Evaluation of effects of physico-chemical factors on chlorophyll-a in Shadegan International Wetland-Khouzestan Province - Iran

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Abstract
Measurement of chlorophyll-a can be considered important to investigate the primary production of an ecosystem. This study aimed to investigate chlorophyll-a concentration, primary production and the factors affecting them in Shadegan Wetland located in Khouzestan Province - Iran. Sampling for measuring (NO3, PO4, BOD5, DO, pH, EC, salinity, temperature) was performed seasonally at five sampling stations of the wetland (Canal, Ragbeh, Khorooosi, Mahshahr and Atish), from March 2013 – through February 2014. Results showed that chlorophyll-a concentrations in Canal station were significantly higher than that in the other stations; however, there were no significant differences among the other sampling stations (p>0.05). The maximum and minimum primary production (and chlorophyll-a) (62, 1.14 mg/m³) were observed at Canal and Ragbeh stations, respectively. Annual average chlorophyll-a concentration was 10.28 mg/m³, and there was no significant seasonal difference (p>0.05). The maximum value of chlorophyll-a was observed in the fall (29.63 mg/m³), whereas, the minimum value was related to the spring (4.07 mg/m³). Among the water physicochemical parameters, nitrate had a significant effect on chlorophyll-a concentrations. According to trophic state index (TSI), Shadegan Wetland is mesotrophic in the spring and winter, whereas it is eutrophic in the summer and fall. Overall, the lake is suitable for warm water species.

Keywords: Chlorophyll-a, Primary production, Shadegan Wetland, Trophic state index

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Introduction
Phytoplankton communities are the most dominant aquatic species that are able to photosynthesis as the first segment of food chain. Many phytoplankton are the biological indices of water resources illustrating the ecological state of environment (Gibson et al., 1990). Also, their assemblages are very sensitive to the environmental changes; and any change in the environment results in the change in their diversity and domination. (Jamil, 2001). Phytoplankton show spatial, vertical and seasonal distribution. Considering its temporal distribution in freshwater, phytoplankton production shows great horizontal variations, which can be due to different physico-chemical and biological characteristics of water, and the influx of phytoplankton-poor water to phytoplankton-rich ones or reverse fluxes. Also, this can be the result of water nutrient variations (Graham and Wilcox, 2000). In vertical distribution, phytoplanktons are mainly concentrated in the surface light-rich layers, and their population decreases with the increase in water depth. Due to the harmful effects of sun light, production mainly occurs in sub-surface layers (Graham and Wilcox, 2000). The seasonal cycle of phytoplankton biomass is correlated by seasonal changes in some parameters such as light and nutrients (Harrison, 1992). Light, water flow, temperature, water chemistry, nutrients and nutrient utilization are the main factors potential to have potential effects on phytoplankton communities (Hynes, 1988). It is possible to determine the effect of one or more of these factors on phytoplankton communities by appropriate comparisons among the different regions and seasons. To avoid drastic changes in aquatic ecosystems, trophy index that is used on a variety of programs can be set to the ecosystem.

Materials and methods
Having a great biodiversity, Shadegan Wetland is the largest one in Iran and 34th wetland of 1201 ones recorded in the Ramsar Convention. This wetland has an area of 537700 ha (the area differs in different years) and include 34% of total Iranian wetland area recorded in Ramsar Convention. Shadegan Wetland is located at 48°17' to 48°50' E and 3°17' to 3°48'N, in Persian Gulf northwest, 40 km far from Ahvaz City- Khouzestan Province capital (Nabavi et al., 2006). Shadegan Wetland is located in terminal part of Jarrahi River. Jarrahi and Karoon Rivers and the Persian Gulf tidal flow are the main water source of Shadegan Wetland (Lotfi et al., 2002). Different phenomenon such as Iran-Iraq war, constructions after the war and sewage discharge can be of the factors affecting the wetland phytoplankton and ecosystem structure (Nilsaz et al., 2010).
Shadegan Marshes and mudflats of Khor-al Amaya and Khor Musa;
Khuzestan Province; 400,000 ha; 30°30’N 048°45’E. Added to the Montreux list, 16 June 1993, As a Wildlife Refuge. An extensive delta on the border with Iraq, forming part of the largest lowland in Iran, and composed of the floodplains of major rivers draining 11.5 million hectares. The station includes fresh and brackish sedge marshes, tidal flats, creeks, sandbanks and a low island. The delta is fed by overflow channels of the Karun River, irrigation canals and local rainfall. The area is important for breeding and wintering water birds and is possibly the most important wintering station in the world for Marbled Teal. The wetland is bordered by salt flats, rice fields, date palms and human settlements. The station was placed on the Montreux Record in 1993 because of chemical pollution from the Iran-Iraq war.

**Sampling**

Sampling was performed randomly and seasonally, from March 2013 to February 2014. Five sampling stations were chosen including: Canal or Salmaneh (30° 40' N; 48° 28' E), Mahshahr (30° 52' N; 48° 30' E), Ragbeh (30° 41' N; 48° 40' E), Khoroozi (30° 39' N; 48° 40' E) and Atish (30° 54' N; 48° 40' E).

**Analysis**

To determine the physico-chemical parameters in water, three samples were collected from each sampling station, seasonally. All samplings were performed between 10:00 and 14:00 hours. One liter water was collected from each sampling station and transferred to the laboratory under light- and heat-deprived conditions. Water temperature and pH were determined at the time of sampling using Multi-parameter meter (Hach, Model: WTW 2000). Electric conductivity was determined using a spectrophotometer (Model: JENWAY). Total dissolved solids was determined by drying 50 cc of water at 80°C (Clesceri et al., 1989). Nitrate was colorimetrically determined by cadmium reduction method according to APHA (1992). Phosphate was colorimetrically by the molybdate method (Healey, 1987).

Trophic conditions of wetlands are determined using Trophy State Index (TSI) (Zhou and Xu, 2006). This method uses a numerical scale (0-100) to classify the trophic state of lakes. Trophic state of the wetland was determined using the Carlson model, following the determination of chlorophyll-a concentration. In this model, TSI is defined as follows:

$$\text{TSI} (\text{CHL } a) = 9.81 \ln (\text{CHL}_a) + 30.6$$

Where, CHL$_a$ is chlorophyll-a concentration in mg/m$^3$.

Comparison of primary production among the stations and seasons was performed using a completely randomized blocks (sampling stations) design (Zar, 1984). The data were analyzed by two way ANOVA and Duncan's test at $\alpha=0.05$. PCA test was used to analyze the main physico-
chemical factors, whereas, stepwise regression was applied to investigate the correlation between chlorophyll-a concentration and environmental factors. All analyses were performed using SAS software (SAS, 9.3).

Results
There was no significant difference in mean annual concentrations of chlorophyll-a in the seasons (p>0.05; Fig. 1). The maximum chlorophyll-a value was related to the summer, while the minimum value was observed in the winter.

Mean annual chlorophyll-a concentrations of Canal station were significantly higher than that in the other stations (p>0.05). The maximum and minimum value was related to Canal and Ragbeh stations, respectively (Fig. 2).

There was no significant difference in the physico-chemical parameters among the seasons (p<0.05). Overall mean dissolved oxygen was 5.08 ± 1.42 mg/l. Mean annual BOD5 (measured each season) was 2.48 ± 0.22 mg/l, and the maximum and minimum BOD5 values were recorded in the fall and summer, respectively. Mean annual pH was 7.99 ± 0.15, and the maximum and minimum value was observed in the winter and fall, respectively. The maximum and minimum temperature was recorded in the summer and winter, respectively. Mean annual temperature was 17.10 ± 5.95 °C. Lakes are divided into three trophic states (oligotrophic, mesotrophic and eutrophic).

A PCA analyses was first performed to determine the physico-chemical parameters in water affecting the chlorophyll-a concentration.

According to the Table 2, 67.07% of total variance was related to the aforementioned factors, thus they were considered as the main factors. The main factors resulting from PCA analyses were used in stepwise regression to obtain appropriate correlation among the main factors. Accordingly, dissolved oxygen, pH, nitrate, EC and TDS had a higher cumulative variance compared to the other factors.

According to the above information, stepwise regression was performed showing that only nitrate concentrations affected chlorophyll-a concentrations in different sampling stations, significantly.

\[
\text{Chlorophyll-a} = 35.73 - 4.89 \text{NO}_3, \\
R = 0.46
\]

The results showed that Shadegan Wetland is categorized as the wetlands in which nitrate is the limiting factor for production.
Table 1: Physico-chemical parameters ± Standard Error, trophic and Carlson index (TSI) in different seasons in Shadegan Wetland (2013 - 2014).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Spring</th>
<th>Summer</th>
<th>Fall</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO (ppm)</td>
<td>2.66±5.22</td>
<td>1.82±3.77</td>
<td>2.43±4.44</td>
<td>0.79±7.01</td>
</tr>
<tr>
<td>BOD5 (mg/L)</td>
<td>1.24±2.44</td>
<td>1.30±2.22</td>
<td>0.47±2.72</td>
<td>0.65±2.61</td>
</tr>
<tr>
<td>Pri.Product. (gC.m².day)</td>
<td>4.73±7.60</td>
<td>0.65±1.39</td>
<td>1.07±2.04</td>
<td>0.48±1.31</td>
</tr>
<tr>
<td>pH</td>
<td>0.37±7.42</td>
<td>0.41±7.92</td>
<td>0.40±8.04</td>
<td>0.52±8.16</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>1.14±19.14</td>
<td>1.11±23.08</td>
<td>0.55±15.04</td>
<td>1.48±9.81</td>
</tr>
<tr>
<td>Sal. (ppt)</td>
<td>6.18±10.81</td>
<td>20.49±26.41</td>
<td>8.14±10.06</td>
<td>0.55±5.06</td>
</tr>
<tr>
<td>NO₃ (ppm)</td>
<td>0.42±4.91</td>
<td>0.76±4.93</td>
<td>0.42±4.90</td>
<td>0.27±4.21</td>
</tr>
<tr>
<td>EC</td>
<td>47.17±50.47</td>
<td>26.16±36.49</td>
<td>11.66±20.05</td>
<td>24.19±41.81</td>
</tr>
<tr>
<td>TDS</td>
<td>20.17±30.94</td>
<td>21.15±30.11</td>
<td>4.27±12.72</td>
<td>20.19±24.61</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>0.32±2.31</td>
<td>0.43±1.76</td>
<td>0.31±2.26</td>
<td>0.22±2.22</td>
</tr>
<tr>
<td>TSI</td>
<td>40.85</td>
<td>52.44</td>
<td>50.72</td>
<td>46.35</td>
</tr>
</tbody>
</table>

(Mesotrophic) (Eutrophic) (Eutrophic) (Mesotrophic)

Table 2: The result of PCA test for decrease in physico-chemical characteristics in Shadegan Wetland (2013-2014).

<table>
<thead>
<tr>
<th>Component</th>
<th>Total</th>
<th>Percentage of variance</th>
<th>Cumulative variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.596</td>
<td>24.94</td>
<td>24.936</td>
</tr>
<tr>
<td>2</td>
<td>2.094</td>
<td>23.62</td>
<td>48.557</td>
</tr>
<tr>
<td>3</td>
<td>1.687</td>
<td>18.51</td>
<td>67.07</td>
</tr>
</tbody>
</table>

Table 3: Cumulative percentage of physico-chemical parameters of Shadegan Wetland (2013-2014)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Cumulative percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO</td>
<td>0.78</td>
</tr>
<tr>
<td>BOD5</td>
<td>0.69</td>
</tr>
<tr>
<td>pH</td>
<td>0.72</td>
</tr>
<tr>
<td>Temp</td>
<td>0.41</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.52</td>
</tr>
<tr>
<td>NO₃</td>
<td>0.72</td>
</tr>
<tr>
<td>PO₄</td>
<td>0.54</td>
</tr>
<tr>
<td>EC</td>
<td>0.96</td>
</tr>
<tr>
<td>TDS</td>
<td>0.93</td>
</tr>
<tr>
<td>Depth</td>
<td>0.62</td>
</tr>
<tr>
<td>TSS</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Figure 1: Mean natural log of chlorophyll-a (mg/m³) in different seasons in Shadegan Wetland (2013-2014).

Figure 2: Chlorophyll-a concentration (mg/m³) at different stations of Shadegan Wetland (2013-2014).

Discussion
Wetlands are economically and ecologically important. From the aspect of fisheries, wetlands are stations for early growth, sexual maturation, spawning, death, and stock rehabilitation of phytophilus fish (pseudo-sardine). There are no coherent studies on biological and ecological parameters of Shadegan Wetland. Background studies and research on wetlands are limited and just a few studies were recently conducted. Comparison of chlorophyll-a concentrations obtained in this study with those obtained by Nilsaz et al. (2010) showed that chlorophyll-a concentration has increased in the
Shadegan Wetland. Nilsaz et al. (2010) found that mean annual chlorophyll-a concentration was 6.28 mg/m³ with the maximum and minimum values of 15.14 and 2.15 mg/m³, respectively.

The lack of significant differences in chlorophyll-a concentrations among sampling stations could be due to stability of environmental factors affecting phytoplankton frequency and variation (Nilsaz et al., 2010). Primary production and chlorophyll-a are not often variable, annually; particularly, in the regions with narrow annual climatic variations. Generally, in the region with low climatic changes, it seems that phytoplankton communities show a minimum from mid-winter to early spring, and a maximum in the late spring or fall (Naz and Turkman, 2005). These results corresponded to the results presented in this study.

Nejatkhah Manavi et al. (2001) studied algae blooms in Anzali Wetland and its toxic algae finding two annual phytoplankton peaks; the first from late spring to early summer and the second in the fall. The result of the present study showed that Canal or Salameh station had relatively high production in different seasons. Presence of water in this station compared to the other stations, that were dry sometimes, resulted in phytoplankton stocks that increase their populations when environmental conditions turn suitable increasing primary production (Fig. 2).

In the studies on Shadegan Wetland, Nilsaz et al. (2010) reported that Atish and Canal stations are more productive than the other stations. The study of Nilsaz et al. (2010) showed that the turbidity of Shadegan Wetland is derived from its water influx (Jarrahi River). Therefore, the wetland turbidity increases during rainy seasons. High water suspended solid affects light penetration in the water resulting in low primary production (Ramont, 1980). This phenomenon was particularly observed in the stations near the Jarrahi River mouth, such as station Ragbeh that had low primary production of algal community. This was in agreement with the results presented in this study.

In the present study, despite the presence of desirable light, the primary production was low in the late spring compared to the other seasons, which can be due to rotifer and other zooplanktons blooms. High grazing pressure may decrease phytoplankton biomass against light increment (Whitton and Potts, 2000). Also, as Nilsaz et al. (2010) reported the highest zooplankton frequency was in station Ragbeh, therefore, grazing can be a reasonable cause of decrease in phytoplankton abundance. The other reason can be due to decrease in phosphate (0.32 ppm) and nitrate (4.16 ppm) concentrations in water, particularly due to water evaporation in the summer. Generally, nitrate is rarely a limiting factor for phytoplankton growth in freshwater, but phosphorus is (Redfield, 1990).

It seems that salinization of Shadegan Wetland water compared to
the previous two decades resulted in increase in the effect of nitrate concentrations in water on phytoplankton frequency and variation. According to chlorophyll-a concentration (Table 1), the wetland is categorized as mesotrophic, and in some seasons as eutrophic. Overall, it is concluded that Shadegan Wetland is not potentiated for eutrophication due to the lack of cyanophycea blooms, floating lemnaacea, macrophytes, low water turbidity, increase in plankton variation and lack of piscivorous (Janse, 2005), however, it should be noted that the wetland is potentiated for eutrophication in the summer, and appropriate contrivances should be considered to avoid it.

References
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