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# Research Article Evaluating limnological condition of Anzali international wetland during 32 years period using nutrient parameters and TSI indices

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# Abstract

Determining trophic condition of a wetland is an important step in scientific assessment of the aquatic ecosystem. High levels of nitrogen and phosphorus, is mainly the result of human activities in agriculture, industry, etc. Increasing levels of nutrients result in eutrophication of water ecosystems. Regarding the importance of Anzali international wetland, the present study conducted several models of Carlsons on trophic status of the wetland through 1985, 2007, 2014 and 2017. The evaluated stations include, Bandar Anzali under bridge, Sorkhankal, PirBazar, Siadarvishan, Nokhaleh, east of wetlands, Hendkhaleh, and west of wetland. The results of Analysis of Variance indicated significant difference (p<0.05) among values of the indicators listed in stations and different seasons. Assessment of trophy index (TSI) on total phosphorus (TP) and total nitrogen (TN) in order to reveal wetland condition determined oligotrophic and mesotrophic conditions in 1985 to acute hypertrophy in recent years. The results showed increase in the amounts of phosphorus and nitrogen during 1985 to 2017.

Keywords: Anzali international wetland, Carlson's indices, Eutrophication, Nutrient

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## Introduction

Eutrophication or nutrient enrichment is a problem of wetlands, rivers, streams and lakes all over the world (Neverovaand Kowalewski, Dziopak 2013). Eutrophication importance in wetlands and shallow lakes seems to be similar, but hydro logically variety of wetlands showed a remarkable difference to shallow lakes (Angeler et al., 2003, Ansari et al., 2014). Nutrient richness has been shown to influence all trophic levels within a wetland ecosystem and effects include variation in species abundance, species movement, species diversity reduction, and alteration in community structure and composition (Piceno and Lovell, 2000). Changes in biological assemblage structure in response to nutrient enrichment can result in physical and hydrologic changes to wetlands (Sievers et al., 2018). Determining trophic status of a wetland is a main step in scientific assessment of an aquatic ecosystem (Matthews et al., 2002), because it enables us to determine abiotic and biotic status of water bodies; chemical and biological relationships and lake condition in relation to human needs or values (Carlson and Simpson 1996, Najeeb et al., 2014). Trophic status determination is a multidiscipline evaluation of several criteria, including nutrients capacity for production, faunal and floral quality and quantity, and morphology. Unfortunately, a multi parameter trophy index is difficult in usage. Therefore, Carlson formulated trophic state indices (TSIs) as a quantitative measure of the grade of aquatic ecosystem eutrophication (Jarosiewicz et al., 2011). A research conducted on Anzali wetland has shown that water quality is deteriorating continuous through inflow wastewater derived from household, industrial, and agricultural activities. It is generally believed that Anzali wetland is an over polluted ecosystem (Tahershamsi et al., 2009). Incidence of eutrophication in Anzali international wetland was first reported by Kent Kimball and Sarah Kimball (Kimball and Kimball, 1974). Study of Mirzajani et al. (2010) between 1992-2002 indicated the incidence of atrophy in the wetland, similar to the studies of Kimball and Kimball (1974) and Darvishsefat et al. (1999). Anzali international wetland is situated in southern coast of Caspian Sea, in Gilan Province in north of Iran with the area of 193 km<sup>2</sup>. It is internationally well known as an important migration habitat for migratory birds, and is registered in Ramsar Convention in June 1975, and subsequently added to the Montreux Record of wetlands being degraded by human activities (Azimi-Yancheshmeh et al., 2017). Environment of the wetland is deteriorating due to inflow of wastewater, solid waste and sediments from watershed (JICA, 2005). Anzali international wetland is under several stresses, such as sea water level fluctuation, different human activity exotic species impacts. and eutrophication which are causes of over deterioration of wetland the (Khodaparast, 2003). Eutrophication

process rate is influenced by watershed hydrology, which in the case of Anzali wetland; include 52% of drainage Siadarvishan into central through region, 44% via Pirbazar, Khomamrud River into eastern region and 4% through other rivers (Azimi Yancheshmeh et al., 2017). Waters of eastern and central regions drain faster into the Caspian Sea than western basin which has more conserved conditions (JICA, 2005). This study is conducted to determine the trophy status of Anzali international wetland based on different models of Carlson's index (TSI), and to compare the trophy levels using the three mentioned indices in years 1985, 2007, 2014 and 2017.

# Materials and methods

#### Study Area

Anzali international wetland watershed is located between approximately 36°55' to 37°32' N and 48°45' to 49°42' E and is situated in northern part of Iran, at the coast of Caspian Sea (Fig. 1).

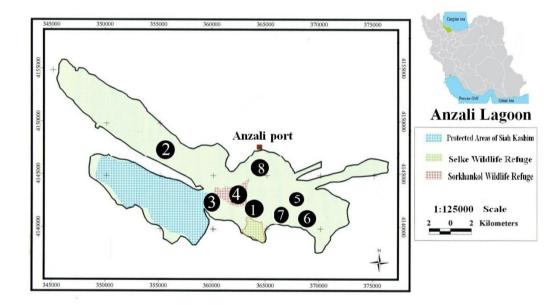


Figure 1: Map of Anzali wetland and sampling locations.

#### Sample collection and preparation

Anzali international wetland trophy evaluation process was carried out using historic data from 1985 and 2007 (with the same sampling time and location) and was compared with data of 2014 and 2017. Trophy data for 1985 and 2007 were obtained from Department of the Environment (DOE) of Iran. Stations Nahang Rogha, Sorkhankal, Pirbazar, Siadarvishan, Hendkhaleh, Nokhaleh, east and west of wetlands were selected (Table 1). They were split geographically between west, east, and center of the wetland. At each station, samples were collected every 45 days from March 2014 to March 2015 and March 2017 to March 2018 respectively, with 3 replicates. At each site, water samples were collected from the middle area of the wetland. For chemical and bacteriological analysis, samples were stored in bottles and sterile glass flask, respectively and processed within 24 hours upon collection.

Table 1: Geographical coordinates of the stations studied in Anzali International wetland (UTM).
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No.	Station	X	Y	Location
1	Hendkhaleh	363170	4140191	Center
2	West of wetland	358977	4145240	West
3	Siadarvishan	360645	4144411	West
4	Sorkhankal	360903	4140191	Center
5	East of wetland	367652	4142686	East
6	Pirbazar	367413	4140646	East
7	Nokhaleh	367267	4140676	East
8	Nahang Rogha	364257	4147399	Center

# Analytical procedures

Total nitrate and total phosphorus were analyzed after transferring the samples to the laboratory. All parameters were analyzed according to standard procedures (APHA, 1998).

#### Method to calculate the TSI

Trophic state indices characterize an aquatic ecosystem based on nutrients concentrations, chlorophyll *a* concentration, secchi disk transparency and other parameters. The most widely known is Carlson's Trophic State Index (TSI) which compares chlorophyll *a* concentration, secchi disk transparency, and total phosphorus concentration (Carlson and Simpson, 1996).

Considering unavailability of data of chlorophyll *a*, transparency and secchi disk, only values of phosphorus and nitrogen have been taken into consideration for calculating TSI index. The formula used to calculate phosphorus index is shown below: TSI(TP) = 14.42Ln(TP) + 4.15 (total phosphorus, in mg/L)

It is suggested that a nitrogen index to be used when water bodies are nitrogen limited (Kratzer and Brezonik 1981). The formula used to calculate nitrogen index is shown below:

# TSI(TN) = 14.43Ln(TN) + 54.45(Total nitrogen, in mg/L)

Carlson's index ranges from 0-100 and each 10-unit division of the index corresponds to a halving or doubling of secchi depth. In addition to trophic state indices, an index based on ratio of total phosphorus concentration to nitrogen was used; the formula used to calculate this index is shown below (Kratzer and Brezonik, 1981),

 $TSI(PN) = 9.81Ln(10^{PN}) + 30.6$  $Log_{(PN)} = 1.25Log_{(XPN)}$ 

$$XPN = \left[ P^{-2} + \left[ \frac{N - 150}{12} \right]^{-2} \right]^{-0.05}$$

Variations in this index as well as Carlson's phosphorus index simultaneously might indicate that neither nitrogen nor phosphorus are while variations in only limiting. Carlson's phosphorus index might indicate nitrogen limitation (Carlson and Simpson, 1996). Each 10 units within this system represents a half decrease in Secchi depth, a one-third increase in chlorophyll concentration and a doubling of total phosphorus amount (Dodds et al., 1998). Generally, value of TSIs below 40 correspond oligotrophy, between 40 and 60 mesotrophy, from 60 to 80 - eutrophy, and above 80 - hypertrophy of the aquatic ecosystem (OECD, 1982).

Finally, after calculating the three mentioned trophy indices, trophy of Anzali wetland was compared based on these indices during 1985, 2007, 2014 and 2017. Mapping of indices of this study were also prepared by Arc GIS 10.3.

# Statistical analysis

One-way ANOVA test was used to determine significant differences among mean values of trophy indices in years 1985, 2007, 2014 and 2017. Then Duncan's multiple range tests was performed when significant difference ANOVA found in results. was Differences were considered significant at p values <0.05. All statistical analyses were performed using SPSS statistical package (version 16). All data were reported as Mean±SE (standard error).

# Results

Estimated trophy indices (TSI) based on nutritious of phosphorus and nitrogen of stations during seasons is Figures 2 presented in and 3: phosphorus and nitrogen concentrations (milligrams per liter) are shown in Figures 5 and 6. Based on phosphate concentration. the phosphorus concentration index value TSI (TP) was the least in 1985 and most of stations were in mesotrophic to eutrophic situation (Figure 2). Whereas TSI index in 2007 and 2017 showed hypertrophy situation of the wetland. According to results of TSI based on phosphorus concentration index (TP), the greatest value for the index was within center in fall 2007 as well as east in summer 2017. On the other hand, the smallest value for the index was shown within west in winter 1985. Based on nitrogen concentration TSI (TN) results. maximum and minimum index values were measured along east in summer 2017 and center in summer 1985, respectively (Fiure 3). Overall, eutrophication process succeeded from mesotrophy to acute hypertrophy during 1985 to 2017. The ratio of phosphorus to nitrogen (PN) was in the range of (40.15-30.4) over the years. According to this range, phosphorus is considered to be limiting factor in Anzali wetland, which appears in mesotrophic wetlands (Fig. 4). Throughout freshwater ecosystems nitrogen abundance is more than phosphorus, phosphorus so productivity is limiting factor (Wetzel, 2001).

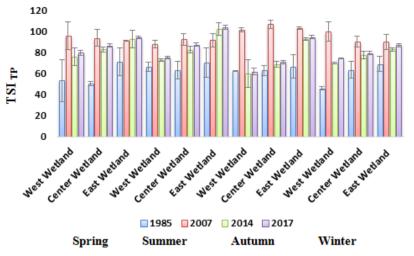
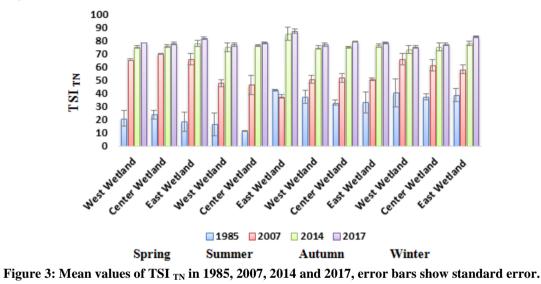


Figure 2: Mean values of TSI TP in 1985, 2007, 2014 and 2017, error bars show standard error.



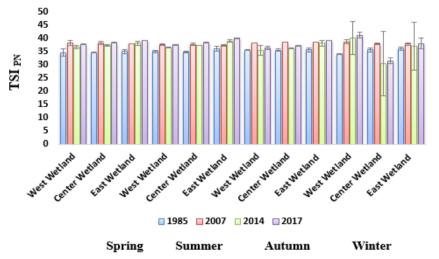
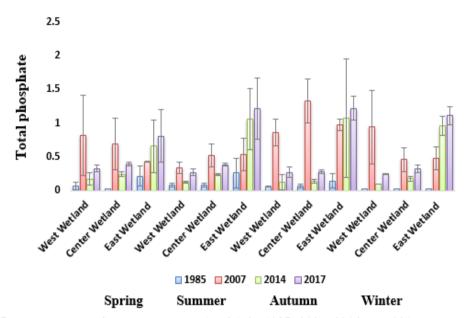
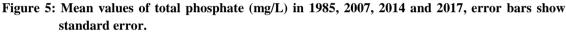


Figure 4: Values of TSI PN (Mean) in 1985, 2007, 2014 and 2017, error bars show standard error.

The maximum total phosphorus and nitrogen were measured during 2007

and 2017, respectively (Figs. 5 and 6).





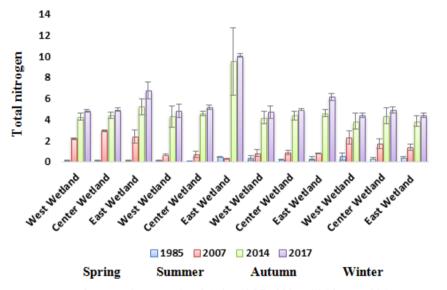


Figure 6: Mean values of total nitrogen (mg/L) in 1985, 2007, 2014 and 2017, error bars show standard error.

The maximum phosphorus concentration was measured at central region in fall 2007 and eastern region in fall 2017. However, the minimum phosphorus concentration was measured at western region in winter 1985. The highest and lowest amounts of nitrogen were measured at eastern region in summer 2017 and at central in summer 1985, respectively. According to During past decades the wetland has been threatened and destroyed through seven pollution points including rivers, municipal, agricultural and industrial sources as well as mines, ecotourism and hospitals (Jafari, 2009). Almost 50% of total organic contamination load flowed into the Wetland from Pirbazar River. It means that the Wetland water quality is extensively affected by domestic wastewater discharges from Rasht citv (Tahershamsi et al., 2009). The analysis of variance results revealed significant differences (p < 0.05) in all parameters listed in different years and at different stations and seasons (Table 2).

Table 2: ANOVA (P value) amounts to $95\%$ of the stations studied (Mean±SE, n=24)*.						
	1985	2007	2014	2017	F	P value
					Value	
Р	$0.903 \pm 0.150^{a}$	$0.689 \pm 0.45^{b}$	$0.455 \pm 0.108^{d}$	$0.568 \pm 0.31^{\circ}$	15.449	<i>p</i> < 0.05
Ν	$0.251 \pm 0.035^{\circ}$	$1.414 \pm 0.16^{b}$	$4.864 \pm 0.389^{a}$	$5.505 {\pm} 0.27^{a}$	94.719	<i>p</i> < 0.05
TSI(P)	$62.788 {\pm} 2.68^{\circ}$	$95.26 \pm 1.74^{a}$	$81.426 \pm 2.64^{b}$	$84.945 \pm 1.14^{b}$	46.777	<i>p</i> < 0.05
TSI(N)	$29.58 \pm 2.25^{d}$	$55.81 \pm 1.94^{\circ}$	$74.966 \pm 1.21^{b}$	$80.241 \pm 0.73^{a}$	165.172	<i>p</i> < 0.05

\*Different letters indicate statistical significance (Duncan, p < 0.05).

# Discussion

Over the recent century, eutrophication has been the common phenomenon for internal water ecosystems (Fang et al., 2004). This phenomenon is usually caused by increased amounts of nutrients (Zhang et al., 2008). Any change in phosphorus concentration of fresh water ecosystems can also alter its trophic status (Carvalho and Kirika, 2003). Kleeberg and Dudel (1997) and Li et al. (2017) have advocated the direct role of phosphorus in eutrophication of water bodies.

Anzali international wetland is not an exception to this rule and has experienced over-production trend with a higher rate. Based on investigations the most important external source, 54% of contribution, that has brought enrichment of about Anzali international wetland is litter and humus of forests and grassland of the wetland watershed, introduced to the wetland through surface currents caused by rainfalls (Darvishsefat et al., 1999). addition. the wastewater In of households and adjacent residential areas, the waste produced by food industries and animal husbandries. entered into the rivers associated with the wetland with no treatment, have a high contribution in intensification of eutrophication trend in Anzali international wetland (JICA, 2005). Furthermore, the wetland's wide area facilitating entrance of organic and inorganic materials to the wetland is considered among the most important of reasons eutrophication intensification. In this regard, three major nutrient sources are: fertilizers used in agricultural sector, household wastewaters. and livestock wastes. Therefore, agricultural uses in conjunction with uses urban are considered the main factors for concentrations higher than allowable

limits for some nutrients, especially nitrogen and total phosphorus compounds (Sakizade, 2003). The nitrogen to phosphorus ratio (N: P) in 2014 and 2017 has reached beyond 10 in the majority of regions, indicating the limitedness of phosphate in the wetland (Fig. 7).

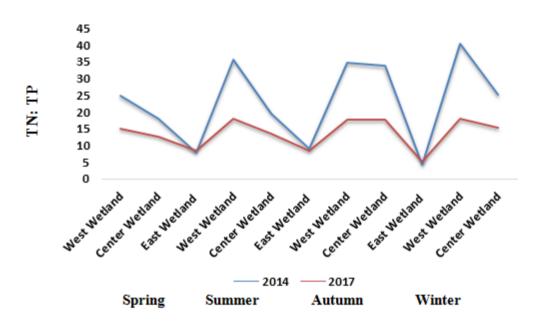


Figure 7: Values of TN: TP ratio during different seasons of 2014 and 2017.

Based on Liebig's law of the minimum, as the limiting element has a major role in proceeding of the production process (Wetzel, 2001), hence in this investigation, estimation of the trophic index based on phosphate concentration is more reliable, and can account for the developed changes with a higher reliability. Average values of TSI  $_{TN}$  and TSI  $_{TP}$  indices for 1985-2017 are mapped in Figure 8.

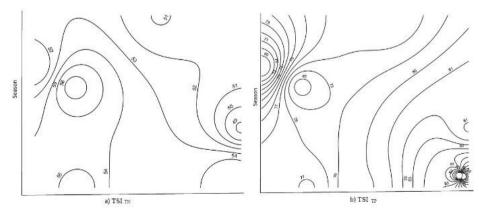


Figure 8: TSI <sub>TN</sub> (a) and TSI <sub>TP</sub> (b) mapping along Anzali wetland during 1985-2017, distance from stations (km).

Carlson's trophic index based on total phosphorus content has placed the wetland's trophic state within the groups of acute mesotrophic and hypertrophic in 1985 and 2017. respectively. According to the total nitrogen, during the same years, oligotrophy has been changed to eutrophy. These estimations represent dramatic development of the wetland's eutrophication process during the studied vears. In addition. since eutrophication follows a geometrical developmental trend, thus its long-term continuation is very serious and dangerous. Similar studies have shown the role of nutrients in the formation and development of eutrophication process in aquatic ecosystems (for example Lenard and Solis, 2009; Ejankowski and Lenard 2014). Levels of phosphorus and nitrogen in this study in comparison with nitrogen standard of lower than 1 mg/L in lakes and wetlands (Sakamoto, 1966) as well as the standard level of phosphorus, lower than 0.1 mg/L, in lakes and wetlands (Vollenweider, 1968) represent that the measured values are higher across all studied points suggests and an ascending trend in recent years (Table 3).

Table 3: Standard levels of eutrophication of phosphate and nitrogen.

	eutrophy	mesotrophy	oligotrophy	References
P (mg/L)	0.030 - 0.100	0.010 - 0.030	0.005 - 0.010	Vollenweider 1968
N (mg/L)	0.500 - 1.300	0.100 - 0.700	0.020 - 0.200	Sakamoto 1966

Also phosphate trend as compared with OECD standard  $(mg/m^3)$  is shown in Figure 9, which is based on wetland in hypertrophy status.

Kimball and Kimball study in 1974 was the first for Anzali international wetland for plant growth. Trophic status of Anzali wetland for 1992 to 2002 period is shown by Mirzajani et al. (2010). Research of Darvishsefat et al. (1999) in terms of chlorophyll-a of Anzali wetland showed that wetland shifts trophy based on total phosphate towards hypertrophy during 1991 to 1995. Nezami (1995) studies in Anzali international wetland on bacterioplankton was based trophic status.

Results of the survey plant on communities of Europe's water resources (Schneider, 2007) showed some species to be eutrophic and some oligotrophic water index. In Anzali international wetland presence and deployment Azolla filiculoides after 1991 was dramatic and had much negative impact on Anzali international wetland and increased nutrients and as a result eutrophication (Mirzajani et al., 2010).

A study on Phosphorus decreasing effects of 5 species submerged in a lake in China showed that comparing growth rate and the rate of decline in phosphorus *Ceratophyllum demersum* is 92% more effective.

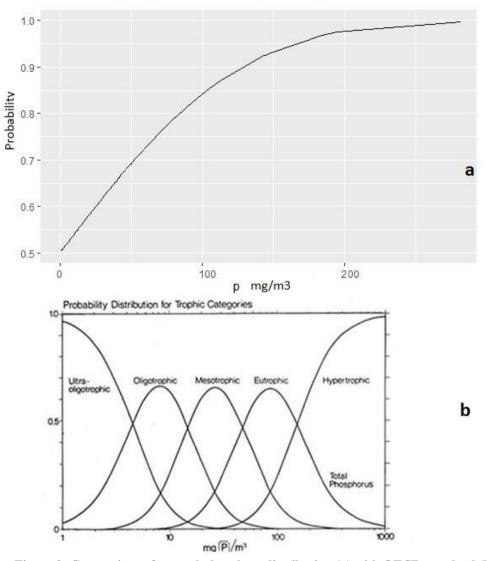


Figure 9: Comparison of normal phosphate distribution (a) with OECD standard (b).

*Ceratophyllum demersum* is dominant in eutrophic lakes and lakes that are seriously polluted and can reduce phosphate and nitrogen of water (Gao *et al.*, 2009). *Ceratophyllum demersum* is one of the most abundant aquatic plant species in most parts of Anzali international wetland (JICA, 2005). The wetland capacity to absorb and digest human waste of current population of the catchment area is limited, so the nutrients load should be reduced at least as a measure that should be used to stop

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wetland trophic change. This procedure applied to the management of Lake Balaton in Hungary in the mid-1980s with waste water control as much as 50% reduction and the lake's nutrient changed trophic to mesotrophic state (Istvánovics *et al.*, 2002).

The changes in concentration of nutrients and TSI index within the time period of 1985 to 2007 and until 2017 represent the ascending trend of this phenomenon, such that it has pushed the wetland's trophic position from oligotrophic to mesotrophic, to the eutrophic to hypertrophic stage. Under these conditions, gradually, the organic load of the materials in the wetland would exceed the wetland's selfpurification capacity and meeting oxygen demand for decomposing and mineralizing these materials. In this regard, adoption proper managerial methods for а comprehensive environmental assessment together with controlling pollutants with human origin as well as reducing organic loads of industrial, urban, and agricultural wastes, qualitative monitoring of water during the year and finally wetland's restoration are recommended.

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