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Article

The Eutrophication Status of Aquaculture Areas in the Black Sea (Sinop) Using TRIX, UNTRIX, and TQRTRIX Indices

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Abstract: Eutrophication, resulting from the environmental impacts of aquaculture activities and the presence of excess nutrients, is particularly significant for aquatic ecosystems. The present study aims to evaluate the eutrophication and trophic status of an aquaculture zone along the southern coast of the Black Sea by employing various indices and comparing the findings with established threshold levels for coastal waters. Water quality parameters-including chlorophyll-a, dissolved oxygen, dissolved inorganic nitrogen, and total phosphorus-were measured at 16 aquaculture production sites located along the southern Black Sea coast. Based on these measurements, TRIX, UNTRIX, and TQRTRIX values were calculated. The average temperature at the production sites was determined to be $21.72 \pm 0.11^\circ\text{C}$ ($p > 0.05$), the average salinity was $\text{‰}16.84 \pm 0.05$ ($p > 0.05$), the average pH was 8.28 ± 0.03 ($p > 0.05$), and the average dissolved oxygen concentration was $8.69 \pm 0.06 \text{ mg/L}$ ($p < 0.05$). The TRIX, UNTRIX, and TQRTRIX values ranged from 2.48 to 4.17, 1.48 to 3.51, and 0.31 to 0.88, respectively. When the water quality parameters and index values from the sampling stations were compared with reference thresholds, variations in trophic status were observed among the sites.

Keywords: black sea; aquaculture; water quality; environmental impacts; eutrophication; trophic index

Key Contribution: In this study, eutrophication and trophic status of aquaculture areas along the southern coast of the Black Sea were evaluated

1. Introduction

Over the past 40 years, the global aquaculture sector has experienced rapid growth as countries have sought to meet the rising demand for aquatic products. While global capture fisheries have declined in both species diversity and total catch due to overfishing and other factors, and harvest levels have remained relatively stable since the 1980's-aquaculture production has continued to grow steadily. This trend suggests that aquaculture, alongside capture fisheries, can play a crucial role in meeting the growing global demand for seafood [1].

According to the FAO's 2024 report, global aquatic production reached 185.4 million tons in 2022, with aquaculture accounting for 50.9% of the total [2]. In Turkey, aquaculture production has increased significantly over the past 25 years, rising from 18,150 tons in 1997 to 556,287 tons in 2023 [3]. While aquaculture holds substantial potential for Turkey, its productive and sustainable development necessitates environmentally responsible practices.

Numerous factors, including competition over limited resources, influence the development and implementation of intensive aquaculture systems. Among these, environmental impacts are particularly critical. During production, uneaten feed, fish excreta, and other waste materials can have an adverse effect on aquatic ecosystems, potentially accumulating over time and contributing to cumulative environmental stress.

Although numerous studies acknowledge the ecological consequences of aquaculture, some have specifically documented environmental degradation at particular facilities [4,5]. The increasing pollution in aquaculture production areas has become a global concern for the health and resilience of ecosystems. As the industry expands, these environmental impacts are expected to intensify [6]. In China, for instance, pollution from fish farming has already become a serious issue in coastal waters [7–10]. Similarly, in Australia and New Zealand, environmental concerns have been cited as major constraints to the further development of cage aquaculture [11].

The environmental impacts of aquaculture vary depending on the culture method, production capacity, hydrographic characteristics of the area, stocking density, farm management practices, feed composition, and the biological, chemical, and physical features of the farming site [12,13]. A measurable increase in the concentration of dissolved nutrients is referred to as eutrophication. In stagnant or semi-enclosed water bodies, excessive accumulation of nitrogen and phosphorus can shift the trophic status from oligotrophic to mesotrophic, eutrophic, and ultimately hypertrophic conditions [14,15]. When nutrient input exceeds the natural assimilative capacity of aquatic ecosystems, it may lead to eutrophication, oxygen depletion, and shifts in biodiversity, both in the water column and in sediments [16].

To assess the environmental impacts of waste from feed and metabolism in aquaculture, factors such as the settling rate of feed and feces, sediment composition and grain size, and changes in natural species composition must be taken into account. Sediment degradation and eutrophication driven by nutrient loading in the water column are key environmental issues. Evaluating the risks and consequences of eutrophication in estuarine and coastal ecosystems is a central aspect of environmental management [17].

One of the key tools used to evaluate the eutrophication status of aquaculture areas is the Trophic Index (TRIX). Owing to its simplicity, TRIX has been widely adopted across various European countries for classifying the trophic status of water bodies [18]. It has been applied in the Adriatic and Tyrrhenian Seas [19–21], northern European seas [22], the Black Sea [23,24], the Baltic coastal waters [25], the Ionian Sea [26], and the Venice Lagoon [27].

In Turkey, legislation enacted in 2022 established parameters and criteria for the siting of fish farms in enclosed bays and gulfs. These regulations assess the environmental impact of aquaculture on aquatic ecosystems using the TRIX index values and the degree of eutrophication [28].

Although TRIX offers valuable insights for environmental decision-making, it must be integrated with other data sources to form a comprehensive understanding necessary for adaptive coastal water management [29–31]. TRIX and its adapted version, TRIXcs, have also been used in combination with chemical and biological indicators—such as phytoplankton abundance in the Caspian Sea—to assess water quality [32].

The Black Sea, bordered by Bulgaria, Romania, Ukraine, Russia, Georgia, and Turkey, has been subjected to multiple stressors since the 1960's due to intensive anthropogenic activity. These include eutrophication caused by nutrient pollution, overfishing, the introduction of invasive species, coastal erosion resulting from construction, and climate change, all of which have severely impacted the region's ecological balance [33]. The sea has experienced nearly every known anthropogenic pressure, including marine litter [34,35]; nutrient enrichment, eutrophication, and hypoxia [36]; coastal erosion and abrasion [37,38]; and biological invasions [39].

Although there is a substantial body of research on the ecological impacts of aquaculture [18,40–44], studies specifically classifying the trophic status of aquaculture zones along the Black Sea coast remain scarce. This renders the present research distinctive.

The present study aims to assess the eutrophication and trophic status of aquaculture areas along the southern coast of the Black Sea using a range of indices and to compare the resulting values with threshold levels established for coastal waters.

2. Materials and Methods

The Black Sea is one of the major fishing grounds, possessing substantial potential for the exploitation of aquatic resources. In addition to capturing fisheries, cage-based aquaculture is carried out in designated zones within the Black Sea. The present study assessed the eutrophication status of 16 aquaculture production sites located along the southern coast of the Black Sea (Figure 1).



Figure 1. Study Area.

Aquaculture facilities along the southern coast of the Black Sea employ cage systems for production (Figure 2).

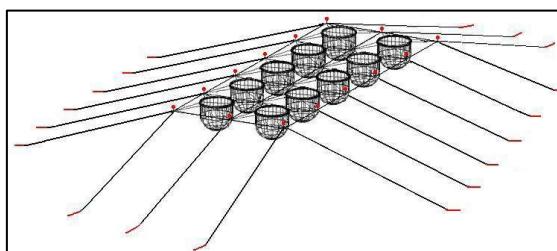


Figure 2. Cage systems.

Measurements and samplings were conducted instantaneously at seven points designated within the aquaculture areas (500m and 50m upstream from the production area against the current; 50m, 100m, and 200m downstream in the direction of the current; and 50 m from the shallow and deep zones of the production area) [28] (Figure 3).

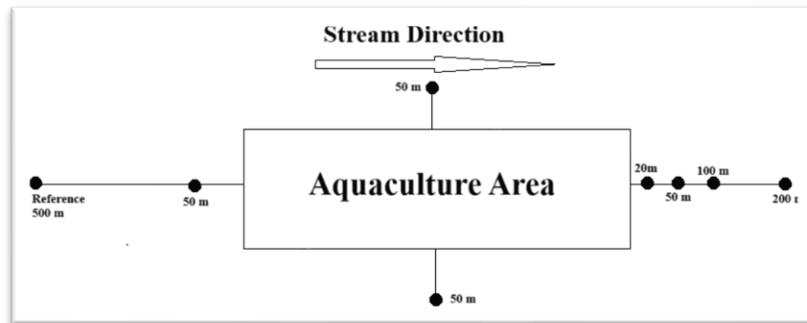


Figure 3. Sampling Points of the Aquaculture Sites.

2.1. Water Quality Measurement

Water quality parameters—specifically temperature, salinity, pH, and dissolved oxygen levels (both percentile saturation and mg/L)—were measured *in situ* at the sampling stations using a YSI 556 MPS multiparameter probe system.

Chlorophyll-a, dissolved inorganic nitrogen, and total phosphorus concentrations in the production areas were determined through laboratory analysis of water samples. These analyses were conducted in a laboratory accredited by the Turkish Accreditation Agency under the TS EN ISO/IEC 17025 standard. They were performed in accordance with ISO, EPA, TS, and EN protocols.

2.2. Trophic Index (TRIX), UNTRIX and TQRTRIX

To determine the eutrophication risk scale of the aquaculture sites, three metrics were employed: the Trophic Index (TRIX), the Unscaled Trophic Index (UNTRIX), and the Quartile Range of the Trophic Index (TQRTRIX). The TRIX index provides a valuable tool for assessing the trophic status of coastal waters [19]. UNTRIX has been proposed as an alternative approach for classifying trophic status using unscaled data. Both TRIX and UNTRIX are based on four state variables that are strongly associated with nutrient-related productivity: chlorophyll-a, dissolved oxygen (% saturation), dissolved inorganic nitrogen (DIN), and total phosphorus (TP). TQRTRIX, in contrast, is derived from a scale developed through the evaluation of UNTRIX values, incorporating data from both reference sites and aquaculture production areas [45].

The formulas used for calculating TRIX, UNTRIX, and TQRTRIX are as follows [19,45]:

$$\text{TRIX} = [\log (\text{Chl-a} \times \text{DO}_2 (\%) \times \text{DIN} \times \text{TP}) - (-1,5)] / 1,2, \quad (1)$$

$$\text{UNTRIX} = \log (\text{Chl-a} \times \text{DO}_2 (\%) \times \text{DIN} \times \text{TP}), \quad (2)$$

$$\text{TQRTRIX} = 50\text{th UNTRIXref} / 75\text{th UNTRIXsite}, \quad (3)$$

Chl-a: Chlorophyll-a concentration ($\mu\text{g L}^{-1}$).

DO₂ (%): Absolute deviation of measured dissolved oxygen content in % from 100% saturation.

TIN: Concentration of total N ($\mu\text{g L}^{-1}$).

TP: Concentration of total P ($\mu\text{g L}^{-1}$).

The comparative evaluation of the TRIX index results was conducted based on the table below (Table 1), which presents the classification of the aquaculture area according to the trophic index.

Table 1. “Eutrophication Risk Scale Table” from the “Environmental Management Regulation for Fish Farms Operating in Marine Areas” [28].

TRIX Value	Eutrophication Status / Class Definition	Explanation
< 4*	No Eutrophication Risk / High quality	Aquaculture is permitted
4–5*	Low Eutrophication Risk / Good quality	Aquaculture is allowed for existing facilities, but no new facilities are permitted
5–6*	Eutrophication Risk Present / Moderate quality	No new aquaculture facilities are allowed; restrictions are imposed on existing facilities
> 6*	High Eutrophication Risk / Bad quality	Aquaculture is not permitted, and existing facilities must cease operations

¹ For the Black Sea, an additional +1 is applied.

This ratio may range from 0 to 1; the higher the value, the more similar the site is to the reference site. The trophic scale according to TQRTRIX is presented in Table 2 [45].

Table 2. Trophic status of coastal waters based on the TQRTRIX trophic scale.

TQRTRIX value	Trophic classification
0.00–0.29	Bad
0.30–0.49	Poor
0.50–0.69	Moderate
0.70–0.84	Good

0.85–1.00

High

2.3. Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted to evaluate differences in water quality parameters among the stations. Following ANOVA, Tukey's HSD post hoc test was applied to identify statistically significant differences at the levels of $p<0.05$, $p<0.01$, and $p<0.001$.

3. Results

The aquaculture site examined in the present study is located in the southern part of the Black Sea and exhibits the characteristics of a semi-enclosed gulf. The average depth of the production area ranges between 40 and 78 meters. A total of 33 aquaculture enterprises operate within this region. While the total licensed production capacity of these enterprises is 38,880 tons per year, the actual production volume in 2023 was approximately 26,700 tons. In addition to the impacts associated with aquaculture activities, urban and industrial influences are also present in and around the production area.

3.1. Water Quality Parameters

The average temperature across the stations was measured at $21.72\pm0.11^\circ\text{C}$ ($p>0.05$), the average salinity at $‰16.84\pm0.05$ ($p>0.05$), the average pH at 8.28 ± 0.03 ($p>0.05$), and the average dissolved oxygen concentration at $8.69\pm0.06 \text{ mg/L}$ ($p<0.05$).

The mean depths of the sampling stations ranged from 42.1 to 77.2 m, while Secchi disk depths were recorded between 3.0 and 14.8 m. Chlorophyll-a concentrations ranged from 0 to 0.5 $\mu\text{g/L}$, and dissolved oxygen saturation varied between 88.07% and 116.83%. Total dissolved inorganic nitrogen (TIN) concentrations in the production areas ranged from 0.073 to 0.404 mg/L , whereas total phosphorus (TP) values were recorded between 0.022 and 0.05 mg/L (Table 3).

Table 3. Coordinates, Depth, Secchi Disk Depth, Chlorophyll-a, Dissolved Oxygen (%), TIN, and TP Values of Sampling Stations.

Station	Coordinates	Depth (m)	Secchi Disk (m)	Chlorophyll-a	Dissolved Oxygen (%)	TIN	TP
1	41°50'48.23-35°13'47.09	57.5	3	0.5<	98.57±2.16	0.081±0.006	0.014±0.002
2	41°54'04.63-35°11'23.91	53.8	3	0.1<	106.82±2.18	0.331±0.01	0.005±0.001
3	41°54'40.19-35°09'48.45	49.6	3	0.1<	112.00±2.11	0.333±0.02	0.005±0.001
4	41°53'56.33-35°10'32.72	48.7	5.7	0.1<	106.96±2.13	0.351±0.02	0.005±0.001
5	41°52'15.59-35°12'38.73	48.0	11	0.5<	99.53±0.03	0.096±0.01	0.020±0.001
6	41°52'06.89-35°12'49.84	47.4	11	0.5<	99.40±0.06	0.106±0.01	0.020±0.001
7	41°43'42.22-35°23'39.96	46.4	11	0.5<	99.43±0.04	0.101±0.02	0.019±0.001
8	41°52'04.00-35°12'43.06	42.1	5.7	0.5<	99.52±0.02	0.106±0.01	0.020±0.001
9	41°45'38.14 35°21'14.32	76.0	10.7	0.5<	88.45±3.21	0.073±0.005	0.012±0.002
10	41°45'28.15-35°23'36.03	77.2	11	0.5<	88.07±2.91	0.087±0.004	0.011±0.003
11	41°43'51.23-35°20'22.09	58.0	11	0.5<	99.17±2.96	0.079±0.003	0.015±0.002
12	41°43'28.62-35°23'33.40	54.0	11	0.5<	99.60±2.75	0.097±0.004	0.022±0.003
13	41°42'54.32-35°27'41.20	59.0	13	0.5<	97.18±3.77	0.158±0.078	0.010±0.003
14	41°41'51.20-35°30'15.41	54.2	14.8	0.5<	116.83±2.98	0.398±0.065	0.010±0.002
15	41°41'42.30-35°27'33.61	57.4	12.3	0.5<	99.75±3.45	0.113±0.059	0.022±0.003
16	41°40'55.72-35°30'04.25	57.4	14.1	0.5<	102.73±1.06	0.404±0.063	0.010±0.001

Dissolved oxygen values ranged from a minimum of 88.07% to a maximum of 116.83%. Total dissolved inorganic nitrogen (TIN) concentrations varied between 0.073 and 0.404 mg/L . Total phosphorus (TP) values were recorded between a minimum of 0.005 and a maximum of 0.022 mg/L . Chlorophyll-a concentrations were generally observed to be below 0.5 $\mu\text{g/L}$.

3.2. TRIX, UNTRIX, and TQRTRIX Values

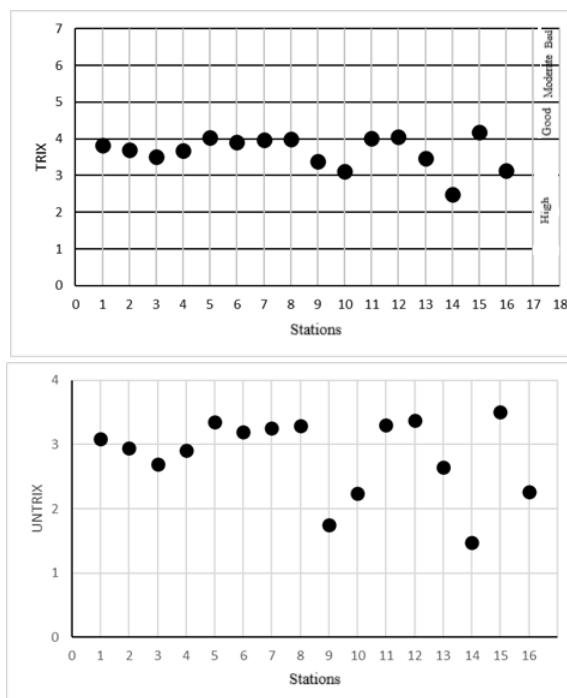
The TRIX, UNTRIX, and TQRTRIX values calculated from the data collected in the production area are presented in Table 4.

Table 4. Calculated TRIX, UNTRIX, and TQRTRIX Values.

Station	Coordinates	TRIX	Trophic status	UNTRIX	TQR _{TRIX}	Trophic status
1	41°50'48,23-35°13'47,09	3,83±0,06	High	3,09±0,08	0,57±0,02	Moderate
2	41°54'04,63-35°11'23,91	3,71±0,05	High	2,95±0,06	0,64±0,03	Moderate
3	41°54'40,19-35°09'48,45	3,50±0,05	High	2,70±0,05	0,68±0,01	Moderate
4	41°53'56,33-35°10'32,72	3,68±0,04	High	2,91±0,05	0,64±0,01	Moderate
5	41°52'15,59-35°12'38,73	4,04±0,02	Good	3,35±0,03	0,59±0,05	Moderate
6	41°52'06,89-35°12'49,84	3,92±0,01	High	3,20±0,02	0,60±0,04	Moderate
7	41°43'42,22-35°23'39,96	3,97±0,02	High	3,26±0,03	0,78±0,05	Good
8	41°52'04,00-35°12'43,06	4,00±0,02	Good	3,29±0,04	0,61±0,03	Moderate
9	41°45'38,14 35°21'14,32	3,39±0,08	High	1,75±0,15	0,67±0,04	Moderate
10	41°45'28,15-35°23'36,03	3,12±0,11	High	2,24±0,11	0,88±0,03	High
11	41°43'51,23-35°20'22,09	4,01±0,05	Good	3,31±0,07	0,77±0,01	Good
12	41°43'28,62-35°23'33,40	4,06±0,03	Good	3,37±0,01	0,75±0,01	Good
13	41°42'54,32-35°27'41,20	3,46±0,15	High	2,65±0,22	0,64±0,08	Moderate
14	41°41'51,20-35°30'15,41	2,48±0,18	High	1,48±0,19	0,66±0,04	Moderate
15	41°41'42,30-35°27'33,61	4,17±0,04	Good	3,51±0,06	0,31±0,02	Poor
16	41°40'55,72-35°30'04,25	3,13±0,09	High	2,26±0,10	0,54±0,06	Moderate

The TRIX values for all production areas ranged from 2.48 to 4.17. According to the TRIX index scale provided in Table 1, stations with TRIX values below 4 were classified as having "No Eutrophication Risk." In contrast, those with values between 4 and 5 were categorized as having a "Low Eutrophication Risk." The UNTRIX values in the production areas were found to range from 1.48 to 3.51. Overall, a general consistency was observed between the TRIX and UNTRIX values calculated for the stations within the production area. The TQRTRIX values ranged from 0.31 to 0.88.

The TRIX, UNTRIX, and TQRTRIX values calculated from the production area data are illustrated in Figure 4.



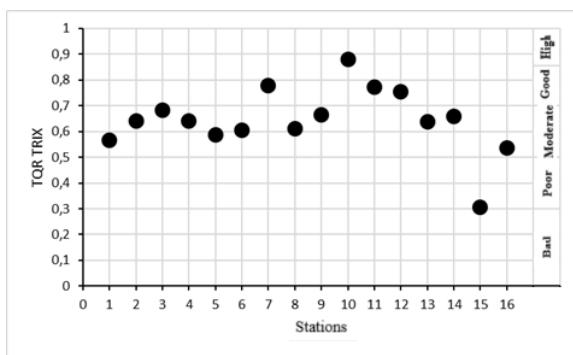


Figure 4. TRIX, UNTRIX, and TQRTRIX values.

Among the calculated TRIX and UNTRIX values for the production area, the best value was identified at Station 14, which also had the highest Secchi disk depth (14.5 m). In terms of the calculated TQRTRIX values, the best result was found at Station 10.

The statistical analysis conducted in the study revealed significant differences among the sampling areas of the production zones in terms of Chl-a, TP, depth, Secchi disk depth, and TRIX values (Table 5).

Table 5. Values of one-way analysis of aquaculture area.

Variables	Sampling area		
	d.f.	F	p level
Chl-a	16	5,28	*
DO	16	3,28	ns
DIN	16	3,20	ns
TP	16	4,19	*
Depth	16	8,06	**
Secchi disk depth	16	6,80	**
TRIX	16	27,63	***
UNTRIX	16	1,66	ns
TQRTRIX	16	1,37	ns

*p<0.05, **p<0.01, ***p<0.001. ns: not significant.

4. Discussion and Conclusions

According to the European Water Framework Directive (WFD), the classification of coastal waters serves as the basis for assessing ecological status [46–48]. Marine and coastal environments are influenced by numerous factors that can lead to eutrophication. In recent years, changes in marine water parameters have altered trophic status by increasing phytoplankton abundance, thereby imposing stress and pressure on local species [49]. Moreover, coastal waters are characterized by rapid temporal fluctuations in nutrient concentrations and chlorophyll-a levels, rendering them highly dynamic systems [20]. To understand the dynamics of marine ecosystems, trophic status can be evaluated using various indices [50]. However, in addition to monitoring eutrophication-related variables, reference data are also essential for assessing trophic conditions [51].

Aquaculture is particularly sensitive to pollution and requires a cleaner environment compared to other coastal activities. Therefore, all factors that may negatively impact the ecosystem must be thoroughly examined [52]. In this context, the present study evaluated the eutrophication and trophic status of the aquaculture area using the TRIX, UNTRIX, and TQRTRIX indices and compared the results with the desired values for coastal waters.

According to the TRIX index proposed by Vollenweider et al. [19] and the reference classification table of the Ministry of Environment and Urbanization [28], TRIX values for the production area in the present study ranged from 2.48 to 4.17, indicating a High–Good trophic status. In the present

study, 31.25% of the aquaculture area exhibited TRIX values between 4 and 5, corresponding to a “Low Eutrophication Risk.”

Giovanardi and Vollenweider [20] reported that TRIX values exceeding 6 for coastal waters indicate potential eutrophication, while values below 4 suggest rare eutrophication and values below 3 correspond to areas with very low productivity and low phytoplankton abundance.

Trophic status classification based on TRIX is as follows: 2–4 Good, 4–5 Moderate, 5–6 Poor, and 6–8 Bad (dlgs. 152/99) [21].

Aertebjerg et al. [53] noted that the trophic status of temperate waters is strongly influenced by seasonal variations, displaying tendencies toward both low and high trophic conditions during winter and spring within the same year. Saravi [54] indicated that even in the presence of high nutrient concentrations, TRIX values may remain low under low-temperature conditions. As the Black Sea is not classified as a temperate sea, seasonal changes are expected to have a limited impact on its trophic status. The present study was conducted during the summer, when seawater temperatures were relatively high, and differences in TRIX values were observed among the stations.

An inverse relationship was identified between TRIX values and the depth of the production areas, although depth alone is not an isolated influencing factor. This finding aligns with the assessments of Saravi [54], Shahrban and Etemad-Shahidi [55], and Saravi et al. [56]. It has also been reported that nearshore marine areas experience more rapid degradation in water quality and are more susceptible to eutrophication [57]. Coastal zones located closer to land are directly influenced by terrestrial runoff [45].

The ongoing development of eutrophication assessment tools highlights the need to strengthen the linkage between nutrient-related variables and both the direct and indirect effects of eutrophication [58]. According to the TQRTRIX values and reference classification proposed by Pettine et al. [45], the trophic status of the production area in the present study was classified as Poor-High (TQRTRIX values: 0.31–0.88). Additionally, UNTRIX values for the production area ranged from 1.48 to 3.51.

Various studies have demonstrated that different trophic indices used for classifying coastal water trophic status are generally compatible and consistent, thus supporting their application in trophic classification [18,45]. However, other studies have indicated that TRIX and UNTRIX values are not always consistent or meaningful and that variations in trophic status may be influenced by nutrient loads, particularly nitrogen and phosphorus concentrations [56,59].

In the present study, the TQRTRIX index, based on TRIX and UNTRIX, was utilized to determine the trophic status of the production area. Based on average TRIX and TQRTRIX values, the area was found to exhibit a low trophic level and a low risk of eutrophication, considering seasonal variation. When compared with established reference thresholds, the results remained below legal limits [28]. TRIX values likely remained below threshold levels because aquaculture operations in the region were not functioning at full capacity. Given the recent increase in aquaculture activity in the Black Sea, continuous monitoring and evaluation of production sites based on reference thresholds is essential. Furthermore, it was observed that, in addition to the main components of the trophic indices (chlorophyll-a, %DO, TIN, TP), both the average depth of the production area and Secchi disk depth were correlated with the index values.

The findings of the present study demonstrate that all stakeholders involved in aquaculture operations must employ continuous monitoring through TRIX applications and systematically analyze the resulting data to ensure the sustainable management of coastal environments.

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