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Eutrophication and trophic status using different indices: A study in the Iranian coastal waters of the Caspian Sea

Nasrollahzadeh Saravi H.[1*]; Pourang N.[2]; Foong S.Y.[3]; Makhlough A.[1]

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Abstract

The aim of this study was to assess the eutrophication and trophic status of the Caspian Sea based on different indices and also compare its status with the reference threshold values in the Iranian coastal waters of Caspian Sea (hereafter ICWCS). The water samples (three replicates) were collected along four transects (off the coast from Anzali, Tonekabon, Nowshahr and Amirabad) between spring 2013 and winter 2014. The results show marked significant differences between the mean concentrations of DIN, TP, DO and Chl-a in different seasons (p<0.01) and high significant correlation with different indices in the ICWCS. The mean of Trophic Index of Vollenweider ($TRIX_{vollen.}$), Trophic Index of the Caspian Sea (TRIXcs) and Technique Quartile Range of Trophic Index (TQR_{TRIXCS}) values ranged from 4.43 to 5.05 (good, high eutrophication risk), 5.21 to 6.00 (moderate, high eutrophication risk) and 0.58 to 0.95 (moderate to high) in different seasons for these three indexes, respectively. The results of nutrients and chlorophyll-a concentrations at the four sampling locations when compared with the reference threshold value indicated that the trophic status of this ecosystem changed from oligotrophic to mesotrophic/meso-eutrophic in 2013-2014. All there indexes (TRIXcs, TQR_{TRIXCS} and Reference Threshold Value methods) produced similar results and therefore, could be used interchangeably for the study area.

Keywords: Eutrophication, Trophic indices, Iranian coast, Caspian Sea

1-Caspian Sea Ecology Research Center (CSERC), Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Sari, Iran.

2-Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.

3-School of Biology, University Science Malaysia, Penang, Malaysia.

*Corresponding author's Email: hnsaravi@gmail.com

Introduction

For the past ten years or so, the Caspian Sea has undergone environmental changes mainly from high level nutrient inputs from anthropogenic sources of the catchment area such as agriculture, industry, tourism, fishing, and fisheries (Dumont, 1998). The combined effects of internal and external loadings lead to eutrophication of coastal waters, which is considered to be one of the greatest threats to the health of this marine ecosystem (Nasrollahzadeh Saravi *et al*., 2013). Besides eutrophication, the dynamic Caspian Sea is enduring other forms of threats such as rapid decline in its sea level, changes in the hydro-chemical regime and alteration of dominant phytoplankton species which is under the influence of increasing anthropogenic loads (Makhlough *et al*., 2011). These imminent threats highlight the need for reliable quantitative and qualitative assessments of the trophic status, suitable for different parts of the Caspian Sea.

Numerous methods have been developed for the quantitative assessment of eutrophication: statistical techniques, simulation models and water quality indicators are the most widely used techniques to assess trophic levels. The objectives of these methods are mainly to evaluate the impacts from high nutrient/phytoplankton biomass input on environment. Water types are usually categorized as being oligotrophic, mesotrophic or eutrophic. This classification is valuable to assess environmental quality and subsequently provide guidelines to coastal managers and planners in determining the best practice for the ecosystem (Karydis, 2009).

Many researchers apply Trophic Index (TRIX) and modified TRIX to gauge the status of coastal water ecosystem. The trophic levels on average were "good"(TRIX annual mean = 4.7) and the probability of exceeding the critical levels of 5 and 6 TRIX units was 37% and 5% in the Emilia-Romagna coastal area of the NW Adriatic Sea and the Tuscany littoral of the Northern Tyrrhenian Sea, respectively (Giovanardi *et al*., 2006). The use of the composite TRIX produced mean TRIX values of 3.6 for the aquaculture area and 2.5 for areas of the outer Bay of Izmir where no aquaculture takes place, this indicated no risk of eutrophication under the Turkish law. In the heavy urbanized inner Izmir Bay area, the mean TRIX value of 4.3 was above the threshold of four for a typical high eutrophication risk zone. TRIX has also been used in the Ionian Sea to appraise the impact of a wastewater treatment plant in its local modified form where Kalamitsi Trophic Index (KALTRIX) and Unscaled TRIX (UNTRIX) were applied. The results showed that the operation of a wastewater treatment plant did not affect the water quality in Kalamitsi (Nikolaidis *et al*., 2008). However, at times these indexes (original or site specific) were unable to provide unanimous interpretations. For instance, Kaptan (2013) reported that the nearshore zone of Mersin Bay in the northeastern Mediterranean Sea was “eutrophic” whilst the offshore waters displayed "oligotrophic" properties. Comparison of the TRIX, TRIX-IMS (modified TRIX with site specific 'a' and 'b' coefficients) and UNTRIX classification approaches revealed that the original TRIX index was not able to demonstrate impacts of excessive nutrient loads on the oligotrophic waters. Whereas the TRIX-IMS and UNTRIX approaches categorized the inner bay water body to be "eutrophic" as compared to the "oligotrophic" waters of the outer bay. In the case of the southern Caspian Sea, the surface water values according to the $TRIX_{CS}$, varied between 4.00 and 5.00 during 1995-96 and increased to 5.31 in 2005 (Nasrollahzadeh Saravi *et al*., 2008). On the other hand, Shahrban and Etemad-Shahidi (2010) who used NTRIX (New trophic index) reported an annual mean values of the coastal water of southern Caspian Sea to range from good to poor trophic status.

The European Environmental agency has evaluated TRIX and suggested that this index scale should be developed at regional levels. In addition, Primpas and Karydis (2011) reported that it is questionable whether TRIX index can form a universal index of eutrophication or the scaling of TRIX should be region specific. Therefore, the aim of this study was to assess the eutrophication and trophic status based on different indices ($TRIX_{vollen,}$ $TRIX_{CS}$ and TQR_{TRIXcs}) and also comparing the status with the reference threshold values to characterize oligotrophic, mesotrophic, and eutrophic conditions in the ICWCS.

Materials and methods

Study area

This study was carried out in the framework of a multidisciplinary research project during 2013 and 2014. The water samples (three replicates) were collected seasonally (May13, August13, November13 and February14) using Niskin bottle (1.7 L) along four transects (Anzali, Tonekabon, Nowshahr and Amirabad) in the ICWCS. Along each transect, samples were collected at 3, 5, 10 and 20 m depths from the surface, 5, 10 and 20 m layers (Fig. 1). The samples were kept in 1L polyethylene bottles and placed on ice. In the laboratory the samples were kept frozen in the dark (-20 ºC) until they were analyzed. Besides this, sample collections were performed in all four seasons because of the significant difference of environmental and biological parameters between seasons in the ICWCS (Nasrollahzadeh, 2008).

Figure 1: Map of the sampling stations in the Iranian coasts of Caspian Sea.

Physico-chemical and biological measurements

Water transparency was measured using a Secchi disc depth (SDD). Nitrate and nitrite were determined using the reduction column and the standard pink azo dye method, respectively while ammonia was analyzed by the hypo-phenol oxidation blue dye method (dissolve inorganic nitrogen, DIN=NO_2+NO_3+NH_4). Digestion of samples for the determination of TP (Total Phosphorous) was performed following the persulfate digestion procedure of Valderrama (1981) and level of TP was determined using the standard molybdenum blue method. All the methods and procedures were in accordance with those recommended by the APHA-standard method (APHA, 2005) and the Russian standard (Sapozhnikov *et al*., 1988). Three replicates were used in every chemical analysis. The determination of DO (Dissolved Oxygen) was carried out using the Winkler method according to Bryan et al. (1976). Dissolved oxygen saturation (DO %) was calculated using the standard table for the Caspian Sea (Sapozhnikov *et al*., 1988). Water samples for chlorophyll-a (Chl-a) determination were filtered through Whatman GF/C glass fiber filters and pigment extraction was performed using 90% acetone. Pigment concentration was measured by spectrophotometry and calculations were done according to APHA (2005).

Eutrophication and trophic Status

The TRIX index integrates chlorophyll a (as a proxy of phytoplankton biomass), oxygen saturation, dissolved inorganic nitrogen and phosphorus. TRIX equation 1 as proposed by Vollenweider *et al.* (1998) is ($TRIX_{vollen.}$=Trophic Index of Vollenweider):

$TRIX_{Vollen}$ = [log (Chl-a * aD%O * DIN* TP)-(-k)]/m………….. (1)

Each of the four components in $TRIX_{Vollen}$: Chl-a =chlorophyll a concentration (µg/L), aD%O=oxygen as absolute % deviation from saturation, DIN = (NO_3-N)+(NO_2-N)+(NH_4-N), as µg L^{-1}, TP= total phosphorus, as µg L^{-}

[1]), scaling factors of k (1.5) and m (1.2) were based on an extended dataset concerning the northern Adriatic Sea. In addition, scaling factors regarding the $TRIX_{CS}$ (Trophic Index of Caspian Sea) (k=1.03, m=0.93) were also applied in this study (Nasrollahzadeh Saravi *et al*., 2008). Long term data from the same transects and area were adopted from Nasrollahzadeh Saravi (2008).

Based on the data in the Italian seas, five trophic categories were defined (Pettine *et al*., 2007). This method expresses a simple number which can be used for trophic status classification (Table 1). Therefore, Pettine *et al*. (2007) proposed the calculation of an unscaled TRIX (UNTRIX), as shown in equation 2:

UNTRIX= log (Chl-a * aD%O * DIN* TP) ………….. (2)

One alternative option for assessing the trophic status by using the UNTRIX is based on the TQR_{TRIX} trophic index and a related trophic scale. TQR_{TRIX} trophic index is given by the ratio between the median UNTRIX value in the specific reference site (in the current study median of data in 1995-1997 at the ICWCS was considered as a $50^{th}UNTRIX_{reference}$) and the 75^{th} percentile UNTRIX in an impacted site, according to equation 3 (TQR_{TRIXCS} =Technique Quartile Range of Trophic Index):

$TQR_{TRIX}=50^{th}UNTRIX_{reference}/75^{th}UNTRIX_{site}$………….. (3)

This ratio may vary in the range of 0-1; the higher the value of the ratio, the more similar is the site to the reference site. The trophic scale is given in Table 1 according to TQR_{TRIX} (Pettine *et al*., 2007).

In 2007, Turkey enacted a new environmental legislation for the purpose of protection of water from pollution in its enclosed bays and gulfs. This legislation uses TRIX to define eutrophication for aquaculture activity according to the indices: TRIX<4 means no eutrophication risk; 4≤TRIX≤ 6 is high eutrophication risk; and TRIX>6 is eutrophic (MEF, 2007).

Table 1: Eutrophication scale and trophic status according TRIX and TQR_{TRIX}. (MEF, 2007; Pettine *et al*., 2007)

TRIX	Eutrophication status	TRIX	Trophic status	TQR_{TRIX} value	Trophic status
TRIX<4	No eutrophication risk	2>TRIX	Excellent	0.00–0.29	Bad
4≤TRIX≤6	High eutrophication risk	2<TRIX≤4	High	0.30–0.49	Poor
TRIX>6	Eutrophic	4<TRIX≤5	Good	0.50–0.69	Moderate
		5<TRIX≤6	Moderate	0.70–0.84	Good
		6<TRIX<8	Poor	0.85–1.00	High

Excellent: Ultra-oligotrophic, High: Oligotrophic, Good: Mesotrophic, Moderate: Meso-eutrophic, Poor: Eutrophic, Bad: Hypertrophic

Most of the time, it is difficult to discriminate whether the source of nutrient enrichments in coastal environments is from natural or anthropogenic sources (OECD, 1982). However, we can always use a similar ecosystem which is still pristine as a reference site to compare purposes by looking into variables that are related to eutrophication and trophic status. These unimpacted ecosystems are characterized by low anthropogenic influences and are categorized as oligotrophic systems (Vounatsou and Karydis, 1991). The main water body of the southern Caspian Sea was oligotrophic during 1995-1997 as indicated by many authors (Pourgholam, 1998; Nasrollahzadeh Saravi, 2008; Nasrollahzadeh Saravi *et al*., 2008; Ganjian *et al*., 2010a; Hosseini, 2011; Nasrollahzadeh Saravi *et al*., 2013). After a quarter of a decade, the present study intends to review the trophic status of the Caspian Sea water body focusing on comparing the nutrient levels with those of 2013/14, and Chl-a and water transparency values with oligotrophic reference values of 1995/97 (low nutrient levels, impoverished phytoplankton populations) as shown in Table 2. Outliers were removed from each variable to normalize data. A detailed work on nutrient and other parameters scaling has been mentioned by Ignatiades *et al*. (1992).

Table 2: Reference threshold values of some variables related to eutrophication and trophic assessment from oligotrophic waters in the Iranian coastal of the Caspian Sea.

SDD (m)	Chl-a (µg L^{-1})	DIP-P (µg L^{-1})	NO_3-N (µg L^{-1})	NO_2-N (µg L^{-1})	NH_4-N (µg L^{-1})	Reference
6.13 (N=154)	1.07 (N=81)	10.23 (N=567)	6.02 (N=457)	1.26 (N=567)	8.96 (N=567)	1995-1997 (Pourgholam, 1998)

Data analysis

One-way analysis of variance (ANOVA) was used to analyze effects of seasons, depths and stations on variations in the physic-chemical and biological parameters. Relationships among the trophic state parameters were assessed by Pearson's correlation test with statistical significance set priori at $p<0.05$. Stepwise regression analyses were executed to determine the combined influences of each variable on trophic state parameters of the ecosystem. Outliers of all data were removed from each variable.

Results

The mean concentration of DIN, TP and Chl-a fluctuated around 36 to 58, 28 to 33, and 1.84 to 2.76 µg L^{-1}, respectively at different transects (Table 3). The mean concentration of DIN, TP and Chl-a varied around 49 to 57, 28 to 29, and 1.99 to 2.72 µg L^{-1}, respectively at different depths (Table 3). The DO concentration was around 6.5 to 6.9 ml L^{-1} at different depths and transects. Results also show that the mean concentration of DIN was significantly lower in the Amirabad transect as compared with the other three transects and Chl-a concentration was significantly greater in the Anzali transect compared with the other transects (Table 4, $p<0.01$). The analysis of the data shows that there were no significant differences between the mean concentrations of DIN, TP, DO and Chl-a at different depths (Table 4, $p>0.05$).

Table 3: Mean (±SE) of components of trophic index in ICWCS (2013-2014).

Depths (m)	DIN ($\mu g\ L^{-1}$)	TP ($\mu g\ L^{-1}$)	DO ($ml\ L^{-1}$)	Chl-a ($\mu g\ L^{-1}$)
5	58.9±1.9	28.9±1.8	6.69±0.26	1.99±0.30
10	49.6±2.4	28.3±1.4	6.70±0.19	2.71±0.36
20	49.0±1.8	29.1±1.0	6.65±0.17	2.15±0.22
Transects				
Anzali	58.1±1.3	28.6±1.2	6.93±0.15	2.76±0.35
Tonekabon	52.3±1.5	27.0±1.3	6.84±0.21	2.24±0.29
Nowshahr	55.7±1.7	27.6±1.0	6.56±0.24	2.62±0.36
Amirabad	36.3±2.1	33.2±2.2	6.51±0.27	1.84±0.17

A comparison of the seasonal changes in the mean concentrations of dissolved inorganic nitrogen (DIN), total phosphorous (TP), dissolved oxygen (DO) and Chl-a are presented in Fig. 2(a) and Fig. 2(b). The mean (±SE) annual concentrations of DIN, TP, DO and Chl-a were 50.48±1.24 $\mu g\ L^{-1}$, 28.84±0.71 $\mu g\ L^{-1}$, 6.67±0.11 $ml\ L^{-1}$ and 2.31±0.17 $\mu g\ L^{-1}$, respectively. Analysis of the data shows significant differences between the mean concentrations of DIN, TP, DO and Chl-a in different seasons (Table 4, $p<0.01$).

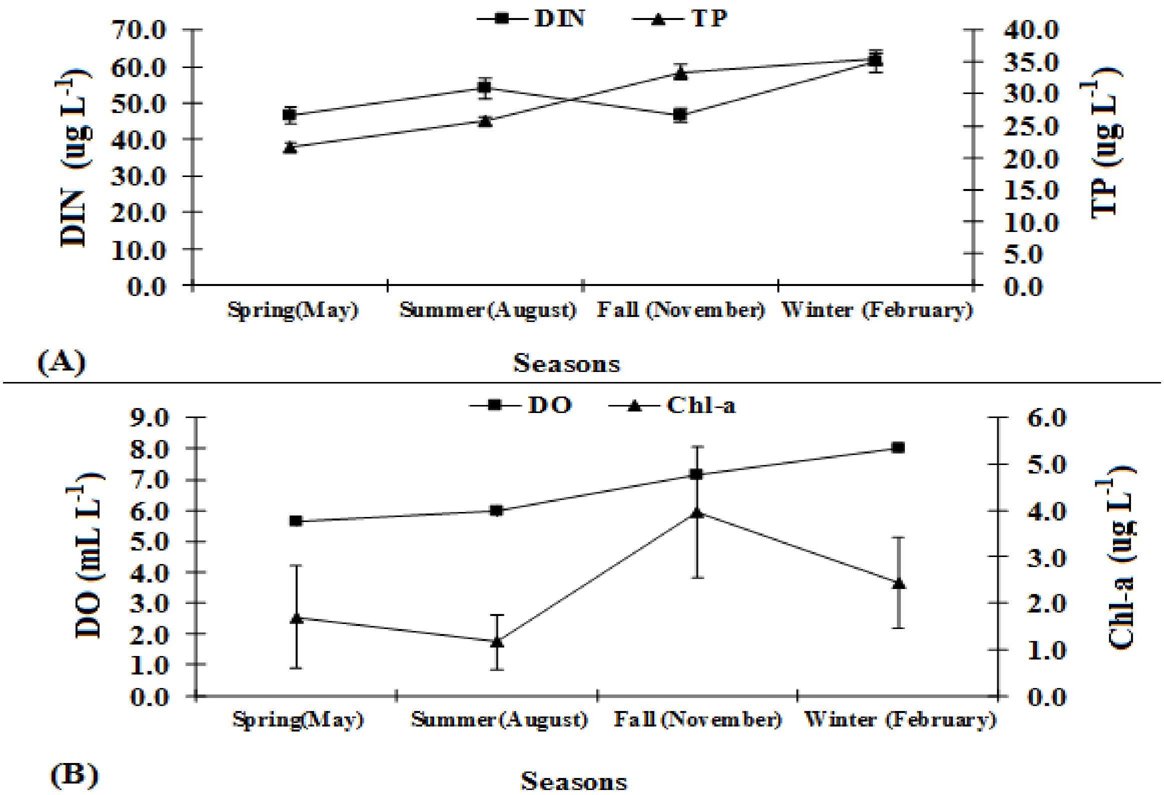

Figure 2: Seasonal changes in the mean concentrations of environmental parameters collected from four transects in ICWCS (2013-2014). (A) DIN and TP; (B) DO and Chl-a.

Table 4: Values of one-way analysis of variance for all sampling periods in different areas of ICWCS (2013-2014).

	Depths			Transects			Seasons		
Variables	d.f.	*F*	*p* value	d.f.	*F*	*p* value	d.f.	*F*	*p* value
$TRIX_{vollen.}$	2	0.23	ns	3	2.13	ns	3	6.00	**
$TRIX_{CS}$	2	0.24	ns	3	2.15	ns	3	5.68	**
Chl-a	2	0.64	ns	3	4.87	**	3	25.76	***
DIN	2	2.36	ns	3	13.61	***	3	8.51	***
TP	2	0.52	ns	3	2.20	ns	3	51.76	***
DO	2	1.00	ns	3	0.98	ns	3	74.23	***

**$p<0.05$, ***$p<0.001$, d.f.: degrees of freedom, ns: not significant

Mean (±SE), minimum and maximum of different trophic indices of the ICWCS are shown in Table 5. The minimum and maximum values of $TRIX_{vollen,}$ and $TRIX_{CS,}$ were observed in spring and autumn, respectively. However, the minimum and maximum values of TQR_{TRIXCS} were indicated in winter and spring, respectively.

Table 6 presents Pearson correlation coefficients of different indices and variables in the ICWCS. The results show that all indices were significantly correlated with DO and Chl-a ($p<0.01$) and $TRIX_{CS}$ were significantly correlated with DIN ($p<0.05$), while these indices were not significantly correlated with TP ($p>0.05$) (Table 6). Stepwise regression coefficient of different indices and variables were indicated: $TRIX_{vollen.}$=-0.20+0.43Chl-a+0.32DO and $TRIX_{CS}$= -0.28+0.45Chl-a+0.29DO+0.27DIN.

Therefore, the two components of trophic indices, biological response (Chl-a, a proxy for biomass) and environmental disturbance in the water quality (DO), showed highly significant correlation with different indices in the ICWCS.

Table 5: Mean (±SE), minimum and maximum of different trophic indices of ICWCS (2013-2014).

		Spring	Summer	Autumn	Winter	Annual
$TRIX_{vollen.}$	Mean±SE	4.43±0.09	4.61±0.16	5.05±0.10	4.76±0.08	4.76±0.06
	(Min.-Max.)	(3.99-4.93)	(3.46-5.24)	(3.91-5.71)	(3.94-5.40)	(3.46-5.71)
$TRIX_{CS}$	Mean±SE	5.21±0.12	5.43±0.20	6.00±0.13	5.64±0.11	5.63±0.07
	(Min.-Max.)	(4.60-5.90)	(4.00-6.20)	(4.50-6.90)	(4.60-6.50)	(4.00-6.90)
TQR_{TRIXCS}		0.95	0.61	0.63	0.58	0.68

Table 6: Pearson correlation coefficients of different indices and variables in ICWCS (2013-2014).

	Chl-a	DIN	TP	DO
$TRIX_{vollen.}$	0.394**	0.252	0.09	0.374**
$TRIX_{CS}$	0.387**	0.272*	0.09	0.388**

**Correlation is significant at the 0.01 level (2-tailed),
*Correlation is significant at the 0.05 level (2-tailed)

In the present study, the concentrations of NH_4-N, NO_2-N, NO_3-N, DIP-P, Chl-a and SDD were measured as 22.40, 0.98, 25.20, 13.02, 2.48 µg.L^{-1} and 3.49 m, respectively in the ICWCS. Based on these results all parameters (except NO_2-N, SDD) were more than the reference threshold values presented in Table 2 showing that the trophic state of the ICWCS has deviated from oligotrophic status.

Discussion

We could rely on trophic conditions to obtain useful information on the dynamics of certain phenomena and in the meanwhile carry out comparisons between different sites (Cognetti, 2001). However, in addition to monitoring variables related to eutrophication, there is a need for a system to develop robust qualitative-quantitative indicators for assessing the trophic conditions (Izzo *et al*., 1997). Therefore, in this study, different trophic indices ($TRIX_{vollen.}$, $TRIX_{CS}$ and TQR_{TRIXcs}) in line with reference threshold value of the variables are evaluated to characterize oligotrophic, mesotrophic, and eutrophic condition in the ICWSCS over a period of time.

According to the $TRIX_{vollen}$. as proposed by Vollenweider *et al*. (1998) and the corresponding status made by Pettine *et al*. (2007) and MEF, (2007) (Table 1), the condition of the ICWSCS during different seasons corresponded to a good and moderate state and had the tendency toward the good state, which was characterized by moderate to low productive waters (seasonal mean of the TRIX: 4.43–5.05, annual mean; 4.76). According to the TRIXcs as proposed by Nasrollahzadeh Saravi et al. (2008), the condition of the ICWCS was considered moderate (seasonal mean of the TRIXcs: 5.21–6.00) with a yearly mean trophic status which was moderately productive (TRIXcs: 5.63). The mean value of the TQR_{TRIXcs} was calculated as 0.68 which resulted in a moderate trophic status at all seasons (except in spring) (Table 7). The results of TRIXcs are consistent with those of the actual potentialities of TQR_{TRIXcs} in the investigated areas.

Table 7: An inter comparison of mean values of trophic status in ICWCS (2013-2014) according to $TRIX_{Vollen}$, $TRIX_{CS}$ and TQR_{TRIXcs}.

	Spring	Summer	Autumn	Winter	Annual
$TRIX_{vollen.}$ scale	4.43	4.61	5.05	4.76	4.76
	Good	Good	Moderate	Good	Good
	High eutrophication risk	High eutrophication risk	High eutrophication risk	High eutrophication risk	High eutrophication risk
$TRIX_{CS}$ scale	5.21	5.43	6.00	5.64	5.63
	Moderate	Moderate	Moderate	Moderate	Moderate
	High eutrophication risk	High eutrophication risk	High eutrophication risk	High eutrophication risk	High eutrophication risk
TQR_{TRIXcs} scale	0.93	0.61	0.63	0.58	0.68
	High	Moderate	Moderate	Moderate	Moderate

In the current study, the value of $TRIX_{CS}$ varied from 4.00 to 6.50 and the critical level of 5 and 6 TRIX units was around 86% in this area (Fig. 3). Giovanardi and Vollenweider (2004) defined the TRIX values exceeding 6.00 as typical of highly productive coastal waters. Values lower than 4.00 TRIX units reflect scarcely productive coastal waters, while values lower than 3.00 are usually found in the open oceans where the productivity is low. In the case of ICWCS in the current study period, the $TRIX_{CS}$ value was only between 6.00 and 6.50 (<10% of whole data) in the autumn season (corresponding to high abundance of phytoplankton (Makhlough *et al*., 2013) strongly revealing an internal factor influencing the productivity of the area (Fig. 3). Long term changes in the trophic index showed that TRIXcs was lower than 5 in the year of 1995-2000 (before introduction of the *Mnemiopsis leidyi* ctenophore) and was more than 5 after the introduction of the ctenophore (Fig. 3). As Nasrollahzadeh Saravi *et al*., (2008) noted the $TRIX_{CS}$ increased significantly after the introduction of *M. leidyi* in 2005-06 (p<0.01). The increase was attributed to *M. leidyi* as it has been shown to directly affect the levels of various hydrochemical parameters (Shiganova *et al*., 2003). Meanwhile, the increase of $TRIX_{CS}$ might be due to the increase of anthropogenic impacts in this area that happened concurrently (Ganjian *et al.*, 2010b; Bagheri *et al*., 2012).

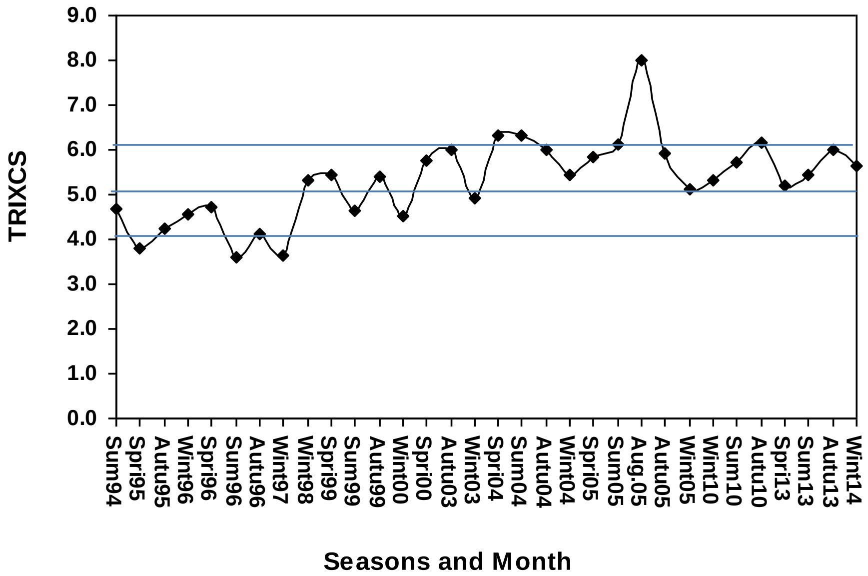

Figure 3: Long-term changes in the trophic index of ICWCS based on $TRIX_{CS}$ values. Long term data was adopted from Nasrollahzadeh Saravi (2008).The data for August 2005 was adopted from Soloviev (2005). Note the high peak of $TRIX_{CS}$ was in August 2005 when one species of Cyanophyta bloomed in the area.

Aertebjerg *et al*. (2001) reported that temperate waters tend to show strong seasonal variations in trophic status, ranging from low to high trophic status in winter and spring, respectively. Nasrollahzadeh Saravi (2008) reported that even if the nutrient concentrations are high in winter, production can be low, resulting in low values of the $TRIX_{CS}$. This trend was observed in the Caspian Sea during 1996-97 (before the introduction of the ctenophore), but during 2005 (after the introduction of the ctenophore), seasonal variations in the $TRIX_{CS}$ value were less obvious with the minimum recorded in winter and the maximum observed during autumn. In the current study, $TRIX_{CS}$ ranged from low to high trophic status in spring and autumn, respectively.

These results show that the maximum trophic status in the current study and in the year of 2005 (Nasrollahzadeh Saravi, 2008) were similar but the minimum values in the two studies were observed in different seasons. Coastal waters are very dynamic with fast changing temporal variations of nutrients and phytoplankton (Chl-a) (Giovanardi and Vollenweider, 2004). It is therefore obvious that trophic status could vary over space and time as discussed above.

The $TRIX_{CS}$ values were found to decline from 5 m (5.76±0.12) depths to 20 m (5.62±0.12) in all seasons and transects (p>0.05, Table 3, 4). Higher $TRIX_{CS}$ values at 5 m depths were probably associated with impacts of the Anzali Wetland, small rivers and rainfall in the near shore zone. $TRIX_{CS}$ values showed a decreasing trend from the Anzali transect (5.47±0.12) (western part) to Amirabad (5.34±0.10) (eastern part) (p>0.05, Table 3, 4) and this may be due to the presence of the Anzali Wetland and Sefidrud River in the western part. This finding agrees with studies by Nasrollahzadeh Saravi *et al*. (2008) and Shahrban and Etemad-Shahidi (2010) in the southern coasts of Caspian Sea which confirms the aforementioned discussion.

Results of reference threshold values (years of 1995-97) and prior monitoring studies showed that two variables NO_2-N and DIP-P had little changes (except in 1999-2000), but the other three variables had significant changes (Table 8). Ammonium-nitrogen and nitrate-nitrogen values were 3 to 5 fold higher and SDD had declined by almost 2 times as compared with reference threshold values. Moreover, the existing Chl-a value (2004-2014) was at least two times higher as compared with the reference threshold value. It is obvious that NH_4-N, NO_3-N, SDD and Chl-a values deviated from or exceeded the reference threshold values for the ecosystem as proposed by Nasrollahzadeh Saravi *et al*., 2008; Farabi, 2009; Shahrban and Shahidi, 2010; Nasrollahzadeh Saravi, 2010, 2011(Table 8). Therefore, based on these results, trophic state of the ICWCS has deviated from its oligotrophic status. Nasrollahzadeh Saravi (2008) and Soloviev (2005) reported mean values of NH_4-N, NO_3-N, DIP-P and Chl-a as 24.08, 25.90 and 22.32, >50 µg L^{-1}, respectively during August 2005 when cyanophyta species bloom occurred in the Caspian Sea. Izzo *et al*. (1997) noted that the reasonable assessment of the trophic situation was achieved based on historical records of the observed biological effects. So if these results (Soloviev, 2005; Nasrollahzadeh Saravi, 2008) concluded at an eutrophic condition for the southern Caspian Sea as compared to other studies (from 1999 to 2014), the results of the present study may conclude that the trophic status of this area is mesotrophic/meso-eutrophic. Results show that trophic state of reference threshold values and TRIXcs as proposed by Nasrollahzadeh Saravi *et al*. (2008) were similar in the ICWCS.

As Primpas and Karydis (2011) noted, the $TRIX_{vollen}$. (original TRIX) used for the assessment of trophic status of coastal waters has been applied in many European seas (Adriatic, Tyrrhenian, Baltic, Black Sea, and North Sea) and it is questionable whether this index can form a universal index of eutrophication or the scaling of TRIX should be region specific. Results of Table 7 show that trophic state of $TRIX_{vollen.}$ and TRIXcs were not similar (except in autumn) for this area. In the current study, it was found that the used scale (TRIXcs as proposed by Nasrollahzadeh Saravi *et al*. (2008)) is in good agreement and reliable for the region of the ICWCS. In addition, based on the trophic index of $TRIX_{vollen.}$ and TRIXcs the corresponding status made by Pettine *et al*. (2007) and MEF (2007) (Table 1), the condition in the ICWCS during different seasons was known to be at a high eutrophication risk according to Table 7.

Table 8: Mean and reference threshold values of the variables related to eutrophication and trophic assessment in ICWCS.

Reference	SDD (m)	Chl-a (µg L^{-1})	DIP-P (µg L^{-1})	NO_3-N (µg L^{-1})	NO_2-N (µg L^{-1})	NH_4-N (µg L^{-1})	Year of sampling
Reference values	6.13 (N=154)	1.07 (N=81)	10.23 (N=567)	6.02 (N=457)	1.26 (N=567)	8.96 (N=567)	1995-1997
Shahrban and Shahidi, 2010	-	1.14 (N=72)	44.95 (N=72)	19.32 (N=72)	3.22 (N=72)	16.38 (N=72)	1999-2000
Nasrollahzadeh Saravi *et al*., 2008	3.99 (N=96)	2.14 (N=96)	22.94 (N=264)	27.44 (N=264)	0.98 (N=264)	20.44 (N=264)	2004-2005
Farabi, 2009	4.61 (N=154)	-	6.51 (N=474)	18.34 (N=480)	0.84 (N=389)	12.46 (N=380)	2008-2009
Nasrollahzadeh Saravi, 2010	3.82 (N=150)	-	7.13 (N=446)	15.12 (N=400)	0.84 (N=420)	40.46 (N=464)	2009-2010
Nasrollahzadeh Saravi, 2011	4.61 (N=154)	2.19 (N=57)	9.30 (N=437)	17.64 (N=382)	0.98 (N=418)	15.12 (N=407)	2010-2011
Present study	3.49 (N=46)	2.48 (N=80)	13.02 (N=95)	25.20 (N=95)	0.98 (N=95)	22.40 (N=96)	2013-2014

N=number of observations, SDD: Secchi disc depth (dash means no data available)

The reference threshold values from this study were proposed in the ICWCS. They can also serve as a reference for researchers of other Caspian ecoregion countries to make new limits and ranges for the Caspian Sea to cover a wide spectrum of possible situations.

The present study attempts to re-visit the trophic index of the Caspian Sea ($TRIX_{CS}$) proposed by Nasrollahzadeh Saravi *et al*. (2008) to assess the trophic status along the ICWCS during 2013-2014. In addition, we present new reference threshold values of the variables in line with TQR_{TRIXcs} (in the current study median of data in 1995-1997 was considered as a $50^{th}UNTRIX_{reference}$) and test their potentialities in the classification of the ICWCS into trophic status and evaluated eutrophication risk of water types. According to the annually mean values of $TRIX_{CS}$ and TQR_{TRIXcs}, the study area has moderate trophic status (meso-eutrophic) which in certain seasons poses a high eutrophication risk. Results of reference threshold values (years of 1995-97) showed that the area of the Caspian Sea has shifted from oligotrophic (high) to the meso-eutrophic (moderate) status. Therefore, results of TRIXcs and TQR_{TRIXcs} are consistent with the actual potentialities of the reference threshold values in the investigated areas. In addition, based on the regression equation the two components of trophic indices, biological response (Chl-a, a proxy for phytoplankton biomass) and environmental disturbance in the water quality (DO%), showed significant correlation with TRIXcs in the ICWCS.

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