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Aquatic Sciences and Engineering is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of İstanbul University Faculty of Aquatic Sciences and it is published quarterly on January, April, July, and October. The publication language of the journal is English and continues publication since 1987.

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.

The scope of the journal includes but not limited to; aquaculture science, aquaculture diseases, feeds, and genetics, ecological interactions, sustainable systems, fisheries development, fisheries science, fishery hydrography, aquatic ecosystem, fisheries management, fishery biology, wild fisheries, ocean fisheries, biology, taxonomy, stock identification, functional morphology freshwater, brackish and marine environment, marine biology, water conservation and sustainability, inland waters protection and management, seafood technology and safety.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of aquatic sciences.

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Contents

Research Articles

Review Article

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Research Article

Water Quality Assessment of Derinçay Stream (Çorum) by Biotic Indices

Süleyman İpek[1], Faruk Maraşlıoğlu[2]

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ABSTRACT

In this study, counting and identification were performed on epiphytic diatom samples taken from 6 sampling stations located on the Derinçay Stream (Çorum). In the study, 42 taxa belonging to Bacillariophyta were identified, and the dominant taxon of the stream was *Nitschia palea.* According to some biotic indices such as the Trophic Diatom Index (TDI) and Saprobic Index (SI), it was determined that the Derinçay Stream has a hypertrophic trophic structure corresponding to the "bad" ecological status with an average TDI value of 79.8, and according to the SI, the upper stations (St1, St2) were organically slightly polluted corresponding to β-mesosaprobic, while the other stations were determined to be heavily polluted corresponding to α-mesosaprobic. When looking at the similarities of the stations, the stations with the highest similarity values were the 4th and 6th stations, while the similarity of the 1st and 6th stations was low. When Shannon diversity index (H') averages are examined, the stream is classified as "low diversity" and "heavily polluted". According to the Pielou evenness index (J'), the community balance at the epiphytic flora stations was better in other months, except February 2021. According to Margalef's species richness index (d), species richness decreases upstream from downstream. When the data in the study area are evaluated, it was seen that the stream is under the threat of pollution, and the SI results represented the ecological structure of the stream better than the TDI results.

Keywords: Diatom, Stream, Biotic Index, Water Quality

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INTRODUCTION

Despite the positive effects of developing technology and industrialisation that make human life easier, their negative effects on the environment cannot be ignored (Sun et al., 2019). Changes in consumption habits due to technological developments, waste of natural resources, rapid population growth, irregular urbanisation due to irregular migration and damage caused by industrial establishments lead to the deterioration of the ecological balance and cause irreparable consequences (Karagözoğlu, 2020). Pollutants from point or nonpoint sources originating from agricultural, urban, and/or industrial activities, particularly in lower river basins, negatively impact water quality and alter the structure of algal communities in aquatic ecosystems. Spatial and temporal variations in algal communities are excellent indicators of environmental changes, as algae offer comprehensive and descriptive insights into water quality by immediately reflecting physicochemical parameters (Taş et al., 2019). Water resources are classified into physicochemical and biological classes. Therefore, biological approaches must be used to support chemical assessments (Çiçek & Ertan, 2015). The number of studies on the biological classification of water quality has increased with the publication of the EU Water Framework Directive (WFD, 2000/60/EC), and various studies abroad have suggested that the trophic status of updated diatoms can be used as an indicator of water quality (Eassa et al., 2015; Liu et al., 2020). In parallel with studies in Europe, monitoring of the ecological status of determining water quality biologically has also increased over time in our country (Kalyoncu et al., 2009; Gürbüz & Kıvrak, 2002; Solak, 2011; Maraşlıoğlu et al., 2020; Sevindik et al., 2023).

In recent years, after the establishment of relationships between diatoms and environmental factors, the number of studies on determining river water quality using diatoms has significantly increased (Richards et al., 2020; Masouras et al., 2021; Dalu et al., 2022). Many diatom indices have been developed in various countries worldwide, especially in Europe, to assess the water quality and pollution status of rivers. Some of these indices include the following: CEE (CEC): Descy and Coste Diatom Index (Descy & Coste, 1991); GDI: Generic Diatom Index (Coste & Ayphassorrho, 1991); SHE: Steinberg and Schiefele Index (Steinberg & Schiefele, 1988); and TDI: Trophic Diatom Index (Kelly & Whitton, 1995; Kelly, 1998).

The first study on the biological assessment of river water quality in Turkey were conducted by Girgin and Kazancı (1994). In this study, Girgin and Kazancı (1994) evaluated the water quality of the Ankara Stream not only based on physicochemical parameters but also using biological indices of benthic macroinvertebrates. The use of diatom indices for assessing water quality in rivers in our country has been recently reported, and the number of studies is quite limited. Barlas et al. (2001) assessed the water quality of Sarıçay and Akçapınar Stream using epilithic diatoms according to the Sládeček Index (SLA) index. Gürbüz and Kıvrak (2002) evaluated the water quality of the Karasu River based on epilithic diatoms using the Generic Index (GI), Trophic Diatom Index (TDI), Sládeček Index (SLA), and Diatom Association Index for Organic Pollution (DAIpo). The water quality of streams and rivers around Isparta (Isparta, Aksu, Darören) was assessed based on physicochemical data and diatom indices, including the Swiss Diatom Index (DI-CH), Trophic Index (TI), and Saprobic Index (SI) (Kalyoncu & Barlas, 1997; Kalyoncu et al., 2009). The water quality of streams and rivers in western Turkey was evaluated based on physicochemical data and diatom indices using Omnidia software (Solak, 2011).

The aim of this study was to determine the water quality of the Derinçay Stream both in terms of physicochemical and biological aspects, identify pollution sources along the river and their impacts on the system, evaluate the stream water, and obtain some findings that could be useful in taking measures to maintain balance in the system. For this purpose, the relationship between the benthic diatom community and the water quality of the Derinçay stream was examined, and the stream water quality was assessed using the Saprobic Index (SI) and the trophic diatom index (TDI). Additionally, the relationship between the diatom indices and the physicochemical properties of stream water was investigated.

MATERIAL AND METODS

Study sites and sampling points

The stations were selected so that they could both provide access to the stream and offer representative samples of water quality along the stream length. For this purpose, six stations were selected before and after evaluating various businesses and facilities on Derinçay Stream (Fig. 1). The stream was classified into three sections: upstream, stations from St1 to St2, midstream, stations from St3 to St4, and downstream, stations from St5 to St6. The first (St1) and second (St2) stations are connected to an agricultural area, while St2 is also associated with industrial and livestock activities. The third station (St3), which is within the vicinity of intensive industrial settlements and urban areas, was selected from the stream midstream reaches. It should be mentioned that the third station (St3) was affected mainly by farming, large factories, and industrial zones. Most of the waste from these facilities is discharged into the stream without any treatment during the study period. The Urban Wastewater Treatment Plant of Çorum located in Karacaköy fed the fourth sampling point (St4) with wastewater. The fifth station (St5) was chosen near the Sarılık village and is located after the discharge point of the Çorum Sugar Factory. Finally, the St6 sampling site, which had no specific industrial activity, was at a point after the Alaca Creek flows into the Derinçay Stream.

Samplings and analyses

Samples of equal amounts each time were collected monthly from the stems and leaves of *Cladophora glomerata* at stations 1, 2, and 5, *Phragmites australis* at stations 3 and 4, and *Typha latifolia* at station 6 to examine epiphytic algae in the Derinçay Stream. Phytobenthos sampling was conducted using standard methods. Because there were not many stone samples found at the river stations during phytobenthos sampling, the epiphytic algae community distributed on the plant surfaces varied according to the stations. The collected plant samples were washed with distilled water and scraped to collect the organisms living on them into containers. After being brought to the laboratory, the samples were transferred to measuring cups and sedimentation was performed. The sedimented parts of the samples were

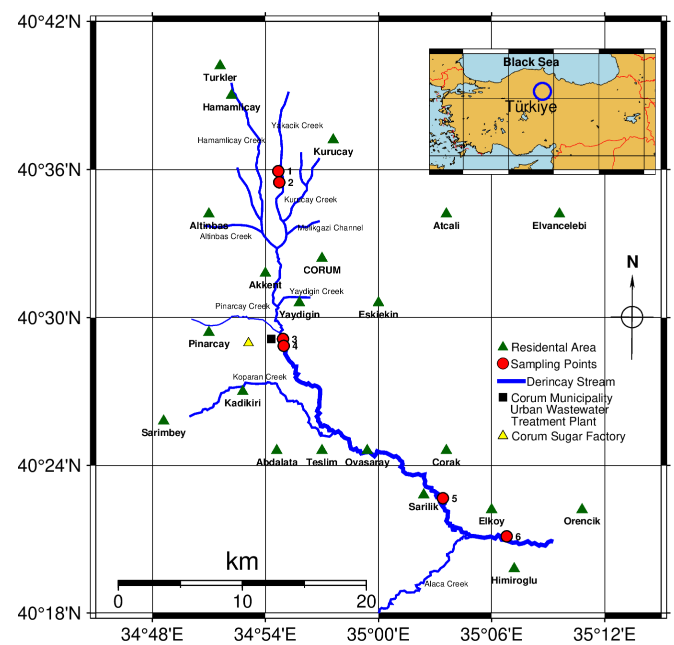

Figure 1. Water quality monitoring stations in the Derinçay Stream (Wessel et al,. 2019).

transferred to centrifuge tubes, and successive treatments with HCl, centrifugation, treatment with H_2O_2 in a hot water bath, and centrifugation were performed (Swift, 1967). Thus, diatom species with only siliceous cell walls (valves) were recovered from organic matter, and permanent preparations were made with "Entellan" mounting medium (Hasle, 1978). Preparations were examined using an Olympus BX51 research microscope at 400x magnification. In each examined preparation, 400 diatom valves (frustules) were counted, and taxa were identified. Krammer and Lange-Bertalot (1991a, 1991b, 1999a, 1999b), Round et al. (1990), and Hartley et al. (1996) were used to diagnose diatoms.

The temperature, pH, electrical conductivity, and dissolved oxygen concentration of the stream water were measured at the time of sampling using a portable YSI Proplus multiparameter measurement device. The measurements of biochemical oxygen demand (BOD5), chemical oxygen demand (COD), orthophosphate (PO_4-P), total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH_4-N), and nitrate nitrogen (NO_3-N) concentrations were conducted through service procurement at Çorum Food Control Laboratories according to the standard methods recommended by APHA (2005).

Trophic and Organic Pollution Indices

The first version of the TDI was empirically derived from graphs summarising the percentage of occurrence against dissolved phosphorus concentrations for 86 taxa (genera and key indicator species: Kelly & Whitton, 1995). This value ranged from 1 (low nutrient concentration) to 5 (very high nutrient concentration). However, the research staff later expressed a clear preference for an index that produces integer values over a wider numerical range. Therefore, the TDI was modified to extend from 0 (low nutrient concentrations) to 100 (very high nutrient concentrations) (Table 1). These calculations were performed using the following equations.

$$TDI = (WMS \times 25) - 25$$

TDI stands for Trophic Diatom Index, and WMS is calculated as weighted wean sensitivity.

$$WMS = \Sigma\, ai \times si \times vi \,/\, \Sigma\, ai \times vi$$

where ai represents the abundance of species *i* in the sample, *si* represents the sensitivity of species *i* to pollution (1-5), and *vi* represents the indicator value (1-3).

The organic pollution level at each station was calculated using the saprobic index formula proposed by Pantle and Buck (1955). The saprobic index (SI) was calculated using the following formula: the sum of saprobic values for all identified indicator species divided by the sum of frequency values for all indicator species.

$$S = \Sigma\, si \times hi \,/\, \Sigma\, ai$$

S is the saprobic index of the algal community; *si* represents the ecological indicator value of freshwater diatoms (Van Dam et al. (1994) for each species; *hi* represents the frequency value of species *i* (three-scored; rare = 1, common = 3, abundant = 5), *and ai represents the abundance* of species *i*.

Diversity and Evenness Indices

Diversity, evenness, and species richness indices are commonly used to determine species diversity and distribution in aquatic ecosystems. For each month at the specified stations in Derinçay, species counts and individual counts of each species were determined. Using the obtained data, the Shannon diversity index (H'), Pielou evenness index (J'), and Margalef species richness index (d) were calculated using the Primer software package programme (Clarke & Ainsworth, 1993). Additionally, to describe a community in terms of diversity and similarity and to compare it with another community, it is necessary to count the species and individuals belonging to the community one by one in the community. For this purpose, the degree of similarity among the sampling stations was calculated using the Sorensen similarity index (Kocataş, 1994).

RESULTS AND DISCUSSION

Physical and chemical variables

The water quality parameters affecting the periphyton community, including temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), ammonium (NH_4^+), nitrate (NO_3^-), orthophosphate (o-PO_4), total nitrogen (TN), and total phosphate (TP), are presented in Table 3. The values of some physical variables (temperature, pH) were at a good quality standard for periphyton growth, but the other parameters had values that exceeded the quality standard at certain seasons. Levels of oxygenation and nutrient parameters in stations that exceed the quality standard are attributed to agricultural activities, domestic waste and industrial waste around the location. However, in the Brantas River (Indonesia), ammonia and total organic matter exceeded accepted levels, but other water quality parameters were within surface water quality standards (Arsad et al., 2021).Water quality parameters showed significant spatial variations, increasing at St4 and St5 and then slowly decreasing with the contribution of Alaca Creek at St6 (Table 3). Regarding water quality classifica-

Table 1. TDI-Trophic Diatom Index scale (Kelly & Whitton, 1995).

Index Range (0–100)	Water Quality Class	Ecological Status	Trophic Status
< 35	I	Very Good	Oligotrophic
35 - 50	II	Good	Oligo- Mesotrophic
50 - 60	III	Moderate	Mesotrophic
60 - 75	IV	Poor/Low	Eutrophic
> 75	V	Bad	Hypertrophic

tion, the physical variables (temperature, pH, EC) in the stream showed no significant spatial variations, whereas there were notable temporal and spatial variations in DO, BOD, COD, NH_4^+, NO_3^-, TN, TP, and o-PO_4 environmental variables. Among the in situ measurements, water temperature showed similar seasonal patterns along the stream length, except upstream, with a slightly prolonged winter cold period. During the study conducted in Derinçay Stream, the lowest water temperature was measured at 3.7°C at the 1st station in November 2020, while the highest temperature was recorded at 23.4°C at the 4th station in August 2020. It was observed that the annual variation in water temperature in the Derinçay Stream follows seasonal averages. According to the Surface Water Quality Regulation (2021), Derinçay Stream has Class I water quality in terms of average water temperature. The average pH values measured at the Derinçay stream stations were 8.22, 7.97, 8.02, 8.03, 8.06, and 8.11, respectively, indicating that the river has slightly alkaline properties. It is known that some diatoms such as *Fragilaria*, *Nitzschia*, and *Cyclotella* prefer slightly alkaline waters (Reynolds, 1993), and these genera have been frequently encountered in our study area as well. Additionally, according to the Surface Water Quality Regulation (2021), Derinçay Stream has Class I water quality in terms of average pH values. The opposite relationship was observed for EC, which decreased upstream (1145 μS/cm) and increased midstream (1427 μS/cm). The conductivity of water is directly related to its salinity, density, and ion concentration (Cirik & Cirik, 2005). In the study area, the average electrical conductivity value was measured as 1277 μS/cm. This indicates that the water quality is low, and Derinçay Stream has Class III water quality in terms of average conductivity according to Surface Water Quality Regulation (SWQR, 2021). The dissolved oxygen value is crucial for aquatic organisms (Çıtakoğlu & Özeren, 2021). DO showed increasing values both upstream and seasonally in cold months, with a maximum value of 6–8 mg/L, while the lowest values were recorded in autumn months. According to the classification of dissolved oxygen values by SWQR (2021), St1 had Class II water quality, while the other stations in the Derinçay Stream had Class III water quality. The biological oxygen demand (BOD) is considered a measure of organic pollution in water environments (Atay & Pulatsü, 2000). The BOD and COD values were reduced at St1 and St2, which corresponded to the upstream (6 mg/L and 16.1 mg/L, respectively) and reached their highest values at St4, which corresponded to the after wastewater treatment (110 mg/L and 117 mg/L, respectively). According to SWQR (2021), in terms of BOD values, the 1st station has Class I, the 3rd station has Class II, and the other stations (St2, St4, St5, St6) have Class III water quality. The chemical oxygen demand (COD) had the lowest value at the 1st station (8.91 mg/L) and the highest value at the 4th station (117 mg/L. According to the SWQR (2021), stations 1, 2, and 3 have Class I water quality, and stations 4, 5, and 6 have Class III water quality in terms of COD. Nutrients (TP, TN, NO_3^- and NH_4^+) reached their highest concentrations at St5 and lowest concentrations at St2. Based on SWQR (2021), the water quality of the Derinçay Stream for ammonium and nitrate is classified as Class I at St1 and St2, Class II at St3 and St4, and Class III at St5 and St6. In terms of total nitrogen (TN) values, St1 and St2 have

Table 2. Saprobic index (Pantle & Buck, 1955).

Index Range (1–4)	Water Quality Class	Ecological Status	Sabrobity
1 - 1.5	I, I-II	Clean	Oligosaprobity
1.5 - 2.5	II	Moderately polluted	β-mesosaprobity
2.5 - 3.5	III-IV	Heavily polluted	α-mesosaprobity
3.5 - 4	IV	Excessively polluted	Polysaprobity

Table 3. Mean values of physicochemical parameters according to station.

Parameters	St1	St2	St3	St4	St5	St6
General parameters						
Temperature (ºC)	11,6	11,7	14,1	17,3	14,7	13,9
pH	8,22	7,97	8,02	8,03	8,06	8,11
EC (μS/cm)	1120	1169	1449	1404	1356	1085
Oxygenation parameters						
DO (mg/L)	6,0	5,3	3,3	1,9	3,4	3,7
BOD (mg/L)	1	11	6	110	32	21
COD (mg/L)	8,9	23,3	15,6	117	55,7	54
Nutrient Parameters						
NH_4^+ (mg/L)	0,1	0,2	0,63	0,69	16,4	9,95
NO_3^- (mg/L)	2,6	1,6	4,2	7,3	21,4	13,6
TN (mg/L)	2,8	1,9	4,9	12,6	17,2	9,85
TP (mg/L)	0,1	0,4	2,8	3,5	3,9	2,4
o-PO_4 (mg/L)	0,2	1,25	8,7	10,5	11,3	7,2

Class I water quality, St3 and St6 have Class II, and St4 and St5 have Class III water quality according to SWQR (2021). Regarding total phosphorus (TP) values, St1 had Class II water quality, while the other stations had Class III water quality according to the SWQR (2021). The orthophosphate concentration started to increase at St4 and peaked at St5 (11.3 mg/L). In the following section of the stream, the trend is decreasing. A similar classification TP was observed for the orthophosphate values, where the stream was classified as Class III for all stations, indicating poor water quality.

Distribution of Epiphytic Diatoms

The dominant epiphyton species in the Derinçay Stream were observed to be the same in the middle and lower sections of the stream. The relatively less polluted St1 was characterised by *Fragilaria tenera* and the partially polluted St2 was characterised by *Ulnaria ulna*. On the other hand, the highly polluted midstream and downstream sites (St3, St4, St5 and St6) were characterised by *Nitzschia palea*. Bere and Tundisi (2011) reported a similar result for the Monjolinho River. However, a study on the temporal and spatial distribution of epiphytes in the Diyala River identified some epiphytic species, including *Gomphonema parvulum*, *Ulnaria acus*, *Cyclotella meneghiniana*, *Navicula gregaria*, and *Nitzschia amphibia*, as bioindicator species of pollution (Hassan et al., 2023). The subdominant species at the stations were as follows: *Navicula phyllepta* and *Ulnaria ulna* at St1, *Rhoicosphenia abbreviata*, *Navicula cryptotenella*, and *Diatoma moniliformis* at station 2, Navicula veneta and Navicula lanceolata at station 3, *Navicula veneta* and *Rhoicosphenia abbreviata* at St4, *Nitzschia littoralis* at St5, and *Craticula cuspidata* at St6. In the entire study area, *Nitzschia palea* and *Fragilaria tenera* were the dominant species, while *Navicula veneta* and *Ulnaria ulna* were the subdominant species. Dominant and subdominant taxa comprised 53% of the epiphytic community in the study area.

Diatome indices

According to the Trophic Diatom Index calculated using epiphytic diatoms identified in Derinçay, the lowest average TDI value was 55.3 at St1. The highest average TDI value was determined to be 93.7 at St6. When we examined the monthly TDI values of the six sampling stations; at St1, the highest TDI value was determined to be 81.6 in October 2020, and the lowest was 31.5 in February 2021. The annual TDI at St1 was 55.3. At St2, the highest and lowest values were 91.7 and 66.7, respectively, indicating generally eutrophic water quality. The annual TDI at St2 was 72.3. The annual TDI at St3 was calculated as 81.3, with the highest monthly TDI value of 87.5 in October 2020 and the lowest TDI value of 65.6 in July 2021. The TDI at St4 was determined to be 86.7. The highest TDI value was 97.5 in November 2020, and the lowest TDI value was 58.3 in October 2020. The TDI value at St5 was determined to be 89.7, with the highest monthly TDI value of 98.6 in July 2021 and the lowest value of 75 in June 2021. St6 had the highest annual TDI of 93.7, with the highest monthly TDI of 100 in April 2021 and the lowest of 37.5 in December 2020 (Table 4).

Low TDI values indicate low nutrient levels, while high index values indicate eutrophic conditions in the water (Kelly, 1998). Accordingly, Derinçay Stream has an average TDI value of 79.8, indicating a hypertrophic trophic structure corresponding to the "poor" water quality class. According to the TDI results determined based on the epiphytic flora in phytobenthos, except for station 1 (moderate water quality), the water quality conditions at other stations are "poor" or "bad". Solak (2011) stated in a study conducted in the Upper Basin of the Porsuk River that, similar to Derinçay, areas close to the source were of Class II (oligo-mesotrophic) water quality, while the region near the Kütahya exit was of Class V (hypertrophic) water quality based on TDI values. When we examined the TDI results of the Derinçay Stream seasonally, although there was partial improvement in water quality in the winter season (weak ecological structure), we generally observed that the water quality is "poor" in all four seasons. In February 2021, there was a sudden increase in the number of organisms of *Fragilaria tenera* species with low pollution sensitivity (S: 2) at station 1, contributing to the partial improvement in water quality during the winter season. In the spring, which is the season when water quality is worst, increases in the numbers of species with high pollution sensitivity (S: 4/5), such as *Nitzschia palea*, *Navicula lanceolata*, and *N. veneta*, have contributed to the decrease in water quality. Despite the low number of organisms recorded in phytobenthos, especially in the summer and spring months, the fact that the existing species have high pollution sensitivity indicates that the ecological structure of the area is in very poor condition. The situation where TDI values calculated based on the low number of diatoms in the phytobenthos flo-

Table 4. Seasonal variation of the Trophic Diatom Index by sampling station.

TDI	Aug 20	Sep 20	Oct 20	Nov 20	Dec 20	Jan 21	Feb 21	Mar 21	Apr 21	May 21	Jun 21	Jul 21	Mean
St1	*	79,3	81,6	61,5	54,9	45,8	31,5	69,1	59,2	*	*	*	55,3
St2	*	*	*	85,7	66,7	91,7	70,8	69,1	75	*	*	*	72,3
St3	70	68,8	87,5	75	70,9	75,7	73,8	82,2	84,9	83,6	84,5	65,6	81,3
St4	91,7	93,8	58,3	97,5	90	85	78,3	92,9	92,5	81,7	77,8	91,7	86,7
St5	87,5	87,5	81	93,8	90	90	89	83,5	94,5	86,5	75	98,6	89,7
St6	65,8	81,3	58,5	71,5	37,5	88,5	98	88,8	100	83,5	75	80	93,7

*Water shortage

ra do not fully reflect the actual water quality level and trophic structure of the environment was also reported by Temizel (2022) in a study on benthic algae in the Harşit Stream.

According to the Saprobic Index (SI), stations 1 and 2 represented Class II water quality (slightly polluted), while stations 3, 4, 5, and 6 represented Class III-IV water quality (heavily polluted). When the SI results are analysed seasonally, it was observed that at St1, except for August, samples taken during the autumn, winter, and spring seasons were characterised by β-mesosaprobic (moderately polluted) conditions. In contrast, α-mesosaprobic (heavily polluted) conditions mostly characterized the samples taken during the winter and spring seasons at St2. At St3 (in the middle part of the stream, except for August and September, α-mesosaprobic (heavily polluted) conditions were dominant in all months. At St4, although there were fluctuations in the saprobic index values on a monthly basis, rises and falls were observed in September, December, and July (polysaprobic), while decreases were observed in October and June (β-mesosaprobic). However, the overall dominant condition was α-mesosaprobic (heavily polluted), as seen in most stations. A similar situation was observed at St5, where polysaprobic (extremely polluted) conditions prevailed in August, September, December, April, and July, while α-mesosaprobic (heavily polluted) conditions dominated the other months of the year. At St6, fluctuations in the sabrobic index values on a monthly basis were also observed, with increases in September and April (polysaprobic) and decreases in August, October, and March (β-mesosaprobic). However, the general dominant condition at this station, as with most stations, was α-mesosaprobic (heavily polluted) (Table 5).

It was observed that the SI results calculated using epiphytic diatoms at the stations of the Derinçay Stream better represented the ecological structure of the stream than the TDI results. However, in a study conducted by Kalyoncu et al. (2009) to determine the water quality of Aksu Stream based on biological indices, it was reported that the SI index better reflects pollution than the TDI index. Solak (2011) reported that water quality categories determined based on diatom indices vary between stations, which could be due to differences in diatom communities used in each index. Although the water quality classes determined based on physicochemical analysis corresponded to the diatom indices at some stations, deviations were observed at other stations. The water quality grade determined by the Saprobic Index showed a half-step positive deviation from the water quality classes determined by Klee (1991) at all stations. Within the scope of the Trophic Diatom Index, the water quality classes at stations 1, 2, 3, and 4 perfectly matched the water quality steps determined by Klee (1991), whereas stations 5 and 6 showed a half-step negative deviation. It has been noted by various researchers (Kalyoncu et al., 2009; Gómez & Licursi, 2001) that discrepancies can occur between water quality steps determined by physicochemical analysis and those determined by biological analysis.

Statistical analysis

Similarities between epiphytic algae

According to the similarity analysis results of epiphytic algae at the sampling stations of Derinçay Stream, the highest value (0.75) was observed between stations 4 and 6, whereas the lowest value (0.31) was observed between stations 1 and 6. It was found that high values in the similarity analysis of epiphytic algae at the sampling stations do not reflect the reality very well, but there are significant differences in organism numbers and species diversity between stations where similarity is reflected as low (Table 6).

Diversity and evenness of epiphytic algae

Diversity index measurements can be used to indicate the status of the ecological system and habitat characteristics. Diversity depends on the ecological roles of competition, predation, and succession (Valiente-Banuet et al., 2015). Diversity is defined as the number of different individuals in an ecosystem relative to the total number of all individuals in the ecosystem (Herawati et al., 2019). Changes in the diversity index reflect the effects of environmental changes on aquatic ecosystems (Ye et al., 2017).

Throughout the study period, the station with the richest species diversity according to the Shannon diversity index was St2, which was obtained in March 2021 with a coefficient of 1.042 bits.org^{-1}. The lowest index value was measured at St4 in November 2020 (0.2606 bits.org-^{1}). The highest value at St1 was 1.029 in March 2021, and the lowest was 0.3486 in February 2021. At St2, the highest value was 1.042 in March 2021, and the lowest was 0.4581 in December 2020. The highest value was 1.009 at St3 in January 2021, and the lowest was 0.301 in October 2020. At St4, the high-

Table 5. Seasonal variation in saprobic index values among sampling stations.

SI	Aug 20	Sep 20	Oct 20	Nov 20	Dec 20	Jan 21	Feb 21	Mar 21	Apr 21	May 21	Jun 21	Jul 21	Mean
St1	*	2,8	2,4	2,4	2,3	2,3	2,3	2,2	2,2	*	*	*	2,4
St2	*	*	*	2,9	3,3	3,2	2,5	2,2	3,2	*	*	*	2,3
St3	2,3	2,3	3,5	3,5	2,6	2,9	2,7	3	3,1	3,1	3,4	2,7	2,7
St4	3	3,7	2,5	3,3	3,7	3,3	2,6	3	3,4	3,1	2,5	3,7	2,9
St5	3,7	3,7	2,7	3,3	3,7	3,3	3,1	2,8	4	3	2,8	3,8	2,8
St6	2,3	3,7	2,3	3	3,3	2,9	3,3	2,5	4	2,8	3	3,5	2,96

*Water shortage

Table 6. Similarity values of the stations in the Derinçay Stream determined from epiphytic diatoms.

	St1	St2	St3	St4	St5	St6
St1	1	0,68	0,65	0,36	0,35	**0,31**
St2		1	0,6	0,48	0,41	0,45
St3			1	0,63	0,66	0,57
St4				1	0,64	**0,75**
St5					1	0,72
St6						1

est value was 0.9319 in February 2021, and the lowest was 0.2606 in November 2020. The highest value at St5 was 0.8278 in March 2021, and the lowest was 0.2975 in July 2021. At St6, the highest value was 0.7952 in January 2021, and the lowest was 0.2764 in April and July 2021 (Fig. 2). Based on the data obtained regarding epiphyton in Derinçay Stream, it can be concluded that the conditions of the stream water are classified as heavily polluted (low diversity). This classification is supported by data on the diversity of epiphytic diatoms, which is classified as being at values < 1. According to previous research conducted by references (Wilhm, 1975; Soeprobowati et al., 2021), the value of epiphyton diversity can be grouped into three criteria: H' < 1 indicates heavily polluted water quality, 1 ≥ H' ≤ 3 indicates moderately polluted water quality, and H' > 3, clean water quality. However, Arsad et al. (2021) reported that the diversity index value obtained from the Brantas River in Indonesia is included in the moderate diversity group, which indicates that it exhibits less stable ecological pressure or an unhealthy (moderately polluted) aquatic environment. It was also pointed out by Hassan et al. (2023) that the Shannon-Weaver index values of epiphytic algae on *Phragmites* and *Ceratophyllum* plants were <4 and thus no serious pollution was recorded in the Diyala River. Considering the composition of epiphytic diatom communities and other indices, it was observed that these index results did not reflect the water quality and pollution levels of the Derinçay Stream stations very well.

According to changes in the Pielou evenness index, the highest value of 1 was observed at St3 in August 2020, October 2020, November 2020, and July 2021, at St4 in August 2020, October 2020, June 2021, and July 2021, and at St6 in August 2020, September 2020, October 2020, and December 2020. The lowest value of 0.2895 was recorded at St1 in February 2021. At St1, the highest value was 0.8201 in March 2021, and the lowest was 0.2895 in February 2021. At St2, the highest value was 0.9602 in December 2020, and the lowest was 0.8458 in November 2020. At St3, the highest value was 1 in August 2020, October 2020, November 2020, and July 2021, and the lowest was 0.4477 in June 2021. At St4, the highest value was 1 in August 2020, October 2020, June 2021, and July 2021, and the lowest was 0.5463 in November 2020. At St5, the highest value was 0.9796 in March 2021, and the lowest was 0.4941 in July 2021. At St6, the highest value was 1 in August 2020, September 2020, October 2020, and December 2020, and the lowest was 0.5247 in February 2021 (Fig. 3). Hassan and Shaawiat (2015) reported that an evenness index value of the Evenness index was <0.5, it represents the homogenisation of species. Besides, the evenness index of epiphytic algae in the Diyala River was found to be <0.5, indicating environmental stress in this river (Hassan et al., 2023).However, the study results of the Pielou evenness index (J') in the Derinçay stream ranged between 0.5 and 1 unit, indicating that the community balance in the epiphytic flora was good in most months.

According to the Margalef species richness index (d), the highest species richness value of 3.8 was observed in November 2020 at station 1 with 19 taxa, while the lowest value of 0.8 was recorded in November 2020 at St4 and in May 2021 at St5 with 2 taxa. At St1, the highest value was 3.815 in November 2020, and the lowest was 2.338 in December 2020. At St2, the highest value was 3.474 in March 2021, and the lowest was 1.243 in December 2020. At St3, the highest value was 3.391 bp in February 2021, and the lowest was 1.443 bp in October 2020. At St4, the highest value was 3.622 in February 2021, and the lowest was 0.8341 in November 2020. At St5, the highest value was 3.753 in March 2021, and the lowest was 0.8247 in May 2021. At St6, the highest value was 2.791 in August 2020, and the lowest was 0.9102 in April and July 2021 (Fig. 4).When

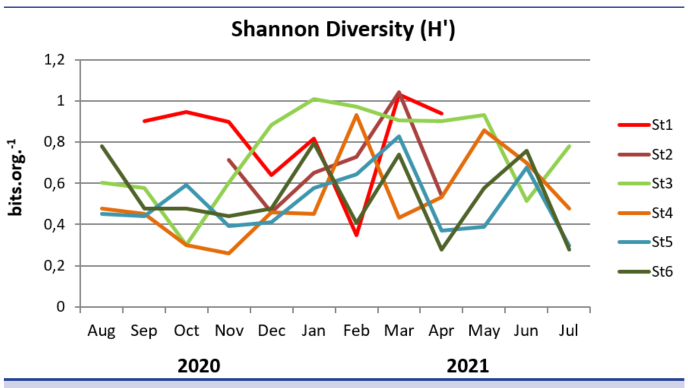

Figure 2. Shannon diversity index of epiphytic algae in the Derinçay stream.

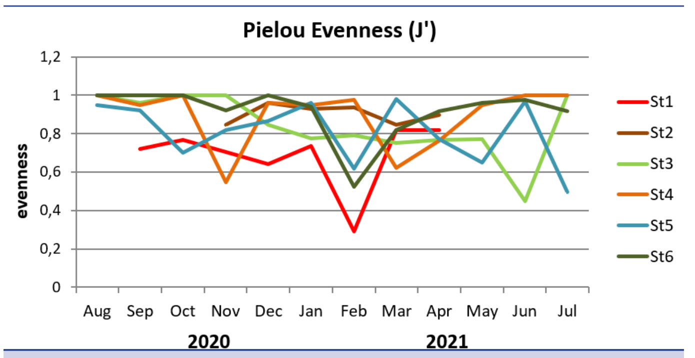

Figure 3. Pielou evenness of epiphytic algae in the Derinçay stream.

we examined the Margalef species richness seasonally, except for the 1st station, it was observed that autumn, spring, and summer had low species richness, while winter had high species richness. When evaluated on station level, the richest station in terms of species was the 1st station, and the lowest was the 5th station. The different values of the richness index revealed the variety of sites according to pollution impact. There was a significant difference between the upstream site and other sites due to the impact of pollution. Similar results were found in the Diyala River (Hassan et al., 2023) and Monjolinho River (Bere & Tundisi, 2011).

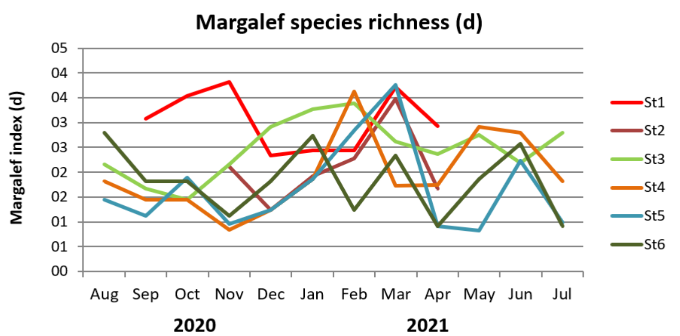

Figure 4. Margalef species richness of epiphytic algae in the Derinçay stream.

CONCLUSION

In this study, the distribution of epiphytic diatom species in the Derinçay stream and the physicochemical properties of water that influence this distribution were determined. It was found that most epiphytic algae commonly found in the Derinçay Stream comprise pollution-tolerant and facultative species. To assess the water quality of the Derinçay Stream, the Saprobic Index (SI), in which epiphytic species are classified, has been successful in reflecting organic pollution in certain stations in our study area, but deviations of the half-step positive direction were observed from the determined water quality classes. Another water quality assessment metric used in this study is the Trophic Diatom Index (TDI), calculated based on epiphytic diatoms in phytobenthos. While TDI has been successful in terms of overall results, it has been observed that it does not reflect the ecological structure of an area very well if there are not many organisms recorded in the area. In conclusion, despite these partial disadvantages, the phytobenthos-based SI and TDI, among the biological indices used to assess the water quality of Derinçay Stream, have provided good results in reflecting the current ecological structure of the stream, similar to physicochemical analyses and interpretations based on indicator diatom species.

Conflict of Interest: The authors declare that they have no competing interests.

Ethics Committee Approval: Ethics approval was not required for this study.

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Research Article

Frequency of Antibiotic-Resistant Bacteria isolated from the Kınalıada Coastal Areas of the Sea of Marmara, Türkiye

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ABSTRACT

In the process of global climate change, the negative effects of anthropogenic activities on microbial interactions have become more visible in coastal areas. Because island coastal ecosystems are fragile ecosystems that are open to dynamic environmental variables, it is important to determine bacteriological signals in these regions. The frequency of bacterial antibiotic resistance in aquatic ecosystems is a micro-marker of human activity. The frequency of antibiotic-resistant bacteria was investigated in surface water samples collected from the coastal areas of Kınalıada Island in the Sea of Marmara between 2018 and 2019. The bacteria isolated from the sea water were screened against: spectinomycin (SC300), nitrofurantoin (F50), Rifampicin (Rd2), tetracycline (TE30), ampicillin (AMP10), and oxytetracycline (OT30) using the disk diffusion technique. The frequencies of antibiotic-resistant faecal coliform, total coliform, intestinal enterococcus, and heterotrophic aerobic bacteria were evaluated according to the Clinical Laboratory Standard Institute (CLSI). The antibiotics to which all bacterial isolates showed the highest resistance were tetracycline and oxytetracycline (98.7% The frequency of resistant heterotrophic aerobic bacteria was recorded at 100% against all tested antibiotics. All bacterial isolates showed resistance to more than three antibiotic derivatives, and the Multiple Antibiotic Resistance (MAR) index was determined to be in the range of 0.67–1. The findings of this study provide regional evidence of the influence of anthropogenic pollution on the spread of antibiotic resistance. The detection of high levels of antibiotic-resistant bacteria indicated that the coastal areas of Kınalıada are at potential risk for the global spread of resistant bacteria, human health, and ecosystem function.

Keywords: The Sea of Marmara, Kınalıada Island, bio-indicator bacteria, antibiotic resistant bacteria

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INTRODUCTION

The marine environment harbours bacteria that play an important role in the decomposition of organic matter in the ecosystem, as well as pathogenic bacteria that enter the marine environment via human activities. Antibiotic pollution caused by anthropogenic activities domestic and industrial wastewaters, and livestock farming, cause the spread of bacterial antibiotic resistance in aquatic areas. Among these pollutants, antibiotic derivatives regional antibiotic resistance in aquatic areas serves as a reservoir for the spread of global bacterial resistance. The occurrence of antibiotic-resistant bacteria in aquatic areas has been documented to be associated with anthropogenic activities (Yang et al., 2013; Terzi and Isler 2019). Furthermore, altered bacterial metabolism under the influence of environmental stress favours the spread of antibiotic-resistant pathogens due to global climate change (MacFadden et al., 2018, Cavicchioli et al., 2019).

Consideration of the relationship between humans, animals, and the environment to prevent the global problem of antimicrobial resistance (AMR) is increasingly recognised worldwide.

Guidelines explaining this in a one-health approach have been strengthened by scientific studies on this topic (McEwen and Collignon 2018; Rüegg et al., 2018; Larsson et al., 2023). Bacterial resistivity data are important for defining the global health dimension of the regular functioning of marine ecosystems. Marine environments offer a unique laboratory for understanding how microorganisms will be affected by global climate change and increasing human activities (Cavicchioli et al., 2019). The natural environment is a genetic resource for the global distribution of antibiotic-resistant bacteria, which poses a threat to humans, animals, and certain crops in the treatment regime of infectious diseases and thus hosts the transition of susceptible microorganisms to resistance (Larsson et al., 2023). The presence of bacteria in the marine environment is not stable and varies according to the conditions. Bacteria can grow in different environments, such as seawater, sediment, and the biota, according to their life requirements. Both planktonic and attached bacteria may change their number in seawater due to the effects of human activities. In marine environments, bacteria can develop different resistance mechanisms and adaptations according to the pollutants they are exposed to. Heterotrophic bacteria convert organic matter into forms that other organisms can use to generate energy and metabolic processes. Intensive human activities in coastal areas create a favourable environment for bacterial growth. The presence of indicator bacteria in marine coastal areas varies depending on the success of anthropogenic pollution control.

However, higher numbers of indicator bacteria were reported in coastal areas. (Fernandes Cardoso de Oliveira 2010; Cicin-Sain et al.2011).

The spread of antibiotic-resistant bacteria in natural environments leads to the ineffective use of antibiotics for the treatment of infectious diseases. The increase in global antibiotic resistance triggers a vicious cycle of continuous development of new antibiotic derivatives. Therefore, it is important to determine the frequency of bacterial antibiotic resistance characterised by coastal areas, especially considering that aquatic areas are reservoirs for the global distribution of antibiotic resistance. The Sea of Marmara is under the effects of various environmental pressures and is one of the most important waterways in the world. Ship ballast water is one such pressure, and high antibiotic resistance has been reported in bacteria isolated from the ballast tanks of ships entering the Marmara Sea from different geographical areas (Altuğ et al., 2012). The frequency of antibiotic-resistant bacteria in the Sea of Marmara, particularly in the coastal area of Istanbul Province, has been reported in different studies. The data obtained indicate the presence of antibiotic-resistant bacteria in the Turkish Strait System, including the Istanbul Strait (Bosphorus) and Çanakkale Strait (Dardanelles), which connect the Marmara Sea to the Black Sea and Mediterranean (Altuğ and Balkıs 2009; Sivri and Akbulut 2016; Çardak et al., 2016; Kimiran et al 2007).

Island ecosystems have a more sensitive position as specialised ecosystems in marine environments. In a previous study conducted on seawater samples taken from the coastal areas of Kınalıada, the first bacteriological data on indicator bacteria, which provide clues to the presence of pathogenic bacteria, were reported above national and international standards (Karaman-Baş and Altuğ, 2022). Several daily island ferry movements from Istanbul increase the population density of the region during the summer months. This situation adds to the fragile structure of the region.

In this study, the frequency of antibiotic-resistant faecal coliforms, total coliforms, intestinal enterococci, and heterotrophic aerobic bacteria against spectinomycin, nitrofurantoin, rifampicin, tetracycline, ampicillin, and oxytetracycline was investigated in surface water samples taken from Kınalıada coastal areas to investigate land-based pollution effects on the island ecosystem in the period between June 2018 and May 2019.

MATERIAL AND METHODS

Study area

The group of islands in the province of Istanbul consists of nine separate islands called the Prince Islands. Büyükada, Heybeliada, Burgazada, and Kınalıada are inhabited, and these islands host intensive touristic activities in the summer months. Kınalıada Island, among the Prince Islands hosted by the Sea of Marmara, is the closest island to Istanbul. The coastal area of Kınalıada, which is located in the Sea of Marmara, hosts domestic and industrial activities as well as intensive touristic use for swimming and boat tourism in spring and summer. Kınalıada is located 7 miles south of Istanbul.

Sampling

Seawater samples were collected under aseptic conditions in brown sterile glass bottles that do not transmit sunlight and were delivered to the laboratory on the same day using a cold chain (APHA, 2012). Surface water (0-30 cm) sampling was carried out monthly in summer months (June 2018, July 2018, August 2018) when bacterial activity was high, and seasonally in autumn (November 2018), winter (February 2019), and spring (May 2019) at ten stations of Kınalıada Island, the Sea of Marmara. Sampling was carried out within the scope of the project titled "Investigation of Bio-Indicator Bacteria Levels in Kınalıada Coastal Area" (Karaman-Baş and Altuğ 2022).

The sampling stations in the study areas are shown in Figure 1.

The locations of the stations are listed in Table 1.

Isolation of heterotrophic aerobic bacteria (HAB) and bio-indicator bacteria

Bacterial antibiotic resistance was tested in heterotrophic aerobic bacteria and indicator bacteria (faecal coliform, total coliform and faecal and intestinal enterocci) isolated from the surface water of the coastal area of Kınalıada Island. Heterotrophic aerobic bacteria isolation were performed according to the method of Austin (1988); bioindicator bacteria analyses consisting of faecal coliform, total coliform, and intestinal enterococcus were performed according to APHA (2012), as detailed in our previous study (Karaman Baş, Altuğ 2022).

Frequency of antibiotic-resistant bacteria

In the antibiogram test Oxoid (UK) discs of spectinomycin (SC300), nitrofurantoin (F50), rifampicin (RD2), tetracycline (TE30), ampicillin (AMP10), and oxytetracycline (OT30) were used. The antibiotic derivatives and doses is summarised in Table 2.

The frequency of antibiotic-resistant bacteria was investigated using the Kirby-Bauer disk diffusion method. The selected 2-3 colonies from dishes were maintained at room temperature for 5–10 min and spread on the surface of Petri dishes containing Mueller-Hinton Agar. Discs containing different antibiotics at various concentrations were placed on the surface of the agar with sterile forceps and incubated at 25 °C for heterotrophic aerobic bacteria and at 37 °C for 24-48 hours for faecal coliform, total coliform, and intestinal enterococci. At the end of incubation, the diameters of the zones around the disks were measured millimetrically to determine whether they were resistant, sensitive, or suspicious. Isolates with full growth on the medium were recorded as resistant. Isolates that formed a clear zone around the disk were recorded as antibiotic-sensitive or susceptible. The plate observations were evaluated in accordance with the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2017).

The percentage distribution of the number of resistant isolates according to the total number of isolates was calculated, and the results are presented as "% frequency of antibiotic-resistant bacteria". In total, 150 bacterial isolates, including total coliforms, faecal coliforms, and intestinal enterocoliforms enterococci, were tested.

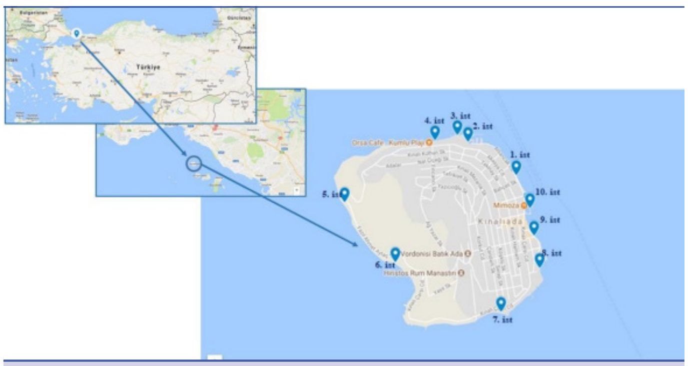

Figure 1. Study Area (Google Maps).

Multiple Antibiotic Resistance (MAR)

The resistance of the isolates to more than one antibiotic was calculated using the multiple antibiotic resistance (MAR) index using the following equation.

MAR index = a/bxc

a = total antibiotic resistance score of all isolates;

b = total number of isolates

c = number of isolates in the sample (Krumperman, 1983).

Bacterial isolates resistant to three or more antibiotic derivatives were defined as multi- antibiotic resistant (ranging from two to ten).

RESULTS AND DISCUSSION

The distribution of antibiotic resistance of bacteria isolated from seawater by faecal coliform, total coliform, intestinal enterococci, and heterotrophic aerobic bacteria, percent resistance of bacteria by antibiotic derivative, and MAR index data showing multiple antibiotic resistance are presented below. The numbers of faecal coliform, total coliform, intestinal enterococci, and heterotrophic aerobic bacteria in the strains tested against the six antibiotic derivatives are shown in Table 3.

The mean antibiotic resistance frequency of all isolates was 95.46%. Heterotrophic aerobic bacteria showed the highest antibiotic resistance (100%) followed by intestinal enterococci, total coliform, and faecal coliform bacteria.

Table 1. Names and coordinates of the stations where sea water samples were collected.

Station No	Sampling Name	Latitude-Longitude	
1	Public Beach I	*40°91′27.39″N*	*29°05′28.87″E*
2	Water Club Port Inner	*40°91′40.42″N*	*29°05′05.23″E*
3	Water Club Pier	*40°91′45.53″N*	*29°05′07.27″E*
4	Public Beach II,	*40°91′40.99″N*	*29°04′80.34″E*
5	Reference Station	*40°91′02.39″N*	*29°04′01.16″E*
6	Special Beach I:	*40°90′64.43″N*	*29°04′43.97″E*
7	26 Number Beach,	*40°90′34.52″N*	*29°05′32.91″E*
8	Special Beach II	*40°90′62.18″N*	*29°05′65.74″E*
9	Kınalıada-Police Centre	*40°90′80.91″N*	*29°05′61.45″E*
10	Marine Taxi Pier	*40°90′99.02″N*	*29°05′57.59″E*

Table 2. Antibiotic derivatives and doses used in the tests.

Antibiotic Discs (Oxoid, UK)	Antibiotic Code	Antibiotic Dose (µg)	Antibiotic Discs (Oxoid, UK)	Antibiotic Code	Antibiotic Dose (µg)
Ampicillin	AMP10	10	Nitrofurantoin	F50	50
Spectinomycin	SC300	300	Rifampicin	RD2	2
Tetracycline	TE30	30	Oxytetracycline	OT30	30

Resistance reactions of bacteria to the six tested antibiotic derivatives are shown in the Table 4.

Bacterial antibiotic resistance varied from 87.3% to 98.7%. The highest resistance was recorded against tetracycline and oxytetracycline (98.7%. The highest frequency of antibiotic-resistant faecal coliform was recorded against ampicillin and tetracycline at 100%, whereas the highest frequency of antibiotic-resistant total coliform was recorded against oxytetracycline at 100%. The highest frequency of antibiotic-resistant intestinal enterococci was recorded for oxytetracycline and spectinomycin. The frequency of antibiotic-resistant heterotrophic aerobic bacteria was 100% against all tested antibiotics.

The frequency of bacteria resistant to specific antibiotics (%) in seawater is shown in Figure 2.

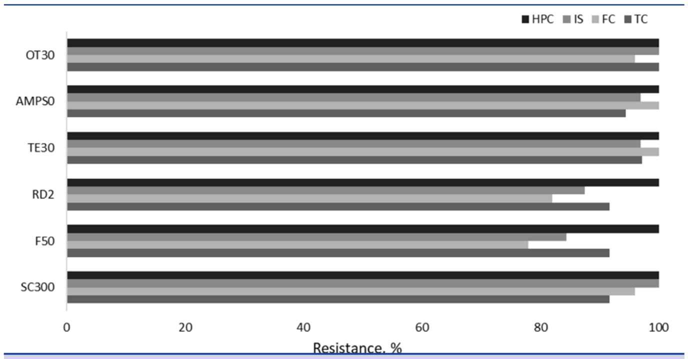

Figure 2. The frequency of bacteria resistant to certain antibiotics (%) in seawater.

95.46% of the 150 isolates were resistant to the tested antibiotics. Isolates showed resistance between 98% and 87.3%. The antibiotics to which the isolates showed the highest resistance (98.7%) were oxytetracycline and tetracycline. The lowest resistance was observed against nitrofurantoin (87.3%. Multiple antibiotic resistance indexes and resistance ratios are presented in Table 5.

The multiple antibiotic resistance (MAR index) of each antibiotic-resistant bacteria was calculated. A MAR index lower than 0.2 indicates a nonpoint source of pollution. A MAR index >0.2 indicates a specific pollution focus and high risk of antibiotic contamination (Nies,1999). In this study, all bacterial isolates exhibited resistance to more than three antibiotic derivatives, and the MAR index was determined to be in the range of minimum 0.67 to maximum 1.

Studies conducted in marine environments across different geographical regions revealed the existence of a marine antibiotic resistance reservoir. While studies conducted around fish farms pose a potential risk for aquatic food supply security, in areas used for recreational purposes, this situation has emerged as a threat affecting human health and, basically, ecosystem functions (Al-Bahry et al., 2009; Fernandes Cardoso de Oliveira et al., 2010; Di Cesare et al. 2012; Mulamattathil et. al., 2014; Altuğ et al., 2020). Antibiotic resistance is therefore one of the greatest challenges facing modern medicine worldwide (Andersson et al., 2020). In this study, the recorded frequency of resistance of bacteria isolated from seawater samples against specific antibiotics showed a high distribution in the range of 5%–50%. This high frequency of resistance detected at the local level indicates once again the importance of protecting the seas, which are reservoirs in the global distribution of antibiotic resistance, from the pressure of human activities.

Table 3. Numbers of antibiotic-resistant faecal coliform (FC), total coliform (TC), and intestinal enterococcus (IE), heterotrophic aerobic bacteria (HAB), and mean resistivity frequency (%).

Bacteria	Number of strains tested	Mean resistivity frequency %
FC	50	92.00
TC	36	94.40
IE	32	94.78
HAB	32	100
Total number of strains	150	95.46

Table 4. Resistance reactions of bacteria to the tested antibiotic derivatives.

*Antibiotics	Dose (µg)	TC (N=36)	FC (N=50)	IE (N=32)	HAB (N=32)	Resistant Bacteria (Σ)	Resistance (%)
		Numbers of Resistant Bacteria					
OT30	30	36	48	32	32	148	98.7
AMP10	10	34	50	31	32	147	98.0
TE30	30	35	50	31	32	148	98.7
RD2	2	33	41	28	32	134	89.3
F50	50	33	39	27	32	131	87.3
SC300	300	33	48	32	32	145	96.7

*OT30: oxytetracycline, AMP10: ampicillin, TE30: tetracycline, RD2: rifampicin, F50: nitrofurantoin, SC300: spectinomycin. TC: Total Coliforms, FC: Faecal Coliforms IE: Intestinal Enterococci, HPC: Heterotrophic Aerobic Bacteria

Table 5. Multiple Antibiotic Resistance Indexes and Resistance Ratios of Isolates.

Number of antibiotics	Numbers of bacteria resistant to antibiotics	MAR Index	Resistance %	p-value
1	0	0.00	0.00	0.00
2	0	0.00	0.00	0.00
3	0	0.00	0.00	0.00
4	13	0.67	8.67	0.1625
5	21	0.83	14.00	0.3935
6	116	1.00	77.33	0.4384

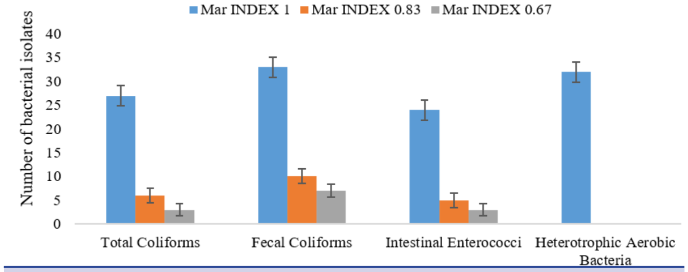

Figure 3. Multiple Antibiotic Resistance Levels of TC, FC, IE and HAB.

In several studies antibiotic resistant bacteria data were reported from various parts of the Sea of Marmara (Altuğ and Balkıs 2009; Sivri and Akbulut 2016; Çardak et al., 2016; Kimiran et al 2007). The MAR index of bacteria isolated from the Sea of Marmara was reported to be between 0.30 and 0.34, which was higher than that of the isolates obtained from the Canakkale Strait (Çardak et al., 2016). In this study, the first regional MAR value was detected to be at least two times higher than that in previous studies. This situation constitutes evidence of the anthropogenic pressure on the Kınalada coastal area of Kınalıada is exposed in terms of bacterial antibiotic resistance.

CONCLUSION

A spontaneous or induced mutations or by the transfer of resistance genes from other bacteria, acquired genes responsible for the development of resistance to antibiotics in bacteria. In the case of exposure to antibiotics, resistance genes are naturally selected because bacteria carrying them have a better chance of survival, and the space occupied by bacteria carrying them in the ecosystem increases. Aquatic areas are considered reserves in the distribution of global antibiotic resistance. Although the concept of "one health" increases awareness of antibiotic resistance by offering holistic approaches to environmental, human, and animal health, bacterial resistance continues to pose a risk for the whole world. Therefore, it is important to conduct inventory studies on antibiotic resistance in marine areas.

Although the levels of antibiotic-resistant bacteria in aquatic areas fluctuate depending on geographical antibiotic exposure, aquatic environments pose a global threat to the spread of antibiotic resistance to a greater or lesser extent. Therefore, it is important to monitor the frequencies of antimicrobial resistance in areas subjected to different anthropogenic pressures, especially coastal areas, through long-term studies.

The coastal areas of Kınalıada were under anthropogenic pressure based on detecting high levels of antibiotic-resistant bacteria. This indicates that the region is at potential risk to human and ecosystem health due to the presence of antibiotic-resistant bacteria. Considering that the global climate change process negatively affects bacterial antibiotic resistance in aquatic environments, it is important to inventory bacterial antibiotic resistance in marine environments to mitigate climate resistance. This research highlights the effects of anthropogenic impacts on marine areas on the spread of antibiotics and the development and spread of antibiotic-resistant bacteria and genes. The data obtained in this study contribute to bacterial data on regional antibiotic resistance, but monitoring studies are needed to ensure that the data are continuously updated.

The high frequency of bacterial antibiotic resistance and multiple antibiotic resistance levels detected for the first time in the coastal area of Kınalıada in this study necessitate the monitoring of the region for studies to be compared with the entire Marmara Sea. The follow-up of these basic data with long-term monitoring studies will provide the necessary data for the development of measures in this regard.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Committee Approval: The authors declare that this study did not include any experiments with human or animal subjects.

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Research Article

Seasonal Length-weight Relationship of *Puntius terio* (Hamilton, 1822) in West Bengal, India

Godhuli Sit[1], Arun Jana[1], Angsuman Chanda[1]

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ABSTRACT

The present study aims to analyse the association among length, weight, condition factor, and relative condition factor of *Puntius terio* (Hamilton,1822), with a focus on seasonal change. The results indicate that the species did not strictly follow the anticipated cube law and had allometric development in all seasons. The length-weight relationship value (b) ranged from 2.14 to 3.21; the condition factor varied between 0.62 and 2.65, and the relative condition factor ranged from 0.61 to 2.98 for *P. terio* in the study area. The average values of 'b' were highest during the monsoon, but the condition factor (K) was highest during the winter. The seasonal association between length, weight, condition factor, and relative condition factor was significant (P<0.05) according to the Post Hoc test. The current research will support the development of sustainable management strategies for *P. terio* in its habitats by fishery managers.

Keywords: Fishery, Length-Weight, *Puntius terio*, West Bengal

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INTRODUCTION

Length and weight, both at the individual and population levels, are two crucial components of species biology. This is particularly important for effectively managing and developing fish populations (Anene, 2005). For stock management and long-term stock utilisation, understanding their biology is crucial. The length-weight relationship is one of the most popular techniques for gathering reliable biological data. In addition to its primary use in converting length to weight and vice versa, the length-to-weight ratio can change within a population. These results are important for fishery biology and management.

The condition factor (CF) is an index of how abiotic and biotic components interact to affect a fish's physiological state. It depicts the health of the population at various stages of life. This relationship allows for comparisons of the fish life cycles between species and populations, as well as an estimate of the fish population's health (Kara & Bayhan, 2008). Studying conditions is necessary to understand the life cycles of fish species, which also contributes to ecological balance and sustainable species management. It also aids in determining the reproductive seasons of fish species without affecting the species, making it a useful tool in developing programmes for monitoring species-specific fisheries and culture (Arellano-Martinez & Ceballos-Vazquez, 2001). The relative condition factor examination is equally important because it reveals a fish's health and resilience. *P. terio* is a small ornamental and food fish species in Asia, specifically India, Bangladesh, Pakistan, and Mayanmar (Talwar & Jhingran, 1991 and Menon, 1999). This species has been gradually declining due to pollution, habitat destruction, and selective captive breeding of commercial fish species. In the world, no study has been conducted on any aspect of *Puntius terio*. In India, Sandhya *et al.* (2020), studied the 7 species in Charkhana, including *P. terio*, but did not observe seasonal variation between the length and weight of these species. In West Bengal, India, there are some aspects (diversity of fish, length-weight relationship, feeding behaviour, reproduction) of various small indigenous fish species that have been studied by different researchers (Sani & Gupta., 2010; Lim et al.,2013; Palaniswamy et al.,2012; Pal et al., 2013; Gupta & Tripathi, 2017; Jana et al., 2021a; Jana et al., 2021b; Jana et al., 2022a; Jana et al., 2022b; Sit et al., 2020; Chanda & Jana., 2021; Sit et al., 2022a; Sit et al., 2022b; Sahil et al., 2022; Sit et al., 2023a; Sit et al., 2023b; Jana et al., 2024a & Jana et al., 2024 b) but yet to observed length, weight, condition factors of the current studied species *Puntius terio*. Therefore, the present study aimed to determine the length, weight, condition factor, and relative condition factor and analyse the association among length, weight, condition factor, and relative condition factor of *Puntius terio* (Hamilton,1822), focusing on seasonal change. The length-weight and relative condition factors determine the studied fish's proper growth and health.

MATERIALS AND METHODOLOGY

Sampling

Specimens have been collected monthly from selected areas (eight sites) in the Paschim Medinipur district during the Summer (March-June), Monsoon (July-Oct), and Winter (Nov-Feb) seasons from March 2022 to February 2024 (Figure 1).

Length & Weight measurement: Seasonally, the total length (TL or L) is measured using a digital slide calliper with ±0.01 mm accuracy and weighted (TW or W) using a digital balance with ±0.01 g accuracy.

Length-Weight relationship: The adjusted formula of Le Cren (1951) was used:

$W=aL^b$

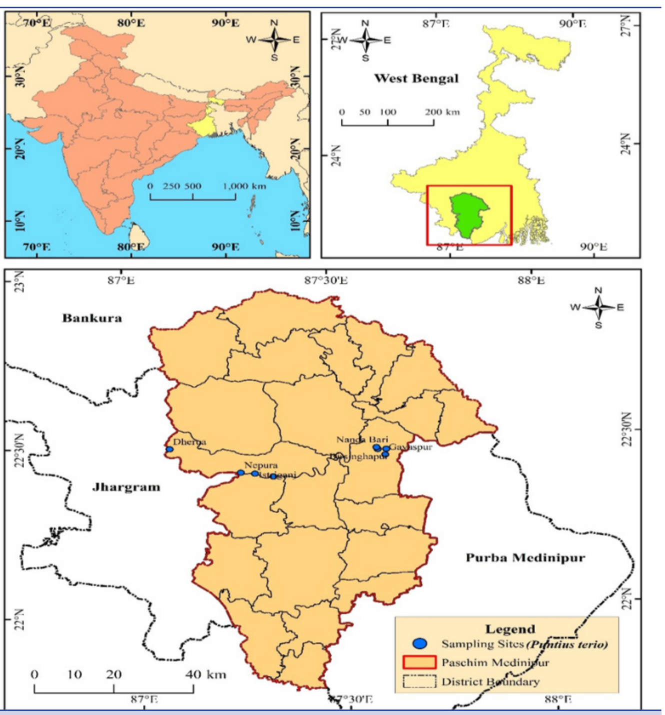

Figure 1. Study area and sampling locations.

Here, W denotes Fish weight (g); L denotes total length of fish (cm); 'a' denotes Intercept and 'b' denotes growth coefficient.

The logarithmic equation is represented as Log W = Log a + b Log L.

Condition factor (K): The following formula of Fulton (1904) was used:

K =100×(W/L3)

Here, W denotes Weight in gram and L denotes total length in cm.

Relative Condition Factor (Kn): The following formula of Fulton (1904) was used:

$Kn=W/aL^b$ Fulton (1904)

Here, W denotes weight in gram, L denotes total length in cm, and a and b denote regression parameters.

Analysis of Data

Data have been analysed (Descriptive statistics, Post Hoc test, Pearson's Correlation and Regression) by SPSS (2021), Microsoft Excel (2019), and Origin Pro (2023) software. Pearson Correlation Coefficient between 1 to + 1; ± 0- 0.10= Markedly low and negligible positive/negative, ± 0.10-0.30= Very low positive/negative, ± 0.30-0.50= Low positive/ negative, ± 0.50-0.70 = Moderate positive/negative, ± 0.70-0.90 = High positive/negative, ± 0.90-0.99 = Very high positive/negative, ±1 = Perfect positive/negative

RESULTS AND DISCUSSION

P. terio's overall size and weight varied from 6.45 ±0.546 to 7.99 ±0.918 cm and 4.26 ±1.36 to 8.46 ±3.87 g, respectively. Table 1 and Figure 2 present the lowest, maximum, and average length and weight data for males and females of *P. terio* for each season. In the current investigation, 'K' and 'Kn' values were 1.31±0.342 to 1.51±0.333 and 1.27± 0.231 to 1.72±0.390, respectively (Table 2). The average 'K' value is highest during the winter season, and 'Kn' is highest during the summer season in *P. terio* (Figure 3). When the fish has 'Kn' values greater than 1 suggests a good nutritional status. On the other hand, the relative condition component remained mostly stable in heavier fish, indicating the fish's health and general well-being. The current results show that 'Kn' is greater after the Monsoon season, but the length-weight ratio is higher in the monsoons, suggesting that the species is not in good health during the Winter period. During the Monsoon season, the highest lengths and weights were observed (Figure 3). The '*b*' and 'R^2' values fluctuated seasonally from 2.138 to 3.216 and 0.510 to 0.756, respectively (Table 3 and Figure 4). The '*b*' value is at its maximum during the monsoon season and lowest during the winter. The parametric and logarithmic length-weight relationships of *P. terio* are shown in Table 4. The r^2 values demonstrate a year-round positive relationship between length and weight. Length has a moderately significant positive correlation with weight, and K has a very low negative and low positive significant correlation with total length and weight, respectively (Table 5). Kn has a high positive significant relationship with K and a very low positive significant relationship with total weight. The post hoc test depicts a significant difference in the total body length, weight, and condition factor (K)

Table 1. Length and weight of *Puntius terio*.

	Length(cm)				Weight (g)			
	Mn	Mx	Mean	SD	Mn	Mx	Mean	SD
Summer								
Combined	5.20	7.90	6.86	±0.798	2.18	10.0	4.86	±1.75
Female	5.30	7.90	7.11	±0.895	2.65	10.0	5.01	±1.91
Male	5.20	7.30	6.66	±0.456	2.22	8.95	4.54	±1.45
Monsoon								
Combined	5.80	9.20	7.99	±0.918	3.47	19.11	8.43	±3.69
Female	5.90	9.20	8.11	±0.921	4.32	19.11	8.46	±3.87
Male	5.60	8.40	7.44	±0.812	3.88	15.62	7.99	±2.99
Winter								
Combined	5.60	8.80	6.75	±0.684	3.01	8.69	4.66	±1.52
Female	5.30	8.80	7.11	±0.724	3.12	8.70	4.99	±1.87
Male	5.10	7.10	6.45	±0.546	2.09	7.76	4.26	±1.36

N=480

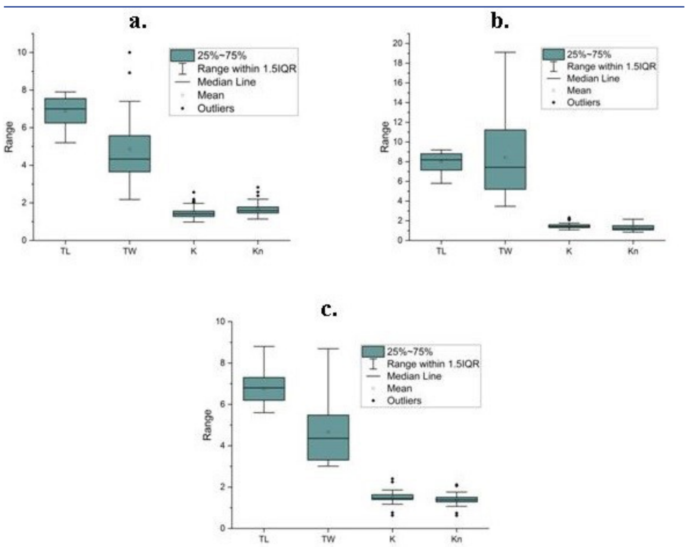

Figure 2. Length, Weight, K, K_n of *Puntius terio*: a. summer; b. Monsoon; c. Winter

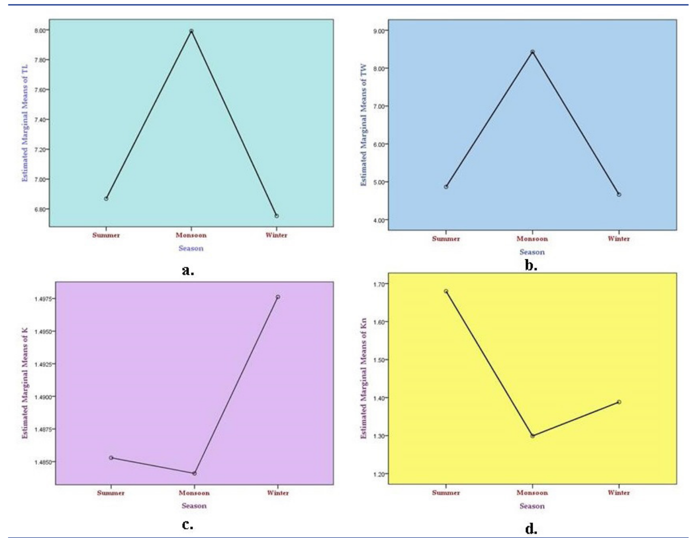

Figure 3. Length, Weight, K, K_n of *Puntius terio*: a. summer; b. Monsoon; c. Winter

of *P. terio* during the summer, monsoon, and winter seasons. Total length and weight significantly differ between summer, monsoon, and monsoon winter; "Kn" is found to differ significantly between summer and monsoon and summer and winter seasons, but not between Monsoon and Winter (Table 6). *P. terio* shows a negative allometric growth pattern (negative) except for the monsoon season. Negative allometric growth may be noticed if food deficiency and the surrounding environment are not suitable for breeding and growth development (Le Cren, 1951; Soni and Kathal, 1953; Weatherly, 1972; Deka and Bura Gohain, 2015).

However, positive allometric growth patterns of various tiny fish species, including *Puntius*, were documented by Sani et al. (2010), Palaniswamy et al. (2012), Pal et al. (2013), Lim et al. (2015), Hossain et al. (2015), and Sahil et al. (2022). Certain *Puntius* species exhibit negative allometric growth patterns, as reported by Bahuguna et al. (2021), Khan et al. (2021), Sarkar et al. (2013), Manorama and Ramanujam (2014), and Shafi and Parveen (2012). Hossain *et al.* (2012) reported the isometric growth pattern of *P. sophore* in Bangladesh. Sandhya *et al.* (2020) studied the length-weight relationship of 18 species of freshwater fish; here, four species belonged to the isometric growth pattern, and the other 14 species had equally positive and negative allometric growth patterns, among them, P. *terio*'s length was 2.6 cm 5.8 cm, weight 0.3-3.01 g, *b* value 3.147, R^2 value between total length and total weight is 0.974. Sahil *et al.* (2022) reported length 1.2-9.8 cm, total weight 0.30-7.18 g, *b* value of 1.86, R^2 value of 0.7270 between length and weight, and k value of 2.06 for *P. terio* in North Bihar. In the present study, except during the Monsoon season, *P. terio* exhibited allometric growth patterns (nega-

Table 2. K and K_n of *Puntius terio.*

	K				K_n			
	Mn	Mx	Mean	SD	Mn	Mx	Mean	SD
Summer								
Combined	0.98	2.56	1.48	±0.353	1.14	2.83	1.68	±0.380
Female	0.99	2.65	1.48	±0.371	1.19	2.98	1.72	±0.390
Male	0.97	2.43	1.31	±0.342	1.13	2.82	1.63	±0.373
Monsoon								
Combined	1.08	2.32	1.48	±0.280	0.84	2.15	1.29	±0.311
Female	1.09	2.41	1.49	±0.291	0.86	2.41	1.33	±0.354
Male	1.07	2.26	1.36	±0.273	0.82	2.02	1.27	±0.322
Winter								
Combined	0.63	2.40	1.49	±0.302	0.63	2.12	1.38	±0.272
Female	0.64	2.54	1.51	±0.333	0.66	2.31	1.43	±0.291
Male	0.62	2.43	1.46	±0.299	0.61	2.09	1.27	±0.231

N=480

Table 3. Seasonal regression parameters of *Puntius terio.*

Season	Sex	a	b	R^2
Summer	Combined	0.0643	2.223	0.548
	Female	0.0653	2.226	0.556
	Male	0.0637	2.201	0.534
Monsoon	Combined	0.00971	3.197	0.751
	Female	0.00983	3.012	0.756
	Male	0.00966	3.216	0.750
Winter	Combined	0.0747	2.142	0.510
	Female	0.0751	2.143	0.520
	Male	0.0742	2.138	0.510

N=480

Table 4. Parabolic and logarithmic length weights of the *Puntius terio.*

Season	Sex	Parabolic	Logarithmic
Summer	Combined	W=0.0643^2.223	Log-W=-1.191+2.223logL
	Female	W=0.0653^2.226	Log-W=-1.185+2.226logL
	Male	W=0.0637^2.201	Log-W=-1.195+2.201logL
Monsoon	Combined	W=0.0097L^3.197	Log-W=-2.012+3.197logL
	Female	W=0.00983L^3.197	Log-W=-2.007+3.012logL
	Male	W=0.00966L^3.216	Log-W=-2.015+3.216logL
Winter	Combined	W=0.0748L^2.142	Log-W=-1.126+2.142logL
	Female	W=0.0751L^2.143	Log-W=-1.124+2.143logL
	Male	W=0.0742L^2.138	Log-W=-1.129+2.138logL

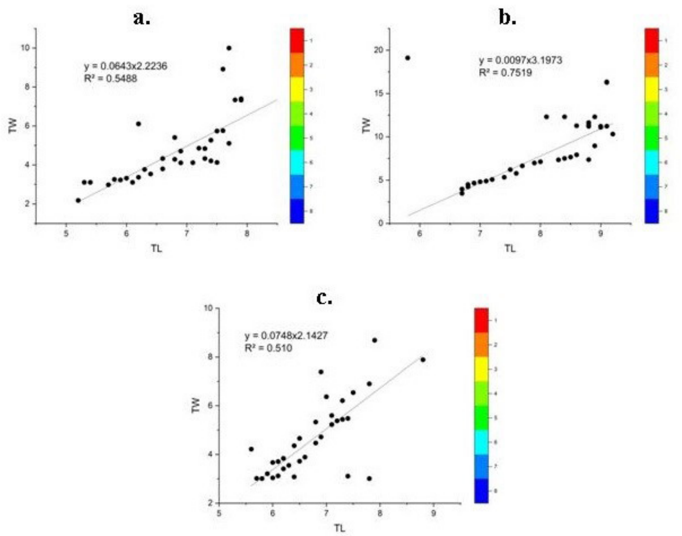

Figure 4. Length-Weight relationship of *Puntius terio,* a. Summer; b. Monsoon; c. Winter

Table 5. Pearson's correlation among Length, Weight, K, and K_n of *Puntius terio.*

	Season	TL	TW	K	Kn
Season	1	-0.048	-0.027	0.016	-0.330^{**}
TL	-0.048	1	0.684^{**}	-0.153^{*}	-0.083
TW	-0.027	0.684^{**}	1	0.388^{**}	0.263^{**}
K	0.016	-0.153^{*}	0.388^{**}	1	0.844^{**}
K_n	-0.330^{**}	-0.083	0.263^{**}	0.844^{**}	1

N=480; ** 0.01, level of significance; 0.05, level of significance; 0.05, level of significance

Table 6. Post-hoc test seasonally comparisons of length, weight, K, and K_n of the *Puntius terio.*

Dependent Variable	(I) Season	(J) Season	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TL	Summer	Monsoon	-1.1234*	0.14254	**0.001**	-1.4602	-0.7867
		Winter	0.1156	0.14254	0.697	-0.2211	0.4524
	Monsoon	Summer	1.1234*	0.14254	**0.001**	0.7867	1.4602
		Winter	1.2391*	0.14254	**0.001**	0.9023	1.5758
	Winter	Summer	-0.1156	0.14254	0.697	-0.4524	0.2211
		Monsoon	-1.2391*	0.14254	**0.001**	-1.5758	-0.9023
TW	Summer	Monsoon	-3.5641*	0.44511	**0.001**	-4.6156	-2.5126
		Winter	0.2042	0.44511	0.891	-0.8473	1.2557
	Monsoon	Summer	3.5641*	0.44511	**0.001**	2.5126	4.6156
		Winter	3.7683*	0.44511	**0.001**	2.7168	4.8198
	Winter	Summer	-0.2042	0.44511	0.891	-1.2557	0.8473
		Monsoon	-3.7683*	0.44511	**0.001**	-4.8198	-2.7168
K	Summer	Monsoon	0.0012	0.05542	1.000	-0.1297	0.1321
		Winter	-0.0123	0.05542	0.973	-0.1433	0.1186
	Monsoon	Summer	-0.0012	0.05542	1.000	-0.1321	0.1297
		Winter	-0.0135	0.05542	0.968	-0.1445	0.1174
	Winter	Summer	0.0123	0.05542	0.973	-0.1186	0.1433
		Monsoon	0.0135	0.05542	0.968	-0.1174	0.1445
Kn	Summer	Monsoon	0.3809*	0.05738	**0.001**	0.2453	0.5164
		Winter	0.2916*	0.05738	**0.001**	0.1561	0.4272
	Monsoon	Summer	-0.3809*	0.05738	**0.001**	-0.5164	-0.2453
		Winter	-0.0892	0.05738	0.268	-0.2248	0.0463
	Winter	Summer	-0.2916*	0.05738	**0.001**	-0.4272	-0.1561
		Monsoon	0.0892	0.05738	0.268	-0.0463	0.2248

tive). These findings contrast those of Sani and Gupta (2010), Rahman et al. (2012), Palaniswamy et al. (2012), Lim et al. (2013), Hossain et al. (2015), Kaushik and Bordoloi (2015), Muhammad et al. (2016), and Gupta and Tripathi (2017). The results demonstrate observations similar to those of Manorama and Ramanujan (2011), Shafi and Yousuf (2012), Sarkar et al. (2013), Vishal and Gaur (2015), Khan et al. (2021), Bahuguna et al. (2021), and Moglekar et al. (2022). These discrepancies can be explained by several factors, including sample size structure, reduced feeding proficiency, gonad maturity, sex, and a high proportion of small specimens (Franco *et al.*, 2014; Froese, 2006). Seasonal variation in the condition factors and relative condition factors for this species was supported by the study of Manorama and Ramanujan (2014). Therefore, fluctuations in growth factors in different seasons are an important concern for the maintenance of these two species populations in the study area.

CONCLUSION

The results indicate that the species did not strictly follow the anticipated cube law and had allometric development in all four seasons. The objectives of this study were met, and the information gathered can be used to guide the creation of future biometric research studies for other fish from the study region. Fishery managers will be able to create growth management strategies for *P. terio* in their habitats using only current findings.

Conflict of Interest: The authors declare no conflicts of interest.

Ethics Committee Approval: This Ethical clearance from the IAEC (Approval no. 08/1AEC(1)/S/RNLKWC/2023, dated-15/06/2023.

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Research Article

Impact of Various Carbon Sources on the Growth Efficiency, Antioxidant Efficacy, and Immunity of Clariid Catfish *Clarias gariepinus* (Burchell 1822)

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ABSTRACT

The study examined the effects of various carbon sources on *Clarias gariepinus* (6.18±0.2g and 8±0.13 cm) growth efficiency, antioxidant efficacy, and immune function. The bioflocs system was developed using four carbon sources: tapioca, cassava flour, rice bran, and molasses. The fish were raised in concrete tanks for 10 weeks, and their water quality was monitored, after which they were challenged. The results demonstrated that the treatments significantly differed in terms of both survival rate and water quality parameters. Fish reared in biofloc systems exhibited significantly higher total cholesterol, total protein, superoxide dismutase (SOD), Lyzosome (LYZ), and Myeloperoxidase (MPO) activities compared with the control group. However, there was a reduction in the activities of ALT, AST, total glucose, and antiprotease in biofloc-treated C. gariepinus compared with the control. Expression of the IL-1 gene in the intestines was significantly elevated in fish raised in biofloc. Similarly, the transcription of GPX, GSR, IL-1, and IL-8 genes in the gut and liver of biofloc-treated fish was considerably enhanced. Applying biofloc to the rearing medium can enhance fish growth efficiency, immune system response, and the transcription of genes associated with immunity and antioxidant activities in *C. gariepinus*. The degree of immune system stimulation by the BFT system is impacted by the carbon source.

Keywords: Carbon sources, biofloc, African catfish, immunity, oxidative stress, gene expression

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INTRODUCTION

Food, fuel, shelter, clothes, and energy have been produced for human use by harvesting, farming, or cultivating the Earth's natural resources (Béné et al., 2016; Chan et al., 2019). However, these resources decline proportionately with population growth, leading to degradation of the environment, biodiversity loss, resource extinction, and climate change (Shah & Mraz, 2020). It is imperative to ensure that the supply of these resources occurs at a faster rate than consumption to guarantee sustainability. Aquaculture has been reported to provide more than 50% of fish production, and it is assumed that aquaculture production will expand to reach 202 million tons in 2030 aside from the production from algae (Ndondo, 2023)

Aquaculture systems result in the accumulation of inorganic nitrogen compounds, which are the primary excretory products of aquatic life. Ammonia, a product from the aquaculture system, has been reported to cause gill injury and predispose the cultured fish to disease stress, thereby affecting growth, reproductive patterns, oxygen expenditure, and death (Abdel Rahman et al., 2019; El-Sayed, 2020). Water exchange, bio-filtration systems, Recirculating Aquaculture Systems (RAS), cutting back on or stopping feeding, flushing ponds with fresh water, lowering stocking density, and aerating the pond are some of the techniques used to remove ammonia from aquaculture systems (Popoola & Miracle, 2022; Upadhyay et al., 2022). Unfortunately, the majority of these techniques are costly, time-consuming, or harmful to the animal being raised.

Biofloc technology, a system that promotes the growth of microorganisms in aquaculture, has been explored as a potential feed source for ridding off ammonia in fish-rearing media (Abdel Rahman et al., 2019; El-Sayed, 2020). Biofloc can be cultivated using carbon sources such as molasses, wheat bran, maize bran, rice bran, and other organic substrates (Ekasari et al., 2014; Popoola & Miracle, 2022). The biofloc composition and carbon sources used could influence the fish's immunity and ability to withstand disease. Studies have demonstrated that biofloc, which contains vitamins, microbes, and other bioactive substances, can improve fish immune responses. In addition, its diets stimulate the fish's immune system, increasing disease resistance (Ahmad et al., 2017; Lumsangkul et al., 2021).

Assessing the expression of antioxidant enzymes (AOE) in response to environmental stressors, such as diseases and infections, is an extremely useful method for studying how aquatic species react to stress (Zhang et al., 2016).

To enhance comprehension of the immune system's impact on juvenile *Clarias gariepinus* raised in biofloc systems with varying carbon supplementation, this study examined the implications of four distinct carbohydrate sources as biofloculating agents, along with the growth performance and survival of *Clarias gariepinus*. Additionally, the study examined the transcription of immune- and antioxidant-associated genes in biofloc-reared *Clarias gariepinus* treated with *Aeromonas hydrophila*.

MATERIALS AND METHODS.

The Fisheries and Aquaculture Department of the Federal University of Technology, Akure (FUTA) in Nigeria provided the necessary resources for the research.

About 2,000 African catfish (*Clarias gariepinus*) juveniles (6.18±0.2g and 8±0.13 cm) were procured from a farm in Ondo Town and transferred to the Federal University of Technology Akure's teaching and research farm. The fish were maintained in 14-day mildly aerated rectangular concrete acclamation tanks with dimensions of 2m (length) x 1m (width) immediately after stocking. The fish were given commercially available feed with 35 % crude protein during acclimation at 08.00 and 17.00 h daily.

Following a completely randomized design, fifteen concrete tanks measuring 1 x 2 x 1 m were divided into five experimental groups based on the carbon sources (rice bran, molasses, tapioca, and cassava peel flour) and in triplicate. A week before the trials, concrete tanks were prepared, and two days before stocking, carbon sources were added. Daily additions of organic carbon sources were made following the anticipated 20:1 carbon-to-nitrogen ratio in the rearing medium and the approximate amount of feed nitrogen supplied to the culture tank (De-Schryver et al., 2008). Each tank held 120 pieces of *C. gariepinus*, vigorously aerated for 10 weeks using an air blower at 5 L/min per line (15 lines). During this period, conventional fish feed was provided to the fish (35% Crude Protein) twice daily, between 08:00 and 10:00 hours in the morning and 15:00 and 17:00 hours in the evening at 5% body weight.

The water parameters like temperature, dissolved oxygen (DO), biological oxygen demand (BOD), pH, salinity, alkalinity, and TDS were measured on a weekly basis using a multi-sensor EXTECH instrument ExStik II following the manufacturer's procedure.

After 10 weeks of stocking, surviving fish were counted and various growth parameters were determined.

The total weight gain was obtained as;

TWG (g/fish) is equal to (W_F–W_I), $\mathrm{TWG}\left(\frac{\mathrm{g}}{\mathrm{fish}}\right) = \mathrm{WF} - \mathrm{WI}$

where W_F is the fish's weight in grams at the end of the trial (Final) and W_I is its initial weight in grams.

The survival rate (SR) was calculated as

SpecificGrowthRate(SGR) $\mathrm{SGR}\left(\frac{\%}{\mathrm{day}}\right) = \frac{\ln \mathrm{WF} - \ln \mathrm{WI}}{\mathrm{trial}}$ $\mathrm{duration\ in\ days} * 100$

where ln is the natural logarithm and $W_{F,}$ W_I represents the final and initial weight.

The feed conversion ratio (FCR) is obtained as;

Feed conversion ratio (FCR)=Weight Gain (g)/Feed Intake (g) x 100

Survival rate (SR)=total fish harvested /total quantity of fish stocked x100

The Animal Care Laboratory, Ogere, identified the pathogenic strain of *A. hydrophilia* (MPSTR 2143) and used it to feed the control fish and those in various biofloc systems. The bacteria were cultured in a 100-mL conical flask containing 30-mL autoclaved tryptic soy broth (TSB; Merck) as it progressed to a log phase. To obtain bacterial pellets, the culture was centrifuged for 20 min at 3500 x g and 4°C. The bacterial pellets were then cleaned using a sterile 0.15 M phosphate-buffered saline (PBS) with a pH of 7.2. Using the findings of the lethal dosage 50% (LD_{50}) trial, the pellets were redissolved in PBS, and the concentration was adjusted to 1.5 × 108 cfuml^{-1} (Popoola et al., 2023). The 50 fishes (10 fish/treatment) were challenged via intraperitoneal injection, and mortality and survival were monitored and documented for further analysis.

Following confirmation of *A. hydrophila* as a source of mortality via challenge, dead and sick fish were collected. The challenging strain was then aseptically isolated and identified from samples collected from the kidneys, liver, intestines, and gills.

With the aid of a 2 ml heparinized syringe, blood was drawn from the caudal vein after 50 µl of clove oil was used to anesthetize the fish (Malick et al., 2020) and poured into a citrate-treated anticoagulant-coated test tube. Serum was isolated from blood and collected without the use of an anticoagulant. After centrifuging the blood for 15 min at 3,500 rpm and 4°C, the straw-colored serum was recovered and stored for later use at 20°C.

Total counts for leucocytes and erythrocytes were obtained using the Schaperclaus et al. (1991) method involving combining 20 µL of the blood sample with 3,980 µl of WBC and RBC diluting solution in a sterile vial, respectively. Using a Neubauer hemocytometer (Rohem, India), the diluted fluids were examined, and the number of cells was recorded. The packed cell volume was determined using capillary action to pull non-dotted blood into the microhaematocrit tubes. A microhaematocrit reader was used to measure the PCV, which was then reported as a percentage (%). Using Drabkin's Fluid (Qualigens, India), the cyanomethemoglobin technique was used to determine blood hemoglobin levels. This involved mixing 5 mL of Drabkin's working solution and 20 µ blood and the spectrophotometer (Thermo Electron, Merck) was used to detect the absorbance at 540 nm. The hematological characteristics of the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formula provided by Haney et al. (1992).

MCV (fl) = Hct x 10/RBC, MCH (pg/cell) = Hgb (gm/dL) x 10/RBC, MCHC (g / dL) = Hgb (g/dL) / Hct.

With the aid of a BioVision USA SOD activity assay kit, the inhibition rate of superoxide anion in serum obtained from *A. hydrophila*-dosed *Clarias gariepinus* fish (control and biofloc raised) was calculated to determine the amount of superoxide dismutase (SOD). The Assay Kit for Lysozyme (USA: Sigma-Aldrich) was utilized to detect lysozyme activity by the instructions provided by the manufacturer. The serum concentration resulting in a 0.001-min decrease in absorbency at 450 mM was considered a single lysozyme activity unit. The techniques of (Quade & Roth, 1997) were used to measure the serum level of myeloperoxidase function (MPO) at 450 mM absorbance. The trypsin inhibition rate was used to indicate antiprotease activity according to the technique of Heo et al. (2013). The following formulas were used to determine the trypsin inhibition rate: (Control OD−Sample OD)/Control OD × 100, with PBS serving as the control. Serum levels of total glucose, cholesterol, and protein, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using a clinical chemistry analyzer, Fuji DRI-CHEM 3500i (Fuji Film, Japan),

Observing the guidelines provided by the manufacturer for the Assay kit TRIZOL (Invitrogen, Carlsbad, CA, USA), with a few adjustments, 1 mL of TRIZOL reagent was used to extract total RNA from organs like the liver, muscle, and gills from five distinct juvenile C. gariepinus individuals. Total RNA was evaluated using a Nano-photometer (Implen®, CA, USA), and an optimal purity threshold of 1.8 and 2.0 was selected. With adherence to the manufacturer's instructions, upon diluting a sample of the total RNA to 300 ng µL−1, DNAse I (1 U/µL, Sigma-Aldrich, Missouri, US) was added to transcribe the cDNA. Reverse transcriptase (Promega, WI, USA) with oligo (dT20) primers was used according to the manufacturer's recommendations to synthesize cDNA from 1.0 µg of RNA (10 µL). Before qPCR analysis, the cDNA was kept at − 70 °C after dilution 10 times with ddH2O. For each qRT–PCR reaction, 2.5 L of this cDNA dilution served as the template. With SYBR Green PCR Reagents from Applied Biosystems and β-action as a housekeeping gene, real-time PCR was performed to evaluate the transcription of the selected genes (Table 1). The primer synthesis was designed based on the reference's

Table 1. Primers and sequences used for gene transcription.

Gene of Interest		Target sequence	Optimum Tm	References
GSR	F	GACTGGTTCCCAACGTGTCA	60.2	XM_034139725
	R	CGCCACCTATCACCAGGAAG		
GPX	F	ACTGCACACTCATGGGAACA	60.2	DQ355022
	R	TTAAAAGCCAGCGGATTGAC		
IL-8	F	CTGTTCGCCACCTGTGAAGG	61.2	NM_001279704
	R	ATGTGGCGGCCAATAGGTTT		
IL-1	F	GTCTGTCAAGGATAAGCGC	59.3	XM_019365844
	R	ACTCTGGAGCTGGATGTT		
β-actin	F	CTACGAGGGTTATGCCCTGC	62.0	XM_003443127.5
	R	ATGTCACGCACGATTTCCCT		

GPX, glutathione peroxidase; GSR, glutathione-disulfide reductase; IL-8, interleukin 8; IL-1, interleukin 1.

Table 2. Water quality parameters of the biofloc systems rearing *Clarias gariepinus*.

	Control	Molasses	Tapioca	cassava flour	Rice bran
Temperature (°C)	27.23±0.14	28.94±0.19	28.37±0.11	28.11±0.41	29.01±0.10
DO (mg/ L)	5.30±1.02	4.70±0.23	4.81±2.17	4.91±1.76	4.60±0.92
BOD (mg /L)	2.52±1.05	2.87±1.02	2.81±1.14	2.91±0.82	2.83±0.82
pH	6.80± 0.98	6.71±1.45	6.74±0.94	6.84±1.23	6.80±0.39
Salinity (g/L)	31.00±1.04	32.02±0.97	31.02±1.23	31.64±1.43	30.85±2.10
Alkalinity (mg/ L)	121.00± 3.23	114.17±4.22	117.10±1.33	119.01±2.35	114.21±3.12
TDS (mg/ L)	93.23±4.23	180.14±5.13	155.19±3.17	160.24±3.12	184.19±2.19

The growth characteristics of *Clarias gariepinus* cultured in systems using biofloc technology with various carbon sources were measured, and mean values ± standard deviation were recorded for each of the six individuals. Values for the same variable show significant variations (P < 0.05), denoted by an alternate superscript letter.

sequences obtained from GenBank sequences. According to (Livak & Schmittgen, 2001), the transcription levels of the indicated transcripts were evaluated for changes using standard curves and the 2−ΔΔCt approach.

Minitab version 14 was used to evaluate the study data following any necessary log transformation. To determine whether or not there was a difference of statistical significance at P<0.05, The New Duncan Multiple Range Test (NDMRT) was employed to differentiate the mean differences between the treatment groups. A descriptive statistic was used for gene expression analysis.

RESULT AND DISCUSSION

Water quality parameters

Salinity, BOD, and total dissolved solids were found to be significantly lower in the control group than in the biofloc group, according to the physicochemical parameters of the trial media. It was observed that the molasses medium had the highest salinity value, whereas the control group had the lowest. The control group had greater values for alkalinity and dissolved oxygen than the biofloc groups. However, there were variations in each treatment's pH, and these variations did not follow a consistent pattern. The study exhibited sustained differences in pH, dissolved oxygen, total dissolved solids, and salinity between the control and biofloc media treatments, all of which were suitable for the growing of *Clarias gariepinus.* (Table 1).

According to Gallardo-Collí et al. (2019), BFT is dubbed the "new blue revolution" since it uses microorganisms (bacteria, fungus, microalgae, and zooplankton) to proliferate and preserve water quality. The recommended values for *C. gariepinus* were met by the water quality indicators used in this investigation.

Growth and survival of experimental fish

Fish grown in biofloc systems showed considerably higher WG and survival values relative to the group under control (p<0.05) (Fig. 1a). In the meantime, there were no discernible differences in survival between fish receiving biofloc treatments for survival (p>0.05). Notable differences were observed among the biofloc treatments, with the control group exhibiting a significantly lower SGR than the other groups (p<0.05). Although tapioca and cassava flour exhibited no appreciable change, the FCR in the systems (tapioca and cassava flour) was higher than that of the other treatments, including the control. However, there was no statistical difference (p>0.05) between the control, molasses, and rice bran, but the FCR was the lowest in the control. Growth indicators and the survival rate of *C. gariepinus* after a 10-week feeding period showed significant differences (p<0.05) across all treatments (Fig. 1b).

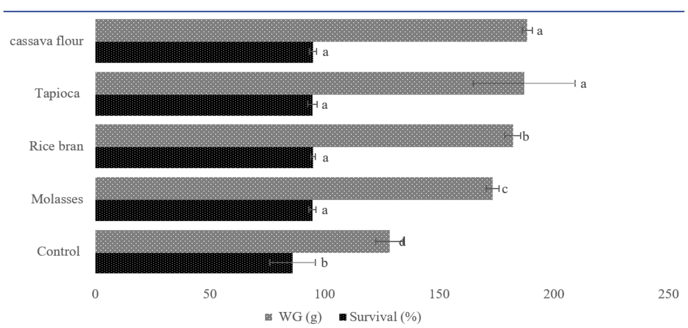

Figure 1a. Survival and weight gain in *Clarias gariepinus* raised under different carbon biofloc system.

Within the same row, mean values (Means, n =5) with different letters exhibit significant differences (P < 0.05).

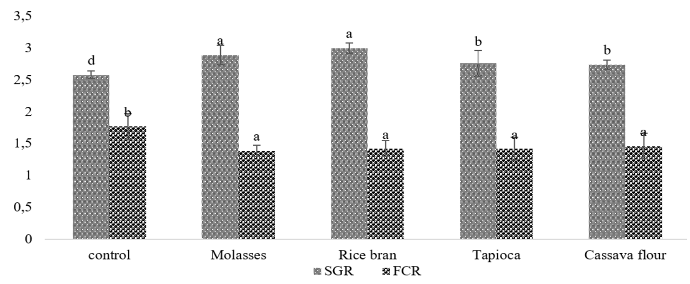

Figure 1b. Growth parameters of *Clarias gariepinus* reared in different carbon bioflocs system.
SGR-Specific Growth Rate; FCR- Feed Conversion Ratio

In the present study, fish reared within the biofloc performed better than fish kept in normal water devoid of carbon. This was revealed in the FCR of the BF groups, which was lower than that of the control groups. This implies that *Clarias gariepinus* could be produced more intensively using BF with less feed. Several factors could account for the beneficial features of biofloc technology, particularly its growth efficiency and feed utilization of *Clarias gariepinus*. According to several studies (Crab et al., 2012, Wang et al., 2015), biofloc improves water quality and stability, and fish can consistently obtain Flocs as a rich nutrient source (Fauji et al., 2018; Bakhshi et al., 2018). In addition, fish resilience to stress is increased by the environment in which they live and by consuming biofloc. (Fauji et al., 2018, Liu et al., 2018). The probability that the raised fish in this study consumed biofloc could be linked with the decreased values of FCR in the BFT groups. According to Yi et al. (2018), the FCR of biofloc groups was considerably lower than that of the control group. Further research has revealed that the biofloc improves fish's feed utilization while both the control and BF groups showed improved percentage survival rates, whereas the BF groups showed greater percentage survival rates; this finding may be related to the biofloc's immunostimulatory effects against stress (Keiko et al. 2015). Improvements in the aquatic environment that decreased stress and supplementation of nutritional components, essential amino acids, and fatty acids available in biofloc may have contributed to the dramatic increase in survival rate observed in this study.

Mortality-Disease resistance

At the end of the challenge experiment, the number of fish that died was deducted from the treated fish to determine whether the survival of fish reared in biofloc media with various carbon sources was significantly higher (P < 0.05) than that of fish kept in regular water. (Fig. 2).

An important measure of the wellness of fish is their survival after a pathogenic bacteria challenge test (Ringø et al., 2010). Qiao et al., (2018) reported that biofloc provides reliable fish immunity to diseases. According to the current investigation, the different carbon sources offered different immune capabilities to *C. gariepinus* and increased survival after challenge testing with *A. hydrophila* and *C. gariepinus*. Liu et al. (2018) observed comparable outcomes, noting that O. niloticus infected with *V. harveyi* and cultivated with biofloc exhibited a greater survival rate compared with the untreated group, suggesting that stimulation of immunity is caused by biofloc coupled with different materials used as flocculating agents. Kishawy et al. (2020) reported that *O. niloticus* infected with *A. hydrophila* had a higher chance of survival in groups using biofloc as the carbon source. Furthermore, Kishawy et al. (2020) argued that by increasing fish immunity and infection tolerance through the use of mannan oligosaccharides as a source of carbon in biofloc, the percentage of fish that survive is increased. Haridas et al. (2017) also discovered that *O. niloticus* raised with biofloc had a good survival rate when infected with *A. hydrophila*, supporting the positive effects on infection resistance. According to Fauji et al. (2018), *C. gariepinus* grown in biofloc cultures exhibited a much higher survival rate following *A. hydrophila* infection. According to Verma et al. (2016), *L. rohita* raised with biofloc had a greater survival probability for *A. hydrophila* infection than the control group. A high survival rate of *C. carpio* reared in biofloc from *A. hydrophila* infection *L.* was also noted by Bakhshi et al. (2018). Kim et al. (2020) found that *P. olivaceus* with an *E. tarda* infection that was farmed in biofloc had a significantly higher chance of survival. This was attributed to the biofloc's capacity to boost immunological function, thereby enhancing disease resistance.

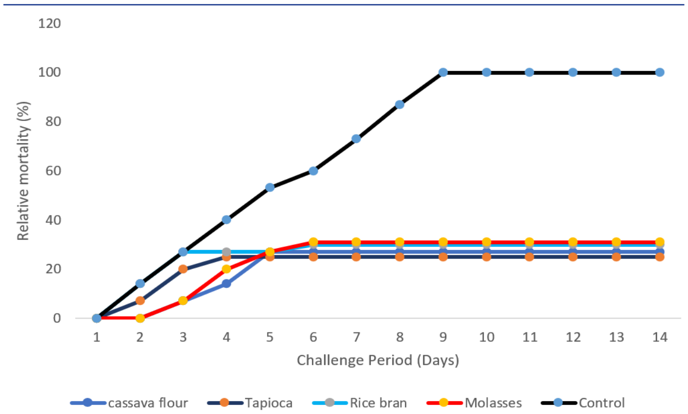

Figure 2. Relative mortality of *C. gariepinus* in different carbon biofloc systems against *A. hydrophila* compared with the control treatment.

Table 3 presents the changes in various Serum non-specific immune factors in *C. gariepinus* raised under different carbon biofloc systems for 10 weeks. Significant differences ($P < 0.05$) were observed for every parameter and treatment group (bioflocs and control). For SOD, lysosome, and MPO, compared with other treatments, the control group's levels were noticeably lower (biofloc) unlike the antiprotease that was highest in levels in control when compared with other treatments.

Immune -antioxidant response

Superoxide dismutase (SOD) (% superoxide inhibition). Lysozyme is the Activity of serum lysozyme. Myeloperoxidase activity (MPO) was measured at 450 nm. An antiprotease is a percentage of trypsin suppression.

In contrast to AST, total glucose, and total cholesterol, which exhibit substantial differences between the carbon sources (biofloc) and control (Table 4), serum biochemical parameters, including ALT and total protein, were not significantly affected by biofloc treatment ($P > 0.05$).

Table 3. Serum non-specific immunological markers of *C. gariepinus* cultured in various carbon biofloc systems.

	SOD (UL^{-1})	Lysosome (mg ml^{-1})	MPO (UL^{-1})	Antiprotease UmL^{-1}
Control	35.95±2.21^{c}	0.51±0.03^{a}	0.78±0.03^{e}	50.17±3.14^{a}
Molasses	55.64±0.50^{ab}	0.62±0.00^{ab}	0.82±0.03^{d}	43.18±2.27^{b}
Tapioca	56.14±0.54^{a}	0.65±0.01^{b}	0.89±0.05^{c}	41.73±1.28^{bc}
cassava flour	43.64±0.36^{b}	0.69±0.02^{b}	1.06±0.01^{a}	41.27±2.01^{c}
Rice bran	55.76±0.05^{a}	0.75 ±0.11^{c}	0.90±0.02^{b}	42.56±1.38^{b}

The results show the average ± standard deviation of three duplicate observations. Values for the same variable show significant variations ($P < 0.05$), denoted by an alternate superscript letter.

Table 4. Biochemical parameters of *C. gariepinus* grown under different carbon biofloc systems.

	ALT (UL^{-1})	AST (UL^{-1})	Total glucose level (mg dl^{-1})	Total cholesterol level (mg dl^{-1})	Total protein level (mg ml^{-1})
Control	13.30±1.01^{a}	17.03±0.20^{a}	45. 73±2.06^{a}	119±5.81^{d}	6.71±0.31^{c}
Molasses	12.15±1.13^{b}	15.65±1.32^{b}	42.66±2.17^{d}	129±7.41^{c}	7.21±0.14^{b}
Tapioca	11.35±1.52^{c}	15.87±2.11^{b}	44.90±3.17^{b}	138±4.21^{a}	7.46±0.43^{a}
Cassava flour	11.16±1.11^{c}	15.15±1.02^{c}	43.67±3.31^{c}	128±2.44^{c}	7.23±0.13^{b}
Rice bran	12.13±1.22^{b}	15.21±2.11^{c}	44.75±2.17^{b}	133±3.18^{b}	7.28±0.26^{b}

Three replicates' mean ± standard deviation of three replicates is represented by the values. Values for the same variable show significant variations ($P < 0.05$), denoted by an alternate superscript letter.

The main antioxidant enzyme found in fish, superoxide dismutase (SOD), functions as a primary defense against oxidative damage by converting superoxide anion (O_2^-) into hydrogen peroxide, shielding fish from damage caused by reactive oxygen compounds, and maintaining the metabolic equilibrium of ROS (Kim et al., 2019). When *C. gariepinus* was cultivated in biofloc, it was discovered that the SOD and CAT values were amplified. This conclusion aligns with research by Mansour & Esteban, (2017) and Shourbela et al. (2021), who observed a significant increase in the serum SOD and CAT activity of *O. niloticus* grown in biofloc. Furthermore, *O. niloticus* cultivated with biofloc exhibited increased serum SOD and CAT activity. The reduction in oxidative stress, consequentially leading to fish health improvement recorded in this study was earlier observed by Shourbela et al. (2021). According to Menaga et al., (2019), biofloc may increase fish SOD and CAT activity levels, and low levels of these enzymes may cause high concentrations of free radical accumulation in cells, leading to cell damage. The explanation of the increased antioxidant capacity is believed to be an increase in the antioxidant enzyme that inhibits lipid peroxidation, as reported by Youse et al. (2020), who also observed a discernible increase in the blood concentrations of SOD and CAT in *C. carpio* cultured in biofloc. According to Popoola & Miracle, (2022), *C. gariepinus* cultured in biofloc medium with high stocking density exhibited better growth performance because of higher levels of SOD and CAT. Yu et al. (2020) found that fish grown with biofloc exhibited increased levels of SOD and CAT activity. Lipid peroxidation levels decreased as a result, and the body's ability to combat free radicals was enhanced. *P. hypophthalmus* cultivated in a biofloc environment exhibited a significant increase in SOD and CAT activities, according to (Nabi, 2021). The implication of this is that the biofloc environment provides fish with a high level of resistance to oxidative stress and acts as an effective antioxidant. However, Haridas et al. (2017) found that *O. niloticus* cultivated with biofloc had lower liver tissue SOD and CAT activity. They speculated that this could be because the bioactive chemicals decreased the production of SOD and CAT. This implies that antioxidant enzymes are not stimulated by reduced levels of oxidative stress. Yu et al. (2020) reported that fish health, environmental stress, and exposure to toxins can affect the activity of lysozyme, which acts as the first line of defense against various diseases, including bacteria, viruses, and parasites. Leukocytes in the blood produce an enzyme called lysozyme, which breaks down bacterial cell walls and promotes the formation of phagocytes to fight infections and other harmful diseases (Hwihy et al., 2021). Biofloc showed noticeably more LYZ activity than the control. According to Ali et al. (2018), consumption of the floc may have led to increased LYZ levels, which are indicative of *C. gariepinus* supplementation. Innate immunity in fish is measured by analyzing LYZ activity (Skouras et al., 2003). The outcome showed that elevated serum LYZ activity in C. *gariepinus* fed biofloc would have strengthened the fish's immune system. This finding is similar to the findings by Mansour & Esteban, (2017), who found that *O. niloticus* cultivated with biofloc exhibited a substantial increase in lysozyme levels, indicating improved immunity. Several researchers have observed this increase, and it has been suggested that the fish's defense mechanism is stimulated by bioactive chemicals found in biofloc, especially carotenoids, endogenous microbes, and fat-soluble vitamins (Long et al., 2015; Hwihy et al. (2021). Verma et al. (2016) reported that when *L. rohita* was grown on biofloc, lysozyme activity increased considerably, indicating that non-specific immunity can be improved by raising fish within a biofloc-based system. According to Yu et al. (2020), diverse species of fish, including *O. fopingensis, C. auratus*, and *C. argus*, exhibit increased lysozyme activity when reared with biofloc. Accordingly, fish may obtain protein from microbial flocs to support their immune system.

According to Borgia et al. (2018), serum antiprotease is essential for preventing bacterial infection in host cells from invading and multiplying. Therefore, a notable increase in the serum antiprotease activity of *C. gariepinus* cultured in biofloc prompted the prevention of *A. hydrophila* proliferation.

One of the peroxidases that is most frequently expressed in neutrophil granulocytes is myeloperoxidase (MPO). This ferrous lysosomal polypeptide is found in myeloid cells and is composed of monocytes and neutrophils. MPO causes hydrogen peroxide (H_2O_2) and chloride anions (Cl^-) to release hypochlorous acid (HOCl) during neutrophil respiratory bursts. Sontakke et al. (2018) reported that MPO is mostly found in basic neutrophil granules, where it combines with phagocyte packets to expedite the killing of pathogens and then transports these materials to invasive pathogens. To provide a potent antibacterial response, HOCl is generated (Kumar et al., 2005). Menaga et al. (2019) reported a large increase in MPO in *O. niloticus* cultivated in biofloc cultures, which they attributed to the fish's immune system being stimulated or triggered. Verma et al. (2016) reported that *L. rohita* cultivated in biofloc exhibited increased MPO, although the level of stimulation differed depending on the carbon source used to create the floc. Some carbon sources produced biofloc with a significant decrease in MPO, whereas biofloc made from additional carbon sources demonstrated a notable increase in MPO. This suggests that carbon sources have strong antimicrobial effects because they produce hypochlorous acid during respiratory bursts (Verma et al. 2016).

The AST and ALT enzymes were observed to be significantly low in all carbon-containing BFT treatments, whereas ALP showed no significant difference between BFT and control. These results demonstrate the stress-releasing effect of BFT in cultured *C. gariepinus*. Similar observation was reported by Abduljabbar et al. (2015) on Tilapia reared in a biofloc system. From another perspective, it could be mentioned that BFT systems in zero water exchange systems are suitable for Catfish culture and comparable with common intensive culturing methods.

A prior study found that when crucian carp were fed a supplemented diet, the levels of glucose were lower and the levels of total protein were much higher (Tan et al., 2018). Analogously, in our investigation, the biofloc system worked regardless of the carbon source employed to raise total protein and glucose. Serum components, including total cholesterol, have been widely established to be associated with immunological function and overall health (Zhou et al., 2012). The current study found that fish produced in biofloc had higher total cholesterol levels than those raised in a control group, and the total cholesterol levels of fish raised in different carbon inclusion environments varied.

Gene Expression Profiling

The significant effects of carbon sources on transcription of the GPX, GSR, IL1, and IL8 genes in *C. gariepinus* livers are illustrated in Fig. 3. Both IL-1 and IL-8 expression levels were significantly increased in the biofloc system, and there was a significant ($p < 0.05$) variance in their expression. There is upregulation of IL-1 and IL-8 in fish reared in all biofloc systems (molasses, tapioca, rice bran, and cassava flour), with the highest levels observed in tapioca, which is included in biofloc. Comparing fish in biofloc to those in other treatments and the control, we observed that the fish with molasses included in biofloc had significantly greater expression levels of GSR genes ($p < 0.05$).

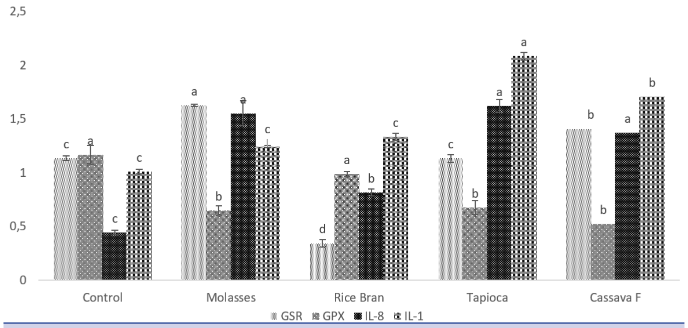

Figure 3. The liver of *Clarias gariepinus*, grown in various carbon-induced biofloc systems, expresses genes associated with immunity (interleukin 1, IL1; interleukin 8), as well as antioxidants (glutathione-disulfide reductase, GSR; glutathione peroxidase, GPX). (n = 5). Significant variations ($p<0.05$) are designated by superscripts in identical columns.

There were notable variations existing in GPX and GSR expression in fish raised in biofloc and in control *C. gariepinus* guts (Fig. 4). The transcription levels of the selected genes in the intestines of *C. gariepinus* (IL1, IL8, GPX, and GSR) displayed significant changes ($p < 0.05$) between the biofloc-treated and untreated groups (Fig. 4). Nevertheless, there was no discernible variation in GSR transcription levels among the fish raised in the Molasses, Tapioca, and control groups, respectively. In addition, the expression of IL-8 was not significant in rice bran, tapioca, and cassava flour, however, IL-1 was clearly upregulated among the biofloc groups.

Wang and Secombes (2013) asserted that cytokines; a byproduct of white blood cells, are essential for controlling and establishing connections between specific and non-specific immune systems. The current investigation showed that when *C. gariepinus* was challenged with *A. hydrophila* and raised in a biofloc system, there was a markedly elevated IL-1 and IL-8 levels. These important cytokines are expressed in fish in response to pathogenic pathogens, as reported by Sakai et al. (2021) in aquatic species.

De Schryver et al. (2010) and Ray et al. (2010) speculated that the significantly improved immunity shown by *C. gariepinus* in the present study may be related to the biologically active ingredients (taurine, carotenoids, phytosterols, and polysaccharides) available in the flocs, which contain a significant amount of possible prebiotics. Popoola and Miracle (2022) reported that biofloc carotenoids perform bioactive physiological tasks, strengthen fish immunity, and supply vital nutrients.

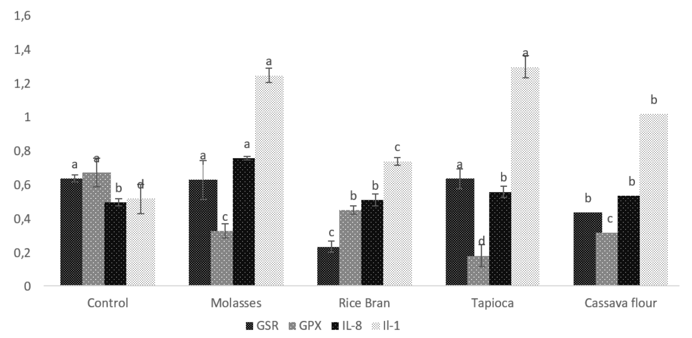

Figure 4. In the gut of *Clarias gariepinus* cultured in various carbon-induced biofloc systems, the expression of genes linked to immunity (interleukin 1 and IL1; interleukin 8) and antioxidants (Glutathione peroxidase, GPX, and glutathione-disulfide reductase, GSR). Significant variations ($p<0.05$) indicated by superscripts in identical columns.

Bioactive compounds with specific antagonistic uses against pathogens have been reported to be present in biofloc, which can control disease occurrence in addition to boosting the immunity of farmed fish (Yu et al., 2023). The probiotic Bacillus, which is the main bacterium in the biofloc and increases fish immunity and resistance to infections, is beneficial to fish farming. Together, GPx and GSR remove hydrogen peroxide (H_2O_2) through a glutathione protection mechanism. Through the oxidation of glutathione (GSH) to glutathione disulfide (GSSG), GPx converts H_2O_2 into water. GSR uses the oxidizing reduction of NADPH to restore GSH after it has been oxidized (Imai and Nakagawa, 2003). Phase II xenobiotic metabolic catalyst glutathione S-transferase (GST) builds larger endogenic compounds through phase I reactions and is easily discharged through the kidney or bile (Diamond, 1993). Based on this study, it is possible that *C. gariepinus* raised in biofloc were fed supplemental diets from rearing medium that significantly increased GSR and GPX transcription in their livers.

Compared with controls, fish in the biofloc group had a greater capacity for defense against infection. These findings may be attributed to the antioxidant activity of the experimental fish. In a related study, Kheti et al. (2017) fed the Rohu microbial floc to their diets and found that this boosted the survival rate of the animals after contracting *Edwardsiella tarda*.

Additionally, it has been determined that the floc components of biofloc contribute to its immune-boosting and antioxidant qualities by scavenging its oxidative activity and enhancing fish immunity (Ahmad et al., 2016: Van Doan et al., 2018; Popoola et al., 2023).

It is interesting to note that in fish raised on biofloc, the expression of the GSR, IL-1, and IL-8 genes was higher in the liver than in the intestine. (Lumsangkul et al., 2021) additionally observed that fish livers exhibit markedly higher relative immunological and antioxidant gene transcription. but not even a discernible variation was observed in *C. gariepinus* intestines other than IL-1. Differences in the quantity of immune cells in each tissue (Lumsangkul et al., 2021) as well as the types of carbon sources that support floc development (Popoola and Oyelade 2021) may explain the variation in relative immune gene expression. Comparable results were noted for antioxidant gene expression in common carp, with increased expression of these genes in the liver rather than the gut. This phenomenon could be explained by the expression of antioxidant genes differently in different tissues under oxidative stress (Lumsangkul et al., 2021). According to Hermesz and Ferencz (2009), oxidative stress enhances the expression of antioxidant genes in the liver of carp and lowers their transcription in other organs.

CONCLUSION

Because fish produced in biofloc exhibit better growth and survival rates, they can help increase output in the aquaculture sector. In comparison with the control, fish species grown in biofloc exhibited improved physiological indicators and reduced stress in biochemical measures, such as total protein, glucose, cholesterol, aspartate transaminase, and alanine transaminase. Fish grown on biofloc exhibited increased antioxidant responses, such as SOD and CAT, indicating a greater capacity to eliminate reactive oxygen species (ROS) resulting from environmental stress. Significant immune responses, lysozyme activity, and MPO were activated in a biofloc-reared fish species. In studies of *A. hydrophila* disease resistance, fish reared in biofloc showed a greater level of resistance. In conclusion, various carbon sources, particularly Molasses, Tapioca, cassava flour, and rice bran, could be successfully used to induce flocs in a rearing medium, and any of them could be used to improve fish immunity against pathogenic organisms while at the same time lessening production stress.

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Ethics Committee Approval: The ethical requirements of the Experimental Animal Welfare Ethics Committee of the Federal University of Technology, Akure, Nigeria were strictly adhered to during the conduct of this study. On the other hand, every attempt was made to reduce the experimental fish's pain and anguish.

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Research Article

Evaluation of Some Aspects of the Growth Parameters and Exploitation Rates of Deep-Water Rose Shrimp (*Parapenaeus longirostris*) from the Sea of Marmara

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ABSTRACT

The deep-water rose shrimp, *Parapenaeus longirostris* (Lucas, 1846) (Decapoda, Penaeidae) is economically significant due to its distinctive long rostrum and attractive pink-red colouring, playing a crucial role in marine ecosystems. This study investigated the length frequency, growth pattern, sex ratio, condition factor, von Bertalanffy growth parameters (L_∞, k, t_0), growth performance index (φ), exploitation rate, and mortality rates of deep-water rose shrimp. Samples were collected from commercial fishermen using beam trawl nets at depths of 60–110 m around the Kapıdağ Peninsula in the Sea of Marmara from September 2021 to April 2022 (excluding January). The minimum total lengths recorded were 58 mm for females and 68 mm for males. The total length-weight relationship exhibited positive allometry, whereas the carapace length-weight relationship showed negative allometry. The sex ratio did not deviate significantly from 1:1. The condition factor of females was significantly higher than that of males. The overall growth parameters, based on total length, were L_∞ = 225.69 mm, k = 0.14 year^{-1}, t_0 = -0.17 year, and φ = 3.88. The estimated mortality rates were: total mortality = 0.83 year^{-1}, fishing mortality = 0.65 year^{-1}, natural mortality = 0.18 year^{-1}, and exploitation rate = 0.78. These findings provide valuable insights for the sustainable management of deep-water rose shrimp in the Sea of Marmara.

Keywords: Decapoda, Exploitation rate, Length-weight relationships, mortality rate, Türkiye

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INTRODUCTION

The Sea of Marmara is a unique geographical feature, acting as a relatively shallow (average depth: 494 metres) semi-enclosed sea, with a maximum depth reaching 1350 m in some areas (Özsoy et al., 2016). It serves as a critical transitional zone between the saltier Mediterranean Sea, connected via the Çanakkale Strait, and the fresher Black Sea, linked by the Istanbul Bosphorus Strait (Öztürk, 2021). This strategic location designates the Sea of Marmara as a vital migration route for commercially valuable species of Atlantic-Mediterranean origin (Öztürk, 2021). These species, found in both the Aegean and Mediterranean Seas, utilise the Sea of Marmara as a corridor to reach the Black Sea (Kocataş et al., 1993).

The deep-water rose shrimp (Penaeidae: *Parapenaeus longirostris* Lucas, 1846) has a broad distribution in the Eastern Atlantic from Portugal to Angola and across the entire Mediterranean. It is also found in the Western Atlantic, ranging from Massachusetts, USA, to French Guiana (Ungaro & Gramolini, 2006). This species inhabits depths of 20 to 700 m, typically between 150 and 400 m, on muddy or muddy sand bottoms. They display sexual size dimorphism; typically, females grow larger than males, with females reaching up to 186 mm and males usually measuring between 140 and 160 mm (FAO, 1980). Although some variation exists, female deep-water rose shrimp across their range share a common reproductive pattern: extended spawning (1-3 peaks) in spring-summer and autumn-winter (location, temperature, and size dependent), with batch-produced eggs and a fecundity range of 20,000-400,000 (Bianchini et al., 2010).

The deep-water rose shrimp is a commercially valuable species, particularly prominent along the Mediterranean Sea coastline (FAO, 2008; Kocataş, 1981; Sbrana et al., 2006). Notably, the Kapıdağ Peninsula is recognised as the Sea of Marmara's most productive fishing ground for shrimp. Beam trawling, the primary method used to harvest crustaceans in the Sea of Marmara, is typically conducted with boats ranging from 7 to 13 m in length (Zengin et al., 2004). According to TÜİK data, 234 beam trawl and dredging vessels operating in the Sea of Marmara harvested 2,129 tonnes of shrimp in 2021 (TÜİK, 2021). The quantity of deep-water rose shrimp caught in the Sea of Marmara surpasses that of the Aegean and Mediterranean Seas, as illustrated in Figure 1, which depicts a 12-year (2011-2022) catch record of the species across these Turkish seas.

Despite its commercial value, the deep-water rose shrimp faces exploitation pressure throughout its range from large fishing fleets in the Mediterranean and Eastern Atlantic, leading to declining stocks (Arculeo et al., 2014). This decline is further exacerbated by pollution and unsustainable fishing practises like beam trawling. The scarcity of recent data on the growth parameters and exploitation rates of deep-water rose shrimp collected from the Sea of Marmara necessitates further research. Consequently, this study, investigated: 1) length frequency, 2) growth pattern, 3) sex ratio, 4) condition factor, 5) von Bertalanffy growth equation parameters, 6) growth performance index (φ), 7) mortality and 8) exploitation rate of deep-water rose shrimp in the Sea of Marmara. The results of this study should contribute to the scientific literature on sustainable stock management for the region.

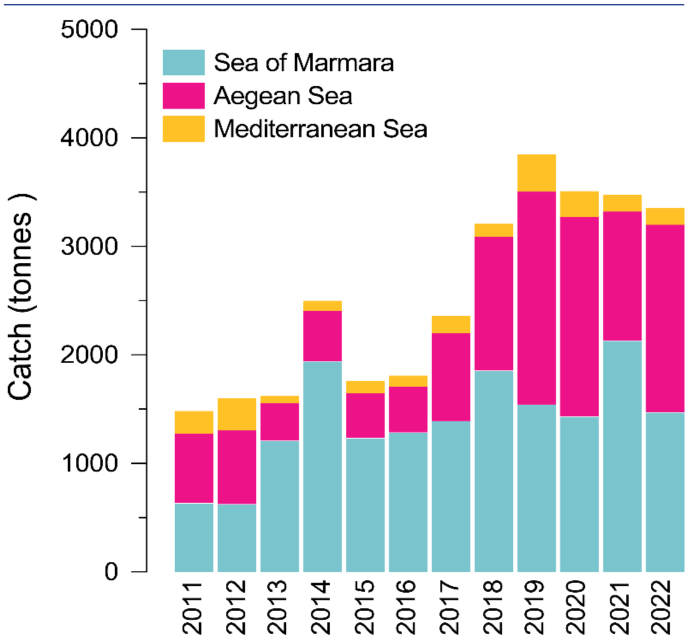

Figure 1. Catches (tonnes) of deep-water rose shrimp (*Parapenaeus longirostris*) in three Turkish seas over 12 years (2011-2022). Data source: TUIK, 2023.

MATERIALS AND METHODS

Sampling

The samples of deep-water rose shrimp were collected during the fishing season from September 2021 to April 2022 by commercial fishermen using beam trawl nets around the Kapıdağ peninsula in the Sea of Marmara at depths ranging from 60 to 110 m. Sampling was not conducted in January because of the closed fishing season. Samples were obtained from regions with high fishing intensity (Figure 2).

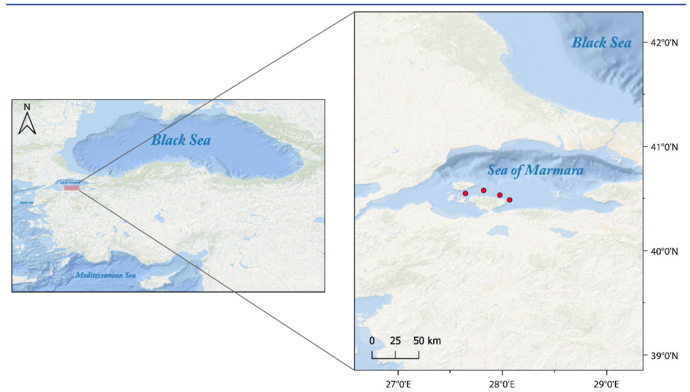

Figure 2. Sampling area in the Kapıdağ Peninsula, Sea of Marmara, for deep-water rose shrimp (*Parapenaeus longirostris*). Sources: QGIS 3.36.2, www.qgis.org

Laboratory work

In the laboratory, 932 deep-water rose shrimp individuals were examined. Each individual underwent the following measurements:

- **Weight (W):** Determined using a balance with a precision of 0.01 g.
- **Total Length (L_T):** Measured in millimetres (mm) from the tip of the rostrum (beak-like projection) to the end of the telson (tail fan) using a ruler with 1 mm precision.
- **Carapace Length (L_C):** Measured using callipers with an accuracy of 0.1 mm. The carapace refers to the hard upper shell covering the head and thorax of the shrimp.

In addition, the sex of each individual was determined through macroscopic observation. This involved examining the shrimp with the naked eye or a magnifying glass to identify any visual differences between males and females.

Statistical analysis of the data

Sex Ratio

The sex ratio of deep-water rose shrimp was investigated using the Chi-square (χ^2) test to determine whether it differed significantly from a 1:1 ratio.

Length-weight relationships

The relationships between the body weight of deep-water rose shrimp and their lengths (either L_T or L_C) were determined using a simple power function (Dürrani, 2023):

$$W = a \times L_T^b \quad (1a)$$

$$W = a \times L_c^b \quad (1b)$$

The estimated value of *b* indicates the growth pattern of deep-water rose shrimp: isometric growth (b ≈ 3), positive allometric growth (b > 3), or negative allometric growth (b < 3)

Condition factor

The condition factor (C_F) was computed using the following function (Htun-Han, 1978).

$$C_F = \frac{W \cdot 100}{{L_T}^3} \quad (2)$$

von Bertalanffy Growth Function

Age classes were determined using the Bhattacharya method in the FISAT (FAO-ICLARM Stock Assessment Tools) programme. The von Bertalanffy growth function was used to determine the size-at-age relationship for deep-water rose shrimp (Bal et al., 2018):

$$L_T = L_{T\infty}(1 - e^{-k(t-t_o)}) \quad (2a)$$

$$L_c = L_{c\infty}(1 - e^{-k(t-t_o)}) \quad (2b)$$

where L_T and L_c are the total length and carapace length at age t, $L_{T\infty}$ and $L_{c\infty}$ are the asymptotic total length and carapace length, k is the growth coefficient, t_0 is the hypothetical age at which the length (total length or carapace length) is zero.

Growth performance index

The growth performance index (φ) was estimated using the Munro and Pauly (1983) equation:

$$\varphi = 2 \times \log_{10}(L_\infty) + \log_{10}(k) \quad (3)$$

Mortality rates

- **Total mortality (Z):** It was calculated using Pauly's method (1984):

$$\log_{10}(Z) = \log_{10}(M) + \log_{10}(F) \quad (4a)$$

- **Natural mortality (M):** It was estimated using Pauly's empirical relationship (1980):

$$\log_{10}(M) = -0.0066 - 0.279 \times \log_{10}(L_\infty) + 0.6543 \times \log_{10}(k) + 0.4634 \times \log_{10}(T) \quad 4b$$

where *T* is the annual average water temperature in degrees Celsius (℃). The average temperature values measured during the fishing period were obtained from the official website of the General Directorate of Meteorology of the Republic of Türkiye. Between September 2021 and April 2022, the annual average water temperature was 14.98℃.

- **Fishing Mortality (F):** It was determined by the difference between total mortality (Z) and natural mortality (M) Gulland (1971):

$$F = Z - M \quad (4c)$$

- **Exploitation rate:** The exploitation rate (E) of the stock was calculated as follows Gulland (1971):

$$E = \frac{F}{Z} \quad (4d)$$

All statistical analyses, including data processing, visualisation, and hypothesis testing, were performed using R software.

RESULTS

Sex ratio

The examination of 932 deep-water rose shrimp specimens revealed a near parity in sex ratio with 49% (n = 458) males and 51% (n = 474) females, translating to a ratio of approximately 1 male:1.03 females. The Chi-Square test also confirmed the absence of a statistically significant difference between male and female counts ($\chi^2 = 0.137$, $P = 0.711$).

Size and Growth

Length frequency distribution

The deep-water rose shrimp exhibited variation in both L_T and L_C, with L_T ranging from 58 to 155 mm for females and 68 to 156 mm for males; and L_C varying from 13.0 to 38.0 mm for females and 12.0 to 38.0 mm for males. The average L_T was 105.9 mm for females and 98.6 mm for males, with average L_C of 21.5 mm and 23.4 mm, respectively. The length frequency distribution based on L_T is provided in Figure 3.

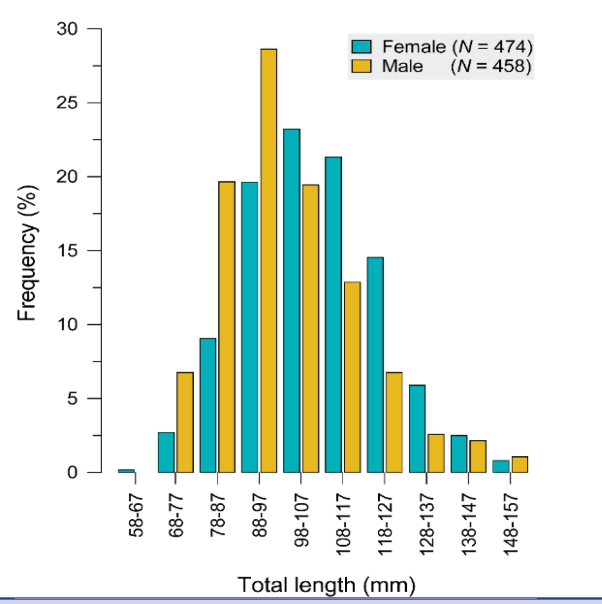

Figure 3. Length frequency distribution of female and male deep-water rose shrimp (*Parapenaeus longirostris*) collected from the Kapıdağ Peninsula, Sea of Marmara

Length-weight relationships

The relationships between the total length–weight and carapace length–weight are illustrated in Figure 4. The results of Student's *t*-test revealed significant deviation from isometric growth for the total length-weight relationship. This indicates positive allometry: deep-water rose shrimp exhibit a trend of becoming relatively heavier than its total length increases. Conversely, for carapace length, weight increases more slowly than increases in carapace length (Figure 4).

Condition factor

It was determined that the difference between men and women in terms of condition factors was significant, and the female condition factor was significantly higher than the male ($t_{(930)}$= 2.67, P < 0.001). The monthly variation graph of the condition factor is provided in Figure 5.

von Bertalanffy growth parameters

For the total samples in the study area, asymptotic total lengths, growth coefficients, age at which the size is 0, and growth performance index were calculated as $L_{T\infty}$ = 225.69, k = 0.140, t_0 = −0.170 and φ =3.884, respectively. The maximum age was deter-

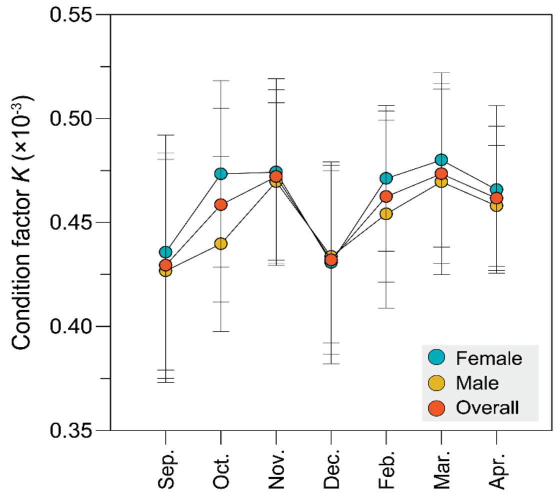

Figure 5. Monthly variation in the condition factor (mean ± s. d.) of deep-water rose shrimp (*Parapenaeus longirostris*) collected from the Kapıdağ Peninsula, Sea of Marmara

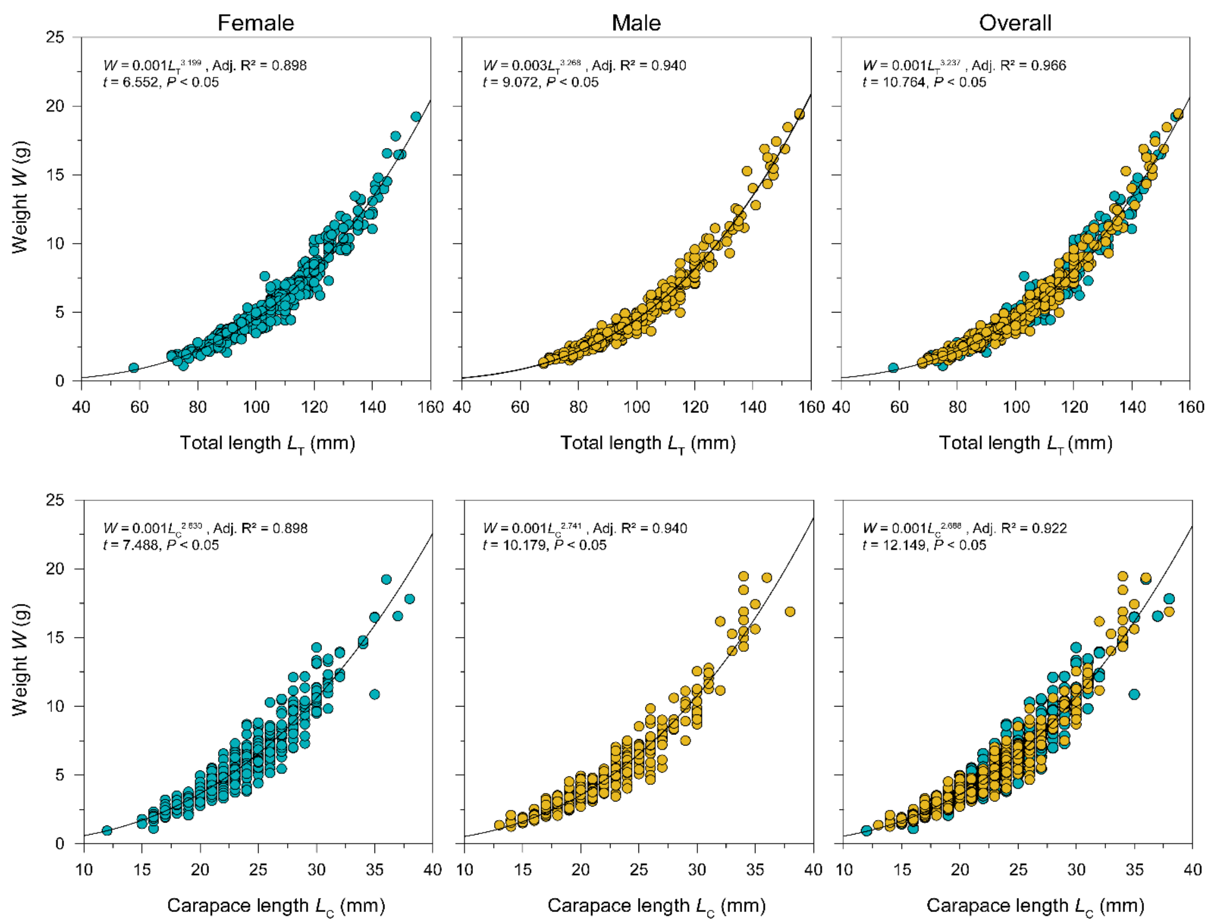

Figure 4. Length-weight relationships of deep-water rose shrimp (*Parapenaeus longirostris*) collected from the Kapıdağ Peninsula, Sea of Marmara. The solid line represents the fitted power regression function. The results of Student's t-test are provided as t-value and P-value, indicating whether the estimated slope b of the power regression function deviates significantly from the isometric growth value.

mined as 5 years for females and 4 years for males. When applying carapace length in the von Bertalanffy growth function, for males, the parameters were determined as $L_{c\infty}$ = 66.08 mm, k = 0.11 year^{-1}, and t_0 = −1.66 years. Meanwhile, for combined male and female data, the parameters were $L_{c\infty}$ = 110.09 mm, k = 0.05 year^{-1}, and t_0 = −2.07 years. However, the von Bertalanffy growth function parameters obtained for the female samples using carapace length fell outside an acceptable range and are therefore not included in the analysis. The growth curve drawn according to the von Bertalanffy growth function parameters is provided in Figure 6.

Mortality and Exploitation Rate

Mortality rate parameters were estimated separately for males, females, and all samples combined. For males, the natural mortality rate was estimated to be 0.23, the fishing mortality rate was 0.56, and the total mortality rate was 0.79. This resulted in an exploitation rate of 0.70. Similarly, for females, the estimated values were as follows: natural mortality rate = 0.11, fishing mortality rate = 0.49, total mortality rate = 0.60, and exploitation rate = 0.81. When considering the overall population, the estimated rates were as follows: natural mortality rate = 0.18, fishing mortality rate = 0.65, total mortality rate = 0.83, and exploitation rate = 0.78. These rates indicate different dynamics between male and female deep-water rose shrimp. Males exhibited a higher natural mortality rate than females (0.23 vs. 0.11), but a slightly lower exploitation rate (0.70 vs. 0.81). The overall exploitation rate of 0.78 suggests that a substantial portion of the population is being harvested.

DISCUSSION

This study explored the length-weight relationship of deep-water rose shrimp using both total length and carapace length. While carapace length indicated negative allometry (b<3), consistent with previous research (e.g., Tosunoğlu et al., 2009), total length revealed positive allometry (b>3) for both sexes. This finding emphasises the importance of considering both metrics for accurate growth assessments. The sex ratio in this study was 1:1.03, consistent with some previous studies (Demirci & Hoşsucu, 2007; İhsanoğlu & İşmen, 2020; García-Rodríguez et al., 2009), but differing from others (Dereli, 2010; Çiloğlu & Ateş, 2022; Kapiris et al., 2013), suggesting variations due to factors like mortality differences and behavioural traits such as migration (Amin et al., 2009).

This study found the condition factor C_F to be highest in March (spring) and lowest in September (autumn), which is in line with observations by Arslan İhsanoğlu and İşmen (2020) in the Sea of

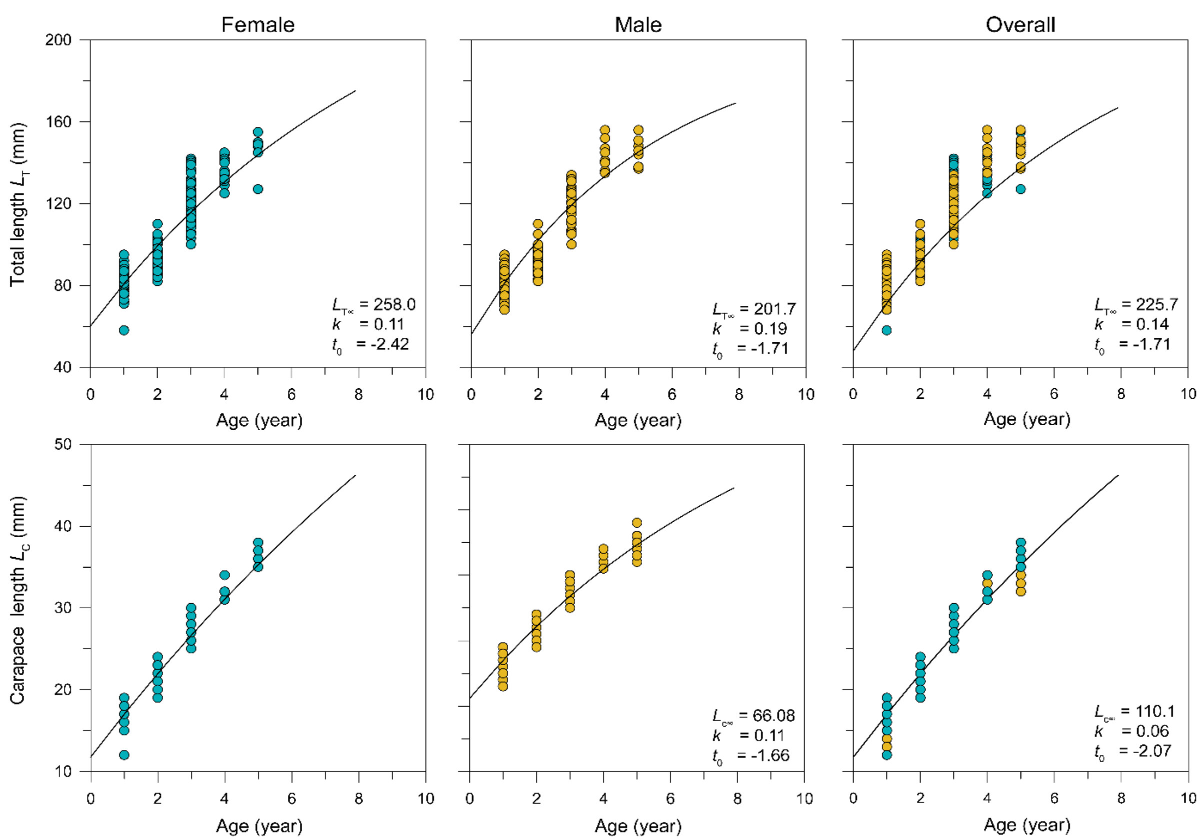

Figure 6. The von Bertalanffy growth function fitted to the size-at-age relationship for deep-water rose shrimp (*Parapenaeus longirostris*) collected from the Kapıdağ Peninsula, Sea of Marmara. The curve illustrates the predicted growth trajectory derived from the estimated parameters of the function for male and combined samples. The curve for females was provided using the estimated parameters of the overall samples.

Marmara (2011-2014). Piper (1972) found C_F to be stable with consistent water temperature and feeding rates, further supported by Ahmad & Ahmed (2019), who observed that seasonal changes in feeding intensity and reproduction likely drive the observed C_F variations.

In this study, the maximum age observed was 5 years, consistent with a study conducted in Sığacık Bay, documenting a maximum age of 6 years (Tosunoğlu et al., 2009). However, previous studies in the Mediterranean region reported lower maximum ages of 4 and 3 years (Demirci & Hoşsucu, 2007; Manaşırlı et al., 2008), and another study in the Sea of Marmara reported a maximum age of 3 years (Zengin et al., 2004). The estimated von Bertalanffy growth function parameters using total length differed from those reported by Zengin et al. (2004). Their study found female and male $L_{T\infty}$ values of 170.2 mm and 157.9 mm, respectively, with corresponding k values of 0.58 and 0.38 (Table 1). In contrast, this study estimated a larger $L_{T\infty}$ for both females (258.0 mm) and males (201.7 mm), suggesting a greater potential size for this shrimp in the studied area. Additionally, the k values obtained in this study were considerably lower (females: 0.11, males: 0.19) than those obtained by Zengin et al. (2004), indicating a slower growth rate in the studied population. Notably, both studies estimated negative t_0 values (females: −2.42 years, males: −1.71 years; overall: −1.71 years), which is typical for the von Bertalanffy growth function. Compared to using total length, carapace length L_c has been applied by several studies to fit the carapace length-at-age relationship (Table 1). The lowest calculated $L_{c\infty}$ for individuals caught in the Sea of Marmara was 16.28 mm (Deval et al., 2006), whereas the highest $L_{c\infty}$ was recorded on the Mediterranean coast of Morocco at 52.87 mm (Awadh & Aksissou, 2020). In this study, $L_{c\infty}$ was determined to be 66.1 mm for males, while no estimation was performed for the female samples. Furthermore, the estimated $L_{c\infty}$ from combined data was 110.1 mm, which could be the highest estimation recorded to date. Consequently, the estimation of the carapace length-at-age relationship with von Bertalanffy growth function in this study might need further confirmation.

The growth performance index in this study was generally similar to the results of previous study for both gender groups. However, in a study conducted on the Balearic Islands, the growth index was calculated as 6.88 for males, 7.40 for females, and 7.20 for all samples (Guijarro et al., 2009). The exploitation rate was determined to be much higher than that in previous studies conducted in the Sea of Marmara and the Aegean Sea, indicating significant catch pressure on the species (Demirci & Hoşsucu, 2007; Tosunoğlu et al., 2009; Dereli, 2010; Çiloğlu & Ateş, 2022; İhsanoğlu & İşmen, 2020). In contrast, the exploitation rate found in this study was higher than that found in other previous studies, indicating high fishing pressure on the species.

CONCLUSIONS

This study elucidates the growth and population dynamics of the deep-water rose shrimp in the Sea of Marmara. By analysing both the total length and carapace length, this study revealed a

Table 1. Length-weight parameters, growth types, sex ratios, and growth parameters of deep-water rose shrimp (*Parapenaeus longirostris*) from this study and existing literature.

Sex	n	Sex ratio	Length-weight relationship				von Bertalanffy growth parameters				Mortality rates				φ	Location	References
			a	*b*	r^2	GT	$L_{T\infty}$	$L_{c\infty}$	*k*	t_0	F	NM	Z	E			
M	923		0.002	2.625	0.91			31.2	0.76	−0.39	3.86	1.31	5.17	0.74	2.87	Babadillimanı	Manaşırlı et al. (2008)
F	2859		0.001	2.819	0.95			32.3	0.77	−0.39	2.12	1.29	3.41	0.62	2.90		
O	3886		0.001	2.795	0.95			32.1	0.76	−0.39	2.71	1.29	4.00	0.67	2.89		
M	1313		0.001	2.690	0.94			35.0	0.41		0.54	0.67	1.21	0.45		Aegean Sea	Dereli (2010)
F	2456		0.001	2.700	0.95			41.3	0.31		1.71	0.77	2.48	0.69			
O	3768	1:09	0.001	2.700	0.95												
O	2238	1:1.2	1.871	1.843				34.6	0.48	−1.01	0.34	1.29	1.63	0.21	2.76	Northeastern Mediterranean	Demirci & Hoşsucu (2007)
M	1073		0.011	2.590	0.93												
F	869		0.006	2.950	0.95											Sea of Marmara	Yazıcı (2004)
M	1076		0.006	2.862	0.96											Sea of Marmara	
F	2679		0.003	3.130	0.98												Bayhan et al. (2005)
O	3755		0.003	3.160	0.97												
O	12932							16.3	0.49		1.86	0.97	2.83			Sea of Marmara	Deval et al. (2006)

Table 1. Continue.

Sex	n	Sex ratio	Length-weight relationship				von Bertalanffy growth parameters				Mortality rates				φ	Location	References
			a	*b*	r^2	GT	$L_{T\infty}$	$L_{c\infty}$	*k*	t_0	F	NM	Z	E			
M	1416		0.012	2.610		A–		28.6	0.95	−0.76	0.71	1.33	2.04	0.35	2.89	Sea of Marmara	Çiloğlu & Ateş (2022)
F	2410		0.010	2.687		A–		32.5	0.76	−0.23	1.20	1.09	2.29	0.52	2.91		
O	3826	1:1.7															
F	20384		0.002	2.500	0.94	A–		41.0	0.27	−0.78	0.57	0.52	1.09	0.52	2.66	Sea of Marmara	İhsanoğlu & İşmen (2020)
M	15904		0.003	2.430	0.89	A–		36.8	0.37	−0.69	1.56	0.66	2.22	0.70	2.70		
O	36288	1:1.3	0.002	2.520	0.94	A–		42.0	0.35	−0.69	0.99	0.61	1.60	0.62	2.79		
F	2456		0.0001	2.840	0.95	A–		41.3	0.31	1.039	1.71	0.77	2.48	0.69	2.73	Aegean Sea (Sığacık Bay)	Tosunoğlu et al. (2009)
M	1313		0.0001	2.760	0.94	A–		35.0	0.41	1.016	0.54	0.67	1.21	0.45	2.70		
O	3768		0.0001	2.830	0.95	A–		42.0	0.5		1.18	0.77	1.95	0.60	2.95		
F								34.7	1.05	−0.95			1.19		3.14	Aegean Sea (Saros Bay)	Bilgin et al. (2009)
M								27.0	1.49	−0.88			0.88		3.00		
O																	
F	2483		0.005	2.930	0.95		170.2		0.581	0.96					4.23	Sea of Marmara	Zengin et al. (2004)
M	1964		0.009	2.700	0.91		157.9		0.380	1.42					3.98		
O	4447		0.005	2.970	0.95												
M	214		0.002	2.530	0.97			31.3	1.00	0.49					6.88	Balearic Island	Guijarro et al. (2009)
F	593		0.002	2.560	0.98			44.0	0.67	0.21					7.16		
O	807		0.002	2.560	0.98			40.0	0.84	0.49					7.20		
M								33.1	0.93	0.05					3.01	Tiran Sea	Ardizzone et al. (1990)
F								44.4	0.74	0.13					3.16		
O			0.006	2.270				30.5	0.63	0.190			1.23		2.77	Sicilian Channel	Levi et al. (1995)
O	1510		0.003	2.312	0.97	A–		52.8	0.39	−0.35			3.49	0.68		Morocco	Awadh & Aksissou (2020)
M	866		0.003	2.380	0.94										2.87	Ionian Sea	Kapiris e al. (2013)
F	1282		0.001	2.620	0.91			37.2							2.87		
O	2148	1:0.6													3.02		
M	4948		0.003	2.490	0.91	A–		36.0	0.49	0.07						Gulf of Alicante	García-Rodríguez et al. (2009)
F	6665		0.002	2.560	0.96	A–		47.0	0.43	0.13							
O	11613	1:1.3	0.002	2.610	0.96	A–		45.0	0.39	0.10							
M	458		0.003	3.260	0.93	A+	201.7		0.19	−1.71	0.56	0.23	0.79	0.80	3.89	Sea of Marmara	Present study
F	474		0.001	3.190	0.89	A+	258.0		0.11	−2.42	0.49	0.11	0.60	0.81	3.87		
O	932	1:1.03	0.001	3.230	0.96	A+	225.6		0.14	−1.71	0.65	0.18	0.83	0.78	3.85		
M	458							66.1	0.11	−1.66							
F	474																
O	932							110.1	0.06	−2.07							

M = male, F = female, O = overall (F+M); n = size; a = regression constant, b = coefficient of allometry; GT = growth type: A- = negative allometry, A+ = positive allometry; $L_{c\infty}$ = maximum theoretical size of carapace length (mm); $L_{T\infty}$ = maximum theoretical size of total length (mm); NM: natural mortality; φ = growth performance index larger potential size, but slower growth rate compared with prior studies. The observed differences in growth patterns between length measures emphasise the importance of using appropriate metrics. Additionally, the estimated maximum age, exploitation rate, and sex ratio provide valuable insights for stock assessment and management. However, some extreme values, particularly in carapace length, necessitate further investigation. In sum, this study demonstrates the complex interplay of biology and environment on deep-water rose shrimp, emphasizing the imperative for ongoing research and implementation of sustainable management practises.

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Research Article

Effects of Bacterial-Fungal Coinfection on Biochemical Parameters and Organ Structure in African Catfish (*Clarias gariepinus*)

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ABSTRACT

Five infected African catfish (605±45.98 g) from the Experimental Aquaculture Base at Trakia University exhibited abnormal behaviours, including sluggish movements, lethargy, skin haemorrhages, and fin bleeding. Samples from the lesions were cultured on Sabouraud Dextrose Agar (SDA), and the fungus was identified as *Rhizopus* sp. based on colony morphology and microscopy. Bacteriological samples from the kidney, spleen, liver, and blood were cultured on Tryptic Soy Agar (TSA) and Brain Heart Infusion Agar (BHIA), revealing the presence of *Yersinia ruckeri, Aeromonas hydrophila*, and *Pseudomonas aeruginosa* based on their physiological, morphological, and biochemical traits. Significant differences ($p<0.01$) were observed in the serum protein profiles, including total protein, albumin, globulin, and glucose levels. Histological examination showed pathological changes in the liver, kidneys, and gills, as well as haemorrhages and polymorphonuclear leukocyte infiltration in the skin lesions, with intense muscle necrosis. These biochemical and histopathological findings are valuable for monitoring health and diagnosing fungal diseases in African catfish.

Keywords: African catfish, biochemical parameters, pathogen bacteria, *Rhizopus* sp.

INTRODUCTION

The African catfish (*Clarias gariepinus*) is an omnivorous freshwater fish and is widely introduced in Africa, Asia and Europe. It was introduced for fish farming and aquaculture from Africa to Europe several times in the early 1970s. For the first time in Bulgaria was introduced in 2007 at the Ovcharitza Reservoir for aquaculture (Bozhkov et al., 2023). It is a preferred aquaculture fish species because of its rapid growth rate, ability to survive in unsuitable environments, and resistance to diseases and stress (Ikpegbu, Ezeasor, Nlebedum & Nnadozie, 2013). Fungal diseases are the most recurrent type of disease problem, which cause low productivity of fish and serious economic losses in aquaculture (Bangyeekhun & Sylvie, 2011). Fungal infections occur in all life stages of fish and are mainly caused by immune suppression. *Rhizopus stolonifer* is one of the most common and fastest-growing species in the *Zygomycota* phylum and is commonly known as black bread mould, a worldwide distributed species, which may cause disease, especially under favourable predisposing conditions, resulting in unacceptable mortality (Bautista-Baños, Bosquez-Molina & Barrera-Necha, 2014). *Rhizopus* sp. was isolated and identified from different fish species (Rashmi & Chandan, 2015). Bulging eyes, loss of colour, and ulcers (cysts) in internal organs have been reported in catfish infected by *Rhizopus stolonifer*. In addition, the pathogen can cause damage to multiple body systems, such as the liver, kidney, and brain. Over 70% of these infections are polymicrobial, as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aeromonas hydrophila* and *Escherichia coli* are the most commonly co-isolated bacterial species (Kumar & Prakash, 2020). Despite significant interest in African catfish aquaculture, knowledge about fungal pathogens and mixed infections in farmed fish remains limited. This study reports the first case of *Rhizopus* sp. and opportunistic bacterial infections in Bulgaria. This paper details the biochemical changes and histological features of internal organs, offering important baseline information for diagnosing and treating disease agents in cultivated African catfish (*Clarias gariepinus*).

MATERIALS AND METHODS

Fish sampling and clinical examination

Five African catfish (605±45.98g) from the Experimental Aquaculture Base, Trakia University in Stara Zagora, Bulgaria - 42°23'32.39" N 25°34'10.19" E were investigated. The medical history of the diseased fish (anamnesis vitae and anamnesis morbi) was taken, and the clinical findings of haemorrhage and white lesions on the whole body surface (Figure 1b, c) and haemorrhagic fin (Figure 1d) were observed in the physical examination. The rearing conditions were as follows: photo-period 8 h L: 16 h D with low light intensity (40 lux) in RAS with low water exchange. Fish are fed twice daily (3% body weight) with commercial pellets (Aqua Wels Swim, Garant-Tiernahrung GmbH, Austria).

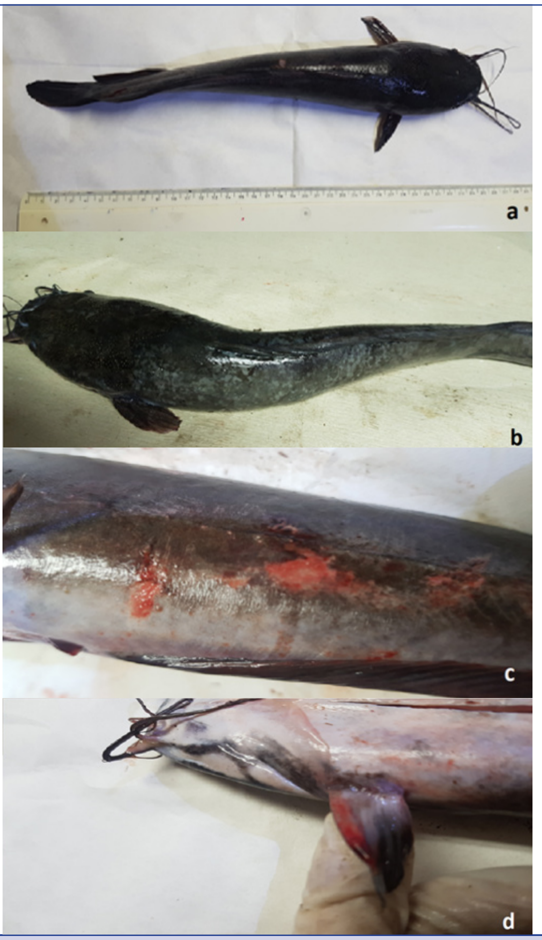

Figure 1. Control fish (a), white (b), and haemorrhagic lesions (c) on the skin, haemorrhagic fins (d).

Hydrochemical analyses

Water samples were collected 80 cm below the water surface in aseptic containers and immediately shipped to the laboratory in a cold environment. The hydrochemical parameters (SO_4-, NO_3-N, NO_2-N, NH_3-N, PO_4- and permanganate oxidizability) were measured using a colorimeter (Hach DR/850, USA) and compatible reagents (HACH Company, Loveland, USA) according to the operating manual. The values of sulphates were 50.75±1.48 mg L^{-1}, nitrate nitrogen 6.42±1.15 mg L^{-1}, nitrite nitrogen 0.160±0.02 mg L^{-1}, nitrogen 0.515±0.08 mg L^{-1}, phosphates 1.37±0.24 mg L^{-1}, permanganate oxidizability 1.33±0.08 mg L^{-1} respectively.

Biochemical analysis

Blood for analysis (approximately 1.2 ml) was drawn from the caudal vein using an aseptic technique in plain containers for biochemical analysis (K_2EDTA). Selected serum biochemical parameters were analysed using Auto Chemistry Analyser (Mindray BS-240VET, Mindray Bio-Medical, China). Plasma cortisol was assayed using a laser fluorescence reader (i-chroma TM Reader, Boditech Med, Korea). The fibrinogen concentration was measured using the BN2 System (Siemens Healthcare Diagnostics).

Parasitical and fungal examination

The presence of external parasites was investigated by examining fresh samples from haemorrhagic and white skin lesions (Buchmann, 2007). The samples were taken from diseased fish's white lesions and inoculated onto Sabouraud Dextrose Agar (SDA) plates for fungal analysis (Bautista-Baños et al., 2014). The choice of culture media was based on our knowledge that *Aspergillus* sp. and *Mucor* sp. showed very good growth on the three culture media but *Rhizopus* sp. grew only on PDA (Potato Dextrose Agar) and SDA. Fungi recovered from the skin lesions of diseased catfish were identified according to their microscopic character and colony structure.

Bacterial identification

Bacteriological samples were taken from the liver, spleen, kidney, and blood and inoculated onto Tryptic Soy Agar (TSA) and Brain Heart Infusion Agar (BHIA). The isolated bacteria were identified using the conventional bacteriological method (Gram staining, motility, cytochrome oxidase and catalase activity, fermentative degradation of glucose (O/F), O/129 (2,4-diamino-6,7-diisopropyl pteridine) sensitivity,beta-galactosidase), and API 20E (Austin & Austin, 2016).

Histopathological examination

The changes induced by the detected disease agents in the tissues were examined using routine histological methods Culling (1963). The fish were sacrificed by concussion according to Council Regulation (EU) 1099/2009 and autopsied. The tissue samples from the skin, gill, kidney, liver, and spleen were immediately fixed in 10% buffered formalin at room temperature for 24 h, dehydrated with graded series of ethanol, treated with xylene, and processed for paraffin embedding. Paraffin blocks were sectioned (4-5 µm thickness) on a microtome Leica RM 2125 (Leica Microsystems GmbH, Austria), dewaxed and stained with haematoxylin and eosin (H&E), and the slides were examined under an Olympus BX-51 light microscope equipped with an Olympus

DP72 digital camera (Culling, 1963).

Statistical analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA). The results are presented as the mean and standard deviation of the mean (Mean ± SD). Statistical significance is indicated by $p < 0.05$* and $p < 0.01$ **.

RESULTS AND DISCUSSION

External examination

During the physical examination, it was observed that the fish presented with lethargic and erratic swimming activity. It may be hypothesised that low water temperature, and the increased glucose levels found at a later stage, generally affected the behaviour of the fish. According to Barton (2002), the changes in fish behaviour and disease resistance may result directly or indirectly from the primary and secondary responses in the organism.

Affected fish demonstrated clinical signs of haemorrhage and white lesions were present on the surface of the skin and haemorrhagic fin, however clinical findings such as bulging eyes and loss of colour described by Kumar & Prakash (2020) or deeply cottony surface texture reported by Adamu, Muhammad, Ahmad, Ahmad, & Yakubu (2020) have not been observed. The described symptomatic fungal lesions were not only superficial but also penetrated the fish muscles (Figure 1). These results are in agreement with those of Haroon et al. (2014) where the posterior part, which includes all fins and body of the fish, had a significantly higher infection rate (53%) as compared to the anterior part of the fish.

The morphological features of the colony included a cottony texture with a white to grey-brown colour on the top and a pale white colour on the reverse of the plate. The microscopic photograph shows the shape and arrangement of the sporangium. The microscopic examination was used for further identification of isolates up to the genus level. The colony structure and microscopic characteristics of fungal isolates from the skin lesions of moribund catfish were very similar to *those of Rhizopus* sp. (Bautista-Baños et al., 2014; Rashmi & Chandan, 2015) (Figure 2). Based on the body of the mycelia and the types of hyphae, sporangiophores were identified *as Rhizopus stolonifer* as the cause of the infection. The results align with previous studies that identified the same fungal species in catfish (Kalan et al., 2016).

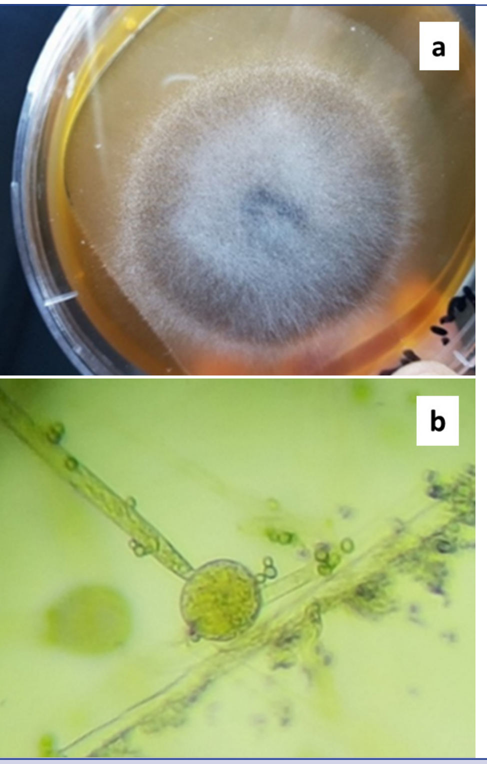

Figure 2. *Rhizopus* sp. on the SDA (a), Microscopic character of *Rhizopus* sp. with its pin head sporangium (b).

Condition and hydrochemical analysis

From the fish farm hygiene point of view, some of the prerequisites that increase the threat of fungal infection are hydrochemical parameters, water temperature, moisture of feed (above 14%), etc. The current fungal species are determined to be infectious and may spread through the contamination of feed (Ikpegbu et al., 2013). The development of fungal infection as well as mycotoxin production is environment sensitive, and has suggested the huge humidity in aquaculture to be a prerequisite for the flourishing of them in feed. To rule out the probability that the disease was due to the presence of mycotoxins with a sufficient level of certainty, a sample was sent to an approved laboratory, where they were evaluated for AFB1; DON; 3-Acetyl-DON; 15-Acetyl-DON; DON-3-glucoside; OTA; T-2/TH-2; FB1; FB2 and ZEA using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (TSQ Quantum Access MAX, Thermo Fisher Scientific Inc, USA). The final result from the analysis (Protocol A3694) does not involve positive data.

Poor water quality is one of the most important factors favouring the fungus growth. Kumar & Prakash (2020) reported that one of the main reasons for the development of infection in African catfish (*Clarias batrachus*) is cultivated in substandard water quality or an overstocked fish tank. In the current case, except for the water temperature (at the time of sampling was 19.3°C), the physical-chemical parameters of the water as well as the feed were according to the standards for cultivation and requirement of the species. Hence, it can be concluded that the low temperature of water was the main prerequisite for the occurrence of the fungal infection.

Internal examination

Rhizopus spp. have been considered as opportunistic pathogens and they may cause disease under favourable predisposing conditions (Refai, Laila, Amany & Shimaa, 2010). Additionally, the microbial invasion with pathogen bacteria may be facilitated by the fungal agent, *Rhizopus* sp., which causes skin and muscle damage and creates a gateway for infection. This earlier finding is in agreement with the recent findings by Adamu et al. (2020), who reported that *Rhizopus* sp. was the most frequent isolate and may easily cause secondary bacterial infection. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* are the most commonly co-isolated bacterial species (Kumar & Prakash, 2020).

After the incubation of the bacteriological inoculations from the visceral organs, three different bacterial colonies on TSA and

BHIA were observed at 24-25 °C for 48 h. The three different types of bacteria have Gram-negative and bacillus forms; It has been determined that one bacterial species gives a negative reaction to cytochrome oxidase, unlike other bacterial species. According to the API profile results, the bacteria isolated from the catfish were identified as *Y. ruckeri* (530510057), *A. hydrophila* (724752656), and *P. aeruginosa* (220600403). There have been reports that these pathogenic bacteria were isolated from diseased catfish (Magdy, El-Hady, Ahmed, Elmeadawy & Kenwy, 2014).

Biochemical analysis

The results of the biochemical analysis are presented in Table 1.

Table 1. Blood plasma biochemical parameters of infected African catfish (Mean±SD).

Parameters	Infected	Healthy
Total Protein, g/L	33.07±0.35	41.53±2.91**
Albumin, g/L	13.83±0.23	16.89±1.61**
Globulins, g/L	19.16±0.35	24.79±3.06**
A/G ration	0.72/1	0.68/1
Glucose, mmol/L	13.09±0.23	5.19±2.25**
Triglycerides, mmol/L	2.32±0.11	2.46±2.07
Total cholesterol, mmol/L	3.44±0.37	5.45±2.08
AST, U/L	94.5±34.9	76±2.12
ALT, U/L	23.67±7.77	24.1±2.54
Alkaline phosphatase, U/L	11±2.41	27.95±16.82*
Creatinine, µmol/L	21.16±7.86	19.01±1.79
Urea, mmol/L	0.66±0.11	0.57±0.19
Uric acid, µmol\l	23.17±11.83	8.4±4.11*
Calcium, mmol/L	6.12±1.47	4.55±0.83*
Phosphorus, mmol/L	2.88±0.07	2.73±0.35

Different superscripts *(p < 0.05) and **(p < 0.01) indicate significant differences

Aquaculture conditions expose fish to several environmental and husbandry-related stressors such as salinity, hypoxia, and temperature. One of the main stressors in catfish is cold-shock stress because it cannot tolerate cold water. In African catfish, the immune system slows down in exposition to low water temperature (less than 20°C) and eventually leads to illness/death. Stressed fish will have a disturbance in homeostasis and reduced endurance, making them vulnerable to coinfection, whether caused by parasites, bacteria, fungi, or viruses. In addition, stress caused by fungal infection leading to haemostatic imbalances in fish affects the blood biochemical profile of infected fish. An indication that fish are stressed is an increase in blood glucose levels. Results of plasma glucose values revealed a significant elevation ($p<0.05$) in the diseased fish compared with the healthy fish. These results are in agreement with those of Reid et al., (2022), who explained that acute cold stress led to increased blood glucose concentrations and suppression of antioxidant enzyme activity in the kidneys, liver, and gills, whereas chronic cold stress led to decreased blood glucose, enlarged liver protein content, and elevated lipid peroxidation in the same internal organs. Regarding chronic cold stress, the last one is not accepted as a rule, as it can vary among fish species and duration of the stressor.

In the veterinary practise, some biochemical parameters are used to measure kidney function, including creatinine, urea, and uric acid. The rising concentrations of creatinine and urea in the blood are signs of kidney damage. In the present case, the urea accumulated in the plasma was relatively the same as that in other airbreathers' teleosts, and the rate of urea excretion was unchanged via the ornithine-urea cycle (OUC). These results are in agreement with those of Ip et al. (2004) who described the absence or low activities of hepatic OUC enzymes, especially ornithine transcarbamylase in the liver of the adult *C. gariepinus*. In contrast, the uric acid showed a significant increase ($p<0.05$) in the diseased fish compared to the healthy ones. We sought an explanation for these results, in renal impairment, particularly with regard to the process of excretion (Figure 3a).

In declining renal function accompanied by an excess of purine precursors, higher uric acid concentrations may be observed. Regarding the data of AST in our study, it may be suggested that activities were elevated because of compensation by providing alpha-ketoglutarate due to a positive correlation between am-

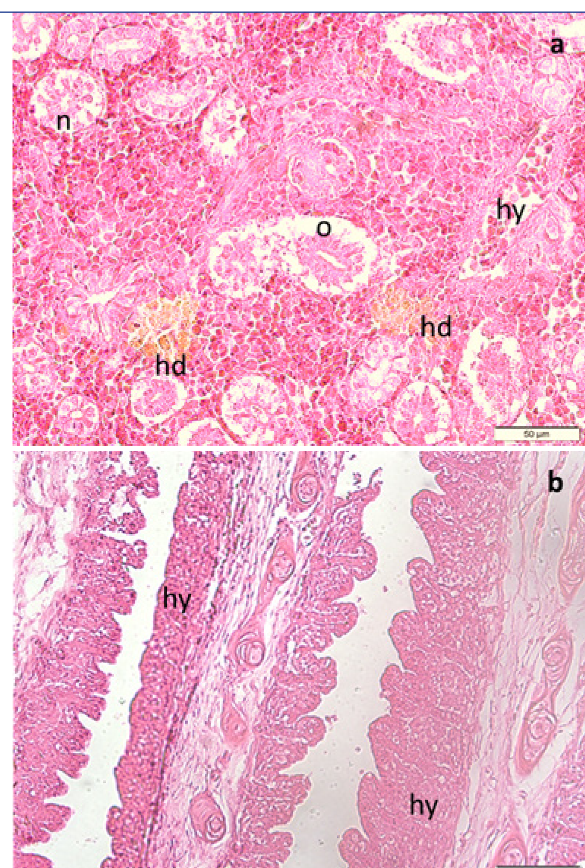

Figure 3. Necrosis of the renal tubules, hyperaemia (hy), peritubular oedema (o), and hemosiderin deposits (hd) in the kidney (a); hyperplasia (hy) between the primary gill lamellae (b) (H&E).

monia components and enzyme level. Considering that more than 80% of the nitrogen excretion in the teleost is by passive diffusion through the gills, with only a small amount excreted by the kidney, it may be reasonable to assume that the pathological changes in the gills also affect the blood plasma concentrations (Figure 3b). Separately, the hydrochemical parameters, especially NH_3 near the gill surface (present case 80 cm), are also important for ammonia excretion, but in the current case, except for the water temperature, all the measured parameters were according to the standard for the cultivation of the species. Moreover, gill hyperplasia is a pathological condition of gill tissue caused by disease, poor water quality, or gill injury. It is thought that the hyperplasia detected in this study may have been caused by secondary infection (fungi and bacteria).

The values obtained for total protein, albumin, and globulin in this current investigation were slightly lower than those for healthy catfish but within the reference value for species as reported by Secer et al. (2018). Also, the present study documents that a decrease in total protein, albumin, and globulin levels may be due to the activation of a humoral immune response against fungal invasion at cold water temperatures.

Certain enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are used as indicators of tissue damage and liver dysfunction. In general, blood ALP levels increase in the fish during stress and parasitic infection, but the pathophysiological reason for the elevation is still unclear. In this case, the blood levels of ALP were twice as high as with an AST/ALT fluctuation that was never above the reference range. An opportunistic infection can be assumed to have caused the obstruction and cholestatic liver injury, which is demonstrated by papillary stenosis or intrahepatic or extrahepatic biliary strictures (Figure 4a, b). All of this makes it likely that *Rhizopus* sp. is the primary factor in increasing ALP.

In the current study, the hemosiderin deposits around the red pulp and discharge in the white pulp was observed in the spleen tissue. Hemosiderin, which appears brown with the H&E staining method, is the pigment found in MAs (Macrophage aggregates). Hemosiderosis is known to be caused by an increased rate of destruction of erythrocytes in the spleen. This pathological condition may be caused by alpha- and beta-haemolytic bacteria isolated from the diseased catfish in this study.

When tissue samples taken from the lesional skin were examined histopathologically, the presence of club cells in the epidermis, haemorrhage, enlargement of the stratum spongiosum of the dermis, and infiltration of polymorphic nuclear leukocytes were detected. In the current case, haemorrhage and polymorphonuclear leukocyte infiltration in the skin lesions and intense necrosis in the muscles were observed (Figure 5a, b, c, d). Most fungi are characterised to attack the external tissues and only a few species infect the internal organs of fish. In this study, the bacteria-accompanying fungal infection were the bacteria that cause septicaemia. For this reason, more lesions (haemorrhage, polymorphous nuclear leukocytes) may have occurred on the skin.

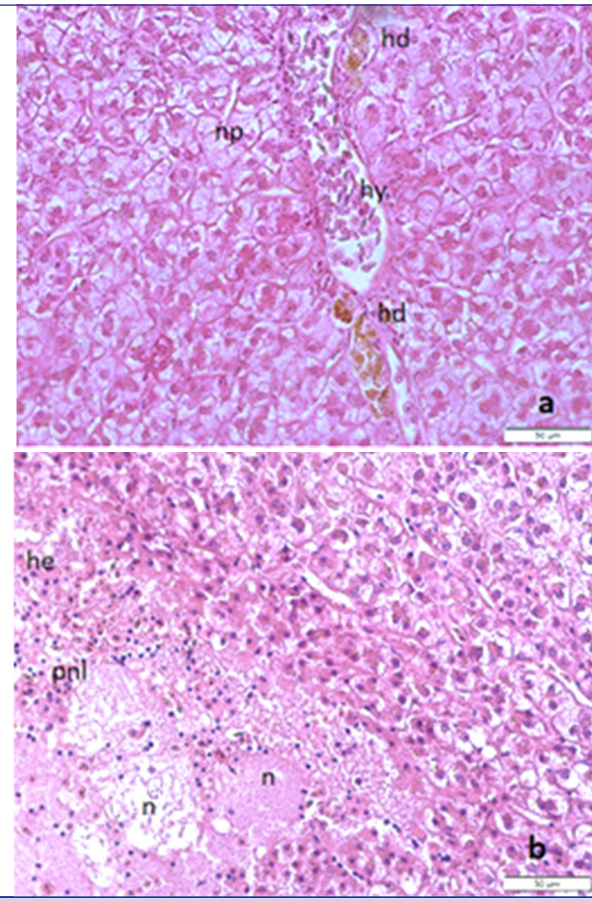

Figure 4. Deposition of hemosiderin (hd) around the blood vessels, hyperaemia (hy) and necrotic hepatocytes (np) (a), necrosis (n), polymorphous nuclear leukocytes (pnl) infiltration, haemorrhages (he) (b) (H&E).

Treatment

Currently, there is no known cure for *Rhizopus* sp. co-infection in fish. The available scientific literature, advances in research and new medicines being developed, obliges us to a certain extent to try to present starting points for achieving adequate treatment. We will first focus on external damages. The haemorrhagic fins are considered less serious damages and after sodium chloride (NaCl) or broad-spectrum antibiotic treatment (Optimal dose/3 days) frequently complete recovery and fish themselves survive. However, severe cases of fungal infection may lead to complete erosion of the fins. In addition, accompanied by a disseminated infection in the gills and eyes, they become fatal. In such a condition, medical treatment is not applicable and the patients die.

Second, certain amino acids, such as L-alanyl-L-glutamine (Recommended dosage 1-4 g/kg) and arginine (1.5-5 g/kg) supplemented by feed in high-fat diets with prebiotics for African catfish, may improve intestinal metabolism and antioxidation mechanism even at low water temperatures (Hu, Zhao, Wang, Sun & Wang, 2022).

Third, nowadays, antifungal antibiotics mainly target the cell wall or cellular membrane metabolism, and their effect is sometimes

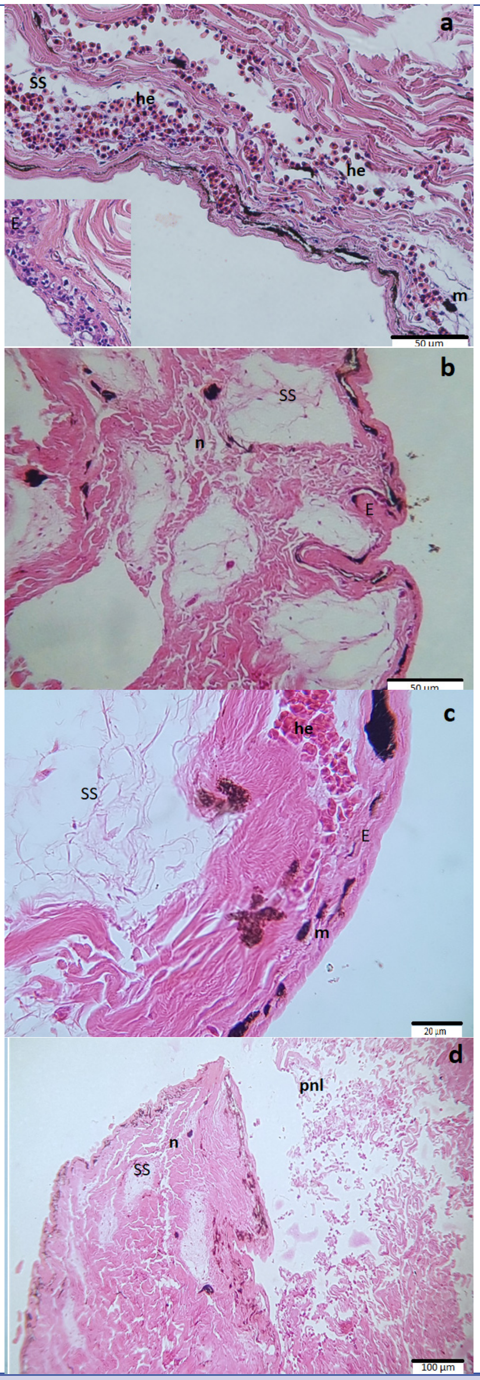

Figure 5. Club cells in the epidermis (E), haemorrhage (he) in the dermis layer (a,c), enlargement in the stratum spongiosum (SS) of the dermis (b,c), polymorphic nuclear leukocyte (pnl) infiltration in the lesioned area (d) (m: melanocyte)

controversial. In addition, the emerging antibiotic resistance represents a serious problem as well. One of the commonly applied antifungal antibiotics is itraconazole (40 mg/kg feed/7 days), which is prepared by mixing the agent with commercial feed pellets. Considering that *P. aeruginosa* inhibits the germination and therefore the virulence of *Rhizopus microsporus* by iron-chelating molecules, it would be more appropriate to administer the antibiotic at a later stage. Iron is essential for the survival and pathogenicity of *R. microsporus*; therefore, it is desirable to first apply pyoverdine (80μg ml^{-1}) (Kousser, Clark, Sherrington, Voelz & Hall, 2019).

CONCLUSIONS

This research is the first to report the pathogenic effects of *Rhizopus* sp. in conjunction with *Y. ruckeri, A. hydrophila*, and *P. aeruginosa* in RAS-farmed African catfish (*C. gariepinus*). Our findings could help develop better disease control and treatment strategies in aquaculture and prevent outbreaks in poor conditions. Additionally, fungi affecting fish can also cause serious human infections due to similarities in their epidemiology and lesions. Although the list of zoonotic fungal agents in aquaculture is limited, Mucorales fungi like *Rhizopus* sp. pose significant public health risks.

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethics Committee Approval: The authors affirm that ethical approval is unnecessary for this study.

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Research Article

Age, Growth and Mortality Rates of *Symphodus tinca* (Osteichthyes: Labridae) in Izmir Bay (NE Aegean Sea)

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ABSTRACT

A total of 373 *Symphodus tinca* specimens were obtained from small-scale fisheries in January 2017 - December 2018 off Urla coasts (Izmir Bay), north-eastern Aegean Sea. The length and weight distribution of all *S. tinca* specimens ranged from 7.8 to 24.5 cm TL (mean: 14.4 ± 0.12) and from 6.6 to 209.3 g (mean: 43.2 ± 1.30), respectively. The female – male ratio was 1:0.41. The weight-length relationship parameters (*a, b,* r^2) were calculated to be 0.0175, 2.9023 and 0.9629, respectively. The age of *S. tinca* was determined to be between 0 and V years. The von Bertalanffy growth parameters were L∞ = 39.06 ± 23.57 cm, K = 0.14 ± 0.14, and t_0 = -1.77 ± 0.77. In addition, the Von Bertalanffy Growth Formula for length is given by the following equation: $Lt = 39.06\,[1 - e^{-0.14\,(t + 1.77)}]$. Thus, the growth performance (Φ`) was estimated as 2.32. The values of mortality and exploitation rates of *S. tinca* are 1.015 (Z), 0.380 (M), 0.635 (F) and 0.626 (E), respectively. These results indicate that stocks of *S. tinca* in the Bay of Izmir are overexploited.

Keywords: East Atlantic peacock wrasse, fishing, length-weight, exploitation, Mediterranean

INTRODUCTION

The East Atlantic peacock wrasse, *Symphodus tinca* (Linnaeus, 1758) is a marine reef-associated fish that inhabits depths ranging from 1 to 50 m (Froese & Pauly, 2024). It has been observed to occasionally enter salty lagoons (Quignard & Pras, 1986). The male constructs and maintains a nest of seaweed following the spawning of one or more females. It reproduces from April to July along the Mediterranean coasts of France, and from March to June along the Mediterranean African coast. The growth rate is relatively slow (Quignard & Pras, 1986). The species is known to feed on benthic invertebrates, with crustaceans, molluscs and echinoderms representing the majority of its diet (Golani et al., 2006). It has very distinct sexual dimorphism, and has partially protogynic hermaphroditic. *S. tinca* reaches sexual maturity after two years (Golani et al., 2006). Its geographic distribution spans from Spain to Morocco, encompassing both the Mediterranean and the Black Sea (Quignard & Pras, 1986).

There are 20 labrid species in eastern Mediterranean (Golani et al., 2006) of which 19 occurring in Turkish seas (Mater et al., 2002). Nevertheless, Lök & Gül (2005) and Çoker & Mater (2006) have documented the presence of 14 species in the Bay of Izmir. However, labrid species are frequently caught by gillnets, and occasionally by bottom trawl nets (Pallaoro & Jardas, 2003; Özaydın et al., 2007) and beach seine nets (Akyol, 2003; Skeljo & Ferri, 2012) in Turkish seas like the Mediterranean basin, and they are usually discarded due to a lack of commercial value (Gordoa et al., 2000; Akyol, 2003; Aydın et al., 2007).

The bio-ecological studies of labrid species has not been a prominent area of interest within the academic community to date. It is evident that there is a need for further research to deter-

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mine population parameters in *S. tinca* and other labrids. While some studies have been made on reproduction, diet composition, otolithometry, morphometry, growth and length-weight relationships of *S. tinca* (Boughamou et al., 2014, 2015; Ouannes-Ghorbel & Bouain, 2006; Skeljo & Ferri, 2012; Shili et al., 2018; Kasapoglu et al., 2016; Gordoa et al., 2000; Pallaoro & Jardas, 2003; Petrakis & Stergiou, 1995; and references herein), there is still a paucity of information on the age, growth and mortality of *S. tinca* in the Mediterranean basin. The available literature on the age, growth and mortality rate of *S. tinca* in the Mediterranean Sea is limited. Accordingly, the study presents the first data on the age, growth, and mortality rates of *S. tinca* in Izmir Bay, North-eastern Aegean Sea.

MATERIALS AND METHODS

A total of 373 specimens of *S. tinca* were intermittently collected from Small-scale fisheries (SSF) off Urla coasts of Izmir Bay, Aegean Sea (Fig. 1) between January 2017 and December 2018.

The sex of the fish was macroscopically identified by means of gonads. The sagittal otoliths were used for the purpose of determining the ages. For age reading, a total of 217 sagittal otoliths was utilized under a binocular microscope (SOIF XSZ-7GX) at 10× magnification.

The specimens were measured both total length (TL, ±0.1 cm) and the wet weight (W, ±0.1 g). The length–weight relationship (LWR) was estimated with the formula: $W = a \times L^b$; where, W is weight (g), TL is total length (cm), *a* is the intercept and *b* is the slope.

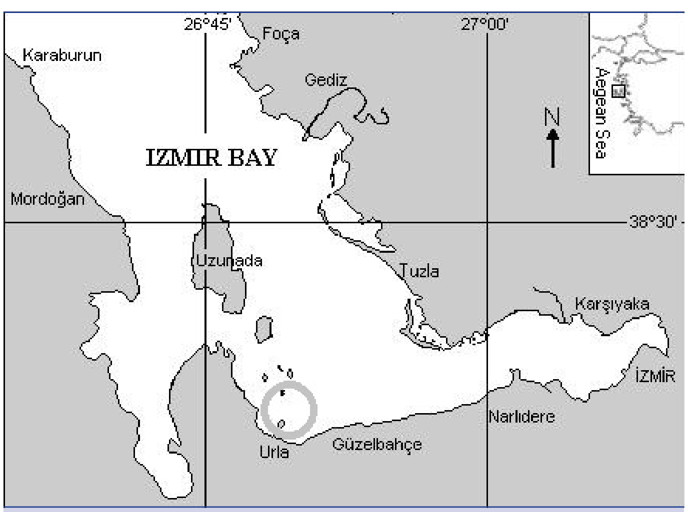

Figure 1. Sampling area (grey circle).

The von Bertalanffy growth parameters (VBGP, L_∞, K, and t_0) were computed with the von Bertalanffy growth formula (VBGF) by using the FISAT II (FAO–ICLARM Stock Assessment Tools, Gayanilo et al., 1994). The VBG equation (VBGE) is represented by the following formula: $L_t = L_\infty [1 - e^{-K(t - t_0)}]$; where, L_∞ is the asymptotic length, K the growth curve parameter, and t_0 the theoretical age when fish length would have been zero. The growth performance was calculated using the phi-prime index (Φ'), which is defined as $\Phi' = \log K + 2\log L_\infty$ (Pauly & Munro, 1984).

The natural mortality (M) of *S. tinca* was calculated using the multiple regression formula proposed by Pauly (1980), which is given by $\ln M = -0.0152 - 0.2779 \ln(L_\infty) + 0.6543 \ln(K) + 0.463 \ln(T)$. The mean annual temperature for the Aegean Sea, as estimated by the General Directorate of Meteorology (Anon., 2018) is assumed to be 18.7°C.

The total mortality (Z) was estimated using the average size of the catch (Beverton & Holt, 1957). The mean total mortality rate (Z) can be estimated from the mean length of individuals in a population by the following formula: $Z = K (L_\infty - L_{mean}) / (L_{mean} - L_c)$, where L_∞ and K are parameters of the VBGE. In the absence of L_c, Erkoyuncu (1995) proposed that L' can be used in the formula instead, that is to say, $L_c = L'$. The mean length (L_{mean}) is calculated from L' upwards, with L' representing a length not smaller than the smallest length of fish fully represented in catch samples (Pauly & Soriano, 1986).

The mortality rate of fishing (F) can be estimated from $F = Z - M$. Once values of F and M are available, an exploitation ratio (E) can be calculated using the equation $E = F / Z$ (Sparre & Venema, 1992). This allows for an evaluation of whether a stock has been overfished, on the assumption that the optimal value of E is approximately equal to 0.5 (Pauly, 1980). The means were presented with their standard error (± s.e.).

RESULTS AND DISCUSSION

The length / weight distribution of all samples exhibited a range of 7.8 to 24.5 cm (average: 14.4 ± 0.12 cm) and from 6.6 to 209.3 g (average: 43.2 ± 1.30 g), respectively (Table 1). The length frequency in overall is illustrated in Figure 2. The length range of 11 - 15 cm exhibited the highest rate, approximating 85%. The female : male ratio (F : M) was 1 : 0.41. The F : M ratio according to the age groups was tested by χ2 of independence, which revealed a statistically significant difference between F : M ratios in all age groups ($p < 0.05$).

The LWR parameters (*a, b, r*²) were calculated as 0.0175, 2.9023 and 0.9629, respectively. And also, the LWR is indicated in Figure 3.

Of the individuals whose age was estimated, 128 were female, 75 were male, and 14 were of unknown gender. The age of *S. tinca* was estimated to be between 0 and V. The estimated VBGP were

Table 1. Length and weight range and means with standard error (s.e.) for *Symphodus tinca* in Izmir Bay, NE Aegean Sea.

Sex	n	L_{min} - L_{maks} (cm)	W_{min} - W_{maks} (g)	L_{ort} ± s.e.	W_{ort} ± s.e.
♀	250	8.7 – 20.6	11.3 – 113.0	13.9 ± 0.12	39.8 ± 1.20
♂	103	11.3 – 24.5	17.4 – 209.3	15.4 ± 0.28	52.4 ± 3.46
?	20	7.8 – 17.5	6.6 – 71.9	14.0 ± 0.61	40.1 ± 4.46
∑	373	7.8 – 24.5	6.6 – 209.3	14.4 ± 0.12	43.2 ± 1.30

L_∞ = 39.06 ± 23.57 cm, K = 0.14 ± 0.14, and t_0 = -1.77 ± 0.77. The VBGF for length was found to be $L_t = 39.06\ [1 - e^{-0.14\ (t + 1.77)}]$. The observed lengths of *S. tinca* assigned to each age group were employed in the fitting of the VBGF (Fig. 4). Moreover, the index of Φ` was estimated to be 2.32.

Table 2 presents the total (Z), natural (M), and fishing (F) mortalities of *S. tinca*. Furthermore, the exploitation rate was calculated to be 0.63.

The length frequency distribution of the sampled fish was found to encompass a range of 7.8 to 24.5 cm total length (TL). This length range encompasses the normal distribution of 12 - 25 cm, which is the common length observed throughout the Mediterranean (Golani et al., 2006; Froese & Pauly, 2024). Previously, various studies on *S. tinca* have provided length records (with VBGP, phi-prime index, and parameters of LWR), which are presented in Table 3. In a recent study, Aydın & Karadurmuş (2023) reported the largest specimen of *S. tinca* (male, 316 mm TL, 430.4 g) from the Turkish Black Sea coast. Furthermore, maximum length and maturity length have been documented as 44.0 cm SL for male specimen and 11.7 cm in FishBase, respectively (Froese & Pauly, 2024). Consequently, only 5.4% of fish is under first maturity length in this study. Due to the slow growth rate (Froese and Pauly, 2024), the sustainability of these fish can be achieved with low fishing pressure. The Izmir Bay population of *S. tinca* exhibits the lowest growth performance (Φ`= 2.32), while the Spanish coast population demonstrates the highest growth rate (2.88) (Gordoa et al., 2000) (Table 3).

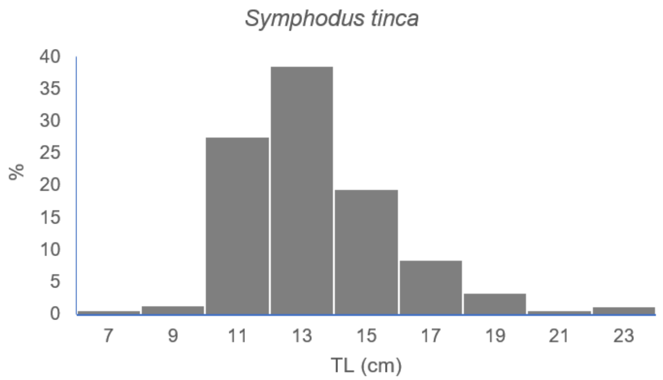

Figure 2. Length frequency distribution of *Symphodus tinca* in Izmir Bay, NE Aegean Sea.

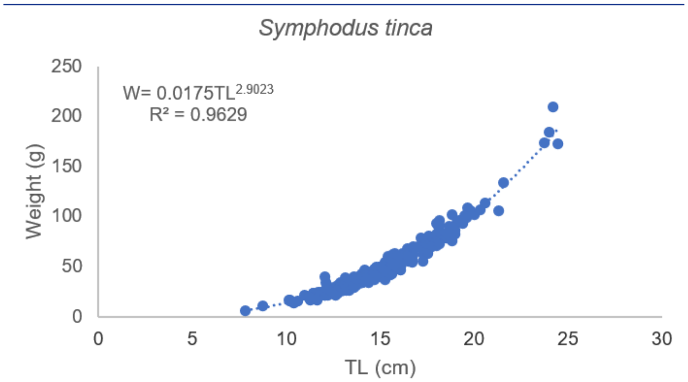

Figure 3. Length-weight relationship of *Sympodus tinca* in Izmir Bay, NE Aegean Sea.

The data indicated a negative allometric growth pattern for *Symphodus tinca* (*b* = 2.902). The preceding data on LWRs in the Mediterranean Sea are presented in Table 3. The *b* values observed in the study were found to be generally in agreement with the results of previous studies.

A limited number of studies (Table 3) have calculated the VBGP for *S. tinca* to be between 26.46 and 42.24, 0.21 and 0.81, -0.12 and -0.78 for L_∞, K and t_0, respectively (Pallaoro & Jardas, 2003; Gordoa et al., 2000; Boughamou et al., 2014). The VBGP and mortality rates presented in this study are the first to be reported for the Turkish seas.

The determination of mortality rates is a crucial aspect in the assessment of population abundance. The given values of mortality and E rates of *S. tinca* are 1.015 (Z), 0.380 (M), 0.635 (F) and 0.626 (E), respectively. The results demonstrate that the *S. tinca* stocks in the Bay of Izmir have been subjected to over exploitation. Although *S. tinca* is not a target species, its high exploitation rate may be attributed to the high rate of catch of this spe-

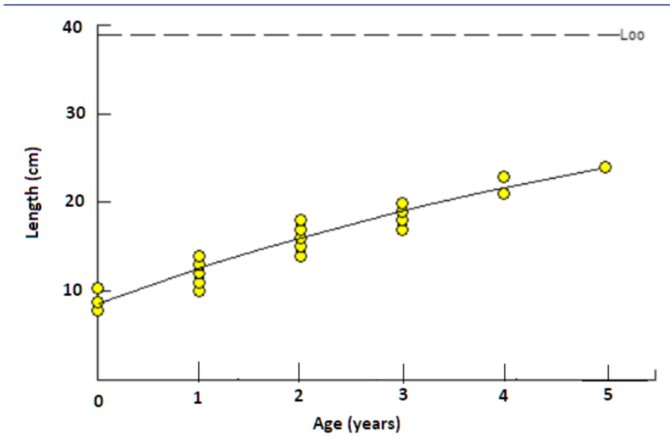

Figure 4. Von Bertalanffy growth curve fitted by length-at-age for *Symphodus tinca* in Izmir Bay, NE Aegean Sea.

Table 2. Mortalities (M, F, Z) and exploitation rate (E) of *Symphodus tinca* in Izmir Bay, NE Aegean Sea.

Total mortality Z $year^{-1}$	Natural mortality M $year^{-1}$	Fishing mortality F $year^{-1}$	Exploitation rate E $year^{-1}$
1.015	0.380	0.635	0.626

Table 3. Previous records on the age, length, weight, VBGP, phi-prime index, and parameters of LWR of *Symphodus tinca* in the Mediterranean Sea.

Authors	n	L_{min} - L_{max}	W_{min} - W_{max}	L_∞	K	t_0	Φ`	Age	*a*	*b*	r^2
Petrakis and Stergiou (1995)	31	12.7 – 20.8	-	-	-	-	-	-	0.00001	3.068	0.970
Dulčić & Kraljević (1996)	100	12.7 – 30.2	15.0 - 305	-	-	-	-	-	0.0003	2.726	0.905
Can et al. (2002)	10	12.1 – 17.2	-	-	-	-	-	-	0.002	3.675	0.997
Morey et al. (2003)	375	4.7 – 30.1	1.2 – 356	-	-	-	-	-	0.018	2.876	0.991
Pallaoro and Jardas (2003)	595♀ 848♂	8.6 - 42.5	7.9 - 764.2	28.18 42.24	0.290.21	-0.78 -0.63	2.26* 2.57*	I-XII I-XIII	0.022	2.815	0.983
Valle et al. (2003)	56	11.4 - 30.4	-	-	-	-	-	-	0.026	2.788	0.976
Karakulak et al. (2006)	248	10.0 - 26.8	-	-	-	-	-	-	0.011	3.046	0.974
Özaydın et al. (2007)	89	6.7 - 23.0	-	-	-	-	-	-	0.018	2.905	0.984
Uçkun İlhan et al. (2008)	277	6.7 - 24.3	4.3 - 185.2	-	-	-	-	-	0.018	2.907	0.984
Keskin & Gaygusuz (2010)	41	2.1 - 15.5	-	-	-	-	-	-	0.011	3.098	0.992
Gordoa et al. (2000)	291	-	-	30.65	0.81	-0.32	2.88*	0-VIII	0.290	2.795	0.960
Gurkan et al. (2010)	10	4.7 - 10.5	1.0 - 12.1	-	-	-	-	-	0.013	2.893	0.965
Boughamou et al. (2014)	277♀ 209♂ 232♀ 173♂	4.9 – 31.3	1.4 – 400.6	26.46[o] 32.32[o] 26.61[s] 32.50[s]	0.79 0.54 0.61 0.48	-0.12 -0.22 -0.45 -0.31	2.74 2.75 2.63 2.70	I-V	-	-	-
Boughamou et al. (2014)	303♀ 227♂	4.9 -31.3	1.4 – 400.6	-	-	-	-	-	0.010 0.020	3.010 2.860	0.920 0.910
Bilge et al. (2014)	110	6.6 - 22.0	-	-	-	-	-	-	0.018	2.924	0.969
Altın et al. (2015)	27	3.0 - 18.5	0.2 - 77.2	-	-	-	-	-	0.007	3.269	0.995
Dimitriadis & Fournari-Konstantinidou (2018)	83	12.4 - 25.3	-	-	-	-	-	-	0.026	2.760	0.976
Miled-Fathalli et al. (2019)	60	11.6 - 25.0	22.0 - 186	-	-	-	-	-	0.019	2.848	0.990
Cengiz (2021)	22	10.5 - 17.1	20.3 - 80.6	-	-	-	-	-	0.026	2.810	0.950
Onay (2021)	17	6.5 - 12.8	3.9 - 36.1	-	-	-	-	-	0.016	2.990	0.910
This study	373	7.8 - 24.5	6.6 - 209.3	39.06	0.14	-1.77	2.32	0 - V	0.018	2.902	0.963

*calculated by us; o: otolith, s: scale

cies due to intensive coastal fishing. A comparison between the mortalities of the species studied in this work and those presented in the literature is not possible due to the unavailability of the requisite data. The only other source to report a survival rate (S) is Pallaoro & Jardas (2003), who found it to be 0.80 for males and 0.76 for females.

CONCLUSION

The *S. tinca* is subjected to considerable fishing pressure, and the implementation of effective management measures is imperative. Rather than being discarded at sea, these species could be relocated to marine aquariums (Türkmen et al., 2011). It is a species that is sought after for marine aquariums, as are other Labrids. This is due to a combination of factors, including its attractive appearance and its status as a natural cleaner that consumes ectoparasites found on other fish species (Cato & Brown, 2003; Wabnitz et al., 2003; Rhyne et al., 2012). This approach could allow fishers to increase their income while ensuring the survival of these fish in marine aquariums. However, in order to prevent this situation from leading to overfishing of this species, protective measures such as appropriate size, closed and open seasons in a specific area, catch quotas, etc. should be planned by the fisheries authority.

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Review Article

A Review of Fish Anomalies in Türkiye's Waters

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ABSTRACT

In recent years, anomaly studies have increased in number, covering both individual cases and their effects on natural populations and farmed species. However, in the literature review, no current study has been found to compile the types and cases of anomalies encountered in species found in Türkiye's waters. In the context of this review, 34 articles and symposium proceedings were found to belong to fish anomalies in Türkiye's waters. In these studies, 62 individuals from 38 species and 20 families from different water bodies were detected to have at least one anomaly type that is included in this review. According to the literature, possible factors causing anomalies can be discussed under four general headings; environmental (anthropogenic factors, industrial activities, industrial chemicals, trace elements, pollution, light intensity, pesticide usage), biological (endocrine system, genetic, teratological cases, epigenetic, nutritional problems, parasitic or physiologic reasons, oxidative stress, pigment deficiency), ecological (attacked by carnivores, competition, changes in water parameters), and fishery-related (ghost fishing). This review also presents some possible causes of particular anomaly types in detail. It is thought that with the information provided, this review may establish an up-to-date basis for future studies.

Keywords: Fish anomaly, environmental factors, genetics, nutrition

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INTRODUCTION

The deterioration of water resources due to urbanization and intensification of anthropogenic activities have raised global concerns regarding environmental sustainability (Giora et al., 2022). The health of the organisms in an ecological system typically determines the biotic entirety of the system. Species in aquatic ecosystems, particularly those at the top of the food chain, are often seen as representative indicators of the overall health of the ecosystem. Fish contribute to the nutrition of billions of consumers by providing rich sources of vitamins, minerals, fatty acids, and high-quality proteins (Belton et al., 2018).

In addition, because of their location in the food chain, fish combine the effects of many biotic and abiotic variables in the system and represent the secondary effects of chronic stress transferred through the food chain (Adams & McLean 1985). Unusual developmental disorders associated with deteriorating environmental conditions threaten the marine environment, fisheries, and community health (Mavruk et al., 2015).

Fish anomalies were identified and documented for the first time in a three-volume bibliography published by Dawson (1964; 1966; 1971 in Ugbomeh et al., 2022). There are two main types of anomalies in fish: severe, which can affect the condition of the animal, and minor anomalies, which have little or no effect on the survival of the fish (Jawad & Akyol, 2018). The dominant morphological abnormalities in fish may be divided into a few major classes, including the skeleton, body shape, scales, otoliths, and pigmentation (Uzer & Karakulak, 2022).

Aquaculture Anomalies

Morphological and skeletal abnormalities observed in aquaculture are the main problems because they affect the esthetic appeal of fish, reduce their market value by affecting their growth and survival, and decrease the performance of fish grown in hatcheries, such as swimming ability, feed consumption, and susceptibility to pathogens (Çoban et al., 2016). Malformations in fish are caused by environmental factors, nutritional deficiencies, livestock conditions, and genetic factors (Lewis et al., 2004).

The literature defines three primary spinal conditions: lordosis (dorsal deformity, V shape), scoliosis (lateral deformity, zig-zag shape), and kyphosis (ventral deformity, Λ shape) (Afonso et al., 2000). In reared fish, vertebral column deformity is an important factor in economic loss because it adversely affects the external morphology, growth, and survival of the fish, reducing the quality of the fish. The survival of most fish species is largely influenced by their swimming performances (Plaut, 2001). The relationship between lordosis severity and swimming performance in juvenile sea bass was investigated by Başaran et al. (2009), who demonstrated that spinal angles affect swimming performance in these species. Abnormal lordosis is frequently observed in dense cultures of *Sparus aurata*, and it affects biological functions such as swimming speed during feeding (Ortiz-Delgado et al., 2014). Scoliosis and lordosis are some of the most common problems in sea bass larvae and fry rearing in Türkiye (Tan et al., 2015). It has been reported that the prevalence of kyphosis is lower than that of lordosis (Jawad et al., 2017a).

Otoliths are required for hearing and hydro-dynamic balance (Popper et al., 2005). The otolith morphology, especially the sagittal otolith, is genus- and species-specific. It is widely used in taxonomic research and for the formation of phylogenetic relationships among different fish groups (Teimori et al., 2012). Abnormal otoliths have been reported in various fish from both marine and freshwater ecosystems (Sweeting et al., 2004). Manizadeh et al. (2018) examined fish samples from 83 species and 33 families from the Persian Gulf and the Gulf of Oman and found that 4.8% of the fish had abnormal otoliths. Ecologically, the Persian Gulf has been recognized as one of the most stressful environments in terms of salinity fluctuation (>39‰), acidification, and water temperature (12 to 38°C) (Bauman et al., 2013). These stressors likely affect otolith formation during the larval stages of these fish and have been accepted as possible causes of the abnormalities observed in the study.

Another anomaly observed in fish concerns pigmentation. The role of skin pigmentation is significant in camouflage, thermoregulation, photoprotection, and mate selection; nonetheless, it is one of the quality criteria that determines its market value for both human consumption and ornamental use (Cal et al., 2017). The pigmentation pattern of fish can be altered by nutritional quality, UV light incidence, light intensity, and social interactions (Kumar et al., 2023). The first documented abnormal pigmentation in the Aegean Sea was reported by Akyol and Şen (2012).

İlkyaz and Tosunoğlu (2019) reported findings of partial albinism observed in a blue crab sampled at Köyceğiz Lagoon (Muğla, Türkiye). The authors mentioned that the underlying cause of the incident might be genetic, leading to the absence of pigments.

It is unclear why pigment abnormalities occur, but the most likely cause is the interaction between genetic and environmental factors (Kumar et al., 2023).

It has been indicated that abnormalities in some fish species can be reduced through rearing practices, such as the application of appropriate feeding regimes and optimization of environmental conditions (Fjelldal et al., 2006). It is emphasized that developing protocols for the early identification of this ailment will enhance quality monitoring in commercial hatcheries because the majority of skeletal malformations occur in the larval and juvenile stages (Çolak & Çanak 2020).

Anomalies in Natural Environments

Natural aquatic environments pose various conditions (temperature fluctuations, decrease in the amount of dissolved oxygen, food shortage, etc.) that can challenge fish. The presence of anomalies in fish has been a source of interest for researchers since the sixteenth century, and since then, several studies have documented the presence of different types of anomalies in wild fish (Rutkayová et al., 2016). Abnormalities in wild fish are usually encountered because of fishing activities and scientific research. The high incidence of abnormal fish in polluted areas can serve as a sign of water pollution (Jawad et al., 2018a).

Dağlı (2008) reported that the ventral fin of *Capoeta damascina* from the family Cyprinidae is reduced and this region is completely covered with scales. In addition, in *Garra variabilis* belonging to the same family, he determined that while the rays of the left ventral fin developed in the form of protrusions, those of the right ventral fin were underdeveloped and completely covered with scales. It is thought that the underdevelopment of the ventral fins and arches of *C. damascina* may be due to mutations, whereas the partial underdevelopment of the ventral fins of *G. variabilis* may be due to parasitic or physical causes.

İnnal et al. (2019) aimed to determine skeletal deformations in natural populations of *Barbus pergamonensis* inhabiting the Dalaman Stream. According to the study, kyphosis can be induced in natural populations of *B. pergamonensis* by exposure to environmental pollutants.

Heavy metals are considered the main cause of many fish anomalies (Jawad & Ibrahim, 2021). The aquatic environment and its inhabitants are threatened when heavy metal concentrations exceed safe limits. Heavy metal toxicity has been linked to decreased gonadosomatic index values, fertility, hatchability, fertilization success, abnormal reproductive organ shape, and reproductive failure in fish (Taslima et al., 2022).

The aforementioned deformities are linked to genetic and epigenetic factors (Nguyen et al., 2016). Environmental parameters such as salinity, fluctuations in water temperature, organic compounds, oxygen, radiation, heavy metals, wastes of industrial origin, stress, food deficiency, parasitism, and other pollutants are among the remarkable epigenetic factors (Kolarevic et al., 2013). Moreover, pollution is a valuable indicator of the frequency of deformities in fish (Boglione et al., 2013).

The cited literature contains many terms (anomaly, abnormality, aberration, malfunction, malformation, etc.) that can be confusing for readers in certain situations. To prevent term chaos and make it easier to read the text, these terms were grouped together as "anomaly" in the entire text. In this study, we reviewed studies on fish anomalies and the causes of anomalies in Türkiye's marine, fresh, and brackish waters. To understand the diverse fish anomalies that can be encountered in Türkiye's waters and their differences from healthy individuals, visual materials from the literature were collected.

MATERIAL AND METHODS

The literature was separated into three sections by marine, freshwater, and brackish water and scanned with keywords such as fish anomaly, abnormality, aberration, malfunction, malformation, Türkiye, Anatolia, Black Sea, Aegean Sea, Sea of Marmara, and Mediterranean. References from the literature were also sought. Species, anomalies, possible causes, regions, and fishing gear in the papers were detected and ordered.

RESULTS AND DISCUSSION

In the literature, 34 articles and symposium proceedings were found relating to fish anomalies in Türkiye's waters. According to studies, 38 species, 20 families, and 62 individuals from different water bodies were detected (Table 1).

In these articles, 62 anomaly records were provided by researchers from Türkiye's marine, freshwater, and brackish waters. The Aegean Sea was considered the location of most detected anomalies (20 records). The Black Sea (5 records), Mediterranean (5 records), and Sea of Marmara (4 records) also had certain cases of fish anomalies. On the other hand, while 12 records concerning freshwater species have been found that belong to 7 studies, only one crustacean (*Callinectes sapidus*) has been reported from brackish water (Figure 1).

According to the literature, Soleidae had the most anomaly (12 records) records among marine fish families. On the other hand, in freshwater, Cyprinidae dominated (9 records) the other freshwater families in terms of anomalies presence (Figure 2).

According to the literature, the most anomalous finding was found in *Solea solea* (8 records). Additionally, *Barbus xanthos* has the most anomaly studies in Türkiye's freshwater (Figure 3).

In addition, 10 different anomaly types were determined. The most frequently observed anomaly type is an otolith anomaly (12 records). It was followed by skeletal (11 records), pigmentation anomalies (8 records), and head (7 records) (Figure 4).

Studies have shown that trawling and trammel nets are the most common methods to come across individuals with anomalies, respectively (Figure 5).

Fish can be affected by different diseases and disorders caused by either living or non-living agents. Infectious organisms that are both communicable and pathogenic are among the living agents of diseases. Bacterial, fungal, viral, and parasitic organisms commonly cause diseases in fish. Fish health can be negatively impacted by non-living agents that may come from the fish's interior or exterior. Normal physiological processes can be

Table 1. Literature summary of anomalies in fish species and their regions.

The order of studies in Figure 1	Species	Types of anomalies/ abnormalities/ deformities	Possible causes	Region	Fishing gear	Researcher(s)
			Marine			
1	*Solea solea*	Abnormal pigmentation (Ambicoloration)	Light intensity, Feeding during the larval stages Neurological aspects such as hormones (i.e. endocrine system) Genetic factors Environmental stressors Environmental contamination of sediments (Anthropogenic and industrial activities)	İzmir Bay, Aegean Sea	Trammel net	Akyol and Şen (2012)
2	*Raja clavata*	Nose and tail deformation	Ghost fishing	Çandarlı Bay, Aegean Sea	Trawl	Akyol and Aydın (2018)
3	*Liza ramada*	Hermaphroditism	Industrialization Pesticide usage Environmental pollution (effect on hormonal balance)	İzmir Bay, Aegean Sea	Gillnet	Bayhan and Acarlı (2006)
4	*Liza ramada*	Caudal fin anomaly	Domestic and industrial chemicals	İzmir Bay, Aegean Sea	Gillnet	Bayhan et al. (2010)

Table 1. Continue.

The order of studies in Figure 1	Species	Types of anomalies/ abnormalities/ deformities	Possible causes	Region	Fishing gear	Researcher(s)
			Marine			
5	*Raja clavata* *Raja clavata*	Disc asymmetry Caudal fin anomaly	Fishery related Trace elements Teratological cases Accidental (in early life stages)	İzmir Bay, Aegean Sea	Trawl Trawl	Capapé et al. (2018)
6	*Solea solea*	Hypomelanosis	Environmental and/or anthropogenic causes	Güllük Bay, Aegean Sea	Trammel net	Cerim et al. (2016)
	Solea solea	Dorsal fin anomaly			Trammel net	
7	*Pagellus erythrinus*	Saddleback syndrome Pughead	Environmentally induced stress	Gerence Bay, Aegean Sea	Trawl	Jawad et al. (2017b)
8	*Atherina boyeri*	Lordosis Kyphosis	Genetics	İzmir Bay, Aegean Sea	Beach seine	Jawad et al. (2017a)
9	*Conger conger*	Caudal fin deformity	Viral, bacterial, or environmental pollution	Çandarlı Bay, Aegean Sea	Trawl	Jawad et al. (2018a)
10	*Mullus barbatus*	Vertebral coalescence Vertebral deformity Hyperostosis	Environmental and genetic factors	İzmir Bay, Aegean Sea	Trammel net	Jawad and Akyol (2018)
11	*Trachurus mediterraneus*	Conjoined twinning	Pollution	Bandırma Bay, Sea of Marmara	Hensen Net (500µm)	Mavruk et al. (2015)
12	*Raja polystigma*	Morphologic deformation (head)	?	İzmir Bay, Aegean Sea	Trawl	Metin et al. (2009)
13	*Lophius budegassa*	One-eyed	Environmental or genetic factors Attacked by other carnivores (in early life stages)	Karaburun offshore, Aegean Sea	Trawl	Şenbahar and Özaydın (2019)
14	*Mullus surmuletus*	Abnormal pigmentation	Environmental contamination of sediments (Anthropogenic and industrial activities)	İzmir Bay, Aegean Sea	Trawl	Tokaç et al. (2013)
15	*Dicologolossa cuneata*	Albinism	?	İzmir Bay, Aegean Sea	Trammel net	Ulutürk et al. (2015)
		Xanthochroism	?		Trawl	
	Scophthalmus maeoticus	Ambicoloration	?	Black Sea coast of Istanbul	Gillnet	
16	*Merluccius merluccius*	One-eyed	Fishery related Competition during the early life stages Genetics Pollution	İzmit Bay, Sea of Marmara	Gillnet	Uzer and Karakulak (2022)
17	*Lophius budegassa*	Aberrant otolith morphology	Changing water parameters Pollution	Sea of Marmara	Angling	Yedier and Bostanci (2019)

Table 1. Continue.

The order of studies in Figure 1	Species	Types of anomalies/ abnormalities/ deformities	Possible causes	Region	Fishing gear	Researcher(s)
			Marine			
18	*Pagellus acarne*	Aberrant otolith morphology	Pollution	Sea of Marmara	Angling	Yedier and Bostanci (2020)
	Trachurus mediterraneus			Black Sea	Angling	
	Diplodus puntazzo			Aegean Sea	Angling	
	Merlangius merlangus			Black Sea	Angling	
19	*Dicentrarchus labrax*	Gonadal anomaly	Environmental pollution Genetics	Güllük Bay, Aegean Sea	Trammel net	Cerim et al. (2018)
	Solea solea				Trammel net	
20	*Solea solea*	Lateral line anomaly	?	Güllük Bay, Aegean Sea	Trammel net	Cerim et al. (2021)
	Solea solea	Caudal fin anomaly			Trammel net	
21	*Microchirus ocellatus*	Otolith deformation	Environmental and/or anthropogenic causes	Güllük Bay, Aegean Sea	Trawl	Cerim et al. (2019)
	Solea solea				Trawl	
28	*Merluccius merluccius*	Pughead deformity	Genetic and epigenetic causes		Trawl	Jawad et al. (2018b)
32	*Serranus hepatus*	Pugheadedness and ankylosis	Environmental factors	Sea of Marmara	Trawl	Jawad et al. (2022a)
	Mullus surmuletus	Lordosis-kyphosis			Trawl	
	Merluccius merluccius	Pugheadedness			Trawl	
	Trachurus trachurus	Abnormal body			Trawl	
	Trachurus trachurus	lordosis-kyphosis			Trawl	
	Trachurus trachurus	Pugheadedness			Trawl	
33	*Lepidorhombus boscii*	Abnormal otoliths	Pollution, nutritional problems, stress, and environmental factors	Aegean Sea	Trawl	Yedier et al., (2023)
	Platichthys flesus			Black Sea	Trawl	
	Solea solea			Mediterra-nean Sea	Trawl	
	Pegusa lascaris			Black Sea	Trawl	
29	*Champsodon nudivittis*	Otolith anomaly	Environmental factors	Aegean Sea	Trawl	Cerim et al., (2022)
	Mullus surmuletus	Skeletal anomaly	Environmental factors		Trawl	
34	*Chelon auratus*	Otolith asymmetry	Ecological issues, like water temperature, salinity, and pollut-ants.	Köyceğiz Lagoon, Muğla	Fish bar-riers	Reis et al. (2023)
	Chelon labrosus					
	Chelon saliens					
	Mugil cephalus					

Table 1. Continue.

The order of studies in Figure 1	Species	Types of anomalies/ abnormalities/ deformities	Possible causes	Region	Fishing gear	Researcher(s)
			Freshwater			
22	*Garra variabilis*	Ventral fin anomaly	Genetics Parasitic or physical reasons	Balıksuyu creek, Kilis	?	Dağlı (2008)
	Capoeta damascina			Afrin creek, Kilis	?	
23	*Barbus pergamonensis*	Kyphosis	Environmental pollutants	Dalaman Stream, Burdur	Electro-fishing	İnnal et al. (2019)
24	*Alburnus tarichi*	Abnormal ovary	Oxidative stress	Lake Van	Cast nets	Özok et al. (2017)
25	*Chalcalburnus tarichi*	Abnormal gonads	Polluting chemicals or other unknown factors (such as global warming)	Karasu river and Lake Van	?	Ünal et al. (2007)
30	*Salaria fluviatilis (3 specimens)*	Pectoral and pelvic fin deformity	Environmental factors	Kızılırmak River	Electro-fishing	Jawad et al., (2022b)
31	*Barbus xanthos (4 specimens)*	Skeletal deformities	Environmental factors	Dalaman River	Electro-fishing	Jawad and Güçlü (2022)
			Crustacea			
26	*Callinectes sapidus*	Albinism	Pigment deficiency, genetic origin, or both.	Köyceğiz Lagoon, Muğla	Trap	İlkyaz and Tosunoğlu (2019)
27	*Pontastacus leptodactylus*	Albinism	Genetics	Atikhisar Reservoir, Çanakkale	Fyke net	Kale et al. (2020)

affected by external factors, such as environmental conditions, resulting in disease. Endogenous factors, like their genetic composition, may have an impact on fish's susceptibility to less-optimal environmental conditions or infectious agents. different fish species, as well as specific strains within a particular species, exhibit diverse tolerance to different environmental conditions and pathogens (Wiegertjes et al., 1996).

The changes in biology/physiology of fish due to the effectors briefly mentioned above may reflect on the phenotype of fish either as an unusual appearance or an altered behaviour. As a very well-established example, disorders affecting the coloration of fish and other water-related species can be described. Coloration plays a crucial role in the connection between an organism and its environment. It serves as a means of communication, protection from predators, defense against parasites, regulation of body temperature, and protection against UV light, bacteria, and physical damage (Cuthill et al., 2017). The cases included in the current study display various types of coloration-related anomalies, such as ambicoloration (*S. solea*, *Scophthalmus maeoticus*), albinism (*Dicologolossa cuneata*), xanthochroism (*D. cuneata*), and abnormal pigmentation (*Mullus surmuletus*). Disorders involving the melanin pigmentary system are often linked to genetic abnormalities (Muto et al., 2013), specifically arising from mutations in the tyrosinase gene family. These mutations can affect the enzymes responsible for the production of melanin, leading to an imbalance in the body's pigment metabolism (Wang et al., 2007).

Champsodon nudivittis, *Chelon auratus*, *Chelon labrosus*, *Chelon saliens*, *Mugil cephalus*, *Lepidorhombus boscii*, *Platichthys flesus*, *S. solea*, *Microchirus ocellatus*, *Lophius budegassa*, *Pagellus acarne*, *Trachurus mediterraneus*, *Diplodus puntazzo*, *Merlangius merlangus*, and *Pegusa lascaris* are species with otolith anomalies (most of which being otolith asymmetry) cases covered in the context of this study. The formation of otoliths in embryos occurs as a cluster of precursor particles attached to saccular epithelial tissue (Riley et al., 1997). The cumulative nature of the increment bands can be used to determine the age and changes in somatic growth of individual fish using increment numbers and widths (Campana & Neilson, 1985). The process of validating the deposition of annual and daily increments is a fundamental component of effective fisheries management, as well as age and growth studies (Devries & Frie, 1996). The expression of otolith proteins and increment growth are influenced by thyroid hormone activity, as shown in numerous studies (Coffin et al., 2012). On the other hand, carbonic anhydrase activity is considered to have a regulatory function in otolith growth in terms of both size and symmetry (Thomas & Swearer, 2019). Trace and minor elements as constituents of their aquatic environment also form the inorganic part of the otoliths, and these components may hint at the physicochemical nature of the organism's surroundings (Campana & Thorrold, 2001).

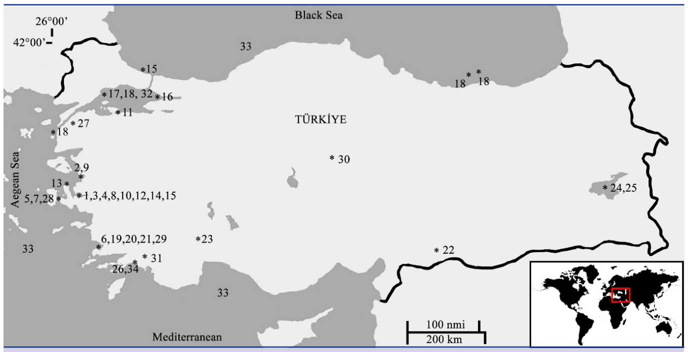

Figure 1. Anomaly studies in Türkiye 1- Akyol and Şen (2012), 2- Akyol and Aydın (2018), 3- Bayhan and Acarlı (2006), 4- Bayhan et al. (2010), 5- Capapé et al. (2018), 6- Cerim et al. (2016), 7- Jawad et al. (2017b), 8- Jawad et al. (2017a), 9- Jawad et al. (2018a), 10- Jawad and Akyol (2018), 11- Mavruk et al. (2015), 12- Metin et al. (2009), 13- Şenbahar and Özaydın (2019), 14- Tokaç et al. (2013), 15- Ulutürk et al. (2015), 16- Uzer and Karakulak (2022), 17- Yedier and Bostanci (2019), 18- Yedier and Bostanci (2020), 19- Cerim et al. (2018), 20- Cerim et al. (2021), 21- Cerim et al. (2019), 22- Dağlı (2008), 23- İnnal et al. (2019), 24- Özok et al. (2017), 25-Ünal et al. (2007), 26- İlkyaz and Tosunoğlu (2019), 27- Kale et al. (2020), 28- Jawad et al. (2018b), 29- Cerim et al. (2022), 30- Jawad et al. (2022b), 31-Jawad and Güçlü (2022), 32- Jawad et al. (2022a), 33- Yedier et al., (2023), 34- Reis et al. (2023).

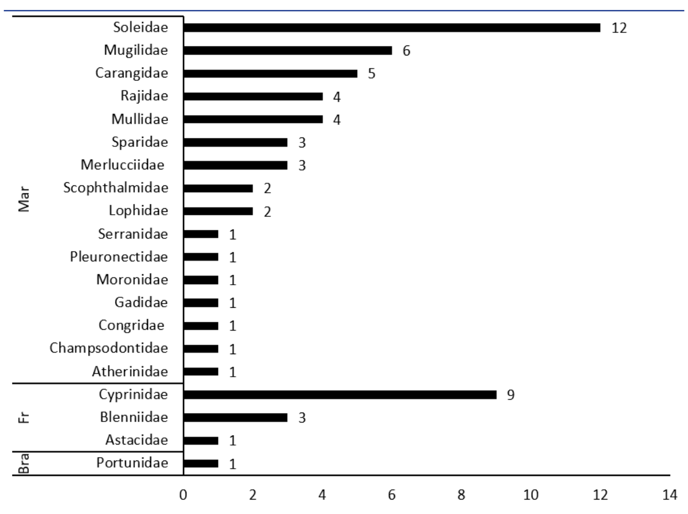

Figure 2. Number of anomaly studies in Türkiye's waters by family (Mar; Marine, Fr; Freshwater, Bra; Brackish water).

Raja clavata, *Liza ramada*, *S. solea*, *Pagellus erythrinus*, *Atherina boyeri*, *Conger conger*, *Mullus barbatus*, *Raja polystigma*, *Merluccius merluccius*, *Serranus hepatus*, *M. surmuletus*, *Trachurus trachurus*, *G. variabilis*, *C. damascina*, *B. pergamonensis*, *Salaria fluviatilis* (3 specimens), and *B. xanthos* (4 specimens) are speci-

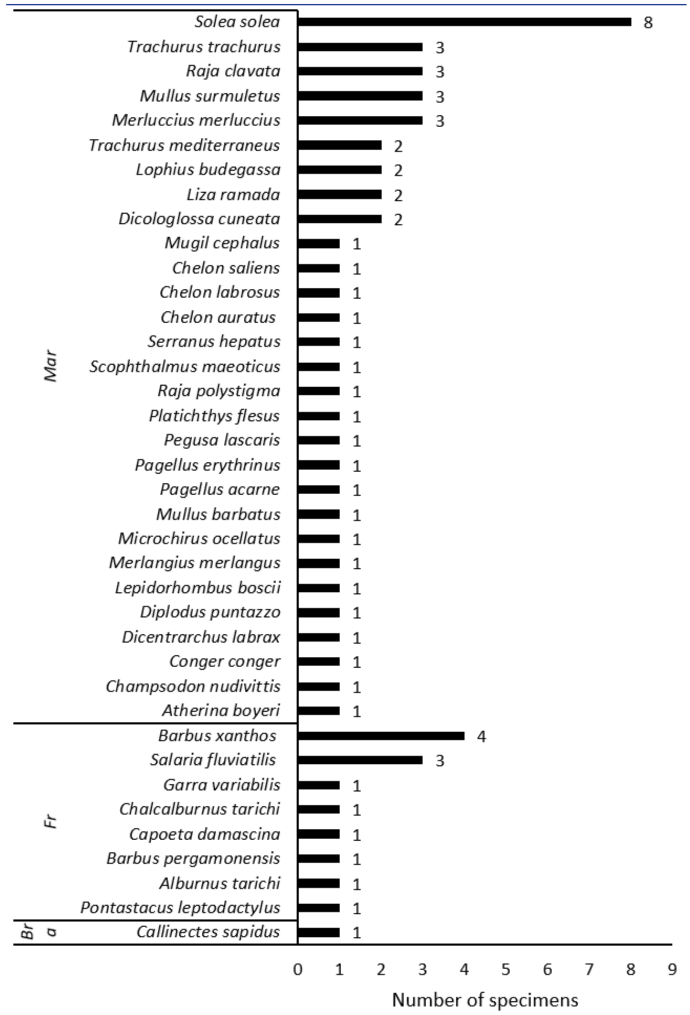

Figure 3. Number of anomaly studies in Türkiye's waters by species (Mar; Marine, Fr; Freshwater, Bra; Brackish water).

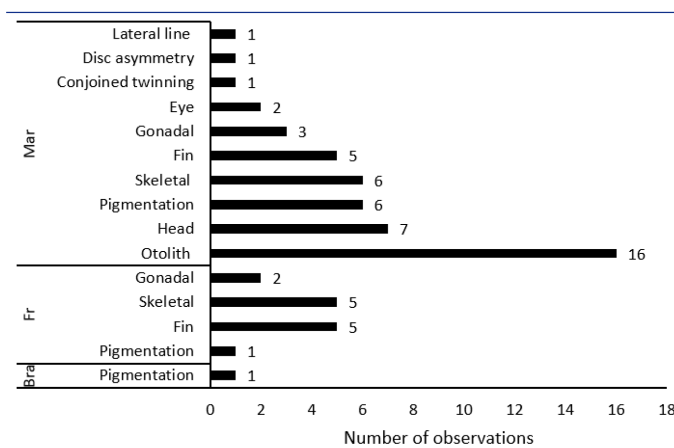

Figure 4. Anomaly types in Türkiye's waters (Mar; Marine, Fr; Freshwater, Bra; Brackish water).

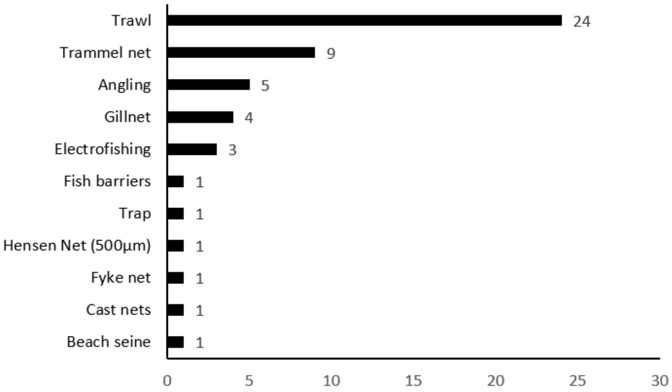

Figure 5. Number of capture methods used in the studies.

mens reported to have hard tissue deformities. These deformities are identified as nose and tail deformities, fin anomalies, vertebral deformities, pigheadedness, lordosis-kyphosis and hyperostosis. The etiology of skeletal anomalies, in general, is mentioned to have a relationship with incubation temperature (Sfakianakis et al., 2004), toxins like trifluralin (Wells & Cowan, 1982), treatment reagents like oxytetracycline (Toften and Jobling, 1996), infections (Madsen et al., 2001), deficiencies of or over-exposure to vitamins (vitamin C: Halver & Hardy, 1994) and minerals (selenomethionine: Kupsco & Schlenk, 2016), and genetic factors (Sadler et al., 2001).

Alburnus tarichi, *Chalcalburnus tarichi*, *T. trachurus*, *Dicentrarchus labrax*, *S. solea*, *M. merluccius*, *T. mediterraneus*, *R. polystigma*, *L. budegassa*, *and L. ramada* are reported to have various anomalies concerning the external and internal structures of specimens. Abnormal gonads and ovaries, abnormal body shape, lateral line anomaly, one-eyedness, conjoined twinning, head with morphological deformation, and hermaphroditism (intersex) are the deviations observed in these species. In the study of Jobling and Tyler (2003), the disruption of the endocrin system by chemicals, sub-optimal temperatures, food supply shortages, low pH, environmental pollutants, and parasites may alter the reproductive biology of fish. The masculinization of wild fish, meaning that female fish develop secondary sex characteristics to male fish, and other abnormal reproductive and developmental problems are some of the consequences of endocrine disorders in fish. The emergence of intersex cases throughout the fish populations is reported to alter sex steroid hormone profiles, thus affecting spawning times and resulting in reduced sperm production (Jobling et al., 2002). One-eyedness is also another case for the fish, and it is mentioned that in the development phase of fish embryos exposure to alkaloids might cause loss of eye(s) (McClendon, 1912). A more recent publication (Jones et al., 2017) on *Carcharhinus leucas* showed that postnatal trauma might be the background for lost eyes. Species' interactions with fishing gear or other sharks may also explain the loss of external structures.

The frequent occurrence of anomalies in certain families does not necessarily imply that the species are more exposed to the conditions that cause anomalies. According to the findings, a more logical explanation may be that species belonging to the Soleidae family (especially *S. solea*) have significant economic value, are constantly captured, and are thus seen in catch, and that many anomalies have been detected in these species. On the other hand, it can be said that species found in fresh water are exposed to more factors because they are found in perturbational, narrow, and specific water bodies, such as marine areas.

It can be seen that the majority of anomaly studies belong to studies conducted in the western regions of Türkiye (Figure 1). The reason for this may be more likely the capture method. Trawl nets allow for the catch of different sizes and larger amounts of fish compared to other fishing gear. For this reason, it is logical that in trawling, the number of individuals with anomalies in the trawl net coded is higher than that in other capture methods (i.e. mass capture of the species). Trawl nets and trammel nets are fishing gear generally used by commercial fishermen. Researchers either participate in fishing operations with commercial boats or perform these operations with their own research vessels on which trawl nets are generally used. On the other hand, although electrofishing and beach Seine are prohibited in Türkiye, researchers are allowed to do this for research projects with the permission of the Ministry of Agriculture and Forestry. In addition, Hansen net is also used for research.

CONCLUSION

According to the literature, possible factors causing anomalies can be discussed under four general headings; environmental (anthropogenic factors, industrial activities, industrial chemicals, trace elements, pollution, light intensity, pesticide usage), biological (endocrine system, genetic, teratological cases, epigenetic, nutritional problems, parasitic or physiologic reasons, oxidative stress, pigment deficiency), ecological (attacked by carnivores, competition, changes in water parameters), and fishery-related (ghost fishing). However, although the factors that cause anomalies are stated as is, further studies should be conducted with the mentioned factors to better understand their effects and consequences. If the origin of the factors is identified, some precautions can be taken to improve the health of the population.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethics Committee Approval: Ethics approval was not required for this study.

Financial Disclosure: The authors declare no relevant financial or non-financial interests.

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Further to the above, The Aquatic Sciences and Engineering provides a platform for the open public discussion of the journal contents. To secure accountability and to encourage sincere professional inputs without incivilities the system is set up to require registration and logging for recording of inputs. Some of the website contents will be available without logging but no peer review comments can be posted on the website without the disclosure of the reviewer identity to the journal editors.

It is necessary to agree upon standards of expected ethical behavior for all parties involved in the act of publishing: the author, the journal editor, the peer reviewer and the publisher.

Our ethic statements are based on COPE's Best Practice Guidelines for Journal Editors.

Publication Decisions

The editor of the TJAS is responsible for deciding which of the articles submitted to the journal should be published. The editor may be guided by the policies of the journal's editorial board and constrained by such legal requirements as shall then be in force regarding libel, copyright infringement and plagiarism. The editor may confer with other editors or reviewers in making this decision.

Fair Play

An editor at any time evaluate manuscripts for their intellectual content without regard to race, gender, sexual orientation, religious belief, ethnic origin, citizenship, or political philosophy of the authors.

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The editor and any editorial staff must not disclose any information about a submitted manuscript to anyone other than the corresponding author, reviewers, potential reviewers, other editorial advisers, and the publisher, as appropriate.

Disclosure and Conflicts of Interest

Unpublished materials disclosed in a submitted manuscript must not be used in an editor's own research without the express written consent of the author.

Research Ethics

An approval of research protocols by the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net) is required for experimental, clinical, and drug studies. If required, ethics committee reports or an equivalent official document will be requested from the authors.

For manuscripts concerning experimental research on humans, a statement should be included that shows the written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. Information on patient consent, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the authors' responsibility to carefully protect the patients' anonymity. For photographs that may reveal the identity of the patients, signed releases of the patient or of their legal representative should be enclosed.

Aquatic Sciences and Engineering requires experimental research studies on vertebrates or any regulated invertebrates to comply with relevant institutional, national and/or international guidelines. The journal supports the principles of Basel Declaration (basel-declaration.org) and the guidelines published by International Council for Laboratory Animal Science (ICLAS) (iclas.org). Authors are advised to clearly state their compliance with relevant guidelines.

Aquatic Sciences and Engineering advises authors to comply with IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora for research involving plants.

Human Subjects and Animal Use in Research, Ethics Committee Approval and Informed Consent

The Journal takes as principle to comply with the ethical standards of World Medical Association (WMA) Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects and WMA Statement on Animal Use in Biomedical Research.

An approval of research protocols by the Ethics Committee in accordance with international standards mentioned above is required for experimental, clinical, and drug studies and for some case reports. If required, ethics committee reports or an equivalent official document will be requested from the authors. For manuscripts concerning experimental research on humans, a statement should be included that shows that written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. For studies carried out on animals, the measures taken to prevent pain and suffering of the animals should be stated clearly. Information on patient consent, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the authors' responsibility to carefully protect the patients' anonymity. For photographs that may reveal the identity of the patients, signed releases of the patient or of their legal representative should be enclosed.

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The journal requires the authors and all individuals taking part in the evaluation process to disclose any existing or potential conflict of interest (such as financial ties, academic commitments, personal relationships, institutional affiliations) that could unduly influence one's responsibilities. To disclose potential conflicts of interest, the ICMJE Potential Conflict of Interest Disclosure Form should be filled in and submitted by authors as explained in the Author Form of the journal. Cases of a potential conflict of interest are resolved within the scope of COPE Conflict of Interest Flowcharts and ICMJE Conflict of Interest guidelines

Besides conflict of interest, all financial support received to carry out research must be declared while submitting the paper.

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Authors are asked to provide the raw data in connection with a paper for editorial review, and should be prepared to provide public access to such data (consistent with the ALPSP-STM Statement on Data and Databases), if practicable, and should in any event be prepared to retain such data for a reasonable time after publication.

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By submitting articles to Turkish Journal of Aquatic Sciences, the author attest the following:

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- Proper reference is provided for all contents extracted from other sources

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An author should not in general publish manuscripts describing essentially the same research in more than one journal or primary publication. Submitting the same manuscript to more than one journal concurrently constitutes unethical publishing behaviour and is unacceptable.

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If the work involves chemicals, procedures or equipment that have any unusual hazards inherent in their use, the author must clearly identify these in the manuscript.

Disclosure and Conflicts of Interest

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When an author discovers a significant error or inaccuracy in his/her own published work, it is the author's obligation to promptly notify the journal editor or publisher and cooperate with the editor to retract or correct the paper.

Responsibility for the Editor and Reviewers

General duties and responsibilities for editor;

- Actively seek the views of authors, readers, reviewers and editorial board members about ways of improving their journal's processes
- Encourage and be aware of research into peer review and 'journalology' and reassess journal processes in the lightof new findings
- Work to persuade their publishers to provide them with appropriate resources, guidance from experts (e.g. designers, lawyers) and adequate training to perform their role in a professional manner and raise the quality of their journal
- Support initiatives designed to reduce academic misconduct
- Support initiatives to educate researchers about publication ethics
- Assess the effects of their journal policies on author and reviewer behaviour and revise policies, as required, to encourage responsible behaviour and discourage misconduct
- Ensure that any press releases issued by the journal reflect the message of the reported article and put it into context

Duties of Reviewers;

- **Contribution to Editorial Decisions:** Peer review assists the editor in making editorial decisions and through the editorial communications with the author may also assist the author in improving the paper.
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Peer Review Policies

Only those manuscripts approved by its every individual author and that were not published before in or sent to another journal, are accepted for evaluation.

Submitted manuscripts that pass preliminary control are scanned for plagiarism using iThenticate software. After plagiarism check, the eligible ones are evaluated by editor-in-chief for their originality, methodology, the importance of the subject covered and compliance with the journal scope.

The editor hands over the papers matching the formal rules to at least two national/international referees for double-blind peer review evaluation and gives green light for publication upon modification by the authors in accordance with the referees' claims.

Responsibility for the Editor and Reviewers

Editor-in-Chief evaluates manuscripts for their scientific content without regard to ethnic origin, gender, citizenship, religious belief or political philosophy of the authors. Editor-in-Chief provides a fair double-blind peer review of the submitted articles for publication and ensures that all the information related to submitted manuscripts is kept as confidential before publishing.

Editor-in-Chief is responsible for the contents and overall quality of the publication. He/She must publish errata pages or make corrections when needed.

Editor-in-Chief does not allow any conflicts of interest between the authors, editors and reviewers. Only he has the full authority to assign a reviewer and is responsible for final decision for publication of the manuscripts in the Journal.

Reviewers must have no conflict of interest with respect to the research, the authors and/or the research funders. Their judgments must be objective.

Reviewers must ensure that all the information related to submitted manuscripts is kept as confidential and must report to the editor if they are aware of copyright infringement and plagiarism on the author's side.

A reviewer who feels unqualified to review the topic of a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process.

Peer Review Process

Only those manuscripts approved by its every individual author and that were not published before in or sent to another journal, are accepted for evaluation.

Submitted manuscripts that pass preliminary control are scanned for plagiarism using iThenticate software. After plagiarism check, the eligible ones are evaluated by Editor-in-Chief for their originality, methodology, the importance of the subject covered and compliance with the journal scope.

Editor-in-Chief evaluates manuscripts for their scientific content without regard to ethnic origin, gender, citizenship, religious belief or political philosophy of the authors and ensures a fair double-blind peer review of the selected manuscripts.

The selected manuscripts are sent to at least two national/international external referees for evaluation and publication decision is given by Editor-in-Chief upon modification by the authors in accordance with the referees' claims.

Editor-in-Chief does not allow any conflicts of interest between the authors, editors and reviewers and is responsible for final decision for publication of the manuscripts in the Journal.

Reviewers' judgments must be objective. Reviewers' comments on the following aspects are expected while conducting the review.

- Does the manuscript contain new and significant information?
- Does the abstract clearly and accurately describe the content of the manuscript?
- Is the problem significant and concisely stated?
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- Are the interpretations and consclusions justified by the results?
- Is adequate references made to other Works in the field?
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Reviewers must ensure that all the information related to submitted manuscripts is kept as confidential and must report to the editor if they are aware of copyright infringement and plagiarism on the author's side.

A reviewer who feels unqualified to review the topic of a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process.

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The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2017 - http://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with the CONSORT guidelines for randomized research studies, STROBE guidelines for observational studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, TREND guidelines for non-randomized studies, and COREQ guidelines for qualitative studies.

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- Copyright Agreement Form,
- Author Contributions Form, and
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors)

Preparation of the Manuscript

Title page: A separate title page should be submitted with all submissions and this page should include:

- The full title of the manuscript as well as a short title (running head) of no more than 50 characters,
- Name(s), affiliations, and highest academic degree(s) of the author(s) and ORCID ID (orcid.org)
- Grant information and detailed information on the other sources of financial support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,

- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfil the authorship criteria.

Abstract: A Turkish and an English abstract should be submitted with all submissions except for Letters to the Editor. Submitting a Turkish abstract is not compulsory for international authors. Please check Table 1 below for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract.

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The main text should contain Introduction, "Materials and Methods", "Result and Discussion", "Conclusion" and "References" sections.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

After the Conclusion section and before references list, information regarding conflict of interest, financial disclosure, ethics committee approval and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

Conflict of interests: When you (or your employer or sponsor) have a financial, commercial, legal or professional relationship with other organizations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.

Ethics committee approval: Ethical committee approval is routinely requested from every research article based on experiments on living organisms and humans. Sometimes, studies from different countries may not have the approval of the ethics committee, and the authors may argue that they do not need the approval of their work. In such situations, we consult COPE's "Guidance for Editors: Research, Audit and Service Evaluations" document and evaluate the study at the editorial board and decide whether or not it needs approval.

Financial disclosure: If there is any, the institutions that support the research and the agreements with them should be given here.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Review Articles: Reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. These authors may even be invited by the journal. Reviews should describe, discuss, and evaluate the current level of knowledge of a topic in researches and should guide future studies. The main text should start with Introduction and end with "Conclusion" and "References" sections. Authors may choose to use any subheading in between those sections.

After the Conclusion section and before references list, information regarding conflict of interest and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

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Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Short Communication" Readers can also present their comments on the published manuscripts in the form of a "Short Communication". The main text should contain Introduction, "Materials and Methods", "Result and Discussion", "Conclusion" and "References" sections.

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Ethics committee approval: Ethical committee approval is routinely requested from every research article based on experiments on living organisms and humans. Sometimes, studies from different countries may not have the approval of the ethics committee, and the authors may argue that they do not need the approval of their work. In such situations, we consult COPE's "Guidance for Editors: Research, Audit and Service Evaluations" document and evaluate the study at the editorial board and decide whether or not it needs approval.

Financial disclosure: If there is any, the institutions that support the research and the agreements with them should be given here.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤20	250	40
Review Article	≤25	250	60
Short Communication	≤5	250	20

Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

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When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

While citing publications, preference should be given to the latest, most up-to-date publications. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. List references in alphabetical order. Each listed reference should be cited in text, and each text citation should be listed in the References section. The reference styles for different types of publications are presented in the following examples.

Reference Style and Format

Aquatic Sciences and Engineering complies with APA (American Psychological Association) style 6th Edition for referencing and quoting. For more information:

- American Psychological Association. (2010). Publication manual of the American Psychological Association (6th ed.). Washington, DC: APA.
- http://www.apastyle.org

Accuracy of citation is the author's responsibility. All references should be cited in text. Reference list must be in alphabetical order. Type references in the style shown below.

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Citations must be indicated with the author surname and publication year within the parenthesis.

If more than one citation is made within the same paranthesis, separate them with (;).

Samples:

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(Akyolcu, 2007)
Citation with two authors;
(Sayıner & Demirci, 2007)
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First citation in the text: (Ailen, Ciambrune, & Welch, 2000) Subsequent citations in the text: (Ailen et al., 2000)
Citations with more than six authors;
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Major Citations for a Reference List

Note: All second and third lines in the APA Bibliography should be indented.

- **A book in print:** Baxter, C. (1997). *Race equality in health care and education*. Philadelphia: Ballière Tindall. ISBN 4546465465
- **A book chapter, print version:** Haybron, D. M. (2008). Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (pp. 17-43). New York, NY: Guilford Press. ISBN 4546469999
- **An eBook:** Millbower, L. (2003). *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song*. Retrieved from http://www.amacombooks.org/ (accessed 10.10.15)
- **An article in a print journal:** Carter, S. & Dunbar-Odom, D. (2009). The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy, 14*(1), 38-48.
- **An article with DOI:** Gaudio, J. L. & Snowdon, C. T. (2008). Spatial cues more salient than color cues in cotton-top tamarins (saguinus oedipus) reversal learning. *Journal of Comparative Psychology*, https://doi.org/10.1037/0735-7036.122.4.441
- **Websites - professional or personal sites:** The World Famous Hot Dog Site. (1999, July 7). Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.15)
- **Websites - online government publications:** U.S. Department of Justice. (2006, September 10). Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.15)
- **Photograph (from book, magazine or webpage):** Close, C. (2002). *Ronald*. [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.15)
- **Artwork - from library database:** Clark, L. (c.a. 1960's). *Man with Baby*. [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor
- **Artwork - from website:** Close, C. (2002). *Ronald*. [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.15)

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

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