Effect of pH on Egg Hatching, Larval Growth Performance, and Enzymatic Activity of the Nile tilapia *Oreochromis niloticus*

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ARTICLE INFO
Article History:
Received: Oct. 11, 2022
Accepted: Dec. 24, 2022
Online: Dec. 30, 2022

**Keywords:**
*Oreochromis niloticus*,
Egg hatching,
Enzyme activity,
Nile tilapia

**ABSTRACT**
The present study aimed to show the effect of pH on egg hatching, growth performance, and enzymatic activity of the Nile tilapia *Oreochromis niloticus* larvae. The results showed that the percentage of mortality of the newly hatched larvae increased with increasing pH for a period of 96h of incubation. However, the lowest value of mortality rate was recorded between the percentage of control and that treated with pH 7.5. In comparison with the abnormalities between the groups of the control group and those exposed to the different degrees of pH, significant differences were recorded (*P* >0.05). However, a non-significant difference was recorded between the control group and that exposed to pH 7.5 (*P* <0.05). The effect of pH on the incubation time showed that a considerably long period of incubation was observed towards the high acidity and high alkalinity of pH. The effect of pH on the growth of larvae is more significant after 35 days of post-hatching than that recorded after 20 days of post-hatching (*P* >0.05). The enzymatic activity is more affected towards low pH (4-5) or towards the more alkaline medium. Finally, the best concentration of pH on enzyme activity and growth performance ranged from 7.5 to 8.5.

**INTRODUCTION**
The Nile tilapia has a resistance against any bad changes in the environmental conditions of water. Thus, it is highly preferred to be cultured in Egypt and all over the globe. It contains a large amount of protein and amino acids. It is cultured in a large area in fresh water fish farms and has a long semi-natural spawning which extends from April to September. The larvae of fishes resulting from the spawning face several problems at the first days of early life stages, leading to high mortality (Watinabe & Kirm, 1994; El-Greisy & El-Gamal, 2012; El-Greisy & El-Gamal, 2016; Barakat et al., 2021). The effect of low pH on adult fish has been extensively studied and many reviews are available (Hienes, 1981; Dilon et al., 1984). Furthermore, acidification has been reported to decrease the hatchability of eggs as described by Trojnar (1977) and consequently reduce the egg’s viability (Beamish, 1976; Menedez, 1976; El-Greisy et al., 2016).
Many researchers have focused on the acidic and alkaline limit at which fish grow and reproduce rapidly (Desilva & Anderson, 1995).

In the last two decades, few studies were carried out on the digestive ability and specific nutritional requirement of fish larvae and juveniles (Cahu & Zamborino, 2001). In this respect, few studies have described the relation between the pH and the digestive of enzymatic secretion during larval stages. In this respect, Ye et al. (2013) studied the pH with ranges from 6 to 10 and its effect on growth performance and enzymatic activities.

However, few studies were conducted on the relation between the pH, the growth performance and the enzyme activities of the fish. Therefore, the present study aimed to cover these topics. While, further studies are needed to detect the relation between the age of larvae, the type of secreted enzyme as well as the type of food for each age group of fish larvae.

**MATERIALS AND METHODS**

**Source of eggs and newly hatched larvae**

The experiment started on the 15th of April 2021 and continued for 40 days of post-hatching. This study was carried out at El-Serw fish Research Station of the farm that is located in the North of Dakahlia Governorate, Egypt. The eggs and larvae were collected from the hatchery of tilapia, Oreochromis niloticus under semi-natural spawning as reported by El-Greisy et al. (2016).

**Preparation of brood stock for spawning**

Both healthy males and females were selected and separated into two large earthly ponds as the follow system occurred at the hatchery of El-serv fish farm for a period of 15 days and were fed daily. One male was mated with two or three females according to the practiced sex ratio and remained for two days later.

**A. Effect of pH on the fertilized eggs and early life stage larvae**

After spawning, the hatched eggs and early larvae were collected, transported, and then stocked in six small round containers made from clay-pots. Each container contained 3 liters of dechlorinated water. About 100 eggs were put in the well-aerated water in each container. The pH were adjusted to a range that varied from 5.5-9.5 by using pH meter model (3050) 1 mol / L HCl and 1 mol/L NaOH, and the variation of pH rarely exceeded ±0.2 according to Ye et al. (2013) and extended to 96 hours after fertilization.

The experiment was designed using six small round 3L containers made from clay pot, and the hatched eggs were distributed in a rate of 100 fertilized eggs in each container.

1- The pH of the first container was adjusted to 5.5
2- The pH of the second container was adjusted to 6.5
3- The pH of the third container was adjusted to 7.5
4- The pH of the fourth container was adjusted to 8.5
5- The pH of the fifth container was adjusted to 9.5
6- Finally in the control container, the pH mimics the natural conditions in the environment and ranged from 7.5 - 7.8.
The white eggs appeared on the surface of water, while deformed eggs must be removed. The water in each container was totally changed daily, and the pH in each container was adjusted according to the designed experiment. In order to examine the effect of pH on fertilized and hatched eggs, the fertilized eggs were fixed in the serous fluid to clarify the embryo inside the eggs. The serous solution (600 ml of 95% alcohol +300 ml formaldehyde + 100 ml of acetic acid) was prepared according to Szezebik et al. (2008) and El-Greisy and El-Gamal (2016).

B. Effect of pH on the larvae after 35 days of post-hatching (DPH)

About 1800 newly hatched larvae at an age of 5 days, when yolk sacs were completely absorbed. These larvae were stocked in a large tank, each of which contained 300L of dechlorinated water for a period of two days later. During that period, the larvae were fed 40% of protein diet five times daily as the same used in feeding larvae of El-Serw hatchery (El-Greisy et al., 2016; Barakat et al., 2021).

During this period, the condition of the tank was adjusted to pH ranging from 5.5-9.5, dissolved oxygen from 5.5-6.5 mg/L, and the temperature of water fluctuating from 23-26.5°C. The pH was daily adjusted in the early morning by using pH meter with 1 mol/L of HCL and 1 mol/l of NaOH. The variation of pH in each measuring rarely exceeded ±0.21 as described by Ye et al. (2013). The experiment continued for 35 days of post-hatching larvae for studying the effect of pH on growth and 40 days for the secreted enzymes. About 900 healthy larvae were selected and divided into six glass tanks, with a capacity of 50L of dechlorinated water in each tank and then duplicated.

The first glass tank had a pH = 5.5; the second pH = 6.5, the third pH=7.5; the fourth pH=8.5, fifth pH= 9.5, while the sixth glass tank was used as control, where the pH of the natural environmental conditions was used and adjusted to 7.5 -7.8.

In each glass tank, the dissolved oxygen was kept at 5.5 mg/L, and the water temperature ranged from 23-26°C. Each glass tank was well aerated with a central air pump used inside the hatchery of El-Serw. Half of water in each tank was daily changed, and the pH in each tank was adjusted according to the requirement for each tank.

Collection of samples
The larvae were collected after 10, 20, and 35 days of post-hatching. The collection of samples after 40 days was used to study the enzyme activity (lipase, amylase and alkaline phosphates). The length of larvae was measured to the nearest mm, and the weight was measured to the nearest mg.

Calculation formula
The data on Odontobutis obscures were calculated in detail according to Ye et al. (2013) as follows:

Survival rate % = number of fish surviving / total number of fish X 100
Average of final weight (AFW) = Tw/n
Weight gain (WG) = W₂ - W₁
Weight gain rate (WGR) = W₂ - W₁ / W₁
where W₁, W₂ are the preliminary weight and final weight in mg, respectively, and n is the number of fish.
The pH values and behavior of newly hatched larvae

The effect of the high acidity of pH in less than 5 and high alkalinity of pH in more than 10 was recorded. The behavior of larvae was observed, and the mortality rate was calculated.

The condition factors

The condition factor was calculated after 35 days of post hatching, using the succeeding equation:

\[ \text{condition factors} = \frac{W}{L^3} \times 100 \]

Where, W weight of fish in mg, and L is the total length of fish in (mm)

Preparation of enzyme fluid

About 1500 small healthy larvae were distributed in small ponds for 40 days to determine the enzyme activity. The PH was adjusted and ranged from 5 to 9. After 40 days the larvae were collected and furtherly studied.

In a detail process, 25 -30 healthy larvae were rinsed in distilled water and then dried with paper toweling and freeze at -20°C until analysis according the method of Golchinfar et al. (2011).

In order to obtain the supernatant fluid, the frozen larvae were thawed at room temperature, weighed and homogenized on ice, with volumes of 0.2 M NaCl (w/v) using homogenizer. The suspension was centrifuged (5000 r/pm) for about 30min under 4°C. The supernatant was stored at -20°C until analysis according to Golchinfar et al. (2011).

Determination of enzymatic activity

A- Quantitative determination of lipase

Lipase enzyme was determined using SPINREACT quantitative method as described in the studies of Me Need et al. (1984) and Buritis et al. (1999).

b- Quantitative determination of amylase

Amylase enzyme was quantitatively determined according to Winn Deen et al. (2005) and Béraud-Dufour et al. (2010).

C- Quantitative determination of alkaline phosphates ALP

Alkaline phosphatase enzyme was determined quantitatively according to Young (1997).

Statistical analysis

The effects of pH on different groups of larvae were determined using SPSS 170 software, while \( P< 0.05 \) was considered as a significant level. All measurements were carried out and the results were given as Average ± SD according to Golchinfar (2011) and Ye et al. (2013).

RESULTS

The pH values and mortality rates of newly hatched eggs and fry after 96 hrs of post-hatching (LC50)

The effect of pH on the mortality rates in both of the yolk sac fry and fertilized eggs were recorded after 96 hrs (LC50) of post-hatching larvae as shown in Table (1). In comparison, these effects considering various degrees of pH in untreated group (control) showed that, the percentage of mortality rate increased with increasing pH for a period of 96 hrs in incubation. The lowest value of mortality rate was recorded between the
percentage of control of untreated group and that of treated with pH 7.5 and showed non significant difference ($P<0.05$). However, the highest percentage values were recorded in the control group and that of pH 5.5, 6.5 and 9.5, respectively, and significant differences were recorded ($P>0.05$). The effect of pH towards the acidity from 4 to 5 or alkalinity form 9.5 to 10.5 or more showed non significant differences ($P<0.05$), compared to the control group or treated group with pH 7.5 as shown in Table (1). The most newly hatched fry died and was lost in high acidity (4 or more) and high alkalinity (Table 1 & Fig. 1).

**Effect of pH on hatching rate and abnormalities of fry after 45 hours of incubation**

The effect of pH 5.5 on the percentage of hatched eggs and abnormalities of fry showed that the percentage of hatched eggs decreased and recorded a value of 35+1.63. The high alkalinity (more than pH 9.5) had more impact on the newly hatched eggs (34 +0.81). On the other hand, more hatching rate showed highly non-significant difference after the fry was exposed to 7.5 or in control group in natural condition state (pH 7.5-7.8). Compared to the abnormalities between the groups and those exposed the different degrees of pH, significance differences were recorded ($P>0.05$). Nevertheless, no significant difference was recorded between the control (untreated) group and that exposed to pH 7.5 as shown in Table (2). The effect of pH on the incubation time showed that the incubation time is short in control group and after exposing the hatched eggs to pH 7.5. However, a long period of incubated times was observed towards the high acidity (pH 5.5 or more) and high alkalinity if pH was 9.5 (Table 2).

**Effect of pH on growth of larvae after 20 days of post-hatching**

The effect of pH on growth in length for larvae was more significant than that of the growth in weight, as shown in Table (3). No significant difference occurred between the different effects of pH on weight and lengths of newly hatched larvae. All the hatched larvae died after exposing to the pH 5.5 and in alkalinity of 10.5. The survival rate increased in control group under natural condition or after the larvae were exposed to pH 7.5. The survival rate recorded an average of 73.33+3.85. These values decreased and reached 68.66+2.66 and 63.66+2.49 after the larvae were exposed to pH ranging from 8.5 to 9.5, respectively, as shown in Table (5).

**Effect of pH on growth of larvae after 35 days of post-hatching**

The effect of pH on growth in length of larvae was more significant than at the earlier stage (20 days of post hatching, Table 4). Remarkably, no significant difference was recorded between the mean of lengths in the larvae exposed to pH 7.5 and 8.5, for those measured 2.16 mm + 0.79 and 2.10 +0.44, respectively.

However, there was a significant difference ($P>0.05$) after the larvae were exposed to pH 5.5 and 9.5 and that treated with pH 7.5 and control groups. Towards the high acidity (pH 4-5) and more alkalinity (pH 9.5-10.5), the larvae were more affected. The effect of pH on the survival rate of larvae that ranged from 6.5 to pH 9.5 showed that, in pH 6.5, the survival rate was 63.33 as an average (Table 5).
percentage increased and reached a value of 71.66+0.81 for an average. All larvae that exposed to pH 5.5 died before five days of the sample collection.

**Effect of pH on the behavior of newly hatched larvae**

After the larvae were exposed to high pH for a short period, clinical signs appeared in abnormalities in yolk sac fries, and shortening in the body lengths of larvae after 35 days of post-hatching. The mortality rate of larvae and the morphology of skin changed into dark black color, they lost equilibrium and were slow in swimming on the surface of water and then died. However, the larvae that live in natural condition under control group (pH 7.5–7.8) were able to swim and were fed daily at least three times with artificial food containing 40% of protein.

**Condition factor (Kn)**

In the control group, the condition factor was 2.040+0.87 and then decreased to 1.602+1.39 with pH of 6.5. This value increased after the larvae were exposed to pH 7.5 and reached 2.09+0.84 and then decreased to the minimum value of 1.33+0.24, with pH 9.5 (Table 4).

**pH and enzymatic activity of larvae**

**A. The enzymatic activity of amylase**

As shown in Table (6) and Fig. (2a), when pH ranged from 5.5 and 9.5, the amylase activity was primarily increased after the larvae were exposed to pH 6.5 and measured 5.5+0.08mg/ l; these values increased gradually and reached the maximum value at pH 7.5 and 8.5 and measured 8.26+0.16 and 4.20+0.20mg/ l, respectively.

Therefore, the appropriate pH ranged from 6.5- 8.5 and the suitable pH was 7.5 for *O. niloticus*. However, the lowest value of enzyme activity was recorded after pH decreased and reached its minimum value at high acidity in pH 5.5 (1.5 mg/g) or towards the high alkalinity at pH of 9.5 or more (1.70 mg/l).

**B. Enzymatic activity of pH for alkaline phosphatase (Alk phosphatase)**

As shown in Table (6) and Fig. (2b), when pH ranged between of 5.5 and 9.5, the alkaline phosphatase activity was initially increased when pH was 6. 5(0.81 +0.08 mg/l) and then increased and reached its the maximum value at pH 7.5 (0.95 +0.01). A suitable pH ranged from 6.5 to 7.5 (0.81– 0. 95mg/ l), respectively, for *O. niloticus* larvae. However, the enzymatic activity decreased and reached its minimum value either for the larvae exposed to high acidity of pH 5.5 or in high alkalinity when pH was 9.5 and measured 0. 14 and 0.04mg/l, respectively.

**C. Enzymatic activity of lipase**

As shown in Table (6) and Fig. (2c), when pH ranged from 5.5 and 9.5, the lipase activity increased when pH reached 6.5 and measured 6.28 + 0.06mg/ l. These values increased and reached their maximum values at pH 7.5 and measured 7.16mg/l, then decreased and reached values of 4.53+ 0.20mg/ l at pH 8.5. The lowest value was recorded after the larvae were exposed to pH 9.5 and measured (2.46+ 0.124 mg/l).
Therefore, the appropriate pH for *O.niloticus* larvae ranged from 6.5 to 8.5, and the suitable pH was 7.5 as shown in Table (6) and Fig. (2c).

Table 1. Effect of pH on the mortality rate of yolk sac fry and fertilized eggs of *Oreochromis niloticus* after 96 hours of post-hatching (LC$_{50}$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH</th>
<th>Time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (hours)</td>
<td>Control</td>
<td>4</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>75</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>96</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

Mortality rate %: 37 100 55 50 35 42 90 100

Number of fertilized eggs in the different tests was 10

Table 2. Influence of various degrees of pH values on the hatching rate and abnormalities in fry after 45- hour incubation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH (Avg±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (hours)</td>
<td>Control</td>
</tr>
<tr>
<td>Hatching rate %</td>
<td>71.66 ± 0.12</td>
</tr>
<tr>
<td>Abnormalities rate %</td>
<td>24.66 ± 2.05</td>
</tr>
<tr>
<td>Incubation time (hrs)</td>
<td>22.33 ± 2.05</td>
</tr>
</tbody>
</table>

Table 3. Influence of pH on the means of growth in length (mm) and weight (mg) after 20 days of post-hatching

<table>
<thead>
<tr>
<th>Variable</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of length (mm)</td>
<td>Control</td>
</tr>
<tr>
<td>(mm)</td>
<td>1.59 (-0.25)</td>
</tr>
<tr>
<td>Mean of weight (mg)</td>
<td>Control</td>
</tr>
<tr>
<td>(mg)</td>
<td>0.051 (+0.021)</td>
</tr>
<tr>
<td>Survival rate %</td>
<td>Control</td>
</tr>
<tr>
<td>(%)</td>
<td>77.33 (+2.52)</td>
</tr>
</tbody>
</table>

Number of measured larvae in each treatment was 30 larvae; standard deviation in each treatment is put between brackets.
Table 4. Influence of pH on the means of length (mm) and weight (mg) and survival rate of larvae after 35 days of post-hatching

<table>
<thead>
<tr>
<th>Variable</th>
<th>pH</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>5.5</td>
<td>6.5</td>
<td>7.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>1.87(+)</td>
<td>1.65(+)</td>
<td>2.16(+)</td>
<td>2.1(+)</td>
<td>1.78(+)</td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>0.12(+)</td>
<td>0.083(+)</td>
<td>0.20(+)</td>
<td>0.13(+)</td>
<td>0.07(+)</td>
</tr>
<tr>
<td>Survival rate %</td>
<td>75(+)</td>
<td>65.33(+)</td>
<td>72.00(+)</td>
<td>69.33(+)</td>
<td>64.33(+)</td>
</tr>
<tr>
<td>Condition factor</td>
<td>2.040(+)</td>
<td>1.602(+)</td>
<td>2.099(+)</td>
<td>1.635(+)</td>
<td>1.334(+)</td>
</tr>
</tbody>
</table>

Table 5. Effect of different degrees of pH on survival rates of larvae after 20 and 35 days of post-hatching

<table>
<thead>
<tr>
<th>pH</th>
<th>20 DPH Avg ± SD</th>
<th>35 DPH Avg ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.33 ± 2.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.00 ± 3.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5.5</td>
<td>7.00 ± 1.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.5</td>
<td>50.67 ± 4.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>65.33 ± 2.52&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>7.5</td>
<td>73.33 ± 4.73&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>72.00 ± 1.00&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>8.5</td>
<td>68.67 ± 3.51&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>69.33 ± 2.52&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>9.5</td>
<td>63.67 ± 3.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>64.33 ± 3.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in the same column with different superscripts are significantly different at $P < 0.05$. 

Number of measured larvae in each treatment was 30; standard deviation in each treatment is put between brackets.
**Table 6.** Influence of pH on the mean of secreted enzyme (µg/l) for larvae after 40 days of post-hatching

<table>
<thead>
<tr>
<th>pH</th>
<th>Amylase</th>
<th>Alk phosphatase</th>
<th>Lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg ± SD</td>
<td>Avg ± SD</td>
<td>Avg ± SD</td>
</tr>
<tr>
<td>control</td>
<td>10.20 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.82 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.67 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5.5</td>
<td>1.50 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.67 ± 0.32&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.5</td>
<td>5.50 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.81 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.27 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7.5</td>
<td>8.27 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.90 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.17 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>8.5</td>
<td>4.27 ± 0.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.22 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.53 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>9.5</td>
<td>1.70 ± 0.10&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.04 ± 0.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.47 ± 0.15&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in the same column with different superscripts are significantly different at $P < 0.05$.

The concentration of amylase, alkaline phosphates and lipase enzymes were calculated in µg/l and replicated three times and the means were recorded.
Fig. 1. Influence of different degrees of pH on fertilized eggs of early life stage larvae; the pictures were picked by a digital camera.

a) Unfertilized eggs. normal eggs X16
b) fertilized eggs under pH (5.5) newly hatched larvae X16
c) fertilized eggs under pH (7.5) the fry appeared in normal state X16
d) fertilized eggs under pH (6.5) the mouth is closed and the eye is not completely formed
e) fertilized eggs under pH (8.5) the fry appeared in deformed state X16
f) fertilized eggs under pH (9.5) the fry was shortening in the body length X16
Fig. 2. The relation between the concentrations in (µg/l) of (a) Amylase (b) Alkaline phosphatase, and (c) Lipase enzymes and different degrees of pH values.
The pH value is an important indicator expressing the strength of acidity and alkalinity in water. The variation of pH has a direct and/or indirect influence on the survival rate and growth rate, as well as the digestive enzyme activity of aquatic organisms (Ye et al., 2013). Therefore, the present study concerned the effect of pH on growth performance, mortality and hatched rates in the early life stage larvae and their effect on secretion of enzymes. The recent studies on the larvae of *O. niloticus* showed that the percentage of mortality rate of early life stage larvae increased with increasing pH after a period of 96hrs of incubation. In this respect, Nchedo and Chjiioke (2012) studied the hatching success of *Clarias gariepinus* larvae and found that the larval activity decreased at low and high pH, whereas the larvae are very active at pH 7.5 and 8.5. Similar results were obtained in the present study in which the lowest value of mortality was recorded between the percentage of control group and that found in the treated group with pH 7.5 (*P*< 0.05). The most newly hatched fry died after being exposed to high acidity (4-5) or in more alkaline water at pH more than 10.5. The problems at the present days is the inadequate supply of fish seed to fish farmers due to a large-scale of mortalities of fish occurring in the early-life stage (Nchedo & chajioke, 2012). In the present study, the hatchability decreased after the eggs and newly hatched larvae exposed to high acidity (pH 4 or more). Similar results have been reported when high pH decreased the hatchability in the study of Trajnar (1977) and reduction in eggs viability as stated by Menendez (1976) and Beamish (1976). In the present study, the effect of pH on the incubation time of newly hatched larvae showed that, the incubation time is short in the hatched eggs of control group or after exposed to pH 7.5-7.8. Other studies were carried out on the effect of pH on the time required for development and the hatching in eggs of *clarias gariepinius*. It was noted that, when the incubation time exceeded 17 hours at pH 4.5 and 9.5, no hatching occurred at pH 4 and pH 10 (Nchedo & Chjiioke, 2012).

However, the results obtained by Carriek (1979) also showed no relationship between pH and the onset of hatching eggs of various salmonids including brown trout. The effect of low pH (4) in our study on hatching success did not explain this phenomenon; however, other reseachers found that both the activities of the hatching enzyme (chorinase) in the larvae are limited at low pH (Haya & Wainwood, 1981). While, Rask (1983) studied the effect of low pH on the development of perch and found that, there was no eggs’ mortality rate at pH 4- 6. The author added that, in pH 5, the mortality rate was less than 10.0 %; at pH 4.0, the mortality rate was 59%. The reason for longer development times may be attributed to the inhibition of hatching enzyme (chorionase) due to the effect on pH (Hagenmaier, 1974).

In the present study, the reason for longer development times may result from the effect of low pH (4.5, 5). However, the mortality rate decreased after the fertilized eggs were exposed to pH 7.5, and these reasons were not explained as described before. In
addition, lower activity of the alvins low pH may result in failure to rupture of the chorion, and low pH may reduce some damage in the structure of the chorion, making it more difficult to break (Peterson et al., 1980).

The present results showed that, the effect of pH on growth in length in the larvae after 35 days of post-hatching is more significant than the earlier stage of larvae 20 days of post-hatching.

The pH of a solution is among the many abiotic factors affecting the survival rate growth, reproduction and distribution of aquatic animals including fish. In the present study, the low pH towards the acidity (5.5) or towards alkalinity (9.5) is more effective on the growth in length and weight of fish. Whereas, the pH of 7.5 and pH in the untreated group (controls) are less effective, regarding the growth and the survival rate. It was assumed that, among the causes of fish death in very acidic water is the failure to regulate their internal ions content associated with a reduction in ions uptake rates (laurent et al., 2000).

It seems that, pH tolerance in fishes varies by species. For Oreochromis niloticus, under the present study, the pH ranged from 6.5 to 7.5. However, in the other species, the tolerance ranges are different; e.g. for sticklebacks, pH 4.5, Cichlids 6.5-7.2, perch 4.5 - 6.9, and for Clarias gariepinus, it ranges from 6.5- 8. Ndubuisi et al. (2015) found that, the decline in growth rate for the fry of Clarias gariepinus in pH treatment (5,8 and 9) could be attributed to the imbalance in homeostasis, since low or high pH is not directly lethal on fish growth and reproduction. In this context, after the larvae were exposed to high pH for a long period in the current study, clinical signs appeared in shortening in the body lengths. The mortality rate increased and the skin changed into dark black color and finally the larvae died. These observations coincide with those of El-Greisy et al. (2016) who recorded similar changes on the larvae of Oreochromis niloticus after exposed with ammonia for 30 days of post-hatching. In this respect, the deformities which are observed in pH 6.5 - 8.5 may be due to the spinal damage of the larvae, spinal flexures of the larvae and stresses during ontogenic development as described in the study of Onuoha and Nwadukwe (1990).

Many researches have been conducted on enzymes in the last two decades. In this respect, Cahu and Zambonimo (2001) studied the digestive ability and specific nutritional requirement of fish larvae and juveniles. Ontogenesis of digestive enzymes and development features of digestive tract have been well documented in several species such as sole (Ribeiro et al., 1999), red drum (Buchet et al., 2000) and Turbat (Hochne-Reitan et al., 2001).

Unfortunately, few studies were carried out on the enzymatic secretions and their relation with the effect of different degrees of pH. As in the present study, the effect of low pH (5.5) affected the secretion of amalyses enzyme (1.50+0.081 mg/l). This value increased and reached its maximum in secretion after being impacted with pH 7.5, and then decreased and reached 1.70+ 0.08 after pH 9.5. In the respect to the alkaline
phosphatase which plays a vital role in digestion, absorption and transition of nutrients as described by Swarup et al. (1981) and forms an important regulative enzyme in biometabolic process Satyayaneson (1985).

Identical to the present study, the alkaline phosphate (Alk. phosphatase) is secreted in small amounts in low pH 5.5. This value increased and reached the maximum value after affected (the larvae at 40 days of old) with pH 6.5 and 7.5. This value of Alk.ph reached the minimum value after pH was 9.5. Feng et al. (2003) found that, the alkaline phosphatase distributed in the shallow and striated border in fish intestinal of epithelial cells. The present study did not include the histological study to declare the enzymatic activity inside the cells, and in the future study, the work would be concerned with this important point. The lipase activity of larvae reached its highest value at four days of old, which may be related to the absorption of the yolk sac (Guan et al., 2006; Wu et al., 2007).

In the present study, the lipase activity secreted in small amounts after treated with pH 5.5 and increased, reaching the maximum value after pH was 7.5 and measured 7.16 ± 0.124mg/ l. Few studies have been conducted on the influence of pH on digestive of enzyme activity of fishes. In this respect, Ye et al. (2013) addressed Odontobus obscure and found that, when pH was between 6 and 10, the protease activity, amylase activity and lipase activity, all presented a trend of first increase, followed by a decrease with increasing the pH value. The authors also added that, the pH didn't only directly influenced the growth and digestive enzyme activity but also the metabolic of larvae.

**CONCLUSION**

It can be concluded that, the effect of pH on hatching success and mortality rate of larvae were recorded during the period of incubation time. Acidification delayed the incubation time of hatching and reduction on eggs viability. The mortality rate and deficiency in weights increased after the larvae were exposed to low pH (4-5) or more alkalinity (pH 10 or more). The enzymatic activity (amylase, lipase and alkaline, phosphatase) is more affected by the low pH (4-5) or towards more alkalinity (pH 10 or more). The best results of pH on enzyme activity and growth performance ranged from 7.5 to 8.5 as previously explained in the present study.

**REFERENCES**


