Effect of the selected physico-chemical parameters on growth of rainbow trout (*Oncorhynchus mykiss*) in raceway system in Iran

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Abstract
A study was conducted to evaluate the correlation between selected physico-chemical parameters of water and their effects on growth of Rainbow trout (*Oncorhynchus mykiss*) in raceway system in the area of Sarab Gerdu, Iran. The samples were collected from twenty raceways (270-day old fish, surface area 55m²; flow rate 150 l·s⁻¹; total production 15 T). Physico-chemical parameters were the fluctuations of total ammonia nitrogen, dissolved oxygen, turbidity, nitrogen (nitrates), phosphorous (phosphates), total dissolved solids, electrical conductivity, fluoride, boron, pH, temperature, total hardness, and alkalinity. Multivariate analyses were conducted employing three categories of parameters including A, B and C. The results of the study showed that temperature and pH variations were similar. Dissolved oxygen with negative similarity coefficient, _0.618_ was located in the third category while positive coefficient, 0.479 of other parameters except for pH and temperature (second category) and dissolved oxygen (third category) was located in the first one. pH value was not in a standard range due to increase in water temperature. This, in turn had impact on other parameters, such as unionized ammonia, a toxic chemical, to fish. Our result demonstrated that nitrate concentration is the most significant chemical parameter for fish growth in this area. Similarly, increase in concentration of dissolved oxygen in the water (from 3.30 mg·l⁻¹ up to 12 mg·l⁻¹) was necessary to promote productivity.

Keywords: multivariate analyses; raceway system; aquaculture; rainbow trout.

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Introduction
Emphasis on aquatic protein consumption in the 4th national five-year economic, social and cultural development plans, due to human health benefits and food stuff values encourage supplement of terrestrial protein production systems with aquatic protein production facilities (Anon., 2005a). Water quality determines to a great extent the success or failure of a fish culture operation (Piper et al., 1982). Water is an essential requirement for fish farming, so any proper-prepared plan for aquaculture must describe the quality and quantity of water available for this purpose (Summerfelt, 2000). The achievement of rainbow trout as an aquaculture species is dependent on technological advances and production of fish with great amounts of high quality lean muscle (Johansen & Overturf, 2005, Cohen et al., 2005). Moreover it has lately been accepted that the welfare of farmed fish merits consideration, where welfare can be considered to represent the physical and mental state of animal is relative to its environment. Proper stocking density has been emphasized due to an apparent increasing on mortality in the likelihood of poor welfare at higher stock densities. Stocking density practices differed noticeably between individual farms with maximum densities varying from <20 to >80 kgm\(^{-3}\). Stocking density was linked to type of production, with farms producing fish for the table market operated at higher densities than farms rearing fish for the restocking/fisheries market (Ellis, et al., 2002; North et al., 2006). The main source of ammonia in water of a high stocked density cultured pond occurred in the effluent of a raceway is excreted from fish, typically via their gills. Animals produce ammonia as a byproduct of protein metabolism (Hargreaves et al., 2005). What is measured by chemical analysis is called total ammonia nitrogen (TAN) because it includes two forms of ammonia: unionized form (NH\(_3\)), and ammonium ion (NH\(_4^+\)). Unionized ammonia is toxic to fish (Summerfelt., 2000). In other words, studies on spatial changes in physical and chemical characteristics of water which is used for aquaculture by raceway system present essential information for best management options at all time. Moreover in restricted exchange environments, there is a risk of high levels of nutrients accumulating in one area, causing hypernutrification and potentially creating undesirable effects (Midlen & Redding, 1998; MacGravin, 2000; Carrol et al., 2003). The objective of this work was to evaluate important water physical and chemical factors in rainbow trout culture. Although the quantity of water available is of importance, only water quality parameters are considered here.

Materials and methods
The area of study is placed in Lorestan Province (Fig. 1). It lies between latitudes of 46°, 50’ to 50°, 01 and longitudes of 32°, 40’ to 34°, 32’. Temperature of the area is between a maximum of 20.4°C and a minimum of
7.6°C and average temperature is 14°C. Annual precipitation is 612.7 mm and average sunny hours per month is 268h. The number of icy days in the study field is 111 per year (Anon., 2005).

Initially water flow delivered to the farm raceways was determined. Water samples were collected from four sites inside and outside raceways. Raceways were located in four rows up – down. The last station was in the river that receives aquaculture waste water. Selected parameters were: temperature, and pH were measured by YSI model. Nitrate was determined using the reduction column while ammonium was measured using Oriole model EA 940.
Ion analyzer and 95-12 ammonia electrode, standard method 4500-NH₃. Level of PO₄³⁻ was determined using the standard molybdenum blue method. The determination of DO was carried out using the Winkler method according to Strickland & Parsons (1972) and Bryan and Williams (1976). In order to measure the amount of boron and fluoride, they were analyzed using ICP with mass spectrometry. Free chlorine residue was determined using chlorometer model 1000-PT. Total hardness was measured using APHA 3111B method while electrical conductivity (EC) was determined using APHA 2510B method. Alkalinity measured by titration. Furthermore TDS and turbidity were determined using APHA 2540C and APHA 1992 method. Each experiment was conducted in three replications. Statistical analyses (mean water quality parameters, standard deviations and correlation coefficients) were evaluated in Explore software package. Data were analyzed by weighted pair group multivariate Cluster analysis (Davis, 1973).

**Result**

Water sample analyses data were summarized in Table 1. Spatial variation of the parameters implied that the third row of raceways had more critical condition compared to the first row. The rate of change and similarity between the parameters were given in Fig. 2. The measured parameters were compared with water quality standards (Table 3). Tables 1 and 2 showed the temperature in raceways varied between 13.00 °C up to 14.10 °C (13.80 ±0.51). The results of multivariate analysis are in Fig. 2. This dendogram separates dissolved oxygen from other parameters, showing its crucial role in raceway aquaculture. Temperature and pH in one group were placed next in importance. Finally nitrogen showed a small difference from other parameters. So after DO, temperature, pH, Cl and nitrogen can be recognized as an influencing parameter in raceway management. Other parameters were similar, therefore were considered as one group.
Table 1: Summary of water quality data for aquaculture in the present study

<table>
<thead>
<tr>
<th>Parameter:</th>
<th>Temperature (°C)</th>
<th>Turbidity (NTU)</th>
<th>Total Ammonia Nitrogen (mg/l)</th>
<th>Phosphorus (phosphate) (mg/l)</th>
<th>Alkalinity (mg/l)</th>
<th>Dissolved Oxygen (mg/l)</th>
<th>Nitrogen (nitrate) (mg/l)</th>
<th>Free Chlorine residue (mg/l)</th>
<th>Total hardness (mg/l)</th>
<th>Total Dissolved Solids (mg/l)</th>
<th>Fluoride (mg/l)</th>
<th>Boron (mg/l)</th>
<th>pH</th>
<th>Electric Conductivity (dsm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>min valve</td>
<td>13.00</td>
<td>Very low</td>
<td>1.25</td>
<td>0.06</td>
<td>250</td>
<td>2.60</td>
<td>4.40</td>
<td>8.85</td>
<td>122.50</td>
<td>218.24</td>
<td>0.002</td>
<td>0.001</td>
<td>8.27</td>
<td>341</td>
</tr>
<tr>
<td>max valve</td>
<td>14.10</td>
<td>Very low</td>
<td>4.63</td>
<td>0.09</td>
<td>259</td>
<td>3.90</td>
<td>4.90</td>
<td>23.01</td>
<td>203.50</td>
<td>286.72</td>
<td>0.004</td>
<td>0.001</td>
<td>8.44</td>
<td>448</td>
</tr>
<tr>
<td>mean value</td>
<td>13.80</td>
<td>Very low</td>
<td>2.20</td>
<td>0.07</td>
<td>255</td>
<td>3.30</td>
<td>4.55</td>
<td>17.70</td>
<td>154.30</td>
<td>237.28</td>
<td>0.0025</td>
<td>0.001</td>
<td>8.38</td>
<td>371</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.51</td>
<td>0.00</td>
<td>1.62</td>
<td>0.01</td>
<td>13.98</td>
<td>0.57</td>
<td>0.24</td>
<td>6.29</td>
<td>34.58</td>
<td>33.11</td>
<td>0.001</td>
<td>0.00</td>
<td>0.07</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 2: Comparison between water quality standards and measured values for rainbow trout culture in the present study (mg/l)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ground water⁷</th>
<th>Ground water in the Sarab Gerdu</th>
<th>Raceways (Sarab Gerdu)</th>
<th>Best exposure Levels⁸</th>
<th>Acceptable Continuous exposure levels⁹</th>
<th>Levels associated with fish kill²⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>Varies latitudinal and by depth of well</td>
<td>13.0</td>
<td>13.95</td>
<td>15.0</td>
<td>10 to 16</td>
<td>&gt;25.7</td>
</tr>
<tr>
<td>Turbidity⁷</td>
<td>Low(clear water)</td>
<td>Low(clear water)</td>
<td>Low(clear water)</td>
<td>Low(clear water)</td>
<td>&lt;25</td>
<td>It has no significant effect</td>
</tr>
<tr>
<td>UIA</td>
<td>0</td>
<td>0.0800</td>
<td>0.0950</td>
<td>0</td>
<td>0.0125 to 0.0200</td>
<td>&gt;0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 to 0.02 tolerance level</td>
</tr>
<tr>
<td>Phosphate</td>
<td>&lt;surface water</td>
<td>0.060</td>
<td>0.065</td>
<td>0</td>
<td>0.0125 to 0.0200</td>
<td>&gt;0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02 to 4 phosphorus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.1 phosphate</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>Low in granitic or shale, medium to high in limestone aquifers</td>
<td>250.0</td>
<td>253.5</td>
<td>181.0</td>
<td>20 to 300</td>
<td>250</td>
</tr>
<tr>
<td>DO</td>
<td>&gt;1</td>
<td>3.90</td>
<td>3.35</td>
<td>7 to 13</td>
<td>&lt;16 to &lt;6</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Variable</td>
<td>4.5</td>
<td>4.4</td>
<td>&lt;0.55</td>
<td>&lt;50</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Total hardness</td>
<td>50 to 250</td>
<td>122.50</td>
<td>145.75</td>
<td>50 to 400</td>
<td>&gt;20</td>
<td>&gt;800</td>
</tr>
<tr>
<td>TDS</td>
<td>Variable</td>
<td>218.24</td>
<td>222.08</td>
<td>6.5 to 8.5</td>
<td>5.5 to 9</td>
<td>&lt;5.5 or &gt;9</td>
</tr>
<tr>
<td>pH</td>
<td>&lt;7</td>
<td>8.27</td>
<td>8.42</td>
<td>6.5 to 8.5</td>
<td>5.5 to 9</td>
<td></td>
</tr>
</tbody>
</table>

a: summerfelt, 2000  
b: Boyd & Tucker, 1998  
c: Karim, 1997
Discussion

According to Boyd and Tucker (1998) best growth of rainbow trout occurs in a temperature range of 10 °C up to 16 °C. pH varied from 8.27 to 8.44. It seems that temperature is more critical than pH in water management of these raceways. DO concentrations need to be increased? Increasing in nitrates and phosphates due to their adverse impact in the environment also has to be monitored (Lin et al., 2001; Bricker et al., 2003; Ferreira et al., 2007). Turbidity is in allowable range while sun rays directly contact fish skin through the water. According to the dendogram in Fig.2, the similarity coefficient between EC and TDS was 1, which means EC changes due to environmental fluctuations the same as TDS. The similarity coefficient of alkalinity with these two parameters was 0.998. TAN with similarity
coefficient equal to 0.995 joints with previous parameters. As it is illustrated in Fig.2 the similarity coefficients of total hardness, phosphorous, nitrogen with previous parameters were equal to 0.968, 0.962 and 0.902 respectively. The similarity coefficient of chlorine was 0.479 which has a moderate coherence (not high) with seven previous parameters. These eight parameters gather together in a cluster named “A”. In this analysis the similarity coefficient between pH and temperature was 0.997 and these two parameters made the second cluster named “B”. pH and temperature have a high similarity coefficient and their response to environmental stimulus is very similar. The similarity coefficient of clusters “A” and “B” was 0.239 showing these two clusters do not have a noticeable similarity. In the third cluster there was only one parameter (DO), which had a negative similarity coefficient. The similarity coefficient of this cluster with other two clusters was -0.618. It could be implied that changes of DO concentration against the other environmental parameters is in a reverse phase, i.e., an increase in concentrations of the parameters in “A” and “B” clusters will cause a decrease in DO. Best pH is registered from 6.5 through 8.5 (Boyd, & Tucker, 1998). The reason behind presence of temperature and pH in one cluster is that an increase in temperature would enhance pH value and consequently pH can become out of recommended range. Sudden changes in pH of 1.7 would result in fish mortality (Summerfelt, 2000). Furthermore, many studies have been conducted to investigate the basic physiological requirements of fish seed including water quality management during transport. These compose lowering of temperature (Phillips & Brockway, 1954; Norris et al., 1960; Sing et al., 2004). Toxicity of other parameters such as unionized ammonia and chlorine would be affected by pH. Department of the Environment of Iran (DOE) standards allows a pH of 6 to 8.5 in agricultural waters (Anonymous, 2005b). The turbidity of water is minuscule in these raceways, therefore sun lights passed deeply through the water. For prevention of sunburn injuries to the fish stock, it was necessary to protect them from the direct sunlight, especially during the hot summer hours. The minimum concentration of DO for fish health is about 6 mg l\(^{-1}\) (Summerfelt, 2000). The results of the present study showed that the concentration of DO varied from 2.6 to 3.9 mg l\(^{-1}\) with an average of 3.3 mg l\(^{-1}\) and a standard deviation of 0.57. Mortality of rainbow trout may initiate below 3.0 mg l\(^{-1}\) (Summerfelt, 2000). It is essential to install aeration system in these raceways because the minimum dissolved oxygen was below the mortality limit. Lowering the stock density could prevent stress on fish. Furthermore correlation coefficient of DO with other parameters was negative. It means that increasing in concentrations of these parameters would decrease the concentration of DO. In fact concentration of DO was the most critical parameter for water quality management. Total ammonia nitrogen varied from 1.25 mg l\(^{-1}\) to 4.63
mg/L with an average of 2.20 and a standard deviation of 1.62. According to Iran's DOE standards for total ammonia nitrogen, the concentration of this parameter in waters that use for agriculture has to be 0 (Anonymous, 2005b). Continual cleaning of raceways from fish excretion cause fish stocks to be healthy. On the other hand, establishment of a proper waste water management system can decrease adverse environmental impacts of aquaculture in the area. Phytoremediation offers a safe alternative to conventional cleanup technique. Furthermore increase in ammonia, enhance the water pH and therefore pH could go out of allowable range. With an increase in these (pH or ammonia) parameters concentration of DO decreases and the condition can become hazardous for fish health. Alkalinity varied from 250 mg/L to 259 mg/L with an average of 255 and a standard deviation of 13.98. The best concentration of alkalinity for trout farming is reported to be 181 mg/L (Table 2), suggesting that there is no risk to the water quality as far as alkalinity is concerned. Total hardness varied from 122.5 mg/L up to 203.5 mg/L (with an average of 154.3 and a standard deviation of 34.58). The best total hardness for rainbow trout is equal to 50 up to 400 mg/L (Karbassi, et al., 2007). As it could be seen, hardness in the study area was more than ideal hardness, but results of the study showed that it is not considered to be a limiting parameter. TDS in the ground water which is used for aquaculture can be more than 1500 mg/L NaCl (Summerfelt, 2000). Results of the present study showed a TDS range from 218.24 mg/L to 286.72 mg/L with an average of 237.28 and a standard deviation of 33.11. Average concentration of fluoride was 0.0025 mg/L with standard deviation of 0.001 and average concentration of boron was 0.001 mg/L with standard deviation of 0.000. The necessary concentration of boron for rainbow trout culture is equal to 0.2 up to 0.6 mg per Kg weight of fresh fish (Eisler, 1990). According to the DOE standards of Iran allowable concentration of boron is equal to 1 mg/L in water resources that are used for agriculture. As a result it could be determined that boron should be added to the fish food. The best concentration of EC for rainbow trout culture is equal to 432 dS/m (Karami, 1997) and results of the present study showed EC to be varied from 341 up to 448 dS/m (with an average of 371 and standard error of 0.05). The results imply that this resource initially is suitable comparing with other groundwater resources but dissolved oxygen concentration have to be increased in water. Moreover control of an increase in temperature and pH due to their adverse impact on fish health and their effect on other parameters is important.

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