-

Table 7.	Quinquennial eroded and accreted areas
	during wet seasons.

Year	Area (m²)		
rear	Eroded	Accreted	
2000 - 2004	66,947.998	90,439.494	
2004 - 2011	111,566.904	67,997.057	
2011 - 2015	62,824.000	76,124.136	
2015 - 2020	44,533.3268	78,147.671	

ing. For the municipality of Palo, with an area of 65.34 sq. km., it is identified that 53.26% will be able to encounter flood levels of less than 0.20 m, 11.10% of the area will experience flood levels of 0.21 to 0.50 m, while 2.25%, 0.27%, and 0.097% of the area will experience flood depths of 0.51 to 1 m, 1.01 to 2 m, and more than 2 m, respectively. Apart from that, the said river also has the largest area with serious incidents of erosion comprising about 1,387 ha. among the principal river basins and minor watersheds of Cluster 5 (College Forestry and Natural Resources, n.d.). These climate trends and natural hazards paved the way for some morphological changes to occur in the Binahaan River, Palo.

Impact of human activities

Anthropogenic influences are thought to exacerbate erosion and sedimentation in the Binahaan River, Palo. In the Binahaan River, Palo, black sand mining is an essential livelihood for the households residing near the river and a source of sediment for local construction. Black sand mining activities are particularly prominent near the mouth of the river, wherein machinery like grapple trucks excavate the area. However, locals also individually mine points along the river by manually scooping up sand from the riverbed. This method of mining alters the planforms and composition of the river. Although black sand mining is an important element in local commerce, it disturbs the riverine ecosystem and makes the river more susceptible to erosion and as a result, other associated geohazards. It not only increases erosion by directly removing sand but also by disrupting the riverbed sediment distribution (Chaussad & Kerosky, 2016). As such, erosion may continue to affect adjacent areas even decades after the mining activity.

CONCLUSION

Several morphological changes were observed over the course of two decades. The river exhibited significant changes in channel width and area; however, no consistent trend of increase or decrease was found due to the dynamic nature of the river. The right bank consistently measured greater sinuosity than the left bank line, suggesting that the right bank is more susceptible to erosion and accretion. Furthermore, a steady increase in sinuosity can be observed during recent years that contribute to the lengthening of the bank lines. The channel shifting and lengthening bank lines indicate an increase in the amount of land erosion and accretion that the river has been experiencing. This was supported by the erosion and accretion rates of the river. Seasonally, the river is more prone to accretion which led to the narrowing of channel widths and decreasing channel areas between dry and wet seasons. In the quinquennial comparison during the dry seasons, the river was mostly dominated by erosion. On the other hand, in the quinquennial comparison during wet seasons, the river was more prone to accretion. In terms of change in the area whether by loss or gain of land, the river consistently displayed increasing change from 2000 to 2020.

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Ethics committee approval: Ethics committee approval was not required.

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Research Article

Nutritional Quality, Proximate and Fatty Acid Compositions of Commercially Important Fish from Different Rivers in SE Türkiye: A Comparative Research

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ABSTRACT

Nutritional quality, proximate and fatty acid compositions of fish species from the Khabur, Ambar and Tigris Rivers in SE Türkiye were investigated for the first time. The fish with the lowest total lipids were *Mastacembelus mastacembelus* (0.93%) and *Carasobarbus luteus* (0.99%), and the fish with the highest total lipid was *Chondrostoma regium* from the Tigris River (7.47%). The highest cholesterol content was in *Barbus lacerta* (26.3 mg/100 g) and *Capoeta umbla* (29.98 mg/100g) of the Ambar Stream. However, the Tigris River *Cyprinus carpio* (7.9 mg/100g) and *C. luteus* (7.91 mg/100g) had the lowest cholesterol. The results showed that all species are good sources of ΣSFA and ΣMUFA, specifically C14:0, C16:0, C18:0, C16:1ω7 and C18:1ω9. However, the fish were poor for ω6 and ω3, particularly C20:4ω6, C20:5ω3 and C22:6ω3, probably due to hot water adaptation in summer. Nevertheless, *C. carpio* (Tigris River) and *A. mossulensis* (Khabur River) had relatively high ΣPUFA. Among all the fish, *C. regium* and *A. mossulensis* from the Khabur River were good for protein, and *M. mastacembelus*, *C. luteus*, *C. carpio* and *C. trutta* from the Tigris River can be recommended as lean fish. Finally, the results could be useful for fisheries industries and they could also guide studies of nutrition and fish physiology.

Keywords: Fish, Nutritional quality, Khabur River, Ambar Stream, Tigris River

INTRODUCTION

Since the consumption of fish is an important part of a diet that benefits human health and nutrition, studies on the nutritional components of fish have been around for a long time. There are many studies on the fatty acid and proximate composition of both freshwater and marine fish. In most of these studies, the effects of internal and external factors on these biochemical components were investigated. The results of these studies typically show that the fatty acid and proximate composition of fish species differ according to the physiology of the species, seasons, feeding locations, water temperature, water pollution, diet, geographical conditions, ambient temperature, sex and body parts (Citil et al., 2014; Özoğul et al., 2007).

Fatty acids perform many functions in the human body (Bazinet & Layé, 2014; Swanson et al., 2012) and are mainly divided into three groups such as saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). PUFA is the most important group and has great importance for human health (Bazinet & Layé, 2014; Swanson et al., 2012). According to the position of the last double bond relative to the terminal methyl end of the molecule, the PUFA is divided into three classes: ω 9, ω 3 and ω 6. Among them, ω 3 and ω 6 PUFAs are essential fatty acids that cannot be synthesized in mammals (Zhang et al., 2020). Marine fish con-

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tain mainly ω 3 PUFAs, while freshwater fish contain relatively high levels of ω 9 and ω 6, and low levels of ω 3, particularly C20:5 ω 3 (Eicosapentaenoic acid, EPA) and C22:6 ω 3 (Docosahexaenoic acid, DHA) when compared with marine fish (Özoğul et al., 2007). Freshwater fish are generally characterized by high levels of ω 6 PUFA, specifically C18:2 ω 6 (Linoleic acid, LA) and C20:4 ω 6 (Arachidonic acid, AA) (Özoğul et al., 2007).

Some studies found that the intake of ingredients containing PUFA plays a significant part in lowering the prevalence of diabetes, reducing the hazard of coronary heart disease, treating high blood pressure and cardiac arrhythmia, lowering the symptoms of rheumatoid arthritis, Alzheimer's and schizophrenia, and reducing most cancers and hyperactivity problems (Bazinet & Layé, 2014; Citil et al., 2014; Tapiero et al., 2002). Since the medicinal effects of C20:5w3 and C22:6w3 of fish are known, these fatty acids are used for medicinal purposes such as treating migraines, heart attacks, depression, rheumatic fever, some types of cancer, diabetes, high cholesterol, high blood pressure, and cardiovascular diseases (Bazinet & Layé, 2014; Citil et al., 2014; Swanson et al., 2012; Tapiero et al., 2002). Moreover, C20:5w3 and C20:4w6 can also be metabolized to various eicosanoids that act as hormonal agents (Satar et al., 2012; Swanson et al., 2012). Longchain ω 3 PUFA cannot be synthesized by the human body and must be obtained through the diet (Alasalvar et al., 2002; Swanson et al., 2012); therefore w3 and w6 PUFAs are considered essential fatty acids (Satar et al., 2012).

Despite the great diversity of the ichthyofauna of the Khabur, Ambar and Tigris Rivers, detailed information on the proximate composition and fatty acid content of fish species in these rivers is lacking. The purpose of this study was to determine and evaluate the profiles of fatty acids and nutrients such as total lipids, crude protein, cholesterol, ash and moisture in the dorsal muscle of eighteen commercially important fish species living in the Khabur, Ambar and Tigris Rivers.

MATERIALS AND METHODS

Sample collection and preparation

For the purposes of this study 12 different species of economically valued and frequently consumed fish (a total of 18 fish species) from SE Türkiye were chosen. The gathering of samples took place in July 2021 and included freshwater fish Capoeta umbla (Sarı balık, Şah balığı), Barbus lacerta (Benekli bıyıklı balık), Squalius break (Tatlısu kefali), Chondrostoma regium (Zereke, Karaburun) and Alburnus mossulensis (Gümüş balığı) from the Khabur River (Yeşilöz Deresi), taken from under the Khabur II Bridge, near the Khabur Gendarmerie Station, Uludere, Şırnak (37° 22' 1" N: 43° 4' 37" E, water temperature 22 °C); Mastacembelus mastacembelus, (Mezopotamya yılanbalığı), B. lacerta, C. umbla, Cyprinion macrostomus (Bunni balığı) and Capoeta trutta (Karabalık, Berat) from the Ambar Stream, between Yayvan and Soylu Villages, Kocaköy, Diyarbakır (37° 51′ 6″ N: 40° 32′ 20″ E, water temperature 22 °C); C. umbla, Cyprinus carpio (Sazan), C. trutta, S. break, C. regium, Carasobarbus luteus (Himri, Karagöz), Carassius gibelio (Gibel sazanı) and Silurus triostegus (Mezopotamya yayın balığı) from the Tigris River, taken from under the Sadi Bridge, at a distance of 2 km from the center of Diyarbakır (37° 54′ 57.2436" N: 40° 13′ 32.2320" E,

water temperature 21 °C). Once collected, the fish were transported within 30 minutes to the laboratory on ice with a fish/ice ratio of 1:2 (w/w) in a styrofoam box, and morphometric measurements of wet weight (WW) and length (cm) were calculated. Identification of the fish species was performed by ichthyologists in the Department of Biology, Dicle University, Türkiye. Averages of 20 species of similar size were caught for each species, and 6-8 species of similar size (female) were selected and analyzed. The size of the selected fish was determined by the size of the fish caught at the collection point and sold in the market. The fish were prepared using processes such as gutting, deboning, filleting and washing. From each specimen, the dorsal muscle portion between the dorsal fin and the head was removed and stored frozen at -20 °C for a month until analysis. The characteristics of fish species are given in Table 1.

The care and use of experimental animals complied with Ministry of Agriculture and Rural Affairs of Türkiye fish welfare laws, guidelines and policies as approved by (Communique No: 2008/48).

Analysis of fatty acid methyl esters (FAME) and Cholesterol and GC-FID conditions

Total lipid extraction was performed according to the method of Bligh and Dyer (Bligh & Dyer, 1959). Samples containing total muscle lipids were trans-esterified with acidified methanol (Stanley-Samuelson & Dadd, 1983).

Gas Chromatography (Agilent 7820A): GC oven: Thermostat controlled for columns that can operate with an accuracy of ± 0.1 °C. Flame Ionization Detector (FID). Capillary column (silica capillary column, DB-23): 60 m long, 0.25 - 0.32 mm inner diameter, 0.10 - 0.30 μ m film thickness. Conditioning of the column: The column was conditioned by the GC-oven temperature program, starting from the ambient temperature, and increased by 3 °C/ min, up to a temperature of 10 °C. Conditioning was continued until the baseline became linear without any peaks and with no deviation from the baseline. After the baseline became linear, it was kept at that temperature for one hour. Gas chromatography injector: 10 μ L (0.1 μ L graduated). Carrier gas: Inert gas nitrogen, (flow rate 1 mL/min). Auxiliary gases: high purity hydrogen (purity \geq 99.9%) and high purity dry air. Oven temperature: After waiting for 15 minutes at the initial temperature of 165 °C, it was increased to 200 °C with an increase in temperature of 5 °C/min. Injection temperature: 250 °C. Detector temperature: constant, maximum temperature is 260/280 °C. The flow rate of the carrier gas: 1.2 mL/min. Amount of substance injected: 1 μ L. Split ratio: 1:20 Optimized by sample. Sample amount: 0.1-0.2 μ L. The sample amount was increased up to 10 times when the trace amount of substances was analyzed. After the oven temperature was programmed, the separation was continued at a constant temperature until all the peaks came out. Since the sample contained fatty acids lower than 12 carbons, the injection was done at 100 °C and the temperature was immediately increased to the optimum temperature at a rate of 4-8 °C/min. The program was continued at a constant temperature until all components were separated. The number of trans-isomers of fatty acids with carbon numbers between 10 and 24 was determined using capillary columns of a certain polarity. Capillary column for trans-isomers: Silica coated with cyanopropsilicon, 60 m long, 0.25-0.32 mm inner diameter,

Fish samples	Mean standard length (cm) mean ± SE	Mean total weight (g) mean ± SE	Sex of fish	Number of samples	Studied part
Khabur River					
Capoeta umbla	28±5.45	250±12.30	Female	7	Dorsal muscle
Barbus lacerta	20±4.10	92±6.40	Female	8	Dorsal muscle
Squalius berak	21±4.20	120±7.90	Female	7	Dorsal muscle
Chondrostoma regium	24±4.90	145±10.45	Female	7	Dorsal muscle
Alburnus mossulensis	19±3.34	73±8.43	Female	8	Dorsal muscle
Ambar Stream					
Mastacembelus mastacembelus	42±6.20	109±9.30	Female	7	Dorsal muscle
Barbus lacerta	24±5.15	103±9.25	Female	7	Dorsal muscle
Capoeta umbla	25±6.24	190±10.34	Female	7	Dorsal muscle
Cyprinion macrostomus	18±4.34	80±8.43	Female	8	Dorsal muscle
Capoeta trutta	25±3.42	150±8.75	Female	7	Dorsal muscle
Tigris River					
Capoeta umbla	33±4.60	420±15.10	Female	7	Dorsal muscle
Cyprinus carpio	42±6.25	910±20.25	Female	7	Dorsal muscle
Capoeta trutta	28±7.20	228±11.50	Female	7	Dorsal muscle
Squalius berak	30±6.40	293±13.15	Female	7	Dorsal muscle
Chondrostoma regium	28±5.30	205±11.10	Female	7	Dorsal muscle
Carasobarbus luteus	25±4.85	250±16.45	Female	7	Dorsal muscle
Carassius gibelio	23±4.34	251±13.30	Female	7	Dorsal muscle
Silurus triostegus	85±10.20	2050±55.40	Female	6	Dorsal muscle

 Table 1.
 The characteristics of fish species from the Khabur River, Ambar Stream and Tigris River.

Values are given as mean \pm SE (standard error) from 6-8 different fish measurements

 $0.10-0.30 \ \mu m$ film thickness. The FAMEs were identified using retention times compared with those of standard purified FAMEs (Sigma Chemical Co., St. Louis, MO, USA). Results were expressed as FID response area relative percentages. The proportions and spectra of FAMEs were obtained with Hewlett-Packard 3365 Chem-Station computer program.

Cholesterol determination was made according to the AOAC method (Helrich, 1990). The standard curve for cholesterol quantification was built using cholesterol standards at values of 0.0125, 0.025, 0.05, and 0.1 mg/mL. In order to standardize injection mistakes, a correction factor based on an internal standard called 5a-cholestane (Sigma-Aldrich, MO, USA) was applied. A high-grade toluene solution (Sigma-Aldrich) was used to dilute all standards. To each of the control samples 1 mg of free cholesterol (Sigma-Aldrich) was added, as also to the samples utilized for the recovery test, which were designated with the letter "R." Each set of samples used for validation had three replicates taken out, with a sample size of 1 g. The addition of free cholesterol (Sigma-Aldrich) to sample matrices was used to assess the method's accuracy. Utilizing a dilution of the standard solution, the detection limit was established. Once the response signal was twice as strong as the noise signal and observable during the retention time corresponding to the free cholesterol standard, the cholesterol standard was diluted and subjected to GC analysis. The cholesterol detection threshold was determined by assuming that the cholesterol came from 1 g of fish tissue.

Fish muscle tissue samples were properly weighed to 1 g and deposited in a 125 mL boiling flask; then 2 mL of 50% potassium hydroxide in water and 10 mL of 95% ethanol were added to the flask. For 80 minutes, the mixture was stirred, boiled, and refluxed. After cooling the boiling liquid to room temperature (25°C), 10 mL of high-grade toluene (Sigma-Aldrich) was added. The solution had to be mixed for 30 seconds before being transferred to a 250 mL separatory funnel. To eliminate the aqueous components, at least five washes of toluene extract were conducted. The amounts of wash solutions (1.0 N, 0.5N KOH, and distilled water) were significantly reduced due to the reduction of the toluene solvent used. It was critical to allow the toluene layer to completely separate before discarding the aqueous layer in all washes. To eliminate any moisture connected with the toluene, the mixture of toluene and anhydrous sodium sulfate was shaken. In a 2.0 mL flask, 0.5 mL of crystal-clear toluene solution containing extracted cholesterol was combined with 0.5 mL of internal standard solution before being run through the GC apparatus. The Agilent 7820A gas chromatographic system and the DB-17 capillary column (30 m ×0.250 mm×0.15 mm, Agilent Technologies Inc., CA, USA) were used to measure the cholesterol. Gas chromatography for cholesterol: Oven temperature: 260±5°C. Injection temperature: 280 °C. Detector temperature: 300 °C. Velocity of carrier gas: Helium 20-35 cm/s, Hydrogen 30-50 cm/s. Split ratio: 1:50. Injection volume: 0.5-1 µL. Using a 10 µL micro-injector, 1 μ L of hexane was taken, 0.5 μ L of air was drawn into it, followed by 0.5-1 μ L of the sample. The identification and

retention times of each peak were made by comparing the retention times of cholesterol (Δ -5-cholesten-3 β -ol) and the standards analyzed under the same conditions.

Analysis of Proximate Composition

The proximate composition of the fish was determined according to the official method of the AOAC (Helrich, 1990). The nitrogen content was measured by the Kjeldahl method, and the percentage (%) of crude protein was calculated from the nitrogen content. In the Kjeldahl method, the entire organic nitrogen is converted to ammonium sulfate after being digested in concentrated sulfuric acid. Under alkaline circumstances, ammonia is created and then distilled into a boric acid solution. The amount of nitrogen in the borate anions, which represents the amount of crude protein in the sample, is estimated by titrating them with standardized hydrochloric acid. Moisture content (%) was calculated by drying the sample in an oven (WISD/WON105) at 103 °C for 18 hours, and the ash content (%) was calculated by direct analysis in a GC-oven (Prothem/PLF 110/15) at 550 °C for 12 hours.

Statistical Analyses

All data are presented as mean \pm standard deviation (SD). For analysis data, a statistical program (SPSS 16.0) was used. Statistical analyses of fatty acid levels and proximate compositions were performed by analysis of variance (ANOVA), and mean comparison was performed by Tukey's test. Means were obtained in triplicate and statistically significant differences were reported at ($P \le 0.05$).

RESULTS AND DISCUSSION

Proximate composition

The proximate compositions of all fish are presented in Table 2. Although the total lipid contents were similar in a few species, significant differences ($P \le 0.05$) were observed between the species. For example, in the Ambar Stream, in M. mastacembelus, the total lipid level was found to be very low at 0.93%, while in B. lacerta and C. umbla, it was found to be close to each other and relatively high at 5.58% and 5.74%, respectively. The total lipid content was found to be the highest in C. umbla with 6.7% and the lowest in B. lacerta with 3.72% in fish from the Khabur River. Among the Tigris River fish, total lipid was found to be the lowest in C. luteus with 0.99% and the highest in C. regium with 7.47%. There are many studies on the total lipid content of freshwater fish. In most of these studies, the total lipid level showed differences as in the current study. For example, in a study on the Indus River fish, the total lipid levels were reported to vary between 0.85% and 18.32% (Memon et al., 2010). In a study of 20 freshwater fish species collected from Malaysian freshwaters, it was reported that the total lipid content of fish (between 1.17 - 34%) varied widely (Rahman et al., 1995). In another study on freshwater fish from Seyhan Dam Lake, it was reported that Sander lucioperca had the lowest total lipid content of 0.39% and Clarias gariepinus had the highest as 3.21% (Özoğul et al., 2007). The total lipid level in C. carpio fish, which was also examined in the current study, was stated as 0.88% (Özoğul et al., 2007). In another study, the total lipid level of C. carpio and Sander lucioperca L. collected from Beyşehir Lake was reported as 3.33 and 1.73%, re-

spectively (Öksüz et al., 2019). Another study reported that the total lipid content of the tissue of M. mastacembelus (Atatürk Dam), which was also investigated in the current study, changed seasonally from 0.50% to 3.59%, and it was also emphasized that the total lipid level was as low as 0.77% in July (Kaçar et al., 2018). In the present study, the total lipid content of M. mastacembelus from the Tigris River was also found to be as low as 0.98% in July. Another study showed that the total lipid content of S. triostegus, male and female, collected from the Atatürk Dam changes seasonally from 0.63% to 1.32% and from 0.45% to 1.83%, respectively (Kacar et al., 2016). Additionally, the same study emphasized that the lipid of female S. triostegus decreased to a minimum in July (Kaçar et al., 2016). In the current study, the total lipid level of S. triostegus was found to be 2.93%. This result was not in agreement with the findings of S. triostegus from Atatürk Dam. Atatürk Dam Lake and the Tigris River conditions differ from each other. This is because fish are exothermic and water temperature is one of the most important abiotic factors affecting the growth and survival of aquatic animals. Any change in optimum water temperature has a marked and direct effect on many of the basic physiological processes, especially lipid content (Fatma & Ahmed, 2020).

Fish are typically divided into four groups according to their total lipid content: lean fish (lipid less than 2%), low-fat fish (lipid 2-4%), medium-fat fish (lipid 4-8%) and high-fat fish (lipid more than 8% by weight) (Ackman, 1994). In the current study, *M. mastacembelus, C. luteus, C. carpio* and *C. trutta* (Tigris River) were lean fish. *C. trutta* (Ambar Stream), *S. berak* (Tigris River), *C. gibelio*, and *S. triostegus* were low-fat fish, and the other species were medium-fat fish.

The cholesterol levels of the Ambar Stream fish were found to be the lowest in M. mastacembelus with 11.45 mg/100 g and the highest in C. umbla with 29.98 mg/100 g. In the Khabur River fish, the lowest level was detected in A. mossulensis with 12.64 mg/100 g and the highest in B. lacerta with 20.2 mg/100 g. In the Tigris River fish, the lowest cholesterol content was found in C. carpio with 7.9 mg/100 g, and the highest in C. umbla with 18.97 mg/100 g. In a study on the cholesterol content of three freshwater fish, the amount of cholesterol was found to be in the range of 40.99-52.79 mg/100 g (Moreira et al., 2001), and the amounts were found to be significantly higher than the cholesterol content of the species in this study. In another study, it was reported that the cholesterol content of fish species living in the Porsuk Dam ranges from 94.68 to 179.84 mg/100g (Donmez, 2009). The lipid and cholesterol content of fish depends on age, spawning period, sex, season, geographical conditions and their nutrients and feeding types (Memon et al., 2010). Age variation and sexual maturity in the same species also cause significant differences in total lipid and cholesterol content (Memon et al., 2010). Fish caught during the spawning season or in waters with scarce food sources have lower lipid and cholesterol content than normal seasons, and reducing PUFA in the diet raises cholesterol content in the fish (Donmez, 2009).

In all studied fish, *C. regium* (Khabur River) had the highest protein content with 20.76% and *C. umbla* (Tigris River) had the lowest with 16.64%. Crude protein levels of the same species in difTable 2.

Proximate composition of the dorsal muscle of fish from the Ambar, Khabur and Tigris Rivers (%, wet basis). Cholesterol is mg/100 g of dorsal meat of the fish.

Species	Total Lipid (%)	Crude Protein (%)	Cholesterol (mg/100g)	Moisture (%)	Ash (%)
Ambar Stream fish					
Mastacembelus mastacembelus	0.93±0.02a	17.74±0.35a	11.45±0.26a	80.14±0.45a	1.19±0.03a
Barbus lacerta	5.58±0.08b	19.02±0.56b	26.30±0.35b	74.25±0.40b	1.15±0.02a
Capoeta umbla	5.74±0.09b	17.56±0.36a	29.98±0.42c	75.55±0.38b	1.15±0.02a
Cyprinion macrostomus	4.56±0.07c	18.45±0.55b	22.16±0.39d	75.8±0.41b	1.19±0.03a
Capoeta trutta	2.32±0.05d	18.98±0.48b	16.37±0.35e	77.57±0.47ab	1.13±0.02a
Khabur River fish					
Capoeta umbla	6.70±0.09e	17.92±0.31a	12.94±0.23a	74.22±0.57b	1.16±0.02a
Barbus lacerta	3.72±0.0cd	17.86±0.45a	20.20±0.29d	77.31±0.63ab	1.11±0.03a
Squalis berak	4.16±0.08c	19.32±0.41b	13.64±0.18a	75.33±0.54b	1.19±0.03a
Chondrostoma regium	6.02±0.06b	20.76±0.38c	16.85±0.17e	72.10±0.49c	1.12±0.02a
Alburnus mossulensis	4.27±0.07c	19.91±0.29bc	12.64±0.13a	74.72±0.57b	1.10±0.03a
Tigris River fish					
Squalis berak	2.14±0.05d	17.45±0.21a	17.67±0.22e	79.29±0.65a	1.12±0.06a
Capoeta trutta	1.63±0.07ad	17.39±0.23a	10.15±0.12a	79.68±0.59a	1.30±0.08a
Cyprinus carpio	1.37±0.09ad	17.28±0.32a	7.90±0.08f	80.2±0.73a	1.15±0.07a
Capoeta umbla	5.41±0.11b	16.64±0.14d	18.97±0.19de	76.77±0.66b	1.18±0.08a
Chondrostoma regium	7.47±0.16e	17.76±0.17a	11.87±0.31a	73.63±0.81bc	1.14±0.09a
Carasobarbus luteus	0.99±0.08a	17.75±0.15a	7.91±0.11f	80.15±0.58a	1.11±0.06a
Carassius gibelio	2.81±0.11d	18.61±0.16b	11.15±0.12a	77.45±0.70ab	1.13±0.05a
Silurus triostegus	2.93±0.12d	18.62±0.19b	8.61±0.09f	77.29±0.68ab	1.16±0.09a

* The values are means ± SD (standard deviation). Results are expressed as a percentage of total lipid, crude protein, moisture and ash, and mg/100g of cholesterol.

**Means followed by different letters in the same column are significantly different (P \leq 0.05).

ferent rivers differed slightly. For example, crude protein contents of *B. lacerta* from the Ambar Stream and Khabur River were found to be 19.02% and 17.86%, respectively ($P \le 0.05$). The crude protein levels of C. umbla from the Ambar Stream, Khabur and Tigris River were 17.56%, 17.92% (P>0.05) and 16.64% respectively. C. regium from the Khabur and Tigris Rivers had 20.76% and 17.76% of crude protein, respectively ($P \le 0.05$). The crude protein content of Indus River fish species was reported to be between 17% and 20.09% (Memon et al., 2010) and the results of the study partly conform with the current study. In another study on five freshwater fish, the crude protein levels were reported to be between 13.93% and 15.41% (Paul et al., 2019) and the results were lower than those in the current study. The crude protein percentages for C. carpio and S. lucioperca, both of which were taken from Beyşehir Lake, were reported to be 17.40% and 18.97%, respectively (Öksüz et al., 2019). The protein requirements of fish depend on many biological and environmental circumstances (sexual maturity, sex, age, feeding frequency, nutritive conditions, water temperature, non-protein diet amount and dietary protein quality, etc.) (Fatma & Ahmed, 2020).

The findings of our study showed that the moisture content of the Ambar fish was the lowest in *B. lacerta* with 74.25% and the highest in *M. mastacembelus* with 80.14%. Moreover, the moisture content of the Khabur fish varied between 72.10% (*C. regium*) and 77.31% (*B. lacerta*). In the Tigris River fish, the moisture content was the lowest in *C. regium* with 73.63% and the highest in *C. carpio* with

80.20%. However, the ash content was more or less similar (P>0.05) in 18 fish species and varied between 1.11% and 1.30% The reason for similar ash content is probably related to the fact that the skeletons of the fish were not used in the ash analysis. However, ash content differs proportionally in some literature. For example, the ash content of eight fish species from the Indus river varied between 0.05% and 4.95% (Memon et al., 2010). The reason for the low level was attributed to the minimal skeleton in small indigenous fish species (Memon et al., 2010). In the same study, the moisture content was found to be between 78.80% and 59.95% (Memon et al., 2010). In another paper on five freshwater fish, the ash content was reported to be between 1.88% and 2.57%, and the moisture content was between 70.82% and 76.11% (Paul et al., 2019). In most studies, significant differences were not observed, and the results are compatible with the current study. In addition, it was noticed that the moisture content of the fish with low total lipid content was high, and an inverse relationship was determined. For example, in M. mastacembelus the total lipid content was 0.95% and the moisture was 80.14%; in C. luteus the total lipid was 0.99% and the moisture was 80.15%; in C. carpio the total lipid level was 1.37% and the moisture content was 80.20%. Similar findings have been highlighted in other studies as well (Memon et al., 2010; Özyurt & Polat, 2006).

Fatty acid composition

The fatty acid compositions of fish taken from the Ambar Stream, the Khabur River and the Tigris River are presented in Tables 3, 4 and 5, respectively. In the analysis, the presence and amount of

37 types of fatty acids from 18 fish species were examined. The predominant fatty acids identified in all fish were C16:0 (Palmitic acid), C16:1w7 (Palmitoleic acid) and C18:1w9 (Oleic acid). Moderately detected fatty acids were C14:0 (Myristic acid) and C18:0 (Stearic acid). C16:0 was the primary saturated fatty acid, the level of which was found to be between 32.53% and 40.66% in the Ambar Stream fish, between 26.98% and 33.65% in the Khabur River fish, and between 30.2% and 43.03% in the Tigris River fish. The primary MUFA C16:1 ω 7 level was found to be between 18.13% and 27.42% in the Ambar Stream fish, between 13.42% and 27.14% in the Khabur River fish, and between 8.08% and 26.92% in the Tigris River fish. The level of C18:1w9 varied between 12.71% and 24.1% in the Ambar Stream fish, between 19.21% and 36.99% in the Khabur fish, and between 14.51% and 30.66% in the Tigris River fish. Notably, C. umbla was collected from the Ambar, Khabur and Tigris Rivers; B. lacerta from the Ambar and Khabur Rivers; C. trutta from the Ambar and Tigris Rivers and S. berak and C. regium from the Khabur and Tigris Rivers. When the same species of fish living in different rivers were compared, it was observed that there were some similarities and differences in the levels of fatty acids. For example, the predominant fatty acid profile of C. umbla was similar to each other (P>0.05) for three rivers except that C18:1w9. C16:0, C16:1w7 and C18:1w9 in C. umbla from the Khabur River were found to be 33.65%, 23.24% and 23.11%, respectively. C16:0, C16:1w7 and C18:1w9 levels of C. umbla in the Ambar Stream were found to be 34.04%, 26.53% and 17.24%, and C16:0, C16:1w7, C18:1w9 levels in the Tigris River were found to be 33.71%, 24.43%, and 20.79%, respectively. In the same way, C14:0 and C18:0 were detected in close levels in C. umbla. Likewise, the dominant and moderate fatty acid profiles of B. lacerta from both the Khabur River and the Ambar Stream were mostly similar except for C16:1w7 and C18:0. For example, the levels of C16:0, C16:1w7, C18:1w9, C14:0, C18:0 in B. lacerta from the Ambar Stream were 32.53%, 24.29%, 22.02%, 5.99%, 5.52%, and in B. lacerta from the Khabur River the levels were 30.63%, 20.16%, 22.41%, 5.53% and 6.84%, respectively. It was also observed that the fatty acid profile of C. trutta was close to each other. The levels of C16:0, C16:1w7, C18:1w9, C14:0, C18:0 for C. trutta from the Ambar Stream, and Tigris River were 36.32%, 27.42%, 12.71%, 10.72%, 4.71%, and 34.17%, 26.92%, 14.71%, 8.27%, 5.98%, respectively. The levels of C16:0, C16:1w7, C18:1w9, C14:0, C18:0 for S. berak from the Khabur and Tigris River were also similar. It was only the level of C18:1w9 that was higher in S. berak from the Khabur River than that from the Tigris River ($P \le 0.05$). Although C16:0 levels were close to each other in C. regium (31.17% for the Khabur River, 33.36% for the Tigris River), differences were observed in the levels of other dominant fatty acids ($P \le 0.05$). Consequently, it was observed that the fatty acid profiles of the same fish in different rivers were roughly similar. Minor level differences of the specific fatty acids are possibly due to diet, age, food, water temperature or geographic differences. It is a fact that the same species generally have the same physiological characteristics. It is normal for this similarity to occur because the same species have the same feeding type and prefer similar foods and tastes.

In most of the studies on freshwater fish, C16:0, C18:0, C16:1 ω 7 and C18:1 ω 9 were determined as the dominant component and

high levels of these fatty acids have been described as a characteristic of freshwater fish (Aras, 2003; Cengiz et al., 2010; Kaçar et al., 2016, 2018; Kaçar & Başhan, 2016; Memon et al., 2010; Osman et al., 2001; Özoğul et al., 2007; Satar et al., 2012; Vasconi et al., 2015). In one study, the main fatty acids of freshwater fish were highlighted as C16:0 (15.9-20.5%), C16:1w7 (2.51-10.9%), C18:0 (5.63-14.8%), C18:1w9 (3.46-15.9%), and C22:6w3 (6.72-24.8%) (Özoğul et al., 2007). In another study, C16:0 (25.39%), C16:1w7 (5.63%), C18:0 (5.91%), C18:1w9 (20.63%) and C22:6w3 (21.42%) were reported as dominant fatty acids in a freshwater Salmo trutta labrax muscle tissue (Aras, 2003). Furthermore, for the freshwater fish Rainbow Trout (Oncorhynchus mykiss), the dominant fatty acids detected in the muscle of the fish were C16:0 (21.3%), C16:1w7 (4.16%), C18:0 (6.79%), C18:1w9 (22.2%), C18:2w6 (10.4%) (22.2%) and C22:6w3 (22.7%) (Haliloğlu et al., 2004). In a paper describing the fatty acid compositions of 9 freshwater fish species from the Tigris River (some of them are the same fish as in the current study, such as A. mossulensis, C. regium, C. luteus, C. macrostomus and S. triostegus) predominant fatty acids were C16:0, C16:1w7 and C18:1w9 as in the current study.

In all studied fish, w3 and w6 PUFAs particularly C18:2w6, C18:3w6 (y-Linolenic acid, GLA), C20:4w6, C20:5w3 and C22:6w3 levels were found to be quite low. Among the PUFAs, C18:2w6 had the highest level, with 5.91% for A. mossulensis. The level of C20:4w6, C20:5w3 and C22:6w3 was determined to be below 1%. On the contrary, a high content of C20:4w6 (between 0.75-12.27%), C20:5w3 (between 0.65-20.15%) and C22:6w3 (between 0.72-26.89%) was reported in some freshwater fish such as C. regium, B. rajonorum, C. luteus, Leuciscus lepidus, Acanthobrama marmid, C. macrostomus, and S. triostegus (Cengiz et al., 2010). In a study conducted on the seasonal fatty acid composition of C. carpio from Beysehir Lake, the levels of C18:2w6 (8.82%), C20:4w6 (6.99%), C20:5w3 (4.72%) and C22:6w3 (11.03%) were reported high in the summer season (Guler et al., 2008). However, as in the present paper, some studies reported that the levels of C20:4 ω 6 and C20:5w3 components in freshwater fish were below 1% (Citil et al., 2014; Kaçar et al., 2018; Łuczyńska et al., 2014; Paul et al., 2019). Interestingly, another study revealed that C. carpio, also used in the current study, contained very different C20:5w3 levels in two different dam lakes (Işıklı and Karacaören Dam lakes 0.56% and 20.91%, respectively) (Citil et al., 2014). This shows that even in the same species, the level of C20:5 ω 3 may differ significantly due to environmental conditions. PUFA levels of fish typically decrease in the summer season (Kaçar et al., 2016; Satar et al., 2012) and fish living in cold waters accumulate more ω 3 PUFAs to respond to their physiological requirements (Özoğul et al., 2007). All fish examined in the current study were collected in July during the hottest month of the year. The breeding period of the studied fish is between April and June. It is estimated that the PUFA levels of the fish may decrease after spawning and breeding. Species diversity is the most important cause of variation in fatty acid profiles of fish. Moreover, fatty acid compositions of fish with high species diversity may differ due to many factors. Living in aquatic environments with different ecological conditions, differences in feeding components, particularly whether a species is herbivorous, omnivorous or carnivorous are consid-

Table 3.Fatty a	cid composition of the t	otal lipid from dorsal	muscle of the Amba	ar Stream fish (%, fatty	acids).
Fatty acids (%)	Mastacembelus mastacembelus	Barbus lacerta	Capoeta umbla	Cyprinion mac- rostomus	Capoeta trutta
C4:0	nd	nd	nd	nd	0.07±0.01
C6:0	0.10±0.02a	nd	nd	0.05±0.01a	0.07±0.02a
C8:0	0.05±0.01a	0.07±0.02a	0.16±0.04b	0.08±0.02a	0.05±0.01a
C10:0	nd	nd	nd	nd	nd
C11:0	nd	nd	nd	nd	nd
C12:0	0.75±0.07a	0.66±0.06a	0.15±0.02b	0.13±0.02b	0.19±0.03b
C13:0	0.07±0.01a	nd	nd	0.05±0.01a	0.15±0.02b
C14:0	6.56±0.05a	5.99±0.06a	9.54±0.08b	6.32±0.05a	10.72±0.20b
C15:0	0.86±0.07a	0.59±0.05a	0.65±0.06a	0.76±0.07a	1.34±0.11b
C16:0	33.68±0.61a	32.53±0.78a	34.05±0.60a	40.66±0.71b	36.32±0.47ab
C17:0	0.91±0.07a	0.49±0.03b	0.24±0.02c	1.12±0.09a	0.54±0.06b
C18:0	7.62±0.25a	5.52±0.21b	3.04±0.19c	6.22±0.37ab	4.71±0.10bc
C20:0	0.36±0.03a	0.29±0.03a	0.12±0.01b	0.16±0.02b	0.24±0.03a
C21:0	nd	nd	nd	nd	nd
C22:0	0.22±0.02a	0.05±0.01b	nd	nd	0.06±0.01b
C23:0	nd	nd	nd	nd	nd
C24:0	0.14±0.03a	0.06±0.01b	0.05±0.01b	nd	nd
C14:1ω5	0.31±0.02a	0.25±0.02a	0.26±0.02a	0.25±0.03a	nd
C15:1ω5	nd	nd	nd	nd	nd
C16:1ω7	18.44±0.33a	24.29±0.28b	26.53±0.31c	18.13±0.22a	27.42±0.44c
C17:1ω7	0.98±0.09a	1.75±0.08b	1.73±0.08b	1.13±0.10ab	1.15±0.11ab
C18:1ω9	24.10±0.37a	22.02±0.28a	17.24±0.19b	22.06±0.21a	12.71±0.13c
C20:1ω9	1.65±0.12a	1.21±0.10a	1.98±0.12a	1.44±0.09a	2.20±0.10b
C22:1ω9	nd	nd	nd	nd	nd
C24:1ω9	0.23±0.06a	0.05±0.01b	0.10±0.03c	nd	nd
C18:2w6-cis	1.06±0.05a	1.55±0.09a	1.05±0.07a	0.51±0.03b	0.38±0.04b
C18:2w6-trans	0.22±0.03a	0.11±0.02b	0.06±0.01c	0.10±0.01b	0.38±0.03d
C18:3ω3	nd	0.07±0.01a	0.14±0.02b	nd	nd
C18:3ω6	0.57±0.03a	0.65±0.05a	0.93±0.08a	0.20±0.01b	nd
C20:2w6	0.08±0.01a	0.08±0.01a	0.07±0.01a	nd	nd
C20:3ω3	0.09±0.02a	0.14±0.02a	nd	0.07±0.01a	0.10±0.03a
C20:3w6	nd	nd	nd	nd	nd
C20:4w6	nd	nd	nd	nd	nd
C20:5ω3	nd	0.48±0.03a	0.52±0.04a	nd	nd
C22:2w6	0.74±0.06a	0.65±0.04a	0.91±0.09b	0.95±0.09b	1.15±0.15b
C22:6w3	nd	0.10±0.02a	0.08±0.01a	nd	nd
ΣSFA	51.32±1.34a	46.25±1.12b	48±1.32c	55.55±1.56d	54.46±1.47d
ΣMUFA	45.70±1.10a	49.57±1.12b	47.77±1.17b	43.01±1.05c	43.48±1.06c
ΣPUFA	2.76±0.13a	3.83±0.17b	3.76±0.21b	1.83±0.09c	2.01±0.10a
<u>Σ</u> ω 6	2.67±0.11a	3.04±0.21b	3.02±0.23b	1.76±0.12c	1.91±0.11c
Σ ω 3	0.09±0.03a	0.79±0.05b	0.74±0.05b	0.07±0.04a	0.10±0.02a
ω6/ω3	29.67	3.85	4.08	25.14	19.10

*Values are means \pm SD (standard deviation) for 3 replicates. Results were expressed as a percentage of total fatty acid methyl esters. **The mean values of different characters on the same line vary significantly (*P*≤0.05). (Results are expressed as % fatty acids of total lipids). Abbreviations: SFA - saturated fatty acid; MUFA - mono-unsaturated fatty acid; PUFA - polyunsaturated fatty acid; nd - not detected.

ered to be the most important reasons for proximate and fatty acid variations. The season when fish are caught, as well as the size and reproductive status of individuals of the same species living in a certain area, also affect the variations. Additionally, the level of ω 3 fatty acids, especially C20:5 ω 3 and C22:6 ω 3 are high in fish living in cold climates and vice versa (Çelik et al., 2005). Another reason for the low rate of ω 3 and ω 6 PUFAs accumulation in the fish of the current study may be related to the low level of

Table 4. F	Canacta Chandractama Albu					
Fatty acids (%)	Capoeta umbla	Barbus lacerta	Squalius berak	Chondrostoma regium	Alburnus mossulensis	
C4:0	0.05±0.01	nd	nd	nd	nd	
C6:0	nd	nd	nd	nd	0.07±0.02	
C8:0	0.16±0.02a	0.10±0.02a	0.16±0.03a	0.06±0.01b	0.22±0.04c	
C10:0	nd	0.12±0.02a	0.20±0.03b	0.05±0.01c	0.13±0.03a	
C11:0	nd	0.06±0.01	nd	nd	nd	
C12:0	0.10±0.02a	1.61±0.05b	0.96±0.03c	0.52±0.03d	1.22±0.05b	
C13:0	nd	0.08±0.02a	0.06±0.01a	0.15±0.03b	nd	
C14:0	8.21±0.12a	5.53±0.11b	3.95±0.07c	7.08±0.08ab	3.17±0.04c	
C15:0	0.47±0.04a	0.68±0.06a	0.51±0.05a	0.84±0.08b	0.45±0.04a	
C16:0	33.65±0.66a	30.63±0.61b	28.89±0.56c	31.17±0.59b	26.98±0.49d	
C17:0	0.56±0.06a	0.66±0.07a	0.59±0.06a	0.50±0.05a	0.61±0.08a	
C18:0	4.86±0.16a	6.84±0.14b	7.51±0.19b	4.46±0.14a	5.98±0.29ab	
C20:0	0.11±0.03a	0.35±0.04b	nd	0.24±0.03ab	0.38±0.03b	
C21:0	nd	nd	nd	nd	nd	
C22:0	nd	0.10±0.04a	0.09±0.02a	0.07±0.01a	0.11±0.03a	
C23:0	nd	nd	nd	nd	0.41±0.04	
C24:0	nd	nd	nd	0.05±0.01a	0.10±0.02b	
C14:1ω5	0.25±0.04a	2.30±0.10b	0.18±0.04a	0.20±0.04a	0.11±0.02c	
C15:1ω5	nd	nd	nd	nd	nd	
C16:1ω7	23.24±0.44a	20.16±0.40b	13.42±0.37c	27.14±0.54d	14.70±0.31c	
C17:1ω7	1.56±0.12a	1.14±0.13a	0.69±0.08b	2.07±0.15c	1.06±0.09a	
C18:1ω9	23.11±0.47a	22.41±0.45a	36.99±0.81b	19.21±0.39c	34.21±0.78d	
C20:1ω9	0.12±0.02a	1.49±0.07b	nd	1.04±0.07b	0.94±0.06ab	
C22:1ω9	nd	nd	nd	0.06±0.01a	0.14±0.03b	
C24:1ω9	nd	0.11±0.03	nd	nd	nd	
C18:2ω6-cis	0.80±0.07a	3.06±0.16b	3.60±0.17b	2.03±0.11c	5.91±0.18d	
C18:2ω6-trans	0.06±0.01a	0.16±0.03b	0.13±0.03b	0.08±0.02a	0.11±0.03b	
C18:3ω3	0.08±0.01a	nd	nd	0.10±0.02a	nd	
C18:3ω6	1.13±0.04a	0.74±0.05b	0.59±0.04b	0.65±0.06b	1.15±0.09a	
C20:2ω6	0.05±0.01a	0.12±0.03b	0.19±0.03b	0.15±0.02b	0.37±0.04c	
C20:3ω3	nd	0.29±0.03	nd	nd	nd	
C20:3w6	nd	nd	nd	0.10±0.02a	0.10±0.03a	
C20:4ω6	0.10±0.03a	0.14±0.04a	0.05±0.01b	0.08±0.01b	0.13±0.03a	
C20:5ω3	0.30±0.04a	0.28±0.04a	0.07±0.02b	0.87±0.07c	0.68±0.05c	
C22:2ω6	0.67±0.04a	0.52±0.03a	0.62±0.04a	0.65±0.05a	0.30±0.03b	
C22:6ω3	nd	nd	0.20±0.03a	0.10±0.03b	0.08±0.01b	
ΣSFA	48.17±1.21a	46.76±.1.18b	42.92±1.14c	45.19±1.13b	39.35±1.09d	
ΣMUFA	48.28±1.30a	47.61±1.27a	51.28±1.39b	49.72±1.40c	51.16±1.45b	
ΣPUFA	3.19±0.08a	5.31±0.09b	5.45±0.10b	4.81±0.08b	8.83±0.18c	
Σω 6	2.81±0.06a	4.74±0.11b	5.18±0.14b	3.74±0.09c	8.07±0.21d	
Σω 3	0.38±0.03a	0.57±0.05a	0.27±0.02a	1.07±0.09b	0.76±0.08c	
ω 6/ω3	7.39	8.32	19.19	3.50	10.62	

*Values are means \pm SD (standard deviation) for 3 replicates. Results were expressed as a percentage of total fatty acid methyl esters. **The mean values of different characters on the same line vary significantly ($P \le 0.05$). (Results are expressed as % fatty acids of total lipids). Abbreviations: SFA - saturated fatty acid; MUFA - mono-unsaturated fatty acid; PUFA - polyunsaturated fatty acid; nd - not detected.

C18:3 ω 3 and C18:2 ω 6 that enable the synthesis of C22:6 ω 3, C20:4 ω 6 and C20:5 ω 3. The higher concentration of ω 6 fatty acids in freshwater fish could be attributed to dietary precursors such as freshwater algae, insect larvae and crustacean that are rich in

C18:2 ω 6 and C18:3 ω 6 (Kaçar & Başhan, 2016; Steffens, 1997). However, because of the absence of both D12 and D15 desaturase, fish cannot convert C18:1 ω 9 to C18:2 ω 6 and further to C18:3 ω 3 (Tian et al., 2016). Thus, fish are considered to have an

Table 5.	Fatty acid composit	ion of the total lipic	d from dorsal muscl	e of the Tigris River	Fatty acid composition of the total lipid from dorsal muscle of the Tigris River fish (%, fatty acids).			
Fatty acids	Squalis berak	Capoeta trutta	Cyprinus carpio	Capoeta umbla	Chondrostoa regium	Carasobarbus Iuteus	Carassius gibelio	Silurus triostegus
C4:0	0.05±0.01a	0.09±0.01a	0.03±0.01a	pu	0.06±0.01a	pu	pu	nd
C6:0	0.07±0.02a	0.07±0.01a	0.06±0.01a	hd	0.07±0.01a	0.09±0.01a	0.09±0.02a	0.07±0.01a
C8:0	0.28±0.04a	0.21±0.03a	0.12±0.02b	0.06±0.01 c	0.16±0.03b	0.54±0.04d	0.10±0.03b	0.07±0.01c
C10:0	0.07±0.01a	0.07±0.01a	0.03±0.01b	hd	0.05±0.01 ab	nd	pu	nd
C11:0	pu	pu	pu	pu	pu	pu	pu	pu
C12:0	0.55±0.07a	0.24±0.04b	0.43±0.05a	0.14±0.03c	0.24±0.03b	0.07±0.01d	0.80±0.08e	0.40±0.05a
C13:0	0.09±0.02a	0.16±0.04b	0.10±0.03a	hd	0.10±0.02a	0.07±0.01a	0.15±0.04b	0.50±0.07c
C14:0	4.88±0.21a	8.27±0.30b	4.68±0.22a	8.39±0.35b	6.62±0.23ab	3.68±0.12a	4.86±0.27a	5.19±0.12ab
C15:0	1.07±0.08a	1.90±0.07a	1.52±0.05a	0.70±0.04b	1.28±0.06a	0.91±0.04a	1.35±0.05a	0.84±0.04ab
C16:0	30.26±0.50a	34.17±0.66b	35.65±0.75c	33.71±0.52b	33.66±0.69b	43.03±0.72d	30.20±0.57a	33.53±0.62b
C17:0	0.94±0.09a	0.49±0.05b	2.22±0.10c	0.31±0.04b	0.94±0.06a	0.70±0.05a	1.50±0.08ac	0.71±0.05ab
C18:0	6.74±0.45a	5.98±0.32a	10.91±0.15b	3.31±0.11c	5.24±0.15a	6.68±0.21a	7.55±0.19a	10.19±0.20b
C20:0	0.22±0.04a	0.20±0.03a	0.55±0.06b	0.12±0.02c	0.27±0.03a	0.10±0.03c	pu	0.32±0.03ab
C21:0	pu	pu	pu	pu	pu	pu	pu	pu
C22:0	0.06±0.01a	0.10±0.03b	0.34±0.05c	pu	0.07±0.02a	pu	0.12±0.03b	0.09±0.02b
C23:0	pu	pu	pu	pu	pu	pu	pu	pu
C24:0	pu	0.11±0.03a	0.12±0.03a	0.05±0.01b	0.06±0.01b	pu	pu	pu
C14:1w5	0.23±0.07a	0.19±0.04a	0.49±0.05b	0.69±0.04b	0.85±0.07 c	pu	0.67±0.06b	0.13±0.02a
C15:1ω5	0.09±0.02a	pu	0.13±0.03a	pu	pu	pu	0.24±0.03b	0.08±0.01a
C16:1w7	15.85±0.21a	26.92±0.26b	9.67±0.19c	24.43±0.26d	22.74±0.32e	8.08±0.13c	11.60±0.16d	15.25±0.15a
C17:1w7	1.04±0.06a	2.13±0.09b	0.94±0.05a	1.61±0.07a	1.06±0.05a	0.47±0.03c	1.24±0.05a	0.58±0.04c
C18:1w9	30.66±0.67a	14.51±0.45b	21.92±0.49c	20.79±0.52c	22.16±0.54c	28.81±0.61a	29.13±0.70a	28.19±0.65a
C20:1w9	pu	2.29±0.09a	1.35±0.06b	1.83±0.07b	1.45±0.06b	1.71±0.08b	3.34±0.16c	1.97±0.08ab
C22:1w9	pu	pu	pu	pu	pu	pu	pu	pu
C24:1w9	0.08±0.02a	pu	0.14±0.03b	pu	pu	pu	pu	pu
C18:2w6-cis	3.59±0.10a	0.37±0.05b	3.47±0.09a	1.36±0.06c	0.53±0.07b	1.58±0.09c	3.72±0.09a	0.71±0.05b
C18:2w6- trans	0.25±0.04a	0.07±0.01b	0.43±0.06a	0.09±0.02b	0.25±0.05a	0.21±0.04a	0.40±0.05a	0.71±0.06c
C18:3ω3	0.07±0.01a	nd	nd	0.09±0.02a	pu	nd	pu	nd
C18:3w6	1.01±0.08a	0.18±0.04b	2.47±0.09c	0.72±0.05a	0.96±0.08a	1.10±0.05a	pu	0.25±0.04b
C20:2w6	0.28±0.03a	0.05±0.01b	1.35±0.08c	0.06±0.01b	0.11±0.03b	0.17±0.03ab	0.52±0.04a	pu
C20:3ω3	0.10±0.03a	0.08±0.02a	0.34±0.04b	0.09±0.02a	h	0.36±0.05b	0.50±0.06b	рц
C20:3w6	0.10±0.03a	pu	pu	pu	pu	pu	0.07±0.01a	pu
C20:4w6	0.10±0.02a	pu	0.09±0.01a	0.50±0.06b	pu	hd	0.22±0.04c	nd

Table 5.	Continue.							
Fatty acids	Squalis berak	Capoeta trutta	Cyprinus carpio	Capoeta umbla	Chondrostoa regium	Carasobarbus luteus	Carassius gibelio	Silurus triostegus
C20:5w3	0.12±0.04a	0.18±0.03a	0.11±0.06a	0.27±0.07b	pu	pu	0.12±0.03a	pu
C22:2w6	0.57±0.06a	0.75±0.07a	0.59±0.05a	0.59±0.06a	0.57±0.05a	0.47±0.04a	0.63±0.06a	0.83±0.07a
C22:6w3	0.06±0.01a	nd	0.24±0.03b	pu	pu	0.20±0.03b	0.20±0.04b	nd
ΣSFA	45.28±1.15a	52.06±1.46b	56.76±1.49c	46.79±1.25a	48.82±1.35d	55.87±1.50c	46.72±1.34a	51.91±1.41b
ΣMUFA	47.95±1.32a	46.04±1.21b	34.64±1.12c	49.35±1.33a	48.26±1.34a	39.07±1.13d	46.22±1.20b	46.20±1.23b
ΣΡυξΑ	6.25±0.12a	1.68±0.09b	9.09±0.19c	3.77±0.09d	2.42±0.07e	4.09±0.11d	6.38±0.15a	2.50±0.08e
Σω 6	5.90±0.11a	1.42±0.06b	8.40±0.18c	3.32±0.09d	2.42±0.08e	3.53±0.10c	5.56±0.15a	2.50±0.09e
Σw 3	0.35±0.03a	0.26±0.02a	0.69±0.05b	0.45±0.04c	pu	0.56±0.04c	0.82±0.07d	nd
w 6∕ w3	16.86	5.46	12.17	7.38		6.30	6.78	
*Values are mea	Values are means ± SD (standard deviation) for 3 replicates. Results were expressed as a percentage of total fatty acid methyl esters. **The mean values of different characters on the same line vary significantly	n) for 3 replicates. Results	were expressed as a perc	centage of total fatty acic	ł methyl esters. **The me	an values of different cha	racters on the same line	vary significantly
(P≤0.05). (Resul:	(P<0.05). (Results are expressed as % fatty acids of total lipids). Abbreviations: SFA - saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid; nd - not detected.	acids of total lipids). Abbre	eviations: SFA - saturated	fatty acid; MUFA - mono	unsaturated fatty acid; Pl	JFA - polyunsaturated fat	ty acid; nd - not detecte	q.

absolute requirement for the essential fatty acid ω 6 and ω 3 PU-FAs that must be provided in the diet (Tian et al., 2016). Furthermore, fish accumulate ω 3 PUFAs in their bodies by consuming zooplankton and phytoplankton or through consuming smaller fish (Balıkçı, 2021). Therefore, the level of ω 3 PUFA in fish muscle is based on nourishment and their variations may be due to differences in the fish's dietary habits (Balıkçı, 2021).

Several studies have reported that zooplankton and phytoplankton abundance is highest in spring and lowest in winter (Balıkçı, 2021). Naturally, zooplankton and phytoplankton diets provide physiologically essential PUFAs such as C22:6w3 and C20:5w3 (Balıkçı, 2021). The low w3 PUFA content of the species studied in the summer is probably due to lower consumption of freshwater phytoplankton and zooplankton compared to other seasons. The quantity of planktonic organisms in the rivers during the summer can affect post-spawning fatty acid profiles and the post-ovulation interval raises the possibility of low fatty acid levels. In order to understand the relationship between the fatty acid levels of fish and their diets, the planktonic levels of the collection regions should be investigated. Additionally, it is wellknown that during the summer when fish are active and need more energy, they directly reduce their fat reserves by using the fat that has been deposited.

In the comparison of Σ SFA, Σ MUFA and Σ PUFA levels of the Ambar Stream fish, there were some significant differences ($P \le 0.05$). The highest Σ SFA level was found in *C. macrostomus* with 55.55%, and the lowest ∑SFA level was found in *B. lacerta* with 46.25%. However, the highest 5MUFA level was in *B. lacerta* with 49.57% and the lowest SMUFA was detected in C. macrostomus with 43.01%. The Σ PUFA level varied between 1.83% and 3.83% and the highest ratio of $\omega 6/\omega 3$ was observed in *M. mastacembelus* with 29.67. For the fish of the Khabur River, the ∑SFA level ranged from 39.35% (A. mossulensis) to 48.17% (C. umbla). Their 5MUFA levels were between 47.61% (B. lacerta) and 51.28% (S. berak). The highest Σ PUFA level was detected in A. mossulensis with 8.83% and the lowest in C. umbla with 3.19%. The highest ratio of $\omega 6/\omega 3$ was found in S. berak (19.19). In the Tigris River fish, Σ SFA levels were high in C. carpio (56.76%) and C. luteus (55.87%), whereas in S. berak (45.28%), C. umbla (46.79%) and C. gibelio (46.72%) the ∑SFA were low. The lowest SMUFA level was in C. carpio with 34.64% and the highest in C. umbla with 49.35%. The ∑PUFA levels varied between 1.68% (C. trutta) and 9.09% (C. carpio). The highest ratio of $\omega 6/\omega 3$ was found in S. berak with 16.86. Omega-3 fatty acids were not detected in C. regium and S. triostegus. In general, fatty acid composition and $\omega 6/\omega 3$ ratio of fish depends upon the composition of consumed feed (Guler et al., 2008). In consequence, Σ SFA and Σ MUFA levels were significantly higher than Σ PUFA levels in all 18 fish species. This is characteristic of freshwater fish living in warm waters (Kaçar et al., 2016; Kayhan et al., 2015). In many studies, it has been reported that levels of the Σ SFA, Σ MUFA and Σ PUFA differed according to season, habitat or species. For instance, in a study on 9 wild-caught freshwater fish, ∑SFA (27% -36.2%), ∑MUFA (21.83% - 50.53%) and ∑PUFA (19.43% - 45.60%) were reported at high levels (Zhang et al., 2020). Similarly, in a paper on the fatty acid composition of the muscle lipids of five dam fish species from Türkiye, the Σ SFA, Σ MUFA and Σ PUFA levels were

found to be high, (24.95% and 35.34%), (26.08% and 38.17%) and 30.73% and 47.71%, respectively (Citil et al., 2014). In the muscle tissue of S. trutta labrax from Kazandere Creek, it was reported that Σ SFA was 37.21%, Σ MUFA was 26.76%, Σ PUFA (ω 6) was 28.85%, and Σ PUFA (ω 3) was 3.98% (Aras, 2003). In another article about Brazilian freshwater fish both from farms and wildlife habitats, results of 18.76% for Σ PUFA and 38.83% for Σ SFA for farmed fish, and 12.02% for Σ PUFA and 41.86% for Σ SFA for wild fish were reported (Moreira et al., 2001). Like the studies above, it was emphasized in some other studies (particularly in cold freshwater fish), that the Σ PUFA levels were higher than the current study (Cengiz et al., 2010; Kaçar & Başhan, 2016; Kayhan et al., 2015; Özoğul et al., 2007). However, in the studies on fish collected in warm freshwaters, it was reported that the Σ PUFA levels were low. For example, in a study about the fatty acid profile of male and female S. triostegus from Atatürk Dam Lake, the SSFA (36.58% for females and 38.02% for males) was found to be higher than the Σ MUFA and Σ PUFA in July; however, the Σ PUFA (35.57% for female and 37.69% for male) level was high in January (Kaçar et al., 2016). Naturally, levels of Σ PUFA in fish muscle are mostly dependent on dietary precursors and environmental temperatures (Aras, 2003; Guler et al., 2017; Sargent, 1997). Variations in fatty acid profiles especially in PUFAs might be related to the changes in the nutritional habits of the fish and the temperature of their habitats. For example in some studies on cultured fish, the ω 3 PUFA was generally lower than that of wild fish possibly due to the lack of components originating from phytoplankton and aquatic organisms in cultured diets (Guler et al., 2017). It is well known that fish tend to accumulate PUFAs in cold waters and SFAs in warm waters (Kaçar et al., 2016). The results and fish samples of the present study were obtained in July (the hottest month of south Anatolia) and it is both normal and possible for the fish to have low PUFAs, and high MU-FAs and SFAs levels.

CONCLUSION

The results of this informative study show that 18 species of freshwater fish are good sources of Σ SFA and Σ MUFA, specifically C14:0, C16:0, C18:0, C16:1w7 and C18:1w9. However, fish (mainly consumed in summer when there is no fishing ban and water levels are low enough to be fished) were observed to be poor in terms of $\omega 6$ and $\omega 3$, particularly C20:4 $\omega 6$, C20:5 $\omega 3$ and C22:6 $\omega 3$. Consumption of those fish in the summer will now no longer offer a good deal of gain as regards <code>SPUFA</code>. Nevertheless, *C. carpio* (Tigris River) and A. mossulensis (Khabur River) had relatively high **SPUFA** levels compared to other fish due to their partly higher C18:2w6 and C18:3w6 levels. Among all the fish, C. regium and A. mossulensis from the Khabur River were discovered to be good sources of protein, and M. mastacembelus, C. luteus, C. carpio and C. trutta from the Tigris River may be recommended for consumption as a lean fish category. C. umbla and B. lacerta from the Ambar Stream were high in cholesterol, while C. carpio, C. luteus and S. triostegus from the Tigris River were low in cholesterol. As a result, the data may be useful to the food and fisheries industries and they may guide studies regarding the nutritional quality, physiology and biochemistry of fish.

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Ethical Approval: Animal care and experiments were carried out in accordance with national and /or international guidelines.

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Research Article

Sea Cucumber Trade and Sustainability in Türkiye: Progress, Problems, and Opportunities

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ABSTRACT

Türkiye is rich in sea cucumber stocks and therefore offers ample opportunities for becoming the leading exporter in the world. This study aims to explore sea cucumber export trends in Türkiye for the first time using annual series data. Export-related information has been compiled from official data records and interviews with the managers of exporting companies. The data set includes the sea cucumber export process in Türkiye, export destinations, temporal shifts in export, and export tendency according to Harmonized System codes. Türkiye is in a competitive and advantageous position in the global market. This study has revealed that the export of sea cucumber products from Türkiye has performed well and seems to have grown well over the years, mainly when exporting to high-demand countries. Investing in the sea cucumber sector will also pay off through long-run economic growth and development. COVID-19, high commodity prices and inflation, and the Russia-Ukraine conflict have been identified as problems in sea cucumber exports. To reach long-term export targets, Türkiye should develop trading policies toward export performance and sustainable stock management. Maintaining exports in high-value-added markets and increasing financial support for exporters have been determined as the fundamental elements of these policies.

Keywords: Beche-de-mer, Marine policy, Marketing, Sea cucumber, Türkiye

INTRODUCTION

The expansion and growth of the fishery sector is a crucial part of economic development for countries. International trade of fishery products is an essential source of foreign exchange earnings for countries, while providing income and employment to millions of families (FAO, 2020). The fishery sector is one of the mainstays of Türkiye's exports because it is a locomotive force of industrial and national economic growth (OECD, 2021). The source of this force is that Türkiye is a country with a coastline of 8,333 km and waters of the Exclusive Economic Zone (EEZ) covering an area of 462,000 km². Among the European Union (EU-28) countries, Türkiye is among the top three leaders in fisheries production, allowing it to compete with other countries in international markets (FAO, 2020).

After the World Trade Organization (WTO) agreements, Türkiye quickly adapted to Hazard Analysis and Critical Control Point (HACCP) standards and EU norms to maintain the quality of seafood exports and reach the desired levels in international trade. Lastly, strategies to increase the export of fishery products in the National Eleventh Development Plan (2019-2023) contributed to this development. In fact, with the rapidly developing fishing industry in Türkiye, a record export of 1.4 billion US\$ was realized in 2021, and this amount constitutes 40% of Türkiye's total animal and animal product exports (TIM, 2022).

Beche-de-mer has high commercial importance because of its rich nutrient content (Aydın, Sevgili, Tufan, Emre, & Köse 2011), and they are a tradition worldwide and generally a luxury

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food item (Fabinyi, 2012). Although the prices of processed dried sea cucumbers at sales points in China vary according to species and body size, the average market price can reach 385 US\$ per kg for Holothuria lessoni (Purcell, 2014). Sea cucumbers are gathered and traded in more than seventy countries worldwide (Purcell, Samyn, & Conand, 2012; FAO, 2023). Three million people worldwide are employed in the sea cucumber sector, and it is the main source of income for small-scale fishers (Purcell et al., 2013; Rahman et al., 2020). Harvesting of sea cucumbers is done mainly to meet seafood demand in the Far East and Asian countries (FAO, 2023). The main import markets are traditionally Hong Kong, mainland China, Singapore, and Taiwan; recently, demand has also increased in the Asian diaspora and other Southeast Asian countries (Purcell, 2010). The demand for and value of sea cucumber products in the international market have risen globally in recent years (Rahman et al., 2020).

Interest in sea cucumber fisheries in Türkiye started in 1996. Since 2011, there has been a significant increase in Türkiye's sea cucumber production, and it is still an essential source of income for local communities. Artisanal fishers, processors, intermediaries, or local traders along the coastline in the Aegean are heavily dependent on sea cucumber fishery. Commercial sea cucumber species in Türkiye are not consumed domestically; all the harvested sea cucumbers in Türkiye are exported to international seafood markets (Aydin, 2017; Dereli, & Aydın, 2021). Depending on the buyer's request, products can be shipped fresh, frozen, salted, cooked, dried, as cooked calcium-removed products, or as a combination of these (Aydın, 2017). Measures to regulate sea cucumber fishing (e.g., limitations, regulations, licensing) are the responsibility of the Ministry of Agriculture and Forestry, and the regional exporters' associations report data on trade in sea cucumber.

The international market price of sea cucumbers has increased in recent years, and producers have shown more interest in the products. Poorly managed fisheries in many countries have contributed to increased fishing pressure (Cánovas-Molina, García-Charton, & García-Frapolli, 2021). Many countries have begun developing the fisheries and aquaculture of sea cucumbers to enhance the livelihoods of coastal societies (Buonfiglio, & Lovatelli, 2023). Evaluation and good management of the countries' sea cucumber trade are necessary for their management policies. The status and significance of the worldwide beche-demer trade have been extensively reported in Food and Agriculture Organization (FAO) reports (Lovatelli et al., 2004; Toral-Granda, Lovatelli, & Vasconcellos, 2008; Purcell, 2010; Purcell et al., 2012) and by several researchers (Purcell, 2014; Mangubhai et al., 2016; Vidal-Hernández et al., 2019; Louw, & Bűrgener, 2020; Wirawati, Jasmadi, Pratiwi, Widyastuti, & Ibrahim, 2021). While sea cucumber exporters in Türkiye submit their activity reports to the Exporters' Associations, a member of the FAO, the data on the sea cucumber trade has never been compiled, analyzed, or used to manage the sea cucumber trade.

This study aims to reveal the structure of Türkiye's sea cucumber trade, perform a competitiveness analysis, and put forward policy proposals for the sector. The study evaluates any tendencies that may aid the regulation of sea cucumber fishery and trade in Türkiye. In addition, competitiveness with RCA analysis was used methodologically for the sea cucumber trade for the first time. The study also helps us understand the contribution and future potential of Turkish sea cucumber exports to the international market, where the demand for seafood is constantly increasing with the growing population. The outputs will contribute to policymakers, international seafood reports, and further studies.

MATERIALS AND METHODS

Data were obtained in two stages. First, Türkiye's sea cucumber export statistics and details were compiled from the official records obtained from the Aegean Exporters' Associations and the Ministry of Agriculture and Forestry. Second, interviews (face-toface or online) were conducted with the managers of four exporting companies (hereinafter referred to as traders) of sea cucumber in Türkiye in 2021. Afterward, these data formed the basis for the competitiveness analysis, and Balassa's Revealed Competitive Advantage (*RCA*) calculation and analysis method was used (Balassa, 1965). Finally, a multi-method study was carried out in which qualitative and economic models were used together.

Türkiye's official trade records from 1996 to 2021 were included in the data set without undergoing any statistical analysis before the procedure. Since Türkiye has no sea cucumber consumption habit (Aydın, 2008), the supply is not formed. As a result, all the collected product is exported to foreign countries. Therefore, the dataset does not include import data. Yearly FAO statistics for catches or trades (both export and import) are the primary source of knowledge but are occasionally challenging to use as they rely on the declaration of the countries. Production data (in kg) has been available since 1996, and export values have only been recorded since 2014 in Türkiye. In the raw data, the export values for 2014 and 2015 are unreliable. The data are only approximate (the beginning of the transition to the national data recording system); therefore, only export figures after 2016 were included in the data set. This study focuses on the following information from the data set: the change in sea cucumber trade, current situation, market industry, trade network, and pricing. While the findings related to the data set based on national statistics are given in the Results section, the Discussion section was synthesized in line with the interviews with the local traders. The representativeness of the data and the sample size were considered to ensure the quality of the data analysis. While 16 companies that export sea cucumbers and are members of the Aegean Exporters' Association created the list of potential participants, the interviews were held with four companies that make up 70% of the total exports in order to increase the representative power of our data. To ensure the validity and reliability of the interview results, the data were interpreted by avoiding common biases in their communication.

Türkiye was included in the General Agreement on Tariffs and Trade (GATT) in 1951 and has been a member of the WTO since 1995. The data set was extracted and coded according to the coding list based on the Trade Data Categorized by Harmonized System Codes (HS), which is developed by the WTO to enable a systematic description of detailed analyses. The codes are internationally harmonized according to specific tariffs and statistical requirements that countries can decide to apply unilaterally. Türkiye uses a 12-digit HS code to classify products for export, with the first six digits being the HS code (No 31706 dated 31 December 2021 in Official Gazette) (Table 1).

Table 1.		stem (HS) codes used to cumbers in Türkiye.
HS codes [†]	Descriptio	ns
030811 0090	00 Aquatic inv live, fresh,	vertebrates; sea cucumbers; or chilled
030812 0000	00 Aquatic inv frozen	vertebrates; sea cucumbers;
030819 0000)() '	vertebrates; sea cucumbers; ed or in brine, or smoked
030819 9000	00 Aquatic inv others	vertebrates; sea cucumbers;
+ D .		

⁺ Bold parts represent harmonized six-digit codes by the World Trade Organization

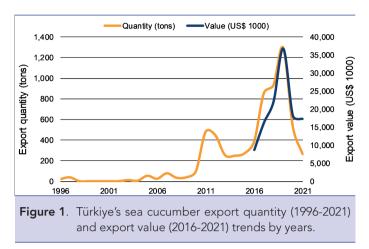
Calculating competitiveness was based on the Balassa Index (Balassa, 1965), also popularly known as Revealed Comparative Advantage (*RCA*).

$RCA = (X_{ij}/X_{it}) / (X_{wj}/X_{wt})$

 X_{ij} is the sea cucumber exports of Türkiye, X_{it} total exports of Türkiye (all products), X_{wj} total sea cucumber exports worldwide, and X_{wt} total worldwide exports (all products). The *RCA* index of less than one can be assessed as a disadvantage. An *RCA* value greater than 1 is a higher advantage for a country exporting a particular category of commodities. The coefficient of variability (*CV*) was calculated to indicate the volatility of the competitiveness values over the years. If this coefficient is below the value of 15, it can be said that the course of the scores related to competitiveness is stable (Daly, 2008). The "*In*" values of the *RCA* coefficients were translated to make the competitiveness values symmetrical. Sea cucumber export quantities (ton) were presented up to two decimal places, while export values (US\$ 1,000) were rounded to an integer throughout the paper.

RESULTS AND DISCUSSION

Sea cucumber fisheries in Türkiye started in 1996 with the export of *Stichopus regalis* with 19.87 tons of export, which was caught as a by-catch in shrimp trawl. Although sea cucumber exports showed a slight increase in the following 10 years after 2000, the data were erratic and volatile. Sea cucumber production in Türkiye increased from 97.18 tons to over 384.65 tons per year between 2010 and 2016. The sea cucumber harvests between 2017 and 2019 provided tremendous export value to Türkiye. Total sea cucumber exports, which were 8,779,000 US\$ in 2016, increased by more than 300% and reached 36,720,000 US\$ in 2019. The export volume, which increased rapidly during the previous period, declined sharply from 2019 to 18,154,000 US\$ in 2020 and then to 17,346,000 US\$ in 2021 (Figure 1).



Between 2016 and 2021, 83.5% of sea cucumber exports were to China (both China mainland and Hong Kong), with a total export value of 99,852,000 US\$. The USA was in second place, receiving 12,973,000 US\$ in exports, and Vietnam, Malaysia, and Canada followed it (Figure 2). China has always been the leading supplier of sea cucumber imports from Türkiye at all times. Whereas the value of exports to China rose by 118.9% in 2019, exports to the USA and Canada decreased (-65.8% and -69.4%, respectively). In the same year, exports to Vietnam stopped entirely, and there is still no export. Malaysia entered the list of countries to which sea cucumbers are exported in 2020, at 474,000 thousand US\$, and commercial relations continue (Figure 3). While the sea cucumber trade was carried out with 16 countries between 2016 and 2021 (Figure 4), exports stopped to most countries except China, America, Malaysia, and Canada in 2021. In 2021, China imported approximately 90% of Türkiye's total sea cucumber production.

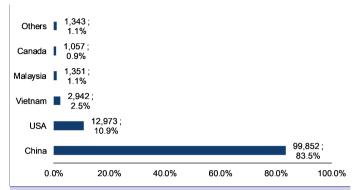
Frozen products accounted for 81.5% (3,474 tons) of total sea cucumber exported from Türkiye from 2016 to 2021 (Table 2). The increase in unit price was particularly evident for China (72 US\$ per kg) and USA (70 US\$ per kg) exports. The average price of the product exported to Malaysia was 17-25 US\$ per kg due to the export of frozen products, which is cheaper than other processed products (Figure 5). The percentage share revealed that frozen sea cucumber was the major exporting commodity during the years, but in 2021, its share fell from 79.2% to 34.8%. The same condition was true for Vietnam between 2016-2018; almost all exported products (99.2%) were frozen products. Prices of sea cucumber in 2021 increased slightly to 66 US\$ per kg from 20-35 US\$ in previous years because the countries had turned to dried, salted, brined, or smoked products, which have the highest unit price (Figure 5).

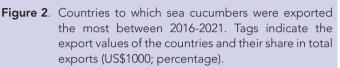
The historical development of export

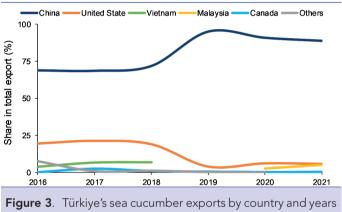
Orientation in sea cucumber fisheries in Türkiye began in 1996 with the fishing of *S. regalis*, a bycatch species of a shrimp beam trawl (Aydın, 2008). Fishers only became aware of sea cucumber fisheries and exports in the 2000s. Consequently, *Holothuria mammata* and *H. tubulosa* became Türkiye's two primary commercial products (Aydın, 2017). Their production and trade were first regulated legally in 2007 (Dereli & Aydın, 2021), so data from previous years were utterly absent or partially invalid. After

Table 2.		sea cucum s between			ding to
HS Codes	030811009000	030812000000	03081900000	030819900000	Total
2016 Quantity (tons) Value (US\$ 1000)	0.42 7	306.75 5,011	-	77.48 3,761	384.65 8,779
2017 Quantity (tons) Value (US\$ 1000)	30.68 36	694.24 10,693	129.78 5,564	0.28 13	854.97 16,306
2018 Quantity (tons) Value (US\$	-	791.74	142.24	-	933.98
1000) 2019 Quantity (tons)	-	15,322 1172.86	6,890 124.10	-	22,212 1296.97
Value (US\$ 1000) 2020 Quantity	-	29,331	7,389	-	36,720
(tons) Value (US\$ 1000) 2021	-	416.57 10,626	109.31 7,528	-	525.88 18,154
Quantity (tons) Value (US\$ 1000)	-	91.86 2,282	171.81 15,064	-	263.67 17,346
Total (2016- 2021) Quantity (tons)	31.10	3474.03	677.25	77.76	4260.13
Value (US\$ 1000) Unit price (US\$ per kg)	43 1.4	73,264 21.1	42,435 62.7	3,775 48.5	119,517 28.1
(030811009000: li dried, salted or ir					00000:

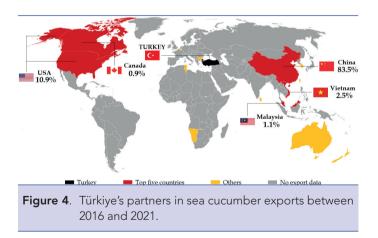
2011, there was a significant increase in Türkiye's sea cucumber production. Aydın (2017) attributes this rise to the increase in global demand and the beginning of the commercial harvest of *H. poli* in Türkiye. After 2017, local fishers and traders could economically benefit very significantly from their products, and production started to increase (Figure 1). The production



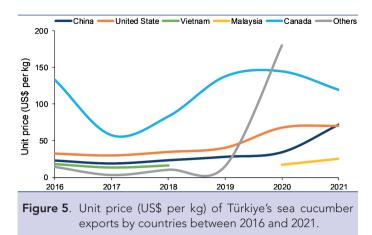




(2016-2021).



quantities indicated here (from 1996 to 2016) represent the amount of processed sea cucumber exported by local traders based on the Ministry of Agriculture and Forestry database. Traders and previous research (Aydın, 2008; Dereli, Çulha, Çulha, Özalp, & Tekinay, 2016; Aydın, 2017) confirm the accuracy of these data. Based on the data of Turkish Exporters' Associations, the monetary equivalent of exports is only possible after 2016, and these data are shown in national (e.g., Turkish Statistical



Institute, Exporters' Associations) and international (e.g., FAO, WTO) statistical reports.

Current situation in exports

Since 2016, the export of sea cucumber in Türkiye has increased exponentially, making Türkiye a voice in the international market. Export figures (between 2016 and 2019) show that Türkiye is the fourth biggest sea cucumber exporter worldwide. Traders reported that the higher volumes of exported sea cucumber products result from national policies implemented to expand export volumes and maintain sea cucumber fisheries. In the same period, traders' investments in processing facilities gained momentum, and government support (e.g., investment and business loans, fuel support without excise tax) increased. Local traders expanded their investments by benefiting from the financial support of the regional development agencies and the Small and Medium Enterprises Development Organization of Türkiye. The processing facilities have been integrated with HACCP and EU standards with the investments made. The confidence in the processed sea cucumber exported from Türkiye has increased in the international market. Local traders have participated in food fairs in the international market to promote their products. There is no doubt that scientific studies on production and management activities (Aydın, 2008; González-Wangüemert, Aydın, & Conand, 2014; Aydın, & Erkan, 2015; Tolon, Emiroğlu, Günay, & Saygı, 2015; Dereli et al., 2016; Aydın, 2017; Aydın, 2019; Dereli, & Aydın, 2021) have also contributed to this development. These studies have helped traders to understand natural resources and their condition better and have guided policymakers. The traders said that "scientific studies and the support of national authorities have made a significant contribution to both production and the sustainable management of sea cucumber stocks in Türkiye."

With time, fishers and traders have realized the income potential of sea cucumber fishing and begun overfishing. Uncontrolled growth and increased fishing activities brought new responsibilities. In 2020, the Ministry of Agriculture and Forestry issued a memorandum containing a series of legal measures to prevent illegal fishing and possible reduction in stocks (No 2019/39 dated 27 September 2019 in the Official Gazette). In the memorandum, provisions were made to determine a quota for sea cucumber fishing, operating fishing and the Total Allowable Catch (TAC) and transporting and assessing the produce from fishing carried

out within the TAC. With the memorandum, the TAC amount was determined to be 2,500 tons of gutted weight, and only fishing boats holding a License for Sea Cucumber could benefit from this TAC. This regulation explains the sharp decline in export figures in 2020 and 2021 (Figure 1). Although the stocks did not collapse, signals about overfishing were considered in fishing regions. It has been emphasized that most sea cucumber stocks suffer from overfishing and collapse (Conand, 1990, Rogers, Hamel, Baker, & Mercier, 2018). Overfishing in recent years has led to the extinction of high-value species in some areas and the closure of many national fisheries (Purcell, 2010). Bruckner (2005) stated that guota enforcement is an appropriate regulatory method when stock levels are in good standing but less appropriate when stocks are depleted. No risks have yet been reported for sea cucumber stocks on the coasts of Türkiye, and these regulations are purely a result of sustainable resource management efforts. This management strategy is the first policy to protect sea cucumber stocks in Mediterranean countries. Traders welcome these practices and say, "We recognize that sustainable sea cucumber management largely depends on these regulations, and we want this management policy to continue."

Sea cucumber market and industry

The questionnaires revealed there are two leading players: (1) fishers (hookah divers) and (2) local traders who are also processors and exporters. Exporter agents that buy, sell, and process sea cucumbers are collectively referred to as local traders. The Aegean Sea is one of the central regions for sea cucumber products and is a strategic location for beche-de-mer exporters in Türkiye. Most of the exporters (11 out of 17) are based in Izmir (the Aegean region was close to legal fishing areas). In contrast, fewer exporters are based far from the fishing area, considering that there are two companies each in Balıkesir and Istanbul, and one each in Çanakkale and Samsun. In line with the circular, 327 boats were legally allowed to harvest sea cucumbers in 2020. Exporters purchase harvested products such as gutted sea cucumber directly from local fishers. Exporters sell to buyers directly, but they do not deal through wholesale markets or auctions, or do not use agents. Local traders heavily finance fishers and this support gives them confidence for sustainable production and export. Four species of sea cucumber (H. tubulosa, H. mammata, H. poli, and lastly, H. sanctori) are harvested legally in Türkiye and have significant value in international markets. All the species are listed as Least Concern (LC) according to the IUCN Red List of Threatened Species (IUCN, 2022). Harvested products are processed and marketed as fresh, frozen, dried, and a small amount decalcified. Exports shifted significantly to dried products in 2021.

Trade network and export pricing

The export network and price analyses in Türkiye mainly focus on the Asian markets (Hong Kong and China mainland). Traders have traded sea cucumbers with 16 countries so far. Traders who took their place in international fairs found a place in the market due to their promotional activities, but the trade relations were not permanent. Traders define *trade relations* as temporary outside of China, America, Malaysia, and Canada. While Türkiye exports primarily to mainland China and Hong Kong, it also maintains its trade network with other countries in smaller volumes, because they report that the main factors that increase the unit price and profit margin of the product are product quality and pricing, not the trade network. Traders stated that "Our commercial relations with China are well developed. The ever-increasing demand and high unit price result in high profits for our companies. We aim to be permanent in the Chinese market in the future."

Pricing can be the most challenging due to different market forces and pricing structures worldwide. As discussed in this chapter, the key elements include assessing foreign market objectives, market demand, product-related costs, and competition. The price of a sea cucumber varies according to its moisture content, physical damage, body size, and species. It is especially preferred in a dried form in the market (Rahman, & Yusoff, 2017). Dried products are traded at a higher price than other marketing methods (fresh or frozen). The dried product corresponds to the HS code 030819000000 in the data set. The Asian market previously demanded frozen sea cucumbers; this demand shifted to thoroughly dried products in 2021 (~87% of total export value). Traders said that "the transition from frozen product to the dried product was not our own sales policy, it was processed and produced according to the demand of the Asian market." Traders hope to export their products dried in the coming years. Because the dried product brought almost twice as much income as the frozen ones, this shift was positively reflected in the unit price (Figure 5) and HS code-based export figures (Table 2). Exporters who also benefited from currency fluctuations against the US dollar in 2021 with the increase in the product's unit price increased their revenues considerably. The hopeful thing is that the very rapid response in supply to sudden changes in demanded products reflects the success of traders in exports and processing. The degree of dryness and product guality of commercial sea cucumbers vary from species to species, and therefore the price of the product is variable. Sea cucumbers harvested and processed in Türkiye are largely considered to be of medium quality in Asian countries (according to food quality analysis reports of traders), which results in estimated losses of approximately 80% for the final dried product. Relatively low-quality products and fluctuations in product quality cause differences in market price between 5-20% (Ram, Chand, & Southgate, 2014). Thus, to receive a higher income from Asian markets, improving product quality will be a key issue for local exporters. Labor, packaging, storage, and marketing costs are considered for sea cucumber exporters. Freight costs vary by supplier and destination. Variable tariffs and exchange rates contribute to the constant fluctuation of especially freight operations. Sea shipment is the most typical transportation form for sea cucumber exports in Türkiye, although some countries, such as Canada, transport via airways. Sea cucumbers exported to Canada were priced well above the average market price each year (2016 to 2021). This increase in unit price was attributed to the fact that all shipments to Canada were conducted by airways. Trade contracts are for one year or longer and cover one fishing season in Türkiye. Buyers are typically offered higher discounts on the regular supply provided by a longer-term contract (the six months throughout the fishing season). Exporters report that there is a slight variation in the duration and structure of trading contracts, and this

change does not affect price trends too much. Food safety is one of the essential non-tariff obstacles to international trade, and it plays an essential role in exports. Türkiye has complied well with food safety standards such as HACCP and EU norms, and its products have been accepted in international trade. Traders reported that their production facilities are produced according to these standards and are regularly inspected by ministry officials. Traders stated, "As the sea cucumber started to be produced following food safety standards, our export figures increased, and our products became more accepted in the international market."

According to the Balassa Index analysis results (the five-year *RCA* average is 7.12, > 1), Türkiye has an advantage in global sea cucumber exports (Table 3). The five-year *RCA* trend and coefficient of variation (CV = 0.32, <15) refer to the stability of competitiveness (Figure 6). It means that Türkiye has an excellent opportunity and competitiveness in exporting sea cucumber, and the ongoing trade relations between partners and Türkiye prove this harmony.

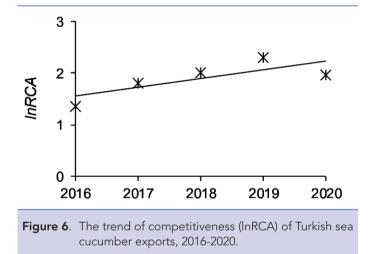
Problems, opportunities, and future policy recommendations

The coronavirus disease (COVID-19) caused tremendous pressure on global trade, causing exceptional worries to supply chains and trade collaboration (WTO, 2021). The lockdown of markets, borders, and exports limited fishing and cultivation activities in companies and countries (UNCTAD, 2022). The impact of the pandemic on economic activity in Türkiye unfolded later than in other countries but was sharp (OECD, 2021). The initial economic impacts of COVID-19 were the deep recession and higher inflation. Globally, commodity prices have pushed the cost of essential inputs higher (e.g., energy, oil prices, food). Prices have been accelerating faster in Türkiye, experiencing higher inflation before the pandemic due to exchange-rate pressures (UNCTAD, 2021). The Turkish Lira (TL) depreciated to record lows, and inflation grew (the TL peaked at 26.00 against the US dollar, and inflation reached 69.97% annually in April 2022). Traders reported that their exports were significantly affected in this period and that mainly rising costs contributed to this effect. The COVID-19 effect and high inflation are now factors weighing on world trade. The Russia-Ukraine conflict that started in February 2022 put global trade recovery, which was already fragile, at risk. The first economic effect of the conflict has been an intense increase in commodity prices in Türkiye as well as around the world. The war threatened the supply of essential goods, including food, energy, and supplies. The war is expected to disrupt Türkiye's energy, transportation, and agricultural trade (FAO, 2022). Economic integration makes Türkiye more dependent on overseas trade networks and more exposed to successive risks and threats. Undoubtedly, as in all international trade, it is thought that the export of sea cucumber will also experience its share of this turmoil. Traders are uneasy about the possible effects of the conflict, and they say that they do not have any countermeasures. They expect to have sales prices at a high level and keep their profitability at a stable level, if not at the maximum.

The international market price for beche-de-mer has increased in recent years, contributing to increased fishing pressure on many natural stocks (Purcell et al., 2018). Cultivating sea cucumbers in aquaculture systems can reduce the exploitation Table 3.

3. Analysis of Türkiye's rivalry in the sea cucumber market yearly, according to the Revealed Comparative Advantage (RCA) index.

Years	Sea cucumber exports (US\$ 1000)		Total expo	Total exports (US\$ 1000)		CV	Rivalry
	Türkiye	Global	Türkiye	Global			power
2016	8,779	142,606,247	252,740	15,926,982,653	3.88		\uparrow
2017	16,306	156,992,940	297,186	17,568,188,372	6.14	0.00	\uparrow
2018	22,212	167,923,862	340,364	19,332,363,800	7.51	0.32 Stable	\uparrow
2019	36,720	180,870,841	381,210	18,763,129,315	9.99	Stable	\uparrow
2020	18,154	169,657,940	264,724	17,499,013,461	7.07		\uparrow
Five-year total	102,171	818,051,830	1,536,224	89,089,677,601	7.24		\uparrow



of wild stocks while providing benefits for meeting increasing demand and maintaining the sustainability of natural stocks. Sea cucumber cultivation is not yet an active industry in Türkiye. However, there are several successful laboratory-scale attempts to culture Mediterranean species such as H. tubulosa (Tolon et al., 2015; Han, Keesing, & Liu, 2016; Rakaj et al., 2018). If these attempts are successful, H. tubulosa culture can be carried out as a profitable business in Türkiye. Nevertheless, to be successful, the technical aspects of nutrition, rearing, and reproduction must be clarified before sea cucumber can be developed as a farming activity. An attempt was made within the scope of an EU project for sea cucumber cultivation with the cooperation of FAO, the Minister of Agriculture and Forestry of the Republic of Türkiye, and national traders. However, the results were not sufficient for sea cucumber cultivation in Türkiye. The government should support more scientific studies of sea cucumber cultivation and might subsidize the establishment of sea cucumber farms. In addition, government and fishery departments could encourage inward foreign direct investment and develop policies to sustain the growth of the sea cucumber industry. Transforming sea cucumbers into highvalue-added products for use in the cosmetics and pharmaceutical industries should become a national policy in Türkiye.

Finally, regular and systematic recording of production as well as export data is essential for managing market networks and pricing in the sea cucumber trade in the future. Although Türkiye started to record regular export data in 2014, only after 2016 were data considered reliable. Economic analysts and policymakers need to guide their sea cucumber policies using a combination of production data and export figures. This study improves Türkiye's sea cucumber trade, relying heavily on export data.

While the results give some intriguing insights into Türkiye's sea cucumber export industry, it is also valuable to consider their broader implications for international trade, particularly in poor countries. A successful increase in sea cucumber exports can help improve a country's trade balance, especially if it has a competitive advantage in the fishery sector. Successfully penetrating international fish markets can pave the way for broader access to global markets, and open doors to explore export opportunities for other agricultural or food products. Perhaps one of the key issues is that successfully exporting fish products can contribute to building a positive brand image and reputation for a country or company. This positive perception can extend to other sectors, enhancing the country's overall competitiveness and attractiveness for international trade. Consequently, it is important to note that the applicability of results to broader international trade depends on various factors such as market dynamics, sector-specific considerations, and geopolitical factors.

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